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Caractérisation des composés volatils responsables des qualités odorantes du saumon fumé (*Salmo salar*) et évaluation des contaminants du fumage (Hydrocarbures Aromatiques Polycycliques)

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THÈSE DE DOCTORAT
Discipline : Sciences de l'aliment
Spécialité : Chimie des arômes

Présentée
et soutenue publiquement par

Vincent VARLET

Le 25 octobre 2007, devant le jury ci-dessous

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Les résultats obtenus au cours de cette thèse ont été valorisés sous la forme de publications, communications et participations à congrès suivantes :

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VARLET, V., SEROT, T., KNOCKAERT, C., CORNET, J., CARDINAL, M., MONTEAU, F., LE BIZEC, B., PROST, C. (2007). Organoleptic characterization and PAH content of salmon (*Salmo salar*) smoked according to four industrial smoking techniques. *Journal of Science and Food Agriculture*, 87(5), 847-854.

VARLET, V., PROST, C., SEROT, T. (2007). Volatile aldehydes in smoked fishes : analysis methods, occurence and mechanisms of formation. *Food Chemistry*, 105, 1536-1556.

VARLET, V., SEROT, T., MONTEAU, F., LE BIZEC, B., PROST, C. (2007). Determination of PAH profiles by GC-MS/MS in salmon muscle processed according to four different smoking techniques. *Food Additives and Contaminants*, 24(7), 744-757.

VEYRAND, B., BROSSEAUD, A., SARCHER, L., **VARLET, V.,** MONTEAU, F., MARCHAND, P., ANDRE, F., LE BIZEC, B. (2007). Innovative method for determination of 19 polycyclic aromatic hydrocarbons in food and oil samples using gas chromatography coupled to tandem mass spectrometry based on an isotope dilution approach. *Journal of Chromatography* A, 1149(2), 333-344.

VARLET, V., SEROT, T., CARDINAL, M., KNOCKAERT, C., PROST, C. (2007). Olfactometric determination of the most potent odor-active compounds in salmon muscle (*Salmo salar*) smoked by using four smoke generation techniques. *Journal of Agricultural and Food Chemistry*, 55, 4518-4525.

VARLET, V., SEROT, T., CARDINAL, M., COURCOUX, P, KNOCKAERT, C., CORNET, J., PROST, C. (2008). Relationships between sensorial characteristics and the most odorant volatile compounds of salmon smoked by four industrial smoking techniques. *Food Chemistry*, soumise.

VARLET, V., SEROT, T., PROST, C. (2008). Innovative gas chromatography-olfactometric method : gas chromatography-Concentration/Omission of Odorants at Liquid state (GC-COOL). Application to smoked salmon (*Salmo salar*) treated by liquid smoke. En rédaction.

Chapitres d'ouvrages :

VARLET, V., PROST, C., CARDINAL, M., CORNET, J., KNOCKAERT, C., SEROT, T. (2007). Influence of smoking processes on odorant properties of smoked salmon. Proceedings of 5th International Congress of Food Technology : Consumer protection through food process improvement & Innovation in the Real World », E.S. Lazos, Eds., vol. 2, 140-147.

VARLET, V., SEROT, T., CARDINAL, M., COURCOUX, P., CORNET, J., KNOCKAERT, C., PROST, C. (2007). Relationships between odorant characteristics and the most odorant volatile compounds of salmon smoked by four industrial smoking techniques. Proceedings du congrès : "14th EUROFOOD CHEM : Food quality, an issue of molecule based science", H. This & T. Eklund, Eds., vol.1, 156-159.

VARLET, V., SEROT, T., PROST, C. (2008). Additives : smoke flavorings. In : *Handbook of Seafood and Seafood Products Analysis* (Nollet, L & Toldra, F., Eds). CRC Press (Francis & Taylor). En rédaction.

Communications orales :

VARLET, V. Caractérisation aromatique du fumage. « séminaire GEPEA-UMR CNRS 6144 », Piriac/mer (France), 30-31 Mai 2005.

VARLET, V. Caractérisation odorante et sanitaire des différents types de fumage industriel : Application au saumon fumé. « Forum National des Doctorants de l'école doctorale de Chimie-Biologie », Nantes (France), 30 Mai 2006.

VARLET, V., PROST, C., CARDINAL, M., CORNET, J., KNOCKAERT, C., SEROT, T. Influence of smoking processes on odorant properties of smoked salmon. « 5th International Congress on Food Technology », Thessalonique (Grèce), 9-11 Mars 2007.

VARLET, V., PROST, C., VEYRAND, B., MONTEAU, F., LE BIZEC, B., SEROT, T. The ambiguity of liquid smoke condensates use in food processes. « 5th International Congress on Food Technology », Thessalonique (Grèce), 9-11 Mars 2007.

VARLET, V., SEROT, T., CARDINAL, M., COURCOUX, P., CORNET, J., KNOCKAERT, C., PROST, C. Relationships between odorant characteristics and the most odorant volatile of salmon smoked by four industrial smoking techniques. "EUROFOOD CHEM XIV", Paris (France), 29-31 Août 2007.

Communications par affiches :

MONTEAU, F., SARCHER, L., **VARLET, V.,** MAUME, D., ANDRE, F., LE BIZEC, B. Compared mass spectrometry approches for PAH determination in food. « Dioxin 2005/ISPAC 20 congress », Toronto (Canada), 21-26 Août 2005.

VARLET, V., SEROT, T., MONTEAU, F., LE BIZEC, B., PROST, C. Evaluation par GC-MS/MS des hydrocarbures aromatiques polycycliques dans le saumon fumé par différentes techniques de fumage. « 23èmes Journées Françaises de Spectrométrie de

Masse/4èmes Journées Françaises des Isotopes Stables », Nantes (France), 11-14 Septembre 2006.

VARLET, V., SEROT, T., CARDINAL, M., KNOCKAERT, C., MONTEAU, F., LE BIZEC, B., PROST, C. Olfactometric characterization and PAH occurrence in smoked salmon by four different techniques of smoke production. «13th IUFOST congress », Nantes (France), 17-21 Septembre 2006.

VARLET, V., PROST, C., CARDINAL, M., KNOCKAERT, C., CORNET, J., MONTEAU, F., LE BIZEC, B., SEROT, T. Odorant and Sanitary Differences between Salmon Smoked Traditionally and Salmon Treated by Liquid Smoke. « 5th International Congress on Food Technology », Thessalonique, (Grèce), 9-11 Mars 2007.

VARLET, V., SEROT, T., PROST, C. (2007). Un nouveau couplage de chromatographie en phase gazeuse : le système GC-COOL (Gas Chromatography-Concentration Omission of Odorants at Liquid state). « Forum National des Doctorants de l'école doctorale de Chimie-Biologie », Nantes (France), 5 Juin 2007.

Autres collaborations / expertises nationales et internationales :

Audité par : Arômes Ingrédients et Additifs (AIA) (Paris, France).
PETITDIDIER, C. Dossier : Fumées liquides et arômes de fumée. Arômes Ingrédients et Additifs, N°64- Juin/Juillet 2006, 60-62.

Audité par : Feedinfo News Services (Global Data Systems) (Labege, France).
VARLET, V., PROST, C., SEROT, T. (2006). New method for the evaluation of representativeness of aromatic extracts from processed food as smoked salmon. Feedinfo News Service 2006, feedinfo.com, Scientific Reviews.

Audité par : La Revue de l'Industrie Agroalimentaire (RIA) (Paris, France).
MEYER, H.L. Fumage: les procédés classiques rattrapés par la brumisation. Revue de l'Industrie Agroalimentaire, N° 674- Décembre 2006, 46-48.

Expertise et participation : The proposed draft code of practice for the reduction of contamination of food with polycyclic aromatic hydrocarbons (PAH) from smoking and direct drying processes (FAO-WHO CX/CF 07/1/16 (E)). JOINT FAO/WHO FOOD STANDARDS PROGRAMME CODEX COMMITTEE ON CONTAMINANTS IN FOODS. First session, Beijing, Chine, 16-20 Avril 2007.

Participations à congrès sans présentation :

Congrès « La Recherche et l'Entreprise », Ecole Centrale de Nantes, Nantes (France), 14-15 Février 2006.

Congrès « Arômes Alimentaires » DGCCRF, Université de Provence, Marseille (France), 5 Décembre 2006.

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Ce travail vous est dédié.

LISTE DES ABBRÉVIATIONS

ABVT : Azote Basique Volatil Total

AED : Atomic Emission Detector (Détecteur d'Emission Atomique)

AEDA : Aroma Extract Dilution Analysis

AFSSA : Agence Française de Sécurité Sanitaire des Aliments

AG : Acides Gras

AGI : Acides Gras Insaturés

AGPI : Acides Gras PolyInsaturés

AGS : Acides Gras Saturés

AMP : Adénosine MonoPhosphate

ASE : Accelerated Solvent Extraction (Extraction Accélérée par Solvants)

CHARM : Combined Hedonic Aroma Response Measurement

COV : Composés Organiques Volatils

DGCCRF : Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes

DLC : Date Limite de Consommation

DMA : Diméthylamine

DSV : Distillation Sous Vide

EDS : Extraction Distillation Simultanée

FD : Fluorimetric Detector (Détecteur Fluorimétrique)

FID : Flame Ionisation Detector (Détecteur à Ionisation de Flamme)

GC : Gas Chromatography (Chromatographie en phase Gazeuse)

GC-COOL : Gas Chromatography-Concentration Omission of Odorants at Liquid state

GC-GOOD : Gas Chromatography-Global Odorants Omission Detection

GC-FID : Gas Chromatography- Flame Ionisation Detector (Chromatographie en phase Gazeuse couplée à un Détecteur à Ionisation de Flamme)

GC-MS : Gas Chromatography-Mass Spectrometry (Chromatographie en phase Gazeuse couplée à la Spectrométrie de Masse)

GC-MS/MS : Gas Chromatography-Tandem Mass Spectrometry (Chromatographie en phase Gazeuse couplée à la Spectrométrie de Masse en Tandem)

GC-O : Gas Chromatography-Olfactometry (Chromatographie en phase Gazeuse couplée à l'Olfactométrie)

GC-MS/O : Gas Chromatography-Mass Spectrometry and Olfactometry (Chromatographie en phase Gazeuse couplée à la Spectrométrie de Masse et à l'Olfactométrie)

HACA : Hydrogen abstraction / ACetylen Addition (Abstraction d'un atome d'Hydrogène / Addition d'un groupement ACétylène)

HAP : Hydrocarbures Aromatiques Polycycliques

INERIS : Institut National de l'Environnement industriel et des RISques

ITD : Ion Trap Detector (Détecteur à Trappe Ionique)

LC : Liquid Chromatography (Chromatographie en phase Liquide)

LC-FD : Liquid Chromatography- Fluorimetric Detector (Chromatographie en phase Liquide couplée à un Détecteur Fluorimétrique)

LC/GC-MS : Liquid Chromatography/Gas Chromatography-Mass Spectrometry (Chromatographie en phase Liquide couplée à la Chromatographie en phase Gazeuse couplée à la Spectrométrie de Masse)

LC/LC : Liquid Chromatography/Liquid Chromatography (Chromatographie en phase Liquide couplée à la Chromatographie en phase Liquide)

LC/LC-FD : Liquid Chromatography/Liquid Chromatography-Fluorimetric Detector (Chromatographie en phase Liquide couplée à la Chromatographie en phase Liquide couplée à un Détecteur Fluorimétrique)

LC/LC-UV : Liquid Chromatography/Liquid Chromatography-UltraViolet (Chromatographie en phase Liquide couplée à la Chromatographie en phase Liquide couplée à un détecteur Ultraviolet)

LC-MS : Liquid Chromatography-Mass Spectrometry (Chromatographie en phase Liquide couplée à la Spectrométrie de Masse)

LC-MS/MS : Liquid Chromatography-Tandem Mass Spectrometry (Chromatographie en phase Liquide couplée à la Spectrométrie de Masse en Tandem)

LC-UV : Liquid Chromatography-UltraViolet (Chromatographie en phase Liquide couplée à un détecteur UltraViolet)

MS : Mass Spectrometry (Spectrométrie de Masse)

MS/MS : Tandem Mass Spectrometry (Spectrométrie de Masse en Tandem)

NIF : Nasal Impact Frequency

OTMA : Oxyde de Triméthylamine

PFPD : Pulsed Flame Photometric Detector (Détecteur Photométrique à Flamme Pulsée)

PLE : Pressurised Liquid Extraction (Extraction par Solvants sous Pression)

PLS : Partial Least Squares

POP : Polluants Organiques Persistants

RMN : Résonance Magnétique Nucléaire

SBSE : Stir Bar Sorptive Extraction (Extraction par Barreau Aimanté Absorbant)

SFE : Supercritical Fluid Extraction (Extraction par Fluide Supercritique)

SNIF : Surface of Nasal Impact Frequency

SPE : Solid-Phase Extraction (Extraction en Phase Solide)

SPME : Solid-Phase MicroExtraction (Microextraction en Phase solide)

SRM : Single Reaction Monitoring

TEF : Toxic Equivalent Factor (Facteur d'Equivalence Toxique)

TEQ : Toxic Equivalent Quantity (Quantité d'Equivalence Toxique)

TMA : Triméthylamine

TOF : Time-Of-Flight detector (Détecteur à Temps de vol)

UV : UltraViolet

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PRÉAMBULE

Ces travaux de recherche ont été menés dans le cadre de ma thèse de doctorat de Chimie-Biologie en Sciences des Aliments au sein du Laboratoire de Biochimie Alimentaire et industrielle (LBAI) de l'Ecole Nationale des Ingénieurs des Techniques pour les Industries Agricoles et Alimentaires de Nantes (ENITIAA) dont je suis sorti diplômé en 2004. La réputation de ce laboratoire a été construite sur l'étude de la qualité aromatique des produits de la mer. Depuis que le Pr Carole PROST en a pris la direction en 2004, les thématiques se sont élargies et organisées en trois axes de recherche (analyse olfactométrique et influence des composés volatils sur la perception odorante, interactions propriétés sensorielles/arômes et interactions procédés/produits) déclinés sur des produits alimentaires tels que les produits de la mer, les végétaux ou les produits céréaliers, d'où l'intégration du LBAI ENITIAA à l'UMR CNRS 6144 GEPEA. Au cours de l'élaboration de ma thèse, j'ai collaboré avec le laboratoire de Sciences et Techniques des Aliments Marins (STAM) du centre nantais de l'Institut Français de Recherche pour l'Exploitation de la MER (IFREMER) pour tout ce qui concernait les procédés de fumage et caractéristiques sensorielles du saumon fumé. J'ai également travaillé avec le LABoratoire d'Etudes des Résidus et Contaminants dans les Aliments (LABERCA) de l'Ecole Nationale Vétérinaire de Nantes (ENV) pour l'analyse des contaminants du fumage dans le saumon fumé.

Les résultats présentés dans ce mémoire sont donc issus de projets réalisés lors de ces trois dernières années, la plupart en collaboration avec le laboratoire STAM et le LABERCA, et dont certains ont nécessité l'encadrement d'étudiants de premier et de second cycle universitaire et d'élèves-ingénieurs de l'ENITIAA. Chaque projet ayant fait l'objet de publications scientifiques, nous avons d'un commun accord décidé avec mes encadrants, le Pr Thierry SEROT et le Pr Carole PROST, de traiter la « caractérisation aromatique et sanitaire du saumon fumé » sous la forme d'une thèse doctorale organisée sur publications.

INTRODUCTION

Le fumage du poisson est un procédé ancestral dont l'objectif premier est la conservation. De nos jours, la consommation de ces produits revêt un caractère plus festif et la demande des consommateurs est très variable vis-à-vis de la perception du caractère « fumé ». En effet, aujourd'hui, le fumage est plus utilisé pour les qualités organoleptiques (arômes, saveurs, texture, …) qu'il confère aux aliments subissant ce procédé que pour ses propriétés de conservation. Le problème posé aux industriels est donc de fabriquer une gamme de produits répondant à l'attente des consommateurs tout en assurant des garanties de conservation. Les professionnels du fumage sont également confrontés à d'autres problèmes. En effet, en dépit d'améliorations importantes des procédés industriels de fumage qui ont conduit à une réduction significative dans les aliments fumés des hydrocarbures aromatiques polycycliques (HAP), contaminants génotoxiques et carcinogènes formés lors du fumage, il subsiste toujours un doute sur l'innocuité de tels aliments pour la santé. Un autre problème majeur est lié à des contraintes environnementales. Les directives européennes tendent à imposer la diminution des rejets de composés organiques volatils (COV) dans l'atmosphère. Ainsi, dans certains pays, pour réduire les émissions de fumée, les industriels utilisent de plus en plus des procédés de fumage à l'aide de condensats de fumée liquide. Cependant, si les taux de HAP dans les fumées liquides sont aujourd'hui connus, celui dans les aliments traités par fumée liquide l'est beaucoup moins, tout comme l'impact organoleptique que confère ce type de condensats à l'aliment. Dans ce climat d'incertitude, la législation a du mal à statuer sur ces procédés émergents.

Toutefois, il apparaît que dans un avenir plus ou moins proche, le contexte sera peu favorable au fumage traditionnel tel qu'il est pratiqué en France. Cependant, la France est le premier producteur européen de saumon fumé, il est donc important pour ce secteur industriel d'anticiper cette évolution par la mise en place d'une recherche active soit pour le développement et la maîtrise de procédés nouveaux, soit pour l'amélioration des procédés traditionnels afin de satisfaire aux contraintes environnementales et sanitaires tout en répondant favorablement à la demande variée des consommateurs quant aux caractéristiques organoleptiques du saumon fumé.

Il convient donc de caractériser et quantifier les composés volatils contribuant à la perception de l'odeur de la chair de saumon fumé. Après avoir identifié les précurseurs et les mécanismes de formation de ces composés, les différentes méthodes de fumage industriel pourront être comparées dans la perspective de leur optimisation quant aux exigences organoleptiques attendues. Simultanément, il est nécessaire d'évaluer la contamination en HAP dans les produits fumés engendrée par les différentes techniques de fumage.

L'ensemble de ces travaux devrait donc permettre d'une part de contribuer à la détermination des teneurs et de la nature des composés contribuant à la flaveur du saumon fumé selon les différentes méthodes de fumage et d'autre part de cartographier la contamination en HAP apportée par les différents types de fumage. L'étude individuelle ou en mélanges des composés volatils odorants du saumon fumé permettra de caractériser les interactions odorantes entre eux et avec la matrice afin d'orienter certains paramètres de fumage impliqués dans leur formation. Ainsi, on pourra favoriser le dépôt de composés ayant un impact organoleptique recherché tout en limitant la formation des HAP dans l'objectif d'élaborer ainsi des produits répondant mieux aux attentes des consommateurs au niveau de la flaveur « fumé ». Les résultats obtenus permettront ainsi d'aborder d'une manière plus rationnelle la mise en œuvre des procédés de fumage dans la filière halieutique, conduisant à une meilleure maîtrise des procédés pour anticiper les évolutions légales en matière d'environnement et de sécurité alimentaire. Ces résultats pourront également constituer une base de connaissance sur les différentes méthodes de fumage applicables à d'autres produits fumés comme ceux de la filière carnée.

ETUDE BIBLIOGRAPHIQUE

1.1 Saumon et fumage

1.1.1 Marché et contexte économique

Le marché des salmonidés s'est développé très rapidement au cours des dernières décennies. Le moteur de cette croissance a été l'aquaculture dont la production est passée de moins de 15 000 tonnes de saumon en 1980 à 1 200 000 tonnes en 2002 (OFIMER, 2004). Outre ses qualités organoleptiques, le succès du saumon provient de la multitude de ses modes de commercialisation (frais, congelé, fumé, en conserve, …). Les principaux producteurs de saumon de pêche sont les Etats-Unis, le Japon et la Russie, assurant 97 % de la production mondiale avec 785 200 tonnes environ en 2002. La production de saumon d'élevage est essentiellement assurée par la Norvège, le Chili, le Royaume-Uni et le Canada avec 91 % de la production mondiale soit 945 000 tonnes environ en 2002.

Le premier importateur de saumon frais est constitué par les Etats-Unis qui achètent principalement des filets en provenance du Chili. La France constitue le second importateur mondial et, comme le Danemark (troisième importateur), achètent surtout du saumon entier frais, essentiellement en provenance de Norvège. La Norvège est ainsi responsable de la moitié des exportations mondiales de saumon frais en vendant à l'Union Européenne (65 %) et au Japon (11 %).

Le marché du saumon congelé concerne surtout le Japon, principal acheteur avec 160 000 tonnes devant la Chine (43 000 tonnes) et la France (10 000 tonnes). Le plus gros exportateur de saumon congelé est le Chili destinant sa production au Japon mais également de plus en plus aux Etats-Unis.

Les échanges de saumon en conserve sont en baisse alors qu'ils étaient historiquement la principale forme d'échange de saumon. Le premier acheteur de conserves de saumon est le Royaume-Uni (41 000 tonnes en 2001), devant le Canada (17 000 tonnes) et l'Australie (10 000 tonnes), les trois quarts de ces conserves étant produites par les Etats-Unis.

Compte-tenu des volumes d'échange et de la forte valeur ajoutée conférée à ce produit, le marché du saumon est en pleine expansion. Le saumon fumé constitue essentiellement un marché européen (OFIMER, 2004). En effet, les importations des Etats-Unis sont destinées à la consommation à l'état frais ou congelé principalement, ou à la transformation en conserves. Le saumon fumé est consommé aux Etats-Unis sous la forme de poissons fumés à

chaud. En outre, les Américains sont très friands du goût fumé, d'où l'emploi massif et le développement de fumées liquides.
L'Allemagne est le plus gros acheteur de saumon fumé (8 500 tonnes en 2001), devant l'Italie (6 500 tonnes) et la France (3 000 tonnes). En 2001, la France était l'un des leaders des pays producteurs de saumon fumé (19 000 tonnes) suivie par le Danemark (15 660 tonnes), l'Allemagne (15 100 tonnes) et la Grande-Bretagne (10 250 tonnes) (EUROSALMON, 2003). En 2003, le Danemark constituait le premier fournisseur de saumons fumés en réalisant la moitié des exportations mondiales. Il est à souligner que la France réalise la moitié de ses exportations sous la forme de saumons fumés et reste parmi l'un des premiers fournisseurs de saumon fumé, saumon provenant presque exclusivement de l'importation. Le fumage entraîne une plus value sur la valeur ajoutée du saumon. Ainsi, le saumon occupe une place de choix dans la filière halieutique mondiale et alimente un marché européen du poisson fumé très dynamique. Compte tenu de l'engouement européen pour ce type de produits, la filière « saumon fumé » est en pleine expansion (EUROSALMON, 2003). Des développements technologiques ont vu le jour, accompagnés de nouvelles législations garantissant la sécurité sanitaire, permettant de pluraliser l'offre en saumons fumés. Il est donc primordial de contrôler ces procédés et leurs effets organoleptiques et sanitaires.

1.1.2 Le produit saumon fumé

En France, le produit alimentaire « saumon fumé » le plus vendu en supermarchés est le saumon fumé à froid présenté en tranches (*Salmo salar*), emballé sous vide. A l'étal du poissonnier, on peut retrouver des produits plus artisanaux. Le fumage est alors réalisé par le commerçant et s'avère de qualité moins ocntstante que le fumage industriel.
Les saumons proviennent de Norvège, d'Ecosse ou d'Irlande principalement et sont fumés en France. Les différentes origines de la matière première, associées aux diverses techniques de salage et de fumage utilisées dans l'industrie, permettent de proposer sur le marché une large gamme de produit. Le consommateur dispose tout au long de l'année d'un choix important de produits, ce qui contribue progressivement à banaliser le saumon fumé. Il reste cependant difficile pour le consommateur d'identifier des critères lui permettant de choisir un produit en fonction de ses préférences. Les industriels sont conscients de cette situation et la baisse de la qualité du saumon fumé a également été rapportée par des enquêtes auprès des consommateurs et des études scientifiques. Les principales critiques des consommateurs

européens concernant la qualité du produit font référence à l'aspect, la texture en relation avec la teneur en lipides, le niveau de sel ou encore le goût sans toutefois que les défauts soient toujours identifiés. D'un autre côté, les consommateurs américains ont fait part de leurs préférences pour des odeurs et des goûts fortement fumés.

Face à ces exigences, l'industrie du saumon fumé doit faire face à un nouveau challenge : produire un aliment de qualité organoleptique prédéfinie par les goûts des consommateurs. Ceux-ci étant très variés, l'industrie du fumage doit être en mesure de s'adapter et de proposer une diversité de produits équivalente. Ainsi, une synthèse des effets des procédés de fumage sur le saumon devait être entreprise.

1.1.3 Le saumon

Le saumon est un poisson carnivore de la famille des Salmonidés. On distingue trois genres : le genre *Salmo salar* et le genre *Salmo oncorhynchus* et le genre *Salmo salvelinus*. *Salmo salar* est présent dans l'océan Atlantique et *Salmo oncorhynchus* dans l'océan pacifique. Parmi les saumons de l'Atlantique, en fonction du mode d'élevage (et donc de leur teneur en matières grasses), on distingue le saumon écossais, le saumon canadien et le saumon norvégien.

Le saumon est un poisson marin qui vit dans les zones tempérées de l'hémisphère nord (température de l'eau inférieure à 16 °C) dont le mode de vie est qualifié d'anadrome, c'est-à-dire qu'il implique une migration de la mer vers les cours d'eau pour une reproduction en eau douce. Ainsi, le juvénile d'eau douce à l'état d'alevin, va grandir jusqu'au stade tacon (de 1 à 7 ans en fonction de la zone géographique et de l'alimentation) pour enfin effectuer une migration vers la mer, précédée d'une adaptation anatomo-morphologique importante consécutive à ce bouleversement que l'on appelle « smoltification » (Ytrestøyl, T. et al., 2004).

Morphologiquement, la musculature développée du saumon lui donne une allure allongée et fuselée. Sa peau est recouverte d'écailles, de couleur bleue sombre sur le dos et argentée sur le ventre et les flancs. Sa chair est rosée. La couleur typique du saumon provient de son régime alimentaire basé sur les poissons et crustacés riches en caroténoïdes. En élevage, en plus des farines de poissons, il convient de supplémenter son alimentation par des caroténoïdes tels que la canthaxanthine ou l'asthaxanthine (Gordon Bell, J. et al., 1998). L'alimentation étant considérée comme un précurseur important des composés volatils

odorants du poisson, une attention particulière est portée à la composition biochimique de la nourriture (Sérot, T. et al., 2002).

Anatomiquement, le squelette du saumon est constitué de cinq rangées de grosses arêtes fixées sur les vertèbres de l'arête centrale. Une fois découpé, les filets mesurent entre 30 et 60 cm de long sur 16 à 17 cm de large environ pour un poids compris entre 600 et 1700 g pour des saumons entiers commercialisés (de 1 à 5 kg).

On distingue deux types de muscles :

_Un muscle brun dit muscle de nage, situé le long de la ligne latérale et autour de la nageoire, représentant 2 à 3 % de la masse musculaire totale.

_Un muscle rose constitué de bandes transversales entrelacées par de fines couches graisseuses.

La majorité du saumon destiné au fumage provient d'élevages de saumons. Le saumon d'élevage est plus gras que le saumon sauvage. Sa teneur en matières grasses varie de 6 à 22 % de la matière totale environ avec une moyenne de 14 %. Le taux lipidique du saumon (Sheehan, E.M. et al., 1996 ; Bencze Røra, A.M. et al., 2005) mais aussi d'autres poissons comme le turbot (Sérot, T. et al., 2001) est fortement influencé par l'apport en matières grasses dans son régime alimentaire (Nordgarden, U. et al., 2002 ; Berrill, I.K. et al., 2004), par la vitesse de croissance de l'animal, par son âge, sa maturité sexuelle, la quantité d'exercice physique effectué, … Ainsi, à la maturité sexuelle, de nombreux changements surviennent, notamment une augmentation de la proportion d'eau et une diminution des proportions en protéines et en lipides. Ceci peut entraîner une modification significative de l'odeur (Josephson, D.B. et al., 1991a), de la flaveur et de la texture.

Les teneurs en lipides et en protéines sont très importantes car elles influencent la texture du poisson frais et du poisson fumé (Aursand, M. et al., 1994 ; Robb, D.H.F. et al., 2002). Les constituants lipidiques et protéiques peuvent également interagir avec les composés volatils de la fumée.

En résumé, nous obtenons une composition moyenne pour la chair de saumon fumé :

- Humidité : 67 %
- Lipides : 14 %
- Protéines : 17,5 %
- Eléments minéraux : 1,5 %

Acides gras	Pourcentage
C 16	17,07
C 18:1n-9	14,61
C 22: 6	9,46
C 22:1n-9	8,16
C 20:1n-9	7,6
C 16:1	7,57
C 20:5	7,38
C 14	6,15
C 18:1n-7	3,18
C 22:5	2,97
C 18	2, 91
C 18:2	2,64
C 18:4n-3	2,34
C 18:3	1,26
C 20:1n-7	0,12
C 15	0,08

Autres

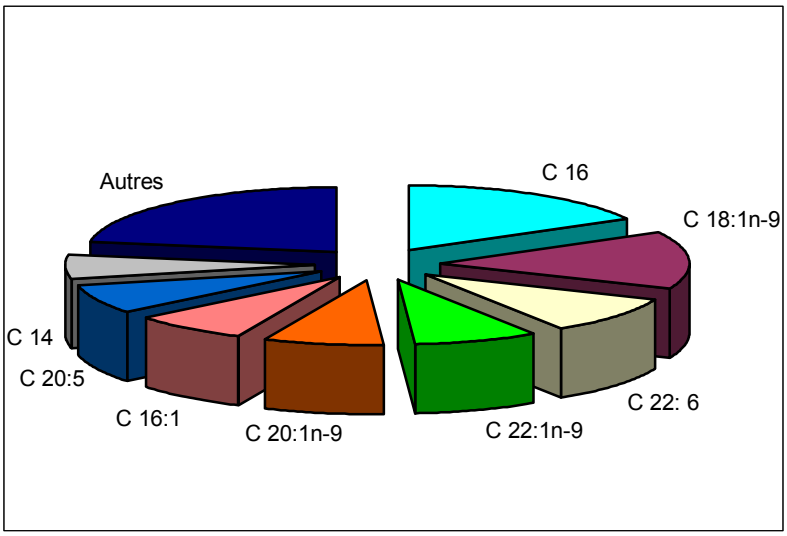


Figure 1. Proportion relative des acides gras dans la chair de saumon fumé (d'après Sainclivier, M., 1985)

Plusieurs travaux ont déjà été menés sur la composition lipidique du saumon fumé (Espe, M. et al., 2002 ; Espe, M. et al., 2004) ou non fumé (Porter, P.J. et al., 1992 ; Refsgaard, H.H.F. et al., 1998). Il y a donc dans le saumon une part très importante d'acides gras insaturés (AGI), environ 74 % des acides gras (AG) totaux et une part d'acides gras saturés (AGS) plus faible (26 %). Les acides gras polyinsaturés (AGPI) représentent environ 20 % de la part des AG totaux (Figure 1). Ces proportions sont caractéristiques des poissons. Ces AG possèdent jusqu'à 7 doubles liaisons maloniques ce qui les rend très sensibles à l'oxydation et sont d'importants précurseurs des composés volatils odorants du saumon. Ainsi, en faisant varier la composition lipidique des aliments des poissons, on peut faire varier les composés volatils odorants présents dans l'arôme des poissons comme l'ont montré des travaux réalisés sur la truite (Sérot, T. et al., 2002).

1.2 Le fumage

1.2.1 Les étapes du fumage

Hormis l'échelle de production consécutive à la mécanisation et la diversification des produits fumés, le principe du fumage reste peu éloigné de celui pratiqué depuis l'Antiquité. Il consiste, après un salage préalable dans la plupart des cas puis un séchage, à imprégner la chair de poisson avec la fumée issue de la combustion lente de bois. Le terme saurissage serait toutefois plus adapté puisqu'il englobe le salage (ou saumurage), le séchage et le fumage mais nous désignerons sous le nom de produit fumé un produit ayant subi ces trois procédés (salage, séchage, fumage).

1.2.2 L'étape de salage

Le chlorure de sodium agit comme un agent antibactérien indirect. En effet, son action a pour principale conséquence de diminuer l'activité de l'eau (Aw) et permet ainsi d'inhiber la prolifération de microorganismes d'altération (Goulas, A.E. & Kontominas, M.G., 2005).
On distingue principalement trois types de salage : à sec, en saumure ou par injection (Sigurgisladottir, S. et al., 2000 ; Gallart-Jornet, L. et al., 2007). Dans tous les cas, la pénétration du sel dans la chair du saumon se fait par un mécanisme d'osmose ce qui

provoque la migration d'eau de l'intérieur vers l'extérieur des cellules des tissus du poisson et une migration de sel de la surface des fillets jusque dans le muscle.
Dans le cas du salage à sec, il s'en suit une perte de poids puis le taux de diffusion de l'eau hors du poisson ralentit jusqu'à l'équilibre. Dans le cas du salage en saumure (solution aqueuse de sel), il y a une absorption de saumure qui entraîne un gain de poids. Enfin, le salage par injection qui consiste à faire pénétrer la saumure directement au cœur du produit à traiter à l'aide d'aiguilles, peut entraîner un feuilletage c'est-à-dire une dissociation des fibres musculaires constitutives du filet et donc avoir un impact négatif sur la texture.
L'action physique du sel est accompagnée simultanément de nombreux phénomènes chimiques. Ceux-ci concernent notamment les constituants protéiques et lipidiques de la chair de poisson, à la fois précurseurs de certains composés de l'odeur finale du poisson fumé et à la fois responsables des principales qualités organoleptiques du produit notamment la texture. Ces réactions entraînent une modification de solubilité et une dénaturation des protéines ainsi que des phénomènes de lipolyse et d'oxydations des lipides (ces mécanismes seront détaillés dans le manuscrit ultérieurement). Pour des produits industriels, la teneur cible en sel se situe aux alentours de 2,5 ~ 3 % (Knockaert, C., 1990).
Par l'élimination d'une partie de l'eau de constitution du produit, le salage provoque un raffermissement des chairs et affecte les constituants de la surface du filet afin de préparer le muscle au procédé de fumage pour que les effets de la fumée sur le produit soient optimisés. D'un point de vue sanitaire, le sel a une action antimicrobienne puisqu'il réduit l'activité de l'eau qui n'est plus disponible pour la croissance des microorganismes. D'un point de vue organoleptique, le salage empêche la décoloration et confère un goût salé au poisson.

1.2.3 L'étape de séchage

L'étape de séchage consiste en une dessiccation par évaporation de l'eau. Cette étape doit être suffisamment rapide pour empêcher le développement de microorganismes mais pas trop pour éviter un durcissement de la chair. Le principe le plus communément employé est le séchage par entraînement sous courant d'air chaud le plus sec possible. L'humidité, la température, la vitesse de l'air, le temps d'exposition et la disposition des filets sont autant de paramètres à contrôler pour diminuer la couche hydrophile formée à la surface des filets pouvant apparaître après le salage. Cette pellicule pourrait nuire au dépôt des composés de la fumée sur le filet et

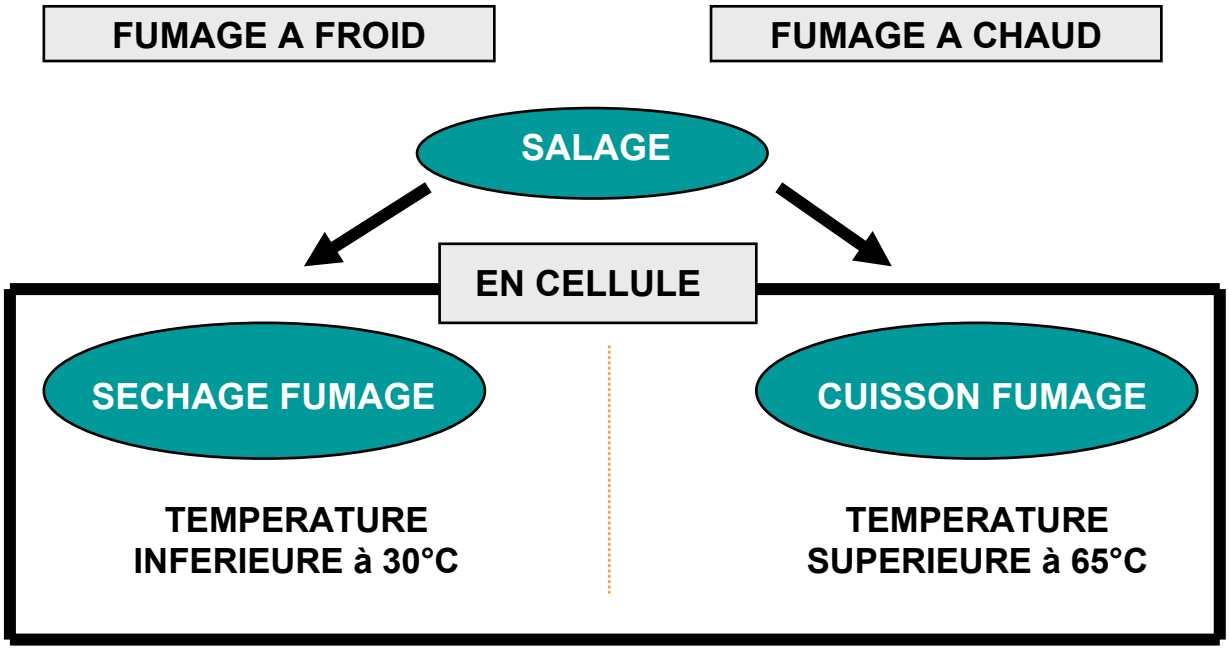


Figure 2. Diagramme de fabrication pour le fumage à froid et le fumage à chaud

avoir un impact négatif sur la conservation et les qualités organoleptiques finales du produit final.

Le séchage s'effectue uniquement juste avant le fumage à froid. Il est le plus souvent réalisé par ventilation mécanique dans l'enceinte de fumage. La température de séchage est comprise entre 17 et 25°C pour une humidité ambiante inférieure d'environ 10 % par rapport à celle de la chair dans le but d'éviter tout croûtage de la chair (Siskos, I. et al., 2005). Le croûtage correspond à la formation d'une pellicule solide formée par l'asséchement poussé du filet conduisant à une texture craquante de la surface du produit. Il est le plus souvent à éviter mais peut être parfois recherché lors du fumage à chaud.

1.2.4 L'étape de fumage

Le fumage proprement dit consiste à soumettre le produit à l'action de la fumée provenant de la combustion incomplète de bois. Les essences utilisées varient selon les pays en fonction des variétés de bois les plus présentes sur chaque territoire. De manière générale, on utilise en France le hêtre, le chêne ou le noyer. Ces matières premières seront plus détaillées dans le chapitre traitant de l'influence du bois sur la génération de composés volatils. Durant le fumage, le poisson continue de se déshydrater en même temps qu'il s'imprègne des composés volatils de la fumée. Selon la température de la cellule de fumage, on distingue deux modes de fumage : le fumage à froid et le fumage à chaud (Figure 2).

Le fumage à froid consiste en deux phases principales : une phase de séchage où le poisson doit rester cru et une phase de fumage comprise entre 17 et 28°C environ (Birkeland, S. et al., 2004). Selon les poissons, il peut durer de quelques heures à plusieurs jours. Les poissons les plus consommés dans les pays européens sont ceux fumés par cette technique excepté les pays nordiques.

Les produits fumés à chaud subissent eux aussi deux phases. La première consiste à exposer le produit à des températures aux alentours 30~40°C pendant 0,5 à 1,5 h. La seconde permet de cuire et de fumer le poisson en lui appliquant un gradient de température jusqu'à avoisiner les 70~80°C (Kołodziejska, I. et al., 2004). Cette technique est principalement utilisée dans les pays nordiques européens (Danemark, Suède, Finlande) ainsi qu'aux Etats-Unis.

Les composés de la fumée se déposent sur les filets par gravité et grâce aux courants d'air créés dans et par la géométrie de l'enceinte. Des innovations technologiques sont apparues pour accélérer ce dépôt et réduire les temps de fumage. C'est le cas du fumage par déposition

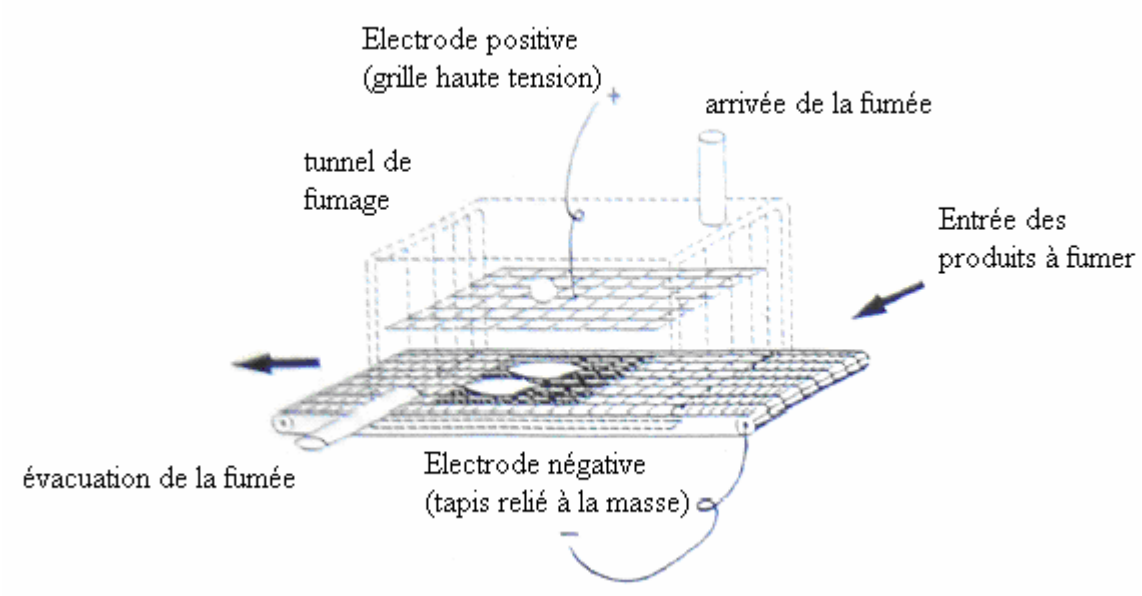


Figure 3. Fumage par déposition électrostatique

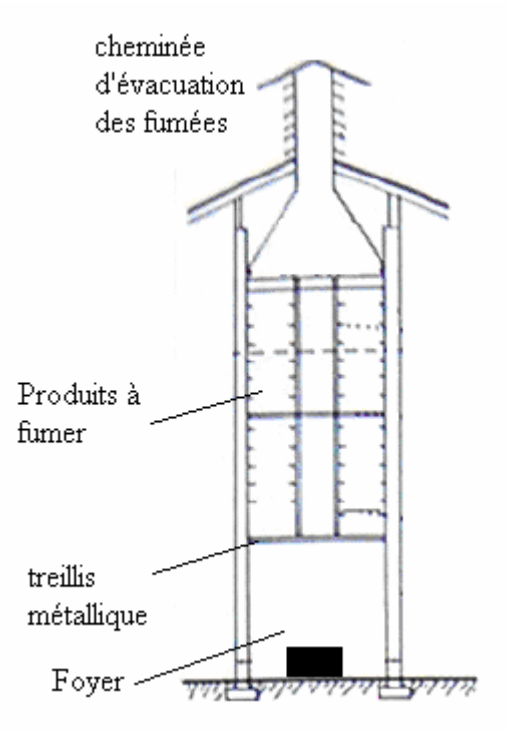


Figure 4. Générateur à exposition directe (armoire monobloc)

électrostatique (Girard, J.P. et al., 1982). Il consiste à faire passer les poissons à fumer sur un tapis de convoyage constituant la masse, tandis qu'au-dessus d'eux est disposée une électrode chargée positivement (Figure 3). Pendant toute la durée du fumage, une différence de potentiel de 20 à 50 kV est appliquée pour des intensités d'environ 1 mA. Les particules composant la fumée se chargent donc positivement au voisinage de l'électrode puis se précipitent vers le produit à fumer puisqu'il est relié à la masse. Cette technique n'a été développée qu'à l'état de prototype notamment par l'IFREMER en France. En effet, les défauts organoleptiques et l'absence de données sur la contamination chimique de produits fumés par cette méthode sont telles que cette technologie ne peut être utilisée aujourd'hui en industrie.

1.2.5 Les modes de production de la fumée

En France, on distingue cinq grands types de techniques de fumage tirant leur nom du mode de générateur employé pour produire la fumée. Ainsi, on répertorie quatre modes de génération de fumée où fumoir et générateur sont séparés (Knockaert, C., 1993) : générateur à autocombustion, à plaques thermostatées, à friction et la revaporisation de fumée liquide dans le fumoir. Dans le cas des générateurs à exposition directe, le fumoir et le générateur constituent une seule et même armoire dite monobloc (Tinkoudgou Kabré, A. et al., 2003).
En 2002, en Europe, les poissons issus de la filière industrielle étaient traités par de la fumée produite à 65 % par autocombustion, 30 % par plaques thermostatées et le reste par fumée liquide. Cependant, les condensats de fumée sont de plus en plus utilisés en Europe comme alternative au fumage industriel. Ainsi, ces changements de pratique illustrent la nécessité d'étudier l'influence de ces procédés sur les qualités organoleptiques et sanitaires des produits fumés.

1.2.5.1 Le fumage par exposition directe ou fumage artisanal

Le fumage à exposition directe correspond au fumage artisanal encore largement pratiqué. La fumée obtenue par lente pyrolyse de sciure de bois depuis le foyer (faisant également office de cendrier puisque cendres et sciures sont mélangées) vient imprégner les filets de poissons pendus par des esses (Figure 4). Le fumoir n'est pas thermostaté mais on opère généralement

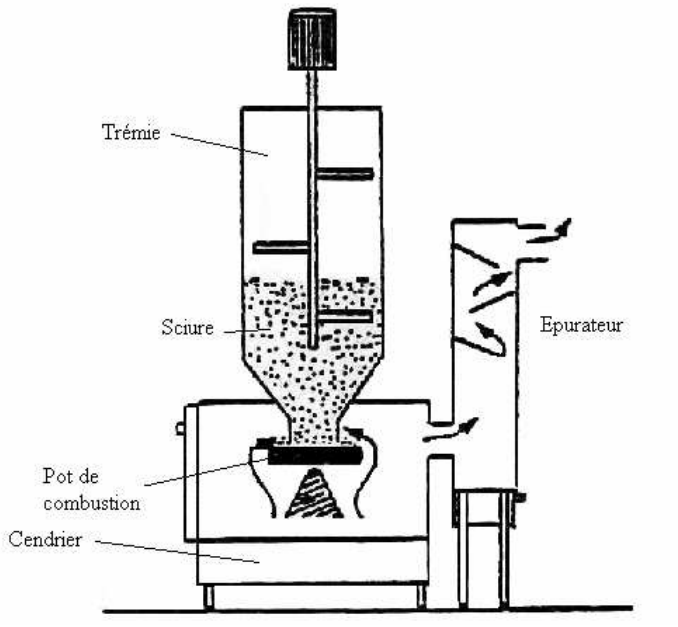


Figure 5. Générateur à autocombustion

Figure 7. Générateur à friction

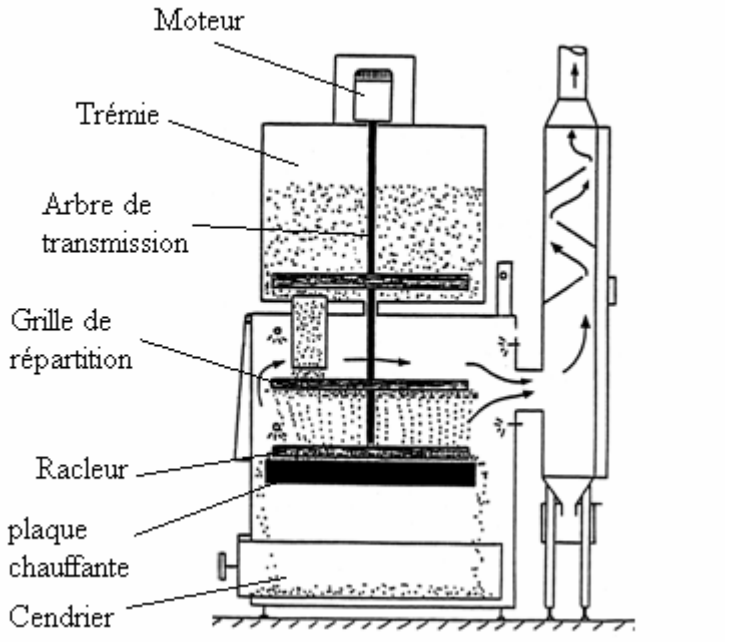


Figure 6. Générateur à plaques thermostatées

avec une température n'excédant pas 30 °C dans le fumoir. Du fait du manque de maîtrise entre les différents paramètres de fumage utilisés par les artisans (sciures, humidité, …), le fumage par exposition directe ne pourra être étudié lors de cette étude. Toutefois, les analyses que nous avons effectuées ont montré une contamination en HAP dans le produit fini supérieure aux autres procédés de fumage du fait de la proximité du générateur et du fumoir (tout en restant conforme à la législation). Ces résultats illustrent la nécessité de s'intéresser et d'harmoniser les pratiques avec ce procédé.

1.2.5.2 Le fumage par autocombustion

La fumée est produite par pyrolyse de sciure de bois tombant périodiquement par gravité depuis une trémie jusque sur une couronne chauffée électriquement (Sainclivier, 1985). La couronne est chauffée uniquement durant la période d'ignition puis la pyrolyse est entretenue par impulsions électriques (Figure 5). Les cendres sont poussées périodiquement par les nouvelles arrivées de sciure sur la couronne et sont recueillies dans un cendrier situé sous le foyer. La pyrolyse est maintenue par une entrée d'air commandée par une turbine alimentant le foyer. Les températures de pyrolyse communément utilisées sont comprises entre 400 et 450 °C. La fumée est alors poussée par la turbine du foyer et aspirée par effet venturi de la cellule de fumage à travers une conduite jusqu'à la cellule de fumage.

1.2.5.3 Le fumage par plaques thermostatées

La fumée est créée par pyrolyse de copeaux de bois tombant périodiquement par gravité depuis une trémie jusque sur une plaque circulaire chauffée électriquement (Figure 6). La plaque est généralement maintenue à des températures voisines de 500 °C. Celle-ci est nettoyée par un râteau raclant périodiquement la plaque et entraînant les résidus de pyrolyse dans un cendrier. Avec ce système, il est à noter qu'un volume très limité de cendres est obtenu.

1.2.5.4 Le fumage par friction

La fumée est générée par la pression d'une bûche sur une roue crantée tournant à grande vitesse. Cette production est réalisée en réalisant des cycles de pression et de repos (Girard,

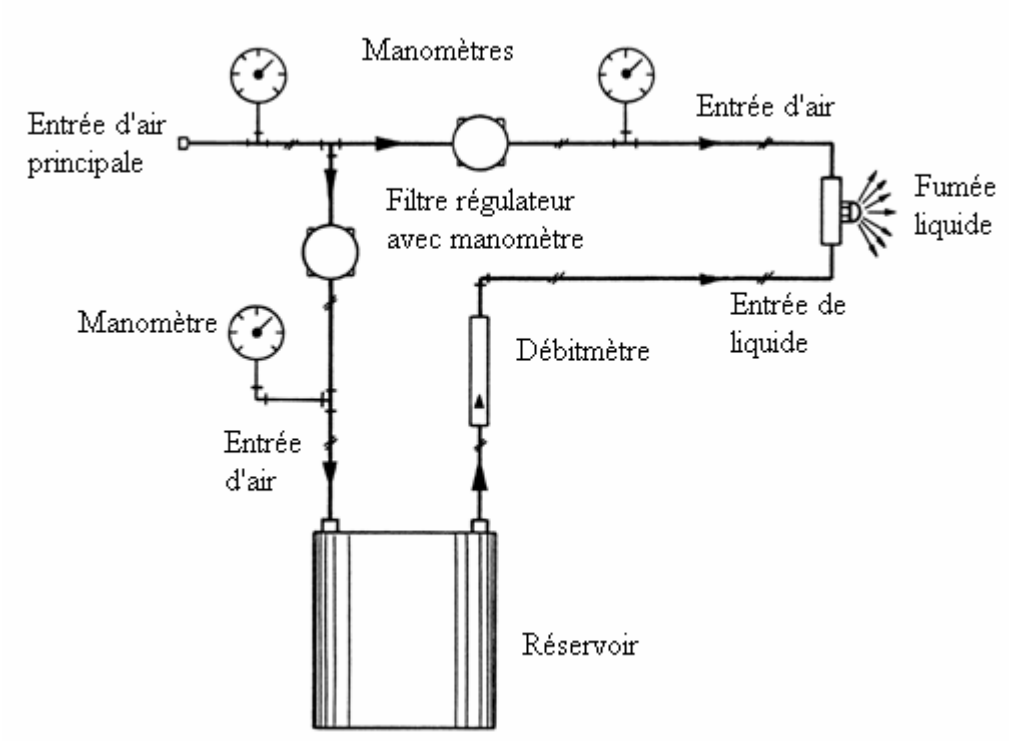


Figure 8. Principe d'une installation de brumisation de fumée liquide

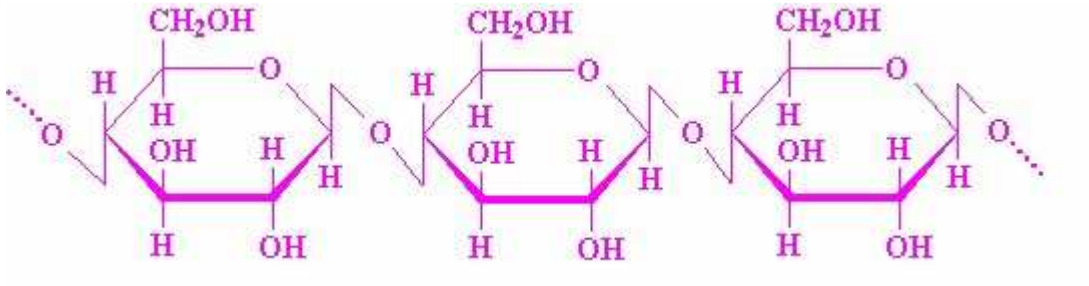


Figure 9. Motif de cellulose

J.P., 1988). La température de pyrolyse est d'environ 380 °C mais la quantité de cendres générée reste conséquente (Figure 7).

1.2.5.5 La revaporisation de condensats de fumée

Cette technique consiste à revaporiser ou encore atomiser de la fumée liquide dans le fumoir. La fumée liquide est obtenue par condensation et purification de fumée produite à partir de bois n'ayant pas été traité à l'aide de produits chimiques pour améliorer sa conservation (2065/2003/EC). Les procédés d'obtention et de formulation de ces fumées restent pour la plupart mal connus du fait du secret industriel. Toutefois, on sait que du bois est pyrolysé dans des fours spéciaux permettant le recyclage des goudrons. La fumée est ensuite condensée dans des colonnes de refroidissement dans de l'eau ou par solvants. Après des étapes de filtration, purification et décantation, on obtient divers types de condensats. La plupart des condensats aqueux peuvent alors être utilisés en fonction de leur composition biochimique pour la revaporisation dans un fumoir. La fumée liquide est donc pulvérisée dans le fumoir par de l'air comprimé (Figure 8). Toutefois, l'interprétation légale de la revaporisation de fumée liquide dans une enceinte demeure ambigue car cette technique peut être considérée comme une méthode de fumage (le brouillard de fumée généré est physiquement proche de la fumée naturelle (granulométrie, …)) ou comme une méthode d'aromatisation car il s'agit également d'une brumisation de fumée liquide sur la surface des filets. En effet, les autres modes d'application des condensats (douchage, trempage, incorporation directe), qu'ils soient sous forme aqueuse (fumées liquides), d'huiles de fumée ou de poudres, relèvent de l'aromatisation car l'ajout de ces condensats a lieu au cours de la fabrication du produit.

1.3 La fumée

1.3.1 Composition chimique du bois

Le bois est essentiellement composé de deux polyoses qui sont : la cellulose et l'hémicellulose, et de lignine dans des proportions 2-1-1 qui constituent 95 % de la matière sèche (Alén, R. et al., 1995). Les bois durs (hêtre, frêne, chêne, noyer,…) sont préférés aux

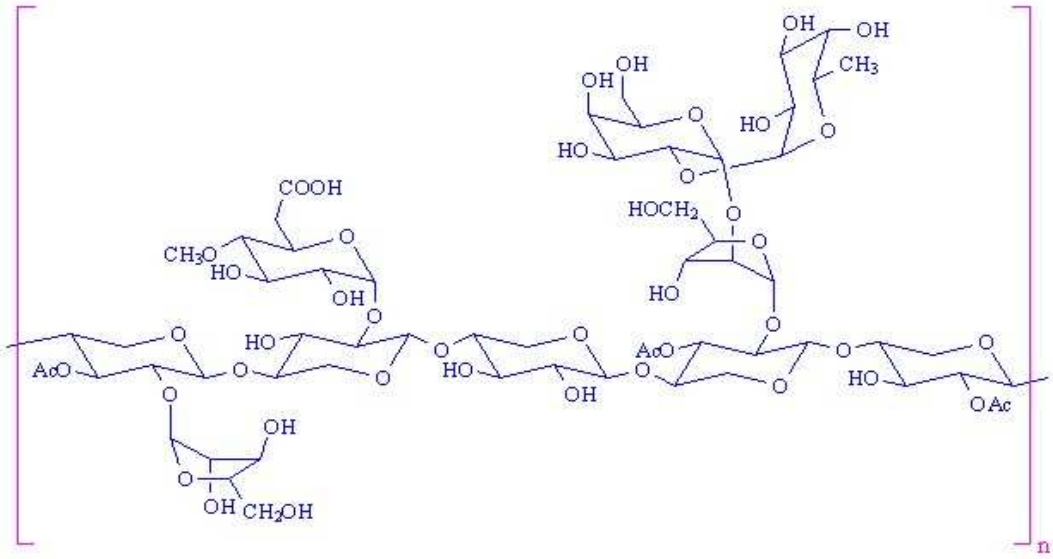
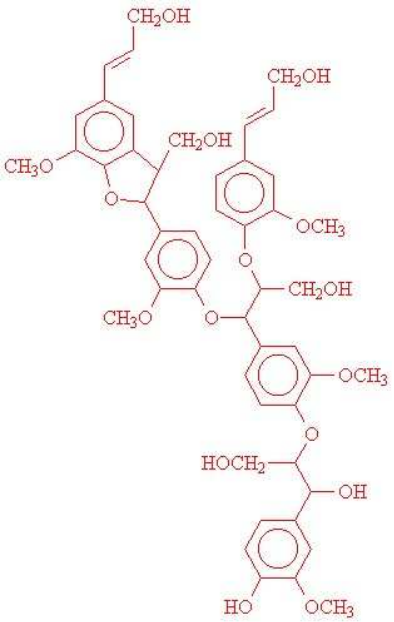
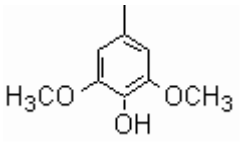
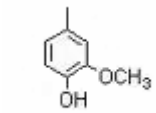


Figure 10. Motif d'hémicellulose (Ac : groupement acétyl)





Noyau syringyl



Noyau guaiacyl

Figure 11. Motif de lignine simplifié et noyaux guaiacyl et syringyl

bois tendres (peuplier, bouleau, conifères…). Il y a généralement plus d'hémicellulose dans les bois durs que dans les bois tendres et plus de lignine dans les bois de conifères.
La cellulose est un homopolymère du D-glucose lié en β 1-4 (Figure 9). Sa structure est globalement la même dans tous les bois.
Les hémicelluloses sont des hétéropolysaccharides (Figure 10), les monomères de la chaîne pouvant être méthylés, acétylés ou carboxylés. Le degré de polymérisation serait de l'ordre de 100 à 400. Dans le bois, on les rencontre soit sous forme linéaire de monomères de configuration β le plus souvent, soit ramifiés à branches courtes, entourant la cellulose. Selon le bois, la structure polymérique des hémicelluloses peut être différente. Les bois durs sont riches en pentosanes tandis que les bois plus tendres (conifères) sont riches en hexosanes. Ce sont les pentosanes qui sont le plus thermosensible.
La structure de la lignine est encore mal définie (Figure 11). Elle est formée essentiellement à partir de deux principaux cycles : le noyau guaïacyl-propane et le noyau syringyl-propane. Elle est ainsi définie comme un polymère de composés phénoliques.

1.3.2 Composition physique de la fumée

On peut définir la fumée comme un système biphasique en équilibre qui évolue en permanence. Elle se compose d'une phase continue, établie sous forme gazeuse, porteuse d'une phase particulaire ou dispersée constituée de fines gouttelettes en suspension (composés moins volatils, goudrons,…) (Foster, W.W. et al., 1961). Les composés créés lors de la combustion du bois se répartissent donc dans ces deux phases en fonction de leur solubilité, de leur point d'ébullition. Ainsi, plus on s'éloigne du foyer, plus la fumée se refroidit et les composés les moins volatils se condensent pour former la phase particulaire de la fumée alors que les composés les plus volatils forment la phase gazeuse.
Il est important de noter que la fumée évolue en fonction du temps et lorsque des composés disparaissent en se fixant sur le produit à fumer, la phase particulaire sert de pool de réserve en éléments volatils à la phase gazeuse. La phase particulaire est donc très évolutive puisque la concentration dans la phase gazeuse est maintenue en équilibre.

1.3.3 Combustion de bois et composition chimique de la fumée

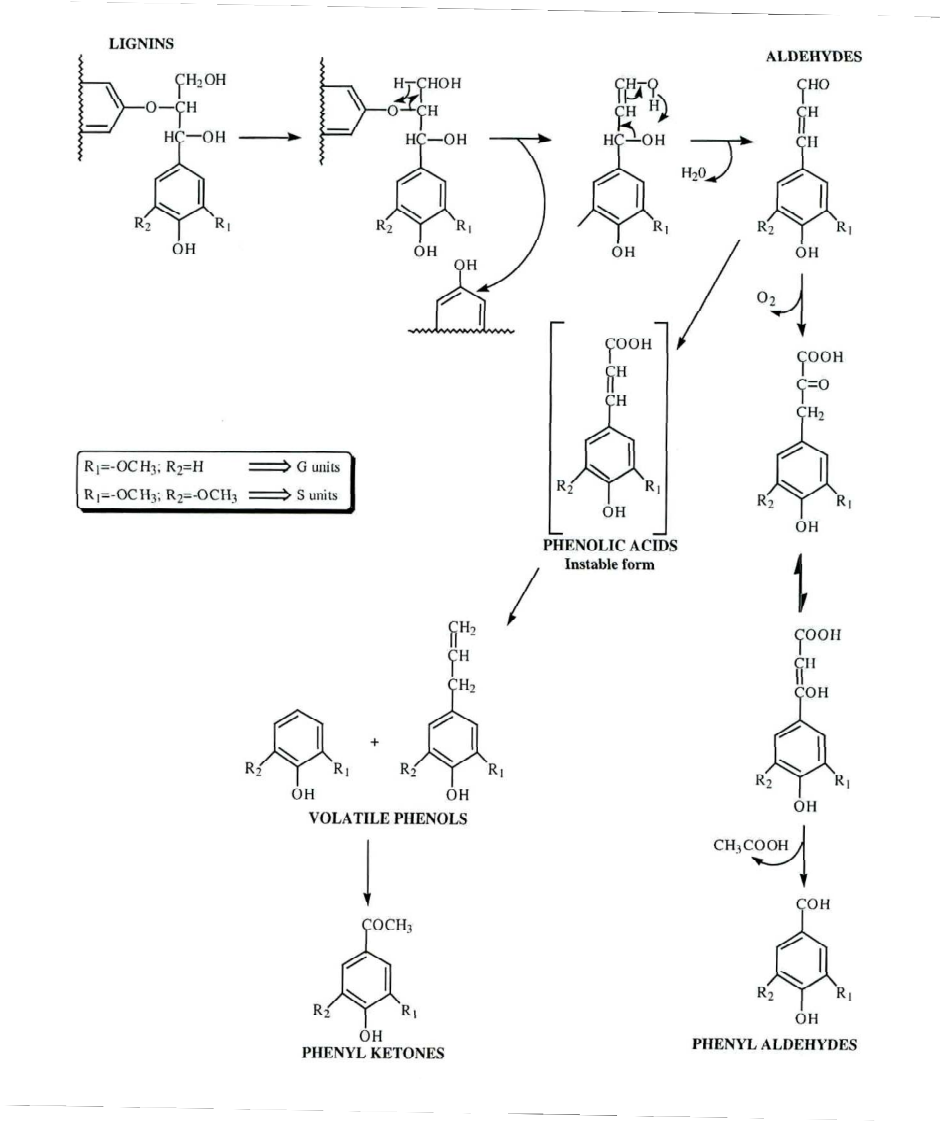


Figure 12. Dégradation de la lignine (d'après Nonier et al., 2005)

La combustion du bois conduit à un processus en deux temps. La première transformation correspond à une destruction thermique sans présence d'oxygène des constituants du bois qui produit des matières volatiles et du charbon. L'humidité du bois joue un rôle important dans la diversité de composés volatils créés. En effet, dans la période de destruction thermique sont essentiellement produits du CO et du CO_2 ainsi que quelques acides volatils à courte chaîne. La seconde transformation a lieu dans la zone de diffusion proche de l'incandescence ou zone d'oxydation. Le bois devient sec et la température augmente très rapidement. A ce moment, les matières volatiles atteignent l'oxygène de l'air et de nouveaux composés sont créés. Ainsi, la production de composés volatils se diversifie dans le temps et dans l'espace en fonction de la distance de la fumée au foyer.

La décomposition thermique de la cellulose se fait donc en deux étapes. Tout d'abord, une hydrolyse acide du glucose suivie d'une déshydratation conduit à la formation de 1-6 anhydroglucose (β glucosane). La deuxième étape immédiatement consécutive à la première consiste en une pyrogénation secondaire conduisant à la production d'acide acétique et ses homologues, de l'eau, ainsi que des traces de furanes et de petites quantités de composés phénoliques.

Les hémicelluloses se décomposent en furanes et dérivés et en acides carboxyliques (surtout aux basses températures de combustion). Les bois durs, riches en pentosanes, conduisent à des quantités d'acides supérieures aux bois tendres. Les hexosanes des bois tendres se décomposent en α-cellulose dont la décomposition a été présentée ci-dessus et fournissent plus de HAP que les bois durs (Simon et al., 2006).

La dégradation de la lignine est complexe (Figure 12). Elle donne un mélange de composés phénoliques et d'éthers phénoliques autrement appelés alkyls aryls éthers (Kjällstrand, J. et al., 2000). Les bois durs fournissent un mélange de guaïacol et de syringol alors que les bois tendres fournissent essentiellement du guaïacol. A ces composés phénoliques doivent s'ajouter d'autres produits comme le méthanol, l'acétone, divers acides organiques simples pouvant s'oxyder en composés carbonylés.

La composition de la fumée est très complexe (plus de quatre cent composés). L'étude des composés volatils de la fumée a fait l'objet de nombreuses recherches qui ont permis de distinguer plusieurs familles de composés qu'ils soient odorants ou non. En effet, les composés volatils de la fumée de bois sont à l'origine (par simple déposition ou par réaction avec les constituants de la chair de poisson) de nombreux composés volatils odorants de la chair de poisson fumé. Ces mécanismes seront présentés ultérieurement.

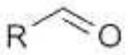
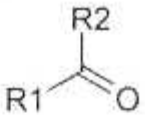
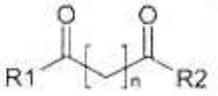
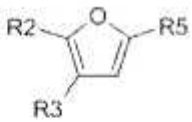
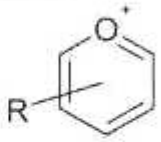
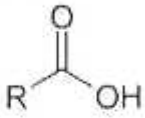
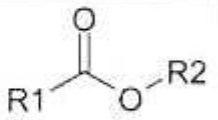
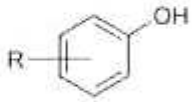
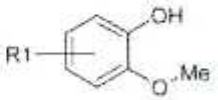
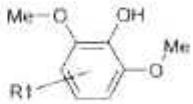
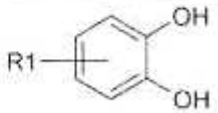
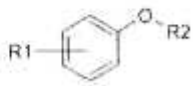
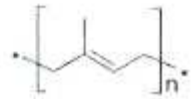
Aldehydes		Ketones	
Diketones ($n = 0 - n$)		Furanes	
Pyranes		Carboxylic acids	
Carboxylic esters		Phenols	
Guaiacols		Syringols	
Pyrocatechols		Alkyl aryl ethers ($R2 = C_nH_{2n+1}$)	
Alcohols		Terpenes ($n > 2$)	

Tableau 1. Groupes et structures chimiques des composés identifiés dans la fumée de bois (d'après Simon, R. et al., 2005)

Ainsi, l'analyse des composés volatils de la fumée, à l'état gazeux, à l'état liquide (fumée condensée) ou solide (poudre de fumée) (Guillén, M.D. & Manzanos, M.J., 1996) a permis de distinguer plusieurs familles chimiques : composés cycliques (phénoliques, benzéniques, furaniques, pyraniques, …) et aliphatiques présentant plusieurs fonctions chimiques : alcools, carbonyles, acides,… résumés dans le tableau 1.

Certains goudrons volatils peuvent également se retrouver dans la fumée sans oublier l'eau, le CO_2, des traces d'hydrogène et d'oxyde de carbone et enfin des éléments (aluminium, silice, phosphore, …) (Hedberg, E. et al., 2002).

L'essence du bois influence grandement la composition chimique de la fumée générée (Maga, J., 1987). Ainsi, les fumées de bois de hêtre (Guillén, M.D. & Ibargoitia, M.L., 1996a ; Guillén, M.D. & Ibargoitia, M.L., 1999a), de chêne (Guillén, M.D. & Manzanos, M.J., 2002 ; Guillén, M.D.& Manzanos, M.J., 2005), de bouleau (Barrefors, G. & Petersson, G., 1995a et b ; Barrefors, G. et al., 1996), de noyer (Lustre, A.O. & Issenberg, P., 1969), de sapin (Barrefors, G. & Petersson, G., 1995a ; Kjällstrand, J. et al., 1998), de paille d'orge (Barrefors, G. & Petersson, G., 1995a), de pin (Barrefors, G. & Petersson, G., 1995b ; Simoneit, B.R.T. et al., 2000), de thym (Guillén, M.D. & Manzanos, M.J., 1999b) de mélanges de bois durs (Barrefors, G. & Petersson, G., 1995b ; Lustre, A.O. & Issenberg, P., 1969) ont été principalement étudiées.

1.3.3.1 Les composés phénoliques

De nombreux travaux ont porté sur l'évaluation et l'identification des composés phénoliques de la fumée car cette famille de composés constitue un bon traceur du procédé de fumage (Sérot, T. et al., 2004). En effet, ces composéssont considérés comme étant responsables de la flaveur « fumée » (Maga, J.A., 1987). Le suivi de leur concentration peut ainsi rendre compte de l'intensité du procédé de fumage. La structure de ces composés comportent essentiellement des noyaux guaïacol, syringol ou encore catéchol (Kjällstrand, J. et al., 1998 ; Guillén, M.D. & Ibargoitia, M.L., 1999b). On distingue le groupe « phénol et dérivés », le plus souvent alkylés comme les crésols, le groupe « guaiacol et dérivés » qui comprend notamment l'eugénol, les isoeugénols, les alkyls guaiacols et dérivés de la vanilline, le groupe « syringol et dérivés » et le groupe « catéchol et dérivés ». Les rapports Guaiacol / Syringol (G/S) et Guaiacol / Phénol (G/P) ont été employés par plusieurs auteurs pour identifier la nature du bois.

Composés phénoliques	Bois (%)					
	Quercus acuta	Quercus seriata	Cerisier	Bambou	Pin	Cèdre
o-crésol	24	23	39	40	40	45
Guaiacol	45	40	30	36	26	24
p-crésol	10	10	13	9	15	16
4-methylguaiacol	12	12	9	7	10	11
4-ethylguaiacol	2	2	1	2	1	2
Syringol	4	4	4	3	4	0

Tableau 2. Composés phénoliques associés à certains bois utilisés pour le fumage (d'après Maga, J.A., 1987)

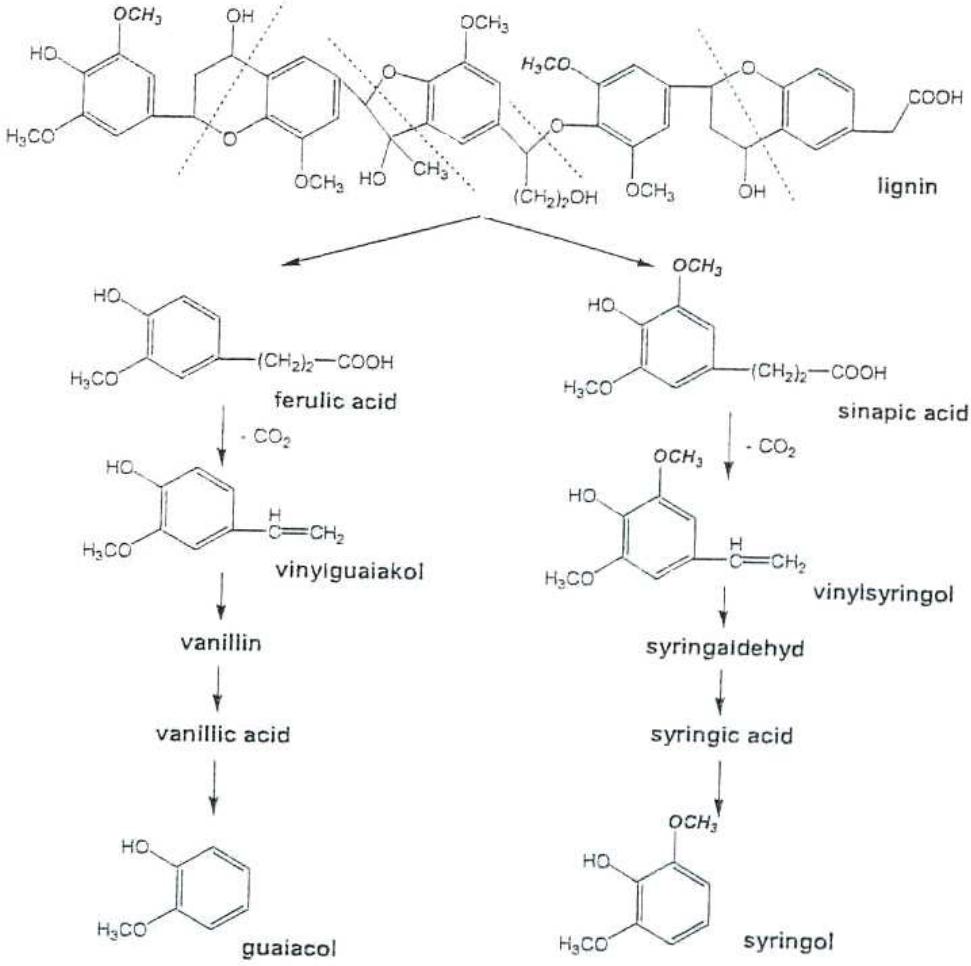


Figure 13. Pyrolyse de la lignine (d'après Jira, W., 2004a).

Par exemple, les bois durs présentent un ratio G/S proche de 1,5 et un ratio G/P proche de 2 (Guillén, M.D. & Manzanos, M.J., 2005). Le tableau 2 présente les principaux composés phénoliques retrouvés dans la fumée des bois les plus utilisés pour le fumage. Les épices parfois employées lors du fumage constituent également une source de composés phénoliques très variés. Parmi les composés phénoliques, on retrouve également des composés phénoliques carbonylés, des acides phénoliques et des esters phénoliques. En effet, la dégradation de la lignine conduit à des acides phénoliques, précurseurs des phénols alkylés et des composés phénoliques carbonylés. Ainsi, la vanilline (4-hydroxy-3-methoxybenzaldehyde) fréquemment rencontrée dans la fumée de bois conduirait à l'acide vanillique (l'acide 4-hydroxy-3-methoxybenzoïque), puis par décarboxylation, au guaïacol (Figure 13).

1.3.3.2 Les autres composés cycliques

La décomposition des constituants du bois conduit également à une quantité non négligeable d'éthers phénoliques parfois appelés alkyls aryl éthers. Pour cette raison, nous n'avons pas voulu les classer parmi les composés phénoliques simples tels ceux définis dans le paragraphe précédent. De plus, ces alkyls aryls éthers (dimères et trimères de lignine (Guillén, M.D. & Ibargoitia, M.L., 1998)) sont considérés comme des précurseurs des composés phénoliques. Ces composés comme les diméthoxybenzènes et diméthoxytoluènes se forment lors de la scission des liaisons carbone-carbone de la lignine (Guillén, M.D. & Ibargoitia, M.L., 1999). Cependant, ils ne sont pas majoritaires mais peuvent servir à identifier l'essence de bois ayant été utilisée pour fumer le produit analysé.

De même, les « enolones », dérivés de la cyclopentenone, sont également présentes dans la fumée de bois et sont suspectés provenir de la dégradation de la cellulose et de l'hémicellulose ou encore de réactions de Maillard dans la fumée elle-même, en dépit de la faible quantité de matière azotée (Guillén, M.D. et al., 2001 ; Nonier, M.F. et al., 2005).

Des composés terpéniques cycliques et leurs dérivés sont également présents dans la fumée de bois, surtout si celle-ci est produite avec l'ajout d'aiguilles, d'herbes aromatiques ou d'épices (Guillén, M.D. & Manzanos, M.J., 1999). Le terpinéol, le linalool sont fréquemment rencontrés dans la fumée de bois mais, comme tous les composés terpéniques, en faibles quantités.

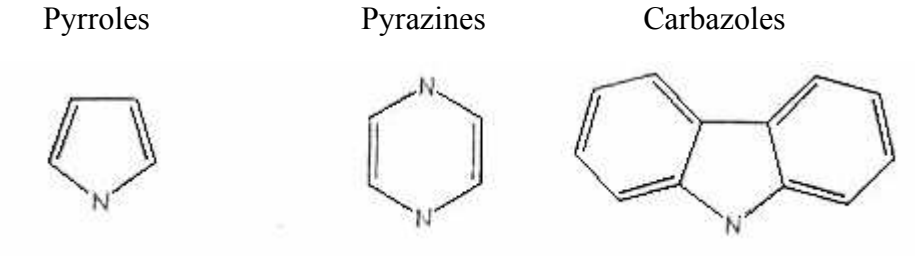


Figure 14. Hétérocycles azotés de la fumée (d'après Jira, W., 2004a)

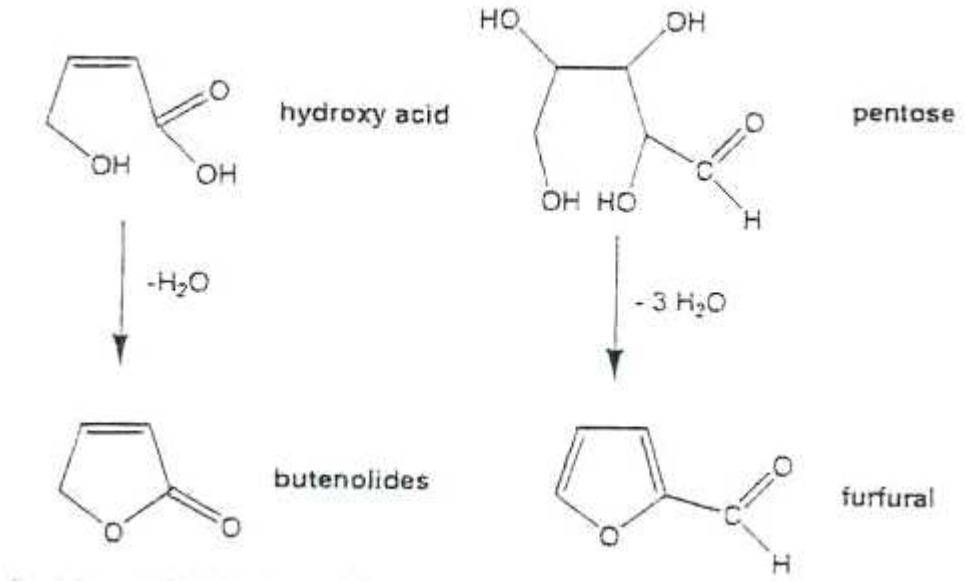


Figure 15. Formation des lactones et furfurals dans la fumée de bois (d'après Jira, W., 2004a)

1.3.3.3 Les composés hétérocycliques

Les composés pyranniques et furaniques proviennent de la thermodégradation des polysaccharides du bois en particulier de l'hémicellulose (Nonier, M.F. et al., 2005). Ils représentent une classe de composés constitués de cycles comportant un atome d'oxygène et respectivement 4 et 5 atomes de carbones. Le maltol apparaît comme le principal composé pyranniques dans la fumée de bois. Ces composés ont été étudiés en raison de leur thermosensibilité, leurs propriétés organoleptiques et de leur présence constante en tant que composés volatils de la fumée (Guillén, M.D. et al., 1995 ; Barrefors, G. et al., 1996 ; Jira, W., 2004a). Ainsi, le furfural, l'alcool furfurylique et leurs dérivés constituent le deuxième groupe de composés volatils de la fumée. Ils sont principalement formés par déshydration des pentoses provenant de la décomposition de l'hémicellulose (Sanders, E.B., et al., 2003).
Malgré la faible quantité d'azote dans le bois, quatre principaux types d'hétérocycles azotés ont été identifiés la fumée : les pyrroles, les pyrazines, les pyridines et les carbazoles (Figure 14). D'autres précurseurs ont également été proposés pour la présence des pyridines notamment les alcaloïdes (Guillén, M.D. et al., 2001). Cependant les hétérocycles azotés sont retrouvés en faibles quantités dans la fumée de bois. En revanche, les lactones sont assez nombreuses, principalement dérivées du butyrolactone et 2-buténolide (Maga, J., 1987). Elles sont formées par la déshydration des hydroxyacides correspondants (Figure 15).

1.3.3.4 Les composés aliphatiques

Les composés aliphatiques ne sont pas majoritaires dans la composition de la fumée. Ils sont souvent de petites tailles, considérés comme des co-produits de dégradation des constituants du bois. On distingue cependant les alcools, les composés carbonylés, les acides carboxyliques et les hydrocarbures (Barrefors, G. & Petersson, G., 1995a et b ; Guillén, M.D. & Ibargoitia, M.L., 1998). Les alcools primaires, secondaires ou tertiaires sont fréquemment oxydés dans la fumée pour former les composés carbonylés correspondants. Ceux-ci sont en très faibles quantités dans la fumée de bois car ils se transforment très rapidement en acides carboxyliques correspondant dans ces conditions très oxydantes. Les acides carboxyliques rencontrés sont dispersés dans les deux phases de la fumée. Dans la phase gazeuse, ce sont des acides simples de 1 à 4 atomes de carbone (acide formique, acétique, propionique,…). Les

chaînes plus longues de 5 à 10 atomes de carbone se trouvent dans la phase particulaire (acide valérique, isovalérique, caproïque, caprylique, …) (Sainclivier, M., 1985).

1.3.4 Paramètres d'influence sur la composition de la fumée lors du fumage

La composition de la fumée résulte de combinaisons entre de nombreux paramètres du fumage. Par conséquent, la température de pyrolyse, l'essence du bois, sa granulométrie, son humidité, la vitesse de l'air, son humidité sont autant de paramètres à contrôler pour obtenir une fumée de qualité.

En effet, pour la production d'une fumée de qualité « alimentaire » non toxique et efficace, il est nécessaire d'obtenir une température de combustion de l'ordre de 400~450 °C. La création des composés volatils est fortement dépendante de la température de pyrolyse du bois (Guillén, M.D. & Ibargoitia, M.L., 1996b). Ainsi, en fonction du gradient de température établi par la distance au foyer, différents groupes de composés sont formés. La teneur en composés carbonylés augmente constamment avec la température de 200 à 600°C. La teneur en acides est plus grande à basse température (< 300°C) puis elle diminue après 300°C avec l'augmentation de la température. De 200 à 600°C, la quantité de composés phénoliques augmente avec un maximum vers 500°C puis décline progressivement. Cependant les teneurs en composés phénoliques varient individuellement avec l'augmentation de la température. Par exemple, la quantité de phénol est multipliée par 2 entre 450 et 650°C tandis que la quantité de syringol est multipliée par 3. Cependant, une température de 450 à 500°C a été rapportée comme le niveau optimal de création de composés carbonylés, de composés phénoliques et furaniques (Maga, J.A., 1987 ; Alén, R. et al., 1996 ; Nonier, M.F. et al., 2005).

La nature du bois et sa granulométrie sont deux paramètres primordiaux parce qu'ils influencent la température de combustion (plus les particules sont grosses, plus la combustion est rapide) (Rusz, J. & Miler, K.B.M., 1977 ; Clifford, M.N. et al., 1980). Les bois durs (hêtre, frêne, chêne, noyer,…) sont préférés aux bois tendres (peuplier, bouleau, conifères…) même s'ils donnent parfois des couleurs sombres et un goût amer. Les bois durs fournissent plus d'acides en raison de leur plus grande teneur en hémicellulose. Les essences ou plantes aromatiques parfois incorporées ont une influence sur les qualités organoleptiques du produit fini mais font également varier la composition du combustible et donc de la composition de la fumée. L'utilisation de bois dur est également recommandée pour limiter la formation des HAP, et ce, avec une humidité de 17 à 20 % (Guillén, M.D. & Ibargoitia, M.L., 1999a). En

effet, l'humidité du bois est, elle aussi, très importante en raison de son influence sur la combustion et la libération des composés volatils.
La vitesse de l'air doit être ajustée à l'humidité du fumoir ainsi qu'à la fragmentation du bois. La vitesse de l'air joue un rôle indirect mais essentiel que ce soit par la modification de la température du foyer ou par celle de la fumée engendrée en s'y mélangeant et en la diluant (Lantz, A.W. & Vaisey, M., 1970 ; Chan, W.S. & Toledo, R.T., 1975). L'humidité de l'air est aussi primordiale et doit être ajustée avec la vitesse de l'air (Guillén, M.D. & Ibargoitia, M.L., 1999).

1.3.5 Actions de la fumée sur le poisson

1.3.5.1 Actions conservatrices de la fumée

La fumée tire sa fonction de conservation de deux caractéristiques : une action antioxydante (Kjällstrand, J. & Petersson, G., 2001a) et une action antimicrobienne (Niedziela, J.C. et al., 1998).
Les composés de la fumée ayant une action antioxydante sont uniquement ceux possédant une fonction phénolique libre (Kjällstrand, J., Petersson, G., 2001b). L'action antioxydante est d'autant plus prononcée que les composés ont un haut point d'ébullition. Ainsi, les plus actifs sont les polyhydroxyphénols comme le pyrogallol et le résorcinol. Pour les monohydroxyphénols, l'activité dépend de la nature des substitutions. La plus forte activité est donc retrouvée pour le 4-méthylguaïacol, le 4-vinylguaïacol, le 4-trans-propenylsyringol. Le guaïacol, le syringol, le 4-methylsyringol et le 4-vinylsyringol sont moins actifs.
Un oxydant (radical libre par exemple) agit par capture d'électrons et peut nuire à la conservation du produit en initiant une oxydation des lipides. Un composé phénolique va pouvoir donner un électron à l'oxydant qui se rétablira. Le composé phénolique est donc oxydé mais ce déficit électronique est compensé par résonance entre les formes mésomères de la molécule. L'oxydation est par conséquent empêchée. Les composés phénoliques à haut point d'ébullition sont considérés comme synergiques avec les composés phénoliques oxydés. Les composés polyphénoliques prolongent ainsi l'action antioxydante. Il est important de noter qu'à de rares exceptions, à partir d'une teneur trop élevée en composés phénoliques, l'effet antioxydant s'inverse et devient pro oxydant.

L'action antimicrobienne est d'abord due à des paramètres physiques comme la chaleur (dans le fumage à chaud) ou la réduction de l'activité de l'eau (fumage à froid) qui inhibe le développement de microorganismes (Rørvik, L.M., 2000 ; Cornu, M. et al., 2005). L'action antimicrobienne est également due à certains constituants de la fumée qui sont notamment les acides carboxyliques (formique, propionique, acétique,…) et les composés phénoliques (Suñen, E. et al., 2003). Les composés carbonylés et les esters sont beaucoup moins impliqués. Les hydrocarbures n'ont aucune influence.

Par comparaison avec les levures et les moisissures, les bactéries sont les microorganismes les plus susceptibles d'être inhibés par les composants de la fumée. L'action antimicrobienne est plus efficace sur les germes Gram + que sur les Gram – (Suñen, E., 1998) et d'autant plus sur les cellules vivantes que sur les spores.

L'action antimicrobienne est également due au caractère bactériostatique des nitrophénols produits par une nitrosation à partir de composés phénoliques (Leis, J.R. et al., 1998 ; Gonzáles-Mancebo, S. et al., 1999) sous l'action de nitrates/nitrites. Cependant, il est important de souligner que ces nitrocomposés peuvent donner des nitrosamines carcinogènes mais les seuils légaux ne sont jamais dépassés dans le poisson dans la mesure où l'ajout de sels nitrités dans le poisson est interdit en France.

1.3.5.2 Effets sur les qualités organoleptiques

1.3.5.2.1 La couleur

La coloration est très liée au pH, à la température et à la composition biochimique de l'aliment.

Outre la couleur initiale du produit à traiter, la couleur du produit fumé a une origine physique et une origine chimique.

Les condensats de fumée liquide possèdent une couleur marron, allant de jaune orangé à brun noir. Cette couleur est principalement due aux composés phénoliques et furanniques de la fumée. Par simple déposition physique, ces composés peuvent donc colorer le produit (Clifford, M.N. et al., 1980).

La couleur est également due à des réactions du type Réactions de Maillard c'est-à-dire des réactions entre les acides aminés des protéines du poisson et les composés de la fumée issus de la dégradation des polysaccharides du bois et comportant des fonctions carbonyles. Ces

réactions conduisent à des brunissements de la surface du produit d'où une couleur finale pouvant varier du jaune doré au brun foncé (coloration également dépendante du bois utilisé) (Tilgner, D.J., 1977). La lysine, principal acide aminé essentiel du poisson (Huss, 1999), grâce au groupe aminé latéral, est considérée comme la principale source de composés aminés impliqués dans ces réactions, ainsi que l'arginine et l'histidine. Les composés carbonylés de la fumée et du poisson jouent également un rôle important dans la coloration. L'hydroxyacétaldéhyde, le méthylglyoxal et le 2-oxopropanal sont considérés comme d'importants précurseurs de la couleur du poisson fumé (Clifford, M.N. et al., 1980 ; Miller, K.B.M. & Sikorski, Z.E., 1990).

Une part de la couleur finale peut également être attribuée aux composés phénoliques présentant une fonction aldéhyde. Le coniféraldéhyde et le syringaldéhyde de la fumée peuvent se lier irréversiblement aux protéines du poisson et contribuer à la couleur orangée du produit (Clifford, M.N. et al., 1980).

Ainsi, une partie des composés phénoliques de la fumée peut se polymériser en surface et colorer le produit. L'aspect brillant présenté par certains poissons fumés résulte de réactions entre des aldéhydes et des composés phénoliques (Girard, J.P., 1988). Elles conduisent à des substances résineuses appelées phénoplastes. La polymérisation est favorisée par la chaleur et le degré de réticulation de ces molécules varie en fonction du temps.

1.3.5.2.2 La texture

En plus des modifications de texture avérées pendant l'étape de salage et de séchage - entre autres le croûtage (formation rapide d'une pellicule de surface pouvant ralentir les échanges) - les composés volatils de la fumée peuvent également influencer la texture des produits fumés. Par exemple, les poissons fumés à froid restent mous et tendres tandis que ceux fumés à chaud sont plus durs et plus secs (Vasiliadou, S. et al ., 2005) en fonction de la température du fumoir. Les modifications de texture sont donc essentiellement dues à l'influence de la température et de la chaleur sur la coagulation des protéines (constitution d'une pellicule brillante et lustrée en surface) avec comme manifestations : une perte en eau, une fusion variable des matières grasses et la dénaturation des protéines du tissu conjonctif. Le formaldéhyde et les acides volatils sont fortement impliqués dans ces modifications sans oublier l'activité de certaines enzymes notamment protéolytiques (Miler, K.B.M. & Sikorski, Z.E., 1990).

1.3.5.2.3 La flaveur

Depuis les années 1970, les composés phénoliques (notamment les phénols de bas ou moyen point d'ébullition) sont décrits comme étant les principaux responsables de l'odeur et du goût fumé (Hamm, R., 1977). Les composés carbonylés et les acides organiques ont été identifiés avec un rôle secondaire mais cependant nécessaire dans l'expression de la totalité de l'arôme. L'odeur de fumée serait plutôt associée au syringol et au 2,6-diméthoxy-méthylphénol alors que le guaïacol et l'eugénol contribueraient plus à la perception du goût de fumée. Aujourd'hui, s'il est vrai que la globalité des composés phénoliques constituent bien le squelette organoleptique « fumé », les conclusions concernant le rôle individuel des composés phénoliques et des composés carbonylés ont été récemment critiquées (Kostyra, E. & Baryłko-Pikielna, N., 2005). Ainsi, si la composition de la fumée en composés volatils a fait l'objet de nombreux travaux, il n'y a cependant que peu d'études ayant permis d'identifier les composés volatils odorants du saumon fumé. Notre étude a donc pour but d'identifier les composés volatils odorants de la fumée et clarifier leurs contributions dans l'odeur du poisson fumé.

Les composés phénoliques sont connus pour être à l'origine de l'odeur « fumé » de la fumée de bois (Fiddler, W. et al., 1970 ; Ojeda, M. et al., 2002). Grâce aux travaux entrepris dans les années 1970-1980, des catégories de composés phénoliques ont été déterminées selon leur point d'ébulltion. Ces catégories nécessitent d'être vérifiées car la détermination du rôle individuel des composés phénoliques dans l'odeur du poisson fumé semble être beaucoup plus complexe que la simple somme d'odeurs fumées de composés pris individuellement. Les composés phénoliques à bas point d'ébullition (60 à 90°C) étaient reconnus pour leur odeur âcre. Le guaiacol, les crésols et leurs dérivés ont largement été étudiés pour leur contribution odorante à la note « fumée/feu de bois » (Shahidi, F. & Naczk, M., 2003). Les composés phénoliques à point d'ébullition moyen (91 à 132 °C) participent à l'ossature « fumée » de la fumée de bois (Dufour, C., 1991). L'eugénol et leurs dérivés ainsi que la plupart des dérivés benzéniques sont plutôt décrits par des notes épicées, vanillées (Maga, J., 1987). Les phénols à haut point d'ébullition (133 à 200°C) sont considérés comme ayant un rôle négatif car ils contribuent à des propriétés organoleptiques acides défavorables. Dans de nombreuses études olfactométriques réalisées sur des matrices autres que la fumée ou le poisson fumé, les composés phénoliques ont été caractérisés par des descripteurs fumés/brûlés selon les molécules. Les crésols sont caractérisés par des odeurs animales, de cuir et de brûlé (Campo, E. et al., 2005), le guaiacol et le syringol avec des odeurs allant de « fumée » à « brûlé » selon

Composés phénoliques	Seuils de perception odorants		Odeurs	
	Seuils de perception dans l'eau (µg/L)	Références	Descripteurs (matrice)	Références
Phénol	5900	Fazzalari, F.A., 1978	Phénolique (raisin)	López, R. et al., 2004
o-crésol	650	Fazzalari, F.A., 1978	Piquant (fumée)	Maga, J.A., 1987
m-crésol	680	Fazzalari, F.A., 1978	Piquant (fumée)	Maga, J.A., 1987
p-crésol	55	Buttery, R.G. et al., 1988	Animal (fromage)	Franck, D.C. et al., 2004
Guaiacol	3 à 21	Fazzalari, F.A., 1978	Fumé (fromage)	Franck, D.C. et al., 2004
4-methylguaiacol	90	Fazzalari, F.A., 1978	Fumé (fumée)	Maga, J.A., 1987
4-ethylguaiacol	50	Swan, J.S. & Burtles, S.M., 1978	Epicé, noix de coco (vin)	Charles, M et al., 2000
4-vinylguaiacol	3	Buttery, R.G. et al., 1988	Phénolique, synthétique	López, R. et al., 2004
Vanilline	20 à 200	Fazzalari, F.A., 1978	Vanille (raisin)	López, R. et al., 2004
Syringol	1850	Fazzalari, F.A., 1978	Chimique (vin)	Ferreira, V. et al., 2002b
Eugénol	6 à 30	Buttery, R.G. et al., 1990	Phénolique, floral (raisin)	López, R. et al., 2004

Tableau 3. Seuils de perception odorants de quelques composés phénoliques

leurs concentrations (Nonier, M.F. et al., 2005). Le 4-méthylguaiacol et le 4-éthylguaiacol apparaissent avec des odeurs épicées, douces comme la vanille ou la cannelle (Simon, R. et al., 2005). Leurs seuils de perception sont compilés dans le tableau 3.

Les composés phénoliques, bien que très importants, ne doivent cependant pas être considérés comme les seuls composés à potentialité aromatique. Des études mettant en œuvre des solutions standards de composés phénoliques dans les concentrations auxquelles ils sont dosés dans la fumée ont montré qu'ils ne suffisaient pas à exprimer à eux seuls l'odeur de fumée (Daun, H., 1979). Par conséquent, d'autres composés volatils odorants devaient être pris en compte tels que les enolones et les composés furaniques. En effet, les enolones et dérivés ainsi que les composés furaniques sont à rapprocher des odeurs grillées/cuites. Les composés furaniques sont caractérisés par des descripteurs odorants tels que caramel/cuit voire parfois épicés (Maga, J., 1987) alors que les composés carbonylés de la fumée, les enolones principalement, sont définis par des termes comme vert, odeur de pomme de terre ou grillés (Maga, J., 1987 ; Nonier, M.F. et al., 2005). Ces composés participent à la complexité de l'odeur de poisson fumé et notre étude doit permettre de caractériser leur contribution restée jusqu'à présent quasiment inconnue.

Il y a peu de travaux menés sur la nature des composés de la fumée responsables du goût fumé. Nous avons vu précédemment que certains composés phénoliques (guaïacol, eugénol) avaient été identifiés comme participant à la flaveur, mais certaines études ont montré que ni l'essentiel des composés phénoliques (guaïacol, syringol, 4-méthylguaïacol), ni la globalité des composés phénoliques ne restituait la flaveur intégrale. Il fallait également prendre en compte la part de composés carbonylés et d'acides ainsi que la quasi-totalité des constituants de la fumée. Cependant, il a été démontré que les composés phénoliques avaient un impact très important sur le goût fumé des aliments fumés (Maga, J., 1987).

1.4 Les composés volatils de la chair du saumon fumé

1.4.1 Composés volatils odorants ou non odorants de la chair des Salmonidés frais

Les composés volatils de la chair des Salmonidés frais peuvent varier en fonction de l'espèce, de la composition biochimique, de l'âge, du régime alimentaire et d'autres facteurs environnementaux. Ce sont principalement les alcools, les aldéhydes, les cétones et les

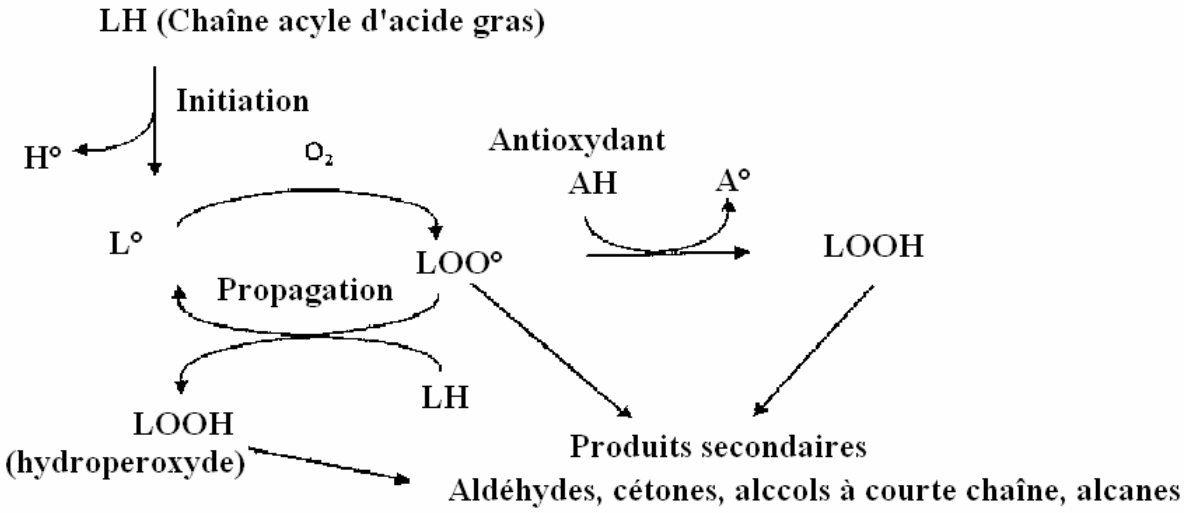


Figure 16. Autoxydation des acides gras

Acide gras	Hydroperoxyde intermédiaire	Produits de dégradation
Acide oléique (C18:1 n-9)	8-hydroperoxyde	Decanal, 2-undecenal
	9- hydroperoxyde	2-decenal, nonanal
	10- hydroperoxyde	Nonanal, octane, 1-octanol
	11- hydroperoxyde	Octanal, heptane, 1-heptanol
Acide linoléique (C18:2 n-6)	9- hydroperoxyde	2,4-decadienal, 2-nonenal
	10- hydroperoxyde	2-nonenal, 2-octene, 2-octen-1-ol
	12- hydroperoxyde	Hexanal, 2-heptenal
	13- hydroperoxyde	Hexanal, pentane, 1-pentanol, pentanal
Acide linolénique (C18:3 n-3)	9- hydroperoxyde	2,4,7-decatrienal, 2,6-nonadienal
	10- hydroperoxyde	2,6-nonadienal, 2,5-octadiene, 2,5-octadien-1-ol
	12- hydroperoxyde	2,4-heptadienal, 2-hexenal
	13- hydroperoxyde	2-hexenal, 2-pentene, 2-penten-1-ol, 2-pentenal
	15- hydroperoxyde	2-butenal, propanal
	16- hydroperoxyde	Propanal, ethane, ethanol
Acide arachidonique (C20:4 n-6)	5- hydroperoxyde	2,4,7,10-hexadecatetraenal, 2,6,9-pentadecatrienal
	6- hydroperoxyde	2,6,9-pentadecatrienal, 2,5,8-tetradecatrien-1-ol
	8- hydroperoxyde	2,4,7-tridecatrienal, 2,6-undecadien-1-ol
	9- hydroperoxyde	2,6-dodecadienal, 2,5-undecadien-1-ol
	11- hydroperoxyde	2,4-decadienal, 2-nonenal
	12- hydroperoxyde	2-nonenal, 2-octene, 2-octen-1-ol
	14- hydroperoxyde	2-heptenal, 2-hexenal
	15- hydroperoxyde	Hexanal, pentane, 1-pentanol, pentanal

Tableau 4. Principaux composés produits par les acides gras dans le poisson (d'après Hsieh, R.J. et al., 1989)

composés soufrés. Parmi ces composés, certains se révèlent comme odorants et sont responsables de l'odeur du poisson.

1.4.1.1 Les composés carbonylés et les alcools

Dans les poissons, ce sont les composés carbonylés qui sont les composés volatils les plus nombreux constituant ainsi l'essentiel de l'arôme du poisson (Josephson, D.B. et al., 1991a).
Les acides gras (AG) sont les précurseurs d'alcools, aldéhydes et cétones volatils, soit par catalyse enzymatique, sous l'effet des lipoxygénases, soit par auto-oxydation (Figure 16). La dégradation des AG conduit à de nombreux alcools et composés carbonylés (Frankel, E.N., 1983 ; Grosch, W., 1987 ; Hsieh, R.J. & Kinsella, J.E., 1989 ; Hutlin, H.O., 1992) (Tableau 4).
Les principaux alcools odorants retrouvés dans l'arôme de poisson Salmonidés sont reépertoriés dans le tableau 5. On y retrouve des composés aux odeurs principalement vertes ou de champignon. La plupart des alcools des Salmonidés proviennent de la dégradation des AG.
Les AG n-3 (C20:5 n-3, C22:6 n-3, …) sont les précurseurs de nombreux alcools volatils comme l'éthanol, retrouvé dans le saumon. Le (E)-2-penten-1-ol est un composé volatil trouvé dans la truite mais aussi dans le turbot (Sérot, T. et al., 2001a ; Sérot, T. et al., 2002), avec une odeur de champignon. L'acide linolénique semble être à l'origine du (E)-2-penten-1-ol tout comme pour le 2,5-octadien-3-ol. Il n'a pas été identifié comme odorant dans le saumon. En revanche, le (Z,Z)-1,5-octadien-1-ol est retrouvé dans les Salmonidés avec une odeur de mousse/champignon comme dans les huîtres fraîches (Piveteau, F. et al., 2000).
D'autres alcools volatils, retrouvés dans les Salmonidés, connus pour provenir d'AG n-3 mais n'ayant pas fait l'objet d'étude olfactométrique ont été perçus dans d'autres produits de la mer comme odorants. C'est le cas du (Z)-3-hexen-1-ol détecté avec une odeur verte/marine également dans les huîtres (Piveteau, F. et al., 2000). Le cyclopentanol quant à lui n'a pas été perçu comme un composé odorant dans la plupart des produits de la mer mais apparaît dans beaucoup de profils de composés volatils de poissons ou coquillages (Cha, Y.J. & Cadwallader, K.R., 1995 ; Chung, H.Y. et al., 2001).
Les AG n-6 sont les précurseurs du 1-pentanol et du 2-octen-1-ol. Le 1-pentanol a déjà été répertorié dans d'autres poissons comme la daurade (Grigorakis, K. et al., 2003) ou le hareng (Cha, Y.J. & Cadwallader, K.R., 1995) mais non odorant. Dans ces poissons, le 2-octen-1-ol,

Composé	Descripteur odorant	Seuil de détection odorant dans l'eau (µg/L)	Poisson	Références
Ethanol	Pas d'étude olfactométrique		Saumon	Josephson, D.B. et al., 1991a
				Girard, B. & Nakai, S., 1994a
1-propanol	Plastique	9 000	Truite	Sérot, T. et al. 2002
2-butanol	Pas d'étude olfactométrique		Saumon	Josephson, D.B. et al., 1991a
1-pentanol	Pas d'étude olfactométrique		Saumon	Josephson, D.B. et al., 1991a
(E)-2-penten-1-ol	Champignon		Truite	Sérot, T. et al. 2002
1-penten-3-ol	Pas d'étude olfactométrique	400	Saumon	Josephson, D.B. et al., 1991a
				Girard, B. & Nakai, S., 1994a
cyclopentanol	Pas d'étude olfactométrique		Saumon	Josephson, D.B. et al., 1991a
1-hexanol	Pas d'étude olfactométrique	2 500	Saumon	Josephson, D.B. et al., 1991a
(E)-2-hexen-1-ol	vert, mousse		Truite	Sérot, T. et al. 2002
(Z)-3-hexen-1-ol	Pas d'étude olfactométrique	70	Saumon	Josephson, D.B. et al., 1991a
1-heptanol	Pas d'étude olfactométrique	3	Saumon	Josephson, D.B. et al., 1991a
Octanol	vert, floral	110 à 130	Truite	Sérot, T. et al. 2002
3-octanol	Pas d'étude olfactométrique		Saumon	Josephson, D.B. et al., 1991a
1-octen-3-ol	Pas d'étude olfactométrique	0,005	Saumon	Josephson, D.B. et al., 1991a
				Josephson, D.B. et al., 1984
				Refsgaard, H.H.F. et al., 1999
2-octen-1-ol	Vert		Truite	Sérot, T. et al. 2002
			Saumon	Josephson, D.B. et al., 1991a
(Z,Z)-1,5-octadien-3-ol	mousse, champignon		Saumon	Josephson, D.B. et al., 1991a
				Josephson, D.B. et al., 1984
			Truite	Sérot, T. et al. 2002
2,5-octadien-1-ol	Pas d'étude olfactométrique		Saumon	Josephson, D.B. et al., 1991a
				Josephson, D.B. et al., 1984
1-nonanol	Citron		Truite	Sérot, T. et al. 2002
			Saumon	Josephson, D.B. et al., 1991a
2-nonanol	plastique, fruité		Truite	Sérot, T. et al. 2002
6-nonen-1-ol	Pas d'étude olfactométrique		Saumon	Josephson, D.B. et al., 1991a
3,6-nonadien-1-ol	Pas d'étude olfactométrique		Saumon	Josephson, D.B. et al., 1991a
4-ethylbenzene methanol	Pas d'étude olfactométrique		Saumon	Girard, B. & Nakai, S., 1994a

Tableau 5. Principaux alcools odorants identifiés dans l'arôme de Salmonidés frais

alcool volatil odorant du saumon et de la truite possède une odeur verte, comme dans le turbot (Prost, C. et al., 1998).

Les AG n-6 comme l'acide arachidonique ou l'acide linoléique sont également connus pour être à l'origine de composés comme le 1-octen-3-ol. Son odeur n'a pas été étudiée dans le saumon mais c'est un composé récurrent dans les produits de la mer, perçu avec une odeur terreuse et des notes de sous-bois.

Le 1-heptanol et le 1-octanol sont produits par les AG n-9 comme l'acide oléique. Si le descripteur odorant du 1-heptanol n'a pas été mis en évidence sur les Salmonidés, il a été perçu avec une odeur verte dans d'autres produits de la mer comme les huîtres (Pennarun, A.L. et al., 2002) et plutôt avec une odeur de pomme de terre dans le turbot (Prost, C. et al., 1998). Le 1-octanol quant à lui, est détecté avec une odeur verte/florale dans la truite. Quant aux autres alcools, si leurs origines ne sont pas formellement identifées, ils ne constituent pas moins d'importants composés volatils présents dans les produits de la mer.

Le 1-hexanol a été identifié comme provenant de la dégradation des AG mais sans information supplémentaire sur le type d'insaturations de ces AG (Pennarun, A.L. et al., 2002). Sa présence a été remarqué dans les moules (Le Guen, S. et al., 2000a), les anchois (Cha, Y.J. & Cadwallader, K.R., 1995) ainsi que la daurade (Grigorakis, K. et al., 2003). Il ne fait pas partie des composés odorants des produits de la mer car il est généralement en quantité plus faible que son seuil de détection (tableau 5). Le 2-butanol a été détecté dans le hareng (Cha, Y.J. & Cadwallader, K.R., 1995), le 3-octanol, dont l'odeur n'a pas été identifiée dans les Salmonidés, a été trouvé dans les huîtres (Pennarun, A.L. et al., 2002) et le jus de moules (Cros, S. et al., 2004) avec une odeur soufrée/mousse. Le 3,6-nonadien-1-ol a été caractérisée dans les huîtres avec un descripteur odorant marin (Pennarun, A.L. et al., 2002). Le 1-propanol, trouvé avec une odeur plastique dans la truite a également été retrouvé dans le turbot et les moules (Prost, C. et al., 1998 ; Le Guen, S. et al., 2000). Le (E)-2-hexen-1-ol détecté avec une odeur verte/mousse dans la truite a également été perçu avec la même odeur dans le turbot (Sérot, T. et al., 2001a). Le 1-nonanol déterminé dans les Salmonidés avec une odeur de citron fait également partie des composés volatils de la daurade (Grigorakis, K. et al., 2003). Enfin, le 2-nonanol, dont l'odeur a été évaluée dans la truite comme plastique/fruité, a également été identifié dans les huîtres avec une odeur de concombre (Pennarun, A.L. et al., 2002) ou verte dans le turbot (Sérot, T. et al., 2001a).

Parmi les aldéhydes volatils, les formes n-alcanals, (E)-2-alcénals et (E,E) ou (E,Z)-alcadiénals sont les plus fréquentes (Josephson, D.B. et al., 1984 ; Girard, B. & Nakai, S., 1994a ; Refsgaard, H.H.F. et al., 1999). Les composés carbonylés proviennent également de

Composé	Descripteur odorant	Seuil de détection odorant dans l'eau (µg/L) *	Poisson	Références
Ethanal	Pas d'étude olfactométrique		Saumon	Girard, B. & Nakai, S., 1991
Propanal	Pas d'étude olfactométrique	9,5 à 37	Saumon	Mansur, M.A. et al., 2003
2-methyl propanal	Pas d'étude olfactométrique		Saumon	Girard, B. & Nakai, S., 1991
2,2-dimethyl propanal	Pas d'étude olfactométrique		Saumon	Girard, B. & Nakai, S., 1991
Butanal	Pas d'étude olfactométrique		Saumon	Girard, B. & Nakai, S., 1991
(E)-2-pentenal	vert, herbacée	1 500	Truite	Sérot, T. et al. 2002
			Saumon	Refsgaard, H.H.F. et al., 1999
				Josephson, D.B. et al., 1991a
Pentanal	Pas d'étude olfactométrique		Saumon	Refsgaard, H.H.F. et al., 1999
(E)-2-hexenal	mousse, sous-bois	17	Truite	Sérot, T. et al. 2002
			Saumon	Refsgaard, H.H.F. et al., 1999
				Josephson, D.B. et al., 1991a
Hexanal	vert, herbe coupée	4 à 5	Truite	Sérot, T. et al. 2002
			Saumon	Josephson, D.B. et al., 1991a
				Refsgaard, H.H.F. et al., 1999
				Josephson, D.B. et al., 1984
				Mansur, M.A. et al. 2003
(E)-2-heptenal	vert, mousse, grillé	13	Truite	Sérot, T. et al. 2002
			Saumon	Refsgaard, H.H.F. et al., 1999
(Z)-4-heptenal	Pas d'étude olfactométrique	0.8 à 10	Saumon	Josephson, D.B.et al., 1991a
Heptanal	Pas d'étude olfactométrique	3	Saumon	Refsgaard, H.H.F. et al., 1999
				Josephson, D.B. et al., 1991a
				Mansur, M.A. et al., 2003
(E,E)-2,4-heptadienal	vert, marin		Truite	Sérot, T. et al. 2002
			Saumon	Refsgaard, H.H.F. et al., 1999
(E)-2-octenal	Pas d'étude olfactométrique	3	Saumon	Refsgaard, H.H.F. et al., 1999
Octanal	gras, vert, fruité	0,7	Truite	Sérot, T. et al. 2002
			Saumon	Refsgaard, H.H.F. et al., 1999
				Josephson, D.B. et al., 1991a
(E)-2-nonenal	Terreux	0,08 à 0,1	Truite	Sérot, T. et al. 2002
			Saumon	Josephson, D.B. et al., 1991a
Nonanal	Pas d'étude olfactométrique	1	Saumon	Refsgaard, H.H.F. et al., 1999
				Josephson, D.B. et al., 1991a
(E,Z)-2,6-nonadienal	vert, concombre, floral	0,01	Truite	Sérot, T. et al. 2002
			Saumon	Refsgaard, H.H.F. et al., 1999
				Josephson, D.B. et al., 1991a
(E,E)-2,4-nonadienal	Pas d'étude olfactométrique		Saumon	Refsgaard, H.H.F. et al., 1999
Decanal	Vert	0,1 à 2	Truite	Sérot, T. et al. 2002
			Saumon	Refsgaard, H.H.F. et al., 1999
(E,E)-2,4-decadienal	Pas d'étude olfactométrique	0,07	Saumon	Refsgaard, H.H.F. et al., 1999
				Josephson, D.B. et al., 1991a

* d'après : http://www.leffingwell.com/odorthre.htm

Tableau 6. Principaux aldéhydes odorants identifiés dans l'arôme de Salmonidés frais

la dégradation des acides gras. Les principaux aldéhydes présents dans l'arôme de Salmonidés sont référencés dans les tableaux 6 et 7. Ils ont en général un faible seuil de perception odorante ce qui fait qu'ils ont une action sur l'arôme global même en très faibles quantités. De plus, ils sont caractérisés par des odeurs vertes mais qui peuvent devenir rapidement désagréables avec des notes huileuses et cuites dès que leurs quantités (liées à l'oxydation des lipides donc à la détérioration du poisson) augmentent. Ainsi, le propanal, le (E)-2-pentenal, le (E)-2-hexenal, le (E,E)-2,4-heptadienal et le (E,Z)-2,6-nonadienal proviennent des AG n-3. Le propanal n'a pas été identifié comme composé volatil odorant dans les Salmonidés. Le (E)-2-pentenal et le (E)-2-hexenal sont des composés volatils odorants de nombreux produits de la mer. Ils ont tous les deux une note odorante verte mais le (E)-2-pentenal semble plus végétal que le (E)-2-hexenal qui apparaît avec des notes de sous-bois/mousse. Le (E)-2-pentenal a déjà été retrouvé dans les anchois (Cha, Y.J. & Cadwallader, K.R., 1995), les huîtres (Piveteau, F. et al., 2000) et la présence du (E)-2-hexenal a déjà été rapportée dans des pétoncles (Chung, H.Y. et al., 2001), le crabe (Chung, H.Y., 1999) ou le turbot (Sérot, T. et al., 2001a). Ces deux composés illustrent bien la variété des seuils de détection odorants retrouvés parmi les composés carbonylés car le (E)-2-pentenal est perçu à partir de 1500 µg/L dans l'eau alors que le (E)-2-hexenal est perçu à partir de 17 µg/L alors que leurs structures sont assez proches. Le (E)-2-hexenal peut également dériver des AG n-6. Le (E,E)-2,4-heptadienal retrouvé avec une odeur verte/marine dans les Salmonidés, est également retrouvé dans les moules (Le Guen, S. et al., 2000a ; Cros, S. et al., 2004) et le crabe (Chung, H.Y., 1999). Enfin, le (E,Z)-2,6-nonadienal a été identifié dans les Salmonidés avec une odeur caractéristique de concombre/vert. C'est un des composés que l'on retrouve de manière récurrente dans les arômes des produits de la mer, que ce soit des poissons comme le turbot (Sérot, T. et al., 2001a) ou les anchois (Cha, Y.J. & Cadwallader, K.R., 1995), que ce soit des crustacés comme le crabe (Chung, 1999) ou des coquillages comme les moules (Le Guen, S. et al., 2000a) ou les huîtres (Pennarun, A.L. et al., 2002).

Les AG n-6 sont également les précurseurs de nombreux aldéhydes. On y retrouve les n-alcanals du pentanal à l'heptanal, les (E)-2-alcènals du (E)-2-hexenal au (E)-2-nonénal ainsi que le (E,E)-2,4-decadienal. Ainsi, le pentanal dont l'odeur n'a pas été évaluée sur le saumon a par ailleurs déjà été identifié comme un composé odorant dans les moules (Le Guen, S. et al., 2000a) avec des descripteurs fruités/chimiques. L'hexanal possède une odeur caractéristique d'herbe coupée. Il fait lui aussi partie des composés odorants retrouvés de manière récurrente dans les produits de la mer comme le turbot (Prost, C. et al., 1998) ou la sardine (Prost, C. et al., 2004). L'odeur de l'heptanal n'a pas été explorée sur les Salmonidés

Aldéhyde (nombre de carbones : insaturations)	Précurseurs	Descripteurs odorants
3 :0	n-3	irritant
5 :0	n-6	amande amère
6 :0	n-6	vert, fruité/amande
7 :0	n-6 / n-9	huileux/savon, fruité
8 :0	n-9	huileux/savon, fruité
9 :0	n-9	suif, savon/fruité
10 :0	n-9	peau d'orange
5 :1 (2-trans)	n-3	peinture, vert/pomme
6 :1 (2-trans)	n-3 / n-6	vert
6 :1 (3-cis)	n-3	feuilles vertes, crudités
7 :1 (2-trans)	n-6	gras, amande amère
7 :1 (4-cis)	n-3	crémeux, vert
8 :1 (2-trans)	n-6	noix, punaise, gras
8 :1 (2-cis)	n-6	gras, noix
9 :1 (2-trans)	n-6	suif/concombre, amidon
9 :1 (3-cis)	n-6	vert, concombre
10 :1 (2-trans)	n-9	suif, orange
11 :1 (2-trans)	n-9	
7 :2 (2-trans, 4-cis)	n-3	friture, pommes pourries
7 :2 (2-trans, 4-trans)	n-3	gras/huileux, noisettes
9 :2 (2-trans, 4-trans)	n-6	gras/huileux
9 :2 (2-trans, 6-cis)	n-3	concombre
9 :2 (2-trans, 6-trans)	n-3	concombre/vert, suif
10 :2 (2-trans, 4-cis)	n-6	friture
10 :2 (2-trans, 4-trans)	n-6	friture
10 :3 (2-trans, 4-trans, 7-cis)	n-3	haricots

Tableau 7. Filiation des aldéhydes provenant de l'autoxydation des acides gras polyinsaturés et caractéristiques odorantes (d'après Grosch, W., 1987).

mais il fait partie de l'arôme de poissons (Cha, Y.J. & Cadwallader, K.R., 1995 ; Sérot, T. et al., 2001a) où il présente une odeur grasse à chimique. Le (E)-2-heptenal est perçu dans les Salmonidés avec une odeur vert/mousse comme dans le turbot (Sérot, T. et al., 2001a) ou les moules (Cros, S. et al., 2004). Le (E)-2-octenal n'a pas été le sujet d'étude olfactométrique chez les Salmonidés mais constitue un composé odorant du turbot (Prost, C. et al., 1998) et des moules (Cros, S. et al., 2004) marqué par des odeurs grasses, vertes parfois fruitées (selon la concentration). Le (E)-2-nonenal a été identifié dans les Salmonidés avec une odeur terreuse comme dans les huîtres (Pennarun, A.L. et al., 2002) ou le turbot (Prost, C. et al., 1998 ; Sérot, T. et al., 2001a) où son odeur peut également être perçue comme verte. Enfin, l'odeur du (E,E)-2,4-decadienal n'a pas été caractérisée dans les Salmonidés mais dans les moules (Cros, S. et al., 2004) et d'autres poissons (Prost, C. et al., 1998), allant d'odeurs vertes à des odeurs de friture en fonction de sa concentration.

Les AG n-9 sont généralement les précurseurs des n-alcanals. Ainsi l'octanal, le nonanal et le décanal sont produits par l'oxydation de l'acide oléique. L'octanal perçu avec une odeur grasse et fruité dans les Salmonidés, est également détecté avec les mêmes descripteurs dans les moules (Le Guen, S. et al., 2000a ; Cros, S. et al., 2004). L'odeur du nonanal n'a pas été évaluée dans les Salmonidés mais elle l'a été dans les moules. Son odeur comporte des notes vertes et grasses selon la concentration. Le décanal quant à lui a été détecté dans les Salmonidés avec une odeur verte. Cependant, dans les huîtres (Piveteau, F. et al., 2000), il a été identifié avec des odeurs plus marines ou florales. D'autres aldéhydes volatils ont également été identifiés dans les Salmonidés. Leurs précurseurs n'ont pas été formellement identifiés ainsi que leurs caractéristiques odorantes n'ont pas été déterminées. Cependant, les acides gras semblent impliqués dans leur génération comme pour le (Z)-4-heptenal retrouvé dans le saumon mais dont l'odeur n'a pas été étudiée sur ce produit. Cependant, ce composé a été détecté avec une odeur de gras/cuit et de pomme de terre dans des coquillages et le cabillaud (McGill, A.S. et al., 1974). L'odeur du butanal n'a pas été déterminée dans les produits de la mer mais il a néanmoins été retrouvé dans d'autres poissons comme les anchois ou le hareng (Cha, Y.J. & Cadwallader, K.R., 1995).

Parmi les cétones, les formes 2-alcénones, 2,3-alcadiones et 1-alcèn-3-one sont les plus rencontrées (Prost, C. et al., 2004 ; Alasalvar, C . et al., 2005) ainsi que la (E,E) ou (E,Z)-3,5-octadièn-2-one (Lindsay, R.C., 1994 ; Aro, T. et al., 2002).

Les principales cétones volatiles présentes dans l'arôme de Salmonidés sont présentées dans le tableau 8. Les cétones sont également produites par l'oxydation des lipides.

Composé	Descripteur odorant	Seuil de détection odorant dans l'eau (µg/L)	Poisson	Références
Acetone	Pas d'étude olfactométrique		Saumon	Mansur, M.A. et al., 2003
2-heptanone	Pas d'étude olfactométrique	140 à 3 000	Saumon	Josephson, D.B. et al., 1991a
3-heptanone	Pas d'étude olfactométrique		Saumon	Refsgaard, H.H.F. et al., 1999
6-methyl-5-hepten-3-one	fruité		truite	Sérot, T. et al. 2002
1-octen-3-one	champignon	0,005	Saumon	Josephson, D.B. et al., 1984
				Refsgaard, H.H.F. et al., 1999
				Josephson, D.B. et al., 1991a
3-octanone	Pas d'étude olfactométrique	28	Saumon	Josephson, D.B. et al., 1991a
(Z,Z)-1,5-octadien-3-one	géranium	$1,2.10^{-3}$	Saumon	Josephson, D.B. et al., 1984
				Josephson, D.B. et al., 1991a
2-nonanone	Pas d'étude olfactométrique	5 à 200	Saumon	Josephson, D.B. et al., 1991a
				Refsgaard, H.H.F. et al., 1999
2-undecanone	Pas d'étude olfactométrique	7	Saumon	Josephson, D.B. et al., 1991a

Tableau 8. Principales cétones odorantes identifiées dans l'arôme de Salmonidés frais

Ainsi, les AG n-3 sont à l'origine de la (Z,Z)-1,5-octadien-3-one identifiée dans le saumon avec une odeur de géranium et dans de nombreux autres produits de la mer (Varlet, V. et al., 2006). Les AG n-6 sont les précurseurs de la 2-heptanone identifiée parmi les composés volatils de nombreux produits de la mer mais son odeur n'a pas été déterminée dans ces produits. Les AG n-6 sont également à l'origine de la 1-octen-3-one retrouvée dans le saumon avec une odeur de champignon. Cette cétone a également été déterminée dans les huîtres (Piveteau, F. et al., 2000) .

L'oxydation des AG est impliquée dans la production d'autres cétones comme la 3-octanone, la 2-nonanone et la 2-undecanone retrouvées dans le saumon (Josephson, D.B. et al., 1991a). Ces composés sont odorants dans d'autres produits de la mer mais leurs odeurs n'ont pas été évaluées dans les Salmonidés. Cependant, ces trois molécules ont déjà été étudiées dans les huîtres (Pennarun, A.L. et al., 2002) et les moules (Le Guen, S. et al., 2000a) avec des odeurs de terre/vert, citron pour la 3-octanone, fruitée/crémeuse pour la 2-nonanone et fruité/floral pour la 2-undecanone.

La dégradation des caroténoïdes pourrait également être à l'origine de la 3-octanone et de la 6-methyl-5-hepten-3-one selon certains auteurs (Pennarun, A.L. et al., 2002).

1.4.1.2 Les composés soufrés

Quelques composés soufrés sont également présents comme le diméthyl disulfide ou diméthyl trisulfide (Girard, B. & Nakai, S., 1994a). Ils sont issus de la dégradation enzymatique ou bactérienne d'acides aminés soufrés comme la cystéine ou la méthionine (Chung, H.Y. et al., 2006). Ils sont responsables d'odeurs soufrées caractéristiques et désagréables. Comme pour les composés carbonylés, ces composés ont des seuils de détection odorants très faibles d'où leurs impacts parfois importants sur l'arôme global. Le diméthyl disulfide possède un seuil de détection odorant dans l'eau situé entre 0,16 et 12 µg/L alors que le diméthyl trisulfide est perçu dans des concentrations dans l'eau allant de 0,005 à 0,01 µg/L.

1.4.1.3 Les hydrocarbures

Les rares hydrocarbures aliphatiques (Girard, B. & Nakai, S., 1994 ; Aro, T. et al., 2002) peuvent soit provenir de l'environnement (Easley, D.M. et al., 1981) soit provenir de

l'oxydation des lipides par la formation de radicaux alkylés ou bien encore de la dégradation des caroténoïdes (Tanchotikul, U. & Hsieh, C.Y., 1989).
Les hydrocarbures cycliques sont plus nombreux, souvent dérivés du benzène ou du toluène (Alasalvar, C. et al., 2005). Ils sont suspectés provenir soit de la dégradation du glucose (Pennarun, A.L. et al., 2002), de la contamination environnementale ou également de la dégradation des caroténoïdes (Josephson, D.B. et al., 1991a).

1.4.1.4 Les composés terpéniques

Concernant les dérivés terpéniques, l'alpha-pinène, le béta-pinène, le limonène le béta-terpinéol sont fréquemment retrouvés parmi les composés volatils des poissons (Grigorakis, K. et al., 2003). Ces composés sont responsables d'odeurs fraîches, florales à fruitées. Ils ont de faibles seuils de détection odorants de l'ordre du µg/L dans l'eau. Par exemple, le limonène a une odeur caractéristique de citron perçue à 10 µg/L dans l'eau. L'environnement et la nourriture semblent être les précurseurs les plus probables de ces composés.

1.4.2 Composés volatils odorants ou non odorants des Salmonidés ayant subi un procédé thermique

Un poisson frais n'a quasiment aucune odeur. Cependant, il constitue une matrice hautement réactive qui peut entraîner très rapidement des processus de dégradation et d'altération. Ainsi, en plus de l'odeur intrinsèque de la matrice fraîche, les procédés thermiques peuvent engendrer la génération de composés volatils odorants. Sous l'effet de la chaleur ou du stockage réfrigéré, des réactions biochimiques peuvent avoir lieu entre les différents constituants de la chair de poisson. On retrouve principalement les mêmes familles de composés que dans le poisson frais mais en nombre plus important ainsi que des composés provenant de l'atération post-mortem du produit comme les amines volatiles. La plupart des composés du poisson cru se retrouvent parmi les composés volatils de l'arôme du poisson ayant subi le traitement thermique.

1.4.2.1 Les amines volatiles

L'ensemble des composés formés par l'ammoniac et diverses amines volatiles constitue l'azote basique volatil total (ABVT). Le dosage des amines basiques volatiles totales est un dosage largement utilisé pour évaluer la qualité des produits de la mer. C'est un terme général qui comprend la détermination de la triméthylamine (produite par les bactéries d'altération), la diméthylamine (produite par les enzymes autolytiques pendant le stockage du poisson congelé), l'ammoniac et d'autres composés azotés volatils basiques associés à l'altération des produits de la mer (produits par décarboxylation des acides aminés) (Huss, H.H, 1999). L'ammoniac est formé par la dégradation bactérienne ou désamination des protéines, peptides et acides aminés. Il est aussi produit pendant l'altération autolytique de catabolites de nucléotides comme l'adénosine monophosphate (AMP) dans les produits glacés de la mer. L'ammoniac a été détecté comme composant volatil dans de nombreux poissons altérés. La triméthylamine (TMA) est une amine volatile à odeur forte souvent associée à l'odeur typique "douteuse" de produit de la mer qui se dégrade. La TMA a un seuil de détection odorant dans l'eau assez faible qui se situe entre 0,37 et 1,06 µg/L. Sa présence dans le poisson en cours d'altération est due à la réduction bactérienne de l'oxyde de triméthylamine (OTMA) (40 à 120 mg/kg chez les poissons de mer). L'OTMA, naturellement présent dans le tissu vivant de plusieurs espèces de poissons marins, est utilisé comme accepteur d'hydrogène. La TMA est, pour de nombreuses espèces, un indicateur d'altération (Timm, M. & Jørgensen, B.M., 2002). La diméthylamine (DMA) est produite par réaction enzymatique lors du stockage à l'état congelé (Lundstrom, R.C. & Racicot, L.D., 1983). Sa production est augmentée par la manipulation brutale et par les fluctuations de température dans les chambres froides. La DMA n'a que peu ou pas d'effet sur la flaveur ou la texture du poisson en soi, mais elle est un indicateur indirect de la dénaturation des protéines qui est souvent due à une mauvaise manutention avant et/ou pendant le stockage à l'état congelé.
La TMA et la DMA dans une moindre mesure participent donc à l'odeur de poisson avarié de l'arôme de poisson. Cependant, ces composés sont en faibles quantités dans les poissons gras comme les Salmonidés.

1.4.2.2 Alcools et composés carbonylés provenant des AG

La chaleur a pour effet d'accélérer l'oxydation des lipides. Ainsi, les AG sont d'autant plus dégradés et des composés carbonylés et alcools sont créés.

Composé	Poisson	Références
2-methyl-1-propanol	Saumon en conserve	Girard, B. & Durance, T., 2000
2-butoxyethanol	Saumon en conserve	Girard, B. & Durance, T., 2000
1-butanol	Saumon en conserve	Girard, B. & Durance, T., 2000
3-pentanol	Saumon en conserve	Girard, B. & Durance, T., 2000
7-octen-4-ol	Saumon en conserve	Girard, B. & Nakai, S., 1994b
decanol	Saumon en conserve	Girard, B. & Durance, T., 2000
1H-pyrrole	Saumon en conserve	Girard, B. & Durance, T., 2000
Benzeneethanol	Saumon cuit	Josephson, D.B. et al., 1991b
2-ethyl-1-hexanol	Saumon en conserve	Girard, B. & Durance, T., 2000

Tableau 9. Principaux alcools odorants ou non, identifiés dans l'arôme de Salmonidés ayant subi un procédé thermique et non identifiés dans les Salmonidés frais.

Composé	Poisson	Références
2-butenal	Huile de saumon	Josephson, D.B. et al., 1991b
	Saumon en conserve	Girard, B. & Durance, T., 2000
2-methylbutanal	Saumon bouilli	Milo, C. & Grosch, W., 1996
	Saumon en conserve	Girard, B. & Nakai, S., 1994b
	Saumon en conserve	Girard, B. & Nakai, S., 1991
	Saumon en conserve	Girard, B. & Durance, T., 2000
3-methylbutanal	Saumon bouilli	Milo, C. & Grosch, W., 1996
	Saumon en conserve	Girard, B. & Nakai, S., 1991
	Saumon en conserve	Girard, B. & Durance, T., 2000
	Truite bouillie	Milo, C. & Grosch, W., 1995
(Z)-2-pentenal	Huile de saumon	Josephson, D.B. et al., 1991b
(Z)-3-hexenal	Saumon bouilli	Milo, C. & Grosch, W., 1996
	Truite bouillie	Milo, C. & Grosch, W., 1995
2,4-hexadienal	Huile de saumon	Josephson, D.B. et al., 1991b
(Z)-2-heptenal	Huile de saumon	Josephson, D.B. et al., 1991b
(E)-4-heptenal	Saumon en conserve	Girard, B. & Durance, T., 2000
(E,Z)-heptadienal	Saumon cuit	Josephson, D.B. et al., 1991b
	Saumon en conserve	Girard, B. & Durance, T., 2000
	Huile de saumon	Josephson, D.B. et al., 1991b
(Z)-2-nonenal	Saumon bouilli	Milo, C. & Grosch, W., 1996
(Z,Z)-3,6-nonadienal	Saumon bouilli	Milo, C. & Grosch, W., 1996
	Truite bouillie	Milo, C. & Grosch, W., 1995
(E)-4,5-epoxy-(E)-2-decenal	Saumon bouilli	Milo, C. & Grosch, W., 1996
2-decenal	Saumon en conserve	Girard, B. & Durance, T., 2000
Undecanal	Saumon en conserve	Girard, B. & Durance, T., 2000
Benzaldéhyde	Saumon frais ou en conserve cuit	Josephson, D.B. et al., 1991b
	Saumon en conserve	Girard, B. & Nakai, S., 1991
	Saumon en conserve	Girard, B. & Nakai, S., 1994b
	Saumon en conserve	Girard, B. & Durance, T., 2000
ethyl benzaldehyde	Saumon en conserve cuit	Josephson, D.B. et al., 1991b
Méthional	Saumon bouilli	Milo, C. & Grosch, W., 1996
	Truite bouillie	Milo, C. & Grosch, W., 1995

Tableau 10. Principaux aldéhydes odorants ou non, identifiés dans l'arôme de Salmonidés ayant subi un procédé thermique et non identifiés dans les Salmonidés frais.

Pour les alcools, la plupart des composés ne sont pas odorants ou leur odeur n'a pas été évaluée sur les Salmonidés (tableau 9). Cependant, ils ont déjà été identifiés dans l'arôme d'autres produits de la mer. En effet, la présence du 1-butanol, du 7-octen-4-ol, du décanol et du 2-ethyl-1-hexanol a déjà été reportée dans le crabe bouilli (Flament, I., 1990) celle du 2-butoxyethanol dans les moules cuites (Le Guen et al., 2000b). Le 1-butanol a déjà été trouvé dans de la sauce de poisson avec une odeur de fromage (Fukami, K. et al., 2002) avec un seuil de détection dans l'eau de 500 µg/L.

Concernant les aldéhydes, la dégradation des AG n-3 conduit à des composés comme le (Z)-2-pentenal, le (Z)-2-heptenal ou le (E,Z)-2,4-heptadienal. Comme pour les aldéhydes issus des AG dans le poisson frais, ces composés ont de faibles seuils de détection odorant dans l'eau et des notes odorantes allant de vert/fruité à gras selon la concentration. Les AG sont également connus pour être à l'origine du 2-butenal ou du (Z,Z)-3,6-nonadienal. Les principaux composés carbonylés (aldéhydes et cétones) retrouvées dans les Salmonidés ayant subi un procédé thermique sont répertoriés dans les tableaux 10 et 11.

Certaisn composés alcooliques proviennent également de réactions de rétroaldolisation desaldéhydes insaturés. L'élévation de la température accélère ces réactions (Josephson, D.B. et al., 1987).

1.4.2.3 Alcools et composés carbonylés provenant des réactions de Maillard et Strecker

De nouveaux composés sont produits par l'intermédiaire de la réaction de Maillard entre les acides aminés et les composés glucidiques (présents en faibles quantités) du poisson puis la dégradation de Strecker.

En effet, la réaction de Maillard intervient entre un substrat carbonylé et un substrat azoté (acide aminé). L'intermédiaire aldosamine ou cétosamine formé peut donner : de petites molécules carbonylées aromatiques par scission, des composés furaniques par déshydratation forte ou des réductones, composés dicarbonylés, par déshydratation douce.

Les aldéhydes ramifiés à courte chaîne peuvent également provenir de réactions purement chimiques de dégradation des acides aminés, telles que les mécanismes de Strecker (Mottram, D.S., 1992), qui mettent en jeu un acide aminé et un composé dicarbonylé. Celui-ci sera souvent la 2,3-butanedione ou la 2,3-pentanedione (Mottram, 1992) mais aussi des produits d'oxydation des lipides, identifiés dans les Salmonidés ayant subi un procédé thermique ainsi que dans d'autres poissons comme le turbot bouilli (Prost, C. et al., 1998).

Composé	Poisson	Références
2-butanone	Saumon en conserve	Girard, B. & Durance, T., 2000
2,3-butanedione	Truite bouillie	Milo, C. & Grosch, W., 1995
	Huile de saumon	Josephson, D.B. et al., 1991b
	Saumon bouilli	Milo, C. & Grosch, W., 1996
3-pentanone	Saumon en conserve	Girard, B. & Durance, T., 2000
2,3-pentanedione	Truite bouillie	Milo, C. & Grosch, W., 1995
	Saumon en conserve	Girard, B. & Durance, T., 2000
	Huile de saumon	Josephson, D.B. et al., 1991b
	Saumon bouilli	Milo, C. & Grosch, W., 1996
1-penten-3-one	Huile de saumon	Josephson, D.B. et al., 1991b
3-methyl-2-butanone	Saumon en conserve	Girard, B. & Durance, T., 2000
3-hexanone	Saumon en conserve	Girard, B. & Nakai, S., 1991
		Girard, B. & Nakai, S., 1994b
3,5,5-trimethyl-2-cyclohexen-1-one	Huile de saumon	Josephson, D.B. et al., 1991b
4-heptanone	Huile de saumon	Josephson, D.B. et al., 1991b
6-methyl-5-hepten-2-one	Saumon en conserve	Girard, B. & Durance, T., 2000
6-methyl-3,5-heptadien-2-one	Huile de saumon	Josephson, D.B. et al., 1991b
2-octanone	Saumon en conserve	Girard, B. & Durance, T., 2000
2,3-octadione	Saumon en conserve	Girard, B. & Durance, T., 2000
	Huile de saumon	Josephson, D.B. et al., 1991b
3-octen-2-one	Huile de saumon	Josephson, D.B. et al., 1991b
3,5-octadien-2-one	Saumon en conserve	Girard, B. & Durance, T., 2000
	Huile de saumon	Josephson, D.B. et al., 1991b
	Saumon cuit	Josephson, D.B. et al., 1991b
Acétophénone	Saumon en conserve	Girard, B. & Durance, T., 2000

Tableau 11. Principales cétones odorantes ou non, identifiées dans l'arôme de Salmonidés ayant subi un procédé thermique et non identifiés dans les Salmonidés frais.

Composé	Poisson	Références
Furanne	Saumon en conserve	Girard, B. & Durance, T., 2000
2-methyl furanne	Saumon en conserve	Girard, B. & Durance, T., 2000
		Girard, B. & Nakai, S., 1991
		Girard, B. & Nakai, S., 1994b
3-methyl furanne	Saumon en conserve	Girard, B. & Durance, T., 2000
2,5-dimethyl furanne	Huile de saumon	Josephson, D.B. et al., 1991b
2-ethyl furanne	Saumon en conserve	Girard, B. & Durance, T., 2000
2-acétyl furanne	Saumon en conserve	Girard, B. & Durance, T., 2000
2-propyl furanne	Saumon frais ou en conserve cuit	Josephson, D.B. et al., 1991b
2-pentyl furanne	Huile de saumon	Josephson, D.B. et al., 1991b
	Saumon en conserve	Girard, B. & Durance, T., 2000
		Girard, B. & Nakai, S., 1991
		Girard, B. & Nakai, S., 1994b
2-furfurol	Saumon frais ou en conserve cuit	Josephson, D.B. et al., 1991b
Pyrazine	Saumon frais ou en conserve cuit	Josephson, D.B. et al., 1991b
Methyl pyrazine	Saumon frais ou en conserve cuit	Josephson, D.B. et al., 1991b
2,6-dimethyl pyrazine	Saumon frais ou en conserve cuit	Josephson, D.B. et al., 1991b
2,5-diethyl-3-methyl pyrazine	Saumon frais ou en conserve cuit	Josephson, D.B. et al., 1991b
4,4-dimethyl-2-buten-4-olide	Huile de saumon	Josephson, D.B. et al., 1991b
2-acétyl-1-pyrroline	Saumon bouilli	Milo, C. & Grosch, W., 1996
dihydro-2-methyl-3(2H)-furanone	Saumon en conserve	Girard, B. & Durance, T., 2000

Tableau 12. Principaux composés furanniques odorants ou non, identifiés dans l'arôme de Salmonidés ayant subi un procédé thermique

Ainsi, le 3-methylbutanal provient de la leucine, le 2-methylbutanal de l'isoleucine et le méthional de la méthionine. Le 3-methylbutanal a déjà été identifié dans la crevette rôtie (Kubota, K. et al., 1986) ou les anchois (Triqui, R. et al., 1999) avec une odeur fruitée/céréale pour un seuil de détection odorant d'environ 1 µg/L. Le méthional possède lui une odeur caractéristique de pomme de terre cuite et un seuil de détection de 0,2 µg/L. Il est retrouvé dans les anchois (Triqui, R. et al., 1999) ou les moules cuites (Le Guen, S. et al., 2000b).Le 1H-pyrrole provient de la dégradation thermique des acides aminés (Yaylayan, A.V. & Keyhani, A., 2001) et fait partie des nombreux composés cycliques formés par la dégradation thermique des constituants de l'aliment. Un processus de séchage peut être à l'origine de sa formation (Kawai, T. et al., 1991).

1.4.2.4 Composés furaniques

Sous l'effet de la chaleur, une déshydratation forte peut conduire à des composés furaniques par la réaction de Maillard dans les Salmonidés (Tableau 12). Cependant, il est à noter que certains composés furaniques peuvent provenir de la dégradation des AG, comme le 2-pentylfuranne. En revanche, les pyrazines et les lactones sont bien produites par la réaction de Maillard. Ces composés possèdent en général des odeurs cuites agréables (chocolat, beurré, grillé) mais aucune étude olfactométrique n'a permis d'identifier leurs odeurs dans les Salmonidés ayant subi un traitement thermique.

1.4.2.5 Composés soufrés

Le catabolisme des acides aminés est aussi essentiel que celui des AG dans la génération de composés volatils, qu'ils soient odorants ou non. La plupart des composés soufrés retrouvés dans les Salmonidés ayant subi un procédé thermique proviennent de la dégradation de la cystine ou de la cystéine. Le procédé thermique agit comme un catalyseur dans la dégradation des constituants du poisson et conduit à la création de composés soufrés, répertoriés dans le tableau 13. Ces composés sont responsables de mauvaises odeurs marquées par des notes soufrées, putrides ou de champignon.

1.4.2.6 Hydrocarbures

Composé	Poisson	Références
Hydrogene sulfide	Saumon en conserve	Girard, B. & Nakai, S., 1991
		Girard, B. & Nakai, S., 1994b
Methane thiol	Saumon en conserve	Girard, B. & Nakai, S., 1991
		Girard, B. & Nakai, S., 1994b
		Girard, B. & Durance, T., 2000
Dimethyl sulfide	Saumon en conserve	Girard, B. & Nakai, S., 1991
		Girard, B. & Nakai, S., 1994b
		Girard, B. & Durance, T., 2000
Methyl ethyl sulfide	Saumon en conserve	Girard, B. & Durance, T., 2000
Dimethyl disulfide	Saumon en conserve	Girard, B. & Durance, T., 2000
2-methyl thiophene	Saumon en conserve	Girard, B. & Durance, T., 2000
		Josephson, D.B. et al., 1991b
3-methyl thiophene	Saumon en conserve	Girard, B. & Durance, T., 2000
		Josephson, D.B. et al., 1991b
Methyl ethyl disulfide	Saumon en conserve	Girard, B. & Durance, T., 2000
2-ethyl thiophene	Saumon en conserve	Girard, B. & Durance, T., 2000
Dimethyl trisulfide	Saumon en conserve	Girard, B. & Durance, T., 2000
dihydro-2H-thiopyran-3(4H)-one	Saumon en conserve	Girard, B. & Durance, T., 2000
Propene sulfide	Saumon en conserve	Girard, B. & Durance, T., 2000
Benzenethiazole	Saumon en conserve	Josephson, D.B. et al., 1991b
4,5-dimethyl thiazole	Saumon en conserve	Josephson, D.B. et al., 1991b
2,5-diethyl-4-methylthiazole	Saumon en conserve	Josephson, D.B. et al., 1991b

Tableau 13. Principaux composés soufrés odorants ou non, identifiés dans l'arôme de Salmonidés ayant subi un procédé thermique

Un grand nombre d'alcanes ramifiés et de composés benzéniques ont été extraits en faibles quantités dans les Salmonidés ayant subi un procédé thermique. Cependant, ils sont très nombreux par rapport à leur occurrence dans les Salmonidés frais (Tableau 14).
Deux raisons peuvent expliquer ce phénomène. La première tient au faible nombre de travaux ayant porté sur la présence d'hydrocarbures dans l'arôme des Salmonidés frais. En effet, il y a en général plus de recherches menées sur les produits de la mer ayant été industrialisés qu'à l'état frais. La seconde hypothèse consiste en une contamination du produit, soit au niveau du procédé, soit au niveau de la matière première. En effet, pour certains composés, une origine alimentaire est envisageable car ils sont rencontrés avec une grande diversité dans les végétaux. Les hydrocarbures benzéniques comme les isomères du xylène sont déjà présents en quantité relativement importante dans les Salmonidés (Josephson, D.B. et al., 1991b).
Ainsi, les procédés thermiques industriels accélèrent la dégradation des constituants du poisson, notamment les acides gras et les acides aminés (Echarte, M. et al., 2001). Parallèlement, sous l'effet de la chaleur, des réactions biochimiques comme celles de Maillard et de Strecker peuvent avoir lieu et complexifient la composition aromatique de l'odeur.

1.4.3 Composés volatils de la chair des poissons fumés

A notre connaissance, aucune étude portant sur la caractérisation de l'odeur du saumon fumé et de poisson fumé en général n'a été réalisée. Cependant, récemment, quelques travaux présentés ci-après ont permis d'identifier certaines molécules volatiles du poisson fumé mais aucune étude exhaustive n'a été entreprise concernant les composés volatils odorants du poisson fumé. L'analyse de l'arôme du saumon fumé est difficile de par la diversité des précurseurs aromatiques qui peuvent provenir du saumon frais, de la fumée de bois, ou de la réaction entre les composés de la fumée et les constituants de la chair de poisson sous l'effet du fumage.
Le fumage, comme tout traitement thermique, provoque donc des modifications physico-chimiques qui affectent la composition biochimique et les qualités organoleptiques du poisson par rapport à son état frais. La connaissance des composés volatils présents dans les arômes de Salmonidés frais ou ayant subi un procédé thermique se révèle une base essentielle pour appréhender l'investigation des composés volatils odorants responsables de la perception de l'odeur des poissons fumés comme le saumon.

Composé	Poisson	Références
Butane	Saumon en conserve	Girard, B. & Nakai, S., 1991
		Girard, B. & Nakai, S., 1994b
3-methyl-1-butene	Saumon en conserve	Girard, B. & Nakai, S., 1991
		Girard, B. & Nakai, S., 1994b
Pentane	Saumon en conserve	Girard, B. & Durance, T., 2000
2,3-dimethyl pentane	Saumon en conserve	Girard, B. & Durance, T., 2000
2,4-dimethyl pentane	Saumon en conserve	Girard, B. & Durance, T., 2000
1-pentene	Saumon en conserve	Girard, B. & Durance, T., 2000
2-pentene	Saumon en conserve	Girard, B. & Durance, T., 2000
Hexane	Saumon en conserve	Girard, B. & Nakai, S., 1991
		Girard, B. & Durance, T., 2000
		Girard, B. & Nakai, S., 1994b
2-methyl hexane	Saumon en conserve	Girard, B. & Durance, T., 2000
Cyclohexane	Saumon en conserve	Girard, B. & Durance, T., 2000
Methylcyclohexane	Saumon en conserve	Girard, B. & Durance, T., 2000
3-ethyl-1,4-hexadiene	Saumon en conserve	Girard, B. & Nakai, S., 1991
		Girard, B. & Nakai, S., 1994b
Heptane	Saumon en conserve	Girard, B. & Nakai, S., 1991
		Girard, B. & Durance, T., 2000
		Girard, B. & Nakai, S., 1994b
Nonane	Saumon en conserve	Girard, B. & Nakai, S., 1994b
		Girard, B. & Durance, T., 2000
Decane	Saumon en conserve	Girard, B. & Nakai, S., 1991
		Girard, B. & Durance, T., 2000
1,3,5-octatriene	Saumon cuit	Josephson, D.B. et al., 1991b
1,3,6-octatriene	Saumon en conserve	Girard, B. & Durance, T., 2000
pentadecane	Saumon frais ou en conserve cuit	Josephson, D.B. et al., 1991b
	Huile de saumon	
heptadecane	Saumon frais ou en conserve cuit	Josephson, D.B. et al., 1991b
1,5-dimethyl cyclopentene	Saumon en conserve	Girard, B. & Nakai, S., 1991
		Girard, B. & Nakai, S., 1994b
benzene	Saumon en conserve	Girard, B. & Nakai, S., 1991
		Girard, B. & Durance, T., 2000
		Girard, B. & Nakai, S., 1994b
Methyl benzene	Saumon en conserve	Girard, B. & Nakai, S., 1991
toluene	Saumon en conserve cuit	Josephson, D.B. et al., 1991b
	Huile de saumon	
	Saumon en conserve	Girard, B. & Durance, T., 2000
		Girard, B. & Nakai, S., 1994b
o-xylene	Saumon en conserve cuit	Josephson, D.B. et al., 1991b
	Huile de saumon	
	Saumon en conserve	Girard, B. & Durance, T., 2000
m-xylene	Saumon en conserve cuit	Josephson, D.B. et al., 1991b
	Huile de saumon	
	Saumon en conserve	Girard, B. & Durance, T., 2000
p-xylene	Huile de saumon	Josephson, D.B. et al., 1991b
	Saumon en conserve	Girard, B. & Durance, T., 2000
trimethylbenzene	Saumon en conserve cuit	Josephson, D.B. et al., 1991b
Ethylbenzene	Saumon en conserve	Girard, B. & Durance, T., 2000
1-ethyl-4-methyl benzene	Saumon en conserve	Girard, B. & Durance, T., 2000
1-ethyl-3-methyl benzene	Saumon en conserve	Girard, B. & Durance, T., 2000
1-methylethylbenzene	Saumon en conserve	Girard, B. & Durance, T., 2000

Tableau 14. Principaux hydrocarbures odorants ou non, identifiés dans les Salmonidés ayant subi un procédé thermique

Quelques travaux ont déjà porté sur l'identification de certains composés volatils des poissons fumés mais avec des objectifs différents de la détermination des composés odorants.

Ainsi l'équipe de Guillén, M.D. a travaillé sur l'extraction par Solid-Phase Microextraction (SPME) des composés volatils de truite, de daurade, d'espadon et de cabillaud fumés (Guillén, M.D. & Errecalde, M.C., 2002 ; Guillén, M.D. et al., 2006). Les molécules retrouvées se différencient en plusieurs familles constituées de composés aliphatiques et de composés cycliques. La plupart des composés aliphatiques proviennent du poisson, que ce soit à travers son alimentation, la dégradation de ses constituants lipidiques ou protéiques. On retrouve des alcools comme le 3-methyl-1-butanol, provenant de la réduction des aldéhydes (nonanal, decanal, 2-decenal, dodecanal, tetradecanal, hexadecanal), des cétones comme la propanone, des hydrocarbures (pentadecane, heptadecane).

Cependant, excepté les acides carboxyliques aliphatiques, la majorité des composés sont de nature cyclique et proviennent de la fumée de bois. On retrouve les dérivés du cyclopenten-1-one, des composés furaniques, des hydrocarbures cycliques et des composés phénoliques. Si l'origine des composés phénoliques et des dérivés du cyclopenten-1-one est aisément identifiée (la fumée de bois), la détermination des précurseurs des composés furaniques ainsi que ceux des hydrocarbures cycliques (alimentation, contamination environnementale, dégradation des caroténoïdes, fumée de bois) n'est pas évidente.

En effet, concernant les composés furaniques, ceux-ci peuvent provenir de la fumée de bois ou de réaction de Maillard entre les constituants du poisson sous l'effet de la température pendant le fumage. Cependant, les dérivés du furfural sont principalement identifiés dans la fumée de bois.

Concernant les hydrocarbures cycliques, certains peuvent provenir de l'environnement mais une autre partie, mal évaluée peut provenir de la fumée. C'est le cas notamment des dimethoxybenzènes et diméthoxytoluènes (Guillén et al., 1999c).

Les travaux de Cardinal, M. et al., en 1997, ont permis de différencier quatre procédés de fumage en fonction de la nature et de la quantité de composés volatils identifiés dans du saumon. Cependant, aucune information sur les potentialités aromatiques des molécules identifiées n'a été présentée. La plupart des composés retrouvés dans le saumon fumé sont ceux précédemment cités dans les travaux de Guillén, M.D. et al.

En 2001, les équipes de Joffraud, J.J. et al. et celles de Jørgensen, L.V. et al. ont étudié les composés volatils du saumon fumé contaminé par des microorganismes. Leurs résultats attestent la présence de composés volatils mais ne peuvent assurer quels en sont les précurseurs entre l'oxydation lipidique, la fumée de bois ou l'activité des microorganismes.

Leurs études étaient essentiellement centrées sur les composés carbonylés et les alcools volatils.

Ainsi, il existe quelques données sur les composés volatils de poisson fumé mais l'identification exhaustive des composés volatils odorants responsables de l'arôme de poisson fumé reste à explorer.

Etant donné le manque d'informations disponibles sur les aldéhydes volatils des poissons fumés en général, l'idée d'une synthèse scientifique des données existantes sur ces composés a abouti à une publication parue dans Food Chemistry. Celle-ci résume les méthodes d'extraction et d'analyse des aldéhydes dans les poissons fumés ainsi que leurs mécanismes de formation, leurs propriétés organoleptiques et leurs toxicités.

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Volatile aldehydes in smoked fish: Analysis methods, occurence and mechanisms of formation

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Abstract

The carbonyl function of volatile aldehydes is discussed from methodological point of view (reactivity and analysis method). From thi presentation, an inventory of volatile aldehydes recovered in smoked fishes are carried out. Then, the different pathways possible for the formation of these molecules are explained in order to better understand their occurence in smoked fish aroma. Maillard reactions for the ''smoked'' aroma and lipid oxidation for ''fishy'' aroma are the two main pathways of creation of odorant volatile aldehydes. Each odorant aldehyde recovered in smoked fish is characterized by its descriptors, its odour thresholds and its origins are investigated. Volatile aldehydes in smoked fishes are also studied according to the others organoleptic roles that they play in this kind of food matrices especially about their contribution in organoleptic properties of smoked products. Finally, the toxicity of several aldehydes identified in smoked fishes is discussed in order to assess their roles in smoked fish safety.

Keywords: Volatile aldehydes; Smoked fish; Maillard reaction; Strecker reaction; Lipid oxidation; Odour thresholds; Organoleptic quality; Aldehyde toxicity

Contents

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1. Introduction

Because of its original flavours and thanks to the technological development in food analysis, the aroma compounds of smoked fishes are very studied, especially salmon. The high value of smoked salmon and the volumes of production – about 88,000 tons produced in Europe in 2003 (Clément, 2004) – can explain the particular attention brought to the smoking process, the storage and the microbiological stability of this product. The world production of farm and fishery salmon has increased from 850,000 tons in 1985 to over 1,800,000 tons in 2001 (Eagle, Naylor, & Smith, 2004). Indeed, salmon seems to be the most investigated smoked fish but herring (Sérot, Baron, Knockaert, & Vallet, 2004; Sérot & Lafficher, 2003), haddock and a lot of other fishes (Guillén & Errecalde, 2002; Guillén, Errecalde, Salmerón, & Casas, 2006) can be also processed by this technique. Smoking is practiced since old times in order to preserve food from the natural degradations and oxidation. Indeed, it consists in the application of smoke produced by the pyrolysis of the wood on the food. This smoke can be at gaseous state the most often, but the development of liquid and solid smokes offers new possibilities of smoking (Guillén & Ibargoitia, 1999; Guillén & Manzanos, 1996; Guillén & Manzanos, 1999). The preservation of the food is guaranteed by the antioxidant and antimicrobial properties of certain molecules (Cornu et al., 2006). For example, phenolic compounds generated by the combustion combined with the temperature and the conditions of smoking can reduce the microbiological development and the oxidation (Kjällstrand & Petersson, 2001). The smoking process is nowadays very looked after for the flavouring of the food and the typical organoleptic qualities that this process confers to the smoked food.

In all the studies which deal with smoked fishes aroma, carbonyl compounds and especially aldehydes form a group very important among the hundreds of volatile components identified. Aldehydes are molecules very interesting because they can be observed as indicators of the oxidative state and of microbiological contamination of the products. Indeed, they can constitute degradation products of microbiological metabolism reactions (Girard & Nakai, 1994b) and can return account of bacterial activity. On smoked salmon, it has been shown that numerous microorganisms as Brochotrix thermosphacta, Photobacterium phosphoreum (Stohr, Joffraud, Cardinal, & Leroi, 2001) and lactic acid bacteria are responsible for the production of typical sour, rancid and sulfurous off odours. These unpleasant odours can be related to the products of the bacterial metabolism which are known to originate from the main catabolic pathways of lipids, carbohydrates and amino acids, then among them, a lot of carbonyls compounds and precursors of aldehydes. They can also constitute degradation products of oxidation reactions from fatty acids and illustrate the level of alteration of a product because they have frequently off-flavour odours (McGill, Hardy, Burt, & Gunstone, 1974). For example, propanal can serve as a reliable indicator of flavour deterioration for fish products and hexanal for meats (Augustin, Sanguansri, & Bode, 2006; Ross & Smith, 2006). These molecules are also very reactive towards oxido-reduction transformations and represent intermediaries in a lot of biochemical reactions. However, numerous aldehydes coming from the wood smoke or created during lipid peroxidation are carcinogenic and can cause mouth, stomach or oesophageal diseases. The genotoxicity and cytotoxicity is especially due to the nucleophilic attack to amino and sulfhydryl functional groups of biomolecules such as proteins, nucleic acids, glutathione and cysteine (Kataoka, Kondo, & Sumida, 1998). For this reason, it is obvious that the fact to survey these molecules is also necessary. For example, a,b-unsaturated aldehydes like 4-hydroxy-(E)-2-hexenal or (E)-2-butenal are cytotoxic because (E)-2-butenal in drinking water was shown to cause liver tumors in rats suggesting that these aldehydes may be potential carcinogens (Chung, Tanaka, & Hecht, 1986; Munasinghe et al., 2003; Witz, 1989). Aldehydes in smoked fishes have also others organoleptic roles in the colour and the texture of the food matrices even if they mainly contribute to the global aroma of food matrices (Sainclivier, 1985).

The aims of this paper are to summarize the information about aldehydes in smoked fish. The chemical properties of aldehydes are firstly presented in order to better understand the ways of extraction and analysis of these molecules in smoked fishes. Then, the different possible pathways for aldehydes formation in smoked fishes will be presented in relation with their origins and odorant properties. We will conclude with the others organoleptic

roles of aldehydes in smoked fishes and their potential toxicity.

2. Analysis of volatile aldehydes

2.1. Extraction methods

Aldehydes are in the same time oxidant and reductor molecules. They can be formed by oxidation of primary alcohols and they can be further oxidized in the correspondent carboxylic acid, then they have a good reductor character. The chemical properties of the carbonyl function imply the aldehydes in a lot of reactions because even if the C=O bond. Indeed, the oxygen atom is more electronegative than the carbon atom ($\chi_O = 3.5 > \chi_C = 2.5$). The inductive attractive effect polarizes the carbonyl system which leads to an important polarity of the carbonyl group even if the alkyl group has a little inductive giver effect. Thus, as there is very little steric obstacle near the functional carbon, the C=O bond is very reactive (Curioni & Bosset, 2002). However, for the extraction, it is a consequent drawback because the compounds can evolve during the extraction.

Extraction methods developed for volatiles aldehydes are mainly those used for volatile compounds and are very linked to the analysis method chosen after the extraction step. Because of the reactivity of aldehyde function, a derivatization step can occur in order to protect the chemical structure. When a derivatization is planned, it is obvious that an extract at liquid state will be easier to derivatize. Then, in this case, extraction by reduce pressure steam distillation is used (Shibamoto & Horiuchi, 1997). According to the results of certain studies, aldehydes can also be extracted by headspace techniques but the aim of these studies was more an identification of the volatile compounds from a food matrix than identification especially focused on volatile aldehydes. Then, solid-phase micro extraction (SPME) and dynamic headspace/static headspace (DHS/SHS) extractions are used on seafood aroma analysis: SHS on salmon (Girard & Nakai, 1991; Girard & Nakai, 1994a, 1994b), DHS on crayfish waste (Tanchotikul & Hsieh, 1989) or sardine (Prost, Hallier, Cardinal, Serot, & Courcoux, 2004), SPME on smoked swordfish and cod (Guillén et al., 2006) or sea fish and prawns (Mansur, Bhadra, Takamura, & Matoba, 2003).

Liquid–liquid extractions (LLE) are not very suitable to recover the volatile aldehydes. However, LLE recent devices like supercritical fluid extraction (SFE) (Aro, Brede, Manninen, & Kallio, 2002) or microwave assisted extraction (MAE) (Grimm, Lloyd, Batista, & Zimba, 2000) are more and more used.

When specific aldehydes are studied, others extraction methods can be developed. It is the case of formaldehyde. All the previously described methods are possible but absorption or adsorption on solid support with a step of solid-liquid extraction with adequate solvents are also used (INERIS, 2004; INRS, 2004a). It consists in trapping the aldehyde at gaseous state on absorbants and in eluting this trap by solvents. The same sampling can be used when aldehydes are at gaseous state. In the case of cooking fumes analysis, aldehydes can also be collected in special bags and directly analyzed at gaseous state (Fullana, Carbonell-Barrachina, & Sidhu, 2004a).

Concerning the efficiency of the extraction methods, headspace techniques allow to recover very volatile aldehydes with low boiling point but these techniques are based on gaseous equilibrium phenomenon. These methods can be criticized about their efficiency because the content of aroma compounds which is moved in the headspace is not totally known. Reduce pressure steam distillation (RPDE) and simultaneous steam distillation solvent extraction (SDE) allow to obtain compounds of higher boiling point (Varlet, Prost, & Sérot, 2006). With reduce pressure steam distillation, the boiling point is reached with lower temperatures than simultaneous steam distillation solvent extraction. Therefore, the possible artefacts generated by thermal degradation can be reduced but this method implies a long time of extraction and a lot of material. SFE seems to be very efficient but it is limited according to the fat matter rate of the food matrix analyzed. MAE is not enough developed for volatile aldehydes analyses and the recovery yields can be very different according to the molecules searched. Moreover, the conditions of extraction are too strong and lead to generation of aldehydes by lipid oxidation.

2.2. Analytical methods

For the analysis of aldehydes, several methods have been used and all almost based on the reactivity of the carbonyl group through derivatization steps and/or colorized products. The analysis method chosen is very linked to the extraction method used. In function of the detector, a derivatization step is applied, especially when the detection occurs optically (spectrophotometry).

Indeed, the carbonyl compounds can be revealed by the action of 2,4-dinitrophenylhydrazine (2,4-DNPH) which leads to an orange precipitate of hydrazones (Kataoka et al., 1998). High performance liquid chromatography with UV detection (HPLC-UV) has been optimized to analyze these aldehydes with a preliminary derivatization step of the aldehydes as their 2,4-dinitrophenylhydrazine derivatives (Wilkes et al., 2000) generally with a wavelength of 360 nm (Jacob, Denis, & Foster, 1998; Pichard et al., 2005). The wavelength can be chosen in a range from 330 to 580 nm in function of the aldehydes (INRS, 2004a). The range from 330 to 390 nm allows generally to obtain the most part of the aldehydes (for example the maximal absorption wavelength of acetaldehyde is 350 nm and 390 nm for furfural). Although HPLC-UV method is very commonly used for the volatile carbonyl compounds, the identification is not easy (formation of both syn and anti forms, difficulties in mixtures analysis). Besides, the preparation of 2,4-DNPH derivatives requires strong acidic

conditions that may cause undesirable reactions, such as decomposition of carbohydrates, which can trouble the analysis. This kind of method has been widely used (Da Cunha Veloso, Da Silva, Vieira Santos, & De Andrade, 2001), even now in qualitative measurements but less and less used for quantifying because of its lack of repeatability.

Others colorimetric methods are individually used for certain aldehydes like formaldehyde which can be analyzed after reaction with chromotropic acid by absorption spectrophotometry because the purple complex formed absorbs at 580 nm (INERIS, 2004). However, these methods can not be apply to all the aldehydes as it is possible with 2,4-DNPH.

Others methods have been developed and also used the derivatization of aldehydes but as their benzyloxime or thiazolidine derivatives. These methods analyze the derivatives with gas chromatography (GC) coupled with specific detector. The benzyloxime or thiazolidine derivatives are very selectively and sensitively detectable by GC-NPD (nitrogen phosphorus detection) (Yasuhara & Shibamoto, 1995). Cysteamine method for aldehyde analysis is simple and specific. Indeed, volatile aldehydes react with cysteamine to form thiazolidines derivatives under mild conditions of neutral pH and room temperature (Fig. 1). The derivatization is nearly complete, fast (15 min) and reaches 95% (Shibamoto & Horiuchi, 1997). There is only one derivative for each aldehyde and an excess of reagent does not interfere with GC analysis. Another detector system can also be coupled after the GC separation. It is the flame photometric detection (FPD) which allows a selective and sensitive determination of saturated and unsaturated aldehydes. The detection limits on a panel of various standard aldehydes at a signal-to-noise ratio of 3 were from 4 to 100 pg injected (Kataoka et al., 1998). GC/MS (mass spectrometry) analysis can also be used, especially to understand the structures of the derivatives. A molecular ion peak $[M]^+$ was observed for each derivative and others decomposition peaks were characteristic for the elucidation like $[M - CH_3]^+$, $[M - SCH_2]^+$, $[M - SCH_2CH_2]^+$, $[M - SCH_2CH_2NH]^+$ and $[M - SCH_2CH_2NHCH]^+$ (Kataoka et al., 1998).

Recently, a new procedure of derivatization was developed for the microscale determination of aldehydes with SPME, especially for aliphatic aldehydes. It consists in a derivatization on-fiber by exposing the fiber to an aqueous solution of (2,3,4,5,6-pentafluorophenyl)hydrazine (PFPH) (Stashenko, Puertas, Salgar, Delgado, & Martinez, 2000). Using GC/MS method after SPME, the mass fragments obtained are very specific according to the aldehydes (Rochat & Chaintreau, 2005). O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine oximes can also be used (PFBO) (Hsu et al., 1999) but molecular ions with this type of derivatization are often missing which limits the specificity. PFPH derivatization seems to be very specific thanks to a more abundant molecular ion. These new types of derivatization are very efficient but only carried out at microscale and with SPME headspace technique (Stashenko & Martinez, 2004). These types of derivatization might be developed for a wider use in food analysis in general and for other extraction methods (Stashenko, Ferreira, Sequeda, Martinez, & Wong, 1997).

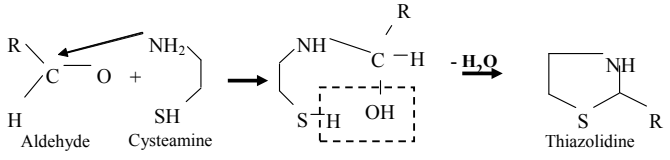

Fig. 1. Reaction mechanism of cysteamine and aldehydes.

With food aroma extraction techniques, the derivatization is not obliged when all the aroma compounds and not especially aldehydes are studied. The detectors used can be various and the most frequently used are MS and FID (Fullana, Carbonell-Barrachina, & Sidhu, 2004b). When particular aldehydes are studied, especially in air analysis, others detectors coupled to GC can be used like pulsed Helium Ionization Detector (pHID). This detector overcomes the insensitivity of FID in the case of the study of ambient formaldehyde (Hopkins et al., 2003). Mass spectrometry was (Josephson, Lindsay, & Stuiber, 1987) and is, especially now, used for the quantification of volatiles in fish and among them aldehydes (Milo & Grosch, 1996; Fukami et al., 2002). This analytical method allows to quantify aldehydes in $\mu g.kg^{-1}$ concentrations.

Finally, aldehydes can be analyzed by nuclear magnetic resonance (NMR). (E)-2-alkenals, n-alkanals, (E,E)-2,4-alkadienals can be particularly measured with this method (Guillén & Ruiz, 2005) but the determination is again qualitative.

3. Identification of volatile aldehydes recovered in smoked fish flesh

Table 1 presents a list of aldehydes the most frequently found in smoked fishes. Table 1 was carried out from seven main teams of scientists, then with different objectives in the investigation of aldehydes. Guillén and Errecalde (2002) and Guillén et al. (2006) identified by SPME the different volatiles present in smoked swordfish, cod, trout and bream. Cardinal, Berdagué, Dinel, Knockaert, and Vallet (1997) investigated the volatile components of smoked salmon in order to characterize four processes of smoking. Jørgensen, Huss, and Dalgaard (2001) and Joffraud, Leroi, Roy, and Berdagué (2001) worked on spoiled smoked salmon. Jørgensen et al. (2001) studied volatile components like aldehydes which are responsible of off-flavours in smoked salmon spoiled with bacteria. These two last references attest the presence of volatiles aldehydes in smoked fish but can not surely insure the origin of the aldehydes (lipid oxidation, wood smoke or production of microorganisms, or both). Headspace extractions are used in the whole works. This extraction method is used especially for the extraction of volatile components with a low boiling point. The group of volatile aldehydes identified corresponds to these properties. Indeed, volatile aldehydes with

Table 1
Volatile aldehydes found in smoked fish

Aldehydes	Smoked fishes				
	Trout	Bream	Salmon	Cod	Swordfish
Ethanal (acetaldehyde)	X(6)				
butanal			X(3)		
3-Methylbutanal			X(2,3,4,7)		
2-Methylbutanal			X(4)		
2-Butenal			X(2)		
2-Methyl-2-butenal			X(4)		
Pentanal			X(2)		
(E)-2-Pentenal			X(3)		
2-Furancarboxaldehyde (furfural)			X(2,3,7)	X(1)	
5-Methyl-2-furancarboxaldehyde (5-methyl-furfural)			X(2,3,7)	X(1)	
Benzaldehyde	X(6)	X(6)	X(3,7)	X(1)	X(1)
4-Methylbenzaldehyde			X(2)		
Benzeneacetaldehyde	X(6)	X(6)	X(7)		X(1)
Hexanal	X(6)	X(6)	X(2,3,4)		
4-Hydroxy-2-(E)-hexenal			X(5)		
Heptanal	X(6)		X(2,7)		
2-Heptenal		X(6)			
Octanal			X(2)		
Nonanal	X(6)	X(6)	X(2,7)		
Decanal	X(6)	X(6)	X(2,4)	X(1)	X(1)
2,4-Hexadienal			X(7)		
2,4-Heptadienal			X(7)		
2,4-Hecadienal		X(6)			
2-Decenal	X(6)		X(2,4,7)	X(1)	X(1)
2-Undecenal	X(6)				
Dodecanal	X(6)	X(6)		X(1)	
Tetradecanal	X(6)	X(6)		X(1)	X(1)
Hexadecanal	X(6)	X(6)	X(7)		X(1)
(1H)-Pyrrole-2-carboxaldehyde			X(7)	X(1)	X(1)

X = presence, in brackets are reported the numbers corresponding to the scientific teams who have worked on the respective kind of smoked fish (1: Guillén et al. (2006); 2: Cardinal et al. (1997); 3: Jørgensen et al. (2001); 4: Joffraud et al. (2001); 5: Munasinghe et al. (2003); 6: Guillén and Errecalde (2002); 7: Varlet et al., 2006, 2006Varlet et al. (2006)).

a diversity of the structure of molecules (aliphatic, aromatic, straight chain, branched chain) from C4 to C16 in smoked fishes are studied. Munasinghe et al. (2003) were only focused on 4-hydroxy-(E)-2-hexenal and used liquid–liquid extraction with dichloromethane. Finally, Varlet et al. (2006) studied odour-active compounds of smoked salmon with SDE extraction. This method allows to obtain volatile compounds with higher boiling point than headspace extractions, but for the aldehydes the results are similar to headspace methods.

Among the volatile aldehydes recovered in smoked fish, several approaches can be considered. A first sorting might be carried out on the basis of their natural origin that is to say if they come from wood smoke or fish flesh. Indeed, there are some compounds met in smoked fishes which come firmly from wood smoke because they are not met in the unsmoked fish flesh and others known to make part of volatile aldehydes involved only in fish metabolism. However, this sorting can be difficult because certain aldehydes could be created by the extraction method and the smoking method (Maillard compounds) or are present in small quantities in unsmoked fish flesh. There are also sometimes ubiquist compounds that is to say, present in wood smoke and unsmoked fish flesh as hexanal. Another characteristic can be used to distinguish volatile aldehydes of smoked fish based on the structure of the aldehydes. Indeed, the aromatic or aliphatic forms of the aldehydes are deeply linked to the pathways of creation. Aromatic aldehydes mainly come from the wood smoke through thermal degradation and aliphatic aldehydes mainly come from the fish flesh through lipid oxidation (Cardinal et al., 1997).

4. Volatile aldehydes of smoked fish coming from Maillard and Strecker reactions

4.1. Maillard reaction mechanism

In smoked fish, the origin of aromatic aldehydes could be attributed to enzymatic reactions but especially to thermal degradations as Maillard reactions (Fig. 2). These reactions occur during thermal process (cooking, smoking, roasting, etc.) and are facilitated when temperature raises (Ames, 1998; Fernandez, Kerverdo, Duñach, & Lizzani-Cuvelier, 2002). The process consists in the formation of a non-enzymatic bound between the carbonyl group of an aldose or ketose and the free amino group of an amino

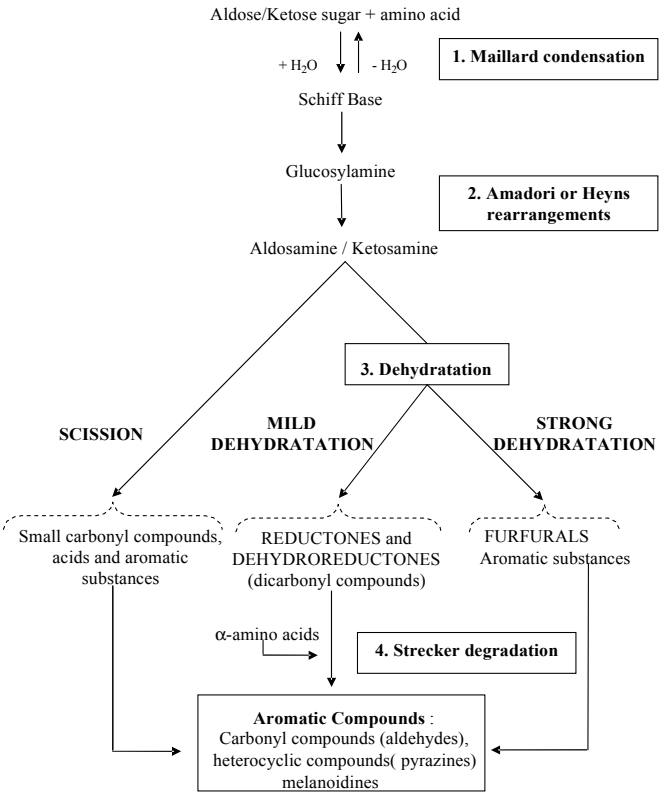

Fig. 2. An example of reaction of Maillard: Principle and different steps for aldehydes formation.

acid. As result, a product very unstable in aqueous solution, by the loss of water molecule can lead to Schiff base. These reactions are reversible. However, a non-reversible isomerisation of Schiff base gives glucosylamines named Amadori products (aldosamines) or Heyns products (ketosamines) (Yaylayan & Huyghues-Despointes, 1994). Thanks to rearrangements, these compounds are more stabilized. The products of these rearrangements can also be transformed. Three cases are possible: a break in the molecule, a soft dehydration or a strong dehydration. The break leads to small carbonylated or acidic molecules (Weenen, 1998). Dehydrations are very important reactions because they produce others intermediaries which are precursors of a lot of aldehydes. Indeed, a sweet dehydration transforms Amadori or Heyns intermediaries in reductones or dehydroreductones which are dicarbonyl compounds. When the dehydration is stronger, reductones and dehydroreductones can form rings and dehydrate to form furfurals and furans very present in liquid smoke used for food flavourings (Guillén & Manzanos, 2005; Simon, De La Calle, Palme, Meier, & Anklam, 2005).

Reductones and dehydroreductones can produce also others aldehydes by retro-aldol reaction reactions which is the reverse of an aldol condensation. Aldol condensation allows the addition of two carbonyl compounds with the fixing of the a-carbon of one on the carbonyl carbon of the other. This product can easily dehydrate in order to form unsaturated carbonyl compounds. In retro-aldol reaction, the unsaturated carbonyl group is hydrated at the double bond to finally form two smaller carbonyl molecules. These reactions are very important and can explain the origin of numerous aldehydes in smoked fish (Josephson & Lindsay, 1987).

4.2. Possibility of aldehydes formation during Maillard reactions

A lot of furannic aldehydes are also found in smoked fishes in important quantities as furfural (2-furancarboxaldehyde) and 5-methyl-2-furancarboxaldehyde (5-methylfurfural) (Guillén et al., 2006; Varlet et al., 2006). These compounds could come only from the wood smoke or could be generated between the wood smoke and the fish flesh through Maillard and Strecker reactions. Indeed, the possibility that furfural and 5-methylfurfural could come from Maillard reactions during smoking process between fish components has not been proven. In wood smoke, these generations are mainly due to the separation of water from pentoses which are degradation products of hemicelluloses (Jira, 2004) whereas Maillard and Strecker reactions have not been undoubtedly proven in wood smoke. However, these pathways could be involved because several products indicators of these reactions are found in liquid smoke and smoked fishes such as pyrazines (Guillén & Ibargoitia, 1996; Guillén, Manzanos, & Ibargoitia, 2001; Varlet et al., 2006). These compounds could be Maillard and Strecker aldehydes created during smoking and could derive from wood smoke (Cardinal et al., 1997; Maga, 1987). Recently, the analysis of the pyrolysis profile of oak has permitted to detect pyrone and furan structures in the smoke (Nonier et al., 2005). These products have been attributed to Maillard reactions. The N-glucoside derivative formed by the reaction of sugars with certain amino compounds leads to an unstable Amadori derivative after several rearrangements which finally generates aromatic compounds with enolone structure when it is heated. The composition in carbohydrates of wood can explain the possibility of the creation of such compounds (Guillén et al., 2001). Indeed, wood is composed of three main polyoses: cellulose (homopolymer of D-glucose with β 1–4 links), hemicellulose (homo or heteropolysaccharides) and lignin. The thermal breakdown of cellulose forms firstly glucose and secondly acetic acid and homologs, water, a few furans and phenolic compounds. The thermal breakdown of hemicellulose produces furans and carboxylic acids. Finally, lignin, whose structure is deeply linked to two main rings (guaïacyl-propan ring and syringyl-propan ring) leads by its combustion to phenolic compounds and phenolic ethers with little amounts of carbonyl, acidic and alcoholic compounds (Sainclivier, 1985; Miler & Sikorski, 1990). Reductor sugars which initiate Maillard reaction are thus very present in wood. The necessary high temperature is also reached by the pyrolysis of the wood. The only disadvantage concerns the essential nitrogen source which is not very clear. Indeed, in the element composition of wood without bark, a ratio of 0.2% of nitrogen by report to the dry matter is observed. This nitrogen is in very weak quantity and depends of the wood type (Alén, Kuoppala, & Oesch, 1996; Alén, Oesch, & Kuoppala, 1995; Maga, 1987). Nitrogen occurence during wood combustion is obligatory because nitrogen oxides (NO_x) and ammonia emissions are found in wood combustion (Welfring & Weidenhaupt, 2000). NO_x are known to be produced during wood combustion from three pathways. Firstly, NO_x can be created from ambient nitrogen (N_2) and oxygen at very high temperatures (>1300 C) obtained very close to the flames (Welfring, Weidenhaupt, Offermann, & Gouy, 2000). The second pathway involves a spontaneous formation in the flames thanks to hydrocarbure radicals. However, these conditions are never reached in smoking process because flames production must be avoided in order to have an adequate wood smoke. The third pathway is the most important and involves the nitrogen of the wood even if atmospheric nitrogen seems also to contribute to Maillard reactions in wood smoke. Nitrogen in wood is present under amine and proteins forms and with oxygen, can form NO_x at temperatures two times lower than the two first pathways (Welfring & Weidenhaupt, 2000; Welfring et al., 2000). Thus, all the conditions are met to allow the aldehydes formation through Maillard and Strecker reaction.

Nevertheless, Strecker degradation and Maillard reactions can also occur during the smoking process between

the components of the fish flesh or between sugars of the wood smoke and amino acids of the fish flesh. Indeed, the quantity of carbohydrates in fish flesh is very low. Therefore, Maillard and Strecker reactions could occur more easily between very abundant sugars of wood smoke and the most important nitrogen source during smoking process that is to say amino acids of the fish flesh. Then, it is difficult to distinguish volatile aldehydes coming from the wood smoke and those coming from the fish flesh. Nevertheless, they can be assessed in function of their origin by comparing individually the quantities of aldehydes present in wood smoke to those present in smoked fish flesh. Besides, the possibility of Maillard and Strecker aldehydes formation during smoking process without involving wood smoke is strengthened by the study of methional case. Methional (3-methylthiopropanal) generally comes from methionine via Strecker degradation and is not present in wood smoke (Maga, 1987) but can be identified in unsmoked seafood flesh like lobster tail (Lee, Suriyaphan, & Cadwallader, 2001), crab (Chung & Cadwallader, 1994; Chung, 1999), shells (Pennarun, Prost, & Demaimay, 2002; Chung, Yung, Ma, & Kim, 2002), turbot (Sérot, Regost, Prost, Robin, & Arzel, 2001) or anchovy (Triqui & Reineccius, 1995). It is important to notice that sometimes the matrix is cooked-processed but sometimes, the product is raw and Maillard and Strecker compounds are also present in both cases. This illustrates the fact that Maillard and Strecker reactions are possible without taking into account wood smoke effects. These compounds could be formed from the amino groups of amino acids and carbonyl groups of reductor sugars presents in small quantities in fish flesh under temperatures of smoking or during the extraction step if the temperature is too important. However, even if sometimes Maillard and Strecker products have been recovered in fish flesh, several studies have permitted to conclude that in general, cyclic compounds such as furfural, 1H-pyrrole-carboxaldehyde, benzaldehyde, 5-methylfurfural (reported in Table 1) and all the derived molecules come from the wood smoke (Cardinal et al., 1997; Guillén et al., 2006; Varlet et al., 2006). Moreover, it is also commonly admitted that Maillard reactions lead to products having caramellic, baked, cooked even roasted odorant descriptors. These descriptors are very often in adequation with those found for wood smoke odorants.

4.3. Volatile Maillard aldehydes in smoked fishes

Maillard compounds such as benzaldehyde and derived compounds are present in smoked fish. However, it is difficult to say if these molecules come from the wood smoke or fish flesh because the two pathways are possible. Indeed, as it is reported in Tables 2 and 3, benzaldehyde and derived compounds have been found in unsmoked fish flesh

Table 2
Occurence of benzaldehyde and methylated derivatives in seafood products

Compound	Odorant descriptor	Extraction method	Matrices	LRI (and column)	References
Benzaldehyde	Fruity, almond, nutty, creamy	SDE	Mussel juices	1524 (Stabilwax)	Cros et al. (2004)
		RPDE	Cooked mussels	1539 (DB-Wax)	Le Guen et al. (2000b)
		SDE	Crab	1530 (Supelcowax 10)	Chung (1999)
		SDE	Scallops	1530 (Supelcowax 10)	Chung et al. (2001, 2002)
		Headspace	Oysters	1525 (DB-Wax)	Piveteau et al. (2000)
		Headspace	Canned salmon	1538 (Supelcowax 10)	Girard and Durance (2000)
		SDE	Anchovy, herring, shrimp	1522 (Supelcowax 10)	Cha and Cadwallader (1995)
		RPDE	Anchovy	960 (DB5-MS)	Triqui and Reineccius (1995)
4-Methylbenzaldehyde	Almond	Headspace	Smoked salmon	1080 (DB5-MS)	Cardinal et al. (1997)
		SDE	Scallops	1654 (Supelcowax 10)	Chung et al. (2001)
4-Ethylbenzaldehyde	Fruity, anised	RPDE	Turbot	1728 (DB-Wax)	Sérot et al. (2001) Cooked
			mussels	1752 (DB-Wax)	Le Guen et al. (2000b)
		SDE	Scallops	1714 (Supelcowax 10)	Chung et al. (2001, 2002)
3,4-Dimethyl benzaldehyde	Not described	SDE	Crab	1790 (Supelcowax 10)	Chung (1999)
3-Methylbenzaldehyde	Not described	SDE	Scallops	1624 (Supelcowax 10)	Chung et al. (2001, 2002)

LRI: linear retention indices, SDE: simultaneous steam distillation – solvent extraction, RPDE: reduce pressure distillation extraction.

Table 3
Occurence of benzaldehyde and methylated derivatives in wood smoke

Compound	Extraction – analysis method	Wood matrix used	References
Benzaldehyde	2,4-DNPH, HPLC/UV	Oak, pine, eucalyptus	Hedberg et al. (2002)
	LLE, GC/MS	Thyme	Guillén and Manzanos (1999)
	Not described	Wood	Maga (1987)
o-Tolualdehyde	2,4-DNPH, HPLC	Oak, pine, eucalyptus	Hedberg et al. (2002)
p-Tolualdehyde	2,4-DNPH, HPLC	Oak, pine, eucalyptus	Hedberg et al. (2002)
2,5-Dimethylbenzaldehyde	2,4-DNPH, HPLC	Oak, pine, eucalyptus	Hedberg et al. (2002)

2,4-DNPH: 2,4-dinitrophenylhydrazine, HPLC/UV: high performance liquid chromatography coupled to ultraviolet detector, LLE: liquid–liquid extraction, GC/MS: gas chromatography coupled to mass spectrometric detector.

and also in wood smoke (Hedberg et al., 2002). Quantities recovered, possibilities of Maillard reactions and cyclic structures can make us to say that these molecules recovered in smoked fishes come from wood smoke (Guillén & Errecalde, 2002; Guillén & Ibargoitia, 1999; Maga, 1987). In fact, it is mainly produced by combustion process (Kat- aoka et al., 1998) like wood combustion (Maga, 1987). Benzaldehyde and derivatives could also be produced, in a weak part, by oxidation or by photochemical degradation of toluene or others hydrocarbons like styrene or methylstyrene. These aromatic hydrocarbons can derive from wood smoke and carotenoids (Josephson, Lindsay, & Stuiber, 1991a). Finally, benzaldehyde and derivatives could also be adsorbed in the flesh fish through its environment because these molecules are lipophilic and can easily cross the biological membranes.

The cyclic aldehydes from wood smoke can be easily absorbed by the fish flesh which is rich in lipids. Indeed, the aromatic structure of the most part of Maillard aldehydes gives to themselves lipophilic properties. Thus, furfurals and aldehyde phenolic compounds (Lustre & Issenberg, 1969) present in important quantities in wood smoke (Guillén & Ibargoitia, 1999; Guillén & Manzanos, 1997; Guillén & Manzanos, 1999; Hedberg et al., 2002) are recovered in smoked fish flesh. Furfurals are known to derive from the strong dehydration of glucosylamines (aldosamine/ketosamine) in Maillard reaction (Fig. 2).

4.4. Strecker degradation mechanism

Reductones and dehydroreductones from Maillard reaction have also another interest due to their dicarbonylated structure. Combined with a-amino acids, dicarbonylated compounds are the seat of Strecker degradation (Rizzi, 1999). This degradation leads to an aldehyde that has one less carbon than the original amino acid and a-amino carbonyl. This reaction consists in a decarboxylation of the Schiff base formed after dehydration, followed by the rehydratation of the molecule. Thus, it has been established for example that 2-methylpropanal (isobutyraldehyde) comes from valine (Scarpellino & Soukup, 1993). Strecker aldehydes are very present in wood smoke (Maga, 1987) even if Strecker degradation is not the only mechanism of generation for these compounds.

4.5. Volatile Strecker aldehydes in smoked fishes

The origin of several aldehydes reported in Table 4 are well known. Ethanal (acetaldehyde) from alanine, 2-methyl-2-butenal from isoleucine, 3-methylbutanal (isovaleraldehyde) from leucine, phenylacetaldehyde (benzeneacetaldehyde) from phenylalanine are volatile aldehydes coming from Strecker degradation (Lee et al., 2001) through Maillard reactions. Formed by the action between a-dicarbonyl compound and an amino acid, all these Strecker aldehydes are very present in many seafood products (Table 4). The attribution of a single origin to these compounds is difficult because even if a lot of Strecker aldehydes are present in wood smoke, another part seems to be linked to the smoking process or to be accumulated by the fish from its environment.

5. Volatile aldehydes of smoked fish coming from lipid oxidation

5.1. Lipid oxidation: aliphatic aldehydes formation

Aliphatic aldehydes are commonly known to derive from lipid oxidation which occurs in fish flesh (Sérot, Regost, & Arzel, 2002). Even if small quantities of aliphatic aldehydes like butanal, pentanal, hexanal have been recovered in wood smoke in weak quantities (Maga, 1987; Guillén & Ibargoitia, 1999; Guillén & Errecalde, 2002; Hedberg et al., 2002), the most part of aliphatic volatile aldehydes in smoked fish comes from the fish flesh lipid oxidation under smoking process conditions. The important quantities of n-alkanals found in smoked fish flesh (from 1.32 equiv of dodecane for heptanal to 34.82 lg equivalents of dodecane for hexadecanal per 100 g in smoked salmon) could be related to the important amount of their lipidic precursors found in unsmoked fish flesh (Varlet et al., 2006). Indeed, it is commonly admitted that saturated or unsaturated aldehydes in fish flesh come from the degradation of the fatty acids and triglycerides by autoxidation (Lindsay, 1994; Farmer, McConnell, & Graham, 1997; Min & Lee, 1999). It is important to notice that autoxidation is a non-enzymatic autocatalytic oxidation reaction resulting in the formation of hydroperoxides. Nevertheless, autoxidation of

Table 4
Occurence of several Strecker aldehydes in seafood products

Compound	Precursors	Odorant descriptor	Extraction method	Matrices	LRI (and column)	References
Phenylacetaldehyde	Phenylalanine	Floral	SDE	Anchovy	1634 (Supelcowax 10)	Cha and Cadwallader (1995)
			SDE	Crab	1652 (Supelcowax 10)	Chung (1999)
			SDE	Scallops	1651 (Supelcowax 10)	Chung et al. (2002)
			RPDE	Anchovy	1046 (DB5-MS)	Triqui and Zouine (1999)
3-Methylbutanal	Leucine	Herbaceous, pungent, cereal, chocolate	Headspace	Smoked salmon	652 (DB5-MS)	Cardinal et al. (1997)
			Headspace	Canned salmon	917 (Supelcowax 10)	Girard and Durance (2000)
			Headspace	Dried squids	622 (DB1)	Kawai et al. (1991)
			RPDE	Anchovy	652 (DB5-MS)	Triqui and Zouine (1999)
			Headspace	Crab meat	911 (supelcowax 10)	Matiella and Hsieh (1990)
			Headspace	Canned salmon	2453 min (Ultra 2)	Girard and Nakai (1991)
			SDE, RPDE, Headspace	Lobster tail	932 (DB-Wax), 651 (DB5-MS)	Lee et al. (2001)
			SDE	Anchovy, herring, shrimps	922 (Supelcowax 10)	Cha and Cadwallader (1995)
2-Methyl-2-butenal	Isoleucine	Green, fruity	SDE	Mussel juices	1082 (Stabilwax)	Cros et al. (2004)
			SDE	Crab	1097 (Supelcowax 10)	Chung (1999)
			SDE	Scallops	1096 (Supelcowax 10)	Chung et al. (2002)
			SDE	Scallops	1096 (Supelcowax 10)	Chung et al. (2001)
			RPDE	Cooked mussels	1101 (DB-Wax)	Le Guen et al. (2000b)
			SDE	Anchovy	1094 (Supelcowax 10)	Cha and Cadwallader (1995)
3-(Methylthio)propanal	Methionine	Cooked potatoe, boiled potatoe	Headspace, SDE, RPDE	Lobster tail	1456 (DB-Wax), 907 (DB5-MS)	Lee et al. (2001)
			SDE	Crab meat	1453 (Supelcowax 10)	Chung and Cadwallader (1994)
			SDE	Scallops	1461 (Supelcowax 10)	Chung et al. (2002)
			SDE	Mussel juices	1482 (StabilWax)	Cros et al. (2004)
			RPDE	Oysters	1451 (DB-Wax)	Pennarun et al. (2002)
			SDE	Crab	1463 (Supelcowax 10)	Chung (1999)
			RPDE	Turbot	1460 (DB-Wax)	Sérot et al. (2001)
			RPDE	Anchovy	911 (DB5-MS)	Triqui and Reineccius (1995)
			SDE	Cooked mussels	1473 (DB-Wax), 1477 (DB-Wax)	Le Guen et al. (2000a, 2000b)

LRI: linear retention indices, SDE: simultaneous steam distillation – solvent extraction, RPDE: reduce pressure distillation extraction.

unsaturated fatty acids can be initiated by a physical catalyst such as light, or by enzymes coming from the fish flesh or microorganisms presents in fish flesh. These hydroperoxides are often transformed in secondary oxidation products as carbonyl compounds. Then, autoxidation of polyunsaturated fatty acids and triglycerides in fish leads to aldehydes which can produce off-flavours according to their concentration but also limit the self-life of the fish. Flavour and odours developed from the autoxidation of lipids give to food globally rancid or oxidized descriptors, depending on the oxidation level reached by the food. One of the aims of smoking process is to prevent

the lipid oxidation by adding to the food some antioxidants compounds presents in wood smoke as phenolic compounds.

Lipid autoxidation is based on fundamental mechanisms involving free-radical chain reactions. Hydroperoxides are formed thanks to the reaction of unsaturated fatty acids and triglycerides with oxygen by a free-radical process involving an initiation, propagation and termination stage (Frankel, 1983; Grosch, 1987). They can also be formed from saturated and monounsaturated fatty acids or esters.

The lipid oxidation begins with an attack of molecular oxygen on the double bonds of polyunsaturated fatty acids (PUFA) and triglycerides which generates free-radicals of PUFA. The PUFA are very sensitive towards molecular oxygen which can be produced thanks to transition metals such as iron (the most present in seafoods) especially haem iron. It can interact with dioxygen to lead to molecular oxygen or hydroxyl radical. The initiation of lipid oxidation with molecular oxygen is autoxidation. Another possible initiator of lipid oxidation is singlet oxygen. Some molecules (sensitizers) are able to absorb light and be converted to a high excited state. These sensitizers can react with molecular oxygen in order to form singlet oxygen (an excited high energy state of molecular oxygen). Singlet oxygen interacts directly on the double bond of PUFA and causes the creation of others degradation products. The oxidation caused by singlet oxygen is photoxidation. Finally, enzymes can incorporate one molecule of oxygen at a position of unsaturated fatty acids. In this case, the oxidation is qualified by enzymatic oxidation. Indeed, enzymes such as lipoxygenase, cyclooxygenase, peroxydase and microsomal enzymes can act as initiators of lipid oxidation in fish tissues. Under all these possible catalysts, free radicals can be produced from unsaturated fatty acids and triglycerides (Hultin, 1994). Differences of volatile carbonyl compounds produced between photoxidized fish and autoxidized or metal-induced oxidized fish have been established. In photoxidized fish, alkanals and 2-alkenals are more present whereas 2,4-alkadienals are more present in autoxidized fish. Nevertheless, hydroperoxides are formed oxidation induced by enzymes, by temperature, by metal or photo-induced oxidation of PUFA and are the main source of off-flavours developed by lipid oxidation.

Hydroperoxides are very unstable and break down readily into many volatile and non-volatile products (Pokorný, 1987). The decomposition products includes: aldehydes, ketones, alcohols, acids, hydrocarbons, lactones, furans and esters. Alkyl hydroperoxides, allyl hydroperoxides and fatty ester hydroperoxides decomposition are accompanied by the formation of a lot of products such as carbonyls compounds, alcohols and acids, esters (Frankel, 1983). Indeed, during hydroperoxide decomposition, secondary alkoxy radicals may cleave to form aldehydes. This reaction is also known as b-scission or elimination (Fig. 3). From the aldehyde, a polymerization can occur or, with dioxygen, it can give alkanes, aldehydes with short chain,

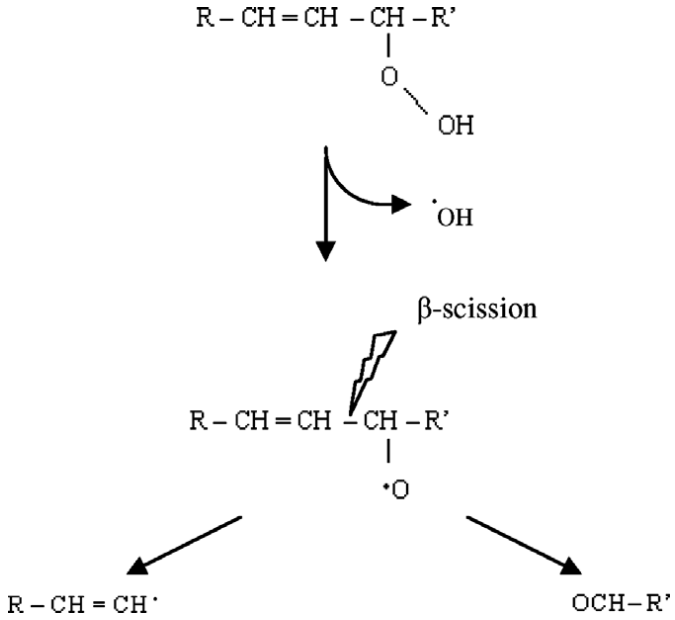

Fig. 3. β - scission of hydroperoxides.

acids and epoxides. From the free radical, several routes are possible: firstly, to keep up the autoxidation, secondly, transformation in hydrocarbons and thirdly, with dioxygen, it can give alkanes, alcohols and aldehydes. A lot of studies have been investigated about the decomposition and the role of hydroperoxides in order to better understand the creation of odours in food (Grosch, 1987; Lindsay, 1994). Indeed, aliphatic aldehydes are the most important breakdown products of hydroperoxides. Table 5 shows the possible origin to some aldehydes obtained from the oxidation of oleic, linoleic, linolenic and arachidonic

Table 5
Possible origin to several aldehydes deriving from PUFA

Fatty acid	Hydroxide position and on hydroperoxides	Aldehyde
Oleic	C11	Octanal
	C8	2-Undecenal
	C9	2-Decenal
	C10	Nonanal
Linoleic	C13	Hexanal
	C9	2,4-Decadienal
	C11	2-Octenal
Linolenic	C16	Propanal
	C14	2-Pentenal
	C12	2,4-Heptadienal
	C13	3-Hexenal
	C11	2,5-Octadienal
	C9	2,4,7-Decatrienal
Arachidonic	C15	Hexanal
	C13	2-Octenal
	C12	3-Nonenal
	C11	2,4-Decadienal
	C10	2,5-Undecadienal
	C7	2,5,8-Tridecatrienal

Table 6
Occurence of several n-alkanals in seafood products

Compound	Main precursors	Odorant descriptor	Extraction method	Matrices	LRI (and column)	References
Butanal	Oleic, linoleic acids	Pungent, green	SDE	Anchovy, herring	874 (Supelcowax 10)	Cha and Cadwallader (1995)
			Headspace	Canned salmon	877 (Supelcowax 10)	Girard and Durance (2000)
Pentanal	n - 6 PUFA	Chemical, wine, sweet, fruity	RPDE	Cooked mussels	980 (DB-Wax)	Le Guen et al. (2000a, 2000b)
			SDE	Scallops	>1000 (Supelcowax 10)	Chung et al. (2001, 2002)
			Headspace	Canned salmon	981 (Supelcowax 10)	Girard and Durance (2000)
			SDE	Crab	1000 (Supelcowax 10)	Chung (1999)
			Headspace	Crab meat	982 (Supelcowax 10)	Matiella and Hsieh (1990)
			Headspace	Smoked salmon	697 (DB5-MS)	Cardinal et al. (1997)
Hexanal	n - 3, n - 6 and n - 9 PUFA	Grass cut, green, pungent	SDE	Mussel juices	1073 (Stabilwax)	Cros et al. (2004)
			RPDE	Turbot	1080 (DB-Wax)	Sérot et al. (2001)
			SDE, RPDE	Cooked mussels	1088 (DB-Wax), 1089 (DB-Wax)	Le Guen et al. (2000a, 2000b)
			SDE	Scallops	1086 (Supelcowax 10)	Chung et al. (2001, 2002)
			SDE	Turbot	1090 (DB-Wax)	Prost et al. (1998)
			Headspace	Smoked salmon	800 (DB5-MS)	Cardinal et al. (1997)
			Headspace, SDE, RPDE	Lobster tail	1083 (DB-Wax), 808 (DB5-MS)	Lee et al. (2001)
			SDE	Crab	1086 (Supelcowax 10)	Chung (1999)
			Headspace	Oysters	1091 (DB-Wax)	Piveteau et al. (2000)
			Headspace	Canned salmon	1092 (Supelcowax 10)	Girard and Durance (2000)
			RPDE	Anchovy	798 (DB5-MS)	Triqui and Reineccius (1995)
			Headspace	Crab meat	1086 (Supelcowax 10)	Matiella and Hsieh (1990)
			RPDE	Anchovy	803 (DB5-MS)	Triqui and Zouine (1999)
			SDE	Anchovy, herring	1081 (Supelcowax 10)	Cha and Cadwallader (1995)
Heptanal	Oleic, linoleic acid	Herbaceous, fishy, fatty, pungent	SDE	Mussel juices	1180 (Stabilwax)	Cros et al. (2004)
			RPDE	Turbot	1198 (DB-Wax)	Sérot et al. (2001)
				Cooked mussels	1195 (DB-Wax)	Le Guen et al. (2000b)
			SDE	Scallops	1189 (Supelcowax 10)	Chung et al. (2001, 2002)
			Headspace	Smoked salmon	900 (DB5-MS)	Cardinal et al. (1997)
			Headspace	Canned salmon	1195 (Supelcowax 10)	Girard and Durance (2000)
			Headspace	Crab meat	1194 (Supelcowax 10)	Matiella and Hsieh (1990)
			SDE	Herring	1184 (Supelcowax 10)	Cha and Cadwallader (1995)
Octanal	Oleic, linoleic acid	Grass, vert, fruité, citron	SDE	Mussel juices	1277 (Stabilwax)	Cros et al. (2004)
			RPDE	Turbot	1296 (DB-Wax)	Sérot et al. (2001)
			SDE, RPDE	Cooked mussels	1301 (DB-Wax), 1303 (DB-Wax)	Le Guen et al. (2000a, 2000b)

(continued on next page)

Table 6 (continued)

Compound	Main precursors	Odorant descriptor	Extraction method	Matrices	LRI (and column)	References
			SDE	Scallops	1293 (Supelcowax 10)	Chung et al. (2001, 2002)
			Headspace	Canned salmon	1323 (Supelcowax 10)	Girard and Durance (2000)
			RPDE	Oysters	1289 (DB-Wax)	Pennarun et al. (2002)
			Headspace	Oysters	1298 (DB-Wax)	Piveteau et al. (2000)
			Headspace	Lobster tail	1263 (DB-Wax), 1005 (DB5-MS)	Lee et al. (2001)
			Headspace, SDE, RPDE	Smoked salmon	1004 (DB5-MS)	Cardinal et al. (1997)
			SDE	herring	1289 (Supelcowax 10)	Cha and Cadwallader (1995)
Nonanal	Oleic, linoleic acid	Green, tallow, citrus, fatty, soapy	SDE	Mussel juices	1387 (Stabilwax)	Cros et al. (2004)
			RPDE	Cooked mussels	1407 (DB-Wax)	Le Guen et al. (2000b)
			Headspace	Smoked salmon	1106 (DB5-MS)	Cardinal et al. (1997)
			Headspace	Canned salmon	1409 (Supelcowax 10)	Girard and Durance (2000)
			SDE	Scallops	1398 (Supelcowax 10)	Chung et al. (2001, 2002)
			SDE	Crab	1399 (Supelcowax 10)	Chung (1999)
			RPDE	Oysters	1392 (DB-Wax)	Pennarun et al. (2002)
			RPDE	Anchovy	1109 (DB5-MS)	Triqui and Reineccius (1995)
			SDE	Herring	1393 (Supelcowax 10)	Cha and Cadwallader (1995)
Decanal	n - 9 PUFA	Green, marine, cucumber, floral	SDE	Mussel juices	1497 (Stabilwax)	Cros et al. (2004)
			RPDE	Turbot	1510 (DB-Wax)	Sérot et al. (2001)
			Headspace	Canned salmon	1505 (Supelcowax 10)	Girard and Durance (2000)
			RPDE	Oysters	1497 (DB-Wax)	Pennarun et al. (2002)
			SDE	Scallops	1503 (Supelcowax 10)	Chung et al. (2001, 2002)
			Headspace	Oysters	1510 (DB-Wax)	Piveteau et al. (2000)
			RPDE	Anchovy	1208 (DB5-MS)	Triqui and Reineccius (1995)
			Headspace	Smoked salmon	1204 (DB5-MS)	Cardinal et al. (1997)

LRI: linear retention indices, SDE: simultaneous steam distillation – solvent extraction, RPDE: reduce pressure distillation extraction.

acids and the respective hydroperoxides formed as intermediaries. Thus, the interpretation of aliphatic aldehydes in smoked fish can be explained by the lipid composition of the fish flesh.

5.2. n-Alkanals

All the alkanals present in smoked fish and also present in seafood products are reported in Table 6. All n-Alkanals are generally produced from PUFA present in fish flesh (Josephson, Lindsay, & Stuiber, 1984; Josephson et al., 1991a, Josephson, Lindsay, & Stuiber, 1991b; Pan, Ushio, & Ohshima, 2005; Stołyhwo, Kołodziejska, & Sikorski, 2006) by oxidation of their double bound carbon–carbon. Alkanals are more produced from n - 6 or n - 9 PUFA or MUFA. The precursors of butanal in fish flesh are mainly oleic (MUFA n - 9) and linoleic acid (PUFA n - 6) or its methyl esters (Grosch, 1987). Aldehydes whose number of carbon is comprised between 4 and 11 can derived from oleic acid with an intermediary 11-hydroperoxide for octanal, 9 or 10-hydroperoxide for nonanal, 8-hydroperoxide for undecanal (all the hydroperoxides coming from oleic acid). They can also be produced from linoleic acid (n - 6 PUFA), especially from propanal to octanal with an intermediary 13-hydroperoxide (coming from linoleic acid) for hexanal. Arachidonic acid is also involved in creation of pentanal and hexanal. The presence of dodecanoic acid, tetradecanoic acid and hexadecanoic acid could be related to dodecanal, tetradecanal, and hexadecanal very present in fish flesh (Varlet et al., 2006).

Docosahexaenoic and eicosapentaenoic acid (n - 3 PUFA) are important fish components compared to linoleic and linolenic acid. Nevertheless, the same kind of hydroperoxides are obtained by oxidation and can lead to the same volatiles aldehydes (Hsieh & Kinsella, 1989). N-alkanals are generally characterized by fat and green descriptors. The odour threshold of butanal is between 9 and 37 ppb in water (Buttery, Teranishi, Ling, & Turnbaugh, 1990) with a pungent and green odour. Except pentanal (odour threshold between 12 and 42 ppb in water (Buttery et al., 1990)) which has an almond, malt and pungent flavour, n-alkanals from hexanal to hexadecanal are qualified with aromatic notes like grass, tallow, green and fat for hexanal (odour threshold at 4.5 ppb in water (Buttery et al., 1990)), with aromatic notes like fat, citrus and rancid for heptanal (odour threshold at 3 ppb in water (Buttery et al., 1990)), with odours like fat, soap, lemon and green for octanal (odour threshold at 0.7 ppb in water (Buttery et al., 1990)) and fat, citrus and green for nonanal (odour threshold at 1 ppb in water (Buttery et al., 1990)). Due to their low odour thresholds, the aliphatic aldehydes play a predominant role in food aromas. For example, decanal, with fat, orange peel and tallow flavour, has an odour threshold from 0.1 to 2 ppb in water, dodecanal (Rychlik, Schieberle, & Grosch, 1998) with lily, fat and citrus flavour has an odour threshold about 2 ppb in water. Even tetradecanal with floral and waxy flavour (Chisholm, Wilson, & Gaskey, 2003) presents a low odour threshold (about 60 ppb in water).

5.3. 2-Alkenals

2-Alkenals are found in a lot of seafood products as presented in Table 7. They are known to be produced from PUFA like n 3 PUFA linolenic acid (Ullrich & Grosch, 1988) especially for aldehydes with short chain (from 2-butenal to 2-heptenal), 2-pentenal deriving from 14-hydroperoxide of linolenic acid. 2-Alkenals from 2-hexenal to 2-undecenal are products of oxidation of oleic acid, by the intermediary of 9-hydroperoxide for 2-decenal and of 8-hydroperoxide for 2-undecenal. They also derive from N - 6 PUFA. Aldehydes as 2-heptenal or 2-octenal are produced from arachidonic acid. (E)-2-alkenals from 2-heptenal to 2-nonenal, 2-nonenal deriving from 9-hydroperoxide-, are generated from linoleic acid. (Z)-2-alkenals as 2-octenal and 2-decenal are also known to derive from linoleic acid (Grosch, 1987; Hsieh & Kinsella, 1989).

Long-chain unsaturated aldehydes have very low odour threshold that is the reason why they are prevalent in seafood products just with small quantities. From 2-pentenal to 2-dodecenal, the odour becomes less citrus and fruity and more fat-like, and the odour threshold falls (Rowe, 1998). For example, (E)-2-pentenal characterized by strawberry, fruity flavour (Rychlik et al., 1998) has an odour threshold at 1500 ppb in water, (E)-2-heptenal characterized by soap, almond flavour (Rychlik et al., 1998) has an odour threshold at 13 ppb in water. The odour threshold is reduced by a factor 100. Similarly, 2-decenal with a tallow flavour (Valim, Rouseff, & Lim, 2003), has an odour threshold close to 0.3 ppb and 2-undecenal with a sweet, fat flavour, has an odour threshold at 5 ppb.

5.4. 2,4-Alkadienals

2,4-Alkadienals are also present in a lot of seafood products (Table 8). 2,4-decadienal has been found in smoked bream and trout (Guillén & Errecalde, 2002) but the configuration is not elucidated. Generally, decadienal and isomers come from n - 6 PUFA like linoleic or arachidonic acid (Piveteau et al., 2000; Lee et al., 2001) and by the intermediary of 9-hydroperoxide deriving from linoleic acid, or 11-hydroperoxide deriving from arachidonic acid.

As long-chain unsaturated aldehydes, decadienal and isomers have very low odour thresholds. They are characterized by fat-like odours. Indeed, (E,E)-2,4-decadienal for example has a fried, fatty and waxy flavour with an odour threshold at 0.07 ppb in water (Buttery et al., 1990; Potter, 1996; Rowe, 1998), (E,Z)-2,4-decadienal is characterized by a fried and fat flavour (Valim et al., 2003).

2,4-Alkadienals may be nonenzymatically derived products of lipid oxidation (Swoboda & Peers, 1977). 2,4-Heptadienal is produced from n 3 PUFA as linolenic acid through 12-hydroperoxide intermediary (Hsieh & Kinsella, 1989). Its odorant threshold is assessed at 10 ppb with fried odour for (E,Z) structure and fatty, nutty odour for (E,E) structure (Ullrich & Grosch, 1988; Triqui & Reineccius, 1995).

2,4-Hexadienal is found in smoked salmon and known as lipid peroxidation product (Claxson et al., 1994). It is characterized by a low odour threshold from 10 to 60 ppb in water (Boehlens & Van Gemert, 1987) and aromatic notes like green and rancid (Qian & Reineccius, 2002).

6. Organoleptic roles of aldehydes in smoked fishes

6.1. Aldehyde participation in flavour of smoked fishes

One of the main organoleptic role of aldehydes is the contribution to the global aroma.

Benzaldehyde and derived compounds are known to have an almond, bitter almond (Triqui & Reineccius, 1995) and nutty odour. Its odorant detection threshold is about from 100 ppb to 4.6 ppm and recognition threshold from about 330 ppb to 4.1 ppm (Burdock, 2002). The great differences of odorant perception are mainly due to the different matrices (water, oil, food, etc.) used to carry out the assessment of the odorant perception.

Furfurals and aldehyde phenolic compounds have characteristic odorant descriptors. For example, furfural is stamped by bread, almond and sweet characteristics (Rychlik et al., 1998). Its odorant threshold is between 280 ppb and 8 ppm in water. Its taste characteristics at 30 ppm are brown, sweet, woody, bread-like, nutty caramellic with

Table 7
Occurence of several 2-Alkenals in seafood products

Compound	Main precursors	Odorant descriptor	Extraction method	Matrices	LRI (and column)	References
2-Butenal		Woody, sulphury	Headspace	Smoked salmon	647 (DB5-MS)	Cardinal et al. (1997)
			SDE	Mussel juices	1002 (Stabilwax)	Cros et al. (2004)
			SDE	Scallops	1042 (Supelcowax 10)	Chung et al. (2001, 2002)
			SDE	Shrimp	1042 (Supelcowax 10)	Cha and Cadwallader (1995)
			Headspace	Canned salmon	1047 (Supelcowax 10)	Girard and Durance (2000)
(E)-2-Pentenal	PUFA n – 3	Herbaceous, green	SDE	Anchovy	1130 (Supelcowax 10)	Cha and Cadwallader (1995)
			SDE	Crab	1132 (Supelcowax 10)	Chung (1999)
			RPDE	Oysters	1124 (DB-Wax)	Pennarun et al. (2002)
			SDE	Scallops	1132 (Supelcowax 10)	Chung et al. (2001, 2002)
			Headspace	Oysters	1138 (DB-Wax)	Piveteau et al. (2000)
			Headspace	Canned salmon	1144 (Supelcowax 10)	Girard and Durance (2000)
(E)-2-Heptenal	PUFA n - 3, n - 6	Mossy, sulphury, cooked fish, roasted	SDE	Mussel juices	1309 (Stabilwax)	Cros et al. (2004)
			RPDE	Turbot	1339 (DB-Wax)	Sérot et al. (2001)
			SDE, RPDE	Cooked mussels	1338 (DB-Wax), 1342 (DB-Wax)	Le Guen et al. (2000a, 2000b)
			SDE	Anchovy	1328 (Supelcowax 10)	Cha and Cadwallader (1995)
				Mussel juices	1425 (Stabilwax)	Cros et al. (2004)
			SDE	Anchovy, herring	1426 (Supelcowax 10)	Cha and Cadwallader (1995)
				Cooked mussels	1451 (DB-Wax)	Le Guen et al. (2000a)
(E)-2-Octenal	PUFA n - 6	Roasted, almaond, cucumber, nutty, fatty	Headspace, SDE, RPDE	Lobster tail	1430 (DB-Wax), 1045 (DB5-MS)	Lee et al. (2001)
			SDE	Turbot	1447 (DB-Wax)	Prost et al. (1998)
			RPDE	Anchovy	1066 (DB5-MS)	Triqui and Zouine (1999)
			Headspace	Oysters	1434 (DB-Wax)	Piveteau et al. (2000)
			Headspace	Canned salmon	1443 (Supelcowax 10)	Girard and Durance (2000)
(E)-2-Nonenal	PUFA n - 6	Earthy, fishy, cucumber, green	SDE	Mussel juices	1534 (Stabilwax)	Cros et al. (2004)
			RPDE	Turbot	1549 (DB-Wax)	Sérot et al. (2001)
			SDE	Cooked mussels	1563 (DB-Wax)	Le Guen et al. (2000a)
			Headspace	Canned salmon	1541 (Supelcowax 10)	Girard and Durance (2000)
			SDE	Crab	1542 (Supelcowax 10)	Chung (1999)
			Headspace	Oysters	1541 (DB-Wax)	Piveteau et al. (2000)
			SDE	Turbot	1555 (DB-Wax)	Prost et al. (1998)
			SDE, RPDE	Lobster tail	1528 (DB-Wax), 1163 (DB5-MS)	Lee et al. (2001)
			RPDE	Anchovy	1166 (DB5-MS)	Triqui and Zouine (1999)
2-Decenal	PUFA n – 6	Tallow, orange	Headspace	Canned salmon	1655 (Supelcowax 10)	Girard and Durance (2000)

LRI: linear retention indices, SDE: simultaneous steam distillation – solvent extraction, RPDE: reduce pressure distillation extraction, PUFA: polyunsaturated fatty acid.

Table 8
Occurence of 2,4-alkadienal isomers in seafood products

Compound	Main precursors	Odorant descriptor	Extraction method	Matrices	LRI (and column)	References
(E)-(Z)-2,4-Decadienal	Arachidonic acid	Green, fatty	RPDE	Anchovy	1302 (DB5-MS)	Triqui and Zouine (1999)
(E,E)-2,4-Decadienal	Linoleic acid	Buttery, green, cut grass, deep fried, cucumber	SDE	Mussel juices	1827 (Stabilwax)	Cros et al. (2004)
			SDE, RPDE	Lobster tail	1883 (DB-Wax), 1275 (DB5-MS)	Lee et al. (2001)
			SDE	Turbot	1828 (DB-Wax)	Prost et al. (1998)
			RPDE	Anchovy	1315 (DB5-MS)	Triqui and Zouine (1999)
			SDE	Herring	1807 (Supelcowax 10)	Cha and Cadwallader (1995)
(E,E)-2,4-Heptadienal	Linolenic acid	Green, marine, fatty, nutty	SDE	Mussel juices	8.20 (Carbowax 20 M)	Cros et al. (2004)
			RPDE	trout	1498 (Supelcowax 10)	Sérot et al. (2002)
			SDE	Cooked mussels	1479 (Supelcowax 10)	Le Guen et al. (2000a)
(E,Z)-2,4-Heptadienal		Not described	EDT	Cooked red salmon	1508 (DB-Wax)	Josephson et al. (1991a)

LRI: linear retention indices, SDE: simultaneous steam distillation – solvent extraction, RPDE: reduce pressure distillation extraction.

a burnt astringent nuance notes (Burdock, 2002). 5-Methylfurfural is characterized by similar descriptors. Its odorant detection is possible at 6 ppm in water with odours of almond, caramel and burnt sugar (Valim et al., 2003) and its taste characteristics at 50 ppm are sweet, brown, caramellic, grain and maple-like.

Volatiles Strecker aldehydes are also known to be potent odorants in foods. For example, acetaldehyde has a pungent flavour but with fruity and green nuance (Scarpellino & Soukup, 1993). Its odorant threshold is comprised between 15 and 120 ppb in water (Buttery, Turnbaugh, & Ling, 1988). 2-Methyl-2-butenal is characterized by a green and fruity flavour (Berger, Drawert, & Kollmannsberger, 1989) and 3-methylbutanal by an almond, chocolate and malt flavour (Beal & Mottram, 1994) with an odorant threshold between 0.2 and 2 ppb in water (Buttery et al., 1990). As the others aldehydes from Strecker reaction, phenylacetaldehyde has also a low odour threshold about 4 ppb in water (Buttery et al., 1988). Phenylacetaldehyde odour is floral-roselike, fruity and green. Its taste characteristics are floral and honey-like with a sweet waxy nuance at 5 ppm (Scarpellino & Soukup, 1993).

Volatile aliphatic aldehydes odours decline important and various aromatic notes which are sometimes wanted and sometimes avoided. Indeed, thanks to their low threshold value, aldehydes produced by lipid oxidation seem to have a wide odour evolution according to their concentration. They can bring sweet, fatty and green aromatic notes but if the lipid oxidation becomes more important, the aromatic notes become stronger, unpleasant and cause undesirable flavours. This is the reason why the aldehydes odour is often smelt with several descriptors combining fatty odours (wax, tallow, butter, etc.) and green odours (floral, fruity, citrus, orange, etc.). Maillard and Strecker aldehydes show also these evolutions of odours because the aromatic notes can evolve from cooked vegetable to burnt sugar, roasty, caramellic in function of their concentrations, food matrix and intensity of process.

It is also very important to note that some phenolic compounds frequently contain aldehyde function which can confers nuanced odours: syringaldehyde, coniferaldehyde, etc. (Clifford, Tang, & Eyo, 1980). These functional groups might strengthen the predominance of the phenolic compounds in smoked fishes flavour. Odour and taste are deeply linked together and gathered under the flavour term. Flavour and odour of smoked fishes have been extensively investigated but to our knowledge, no study has been carried out on the role of aldehydes compounds on smoked fishes taste.

6.2. Others organoleptic roles of aldehydes

Maillard and Strecker aldehydes in a large part are responsible for the colour of the smoked flesh (Sainclivier, 1985). The colour varies from golden yellow to dark brown according to the nature of the wood and the intensity of smoking process. In liquid smoking process, the product must also be placed in a dry and hot ambience during few times in order to favorize colour formation. Thus, an important deposition of Maillard aldehydes leads to a darker colour of fish flesh (Tilgner, 1977). A brown colour can also be caused by an increase of the dehydration step in Maillard reaction drying briefly the fillet after smoke absorption. After scission and dehydration, melanoidines could be created by polymerisation through aldolic condensations. These compounds give to the final product a brown colour (beer, bread, coffee, etc.) (Borrelli et al., 2003). For our knowledge, no study has again dealt with the creation of melanoïdines as colour component in smoked fish flesh.

Carbonyl-amino reactions could play a main role in smoked food products. Protein-bound lysine, the most present essential amino acid in fish (Huss, 1999), thanks

to its terminal amino group, is considered as major source of the amino components in such reactions, but a loss in arginine and histidine is also observed. Methanal is known to be very reactive towards amino groups but the adduct does not seem to be involved in the smoked fishes colour. However, glycolic aldehyde, methylglyoxal and 2-oxopropanal are considered to be important colour precursors (Clifford et al., 1980; Miler & Sikorski, 1990). Then, aldehydes contribute to the colour of smoked fishes but are also responsible of a certain loss about nutritional value by the degradation of amino acids. However, all the aldehydes involved in the smoked fish colour have not all been reported as volatiles aldehydes.

A part of the final colour could derive from phenolic compounds with aldehyde function. Coniferaldehyde and syringaldehyde are considered to be irreversibly bound to proteins and to contribute orange tints to the products (Clifford et al., 1980).

Finally, the glossy aspect noticeable on certain smoked products is the result of reactions between phenolic compounds and aldehydes (Girard, 1988). They lead to resinous substances (phenoplasts). The polymerization is favorized by the heat and the degrees of reticulation of the molecule vary in function of the time (Fig. 4).

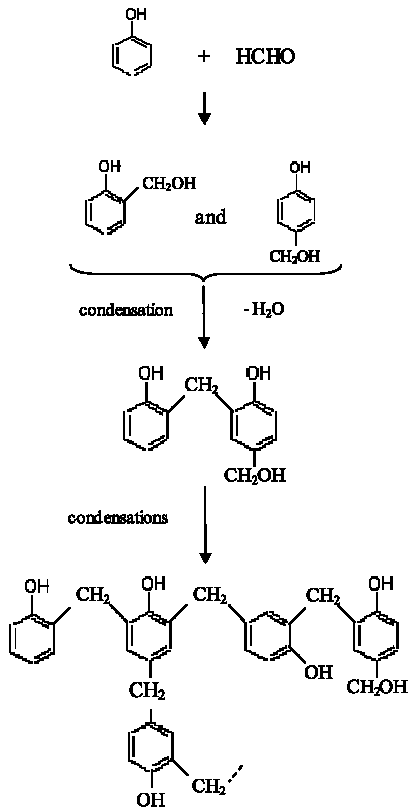

Fig. 4. Reaction between a phenolic compound and an aldehyde (Girard, 1988).

Aldehydes intervention in texture of smoked fishes is unknown. Texture of smoked fishes is due to coagulation of proteins. Formaldehyde which has been reported in wood smoke and smoked meat (Toth & Potthast, 1984) but not in smoked fishes (probably due to the extraction method) can react with proteins. Formaldehyde was shown to react with the amino group of the N-terminal amino acid residue and the side-chains of arginine, cysteine, histidine, and lysine residues (Metz et al., 2004). Formaldehyde seems to be involved in texture of smoked fishes and to be responsible of the dark layer at the dried surface of fishes (Sainclivier, 1985).

7. Toxicity of volatile aldehydes in smoked fishes

Among the aldehydes found in smoked fishes, several can be considered as potentially toxic. Aldehydes effects on human health are not very investigated. Aldehydes could have narcotic properties but at concentrations very higher than those found in smoked fishes.

4-Hydroxy-2-(E)-hexenal has been found in smoked salmon (Munasinghe et al., 2003). It has been reported that its formation occurs during lipid peroxidation of n-3 polyunsaturated fatty acids. It is a a,b-unsaturated aldehyde and therefore suspected to be carcinogen (Chung et al., 1986; Witz, 1989) like 4-hydroxy-2-nonenal found in others smoked products (Munasinghe, Ichimaru, Matsui, Sugamoto, & Sakai, 2003). 4-Hydroxy-2-nonenal has even been reported with the same toxicity as others alkenals such as (E)-heptenal (Nishikawa, Sodum, & Chung, 1992) in rats. The kidney and the liver are known to be the main organs affected by the cytotoxicity of such aldehydes. It is also known that 4-hydroxy-(E)-2-hexenal can react readily with the sulfhydryl groups of proteins (cysteine) and with amino groups of lysine but the toxicity mechanism and aldehyde catabolism decomposition are not still very well investigated. As smoking process implies wood pyrolysis and thus combustion of organic material, a,b-unsaturated aldehydes can be found in smoked fishes. These aldehydes are also considered as endogenously formed substances in animals and humans during lipid peroxidation or arachidonic acid oxidation. They form a group of mutagenic compounds inside which some were shown as carcinogens like 2,4-hexadienal (Picchiottino & Lee, 2002). Their toxic activities are considered as a result of their ability to create exocyclic DNA-adducts, in particular 1,N^2-propanodeoxyguanosine adducts (Eder & Deininger, 2002). This type of adducts is considered as promutagenic DNA-lesion. 2-Butenal, which was identified in smoked fishes, given in drinking water was shown to cause liver tumors in rats (Munasinghe et al., 2003) and 2-heptenal, also found in smoked fishes, have been reported to contribute to a general cancer risk (Eder & Deininger, 2002). Individually, these aldehydes are not

in adequate quantities in smoked fishes to be responsible of toxic activity. However, the effect of mixes of several a,b-unsaturated aldehydes have not been studied and synergic effects could occur between these aldehydes.

Formaldehyde is not found in smoked fishes but is present in wood smoke at levels higher than 200 mg/100 g and in smoked meat products at levels higher than 50 mg/kg (Toth & Potthast, 1984; Maga, 1987). It might be more investigated in smoked fishes because its toxicity was well investigated and assessed (Vargova et al., 1993; Nilsson et al., 1998). Like formaldehyde, acetaldehyde detected in smoked fishes was also shown as toxic aldehyde (Til et al., 1989). On humans, the action of acetaldehyde is nearly unknown on account of the lack of studies. It has only been reported as respiratory and eye irritant (INRS, 2004b). Studies on animals have assessed acetaldehyde toxicity but at high concentrations. A no-observed-adverse-effect level of acetaldehyde is 125 mg/kg body weight/day for rats (Til, Woutersen, & Feron, 1988). Its genotoxicity is assessed on bacteria through DNA alterations and mutagenic action. On rats, carcinogenesis is evaluated from 750 ppm (6 h/j, 5 j/week, during 28 weeks) and reproduction alterations were illustrated by teratogenic effects and hormonal modifications on mice (INRS, 2004b).

Aromatic aldehydes can also be toxic. Benzaldehyde toxicity studies have established its irritant action at the level of the skin and mucous membranes (Pichard et al., 2005). No study about the genotoxicity and effect on reproduction of benzaldehyde on humans is available. However, on mice, the carcinogen activity of benzaldehyde was shown (NCI/NTP, 1990). The main targeted organs of benzaldehyde are brain, kidneys, liver and stomach. Benzaldehyde has been shown as able to specifically inhibit in vitro the glutathione peroxydase. This enzyme plays an important role in the brain protection against antioxidants (Tabatabaie & Floyd, 1996). This reaction illustrates the indirect toxicity that benzaldehyde could have as enzymatic inhibitor. These results can explain the indirect neurotoxicity of benzaldehyde. However, benzaldehyde is found in smoked fishes in quantities very lower than the toxic concentrations.

Furfural is not a carcinogenic compound but it increases carcinogenesis due to polycyclic aromatic hydrocarbons (PAH) as benzo[a]pyrene, known to be carcinogens present in smoked food. It has been reported as respiratory and eyes irritant (INRS, 1999). In France, a threshold value of exposition has been assessed at 2 ppm for workers. This concentration is 1000 times more important than the concentration of furfural found in smoked fishes (Varlet et al., 2006). However, important quantities of furfural have been measured in wood smoke. Toxicity of furfural in wood smoke has not been studied but its concentration makes us to think that it has a potential role in wood smoke toxicity.

Even if some aldehydes found in smoked fishes can be carcinogens, their concentrations are very lower than those necessary to induce toxic effects. The toxicity of smoked fishes is more attributed to PAH content. Nevertheless, even if aldehydes in smoked fishes are found under the individual toxic concentrations, cumulative and synergic effects could be possible and should be better investigated.

8. Conclusion

Volatile aldehydes constitute key molecules for a better comprehension in quality of food products, especially smoked fish. Indeed, volatile aldehydes can be used as indicators of parameters such as intensity of smoking process by the detection of Maillard and Strecker products. Volatile aldehydes can also illustrate the level of oxidation and/or toxicity in smoked fishes. More investigations must be carried out in order to improve the odorant quality of smoked fish because volatile aldehydes will be good organoleptic indicators to optimize a process, to produce food with required odours or to reduce off-odours when once identified, the mechanisms of creation are known. This kind of study might be extended to others food matrices because lipid oxidation and Maillard reaction are very frequent in all industrial food processes. Finally, due to their reactivity and sometimes toxicity, the study of aldehydes in food brings knowledge in health research in order to understand some unexpectable phenomenons.

References

Alén, R., Kuoppala, E., & Oesch, P. (1996). Formation of the main degradation compound groups from wood and its components during pyrolysis. Journal of Analytical and Applied Pyrolysis, 36, 137–148.

Alén, R., Oesch, P., & Kuoppala, E. (1995). Py-GC/AED studies on the thermochemical behavior of softwood. Journal of Analytical and Applied Pyrolysis, 35, 259–265.

Ames, J. M. (1998). Applications of the Maillard reaction in the food industry. Food Chemistry, 62(4), 431–439.

Aro, T., Brede, C., Manninen, P., & Kallio, H. (2002). Determination of semivolatile compounds in Baltic herring (*Clupea harengus membras*) by supercritical fluid extraction-supercritical fluid chromatography-gas chromatography-mass spectrometry. Journal of Agriculture and Food Chemistry, 50(7), 1970–1975.

Augustin, M. A., Sanguansri, L., & Bode, O. (2006). Maillard reaction products as encapsulants for fish oil powders. Journal of Food Science, 71(2), E25–E32.

Beal, A. D., & Mottram, D. S. (1994). Compounds contributing to the characteristics aromas of malted barley. Journal of Agriculture and Food Chemistry, 42, 2880–2884.

Berger, R. G., Drawert, F., & Kollmannsberger, H. (1989). The flavour of Cape Gooseberry (*Physalis peruviana* L.) Z. Lebensm. Unters. Forsch., 188, 122–126.

Boehlens, M. H., & Van Gemert, L. J. (1987). Organoleptic properties of aliphatic aldehydes. Perfumer & Flavorist, 12(5), 31–43.

Borrelli, R. C., Mennella, C., Barba, F., Russo, M., Russo, G. L., Krome, K., et al. (2003). Characterization of coloured compounds obtained by enzymatic extraction of bakery products. Food and Chemical Toxicology, 41, 1367–1374.

Burdock, G. (2002). Feranoli's handbook of flavour ingredients (4th ed.). Boca Raton, FL: CRC Press.

Buttery, R. G., Teranishi, R., Ling, L. C., & Turnbaugh, J. G. (1990). Quantitative and sensory studies on tomato paste volatiles. Journal of Agriculture and Food Chemistry, 38, 336–340.

Buttery, R. G., Turnbaugh, J. G., & Ling, L. C. (1988). Contribution of volatiles to rice aroma. Journal of Agriculture and Food Chemistry, 36(5), 1006–1009.

Cardinal, M., Berdagué, J. L., Dinel, V., Knockaert, C., & Vallet, J. L. (1997). Effet de différentes techniques de fumage sur la nature des composés volatils et les caractéristiques sensorielles de la chair de saumon. Sciences des Aliments, 17, 679–696 [in French].

Cha, Y. J., & Cadwallader, K. R. (1995). Volatile components in salt-fermented fish and shrimp pastes. Journal of Food Science, 60, 19–24.

Chisholm, M. G., Wilson, M. A., & Gaskey, G. A. (2003). Characterization of aroma volatiles in key lime essential oils (*Citrus aurantifolia* Swingle). Flavour and Fragrance Journal, 18(2), 106–115.

Chung, H. Y. (1999). Volatile components in crabmeats of *Charybdis feriatus*. Journal of Agriculture and Food Chemistry, 47, 2280–2287.

Chung, H. Y., & Cadwallader, K. R. (1994). Aroma extract dilution analysis of blue crab claw meat volatiles. Journal of Agriculture and Food Chemistry, 42, 2867–2870.

Chung, H. Y., Yung, I. K. S., Ma, W. C. J., & Kim, J. S. (2002). Analysis of volatile components in frozen and dried scallops (*Patinopecten yessoensis*) by gas chromatography/mass spectrometry. Food Research International, 35, 43–53.

Chung, H. Y., Yung, I. K. S., & Kim, J. S. (2001). Comparison of volatile components in dried scallops (*Chlamys farreri* and *Patinopecten yessoensis*) prepared by boiling and steaming methods. Journal of Agriculture and Food Chemistry, 49, 192–202.

Chung, F., Tanaka, T., & Hecht, S. S. (1986). Induction of liver tumors in F344 rats by crotonaldehyde. Cancer Research, 46, 1285–1289.

Claxson, A. W. D., Hawkes, G. E., Richardson, D. P., Naughton, D. P., Haywood, R. M., Chandler, C. L., et al. (1994). Generation of lipid peroxidation products in culinary oils and fats during episodes of thermal stressing: A high-field ^{1}H NMR study. FEBS Letters, 355, 81–90.

Clément, P. (2004). Le marché du saumon et de la truite fumés en Europe occidentale. UBIFRANCE, OFIMER.

Clifford, M. N., Tang, S. L., & Eyo, A. A. (1980). Smoking of foods. Process Biochemistry (June/July), 8–11.

Cornu, M., Beaufort, A., Rudelle, S., Laloux, L., Bergis, H., Miconnet, N., et al. (2006). Effet of temperature, water-phase salty and phenolic contents on *Listeria monocytogenes* growth rates on cold-smoked salmon and evaluation of secondary models. International Journal of Food Microbiology, 106(2), 159–168.

Cros, S., Lignot, B., Bourseau, P., Jaouen, P., & Prost, C. (2004). Desalination of mussel cooking juices by electrodialysis: Efect on the aroma profile. Journal of Food Engineering, 69(4), 425–436.

Curioni, P. M. G., & Bosset, J. O. (2002). Key odorants in various cheese types as determined by gas chromatography–olfactometry. International Dairy Journal, 12, 959–984.

Da Cunha Veloso, M. C., Da Silva, V. M., Vieira Santos, G., & De Andrade, J. B. (2001). Determination of aldehydes in fish by high-performance liquid chromatography. Journal of Chromatographic Science, 39(5), 173–176.

Eagle, J., Naylor, R., & Smith, W. (2004). Why farm salmon outcompete fishery salmon. Marine Policy, 28, 259–270.

Eder, E., & Deininger, C. (2002). The influence of the solvents DMSO and ethanol on the genotoxicity of a,b-unsaturated aldehydes in the SOS chromotest. Mutation Research, 516, 81–89.

Farmer, L. J., McConnell, J. M., & Graham, W. D. (1997). Flavour characteristics and lipid composition of Atlantic salmon. In F. Shahidi & K. R. Cadwallader (Eds.), Flavour and lipid chemistry of seafoods (pp. 95–109). Washington, DC: American Chemical Society. Fernandez,

X., Kerverdo, S., Duñach, E., & Lizzani-Cuvelier, L. (2002). Les hétérocycles dans la chimie des arômes. L'actualité chimique (Société Francaise de Chimie (Ed.)), Paris (pp. 4–14).

Frankel, E. N. (1983). Volatile lipid oxidation products. Progress in Lipid Research, 22(1), 1–33.

Fukami, K., Ishiyama, S., Yaguramaki, H., Masuzawa, T., Nabeta, Y., & Shimoda, M. (2002). Identification of distinctive volatile compounds in fish sauce. Journal of Agriculture and Food Chemistry, 50(19), 5412–5416.

Fullana, A., Carbonell-Barrachina, A. A., & Sidhu, S. (2004a). Comparison of volatile aldehydes present in the cooking fumes of extra virgin olive, olive, and canola oils. Journal of Agriculture and Food Chemistry, 52(16), 5207–5214.

Fullana, A., Carbonell-Barrachina, A. A., & Sidhu, S. (2004b). Volatile aldehyde emissions from heated cooking oils. Journal of Science and Food Agriculture, 84(15), 2015–2021.

Girard, J. P. (1988). La fumaison. In Technique & Documentation (Ed.), Technologie de la viande et des produits carnés, Paris (pp. 171–214) [in French].

Girard, B., & Durance, T. (2000). Headspace volatiles of sockeye and pink salmon as affected by retort process. Journal of Food Science, 65, 34–39.

Girard, B., & Nakai, S. (1991). Static headspace gas chromatographic method for volatiles in canned salmon. Journal of Food Science, 56(6), 1271–1274.

Girard, B., & Nakai, S. (1994a). Nonparametric discriminant analysis of static headspace volatiles for grading fresh. Pacific Salmon, 409–421.

Girard, B., & Nakai, S. (1994b). Grade classification of canned pink salmon with static headspace volatile patterns. Journal of Food Science, 59(3), 507–512.

Grimm, C. C., Lloyd, S. W., Batista, R., & Zimba, P. V. (2000). Using microwave distillation-solid-phase microextraction–gas chromatography–mass spectrometry for analyzing fish tissue. Journal of Chromatographic Science, 38(7), 289–296.

Grosch, W. (1987). Reactions of hydroperoxides – Products of low molecular weight. In H. W. S. Chan (Ed.), Autoxidation of unsaturated lipids (pp. 95–109). New York: Academic Press.

Guillén, M. D., & Errecalde, M. C. (2002). Volatile components of raw and smoked black bream (*Brama raii*) and rainbow trout (*Oncorhyncus mykiss*) studied by means of solid phase microextraction and gas chromatography/mass spectrometry. Journal of Science and Food Agriculture, 82, 945–952.

Guillén, M. D., Errecalde, M. C., Salmerón, J., & Casas, C. (2006). Headspace volatile components of smoked swordfish (*Xiphias gladius*) and cod (*Gadus morhua*) detected by means of solid phase microextraction and gas chromatography–mass spectrometry. Food Chemistry, 94(1), 151–156.

Guillén, M. D., & Ibargoitia, M. L. (1996). Relationships between the maximum temperature reached in the smoke generation processes from *vitis vinifera* L. shoot sawdust and composition of the aqueous smoke flavoring preparations obtained. Journal of Agriculture and Food Chemistry, 44, 1302–1307.

Guillén, M. D., & Ibargoitia, M. L. (1999). Influence of the moisture content on the composition of the liquid smoke produced in the pyrolysis of *Fagus sylvatica* L. wood. Journal of Agriculture and Food Chemistry, 47, 4126–4136.

Guillén, M. D., & Manzanos, M. J. (1996). Study of the components of a solid smoke flavouring preparation. Food Chemistry, 55(3), 251–257.

Guillén, M. D., & Manzanos, M. J. (1997). Characterization of the components of a salty smoke flavouring preparation. Food Chemistry, 58(1–2), 97–102.

Guillén, M. D., & Manzanos, M. J. (1999). Smoke and liquid smoke. Study of an aqueous smoke flavouring from the aromatic plant Thymus vulgaris L. Journal of Science Food Agriculture, 79, 1267–1274.

Guillén, M. D., & Manzanos, M. J. (2005). Characterisitics of smoke flavourings obtained from mixtures of oak (*Quercus* sp.) wood and aromatic plants (*Thymus vulgaris* L. and *Salvia lavandulifolia* Vahl.). Flavour Fragrance Journal, 20(6), 126–133.

Guillén, M. D., Manzanos, M. J., & Ibargoitia, M. L. (2001). Carbohydrate and nitrogenated compounds in liquid smoke flavorings. Journal of Agriculture and Food Chemistry, 49, 2395–2403.

Guillén, M. D., & Ruiz, A. (2005). Monitoring the oxidation of unsaturated oils and formation of oxygenated aldehydes by proton

NMR. European Journal of Lipid Science Technology, 107, 36–47. Hedberg, E., Kristensson, A., Ohlsson, M., Johansson, C., Johansson, P.

A., Swietlicki, E., et al. (2002). Chemical and physical characterization

of emissions from birch wood combustion in a wood stove. Atmospheric Environment, 36, 4823–4837.
Hopkins, J. R., Still, T., Al-Haider, S., Fisher, I. R., Lewis, A. C., & Seakins, P. W. (2003). A simplified apparatus for ambient formaldehyde detection via GC-pHID. Atmospheric Environment, 37, 2557–2565.
Hsieh, R. J., & Kinsella, J. E. (1989). Oxidation of polyunsaturated fatty acids: Mechanisms, products, and inhibition with emphasis on fish. Advances in Food and Nutrition Research, 233–341.
Hsu, F. F., Hazen, S. L., Giblin, D., Turk, J., Heinecke, J. W., & Gross, M. L. (1999). Mass spectrometric analysis of pentafluorobenzyl oxime derivatives of reactive biological aldehydes. International Journal of Mass Spectrometry, 185(7), 795–812.
Hultin, H. O. (1994). Lipid oxidation in fish muscle. In G. J. Flick, J. R. And, & R. Y. Martin (Eds.), Advances in seafood biochemistry, composition and quality (pp. 99–102). Lancaster, PA: Technomic Publishing company.
Huss, H. H. (1999). La qualité et son évolution dans le poisson frais. FAO Document technique sur les pêches, 348, FAO – Organisation des Nations Unies pour l'alimentation et l'agriculture.
INERIS. Rapport d'étude du 21/12/2004. Exposition par inhalation au formaldehyde dans l'air. Sources, mesures et concentrations. N INERIS-DRC-04-56770-AIRE-n 1057IZd [In French].
INRS (1999). 2-Furaldéhyde. Fiche toxicologique no. 40.
INRS (2004a). METROPOL: Aldehydes. Fiche 001.
INRS (2004b). Aldéhyde acétique. Fiche toxicologique no. 120.
Jacob, V., Denis, I., & Foster, P. (1998). La métrologie des composés organiques volatils (COV): méthodes et difficultés. Analysis Magazine, 26(9), 33–37 (in French).
Jira, W. (2004). Chemical reactions of smoking. Fleischwirtschaft International, 4, 21–25.
Joffraud, J., Leroi, F., Roy, C., & Berdagué, J. L. (2001). Characterisation of volatile compounds produced by bacteria isolated from the spoilage flora of cold-smoked salmon. International Journal of Food Microbiology, 66, 175–184.
Jørgensen, L. V., Huss, H. H., & Dalgaard, P. (2001). Significance of volatile compounds produced by spoilage bacteria in vacuum-packed cold-smoked salmon (*Salmo salar*) analyzed by GC/MS and multivariate regression. Journal of Agriculture and Food Chemistry, 49, 2376–2381.
Josephson, D. B., & Lindsay, R. C. (1987). Retro-aldol degradations of unsaturated aldehydes: Role in the formation of cis-4-heptenal from trans-2, cis-6-nonadienal in fish, oyster, and other flavours. Journal of American Oil Chemical Society, 64, 132–138.
Josephson, D. B., Lindsay, R. C., & Stuiber, D. A. (1984). Variations in the occurences of enzymically derived volatile aroma compounds in salt- and freshwater fish. Journal of Agriculture and Food Chemistry, 32, 1344–1347.
Josephson, D. B., Lindsay, R. C., & Stuiber, D. A. (1987). Influence of processing on the volatile compounds characterizing the flavor of pickled fish. Journal of Food Science, 52(1), 10–14.
Josephson, D. B., Lindsay, R. C., & Stuiber, D. A. (1991a). Carotenoid-related oxidation compounds contributing to cooked salmon flavour. Lebensm-Wiss. Technol., 24, 424–432.
Josephson, D. B., Lindsay, R. C., & Stuiber, D. A. (1991b). Influence of maturity on the volatile aroma compounds from fresh Pacific and Great Lakes salmon. Journal of Food Science, 56(6), 1576–1579.
Kataoka, H., Kondo, T., & Sumida, A. (1998). Gas chromatographic determination of aldehydes in combustion smoke samples. Analytica Chimica Acta, 358, 269–275.
Kawai, T., Ishida, Y., Kakiuchi, H., Ikeda, N., Higashida, T., & Nakamura, S. (1991). Flavour components of dried squid. Journal of Agriculture and Food Chemistry, 39, 770–777.
Kjällstrand, J., & Petersson, G. (2001). Phenolic antioxidants in wood smoke. Science of the Total Environment, 277, 69–75.
Le Guen, S., Prost, C., & Demaimay, M. (2000a). Critical comparison of three olfactometric methods for the identification of the most potent odorants in cooked mussels (*Mytilus edulis*). Journal of Agriculture and Food Chemistry, 48(4), 1307–1314.
Le Guen, S., Prost, C., & Demaimay, M. (2000b). Characterization of odorant compounds of mussels (*Mytilus edulis*) according to their origin using gas chromatography–olfactometry and gas chromatography–mass spectrometry. Journal of Chromatography A, 896, 361–371.
Lee, H., Suriyaphan, O., & Cadwallader, K. R. (2001). Aroma components of cooked tail meat of American Lobster (*Homarus americanus*). Journal of Agriculture and Food Chemistry, 49, 4324–4332.
Lindsay, R. C. (1994). In F. Shahidi & J. R. Botta (Eds.), Flavour of fish in seafoods: Chemistry, processing technology, and quality (pp. 75–84). New York: Blackie Academic and Professional Press.
Lustre, A. O., & Issenberg, P. (1969). Volatile components of hardwood sawdust smoke. Journal of Agriculture and Food Chemistry, 17(6), 1387–1393.
Maga, J. A. (1987). The flavour chemistry of wood smoke. Food Review International, 3(1–2), 139–183.
Mansur, M. A., Bhadra, A., Takamura, H., & Matoba, T. (2003). Volatile flavor compounds of some sea fish and prawn species. Fisheries Science, 69, 864–866.
Matiella, J. E., & Hsieh, T. C. Y. (1990). Analysis of crabmeat volatile compounds. Journal of Food Science, 55, 962–966.
McGill, A. S., Hardy, R., Burt, J. R., & Gunstone, F. D. (1974). Hept-cis-4-enal and its contribution to the off-flavour in cold stored cod. Journal of Science and Food Agriculture, 25, 1477–1489.
Metz, B., Kersten, G. F., Hoogerhout, P., Brugghe, H. F., Timmermans, H. A., De Jong, A., et al. (2004). Identification of formaldehyde-induced modifications in proteins: Reactions with model peptides. Journal of Biological Chemistry, 279(8), 6235–6243.
Miler, K. B. M., & Sikorski, Z. E. (1990). Smoking. In Seafood: Resources, nutritional composition, and preservation. Boca Raton, FL: CRC Press, Inc.
Milo, C., & Grosch, W. (1996). Changes in odorants of boiled salmon and cod as affected by the storage of the raw material. Journal of Agriculture and Food Chemistry, 44, 2366–2371.
Min, D. B., & Lee, H. O. (1999). Chemistry of lipid oxidation. 30 years of progress. In R. Teranishi, E. L. Wick, & I. Horstein (Eds.), Flavour chemistry. Thirty years of progress (pp. 175–187). New York: Kluwer Academic/Plenum Publishers.
Munasinghe, D. M. S., Ichimaru, K., Matsui, T., Sugamoto, K., & Sakai, T. (2003). Lipid peroxidation-derived cytotoxic aldehyde, 4-hydroxy-2-nonenal in smoked pork. Meat Science, 63, 377–380.
Munasinghe, D. M. S., Ichimaru, K. I., Ryuno, M., Ueki, N., Matsui, T., Sugamoto, K., et al. (2003). Lipid peroxidation-derived hepatotoxic aldehydes 4-hydroxy-2E-hexenal in smoked fish meat products. Fisheries Science, 69, 189–194.
NCI/NTP (1990). Toxicology and carcinogenesis studies of benzaldehyde (CAS No. 100-52-7) in F344/N rats and B6C3F1 mice (gavage studies). National cancer institute/National toxicology program. Carcinogenesis technical report series. TR-378.
Nilsson, J. A., Zheng, X., Sundqvist, K., Liu, Y., Atzori, L., Elfwing, A., et al. (1998). Toxicity of formaldehyde to human oral fibroblasts and epithelial cells: Influences of culture conditions and role of thiol status. Journal of Dental Research, 77(11), 1896–1903.
Nishikawa, A., Sodum, R., & Chung, F. (1992). Acute toxicity of trans-5-hydroxy-2-nonenal in fisher 344 rats. Lipids, 27, 54–58.
Nonier, M. F., Vivas, N., Vivas de Gaulejac, N., Absalon, C., Soulié, Ph., & Fouquet, E. (2005). Pyrolysis-gas chromatography/mass spectrometry of *Quercus* sp. Wood. Application to structural elucidation of macromolecules and aromatic profiles of different species. Journal of Analytical and Applied Pyrolysis, 75(2), 181–193.
Pennarun, A. L., Prost, C., & Demaimay, M. (2002). Identification and origin of the character-impact compounds of raw oyster *Crassostrea gigas*. Journal of Science and Food Agriculture, 82, 1652–1660.

Pichard, A., Bisson, M., Diderich, R., Hulot, C., Lacroix, G., & Lefevre, J. P., et al. (2005). Benzaldehyde, Fiche de données toxicologiques et environnementales des substances chimiques. INERIS-DRC-01-25590-01DR026.

Pan, X. Q., Ushio, H., & Ohshima, T. (2005). Comparison of volatile compounds formed by autoxidation and photosensitized oxidation of cod liver oil in emulsion systems. Fisheries Science, 71, 639–647.

Picchiottino, C., & Lee, P. C. (2002). Differential development of aldehyde dehydrogenase in fore- and glandular stomach in postnatal rats. Experimental Biology and Medicine, 227, 554–558.

Piveteau, F., Le Guen, S., Gandemer, G., Baud, J. P., Prost, C., & Demaimay, M. (2000). Aroma of fresh oysters *Crassostrea gigas*: Composition and aroma notes. Journal of Agriculture and Food Chemistry, 48, 4851–4857.

Pokorný, J. (1987). Major factors affecting the autoxidation of lipids. In H. W. S. Chan (Ed.), Autoxidation of unsaturated lipids (pp. 141–206). New York: Academic Press.

Potter, T. L. (1996). Essential oil composition of cilantro. Journal of Agriculture and Food Chemistry, 44, 1824–1826.

Prost, C., Hallier, A., Cardinal, M., Serot, T., & Courcoux, P. (2004). Effect of storage time on raw sardine (*Sardina pilchardus*) flavor and aroma quality. Journal of Food Science, 69(5), 198–204.

Prost, C., Sérot, T., & Demaimay, M. (1998). Identification of the most potent odorants in wild and farmed cooked turbot (*Scophtalamus maximus* L.). Journal of Agriculture and Food Chemistry, 46, 3214–3219.

Qian, M., & Reineccius, G. (2002). Identification of aroma compounds in Parmigiano-Reggiano cheese by gas chromatography/olfactometry. Journal of Dairy Science, 85, 1362–1369.

Rizzi, G. P. (1999). The Strecker degradation and its contribution to food flavour. In R. Teranishi, E. L. Wick, & I. Horstein (Eds.), Flavour chemistry. Thirty years of progress (pp. 335–343). New York: Kluwer Academic/Plenum Publishers.

Rochat, S., & Chaintreau, A. (2005). Carbonyl odorants contributing to the in-oven roast beef top note. Journal of Agriculture and Food Chemistry, 53, 9578–9585.

Ross, C. F., & Smith, D. M. (2006). Use of volatiles as indicators of lipid oxidation in muscle foods. Comprehensive Reviews in Food Science and Food Safety, 5, 18–25.

Rowe, D. J. (1998). Aroma chemicals for savory flavours. Perfumer & Flavorist, 23, 9–14.

Rychlik, M., Schieberle, P., Grosch, W. (1998). Compilation of odour thresholds, odour qualities and retention indices of keyfood odorants. Deutsche forschungsanstalt für Lebensmittelchemie and Institut für Lebensmittelchemie der Technischen Universität München, Garching, Germany.

Sainclivier, M. (1985). Les industries alimentaires halieutiques. Bulletin scientifique et technique de l'école nationale supérieure agronomique et du centre de recherches de Rennes (Vol. II) [in French].

Scarpellino, R., & Soukup, R. J. (1993). Key flavours from heat reactions of food ingredients. In T. E. Acree & R. Teranishi (Eds.), Flavour science: Sensible principles and techniques. ACS Professionnal Refence Book (pp. 309–335). Washington, DC: American Chemical Society.

Sérot, T., Baron, R., Knockaert, C., & Vallet, J. L. (2004). Effect of smoking processes on the contents of 10 major phenolic compounds in smoked fillets of herring (*Cuplea harengus*). Food Chemistry, 85, 111–120.

Sérot, T., & Lafficher, C. (2003). Optimisation of solid-phase microextraction coupled to gas chromatography for determination of phenolic compounds in smoked herring. Food Chemistry, 82, 513–519.

Sérot, T., Regost, C., & Arzel, J. (2002). Identification of odour-active compounds in muscle of brown trout (*Salmo trutt*a) as affected by dietary lipid sources. Journal of Science and Food Agriculture, 82, 636–643.

Sérot, T., Regost, C., Prost, C., Robin, J., & Arzel, J. (2001). Effect of dietary lipid sources on odour-active compounds in muscle of turbot (*Psetta maxima*). Journal of Science and Food Agriculture, 81, 1339–1346.

Shibamoto, T., & Horiuchi, M. (1997). Role of aldehydes in cooked fish flavours. In F. Shahidi & K. R. Cadwallader (Eds.), Flavour and lipid chemistry of seafoods (pp. 20–30). Washington, DC: American Chemical Society.

Simon, R., De La Calle, B., Palme, S., Meier, D., & Anklam, E. (2005). Composition and analysis of liquid smoke flavouring primary products. Journal of Separation Science, 28, 871–882.

Stashenko, E. E., Ferreira, M. C., Sequeda, L. G., Martinez, J. R., & Wong, J. W. (1997). Comparison of extraction methods and detection systems in the gas chromatographic analysis of volatile carbonyl compounds. Journal of Chromatography A, 779, 360–369.

Stashenko, E. E., & Martinez, J. R. (2004). Derivatization and solid-phase microextraction. Trends in Analytical Chemistry, 23(8), 553–561.

Stashenko, E. E., Puertas, M. A., Salgar, W., Delgado, W., & Martinez, J. R. (2000). Solid-phase microextraction with on-fibre derivatization applied to the analysis of volatile carbonyl compounds. Journal of Chromatography A, 886, 175–181.

Stohr, V., Joffraud, J. J., Cardinal, M., & Leroi, F. (2001). Spoilage potential and sensory profile associated with bacteria isolated from cold-smoked salmon. Food Research International, 34, 797–806.

Stołyhwo, A., Kołodziejska, I., & Sikorski, Z. E. (2006). Long chain polyunsaturated fatty acids in smoked Atlantic mackerels and Baltic sprats. Food Chemistry, 94(4), 589–595.

Swoboda, P. A. T., & Peers, K. E. (1977). Volatile odourous compounds responsible for metallic, fishy taint formed in butterfat by selective oxidation. Journal of Science and Food Agriculture, 28, 1019–1024.

Tabatabaie, T., & Floyd, R. A. (1996). Inactivation of glutathione peroxydase by benzaldehyde. Toxicology and Applied Pharmacology, 141, 389–393.

Tanchotikul, U., & Hsieh, T. C. Y. (1989). Volatile flavour components in crayfish waste. Journal of Food Science, 54(6), 1515–1520.

Til, H. P., Woutersen, R. A., & Feron, V. J. (1988). Evaluation of the oral toxicity of acetaldehyde and formaldehyde in a 4-week drinking-water study in rats. Food and Chemical Toxicology, 26(5), 447–452.

Til, H. P., Woutersen, R. A., Feron, V. J., Hollanders, V. H. M., Falke, H. E., & Clary, J. J. (1989). Two-year drinking-water study of formaldehyde in rats. Food and Chemical Toxicology, 27(2), 77–87.

Tilgner, D. J. (1977). The phenomena of quality in the smoke curing process. Pure and Applied Chemistry, 49, 1629–1638.

Toth, L., & Potthast, K. (1984). Chemical aspects of the smoking of meats and meat products. Advance Food Research, 29, 87–158.

Triqui, R., & Reineccius, G. A. (1995). Changes in flavour profiles with ripening of anchovy (*Engraulis encrasicholus*). Journal of Agriculture and Food Chemistry, 43, 1883–1889.

Triqui, R., & Zouine, K. (1999). Sensory and instrumental assessments of the ripening process of anchovy (*Engraulis encrasicholus*). Lebensm.-Wiss. U.-technol., 32, 203–207.

Ullrich, F., & Grosch, W. (1988). Identification of the most intense odour compounds formed during autoxidation of methyl inoleate at room temperature. Journal of American Oil Chemical Society, 65(8), 1313–1317.

Vargova, M., Wagnerova, J., Liskova, A., Jakubovsky, J., Gajdova, M., Stolcova, E., et al. (1993). Subacute immunotoxicity study of formaldehyde in male rats. Drug and Chemical Toxicology, 16, 255–275.

Varlet, V., Knockaert, C., Prost, C., & Sérot, T. (2006). Characterization of odour-active volatile compounds of salmon and smoked salmon by gas chromatography-olfactometry and gas chromatography-mass spectrometry. Journal of Agriculture and Food Chemistry, 54, 3391–3401.

Varlet, V., Prost, C., & Sérot, T. (2006). New procedure for the study of odour representativeness of aromatic extracts from smoked salmon. Food Chemistry, 100, 820–829.

Valim, M. F., Rouseff, R. L., & Lim, J. M. (2003). Gas chromatographic–olfactometric characterization of aroma compounds in two types of cashew apple nectar. Journal of Agriculture and Food Chemistry, 51(4), 1010–1015.

Weenen, H. (1998). Reactive intermediates and carbohydrate fragmentation in Maillard chemistry. Food Chemistry, 62(4), 393–401.

Welfring, J., & Weidenhaupt, A. (2000). Energie du bois. La valorisation thermique des résidus ligneux de l'industrie de la transformation du bois. CRTE, Cahiers de la production propre 01.

Welfring, J., Weidenhaupt, A., Offermann, J., & Gouy, C. (2000). Etude éco-bilan sur l'installation d'un réseau de chaleur alimenté par un mélange de bois provenant d'entreprises de transformation du bois et de travaux d'entretien de forêts. CRTE.

Wilkes, J. G., Conte, E. D., Kim, Y., Holcomb, M., Sutherland, J. B., & Miller, D. W. (2000). Sample preparation for the analysis of flavours and off-flavours in foods. Journal of Chromatography A, 880, 3–33.

Witz, G. (1989). Biological interactions of α,β-unsaturated aldehydes. Free Radical Biology and Medicine, 7, 333–349.

Yasuhara, A., & Shibamoto, T. (1995). Quantitative analysis of volatile aldehydes formed from various knids of fish flesh during heat treatment. Journal of Agriculture and Food Chemistry, 43, 94–97.

Yaylayan, V. A., & Huyghues-Despointes, A. (1994). Chemistry of Amadori rearrangement products: Analysis, synthesis, kinetics, reactions and spectroscopic properties. Critical Review in Food Science, 34(4), 321–369.

1.4.4 Analogie avec la viande fumée

Comme pour le poisson fumé, il y a très peu de données disponibles concernant les composés volatils de la viande fumée et encore moins sur les composés volatils odorants. Les procédés artisanaux et industriels concernant la viande sont beaucoup plus nombreux que dans la filière halieutique. De plus, les pratiques et réglementations sont également différentes. Citons par exemple l'emploi de sels nitrités interdits dans la filière halieutique mais autorisés dans la filière carnée. Les nitrates/nitrites ont une action conservatrice, notamment dirigée contre Clostridium botulinum, responsables du botulisme. Cependant, ces agents minéraux peuvent réagir avec des acides aminés et conduire à la formation de nitrosamines carcinogènes. Ils peuvent également réagir avec des précurseurs aromatiques dans le produit (Timón, M.L. et al., 2004). En conséquence, il n'est pas prudent de comparer les teneurs en composés volatils de viande et de poisson fumé. Cependant, la comparaison entre ces deux matrices permet de cibler les composés volatils communs aux deux matrices et provenant de la fumée.

Comme pour les produits de la mer, nous pouvons distinguer les composés volatils provenant de la matrice initiale, ceux provenant de la fumée et ceux provenant de la réaction entre la fumée et les constituants biochimiques de la matrice. La gamme de composition biochimique est beaucoup plus grande dans les produits carnés que dans les produits de la mer. Elle revêt une importance primordiale pour garantir un fumage de bonne qualité notamment pour appréhender la diffusion des composés volatils de la fumée depuis la surface jusqu'à l'intérieur de l'aliment.

Ainsi, nous pouvons distinguer les composés volatils provenant de l'oxydation des lipides (alcools, composés carbonylés, hydrocarbures aliphatiques et quelques composés furaniques), de la dégradation des acides aminés, de la fermentation des sucres, de l'estérification microbienne, de la fumée, et enfin, selon leur ajout, des épices (Young, O.A. et al., 1997 ; Hierro, E. et al., 2004 ; Sampels, S. et al., 2004). En fonction des pratiques de préparation, des ferments peuvent être ajoutés ce qui diversifie les sources et les quantités de composés volatils dus à une activité métabolique microbienne.

En comparant les profils de composés volatils retrouvés sur ces aliments préparés de différentes façons (salage, séchage, fermentations, fritures, saumurage, …), des points communs apparaissent concernant les molécules issues de mécanisme d'oxydation de la matière première (Shimoda, M. et al., 1993 ; Ansorena, D. et al., 2001). Celles-ci s'avèrent de précieux atouts pour identifier l'origine des composés volatils dans la viande fumée mais on

ne peut pas comparer les teneurs en composés volatils entre un poisson et une viande fumée car les procédés de fumage et les temps d'exposition peuvent être très différents.
Cependant, l'origine des composés phénoliques est attribuée à la fumée. Selon les aliments et les techniques, leur proportion varie très fortement. Dans les spécialités réunionnaises carnées boucanées, le guaiacol, le o-crésol et le phénol représentent 90 % de la fraction phénolique. Ces composés n'avaient été retrouvés que dans des produits carnés fumés à froid (Poligné et al., 2001). Ils sont également retrouvés dans le poisson fumé à froid. De façon générale, les phénols sont absorbés sur la surface de l'aliment carné par la formation de liaisons hydrogène entre leurs groupements hydroxy et les constituants du collagène (Daun, H., 1979).
Ainsi, parmi les composés volatils de viande fumée, il est possible de distinguer ceux provenant principalement de la fumée. En plus des composés phénoliques, des composés dérivés « enolones » ont été identifiés ainsi que des hydrocarbures aromatiques, des composés furaniques (en moindre quantité que dans les poissons fumés), des pyridines et des pyrazines (Hierro, E. et al., 2004). Ces résultats peuvent servir de base scientifique pour appréhender les composés volatils odorants du saumon fumé.

1.4.5 Méthodologies utilisées pour l'extraction et l'analyse des composés volatils de poissons

1.4.5.1 Méthodes d'extraction des composés volatils des produits de la mer

Deux méthodes d'extraction couramment utilisées dans l'extraction de composés volatils de produits de la mer peuvent être employées pour l'extraction des composés volatils du saumon fumé : l'hydrodistillation sous vide (DSV) et l'extraction-distillation simultanée (EDS).
L'hydrodistillation sous vide ou à pression réduite, permet d'obtenir l'ébullition du solvant à faible température et de ne pas créer de nouveaux composés volatils résultant de réactions entre les constituants de la matrice. Les composés volatils sont ensuite condensés dans divers pièges placés à température négative (Le Guen, S. et al., 2001). Les pièges sont alors lavés par des solvants organiques.
Cette méthode apparaît comme l'une des plus efficaces (Stephan, A. et al., 2000 ; Lee, G.H. et al., 2001), mais elle nécessite un temps d'extraction très long et de grands volumes de solvants.

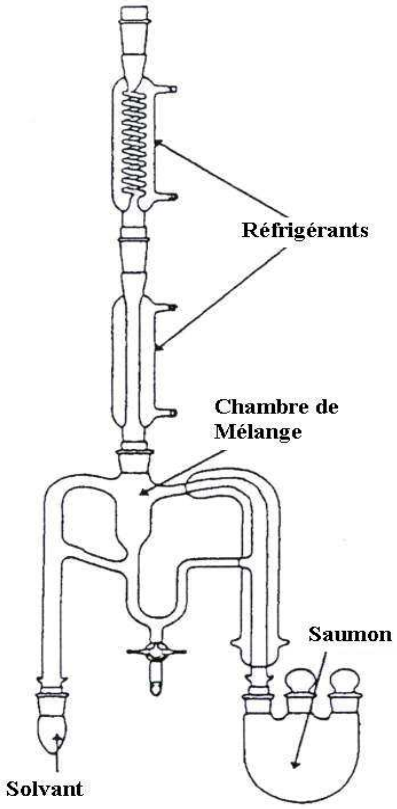

Figure 17. Appareil de Likens-Nickerson (Extraction Distillation Simultanée)

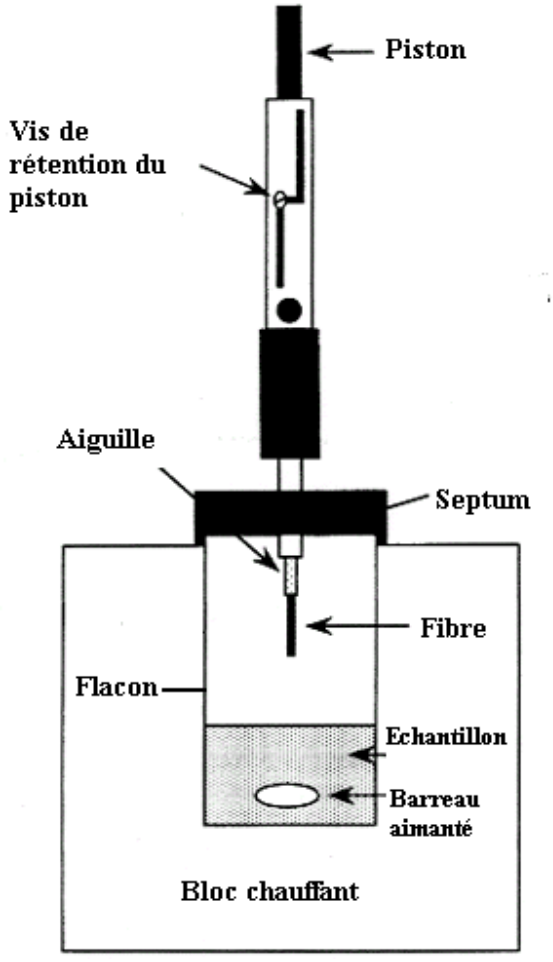

Figure 18. Principe de la MicroExtraction en Phase Solide (SPME)

L'extraction-distillation simultanée consiste à extraire les composés volatils présents dans la vapeur d'un échantillon placé dans de l'eau portée à ébullition par les vapeurs d'un solvant organique volatil porté également à ébullition mais immiscible dans l'eau (Figure 17). Les vapeurs se mélangent et les composés volatils sont captés par les vapeurs organiques puis les vapeurs sont condensées (Chung, H.Y. et al., 2001). On obtient une phase aqueuse appauvrie et une phase organique aromatique. Ce procédé est en circuit fermé ce qui permet de recycler les phases afin de les extraire à nouveau et augmenter le rendement d'extraction. Cette extraction peut se faire à pression réduite également ou à pression atmosphérique. Cette méthode apparaît également comme une des plus efficaces mais elle nécessite également des temps d'extraction longs (quelques heures) et des volumes de solvants non négligeables toutefois bien inférieures à ceux requis par la DSV.

Ces deux méthodes nécessitent des étapes ultérieures de concentration pouvant être à l'origine de perte ou de modification de l'arôme.

Les méthodes d'extraction des composés volatils de poissons fumés ont principalement été réalisées par des techniques dites d'espace de tête. La MicroExtraction en Phase Solide (SPME) a été très expérimentée par Guillén, M.D. et al., en 2002 sur de la daurade et de la truite fumées, en 2006, sur de l'espadon et du cabillaud fumés.

La SPME consiste en une fibre composée d'une phase dont la polarité est variable selon les fibres. Celle-ci est introduite dans un flacon dans lequel on aura pris soin de disposer l'échantillon. Les composés volatils de l'échantillon diffusent dans l'espace de tête (appelé ainsi car il est au-dessus de l'échantillon) jusqu'à un équilibre. La fibre est ensuite introduite et les arômes sont absorbés par la fibre (Figure 18). Cette deuxième étape est dite d'extraction. Pour favoriser la diffusion des composés volatils dans l'espace de tête et l'extraction, la température constitue un paramètre clé sur lequel on peut influer en thermostatant le flacon. La fibre est retirée et peut être désorbée dans l'injecteur d'un chromatographe en phase gazeuse.

Une autre technique d'espace de tête a aussi été employée pour le saumon fumé (Cardinal, M. et al., 1997 ; Jørgensen, L.V. et al., 2001). Il s'agit de la méthode « Purge and Trap » ou extraction d'espace de tête dynamique. L'échantillon est balayé par un flux de gaz vecteur et les composés volatils sont emportés jusque sur un piège absorbant maintenu à température négative tout le temps de l'extraction. La température de l'échantillon peut également être modifiée pour faciliter la diffusion des composés volatils. La méthode « Purge and Trap » est qualifiée de dynamique par rapport à la SPME dite statique car l'espace de tête est

constamment déplacé vers le piège et l'échantillon s'appauvrit au fur et à mesure en composés volatils. Au bout du temps d'extraction, le piège est porté à très haute température et les composés volatils se volatilisent et sont entraînés vers l'injecteur d'un chromatographe en phase gazeuse.

Ces deux méthodes d'espace de tête ont trois avantages principaux.

Le premier est que les temps d'extractions sont généralement courts. En fonction des matrices, on évite cependant de trop chauffer l'échantillon pour ne pas créer de nouveaux composés volatils résultant de réactions entre les constituants de l'échantillon lui-même. Le second tient au fait que les extractions d'espace de tête ne sont pas destructives. Enfin, le troisième avantage consiste en l'absence de solvants d'où des extractions non polluantes du point de vue environnemental.

Cependant, ces méthodes ont également un inconvénient majeur. En effet, l'extraction a lieu sur l'espace de tête et non pas sur le produit lui-même. Le principe d'extraction réside donc sur des changements de phase entre les composés volatils de la matrice solide et l'espace de tête gazeux. L'efficacité de l'extraction est donc supposée mais non vérifiée. Les méthodes d'extraction d'espace de tête s'avèrent adéquates dans le cas de molécules à bas point d'ébullition et pour de la semi-quantification. Les principes d'équilibre qui s'établissent entre l'échantillon et son espace de tête sont très influencés par les paramètres d'extraction (temps, température, …).

D'autres méthodes existent mais apparaissent soit obsolètes, soit inadéquates pour les matrices envisagées.

Par exemple, l'extraction solide-liquide par agitation mécanique n'est pratiquement plus utilisée dans le domaine de l'extraction aromatique, en particulier pour l'extraction de composés volatils de matrices grasses. Cette méthode requiert de grands volumes de solvants pour des rendements plutôt faibles et surtout différents selon les composés volatils. A l'inverse, d'autres méthodes sont apparues comme l'extraction par micro-ondes ou l'extraction par fluide supercritique mais elles restent limitées à des matrices peu grasses (Curioni, P.M.G. & Bosset, J.O., 2002), ce qui n'est pas le cas du saumon fumé.

1.4.5.2 Représentativité des extraits

Dans l'analyse des arômes, bien souvent, ce n'est pas le produit lui-même qui est analysé mais un extrait de celui-ci. Certaines méthodes d'extraction des composés volatils

s'affranchissent de cette étape, notamment les techniques d'espace de tête par exemple. L'espace de tête est supposé représentatif de la matrice mais ne peut être vérifié. D'autres techniques d'extraction comme la DSV ou la EDS conduisent à un extrait aromatique qui doit être aussi représentatif que possible du produit initial. Ainsi, une évaluation sensorielle olfactive de l'extrait préliminaire à l'analyse olfactométrique doit être réalisée dans le but de vérifier l'adéquation odorante entre le produit initial et l'extrait (Moio, L. et al., 1995). Les tests de représentavité permettent ainsi de valider sensoriellement le choix d'une méthode d'extraction lorsque celle-ci conduit à un extrait aromatique de similarité satisfaisante.

L'évaluation olfactométrique ultérieure aura pour but de décrire et de caractériser les composés volatils odorants de l'extrait et par extension du produit analysé.

La présentation des échantillons au jury se fait par l'intermédiaire de flacons lorsque l'extrait est réincorporé dans de l'eau ou dans des solvants neutres. Les échantillons extraits peuvent aussi être présentés sous forme de mouillettes lorsque ceux-ci sont contenus dans un solvant pouvant s'évaporer facilement (Van Gemert, L.J. 1981). Jusqu'à présent, les études de représentativité avaient deux principaux défauts. Le premier était l'emploi de solvants toxiques comme le dichlorométhane, utilisé pour sa forte volatilité et sa semi-polarité. Le second était que l'odeur de l'extrait était évaluée sur des matrices physiquement différentes de celle d'origine. Notre étude devra donc garantir la sécurité des juges par l'emploi final de solvants non toxiques et prendre en compte les éventuelles interactions odorantes entre les composés aromatiques de l'extrait et une matrice de redéposition. Celle-ci devra être la plus représentative physiquement du produit initial mais neutre sur le plan olfactif. Idéalement, il pourrait s'agir de la même matrice mais désodorisée (Tandon, K.S. et al., 2000). Dans le cas d'études de représentativité d'extraits d'aliments ayant subi un procédé industriel, on peut envisager de redéposer l'extrait directement sur l'aliment n'ayant pas subi le procédé. Dans cette optique, les méthodes conduisant à un extrait aromatique sous forme liquide sont plus faciles d'utilisation. Dans notre étude, pour rétablir les éventuelles interactions entre les composés de l'extrait et la matrice, l'extrait aromatique de saumon fumé sera redéposé sur du saumon frais. Ce développement méthodologique n'a jamais été réalisé sur le poisson et constituera l'un des premiers travaux de représentativité permettant de prendre en compte les interactions matricielles. Un point essentiel consiste à déterminer la quantité d'extrait à redéposer sur la matrice pour se placer dans des conditions en d'iso-intensité. Il faut donc calculer la quantité d'extrait pour obtenir des concentrations en composés volatils semblables à celles de la matrice initiale. Ainsi, les méthodes d'extractions des composés volatils devront être le moins sélectif et le plus efficace possible.

Objectif	Tests	Auteurs
Comparaison de techniques d'extraction	test de similarité	Sarrazin, C. et al., 2000
	test de similarité et analyse descriptive	Mehinagic, E. et al., 2000
Optimisation de méthodes	Test triangulaire et de similarité	Etiévant, P.X. et al., 1994
	Rangements, test de similarité et d'appariements	Larráyoz, P. et al., 2000
Comparaison d'échantillons	Test triangulaire et d'appariement, analyse descriptive quantitative	Abbott, N. et al., 1993
	Test triangulaire et analyse descriptive quantitative	Hanaoka, K. et al., 2001

Tableau 15. Synthèse des différentes études de représentativité d'extraits selon l'objectif de l'étude et les tests utilisés

En fonction de l'objectif de l'étude, différentes stratégies de tests de représentativité sont mis en œuvre (Etiévant, P.X. et al., 1994).

Si l'on recherche les origines de différences chimiques entre les caractéristiques odorantes de différents échantillons, on emploiera un test triangulaire suivi d'un test d'appariement. Cela permet de déceler si les différences entre les échantillons se répercutent sur les extraits puis de situer les caractéristiques odorantes de ces extraits par rapport au produit initial (Abbott, N. et al., 1993 ; Bernet, C. et al., 1999).

Si l'on recherche, et ce sera notre cas, à savoir si l'extrait est représentatif du produit initial on procédera avec un test de similarité où les juges évalueront la similitude de l'odeur de l'extrait par rapport à celle du produit initial sur une échelle non structurée en les comparant sur la base de descripteurs judicieusement choisis (Sarrazin, C. et al., 2000 ; Le Pape, M.A. et al., 2004).

Si l'on veut caractériser les extraits, on peut entreprendre des profils sensoriels permettant de statuer sur la présence ou l'absence de composés odorants entre un produit et son extrait (Priser, C., 1997 ; Mehinagic, E. et al., 2003).

Ces tests permettent de comparer des techniques d'extraction, de les optimiser et de les valider en fonction des composés volatils suivis. Ainsi, l'expérimentateur pourra retenir la méthode d'extraction qui permet de conserver le plus possible l'intégrité de l'arôme initial. Enfin, ces tests permettent de comparer facilement deux échantillons. Le tableau 15 évidemment illustratif et non exhaustif, regroupe différents tests sensoriels corrélés à leurs diverses utilisations selon les objectifs d'études.

1.4.5.3 Méthodes d'analyse des composés volatils des produits de la mer

En fonction de la méthode d'extraction, les extraits aromatiques sont soit absorbés sur des supports dans le cas de techniques d'espace de tête, soit à l'état liquide.

Pour séparer les différents composés volatils d'un mélange, la chromatographie en phase gazeuse (GC) s'avère l'outil de séparation le plus utilisé. Pour séparer les constituants, diverses colonnes capillaires de phases variées sont disponibles. Compte tenu de la faible polarité des composés volatils présents dans les poissons fumés (composés phénoliques, furaniques, …), le choix de colonnes apolaires semble plus judicieux (Sérot, T. et al., 2004 ; Guillén, M.D. et al., 2006).

Une fois séparés, les différents composés doivent être identifiés. Deux modes de détection sont largement utilisés : la détection par ionisation de flamme (FID) pour une séparation GC (Prost, C. et al., 1998) et la spectrométrie de masse (MS).

La GC-FID est de moins en moins employée, et essentiellement à des fins quantitatives. En effet, elle nécessite l'injection de standards aromatiques pour l'identification des signaux obtenus. Les programmes de températures et les colonnes ne varient que très peu par rapport à la GC-MS à la différence que la détection se fait à des températures toujours supérieures au moins à 250 °C.

D'autres détecteurs peuvent également être utilisés pour analyser des familles chimiques de composés ciblés. Ainsi, le détecteur photométrique à flamme pulsée (PFPD) et le détecteur d'émission atomique (AED) ont déjà été utilisés pour identifier les composés soufrés du violet (Senger-Emonnot, P. et al., 2006).

Les techniques de séparation par chromatographie en phase liquide (LC) ne sont que très peu utilisées. Les détecteurs utilisés après séparation LC ne sont presque exclusivement que les détecteurs à ultraviolets (UV). La plupart du temps, les composés volatils à analyser sont connus et des étapes de dérivation sont nécessaires (Rauber, C.D.S. et al., 2005 ; Bramanti, E. et al., 2006). Ainsi, l'analyse des aldéhydes peut être développée par LC-UV après la dérivation des aldéhydes en leurs dérivés 2,4-dinitrophenylhydrazine. Ces dérivés sont de couleur orangée et peuvent ensuite être analysés par le détecteur UV (généralement entre 330 et 390 nm) (Jacob, V. et al., 1998 ; Wilkes, J.G. et al., 2000). Les techniques LC peuvent donc être utilisées mais n'ont presque jamais été employées dans la détection des composés volatils des produits de la mer et des aliments en général du fait de l'élution en phase liquide. En effet, ces techniques ne permettent pas une analyse exhaustive des composés volatils d'un produit.

Enfin, parfois, la Résonance Magnétique Nucléaire (RMN) peut également être utilisée en tant que méthode de détection pour étudier la structure de composés particuliers mais pas en technique d'investigation de routine (Silva Elipe, M.V. et al., 2003). Elle suppose une étape de séparation GC ou LC dite préparative afin d'isoler les fractions d'intérêt.

1.4.6 Méthodologies utilisées pour l'analyse de l'odeur des composés volatils des produits de la mer

Les techniques chromatographiques permettent de séparer les constituants d'un mélange de molécules volatiles. Couplés aux techniques chromatographiques, la spectrométrie de masse apparaît comme l'outil d'identification et de quantification des composés volatils le plus

efficace. Les progrès réalisés tant pour l'extraction des composés volatils que pour leur séparation et leur identification ont permis de mettre en évidence souvent plus d'une centaine de composés présents dans un même produit. Etant donné les différences importantes entre les seuils de détection olfactive des composés volatils, tous les composés identifiés par des méthodes instrumentales n'apportent pas la même contribution à l'arôme global du produit. Il s'avère nécessaire d'éliminer ceux qui n'ont pas ou peu d'odeurpour au contraire, privilégier l'étude des composés à seuil de perception très faible. Ainsi, parmi les composés volatils, l'odeur des composés volatils peut être détectée par olfactométrie encore appelée repérage olfactif ou « sniffing ».

Les techniques olfactométriques (GC-O) aussi appelées méthodes de « sniffing » permettent d'identifier olfactivement les composés au fur et à mesure de leur élution. On peut ainsi directement repérer les composés contribuant à l'arôme du produit ainsi que leur odeur propre. Si plusieurs méthodes ont été élaborées pour effectuer ce suivi des molécules odorantes, elles ont toutes un point commun qui est le détecteur final constitué par le nez humain. La limite de détection du nez humain étant de 10^{-19} moles, il satisfait à la détection de l'effluent chromatographique et s'avère d'autant plus adéquat dans le cas d'analyses d'arômes alimentaires dont l'objectif final est de combler les attentes des consommateurs. Notons toutefois les nouvelles conceptions de nez électroniques qui ont pour but de remplacer le nez humain comme détecteur et de s'affranchir des éventuelles erreurs de jugements humains. Cependant ceux-ci sont encore en développement et leurs domaines d'application s'attachent à un but descriptif (Schaller, E. et al., 1998 ; Jonsdottir, R. et al., 2004).

Les diviseurs d'éluats permettent d'obtenir en temps réel l'analyse chromatographique directement corrélée avec l'évaluation olfactive. Une partie de l'éluat est dirigée vers le détecteur analytique, l'autre est dirigée vers une ligne de transfert chauffée (pour éviter la recondensation des composés volatils) aboutissant à un cône nasal où le juge doit disposer son nez. Ce cône est supplémenté en air humidifié pour éviter d'assécher les muqueuses nasales.

Ainsi, la GC-O repose essentiellement sur une évaluation sensorielle olfactive. Celle-ci doit être la plus objective possible en essayant de biaiser le moins possible le jugement c'est pourquoi quelques précautions d'usage doivent être prises. En effet, il ne faut pas oublier que le détecteur est le nez humain, il faut donc pratiquer des temps de pose entre les séances d'olfaction. Le temps d'olfaction joue aussi un grand rôle et doit être optimisé. Un entraînement des juges doit parfois être réalisé. L'environnement des séances d'olfactométrie doit être le plus normalisé possible, c'est-à-dire calme, neutre, à température agréable,

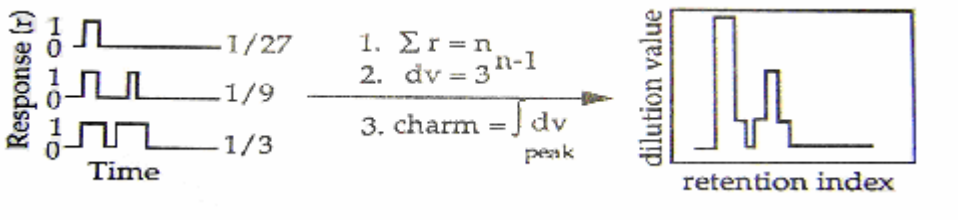

Figure 19. Chromatogramme CHARM

d'une luminosité réglementée et bien ventilé. Le juge doit se sentir calme et à l'aise (Hanaoka, K. et al., 2001).

1.4.6.1 Techniques olfactométriques

Il existe quatre grandes catégories de méthodes de GC-O : les méthodes de dilution apparues les premières dans les années 1980, les méthodes de temps-intensité, d'intensité postérieure et les méthodes de fréquence de détection apparues dans les années 1990.

1.4.6.1.1 Méthodes de dilution

Deux techniques de dilution d'extraits sont principalement et largement employées. Il s'agit de la méthode CHARM (Combined Hedonic Aroma Response Measurement) conçue par l'équipe d'Acree et al., 1984 et la méthode AEDA (Aroma Extract Dilution Analysis) conçue par l'équipe de Grosch (Ullrich, F. & Grosch, W., 1987). Ces deux méthodes consistent à diluer successivement l'extrait jusqu'à ce que le juge ne perçoive plus d'odeur en sorite de colonne (Friedrich, J.E. & Acree, T.E., 1998). La différence fondamentale entre la méthode CHARM et la méthode AEDA réside dans le fait que la méthode CHARM intègre toutes les dilutions et les transforment en surface de pics reflétant l'intensité de l'odeur (Figure 19) alors que dans la méthode AEDA, un composé odorant est caractérisé par le dernier facteur de dilution appliqué à celui-ci (Ferreira et al., 2002a ; Ferreira, V. et al., 2002b).

Ces méthodes présentent néanmoins quelques inconvénients d'où l'avènement de nouvelles techniques. Parmi ces inconvénients, nous pouvons citer l'ambiguïté de ces méthodes vis-à-vis des théories psychophysiques sur la perception de l'odeur. En effet, les méthodes CHARM et AEDA induisent une relation de proportionnalité. Or, plusieurs auteurs ont démontré qu'il n'y avait pas de relation de linéarité entre l'intensité de l'odeur et sa concentration (Le Guen, S. et al., 2003).

Egalement, pour les deux méthodes, le temps d'analyse est très long même si le composé est très bien caractérisé. Cependant, ces techniques sont fastidieuses c'est pourquoi on emploie un nombre limité de juges mais cela augmente la part de spécificité sensorielle propre à chaque individu dans les résultats. L'emploi de seulement un ou deux juges constitue alors la principale faiblesse de ces méthodes.

Enfin, il peut arriver qu'il y ait des trous de dilution c'est-à-dire qu'un juge peut très bien ne pas détecter une odeur à une certaine dilution pour un indice de rétention donné alors qu'il la

détectera à une dilution plus forte. De nombreux biais peuvent alors être introduits dans l'interprétation des résultats.

1.4.6.1.2 Méthodes de temps-intensité

Ces méthodes sont qualifiées de mesure directe de l'intensité odorante. L'extrait n'est évalué qu'à une seule dilution. Le principe repose sur la détection du début, du maximum et de la fin d'une odeur par un juge. Une approche plus automatisée de ce principe a été élaborée sous le nom de « Osme » (odorat en grec) où les juges enregistrent l'intensité des odeurs qu'ils perçoivent au cours du temps à l'aide d'un rhéostat (McDaniel, M.R., Miranda-Lopez, R., Watson, B.T., Michaels, N.J. & Libbey, L.M., 1989) Cette technique a l'avantage de recueillir en temps réel et en un seul chromatogramme l'intensité odorante des composés et de les compiler sur un osmegramme (Van Ruth, S.M., 2001).
Un autre avantage de cette méthode est d'être en accord avec les lois psychophysiques de la perception odorante. De plus, une seule dilution est nécessaire.
Cependant, cette technique présente des inconvénients, notamment les biais qui peuvent être introduits par les juges qui recquièrent un entraînement important. En effet, des trous dans les réponses, des effets contrastes dus à la fatigue où l'intensité odorante d'un composé peut venir perturber la perception du composé suivant, des fortes variabilités entre les juges et entre les notes d'un même juge sont fréquents (Etiévant, P.X. et al., 1999 ; Sérot, T. et al., 2001b).

1.4.6.1.3 Méthodes de fréquence de détection

Ces méthodes sont fondées sur la fréquence de détection des composés odorants par des juges qui indiquent uniquement le début et la fin d'une odeur ainsi que la description du composé détecté. Les réponses des juges sont alors compilées en un aromagramme qui définit en abscisse le temps ou l'indice de rétention et en ordonnées le nombre de juges ayant détecté cette odeur (Charles, M. et al., 2000 ; Carrapiso, A.I. et al., 2002). On travaille uniquement sur une dilution.
Une variante de cette technique est de convertir les hauteurs des pics de fréquence de détection en NIF (Nasal Impact Frequency) où le plus haut pic correspondra à 100 % du NIF ou bien de convertir les surfaces des pics de fréquence de détection en SNIF (Surface of Nasal Impact Frequency) où la plus grande surface correspondra à 100 % du SNIF. En travaillant

Produits : Coquillages	Méthode GC-O	Objectifs	Références
Moules	Fréquence de détection	Expliquer les différences sensorielles entre deux types de moules par les composés odorants	Le Guen, S. et al., 2001
Moules	AEDA, temps-intensité et Fréquence de détection	Comparaison de méthodes olfactométriques	Le Guen, S. et al., 2000
Moules	Fréquence de détection	Identification de composés odorants en fonction de l'origine géographique	Le Guen, S. et al., 2000
Huîtres	Fréquence de détection, temps-intensité	Relier le profil sensoriel avec la composition en composés odorants	Pennarun, A.L. et al., 2002
Moules	Fréquence de détection	Effet d'un procédé de déssalinisation	Cros, S. et al., 2004
Huîtres	Fréquence de détection, temps-intensité	Effet de la supplémentation en lipides de la nourriture	Pennarun, A.L. et al., 2003
Huîtres	Fréquence de détection	Identification de composés odorants	Piveteau, F. et al., 2000
Palourdes	Olfactométrie qualitative	Identification de composés odorants	Tanchotikul, U. & Hsieh, T.C.Y., 1991
Palourdes	AEDA	Identification de composés odorants, effet du stockage	Sekiwa, Y. et al., 1997
Violets	CHARM, temps-intensité	Identification de composés odorants	Senger-Emonnot, P. et al., 2006

Tableau 16. Synthèse des analyses olfactométriques réalisées sur des coquillages

Produits : Crustacés	Méthode GC-O	Objectifs	Références
Homard	AEDA	Identifier les composés les plus odorants	Lee, G.H. et al., 2001
Crabe	AEDA	Comparaison des composés odorants de différents morceaux de crabe	Chung, H.Y. et al., 1995
Ecrevisse	Olfactométrie qualitative	Identification de composés odorants	Vejaphan, W. et al., 1988
Ecrevisse	Olfactométrie qualitative	Identification de composés odorants	Tanchotikul, U. & Hsieh, T.C.Y., 1989
Homard	AEDA	Identification de composés odorants	Cadwallader, K.R. et al., 1995
Crabe	AEDA	Identification de composés odorants	Chung, H.Y. & Cadwallader, K.R., 1994
Ecrevisse	temps-intensité	Effet d'une hydrolyse enzymatique	Baek, H.H. et al., 1996

Tableau 17. Synthèse des analyses olfactométriques réalisées sur des crustacés

avec ces pourcentages de NIF ou de SNIF, les résultats deviennent indépendants du nombre de juges (Pollien, P. et al., 1997 ; Debonneville, C. et al., 2002). Cependant, il faut un nombre de juges minimum (8 personnes).
Les avantages de cette méthode sont multiples. Tout d'abord, la fréquence de détection est en accord avec les lois psychophysiques de la perception odorante. Ensuite, en disposant de plusieurs juges à la fois, sachant que les seuils de perception des individus sont très différents, en compilant les réponses de plusieurs juges à la fois, les résultats seront d'autant plus fiables et les effets contrastes, les trous dans les réponses, les différences inter juges sont minimisés puisque chaque juge ne contribue qu'à 1/n du résultat final (n étant le nombre de juges).

1.4.6.1.4 Méthodes d'intensité postérieure

Ces méthodes sont peu décrites dans la littérature et peu employées. Au lieu de noter en temps réel la courbe de l'intensité odorante caractérisant le composé qu'il perçoit, il est demandé au juge de noter mentalement le pic chromatographique élué (Van Ruth, S.M., 2001 ; Van Ruth, S.M., 2004). La courbe temps-intensité de chaque composé est alors transformée et résumée par un seul chiffre. Cette méthode a été boudée en raison de l'entraînement long et rigoureux que doivent subir les juges afin de rendre la plus objective possible une sensation olfactive très subjective (Acree, T.E. & Barnard, J.C., 1994). Cependant, elle est en évolution à partir des résultats obtenues par fréquence de détection où l'attribution d'une note d'intensité aux pics SNIF pourrait être un moyen de caractériser l'intensité de l'odeur perçue tout en gardant le bénéfice de l'approche par fréquence de détection (Pet'Ka, .,Ferreira,V. & Cacho, J., 2005).

1.4.6.2 Limites et innovations en olfactométrie

Les méthodes olfactométriques permettent ainsi de caractériser l'odeur des composés volatils d'un extrait aromatique. Cependant, dans l'objectif d'être le plus précis possible dans la caractérisation odorante, on utilise un nombre de juges le plus élevé possible. Comme les séances d'olfactométrie ne peuvent se faire qu'en série et non pas simultanément, la longueur du protocole d'analyse constitue un inconvénient majeur.
Récemment des innovations technologiques en GC-Olfactométrie ont permis de distribuer simultanément à plusieurs juges chacun des composés issus d'une séparation chromatographique. Cela permet de faciliter le traitement des données par l'informatisation

Produits : Poissons	Méthode GC-O	Objectifs	Références
Hareng	Méthodes de dilution d'espace de tête	Comparaison de procédés de cuisson, de conservation. Influence de l'origine géographique et de l'âge des poissons	Chung, H.Y. et al., 2006
Sardine	temps-intensité	Effet du temps de conservation	Prost, C. et al., 2004
Saumon fumé	Olfactométrie qualitative	Caractériser l'odeur de composés volatils produits durant le stockage à froid	Jørgensen, L.V. et al., 2001
Anchois	temps-intensité	Etudier le procédé de maturation de l'anchois	Triqui, R. & Reineccius, G.A., 1995a
Morue	Olfactométrie qualitative, nez électronique	Identifier les principaux composés produits par des microorganismes durant le stockage réfrigéré	Olafsdottir, G. et al., 2005
Truite	Fréquence de détection	Effet de la supplémentation en lipides de la nourriture	Sérot, T. et al., 2002
Saumon	Olfactométrie qualitative	Identification des composés volatils et rôles des précurseurs	Josephson, D.B. et al., 1991a
Saumon et morue	AEDA	Effet du stockage	Milo, C. & Grosch, W., 1996
Morue et truite	AEDA	Détection de défauts d'odeurs en relation avec la durée de stockage	Milo, C. & Grosch, W., 1995
Truite	AEDA	Effet du stockage	Milo, C. & Grosch, W., 1993
Sauce de poisson	AEDA	Identification de composés odorants	Fukami, K. et al., 2002
Turbot	Fréquence de détection, AEDA	Identification de composés odorants	Prost, C. et al., 1998
Turbot	Fréquence de détection	Effet de la supplémentation en lipides de la nourriture	Sérot, T. et al., 2001
Saumon	Olfactométrie qualitative	Caractériser l'odeur de composés volatils produits durant le stockage à froid	Refsgaard, H.H.F. et al., 1998
Anchois	AEDA	Etudier le procédé de maturation de l'anchois	Triqui, R. & Zouine, K., 1999
Morue	Olfactométrie qualitative, nez électronique	Identification de composés odorants	Jonsdottir, R. et al., 2004
Anchois	AEDA	Effet de la maturation	Triqui, R. & Reineccius, G.A., 1995b
Thon (sauce)	AEDA	Identification de composés odorants	Cha, Y.J. & Cadwallader, K.R., 1998
Sardine	AEDA	Relier le profil sensoriel avec la composition en composés odorants au cours du vieillissement	Triqui, R. & Bouchriti, N., 2003
Truite	Fréquence de détection, temps-intensité	Identification de composés odorants	Selli, S. et al., 2006
Merlu	AEDA	Effet du stockage	Triqui, R., 2006
Silure	Fréquence de détection, temps-intensité	Effet de conditions d'élevage	Hallier, A. et al., 2006

Tableau 18. Synthèse des analyses olfactométriques réalisées sur des poissons

des compilations des réponses des juges qui étaient réalisées séquentiellement jusqu'alors (Berdagué, J.L. & Tournayre, P. et al., 2004 et 2005).

L'olfactométrie permet d'identifier individuellement l'odeur des composés volatils. En effet, cell-ci est soumise au couplage chromatographique permettant de séparer les composés au cours du temps pour les analyser. Un profil de composés volatils odorants peut donc être établi. Cependant, l'odeur globale du produit ne peut être résumée à la somme de ces composés. En effet, des effets de synergie ou de masque peuvent avoir lieu et générer des interactions odorantes. Dans le but de remettre en mélange les molécules identifiées et séparées, une nouvelle méthode d'investigation a été développée sur le poisson chat européen (*Silurus glanis*). Le principe est fondée sur une analyse par chromatographie en phase gazeuse couplée à de l'olfactométrie globale basée sur des tests d'omission (GC-GOOD). Cette méthode permet d'identifier les familles clés de composés volatils et leurs interactions en retirant certaines familles de composés odorants (Hallier, A. et al., 2004). Notre étude s'appuiera sur ces travaux pour caractériser les rôles individuels des composés volatils sur l'odeur globale du saumon fumé.

1.4.6.3 Applications aux produits de la mer

Etant donné que l'on ne dispose que de peu d'études olfactométriques sur les produits fumés et encore moins sur les poissons fumés, nous relaterons ici que les techniques utilisées sur des matrices qui s'en rapprochent comme les produits de la mer. Ces résultats sont répertoriés dans les tableaux 16, 17 et 18.

Ainsi on remarque que dans certains travaux sur les moules, une comparaison de trois méthodes olfactométriques a été faite et conduisait globalement à préférer la méthode Temps-intensité aux autres méthodes (Fréquence de détection et AEDA) (Le Guen, S. et al., 2000b). Cependant, une très forte corrélation entre les trois méthodes a été mise en évidence (Sérot, T. et al., 2001b).

D'autres études ont montré que la méthode de fréquence de détection GC-SNIF était plus répétable, utilisait plus de juges mais nécessitait moins de temps et ce, sur des échantillons standards (Debonneville, C. et al., 2002). Elle permet d'avoir rapidement la contribution de chaque composé odorant dans l'arôme final et sa note aromatique.

En fonction de l'objectif d'étude, chaque méthode contribue différemment à la caractérisation des composés volatils odorants.

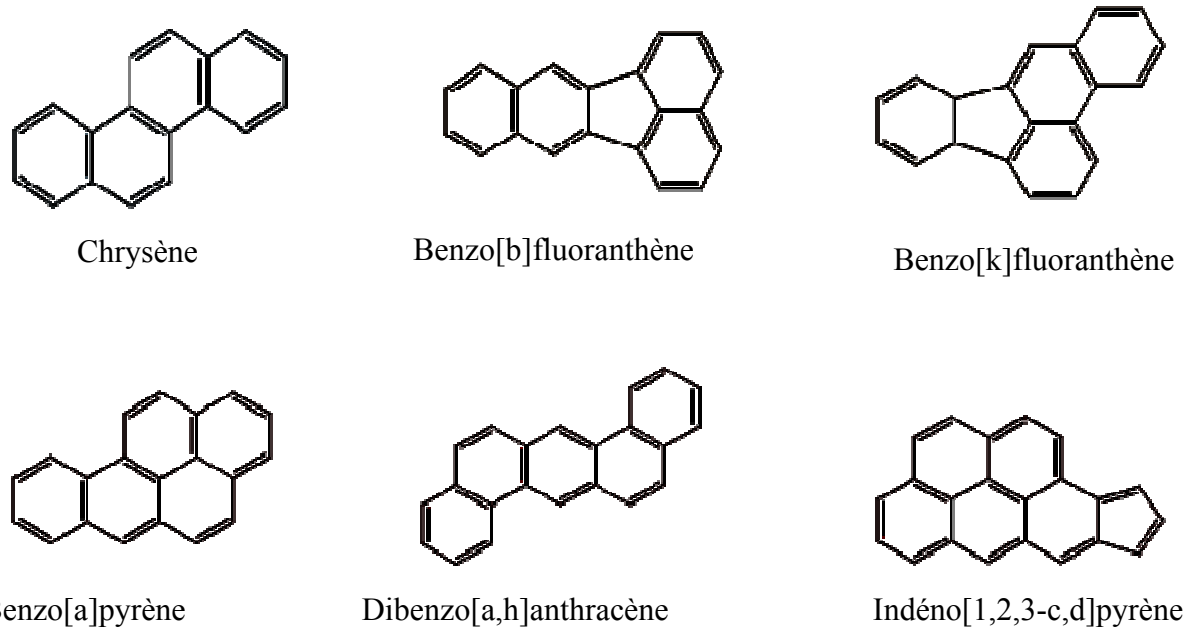

Figure 20. Structures des principaux HAP lourds les plus toxiques

1.5. Les hydrocarbures aromatiques polycycliques

Les différentes techniques de fumage génèrent de nombreux types de composés volatils odorants provenant de la fumée elle-même ou du poisson sous l'effet des paramètres de fumage. Par la maîtrise des paramètres des méthodes de fumage à l'origine de certains composés volatils odorants, il pourrait devenir possible d'orienter l'odeur globale du produit fumé. Cependant, l'élaboration de produits fumés aux qualités organoleptiques maîtrisées doit tenir compte de la création d'hydrocarbures aromatiques polycycliques (HAP), contaminants organiques des produits fumés, créées simultanément aux composés de la fumée lors de la pyrolyse de bois.

Les Hydrocarbures Aromatiques Polycycliques (HAP) sont des composés organiques plus ou moins complexes constitués de deux à sept cycles benzéniques. Ils représentent une famille de plusieurs centaines de molécules. On distingue deux origines pour ces composés : une origine pétrogénique et une origine pyrogénique. Les HAP d'origine pétrogénique sont d'origine naturelle et ont été synthétisés lors de la formation des combustibles fossiles (pétrole et charbon). Ils sont caractérisés par une forte proportion d'hydrocarbures aromatiques ramifiés (groupements alkyles). Les HAP d'origine pyrogénique sont issus de processus pyrolytiques comme les combustions incomplètes de matière organique (sidérurgies, moteurs à combustion, …). Ainsi les sources de pollution par les HAP sont essentiellement anthropiques (transport routier et fluvial, effluents industriels, incinération de déchets, techniques de chauffage domestique…) et prédominent dans l'environnement. Mais elles peuvent être également d'origine naturelle (feux de forêt, éruptions volcaniques).

Selon le nombre de cycles, on distingue les HAP légers (jusqu'à trois cycles) et les HAP lourds (quatre cycles et plus) qui ont des caractéristiques physico-chimiques et toxicologiques différentes. Quelques structures de HAP lourds sont présentées dans la Figure 20.

Ces substances remplissent quatre critères physicochimiques qui permettent de les classer parmi les Polluants Organiques Persistants (POP) au même titre que les dioxines par exemple. Ces critères sont répertoriés dans le protocole d'Aarhus (1998) et la convention de Stockholm (2001) et sont : une toxicité avérée, des capacités de bioaccumulation, de transport longue distance et de grande persistance dans l'environnement.

En ce qui concerne la toxicité des HAP, celle-ci a été rapportée dans de nombreux travaux attestant d'un ou plusieurs impacts prouvés sur la santé humaine (Baird, W.M. et al., 2005).

Les propriétés physico-chimiques des HAP varient selon leur poids moléculaire et leur structure mais ils présentent de manière générale une hydrosolubilité faible et des coefficients

de partage octanol/eau (Kow) relativement élevés s'étalant de 4 à 7. Très liposolubles, ces micropolluants ont tendance, après ingestion ou inhalation, à gagner les tissus riches en lipides. Ces molécules très lipophiles peuvent traverser les membranes cellulaires lipidiques et s'accumuler dans les tissus vivants. Leurs concentrations augmentent le long de la chaîne alimentaire d'où leur propriété de bioaccumulation (Tamakawa, K. 2004). Emis par voies atmosphériques par des sources très diverses, les HAP sont présents dans l'environnement à l'état de traces c'est-à-dire allant du dixième à quelques dizaines de ng/m^3 voire moins dans les matrices alimentaires (ultra-traces). En raison de leur très faible solubilité, ils ont tendance à s'associer aux fines particules en suspension (de 0,3 à 3 µm) qui peuvent avoir un temps de séjour très long dans l'atmosphère. Cette même affinité pour les particules solides est à l'origine de leur forte présence dans les sédiments côtiers et dans l'eau. Dans certains cas, pour diminuer la teneur en HAP de certains produits comme la fumée liquide, les industriels utilisent leurs propriétés d'adsorption. En effet, les HAP peuvent migrer dans les emballages des produits (Guillén, M.D. et al., 2000a ; Šimko, P., 2005) et diminuer la concentration en HAP du produit alimentaire.

Enfin, ils sont très instables dans l'air et peuvent réagir avec d'autres polluants comme l'ozone, le NO2 et le SO2 pour produire des diones, des nitro-HAP et des acides sulfoniques. Leur demi-vie est très variable : de quelques mois à plusieurs années. Ils sont chimiquement stables mais leur persistance serait amoindrie dans l'environnement du fait qu'ils sont photosensibles et dégradés voire détruits par les rayonnements ultraviolets (Šimko, P., 1991). De par leurs propriétés de persistance et de bioaccumulation, ces molécules peuvent se déplacer sur de très longues distances et se déposer loin des lieux d'émission, typiquement des milieux chauds (à forte activité humaine) vers les milieux froids (en particulier l'Arctique).

1.5.1. Toxicité des HAP

Les HAP sont des composés carcinogènes génotoxiques et à ce titre, agents mutagènes. Les premiers travaux portant sur la génotoxicité des HAP sont anciens puisqu'ils remontent aux observations de Sir Percivall Pott (1775) à l'hôpital St Bartholomew de Londres, relatives aux cancers du scrotum causés par les goudrons de la fumée, chez les ramoneurs de cheminées. Puis en 1915, Yamagiwa et Ichikawa (Yamagiwa, K. & Ichikawa, K., 1915) mirent en évidence le pouvoir carcinogène du goudron de charbon, qui, en administration topique était capable d'induire des carcinomes cutanés chez le lapin. En 1930, Kennaway (Kennaway,

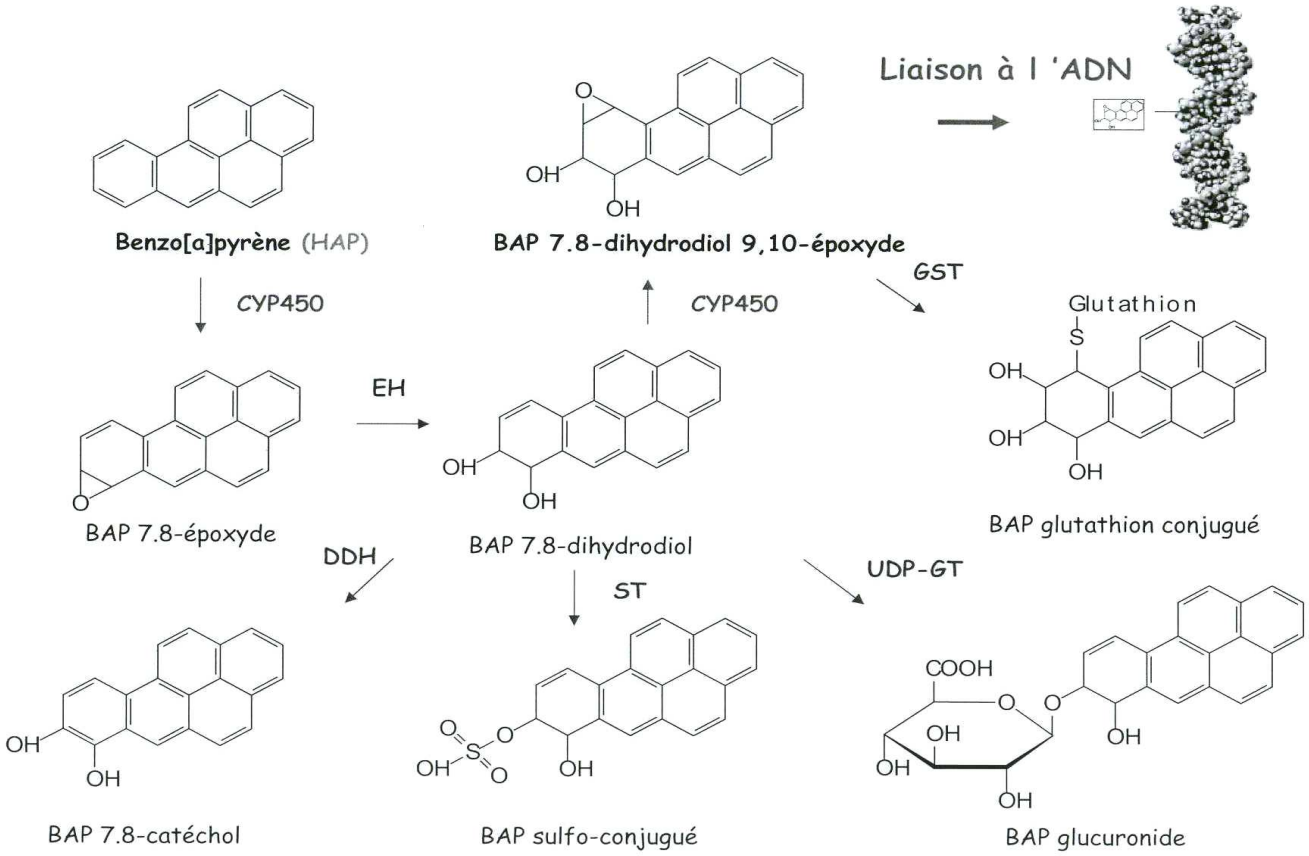

Figure 21. Métabolisation du benzo[a]pyrène : mécanismes d'excrétion et de formation d'epoxydes toxiques (EH : Epoxyde Hydrolase, DDH : Deshydrogenase, GST : Glutathione-S-Transferase, UDP-GT :Uridine DiPhosphate – Glucuronosyl Transferase

E.L., 1924 et 1930) isola pour la première fois le benzo[a]pyrène comme une espèce chimique carcinogène du goudron de charbon. Par la suite, de nombreux travaux ont été menés sur les HAP, notamment le benzo[a]pyrène, qui est encore à ce jour le HAP sur lequel nous disposons le plus de données toxicologiques.

Une caractéristique importante des HAP est que ces produits ne sont pas mutagènes en eux-même. Ils le deviennent chez l'organisme qui les a absorbés, par suite d'une oxydation enzymatique par une monooxygénase, le cytochrome P-450, présent dans les tissus animaux. Le rôle physiologique du cytochrome P-450 est la détoxification des produits xénobiotiques ingérés qui sont ainsi convertis en dérivés hydrosolubles et donc éliminables (Andreoli, R. et al., 1999 ; Simon, P. et al., 2000 ; Ferrari, S. et al., 2002 ; Giessing, A.M.B. et al., 2003).

Plusieurs études sur le métabolisme des HAP ont montré qu'ils sont métabolisés en une grande variété de composés (formes hydroxylées, époxydes, quinones…) par l'action des enzymes à cytochrome P 450 et de l'époxyde hydrolase et qu'ils étaient bio transformés au niveau du foie de façon importante en différents (poly)-hydroxy-HAP (Larsen, J.C. & Larsen, P.B., 1998). La majorité des HAP ingérés peuvent donc être détoxifiés avant d'être excrétés par la bile, les fécès et l'urine sous forme de métabolites conjugués avec l'acide glucuronique et le glutathion. Cependant, une partie des HAP ingérés est transformée en intermédiaires toxiques. Sous l'action du cytochrome P-450, le benzo[a]pyrène peut conduire à la formation d'un benzo[a]pyrène 7,8-dihydrodiol-9,10-époxyde, métabolite mutagène et cancérogène (Figure 21). Les diolépoxydes formés à partir des HAP sont, en tant qu'époxyde, des molécules réactives (Tamakawa, K. 2004). Ils se fixent sur l'ADN par des mécanismes complexes et liaisons covalentes et entraînent des modifications du matériel génétique (mutations) qui sont à la base du processus de cancérogénèse (Baird, W.M. et al., 2005).

Le fait que leur action ait pour cible le matériel génétique confère aux HAP des propriétés génotoxiques. L'expression erronée du matériel génétique consécutive aux mutations peut entraîner des néoplasmes (bénins ou malins) dont la prolifération peut mener à l'apparition de cancers. Les HAP sont donc considérés comme substances carcinogéniques d'où l'attention particulière portée à ces molécules retrouvées dans les produits fumés ou grillés (Kangsadalampai, K. et al., 1996).

1.5.2. Occurrence dans l'alimentation

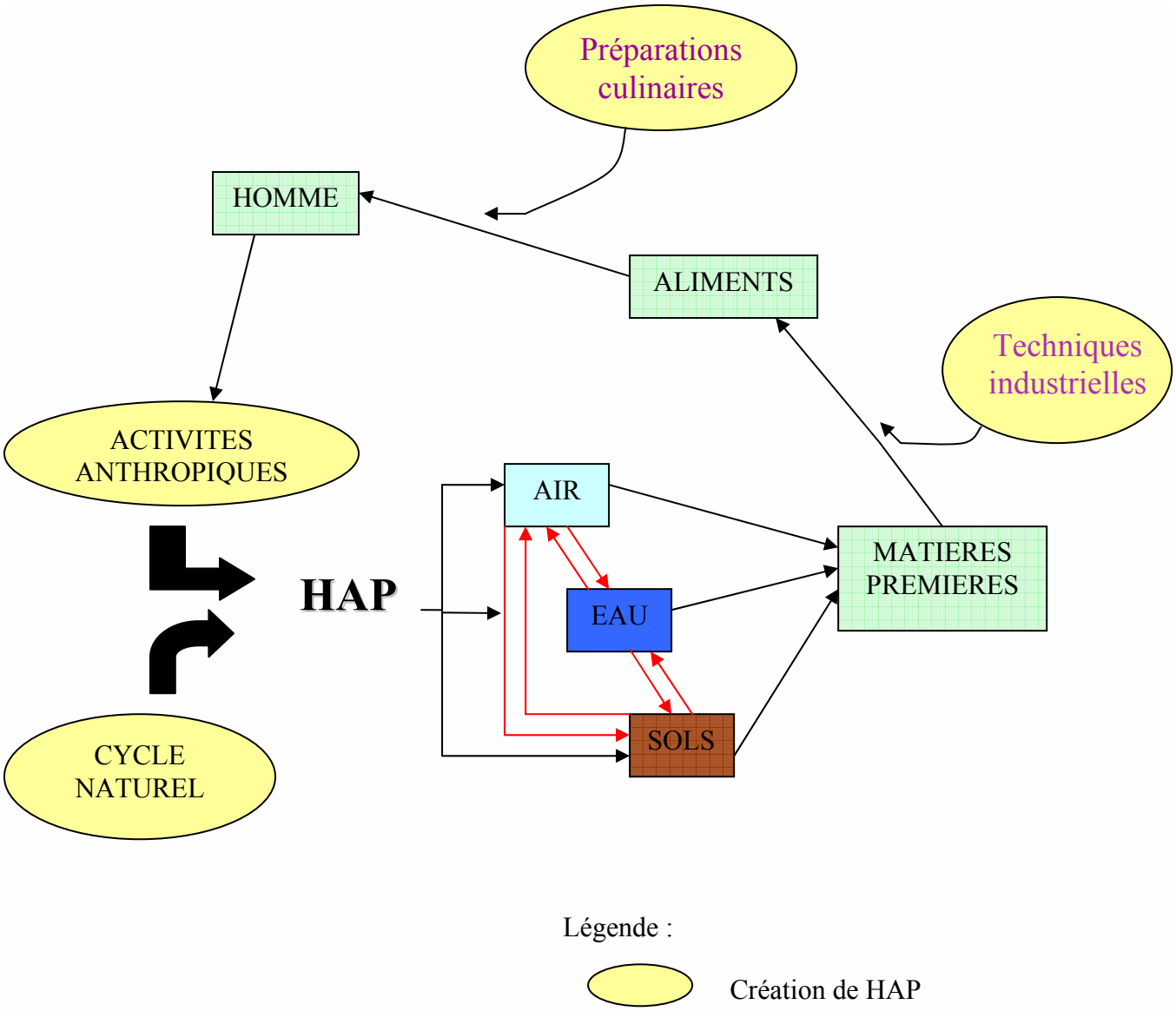

Figure 22. Cycle de contamination de l'alimentation humaine par les HAP

Chez l'homme, les principales voies d'exposition aux HAP sont l'inhalation de l'air à des degrés différents dépendants de l'urbanisation, du trafic et de l'industrialisation (Marvin, C.H. et al., 1999) et l'ingestion alimentaire de produits contaminés par dépôts atmosphériques (Rojo Camargo, M.C. & Toledo, M.C.F., 2003), provenant de sols contaminés (Berset, J.D. et al., 1999 ; Bakker, M.I. et al., 2000), par accumulation chez certaines espèces animale (Speer, K. et al., 1990) ou par des pratiques industrielles ou culinaires particulières (Philips, D.H., 1999). L'importance de l'absorption des HAP par l'organisme dépend très fortement de la granulométrie des particules aéroportant les HAP, de la solubilité des HAP et s'opère par inhalation après adsorption des HAP sur les particules aéroportées. Notons aussi l'absorption par voie cutanée dans certains métiers liés aux combustions de matériel organique. Pour un non fumeur, l'alimentation reste le principal vecteur d'exposition aux HAP. Pour un fumeur, le tabac constitue environ 90 à 95 % de son exposition aux HAP.

La présence de HAP dans les matières premières alimentaires provient essentiellement de la pollution environnementale des biotopes où elles se développent (atmosphère, hydrosphère et géosphère) et des process technologiques humains (aliments transformés par des process industriels ou des pratiques culinaires domestiques) (Figure 22).

1.5.2.1. La contamination environnementale en HAP et des matières premières alimentaires

Dans le cas de matières premières animales, il faut s'intéresser à l'environnement de l'animal, ainsi que son alimentation (Kann, C.A. & Meijer, G.A.L., 2007).

D'une part, l'animal peut être contaminé parce qu'il vit dans un milieu contaminé avec lequel il est en relation constante (Nyman, P.J. et al., 1993 ; Means, J.C., 1998 ; Santana Rodríguez, J.J. & Padrón Sanz, C., 2000 ; Marsili, L. et al., 2001). C'est par exemple le cas des poissons et des fruits de mer vivants dans des eaux polluées, du bétail vivant dans des zones où l'atmosphère est riche en HAP, ou de matières premières végétales extraites de sols pollués. Selon les particularités de chaque organisme, les HAP sont introduits dans l'animal par voie cutanée, par ingestion ou inhalation. Dans les organismes, les HAP, bien qu'hydrophobes, sont généralement peu accumulés parce qu'ils peuvent être métabolisés tout au moins par les vertébrés. Ainsi, la consommation de ces aliments sous forme crue ne comporterait que de faibles ingestions de HAP. La pollution de l'eau par les HAP reste très marginale dans l'alimentation humaine, essentiellement consécutive aux marées noires (AFSSA, 2003). Elle peut s'avérer toutefois un vecteur d'HAP important chez des organismes aquatiques à

HAP	Cancérogénicité	Génotoxicité à court terme	Mutagénicité
Fluorène	I	L	-
Phénanthrène	I	L	+
Anthracène	N	N	-
Fluoranthène	N	L	+
Pyrène	N	L	+
Benzo[a]anthracène	S	S	+
Chrysène	L	L	+
Benzo[b]fluoranthène	S	I	+
Benzo[k]fluoranthène	S	I	+
Benzo[a]pyrène	S	S	+
Indéno[1,2,3-c,d]pyrène	S	I	+
Dibenzo[a,h]anthracène	S	S	+
Benzo[g,h,i]pérylène	I	I	+

Tableau 19. Pouvoirs cancérogène et mutagène de 13 HAP (d'après IARC (1986))

Preuves de cancérogénicité suffisante (S) ou limitées (L) ; données manquantes ou insuffisantes (I) ; non cancérogène (N).

métabolisme filtrant (mollusques) (Andral, B. et al., 2004) et prendre alors une place prépondérante dans la contamination de l'alimentation humaine.

Dans le cas de poissons d'élevage, certains travaux ont été menés dans le but d'étudier l'influence de la contamination de la nourriture sur la teneur en HAP dans le produit final (Easton, M.D.L. et al., 2002). Les taux de HAP de faible poids moléculaires sont beaucoup plus importants que les HAP de haut poids moléculaire plus toxiques. En 1984, l'US EPA (United State Environmental Protection Agency) avait identifié une liste de 16 HAP pour estimer le niveau de contamination des eaux polluées. Ces contaminants, très présents dans l'environnement, sont supposés être les HAP les plus toxiques et présenter un risque pour la santé (INERIS, 2003). Cette liste des HAP les plus couramment rencontrés a servi de base pour diverses études et dosages dans les matrices alimentaires. Le tableau 19 reprend 13 de ces 16 HAP (manquent le naphthalène, l'acénaphtène et l'acénaphtylène). Ainsi, les HAP de deux ou trois cycles benzèniques apparaissent avec une toxicité faible mais leurs fortes concentrations dans l'environnement rend leur surveillance indispensable.

1.5.2.2. La contamination des aliments en HAP par les procédés industriels

Les produits destinés à l'alimentation humaine sont donc déjà contaminés à la base par l'utilisation de matières premières animales ou végétales déjà porteuses d'une contamination en HAP. Cependant, certains procédés industriels et certaines pratiques culinaires induisent également la formation d'HAP dans les aliments au cours de leur élaboration (Figure 19).

Parmi celles-ci nous pouvons citer le fumage de produits de la mer (Kannapan, S. et al., 2000), de produits carnés (Sikorski, Z.E., 1989 ; Janoszka, B. et al., 2004), de fromages (Pagliuca, G. et al., 2003), de marc (Da Porto, C. et al., 2005), la torréfaction (Koffi Houessou, J. et al., 2005), le raffinage des huiles (Pupin, A.M. & Toledo, M.C.F., 1996 ; Moret, S. & Conte, L.S., 2000 ; Barranco, A. et al., 2003 ; Lacoste, F. et al., 2003), ...

Parmi les pratiques culinaires, les barbecues (Wu, J. et al., 1997 ; Mottier, P. et al., 2000), fritures et grillages (Zabik, M.E. et al., 1996) sont à l'origine de contamination aléatoire en HAP pouvant parfois être très importante.

Les HAP sont donc des contaminants ubiquitaires puisqu'ils sont à la fois présents dans l'environnement et dans les aliments. Dans le cas du poisson fumé, l'environnement et la nourriture semblent être à l'origine de l'importance de la concentration des HAP de faible

HAP
Benzo[a]anthracène
Benzo[b]fluoranthène
Benzo[j]fluoranthène
Benzo[k]fluoranthène
Benzo[g,h,i]pérylène
Benzo[a]pyrène
Chrysène
Cyclopenta[c,d]pyrène
Dibenzo[a,h]anthracène
Dibenzo[a,e]pyrène
Dibenzo[a,h]pyrène
Dibenzo[a,i]pyrène
Dibenzo[a,l]pyrène
Indéno[1,2,3-c,d]pyrène
5-methylchrysène

Tableau 20. HAP suspectés d'être carcinogénes dans l'alimentation

poids moléculaire. Cependant, le fumage, selon la technique utilisée, constitue un vecteur important de contamination en HAP considérés en tant que composés néoformés.
Ainsi, de nouvelles dispositions européennes ont vu le jour pour réglementer les taux de HAP dans les produits alimentaires. Dans les aliments fumés, ces teneurs maximales autorisées ne concernent que le benzo[a]pyrène et obligent les industriels à ne pas dépasser 5 µg/kg de poids frais dans le cas de poissons fumés (EC/208/2005, 2005). Concernant les produits alimentaires, l'Union Européenne a également recommandé de se focaliser sur d'autres HAP potentiellement plus toxiques que ceux de l'US EPA en exigeant le suivi de 15 HAP de haut poids moléculaire compilés dans le tableau 20. (205/108/EC, 2005). Une attention toute particulière doit également être portée sur le benzo[c]fluorène (1881/2006/EC, 2006). La récence de ces réglementations justifie la nécessité de travaux sur la contamination en HAP des produits fumés.

1.5.2.2.1. Quantités de HAP dans les poissons fumés

Les concentrations en HAP dans les poissons fumés sont très aléatoires et dépendent du type de poisson ainsi que de sa contamination initiale, de la méthode de fumage et de la méthode d'analyse. Parmi les données disponibles, il n'y a pas d'étude exhaustive rendant compte de la contamination en HAP de saumons ayant été fumés de manière industrielle.
Les données disponibles concernant le saumon fumé sont répertoriées dans le tableau 21. Ainsi, on s'aperçoit que seul le suivi de quelques HAP a été réalisé, principalement le benzo[a]pyrène. Les informations concernant les méthodes de fumage sont très succinctes et partielles. Les concentrations de HAP de haut poids moléculaire sont moins élevées que celles de plus faible poids, ce qui se justifie souvent par une contamination environnementale importante. Les recherches menées sur l'occurrence des HAP dans le saumon fumé n'incluent que très rarement un dosage des HAP sur la matrice non fumée (Zabik, M.E. et al., 1996 ; Easton, M.D.L. et al., 2002). Or, celle-ci s'avère d'une importance capitale car elle peut se révéler très importante et expliquer une grande part des concentrations que l'on aurait tendance à imputer à tort à l'utilisation de tel ou tel générateur (Visciano, P. et al., 2006). D'autres études ont été menées sur le fumage d'autres poissons. Ces données ne sont pas directement transposables au saumon du fait de variations dans la composition biochimique de la matrice mais renseignent sur le niveau de contamination du procédé de fumage.

HAP	Type de fumage	Type de générateur	Concentration (µg/kg de poids frais	Référence
AC	24°C	Autocombustion	10.54 (12.39)[a]	Visciano, P. et al., 2006
FL	24°C	Autocombustion	50.29 (18.38)[a]	Visciano, P. et al., 2006
PHEN	24°C	Autocombustion	28.03 (26.44)[a]	Visciano, P. et al., 2006
	A froid	Externe	7.2	Karl, H., 1997
	A chaud	Exposition directe	46	
AN	24°C	Autocombustion	5.67 (2.81)[a]	Visciano, P. et al., 2006
	A froid	Externe	0.4	Karl, H., 1997
	A chaud	Exposition directe	19	
FA	24°C	Autocombustion	42.64 (57.45)[a]	Visciano, P. et al., 2006
	A froid	Externe	2.3	Karl, H., 1997
	A chaud	Exposition directe	41	
PY	A froid	Externe	0.6	Karl, H., 1997
	A chaud	Exposition directe	22	
B[a]A	A froid		0.94	Yurchenko, S. & Mölder, U., 2005
	A chaud		1.12	
	A froid	Externe	< 0.1	Karl, H., 1997
	A chaud	Exposition directe	3.1	
	24°C	Autocombustion	72.48 (97.28)[a]	Visciano, P. et al., 2006
CHR	24°C	Autocombustion	6.45 (8.74)[a]	Visciano, P. et al., 2006
	A froid	Externe	0.5	Karl, H., 1997
	A chaud	Exposition directe	2.9	
B[a]P	A froid		0.40	Yurchenko, S. & Mölder, U., 2005
	A chaud		0.81	
		Autocombustion	0.77 à 4.7	Sikorski, Z.E., 1989
		Exposition directe	3.6	
			0.47	Lenges, J. et al., 1976
	24°C	Autocombustion	3.20 (3.67)[a]	Visciano, P. et al., 2006
	A chaud	Exposition directe	1.0	Karl, H., 1997
	A froid	Externe	0.04	
B[b+k]F			0.30	Yurchenko, S. & Mölder, U., 2005
	A froid	Externe	0.14	Karl, H., 1997
	A chaud	Exposition directe	1.3	
	24°C	Autocombustion	2.88 (3.67)[a]	Visciano, P. et al., 2006
D[a,h]A	A chaud	Exposition directe	0.1	Karl, H., 1997
B[g,h,i]P	24°C	Autocombustion	4.11 (0.93)[a]	Visciano, P. et al., 2006
	A chaud	Exposition directe	0.5	Karl, H., 1997
PER	A chaud	Exposition directe	0.1	Karl, H., 1997

[a] : la contamination initiale du poisson figure entre parenthèses pour chaque HAP
AC : Acénapthène, FL : Fluorène, PHEN : Phénanthrène, AN : Anthracène, FA : Fluoranthène, PY : Pyrène, B[a]A : Benzo[a]anthracène, CHR : Chrysène, B[a]P : Benzo[a]pyrène, B[b+k]F : Benzo[b+k]fluoranthène, D[a,h]A : Dibenzo[a,h]anthracène, B[g,h,i]P : Benzo[g,h,i]perylène

Tableau 21. Teneurs en différents HAP retrouvés dans le saumon fumé avec différents générateurs de fumée et différentes températures de fumage

Il n'y a en définitive que peu d'études menées sur les HAP mis en exergue par les dernières recommandations européennes (205/108/EC, 2005 ; 1881/2006/EC, 2006). Quelques études d'exposition sont disponibles permettant d'appréhender l'importance du dosage de telles molécules étant donné le régime alimentaire de certaines populations (Europe du Nord) sans pour autant donner d'informations sur la technique de fumage ou la nature du poisson analysé (Reports on Tasks for Scientific Cooperation, 2004 ; Tamakawa, K. 2004). Le benzo[a]pyrène, de par son statut de HAP de référence, a largement été mis en évidence dans les aliments (Šimko, P., 1991 ; Kazerouni, N. et al., 2001). Dans le cas du suivi de quelques HAP uniquement, les informations concernant la méthode de fumage sont plus nombreuses (Chandrasekhar, T .C. & Kaveriappa, K.M., 1976 ; Müller, W.D., 1991). Néanmoins, lorsqu'un dosage plus exhaustif de HAP est réalisé, l'accent est beaucoup moins porté sur les procédés de fumage (Järvenpää et al., 1996 ; Zabik et al.,1996). Parfois, les quantifications sont données sous la forme de somme de concentrations de plusieurs HAP (Duedahl-Olesen, L. et al., 2006).

Concernant les générateurs de fumée, il n'y a pas d'études comparatives exhaustives. Certaines comparent les générateurs externes avec les générateurs à exposition directe mais n'ont été réalisées qu'en suivant quelques HAP et ne concernant pas le saumon (Müller, W.D., 1991), d'autres mettent en évidence la contamination apportée par les fumées liquides, … (Moret, S. et al., 1999 ; Hattula, T. et al., 2001). Ainsi, les informations disponibles sont diffuses et les recoupements entre les travaux assez hasardeux du fait de leur manque d'homogénéité.

1.5.2.2.2. Différents générateurs de fumée

Si l'on connaît encore mal la formation des HAP dans la fumée de bois lors de sa pyrolyse durant le fumage, des procédés ont été néanmoins développés pour éviter leur présence dans le produit fumé final. Ainsi, les différents générateurs de fumée présentés précédemment ont été conçus dans l'optique de réduire la charge en HAP dans la fumée générée. En effet, en externalisant les générateurs, les goudrons et HAP peu volatils doivent parcourir une plus grande distance avant de parvenir à l'aliment par rapport à une exposition directe du produit à la fumée (Poligné, I. et al., 2001). En plaçant des filtres ou des étapes de purification de la fumée, on obtient une fumée plus « propre » arrivant dans la cellule de fumage. L'atomisation de fumée liquide apparaît comme le stade ultime de « fumée propre ». La concentration en

HAP de la fumée liquide est soumise à réglementation afin de pouvoir être utilisée. Il est donc nécessaire pour les industriels de s'assurer de la conformité de leurs fumées liquides et donc d'en assurer la plus grande innocuité vis-à-vis des HAP. Depuis 2003, les fabricants de fumée liquide doivent respecter des taux de benzo[a]anthracène et de benzo[a]pyrène inférieurs à respectivement 20 et 10 µg/kg de fumée liquide (EC 2065/2003, 2003).

Quelques études menées essentiellement sur des produits de la mer sont disponibles en ce qui concerne les générateurs à exposition directe, parfois appelé traditionnels par opposition aux générateurs modernes externes. Celles-ci ont montré que le fumage par exposition directe conduisait à de très fortes et aléatoires teneurs en HAP (Chandrasekhar, T.C. & Kaveriappa, K.M., 1985). Les utilisations du générateur à autocombustion ou de la fumée liquide ont réduit fortement ces concentrations (Karl, H. & Leinemann, M., 1996 ; Karl, 1997 ; Hattula, T. et al., 2001). De manière générale, l'utilisation de générateurs de fumée externes conduit à des produits largement moins contaminés en HAP, tout du moins en benzo[a]pyrène (Müller, W.D., 1991). En effet, les seuls travaux concernant les générateurs de fumée modernes sont uniquement axés sur le benzo[a]pyrène notamment parce qu'il a été et est toujours la substance HAP de référence. Cependant, d'autres études ont montré récemment que d'autres HAP comme les dibenzopyrènes pourraient s'avérer autant voire davantage toxiques que le benzo[a]pyrène (INERIS, 2003). Ainsi, l'absence de données sanitaires plus exhaustives sur la contamination en HAP de produits fumés par les différents générateurs industriels justifie nos travaux.

1.5.3. Formation dans la fumée

La formation des HAP dans la fumée de bois reste encore mal connue. En effet, si la formation de HAP lors de processus pyrolytiques à hautes températures avec formation de flammes a déjà été l'objet d'études, la formation de HAP lors du fumage paraît plus complexe. En effet, il n'y a pas formation de flammes dans le générateur de fumée. Cependant, les mécanismes à l'origine de la création de HAP dans la fumée de bois semblent être similaires. La formation de HAP comporte la génération d'un premier cycle aromatique. Celui-ci peut être obtenu par la dégradation des constituants cycliques du bois ou par assemblage/addition de radicaux aliphatiques.

Les HAP semblent être préférentiellement formés à partir des constituants de la lignine. Durant la pyrolyse, les fissions dues à des thermodégradation affectent les hétérocycles

Arrachement d'un atome d'hydrogène : $C_XH_Y\text{-}H + H \rightarrow C_XH_Y^{\circ} + H_2$

Addition d'une molécule d'acétylène : $C_XH_Y^{\circ} + R\text{-}C_2H_2 \rightarrow R\text{-}C_{X+2}H_{Y+2}^{\circ}$

Figure 23. Réactions du mécanisme HACA à l'origine de la formation d'HAP

furanes et pyranes de la lignine alors que les noyaux aromatiques relativement stables sont uniquement amputés de quelques groupements mais gardent leur intégrité cyclique (Sainclivier, 1985). Ces noyaux aromatiques peuvent alors perdre des atomes d'hydrogène sous l'effet des conditions extrêmes de la pyrolyse et se polymériser.

D'autres mécanismes semblent également être à l'origine de la formation des HAP et notamment de la formation du premier noyau aromatique. Ils font intervenir des réactions d'addition/d'assemblage entre des radicaux alkyls et des dérivés de l'acétylène, produits très facilement à partir de la pyrolyse des constituants du bois. Ces réactions sont regroupées sous l'acronyme HACA (Hydrogen abstraction / ACetylene Addition) (Figure 20) et conduisent à la formation de noyau aromatique (Richter, H. & Howard, J.B., 2000). Dans ce type de mécanisme, il y a deux processus importants, l'arrachement d'un atome d'hydrogène d'une part et l'addition d'une molécule de type acétylénique d'autre part. Ces réactions sont très rapides car les espèces chimiques formées sont très instables et doivent se cycliser pour se stabiliser. La formation de HAP de plus haut poids moléculaire peut également s'envisager à partir de radicaux du naphtalène (Lafleur, A.L. et al., 1996). En effet, le mécanisme HACA permet de rendre compte de la formation du premier noyau aromatique. Il peut ensuite être facilement étendu pour décrire la croissance des HAP. Néanmoins, d'autres mécanismes ont également été avancés pour former un noyau benzénique : le radical cyclopentadienyl et le radical propargyl, radicaux suffisamment stables pour être impliqués dans la formation des HAP. Le radical cyclopentadienyl peut conduire à des radicaux à six atomes de carbone de type fulvène qui peuvent ensuite s'isomériser en benzène. Le naphtalène pourrait alors être obtenu par dimérisation de deux radicaux cyclopentadienyls.

Ainsi, les mécanismes à l'origine des HAP dans la fumée de bois restent encore hypothétiques et sont régis par des paramètres thermodynamiques encore mal définis.

1.5.4. Méthodologies utilisées dans l'extraction et l'analyse des HAP

Compte tenu de l'état physique et de la composition biochimique des matrices, une gamme très variée de techniques d'extractions sont disponibles.

1.5.4.1. Méthodes d'extraction des HAP de la fumée à l'état gazeux

Il faut distinguer l'extraction de HAP de fumée à l'état gazeux de celle à l'état liquide.

Pour extraire les HAP d'une fumée à l'état gazeux, deux principes d'extractions sont employés : l'adsorption des HAP sur des pièges solides suivie de leur extraction de ces dits pièges et le barbotage des fumées directement dans un solvant pour lequel les HAP auront une forte affinité (García-Falcón, M.S. & Simal-Gándara, J., 2005).

L'adsorption de la fumée sur des adsorbants solides est très employée dans l'analyse de fumées (Dos Santos Barbosa, J.M. et al., 2006 ; Sadhra, S. & Wheatley, A.D., 2007). Les techniques divergent selon le mode d'extraction des HAP de ces pièges. En effet, les HAP peuvent en être extraits par extraction liquide-solide (Li, S. et al., 2003), par EDS (Forehand, J.B. et al., 2000) comme dans le cas de fumées de cigarette, par ultrasons (Marvin, C.H. et al., 1999) comme dans le cas d'atmosphères polluées ou encore par Soxhlet (Chen, Y.C. & Chen, B.H., 2003) comme dans le cas de fumées de friture.

1.5.4.2. Méthodes d'extraction des HAP de matrices liquides ou solides

Dans le cas de l'extraction des HAP de matrices liquides ou solides, notamment alimentaires, la composition biochimique de la matrice est un point critique dans le choix de la méthode.

En effet, les HAP, fortement lipophiles sont souvent coextraits avec des composés générant des interférences (Guillén, M.D. et al., 2000a) comme les matières grasses entraînant des problèmes ultérieurs d'identification et de quantification. Le saumon fumé constitue une matrice complexe riche en matières grasses ce qui implique des techniques d'extractions plus douces par opposition aux matrices environnementales (type boues, sols, …) afin d'éviter une dénaturation trop poussée de la matrice et la formation d'artefacts.

L'efficacité de l'extraction des HAP dépend principalement de la polarité du solvant utilisé, de l'état physique de la matrice et de la préparation de l'échantillon. Ainsi, les premières extractions de HAP ont été des extractions liquide-liquide comme dans le cas de la fumée liquide (Guillén, M.D. et al., 2000b et 2000c) ou solide-liquide comme dans le cas de la viande grillée (Rivera, L. et al., 1996). Les solvants utilisés sont pour la plupart des solvants apolaires ou des mélanges avec des solvants plus polaires dans le but d'extraire le maximum de HAP tout en réduisant le taux de matières grasses coextraites. La récupération des HAP est d'autant plus élevée que l'échantillon est soluble dans le solvant organique comme dans le cas de produits laitiers ou de la fumée liquide (Kishikawa, N. et al., 2003). D'autres équipes ont cherché à privilégier une extraction sélective des HAP en augmentant l'affinité des HAP pour un agent d'extraction. La caféine ou les ions argent Ag^+ ont ainsi été utilisés pour complexer

les HAP (Moret, S. & Conte, L.S., 2000 ; Lee, J.H. et al., 2004). Dans le cas de la viande et du poisson, matrices alimentaires solides, les HAP se fixent préférentiellement dans les tissus lipidiques (Stołhywo, A. & Sikorski, Z.E., 2005). Pour les extraire, il faut augmenter leur affinité pour le solvant d'extraction en détruisant la matière grasse. Une étape de saponification est très souvent appliquée (Šimko, P., 2002). Celle-ci n'est pas obligatoire en fonction de la composition biochimique de la matrice d'extraction. En revanche, une étape de purification est quasiment obligatoire. Si au début des purifications sur colonnes d'alumine ou de silice activée étaient les plus utilisées (Stołhywo, A. & Sikorski, Z.E., 2005), la purification sur colonnes d'Extraction en Phase Solide (SPE) s'avère plus aisée et largement répandue aujourd'hui pour ce type d'extraction (Kiss, G. et al., 1996 ; Zamperlini, G.C.M. et al., 2000). Le choix de la nature des cartouches SPE dépend fortement de la méthode d'extraction et de la composition de l'extrait à purifier. La plupart du temps des cartouches apolaires de silice C_{18} sont utilisées mais l'utilisation de phases possédant des similarités de structure avec les HAP donne également de bons résultats. En effet, des cartouches composés de copolymères de styrène - divinylbenzène permet des interactions Π entre les cycles de la phase et les HAP qui augmentent ainsi leur rétention sur la phase par rapport aux matières grasses moins retenues et éliminées lors du nettoyage de la cartouche (Marcé, R.M. & Borrull, F., 2000).

Dans un souci de réduction des composés coextraits, d'autres méthodes ont été optimisées. Citons ainsi l'extraction par ultrasons dans le cas de végétaux (Dugay, A. et al., 2002), l'Extraction Accélérée par Solvants (ASE) dans le cas de viandes fumées qui s'avère moins consommatrice que l'extraction solide-liquide par Soxhlet en terme de volume de solvants pour des résultats tout aussi efficaces (Wang, G. et al., 1999) ou encore l'Extraction par Liquide sous Pression (PLE), une variante de l'ASE (Veyrand, B. et al., 2007). L'Extraction par Fluide Supercritique (SFE) est très utilisée dans le cas d'extractions de HAP de matrices environnementales très contaminées et peu grasses et s'avère plus efficace que l'extraction par solvants liquides. Grâce à ses conditions supercritiques facilement atteintes (Température critique : 31,1°C, Pression critique: 7,38 MPa), le dioxyde de carbone est couramment employé (Järvenpää, E. et al., 1996). Cependant, l'optimisation pour réduire la génération d'artefacts dûe aux lipides coextraits dans le cas de matrices alimentaires reste fastidieuse, mais une fois maîtrisée, permet de s'affranchir de l'étape de purification et de réduire les volumes et les temps d'extraction (Lage Yusty, M.A. & Cortizo Daviña, J.L. et al., 2005).

Dans le cas d'eau polluée, les HAP peuvent également être extraits par adsorption sur des pièges placés au sein du liquide (fibre SPME ou barreau de Stir Bar Sorptive Extraction

(SBSE)) avant d'être désorbés (Popp, P. et al., 2000 ; King, A.J. et al., 2004). Cependant, ces techniques ne sont applicables que dans le cas de matrices liquides peu complexes.

1.5.4.3. Méthodes d'analyse des HAP

Les méthodes de séparation résident dans la chromatographie en phase gazeuse (GC) et la chromatographie liquide (LC).
De nombreux auteurs ont comparé l'efficacité de la séparation GC et LC pour l'analyse des HAP mais ces deux méthodes sont complémentaires et offrent toutes deux des avantages et des inconvénients (Šimko, P., 2002).
La GC possède un pouvoir résolutif plus important que la LC. L'efficacité des colonnes capillaires GC est également plus grande ce qui est un grand intérêt dans le cas de mélanges complexes (Sim, P.G. et al., 1987).
La LC permet des temps d'analyse beaucoup plus courts. La sélectivité est également plus importante qu'en GC puisque l'on peut séparer des isomères ce qui est beaucoup plus difficile en GC (Chiu, C.P. et al., 1997). Ainsi, le choix de la méthode de séparation est guidé par la nature de la matrice d'extraction et les objectifs de l'analyse.

En fonction de la méthode de séparation, différents types de détecteurs sont utilisés permettant de réaliser l'identification et la quantification.
Couplés à la GC, les principaux modes de détection sont la détection à ionisation de flamme (FID) et la spectrométrie de masse (analyseurs trappe d'ion (ITD), quadripôle ou Time-Of-Flight (TOF)) (Šimko, P., 2002). La GC couplée à la spectrométrie de masse a été largement employée pour étudier l'occurrence des HAP dans des matrices telles que l'eau (Davis, S.C. et al., 1999), le poisson (Birkholz, D.A. et al., 1998 ; Pointet, K. & Milliet, A., 2000 ; Vives, I. & Grimalt, J.O., 2002) ou la viande fumée (Yeakub Ali, M. & Cole, R.B., 2001 ; Jira, W., 2004b).
Couplés à la LC, les principaux modes de détection sont la détection Ultraviolets (UV), la détection fluorimétrique (FD) et la spectrométrie de masse (essentiellement des analyseurs quadripolaires). Ainsi, la présence de HAP a déjà été mise en évidence par LC-UV dans des matrices diverses telles que le poisson fumé (Järvenpää, E. et al., 1996), la viande (Chen, B.H. et al., 1996) ou le café (Koffi Houessou, J. et al., 2005). Les longueurs d'onde utilisées sont souvent comprises entre 200 et 400 nm. La fluorimétrie a également été utilisée pour la

détection des HAP dans des matrices marines (Santana Rodríguez, J.J. & Padrón Sanz, C., 2000), le poisson fumé (Storelli, M.M. et al., 2003), la fumée liquide (Simon, R. et al., 2006) ou le chorizo fumé (García-Falcón, M.S. & Simal-Gándara, J., 2005). Les longueurs d'onde d'excitation sont généralement comprises entre 200 et 400 nm et celles d'émission entre 300 et 550 nm (Šimko, P., 2002). Enfin, la spectrométrie de masse a essentiellement été utilisée pour la détection de HAP dans des matrices environnementales (Marvin, C.H. et al., 1999 ; Takino, M. et al., 2001) ou biologiques (urines) (Ferrari, S. et al., 2002).

Certains auteurs ont également développé d'autres modes de détection couplés à la LC comme la détection ampérométrique, notamment pour la détection de HAP dans l'eau potable (Nirmaier, H.P. et al., 1996).

Afin d'obtenir une meilleure séparation, des techniques couplant deux modes de séparation ont été développées comme la chromatographie bidimensionnelle en phase liquide LC/LC couplée à un détecteur UV ou fluorimétrique. La LC/LC-UV a été employée pour la détection des HAP dans le poisson fumé (Moret, S. et al., 1999) et la LC/LC-FD pour l'analyse des HAP dans l'huile alimentaire (Van Stijn, F. et al., 1996). Le passage sur une première colonne permet de sélectionner les zones de coélution afin d'améliorer la séparation sur la deuxième colonne et de s'affranchir des interférences chromatographiques causées par les composés coextraits. De même, des couplages innovants SFE-LC/GC-MS ont également été testés pour l'analyse de HAP dans l'atmosphère (Shimmo, M. et al., 2002)

Ce même objectif est également poursuivi dans le cas d'études mettant en œuvre un couplage impliquant une technique de séparation et deux techniques de détection comme pour la LC-MS/MS ou la GC-MS/MS où deux spectromètres de masse sont placés en série après le système de séparation (Veyrand, B. et al., 2007). La détection des HAP par GC-MS/MS a déjà été mise au point sur du pétrole brut (Munoz, D. et al., 1997) ou des huiles alimentaires (Ballesteros, E. et al., 2006). La deuxième ionisation du second spectromètre de masse permet de fragmenter les molécules interférentes coextraites sans affecter les HAP qui de par leurs structures chimiques sont très stables. La LC-MS/MS a également été utilisée pour l'analyse de métabolites d'HAP (Lintelmann, J. et al., 2006) mais pas à notre connaissance pour l'analyse de HAP.

L'analyse des HAP peut également être menée par des tests immunoenzymatiques. La quantité de HAP est suivie par l'activité enzymatique d'anticorps polyclonaux spécifiques de certains HAP. La technique est très sensible, surtout pour le benzo[a]pyrène mais ne fonctionne pas pour tous les HAP (Roda, A. et al., 1999).

Enfin, la résonance magnétique nucléaire (RMN) est aussi un moyen d'identifier les HAP dans une matrice (Kacker, T. et al., 2002). Cette technique peut être couplée à la LC afin d'effectuer une première séparation et est comparable aux couplages LC-UV, LC-FD ou LC-MS (Weisshoff, H.et al., 2002).

1.6. Conclusion

L'odeur « fumé » du poisson fumé est inhérente au procédé de fumage. La variété des générateurs de fumée et la diversité des paramètres de fumage (bois, paramètres physiques et chimiques, …) multiplient les nuances odorantes du poisson fumé. Certains composés volatils du poisson fumé, imputables à la fumée ou créés lors du fumage entre les composés de la fumée et ceux du poisson sont bien connus. Les composés phénoliques ont largement fait l'objet de travaux d'identification. Cependant il n'y a eu que très peu de quantifications. Quant à la caractérisation du rôle de ces composés volatils dans la perception de l'odeur de produits fumés, elle demeure un domaine quasi inexploré. Ainsi, par une meilleure connaissance des paramètres à l'origine de la création de certains composés odorants, nous pourrons donner des recommandations pour produire la production de poisson fumé avec des caractéristiques odorantes requises tout en favorisant les paramètres minimisant les taux de HAP.

A l'issue de cette étude bibliographique, quatre axes de recherche sont apparus comme essentiels pour appréhender les propriétés organoleptiques et sanitaires du saumon fumé :

- ✓ La mise au point d'une méthode d'extraction quantitative et représentative du saumon fumé et la validation de son efficacité ainsi que de sa capacité à être utilisée en olfactométrie devant être validée.
- ✓ L'influence des paramètres de fumage (générateur de fumée, temps d'exposition, température de fumage) sur les qualités organoleptiques du saumon fumé.
- ✓ L'influence des paramètres de fumage (générateur de fumée, temps d'exposition, température de fumage) sur la contamination en HAP du saumon fumé.
- ✓ L'influence de certains composés odorants dans l'odeur globale du saumon fumé.

Organisation des publications du mémoire de thèse

Travail bibliographique préparatoire

Inventaire des aldéhydes volatils présents dans l'arôme des poissons fumés : méthodes d'extraction et d'analyse, mécanismes de formation, propriétés organoleptiques et toxicité.
VARLET, V., PROST, C., SEROT, T. (2007). Volatile aldehydes in smoked fishes : analysis methods, occurrence and mechanisms of formation. Food Chem., 105, 1536-1556.

Résultats

Partie 1 : Méthodologies d'extraction et validation

1. Mise au point d'une méthode d'extraction quantitative et représentative des arômes du saumon fumé
VARLET, V., PROST, C., SEROT, T. (2007). New procedure for the study of odour representativeness of aromatic extracts from smoked salmon. Food Chem., 100, 820-829.

2. Validation de l'efficacité cette méthode dans l'analyse des composés volatils odorants du saumon frais et du saumon fumé
VARLET, V., KNOCKAERT, C., PROST, C., SEROT, T. (2006). Comparison of odor-active volatile compounds of fresh and smoked salmon. J. Agric. Food Chem., 54, 3391-3401.

Partie 2 : Caractérisation organoleptique et sanitaire du fumage

3. Approche technologique, sensorielle et sanitaire des 4 principales méthodes industrielles de génération de fumée utilisées dans le fumage à froid : Publication introductive / Présentation des méthodes
VARLET, V., SEROT, T., KNOKAERT, C., CORNET, J., CARDINAL, M., MONTEAU, F., LE BIZEC, B., PROST, C. (2007). Organoleptic characterization and PAH content of salmon (Salmo salar) smoked according to four industrial smoking techniques. J. Sci. Food Agric., 87(5), 847-854.

4. Etude sanitaire du saumon fumé par différentes méthodes présentées dans la publication 3 : suivi de la contamination en HAP en fonction de la nature du générateur et des paramètres du fumage.
VARLET, V., SEROT, T., MONTEAU, F., LE BIZEC, B., PROST, C. (2007). Determination of PAH profiles by GC-MS/MS in salmon muscle processed according to four different smoking techniques. Food Addit. Contamin., 24(7), 744-757.

Partie 3 : Caractérisation aromatique du fumage

5. Etude olfactométrique des principaux composés volatils odorants présents dans du saumon fumé par les différentes méthodes présentées dans la publication 3.
VARLET, V., PROST, C., CARDINAl, M., KNOCKAERT, C., SEROT, T. (2007). Olfactometric determination of the most potent odor-active compounds in salmon muscle (Salmo salar) smoked by using four smoke generation techniques. J. Agric. Food Chem., 55, 4518-4525.

6. Corrélation entre les analyses sensorielles et les analyses olfactométriques du saumon fumé par les différentes méthodes présentées dans la publication 3.
VARLET, V., SEROT, T., CARDINAL, M., COURCOUX, P., KNOCKAERT, C., PROST, C. (2007). Relationships between sensory characteristics and the most odorant volatile compounds of salmon smoked by four industrial smoking techniques. Food Res. Int., soumise.

Partie 4 : Rôle des composés volatils odorants dans l'odeur globale

7. Elaboration d'une nouvelle stratégie olfactométrique : étude de l'influence de certains composés volatils odorants dans l'odeur globale de fumée.
VARLET, V., SEROT, T.,CATANEO, C., PROST, C. (2007). Innovative gas chromatography-olfactometric method : gas chromatography-Concentration/Omission of Odorants at Liquid state (GC-COOL). Contribution of odorant volatile compounds of liquid smoke in the overall smoke odour. En rédaction

Figure 24. Schéma de lecture des publications dans l'architecture du manuscrit de thèse

Les résultats de ces recherches ont fait l'objet de publications et seront développées dans les parties suivantes de ce mémoire, organisé selon le schéma ci-contre (Fig. 22)

RESULTATS

PARTIE 1 : Mise au point d'une méthode d'extraction quantitative et représentative pour l'analyse et la caractérisation des composés volatils odorants du saumon frais et du saumon fumé

PARTIE 1 : Mise au point d'une méthode d'analyse quantitative et représentative pour l'analyse et la caractérisation des composés volatils odorants du saumon frais et du saumon fumé

L'étude bibliographique nous a permis de constater les lacunes scientifiques dans la connaissance des composés volatils odorants du saumon fumé. En effet, très peu de travaux ont été menés sur les arômes de tels aliments alors que leur identification et leur dosage constitueraient un atout pour la diversification des poissons fumés et l'amélioration de leur qualité.

L'identification de plusieurs points critiques dans l'élaboration d'une méthode d'analyse nous a permis de définir une stratégie pour extraire les composés volatils odorants du saumon fumé :

- le choix d'une méthode d'extraction la plus performante possible,
- la nécessité de garantir l'innocuité de l'extrait lors des séances sensorielles,
- la nécessité de s'assurer de la représentativité des extraits obtenus,
- la capacité de la méthode d'analyse à identifier et quantifier les composés volatils odorants du saumon frais et du saumon fumé.

Ainsi, la caractérisation des composés volatils odorants du saumon fumé a été planifiée en deux étapes : une première étape de développement méthodologique sur la mise au point de l'extraction des composés volatils odorants sur solutions modèles et une seconde étape de caractérisation des composés volatils odorants du saumon frais et du saumon fumé.

Le développement méthodologique a nécessité une optimisation en trois phases : un premier travail bibliographique sur les méthodes d'extraction des composés volatils d'une telle matrice, un second travail constitué par un développement analytique sur standards pour tester l'efficacité de l'extraction et un troisième travail d'étude de la représentativité odorante des extraits aromatiques obtenus par rapport à la matrice d'origine.

L'étude bibliographique nous a permis de choisir l'extraction-distillation simultanée comme méthode d'extraction des composés volatils du saumon fumé. Nous avons préféré utiliser des méthodes directes plutôt que des méthodes d'espace de tête pour s'assurer de l'extraction de la totalité des composés volatils odorants puisque nous poursuivions un objectif final de leur quantification. Des travaux antérieurs ont montré que cette méthode permettait l'analyse de composés volatils odorants de nombreux produits de la mer (Choi, S.H. et al., 1983 ; Flament, I., 1990 ; Tanchotikul, U. et al., 1991 ; Cha, Y.J. et al., 1995 ; Chung, H.Y., 1999 ; Morita, K. et al., 2001 ; Lee, G.H. et al., 2001 ; Chung, H.Y. et al., 2001 ; Chung, H.Y. et al., 2002). Cette méthode d'extraction a également déjà été utilisée dans notre laboratoire sur du turbot et du hareng fumé

(Prost, C. et al., 1998 ; Sérot, T. et al., 2004). Cependant, cette méthode possède deux inconvénients majeurs. Premièrement, l'extraction étant réalisée à pression atmosphérique, la matrice est portée à des températures importantes qui peuvent favoriser l'éventuelle génération d'autres composés volatils à partir des constituants du poisson et être responsables d'interférences chromatographiques et/ou odorantes. Toutefois, notre travail s'opérant sur une matrice fumée, celle-ci a déjà subi un traitement thermique ce qui pourrait limiter la génération d'éventuels composés lors de l'extraction. Deuxièmement, après l'extraction, une étape de concentration est toujours réalisée afin de réduire le volume de solvant et de concentrer les composés volatils extraits. Etant donné la volatilité des composés aromatiques, il est nécessaire d'utiliser des solvants à bas point d'ébullition pour éviter d'évaporer les molécules extraites. Or, les solvants utilisés sont toxiques, principalement le dichlorométhane. Ainsi, cette méthode pourrait conduire à une extraction non représentative de la matrice et ne pas garantir une sécurité sanitaire totale de nos juges lors de séances sensorielles ou olfactométriques. Pour pallier à ces deux inconvénients, nous avons mis en place un protocole dans l'extraction des composés volatils permettant de s'affranchir de ces problèmes de toxicité. Pour contrôler que le protocole ne créait pas de différences sensorielles, des tests de représentativité ont été mis en place pour s'assurer que les composés volatils odorants quantifiés étaient ceux d'un extrait représentatif de la matrice. De plus, les tests de représentativité ne prennent généralement pas en compte un éventuel effet de la matrice. Or, des interactions odorantes entre les composés de l'extrait et la matrice peuvent avoir lieu. L'extrait obtenu ne devra pas être représentatif du saumon fumé en soi mais représentatif une fois redéposé sur du saumon non fumé. Les tests de représentativité devront donc intégrer l'effet matrice.

Puis, notre objectif final étant l'identification et la quantification des composés volatils odorants du saumon fumé, nous avons appliqué la méthode d'extraction sur matrice réelle. Nous avons caractérisé les molécules odorantes du saumon frais et du saumon fumé. La comparaison des concentrations en composés volatils odorants dans ces deux matrices permeetront de mieux identifier les précurseurs. Pour caractériser les composés volatils odorants de telles matrices, l'analyse par GC-MS/O s'impose. Elle permet la détection des zones odorantes du chromatogramme par les juges et identification simultanée des composés correspondants par spectrométrie de masse.

La mise au point de cette méthode d'extraction des composés volatils du saumon fumé a donc nécessité une validation analytique sur standards aromatiques fréquemment détectés dans les produits de la mer et une validation odorante des extraits par tests de représentativité. Ce

développement méthodologique a été valorisé sous la forme d'une publication dans *Food Chemistry*.

Les résultats obtenus concernant les composés volatils odorants du saumon fumé et non fumé ont été rédigés sous la forme d'une publication parue dans *Journal of Agricultural and Food Chemistry*, constituant l'un des premiers articles dans le domaine.

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Analytical, Nutritional and Clinical Methods

New procedure for the study of odour representativeness of aromatic extracts from smoked salmon

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Abstract

The representativeness of an aromatic extract of smoked salmon obtained from simultaneous steam distillation and extraction with diethyl ether is discussed. After extraction, the extract is diluted in ethanol with an evaporation of diethyl ether, which allows the extract to be redeposited on matrices physically similar to those of the original product. When the shift of the matrix effect is taken into account, the sensorial results are closer to reality and more representative of the real interaction conditions between the matrix and the extract. Several sensory methods are used to describe the representativeness of the smoked salmon extracts, such as triangular and notation tests. Preliminary work is carried out on standards known both to contribute to the aroma of many seafood products and to have a high volatility in comparison with those expected in smoked salmon in order to test the method in difficult conditions of recovery. This enables the recovery yield of the extraction (from 62% for limonene to 97% for 2-methylphenol) to be assessed leading to a better characterization of the representativeness taking into account the odour dilutions. The final aromatic extract is found to be about 70% representative of the original product.

Keywords: Smoked salmon; Odour representativeness; Simultaneous steam distillation-solvent extraction method; Aroma extraction; Sensorial test

1. Introduction

In food aroma analysis, a wide range of extraction methods has been developed (Wilkes et al., 2000). However, when olfactometry is applied after the extraction step, the representativeness of the aromatic extract is not always measured. In the case of headspace extractions, the most volatile compounds are followed but it is not easy to assess the odour representativeness of the extraction because the aromatic extract is in the gaseous state. However, it has become essential to evaluate the representativeness of the aromatic extract before analysing it in order to validate the olfactometric results. The extraction methods, where the final extract is liquid allow the similarity between the extract and the initial product to be assessed. Several studies have already been carried out on representativeness in mussels (Le Guen, Prost, & Demaimay, 2001), oysters (Le Pape, Grua-Priol, Prost, & Demaimay, 2004) and apples (Mehinagic, Prost, & Demaimay, 2003). Nevertheless, the main criticism of studies of odour representativeness is the comparison of odours that might be similar but are smelt on physically different matrices. In other words, most of the studies of odour representativeness do not take into account the shift of the matrix effect due to the potential interactions that may influence the sensory analysis when the mechanisms between the aromatic compounds and the food matrix are not well enough known (Guillard, Le Quere, & Vendeuvre, 1997). Several teams have studied various systems for the assessment of the representativeness of aromatic extracts (Etiévant et al., 1994; Le Guen et al., 2001) but never with a redeposition on a real matrix.

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The difficulty of redepositing the aromatic extract coming from simultaneous steam distillation and solvent extraction (SDE) or vacuum hydrodistillation and solvent extraction (VHDS) methods is due to the final solvent, which is almost always dichloromethane. Dichloromethane is very commonly used in these types of extraction thanks to its low boiling point, which can avoid a loss of the most volatile compounds. However, this solvent is also toxic, which is a drawback in olfactory analysis. It has been assessed by toxicological experiments on rats as a mutagenic agent with carcinogenic properties but with no effect on reproduction (INRS, 1997). In France, the maximum value of exposure for workers has been set at 350 mg/m^3 for dichloromethane. Several techniques have been established to remove dichloromethane after the extraction, for example liquid–liquid extraction on the aromatic extract in dichloromethane after SDE or VDS, but this step further reduces the yield of recovery. Redeposition of the aromatic extract on paper strips has been commonly employed (Pennarun, Prost, & Demaimay, 2002b; Sarrazin, Le Quere, Gretsch, & Liardon, 2000) but the unavoidable evaporation time of the solvent could allow the evaporation of the most volatile compounds and the interactions between the paper strip and the compounds are not representative of the interactions between the food matrix and the compounds. Finally, in odour representativeness studies, it is essential to work in iso-intensity odour conditions or with a measure of the odour dilution because these constitute parameters that are taken into account in the assessment by the judges.

To assess the representativeness of a smoked salmon aromatic extract and take into account all these constraints, the extraction method developed herein consists of an SDE extraction at atmospheric pressure with diethyl ether as the extraction solvent, followed by a dilution in ethanol and, simultaneously, an evaporation of diethyl ether. Thus, the final extract is obtained in a neutral solvent commonly used in sensorial analysis and redeposition on a real matrix is possible. SDE extraction method was chosen because it has already given good similarity results, especially on smoked fish. Moreover, atmospheric pressure SDE has already been found to give more information about the volatile compounds than reduced pressure SDE (Chung & Cadwallader, 1994). Also, the thermally generated compounds created by working at high temperatures in SDE extraction do not affect the final odour because it is an extraction of smoked compounds. Diethyl ether has also been used in SDE extractions from many seafood products (Cha & Cadwallader, 1995; Choi, Kobayashi, & Yamanishi, 1983; Tanchotikul & Hsieh, 1991). After extraction and concentration, the aromatic extract in diethyl ether is diluted in ethanol and diethyl ether is removed because, even though diethyl ether is less toxic (no genotoxic, teratogenic and carcinogenic effects) and more volatile (34.6 C versus 40 C) than dichloromethane (Brondeau et al., 1999), this solvent is not totally safe. The maximum value of exposure for workers in France has been set at 1500 mg/m^3 for diethyl ether, that it is to say dichloromethane is five times more dangerous than diethyl ether. Anaesthesia can be carried out with diethyl ether with 150,000 ppm. Since, in our case, we work with an overall equivalent of about 700 ppm, all the manipulations are safe but need precautions because irritation of the respiratory tract can occur from a level of 200 ppm. All the sensorial tests are safe because the concentration of the aromatic extract leads to an equivalent of 10 ppm (0.5 mL) in ethanol so, after the evaporation of diethyl ether, traces may be present but in quantities much lower than 10 ppm so not in toxic or irritant amounts. During the redeposition of the extract on the food blank matrices, ethanol is evaporated until it is not perceptible. The dilution of the extract in ethanol is chosen because ethanol is totally non-toxic for sniffing analysis (Etiévant et al., 1994) and is a good support for the odorant volatile compounds.

The aim of this paper is to give an alternative to other odour representativeness studies by responding to all these constraints by the redeposition of a representative aromatic extract on a real similar matrix (Tandon, Baldwin, & Shewfelt, 2000). First of all, the method is evaluated in a quantitative way with the measurement of the recovery yields. Then, it is studied in an odorant way because the representativeness of the aromatic extract is assessed by sensorial analysis tests (Mehinagic et al., 2003; Moio et al., 1995). These give information firstly about the adequate amount of matrix that must be used, by studying the impact of the intensity on the similarity. Secondly, they indicate the level of representativeness by comparing a redeposition on a real matrix and on paper strips.

2. Materials and methods

2.1. Materials

Salmons (Salmo salar), reared in Norway, were purchased from seafood wholesaler. The beech smoke was obtained by smouldering. The salmon were smoked for 2 h at a temperature of 24 C in the laboratory of IFREMER (Nantes, France). Standard flavour compounds and collidine were obtained from Aldrich (Steinheim, Germany). The following solvents were used: diethyl ether (purity: 99.5%) from Panreac (Barcelona, Spain), ethanol (purity: 95%) from VWR (Fontenay-sous-bois, France). All water was purified by a MilliQ system. Ethanol was freshly distilled before use.

2.2. Isolation of volatiles

A Likens-Nickerson apparatus was used for the preparation of the SDE extracts. A 500 mL round-bottomed flask was used as the sample flask to contain: 150 mL of purified water, 100 μg of collidine (2,4,6-trimethylpyridine) used as internal standard and, in the case of the study on a salmon matrix, 50 g of smoked salmon, and, in the case of the study

on standards, 150 μL of solution containing all the standards at 1 mg/mL in ethanol. A 30 mL round-bottomed flask containing 30 mL of diethyl ether was linked to the upper arm of the SDE apparatus. The gases were cooled by the circulation of polyethylene glycol at 5 °C. The contents of the sample and solvent flasks were heated to boiling. The temperature of the diethyl ether flask was maintained by a water bath at 50 °C. The distillation–extraction was continued for 3 h. The volume of the extract was reduced to 5 mL by evaporating the solvent using a Kuderna Danish apparatus, and to 2 mL under a gentle cold stream of nitrogen.

2.3. Solvent change

The aromatic extract in diethyl ether was introduced into 10 mL of ethanol and diethyl ether was removed by evaporation under a gentle cold stream of nitrogen for 10 min. As ethanol and diethyl ether are totally miscible, the volatile compounds remain in a liquid state in ethanol, even during the evaporation of diethyl ether at low temperature (40 °C). The evaporation of diethyl ether provides a neutral extract, from a toxic and odorant point of view, which can be redeposited on fish flesh.

2.4. GC-FID analysis

GC analyses were carried out using a gas chromatograph (Star 3900, Varian, Palo Alto, CA, USA) equipped with a split/splitless injector and a flame ionisation detector. The injector was set at 260 °C, the injector at 250 °C and He at a flow rate of 1.0 $mL.min^{-1}$ was used as carrier gas. The volatile compounds were separated on a capillary column (DB-wax, 30 m - 0.25 mm i.d., 0.5 μm thick, J&W Scientific, Folsom, CA, USA) with the following oven temperature programme: from 40 °C (2 min) to 210 °C (2 min) at 5 °C/min. The sample volume injected was 1 μL.

2.5. Assessment of the recovery yields

The recovery yields were characterized using standards well known to participate in the aroma of many seafood products and especially salmon (Table 1). Certain standards that have not been found in salmon (2,5-dimethylpyrazine, 3-octanone) were chosen because it is interesting to obtain the widest range of odorants representing as many chemical groups as possible and odorant ketones and nitrogen-containing compounds are expected in smoked salmon. This assessment was carried out at the concentrations of odorant compounds found in smoked salmon. The ratio between the areas of the peaks of standards after extraction and the standards directly in ethanol gives the recovery yields.

2.6. Sensorial analysis

The SDE extraction was carried out on smoked salmon and the ethanolic aromatic extract obtained was redeposited on the same salmon but not smoked. The odour representativeness was evaluated on the salmon matrix using

Table 1
List of the 11 standards used for the evaluation of the recovery yields

Compounds	Boiling point (C)	Descriptors	Matrix	References
Alcohols				
1-Penten-3-ol	110	Sulfury, spicy, plastic	Smoked salmon	Cardinal et al. (1997)
			Red cooked salmon	Josephson et al. (1991)
			Canned salmon	Girard and Durance (2000)
1-Heptanol	170	Potato	Red cooked salmon	Josephson et al. (1991)
			Canned salmon	Girard and Durance (2000)
Aldehydes				
Nonanal	186	Fatty, green, citrus	Canned salmon	Girard and Durance (2000)
			Red cooked salmon	Josephson et al. (1991)
			Smoked salmon	Cardinal et al. (1997)
(E)-2-hexenal	142	Moss, wood	Canned salmon	Girard and Durance (2000)
Ketones				
3-Octanone	161	Earthy	Mussel juices	Cros et al. (2005)
			Anchovy, herring	Cha and Cadwallader (1995)
Terpenes				
D-Limonene	172	Citrus, green	Smoked salmon	Cardinal et al. (1997)
			Cooked mussels	Le Guen et al. (2000b)
Nitrogen-containing compounds				
2,5-Dimethylpyrazine	150	Nutty	Mussel juices	Cros et al. (2005)
			Roasted shrimps	Kubota et al. (1986)
Furans				
2-Acetylfuran	168	Coffee, burnt	Smoked salmon	Cardinal et al. (1997)
			Canned salmon	Girard and Durance (2000)
2-Furancarbox-aldehyde	157		Smoked salmon	Cardinal et al. (1997)
Phenolic compounds				
2-Methoxyphenol	200	Smoke	Smoked salmon	Cardinal et al. (1997)
			Lobster tail	Lee et al. (2001)
2-Methylphenol	186		Smoked herring	Sérot and Lafficher (2003)

different sensory methods: triangular, descriptive and similarity tests.

The odours of the extracts were assessed by a panel consisting of 16 subjects (4 men, 12 women, from 22 to 49 years old) who were recruited in our laboratory (researchers and students from the Ecole Nationale des Ingénieurs des Techniques Agricoles et Alimentaires of Nantes). They learned to memorize and recognize the basic odours of smoked salmon in order to discriminate the samples. The panellists were trained during two sessions of descriptive analysis, global sniffing and were selected for their interest in the subject and their previous experience in sensory olfactometric evaluation applied to seafood products (Piveteau et al., 2000; Sérot, Regost, Prost, Robin, & Arzel, 2001). The aim of the sensorial tests was to assess safely the representativeness of the aromatic extract obtained by SDE with diethyl ether. Finally, the comparison of the redeposition of the extract on a real matrix or on paper strips was studied in order to measure the gain in representativeness due to redeposition on a real matrix.

2.7. Generation of descriptors

The preliminary training sessions generated the main descriptors of smoked fish flesh. The assessors had to comment on the aroma characteristics using their own descriptors. For this first sensorial session, the panellists (14 judges: 4 men, 10 women, aged from 22 to 49 years old) were asked to give all the descriptors they wanted in order to describe the odour of smoked salmon. They wrote down anonymously the three most pertinent adjectives for the odour they smelt. The seven descriptors most frequently cited were retained for the descriptive tests and the first five descriptors were retained for the similarity tests.

2.8. Sample preparation and presentation to the judges

In both tests, the samples were presented to the panel in 15 mL brown coded flasks during the sessions. In both cases, the reference was smoked salmon. The samples were 1 g cubes of salmon and the aromatic extract was redeposited by gently sprinkling it on the cubes. The extracts were hermetically stored at 4 °C in a fridge then placed at room temperature 3 h before the beginning of each test. All the samples were assessed at room temperature (20 °C) in neutral conditions.

2.9. Descriptive tests

Descriptive tests enabled the samples to be evaluated by their odorant characteristics. The intensity of each descriptor was assessed by giving a mark on an unstructured scale from ''0'' to ''10''. The average of the different marks was used to build the profiles of the reference (smoked salmon) and the extract redeposited on unsmoked salmon. For this characterization, 10 judges were required (seven women and three men, aged from 22 to 49 years old).

2.10. Triangular tests

Triangular tests consist of the presentation to a large number of judges of three samples where one is different from the other two. These tests allowed the link between the real smoked salmon matrix and the aromatic extract redeposited on a blank salmon matrix to be determined. Assessors were requested to smell three samples and to locate the odd sample even if a difference was not perceptible (forced-choice method). Samples were coded and presented to the judges in random order but taking care to have the six permutations at least twice whenever possible (Abbott, Etiévant, Langlois, Lesschaeve, & Issanchou, 1993). The panel was constituted of 16 judges aged from 22 to 49 years old (11 women, 5 men).

2.11. Similarity tests

In similarity tests, the samples are presented to the judges and they must assess the proximity of the extract to the reference by noting the extract on an unstructured 100 mm scale, anchored at the left end with ''odour far from the reference'' and at the right end with ''odour identical to the reference'' (Etiévant et al., 1994; Larráyoz, Ibáñez, Ordóñez, Torre, & Barcina, 2000). There was one scale per descriptor (5) previously set by training sessions. The positions of the extracts on the scale were read as the distance (mm) from the left anchor. For all the similarity tests, eight judges participated (five women, three men). The best quantity of fish flesh required for the sensorial tests was also tested in order to have an extract odour both very close to the matrix and very homogeneous.

2.12. Statistical analyses

All the statistical analyses were performed with STATGRAPHICS Plus 3.1 software (Manugistics, Rockville, Maryland, USA). Multiple-way ANOVA was performed on the descriptors and the judges to determine whether there were significant differences between the marks of the descriptors and thus if the representativeness of the extract was linked with a particular descriptor. Possible significant differences between the response values were evaluated by least significant difference (LSD) multiple comparison tests with a confidence level of 95%.

3. Results and discussion

3.1. Recovery yields

The work on standards was carried out at concentrations equivalent to those found in smoked salmon, especially the volatile phenolic compounds. The mean quantity of volatile phenolic compounds in smoked salmon is about 1 mg/kg (Sérot, Baron, Knockaert, & Vallet, 2004). In the extraction, only 50 g of smoked salmon was used, corresponding to a quantity of 0.05 mg of volatile phenolic compounds. In

order to both be representative of real conditions and obtain a significant chromatographic response, the work on standards was performed with 150 μg of each standard.

Table 2 shows the recovery yields obtained after three extractions and concentrations. Each recovery yield of a compound is the result of the mean of three injections.

As the coefficients of variation of each compound in the three extractions are less than 8%, the method is reproducible. There are no significant differences between the three injections of one extraction, or between the three extractions. Moreover, except for the limonene extracted with a recovery yield of about 62%, the recovery yields of the other compounds are between 70% and 97%, which is quite good with this kind of apparatus.

The loss of volatile compounds created by the total evaporation of diethyl ether is evaluated at 6% on all the standard compounds and at about 20% on the real matrix (mean of the difference of all the areas of the peaks of the compounds injected in diethyl ether and after dilution in ethanol). Phenolic compounds and heavy compounds are the least affected by the evaporation of ether with a loss of about 10%. There can be a loss of up to 38% of the lightest compounds. It is hoped that this small loss will be compensated by the gain in representativeness due to the direct and safe redeposition of the aromatic extract on the matrix made possible by the change of solvent and the ethanolic recovery.

All the standards chosen are representative of the highest volatilities of the volatile compounds liable to be found in smoked salmon. It is reasonable to assume that the final aromatic extract on real smoked salmon is a good representation of the original product.

To conclude, with regard to the recovery yield, SDE with diethyl ether seems to be a suitable method for the extraction of volatile odorant compounds of smoked salmon.

3.2. Sensorial analysis

The quantity of raw salmon on which the aromatic extract is redeposited is calculated based on the overall recovery yield of the extraction (about 75% except for limonene, all the standards are located between 68% and 95%). The recovery yields must be taken into account in order to be close to iso-intensity conditions or, at least, in the same concentrations of odorant volatile compounds expected in smoked salmon. From a quantity of 50 g of smoked salmon, the compounds are recovered in 10 mL of ethanol with an overall yield of 75%. Thus, in 10 mL of ethanol, there is the equivalent of the volatile compounds found in about 40 g of smoked salmon. To respect the real initial concentrations, 250 μL of the final ethanolic extract must be redeposited onto 1 g of raw salmon. In this way, the shift of odour dilution is avoided by taking into account the recovery yields.

After redeposition of the aromatic extract, a short time is necessary to allow the ethanol to evaporate until it has no impact on the sensorial analysis. Indeed, ethanol can add a slight alcoholic odour, which can interfere with the assessment. This time of evaporation was optimized and established at 4.5 min by giving for the judges some flasks with 250 μL of ethanol onto 1 g of salmon and at different evaporation times. After 4.5 min, 80% of the nine judges questioned did not perceive ethanol. The evaporation occurs with no agitation of the flask; it is just left open after 1 min under a gentle cold stream of nitrogen. Knowing that an evaporation time of 10 min under a nitrogen stream leads to a loss of only 6% of the volatile compounds, it is reasonable to assume that the evaporation time of ethanol does not cause a greater loss than the evaporation time of diethyl ether.

3.3. Generation of descriptors

The five descriptors the most frequently cited to describe the smoked salmon by the 14 assessors are compiled in Table 3. All the descriptors were chosen after a discussion to agree a common definition of each descriptor in order to be sure that each descriptor is perceived in the same way by all the judges and represents the same odour for them. The smoke odour was pointed out by all the judges. It is important to say that they characterized the smoke differently

Table 2
Recovery yields of 11 standards (means and coefficients of variation)

Compounds	RI	Extraction 1		Extraction 2		Extraction 3		Total	
		Mean (%)	CV (%)	Mean (%)	CV (%)	Mean (%)	CV (%)	Mean (%)	CV (%)
1-Penten-3-ol	1135	87.99	2.23	90.35	2.34	89.04	2.27	89.13	0.56
Limonene	1209	47.66	0.96	73.77	1.87	66.01	1.34	62.48	6.32
(E)-2-hexenal	1230	71.48	2.28	75.95	3.50	78.92	2.89	75.45	1.77
3-Octanone	1260	71.75	1.32	87.82	1.40	85.54	0.88	81.70	4.10
2,5-Dimethylpyrazine	1338	83.79	0.57	87.13	1.74	86.30	1.48	85.74	0.82
Nonanal	1400	77.80	0.64	84.73	1.19	86.56	2.40	83.03	2.18
1-Heptanol	1458	95.23	0.96	94.41	1.42	97.72	1.55	95.79	0.81
2-Furancarboxaldehyde	1471	68.56	0.75	69.23	1.23	72.97	0.61	70.25	1.12
2-Acetylfuran	1517	88.21	0.55	88.53	1.40	89.46	1.47	88.73	0.31
2-Methoxyphenol	1849	91.31	0.90	90.40	1.56	95.32	1.91	92.34	1.23
2-Methylphenol	2050	90.39	1.03	89.83	1.57	111.76	7.86	97.33	5.89

Table 3
Odorant descriptors of smoked salmon

Descriptors	Numbers of judges who cited this descriptor (total 14 judges)
Smoke	14
Fish/herring	11
Fatty/buttery	10
Fresh/green	6
Salty/marine	6
Burnt/tobacco	4
Woody/mushroom	3

(smoke of fireplace, cold ashes or soot) but they all noticed a woody or natural origin. The second descriptor was the fishy odour and the fish name most frequently cited to describe the smoked salmon was herring. Fatty and buttery was the third descriptor with 10 judges noticing this odour. The last two descriptors retained for the notation tests were cited with an equal frequency. They were fresh/green and salty/marine.

3.4. Descriptive tests

For the descriptive tests of the intensity of each descriptor, two other descriptors were retained. They were the aromatic notes tobacco/burnt and mushroom/woody-earthy. They were not mentioned enough to be taken into account in the notation tests but their presence was noticed by at least three assessors. Thus, the similarity can be influenced by these last two descriptors but they contribute more to the overall aromatic note of smoked salmon rather than constituting a particular odour of smoked salmon. The average of the different marks for all the descriptors enabled profiles to be built for the reference (smoked salmon) and the aromatic extract redeposited on unsmoked salmon and are presented in Fig. 1. They allow an accurate representativeness to be predicted because the profile of the aromatic extract follows the reference profile but just with slightly lower intensity marks for the descriptors, especially smoke odour. This difference may originate from the specific reactions of each judge towards this descriptor, especially for the extract profile. Indeed, the standard deviation for this descriptor was greater (0.47) for the extracts than for the reference (0.24). The descriptor ''smoke'' is very interesting and subjective because it can be perceived in many varied ways.

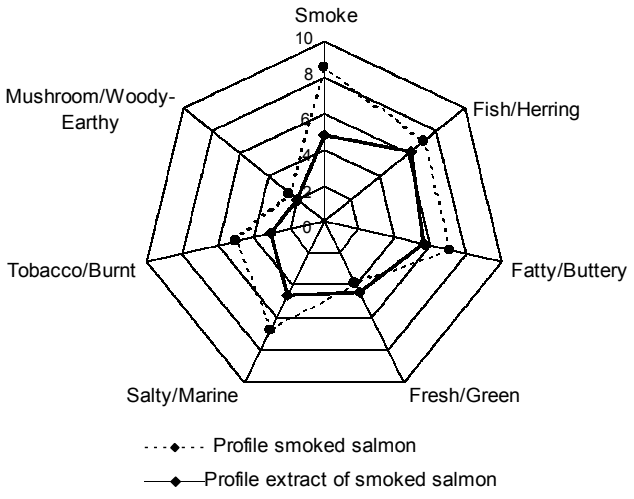

Fig. 1. Profiles in iso-intensity conditions of smoked salmon (reference) and aromatic extract of smoked salmon.

3.5. Triangular tests

Table 4 shows the results of the triangular tests with the levels of significance and the number of correct responses.

It can be observed that there was a significant difference between the aromatic extract and the reference ($p < 0.01$). However, it is important to underline that there were three assessors (out of 16 judges) who correctly identified the odd sample but remarked that there was a problem with the evaporation of the solvent and too weak a perception of smoked salmon in the reference. These phenomena can be explained by the saturation of the headspace in the flasks. Nevertheless, they must be taken into account because the same conditions were applied to all the samples. To avoid these problems, before the notation tests, the assessors must open the flasks then wait for 1 min before smelling. This precaution is sufficient because it was applied to the sensory profiles and nobody noticed a solvent odour. This difference between the aromatic extract and the reference will be evaluated further by the similarity tests.

3.6. Similarity tests

3.6.1. Study of the overall feeling in relation to the quantity of matrix

Assessors were requested to smell a reference of smoked salmon and then to evaluate the proximity of the aromatic extract in terms of odour, firstly based on their overall feeling, on a 100 mm unstructured scale. Secondly, they were asked to judge, on the same scale, the similarity between the reference and the extract according to the five descriptors previously generated.

In order to determine the optimum quantity of salmon flesh on which the aromatic extract must be redeposited, a study of the intensity of similarity marks was carried out. Different amounts of smoked salmon and unsmoked salmon were tested but the redeposition always occurred at the equivalent concentrations of odorant volatile compounds found in smoked salmon. To better understand the behaviour of the judges when they are affected by a variation in the odour intensity, they were asked to do a notation test on the five descriptors where the odour in the samples was (i) the same as in smoked salmon (1 g) (ii) half as concentrated as in smoked salmon (0.5 g of matrix) (iii) twice as concentrated as in smoked salmon (2 g of matrix). In the three tests, the quantities of aromatic extracts corresponded to odour conditions to respect the iso-intensity

Table 4
Results of the triangular test between the reference and the aromatic extract

Samples	Number of assessors	Number of correct responses	Probability levels		
			a = 0.05	a = 0.01	a = 0.001
Aromatic extract versus reference	16	12	10	11	12

principle: 500 μL for 2 g of salmon, 250 μL for 1 g of salmon and 125 μL for 0.5 g of salmon. The results of this study are compiled in Fig. 2 and Table 5 for the 1 g samples and in Fig. 3 and Table 6 for the 2 and 0.5 g samples.

The mean overall apparent feeling found with 1 g of salmon was 59% for the proximity (that is to say 59 mm on a 100 mm unstructured scale) which is quite good because it is statistically proven that, if two identical samples are presented to judges, the total overall mean of proximity is near 80% and not 100% as expected. In our case, when the same two reference flasks were assessed, a similarity mark of 82.8% was found. Thus, the similarity of the aromatic extract must be corrected to 82.8% and not 100% which leads to a real proximity of 70.2% if we relate the overall apparent feeling mean (59%) to the total overall statistical mean (82.8%). The difference noticed in the triangular test was real but it did not affect the representativeness of the extract. So, the similarity marks were satisfactory and in accordance with the results of other studies of representativeness (all on a 100 mm unstructured scale) like Mehinagic et al. (2003), who found scores from 49.1 to 57 mm for fresh apple extracts, Pennarun, Prost, and Demaimay (2002a), who obtained a mean score of 58.9 mm for the aroma extracts of oysters and Le Pape et al. (2004), who found scores from 25.9 to 42.3 mm for edible red algae. Moreover, the representativeness marks illustrate the fact that, even with certain losses caused by the evaporation of diethyl ether, the similarity of the extract is quite correct.

Fig. 3 shows that the similarity is not influenced by the odour intensity variations. Indeed, in the three cases (2, 1 or 0.5 g), the similarity marks are very close in the same concentrations of odorant volatile compounds found in smoked salmon. From 0.5 g, a similarity mark of 59.9% is obtained and, corrected by the total overall statistical mean, this leads to 72.3%. From 2 g, the similarity mark is 59.3% which, when corrected, gives a mark of 71.6%. Both similarity marks are close to 70.2%.

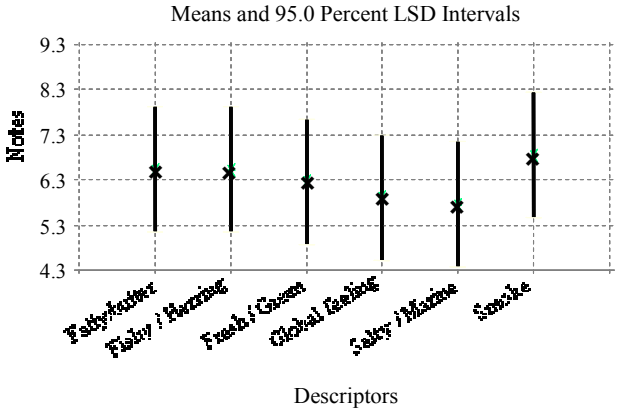

Fig. 2. Marks of proximity between the extract and the reference with 1 g of salmon as redeposition matrix (five descriptors, eight judges) at concentration of odorant volatile compounds found in smoked salmon.

Table 5
Results for the analysis of variance for marks with 1 g of matrix

Source	Sum of squares	Df	Mean square	F-ratio	P-value
Main effects					
A: descriptors	5.22971	4	1.30743	0.15	0.9685
B: judges	76.5795	7	10.9399	1.2	0.3266
Residual	251.789	28	8.99245		
Total (corrected)	333.98	39			

All F-ratios are based on the residual mean square error.

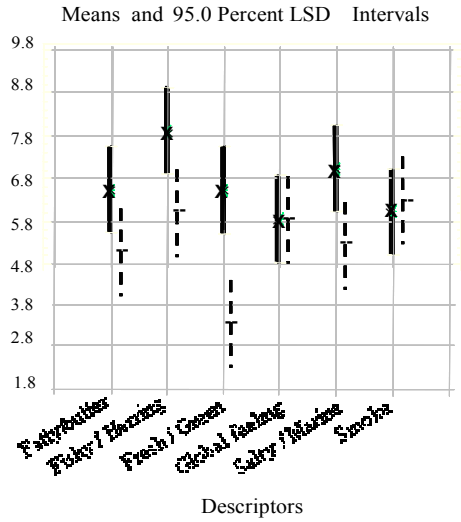

Fig. 3. Marks of proximity between the extract and the reference with variations of the odour intensity (five descriptors, eight judges, full line: 0.5 g of salmon and broken line: 2 g of salmon as redeposition matrix) at concentration of odorant volatile compounds found in smoked salmon.

Table 6
Results for the analysis of the variance for marks of similarity with variations in the odour intensity

Source	P-value (1)	P-value (2)
Main effects		
A: descriptors	0.4898	0.0778
B: judges	0.03	0.0003

(1) With 0.5 g of matrix; (2) with 2 g of matrix.

3.6.2. Study of descriptors in relation to the quantity of matrix

The results of the different mean marks of the proximity on the basis of the descriptors and the overall feeling are presented in Fig. 2. It illustrates the conclusions of the LSD multiple comparison tests, which compare any two means at a confidence level of 95%. It is easy to observe that the overall feeling of proximity is very representative of the means of the five descriptors when 1 g is used. Moreover, the proximity according to the five descriptors was equally assessed because they formed homogeneous groups near the mean of overall proximity, which means that there is not any one descriptor that significantly influences the overall feeling. Thus, the descriptors are very linked to the overall feeling and well chosen to describe the proximity between the aromatic extract and the original product. Thus, the odour of the extract is well-balanced and there was no preferential extraction of one kind of odorant compound.

To study the possible influence of the judges or the descriptors on the different notes, an analysis of variance was also applied. The results are reported in Table 5. As the P-values are always more than 0.05%, it can be concluded that there is no effect of either the judges or the descriptors on the notes.

In the case where 0.5 g of matrix was used, it is interesting to note that all the means of the descriptors are greater than the overall feeling mark as is shown in Fig. 3. The LSD test even discriminates the descriptor ''fishy/herring'' as different from the others because its mean is a little higher. The reduction in the quantity to smell causes an increase in the ''fishy/herring'' odour of the extract. Another remark can be made concerning the descriptors. With 1 g of matrix, the overall feeling was very well represented by all the descriptors. Since with 0.5 g of matrix, all the means of the descriptors are higher than the overall feeling mean, it seems that there is a change in the distribution of the odours in the composition of the overall aroma resulting from a reduction in certain aroma compounds. These compounds cannot be linked to the descriptors because their mean marks are the same, and sometimes even greater than for several descriptors, as the marks obtained with 1 g of matrix. Thus, these fluctuations show that the odour of the 0.5 g sample with the corresponding quantity of aromatic extract is not as well-balanced as that of 1 g of matrix. Nevertheless, these variations are not large enough to be considered as a statistical shift. Indeed, Table 6 presents the results of the analysis of the variance for the similarity marks with both cases of variation of the amount of matrix and of extract. The fluctuations of the descriptors were not detected as a predominant effect (P-value more than 0.05%). However, there is a ''judge'' effect (P-value lower than 0.05%). This can be explained by the different perceptions of the judges when there is too small a quantity of sample to smell. To conclude with this case, when 0.5 g is used, differences in the composition of the overall aroma occur that are deeply linked to the perceptions of the judges, who are not affected by a reduction in the amount of salmon and extract in the same way.

In the case where 2 g of matrix was used, the LSD test found a descriptor different from the others (Fig. 3). All the means of the descriptors are distributed very close to the overall feeling except for ''fresh/green''. When the quantity to smell was doubled, the compounds corresponding to the aromatic note ''fresh/green'' hindered the representativeness because they were not present enough by comparison with the other descriptors. This is the main reason why, in Table 6, a ''descriptor'' effect is detected. Like in the case of 0.5 g of matrix, the sample is not as well-balanced as 1 g of matrix. In Table 6, a ''judge'' effect is also shown and can also be explained by the increase in the quantity to smell. To conclude with this case, when 2 g are used, the increase in the amount to smell causes changes in the composition of the overall odour and can favour the perception or the inhibition of a particular odour, especially due to the different reactions of the assessors towards the variations in amounts. In addition, with the increase in the quantity of the extract redeposited, there is an increase in the quantity of ethanol, which can strongly affect the assessment.

Concerning the study of the odour intensity impact on similarity marks, it can be said that the representativeness is not influenced by the quantity of salmon and extract to smell. However, it can cause a variation in the overall odour, which is derived from the evaluations by the panellists, who do not form a homogeneous group when the quantity is changed. Thus, the best compromise is obtained with 1 g of salmon.

3.6.3. Gain in representativeness from redeposition on a real matrix

A comparison between the odour of the reference and the odour of the aromatic extract redeposited on paper strips was evaluated in order to know the gain in representativeness from redeposition on a real matrix. Indeed, firstly, it is very difficult to work in iso-intensity conditions with paper strips. Secondly, redeposition on paper strips favours the appearance of unexpected aromatic notes, like cardboard, that can affect the evaluation (Pennarun et al., 2002b). Thirdly, the representativeness between the paper strip with the extract and the reference leads to a result of 51.1% and, when corrected by the total overall statistical mean, to a result of 61.6%. Thus, compared to redeposition on a real matrix, redeposition on paper strips causes a loss of about 9% of representativeness. Moreover, even if there was not a ''descriptor'' effect, there was a ''judge'' effect. This effect can come from the very fleeting headspace odour obtained with this method. For example, the descriptor ''fresh/green'' was differentiated by the LSD test from the others because of a too weak intensity.

To conclude, with regard to the results of sensorial analysis tests, the similarity results at the same concentrations of odorant volatile compounds found in smoked salmon are quite acceptable because the assessors evaluated the

extract as 70.2% close to the reference with 1 g of salmon. The gain in representativeness from the redeposition of the aromatic extract on a real matrix instead of paper strips is about 10%.

4. Conclusion

The results reported herein demonstrate that it is possible to develop a safer method than those established until now for the extraction and recovery of the volatile compounds from smoked salmon, and to obtain an aromatic extract representative of the original product.

Diethyl ether allows a good and reproducible extraction and can be easily removed. The recovery in a neutral solvent such as ethanol after the extraction enables a redeposition of the extract on a real matrix and avoids the shift that the matrix could cause if it interacted with the volatile odorant compounds. It also provides a better similarity than the assessment on paper strips. Finally, it opens up new approaches in representativeness studies to take into account the matrix effects and the dilutions of odour caused by the extraction. Concerning the influence of the odour intensity on the assessment of the similarity, it seems that the quantity to smell does not affect the representativeness but creates some variations in the perception of the overall odour. This method can also be extended to other processed foodstuff (smoked food, roasted food, . . .) in order to assess the influence of the industrial or culinary process on the food material. With this procedure, the representativeness of aromatic extracts of altered food can also be considered by the redeposition of spoiled foodstuffs extracts on same fresh initial matrices.

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References

Abbott, N., Etiévant, P. X., Langlois, D., Lesschaeve, I., & Issanchou, S. (1993). Evaluation of the representativeness of the odour of beer extracts prior to analysis by GC eluate sniffing. Journal of Agricultural and Food Chemistry, 41(5), 777–780.

Brondeau, M. T., Falcy, M., Jargot, D., Miraval, S., Protois, J. C., & Reynier, M., et al. (1999). Oxyde de diéthyle. Fiche toxicologique no. 10, INRS, note établie par les services techniques et médicaux de l INRS.

Cardinal, M., Berdagué, J. L., Dinel, V., Knockaert, C., & Vallet, J. L. (1997). Effect of various smoking techniques on the nature of the volatile compounds and on the sensory characteristics of salmon meat. Sciences Des Aliments, 17(6), 679–696.

Cha, Y. J., & Cadwallader, K. R. (1995). Volatile components in salt-fermented fish and shrimp pastes. Journal of Food Science, 60(1), 19–24.

Choi, S. H., Kobayashi, A., & Yamanishi, T. (1983). Odor of cooked small shrimp, *Acetes japonicus* Kishinouye: difference between raw material and fermented product. Agricultural and Biological Chemistry, 47(2), 337–342.

Chung, H. Y., & Cadwallader, K. R. (1994). Aroma extract dilution analysis of blue crab claw meat volatiles. Journal of Agricultural and Food Chemistry, 42(12), 2867–2870.

Cros, S., Lignot, B., Bourseau, P., Jaouen, P., & Prost, C. (2005). Desalination of mussel cooking juices by electrodialysis: effect on the aroma profile. Journal of Food Engineering, 69(4), 425–436.

Etiévant, P. X., Moio, L., Guichard, E., Langlois, D., Lesschaeve, I., Schlich, P., et al. (1994). Aroma extract dilution analysis (AEDA) and the representativeness of the odour of food extracts. In H. Maarse & D. G. Van der Heij, et al. (Eds.), Trends in flavour research (pp. 179–191). The Netherlands: Elsevier Science B.V.

Girard, B., & Durance, T. (2000). Headspace volatiles of Sockeye and Pink salmon as affected by retort process. Journal of Food Science, 65(5), 34–39.

Guillard, A. S., Le Quere, J. L., & Vendeuvre, J. L. (1997). Emerging research approaches benefit to the study of cooked cured ham flavour. Food Chemistry, 59(4), 567–572.

INRS, Institut National de Recherche et de Sécurité, (1997). Fiche toxicologique sur le dichlorométhane no. 34, 1–4.

Josephson, D. B., Lindsay, R. C., & Stuiber, D. A. (1991). Influence of maturity on the volatile aroma compounds from fresh Pacific and Great Lakes salmon. Journal of Food Science, 56(6), 1576–1579.

Kubota, K., Shijiyama, H., & Kobayashi, A. (1986). Volatile compounds of roasted shrimp. Agricultural and Biological Chemistry, 50(11), 2867–2873.

Larráyoz, P., Ibáñez, F. C., Ordóñez, A. I., Torre, P., & Barcina, Y. (2000). Evaluation of supercritical fluid extraction as sample preparation method for the study of Roncal cheese aroma. International Dairy Journal, 10(11), 755–759.

Lee, G. H., Suriyaphan, O., & Cadwallader, K. R. (2001). Aroma components of cooked tail meat of American lobster (*Homarus americanus*). Journal of Agricultural and Food Chemistry, 49(9), 4324–4332.

Le Guen, S., Prost, C., & Demaimay, M. (2000b). Characterization of odorant compounds of mussels (*Mytilus edulis*) according to their origin using gas chromatography–olfactometry and gas chromatography–mass spectrometry. Journal of Chromatography A, 896(1–2), 361–371.

Le Guen, S., Prost, C., & Demaimay, M. (2001). Evaluation of the representativeness of the odor of cooked mussel extracts and the relationship between sensory descriptors and potent odorants. Journal of Agricultural and Food Chemistry, 49(3), 1321–1327.

Le Pape, M. A., Grua-Priol, J., Prost, C., & Demaimay, M. (2004). Optimization of dynamic headspace extraction of the edible red algae *Palmaria palmata* and identification of the volatile components. Journal of Agricultural and Food Chemistry, 52(3), 550–556.

Mehinagic, E., Prost, C., & Demaimay, M. (2003). Representativeness of apple aroma extract obtained by vacuum hydrodistillation: comparison of two concentration techniques. Journal of Food Science, 68(8), 2411–2415.

Moio, L., Chambellant, E., Lesschaeve, I., Issanchou, S., Schlich, P., & Etiévant, P. X. (1995). Production of representative wine extracts for chemical and olfactory analysis. Italian Journal of Food Science, 3, 265–278.

Pennarun, A. L., Prost, C., & Demaimay, M. (2002a). Aroma extracts from oysters *Crassostrea gigas*: comparison of two extraction methods. Journal of Agricultural and Food Chemistry, 50, 299–304.

Pennarun, A. L., Prost, C., & Demaimay, M. (2002b). Identification and origin of the character-impact compounds of raw oyster *Crassostrea gigas*. Journal of the Science of Food and Agriculture, 82, 1652–1660.

Piveteau, F., Le Guen, S., Gandemer, G., Baud, J. P., Prost, C., & Demaimay, M. (2000). Aroma of fresh oysters *Crassostrea gigas*: composition and aroma notes. Journal of Agricultural and Food Chemistry, 48(10), 4851–4857.

Sarrazin, C., Le Quere, J. L., Gretsch, C., & Liardon, R. (2000). Representativeness of coffee aroma extracts: a comparison of different extraction methods. Food Chemistry, 70(1), 99–106.

Sérot, T., Baron, R., Knockaert, C., & Vallet, J. L. (2004). Effect of smoking processes on the contents of 10 major phenolic compounds in smoked fillets of herring (*Cuplea harengus*). Food Chemistry, 85(1), 111–120.

Sérot, T., & Lafficher, C. (2003). Optimisation of solid-phase microextraction coupled to gas chromatography for determination of phenolic compounds in smoked herring. Food Chemistry, 82(4), 513–519.

Sérot, T., Regost, C., Prost, C., Robin, J., & Arzel, J. (2001). Effect of dietary lipid sources on odour-active compounds in muscle of turbot (*Psetta maxima*). Journal of the Science of Food and Agriculture, 81, 1339–1346.

Tanchotikul, U., & Hsieh, T. C. Y. (1991). Analysis of volatile flavor components in steamed rangia clam by dynamic headspace sampling and simultaneous distillation and extraction. Journal of Food Science, 56(2), 327–331.

Tandon, K. S., Baldwin, E. A., & Shewfelt, R. L. (2000). Aroma perception of individual volatile compounds in fresh tomatoes (*Lycopersicon esculentum*, Mill.) as affected by the medium of evaluation. Postharvest Biology and Technology, 20(3), 261–268.

Wilkes, J. G., Conte, E. D., Kim, Y., Holcomb, M., Sutherland, J. B., & Miller, D. W. (2000). Sample preparation for the analysis of flavors and off-flavors in foods. Journal of Chromatography A, 880, 3–33.

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Comparison of Odor-Active Volatile Compounds of Fresh and Smoked Salmon

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The odorant volatile compounds of raw salmon and smoked salmon have been investigated by two gas chromatography−olfactometry methods (frequency detection and odorant intensity) and gas chromatography−mass spectrometry. After simultaneous steam distillation−solvent extraction with diethyl ether and the recovery of the aromatic extract in ethanol, qualitative olfactometric characterization and identification followed by a quantitative assessment of the odorant volatile compounds were carried out. The origin of many odorant compounds of smoked salmon can be attributed to wood smoke. Another part of smoked salmon aroma is due either to the odorant compounds of the raw fish flesh or to an evolution of fish flesh aroma thanks to the smoking process conditions. Forty-nine odorant compounds have been identified in fresh salmon and 74 in smoked salmon. Carbonyl compounds, such as heptanal or (E,Z)-2,6-nonadienal, show a high detection frequency and odorant intensity in unsmoked fish, giving the flesh its typical fishy odor. For smoked salmon, phenolic compounds, such as cresol or guaiacol, and furanic compounds seem to be responsible for the smoked odor.

KEYWORDS: Smoked salmon; olfactometry; odorant volatile compounds; mass spectrometry; SDE extraction; phenolic compounds; wood smoke

INTRODUCTION

The origins of smoked food are lost in antiquity. Initially, the smoking process served primarily to preserve food by hanging it over a fire. Nowadays, this process is widely investigated and controlled. Moreover, much equipment has been developed. The smoking process is preceded by a salting and drying steps, which decrease the water activity and the microbial development in more of the antioxidant, antimicrobial, and flavor characteristics supplied by the wood smoke. With the growth of the smoked salmon industry, several new smoking processes have been developed from the traditional cold-smoking process. Liquid smoking, friction smoking, and hot-smoking have allowed a significant quantity of various products to be targeted. Since then, through these smoking processes, the whole of the salmon industry, from harvest to frozen storage of fish, has been studied and improved to better understand the relationship between the industrial processing of smoked salmon and its sensory characteristics, such as odor and quality (*1*−*5*). The investigation of smoked salmon aroma, to our knowledge, has never been addressed. Many studies are available on the volatile compounds in processed salmon (*6*−*10*) or in raw salmon (*11*, *12*), but very few studies have assessed odorants, and then only in boiled salmon (*13*). Indeed, concerning smoked fish aroma, some studies have characterized volatile compounds (*14*, *15*) or overall odor thanks to sensorial analysis and aromatic profiles (*16*), but very few studies are available concerning the odorant volatile compounds in smoked salmon (*17*). Similarly, although knowledge about wood smoke used in the smoking of salmon is considerable, only volatile compounds have been investigated (*18*−*21*). Nevertheless, more research has been done on the role that several volatiles of wood smoke play in smoke flavor. It is known that in wood smoke, phenolic compounds are antioxidant and antimicrobial agents and carry a "smoky" flavor. These phenolic compounds are also found in smoked fish (*22*). Carbonyl compounds play a role in the color and texture of the final product (*23*) and are more responsible for the "fishy" odor. Thus, the volatile odor-active compounds in smoked salmon are unknown, even though some information is available about the volatile odorant compounds of wood smoke, because no study has yet related the volatile compounds with their odor in smoked salmon. Until now, the olfactive studies have focused on the observation or reduction of off-flavors, and then only on very few compounds and especially on fresh fish. Indeed, they have been investigated

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because they constitute good indicators of the deterioration and spoilage of food because some volatile compounds are produced by microbiological organisms (*17*, *24*, *25*). They have also been studied because certain volatiles, such as phenols, are good indicators of the intensity of the smoking process (*16*), or certain compounds have been investigated to improve smoking techniques by comparing processes or by evaluating food contaminants such as polycyclic aromatic hydrocarbons (*26*, *27*). This knowledge about the volatile odorants of smoked salmon is therefore incomplete. Olfactometry with the frequency detection method and mass spectrometry coupled to gas chromatography will allow their characterization in order to elucidate individually all of the odor-active compounds of smoked salmon.

This study has identified and evaluated the odorant compounds of smoked and fresh salmon and points out the odorant evolution between raw and smoked material. First, the different odorant notes are quantified in smoked salmon, and the origin of the odor-active compounds is discussed according to their presence in wood smoke. Second, odor-active compounds of fresh salmon are studied to explain the origin of odorants in smoked salmon that have not been reported in wood smoke. Finally, a comparison between unsmoked and smoked aroma profiles is carried out and the evolution of odors related to the evolution of the concentrations of odor-active compounds is proposed.

MATERIALS AND METHODS

Reagents. Dodecane came from Aldrich (Steinheim, Germany), diethyl ether from Fluka (Buchs, Switzerland), and ethanol from VWR (Fontenay-sous-bois, France). All water was purified by a Milli-Q system. All standards used for identification were from Aldrich except acetic acid, which was from Panreac (Barcelona, Spain), and phenol, which was from Merck (Darmstadt, Germany).

Fish Processing. Salmon (*Salmo salar*) reared in Norway were purchased from a seafood wholesaler (Nantes, France). The time between their capture and their filleting was not more than 5 days. The beech smoke was obtained by smoldering. Four gutted fishes of 3−4 kg of the same batch were received in a box in ice. They were directly filleted and put in a cold chamber at 3 °C for 2 h. Each of the eight fillets weighed 1 kg. Four fillets were used for aroma analysis on unsmoked salmon, and four fillets were smoked in order to study smoked salmon aroma.

Next, the fillets were hand-salted with refined salt (Salins du Midi, France) for 3 h at 12 °C before being rinsed on grids with water (15 °C) and stored in a cold room at 3 °C for 18 h until smoking (*16*). Smoke was produced by pyrolysis of beech wood sawdust at 400 °C (Thirode, France). The sawdust was wet with water to reach 20% moisture.

The smokehouse was an HMI Thirode (PC90 model) device (Thirode, France), 1500 × 1300 ×2250 mm, with a capacity of 380 kg, mounted on a trolley with 28 grids on which the fillets were deposited. The fillets were placed at the same level (grid 14) at 20 cm of the opening of the door of the smokehouse. The air/smoke circulation was horizontal. The salmon fillets were swept by the smoke for 3 h at a temperature of 32 °C. This temperature was chosen to enrich the odor-active compounds that we could quantify at trace level with cold-smoking at 22 °C. The exhaust valve opening was one-third, and the relative hygrometry was set at 60%. After smoking, the fillets were placed in a cold chamber at 3 °C during one night. The fillets were chopped, and a piece of 100 g taken from the middle of the fillet was put in a polyethylene bag and frozen at −20 °C during one week before extraction. Preliminary biochemical analysis on water and NaCl content was carried out before smoking. The rate of water was 64.57 g/100 g, and the rate of NaCl was 0.23 g/100 g.

Isolation of the Volatiles. A Likens−Nickerson apparatus was used for the preparation of the simultaneous distillation−extraction (SDE) extracts (*28*). A 500 mL round-bottom flask was used as the sample flask to contain 150 mL of purified water and 50 g of salmon or smoked salmon. A 30 mL round-bottom flask containing 30 mL of diethyl ether was linked to the upper arm of the SDE apparatus because the density of diethyl ether is lower than the density of water. The steam was cooled by the circulation of poly(ethylene glycol) at −5 °C. The contents in the sample and solvent flasks were heated to boiling. The temperature of the diethyl ether flask was maintained at 50 °C by a water bath. The distillation−extraction was continued for 3 h. The volume of the extract was reduced to 5 mL by evaporating the solvent using a Kuderna Danish apparatus and to 0.4 mL under a gentle cold stream of nitrogen. The aromatic extract in diethyl ether was introduced into 0.3 mL of ethanol, and diethyl ether was removed by evaporation under a gentle cold stream of nitrogen.

Representativeness of the Extract. SDE with diethyl ether, followed by a step of solvent change to obtain the extract in ethanol by the evaporation of diethyl ether, was carried out. Diethyl ether was used because it is less toxic than dichloromethane and leads to similar recovery yields concerning the smoked salmon matrix. The solvent change step is necessary to remove diethyl ether and obtain the extract in a neutral solvent such as ethanol. Thus, the olfactometric sessions are totally safe. A previous study assessed the representativeness of an aromatic extract of smoked salmon obtained by this method, and it led to a similarity mark of 72% (*28*), that is to say, quite satisfactory.

Gas Chromatography−Olfactometry (GC-O) Analysis. The GC-O system consisted of a 6890N GC (Agilent Technologies, Wilmington, DE) equipped with a FID, a mass detector (5973-Network), and a sniffing port ODP2 (Gerstel, Baltimore, MD) supplied with humidified air at 40 °C. The GC effluent was split 1:1:1 between the FID, the mass detector, and the sniffing port. Each extract (3 μL) was injected in splitless mode into a capillary column (DB-5MS, 30 m length × 0.32 mm id, 0.5 μm thickness) (J&W Scientific, Folsom, CA). The system provides simultaneously a MS signal for the identification and the quantification of the odor-active compounds. The injector and FID detector were set at, respectively, 270 and 280 °C. The flow rate of the carrier gas (helium) was 1.5 mL. min^{-1}. The temperature of the oven was programmed according to the following steps: from 70 °C (1 min) to 80 °C (1 min) at 3 °C..min^{-1}, then to 150 °C at 5 °C.min^{-1}, and, finally, to 280 °C (4 min) at 10 °C.min^{-1}.

Frequency-of-Detection (FDT) and Time−Intensity Methods. The panel was composed of eight judges (five females and three males between 24 and 49 years old) from our department]LBAI-ENITIAA (Laboratoire de Biochimie Industrielle et Alimentaire-Ecole Nationale d'Ingénieurs des Techniques pour les Industries Agricoles et Alimentaires]. They were all previously trained in odor recognition and sensory evaluation techniques and had experience in GC-O. Sniffing of the chromatogram was divided into two sessions of 19 min. Each judge participated in the sniffing of both parts, but during two separate sessions to remain alert. The panelists were asked to describe the odor and to give a mark of intensity to each detected odorant on a scale of 1−9 (1 very weak odor intensity, 9 very strong odor intensity). Detection of an odor at the sniffing port by fewer than three of the eight assessors was considered to be noise. Thus, two responses were followed with two olfactometric methods: first, the FDT method, given by the number of judges who perceived the odor (*29*), allows the selection of the significant odors and, second, the time−intensity (*30*) method, given by the average of the intensity marks attributed in the time by each judge who has smelled the odor.

Gas Chromatography−Mass Spectromety (GC-MS) Analysis. The GC-MS quantification of the compounds was carried out with the same device as described in the GC-O procedure. The injector and detector were set at, respectively, 270 and 280 °C. Helium was used as carrier gas with a flow rate of 0.5 mL.min^{-1}. A quadrupole mass selective detector, with electronic impact ionization (ionization energy 70 eV) operated in scan mode, with a mass range of 30−300 amu, at 2.0 scans/s, was used to detect the ions formed.

Compound identification was based on a comparison of retention indices (RI) (*31*), mass spectra (comparison with standard MS spectra databases: Wiley 6), injection of standards, and odor properties. When possible, the identification was confirmed by detection of the compounds in single ion monitoring (SIM) mode following, for each noticeable odorant, five of the most predominant ions present in their mass spectra. A confirmation of the presence of the compounds

identified was carried out using other GC-MS results obtained with a polar DB-Wax column (30 m length × 0.25 mm i.d., 0.5 μm thickness) and also with a less polar DB1 column (60 m length × 0.32 mm i.d., 0.5 μm thickness) (J&W Scientific, Folsom, CA).

The quantification was performed using dodecane as standard added just before the concentration step. The concentrations of volatile compounds are expressed in microgram equivalents of dodecane for 100 g of salmon.

RESULTS AND DISCUSSION

Odorant Compounds of Smoked Salmon. Eighty-eight odorant areas were detected in smoked fish extract by GC-O, and 74 were identified by GC-MS. Odor descriptions for compounds detected by GC-O in the aromatic extract of fresh salmon and the quantitative results are given in **Table 1**. Among them, 35 were perceived by at least seven of the eight assessors. As has already been reported with an apolar capillary column (*17*), the chromatogram of smoked salmon aromatic extract can be divided in two parts. The first is more characterized by cooked, fishy, and green odorant descriptors, and the second part is more smoked and burnt with the presence of many phenolic compounds.

Odorant Compounds of Smoked Salmon: Phenolic Odorant Compounds in Smoked Salmon. These compounds constitute the main odorant compounds, and they were all detected with an intensity of >5 and perceived by more than five judges with little burnt/roasted and spicy differences. Twelve compounds in particular seem to contribute to the overall odor of smoked salmon: *o*-cresol, *m*-cresol, guaiacol, 4-methylguaiacol, thymol, 4-ethylguaiacol, 4-vinylguaiacol, syringol, eugenol, 4-propylguaiacol, (*Z*)-isoeugenol, and (*E*)-isoeugenol. All of the phenolic compounds found in smoked salmon come from the thermal degradation of wood through the pyrolysis of lignin (*14*). Many studies have indicated that phenolic compounds present in the vapor phase of smoke may contribute to imparting a smoky flavor to foods. Some 85 different phenolic compounds have been characterized in smoke condensate but only 20 phenols in smoked product (*32*). It has been reported that only phenolics with a boiling point of 76−89 °C at 5.33 hPa carry a smoke-like flavor (*33*). Among them, syringol, guaiacol, 4-methylguaiacol, and eugenol may be the dominant contributors to the pleasant smoke-like aroma (*33*). By comparison with these results, we have found that guaiacol, 4-methylguaiacol, syringol, and cresol compounds are especially responsible for the smoke and burnt odor, whereas the other guaiacol derivatives, thymol, eugenol, and isoeugenol compounds, contribute more to spicy notes such as clove, vanilla or curry, licorice, and cinnamon. Guaiacol is the major compound in smoked salmon with a concentration of 345 μg of IS/100 g. It is important to note that odor thresholds of phenolic compounds are very different because thymol and guaiacol were detected by the same number of judges and with the same intensity mark of 5; however, thymol is 70 times less concentrated (4.84 μg of IS/100 g). Other phenolic compounds are also present at a concentration between 20 and 100 μg of IS/100 g: dimethylphenols (such as 3,4-dimethylphenol) or trimethylphenols (such as 2,4,6-trimethylphenol) are also found in smoked salmon and supply phenolic odors such as roasted, smoked, earthy, or burnt. Their odorant role is less obvious because they are perceived with a weaker intensity (from 4 to 6), by a lower number of judges (six or seven), but especially at a lower concentration (under 15 μg of IS/100 g). The exception is 2,5-dimethylphenol, found by seven assessors with an intensity of 7, probably due to its higher concentration of 28.60 μg of IS/100 g. The quantification of phenolic compounds seems to be satisfactory because the coefficients of variation calculated with each mean and standard deviation (SD) value were not often >10%. Nevertheless, for phenolic compounds with weak chromatographic signals such as 2,3-dimethylphenol and thymol, which leads to weak quantities, the SD values are more important because of the difficulty of quantification.

Odorant Compounds of Smoked Salmon: Furanic, Maillard, and Strecker Odorants Compounds in Smoked Salmon. Other compounds of smoked salmon with pleasant roasted notes were detected by the judges. They are furanic compounds created during the smoking process by thermal degradation. In wood smoke (*18*−*20*), furanic compounds such as furfuryl alcohol and furfural are mainly generated by separation of water from pentoses, which are decomposition products of hemicelluloses (*34*). Maillard reactions (and Strecker degradation) could also be proposed as pathways of creation for these compounds because Maillard reactions occur during smoking process and are responsible for the color of many smoked products (*35*). The fact that furanic compounds are not found in unsmoked salmon shows that it is not the extraction method used here but the smoking process that is responsible for the formation of these compounds. They are also present in processed seafood (ripening, curing, roasting) at a lower concentration than in wood smoke (*36*−*38*). The smoking process favors the generation of furanic compounds, which are deposited (if they come from the wood) or eventually generated in the fish flesh. They are all known to give burnt/cooked and roasted aromas to the food. Furanic compounds do not have strong odorant intensity (from 4 to 6) but were perceived by a large number of judges (never fewer than five). Their concentrations are lower than those of phenolic compounds, except for furfural, which is the second most common odorant in smoked salmon (299 μg of IS/100 g), and furfuryl alcohol (143.50 μg of IS/100 g). Nevertheless, the quantifications of furanic compounds in smoked salmon are less precise because of high SD values. As their chromatographic peaks are sufficiently significant, problems of quantification cannot be involved. As the furanic compounds such as furfural seem to derive from wood smoke because they are not found in fresh fish (*17*), heterogeneities of the wood smoke in the smokehouse or differences in the deposition of wood smoke odorants on fish flesh could better explain these variations. Two other furanic compounds are detected in smoked salmon, acetylfuran and 5-methylfurfural, which are often found in wood smoke. Acetylfuran has a strong impact on the smoked salmon aroma because it was found by seven judges with an intensity of 7. The contribution of 5-methylfurfural is lower. 2-Acetyl-5-methylfuran and benzofuran derivatives carry more green/chemical odors, which are sometimes unpleasant like rotten/moss for benzofuran. As for phenolic compounds, the weaker the signal is, the more difficult the quantification is and the higher the variation can be.

Pyrazines and heterocyclic nitrogen compounds could also be generated from Maillard reaction products with roasted, cooked, and smoked odors. Thus, 2-methylpyrazine, 2-acetyl-1-pyrroline, tetrahydropyran-2-one, and 1(*H*)-pyrrole carboxaldehyde with more chemical and resinous notes were identified in smoked salmon. 2-Methylpyrazine and 1(*H*)-pyrrole carboxaldehyde have already been reported as components of smoked fish (*10*, *14*), and 2-acetyl-1-pyrroline has been reported as a component of processed seafood (*39*, *40*). In smoked salmon, these two compounds are almost at trace level, especially 2-acetyl-1-pyrroline, but they have a strong odorant impact because they were perceived by eight judges for 2-acetyl-1-pyrroline and by six judges for (1*H*)-pyrrole carboxaldehyde.

Table 1. Identification and Odorant Charcteristics of Volatile Odor-Active Compounds of Smoked Salmon

compound	LRI (DB5)	means of identification or mass fragments of its mass spectrum[a]	odorant descriptors given by judges	intensity[b]	no. of judges[c]	concentration[d] (mean ± SD)
diacetyl	600	LRI	butter	4	6	Tr
3-methylbutanal	645	LRI, STD	green, coffee	4	4	Tr
acetic acid	680	MS, LRI, STD	sour, garlic, NC[e]	3	4	2.05 ± 1.78
1-penten-3-ol	688	MS, LRI, STD	fat, chemical	2	4	Tr
furfural or isomer	840	MS, LRI, STD	roasted, nutty	3	6	7.83 ± 1.68
furfural	859	MS, LRI, STD	roasted, nutty	4	6	299.02 ± 135.58
2-methylpyrazine	845	MS, LRI, STD	roasted nuts	3	5	5.99 ± 4.14
furfuryl alcohol	875	MS, LRI, STD	cooked	5	8	143.49 ± 57.98
2,4-hexadienal	904	MS, LRI	cooked vegetable, fishy, earthy	7	6	3.24 ± 1.86
tetrahydropyran-2-one	908	MS	cooked, smoked	6	7	0.14 ± 0.16
heptanal	914	MS, LRI, STD	cooked, leather, plastic	6	8	1.32 ± 0.50
2-methyl-2-cyclopentenone	920	MS, LRI, STD	soup, cooked food	7	7	21.72 ± 6.48
acetylfuran	925	MS, LRI, STD	cooked, sweet	7	7	33.68 ± 8.82
2-acetyl-1-pyrroline	935	LRI	oily, roasted, nuts, bread	7	8	Tr
5-methylfurfural	970	MS, LRI, STD	cooked, earthy, coffee	3	5	58.08 ± 14.02
benzaldehyde	980	MS, LRI, STD	floral, fresh, green	5	5	29.49 ± 7.70
phenol	992	MS, LRI, STD	marine, vinegar, metallic, sulfury	5	5	78.28 ± 24.18
benzonitrile	1003	MS, LRI	cooked potato, mushroom, fishy	5	6	0.64 ± 1.12
unknown	1012	39 (47), 41 (85), 67 (50), 69 (100), 112 (80)	NC	4	5	NQ
benzofuran	1015	MS, LRI	rotten	5	6	1.34 ± 2.60
(E,E)-2,4-heptadienal	1019	MS, LRI, STD	plastic, fat	5	5	Tr
1(H)-pyrrole carboxaldehyde	1030	MS	leather, resinous, chemical	5	6	1.78 ± 1.34
2-ethyl-1-hexanol/2-hydroxy-3-methyl-2-cyclopentenone	1038	MS, LRI, STD MS, LRI	spicy, green	5 6	6 6	4.34 ± 4.36 11.39 ± 8.32
2-acetyl-5-methylfuran	1048	MS, LRI	vegetal, solvent	6	7	2.33 ± 2.10
2,3-dimethyl-2-cyclopentenone	1052	MS	moss, woody, burnt rubber	5	8	19.10 ± 4.32
benzyl alcohol	1057	MS, LRI, STD	moss, solvent, chemical	5	6	Tr
benzeneacetaldehyde	1062	MS, LRI, STD	moss, solvent	5	7	2.90 ± 0.52
o-cresol	1068	MS, LRI, STD	smoke, burnt rubber	6	8	50.82 ± 5.50
unknown	1077	39 (18), 43 (25), 95 (100), 108 (15), 138 (25)	woody	5	6	NQ
unknown	1083	55 (95), 77 (90), 95 (75), 105 (100), 109 (85)	woody	4	4	NQ
acetophenone	1086	MS, LRI	vegetal, plastic	5	7	5.43 ± 1.88
p-cresol	1093	MS, LRI, STD	burnt, licorice, medicinal	7	8	67.92 ± 13.54
guaiacol/nonanal[f]	1110	MS, LRI, STD	smoke, vanilla, medicinal	7	8	344.98 ± 40.92
unknown[f]	1123	68 (30), 81 (55), 82 (30), 109 (55), 124 (100)	oily, plastic, earthy	6	7	NQ
2,6-dimethylphenol	1130	MS, LRI	roasted, phenolic, chemical	5	7	16.48 ± 2.60
unknown[f]	1136	55 (60), 79 (65), 91 (95), 122 (85), 126 (100)	earthy, plastic, cucumber	5	6	NQ
unknown	1142	43 (30), 81 (18), 91 (27), 109 (100), 138 (40)	raw vegetable, carrot	5	7	NQ
1,2-dimethoxybenzene	1147	MS, LRI	earthy, moss, woody, mouldy	5	7	7.78 ± 1.48
3-ethylphenol[f]	1153	MS, LRI	moss, earthy, woody, smoke	5	6	5.75 ± 0.38
unknown	1157	77 (75), 79 (95), 122 (80), 135 (100), 136 (100)	moss, cucumber	3	4	NQ
2,4-dimethylphenol[f]	1160	MS, LRI	carrot, green, violet, vanilla	5	6	Tr
2,5-dimethylphenol[f]	1167	MS, LRI		7	7	28.60 ± 3.36
1-methyl-1(H)-indene	1172	MS	plastic, phenolic	6	6	1.51 ± 1.48
3-methoxybenzaldehyde	1176	MS	burnt, amine	6	6	4.02 ± 0.88
3,4-dimethylphenol	1182	MS, LRI	chemical/burnt, smoked, roasted	6	6	11.98 ± 7.90
2,3-dimethylphenol	1184	MS, LRI	burnt, smoke, plastic	6	7	0.98 ± 1.92
4-methylguaiacol	1192	MS, LRI, STD	phenolic, smoke, plastic	5	7	33.66 ± 2.16
2-(2-butoxyethoxy)ethanol	1198	MS, LRI	fruity, plastic	6	6	Tr
naphthalene	1211	MS, LRI, STD	spicy, smoke, cold ashes	6	7	NQ
2,5-diformylthiophen[f]	1220	MS	burnt, smoke, green, earthy	4	5	5.11 ± 0.88
2,4,6-trimethylphenol	1229	MS, LRI	roasted, earthy, burnt, smoke	4	5	4.40 ± 0.42
4-methoxybenzaldehyde	1235	MS, LRI	gasoline, green, cucumber	4	7	Tr
4,7-dimethylbenzofuran	1240	MS	smoke, moss, spicy	6	6	1.01 ± 1.44
2,3-dimethoxytoluene	1247	MS, LRI	aniseed/green, smoke, NC	6	6	9.10 ± 4.92
3-ethyl-5-methylphenol	1260	MS	green/honey, smoke, NC	6	5	1.09 ± 1.54
(E)-2-decenal	1266	MS, LRI	plastic, green, cheese	6	6	Tr
thymol	1272	MS, LRI, STD	spicy, chemical, medicinal	7	8	4.84 ± 2.16
3,5-dimethoxytoluene	1282	MS, LRI	spicy, gasoline, chemical	7	7	11.08 ± 0.46
4-ethylguaiacol/2-undecanone[f]	1287 1296	MS, LRI MS, LRI, STD	peanut, vanilla, camphor, phenolic	5 5	6 4	97.35 ± 9.24 Tr
unknown	1300	58 (86), 115 (50), 134 (80), 145 (100), 160 (55)	sweet, leather, phenolic	4	4	NQ

Table 1. (Continued)

compound	LRI (DB5)	means of identification or mass fragments of its mass spectrum[a]	odorant descriptors given by judges	intensity[b]	no. of judges[c]	concentration[d] (mean ± SD)
4-vinylguaiacol	1330	MS, LRI, STD	medicinal, woody, spicy, smoke	6	8	40.82 ± 11.12
2-methylnaphthalene	1340	MS, STD	moss, plastic	6	7	3.68 ± 1.28
unknown	1352	43 (65), 55 (55), 121 (50), 145 (100), 146 (75)	minty, eucalyptus, citronella	4	4	NQ
2,3-dimethoxybenzaldehyde	1362	MS	spicy, aromatic plant	3	4	2.78 ± 2.10
syringol	1365	MS, LRI	spicy, smoke	5	5	28.78 ± 19.42
eugenol	1370	MS, LRI, STD	vanilla, clove, burnt rubber	6	7	47.60 ± 7.04
4-propylguaiacol	1382	MS, LRI, STD	clove, marine, vanilla, spicy	7	7	21.25 ± 2.84
1,2,3-trimethoxy-5-methylbenzene	1400	MS, LRI	burnt rubber, earthy, spicy	4	5	0.85 ± 0.60
(Z)-isoeugenol	1423	MS, LRI, STD	clove, spicy, coffee, burnt	6	8	19.94 ± 5.06
4-methylindanone	1444	MS, LRI	burnt, plastic, pine	6	5	3.92 ± 1.36
1,6-dimethylnaphthalene	1452	MS	green, burnt, spicy	5	4	NQ
unknown	1465	91 (70), 131 (73), 141 (77), 156 (100), 178 (80)	green, moss, woody, spicy	5	5	NQ
(E)-isoeugenol	1473	MS, LRI, STD	clove, fruity, cinnamon, fat	7	8	48.26 ± 13.48
dodecanol	1490	MS, LRI	raw carrot, medicinal	6	7	Tr
unknown	1500	162 (100), 166 (25), 167 (25), 174 (75), 179 (40)	green, woody, spicy	6	8	NQ
2,3,5-trimethoxytoluene	1527	MS, LRI	solvent, fruity, rubber	5	6	10.89 ± 6.44
dibenzofuran	1545	MS, LRI	rotten, rubber, fat, moss	6	5	3.22 ± 0.96
unknown[f]	1575	41 (60), 57 (100), 175 (75), 181 (73), 190 (55)	aromatic plant, roasted	5	8	NQ
1-hexadecene	1600	MS, LRI	burnt rubber, fat, oily, soup	6	7	3.29 ± 2.52
4-allylsyringol	1615	MS, LRI	burnt rubber, medicinal	6	4	3.81 ± 1.54
unknown	1665	41 (80), 55 (80), 79 (100), 81 (65), 91 (85)	woody, earthy, NC	5	6	NQ
8-heptadecene	1680	MS, LRI	leather	6	4	9.91 ± 1.70
hexadecanal	1808	MS, LRI	leather, burnt rubber, NC	5	4	34.82 ± 31.00
(Z)-9-octadecenol	1880	MS	cooked, leather, bread, wood	5	5	11.66 ± 6.34
unknown	1915	43 (78), 55 (65), 57 (100), 71 (70), 85 (50)	burnt rubber, leather, cooked	3	4	NQ
(Z)-9-octadecenal	1995	MS, LRI	cooked meat, sulfury, leather	5	4	13.58 ± 9.20
unknown	2190	39 (80), 43 (95), 55 (95), 57 (100), 82 (80)	marine, fresh, leather	3	4	NQ

[a] Means of identification: MS, mass spectrum (identified thanks to the mass spectra of the compounds); LRI, linear retention index (when the LRI of the compound identified corresponds to the LRI in the literature); STD, standard (when the retention time, spectrum, and odor description of an identified compound correspond to the retention time, spectrum, and odor description of the injected standard of this compound). For mass fragments, the proportion of the mass fragment is given in parentheses. When only MS or LRI is available for the identification of a compound, it must be considered as an attempt of identification. The odor given corresponds to the odor detected by the judges for its retention time but not surely to the compound that we try to identify. [b] Intensity is rounded to the nearest whole number. An intensity between 3 and 3.5 is rounded to 3 and an intensity between 3.5 and 4 is rounded to 4 (1 very weak odor intensity, 9 very strong odor intensity). [c] Number of judges who detected an odor. [d] In microgram equivalents of dodecane per 100 g of smoked salmon. The mean and the standard deviation are given for each identified and quantifiable compound. Each concentration is the mean of three aromatic extracts injected corresponding to three individual fillets smoked at 32 °C. Tr, trace; NQ, not quantified.[e] NC, not common descriptors. [f] Possibility of coelution.

Products of Strecker degradation were also detected in smoked salmon, such as 3-methylbutanal and benzeneacetaldehyde even if Strecker degradation is not the only origin of these molecules (*17*). 3-Methylbutanal is found at trace level with a weak impact (a frequency of detection of four judges with an intensity of 4) and benzeneacetaldehyde, which provides green aromatic notes perceived by seven panelists with an intensity of 5. Therefore, Maillard reaction and Strecker degradation products could strongly affect the overall smoked salmon aroma by varying the smoky flavor with green, roasted, nutty, earthy, and resinous aromatic notes.

Odorant Compounds of Smoked Salmon: Other Cyclic and Aliphatic Compounds in Smoked Salmon. Numerous cyclic compounds were also perceived in smoked salmon flesh. We can differentiate groups of derivatives of cyclopentenone, benzene, toluene, and benzaldehyde. They have been previously described in wood smoke in various studies (*19*, *20*, *41*). Cyclopentenone derivatives are known to be formed from cellulose pyrolysis and provide aromatic notes such as burnt/sweet and green (*18*). 2,3-Dimethyl-2-cyclopentenone was the most smelled by eight judges but with the weakest intensity of 5. 2-Methyl-2-cyclopentenone and 2-hydroxy-3-methyl-2-cyclopentenone were detected by seven and six assessors, respectively, with higher intensities. The cyclopentenone derivative concentrations range from 10 to 22 μg of IS/100 g and appear as the third most common compound family after phenolic and furanic compounds. Benzene derivatives, such as 1,2-dimethoxybenzene and 1,2,3-trimethoxy-5-methylbenzene, have been identified in smoked salmon, but no studies have reported their odors, which were named earthy/moldy/green for the first compound and more burnt and spicy for the second. The assessment of 1,2-dimethoxybenzene is easily established because of its concentration (7.78 μg of IS/100 g), its frequency of detection (seven judges), and its intensity (mark of 5), but for 1,2,3-trimethoxy-5-methylbenzene it is not obvious because of its low concentration, frequency of detection, intensity, and the absence of odorant descriptor in the literature. It is nearly the same case for toluene derivatives. Indeed, except for 3,5-dimethoxytoluene, the odor of which is in accordance with the literature, it was not possible to find previous odorant descriptors for 2,3-dimethoxytoluene (found with green, smoked, and fruity aromatic notes) and 2,3,5-trimethoxytoluene (found with solvent,

Table 2. Volatile Odor-Active Compounds of Smoked Salmon Present in Wood Smoke, Fresh Seafood, or Processed Seafood

	occurrence in		
compound	wood smoke	processed seafood	fresh seafood
diacetyl	21	38	45
acetic acid	18	14	50
2-pentanol	21	49	50
2,4-hexadienal	18		45
heptanal		38	50
benzonitrile		10	
(E,E)-2,4-heptadienal		38	29
2-ethyl-1-hexanol		38	29
benzyl alcohol	18	38	50
acetophenone	21	49	29
(E)-2-decenal		14	
2-undecanone		38	29
undecanal		48	
dodecanol		51	
hexadecanal		14	

fruity, and chemical aromatic notes). Nevertheless, their presence in smoked salmon was checked by MS spectra and LRI in accordance with the literature. Benzaldehyde and benzaldehyde derivatives exhibit green and spicy pleasant odors, like 4-methoxybenzaldehyde (even when found at trace level), which was perceived by seven judges with an intensity of 4, and 2,3-dimethoxybenzaldehyde. 3-Methoxybenzaldehyde gives more burnt/amine notes, but only an MS spectrum was obtained to assess its presence as 2,3-dimethoxybenzaldehyde.

Aromatic hydrocarbons, such as indene derivatives and naphthalene derivatives, were determined. Indene derivatives, such as 2,3-dihydro-(1*H*)-indene or 4-methylindanone, give a rubber, plastic, and resinous aroma to the smoked flesh. Naphthalene and its derivatives could be interpreted as polycyclic aromatic hydrocarbon contaminants through the smoking process or environmental contaminants through the rearing of the salmon. Naphthalene is responsible for a spicy/smoked odor, whereas 2-methylnaphthalene, described by seven judges with an intensity of 6, and 1,6-dimethylnaphthalene carry more green notes with the burnt/smoke overall odor. Even if naphthalene and its derivatives must be avoided in smoked salmon, they seem to play a role in the smoked aroma. Naphthalene and 1,6-dimethylnaphthalene could not be quantified because of problems of separation from other compounds. However, their odors were assessed by seven and four judges, respectively, with intensities of 6 and 5. Hexadecene was the most smelled aliphatic alkene, whereas 8-heptadecene was perceived by only half of the total number of judges with a leather odor and an intensity of 6. Nevertheless, 8-heptadecene concentration is about 9.91 μg of IS/100 g and that of hexadecene is about 2. Therefore, the odor threshold values of these two similar aliphatic hydrocarbons are very different and an increase in the carbonated skeleton can strongly influence the perception of the odor of the molecule.

It is interesting to note that certain compounds found in smoked salmon have been reported in wood smoke (*21*) or in fresh seafood (*29*), sometimes in both, or also in processed seafood (*38*). They are heterocyclic, such as benzonitrile, or aliphatic compounds, alcohols (2-pentanol, dodecanol), aldehydes (2,4-alkadienals, heptanal), ketones (2-undecanone), and acids (acetic acid). Carbonyls and alcohols, which have low odor threshold values, are detected at trace level except for hexadecanal, which is significantly abundant (34.82 μg of IS/100 g). These ubiquitous compounds are compiled in **Table 2** and illustrate the difficulty in assessing the origins of the odorants in smoked salmon. Indeed, the odorant compounds can derive from common lipid oxidation in unsmoked fish flesh, from lipid oxidation due to the smoking process conditions or from the wood smoke.

It is also important to note that the smoking process favors fatty acid degradation because some compounds not present in fresh salmon and known to derive from fatty acids are present in smoked salmon. This is the case for (*Z*)-9-octadecanol and (*Z*)-9-octadecanal with cooked odors, but more woody/pleasant for the first and more sulfury/leather for the second. Their concentrations are, respectively, 11.66 and 13.58 μg of IS/100 g. They have nearly the same weak frequency of detection (four/five judges), so they may not have a strong effect on the overall aroma. Nevertheless, their intensity is marked for both compounds at 5.

Odorant Compounds of Smoked Salmon: Chromatographic Coelutions and Impact on the Odors. Unexpected odors for several compounds, such as 2,4- and 2,5-dimethylphenol, were detected by the judges. They are identified in the odorant areas of the chromatogram that correspond to green and floral aromatic notes, whereas burnt, spicy/smoky notes were expected as for 2,3- and 3,4-dimethylphenol. This phenomenon can be explained by coelution with (*E*)-2-nonenal, which carries similar green and vegetal odors, observed at the same retention time in unsmoked salmon. It is also the same type of coelution between (*E*)-2-decenal and thymol, where (*E*)-2-decenal, with a plastic, green, and cheesy odor, has a retention time close to that of thymol, marked by spicy and chemical notes. Moreover, 10 of the 14 unknown compounds were assessed with green, floral, woody, and spicy notes. These odors are more similar to the descriptors of odorants of fresh salmon. It can be proposed that these odorants, which often have a low odorant threshold, are the unknown compounds of smoked salmon aroma but cannot be measured because of their very low quantities and so are hidden by the signal given by the important odorless volatiles of smoked salmon. The extraction method could also explain a part of the unknown compounds by the treatment of the sample.

Odorant Compounds of Unsmoked Fresh Salmon. Fifty-eight odorant areas were detected in unsmoked fish extract by GC-O, and 49 were identified by GC-MS in **Table 3**. Among them, 13 were perceived by at least seven of the eight assessors. Carbonyl compounds resulting from lipid oxidation are very present and contribute strongly to the overall fishy odor as *n*-alkanals, 2-alkenals, and 2,4-alkadienals.

Odorant Compounds of Unsmoked Fresh Salmon: *n*-Alkanals. All *n*-alkanals are produced from *n*-6 or *n*-9 polyunsaturated fatty acids (PUFA) present in fish flesh (*6*, *7*, *11*). Indeed, aldehydes from butanal to undecanal could derive from oleic acid (*n*-9 PUFA), detected in a large amount in salmon but with a weak odor of plastic/earth. *n*-Alkanals from hexanal to undecanal were identified in unsmoked flesh. They were detected by at least six judges, except decanal and undecanal, which were perceived by only four judges. Each alkanal was smelled very differently by the judges. Products of lipid oxidation with higher carbon atom number are also present in unsmoked fish flesh. As a result, hydroperoxides and carboxylic acids are created. Thus, tetradecanoic acid (with marine/fatty, cheese aroma) found in fresh salmon at a concentration of 5.83 μg of IS/100 g could be formed from hydrolysis of triglycerides but also from tetradecanal. Although the tetradecanal concentration is not very high (0.37 μg of IS/100 g), it was smelled by seven assessors with an intensity of 5. Following the same scheme of oxidation, hexadecanoic acid

Table 3. Identification and Odorant Characteristics of Volatile Odor-Active Compounds of Unsmoked Salmon

compound	LRI (DB5)	means of identification[a]	odorant descriptors given by judges	intensity[b]	no. of judges[c]	concentration[d] (mean ± SD)
diacetyl	600	LRI	butter	4	6	Tr
1-penten-3-ol	688	MS, LRI, STD	chemical, plastic	2	4	Tr
2-hydroxy-3-pentanone	710	MS, LRI	floral, dusty	3	4	$23.50 \times 10^{-3} \pm 4.76 \times 10^{-3}$
hexanal	805	MS, LRI, STD	cut grass, fruity, plastic	4	6	$380.74 \times 10^{-3} \pm 78.82 \times 10^{-3}$
unknown	835	43 (50), 44 (50), 45 (100), 57 (30), 70 (12)	roasted, burnt rubber	4	5	Tr
(E)-2-hexenal	865	MS, LRI, STD	eucalyptus, mushroom	5	6	1.06 ± 0.47
p-xylene	875	MS, LRI	solvent, phenolic	6	8	$1.24 \pm 43.21 \times 10^{-3}$
m-xylene	885	MS, LRI	plastic, phenolic	6	7	1.32 ± 0.38
o-xylene	900	MS, LRI	cooked vegetable	5	8	$117.66 \times 10^{-3} \pm 28.43 \times 10^{-3}$
heptanal	914	MS, LRI, STD	cooked potato, fat	7	7	2.13 ± 0.18
(Z)-4-heptenal	915	MS, LRI, STD	cooked vegetable, fishy	7	6	$673.37 \times 10^{-3} \pm 95.50 \times 10^{-3}$
methional	925	MS, LRI, STD	cooked potato	7	6	$670.53 \times 10^{-3} \pm 0.1$
2-acetyl-1-pyrroline	935	LRI	roasted, roasted bread/nuts	5	6	Tr
benzaldehyde	980	MS, LRI, STD	fruity, floral	4	5	$547.53 \times 10^{-3} \pm 86.30 \times 10^{-3}$
1-octen-3-ol	990	MS, LRI, STD	mushroom	5	6	$186.72 \times 10^{-3} \pm 24.76 \times 10^{-3}$
phenol	992	MS, LRI, STD	phenolic, sulfury, leather	6	7	$789.25 \times 10^{-3} \pm 0.16$
octanal	1009	MS, LRI, STD	cooked potato, fat, fishy, wax, citrus	6	6	2.23 ± 0.37
thiophenecarboxaldehyde	1012	MS, LRI	sulfury, earthy	5	4	Tr
(E,E)-2,4-heptadienal	1019	MS, LRI, STD	roasted	5	4	2.04 ± 0.25
2-ethyl-1-hexanol	1038	MS, LRI, STD	mushroom, cucumber, cooked vegetable	4	4	$1.20 \pm 92.26 \times 10^{-3}$
limonene	1042	MS, LRI	pine/chemical, floral/fresh	2	4	Tr
benzyl alcohol	1057	MS, LRI, STD	herbaceous, wet wood, floral	3	4	$200.43 \times 10^{-3} \pm 49.57 \times 10^{-3}$
benzeneacetaldehyde	1062	MS, LRI, STD	moss, spicy	3	4	$446.42 \times 10^{-3} \pm 70.45 \times 10^{-3}$
(E)-2-octenal	1076	MS, LRI, STD	moldy, pungent, cucumber/moss	4	5	$937.70 \times 10^{-3} \pm 66.89 \times 10^{-3}$
3,5-octadien-2-one	1098	LRI	plastic	5	5	1.54 ± 0.24
nonanal	1110	MS, LRI, STD	hospital, cucumber, vegetal	6	6	5.09 ± 0.17
(E,E)-2,4-octadienal	1111	MS, LRI, STD	phenolic, roasted/cucumber, cooked, fat	6	7	2.32 ± 0.38
unknown	1121	45 (45), 81 (30), 85 (100), 97 (15), 114 (100)	cooked meat, fat, green	5	7	NQ
menthatriene	1130	MS, LRI	green, cucumber, floral	5	7	$813.10 \times 10^{-3} \pm 0.19$
unknown	1144	40 (80), 43 (65), 57 (100), 119 (88), 133 (70)	roasted, burnt	5	5	NQ
unknown	1153	40 (100), 57 (75), 71 (75), 133 (90), 151 (50)	cut grass, spicy	3	4	NQ
(E,Z)-2,6-nonadienal	1160	MS, LRI, STD	floral, cucumber	6	7	$525.63 \times 10^{-3} \pm 60.09 \times 10^{-3}$
(E)-2-nonenal	1173	MS, LRI, STD	moss, woody, floral	6	6	Tr
decanal	1213	MS, LRI, STD	plastic, fishy, cardboard	4	4	2.89 ± 0.74
benzothiazole	1258	MS, LRI	green, plastic, fruity	4	7	$1.24 \pm 404.31 \times 10^{-3}$
(E)-2-decenal	1266	MS, LRI	cooked, plastic	3	5	$370.42 \times 10^{-3} \pm 66.15 \times 10^{-3}$
decanol	1280	MS, LRI	plastic, fatty,	4	4	Tr
unknown	1289	40 (100), 41 (88), 44 (88), 55 (95), 57 (95)	green, vanilla	6	6	NQ
2-undecanone	1296	MS, LRI, STD	nutty, green, fruity	4	6	$208.79 \times 10^{-3} \pm 48.57 \times 10^{-3}$
undecanal	1319	MS, LRI, STD	herbaceous, aniseed, fruity	4	4	1.31 ± 0.23
(E,Z)-2,4-decadienal	1319	MS, LRI, STD	fishy, medicinal	5	7	$349.06 \times 10^{-3} \pm 76.35 \times 10^{-3}$
1-methylnaphthalene	1325	MS, LRI	vegetal, cooked, green	5	5	$458.17 \times 10^{-3} \pm 0.16$
(E,E)-2,4-decadienal	1330	MS, LRI, STD	cooked, oily, solvent	6	6	$817.38 \times 10^{-3} \pm 0.15$
2-methylnaphthalene	1340	MS, LRI	marine, green, solvent	6	5	$309.90 \times 10^{-3} \pm 0.14$
unknown	1390	39 (75), 40 (60), 43 (100), 55 (100), 69 (80)	minty, green, spicy	5	6	NQ
aromadendrene	1441	MS, LRI	cucumber, vanilla, floral	4	7	$131.36 \times 10^{-3} \pm 39.66 \times 10^{-3}$
unknown	1472	41 (90), 43 (70), 55 (100), 69 (75), 83 (70)	rubber, sugar	4	4	NQ
1-pentadecene	1488	MS, LRI	rubber, plastic	3	4	3.45 ± 0.58
unknown	1540	41 (90), 55 (46), 69 (100), 95 (45), 109 (75)	plastic, fishy, earthy	3	6	NQ
tetradecanal	1625	MS, LRI	wet wood, marine, plastic	5	7	$369.71 \times 10^{-3} \pm 84.03 \times 10^{-3}$
unknown	1672	41 (90), 55 (100), 67 (95), 81 (95), 96 (80)	rubber, amine, algae	5	7	NQ
8-heptadecene	1680	MS, LRI	plastic, moss	6	5	23.96 ± 5.85
unknown	1735	43 (80), 55 (65), 57 (100), 71 (80), 85 (55)	sugar, plastic	4	4	NQ

[a] Means of identification: MS, mass spectrum (identified thanks to the mass spectra of the compounds); LRI, linear retention index (when the LRI of the compound identified corresponds to the LRI in the literature); STD, s tandard (when the retention time, spectrum and odor description of an identified compound corresponds to the retention time, spectrum and odor description of the injected standard of this compound). For mass fragments, the proportion of the mass fragment is given in parentheses. When only MS or LRI is available for the identification of a compound, it must be considered as an attempt of identification. The odor given corresponds to the odor detected by the judges for its retention time but not surely to the compound that we try to identify. [b] Intensity is rounded to the nearest whole number. An intensity between 3 and 3.5 is rounded to 3 and an intensity between 3.5 and 4 is rounded to 4 (1 very weak odor intensity, 9 very strong odor intensity). [c] Number of judges who detected an odor. [d] In microgram equivalents of dodecane per 100 g of unsmoked salmon. The mean and the standard deviation are given for each identified and quantifiable compound. Each concentration is the mean of three aromatic extracts injected corresponding to three individual fillets smoked at 32 °C. Tr, trace; NQ, not quantified. [e] NC, not common descriptor.

(with marine/fatty, fruity notes) could be formed from hexadecanal, identified as a volatile compound in fresh salmon but without odorant properties. Hexadecanal is the second odorant compound in fresh salmon, detected at a concentration of about 18.74 μg of IS/100 g, but it does not have a strong impact on the overall fresh aroma.

Odorant Compounds of Unsmoked Fresh Salmon: 2-Alkenals. 2-Alkenals, from 2-hexenal to 2-undecenal, are products of oxidation of fatty acids such as oleic acid, but they can also derive from *n*-6 PUFA, like arachidonic acid for aldehydes such as 2-heptenal or 2-octenal and like linoleic acid for (*E*)-2-alkenals from 2-heptenal to 2-nonenal and for (*Z*)-2-alkenals such as (*Z*)-2-octenal and (*Z*)-2-decenal (*41*, *42*). Thus, (*E*)-2-hexenal (with green aromatic notes), (*E*)-2-octenal (with less green and more unpleasant odors), (*E*)-2-nonenal (with moss, woody/floral descriptors), and (*E*)-2-decenal (with cooked, plastic odors) have been characterized in fresh salmon. They have very low odor thresholds because they were smelled by five or six judges with medium intensity marks and because they were in very low quantities. The most perceived and the most intense 2-alkenal identified is (*E*)-2-nonenal, which is at trace level. The concentrations of the other 2-alkenals do not exceed 1 μg of IS/100 g. From 2-hexenal to 2-decenal, the odor becomes less citrus and fruity and more fat-like, moldy, and unpleasant when the size of the carbonated skeleton increases.

Odorant Compounds of Unsmoked Fresh Salmon: 2,4-Alkadienals. 2,4-Alkadienals, such as decadienal and isomers, come from PUFA *n*-6 such as linoleic or arachidonic acid. All of these aldehydes give particular notes to the overall aroma. Indeed, they bring a fatty and a floral/fruity note, which decreases with the increase in the number of carbon atoms in the chain of the aldehyde. Except for (*E*,*E*)-2,4-heptadienal, which was detected by only four judges, the other 2,4-alkadienals were described by at least six judges with concentrations of about 2.04 or 2.33 μg of IS/100 g for (*E*,*E*)-2,4-heptadienal and octadienal, respectively. The concentration of (*E*,*E*)-2,4-decadienal is about 0.82 μg of IS/100 g, 2 times more than the concentration of (*E*,*Z*)-2,4-decadienal (0.35 μg of IS/100 g). Nevertheless, the variation of one judge in the frequency of detection or 1 unit in the intensity is not sufficient to show a trend between the concentration and the odorant perception of these compounds.

Odorant Compounds of Unsmoked Fresh Salmon: Other Carbonyl Compounds. Unsmoked salmon aroma is also very marked by (*Z*)-4-heptenal and methional, which provide a strong cooked potato odor. However, (*Z*)-4-heptenal also brings a slight fishy flavor. These two compounds are very close on the chromatogram and were detected by the same number of assessors; they have the same intensity of 7 and similar concentrations of about 0.67 μg of IS/100 g. Thus, their contribution is essential to the overall aroma of unsmoked salmon. Benzothiazole could also be identified as a Strecker degradation compound (like methional) and brings green, plastic, and fruity aromatic notes. (*E*,*Z*)-2,6-Nonadienal, 2-hydroxy-3-pentanone, 3,5-octadien-2-one have been reported in many seafood products with pleasant odors such as cucumber, fruit, and flower. In fresh salmon, several works have suggested that (*E*,*Z*)-2,6-nonadienal plays an important role in fresh fish-like odors due to its low threshold values (*6*, *7*, *13*). Indeed, it is found in unsmoked salmon at a weak concentration; however, it was perceived by seven judges who described it with an intensity mark of 6.

Odorant Compounds of Unsmoked Fresh Salmon: Aliphatic Compounds. The terpenes recovered in fish such as limonene, menthatriene, aromadendrene, and farnesol usually come from the diet. The environment of the salmon is therefore very important for the final odor because contaminants such as naphthalene derivatives and terpenes can be odorants in small quantities. Farnesol and limonene were measured at trace levels in unsmoked fish flesh, aromadendrene was found at 0.13 μg of IS/100 g, and menthatriene was found at about 0.81 μg of IS/100 g. Nevertheless, they were smelled by between four and seven judges for menthatriene and aromadendrene, but their intensities are not very high and, in general, marked at 4. 1-Penten-3-ol and 1-octen-3-ol have a fruity/chemical and a mushroom odor, respectively. These compounds have already been reported in many seafood products (*29*, *38*). Their low odor threshold makes them odorant at low concentrations. Unsmoked salmon aroma is also constituted by 2-acetyl-1-pyrroline with a roasted/nutty aroma. As in smoked salmon, it was detected at trace level but seems to be very predominant in the overall aroma because six panelists marked it with an intensity of 5.

Odorant Compounds of Unsmoked Fresh Salmon: Cyclic Compounds. Aromatic hydrocarbons, such as naphthalene derivatives, have been found olfactively in fresh salmon and could be considered as environmental contaminants. They were detected by five judges with an intensity close to 5/6 and are present in weak concentrations.

Other cyclic compounds were also smelled in fresh flesh in the form of benzaldehyde, benzenemethanol, and benzeneacetaldehyde, which exhibit floral and fruity notes. Their contribution to the fish aroma appears not to be very important because only four/five judges pointed them out and qualified them with intensity no higher than 4. Xylene isomers were strongly perceived by nearly all of the judges. Their intensity at around 5 or 6 seems not to be linked to their concentrations because *o*-xylene was detected at 0.12 μg of IS/100 g, whereas *p*- and *m*-xylene are 10 times more present in unsmoked flesh. These compounds can originate from carotenoid degradation and carry odors defined as plastic/fruity or cooked vegetables according to the geometry of the molecule. They have been reported as odor-active components of many fresh seafood products (*44*, *45*).

The unknown odors were generally marked by pleasant descriptors, but they cannot be identified due to a weak MS signal. Nevertheless, even with a weak signal and low concentrations, these compounds remain very odor-active.

Odorant Compounds of Unsmoked Fresh Salmon: New Compounds of Salmon Aroma. Fresh or processed (boiled, canned) salmon aroma has already been studied. By comparison with these previous works, 16 compounds have been identified in unsmoked salmon that were not identified in the past in this matrix. Nevertheless, some of these compounds were identified in fresh or processed seafood but not especially with odorant properties. Phenol, thiophenecarboxaldehyde, limonene, benzyl alcohol, benzeneacetaldehyde, (*E*,*E*)-2,4-octadienal, tetradecanoic acid, and farnesol have been reported as seafood volatile compounds (*12*, *13*, *29*, *30*, *39*). This is the first time that 2-hydroxy-3-pentanone, menthatriene, aromadendrene, 1-pentadecene, tetradecanal, 8-heptadecene, (*E*)-3-octadecene, and hexadecanal are identified in smoked salmon aroma. The presence of none of these compounds is surprising because alkenes, terpenes, and products of lipid oxidation are commonly found in seafood aroma. They do not have a strong impact on the overall odor because they are not perceived by a high number of judges and have low or medium intensities except for the terpenes and (*E*,*E*)-2,4-octadienal.

Quantitative and Odorant Comparison between Unsmoked and Smoked Salmon: Evolution of Odorant Compounds of Unsmoked Salmon during the Smoking Process. The overall aroma of unsmoked and smoked salmon has already been presented (*28*). Comparison between **Tables 1** and **3** explains the aromatic trends previously observed and sufficiently presents the differences from an "odorant descriptor" point of view. Thus, comparison between the two matrices must be more focused on quantitative and odorant parameters such as the concentration and FDT of the compounds identified. The compounds can be divided into three categories. Indeed, the first possibility is that the FDT of the compound is the same in fresh and smoked salmon. The second possibility is that the FDT varies, and it is possible to link these variations with variations in concentrations. The third category concerns the coelutions of odor-active fresh salmon compounds with weak odor-active or odorless smoked salmon compounds. As a result, odor mixtures, synergic or masked effects, can occur. The smoking process acts very unequally according to the odor-active compounds, and each case must be separately discussed.

Similar Odorants FDT in Smoked and Unsmoked Salmon: Case of Benzaldehyde. Benzaldehyde is perceived by five of the eight assessors in both types of matrix. We can conclude that the smoking process does not affect the odorant perception of these molecules. Nevertheless, the concentrations between the two extracts can be different but the odor descriptors lead to unchanged aromatic notes for both molecules. Thus, even if the concentrations are not the same (benzaldehyde is found in fresh salmon at a concentration of 0.55 μg of IS/100 g of product and at 29.49 μg of IS/100 g in smoked salmon), the smoking process does not create odorant differences for these compounds The variation in the intensity from 4 in unsmoked flesh to 5 in smoked fish is not significant enough to imply an effect of the process on the odor perception of this compound.

Variations in Odorants FDT between Unsmoked and Smoked Salmon and Relationship with Concentration: Cases of 2-Ethyl-1-hexanol, 2-Methylnaphthalene, and Phenol. For some compounds, it is easy to link an increase or a decrease in the frequency of the odorant perception with the variation in its concentration. This is the case for 2-ethyl-1-hexanol, for which the frequency of odorant perception increased from four to six while its concentration increased from about 1.20 to 4.34 μg of IS/100 g of salmon. This observation can be also carried out with 2-methylnaphthalene. The number of assessors who smelled it increased from five to seven between the unsmoked and smoked samples. This can be explained by the creation of aromatic hydrocarbons during the smoking process, which could increase the concentration and odorant perception of 2-methylnaphthalene. Indeed, its concentration was approximately 0.31 μg of IS/100 g of salmon in the fresh matrix and reached 3.68 μg of IS/100 g in the smoked matrix. However, it is important to note that the intensity mark between the two matrices does not vary.

Sometimes, it is difficult to confirm the odorant perception of a compound and to relate it to its concentration. Phenol, for example, was detected in smoked extract by fewer judges than in fresh extract (a decrease from seven to five assessors), whereas its concentration was multiplied by a factor of >100. In general, there is no linear relationship between odorant power and concentration. Besides, for some compounds, the quantification cannot be obvious. Therefore, it leads to higher SD values, which can be related to difference in perception of the judges. It is the case of phenol where the coefficient of variation in unsmoked salmon is 20.27% and that in smoked salmon is 30.89%. Then, the lack of homogeneity of the phenol content in smoked salmon could explain the global decrease of FDT. However, even if a linear relationship between FDT and concentration cannot be proven, global trends can be observed between the evolution of some FDT of compounds with their concentrations.

Coelutions in Smoked Salmon between Odor-Active Compounds from Unsmoked Salmon and Compounds of Smoked Salmon. Another explanation for the FDT decrease of phenol between unsmoked and smoked salmon is the phenomenon of coelution of odorless or odor-active aroma compounds of fresh salmon and smoked salmon. Indeed, there is a proximity of elution between phenol and 1-octen-3-ol. Therefore, their odor can very easily be mixed by the judges. In the salmon aromatic extracts, coelutions can occur under three forms according to the intensity and the odor descriptor of the smoked salmon compound. In the case where the smoked salmon coeluted compound is odorless, it causes only problems of identification. If it is odorant, it can strongly affect the odor. For example, in smoked salmon, dimethylphenols are found but related to unexpected green and fruity aromas. By comparing the aromatic profiles of unsmoked and smoked salmon, we have pointed out that, at the same retention time, there were similar odorant descriptors in fresh salmon but attributed to (*E*,*Z*)-2,6-nonadienal and (*E*)-2-nonenal. In this case, the MS signals of the two aldehydes are two small and hidden by the large signal of dimethylphenols. Nevertheless, thanks to a careful study of the mass spectra through extraction ions from the chromatogram of the SCAN acquisition, we have identified four of the five most important ions of (*E*,*Z*)-2,6-nonadienal [*m*/*z* 39 (25%), 41 (100%), 43 (5%), 69 (40%), 70 (45%)] and four for (*E*)-2-nonenal [*m*/*z* 41 (85%), 43 (100%), 55 (90%), 70 (86%), 83 (68%)] under dimethylphenol peaks. These aldehydes cannot be quantified in smoked salmon due to their too weak concentration. In unsmoked salmon, they have been identified because the noise in the fresh sample is lower than in the smoked sample loaded with many volatile compounds. It leads to mixtures of unexpected odors characterized as spicy by the judges but corresponding to mixtures of green/fruity for the aldehydes and burnt/smoke for the dimethylphenols. It is the same case for (*Z*)-4-heptenal in fresh salmon and 2-methyl-2-cyclopentenone found in smoked salmon. Indeed, 2-methyl-2-cyclopentenone is marked, like the cyclopentenone derivatives, with burnt/sugared aromatic notes in wood smoke but, in smoked salmon, the odorant descriptors become less burnt and more like cooked food. We can suggest an influence of (*Z*)-4-heptenal, which carries cooked vegetable/fishy odors, even if we did not manage to identify this compound under the 2-methyl-2-cyclopentenone peak except on one injection. Finally, the third case is when the smoked salmon coeluted compound is very odorant. As a result, the fresh salmon odorant descriptor can totally disappear (like for nonanal coeluted with guaiacol and 2-undecanone coeluted with 4-ethylguaiacol, where the phenolic odor masks the carbonyl odors).

By the study of the coelutions of smoked salmon chromatogram related to odorant descriptors, it becomes easier to understand how the smoking process influences the evolution of odor-active compounds between unsmoked and smoked salmon.

Creation of Smoked Salmon Odorants from Unsmoked Salmon Precursors. It is also very interesting to note that smoked salmon aroma contains odor-active compounds not detected in fresh salmon but known to derive from odorant or odorless precursors that are present in fresh salmon. Under

smoking process conditions, lipid oxidation continues (*46*). For example, 2,4-hexadienal comes from lipid oxidation and is revealed as odorant in smoked salmon, although it cannot be detected in fresh salmon. Oleic acid oxidation is also noticeable. Oleic acid is present and odorant in unsmoked salmon but is not recovered in the smoked sample, whereas we can observe the creation of the odorants (*Z*)-9-octadecenal and (*Z*)-9-octadecenol. It seems that industrial processes involving heat could affect the odorless (or odor-active) components of fresh salmon, which become the precursors of certain aroma compounds of smoked salmon. This is especially the case for carbonyl compounds (*47*).

Smoked salmon odor is a complex combination between its own odor of smoked salmon, due largely to wood smoke, fresh salmon aroma, and the odors created from fresh salmon components under the conditions of the smoking process. It is sometimes very difficult to suggest a simple origin for an odor-active compound because of the various possible routes of its creation. Moreover, it is not easy to elucidate certain odors that are the result of a combination of several odor-active compounds derived from various origins. The differences between smoked and unsmoked fish flesh involve masking and synergic effects in the odor perception.

Conclusion. Smoked and unsmoked salmon aroma profiles have been carried out, and the odor-active compounds have been individually characterized. The influence of the smoking process has been confirmed and assessed in an odorant way because the aroma compounds have been affected by the process. The concentrations and the odorant occurrences of the odor-active compounds of smoked salmon could be good indicators for the discrimination of all smoking processes. Their study also opens up new possibilities for adapting the process to increase the odors of smoked salmon. Indeed, such modifications of smoking parameters could change the concentrations and the odorant perception of certain aroma compounds of smoked salmon to increase the choice of products for the consumer. Phenolic compounds, in particular, can be followed as indicators of the smoke creation and intensity of the process. When the odorants of smoked salmon and their pathways of creation are known, it will be easier to modify the smoking process. This knowledge could also stimulate the production of smoked salmon with required odors. We can also imagine an application of this study to other smoked products such as fish or meat. However, the overall aroma of smoked salmon is built by complex combinations of odors, which will not be easy to favor without creating polycyclic aromatic hydrocarbons (PAHs) such as naphthalene and its derivatives that we have found in smoked salmon.

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LITERATURE CITED

(1) Sigurgisladottir, S.; Ingvarsdottir, H.; Torrissen, O. J.; Cardinal, M.; Hafsteinsson, H. Effects of freezing/thawing on the microstrucutre and the texture of smoked Atlantic salmon (*Salmo salar*). *Food Res. Int*. **2000**, *33*, 857−865.

(2) Cardinal, M.; Knockaert, C.; Torrissen, O.; Sigurgisladottir, S.; Mørkøre, T.; Thomassen, M.; Vallet, J. L. Relation of smoking parameters to the yield, colour and sensory quality of smoked Atlantic salmon (*Salmo salar*). *Food Res. Int*. **2001**, *34*, 537−550.

(3) Cardinal, M.; Gunnlaugsdottir, H.; Bjoernevik, M.; Ouisse, A.; Vallet, J. L.; Leroi, F. Sensory characteristics of cold-smoked Atlantic salmon from European market and relationships with chemical, physical and microbiological measurements. *Food Res. Int*. **2004**, *37*, 181−193.

(4) Espe, M.; Nortvedt, R.; Lie, Ø.; Hafsteinsson, H. Atlantic salmon (*Salmo salar*, L) as raw material for the smoking industry. II: Effects of different smoking methods on losses of nutrients and on the oxidation of lipids. *Food Chem*. **2002**, *77*, 41−46.

(5) Espe, M.; Kiessling, A.; Lunestad, B. T.; Torrissen, O. J.; Bencze Rørå, A. M. Quality of cold smoked salmon collected in one French hypermarket during a period of 1 year. *Lebensm.-Wiss. Technol*. **2004**, *37*, 627−638.

(6) Josephson, D. B.; Lindsay, R. C.; Stuiber, D. A. Volatile carotenoid-related oxidation compounds contributing to cooked salmon flavor. *Lebensm.-Wiss. Technol*. **1991**, *24*, 424−432.

(7) Josephson, D. B.; Lindsay, R. C.; Stuiber, D. A. Influence of maturity on the volatile aroma compounds from fresh Pacific and Great Lakes salmon. *J. Food Sci*. **1991**, *56* (6), 1576−1579.

(8) Girard, B.; Nakai, S. Static headspace gas chromatographic method for volatiles in canned salmon. *J. Food Sci*. **1991**, *56*, 1271−1274.

(9) Girard, B.; Durance, T. Headspace volatiles of Sockeye and Pink salmon as affected by retort process. *J. Food Sci.* **2000**, *65* (1), 34−39.

(10) Cardinal, M.; Berdagué, J. L.; Dinel, V.; Knockaert, C.; Vallet, J. L. Effect of various smoking techniques on the nature of volatile compounds and on the sensory characteristics of salmon meat. *Sci. Aliments* **1997**, *17*, 679−696.

(11) Josephson, D. B.; Lindsay, R. C.; Stuiber, D. A. Variations in the occurrences of enzymically derived volatile aroma compounds in salt- and freshwater fish. *J. Agric. Food Chem*. **1984**, *32*, 1344−1347.

(12) Refsgaard, H. H. F.; Haarh, A. M.; Jensen, B. Isolation and quantification of volatiles in fish by dynamic headspace sampling and mass spectrometry. *J. Agric. Food Chem.* **1999**, *47*, 1114−1118.

(13) Milo, C.; Grosch, W. Changes in the odorants of boiled salmon and cod as affected by the storage of the raw material. *J. Agric. Food Chem*. **1996**, *44*, 2366−2371.

(14) Guillén, M. D.; Errecalde, M. C. Volatile components of raw and smoked black bream (*Brama raii*) and rainbow trout (*Oncorrhyncus mykiss*) studied by means of solid-phase microextraction and gas chromatography/mass spectrometry. *J. Sci. Food Agric*. **2002**, *82*, 945−952.

(15) Guillén, M. D.; Errecalde, M. C.; Salmerón, J.; Casas, C. Headspace volatile components of smoked swordfish (*Xiphias gladius*) and cod (*Gadus morhua*) detected by means of solid-phase microextraction and gas chromatography−mass spectrometry. *Food Chem*. **2006**, *94* (1), 151−156.

(16) Cardinal, M.; Cornet, J.; Sérot, T.; Baron, R. Effects of the smoking process on odour characteristics of smoked herring (*Cuplea harengus*) and relationships with phenolic compound content. *Food Chem*. **2006**, *96*, 137−146.

(17) Jørgensen, L. V.; Huss, H. H.; Dalgaard, P. Significance of volatile compounds produced by spoilage bacteria in vacuum-packed cold-smoked salmon (*Salmo salar*) analyzed by GC-MS and multivariate regression. *J. Agric. Food Chem.* **2001**, *49*, 2376−2381.

(18) Maga, J. A. The flavor chemistry of wood smoke. *Food ReV. Int*. **1987**, *1*−*2*, 139−183.

(19) Guillén, M. D.; Ibargoitia, M. L. Influence of the moisture content on the composition of the liquid smoke produced in the pyrolysis process of *Fagus sylNatica* L. wood. *J. Agric. Food Chem*. **1999**, *47*, 4126−4136.

(20) Guillén, M. D.; Manzanos, M. J. Smoke and liquid smoke. Study of an aqueous smoke flavouring from the aromatic plant *Thymus Vulgaris* L. *J. Sci. Food Agric*. **1999**, *79*, 1267−1274.

(21) Guillén, M. D.; Manzanos, M. J. Study of the volatile composition of an aqueous oak smoke preparation. *Food Chem*. **2002**, *79* (3), 283−292.

(22) Sérot, T.; Baron, R.; Knockaert, C.; Vallet, J. L. Effect of smoking processes on the contents of 10 major phenolic compounds in smoked fillets of herring (*Cuplea harengus*). *Food Chem*. **2004**, *85* (1), 111−120.
(23) Tilgner, D. J. The phenomena of quality in the smoke curing. *Pure Appl. Chem*. **1977**, *49*, 1629−1638.
(24) Rørvik, L. M. *Listeria monocytogenes* in the smoked salmon industry. *Int. J. Food Microbiol.* **2000**, *62* (3), 183−190.
(25) Joffraud, J. J.; Leroi, F.; Roy, C.; Berdagué, J. L. Characterisation of volatile compounds produced by bacteria isolated from the spoilage flora of cold-smoked salmon. *Int. J. Food Microbiol.* **2001**, *66*, 175−184.
(26) Šimko, P. Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. *J. Chromatogr. B* **2002**, *770*, 3−18.
(27) Stołyhwo, A.; Sikorski, Z. E. Polycyclic aromatic hydrocarbons in smoked fish−a critical review. *Food Chem*. **2005**, *91* (2), 303−311.
(28) Varlet, V.; Prost, C.; Sérot, T. New procedure for the study of odor representativeness of aromatic extracts from smoked salmon. *Food Chem*. **2006**, in press.
(29) Pennarun, A. L.; Prost, C.; Demaimay, M. Identification and origin of the character-impact compounds of raw oyster *Crassostrea gigas*. *J. Sci. Food Agric*. **2002**, *82*, 1652−1660.
(30) Pennarun, A. L.; Prost, C.; Haure, J.; Demaimay, M. Comparison of two microalgal diets. 2. Influence on odorant composition and organoleptic qualities of raw oysters (*Crassostrea gigas*). *J. Agric. Food Chem.* **2003**, *51*, 2011−2018.
(31) Van Den Dool, H.; Kratz, P. D. A generalization of the retention index system including linear temperature programmed gas−liquid partition chromatography. *J. Chromatogr*. **1963**, *11*, 463.
(32) Toth, L.; Potthast, K. Chemical aspects of the smoking of meat and meat products. *AdV. Food Res*. **1984**, *29*, 87−158.
(33) Shahidi, F.; Naczk, M. Contribution of phenolic compounds to the flavor and color characteristics of foods. In *Phenolics in Food and Nutraceuticals*; CRC Press: Boca Raton, FL, 2003; pp 443−481.
(34) Jira, W. Chemical reactions of smoking. *Fleischwirtschaft Int.* **2004**, *4*, 21−25.
(35) Sainclivier, M. L'industrie alimentaire halieutique. Des techniques ancestrales à leurs réalisations contemporaines: salage, séchage, fumage, marinage, hydrolysats. *Bull. Sci. Tech. Ecole Natl. Supérieure Agron. Centre Recherches Rennes* **1985**, 219−285.
(36) Kawai, T.; Ishida, Y.; Kakiuchi, H.; Ikeda, N.; Higashida, T.; Nakamura, S. Flavor components of dried squid. *J. Agric. Food Chem.* **1991**, *39*, 770−777.
(37) Triqui, R.; Zouine, K. Sensory and instrumental assessments of the ripening process of anchovy (*Engraulis encrasicholus*). *Lebensm.-Wiss. Technol*. **1999**, *32*, 203−207.

(38) Chung, H. Y.; Yung, I. K. S.; Ma, W. C. J.; Kim, J. S. Analysis of volatile components in frozen and dried scallops (*Patinopecten yessoensis*) by gas chromatography/mass spectrometry. *Food Res. Int*. **2002**, *35*, 43−53.
(39) Triqui, R.; Reineccius, G. A. Changes in flavor profiles with ripening of anchovy (*Engraulis encrasicholus*). *J. Agric. Food Chem*. **1995**, *43*, 1883−1889.
(40) Lee, G. H.; Suriyaphan, O.; Cadwallader, K. R. Aroma components of cooked tail meat of American lobster (*Homarus americanus*). *J. Agric. Food. Chem*. **2001**, *49*, 4324−4332.
(41) Guillén, M. D.; Manzanos, M. J. Study of the components of a solid smoke flavouring preparation. *Food Chem*. **1996**, *55* (3), 251−257.
(42) Hsieh, R. J.; Kinsella, J. E. Oxidation of polyunsaturated fatty acids: mechanisms, products, and inhibition with emphasis on fish. *AdV. Food Nutr. Res*. **1989**, 233−341.
(43) Grosch, W. Reactions of hydroperoxides−products of low molecular weight. In *Autoxidation of Unsaturated Lipids*; Chan, H. W. S., Ed.; Academic Press: New York, 1987; pp 95−109.
(44) Piveteau, F.; Le Guen, S.; Gandemer, G.; Baud, J. P.; Prost, C.; Demaimay, M. Aroma of fresh oysters *Crassostrea gigas*: composition and aroma notes. *J. Agric. Food Chem*. **2000**, *48* (10), 4851−4857.
(45) Grigorakis, K.; Taylor, K. D. A.; Alexis, M. N. Organoleptic and volatile aroma compounds comparison of wild and cultured gilthead sea bream (*Sparus aurata*): sensory differences and possible chemical basis. *Aquaculture* **2003**, 225, 109−119.
(46) Sampels, S.; Pickova, J.; Wiklund, E. Fatty acids, antioxidants and oxidation stability of processed reindeer meat. *Meat Sci.* **2004**, *67*, 523−532.
(47) Fullana, A.; Carbonell-Barrachina, A. A.; Sidhu, S. Volatile aldehyde emissions from heated cooking oils. *J. Sci. Food Agric*. **2004**, *84*, 2015−2021.
(48) Girard, B.; Nakai, S. Sexual maturity stages of canned chum salmon segregated using static headspace volatile patterns. *Food Res. Int*. **1994**, *27*, 469−476.
(49) Tanchotikul, U.; Hsieh, T. C. Y. Volatile flavor components in crayfish waste. *J. Food Sci.* **1989**, *54*, 1515−1520.
(50) Chung, H. Y. Volatile components in crabmeats of *Charybdis feriatus*. *J. Agric. Food Chem.* **1999**, *47*, 2280−2287.
(51) Flament, I. Analysis of boiled crab meat flavour: identification of new alcoholic constituents. *Lebensm.-Wiss. Technol*. **1990**, *23*, 274−275.

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Bilan

L'efficacité de l'extraction a été évaluée sur solutions modèles constituées de composés connus pour être des composés volatils odorants récurrents des arômes de poissons et produits de la mer. La méthode d'extraction développée permet une extraction relativement indépendante de la nature des composés et a conduit à un rendement moyen de 70 à 75 %. Elle s'avère donc suffisamment quantitative et surtout non sélective (quasiment tous les composés sont extraits de la même façon). Ce travail sur standards a par conséquent permis de valider analytiquement la méthode.

Pour garantir l'innocuité de l'extrait lors des séances d'analyse sensorielle, nous avons choisi comme solvant d'extraction l'éther diéthylique qui a un point d'ébullition encore plus bas que le dichlorométhane (34,5°C versus 39,5°C) et qui est reconnu comme beaucoup moins toxique. Pour se prémunir d'éventuelles traces d'éther diéthylique, un changement de solvant a été réalisé de façon à obtenir l'extrait aromatique dans un petit volume d'éthanol (quelques centaines de µL) dépourvu d'éther diéthylique préalablement évaporé, l'éthanol étant neutre du point de vue toxique pour l'olfaction de tels volumes. Le volume de solvant employé a été optimisé pour garantir à la fois la sécurité de l'opérateur de l'extraction et du juge. Parmi les travaux sur la représentativité odorante d'extraits aromatiques, ce travail constitue l'un des premiers permettant de concilier une extraction-distillation simultanée des composés volatils odorants par solvants organiques avec une évaluation sensorielle de ces extraits totalement dépourvue d'éléments toxiques pour le juge.

Les notes de similarité obtenues entre le poisson fumé initial et son extrait redéposé en iso-intensité sur la même matrice non fumée, c'est-à-dire dans des concentrations équivalentes à celles quantifiées dans le saumon fumé, sont satisafisantes (> 70 %). Les tests de représentativité ont donc permis de valider de manière sensorielle la méthode d'extraction. D'autre part, la méthodologie d'évaluation de la représentativité s'avère également innovante puisque c'est la première fois que nous avons pu mettre en évidence l'importance de prendre en compte la matrice en rétablissant les interactions physico-chimiques entre les composés de l'extrait et les constituants de la matrice. L'apport de la redéposition des extraits aromatiques sur matrice réelle par rapport à des mouillettes en carton a pu pour la première fois être quantifié et améliore la représentativité de 10 %. En faisant varier les quantités de la matrice de redéposition de l'extrait, nous avons pu optimiser les conditions des tests de représentativité. Les notes de similarité obtenues font partie des plus hautes parmi celles retrouvées dans les travaux traitant de la représentativité odorante d'extraits aromatiques.

Ce développement méthodologique nous a permis d'identifier et de quantifier respectivement 74 et 49 composés volatils odorants du saumon fumé et du saumon non fumé. Plus de 84 % des

composés volatils odorants du saumon frais et du saumon fumé ont ainsi été identifiés et quantifiés. La non identification de 16 % des composés volatils odorants du saumon frais et fumé est liée à leur présence en trop faibles quantités. La plupart des composés identifiés ont été reliés à leurs précurseurs. En effet, l'analyse en parallèle des composés volatils odorants du saumon non fumé et du saumon fumé a permis de différencier quatre catégories de composés : ceux propres à la chair de poisson, ceux provenant de la fumée de bois, ceux créés par des réactions entre les constituants de la chair de poisson sous l'effet des paramètres du fumage et enfin, ceux créés entre les constituants de la chair de poisson et la fumée de bois sous l'effet des paramètres du fumage. Les acides gras du saumon et la fumée de bois ont une grande responsabilité dans la création de composés volatils odorants.

Les caractéristiques odorantes de tous les composés volatils odorants ont pu être déterminées par les juges, même pour les composés à l'état de traces. Toutefois, certains composés identifiés furent perçus par les juges avec des odeurs individuelles différentes de celles communément retrouvées dans des extraits aromatiques de matrices alimentaires semblables. Ces différences peuvent être engendrées par des coélutions chromatographiques. L'utilisation de colonnes chromatographiques de polarité différentes a été réalisée dans le but de lever ces coélutions chromatographiques mais n'a pas permis de mieux identifier les composés coélués, sans doute présents à l'état de traces. Une analyse chromatographique bidimensionnelle en phase gazeuse GC/GC-MS/O des extraits aromatiques obtenus serait certainement très utile. En effet, des premiers essais non publiés menés sur les composés volatils d'un extrait aromatique de saumon fumé par GC/GC-MS (TOF) ont permis de se rendre compte de la multitude de coélutions chromatographiques existantes entre les composés d'un extrait aromatique. Dans le cas de composés volatils odorants, une coélution entre un composé chromatographiquement majoritaire mais avec un seuil de perception odorante plus élevé que celui d'un composé à l'état de traces coélué avec lui peut être responsable de la différence de la perception odorante du composé majoritaire. Par exemple, les caractéristiques odorantes des 2,4 et 2,5-dimethylphenols identifiés dans le saumon fumé ne correspondent pas avec celles trouvées dans la litérature. Les juges ne sont pas consensuels sur les descripteurs mais perçoivent tout de même des notes végétales inattendues. Or, dans la même zone chromatographique, le (E,Z)-2,6-nonadienal et le (E)-2-nonenal, composés carbonylés à très faible seuil de perception odorante, ont été identifiés dans le saumon non fumé. Les quantités importantes des diméthylphénols ne permettent pas d'identifier les composés carbonylés mais ces composés carbonylés pourraient être coélués avec les composés phénoliques et leurs seuils de perception odorante faibles leur permettraient d'être odorant même à l'état de traces.

PARTIE 2 : Qualité organoleptique et sanitaire (HAP) du saumon fumé à froid par différents modes industriels de génération de fumée

Partie 2 : Qualité organoleptique et sanitaire (HAP) du saumon fumé à froid par différents modes industriels de génération de fumée

Dans les dernières décennies, des développements matériels et des innovations technologiques ont vu le jour dans le but d'optimiser les procédés de fumage. Ainsi, des générateurs de fumée externes ont été développés, l'utilisation de condensats de fumée s'est généralisée, en particulier aux Etats-Unis. En effet, aujourd'hui, en Europe, dans l'industrie, la fumée pour le fumage de poisson à froid est produite le plus souvent par autocombustion (environ 65 %) et par plaques thermostatées. Viennent ensuite la revaporisation de fumée liquide (essentiellement pour les produits carnés) et la friction. Ces générateurs externes ont été développés notamment pour réduire la concentration des HAP dans la fumée se déposant sur les filets dans la cellule de fumage. La diversité de ces modes de production de fumée a entraîné l'augmentation de la variété des produits fumés mais nécessite d'être étudiée dans le but de maîtriser la contamination de ces aliments. En effet, il n'existe pas de travaux exhaustifs portant sur les conséquences sanitaires de ces différents procédés de fumage alors qu'ils vont être amenés à être de plus en plus utilisés afin de garantir l'innocuité des produits fumés.

De plus, la diversité des générateurs de fumée et des pratiques de fumage conduisent à une large gamme de produits fumés. En effet, dans certains pays, la température minimale du fumoir pour un fumage à froid est de 16°C alors que dans d'autres pays, elle sera de 22°C. De même, les différents générateurs produisent de la fumée à des températures de pyrolyse différentes, ce qui conduit à des compositions de fumées variées. Tous ces développements technologiques doivent donc être étudiés pour connaître leurs influences sur les propriétés sensorielles des produits fumés et améliorer leurs qualités organoleptiques.

Ainsi, une méthode de dosage des HAP a été développé pour caractériser la qualité sanitaire de différents filets de saumon fumés par les quatre principaux modes industriels de génération de fumée. Les équipements de la halle de technologie alimentaire de l'IFREMER nous ont permis de fabriquer ces différents produits dans des conditions similaires à celles utilisées dans les industries, conduisant à des filets de saumon fumé de qualité commerciale. En plus du type de générateur de fumée, nous avons voulu connaître l'influence des paramètres « temps de fumage » et « température de fumage » en faisant varier respectivement le temps de fumage de 1, 2 à 3 heures et la température du fumoir étant fixée soit à 22, soit à 32°C. L'évaluation de la contamination en HAP a été réalisée par le dosage du benzo[a]pyrène dans tous les filets au LABERCA. En effet, la contamination des poissons fumés par les HAP est fixée par une quantité maximale de 5 µg de benzo[a]pyrène /kg de matrice. Une fois l'innocuité des différents produits fumés démontrée, nous avons mis en place un protocole d'analyse sensorielle pour caractériser l'odeur globale ainsi que la texture, la flaveur et

l'aspect de ces différents produits afin d'identifier les conséquences organoleptiques générales des procédés de génération de fumée sur le saumon. Les qualités sensorielles des différents saumons fumés ont été évaluées par un panel de juges entraînés de l'IFREMER. Ainsi, ces premiers résultats nous ont permis de dresser un comparatif entre les différentes méthodes de production de fumée sur des critères organoleptiques et sanitaires.
Par conséquent, les effets de la nature des générateurs et des paramètres des procédés de fumage qu'ils soient d'ordre technique (temps, température) ou économique (consommation de bois, …) sur les qualités sensorielles et sanitaires du saumon fumé (goût, odeur et concentration en benzo[a]pyrène) ont été valorisées sous la forme d'une publication dans *Journal of Food Science and Agriculture*.

Cependant, le suivi du seul benzo[a]pyrène comme HAP de référence ne semble aujourd'hui pas suffisant compte-tenu des récentes études toxicologiques menées sur les effets d'autres HAP de haut poids moléculaires comme les dibenzopyrènes et les éventualités synergiques entre les HAP dans un mélange. Ainsi, le dosage de tous les HAP suspectés d'être carcinogènes par les derniers règlements européens (EC 208/2005, 2005a ; EC 1881/2006, 2006) a été entrepris sur les différents saumons fumés (excepté le benzo[c]fluorène, le règlement 1881/2006 ayant été publié après nos analyses). Un développement méthodologique de caractérisation des HAP a donc été mis en place en conformité avec les recommandations de l'Union Européenne exigées pour le benzo[a]pyrène (EC 10/2005, 2005c). Ce développement méthodologique demeure en partie utilisé dans le protocole de la méthode officielle de dosage des HAP dans les matrices alimentaires. Le point critique dans une telle stratégie analytique est constitué par la composition biochimique de l'aliment. En effet, le saumon est une matrice alimentaire riche en lipides pour lesquels les HAP ont une forte affinité. La méthode d'extraction doit être optimisée de telle sorte à éviter la co-extraction des HAP et des matières grasses. Celles-ci peuvent non seulement gêner l'extraction mais également être responsables de coélutions chromatographiques préjudiciables pour l'identification et la quantification des HAP. Ainsi, l'extraction doit permettre de récupérer les HAP tout en limitant les matières grasses. Toutefois, même dans les meilleures conditions, de faibles quantités résiduelles de lipides peuvent être présentes en fin d'extraction. Afin d'augmenter la précision de détection et de quantification des analytes suivis, l'analyse des extraits obtenus a été réalisée par chromatographie en phase gazeuse couplée à la spectrométrie de masse en tandem. Comme les HAP ont une structure chimique condensée qui les rend très stables, la deuxième ionisation des HAP dans la cellule de collision a pour effet de refragmenter les éventuelles substances coéluantes tout en altérant pas les HAP.

L’étude des effets de la nature des générateurs et des paramètres des procédés de fumage sur les concentrations en HAP suspectés d’être potentiellement toxiques et le développement méthodologique d’analyse des HAP dans une telle matrice a fait l’objet d’une publication parue dans *Food Additives and Contaminants*.

Organoleptic characterization and PAH content of salmon (*Salmo salar*) fillets smoked according to four industrial smoking techniques

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Abstract: Four industrial processes for smoking food were studied through their effects on the organoleptic properties of smoked salmon and on the occurrence of 20 polycyclic aromatic hydrocarbons (PAHs) known as being contaminants of smoking processes. The contamination by PAHs of the food might be measured by their corresponding toxic equivalent quantity (TEQ) expressed in μg kg^{-1}. The results show a significant correlation between the smoking process parameters, the odour of the smoked fish and the presence of PAHs. Smouldering, thermostated plates and friction smoking processes allow smoked fish with very close odorant characteristics to be obtained. However, differences of pyrolysis temperature (between 380 and 500 °C) causes significant differences of PAHs concentration even if the contents are under the legal threshold concerning benzo(a)pyrene (5 μg kg^{-1}). Smoked fish obtained by liquid smoke vaporisation presented the lowest level of PAHs but benzo(a)pyrene concentration is nevertheless important. The odours brought by the liquid smoke process are more 'cold smoke' and 'vegetal/green' than the other techniques, which are smokier and fishier.

Keywords: smoking process; smoke generation; PAH; organoleptic properties

INTRODUCTION

Smoking is one of the oldest food preservation methods. It consists of the application of wood smoke on food. However, nowadays, this process is predominantly used to give food organoleptic characteristics than for food preservation. The industry has developed four main types of smoking process very dependent of the technique for generating wood smoke.[1] In 2002, the proportion of smoking processes for fish in Europe was close to 65% for smouldering, 30% for thermostated plates, and the rest for friction and liquid smoke vaporisation. Smouldering, thermostated plates and friction processes allow smoke to be produced by pyrolysis of wood sawdust, wood chips and wood log, respectively.[2] According to the nature of the wood and to the parameters of pyrolysis (e.g. temperature, moisture, oxygen), the wood smoke is very different from an aromatic point of view.[3] In recent decades, liquid smoke vaporisation was used. It consists in the atomisation (vaporisation) of liquid smoke obtained from condensation of wood smoke. This process leads to an important diversity of products. Additionally, the control of smoke contaminants is easier in liquid smoke in order to avoid the formation of polycyclic aromatic hydrocarbons (PAHs).[4] These carcinogenic molecules are produced during pyrolysis of organic material such as wood. During the production of liquid smoke, some steps allow a reduction of the PAH rate in the final liquid smoke whereas it is not easy to control the PAH rate during traditional methods of smoke generation. Moreover, the storage of liquid smoke in polyethylene flasks can also reduce the concentration of PAHs.[5,6] Nevertheless, the legal threshold of benzo(a)pyrene is lower for the smoke flavouring additives than for the other smoking procedures. With regards to smoking techniques applying wood pyrolysis, modern facilities allow the smokehouse and the wood smoke generator to be separated. However, even if food is placed in a separate chamber from the wood smoke generator and even if wood smoke is cleaned by various electrostatic filters or smoke washing, the occurrence of PAHs can be minimised but not totally controlled and suppressed.[7] Better knowledge of smoking parameters, particularly smoke production parameters, could allow optimisation of the organoleptic characteristics of smoked food and safer products to be obtained. Therefore, the aim of this study was to compare the effects of four smoking techniques on the organoleptic

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characteristics of smoked salmon fillets with the simultaneous evaluation of the PAH content.

MATERIALS AND METHODS

Raw material and reagents

Ultrapure water was obtained with a MilliQ® system. All solvents for PAH analysis were of analytical or HPLC grade and were purchased from SDS (Peypin, France) except toluene and tetrahydrofurane from Fluka (Buchs, Switzerland). The phenyl-2,3-dimethyl-4-amino-5-pyrazolone came from Merck (Darmstadt, Germany) and the phenolic standard solution at 1 mg L^{-1} (Prolabo, Fontenay-sous-bois, France).

ENVI Chrom P cartridges of 6 mL, 0.50 g bonded phase of styrene divinylbenzene resin were obtained from Supelco (Bellefonte, USA). All mixes of PAHs were from LGC Promochem (Wessel, Germany) except benzo(a)anthracene, chrysene and benzo(a)pyrene, which were obtained from Chiron (Trondheim, Norway).

Beechwood sawdusts came from SPPS (Paris, France) and beechwood log from Bourdeau (Nozay, France)

Fish processing

Salmon (*Salmo salar*) reared in Norway were purchased from a seafood wholesaler (Nantes, France). The time between the capture and the filleting was less than 1 week. Nine gutted fishes of approximately 3–4 kg from the same batch were received in a box in ice. They were directly filleted, trimmed and put on grids in a cold chamber at +3 °C for 2 h. All the fillets were about 1 kg. Biochemical analysis of water and NaCl content were carried out before smoking. The rate of water was 65 g 100 g^{-1} and the rate of NaCl was 0.20 g 100 g^{-1}. Dry matter content was analysed by oven drying of 2 g of smoked salmon at 105 °C until a constant weight was reached and salt content was measured with Chloride Analyser 926 (Corning, Halstead, UK). Next, they were hand-salted with refined salt (Salins du Midi, France) and left for 3 h at +12 °C before being rinsed on grids with water (15 °C) and stored at 3 °C for 18 h until smoking.

For the evalution of PAHs, two smokehouse temperatures were studied (32 and 22 °C). Before smoking, a drying step was carried out by putting the fillets in the smokehouse at 18 °C for 15 min in order to standardise the internal temperature at 8 °C for all the samples. Then, at the beginning of the smoking process, fillets had the same inner temperature whatever the smokehouse temperature. After smoking the fillets were stored during less than 1 week at +2 °C before sensory analysis. The medium parts of smoked fillets (about 200 g) were left at −80 °C and freeze-dried for analysis of PAHs.

Smoking equipment and procedures

The smokehouse was an HMI Thirode (PC90 Model) device (Thirode, France), 1500 × 1300 × 2250 mm with a capacity of 380 kg, mounted on a trolley with 28 grids on which the fillets were deposited. For each smoking technique, the fillets were placed at the same level (grid numbers 10, 12 and 14) at 20 cm of the door of the smokehouse. The air/smoke circulation was horizontal. Salmon fillets were swept by the smoke for 1, 2 and 3 h at a temperature of 22 and 32 °C. The exhaust valve opening was 1/3 and closed for liquid smoke and the relative hygrometry was sat at 60%. For each process except liquid smoke, the smoke was introduced in the smokehouse with a flow rate of 90 m^3 h^{-1}.

For the evaluation of PAHs, three fillets were removed from the smokehouse each hour during 3 h in order to measure the formation of PAHs according to the time of smoking and the temperature of the smokehouse. For sensory assessment, only the fillets smoked for 3 h at 32 °C were compared.

The smokehouse consists of a separate chamber from the wood smoke generators. The smoke is purified through filters and introduced into the smokehouse thermostated through a tube that permits a decrease of smoke temperature close to the room temperature.

Smouldering parameters

A generator (Thirode, France) produced smoke by pyrolysis (between 400 and 450° C) of beech sawdust using the smouldering method. The sawdust was poured onto an electrically heated ring and pyrolysed. The pyrolysis temperature was determined with a probe placed on the heated ring. The ring was heated only for the ignition period and was maintained further only by electric pulses. The pyrolysis was also maintained as result of an air intake producing a continuous flow around the heated ring by a fan. The sawdust, which was in a hopper, fell by gravity onto the heated ring. Introduction of sawdust was programmed every 6 min. The sawdust was moistened and homogenised beforehand in order to obtain a moisture rate of 20%.

Thermostated plates parameters

A generator 720 × 1120 × 1730 mm (Thirode, France) produced smoke by pyrolysis (500 °C) of beech chips. The pyrolysis temperature was determined with a probe placed on the plates. The chips were spread mechanically onto thermostated plates and the plates were cleaned after 3 min of combustion. The smoke was pulsed by a ventilator in order to obtain the same flow rate of smoke in the smokehouse than smouldering and friction.

Friction parameters

A generator type FR 1002 (Muvero, the Netherlands) produced smoke by friction (380 °C) by pressing a beech log (8 × 8 × 10 cm) against a rotating friction wheel during 10 s and every 30 s. The pyrolysis temperature was determined with a probe placed into the log. The beech log is pressed pneumatically

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by means of a wood gripper with a pressure of 3.5×10^2 kPa (3.5 bars).

Liquid smoke parameters

Liquid smoke was purchased from a manufacturer of smoke flavourings (France). It is a purified condensate of beech smoke. Liquid smoke is atomised by pressurised air in the smokehouse at ambient temperature. The vaporisation device (Lutetia, France) allows the pressures of air and liquid smoke to be set in order to obtain a consumption of liquid smoke of 1 L h^{-1} as in industrial procedures. Liquid smoke was injected in the smokehouse for 2 min every 3 min. For this type of smoking process, the hygrometry of the smokehouse was sat at 70%.

Sensory evaluation

Sensory evaluation of smoked salmon odours were performed by a panel of 20 subjects recruited in IFREMER laboratories and trained in the sensory analysis of fish. Descriptive tests enabled the samples to be evaluated by their organoleptic characteristics. Sessions were performed in individual partitioned boxes equipped with a computerised system (Fizz, Biosystèmes, Couternon, France). The panellists were asked to evaluate the intensity of organoleptic descriptors (six for odour and seven for taste evaluation) on a continuous scale displayed on the computer screen, from 0 (low intensity to 10 (high intensity). The descriptors used were chosen according to their efficiency to differentiate fish sample characteristics. Products were assigned three-digit numbers and randomised. The average of the different marks was used to build the sensorial profiles of the different smoked salmons. Sensory analysis was carried out only on salmon smoked for 3 h at 32 °C.

Total phenolic compounds analysis

All the procedures for the quantification of total phenolic compounds have been described in previous studies.[8]

PAH analysis

The method for determining PAHs has been described by Monteau *et al*.[9]

Solid–liquid extraction

Two grams of freeze-dried fillet, spiked with a mixture of the 20 ^{13}C-labelled PAHs at a concentration of 1 µg kg^{-1} (as internal standards), were homogenised in 40 mL of cyclohexane/ethyl acetate (50:50, v/v), shaken for 30 min, then centrifuged at $5000 \times g$ for 30 min at 0 °C. The liquid part was carefully isolated and evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 6 mL of cyclohexane. Quantification of PAHs was the result of the mean of measures carried out on three individual fillets smoked under the same conditions.

SPE clean-up procedure

For the purification stage, the solid-phase extraction cartridges ENVI Chrom P cartridges of 6 mL, 0.50 g bonded phase (Supelco, Bellefonte, USA) were conditioned with 5 mL of water, then 5 mL of methanol followed by 5 mL of cyclohexane. Six millilitres of sample in cyclohexane were then introduced onto the cartridge and washed with 3 mL of cyclohexane in order to remove fat matter, which interfers with the results. The PAHs were eluted with 12 mL of a mixture of cyclohexane and ethyl acetate (50:50, v/v) and after, evaporated to dryness under nitrogen stream. The residue was finally dissolved in 40 µL of toluene.

GC/MS/MS analysis

For GC/MS/MS experiments, an HP-6890 gas chromatograph (Agilent Technologies, Palo-Alto, USA) was coupled to a MS–MS Micromass Quattro Micro mass spectrometer (Waters Milford, USA). A glass insert, 4 mm i.d., loosely filled with silanised glass wool was used in the split/splitless mode GC injector (280 °C). The GC column Zebron was a ZB-5MS 30 m × 0.25 mm i.d., film thickness 0.25 µm (Phenomenex, Torrance, USA). The GC oven temperature was maintained at 110 °C for 1 min after injection then programmed at 20 °C min^{-1} to 240 °C, then at 5 °C min^{-1} to 340 °C. Helium was used as the carrier gas. Electron ionisation was used and selected reaction monitoring (SRM) was selected as the acquisition technique. Monitored ions were those of PAHs from fluorene to benzo(g,h,i)perylene listed amongst the US EPA 16 priority pollutants with, in addition, cyclopenta(c,d)pyrene, 5-methylchrysene, benzo(j)fluoranthene and dibenzo(a,l)pyrene, dibenzo(a,e)pyrene, dibenzo(a,i)pyrene, dibenzo(a,h)pyrene. The last added PAHs were those recently recommended by the European Commission.[10]

RESULTS

Economic approach of wood smoke generation

Among the smoke generation techniques studied (Table 1), thermostated plates process is the mode that uses the weakest wood quantity but has the greatest electric consumption (1.8 kW h^{-1}) due to the temperature of the plates which must be hold at 500 °C. The smouldering technique involves more sawdust and generates ash (110 g h^{-1}) but the consumption of electricity is more than two times lower than in thermostated plates technique (0.7 kW h^{-1}). Friction implies a similar quantity of wood pyrolysed but the ash generated is nearly two times lower than in the smouldering mode (60 g h^{-1}). However, the cost of the wood log is higher and the consumption of electricity is also higher because the energy used to press the log against the rotating friction wheel must be constant (1.6 kW h^{-1}). With regard to liquid smoke atomisation, the only cost is

Table 1. Parameters of the four smoking techniques for 3 h of smoke exposition for a smokehouse capacity of 380 kg

Parameter	Smouldering	Thermostated plates	Friction	Liquid smoke
Pyrolysis temperature	400–450 °C	500 °C	380 °C	–
Moisture of sawdust	20%	–	–	–
Quantity of wood pyrolysed (or liquid smoke vaporised)	1.69 kg	1 kg	1.59 kg	3 L
Amount of ash	330 g	–	180 g	–
Price of wood raw material used with smouldering as reference (euros)	1	0.59	3.08	7.62
Electricity consumption (kW h^{-1})	0.7	1.8	1.6	<0.1

that of the liquid smoke used but this process is the most expensive from the smoke production point of view (Table 1).

The friction technique seems to be the more expensive smoking process applying generation of wood smoke by pyrolysis, and smouldering the cheapest. Even though the liquid smoke process does not use electricity, it is the most expensive technique.

Organoleptic profiles and relation with total phenolic content

Odour analysis

Two organoleptic characteristics, odour and taste, were studied and related to the total phenol content. For odour characterisation, six descriptors were chosen for their suitability to describe smoked salmon ('wood fire smoke' odour, cold smoke odour which is a little more bitter than wood fire smoke, butter, vegetal, salmon and herring-like odour).

The results of odour analysis (Table 2) show that, except for the descriptor 'herring-like' odour, the odours of fillets smoked by smouldering, thermostated plates and friction smoking modes are significantly different from the odours of fillets smoked by liquid smoke. These results illustrate the odorant similarity of fillets smoked with the three pyrolysis processes compared to the liquid smoke technique. Nevertheless, odorant differences between the three pyrolysis techniques can be observed especially on cold smoke, butter and salmon-like descriptors. These differences could be due to the differences of the temperature of pyrolysis of the wood.

Table 2. Characterization of the odour of smoked salmon according to the four smoking techniques

	Smoking process			
Odorant descriptor	Smouldering	Friction	Thermo-stated plates	Liquid smoke
Wood fire smoke	5.29[a]	5.67[a]	5.33[a]	2.79[b]
Cold smoke	1.60[b]	1.43[b]	2.38[b]	5.04[a]
Butter	1.33[a]	1.73[a]	1.30[a]	0.24[b]
Vegetal (grass)	0.45[b]	0.32[b]	0.47[b]	1.52[a]
Salmon	3.59[a]	3.46[a]	3.06[a]	1.51[b]
Herring	0.95	0.71	1.00	0.65

Means in the same row with different superscripts are significantly different ($P < 0.05$).

Indeed, according to the parameters of wood smoke generation, the nature and the quantity of odour-active compounds formed could be different. For example, from 400 to 500 °C, the higher the temperature, the stronger the pyrolysis of lignin. Therefore, the formation of phenolic and furannic compounds increases.[11] Similarly, it has previously been reported that the difference in smoke production temperature between friction (350–380 °C) and smouldering (400–450 °C) leads to a less advanced degradation of the wood with friction than with others higher temperature of pyrolysis methods.[12] Therefore, for friction, with a pyrolysis temperature of 380 °C (Table 1), the smoke obtained could contain fewer odorants responsible for the smoke odour and could hide less well the butter odour of the fillets than when thermostated plates (500° C) were used. As result, the butter odour is stronger than when thermostated plates are used.[13] Further, this trend between processes where a high pyrolysis temperature (smouldering, thermostated plates) and those where a lower pyrolysis temperature (friction) is used has been shown to result in a stronger perception of smoke (cold ash, burnt tyre) odour of smouldering product.[14] Similar qualitative results are obtained herein except for the smoke perception. It could be certainly due to a weaker range of pyrolysis temperature because pyrolysis by the friction technique was carried out at 380 °C instead of 350 °C and for smouldering at about 400 °C instead of 450 °C. That is the reason why the perception of a 'wood fire smoke' odour of the three smoke generations by wood pyrolysis processes is close. The relative homogenity in smoky odours for the three processes could be related to the total phenol content. Indeed, quantities of 1.33, 1.29 and 1.17 mg of phenolic compounds reported per 100 g of smoked salmon are found for, respectively, the smouldering, thermostated plates and friction techniques. For liquid smoke, a phenol content of 0.95 mg 100 g^{-1} was obtained. The perception of 'wood fire smoke' in the products treated with liquid smoke is nearly two times lower than for the three other processes.

Among the three processes applying wood pyrolysis, thermostated plates seem to be the most responsible for 'cold smoke' odour. It could be due to the high temperature of the plates (500 °C) which could favour the generation of not only phenolic but also of furannic compounds in wood smoke[15] because the total phenolic content is not sufficient to explain the

small differences and, especially, seems not to act in the perception of cold smoke odour. Indeed, products treated with liquid smoke are at least two times more 'cold smoke' than the other products and although they have the lowest total phenol content. The 'cold smoke' odour intensity of fish smoked by liquid smoke atomisation is very important (5.04) whereas for the pyrolysis techniques the intensity is between 1.43 and 2.38. These observations are strengthened by a previous work[16] which showed that salmon fillets smoked with liquid smoke appeared nearly two times 'sootier' than fillets smoked with the smouldering process and three times 'sootier' than those smoked with the thermostated plates process.

Concerning the 'vegetal' odour, no significant difference is noticeable between fillets smoked according to smouldering, thermostated plates and friction methods. Only liquid smoke is very different. The important vegetal odour of smoked salmon treated by liquid smoke could be explained by the liquid smoke odour because it has been reported that smoke flavourings carry green/almond odour.[17] In smoked salmon treated with liquid smoke, stronger earthy and green odours have already been described by comparison to techniques applying wood pyrolysis.[14] This can strengthen our results because the products smoked by atomisation are found nearly four times more 'vegetal' than the products smoked by pyrolysis smoking techniques.

Finally, concerning the 'salmon-like' odour, no difference can be observed between the odour of fillets smoked with the three pyrolysis techniques even if smouldering seems to be more marked by the salmon-like odour than friction and thermostated plates. This trend has already been noticed in similar pyrolysis temperature conditions but with a smokehouse temperature of 22 °C with smouldering and thermostated plates.[16] As we worked with a smokehouse temperature of 32 °C and observed this trend, the smouldering mode seems to cause odours that are more 'fishy' than do the two others techniques, independently of the smokehouse temperature. For this descriptor, products treated with liquid smoke appear with a significantly weaker 'salmon-like' odour. In a previous study, Cardinal *et al*.[16] have shown that fillets smoked with liquid smoke were characterised by 'salmon-like' odour one time and a half weaker than the odour of fillets smoked by smouldering process (intensity of 2.3 for liquid smoke and 4.3 for smouldering). The other odours carried by liquid smoke to the salmon as 'cold smoke' and 'vegetal' are so strong that they could mask the own fishy odour of the smoked salmon flesh. The same remark could be formulated to explain the weaker perception of 'butter' odour in products smoked with liquid smoke. Moreover, even if the smokehouse temperature was the same, the wood smoke arrives on the fillets at a temperature of about 80 °C before to be diluted in the smokehouse at 32 °C. By comparison with liquid smoke which is vaporised at room temperature, the heat of the smoke used in the techniques applying wood pyrolysis can cause a stronger lipid oxidation during smoking process. This difference could lead to variations in the aroma profile of the smoked salmon obtained.

Liquid smoke gives to the smoked products odour characteristics which seem to be very different from the odours characteristics of products obtained with the three other techniques applying wood pyrolysis. The parameters of the smoking process by atomisation of liquid smoke can be responsible of these odorant differences but the nature of the liquid smoke itself could be more certainly implied in these variations. Indeed, according to the wood nature, the conditions of liquid smoke generation processes, the liquid smokes can be very different in composition and odours.

Taste evaluation

The taste of the different smoked salmons was described with the same descriptors as for the odour characterisation with in more, the descriptor 'salty'. The taste analysis results are presented in Table 3. The results show that odour and taste are closely linked because the intensity marks according to the descriptors and the smoking process evolve similarly. The taste of salmon smoked by liquid smoke are very different from the tastes obtained with techniques applying wood pyrolysis. For each descriptor, the remarks formulated for the odour analysis according to the different smoking techniques could be said again about the taste. The intensities of smoke and salmon-like tastes of products appear globally weaker than those of corresponding odours.

The difference in taste observed between salmons treated with liquid smoke and salmons smoked with the other techniques for the descriptor 'salty' is interesting. Normally, as the salmons were salted according to the same procedure, the salty taste would have been similar for all the salmons. This difference could mean that liquid smoke causes not only odour interactions with the product but also generates physical interactions which influence the

Table 3. Characterisation of the taste of smoked salmon according to the four smoking techniques

	Smoking process			
Taste descriptor	Smouldering	Friction	Thermo-stated plates	Liquid smoke
Wood fire Smoke	5.61[a]	5.71[a]	5.58[a]	3.03[b]
Cold smoke	1.45[b]	1.15[b]	2.10[b]	5.07[a]
Butter	0.93	1.00	1.20	0.46
Vegetal (grass)	0.29[b]	0.55[b]	0.29[b]	1.63[a]
Salmon	3.96[a]	4.04[a]	3.75[a]	2.94[b]
Herring	0.73	0.76	0.85	0.61
Salty	5.72[a]	5.54[a]	6.08[a]	4.64[b]

Means in the same row with different superscripts are significantly different ($P < 0.05$).

salty taste. As for odour analysis, the process of liquid smoke atomisation can be implied but this is especially the composition of the liquid smoke that is responsible of this difference. This result allows the conclusion that wood smoke in the gaseous state, produced according various devices and wood pyrolysis temperatures, seems to have the same effect on the salty taste. More generally, the three smoking processes applying wood pyrolysis lead to products quite similar in odour and taste. It means that whatever the wood pyrolysis, the smoke produced has a similar odour and acts in the same way on the salmon flesh components.

The taste analysis does not bring much additional information by comparison with the odour analysis in order to differentiate the processes. It only strengthens the homogenity of the three techniques applying wood pyrolysis to produce smoke and the marginality of liquid smoke.

PAH assessment

Except for benzo(a)pyrene, the maximum residue limits are not available. Therefore, in order to assess the contamination of a smoked salmon, the toxic equivalent quantity (TEQ) approach was chosen. TEQs are calculated by the multiplication of each PAH concentration by its corresponding toxic equivalent factor (TEF). The TEF consists of giving each PAH a relative toxicity as a fraction of the most toxic one, benzo(a)pyrene. This strategy, chosen by the Association Française de la Sécurité Sanitaire des Aliments (AFSSA) and the Institut National de l'Environnement Industriel et des Risques (INERIS) is based on the principle that the effects of PAHs are additive, but are not antagonist or synergic, and that they have the same mechanism of toxic action.[18] Table 4 shows the TEFs for each of the PAHs targeted in this study. INERIS gives benzo(j)fluoranthene the same TEF as benzofluoranthenes, i.e. 0.1. The different TEQs (calculated from an INERIS table) and benzo(a)pyrene concentrations obtained for each smoking process are listed in Table 5.

Table 4. TEF of the PAHs studied

PAH	TEF (INERIS)	TEF (Larsen *et al*., 1998)[26]
Fluorene	0.001	0.05
Phenanthrene	0.001	0.0005
Anthracene	0.01	0.0005
Fluoranthene	0.001	0.05
Pyrene	0.001	0.001
Benzo(a)anthracene	0.1	0.005
Cyclopenta(c,d)pyrene	0.1	0.02
Chrysene	0.01	0.03
5-methylchrysene		
Benzo(b)fluoranthene	0.1	0.1
Benzo(j)fluoranthene		0.05
Benzo(k)fluoranthene	0.1	0.05
Benzo(a)pyrene	1	1
Indeno(1,2,3-c,d)pyrene	0.1	0.1
Dibenzo(a,h)anthracene	1	1.1
Benzo(g,h,i)perylene	0.01	0.02
Dibenzo(a,l)pyrene		1
Dibenzo(a,e)pyrene		0.2
Dibenzo(a,i)pyrene		0.1
Dibenzo(a,h)pyrene		1

TEF of 5-methylchrysene was only assessed for aerial contamination and remained at 1 (Collins *et al*., 1998). [27]

It is important to note that when smoke comes from wood pyrolysis the maximum residue limit of 5 μg kg^{-1} of benzo(a)pyrene fixed by the European Commission[19] for smoked seafood products is never reached. Friction and smouldering seem to lead to the highest TEQ which are 20 times lower than the European limit value for benzo(a)pyrene concentration. The thermostated plates method leads to lower value of TEQ and has the weakest concentrations of benzo(a)pyrene. For these three smoking processes, benzo(a)pyrene has been evaluated at a concentration of 0.1 μg kg^{-1}. This benzo(a)pyrene concentration is in accordance with others benzo(a)pyrene concentrations found in smoked fish which underlines the coherence of our results.[20–22] The liquid smoke technique allows the less contaminated smoked salmon to

Table 5. Toxic equivalent quantity (TEQ) and concentration of benzo(a)pyrene (μg kg^{-1} of wet weight) for the four smoking techniques Value of TEQ at 22 °C and 32 °C

	Value of TEQ at 22 °C and 32 °C					
	1 h		2 h		3 h	
Smoking technique	22 °C	32 °C	22 °C	32 °C	22 °C	32 °C
Smouldering	0.099	0.112	0.119	0.123	0.106	0.140
[BaP]	**0.080**	**0.079**	**0.083**	**0.077**	**0.072**	**0.092**
Thermostated plates	0.014[a]	0.016[a]	0.096	0.092	0.093	0.100
[BaP]	**nq**[a]	**nq**[a]	**0.072**[b]	**0.065**[b]	**0.069**[b]	**0.070**[b]
Friction	0.093	0.090	0.112	0.107	0.137	0.125
[BaP]	**0.074**[b]	**0.071**[b]	**0.089**[c]	**0.081**[c]	**0.111**[d]	**0.097**[d]
Liquid smoke	0.076	0.102	0.078	0.098	0.060	0.064
[BaP]	**0.047**	**0.085**	**0.064**	**0.084**	**0.047**	**0.047**

[BaP], benzo(a)pyrene concentration; Nq, not quantifiable. The means followed by the same letter are not significantly different at 5%. TEQs were calculated by using the INERIS TEFs (Table 4).

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be obtained. However, the legislation concerning this technique is different. The directive 88/388/EEC has limited the maximum residual levels of benzo(a)pyrene to 0.03 $\mu g\,kg^{-1}$ in liquid smoke flavourings.[23–25] Our results show that if the liquid smoke process reduces the individual PAH concentrations, benzo(a)pyrene concentration can reach, in certain conditions (1 or 2 h of smoke exposure at 32 °C), until three times higher this legal threshold (0.09 $\mu g\,kg^{-1}$).

It can be noted that when thermostated plates process is used, TEQ and benzo(a)pyrene concentration are very low for one hour of smoke exposure, compared to the others processes. This difference is not noticeable for 2 and 3 h of smoke exposure. It is surprising because this process implies the highest pyrolysis temperature (500 °C) and the temperature of wood pyrolysis has already been reported as determinant in PAHs generation in wood smoke. The quantity of PAHs increases linearly according to the pyrolysis temperature between 400 and 1000 °C.[1] In fact, smouldering and friction with a pyrolysis temperature about 400 °C lead to the highest TEQ. The results were similar with thermostated plates but only after more than 1 h of exposure to smoke. This phenomenon could show an inertia in the thermostated plates process for the generation of PAHs which is not found for the other odorants of wood smoke because no consequent odour and taste difference between the three processes using wood pyrolysis is seen.

For the four smoking processes, PAHs were studied at three times of smoking and at two temperatures of the smokehouse. An analysis of the variance was carried out and the results are compiled in Table 5.

For smouldering smoking and liquid smoke process, the time of exposure and the temperature of the smokehouse seem not to influence PAH concentration because the analysis of the variance with TEQ and after with benzo(a)pyrene concentration as variables did not reveal possible impact of these factors at a risk 5%.

For thermostated plates, the low levels of PAHs during the first hour of smoking are also shown in ANOVA results. Indeed, an effect of the time of exposure to the smoke is noticed to explain the differences between the content at 1 h and the contents at 2 and 3 h of smoking. For TEQ and benzo(a)pyrene, the longer is the smoking time, the higher PAHs concentration is. The same remark can be formulated for friction technique but TEQ and benzo(a)pyrene concentration were already high during the first hour of smoking.

The occurrences of PAHs in the four smoking processes are nearly similar but the legislation between liquid smoke technique and the others is different. If liquid smoke is considered as a flavouring process, the benzo(a)pyrene concentration recovered is higher than the legal value. Among the three others techniques, thermostated plates appears as the smoking process that leads to the less contaminated samples.

It is important to note that indeno(1,2,3-c,d)pyrene, dibenzo(a,h)anthracene and all dibenzopyrenes were sometimes detected but it was never possible to quantify them.

CONCLUSION

The four most frequently used smoking processes have shown organoleptic, economic and sanitary differences. It is of considerable interest for processors to know the characteristics brought by a process to the product that they want to smoke. It is important to notice that there is not an ideal smoking process but each smoking process is sufficiently different from the others to allow a choice according to the aims of the processor. According to the three parameters studied (odour, taste, occurrence of PAHs, and cost of the smoke generation), liquid smoke atomisation seems to be insufficiently controlled because the odours carried are very different from those encountered in the other techniques, the costs are the highest and even if the total concentration of PAHs is lower than the other techniques, the benzo(a)pyrene is not under the 0.03 $\mu g\,kg^{-1}$ level. However, industrially, liquid smoke has other benefits for the storage; for example, the decrease of accidents due to fire, and the ease of vaporisation. Among the three processes using wood pyrolysis, the costs do not constitute the critical parameter even if friction is a little more expensive due to the price of logs. From the point of view of PAHs, thermostated plates seem to be the process generating the lowest level of PAHs. Moreover the odorant profiles of smoked products obtained by these techniques are nearly similar. This type of work could be extended to other smoked food in order to better study a possible effect of the food matrix. The creation of odours from smoked products and the occurrence of PAHs might be better understood by a study of their kinetics of deposition according to the parameters of the smoking process.

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REFERENCES

1 Sainclivier M. L'industrie alimentaire halieutique. Des techniques ancestrales à leurs réalisations contemporaines: salage, séchage, fumage, marinage, hydrolysats. *Bulletin scientifique et technique de l'Ecole Nationale Supérieure Agronomique Centre de Recherches de Rennes* 219–285 (1985).

2 Miller KBM and Sikorski ZE. Smoking, in *Seafood: Resources, Nutritional Composition, and Preservation*, ed. by Sikorski ZE. CRC Press Inc., Boca Raton, Florida (1990).

3 Maga JA. The flavor chemistry of wood smoke. *Food Rev Int* **1&2**:139–183 (1987).

4 Guillén MD, Sopelana P and Partearroyo MA. Determination of polycyclic aromatic hydrocarbons in commercial liquid smoke flavorings of different compositions by gas chromatography–mass spectrometry. *J Agric Food Chem* **48**:128–131 (2000).
5 Šimko P and Brunckova B. Lowering of polycyclic aromatic hydrocarbons concentration in a liquid smoke flavour by sorption into polyethylene packaging. *Food Addit Contam* **10**:257–263 (1993).
6 Guillén MD, Sopelana P and Partearroyo MA. Polycyclic aromatic hydrocarbons in liquid smoke flavorings obtained from different types of wood. Effect of storage in polyethylene flasks on their concentrations. *J Agric Food Chem* **48**:5083–5087 (2000).
7 Yurchenko S and Mölder U. The determination of polycyclic aromatic hydrocarbons in smoked fish by gas chromatography mass spectrometry with positive-ion chemical ionization. *J Food Comp Anal* **18**:857–869 (2005).
8 Cardinal M, Gunnlaugsdottir H, Bjoernevik M, Ouisse A, Vallet JL and Leroi F. Sensory characteristics of cold-smoked atlantic salmon (*Salmo salar*) from European market and relationships with chemical, physical and microbiological measurements. *Food Res Int* **37**:181–193 (2004).
9 Monteau F, Sarcher L, Varlet V, Maume D, André F and Le Bizec B. Compared mass spectrometry approches for PAH determination in food. Work presented in the Proceedings of the Dioxin 2005/ISPAC 20 congress, August 21–26, Toronto, Canada. International Society for Polycyclic Aromatic Compounds (ISPAC), CDROM ID : 1100. (2005).
10 European Commission. 205/108/EC Commission Recommendation of 4 February 2005 on the further investigation into the levels of polycyclic aromatic hydrocarbons in certain foods. *Official Journal of the European Union* **L34**:43–45 (2005).
11 Guillén MD and Ibargoitia ML. Relationships between the maximum temperature reached in the smoke generation processes from *Vitis vinifera* L. Shoot sawdust and composition of the aqueous smoke flavoring preparations obtained. *J Agric Food Chem* **44**:1302–1307 (1996).
12 Knockaert C. *Le fumage du poisson. Service de la documentation et des publications*. IFREMER, Brest (1990).
13 Varlet V, Knockaert C, Prost C and Sérot T. Comparison of odor-active volatile compounds of fresh and smoked salmon. *J Agric Food Chem* **54**:3391–3401 (2006).
14 Cardinal M, Cornet J, Sérot T and Baron R. Effects of the smoking process on odour characteristics of smoked herring (*Cuplea harengus*) and relationships with phenolic compound content. *Food Chem* **96**:137–146 (2006).
15 Nonier MF, Vivas N, Vivas de Gaulejac N, Absalon C, Soulié Ph and Fouquet E. Pyrolysis-gas chromatography/mass spectrometry of *Quercus* sp. Wood. Application to structural elucidation of macromolecules and aromatic profiles of different species. *J Anal Appl Pyrolysis* **75**:181–193 (2005).
16 Cardinal M, Berdagué JL, Dinel V, Knockaert C and Vallet JL. Effect of various smoking techniques on the nature of volatile compounds and on the sensory characteristics of salmon meat. *Sci Alim* **17**:679–696 (1997).
17 Kostyra E and Baryłko-Pikielna N. Volatiles composition and flavour profile identity of smoke flavourings. *Food Quality and Preference* **17**:85–95 (2006).
18 Doornaert B, Pichard A and Gillet C. Hydrocarbures aromatiques polycycliques (HAPs). Evaluation de la relation dose–réponse pour des effets cancérigènes: Approche substance par substance (facteurs d'équivalence toxique – FET) et approche par mélanges. Evaluation de la relation dose–réponse pour des effets non cancérigènes: Valeurs Toxicologiques de Référence (VTR). INERIS-DRC-03-47026-ETSC-BDo-N° 03DR177 (2003).
19 European Commission. 208/2005/EC Commission Regulation of 4 February 2005 amending Regulation (EC) No 466/2001 as regards polycyclic aromatic hydrocarbons. *Official Journal of the European Union* **L34**:3–5 (2005).
20 Kazerouni N, Sinha R, Hsu CH, Greenberg A and Rothman N. Analysis of 200 food items for benzo(a)pyrene and estimation of its intake in an epidemiologic study. *Food Chem Toxicol* **39**:423–436 (2001).
21 Storelli MM, Stuffler RG and Marcotrigiano GO. Polycyclic aromatic hydrocarbons, polychlorinated biphenyls, chlorinated pesticides (DDTs), hexachlorocyclohexane, and hexachlorobenzene residues in smoked food. *J Food Protection* **66**:1095–1099 (2003).
22 Stołyhwo A and Sikorski ZE. Polycyclic aromatic hydrocarbons in smoked fish – a critical review. *Food Chem* **91**:303–311 (2005).
23 European Commission. 88/388/EEC Council Directive of 22 June 1988 on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production. *Official Journal of the European Union* **L184**:1–10 (1988).
24 Šimko P. Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavourings additives. *J Chromatogr B* **770**:3–18 (2002).
25 Šimko P. Factors affecting elimination of polycyclic aromatic hydrocarbons from smoked meat foods and liquid smoke flavorings. *Mol Nutr Food Res* **49**:637–647 (2005).
26 Larsen JC and Larsen PB. Chemical carcinogens, in *Air Pollution and Health*, ed. by Hester RE and Harrison RM, 33–56 (1998).
27 Collins JF, Brown JP, Alexeeff GV and Salmon AG. Potency equivalency factors for some polycyclic aromatic hydrocarbons and polycyclic aromatic hydrocarbon derivatives. *Regul Toxicol Pharmacol* **13**:45–54 (1998).

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Determination of PAH profiles by GC–MS/MS in salmon processed by four cold-smoking techniques

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Abstract
An analytical method based on gas chromatography/tandem mass spectrometry (GC–MS/MS) (triple quadrupole device) has been developed for quantification of polycyclic aromatic hydrocarbons (PAHs) in smoked salmon. This method was applied to determine PAH concentrations in smoked fish and assess the impact of four industrial smoking processes on their profiles. Two smokehouse temperatures and three smoke-exposure times were applied. All the smoking techniques used lead to acceptable PAH levels: the quantities recovered are 100 times lower than the legal limit (5 $\mu g\,kg^{-1}$) concerning the principal PAH, i.e. benzo[a]pyrene. To compare different smoking processes, the toxic equivalent quantity (TEQ) approach was chosen. Smouldering leads to the highest TEQ, while liquid smoke leads to the lowest TEQ.

Keywords: PAH, smoked salmon, smoking process, TEQ, purification, tandem mass spectrometry

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic food and environmental contaminants (Mottier et al. 2000; Baird et al. 2005). Environmental exposure can occur through inhalation (Easton et al. 2002; Storelli et al. 2003; Grova et al. 2005) and deposition from air can lead to contamination of cereals and vegetables, resulting in human exposure from uncooked foodstuffs (SCF 2002). However, home-cooking and industrial food processes represent the major source of human exposure from the diet (Zabik et al. 1996; Kannappan et al. 2000; Stolyhwo and Sikorski 2005). Smoked foods (Chen et al. 1996; Jira 2004) have been known for several decades to be a source of PAHs, especially benzo[a]pyrene (Šimko 1991; Kazerouni et al. 2001).

In the smoking process, PAHs are generated during smoke production by wood pyrolysis. Owing to their lipophilic properties ($\log K_{ow}$ range between 4 and 7), PAHs can accumulate in the lipid fraction of food products and are not easily extracted from food with a high fat content, such as smoked salmon. Moreover, many analytical strategies have been developed to detect and measure these compounds (Rivera et al. 1996; Moret et al. 2000) such as de-fatting, which is especially used during the extraction step (Nyman et al. 1993; Yeakub-Ali and Cole 2001; Dugay et al. 2002). Accelerated solvent extraction (ASE), solid-phase extraction (SPE) clean-up on selected cartridges and saponification have been developed to avoid lipids suspected of disrupting the analysis (Wang et al. 1999; Marcé et al. 2000; Kishikawa et al. 2003). All the studies dealing with PAH analysis in smoked food have been carried out with a single detector, and GC/MS (Šimko 2002; Yurchenko et al. 2005) and HPLC/FD (Chen et al. 1996; Koffi Houessou et al. 2005) are the two main analytical methods for their

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measurement at low levels. However, co-elution that could be attributed to co-extracted lipids, as in the case of smoked fish (Wang et al. 1999), cannot be totally removed (Chiu et al. 1997; Moret et al. 1999). Indeed, the lipidic substances of food matrices can disrupt the extraction step but they are also involved in chromatographic coelutions, disturbing the detection of PAHs by creating interferences with the analytes or by increasing global noise. Gas chromatography coupled to tandem mass spectrometry appears to be a candidate method to lower the limit of detection (LOD) of PAHs, even in oily matrices (Munoz et al. 2001; Veyrand et al. 2006), for example, 0.07 µg kg^{-1} for benzo[g,h,i]perylene in oil (Ballesteros et al. 2006). Nevertheless, this detection system has not been used to monitor PAHs in smoked food and, especially, in smoked salmon.

The presence of PAHs, especially benzo[a]pyrene, in smoked fish has previously been reported (Šimko et al. 2002) but little information is available concerning the influence of the smoking processes. Most studies on PAHs and smoked fish have focused on methods of extraction and determination (Järvenpää et al. 1996; Wang et al. 1999; De Boer and Law 2003). Some studies compare modern and traditional kilns (Karl and Leinemann 1996; Karl 1997) but, to our knowledge, there is no comparative study of modern industrial smoking processes for fish with respect to the 20 PAHs suspected to be carcinogens (European Commission 2005b). Moreover, to reduce PAH levels in smoked fish, a liquid smoke atomization process has been developed in recent decades, involving the vaporization of liquid smoke, obtained from a condensation process of wood smoke, onto the fish. However, legal maximum residue limit for PAHs have not been set for this technique. Studies concerning PAHs in liquid smoke have only focused on the PAH composition of liquid smoke itself or the PAH composition of food smoked with this technique (Guillén et al. 2000a,b; Šimko 2005). No comparative studies of liquid smoke with traditional smoking techniques, applying wood pyrolysis, are available.

The aims of this work were, firstly, to assess a GC–MS/MS (SRM acquisition) quantification method for PAHs in smoked salmon compared with GC–MS (SIM acquisition). To carry out PAH quantification, an extraction method for smoked salmon was developed consisting of a liquid–solid extraction followed by an optimized SPE purification step. Secondly, this method was then applied to the determination of PAHs in salmon flesh, smoked according to the four, most common, smoking processes used in the industry. The effect of time of smoke exposure and smokehouse temperatures on PAH content was also assessed.

Materials and methods

Materials and reagents

All solvents for PAH analysis were analytical or HPLC grade and purchased from SDS (Peypin, France), except toluene and tetrahydrofuran which were from Fluka (Buchs, Switzerland).

ENVI Chrom P cartridges of 6 ml, 0.50 g bonded phase were obtained from Supelco (Bellefonte, PA, USA). All PAH standards (fluorene, phenanthrene, anthracene, cyclopenta[c,d]pyrene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-c,d]pyrene, dibenzo[a,e]pyrene, dibenzo[a,i]pyrene, benzo[g,h,i]perylene, dibenz[a,h]anthracene, dibenzo[a,l]pyrene and dibenzo[a,h]pyrene) were from LGC Promochem (Wessel, Germany), except benz[a]anthracene, chrysene and benzo[a]pyrene, which were obtained from Chiron (Trondheim, Norway). All ^{13}C-labelled PAHs were from Cambridge Isotope Laboratories (Andover, MA, USA), except for cyclopenta[c,d]-pyrene, dibenzo[a,h]pyrene and dibenzo[a,l]pyrene for which ^{13}C-labelled standards were not available. Ultrapure water was obtained from a Milli-Q system (resistivity <18 M cm $^{-1}$).

Fish processing

Salmon (*Salmo salar*), reared in Norway, were purchased from a seafood wholesaler (Nantes, France). The time between their capture and filleting was not more than 1 week. Nine gutted fish of 3–4 kg from the same batch were received in a box on ice. They were directly filleted, trimmed and put on grids in a cold chamber at + 3 °C for 2 h. All the fillets were 1 kg. Next, they were hand-salted with refined salt (Salins du Midi, France) and left for 3 h at +12 °C before being rinsed on grids with water (15° C) and stored at 3 °C for 18 h until smoking.

Before smoking, a drying step was carried out by putting the fillets in the smokehouse at 18° C for 15 min. The aim of this step is to dry the product surface for better smoke penetration, according to industrial procedures. Secondly, this step allows the standardization of the internal temperature at 8 °C for all the samples that were previously stored in a cold-room at + 2 °C. Then, at the beginning of the smoking process, smoke was introduced into the cell on fillets that had the same internal temperature whatever the smokehouse temperature. The smoked fillets were stored for less than 1 week at + 2 °C prior to sensorial analysis. The medium parts of smoked fillets (about 200 g) were kept at -80° C and freeze- dried for PAH analysis.

Raw salmon from the same batch were separated to prepare blank matrices for measuring environmental contamination in PAHs in salmon.

Smoking equipment and procedures

The smokehouse was an HMI Thirode (PC90 Model) device (Thirode, France), 1500 × 1300 × 2250 mm with a capacity of 380 kg, mounted on a trolley with 28 grids on which the fillets were placed. For each smoking technique, the fillets were positioned at the same level (grid numbers 10, 12 and 14) 20 cm from the door of the smokehouse. Air/smoke circulation was horizontal. To study the effect of time of smoke exposure and smokehouse temperatures, salmon fillets were swept by the smoke for 1, 2 or 3 h at a temperature of 22 or 32 C.

The exhaust valve opening was 1/3, but was closed for liquid smoke vaporization and the relative hygrometry was set at 60%. For each process, except liquid smoke, the smoke was introduced into the smokehouse at a flow-rate of 90 $m^3 h^{-1}$.

For the production of wood smoke, four processes are used: smouldering, thermostated plates and friction, which generate smoke from wood sawdust, chips and logs, respectively, and atomization of liquid smoke (Varlet et al. 2006).

Smouldering parameters

A generator (Thirode, France) produced smoke by pyrolysis (between 400 and 450° C) from beech sawdust using the smouldering method. The sawdust was poured onto an electrically heated ring and pyrolyzed. Pyrolysis temperature was determined with a probe placed on the heated ring. The ring was heated for the ignition period; further treatment was by electric pulses only. Pyrolysis was maintained through an air intake producing a continuous flow around the heated ring via a fan. Sawdust fell on the heated ring by gravity from a hopper programmed for every 6 min. The sawdust was moistened and homogenized to give a moisture content of 20%.

Thermostated plates parameters

A generator 720 × 1120 × 1730 mm (Thirode, France) produced smoke by pyrolysis (500° C) of beech chips. Pyrolysis temperature was determined via a probe placed on the plates. A system spread the chips on thermostated plates, which were cleaned after 3 min of combustion. Smoke was pulsed by a ventilator to give the same flow-rate of smoke in the smokehouse as in smouldering and friction.

Friction parameters

A generator-type FR 1002 (Muvero, The Netherlands) produced smoke by friction (380° C) by pressing a beech log (8 × 8 × 100 cm) against a rotating friction wheel for 10 s every 30 s. The pyrolysis temperature was determined via a probe placed into the log. The beech log was pressed pneumatically by a wood gripper with a pressure of 3.5 bars.

Liquid smoke parameters

Liquid smoke was purchased from a flavouring manufacturer (France). It was a purified condensate of beech smoke. Liquid smoke is utilised by atomising with pressurized air in the smokehouse at ambient temperature. The vaporization device (Lutetia, France) allows the setting of air and liquid smoke pressures to give a consumption rate of liquid smoke of 1 l h^{-1}, as in industrial smoking procedures. Liquid smoke was injected into the smokehouse for 2 min every 3 min. For this type of smoking process, the hygrometry of the smokehouse was set at 70%.

Solid–liquid extraction of PAH

A 2 g aliquot of freeze-dried fillet spiked with a mixture of 20 ^{13}C-PAHs at 1 μg kg^{-1}, considered as internal standards, was homogenized in 40 ml of cyclohexane/ethyl acetate (50:50; v/v), shaken for 30 min and then centrifuged at 5000 g for 30 min at 0° C. The liquid portion was carefully isolated and evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 6 ml of cyclohexane. Each PAH quantification was the result of the mean of triplicate measurements carried out on three individual smoked fillets.

SPE clean-up procedure

Solid-phase extraction cartridges placed on a Vac Elut system were conditioned with 5 ml of water, then 5 ml of methanol and finally with 5 ml of cyclohexane. A 6 ml sample in cyclohexane was introduced into the cartridge and washed with 3 ml of cyclohexane to remove fat. PAHs were eluted with 12 ml of a mixture of cyclohexane and ethyl acetate (50:50, v/v) then evaporated to dryness under a nitrogen stream. Finally, the residue was dissolved in 40 ml of toluene.

GC/MS/MS analysis

For GC/MS/MS experiments, an HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) was coupled to a triple stage quadrupole Quattro Micro mass spectrometer (Waters, Milford,

MA, USA). A 4 mm I.D. liner, loosely filled with silanized glass wool, was used in splitless GC injector mode (280° C). The Zebron GC column was a ZB-5MS 30 m × 0.25 mm I.D., film thickness 0.25 mm (Phenomenex, Torrance, CA, USA). The GC oven temperature was maintained at 110° C for 1 min after injection then programmed at 20 °C min^{-1} to 240 °C, then at 5 °C min^{-1} to 340° C. Helium was used as carrier gas. Electron ionization was used and SRM (selected reaction monitoring) was selected as acquisition technique. The monitored ions were those listed by the European Commission (European Commission 2005b), in addition to fluorene, phenanthrene, anthracene, fluoranthene and pyrene from the US EPA's list of 16 priority pollutants (INERIS 2003). The mass-to-charge ratio (m/z) ranged 166 (fluorene), 178 (phenanthrene, anthracene), 202 (fluoranthene, pyrene), 226 (cyclopenta[c,d]pyrene), 228 (benz[a]anthracene, chrysene), 242 (5-methylchrysene), 252 (benzo[b], [j] and [k]fluoranthene, benzo[a]pyrene), 276 (benzo[g,h,i]perylene, indeno[1,2,3-c,d]pyrene), 278 (dibenz[a,h]anthracene) and 302 (dibenzopyrenes).

Analytes were identified on the basis of their retention time and at least two transitions. ^{13}C-labelled internal standards, corresponding to each PAH studied, were used for quantification, excepted for cyclopenta[c,d]pyrene reported to ^{13}C-benz[a]anthracene, dibenzo[a,h]pyrene to ^{13}C-dibenzo[a,i]pyrene and dibenzo[a,l]pyrene to ^{13}C-dibenzo[a,e]pyrene.

To express the concentrations in µg $kg^{-1}$of wet tissue, the concentrations expressed in µg kg^{-1} of freeze-dried tissue have been multiplied by an individual freeze-drying factor for each PAH. These factors take into account the loss of weight due to water loss and the loss of analyte signals during freeze-drying. They were determined by the addition of internal standards before and after this step.

GC–MS analysis

For GC–MS experiments, an HP-6890 gas chromatograph was coupled to an HP-5973 (Agilent Technologies, Palo Alto, CA, USA) quadrupole mass spectrometer (low-resolution single MS). Injector and transfer-line temperatures were 250 and 305 °C, respectively, and source and analyzer temperatures were 230 and 150 °C, respectively. A 4 mm I.D. glass insert, loosely filled with silanized glass wool, was used in the split/splitless GC injector (250° C, purge splitless 1.5 min). The GC column was 30 m × 0.25 mm I.D., film thickness 0.25 mm, OV–1 (Ohio Valley). The GC oven temperature was maintained at 110 °C for 10 min after injection, then programmed at 20° C min^{-1} to 160 °C and then at 15 °C min^{-1} to 300° C, which was maintained for 10 min. Helium (N55) was used as carrier gas at 1.1 ml min^{-1}. The acquisition mode was SIM (single ion monitoring). Electron ionization was used at 70 eV.

Statistical analysis

Analysis of variance (ANOVA) was performed on PAH concentration data using Statgraphics Plus 5.1 software (Statistical Graphics Corp., USA). The significant statistical level was set at $P < 0.05$ (Cardinal et al. 2006). Multivariate data processing was performed with Uniwin Plus 5.1 software (Sigma Plus, France). A correspondence factorial analysis (CFA) was carried out on the PAH content according to the smoking parameters (3 h of smoke exposure, 32 °C) and the type of smoking process.

Results and discussion

Efficiency of the analytical method

Extraction method and recovery yields. The main critical factor for PAH analysis in food is their lipophilic properties. Indeed, PAHs are often co-extracted with lipids. To decrease the fat content in the final sample, washing and elution volumes have been precisely chosen to avoid lipid residues after concentration with nitrogen and to prevent rapid contamination of the mass spectrometer. The potential loss caused by the cyclohexane washing step during SPE is compensated by repeatable recovery yields (Table I) for each monitored PAH. Estimation of recovery yields was performed with three replicates on spiked unsmoked freeze-dried salmon (1 ng g^{-1} of each PAH). As the number of rings increases in the PAH molecule, polarity decreases and the particular PAH has a high affinity for the lipid fraction and is less extracted by the solvent mixture (cyclohexane/ethyl acetate). As required for benzo[a]pyrene (European Commission, 2005c), the recovery yields of all PAHs are between 50 and 120% – from 43% (for dibenzo[a,e]pyrene and dibenzo[a,l]pyrene) to 99% (for phenanthrene). These results are acceptable for fatty matrices, such as salmon, in which we have previously measured a fat content of 13% before smoking.

Good reproducibility of extraction can be observed with coefficients of variation less than 10% for all the molecules studied, except dibenzopyrenes. Nevertheless, in all cases, the coefficient of variation is under the 15% required for the analysis of similarly structured contaminant (European Commission 2002/69, 2002).

Table I. Recovery yield (%) of 20 PAHs and coefficient of variation of the extraction method in unsmoked salmon samples.

Compounds	Recovery yield in % (mean of six extractions)	Coefficient of variation in %
Fluorene	95	5
Phenanthrene	99	6
Anthracene	52	6
Fluoranthene	83	2
Pyrene	85	4
Benzo[a]anthracene	57	5
Cyclopenta[c,d]pyrene	60	7
Chrysene	65	6
5-rnethylchrysene	53	7
Benzo[b]fluoranthene + Benzo[j] fluoranthene	53	6
Benzo[k]fluoranthene	59	5
Benzo[a]pyrene	49	6
lndeno[1,2,3-c,d]pyrene	47	5
Dibenzo[a,h] anthracene	48	9
Benzo[g,h,i]perylene	61	10
Dibenzo[a,l]pyrene	43	11
Dibenzo[a,e]pyrene	43	12
Dibenzo[a,i]pyrene	44	11
Dibenzo[a,h]pyrene	40	15

Each recovery yield is the mean of three measurements.

GC–MS/MS approach and comparison with GC–MS (SIM)

GC-MS/MS analysis provides several advantages for PAH detection and quantification in food. Firstly, it allows treatment of the food matrix under gentler extraction conditions than those developed until now, which remove lipids under severe conditions, such as saponification. Extraction must give samples that can be injected after removing lipids and detection is improved owing to further specific monitored transitions, which enables co-extracted lipid substances to be minimized and separated from the PAHs.

The second advantage is that the specificity of GC–MS/MS allows a focus on the transitions of PAHs. It enables extraction to be optimised to improve the signal, especially in the chromatographic zone of the analyte peaks. Consequently, the levels of detection are reduced (Monteau et al. 2005; Veyrand et al. 2006). Comparing GC–MS in the SIM acquisition mode and GC–MS/MS with SRM acquisition (Figure 1), especially for high molecular-weight PAHs, such as benzo[g,h,i]perylene, the signal for GC–MS/MS has less interference and a more stable baseline. Thus, detection limits were significantly improved. For lipophilic PAHs, such as benzo[g,h,i]perylene, suspected of being co-eluted with lipid substances, a level of detection is achieved of 0.5 $\mu g\,kg^{-1}$ with GC–MS (SIM) and $<0.1\ \mu g\,kg^{-1}$ with GC–MS/MS. Therefore, gas chromatography coupled to MS/MS detection shows a five times increase in sensitivity in this type of food matrix, noticeably for the main heavy PAHs. These results are in accordance with other studies applying tandem mass spectrometry to the recovery of PAHs in food oils (Ballesteros et al. 2006).

The improvement in chromatographic signals is also due to the chemical structures of PAHs. Indeed, PAHs are highly condensed and stable, so the $M^{+\cdot} \rightarrow M^{+\cdot}$ transition can be monitored. Their stability increases with the number of rings in the chemical structure. For this reason, the energy in the collision cell is increased from 25 to 35 eV for dibenz[a,h]anthracene, indeno[1,2,3-c,d]pyrene, benzo[g,h,i]perylene and all the dibenzopyrenes to cause a loss of two protons and to generate the M-2 ion (Table II). This second ionization causes a decrease in noise by fragmenting the co-extracted substances co-eluted with PAHs, whereas PAHs are only weakly affected. With GC–MS/MS analysis, owing to the energy of collision and the monitoring of specific transitions, a mass clean-up is carried out that reduces the noise due to lipidic substances, thus increasing simultaneously the signal-to-noise ratio of PAHs.

The method reported here is a suitable analysis of PAHs in investigative studies. It has a faster extraction time and takes place under gentle conditions, which avoid the generation of potential interference or matrix effects. The benefits of the extraction step are only noticeable because the GC–MS/MS analysis has been optimized for monitoring PAHs.

PAH content in smoked salmon according to the smoking process

The analytical method presented above was used to investigate PAHs in salmon smoked by four different industrial smoking processes: smouldering, thermostated plates, friction and liquid smoke. In parallel, PAH quantification in unsmoked salmon is compiled for comparative purposes in the tables of each smoking process to differentiate PAHs coming from the environment and salmon feed and those generated during the smoking process (Tables IV–VII). Environmental contamination due to the bioaccumulated PAHs is in agreement with the literature (Easton et al. 2002). PAHs of low molecular-weight are predominant but in weak concentrations (between 0.06 for fluorene to 0.19 $\mu g\,kg^{-1}$ for pyrene); all PAHs of heavy molecular-weight are below 0.02 $\mu g\,kg^{-1}$.

Regarding the four processes, it should be noted that 5-methylchrysene, indeno[1,2,3-c,d]pyrene, dibenz[a,h]anthracene and all the dibenzopyrenes

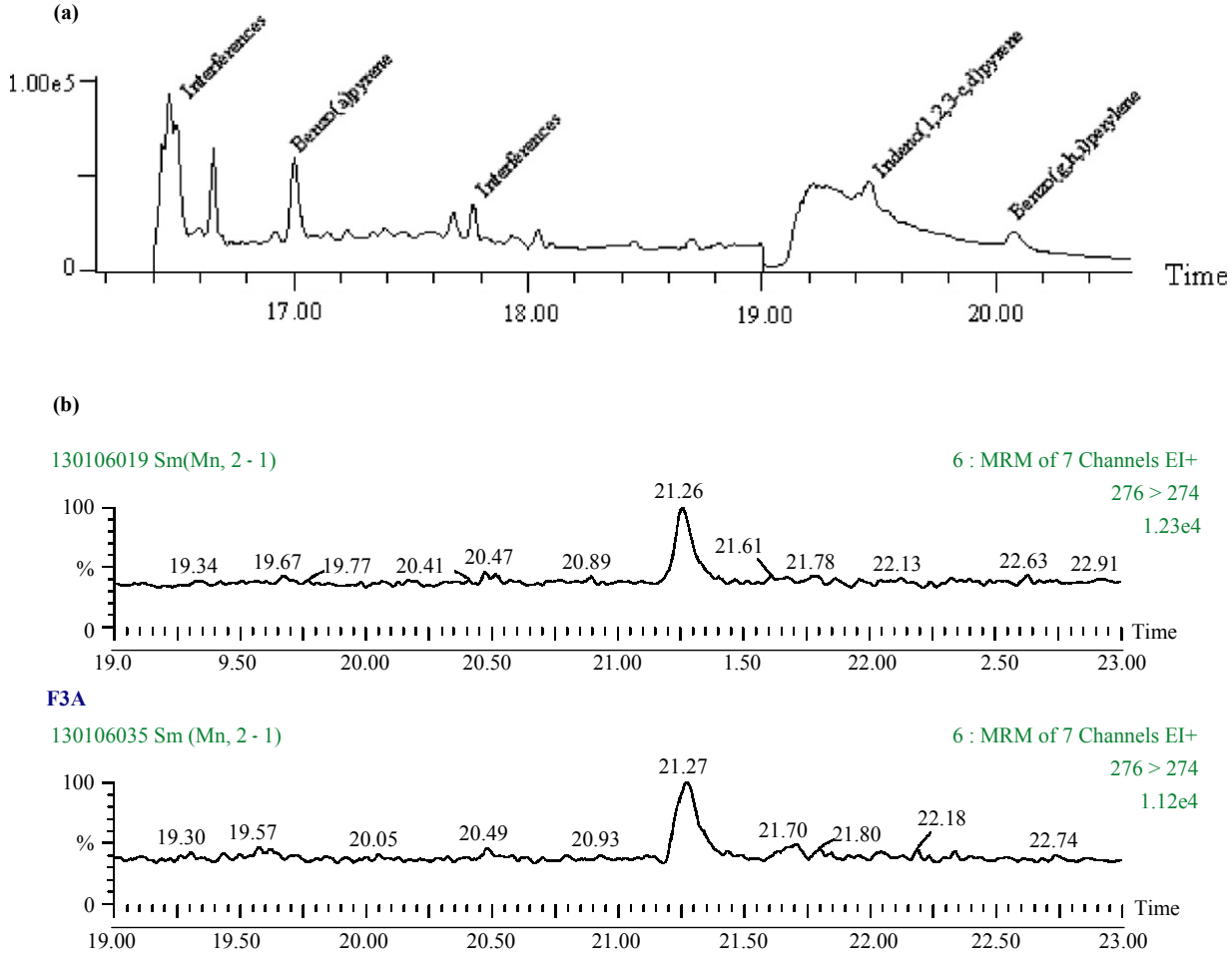

Figure 1. (a) GC–MS, SIM acquisition. Ion chromatograms corresponding to benzo[a]pyrene, indeno[1,2,3-c,d]pyrene and benzo[g,h,i]perylene in commercial smoked salmon extracts (masses monitored: m/z 252 and 276). (b) GC–MS/MS, SRM acquisition. Ion chromatograms corresponding to benzo[g,h,i]perylene in smoked salmon extract (transition 276 ⟶ 274). F1A: Friction technique, 1-h smoke exposure, 22 °C in the smokehouse. F3A: Friction technique, 3-h smoke exposure, 22 °C in the smokehouse.

were not found in samples, whatever the time of smoke exposure or smokehouse temperature. These PAHs are considered much more toxic than low molecular-weight PAHs, such as fluorene.

Since PAHs do not have the same level of toxicity, a TEF (toxic equivalent factor), expressed in comparison to benzo[a]pyrene, was defined for each PAH (Table III). The concentration of each PAH is multiplied by its corresponding TEF and then added to obtain a single value illustrating the toxicity of the foodstuff studied. This value corresponds to the TEQ (toxic equivalent quantity) (AFSSA 2003). The TEQ approach was chosen to express the total PAH contamination of a smoked or unsmoked product. Even if this presentation of PAH content is empirical because the effects of PAHs in a mixture are insufficiently understood, with this approach it is possible to express PAH contamination of food by a single value.

Contamination of samples for each experimental point (one smokehouse temperature and one time of exposure) is summarized as the TEQ in Tables IV–VII. Except for the thermostated plates, analysis of variance of the TEQs does not show a significant influence of time of smoking or smokehouse temperature. The low level of PAHs at 1 h in fish fillets smoked with the thermostated plates could be due to the inertia of the device. Whatever the settings of the parameters, all smoking processes lead to high levels of PAHs of low molecular-weight, which seem to be generated mainly during smoking (Figure 2). The concentrations of PAH from fluorene to fluoranthene varied between 1 and 5 $\mu g\ kg^{-1}$. Smouldering gave the highest and liquid smoke the lowest concentrations of low molecular-weight PAHs. Friction and thermostated plates lead to intermediary and similar levels of contamination, especially for fluoranthene and pyrene (from 0.10 to 0.40 $\mu g\ kg^{-1}$).

Table II. Molecular weights, log K_{ow}, energy of collision used and mass transitions monitored for the 20 PAHs.

Compounds	Mw	Log K_{ow}	Number of rings	Collision cell energy (eV)	Monitored transition
Fluorene	166	4.18	3	20	166 → 166
				25	166 → 164
Phenanthrene	178	4.57	3	20	178 → 178
				25	178 → 176
Anthracene	178	4.45	3	20	178 → 178
				25	178 → 176
Fluoranthene	202	5.10	4	20	202 → 202
				25	202 → 200
Pyrene	202	5.32	4	20	202 → 202
				25	202 → 200
Cyclopenta[c,d] pyrene	226		5	20	226 → 226
				25	226 → 224
Benz[a] anthracene	228	5.63	4	20	228 → 228
				25	228 → 226
Chrysene	228	5.81	4	20	228 → 228
				25	228 → 226
5-methyl-chrysene	242		4	20	242 → 242
				25	242 → 240
Benzo[b] fluoranthene	252	6.57	5	20	252 → 252
				25	252 → 250
Benzo[j] fluoranthene	252	6.11	5	20	252 → 252
				25	252 → 250
Benzo[k] fluoranthene	252	6.84	5	20	252 → 252
				25	252 → 250
Benzo[a]pyrene	252	6.00	5	20	252 → 252
				25	252 → 250
Benzo[g,h,i] Perylene	276	6.63	6	20	276 → 276
				35	276 → 274
Indeno[1,2,3-cd] pyrene	276	6.60	6	20	276 → 276
				35	276 → 274
Dibenz[a,h] Anthracene	278	6.70	5	20	278 → 278
				35	278 → 276
Dibenzo[a,l] pyrene	302		6	20	302 → 302
				35	302 → 300
Dibenzo[a,e] Pyrene	302		6	20	302 → 302
				35	302 → 300
Dibenzo[a,h] Pyrene	302	7.28	6	20	302 → 302
				35	302 → 300
Dibenzo[a,i] pyrene	302	7.28	6	20	302 → 302
				35	302 → 300

These PAHs, and especially phenanthrene whose bioavailability in water is significant, can be an indicator of initial contamination in marine organisms (Pointet and Milliet 2000; Marsili et al. 2001; Easton et al. 2002). It is interesting to note that the predominance of low molecular-weight PAHs is remarkable after the smoking of the fish.

Effects of the time of smoke exposure and smokehouse temperature on the generation of PAHs according to the process used

Smouldering. All fillets smoked according to this technique are acceptable because none of the samples exceeded the legal limit (benzo[a]pyrene concentration <5 mg kg ^{1}) (European Commission 2005a). Under more extreme conditions (3 h, 32° C), benzo[a]pyrene levels reached only 0.05 µg kg^{-1}. No effect of time of smoke exposure or smokehouse temperature was noticeable for this PAH in the smouldering method (see Table IV). In fish fillets smoked by smouldering, there are more low than high molecular-weight PAHs. Analysis of variance showed a significant influence of smokehouse temperature and time of smoke exposure on the quantity of PAHs recovered in salmon fillets, especially phenanthrene and chrysene (P values < 0.05). Increasing the smokehouse temperature and the time of smoke exposure raises the concentration of phenanthrene. For chrysene, only the effect of increasing smokehouse temperature is noticeable, which could be explained by a higher level of lipids in the liquid state at 32 °C than at 22° C. Thus, PAHs that are lipophilic could be better adsorbed in the fillet (Moret et al. 1999). Measurements on a greater number of fillets are needed to confirm this trend.

Table III. Toxic equivalent factors (TEFs) for the studied PAHs.

PAHs	TEF (INERIS)	TEF (Larsen et al. 1998)
Fluorene	0.001	0.05
Phenanthrene	0.001	0.0005
Anthracene	0.01	0.0005
Fluoranthene	0.001	0.05
Pyrene	0.001	0.001
Benz[a]anthracene	0.1	0.005
Cyclopenta[c,d]pyrene	0.1	0.02
Chrysene	0.01	0.03
5-Methyl-chrysene	–	–
Benzo[b]fluoranthene	0.01	0.1
Benzo[j]fluoranthene	–	0.05
Benzo[k]fluoranthene	0.01	0.05
Benzo[a]pyrene	1	1
Benzo[g,h,i]perylene	0.01	0.02
Indeno[1,2,3-cd]pyrene	0.1	0.1
Dibenz[a,h]anthracene	1	1.1
Dibenzo[a,l]pyrene	–	1
Dibenzo[a,e]pyrene	–	0.2
Dibenzo[a,h]pyrene	–	1
Dibenzo[a,i]pyrene	–	0.1

TEF of 5-methylchrysene was only assessed for aerial contamination and set at 1 (Collins et al. 1998).

Thermostated plates. The results presented in Table V show that this method generates low levels of PAHs (benzo[a]pyrene concentration <0.04 µg kg^{-1} whatever the parameter settings). This technique reaches the highest temperature of pyrolysis (500 C) and it has been reported that PAH occurrence is greater at high pyrolysis temperature (Clifford et al. 1980). Thus, the generation of PAHs at high pyrolysis temperature can be compensated

Table IV. Toxic equivalent factors (INERIS) and PAH levels in salmon smoked by smouldering at three different smoke-exposure times and two smokehouse temperatures (in µg kg^{-1} ± standard deviation wet tissue).

		Parameters of smoking					
	Initial contamination	1 h, 22 °C	1 h, 32 °C	2 h, 22° C	2 h, 32 °C	3 h, 22 °C	3 h, 32 °C
Fluorene	0.06 ± 0.041	1.29 ± 0.215	2.38 ± 0.430	2.42 ± 0.331	4.09 ± 0.739	2.84 ± 0.325	5.74 ± 1.511
Phenanthrene	0.04 ± 0.018	1.36 ± 0.353	2.43 ± 0.563	2.28 ± 0.764	3.94 ± 0.735	3.42 ± 0.318	5.20 ± 0.979
Anthracene	0.01 ± 0.005	0.33 ± 0.038	0.50 ± 0.069	0.50 ± 0.076	0.71 ± 0.078	0.54 ± 0.101	0.90 ± 0.147
Fluoranthene	0.075 ± 0.100	0.27 ± 0.043	0.05 ± 0.008	0.37 ± 0.072	0.55 ± 0.113	0.62 ± 0.137	0.63 ± 0.197
Pyrene	0.19 ± 0.015	0.16 ± 0.268	0.14 ± 0.123	0.26 ± 0.266	0.48 ± 0.223	1.00 ± 0.763	0.35 ± 0.092
Benz[a]anthracene	<0.02	0.02 ± 0.005	0.02 ± 0.001	0.02 ± 0.003	0.02 ± 0.004	0.02 ± 0.003	0.03 ± 0.001
Cyclopenta[c,d]pyrene	<0.01	0.01 ± 0.011	0.02 ± 0.004	0.05 ± 0.002	0.05 ± 0.005	0.02 ± 0.002	0.03 ± 0.003
Chrysene	0.01 ± 0.03	0.08 ± 0.010	0.10 ± 0.019	0.08 ± 0.007	0.09 ± 0.002	0.07 ± 0.002	0.09 ± 0.007
Benzo[b]fluoranthene þ Benzo[j]fluoranthene	<0.02	0.03 ± 0.003	0.03 ± 0.003	0.03 ± 0.002	0.03 ± 0.001	0.02 ± 0.006	0.03 ± 0.003
Benz[k]fluoranthene	<0.01	0.01 ± 0.001	0.01 ± 0.003	0.01 ± 0.001	0.01 ± 0.003	0.01 ± 0.003	0.01 ± 0.002
Benzo[a]pyrene	<0.02	0.04 ± 0.003	0.04 ± 0.005	0.04 ± 0.003	0.04 ± 0.009	0.04 ± 0.005	0.05 ± 0.004
Benzo[g,h,i]perylene	<0.01	0.07 ± 0.008	0.10 ± 0.001	0.05 ± 0.011	0.07 ± 0.017	0.07 ± 0.022	0.06 ± 0.014
TEQ (µg kg^{-1})	5.65 10 $^{-4}$	0.055	0.065	0.059	0.065	0.059	0.079

TEQ are calculated with TEFs from INERIS table. TEQ are lower-bound TEQs (PAH contents below the limit of quantitation were not been taken into account). For 1 h, 32 °C, fluoranthene concentration was presumptively quantified due to its very low levels.

Table V. Toxic equivalent factors and PAH levels in salmon smoked by thermostated plates at three different smoke-exposure times and two smokehouse temperatures (in µg kg^{-1} ± standard deviation of wet tissue).

		Parameters of smoking					
	Initial contamination	1 h, 22 °C	1 h, 32° C	2 h, 22 °C	2 h, 32° C	3 h, 22 °C	3 h, 32° C
Fluorene	0.06 ± 0.041	1.43 ± 0.225	2.18 ± 0.615	2.10 ± 0.648	2.63 ± 0.763	2.37 ± 0.621	4.28 ± 0.710
Phenanthrene	0.04 ± 0.018	1.61 ± 0.413	2.16 ± 0.845	1.33 ± 0.353	1.88 ± 0.164	1.54 ± 0.636	3.04 ± 0.748
Anthracene	0.01 ± 0.005	0.29 ± 0.043	0.36 ± 0.011	0.33 ± 0.053	0.41 ± 0.010	0.41 ± 0.122	0.45 ± 0.097
Fluoranthene	0.075 ± 0.100	0.27 ± 0.077	0.37 ± 0.100	0.32 ± 0.069	0.33 ± 0.082	0.27 ± 0.161	0.46 ± 0.107
Pyrene	0.19 ± 0.015	0.19 ± 0.198	0.08 ± 0.019	0.26 ± 0.216	0.18 ± 0.026	0.20 ± 0.262	0.20 ± 0.090
Benzo[a]anthracene	<0.02	0.01 ± 0.005	0.01 ± 0.001	0.01 ± 0.005	0.01 ± 0.003	0.01 ± 0.001	0.01 ± 0.004
Cyclopenta[c,d]pyrene	<0.01	<0.01	<0.01	0.02 ± 0.007	0.02 ± 0.002	0.02 ± 0.005	0.01 ± 0.008
Chrysene	0.01 ± 0.03	0.06 ± 0.006	0.05 ± 0.005	0.07 ± 0.004	0.07 ± 0.003	0.05 ± 0.008	0.06 ± 0.016
Benzo[b]fluoranthene þ Benzo[j]fluoranthene	<0.02	<0.02	<0.02	0.02 ± 0.005	0.02 ± 0.004	0.02 ± 0.004	0.03 ± 0.005
Benzo[k]fluoranthene	<0.01	<0.01	<0.01	0.01 ± 0.003	0.02 ± 0.004	0.01 ± 0.001	0.01 ± 0.002
Benzo[a]pyrene	<0.02	<0.02	<0.02	0.04 ± 0.012	0.03 ± 0.002	0.04 ± 0.009	0.04 ± 0.012
Benzo[g,h,i]perylene	<0.01	0.07 ± 0.064	0.08 ± 0.019	0.08 ± 0.026	0.11 ± 0.064	0.08 ± 0.004	0.08 ± 0.007
TEQ (µg kg^{-1})	5.65 10 $^{-4}$	0.009	0.011	0.052	0.044	0.053	0.056

TEQ are calculated with TEFs from INERIS table. TEQ are lower-bound TEQs (PAH contents below the limit of quantitation were not been taken into account).

for by the device used in smoke production. Below 400 °C, smoke contains very low amounts of PAHs but also fewer odour-active compounds and molecules that influence the required organoleptic characteristics of the final product (Hamm 1977). Clearly, pyrolysis must be optimized to obtain a flavoured smoke while minimizing PAH generation. For anthracene, benzo[b] and benzo[j]-fluoranthene and benzo[a]pyrene, the results of ANOVA show a significant effect of time of smoke exposure with a 5% risk. A significant effect of smokehouse temperature is also noticeable for anthracene.

An increase in the smoking parameters (especially time of smoke exposure) generated PAHs, nevertheless all PAH levels were below the European legal limit for smoked fish. The highest concentration of benzo[a]pyrene was found after 3 h of smoke exposure, reaching 0.04 µg kg^{-1} but still clearly below 5 µg kg^{-1}. In general, there is an important difference between the benzo[a]pyrene concentrations found in smoked salmon whatever the smoking

Table VI. Toxic equivalent factors and PAH levels in salmon smoked by friction at three different smoke-exposure times and two smokehouse temperatures (in μg kg^{-1} ± standard deviation of wet tissue).

		Parameters of smoking					
	Initial contamination	1 h, 22 °C	1 h, 32° C	2h, 22° C	2 h, 32 °C	3 h, 22° C	3 h, 32 °C
Fluorene	0.06 ± 0.041	1.39 ± 0.521	1.60 ± 0.312	1.46 ± 0.652	2.57 ± 0.416	1.99 ± 0.345	3.16 ± 0.65
Phenanthrene	0.04 ± 0.018	1.33 ± 0.202	1.37 ± 0.172	1.82 ± 0.457	2.32 ± 0.282	1.34 ± 0.219	2.73 ± 0.26
Anthracene	0.01 ± 0.005	0.29 ± 0.015	0.35 ± 0.011	0.38 ± 0.035	0.50 ± 0.019	0.33 ± 0.058	0.49 ± 0.08
Fluoranthene	0.075 ± 0.100	0.28 ± 0.045	0.24 ± 0.042	0.30 ± 0.066	0.33 ± 0.040	0.23 ± 0.010	0.33 ± 0.08
Pyrene	0.19 ± 0.015	0.04 ± 0.044	<0.01	0.07 ± 0.056	0.10 ± 0.048	0.05 ± 0.051	0.15 ± 0.09
Benz[a]anthracene	<0.02	0.01 ± 0.003	0.01 ± 0.003	0.01 ± 0.005	0.01 ± 0.002	0.02 ± 0.006	0.01 ± 0.00
Cyclopenta[c,d]pyrene	<0.01	0.02 ± 0.004	0.01 ± 0.003	0.02 ± 0.002	0.02 ± 0.001	0.03 ± 0.006	0.02 ± 0.00
Chrysene	0.01 ± 0.03	0.06 ± 0.009	0.05 ± 0.005	0.05 ± 0.002	0.07 ± 0.012	0.06 ± 0.007	0.06 ± 0.00
Benzo[b]fluoranthene þ Benzo[j]fluoranthene	<0.02	0.01 ± 0.001	0.01 ± 0.002	0.02 ± 0.010	0.01 ± 0.002	0.02 ± 0.003	0.01 ± 0.00
Benzo[k]fluoranthene	<0.01	0.01 ± 0.003	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.002	0.01 ± 0.003	0.01 ± 0.00
Benzo[a]pyrene	<0.02	0.04 ± 0.004	0.04 ± 0.010	0.05 ± 0.006	0.04 ± 0.011	0.06 ± 0.015	0.05 ± 0.00
Benzo[g,h,i]perylene	<0.01	0.07 ± 0.020	0.07 ± 0.023	0.10 ± 0.001	0.09 ± 0.039	0.16 ± 0.012	0.08 ± 0.02
TEQ (μg kg^{-1})	5.65 10 $^{-4}$	0.050	0.050	0.062	0.055	0.074	0.066

TEQ are calculated with TEFs from INERIS table. TEQ are lower-bound TEQs (PAH contents below the limit of quantitation were not been taken into account).

Table VII. Toxic equivalent factors and PAH levels in salmon smoked by liquid smoke at three different smoke-exposure times and two smokehouse temperatures (in μg kg^{-1} ± standard deviation of wet tissue).

		Parameters of smoking					
	Initial contamination	1 h, 22 °C	1 h, 32° C	2 h, 22 °C	2 h, 32° C	3 h, 22 °C	3 h, 32° C
Fluorene	0.06 ± 0.041	0.67 ± 0.372	0.97 ± 0.212	1.48 ± 0.200	1.48 ± 0.249	1.0 ± 0.274	1.93 ± 0.785
Phenanthrene	0.04 ± 0.018	1.25 ± 0.469	1.53 ± 0.279	1.51 ± 0.344	1.05 ± 0.429	1.13 ± 0.455	1.53 ± 0.56
Anthracene	0.01 ± 0.005	0.22 ± 0.076	0.38 ± 0.059	0.36 ± 0.002	0.33 ± 0.089	0.29 ± 0.095	0.37 ± 0.04
Fluoranthene	0.075 ± 0.100	0.31 ± 0.050	0.23 ± 0.041	0.37 ± 0.104	0.25 ± 0.024	0.34 ± 0.094	0.29 ± 0.11
Pyrene	0.19 ± 0.015	0.08 ± 0.110	0.07 ± 0.072	0.45 ± 0.118	0.06 ± 0.023	0.39 ± 0.207	0.20 ±0.25(
Benz[a]anthracene	<0.02	0.01 ± 0.003	0.01 ± 0.003	0.01 ± 0.002	0.01 ± 0.006	0.01 ± 0.003	0.02 ± 0.00
Cyclopenta[c,d]pyrene	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chrysene	0.01 ± 0.03	0.05 ± 0.004	0.05 ± 0.004	0.07 ± 0.007	0.07 ± 0.004	0.06 ± 0.005	0.07 ± 0.00
Benzo[b]fluoranthene þ Benzo[j]fluoranthene	<0.02	0.01 ± 0.001	0.01 ± 0.001	<0.02	<0.02	<0.02	<0.02
Benzo[k]fluoranthene	<0.01	0.01 ± 0.001	0.01 ± 0.001	<0.01	<0.01	<0.01	<0.01
Benzo[a]pyrene	<0.02	0.03 ± 0.001	0.05 ± 0.012	0.03 ± 0.007	0.04 ± 0.004	0.03 ± 0.003	0.03 ± 0.027
Benzo[g,h,i]perylene	<0.01	0.08 ± 0.064	0.12 ± 0.028	0.03 ± 0.016	0.05 ± 0.025	0.09 ± 0.19	0.09 ± 0.049
TEQ (μg kg^{-1})	5.65 10 $^{-4}$	0.037	0.060	0.039	0.048	0.038	0.041

TEQ are calculated with TEFs from INERIS table. TEQ are lower-bound TEQs (PAH contents below the limit of quantitation were not been taken into account).

processes/parameters and the maximum legal value. The heterogeneity in PAH contamination of food in the various European countries and the different diets can explain this high legal limit.

Friction. As with the thermostated plates process, there are half the number of low molecular-weight PAHs than in the smouldering process (Table VI). All PAH levels are generally below those of the thermostated plates. However, the benzo[a]pyrene content is higher than in thermostated plates (0.06 and 0.04 μg kg^{-1}, respectively) and at 32 °C, 3 h of smoke exposure, benzo[g,h,i]perylene concentration is double that in the thermostated plates method. Thus, this process is potentially more hazardous than thermostated plates, due to a higher occurrence of certain high molecular-weight PAHs, even if the PAH content is lower than the smouldering process. Benzo[a]pyrene reaches a level close to 0.06 μg kg^{-1} after 3 h of smoking. This is the highest concentration found for all smoking processes, but is still much lower than the legal limit. A concentration of benzo[a]pyrene of 0.10 μg kg^{-1} has already been reported in commercially smoked fish (Kazerouni et al. 2001), which strengthens our results and shows

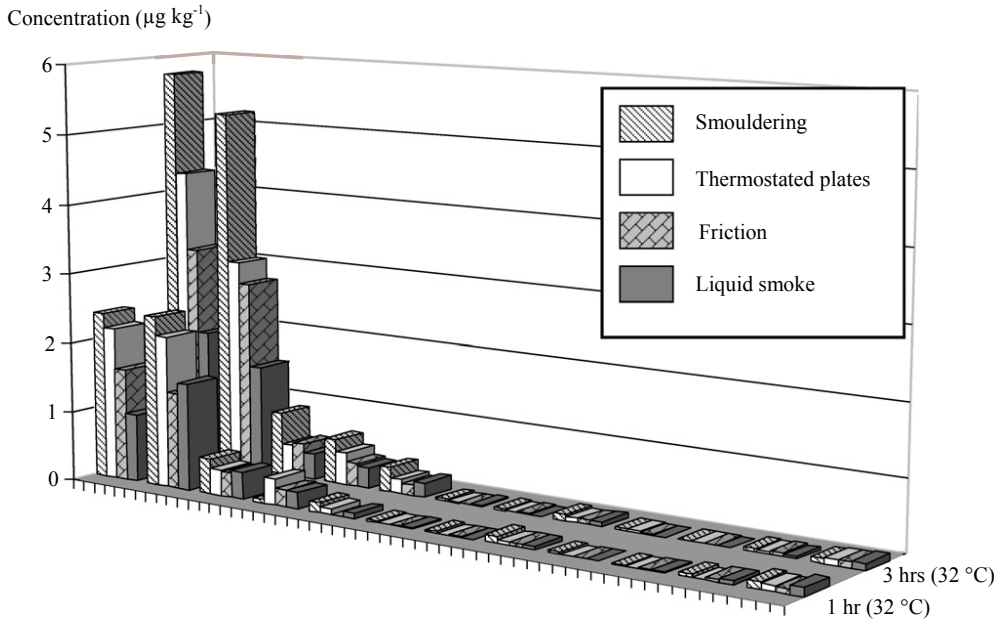

Figure 2. Predominance of PAHs of low-molecular-weight according to the smoking process (1 or 3 h of smoke exposure at 32° C). FL: fluorene; PHE: phenanthrene; AN: anthracene; FA: fluoranthene; PY: pyrene; BA: benzo[a]anthracene; CPP: cyclopenta[c,d]pyrene; CHR: chrysene; B[b+j]F: benzo[b]fluoranthene+benzo[j]fluoranthene; BkF: benzo[i]fluoranthene; BaP: benzo[a]pyrene; BghiP: benzo[g,h,i]perylene.

that the conditions of the smoking process used are similar to those found in the industry. However, greater levels of benzo[a]pyrene have also been reported in commercially smoked herring with 0.5 µg kg^{-1} and eel with 0.3 µg kg^{-1} (Storelli et al. 2003).

It is not surprising to find very low concentrations of PAHs with the friction process as this smoking technique has the lowest wood pyrolysis temperature (380° C). It has already been reported that when the thermal degradation of wood does not exceed 425 °C, PAHs are virtually absent in wood smoke (Sainclivier 1985). Friction is the only modern smoking process that produces wood smoke under 400 °C, which is the natural temperature of wood ignition.

Liquid smoke. This process gave the lowest levels of PAHs (Table VII). As reported in Regulation 2065/2003 (European Commission, 2003), the use of smoke flavourings is generally considered to be of less concern than traditional smoking processes. The low PAH concentrations found in salmon treated with liquid smoke confirm the legislative view and improvements during the process of producing liquid smoke. Nevertheless, liquid smokes can differ considerably in composition according to the production process and the nature of the wood used, not only from an organoleptic but also from a PAH content point-of-view (Hattula et al. 2001). ANOVA results do not show any effects of time of smoke exposure or smokehouse temperature. Thus, the maximum number of PAHs adsorbable from liquid smoke by the product could be considered as reached. The highest benzo[a]pyrene concentration is 0.05 µg kg^{-1}, found for 1 h of smoke exposure and 32° C. If liquid smoke atomization is considered as a flavouring process, this value is 1.5 times the legal limit (0.03 µg kg^{-1}) but, if it is considered as a smoking process, this value is well below the legal limit of 5 µg kg^{-1}. Actually, the use of liquid smoking should be ruled by the European Commission (1988), thus, giving a non-compliant product (benzo[a]pyrene concentration of 0.05 µg kg^{-1} in the product versus the legal limit of 0.03 µg kg^{-1}). However, contamination is below those of traditional smoking processes, whose legal limit has been set at 5 µg kg^{-1} of benzo[a]pyrene (European Commission, 2005a). Clearly, the liquid smoke process needs further investigation. Indeed, for the same product, different liquid smokes exist that lead to different limit values of contamination. Much work has already been done on the volatile composition of liquid smokes (Guillén and Ibargoitia 1996; Guillén and Manzanos 1996, 1999) but not enough on their PAH composition. Thus, similar investigations are needed to assess the PAH content

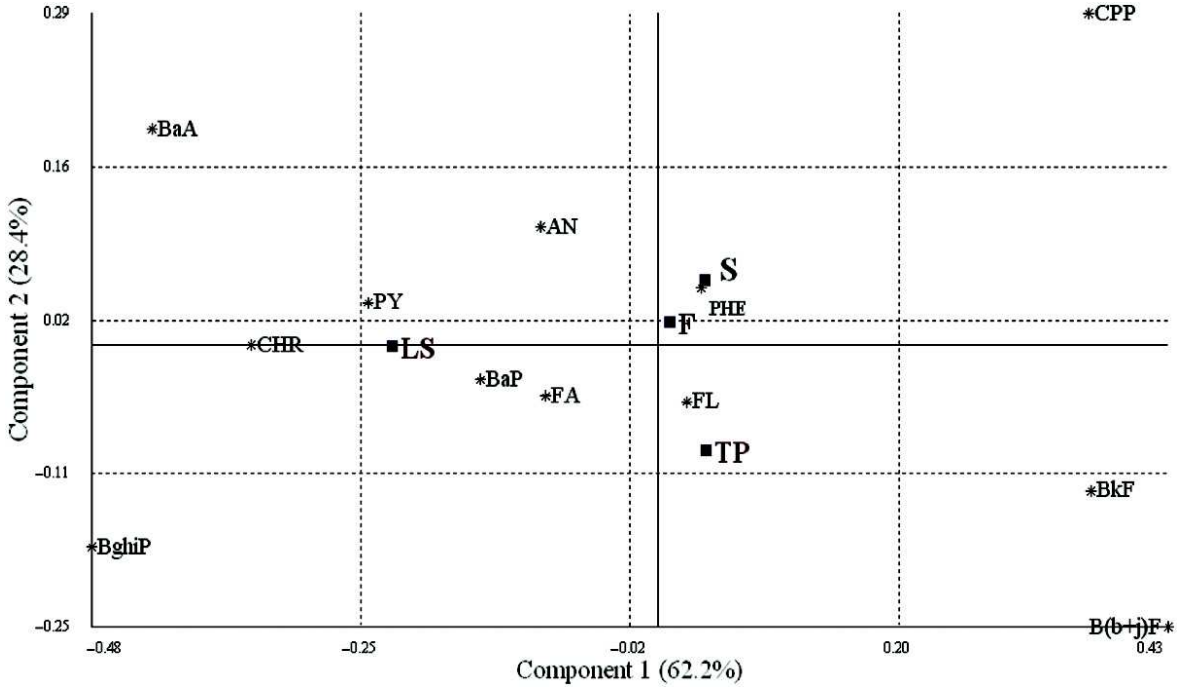

Figure 3. Projection of processes and PAH levels in plane 1–2 of factorial analysis of correspondences. For the smoking processes (in bold), S: smouldering; TP: thermostated plates; F: friction; LS: liquid smoke. FL: fluorene; PHE: phenanthrene; AN: anthracene; FA: fluoranthene; PY: pyrene; BA: benzo[a]anthracene; CPP: cyclopenta[c,d]pyrene; CHR: chrysene; B(b+j)F: benzo[b]fluoranthene + benzo[j]fluoranthene; BkF: benzo[i]fluoranthene; BaP: benzo[a]pyrene; BghiP: benzo[g,h,i]perylene.

of products treated with liquid smokes and to understand the deposition and penetration kinetic of PAHs from smoke flavourings in the products.

Comparison of the four processes. Smouldering is the smoke generation process that leads to smoked fish with the highest TEQs. The variation is caused by the higher levels of low molecular-weight PAHs than in fish smoked by other techniques. Fish smoked by liquid-smoke vaporization gave the lowest TEQs. When friction is used, the smoked fish fillets have the lowest PAH content, but the benzo[a]pyrene concentration is high. Therefore, as the TEF of this PAH is 1, the TEQ is significantly increased. Conversely, when the thermostated plates process is used, individual PAH concentrations are higher, while the benzo[a]pyrene concentration is lower than with the friction technique. Consequently, the TEQs of the thermostated plates method are always lower than the friction technique. Thus, smoke generation by thermostated plates is the wood pyrolysis technique that leads to the lowest TEQs and least contaminated fillets.

A correspondence factorial analysis (CFA) (Figure 3) performed on the PAH content of fish smoked for 3 h at 32 °C shows the main effects of the four smoking processes versus PAH contamination. The three wood-pyrolysis processes seem to form a homogeneous group versus liquid smoke according to their effect on the PAH composition of smoked fish. In fact, the PAH composition of the four groups of smoked fish is very similar, but the fish fillets smoked by processes applying wood pyrolysis showed higher levels of fluorene, phenanthrene cyclopenta[c,d]pyrene and benzofluoranthenes. For a 3-h smoke exposure and smokehouse temperature of 32° C, the concentration of benzo[a]pyrene is 0.03 μg kg^{-1} in fillets treated by liquid smoke, 0.04 μg kg^{-1} in fillets smoked by thermostated plates and 0.05 μg kg^{-1} in fillets smoked by friction and smouldering. After fluorene, phenanthrene showed the highest level in all the smoking processes studied. This result has been reported in previous studies (Karl and Leinemann 1996; Wang et al. 1999; Storelli et al. 2003). This PAH deserves special interest but its toxicity is not very high. However, the determination of phenanthrene content could be an indicator of the intensity of the smoking process or to discriminate between products smoked by wood pyrolysis processes and products treated with liquid smoke, especially with increased smoke-exposure times. Indeed, phenanthrene concentration is the second highest compared to other PAH concentrations in salmon smoked by liquid smoke, but is the lowest (1.53 μg kg^{-1})

compared to the other smoking techniques (from 2.73 μg kg^{-1} with friction to 5.20 μg kg^{-1} with smouldering). The difference also increases with smoke-exposure time.

Conclusions

The method reported here is a suitable analytical technique for PAHs in investigative studies. However, this type of analysis is only possible with technologies, such as GC–MS/MS and the use of ^{13}C-labelled internal standards, which demonstrates their suitability and accuracy for the determination and quantification of contaminants in food. It offers reduced extraction times and takes place under gentle conditions, which avoid the generation of potential interference or matrix effects. The benefits of the extraction step are only noticeable owing to a GC–MS/MS analysis optimized for the monitoring of PAHs.

This study assesses the occurrence of PAHs in salmon smoked with the four commonly used industrial processes. The potential effects of two essential parameters (time of smoke exposure and smokehouse temperature) have been evaluated. Among the three techniques applying wood pyrolysis, smouldering, which is the most common, leads to the more contaminated products. Salmons smoked by the friction method have the lowest levels of PAHs, except for benzo[a]pyrene, which mainly contributes to the increase in TEQ of smoked products. The thermostated plates technique generates higher PAH levels but the benzo[a]pyrene concentration is lower than in the friction method, which gives the lowest overall contamination. To better understand the TEQs, further research is needed to establish the conditions affecting PAH generation in modern smoking processes. Variations in parameter settings, such as moisture of sawdust, alternative smoke-exposure times and smokehouse temperatures and nature of wood, could supply other significant data on PAH generation. This study focused on the impact of various parameters on PAH profiles. Further investigations on the influence of smoking parameters on the deposition kinetics of PAHs (adsorption, migration in the product) during the smoking process are essential. Liquid smoke is a unique case because, when considered as a smoking process, the lowest individual levels of PAHs and TEQ are found. However, when considered as a flavouring process, the benzo[a]pyrene value is higher compared to the legal level; thus, the legislation concerning liquid smoke in the fish-smoking process needs clarification. Finally, the TEQ approach was chosen to express total PAH contamination of a smoked or unsmoked product. This presentation of PAH content is empirical because the effects of PAHs in a mixture are insufficiently understood. Nevertheless, we have shown that the TEQ approach can compare and discriminate products smoked by various smoking processes. However, this type of analysis is only possible with technologies, such as GC–MS/MS and the use of ^{13}C-labelled internal standards, which demonstrates their suitability and accuracy for the determination and quantification of contaminants in food.

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References

AFSSA 2003. Avis de l'AFSSA relatif à une demande d'avis sur l'évaluation des risques présentés par le benzo[a]pyrene B[a]P et par d'autres hydrocarbures aromatiques polycycliques (HAP), présents dans diverses denrées ou dans certaines huiles végétales, ainsi que sur les niveaux de concentration en HAP dans les denrées au-delà desquels des problèmes de santé risquent de se poser. Agence Française de Sécurité Sanitaire des Aliments. Saisine no. 2000-SA-0005.

Baird WM, Hooven LA, Mahadevan B. 2005. Carcinogenic polycyclic aromatic hydrocarbon–DNA adducts and mechanism of action. Environmental and Molecular Mutagenesis 45:106–114.

Ballesteros E, García Sánchez A, Ramos Martos N. 2006. Simultaneous multideterminationof residues of pesticides and polycyclic aromatic hydrocarbons in olive and olive-pomace oils by gas chromatography/tandem mass spectrometry. Journal of Chromatography 1111:89–96.

Chen BH, Wang CY, Chiu CP. 1996. Evaluation of analysis of polycyclic aromatic hydrocarbons in meat products by liquid chromatography. Journal of Agriculture and Food Chemistry 44:2244–2251.

Chiu CP, Lin YS, Chen BH. 1997. Comparison of GC–MS and HPLC for overcoming matrix interferences in the analysis of PAHs in smoked food. Chromatographia 44:497–504.

Clifford MN, Tang SL, Eyo AA. 1980. Smoking of foods. Process Biochemistry June/July:8–11.

Collins JF, Brown JP, Alexeeff GV, Salmon AG. 1998. Potency equivalency factors for some polycyclic aromatic hydrocarbons and polycyclic aromatic hydrocarbons derivatives. Regulatory Toxicology and Pharmacology 28:45–54.

De Boer J, Law RJ. 2003. Development in the use of chromatographic techniques in marine laboratories for the determination of halogenated contaminants and polycyclic aromatic hydrocarbons. Journal of Chromatography A 1000:223–251.

Dugay A, Herrenknecht C, Czok M, Guyon F, Pages N. 2002. New procedure for selective extraction of polycyclic aromatic hydrocarbons in plants for gas chromatographic – mass spectrometric analysis. Journal of Chromatography A 958:1–7.

Easton MDL, Luszniak D, Von der Geest E. 2002. Preliminary examination of contaminant loadings in farmed salmon, wild salmon and commercial salmon feed. Chemosphere 46:1053–1074.

European Commission. 1988. Council Directive No. 88/388 of 22 June 1988 on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production. Official Journal of the European Union L 184:1–10.

European Commission. 2002. Commission Directive No. 2002/69 of 26 July 2002 laying down the sampling methods and the methods of analysis for the official control of dioxins and the determination of dioxin-like PCBs in foodstuffs. Official Journal of the European Union L 209:5-14.

European Commission. 2003. Regulation (EC) No. 2065/2003 of the european Parliament and of the Council of 10 November 2003 on smoke flavourings used or intended for use in or on foods. Official Journal of the European Union L 309:1–8.

European Commission. 2005a. Commission Regulation (EC) No 208/2005 of 4 February 2005 amending Regulation (EC) No 466/2001 as regards polycyclic aromatic hydrocarbons. Official Journal of the European Union L 34:3–5.

European Commission. 2005b. Commission Recommendation No. 2005/108 of 4 February 2005 on the further investigation into the levels of polycyclic aromatic hydrocarbons in certain foods. Official Journal of the European Union L 34:43–45.

European Commission. 2005c. Commission Directive 2005/10/EC of 4 February 2005 laying down the sampling methods and the methods of analysis for the official control of the levels of benzo[a]pyrene in foodstuffs. Official Journal of the European Union L 34 : 15-20.

Grova N, Monteau F, Le Bizec B, Feidt C, André F, Rychen G. 2005. Determination of phenanthrene and hydroxyphenanthrenes in various biological matrices at trace levels using gas chromatography coupled to mass spectrometry. Journal of Analytical Toxicology 29:175–181.

Guillén MD, Ibargoitia ML. 1996. Volatile components of aqueous liquid smokes from *Vitis vinifera* L shoots *and Fagus sylvatica* L wood. Journal of Science and Food Agriculture 72:104–110.

Guillén MD, Manzanos MJ. 1996. Study of the components of a solid smoke flavouring preparation. Food Chemistry 55:251–257.

Guillén MD, Manzanos MJ. 1999. Extractable components of the aerial parts of *Salvia lavandulifolia* and composition of the liquid smoke flavoring obtained from them. Journal of Agriculture and Food Chemistry 47:3016–3027.

Guillén MD, Sopelana P, Partearroyo MA. 2000a. Determination of polycyclic aromatic hydrocarbons in commercial liquid smoke flavorings of different compositions by gas chromatography–mass spectrometry. Journal of Agriculture and Food Chemistry 48:126–131.

Guillén MD, Sopelana P, Partearroyo MA. 2000b. Polycyclic aromatic hydrocarbons in liquid smoke flavorings obtained from different types of wood. Effect of storage in polyethylene flasks on their concentrations. Journal of Agriculture and Food Chemistry 48:5083–5087.

Hamm R. 1977. Analysis of smoke and wood smoke. Pure and Applied Chemistry 49:1655–1666.

Hattula T, Elfving K, Mroueh UM, Luoma T. 2001. Use of liquid smoke flavouring as an alternative to traditional flue gas smoking of rainbow trout fillets (*Oncorhyncus mykiss*). Lebensmittel-Wissenschaft ünd Technologie 34:521–525.

INERIS (Doornaert, B, Pichard, A, Gillet, C.). 2003. Hydrocarbures aromatiques polycycliques (HAPs). Evaluation de la relation dose-réponse pour des effets cancérigènes: Approche substance par substance (facteurs d'équivalence toxique – FET) et approche par mélanges. Evaluation de la relation dose-réponse pour des effets non cancérigènes: Valeurs Toxicologiques de Référence (VTR). INERIS-DRC-03-47026-ETSC-BDo-No. 03DR177.

Järvenpää E, Huopalahti R, Tapanainen P. 1996. Use of supercritical fluid extraction–high performance chromatography in the determination of polynuclear aromatic hydrocarbons from smoked and broiled fish. Journal of Liquid Chromatography and Related Technologies 19:1473–1482.

Jira W. 2004. A GC/MS method for the determination of carcinogenic polycyclic aromatic hydrocarbons (PAH) in smoked meat products and liquid smokes. European Food Research and Technology 218:208–212.

Kannappan S, Indra Jasmine G, Jeyachandran P, Tamil Selvi A. 2000. Polyaromatic hydrocarbons in processed fishery products. Journal of Food Science and Technology 37:596–601.

Karl H. 1997. Influence of the smoking technology on the quality of smoked fish regarding undesirable compounds. In: Luten JB, editor. Seafood from producer to consumer. Integrated approach to quality. Amsterdam: Elsevier. pp 633–639.

Karl H, Leinemann M. 1996. Determination of PAH in smoked fishery products from different smoking kilns. Zeitschrift fur Lebensmittel Unterschung ünd Forschung 202:459–464.

Kazerouni N, Sinha R, Hsu CH, Greenberg A, Rothman N. 2001. Analysis of 200 food items for benzo[a]pyrene and estimation of its intake in an epidemiologic study. Food Chemistry and Toxicology 39:423–436.

Kishikawa N, Wada M, Kuroda N, Akiyama S, Nakashima K. 2003. Determination of polycyclic aromatic hydrocarbons in milk samples by high-performance liquid chromatography with fluorescence detection. Journal of Chromatography B 789:257–264.

Koffi Houessou J, Benac C, Delteil C, Camel V. 2005. Determination of polycyclic aromatic hydrocarbons in coffee brew using solid-phase extraction. Journal of Agriculture and Food Chemistry 53:871–879.

Larsen JC, Larsen PB. 1998. Chemical carcinogens. In: Hester RE, Harrison RM, editors Air pollution and health.: Cambridge University Press. pp 33–56.

Marcé RM, Borrull F. 2000. Solid-phase extraction of polycyclic aromatic compounds. Journal of Chromatography A 885:273–290.

Marsili L, Caruso A, Fossi MC, Zanardelli M, Politi E, Focardi S. 2001. Polycyclic aromatic hydrocarbons (PAHs) in subcutaneous biopsies of Mediterranean cetaceans. Chemosphere 44:147–154.

Monteau, F, Sarcher, L, Varlet, V, Maume, D, André, F, Le Bizec, B. 2005. Compared mass spectrometry approaches for PAH determination in food. Paper presented at the Dioxin 2005/ISPAC 20 Congress, Toronto, Canada.

Moret S, Conte LS. 2000. Polycyclic aromatic hydrocarbons in edible fats and oils: occurence and analytical methods. Journal of Chromatography A 882:245–253.

Moret S, Conte L, Dean D. 1999. Assessment of polycyclic aromatic hydrocarbon content of smoked fish by means of a fast HPLC/HPLC method. Journal of Agriculture and Food Chemistry 47:1367–1371.

Mottier P, Parisod V, Turesky RJ. 2000. Quantitative determination of polycyclic aromatic hydrocarbons in barbecued meat sausages by gas chromatography coupled to mass spectrometry. Journal of Agriculture and Food Chemistry 48:1160–1166.

Munoz D, Doumenq P, Guiliano M, Jacquot F, Scherrer P, Mille G. 1997. New approach to study of spilled crude oils using high resolution GC–MS (SIM) and metastable reaction monitoring GC–MS–MS. Talanta 45:1–12.

Nyman PJ, Perfetti GA, Joe Jr FL, Diachenko GW. 1993. Comparison of two clean-up methodologies for the gas chromatographic/mass spectrometric determination of low

nanogram/gram levels of polynuclear aromatic hydrocarbons in seafood. Food Additives and Contaminants 10:489–501.

Pointet K, Milliet A. 2000. PAHs analysis of fish whole gall bladders and livers from the Natural Reserve of Camargue by GC/MS. Chemosphere 40:293–299.

Rivera L, Curto MJ, Pais P, Galceran MT, Puignou L. 1996. Solid-phase extraction for the selective isolation of polycyclic aromatic hydrocarbons, azaarenes and heterocyclic aromatic amines in charcoal-grilled meat. Journal of Chromatography A 731:85–94.

Sainclivier, M. 1985. L'industrie alimentaire halieutique. Des techniques ancestrales à leurs réalisations contemporaines: Salage, séchage, fumage, marinage, hydrolysats. Bulletin scientifique et technique de l'Ecole Nationale Supérieure Agronomique Centre de Recherches de Rennes. pp 219–285 [in French].

SCF. 2002. Opinion of the Scientific Committee on Food (SCF) on the risks to human health of polycyclic aromatic hydrocarbons in food (expressed on 4 December 2002). SCF/CS/CNTM/PAH/29 final.

Šimko P. 1991. Changes of benzo[a]pyrene contents in smoked fish during storage. Food Chemistry 40:293–300.

Šimko P. 2002. Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. Journal of Chromatography B 770:3–18.

Šimko P. 2005. Factors affecting elimination of polycyclic aromatic hydrocarbons from smoked meat foods and liquid smoke flavorings. Molecular Nutrition and Food Research 49:637–647.

Stolyhwo A, Sikorski ZE. 2005. Polycyclic aromatic hydrocarbons in smoked fish: a critical review. Food Chemistry 91:303–311.

Storelli MM, Stuffler RG, Marcotrigiano GO. 2003. Polycyclic aromatic hydrocarbons, polychlorinated biphenyls, chlorinated pesticides (DDTs), hexachlorocyclohexane, and hexachlorobenzene residues in smoked seafood. Journal of Food Protection 66:1095–1099.

Varlet V, Serot T, Knockaert C, Cornet J, Cardinal M, Monteau F, Le Bizec B, Prost C. 2006. Organoleptic characterization and PAH content of salmon (*Salmo salar*) smoked according to four industrial smoking techniques. Journal of Science and Food Agriculture (accepted for publication).

Veyrand B, Brosseaud A, Sarcher L, Varlet V, Monteau F, Marchand P, André F, Le Bizec B. 2006. Innovative method for determination of 20 polycyclic aromatic hydrocarbons in food and oil samples using GC-MS/MS based on an isotope dilution approach. Journal of Chromatography A (submitted for publication).

Wang G, Lee AS, Lewis M, Kamath B, Archer RK. 1999. Accelerated solvent extraction and gas chromatography/mass spectrometry for determination of polycyclic aromatic hydrocarbons in smoked food samples. Journal of Agriculture and Food Chemistry 47:10626–1066.

Yeakub-Ali MD, Cole RB. 2001. SFE–plus–C_{18} cleanup and selective extraction method for GC/MS quantitation of polycyclic aromatic hydrocarbons in smoked meat. Journal of Agriculture and Food Chemistry 49:4192–4198.

Yurchenko S, Mölder U. 2005. The determination of polycyclic aromatic hydrocarbons in smoked fish by gas chromastography mass spectrometry with positive-ion chemical ionization. Journal of Food Composition and Analysis 18:857–869.

Zabik ME, Booren A, Zabik MJ, Welch R, Humphrey H. 1996. Pesticides residues, PCBs and PAHs in baked, charbroiled, salt boiled and smoked Great Lakes lake trout. Food Chemistry 55:231–239.

Bilan

La garantie de la sécurité sanitaire des saumons fumés fabriqués passe par la surveillance de deux critères : le critère de sécurité alimentaire vis-à-vis des contaminants du fumage (HAP) et le critère de sécurité microbiologique. Ce dernier a été et reste toujours très étudié aujourd'hui, notamment à l'IFREMER, c'est pourquoi il n'a pas fait l'objet d'une étude de notre part (Leroi, F. et al., 1998 ; Cardinal, M. et al., 2004). En effet, les produits commercialisés sont soumis à des règles d'hygiène au cours de leur fabrication permettant de garantir une sécurité microbiologique totale. Nous nous sommes donc imposés ces mêmes règles d'hygiène lors de nos campagnes de fumage. Ainsi, dans notre étude, le critère sanitaire était uniquement ramené au critère de sécurité alimentaire vis-à-vis des HAP.

Au terme des analyses sanitaires, on a donc pu s'apercevoir que les quatre techniques de production de fumée testées conduisaient à des quantités similaires de contaminants, en conformité avec les normes européennes en vigueur. Cependant, on a pu mettre en évidence des imperfections dans la législation concernant le statut de la fumée liquide. En effet, au regard de la législation de la fumaison, tous les produits fumés sont largement conformes puisque l'on atteint des taux de benzo[a]pyrène supérieurs à 0,03 µg/kg dans le saumon traité par cette technique. Pour la fumée liquide, au regard de la législation sur l'aromatisation, les produits fumés ne sont pas conformes. Cependant, en France, le statut de l'atomisation de fumée liquide en cellule de fumage n'est pas clair à savoir s'il doit être considéré comme méthode de fumage ou comme moyen d'aromatisation. Par exemple, dans la filière carnée, la revaporisation de fumée liquide dans un fumoir est assimilée à un fumage classique mais la valeur maximale autorisée en HAP (0,03 µg/kg) ainsi que l'étiquetage (mention « au goût fumé » ou « arôme fumée ») restent ceux en vigueur dans l'aromatisation. Cette technique n'est donc pas comparable au niveau législatif aux autres méthodes de fumage alors qu'elle conduit aux taux les plus faibles de HAP dans les saumons fumés. De plus, malgré des déficiences organoleptiques, cette méthode apparaît comme une alternative au fumage étant donné les restrictions environnementales (rejets de fumées dans l'atmosphère, …) auxquelles seront bientôt soumises les autres techniques.

Des analyses supplémentaires sur la totalité des HAP reconnus potentiellement toxiques par l'Union Européenne ont été réalisées pour élargir le spectre de contamination en HAP de ces produits. En effet, il est nécessaire d'avoir d'une part accès aux quantités de HAP aussi toxiques que le benzo[a]pyrène, ce que ne prennent pas en compte les législations actuelles, et d'autre part aux quantités de HAP apportées par la contamination environnementale initiale qui peut parfois

être très importante notamment en HAP de faible poids moléculaire (Visciano, P. et al., 2006). Dans notre cas, la part des HAP provenant de la contamination environnementale est négligeable. Ainsi, les différents filets de saumons fumés se sont révélés peu contaminés en HAP de haut poids moléculaire et plus fortement contaminé en HAP de faible poids moléculaire. Les procédés de fumage tels que nous les avons pratiqués ont donc été principalement générateurs d'HAP de faible poids moléculaire considérés peu toxiques. Cette conclusion est surtout valable pour les produits fumés par plaques thermostatées, autocombustion et friction. En effet, les produits traités par fumée liquide présentaient certes les plus faibles concentrations en HAP mais étaient particulièrement composés d'HAP de poids moléculaire plus important comme le chrysène ou le benzo[a]pyrène. La traçabilité sur les modes d'obtention des condensats de fumée doit donc être améliorée pour comprendre cette différence de composition.

L'originalité de l'utilisation de la spectrométrie de masse en tandem en mode SRM pour la précision de la détection des signaux des analytes a consisté à jouer sur la forte stabilité des HAP aux fortes énergies d'ionisation pour améliorer le suivi d'ions fils tout en refragmentant d'éventuelles substances résiduelles coextraites avec les matières grasses malgré un protocole d'extraction efficace. En effet, ces matières grasses peuvent être responsables de coélutions chromatographiques. L'utilisation de la spectrométrie de masse en tandem a donc permis de détecter et de quantifier de faibles taux de HAP même dans une matrice alimentaire grasse comme le saumon. Les différents produits étant conformes vis-à-vis de la législation (< 5 µg/kg), nous avons pu les soumettre au protocole d'analyse sensorielle.

Les résultats des analyses sensorielles des différents saumons fumés ont permis de différencier de prime abord les produits fumés par générateur externe de ceux traités par fumée liquide, aussi bien selon des descripteurs d'odeur que de flaveur. En effet, les produits fumés par autocombustion, plaques thermostatées et friction ont été plutôt décrits par des notes élevées et homogènes en ce qui concerne les descripteurs odorants « fumée feu de bois », « saumon » et « gras » et les descripteurs de la flaveur « fumée feu de bois », « saumon » et « salé ». En revanche, les produits traités par fumée liquide ont été plutôt perçus avec des notes élevées pour les descripteurs d'odeurs et de flaveurs « végétal », « fumée froide ». La fumée liquide utilisée semble donc donner aux produits une odeur et une flaveur forte qui cache rapidement les caractéristiques du poisson (gras, saumon, hareng). Les autres méthodes génèrent une fumée dont les propriétés organoleptiques conduisant à une odeur et une flaveur plus équilibrée de poisson fumé en préservant plus les caractéristiques organoleptiques du poisson.

Enfin, la production de saumon fumé par ces différents modes de génération de fumée a permis de réaliser une comparaison technologique et économique. Les plaques thermostatées

consomment la quantité de bois la moins importante mais la consommation électrique est bien supérieure aux autres. A l'inverse, le générateur à autocombustion entraîne une consommation électrique deux fois moins importante mais utilise de plus grandes quantités de sciure de bois et génére des imbrûlés et des cendres. Le générateur à friction emploie la même quantité de bois mais génére moins d'imbrûlés. Cependant, la consommation électrique est presque aussi importante que celle des plaques thermostatées et le coût de la bûche est plus important que celui de la sciure. Enfin, la fumée liquide possède une consommation électrique quasi nulle (vaporisation par air comprimé) mais le prix de la matière première classe ce procédé parmi l'un des plus coûteux. Ainsi, le générateur à autocombustion paraît être le meilleur compromis technologique et économique, ce qui pourrait expliquer qu'il est le plus largement utilisé dans l'industrie. Cependant, l'utilisation de fumée liquide pourrait également être une alternative si l'on prend en compte le coût des polices d'assurance par rapport à la diminution des risques d'incendie engendrée par l'utilisation de cette technique.

PARTIE 3 : Les composés volatils odorants du saumon fumé à froid :

- ✓ ***Influence des paramètres du fumage sur leur génération***
- ✓ ***Contribution à la définition de l'odeur globale du saumon fumé***

PARTIE 3 : Analyse des composés volatils odorants du saumon fumé à froid : influence des paramètres du fumage sur leur génération et leur contribution dans la définition de l'odeur globale du saumon fumé

Les travaux présentés dans la seconde partie ont montré que les différents modes industriels de génération de fumée conduisaient à des saumons fumés aux caractéristiques organoleptiques différentes. En effet, la composition chimique des fumées produites varie selon la température de pyrolyse du bois, la granulométrie du bois, son humidité, la géométrie du générateur, … Ces différences organoleptiques et en particulier odorantes sont à relier à la composition en composés volatils odorants des différents saumons fumés. L'étude des effets des différents modes de production de fumée sur la composition en composés volatils odorants des saumons fumés constitue donc un deuxième niveau d'investigation de l'odeur du saumon fumé. Cette étude est menée avec deux objectifs. L'un, scientifique, correspond à l'identification des composés volatils odorants responsables de la perception de l'odeur de poisson fumé et à la caractérisation de leur contribution dans l'odeur globale. L'autre, industriel, correspond à l'amélioration des connaissances des techniques de fumage afin de renseigner sur les paramètres de fumage conduisant à des produits fumés avec des odeurs prérequises.

Les travaux présentés dans la première partie ont permis de valider une méthode d'extraction quantitative et représentative des composés volatils odorants du saumon fumé. Cette méthode a notamment permis l'identification et la quantification de ces composés volatils odorants extraits de matrice fumée ou non fumée. Nous avons donc utilisé cette même méthode pour caractériser les composés volatils odorants de saumons fumés par différentes techniques de fumage.

Pour analyser l'odeur du poisson fumé, nous avons donc utilisé deux approches : une approche instrumentale, présentée dans la partie 1, permettant de caractériser les composés volatils odorants des différents saumons fumés ; une approche sensorielle, présentée dans la partie 2, permettant de caractériser l'odeur globale des différents poissons fumés. L'approche intrumentale est fondée sur la chromatographie en phase gazeuse couplée simultanément à l'olfactométrie et à la spectrométrie de masse. Ainsi, il est possible d'associer une zone odorante du chromatogramme détectée par olfactométrie avec l'identité des pics repérés dans cette zone par spectrométrie de masse. Cependant, il est beaucoup plus difficile de relier les résultats issus de l'analyse olfactométrique obtenus individuellement grâce à la séparation chromatographique avec les résultats de l'analyse sensorielle obtenus sur la matrice globale. Or, l'odeur globale est constituée des composés volatils odorants de la matrice. En effet, l'analyse des composés volatils odorants peut amener des informations concrètes pour

expliquer les différences odorantes entre les produits testés, notamment entre les saumons traités par fumée liquide et les autres saumons fumés.
Ainsi, les effets de la nature des générateurs sur les concentrations et la nature des composés volatils les plus odorants des saumons fumés produits ont été rédigés dans un article paru dans *Journal of Agricultural and Food Chemistry*.

Afin de relier les données olfactométriques et sensorielles obtenues sur les différents saumons fumés, nous avons effectué des traitements statistiques des résultats obtenus pour synthétiser d'une part les effets des différents générateurs et paramètres de fumage sur les caractéristiques odorantes et sur les concentrations en composés volatils odorants du saumon fumé et d'autre part pour identifier les odeurs, les flaveurs et les composés aromatiques prédominants pour discriminer les différents saumons fumés. Puis, nous avons réalisé un traitement statistique par la méthode des moindres carrés partiels - Partial Least Squares (PLS2) - afin d'expliquer les relations existantes entre les odeurs des saumons fumés et les concentrations en composés volatils odorants déterminés dans chaque produit. Ceci nous a donc permis d'élaborer une stratégie d'identification des composés volatils odorants responsables ou impliqués dans les odeurs des saumons fumés caractérisées par l'analyse sensorielle.
Ces travaux font l'objet d'une publication soumise dans *Food Chemistry*. Ils ont également été présentés lors d'une conférence orale intitulée « relationships between odorant characteristics and the most odorant volatile of salmon smoked by four industrial smoking techniques » au congrès EUROFOOD CHEM XIV à Paris le 29 août 2007.

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Olfactometric Determination of the Most Potent Odor-Active Compounds in Salmon Muscle (*Salmo salar*) Smoked by Using Four Smoke Generation Techniques

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The volatile compounds of salmon fillets smoked according to four smoked generation techniques (smoldering, thermostated plates, friction, and liquid smoke) were investigated. The main odor-active compounds were identified by gas chromatography coupled with olfactometry and mass spectrometry. Only the odorant volatile compounds detected by at least six judges (out of eight) were identified as potent odorants. Phenolic compounds and guaiacol derivatives were the most detected compounds in the olfactometric profile whatever the smoking process and could constitute the smoky odorant skeleton of these products. They were recovered in the aromatic extracts of salmon smoked by smoldering and by friction, which were characterized by 18 and 25 odor-active compounds, respectively. Furannic compounds were more detected in products smoked with thermostated plates characterized by 26 odorants compounds. Finally, the 27 odorants of products treated with liquid smoke were significantly different from the three others techniques applying wood pyrolysis because pyridine derivatives and lipid oxidation products were perceived in the aroma profile.

KEYWORDS: Olfactometry; smoked salmon; odor-active compounds; fish smoking process

INTRODUCTION

Traditionally, smoking was used for the preservation of fish, but for several years, smoked fish was appreciated for its organoleptic quality. A recent study on European consumer preferences showed that these preferences were represented by a whole range of smoke odors and flavors (*1*). The control of the organoleptic characteristics of smoked fish through the control of processes to adapt their products to consumer demand could be a real interest to processors.

Several studies were carried out to characterize the volatile compound compositions of smoked fishes (*2*, *3*) and wood smokes (*4*, *5*) and to assess the effect of smoking parameters, smoke generator, wood species, hygrometry, and temperature of the smokehouse on the deposition of smoke compounds (*6*–*8*). More recently, studies focused on phenolic compounds have shown that their deposition depends on the smoking processes and on the parameters of these processes (*9*–*11*). Actually, phenolic compounds have been presented for a long time as key compounds in the smoked flavor of smoked products (*12*, *13*). Guaiacol and derivatives have been reported as contributors of the smoky taste, and syringol and derivatives are responsible for the smoky odor (*14*). However, the works of Ojeda et al. (*15*) or Cardinal et al. (*16*) have shown that it was not easy to associate the presence of molecules with flavor perception of smoked fishes. All of these works have allowed us to investigate volatile compounds of smoke and smoked fish, but they were not focused on odor-active compounds. Gas chromatography–olfactometry (GC-O) proposed by Fuller as early as 1964 enables odor-active compounds to be distinguished among all of the volatile compounds. Olfactometry was already used to identify raw or processed seafood aroma compounds (*17*–*19*), but for the first time in a recent study performed in our laboratory, the odor-active compounds in fresh salmon and salmon smoked by smoldering were identified by an olfactometric method (*20*). However, today, even if smoldering stays the main smoking technique for fish, other methods such as thermostated plates, friction, or liquid smoke atomization are used (*21*).

The aim of this study was to identify and to compare the odorant volatile compounds of salmon flesh smoked by using four smoke generation processes. To investigate the volatile odor-active compounds of smoked salmons, simultaneous steam distillation–solvent extraction (SDE) was used (*22*). This technique has already been validated to recover the odor-active compounds of fresh and smoked salmon (*20*).

In the first experiment, the odorant similarity of extract and smoked fish was assessed. Then, combining two olfactometric

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techniques, time intensity and frequency of detection methods, the four processes were differentiated and linked to their main detected odor-active compounds. In the second experiment, the perception of odorant compounds was tentatively correlated to their concentration. Finally, we try to establish a relation between the eventual differences in the odorant profiles of the four salmon sets and the smoke generation parameters. The identification of volatile compounds, which really contribute to odor quality of smoked fish as well as to knowledge of the impact of smoke generation conditions on the perception of these compounds, was expected. As determination of odorant compounds of salmon smoked by various methods has never been carried out, this information is essential to understand the role of external smoke generators in the final odor of smoked products.

MATERIALS AND METHODS

Raw Material and Reagents. Ultrapure water was obtained with a MilliQ system. Dodecane was purchased from Aldrich (Steinheim, Germany), diethyl ether (purity, 99.5%) was purchased from Panreac (Barcelona, Spain), and ethanol (purity, 95%) was purchased from VWR (Fontenay-sous-bois, France). Beech wood sawdusts came from SPPS (Paris, France), and beech wood logs came from Bourdeau (Nozay, France). All standards were obtained from Sigma Aldrich (Steinheim, Germany), except all of the dimethylphenols were from Fluka (Buchs, Switzerland) and phenol was from Merck (Darmstadt, Germany).

Fish Processing. Salmon (*Salmo salar*) reared in Norway were purchased from a seafood wholesaler (Nantes, France). The time between their capture and their filleting was not more than 1 week. Nine gutted fishes of 3−4 kg of a same batch were received in a box in ice. They were directly filleted, trimmed, and put on grids in a cold chamber at +3 °C for 2 h. All of the fillets were about 1 kg. Analysis of water and NaCl contents were carried out before salting. The rate of water was 65 g/100 g, and the rate of NaCl was 0.20 g/100 g. Then, fillets were hand-salted with refined salt (Salins du Midi, France) and left for 3 h at +12 °C before they were briefly rinsed on grids with water (15 °C) and stored at 3 °C for 18 h until smoking.

Before smoking, the drying step was carried out by putting the fillets in the smokehouse at 18 °C for 15 min. This step allowed us to also standardize an internal temperature of 8 °C for all of the samples. Then, at the beginning of the smoking process, smoke was introduced in the cell on fillets that had the same inner temperature, whatever the smokehouse temperature. The aim of the drying step was also to dry the product surface for a better smoke penetration according to industrial procedures. After smoking, the fillets were stored at −80 °C before the extraction of volatile compounds.

Smoking Equipment and Procedures. The smokehouse was an HMI Thirode (PC90 model) device (Thirode, France), 1500 mm × 1300 mm×2250 mm with a capacity of 380 kg, mounted on a trolley with 28 grids on which the fillets were deposited. For each smoking technique, the fillets were placed at the same level (grid numbers 10, 12, and 14) at 20 cm from the door of the smokehouse. The air/smoke circulation was horizontal. Salmon fillets were swept by the smoke for 3 h at a temperature of 32 °C. The exhaust valve opening was one-third except for liquid smoke, and the relative hygrometry was set at 60%. For each process except liquid smoke, the smoke was introduced in the smokehouse with a flow rate of 90 m^3/h.

Smoldering Parameters. A generator (Thirode, France) produced smoke by pyrolysis (between 400 and 450 °C) of beech sawdust using the smoldering method. The sawdust was poured onto an electrically heated ring and pyrolyzed. The ring was heated only for the ignition period and was entertained further only by electric pulses. The pyrolysis was also maintained thanks to an air intake around the heated ring by a turbine. The sawdust fell on the heated ring by gravity from a hopper. The introduction of sawdust was programmed every 6 min. The sawdust was wet before and homogenized to obtain a moisture rate of 20%.

Thermostated Plates Parameters. A generator 720 mm×1120 mm × 1730 mm (Thirode, France) produced smoke by pyrolysis (500 °C) of beech chips. A system spread the chips on thermostated plates, and the plates were cleaned by a rake system after 3 min of combustion. The smoke was pulsed by a ventilator to obtain the same flow rate of smoke in the smokehouse as smoldering and friction.

Friction Parameters. A generator type FR 1002 (Muvero, The Netherlands) produced smoke by friction (380 °C) by pressing a beech log (8 cm × 8 cm×100 cm) against a rotating friction wheel for 10 s every 30 s. The beech log was pressed pneumatically by means of a wood gripper with a pressure of 3.5 bar.

Liquid Smoke Parameters. Liquid smoke was purchased from a smoke-flavoring manufacturer (France). Liquid smoke (purified condensate of beech smoke) was atomized thanks to pressurized air in the smokehouse at ambient temperature. The vaporization device (Lutetia, France) allowed us to set the pressures of air and liquid smoke to obtain a consumption of liquid smoke of 1 L/h as in industrial procedure. Liquid smoke was injected in the smokehouse for 2 min every 3 min. For this type of smoking process, the hygrometry of the smokehouse was maintained at 70%.

Extraction of Volatile Compounds. The volatile compounds were extracted by SDE with diethyl ether in a Likens−Nickerson apparatus. A 500 mL round-bottom flask was used as the sample flask to contain 50 g of cubes of smoked salmon, 150 mL of purified water, and 100 μg of dodecane used as an internal standard. A 30 mL round-bottom flask containing 30 mL of diethyl ether was linked to the upper arm of the SDE apparatus because the density of diethyl ether is lower than the density of water. The steams were cooled thanks to the circulation of polyethylene glycol at −5 °C. Contents in the sample and solvent flasks were heated to a boil. The temperature of diethyl ether flask was maintained by a water bath at 50 °C. The distillation−extraction was continued for 3 h. The volume of the extract was reduced to 5 mL by evaporating the solvent thanks to a Kuderna Danish apparatus and to 1 mL under a gentle cold stream of nitrogen. Finally, a solvent change was applied by adding 1 mL of ethanol and evaporating diethyl ether. This solvent change was performed to present to the panel the aromatic extract in a nontoxic solvent for the evaluation of the odor representativeness (*22*).

Representativeness of the Extracts. *Samples Preparation and Presentation to the Judges.* The panel was composed of eight judges from our laboratory (five females and three males between 24 and 49 years old) trained in sensorial characterization of seafood products. Small cubes of salmon flesh of 1 g were placed in 15 mL brown-coded flasks, and the aromatic extract was deposited by softly sprinkling the cube of salmon with respect to the initial volatile compound concentrations of salmon fillets (*22*). The extracts were hermetically stored at 4 °C in a fridge and put at room temperature 3 h before the beginning of each test. All of the samples were assessed at room temperature (20 °C) in neutral conditions.

Similarity Test. To validate the extraction method, the closeness between the odor of the aromatic extracts corresponding to salmon fillets smoked according to the four smoking techniques and the odor of respective salmon fillets was evaluated. Smoked salmon aromatic extracts were deposited on cubes of unsmoked salmon (1 g) as described previously (*22*). Each pair of samples was randomly presented to the judges. They were asked to assess the odor similarity of the extract to the respective reference by noting the extract on an unstructured 100 mm scale anchored at the left end with "odor far from the reference" at the right end with "odor identical to the reference".

GC-MS/O Parameters. The GC-O system consisted of a 6890N GC (Agilent Technologies, Wilmington, DE) equipped with a flame ionization detector (FID), a mass detector (5973-Network), and a sniffing port ODP2 (Gerstel, Baltimore, MD) supplied with humidified air at 40 °C. The GC effluent was split 1:1:1 between the FID, the mass detector, and the sniffing port. Each extract (3 μL) was injected in splitless mode into a capillary column (DB-5MS, 30 m length × 0.32 mm id, 0.5 μm thickness) (J&W Scientific, Folsom, CA). The flow rate of the carrier gas (helium) was 1.5 mL min^{-1}. The temperature of the oven was programmed according to the following steps: from 70 to 85 °C (1 min) at 5 °C min^{-1}, then to 165 °C at 3 °C min^{-1} and, finally, to 280 °C (3 min) at 10 °C min^{-1}. The quantification was carried out by mass spectrometric detection. For GC-MS analysis, a quadrupole mass selective detector, with electron ionization (ionization energy, 70

Table 1. Odor-Active Compounds Found in Salmon Extracts Smoked by Smoldering and Thermostated Plates[a]

				smoldering			thermostated plates		
compounds	LRI (DB5)	means of identification[b]	odorant attributes given by the judges[c]	no. of judges[d]	average intensity[e]	mean ± standard[g]	no. of judges[d]	average intensity[e]	mean ± standard[f]
furfural	859	MS, LRI, STD	smoke, green	6	3	326.18 ± 91.57	(3)	(1)	(807.67 ± 96.83[1])
furfuryl alcohol	875	MS, LRI, STD	cooked/soup, chemical	7	4	161.88 ± 53.23[1]	8	5	544.46 ± 77.49
2,4-hexadienal	904	MS, LRI	cooked vegetable, fatty	(5)	(3)	(2.93 ± 1.11[1])	8	5	4.63 ± 0.73[1,2]
2-methyl-2-cyclopenten-1-one	920	MS, LRI, STD	cooked potato, green	(5)	(3)	(14.85 ± 5.27[1,2])	(5)	(4)	(53.53 ± 6.41)
2-acetylfuran	925	MS, LRI, STD	cooked vegetable, potato	7	5	19.49 ± 10.29[1]	8	6	70.14 ± 5.16[2]
5-methylfurfural	970	MS, LRI, STD	cooked, earthy, green			(58.68 ± 21.16[1])	7	3	163.26 ± 22.24
phenol	992	MS, LRI, STD	marine, metallic, chemical, mushroom	7	4	61.69 ± 21.81[1,2]	8	6	115.6 ± 14.97[2]
2-hydroxy-3-methyl-2-cyclopenten 1-one	1036	MS, LRI	cooked, spicy	(4)	(2)	(7.03 ± 2.82[1])	(5)	(2)	(29.35 ± 10.42[1])
2,3-dimethyl-2-cyclopentenone	1052	MS, LRI, STD	spicy, wood fire, roasty			(13.98 ± 4.35[1])	6	3	42.95 ± 7.49
o-cresol	1068	MS, LRI, STD	chemical, spicy, burnt,	7	4	34.16 ± 12.36[1,2]	8	5	69.14 ± 7.44[2]
p-cresol	1093	MS, LRI, STD	animal, spicy, burnt	8	6	39.67 ± 16.29[1,2]	8	7	66.04 ± 5.57[1,2]
guaiacol	1110	MS, LRI, STD	smoked, vanilla, ink	8	7	327.91 ± 109.15[1]	8	8	755.26 ± 91.56[2]
2,6-dimethylphenol	1130	MS, LRI, STD	chemical, burnt, spicy/woody	7	5	7.11 ± 3.02	6	4	13.89 ± 0.39
2,3,4-trimethylcyclopenten-1-one	1132	MS	cooked, green, spicy	(4)	(4)	(18.77 ± 9.02[1])	(4)	(3)	(70.15 ± 7.16)
3-ethyl-2-hydroxy-2-cyclo-pentenone	1140	MS	solvent, medicinal	(4)	(3)	(3.19 ± 1.45[1])	8	5	5.26 ± 0.36[1,2]
1,2-dimethoxybenzene	1147	MS, LRI	ashes, green	(4)	(2)	(6.38 ± 2.43[1])	(5)	(2)	(16.45 ± 0.88[2])
2,4 and 2,5-dimethylphenol/(E)-2-nonenal	1160–1180	MS, LRI, STD	cucumber, violet, spicy, smoked	7	5	15.41 ± 5.87[1]	8	5	20.68 ± 0.93[1]
4-methylguaiacol	1192	MS, LRI, STD	candy, spicy, smoked	(4)	(4)	(543.42 ± 210.79[1,2])	6	5	893.98 ± 87.67[2]
2,3-dimethoxytoluene	1247	MS, LRI	cooked vegetable, fatty, green	(4)	(2)	(6.55 ± 2.78[1])	7	4	11.02 ± 1.17[1]
3,5-dimethoxytoluene	1282	MS, LRI	burnt, green, chemical	6	3	7.84 ± 3.61[1,2]	7	5	10.86 ± 0.03[2]
4-ethylguaiacol	1287	MS, LRI, STD	green, smoke, vanilla, clove	7	5	84.7 ± 32.44[1]	8	4	190.6 ± 11.68
indanone	1307	MS, LRI	sawdust, rotten, burnt	(3)	(3)	(2.69 ± 0.88[1])	6	4	6.95 ± 0.51
4-vinylguaiacol	1330	MS, LRI, STD	smoke, green, spicy	6	4	36.27 ± 18.59[2,3]	7	5	41.77 ± 2.92[3]
syringol	1365	MS, LRI, STD	burnt rubber, spicy	(5)	(3)	(23.31 ± 18.59[1,2])	(3)	(2)	(25.11 ± 4.77[1,2])
eugenol	1370	MS, LRI, STD	spicy, smoke, clove	6	4	34.84 ± 13.93[1]	8	5	62.68 ± 1.45[1]
4-propylguaiacol	1382	MS, LRI, STD	green, spicy, vanilla	8	5	11.33 ± 4.78[1]	8	5	29.89 ± 2.79
1,2,3-trimethoxy-5-methyl-benzene	1400	MS, LRI	cooked, earthy	(3)	(2)	(1.85 ± 0.79[1])	6	3	59.73 ± 3.49
(Z)-isoeugenol	1423	MS, LRI, STD	burnt rubber, spicy	6	3	11.45 ± 4.17[1,2]	8	5	17.3 ± 1.43[2]
(E)-isoeugenol	1473	MS, LRI, STD	clove, green, roasty	8	6	36.95 ± 18.71[1,2]	8	7	59.73 ± 3.49[2]
2,3,5-trimethoxytoluene	1527	MS, LRI	spicy, woody	(4)	(2)	(9.55 ± 6.38[1,2])	6	4	8.78 ± 2.24[1,2]
4-allylsyringol	1615	MS, LRI	smoke, rotten	8	5	1.19 ± 1.14[1]	7	3	1.35 ± 0.52[1]
8-heptadecene	1680	MS, LRI	animal, roasty, chemical	6	3	2.67 ± 0.93[1]	6	3	4.65 ± 2.62[1,2]

[a] Numbers 1–3, quantities followed by a same number on the same line for all of the tables are not statistically or significantly different at a risk of 5% (ANOVA only carried out on the most potent odorant compounds). Frequency of detection, odor intensity, and quantities of odor-active compounds detected by fewer than six judges are indicated in parentheses. [b] Means of identification: MS, mass spectrum (identified thanks to the mass spectra of the compounds); LRI, linear retention index (when the LRI of the identified compound corresponds to the LRI in the litetrature); and STD, standard (when the retention time, spectrum, and odor description of an identified compound correspond to the retention time, spectrum, and odor description of the injected standard of this compound). When only MS is available for identification, it must be considered as an attempt of identification. [c] The odor given corresponds to the odor detected by the judges during olfactometric analysis for its retention time but not surely to the compound that we try to identify. [d] Number of judges (out of eight) who have detected an odor. [e] The average intensity of the eight judges is rounded to the nearest whole number. An intensity between 5 and 5.5 is rounded to 5 and an intensity between 5.5 and 6 is rounded to 6 (1 very weak odor and 9 very strong odor intensity). [f] In microgram equivalents of dodecane per 100 g of smoked salmon. Means of three fillets.

eV) operated in scan mode, with a mass range of 30−300 amu, at 2.0 scans/s, was used to detect the ions formed.

Compound identification was based on a comparison of retention indices (RI) found in the litterature comparison of their mass spectra with those of standard MS spectra database WILEY 6 and with those of chemical standards, when they were available, injected in the same chromatographic conditions. Comparison of odor description with the literature could be also used to confirm the identification. When possible, the identification was confirmed by detection of the compounds in single ion monitoring mode, for each noticeable odorant, five of the most predominant ions present in their mass - spectra.

The quantification was performed using dodecane as an internal standard. The concentrations of volatile compounds are expressed in µg equivalents of dodecane for 100 g of salmon in **Tables 1** and **2**. However, when standards were available, response factors were calculated. Therefore, the relationship between the perception and the concentrations of odor-active compounds, expressed in µg for 100 g of salmon, can be discussed as compared to the odorant threshold described in litterature.

Olfactometric Methods. The panel was composed of the same judges used for the similarity test. They were all previously trained in odor recognition and sensory evaluation techniques and had experience in GC-O on seafood products. Sniffing of the chromatogram was divided into two sessions of 19 min. Each judge participated in the sniffing of both parts but during two separate sessions in order to remain alert. The panelists were asked to describe the odor that they smelled and to give a mark of intensity to each detected odorant on a scale of 1−9 (1 very weak odor intensity and 9 very strong odor intensity). Thus, two olfactometric methods were used as follows: frequency of detection (FD) and time intensity (TI). For the FD method, the results were expressed as the number of judges who perceived an odor at the same retention time of chromatography (*19*). For this study, a volatile compound was considered as a potent odorant if it was detected by at least six judges. For the TI method, each judge was asked when he/she perceived an odorant zone to assess the intensity of the odor on a scale of 1−9 (1 very weak odor intensity and 9 very strong intensity). The results were expressed as the average intensity computed for all eight judges (*23*).

Table 2. Odor-Active Compounds Found in Salmon Extracts Smoked by Friction and Liquid Smoke[a]

				friction			liquid smoke		
compounds	LRI (DB5)	means of identification[b]	odorant attributes given by the judges[c]	no. of judges[d]	average intensity[e]	mean ± standard[f]	no. of judges[d]	average intensity[e]	mean ± standard[f]
furfural	859	MS, LRI, STD	smoke, green	7	4	751.15 ± 127.79[1]			124.24 ± 63.04
4-methylpyridine	865	MS, LRI	green, milk				7	4	16.55 ± 9.12
furfuryl alcohol	875	MS, LRI, STD	cooked/soup, chemical	7	3	220.55 ± 46.49[1]			42.17 ± 26.25
2,6-dimethylpyridine	890	MS, LRI	roasty, green, milk				6	4	4.27 ± 2.90
2,4-hexadienal	904	MS, LRI	cooked vegetable, fatty	8	5	7.11 ± 3.09[2]	(5)	(2)	(1.33 ± 1.53[1])
2-methyl-2-cyclopenten-1-one	920	MS, LRI, STD	cooked potato, green	7	6	8.26 ± 3.58[2]	6	5	8.37 ± 3.98[1]
2-acetylfuran	925	MS, LRI, STD	cooked vegetable, potato	7	6	48.07 ± 13.10[2]	7	6	20.22 ± 9.34[1]
5-methylfurfural	970	MS, LRI, STD	cooked, earthy, green			(65.74 ± 14.22[1])	(4)	(2)	(24.63 ± 13.50)
phenol	992	MS, LRI, STD	marine, metallic, chemical, mushroom	7	5	31.05 ± 10.48[1]	7	4	65.55 ± 39.97[1,2]
2-hydroxy-3-methyl-2-cyclo-penten-1-one	1036	MS, LRI	cooked, spicy	(4)	(2)	(16.26 ± 5.49[1])	7	4	23.64 ± 18.44[1]
2,3-dimethyl-2-cyclopentenone	1052	MS, LRI, STD	spicy, wood fire, roasty	(5)	(2)	(15.35 ± 5.25[1])	6	3	17.48 ± 8.94[1]
o-cresol	1068	MS, LRI, STD	chemical, spicy, burnt,	8	4	28.72 ± 7.18[1]	8	5	49.74 ± 27.62[1,2]
p-cresol	1093	MS, LRI, STD	animal, spicy, burnt	8	7	25.09 ± 7.18[1]	8	6	74.18 ± 37.53[2]
guaiacol	1110	MS, LRI, STD	smoked, vanilla, ink	8	7	488.72 ± 166.03[1,2]	8	7	360.45 ± 172.07[1]
2,6-dimethylphenol	1130	MS, LRI, STD	chemical, burnt, spicy/ woody	7	5	0.77 ± 0.58[1]	(3)	(2)	(1.27 ± 0.75[1])
2,3,4-trimethylcyclopenten-1-one	1132	MS	cooked, green, spicy	8	6	29.24 ± 12.90[1]	6	5	17.25 ± 10.35[1]
3-ethyl-2-hydroxy-2-cyclo-pentenone	1140	MS	solvent, medicinal	(4)	(2)	(3.05 ± 1.98[1])	7	4	10.49 ± 6.36[2]
1,2-dimethoxybenzene	1147	MS, LRI	ashes, green	(5)	(3)	(6.20 ± 1.87[1])	6	3	11.16 ± 5.50[1,2]
2,4- and 2,5-dimethylphenol/ (E)-2-nonenal	1160–1180	MS, LRI, STD	cucumber, violet, spicy, smoked	8	5	6.69 ± 1.82[1]	8	6	18.96 ± 10.46[1]
4-methylguaiacol	1192	MS, LRI, STD	candy, spicy, smoked	8	6	478.51 ± 65.47[1]	7	5	482.15 ± 243.13[1]
2,3-dimethoxytoluene	1247	MS, LRI	cooked vegetable, fatty, green	(5)	(4)	(8.89 ± 4.65[1])	7	4	6.62 ± 4.12[1]
(E)-2-decenal	1266	MS, LRI	spicy, green, milk	6	4	2.07 ± 0.83[1]	6	3	4.26 ± 1.79[2]
3,5-dimethoxytoluene	1282	MS, LRI	burnt, green, chemical	7	6	7.79 ± 3.56[1,2]	(5)	(3)	(6.25 ± 4.15[1])
4-ethylguaiacol	1287	MS, LRI, STD	green, smoke, vanilla, clove	8	6	68.19 ± 23.44[1]	8	6	86.85 ± 40.97[1]
indanone	1307	MS, LRI	sawdust, rotten, burnt	(4)	(3)	(2.85 ± 0.91[1])	7	4	2.87 ± 1.71[1]
4-vinylguaiacol	1330	MS, LRI, STD	smoke, green, spicy	8	6	19.13 ± 4.79[1,2]	(3)	(2)	(3.24 ± 1.95[1])
(E,E)-2,4-decadienal	1330	MS, LRI, STD	oily, green, fatty	(3)	(2)	(19.13 ± 4.79[1])	7	5	8.82 ± 6.72[2]
syringol	1365	MS, LRI, STD	burnt rubber, spicy	7	3	11.19 ± 2.96[1]	8	5	44.61 ± 22.91[2]
eugenol	1370	MS, LRI, STD	spicy, smoke, clove	8	5	52.02 ± 12.69[1]	8	5	36.51 ± 18.17[1]
4-propylguaiacol	1382	MS, LRI, STD	green, spicy, vanilla	7	5	16.10 ± 6.16[1]	8	5	15.21 ± 7.86[1]
1,2,3-trimethoxy-5-methyl-benzene	1400	MS, LRI	cooked, earthy	7	4	5.05 ± 0.09[2]	(5)	(2)	(2.15 ± 1.15[1,2])
(Z)-isoeugenol	1423	MS, LRI, STD	burnt rubber, spicy	8	5	15.04 ± 6.34[1,2]	6	3	7.40 ± 3.77[1]
(E)-isoeugenol	1473	MS, LRI, STD	clove, green, roasty	7	6	46.23 ± 10.40[1,2]	7	4	24.81 ± 11.35[1]
2,3,5-trimethoxytoluene	1527	MS, LRI	spicy, woody	(3)	(2)	(5.18 ± 1.13[1])	(4)	(2)	(20.55 ± 8.48[2])
4-allylsyringol	1615	MS, LRI	smoke, rotten	8	5	1.67 ± 0.35[1]	7	4	1.23 ± 0.39[1]
8-heptadecene	1680	MS, LRI	animal, roasty, chemical	7	3	2.89 ± 0.65[1]	6	4	6.87 ± 2.68[2]

[a] Numbers 1–3, quantities followed by a same number on a same line for all of the tables are not statistically significantly different at a risk of 5% (ANOVA only carried out on the most potent odorant compounds). Frequency of detection, odor intensity, and quantities of odor-active compounds detected by fewer than six judges are indicated in parentheses. [b] Means of identification: MS, mass spectrum (identified thanks to the mass spectra of the compounds); LRI, linear retention index (when the LRI of the identified compound corresponds to the LRI in the litetrature); and STD, standard (when the retention time, spectrum, and odor description of an identified compound correspond to the retention time, spectrum, and odor description of the injected standard of this compound). When only MS is available for identification, it must be considered as an attempt of identification. [c] The odor given correponds to the odor detected by the judges during olfactometric analysis for its retention time but not surely to the compound that we try to identify. [d] Number of judges (out of eight) who have detected an odor. [e] The average intensity of the eight judges is rounded to the nearest whole number. An intensity between 5 and 5.5 is rounded to 5, and an intensity between 5.5 and 6 is rounded to 6 (1 very weak odor and 9 very strong odor intensity). [f] In micrograms equivalents of dodecane per 100 g of smoked salmon. Means of three fillets.

Statistical Treatments. All of the statistical analyses were performed with STATGRAPHICS Plus 5.1 software (Statistical Graphics Corp., Herndon, United States). One-way analysis of variance (ANOVA) was performed on the odor-active compound quantities to determine whether there were significant differences between the concentrations according to the smoke generation technique. Possible significant differences between the values were evaluated by least significant difference multiple comparison tests with a confidence level of 95%.

RESULTS AND DISCUSSION

Similarity of the Aromatic Extracts. The extraction method has already been presented and justified in previous works (*20*, *22*). The validity of an extraction method of volatile compounds, particularly when an olfactometry technique is applied after the extraction step, is based on assessment of the similarity of the odor of the aromatic extract with the odor of original sample (*24*, *25*). Even if headspace extraction was used by several authors to study the volatile compounds from seafood products (*26*–*29*), the SDE extraction method was chosen because it has previously been used with a good efficiency (*30*–*32*) on seafood products. Moreover, it is easier to assess the odor similarity of liquid extracts than this of extracts in the gaseous state. SDE implies the cooking of the material and can generate artifacts, in particular lipids oxidation products. In our case, we have thought that the eventual thermally generated compounds created by working at high temperatures in the SDE method did not affect the final odor because, first, we were focused on the

extraction of smoke compounds and, second, the material has already been thermally processed.

Our results confirm this hypothesis since we observe a good relationship between the odor properties of the smoked samples and the corresponding extracts. The extracts from salmon smoked by liquid smoke and smoldering smoking techniques present the highest similarity (72 ± 14 and 72 ± 17%, respectively) with the original matrix followed by thermostated plates (66 ± 13%) and friction (60 ± 16%). As compared to other works previously published (*31*, *32*), the aromatic extracts similarity marks are quite acceptable. These results confirm the importance of the deposition on real matrix in the assessment of an odor extract (*22*, *33*). Taking into account the standard deviation, the similarity marks between the four types of smoked salmon aromatic extracts and their respective references are quite homogeneous. Finally. these results justify the determination of odor-active compounds by GC-O.

Olfactometric Results. The most potent odorant compounds detected in aromatic extracts from the four smoked salmon types are compiled in **Tables 1** and **2**. According to the criteria chosen for this study, 18 odor-active compounds have been found in the aromatic extract of salmon smoked by smoldering, 26 in the aromatic extract of salmon smoked by thermostated plates, and 25 and 27 aromatic compounds have been found in friction and liquid smoke extracts, respectively. Odorant compounds were mainly represented by phenolic and furannic compounds.

Phenolic Compounds. Whatever the smoking process, the most part of odor-active compounds detected in each smoked salmon is constituted by phenolic compounds, more particularly, the guaiacol and derivatives (4-ethylguaiacol and 4-propylguaiacol). These compounds are characterized by spicy notes (vanilla, clove, curry, etc.), which could be very important for the final overall odor of the product. Guaiacol is perceived in all of the extracts by the eight judges with an intensity of seven or eight while its concentration in the fish can be very different (from 284 μ g /100 g for smoldering to 653 μ g/100 g for thermostated plates). The fact that guaiacol is perceived similarly even at different concentrations could indicate that the concen- tration of this compound is widely above its odorant threshold. This hypothesis can be checked because the odorant threshold of guaiacol in water (*34*) was assessed between 3 and 21 μg L^{-1}, which is more than 100 times lower than the weakest guaiacol concentration quantified and recovered in salmon smoked, whatever the smoking process. 4-Ethylguaiacol and 4-propylguaiacol are also perceived by the seven or eight judges in all of the extracts. However, these compounds seem to be perceived with a lower intensity than the guaiacol. This difference of perception could be related to a much weaker concentration for these compounds.

Thermostated plates are more characterized by 4-methylguaiacol, 4-vinylguaiacol, and 2,6-dimethylphenol. The 4-methylguaiacol concentration is nearly two times higher than in salmon smoked by friction or liquid smoke and is not found as a potent odorant in salmon smoked by smoldering. This volatile compound is detected in salmon smoked by friction by eight judges and by six judges in extract from salmon smoked by thermostated plates mode while its concentration is lower in the last extract. In a previous work, coelutions phenomena have been reported in smoked salmon extracts and might be responsible for this kind of result (*20*). However, coelutions are not sufficient to explain the fact that 4-methylguaiacol is detected by all of the judges in the friction mode and by only four judges in the smoldering mode whereas its concentration is similar in these two modes. Further investigation will be led in our laboratory to understand these important differences about the perception of this compound. Besides, sometimes, when a compound is detected by judges with a high intensity, the persistence of the odor can affect the assessment of the judges for the odor-active compounds detected after. Therefore, in salmon smoked by smoldering, 4-methylguaiacol could not be perceived due to the high odorant intensity of dimethylphenols previously detected.

Phenolic compounds are produced by thermal degradation through depolymerization/oxidation of lignin (*35*). All of the techniques used to produce smoke lead to phenolic odor-active compounds. However, some differences can be pointed out. Smoked salmon extracts from friction and smoldering processes seem to have a similar composition in odor-active compounds, and concerning the phenolic compounds, isoeugenol isomers contents are particularly important for these techniques rather than for liquid smoke technique. They are also recovered in important amounts for thermostated plates technique. (*E*)-Isoeugenol is detected in salmon smoked by smoldering by eight judges with an odorant intensity of 6 and by seven judges with the same intensity in salmon smoked by friction. The similar odorant perception of (*E*)-isoeugenol can be linked to the similar concentrations of this compound in both smoked salmons fillets (96.81 μg/100 g for smoldering and 121.12 μg/100 g for friction). (*Z*)-Isoeugenol is detected in salmon smoked by friction by eight judges (intensity of 5) vs only six assessors (intensity of 3) in salmon smoked by smoldering, while the concentrations in both salmons are similar (30 μg/100 g for smoldering and 39.4 μg/100 g for friction). This result is not easy to explain because these concentrations are 50 times higher than the odorant threshold of (*Z*)-isoeugenol described in the literature (6 μg L^{-1} in 10% water/ethanol containing 5 g L^{-1} of tartaric acid at pH 3.2) (*36*).

Liquid smoking is responsible for the recovery in smoked salmon of odor-active compounds such as *o*-cresol, *p*-cresol, syringol, and 4-allylsyringol, by comparison with the other techniques. According to some authors, syringol could be mainly implied in the smoky odor of smoked products (*14*) and could be responsible for the cold smoke odors often reported in sensorial analysis applied on fishes treated with liquid smoke (*15*, *21*, *37*). According to our GC-MS/O results, if syringol was consensually detected by the judges with a burnt rubber/spicy aromatic note in all extracts, it is the only smoky phenolic compound whose quantity (close to 79 μg/100 g) in liquid smoke extract is significantly higher than in the three others extracts (close to 40 μg/100 g for thermostated plates and smoldering and 20 μg/100 g for friction) and makes it more odorant. However, the difference of perception of syringol is probably not sufficient to explain the overall cold smoke odor perception of smoked salmon treated with liquid smoke (*8*). The existence of interactions between these volatile compounds must be considered to understand the contribution of syringol to the overall cold smoke aroma. Indeed, cresol isomers, whose contents are important in salmon smoked by liquid smoke (55 μg/100 g for *o*-cresol and 76 μg/100 g for *p*-cresol), are thought to play a role in the overall odor of a such processed product. The other phenolic compounds such as alkyl phenols are not suitable to differentiate the smoking technique of a salmon, but they are responsible for the smoky and spicy smoked salmon aroma.

Furannic Compounds. The second group of most potent odor-active compounds found in smoked salmon aromatic extracts is constituted by the furan derivatives. Furannic compounds are generated by the thermal degradation of wood

polysaccharides (cellulose and hemicellulose) through hexoses and pentoses intermediaries (*38*). Therefore, an increase of the wood pyrolysis temperature could cause a higher thermal degradation and higher quantities of furannic compounds in the aromatic extracts. Indeed, this trend is confirmed by the important number of judges who have detected furannic odor-active compounds in the extracts of salmon smoked with the smoking technique using the highest wood pyrolysis temperature, that is to say thermostated plates (500 °C). Except for 5-methylfurfural, they are detected by the maximum of the assessors with odorant intensity from 5 to 6. This trend is also confirmed by the important quantities of furannic compounds detected with this smoking technique. Except for furfural, present but not classified as a potent odorant (1.018 mg/100 g), the salmon extract smoked by thermostated plates is characterized by high amounts of furannic compounds like 5-methylfurfural (178 μg/100 g), 2-acetylfuran (49 μg/100 g), and furfuryl alcohol (528 μg/100 g). They bring cooked vegetables/green aromatic notes (*15*).

When the wood pyrolysis temperature is lower, close to 400 °C for smoldering and 380 °C for friction, the odorant perception and quantities of furannic compounds found in the smoked salmon extracts decrease. Consequently, these compounds are detected by a fewer number of judges (seven) and fewer odorant intensities. Furfuryl alcohol and 2-acetylfuran were, respectively, two and three times less abundant in friction and smoldering extracts than in thermostated plates. Moreover, 5-methyl furfural, whose concentration is close to 64 μg/100 g in smoldering and friction extracts, is not sufficiently detected to be classified as an odorant according to the retained criteria of this work. These results confirm the impact of the wood pyrolysis temperature. Indeed, a temperature of pyrolysis of 500 °C has already been reported for the optimum yield of total flavor compounds (*39*, *40*), especially furannic and phenolic compounds, in *Vitis Vinifera* L. shoot sawdust (*41*).

Concerning liquid smoke, conclusions are not possible because information about wood pyrolysis temperature was not available. Nevertheless, similarities of concentrations of phenolic and furannic compounds between smoldering and liquid smoke extract could make the wood pyrolysis temperature for liquid smoke obtention close to this of smoldering technique.

Others Compounds. The third class of odorant volatile compounds is the enolones derivatives. Enolone derivatives are very present in liquid smoke (*37*) and could come from heated Amadori derivative from Maillard reaction after several rearrangements (*42*). Even if Maillard reaction occurrence has not been proven during smoking process, the presence of such compounds makes it possible even if the required nitrogenous substances are present in very low quantities. It has been reported that they bring a toasted odor (*42*), but our panel characterized these molecules with cooked and spicy aromatic notes. 2,3,4-Trimethyl-2-cyclopenten-1-one and 2-methyl-2-cyclopenten-1-one are mainly detected in salmon smoked by friction and liquid smoke. Liquid smoke is the only process that leads to the olfactometric detection of 2-hydroxy-3-methyl-2-cyclopenten-1-one in smoked salmon as a potent odorant compound. This compound exhibits a cooked/spicy odor and was smelled by seven judges with an intensity of 4. All of the odorant intensities of the enolones derivatives found in smoked salmon whatever the smoking process are not strong and ranged from 3 to 6, weaker than the odorant intensities phenolic compounds. These differences of odorant intensity are obviously linked to their concentrations but also to the odorant descriptors (cooked, soup), which are less sharp and characteristic than phenolic compounds (smoke/spicy/burnt).

Products treated with liquid smoke have already been reported with an overall green odor (*8*, *21*). This odorant characteristic could be put down to some odorant compounds such as lipid oxidation products and pyridines derivatives, which were only perceived in salmon treated with liquid smoke. Indeed, (*E*,*E*)-2,4-decadienal produced by oxidation of polyunsaturated fatty acids is only detected in the extract of salmon treated by liquid smoke as potent odor-active compounds. The aldehydes produced by fatty acid oxidation are characterized by a low odor threshold. In salmon treated with liquid smoke, this compound present in a small concentration was detected with green and fatty aromatic notes by seven judges. (*E*)-2-Decenal, also produced by fatty acids oxidation, is detected in extracts from salmon smoked with liquid smoke and with smoke produced by friction in the similar conditions of perception by the judges. However, its concentration is three times lower in friction extracts than in liquid smoke extracts. It could mean that the (*E*)-2-decenal concentration is higher than the odorant threshold concentration in both smoked salmons because (*E*)-2-decenal is known to have a low odorant threshold (*20*, *34*) close to 0.3–0.4 $\mu g\ L^{-1}$ in water.

Finally, pyridine derivatives are not compounds commonly found in smoke or smoke flavorings. However, they could be formed during the thermal degradation of nitrogenated derivatives of wood such as alkaloids (*43*, *44*). They have already been reported in coffee, tea, and cocoa flavors with green, bitter, toasted, and burnt aromatic notes (*43*), which is in accordance with their green and roasty odors found in salmon treated with liquid smoke. 4-Methylpyridine and 2,6-dimethylpyridine are only detected in salmon treated by liquid smoke by seven and six judges, respectively, but both with a weak odorant intensity of 4.

Odor-Active Volatiles Differences between the Salmons Smoked by the Four Techniques. Some differences in the odorant composition of the salmons smoked by the four techniques have been highlighted, especially between the products smoked by methods applying wood pyrolysis in situ and the products treated by liquid smoke. The pyrolysis temperature has been previously investigated to explain those differences, but the nature of the wood used for the liquid smoke obtention could also explain the variations between products treated with liquid smoke and the three other techniques. However, the nature of the wood cannot explain the differences between smoldering, thermostated plates, and friction because the same wood is used under different forms (wood sawdust, wood chips, and wood log). Therefore, it is mainly wood pyrolysis temperature, moisture, and granulometry of the wood and the geometry of the smoke generator that are involved in the variations of composition of salmon smoked by smoldering, thermostated plates, and friction as has been already reported for phenolic compounds (*45*). Coelutions could also be responsible for differences of perception because smoked salmon extract is a complex odorant mixture with, hence, complex chromatograms. Differences in the odorant perception of compounds in similar quantities could imply odorant mask or synergic effects between coeluated odorant or not odorant volatiles compounds present in smoke or produced by degradation of constituents of fish flesh under the smoking conditions.

GC-O has permitted us to study smoked salmon aroma and to point out the odorant differences between the samples smoked by four techniques used as industrial smoke generators. Comprehension of smoked food aroma is not easy because of the

diversity of the precursors of the odor-active compounds: wood smoke, fish flesh or evolution of fish flesh during smoking, and smoke action. Moreover, some odorants can be present in very low quantities, and odorant mixes can occur. Nevertheless, the method developed herein seems strong enough to differentiate salmons smoked by four industrial techniques by the analysis of their composition in odor-active compounds. Phenolic compounds are common odorants for the four types of smoked salmon. Furannic, nitrogenated, and Maillard compounds are more specific smoking techniques by applying high wood pyrolysis temperature. More investigations should be led to strengthen the identification of the smoking technique of smoked food thanks to the odor-active compounds analysis. Besides, the production of smoked fishes with required odor should become possible by a better knowledge of the smoking parameters responsible for the generation of certain odorants. GC-O appears as an accurate method to differentiate the smoking technique of smoked food and could be extended to the study of other processed food aroma compounds.

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LITERATURE CITED

(1) Anonymous. European consumer preferences for smoked salmon. Collective report from European project, QLK1-2000-01575, specific RTD programme "Quality of Life and Management of Living Resources"; *Eurosalmon*; Nantes: Adriant, 2004.

(2) Guillén, M. D.; Errecalde, M. C. Volatile components of raw and smoked black bream (*Brama raii*) and rainbow trout (*Oncorhyncus mykiss*) studied by means of solid phase microextraction and gas chromatography/mass spectrometry. *J. Sci. Food Agric*. **2002**, *82*, 945−952.

(3) Guillén, M. D.; Errecalde, M. C.; Salmerón, J.; Casas, C. Headspace volatile components of smoked swordfish (*Xiphias gladius*) and cod (*Gadus morhua*) detected by means of solid phase microextraction and gas chromatography-mass spectrometry. *Food Chem.* **2006**, *94* (1), 151−156.

(4) Guillén, M. D.; Ibargoitia, M. L. New components with potential antioxidant and organoleptic properties, detected for the first time in liquid smoke flavouring preparations. *J. Agric. Food Chem*. **1998**, *46*, 1276−1285.

(5) Guillén, M. D.; Manzanos, M. J. Characterisitics of smoke flavourings obtained from mixtures of oak (*Quercus* sp.) wood and aromatic plants (*Thymus Vulgaris* L. and *SalVia laVandulifolia* Vahl.). *FlaVour Fragrance J*. **2005**, *20* (6), 126−133.

(6) Girard, J. P.; Talon, R.; Sirami, J. Le fumage électrostatique: Son efficacité relativement à deux paramètres. *Sci. Aliment.* **1982**, *2*, 1−15 (in French).

(7) Girard J. P. *La Fumaison. Technologie de la Viande et des Produits Carnés*; Lavoisier; Paris, France, 1988; pp 171−214 (in French).

(8) Cardinal, M.; Berdagué, J. L.; Dinel, V.; Knockaert, C.; Vallet, J. L. Effet de différentes techniques de fumage sur la nature des composés volatils et les caractéristiques sensorielles de la chair de saumon. *Sci. Aliment*. **1997**, *17*, 679−696 (in French).

(9) Guillard, A. S.; Grondin, C. Etude des composés phénoliques de l'arôme des saucisses fumées par micro-extraction en phase solide (SPME). *Bull. Liaison CTSCCV* **2003**, *13*, 13−17 (in French).

(10) Sérot, T.; Baron, R.; Knockaert, C.; Vallet, J. L. Effect of smoking processes on the contents of 10 major phenolic compounds in smoked fillets of herring (*Cuplea harengus*). *Food Chem.* **2004**, *85*, 111−120.

(11) Cardinal, M.; Cornet, J.; Sérot, T.; Baron, R. Effects of smoking process on odor characteristics of smoked herring (*Cuplea harengus*) and relationships with phenolic compound content. *Food Chem.* **2006**, *96*, 137−146.

(12) Fiddler, W.; Wasserman, A. E.; Doerr, R. C. A "smoke" flavor fraction of a liquid smoke solution. *J. Agric. Food Chem*. **1970**, *18* (5), 934−936.

(13) Miler, K. B. M.; Sikorski, Z. E. Smoking. *Seafood: Resources, Nutritional Composition, and PreserVation*; CRC Press Inc.: Boca Raton, Florida, 1990; pp 163−180.

(14) Maga, J. A. The flavour chemistry of wood smoke. *Food ReV. Int*. **1987**, *3* (1−2), 139−183.

(15) Ojeda, M.; Bárcenas, P.; Pérez-Elortondo, F. J.; Guillén, M. D. Chemical references in sensory analysis of smoke flavourings. *Food Chem.* **2002**, *78* (4), 433−442.

(16) Cardinal, M.; Knockaert, C.; Torrissen, O.; Sigurgisladottir, S.; Morkore, T.; Thomassen, M. S.; Vallet, J. L. Relation of smoking parameters to the yield, colour and sensory quality of smoked Atlantic salmon (*Salmo salar*). *Food Res. Int*. **2001**, *34*, 537−550.

(17) Milo, C.; Grosch, W. Detection of odor defects in boiled cod and trout by gas chromatography-olfactometry of headspace samples. *J. Agric. Food Chem*. **1995**, *43*, 459−462.

(18) Prost, C.; Sérot, T.; Demaimay, M. Identification of the most potent odorants in wild and farmed cooked turbot (*Scophtalamus maximus* L.). *J. Agric. Food Chem*. **1998**, *46*, 3214−3219.

(19) Pennarun, A. L.; Prost, C.; Demaimay, M. Identification and origin of the character-impact compounds of raw oyster *Crassostrea gigas*. *J. Sci. Food Agric*. **2002**, *82*, 1652−1660.

(20) Varlet, V.; Knockaert, C.; Prost, C.; Sérot, T. Comparison of odor-active volatile compounds of fresh and smoked salmon. *J. Agric. Food Chem*. **2006**, *54* (9), 3391−3401.

(21) Varlet, V.; Sérot, T.; Knockaert, C.; Cornet, J.; Cardinal, M.; Monteau, F.; Le Bizec, B.; Prost, C. Organoleptic characterization and PAH content of salmon (*Salmo salar*) fillets smoked according to four industrial smoking techniques. *J. Sci. Food Agric.* **2007**, in press.

(22) Varlet, V.; Prost, C.; Sérot, T. New procedure for the study of odour representativeness of aromatic extracts from smoked salmon. *Food Chem.* **2007**, *100* (2), 820−829.

(23) Pennarun, A. L.; Prost, C.; Haure, J.; Demaimay, M. Comparison of two microalgal diets. 2. Influence on odorant composition and organoleptic qualities of raw oysters (*Crassostrea gigas*). *J. Agric. Food Chem*. **2003**, *51*, 2011−2018.

(24) Abbott, N.; Etievant, P.; Langlois, D.; Lesschaeve, I.; Issanchou, S. Evaluation of the representativeness of the odor of beer extracts prior to analysis by GC eluate sniffing. *J. Agric. Food Chem.* **1993**, *41*, 777−780.

(25) Moio, L.; Chambellant, E.; Lesschaeve, I.; Issanchou, S.; Schlich, P.; Etievant, P. X. Production of representative wine extracts for chemical and olfactory analysis. *Ital. J. Food Sci.* **1995**, *3*, 265−278.

(26) Milo, C.; Grosch, W. Changes in the odorants of boiled trout (*Salmo fario*) as affected by the storage of the raw material. *J. Agric. Food Chem.* **1993**, *41*, 2076−2081.

(27) Milo, C.; Grosch, W. Changes in the odorants of boiled salmon and cod as affected by the storage of the raw material. *J. Agric. Food Chem*. **1996**, *44*, 2366−2371.

(28) Cha, Y. J.; Cadwallader, K. R. Volatile components in salt-fermented fish and shrimps pastes. *J. Food Sci.* **1995**, *60* (1), 19−24.

(29) Choi, S. H.; Kobayashi, A.; Yamanishi, T. Odor of cooked small shrimp, *Acetes japonicus* Kishinouye: Difference between raw material and fermented product. *Agric. Biol. Chem.* **1983**, *47* (2), 337−342.

(30) Tanchotikul, U.; Hsieh, T. C. Y. Analysis of volatile flavor components in steam rangia clam by dynamic headspace sampling and simultaneous distillation and extraction. *J. Food Sci.* **1991**, *56* (2), 327−331.

(31) Escudero, A.; Etiévant, P. X. Effect of antioxydants on the flavor characteristics and the gas chromatography/olfactometry profiles of the champagne extracts. *J. Agric. Food Chem*. **1999**, *47* (8), 3303−3308.
(32) Le Guen, S.; Prost, C.; Demaimay, M. Evaluation of the representativeness of the odor of cooked mussel extracts and the relationship between sensory descriptors and potent odorants. *J. Agric. Food Chem*. **2001**, *49* (3), 1321−1327.
(33) Larráyoz, P.; Ibáñez, F. C.; Ordóñez, A. I.; Torre, P.; Barcina, Y. Evaluation of supercritical fluid extraction as sample preparation method for the study of Roncal cheese aroma. *Int. Dairy J*. **2000**, *10* (11), 755−759.
(34) Buttery, R. G.; Turnbaugh, J. G.; Ling, L. C. Contribution of volatiles to rice aroma. *J. Agric. Food Chem*. **1988**, *36*, 1006−1009.
(35) Guillén, M. D.; Manzanos, M. J. Smoke and liquid smoke. Study of an aqueous smoke flavouring from the aromatic plant *Thymus Vulgaris* L. *J. Sci. Food Agric*. **1999**, *79*, 1267−1274.
(36) Campo, E.; Ferreira, V.; Escudero, A.; Marqués, J. C.; Cacho, J. Quantitative gas chromatography-olfactometry and chemical quantitative study of the aroma of four Madeira wines. *Anal. Chem. Acta* **2006**, *563* (1−2), 180−187.
(37) Kostyra, E.; Baryłko-Pikielna, N. Volatiles composition and flavour profile identity of smoke flavourings. *Food Qual. Pref*. **2006**, *17* (1−2), 85−95.
(38) Jira, W. Chemical reactions of smoking. *Fleischwirtschaft Int*. **2004**, *4*, 21−25.
(39) Alén, R.; Kuoppala, E.; Oesch, P. Formation of the main degradation compound groups from wood and its components during pyrolysis. *J. Anal. Appl. Pyrolysis* **1996**, *36*, 137−148.
(40) Simon, R.; De La Calle, B.; Palme, S.; Meier, D.; Anklam, E. Composition and analysis of liquid smoke flavouring primary products. *J. Sep. Sci*. **2005**, *28*, 871−882.
(41) Guillén, M. D.; Ibargoitia, M. L. Relationships between the maximum temperature reached in the smoke generation processes from *Vitis Vinifera* L. shoot sawdust and composition of the aqueous smoke flavoring preparations obtained. *J. Agric. Food Chem*. **1996**, *44*, 1302−1307.
(42) Nonier, M. F.; Vivas, N.; Vivas de Gaulejac, N.; Absalon, C.; Soulié, P.; Fouquet, E. Pyrolysis-gas chromatography/mass spectrometry of *Quercus* sp. wood. Application to structural elucidation of macromolecules and aromatic profiles of different species. *J. Anal. Appl. Pyrolysis* **2006**, *75* (2), 181−193.
(43) Guillén, M. D.; Manzanos, M. J.; Ibargoitia, M. L. Carbohydrate and nitrogenated compounds in liquid smoke flavorings. *J. Agric. Food Chem*. **2001**, *49*, 2395−2403.
(44) Guillén, M. D.; Manzanos, M. J. Study of the volatile composition of an aqueous oak smoke preparation. *Food Chem.* **2002**, *79*, 283−292.
(45) Chan, W. S.; Toledo, R. T. Effect of smokehouse temperature, humidity and air flow on smoke penetration into fish muscle. *J. Food Sci*. **1975**, *40*, 240−243.

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Relationships between sensory characteristics and the most odorant volatile compounds of salmon smoked by four industrial smoking techniques

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Abstract :

Salmon fillets were smoked by using four different smoke generation processes: smoldering, thermostated plates, friction and liquid smoke. Smoked salmon fillets were submitted to sensory analysis and the concentration of odorant volatile compounds were investigated from volatile extracts of smoked salmon by gas chromatography coupled to mass spectrometry and olfactometry (GC-MS/O). Analyses of variance (ANOVA) were performed on the sensory and analytical data in order to study the effects of smoking parameters: three times of smoke exposure (1, 2 or 3 hours) and two smokehouse temperatures (22°C and 32°C). Secondly, Partial Least Squares regression analysis was performed to evaluate the relationships between the sensory attributes of the different smoked salmons and their composition in odorant volatile compounds. Liquid smoke atomisation smoking process led to products described by "cold smoke" and "vegetal" caused by lipid oxidation products, pyridine derivatives, alkyl aryl ethers and several phenolic compounds such as syringol or p-cresol. The other smoked salmons were characterised by "salmon-like" attributes for a short time of process then by "wood fire smoke" attributes. This odorant evolution is due to the increase of the deposition of phenolic and furannic compounds with increases of smoking parameters (time of smoke exposure and smokehouse temperature). The "wood fire smoke" attribute of fillets smoked by smoldering, thermostated plates and friction was caused by high content of phenolic and furannic odor-active compounds.

Keywords : smoked salmon, sensory analysis, olfactometry, Partial Least Squares, Flash table

1. INTRODUCTION

Smoking is a traditional food preservation technique. Nowadays this process is more used for the organoleptic characteristics, appreciated of the consumers. A recent European study on smoked salmon quality (Cardinal, Gunnlaugsdottir, Bjoernevik, Ouisse, Vallet & Leroi, 2004) shows that all consumers do not like the same kind of products. For example, it appears that some consumers require strong smoke odor and flavor, other want a specific "wood fire smoke" note. The control of this smoke characteristic can be of real interest for processors in order to adapt their products to the consumer demand. That requires to have a good knowledge of the compounds responsible for the flavor of the smoked products and of the influence of the smoking process on the flavor of the smoked products. To investigate the odorant quality of food product, two strategies are commonly employed: an approach by sensory analysis or an approach by instrumental analysis. Sensory analysis aims to describe the main odorant characteristics of the odor and flavor of products assessed (Chambers IV & Robel, 1993 ; Morzel, Sheehan, Delahunty & Arendt, 1999 ; Cardinal et al., 2001). Instrumental analysis aims to identify and quantify volatile compounds from an aromatic extract of the product (Girard & Nakai, 1991 ; Girard & Durance, 2000 ; Aro, Brede, Manninen & Kallio, 2002), some of these volatile compounds known as exhibiting an odor (Josephson, Lindsay & Stuiber, 1987 ; Mansur, Bhadra, Takamura & Matoba, 2003). However, the identification of the odorant volatile compounds contributing to food product odor requires use of gas chromatography coupled to olfactometry (GC-O) (Fuller, Steltenkamp & Tisserand, 1964). This technique has been widely used to describe effects of processes, diets or storages on fish odorants compounds. Milo et al., 1996 described the effect of boiling and storage on volatile compounds of trout flesh (Milo & Grosch, 1996); the effect of dietary lipids on odorant compounds of trout and turbot was studied by Sérot et al. (Sérot, Regost, Prost, Robin & Arzel, 2001 ; Sérot, Baron, Knockaert & Vallet, 2003). GC-O was also used to determine the impact of the storage time on odorant volatile compounds of some fish species (Triqui & Reineccius, 1995 ; Prost, Sérot & Demaimay, 1998)). The main odor impact compounds of salted-dried herring were also investigated by means of GC-O (Chung, Yeung, Kim & Chen, 2007). However, even if several works concern the identification and quantification of volatile compounds in liquid smoke (Guillén & Ibargoitia, 1996 ; Guillén, Manzanos & Ibargoitia, 2001) or in smoked fish (Maga, 1987 ; Cardinal, Berdagué, Dinel, Knockaert & Vallet, 1997 ; Sérot et al., 2003) there is no work, to our knowledge except those carried out in our laboratory concerning the identification of odorant volatile compounds of smoked salmon by GC-O.

However as odor characteristic of odorant compounds is individually evaluated at the sniffing port, it is not easy to relate olfactometric analysis data and results sensory analysis data whatever the food matrix. Attempts of explanations of the overall « smoke odor » by a set of phenolic compounds (Cardinal, Cornet, Sérot & Baron, 2006) and studies about odorant volatile compounds of liquid smoke (Kostyra & Baryłko-Pikielna, 2005) have been nevertheless carried out. The increase of off flavors during frozen storage of smoked salmon was tentatively related to the increase of seven odorant volatile carbonyles concentrations coming from lipid oxidation (Refsgaard, Brockhoff & Jensen, 1998). However, the odorant potentiality of these compounds was not studied by olfactometry. Other works performed on anchovy and sea bream attempted to relate the evolution of the overall odor assessed by sensory evaluation and the evolution of odorant volatile compounds concentrations detected by olfactometry (Triqui & Zouine, 1999 ; Grigorakis, Taylor & Alexis, 2003). However, the relationships were not further investigated and the sensory analysis was only focused on overall odor and not on the main odorant characteristics of the product odor. Relationships between sensory characteristics and volatile components of boiled prawns aroma were statistically studied but no information was given concerning the odorant properties of the volatile compounds (Morita, Kubota & Aishima, 2001). However, this study is one of rare studies using Partial Least Squares regression to explain sensory results by analytical data. Indeed, Partial Least Square regression appears as a suitable method (Rosenfeld, Baardseth & Skrede, 1997 ; Tamine, Muir, Barclay, Khaskheli & McNulty, 1997 ; King & Duineveld, 1999) to explain a whole set of sensory variables by a whole set of instrumental variables, and has already been used to relate sensory analysis results obtained on food with analytical results as colorimetric and chemical measurements (Mielnik, Aaby, Rolfsen, Ellekjaer & Nilsson, 2002). From our knowledge, no study concerning the relation between odorant volatile compounds identified by olfactometry and sensory analysis results is available on fish.

The aims of this work were firstly to assess the effect of several smoking parameters as smoking generation technique, time of smoke exposure and smokehouse temperature on the flavor and odor attributes of smoked salmon. Secondly, the impact of these smoking parameters on the perception and content of odorant volatile compounds was evaluated by GC-MS/O. Finally, we attempt to establish relationships between sensory characteristics of smoked fishes and concentrations of odorant volatile compounds by means of Partial Least Squares regression.

2. MATERIAL AND METHODS

2.1. Smoking procedures

36 Salmons (*Salmo salar*) of 4~5 kg reared in Norway were purchased from a seafood wholesaler (Nantes, France). They were all processed according to the same parameters of filleting, salting and drying. Fish were received 5 days after slaughtering were filleted, trimmed, individually vacuum packed, rapidly frozen at –60°C with a cryogenic system and stored at –80°C until smoking step. The freezing of the raw material was chosen in order to work with the same batch of salmons and to manage easily the four smoking techniques. Before smoking, fillets of 1 kg were thawed under running water during 2 hours, hand salted and dried as described by Varlet et al., 2006 (Varlet, Knockaert, Prost & Sérot, 2006). They were smoked in a smokehouse at two temperatures (22 and 32 °C) and for three times of smoke exposure (1, 2 and 3 hours). Four smoke generation techniques were studied: smoldering (smoke produced by pyrolysis of wood sawdust falling on an heated ring at 450°C), by thermostated plates (smoke produced by pyrolysis of wood chips falling on thermostated plates heated at 500°C), by friction (smoke produced the pyrolysis of a beech log against a rotating wheel) and atomization of liquid smoke (wood smoke condensates vaporised in the cell). Smoked fillets were under vacuum packaged and stored five days at +2°C before to be frozen at –60°C with a CO_2 cryogenic system and stored at –80°C until analyses. The time of freezing for smoked fillets did not exceed 2 months. Three fillets were analysed for each condition of smoking. Sensory analysis was performed on the front part of the fillet and a part of 10 cm large was sampled for volatile compounds analysis.

2.2. Raw material and reagents

Ultrapure water was obtained with a MilliQ system (Millipore Division Analytique, Saint Quentin-Yvelines, France) (resistivity <18 $M.cm^{-1}$). Dodecane was purchased from Aldrich (Steinheim, Germany), diethyl ether (purity : 99.5 %) was obtained from Panreac (Barcelona, Spain) and ethanol (purity : 95 %) was purchased from VWR (Fontenay-sous-bois, France). Beech wood sawdust and chips came from SPPS (Paris, France) and beech wood log came from Bourdeau (Nozay, France). All standards were obtained from Sigma Aldrich (Steinheim, Germany) except all the dimethylphenols purchased from Fluka (Buchs,Switzerland) and phenol purchased from Merck (Darmstadt, Germany).

2.3. Extraction of volatile compounds

The volatile compounds were extracted by SDE with diethyl ether in a Likens-Nickerson apparatus (Varlet, Sérot & Prost, 2007a). A 500 mL round-bottom flask was used as the sample flask to contain 50 g of cubes of smoked salmon, 150 mL of purified water, and 100 µg of dodecane used as internal standard. A 30 mL round-bottom flask containing 30 mL of diethyl ether was linked to the upper arm of the SDE apparatus because the density of diethyl ether is lower than the density of water. The steams were cooled down thanks to the circulation of polyethylene glycol at –5 °C. Contents in the sample and solvent flasks were heated to a boil. The temperature of diethyl ether flask was maintained by a water bath at 50 °C. The distillation-extraction was continued during 3 h. The volume of the extract was reduced to 5 mL by evaporating the solvent thanks to a Kuderna Danish apparatus and to 1 mL under a gentle cold stream of nitrogen. Finally, a solvent change was applied by adding 1 mL of ethanol and evaporating diethyl ether). This solvent change was performed in order to have an extract in a non toxic solvent for the evaluation of the odor representativeness by the panel. The high temperature used during the extraction can generate artifacts but smoked salmon is already a thermally processed food matrix. Moreover, in a previous work, the odor of odorant extract was shown similar to the odor of respective smoked salmon fillet from 60 to 72 % according to the smoke production technique (Varlet, Prost, Cardinal & Sérot, 2007b). These satisfactory similarity marks allow to validate the extraction procedure.

2.4. GC-MS/O analysis

The GC-O system consisted of a 6890N GC (Agilent Technologies, Wilmington, DE, USA) equipped with a flame ionization detector (FID), a mass detector (5973-Network) and a sniffing port ODP2 (Gerstel, Baltimore, MD, USA) supplied with humidified air at 40 °C. The GC effluent was split 1:1:1 between the FID, the mass detector and the sniffing port. Each extract (3 µL) was injected in splitless mode into a capillary column (DB-5MS, 30 m length × 0.32 mm id, 0.5 µm thickness) (J&W Scientific, Folsom, CA, USA). The flow rate of the carrier gas (helium) was 1.5 $mL.min^{-1}$. The temperature of the oven was programmed according to the following steps: from 70 °C to 85 °C (1 min) at 5 $°C.min^{-1}$, then to 165 °C at 3 $°C.min^{-1}$ and, finally, to 280 °C (3 min) at 10 $°C.min^{-1.}$

For GC-MS analysis, a quadrupole mass selective detector, with electron ionization (ionization energy, 70 eV) operated in scan mode, with a mass range of 30-300 a.m.u., at 2.0 scans/s, was used to detect the ions formed.

Compounds identification was based on a comparison of retention indices (RI) found in the literature comparison of their mass spectra with those of standard MS spectra database: WILEY 6, and with those of chemical standards, when they were available, injected in the same chromatographic conditions. Comparison of odor description with the literature could be also used to confirm the identification. When possible, the identification was confirmed by detection of the compounds in SIM mode (Single Ion Monitoring) monitoring, for each noticeable odorant, five of the most predominant ions present in their mass spectra.

The quantification was performed using dodecane as an internal standard. The concentrations of volatile compounds are expressed in µg equivalents of dodecane for 100 g of salmon because several standards were not available in order to calculate response factors and to express the concentrations in µg/100 g of smoked salmon. As the concentrations of the odorant volatile compounds must be expressed in the same unity for the statistical treatments, we have chosen to keep them in µg equivalents of dodecane for 100 g.

2.5. Olfactometric methods

The panel was composed of eight judges (6 females and 2 males). They were all previously trained in odor recognition and sensory evaluation techniques and had experience in gas chromatography/olfactometry on seafood products. Sniffing of the chromatogram was divided into two sessions of 19 min. Each judge participated in the sniffing of both parts, but during two separate sessions in order to remain alert. Thus, two olfactometric methods were used as follows: frequency of detection (FD) and time intensity (TI). For the FD method the results were expressed as the number of judges who perceived an odor at the same retention time of chromatography. For this study, only the volatile compounds detected by at least six judges in at least one of the four types of smoked salmons were selected and quantified. For the TI method each judge was asked, when she/he perceived an odorant zone to assess the intensity of the odor on a scale of 1-9 (1= very weak odor intensity and 9 = very strong intensity). The results were expressed as the average intensity computed for eight judges. For an easier reading, the number code corresponding for each odorant volatile compounds was indicated underlined and in brackets.

2.6. Sensory analysis

A descriptive test with conventional profiling (Stone & Sidel, 1998) was carried out to evaluate the sensory characteristics of smoked salmon. Samples were scored by twenty panellists trained on sensory descriptors for smoked salmon. The descriptors related to the appearance, odor, flavor and texture of smoked salmon slices. Only odor and flavor descriptors have been presented here. The panel of 20 trained judges had to assess for odor and for flavor the following attributes: global intensity, wood fire smoke, cold smoke, butter, vegetal, raw salmon-like and herring-like. Sessions were performed in individual partitioned booths equipped with a computerised system (Fizz system, Biosystèmes, Dijon). Panellists rated the sensory attributes on a continuous scale, from low intensity (0) to high intensity (10). An experimental design was built in order to balance the process parameters (kind of smoke generation, smoking and time temperature) associated to the products presented within a session. Four products, assigned 3-digit numbers, randomised for the order of presentation were simultaneously presented at each session. Six sessions were necessary to test all the products. Assessors received, packed in aluminium foil, a smoked salmon slice of 2 cm large taken from the front part of the fillet previously thawed one night at +2°C.

2.7. Statistical treatment

The relationships between the smoking parameters (smoking technique, time of smoke exposure and smokehouse temperature) and firstly, the sensory attributes and secondly, concentrations of odorant volatile compounds were performed by a multi-way analysis of variance (ANOVA). In order to assess the most predominant odorant volatile compounds of the products, one-way analysis of variance (ANOVA) was carried out. The results were presented according to the Flash Table procedure (Schlich, 1998). ANOVA analyses were performed using Statgraphics Plus 5.1 software (Sigma Plus, Paris, France) and Uniwin Plus 5.1 software (Sigma Plus, Paris, France). The relationships between odorant volatile compounds data set and the sensory attributes marks data set were investigated by Partial Least Square regression (PLS2). A PLS1 was carried out to test the model for the prediction of “cold smoke” odorant attribute. All PLS analyses were performed using Unscrambler Windows version 9.1 (Camo A/S, Trondheim, Norway). All PLS models were calculated with cross-validation. The optimal number of components was determined as the number of components used to describe the maximum of variance of variables (between 80 and 90 %).

Table 1. Analysis of variance results with the effects of smoking processes, time and temperature parameters on scores of each attribute given by the 20 panellists

Attribute *	Process **	Time	Temperature	Process-Time	Process - Temperature	Time - Temperature
oglo	+ LS^a, TP^b, F^b, S^b	+ ↗	+ ↗	-	+	-
owf	+ TP^a, F^a, S^a, LS^b	+ ↗	+ ↗	-	-	-
ocs	+ LS^a, TP^b, F^b, S^b	+ ↗	+ ↗	-	+	-
obut	+ F^a, TP^b, S^b, LS^c	-	+ ↘	-	+	-
oveg	+ LS^a, TP^b, F^b, S^b	-	-	-	+	-
osalm	+ TP^a, F^a, S^a, LS^b	-	-	-	+	-
oher	-	-	+ ↗	-	-	-
fglo	+ LS^a, $TP^{a,b}$, $F^{b,c}$, S^c	+ ↗	+ ↗	-	-	-
fwf	+ TP^a, F^a, S^a, LS^b	+ ↗	-	-	-	-
fcs	+ LS^a, TP^b, F^b, S^b	+ ↗	-	-	-	-
fbut	+ F^a, $TP^{a,b}$, $S^{b,c}$, LS^c	-	-	-	-	-
fveg	+ LS^a, TP^b, F^b, S^b	-	+ ↘	-	-	-
fsalm	+ TP^a, F^a, S^a, LS^b	-	-	-	-	-
fher	-	+ ↗	-	-	-	-

+ : Significant effect of the considered factor or interaction at a risk of 5 %.

- : No effect of the considered factor or interaction at a risk of 5 %.

↗ : Increase of scores with the increase of the parameters of the factor considered.

↘ : Decrease of scores with the increase of the parameters of the factor considered.

* : global odor (oglo), wood fire smoke odor (owf), cold smoke odor (ocs), butter odor (obut), vegetal odor (oveg), salmon-like odor (osalm), herring-like odor (oher), global flavor (fglo), wood fire smoke flavor (fwf), cold smoke flavor (fcs), butter flavor (fbut), vegetal flavor (fveg), salmon-like flavor (fsalm), herring-like flavor (fher)

** : Process followed by the same letter on a same line are not significantly different. The processes are ranked from the process where the judges scores are the highest to the lowest. TP : Thermostated Plates, LS : Liquid Smoke, F : Friction, S : Smoldering

3. RESULTS

3.1. Effect of the smoke generation process on organoleptic properties of smoked salmon

The ANOVA performed on sensory attributes identified two groups, one constituted by products smoked by external smoke generators (thermostated plates, friction and smoldering) and the other constituted by salmons treated by liquid smoke. The results of a Fischer's Least Significant Difference (LSD) procedure shows that there is no significant differences at level 5 % between the intensities of odor attributes for all samples smoked by thermostated plates, smoldering and friction wood smoke generators (Table 1). The same test performed on flavor attributes shows that the products smoked by thermostated plates, smoldering and friction have obtained significantly different marks only for the attributes global flavor and butter flavor. It can be noticed that "herring-like" attribute is not very suitable to study the effect of the smoking processes because the scores given by the panelists for this attribute whatever the smoking process are not statistically different at a risk of 5 %.

Products treated with liquid smoke are characterised by high scores for the sensory attributes "cold smoke" and "vegetal" whereas salmons smoked by smoldering, friction or thermostated plates exhibit a "wood fire smoke" and "salmon-like" odor and flavor. The products smoked by friction have been characterised by the highest scores for "butter" sensory attribute.

Products treated by liquid smoke have been already shown with a "cold smoke" and "vegetal" sensory attributes in previous works (Cardinal et al., 1997 ; Kostyra et al., 2005 ; Varlet et al., 2007c) This kind of attributes were also used to characterise the liquid smoke odors which were described with « green » and « died bonfire» odors (Kostyra et al., 2005). The "wood fire smoke" and "salmon-like" odorant attributes of fishes smoked by smoldering, friction and thermostated plates have already been shown (Knockaert, 1990). These sensory attributes were used for the comparison of the level of smoking, evaluated by the odor intensity, on salmons smoked by external wood smoke generators (Cardinal et al., 2006).

3.1.1. Effect of the smoking parameters (smokehouse temperatures and times of smoke exposure) on organoleptic properties of smoked salmon

The effects of time of smoke exposure and smokehouse temperatures on organoleptic properties of smoked salmon are shown in Table 1. A positive effect of the time of smoke exposure can be highlighted by a multi-way ANOVA for “global”, “wood fire smoke” and “cold smoke” odor and flavor and also for “herring-like” flavor. This parameter has no significant effect for the other sensory attributes.

For smokehouse temperature, a positive effect was shown on “global” odor and flavor, on “wood fire smoke”, “cold smoke” and “herring-like” odors of smoked salmon. A negative effect has been found on “butter” odor and “vegetal” flavor.

The results of multi-way ANOVA (Table 1) show that whatever the smokehouse temperature, products treated by liquid smoke exhibit stronger “global”, “cold smoke” and “vegetal” and weaker “butter” and “salmon-like” odors than the products smoked by the other processes. Moreover, whatever the smokehouse temperature, the scores for products treated by liquid smoke were similar for “global”, “cold smoke” and “butter” odors. However, the “vegetal” odor was significantly more perceived by the assessors when products are smoked at 22°C than at 32°C. Concerning the “salmon-like” odorant attribute the scores given by the judges are higher for products processed by liquid smoke atomization and thermostated plates at 22°C than 32°C whereas they are similar for products smoked by friction and smoldering.

The increase of “smoky” sensory attributes intensity (cold smoke, global odor, wood fire smoke) with the increase of the time of smoke exposure could be explained by a greater deposition of the smoke aroma compounds for a longer time in the smokehouse (Sérot, Baron, Knockaert & Vallet, 2004). Concerning the increase of the judges scores for “herring-like” flavor, one hypothesis could be that this odor would be due to a mix of individual odorant volatile compounds of wood smoke and/or fish flesh through lipid oxidation resulting in an increase of “herring-like” flavor perception.

The increases of “global” odor and flavor, “wood fire smoke”, “cold smoke” and “herring-like” odors with the increase of smokehouse temperature could be due to differences of physicochemical properties of the food at 32°C and 22°C. A higher fluidity of the lipid phase of fish flesh has already been purposed to explain the increased deposition of phenolic compounds when temperature increased (Sérot et al., 2004). A high temperature could also prevent condensation of water vapor on the surface of fish fillets resulting in a washing out of deposited smoke compounds (Chan & Toledo, 1975). As result, the notes for “smoky” (related to deposition of wood smoke components) and “fishy” (related to oxidations of fish flesh components) odorant attributes are higher when products are smoked at 32°C.

Table 2. Attributes of smoked salmon odor and flavor of 24 smoked salmon samples according to the Flash Table procedure

	Attributes of smoked salmon odour							Attributes of smoked salmon flavour						
Compounds *	ocs	oveg	obut	owf	oglo	osalm	oher	fcs	fwf	fveg	fglo	fsalm	fbut	fher
F [a]	21,84	8,33	6,12	5,67	4,04	2,99	1,37 (NS)	16,01	7,27	6,59	3,09	1,52 (NS)	1,39 (NS)	0,98 (NS)
G mean [b]	1,91	0,84	1,66	4,01	5,78	3,07	0,43	1,89	4,64	0,77	6,39	3,92	1,17	0,45
SD [c]	2,46	1,47	1,96	2,29	1,69	2,07	0,87	2,53	2,29	1,45	1,37	2,26	1,54	0,83
S 1 22														
S 1 32														
S 2 22														
S 2 32														
S 3 22														
S 3 32														
F 1 22														
F 1 32														
F 2 22														
F 2 32														
F 3 22			3,81 (+)											
F 3 32														
LS 1 22		2,70 (+)							2,30 (-)	2,27 (+)				
LS 1 32														
LS 2 22	5,26 (+)	2,32 (+)						5,15 (+)		2,50 (+)				
LS 2 32	5,30 (+)							5,22 (+)						
LS 3 22	5,56 (+)	2,73 (+)						5,12 (+)						
LS 3 32	5,04 (+)							5,07 (+)						
TP 1 22														
TP 1 32														
TP 2 22														
TP 2 32														
TP 3 22														
TP 3 32														

F [a] : *F* value of Products effect

NS : No Products effect

G mean [b] : Grand mean of the products

SD [c] : Standard Deviation of the 24 products

* : Names of attributes are given in the subtext of Table 1

The increase of “smoky” odor and flavor intensity could be responsible for the decrease of “butter” and “vegetal” flavor and odor perception by masking this note.

3.1.2. Study of the main sensory characteristics of the products

In order to summarize the main differences between samples and to identify rapidly the more discriminated sensory attributes, an analysis of variance was performed with the effects of the products (24 smoked salmon samples: 4 smoking processes × 3 hours of time of exposure (1, 2 or 3) × 2 smokehouse temperatures (22 or 32°C)) on scores of each sensory attribute given by the panelists. The aim of this study is to identify the most discriminative sensory attributes of the smoked products. The results are presented in Table 2 according to a modified Flash table (Schlich, 1998 ; Cardinal et al., 2006) where each group of characteristics (odor and flavor) sensory attributes are sorted into columns by decreasing *F*-value and products are sorted into rows. The grand mean and the standard deviation calculated for the 24 products are also included in the Table 2 and allow a fast analysis of the attributes as main contributors to discriminate the samples. A (+) sign is added when the mean score is higher than the grand mean plus one standard deviation. A (-) sign is added when the mean score is lower than the grand mean minus one standard deviation. To facilitate the reading, only the means corresponding to these criteria are given and the means close to the grand mean are not written in the Table 2.

The “cold smoke” odor and flavor attributes present the highest *F*-values for product effect with a significant *p*-value. It means that great differences exist between the samples for these odors and flavors. Conversely, “herring-like” odor and flavor, “butter” and “salmon-like” flavor attributes have not a significant *p*-value. That means that these sensory attributes are not very useful to describe the different smoked salmons.

Salmons treated by liquid smoke are very different from the other products. These products present very high “cold smoke” odor and flavor. Moreover, they present important “vegetal” odor and flavor when a smokehouse temperature of 22°C is applied whatever the time of smoke exposure. Conversely, salmons treated by liquid smoke present low “wood fire smoke” flavor for 1 hour of smoking at 22°C. When the parameters of smoking increase, this difference with the other processes disappears.

It is interesting to note that salmons smoked by friction are characterised by the judges with a high “butter” odor for 3 hours of smoking at 22°C. The lower quantity of smoky aroma compounds in the wood smoke produced by friction by comparison with those produced by

thermostated plates or smoldering, because of a lower wood pyrolysis temperature, could be responsible for the higher perception of the initial “butter” sensory attributes of salmon.

3.2. Effect of the smoke generation processes on odorant volatile compounds concentrations

An ANOVA was performed on the content of odorant compounds of salmon on the concentrations of all odorant volatile compounds (Table 3) indicated a significant effect of smoke generation processes.

Significant differences can be noted concerning the furannic and enolones derivatives concentrations between fishes processed by thermostated plates and the by the other techniques. Indeed, the concentrations of furfural (1), furfuryl alcohol (3), 5-methylfurfural (8), 2-methyl-2-cyclopenten-1-one (6), 2-hydroxy-3-methyl-2-cyclopenten-1-one (10), 2,3-dimethyl-2-cyclopenten-1-one (11) and 2,3,4-trimethyl-2-cyclopenten-1-one (16) are higher in fillets smoked by thermostated plates. The concentrations of phenolic compounds (phenol (9), o-cresol (12), guaiacol (14), alkyl phenols (15 and 19), guaiacol derivatives (20, 24, 27, 30) and isoeugenol isomers (32, 33)) and alkyls aryls ethers such as 2,3 or 3,5-dimethoxytoluene (21, 22) are also significantly higher in salmons smoked by this technique. Friction and smoldering lead to products with close odor-active compounds contents except for 2-acetylfuran (7), 2,4-hexadienal (5), 4-vinylguaiacol (27) and 4-allylsyringol (35) more concentrated in salmon smoked by friction.

These results confirm previous works carried out in our laboratory (Cardinal et al., 2006 ; Varlet et al., 2007b ; Varlet et al., 2007c) and can be explained by the high wood pyrolysis temperature used for this thermostated plates process (close to 500°C vs 380°C for friction and 450 °C for smoldering). High quantities of aroma compounds are generated at high pyrolysis temperatures. Conversely, lower concentrations of phenolic, furannic and enolones compounds can be observed with the decrease of wood pyrolysis temperature as it is noticeable for smoldering.

Products treated by liquid smoke show the highest concentrations 2-hydroxy-3-ethyl-2-cyclopenten-1-one (17), 2,3,5-trimethoxytoluene (34), syringol (28), p-cresol (13) and 8-heptadecene (36). It can be also noted that some odorant volatile compounds are found only in salmon treated by liquid smoke atomization such as lipid oxidation products or pyridine derivatives (4-methylpyridine (2) and 2,6-dimethylpyridine (4)). More information about the technology used to obtain liquid smoke could allow to explain the differences of composition of odor-active compounds between salmons treated by liquid smoke and those smoked by

Table 3. Analysis of variance results with the effects of smoking processes, time and temperature parameters on the concentration of the odorant volatile compounds

Number code	Compounds	Odor in smoked salmon	Process *	Time	Temperature	Process-Time	Process - Temperature	Time - Temperature
1	furfural	smoke, green	+ TP^a, F^b, S^c, LS^d	+ ↗	+ ↗	+	+	+
2	4-methylpyridine	green, milk	+ LS^a, F^b, TP^b, S^b	+ ↗	-	+	-	-
3	furfuryl alcohol	cooked/soup, chemical	+ TP^a, F^b, S^b, LS^c	+ ↗	+ ↗	+	+	+
4	2,6-dimethylpyridine	roasty, green, milk	+ LS^a, F^b, TP^b, S^b	-	-	-	-	-
5	2,4-hexadienal	cooked vegetable, fatty	+ F^a, TP^b, S^c, LS^d	+ ↗	+ ↗	-	+	-
6	2-methyl-2-cyclopenten-1-one	cooked potato, green	+ TP^a, F^b, S^b, LS^c	+ ↗	+ ↗	+	+	-
7	2-acetylfuran	cooked vegetable, potato	+ F^a, TP^b, S^c, LS^c	+ ↗	+ ↗	+	+	+
8	5-methylfurfural	cooked, earthy, green	+ TP^a, F^b, S^c, LS^c	+ ↗	+ ↗	+	+	+
9	phenol	marine, metallic, chemical, mushroom	+ TP^a, LS^b, S^b, F^c	+ ↗	+ ↗	-	+	+
10	2-hydroxy-3-methyl-2-cyclopenten-1-one	cooked, spicy	+ TP^a, LS^b, F^c, S^d	+ ↗	+ ↗	-	-	-
11	2,3-dimethyl-2-cyclopenten-1-one	spicy, wood fire, roasty	+ TP^a, F^b, S^b, LS^b	+ ↗	+ ↗	-	+	-
12	o-cresol	chemical, spicy, burnt,	+ TP^a, LS^a, F^b, S^b	+ ↗	+ ↗	-	+	+
13	p-cresol	Burnt, animal, spicy	+ LS^a, TP^b, F^c, S^c	+ ↗	+ ↗	-	+	+
14	guaiacol	smoked, vanilla, ink	+ TP^a, F^b, S^c, LS^c	+ ↗	+ ↗	-	-	+
15	2,6-dimethylphenol	chemical, burnt, spicy/woody	+ TP^a, S^b, F^c, LS^c	+ ↗	+ ↗	+	+	+
16	2,3,4-trimethylcyclopenten-1-one	cooked, green, spicy	+ TP^a, S^b, F^b, LS^b	+ ↗	+ ↗	+	-	+
17	3-ethyl-2-hydroxy-2-cyclopenten-1-one	solvent, medicinal	+ LS^a, F^b, TP^b, S^b	+ ↗	+ ↗	+	-	+
18	1,2-dimethoxybenzene	ashes, green	+ TP^a, LS^a, F^b, S^b	+ ↗	+ ↗	-	-	-
19	2,4 and 2,5-dimethylphenol / (E)-2-nonenal	cucumber, violet, spicy, smoked	+ TP^a, LS^a, S^b, F^c	+ ↗	+ ↗	-	+	-
20	4-methylguaiacol	candy, spicy, smoked	+ TP^a, LS^a, S^b, F^b	+ ↗	+ ↗	-	-	-
21	2,3-dimethoxytoluene	cooked vegetable, fatty, green	+ TP^a, F^a, S^b, LS^b	+ ↗	+ ↗	-	-	-
22	3,5-dimethoxytoluene	burnt, green, chemical	+ TP^a, F^b, LS^b, S^b	+ ↗	+ ↗	-	-	-
23	(E)-2-decenal	spicy, green, milk	+ TP^a, F^a, LS^a, S^b	-	-	-	-	-
24	4-ethylguaiacol	green, smoke, vanilla, clove	+ TP^a, F^b, LS^b, S^b	+ ↗	+ ↗	-	+	+
25	indanone	sawdust, rotten, burnt	+ TP^a, F^a, LS^a, S^b	+ ↗	+ ↗	+	+	+
26	(E,E)-2,4-decadienal	oily, green, fatty	+ TP^a, S^b, F^c, LS^d	-	+ ↗	+	-	-
27	4-vinylguaiacol	smoke, green, spicy	+ F^a, LS^b, S^c, TP^c	+ ↗	+ ↗	-	-	-
28	syringol	burnt rubber, spicy	+ LS^a, F^b, TP^b, S^b	+ ↗	+ ↗	-	-	-
29	eugenol	spicy, smoke, clove	+ F^a, $TP^{a,b,}$ $LS^{b,c}$, S^d	+ ↗	+ ↗	-	+	+
30	4-propylguaiacol	green, spicy, vanilla	+ TP^a, LS^b, $F^{b,c}$, S^c	+ ↗	+ ↗	-	+	-
31	1,2,3-trimethoxy-5-methylbenzene	cooked, earthy	+ TP^a, F^b, LS^b, S^b	+ ↗	+ ↗	+	+	+
32	(Z)-isoeugenol	burnt rubber, spicy	+ TP^a, F^a, LS^b, S^b	+ ↗	+ ↗	+	+	+
33	(E)-isoeugenol	clove, green, roasty	+ TP^a, F^a, LS^b, S^b	+ ↗	+ ↗	-	-	+
34	2,3,5-trimethoxytoluene	spicy, woody	+ LS^a, F^b, TP^b, S^b	+ ↗	+ ↗	-	-	-
35	4-allylsyringol	smoke, rotten	+ F^a, $LS^{a,b}$, S^b, TP^b	+ ↗	+ ↗	-	-	-
36	8-heptadecene	animal, roasty, chemical	+ LS^a, F^b, TP^b, S^c	+ ↗	-	-	-	-

external generators. Indeed, we can wonder why pyridine derivatives (2,4), hydrocarbons such as 1,2-dimethoxybenzene (18), 2,3,5-trimethoxytoluene (34) and 8-heptadecene (36) and certain phenolic compounds such as cresol isomers, 4-methylguaiacol (20) but especially syringol (28) are particularly important in liquid smoked salmon fillets. This observation suggests a step of formulation on behalf of the manufacturer during the pyrolysis (by adding other woods), or by recovering the condensates at different temperatures during the condensation of the smoke or after the condensation (by mixing the smoke condensates with other liquid smokes) (Kostyra et al., 2005).

3.2.1. Effect of the smoking parameters (time of smoke exposure and smokehouse temperature) on odorant volatile compounds concentrations

A multi-way ANOVA was performed on the content of odorant compounds of salmon subjected to different smoking conditions of time and temperature. The results (Table 3) indicated that these factors had significant effect on the deposition of odorants compounds in fish fillets ($p < 0.05$). The deposition of compounds was greater when time of smoke exposure increased except for 2,6-dimethylpyridine (4), (E)-2-decenal (23) and (E,E)-2,4-decadienal (26) and when the smokehouse temperature increased (from 22 to 32°C) except for the pyridine derivatives (2,4), (E)-2-decenal (23) and 8-heptadecene (36).

The increase of odorant volatile compounds concentrations with increase of time of smoke exposure seems logically due to higher quantities of compounds recovered when fillets are exposed for a longer time to wood smoke. Concerning the effect of smokehouse temperature, the higher concentrations of odorant volatile compounds recovered at 32°C by comparison with those recovered at 22°C could be related to the observations done on the sensory attributes. A high smokehouse temperature during the smoking step can allow potential compounds with a higher molecular weight, involved in the smoking effect, to remain in the vapor phase and therefore to be deposited in higher quantities (Cardinal et al., 2006). However, it can also be caused by modifications of the fish flesh components as lipids which can diffuse from a solid state in the interior of the fillet to a liquid state at the exterior of the fillet. Therefore, the solubilisation of semi-polar wood smoke odorant volatile compounds at the surface of the fillets is facilitated. The change of state of the lipids can also generate higher quantities of lipid oxidation products as carbonyles compounds. The higher quantities of compounds known as deriving from lipids degradation such as (E,E)-2,4-decadienal (26) and 2,4-hexadienal (5) recovered in fillets smoked at 32°C rather than 22°C seems to confirm

Table 4. Main odor-active volatile compounds of 24 smoked salmon samples according to the Flash Table procedure

Compounds *	31	1	15	6	3	2	7	8	26	24	10	17	4	11	36	34	5	13
F [a]	283	19,25	18,73	14,83	14,67	14,22	14,04	12,94	11,08	10,52	10,05	9,75	9,22	8,93	8,22	7,87	7,77	7,74
G mean [b]	3,96	302,07	3,02	13,24	110,18	2,63	21,58	44,06	5,03	54,45	10,34	1,72	0,9	11,53	5,01	7,11	2,2	24,02
SD [c]	9,82	217,4	3,06	11,15	120,13	5,27	16,23	37,11	6,88	40,69	11,01	2,99	1,76	8,97	2,97	6,59	2,23	20,24
S 1 22						0 (-)			0 (-)		0 (-)	0 (-)	0 (-)					
S 1 32						0 (-)			0 (-)		0 (-)	0 (-)	0 (-)					
S 2 22						0 (-)			0 (-)		0 (-)	0 (-)	0 (-)					
S 2 32						0 (-)			0 (-)		0 (-)	0 (-)	0 (-)		1,38 (-)			
S 3 22						0 (-)			0 (-)		0 (-)	0 (-)	0 (-)					
S 3 32			7,11 (+)			0 (-)			0 (-)				0 (-)					
F 1 22						0 (-)					0 (-)	0 (-)	0 (-)					
F 1 32						0 (-)			13,71 (+)			0 (-)	0 (-)				5,09 (+)	
F 2 22						0 (-)			13,50 (+)			0 (-)	0 (-)					
F 2 32						0 (-)			14,97 (+)			0 (-)	0 (-)				5,76 (+)	
F 3 22						0 (-)			16,39 (+)			0 (-)	0 (-)					
F 3 32		751,14 (+)				0 (-)	48,07 (+)		19,13 (+)				0 (-)				7,11 (+)	
LS 1 22		43,52 (-)	0 (-)			5,83 (+)							3,62 (+)		11,65 (+)		0 (-)	
LS 1 32						9,57 (+)							3,73 (+)		10,16 (+)	15,87 (+)	0 (-)	
LS 2 22		64,75 (-)				8,85 (+)			1,76 (-)			4,74 (+)	3,22 (+)		8,68 (+)	21,23 (+)	0 (-)	
LS 2 32						10,54 (+)						4,20 (+)	3,13 (+)		9,59 (+)		0 (-)	
LS 3 22		66,74 (-)				13,62 (+)						6,6 (+)	4,10 (+)			17,22 (+)	0 (-)	
LS 3 32						16,65 (+)					23,64 (+)	10,49 (+)	4,27 (+)			20,55 (+)		74,18 (+)
TP 1 22						0 (-)			0 (-)			0 (-)	0 (-)					
TP 1 32						0 (-)			0 (-)		22,07 (+)	0 (-)	0 (-)					
TP 2 22						0 (-)			0 (-)			0 (-)	0 (-)					
TP 2 32		630,59 (+)	7,76 (+)	36,28 (+)	387,90 (+)	0 (-)	54,06 (+)	124,59 (+)	0 (-)	148,45 (+)	37,60 (+)	0 (-)	0 (-)	31,24 (+)				47,11 (+)
TP 3 22		558,33 (+)		26,29 (+)		0 (-)	38,03 (+)	92,06 (+)	0 (-)			0 (-)	0 (-)					
TP 3 32	59,73 (+)	807,67 (+)	13,90 (+)	53,53 (+)	544,46 (+)	0 (-)	70,14 (+)	163,36 (+)	0 (-)	190,6 (+)	29,35 (+)	5,26 (+)	0 (-)	42,96 (+)			4,64 (+)	66,04 (+)

F [a] : *F* value of Products effect

G mean [b] : Grand mean of the products

SD c : Standard Deviation of the 24 products

* : Number codes of the compounds are given in Table 3

this hypothesis. Finally, it can be due to biochemical reactions, favorised with the increase of temperature, between the components of the fish flesh themselves or with the smoke Indeed, a positive effect of the smokehouse temperature has already been hypothesized as a parameter favourizing biochemical reactions leading to the generation of aroma compounds (Varlet et al., 2007b). Maillard and Strecker reactions between aminoacids from the fish flesh and carbohydrates from the wood smoke could be facilitated with an increase of the smokehouse temperature. Although Maillard and Strecker reactions occur at high temperatures, it has been shown that they could also take place at low temperatures (Marchand, De Revel & Bertrand, 2000 ; Pripis-Nicolau, De Revel, Bertrand & Maujean, 2000).

3.2.2. Study of the main odor-active compounds of the products

In order to summarize the main differences between the samples and to identify rapidly the more discriminated odorant volatile compounds, an analysis of variance was performed with the effects of the products (24 smoked salmon samples: 4 smoking processes × 3 hours of time of exposure (1, 2 or 3) × 2 smokehouse temperatures (22 or 32°C) on the concentrations of odorant volatile compounds. The results are presented in Table 4 according to a modified Flash table (Schlich, 1998 ; Cardinal et al., 2006) where the odorant volatile compounds are sorted into columns by decreasing *F*-value and products are sorted into rows.

Except 1,2,3-trimethoxy-5-methylbenzene (31) whose concentration is only important for salmons smoked by thermostated plates after 3 hours at 32°C, four homogeneous groups of products have been identified. One group consisted of salmons smoked by thermostated plates exposed during 2 or 3 hours to wood smoke and at a smokehouse temperature of 32°C rather than 22°C. These products show the highest concentrations of odorant volatile compounds especially furannic compounds. This result could be explained by high wood pyrolysis temperature used for thermostated plates. The second group is constituted by the products treated by liquid smoke. These products were characterised by low concentrations of 4-vinylguaiacol (27) and furfural (1) for smokehouse temperature of 22°C whatever the time of smoke exposure. They are also characterised by the presence of pyridine derivatives (2,4) and by high quantities of 3-ethyl-2-hydroxy-2-cyclopenten-1-one (17), 8-heptadecene (36) and 2,3,5-trimethoxytoluene (34) and by the absence of compounds such as 2,4-hexadienal (5). Moreover, after 3 hours of smoking and especially at 32°C, these products exhibit high concentrations of 2-hydroxy-3-methyl-2-cyclopenten-1-one (10), (E)-2-decenal

Table 4 (continued). Main odor-active volatile compounds of 24 smoked salmon samples according to the Flash Table procedure

Compounds *	29	14	12	9	19	27	33	32	16	20	21	25	30	18	28	22	23	35
F [a]	7,74	7,11	6,82	6,55	6,53	6,2	5,99	5,71	5,68	4,5	4,46	4,43	4,38	4,01	3,6	3,44	2,16	1,95
G mean [b]	22,85	249,01	22,43	31,38	7,72	16,93	20,68	6,73	17,09	387,23	4,41	2,22	9,7	5,9	13,71	5,05	3,06	0,77
SD [c]	14,93	167,16	16	26,86	5,78	12,31	14,45	4,37	12,53	202,67	2,88	1,35	7,02	4,27	14,39	2,65	3,09	0,57
S 1 22										163,1 (-)							0 (-)	
S 1 32																	0 (-)	
S 2 22										178,43 (-)		0,81 (-)		1,46 (-)			0 (-)	
S 2 32																	0 (-)	
S 3 22																	0 (-)	
S 3 32				61,69 (+)		36,27 (+)	36,95 (+)	11,45 (+)								7,84 (+)	0 (-)	
F 1 22					1,93 (-)					175,06 (-)				1,50 (-)				
F 1 32																	6,59 (+)	
F 2 22										164,10 (-)								
F 2 32																	6,27 (+)	1,38 (+)
F 3 22																		
F 3 32	52,02 (+)	488,72 (+)					46,23 (+)	15,04 (+)			8,89 (+)					7,79 (+)		1,66 (+)
LS 1 22						0,50 (-)										2,40 (-)		
LS 1 32						2,70 (-)												
LS 2 22						3,61 (-)									34,46 (+)			
LS 2 32																		
LS 3 22						1,21 (-)									34,43 (+)			
LS 3 32			49,74 (+)	65,55 (+)	18,96 (+)	3,24 (-)								11,16 (+)	44,61 (+)		6,25 (+)	
TP 1 22	5,66 (-)																	
TP 1 32																		
TP 2 22																		
TP 2 32	39,24 (+)	556,79 (+)	50,53 (+)	80,12 (+)	16,25 (+)	34,09 (+)	40,57 (+)	13,44 (+)		705,82 (+)	9,32 (+)	3,82 (+)	24,52 (+)	11,51 (+)		8,83 (+)		
TP 3 22						32,34 (+)			27,06 (+)									
TP 3 32	62,69 (+)	755,26 (+)	59,14 (+)	115,60 (+)	20,68 (+)	41,77 (+)	59,73 (+)	17,30 (+)	70,15 (+)	893,98 (+)	11,02 (+)	6,95 (+)	29,9 (+)	16,45 (+)		10,86 (+)		1,36 (+)

F [a] : *F* value of Products effect

G mean [b] : Grand mean of the products

SD c : Standard Deviation of the 24 products

* : Number codes of the compounds are given in Table 3

(23), 1,2-dimethoxybenzene (18) and several phenolic compounds such as cresol isomers (12,13), phenol (9), syringol (28), 2,4 and 2,5-dimethylphenols (19). The third group of products is represented by salmons smoked by friction. This group of products is characterised by particular high amounts of lipid oxidation products such as (E,E)-2,4-decadienal (26) and 2,4-hexadienal (5) by comparison with the other smoked products. After 3 hours of smoking at 32°C, products smoked by friction exhibited high concentrations of furannic compounds such as furfural (1) and 2-acetylfuran (7), high concentrations of alkyls aryls ethers such as 2,3-dimethoxytoluene (21) and 3,5-dimethoxytoluene (22) and phenolic compounds such as eugenol (29), isoeugenol isomers ((E)-isoeugenol (32) and (Z)-isoeugenol (33)), guaiacol (14) and 4-allylsyringol (35). The fourth group is constituted by the products smoked by smoldering. These products are generally characterised by the absence of (E,E)-2,4-decadienal (26) and (E)-2-decenal (23) and low quantities of enolones derivatives such as 2-hydroxy-3-methyl-2-cyclopenten-1-one (10) and 3-ethyl-2-hydroxy-2-cyclopenten-1-one (17). After 3 hours of smoking at 32°C, these products presented high concentrations of 3,5-dimethoxytoluene (22) and phenolic compounds such as 2,6-dimethylphenol (15), phenol (9), 4-vinylguaiacol (27) and isoeugenol derivatives (32,33).
It has been reported that the yields for the targeted organoleptically active compounds as phenolic and other compounds such as furans go through a maximum at 500°C with increasing temperature between 400 and 650°C (Alén, Kuoppala & Oesch, 1996 ; Nonier, Vivas, Vivas de Gaulejac, Absalon, Soulié & Fouquet, 2005 ; Simon, De la Calle, Palme, Meier & Anklam, 2005). The respective wood pyrolysis temperatures applied in thermostated plates (500°C), smoldering (450°C) and friction (380°C) can explain our results about odorant compounds composition of smoked salmons.

3.3. Sensory attributes explained by odorant volatile compounds concentrations

Analysis of the relationships between the sensory variables (notes given by the judges for each sensory attribute) and analytical variables (concentrations of odorant volatile compounds) was performed by means of PLS2 whose results are shown in Fig.1 and 2. The optimum number of components to describe the variables was found to be two to express the maximum of the variance (Fig. 3). Fig. 1 shows the score plot of the samples for the two dimensions and gives the respective location of the products. Fig. 2 shows the loading plot of the sensory and the odorant volatile compounds variables for the two dimensions of the PLS2 analysis. The first PLS-component explained 26 % of the X-block of « odorant compounds »

Figure 1. Score plots of the PLS analysis (S : Smoldering, TP : Thermostated Plates, F : Friction and LS : Liquid Smoke ; 1, 2, 3 are the times of smoke exposure applied ; 22 and 32 are the smokehouse temperatures applied.)

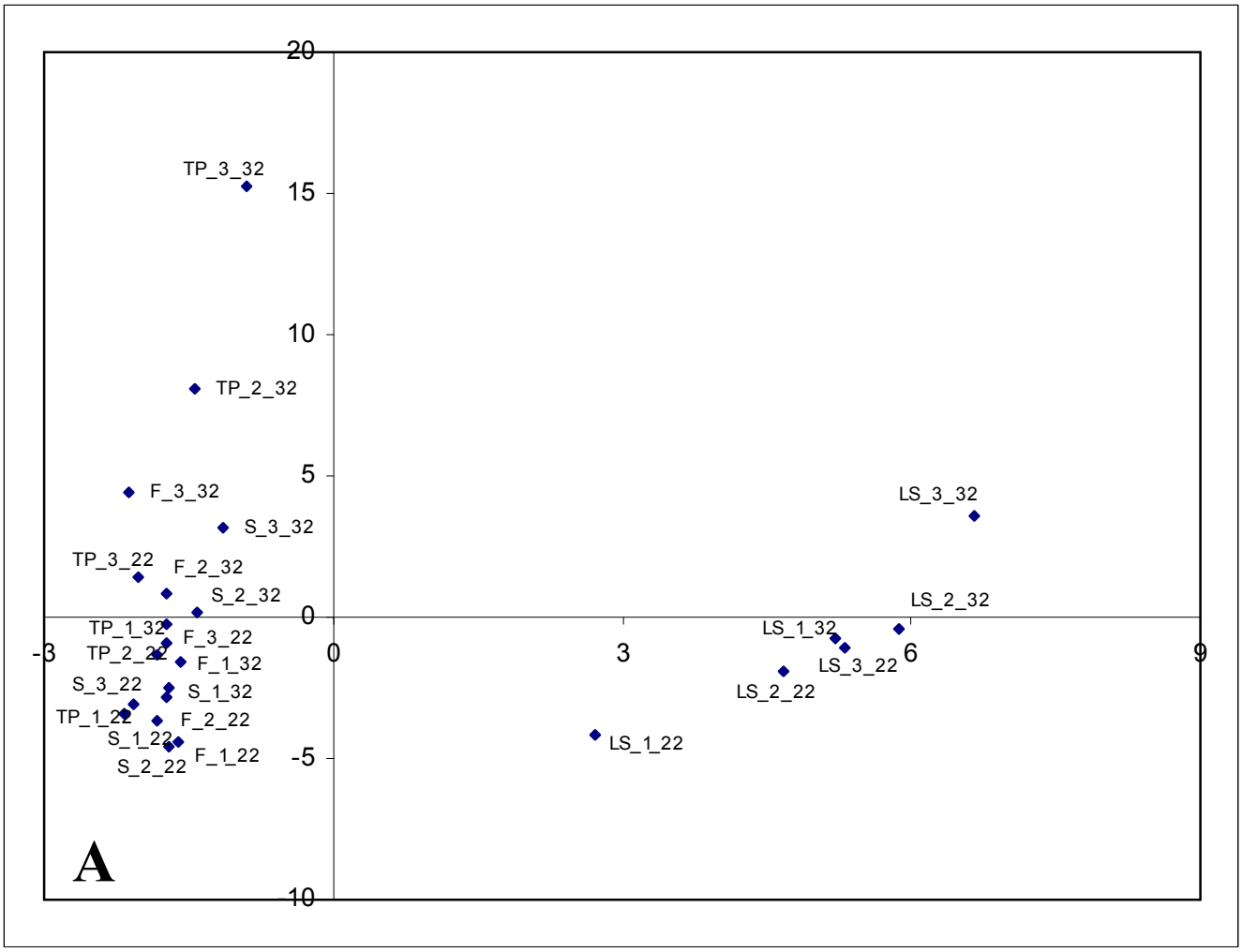


Figure 2. Loading plots of the PLS analysis (odorant volatile compounds are coded by a number related to Table 3 and sensory attributes are coded by letters related to the subtext of Table 1)

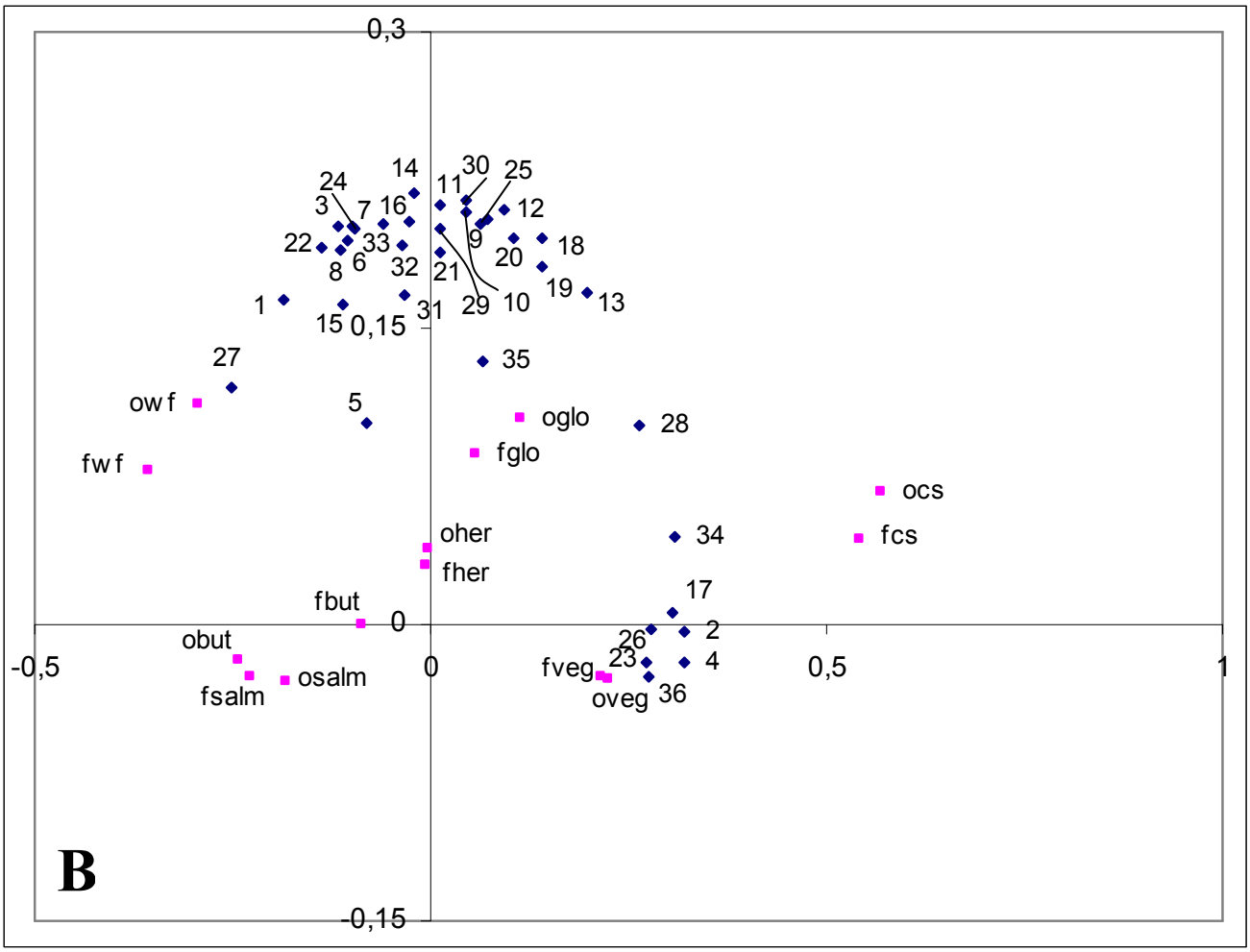
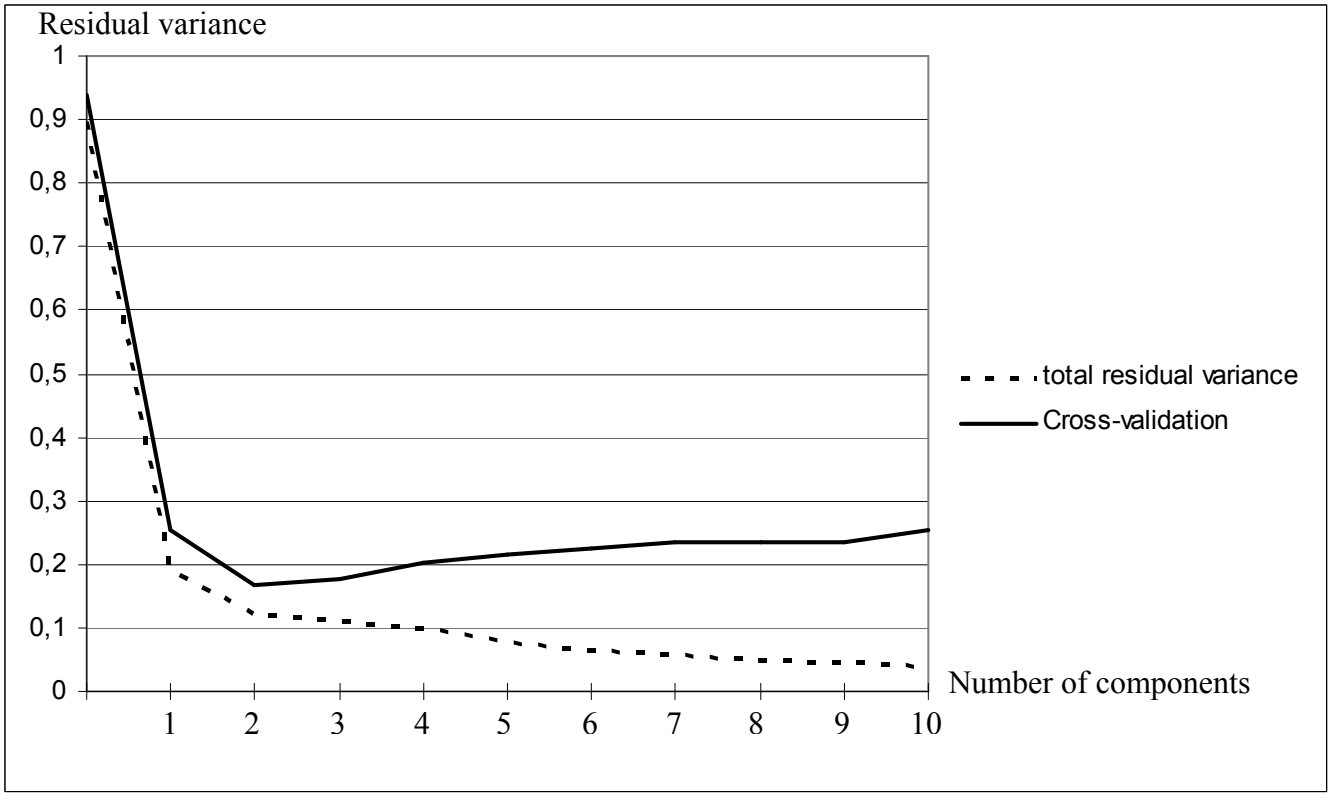


Figure 3. Percentage of residual variance for PLS2 model



variables and 79 % of the Y-block of « sensory » variables. The second PLS-component explained 56 % of the X-block of « odorant compounds » variables and 7 % of the Y-block of « sensory » variables.

The first PLS-component allows to discriminate the products smoked by processes applying wood pyrolysis in situ and the products treated by liquid smoke. Moreover, in the case of liquid smoke, this component is also useful to illustrate the effect of the time of smoke exposure and smokehouse temperature. Conversely, time of smoke exposure and smokehouse temperature effects for the three others smoking techniques are discriminated by the second PLS-component.

3.3.1. Products treated by liquid smoke

The loading plot (Fig. 2) show that the "cold smoke" but especially "vegetal" sensory attributes are explained by eight odor-active compounds. The "vegetal" odor and flavor of smoked salmon could be explained by high contents of lipid oxidation products such as (E)-2-decenal (23) and (E,E)-2,4-decadienal (26), the pyridine derivatives (2,4) and 8-heptadecene. The "cold smoke" odor and flavor can not be explained strictly by odorant volatile compounds. However, it can be noticed that among the odorant volatile compounds identified in smoked salmon, syringol (28) and p-cresol (13) are the most abundant phenolic compounds related to the "cold smoke" sensory attributes. 3-ethyl-2-hydroxy-2-cyclopenten-1-one (17) and 2,3,5-trimethoxytoluene (34) could be considered as intermediaries between "cold smoke" and "vegetal" sensory attributes.

The initial "vegetal" sensory attributes of products treated by liquid smoke could be explained by the individual odors exhibited by pyridine derivatives (2,4), previously found in liquid smokes in which they have been characterised by green, bitter and burnt odors (Guillén et al., 2001) and lipid oxidation products which have already been identified with green odors in smoked salmon (Cardinal et al., 2006). The "cold smoke" sensory attributes could be explained by the individual odors exhibited by syringol (28) found in high concentrations in liquid smoke (results not published here) which has been previously identified as potent odorant volatile compounds in olfactometric studies of salmons treated by liquid smoke with a burnt rubber, spicy aromatic note (Varlet et al., 2006) (Table 3). However, recent studies have attributed to this compound a minor organoleptic role in liquid smoke (Kostyra et al., 2005 ; Cardinal et al., 2006). The "vegetal" and "cold smoke" sensory attributes are simultaneously linked to high contents of 3-ethyl-2-hydroxy-2-cyclopenten-1-

one (17) and 2,3,5-trimethoxytoluene (34) perceived by olfactometry with respectively solvent/medicinal and spicy/woody aromatic notes (Varlet et al., 2006). The fact that odorant volatile compounds could be implied in two different odors shows possibilities of odorant interactions between odorant volatile compounds responsible for the overall odor.

Therefore, the sensory attributes and the odorant volatile compounds cited are commonly found in liquid smoke or products treated by liquid smoke (Cardinal et al., 1997). These results are confirmed by the comparison of the both Fig. 1 and Fig. 2 which shows that the "vegetal" sensory attributes and the related odorant volatile compounds are linked to products treated by liquid smoke for low setting of smoking process (one hour of smoke exposure, 22°C). The "cold smoke" sensory attributes and the related odorant volatile compounds are linked to products treated by liquid smoke for high setting of smoking process (three hours of smoke exposure, 32°C). The effects of the smoking parameters (smokehouse temperature and time of smoke exposure) are remarkable for liquid smoke where smoked salmon odors are characterised by « vegetal » sensory attributes for a short time of smoking and evolve to « cold smoke » with the increase of the smoking intensity. In the case of liquid smoke, the initial odor of the salmon fillet after 1 hour of smoking is not characterised by an expected « salmon-like » odor because liquid smoke confers rapidly to the fish flesh its « vegetal » (after short time of atomization at 22°C) and « cold smoke » (after 3 hours of smoking at 32°C) odors and flavors.

3.3.2. Products smoked by smoldering, thermostated plates and friction

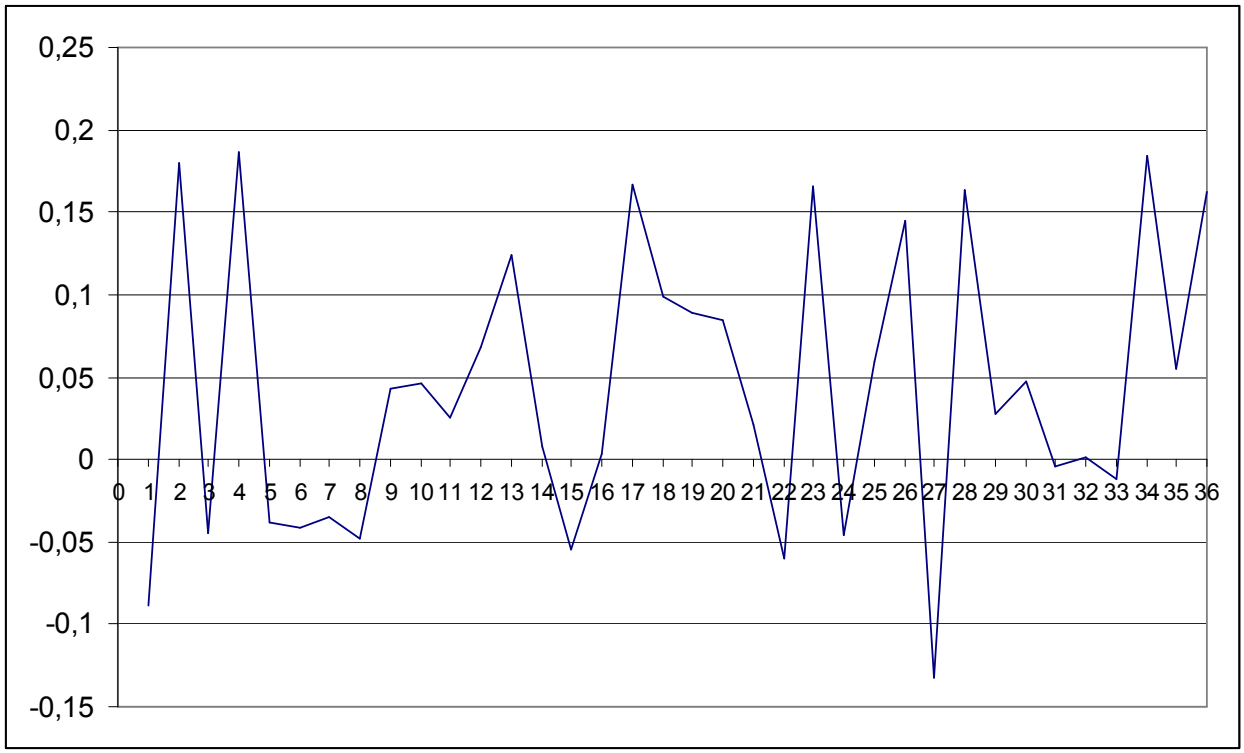
The loading plots (Fig. 2) show that the "wood fire smoke" sensory attributes are explained by all the other odorant volatile compounds especially by high contents of phenolic compounds as 4-vinylguaiacol (27) and furannic compounds such as furfural (1), furfuryl alcohol (3), 2-acetylfuran (7) and 5-methylfurfural (8). Cresol isomers such as p-cresol (13) were found to be the phenolic compounds the most implied in « cold smoke » sensory attributes. The "butter", "salmon-like" and "herring-like" sensory attributes seem to not be linked to odorant volatile compounds concentrations.

The comparison between Fig. 1 and Fig. 2 can explain these results. In the case of smoking methods applying wood pyrolysis in situ, the smoking parameters are responsible for a product characterised by "salmon-like" and "butter" odors and flavors for a short time of smoking and are characterised by "wood fire smoke" sensory attributes when time of smoke exposure and smokehouse temperature increase as it can be visualized on the second PLS-

component. Thus, for a longer time of smoke exposure and a higher smokehouse temperature, there is a greater deposition of aroma compounds coming from the wood smoke, or a greater generation of compounds between the fish flesh components and the wood smoke components under the effect of smoking conditions, which could explain the evolution of the sensory attributes from “raw fish” and “butter” to “wood fire smoke”. Therefore, the “butter”, “salmon-like” and “herring-like” sensory attributes are linked to low content of odorant volatile compounds, especially phenolic and furannic compounds and enolones derivatives. Besides, compared with the concentrations of odor-active compounds, a relation can be noted between low quantities of phenolic and furannic derivatives, higher quantities of carbonyles as 2,4-hexadienal (5), (E)-2-decenal (23) reached after short times of smoking especially at the lowest smokehouse temperature and the “salmon-like”, “butter” sensory attributes. In low quantities at the beginning of smoking, phenolic and furannic compounds are not the main contributors of smoked salmon aroma. Indeed, the odor-active volatile compounds of unsmoked salmon are again present as 2,4-hexadienal (5) (Varlet et al., 2006). But as soon as the smoking parameters increase, the deposition of odorant volatile compounds responsible of “woodfire smoke” sensory perception increases and could gradually mask the initial odor of smoke salmon.

The role played by the phenolic compounds in “wood fire smoke” sensory attributes perception is logical. Indeed, phenolic compounds have been previously found as strongly contributing to the “global” odor and flavor of smoked salmon as previously shown by numerous scientists (Fiddler, Wasserman & Doerr, 1970 ; Guillén et al., 2001 ; Shahidi & Naczk, 2003). The “cold smoke” sensory attributes of salmons processed by smoking techniques applying wood pyrolysis in situ seem to be related to high contents of cresols isomers (12,13). These compounds have been identified with burnt/spicy odors in smoked salmon *(22)* and already correlated to earthy, phenolic/medicinal sensory attributes (Cardinal et al., 2006). It is interesting to note that it is the first time that 4-vinylguaiacol (27) is shown to be strongly linked to “wood fire smoke” odor. This compound is normally described by smoke, green/spicy aromatic notes (Varlet et al., 2006), clove-like, phenolic (Swiegers, Bartowsky, Henschke & Pretorius, 2005) or bitumen (Campo, Ferreira, Escudero, Marqués & Cacho, 2006) according to its concentration. Then, in smoked salmon, this compound could interact with other odorant compounds to play a role in the “wood fire smoke” odor. It is also important to note that the “wood fire smoke” sensory attributes are linked to high concentrations of furannic compounds such as furfural (1), 2-acetylfuran (7), 5-methylfurfural (8) and furfuryl alcohol (3) which confirms a previous olfactometric study

Figure 4. Weighted coefficients of odorant volatile compounds concentrations for PLS1 regression in order to predict « cold smoke » odor



Number codes of the compounds are given in Table 3

where these furannic compounds were found in smoked salmon with « smoked, cooked » odorant attributes (Varlet et al., 2007b).

The "wood fire smoke" sensory attributes are related to high contents of phenolic and furannic compounds and enolones derivatives which correspond to quantities recovered in salmons smoked by external generators especially those applying high wood pyrolysis temperature (Nonier et al., 2005) as thermostated plates. The effects of smoking parameters can also explain can be explained the evolution of sensory attributes from "salmon-like" to "wood fire smoke". Indeed, with increase of time of smoking, salmon fillets are subjected to increasing quantities of "smoky" aroma compounds. Concerning the wood smokehouse temperature, it acts on the food matrix leading to physical or biochemical reactions which could lead to an important variety and quantity of aroma compounds. Indeed, the effect of smokehouse temperature to explain the evolution of sensory attributes from "salmon-like" to "wood fire smoke" has been previously shown on salmons processed by smoldering (Cardinal et al., 2001).

3.4. Prediction of "cold smoke" odor by odorant volatile compounds concentrations

The capacity of prediction of a sensory attribute by all the analytical data was tested. Therefore, we have tested our data in order to predict the « cold smoke » odor by odorant volatile compounds concentrations by means of PLS1 regression.

The results have led to a quite good prediction with a R^2 of 0.93. Then, there was a very good correlation between the « cold smoke » odor observed value and the « cold smoke » odor predicted value. The most influent odor-active compounds implied to predict « cold smoke » odor are given in Fig. 4 which represents the weighted coefficients of the odor-active compounds concentrations. The positive coefficients are those of odorant volatile compounds implied in the « cold smoke » odor and the negative coefficients are those of compounds which presence must be avoided to lead to the « cold smoke » odor. Therefore, the absence of furfural and 4-vinylguaiacol which have the coefficients with the most negative values might be required if a « cold smoke » salmon odor is targeted. Conversely, the smoking parameters must be set in order to favorize the generation of phenolic compounds as syringol (28), p-cresol (13) and of 2,3,5-trimethoxytoluene (34), 8-heptadecene (36) and 2-hydroxy-3-ethyl-2-cyclopenten-1-one (17). Pyridine derivatives (2,4) and lipid oxidation products (23,26) seem also implied in the "cold smoke" odor because they present coefficients with highly positive values in order to predict the « cold smoke » odor. 2,3-dimethyl-2-

cyclopenten-1-one (11), guaiacol (14), 2,3,4-trimethyl-2-cyclopenten-1-one (16), 2,3-dimethoxytoluene (21), 1,2,3-trimethoxy-5-methylbenzene (31) and isoeugenol isomers (32,33) do not play a significant role in the prediction of "cold smoke" odor because their coefficients are close to zero. Thus, the prediction of an odorant attribute by analytical data such as odorant volatile compounds concentration is possible and reliable through this type of analysis. Nevertheless, the unexpected role of lipid oxidation products in the "cold smoke" odor shows that the smoky overall odor of a food matrix is not only composed of the addition of individual smoky odors. It is the result of a complex mix of odorant volatile compounds which exhibit their own odor but which can also interact with the matrix components or between themselves.

4. CONCLUSION

The relationships between the smoking parameters (smoking technique, time of smoke exposure and smokehouse temperature) and sensory attributes in one hand and concentrations of odorant volatile compounds in other hand have been carried out by ANOVA and Flash tables. It has permitted to relate the sensory attributes of the different smoked salmons and their composition in odor-active compounds to the evolutions of smoking parameters and the smoke generation technique. Then, PLS2 statistical treatment has allowed to explain the relationships between the sensory attributes and the concentrations of odorant volatile compounds found in the different samples of smoked salmon. Our results have shown the excellent correlation between the perception of flavor and perception of odour. The same compounds are probably responsible of orthonasal and retronasal perception of smoke. The results of this study confirm also the importance of phenolic and furannic compounds in smoked salmon aroma, particularly for "wood fire smoke" odor and flavor in products smoked by thermostated plates, friction or smoldering. Indeed, smoked salmon is perceived as fishy for short times of smoking with little amounts of phenolic and furannic compounds. Deposited in increasing quantities, the aroma of smoked salmons evolves to "wood fire smoke" after 3 hours of smoking. Liquid smoke characterised by « vegetal » and « cold smoke » odors appears very different from the three other smoking techniques described by « wood fire » odors. Pyridine derivatives and lipid oxidation products are responsible for the « vegetal » odor and syringol and cresols are implied in the "cold smoke" sensory attributes. However, the results concerning the products treated by liquid smoke correspond to only one type of smoke condensates. More investigation about the whole

smoke flavorings must be carried out in order to validate our conclusions. This kind of statistical treatment should be more often used to invest the relationships that link the overall odor of a matrix assessed by sensory analysis and its composition in odorant volatile compounds assessed by olfactometry in order to understand the interactions of the odor-active between themselves, with the matrix, and how they contribute to the overall odor. More investigation on hedonic analyses should be carried out in order to identify the favorite sensory attributes of the consumer. The study of consumer preferences for different kind of products, associated to the identification of the odour-active molecules could allow smoked salmon manufacturers to orientate the final overall odorant composition by acting on the parameters of smoking implied in the generation of required odorant volatile compounds. However, it is important to carry out in parallel a sanitary study because modifications of smoking parameters can cause variations in smoking contaminants contents and can affect the growth of microorganisms because a lot of volatile compounds of wood smoke have antimicrobial effects.

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LITERATURE CITED

Cardinal, M., Gunnlaugsdottir, H., Bjoernevik, M., Ouisse, A., Vallet, J.L. & Leroi, F. (2004). Sensory characteristics of cold-smoked Atlantic salmon (*Salmo salar*) from European market and relationships with chemical, physical and microbiological measurements. *Food Research International*, 37, 181-193.

Chambers IV, E. & Robel, A. (1993). Sensory characteristics of selected species of freshwater fish in retail distribution. *Journal of Food Science*, 58(3), 508-512.

Morzel, M., Sheehan, E.M., Delahunty, C.M. & Arendt, E.K. (1999). Sensory evaluation of lightly preserved salmon using free-choice profiling. *International Journal of Food Science and Technology*, 34, 115-123.

Cardinal, M., Knockaert, C., Torrissen, O., Sigugisladottir, S., Mørkøre, T., Thomassen, M. & Vallet, J.L. (2001). Relation of smoking parameters to the yield, colour and sensory quality of smoked Atlantic salmon (*Salmo salar*). *Food Research International*, 34, 537-550.

Girard, B. & Nakai, S. (1991). Static headspace gas chromatographic method for volatiles in canned salmon. *Journal of Food Science*, 56(5), 1271-1274.

Girard, B. & Durance, T. (2000). Headspace volatiles of sokeye and pink salmon as affected by retort process. *Journal of Food Science*, 65(1), 34-39.

Aro, T., Brede, C., Manninen, P. & Kallio, H. (2002). Determination of semivolatile compounds in Baltic herring (*Cuplea harengus membras*) by supercritical fluid extraction – supercritical fluid chromatography – gas chromatography – mass spectrometry. *Journal of Agricultural and Food Chemistry*, 50, 1970-1975.

Josephson, D.B., Lindsay, R.C. & Stuiber, D.A. (1987). Influence of processing on the volatile compounds characterizing the flavor of pickled fish. *Journal of Food Science*, 52(1), 10-14.

Mansur, M.A., Bhadra, A., Takamura, H. & Matoba, T. (2003). Volatile flavor compounds of some seafish and prawn species. *Fisheries Science*, 69, 864-866.

Fuller, G.H., Steltenkamp, G.A. & Tisserand, G.A. (1964). The gas chromatography with human sensor: perfumer model. *Annals N.Y. Academic Science*, 116, 711-724.

Milo, C. & Grosch, W. (1996). Detection of odor defects in boiled cod and trout by gas chromatography-olfactometry of headspace samples. *Journal of Agricultural and Food Chemistry*, 43, 459-462.

Sérot, T., Regost, C., Prost, C., Robin, J. & Arzel, J. (2001). Effect of dietary lipid sources on odour-active compounds in muscle of turbot (*Psetta maxima*). *Journal of Science and Food Agriculture*, 81, 1339-1346.

Sérot, T., Baron, R., Knockaert, C. & Vallet, J.L. (2003). Effect of smoking processes on the contents of 10 major phenolic compounds in smoked fillets of herring (*Clupea harengus*). *Food Chemistry*, 85 (1), 111-120.

Triqui, R. & Reineccius, G.A. (1995). Changes in flavor profiles with ripening of anchovy (*Engraulis encrasicholus*). *Journal of Agricultural and Food Chemistry*, 43, 1883-1889.

Prost, C., Sérot, T. & Demaimay, M. (1998). Identification of the most potent odorants in wild and farmed cooked turbot (*Scophtalamus maximus* L.). *Journal of Agricultural and Food Chemistry*, 46, 3214-3219.

Chung, H.Y., Yeung, C.W., Kim, J.S. & Chen, F. (2007). Static headspace analysis-olfactometry (SHA-O) of odor impact components in salted-dried white herring (*Ilisha elongata*). *Food Chemistry*, 104(2), 842-851.

Guillén, M.D. & Ibargoitia, M.L. (1996). Relationships between the maximum temperature reached in the smoke generation processes from Vitis vinifera L. shoot sawdust and

composition of the aqueous smoke flavoring preparations obtained. *Journal of Agricultural and Food Chemistry*, 44, 1302-1307.

Guillén, M.D., Manzanos, M.J. & Ibargoitia, M.L. (2001). Carbohydrate and nitrogenated compounds in liquid smoke flavorings. *Journal of Agricultural and Food Chemistry*, 49, 2395-2403.

Maga, J.A.(1987). The flavor chemistry of wood smoke. *Food Reviews International*, 3(1-2), 139-183.

Cardinal, M., Berdagué, J.L., Dinel, V., Knockaert, C. & Vallet, J.L.(1997). Effect of various smoking techniques on the nature of volatile compounds and on the sensory characteristics of salmon meat. *Sciences Alimentaires*, 17, 679-696. (In French)

Varlet, V., Knockaert, C., Prost, C. & Sérot, T. (2006). Comparison of odor-active volatile compounds of fresh and smoked salmon. *Journal of Agricultural and Food Chemistry*, 54(9), 3391-3401.

Cardinal, M., Cornet, J., Sérot, T. & Baron, R. (2006). Effects of the smoking process on odour characteristics of smoked herring (*Cuplea harengus*) and relationships with phenolic compound content. *Food Chemistry*, 96, 137-146.

Kostyra, E. & Baryłko-Pikielna, N. (2005). Volatiles composition and flavour profile identity of smoke flavourings. *Food Quality and Preference*, 17(1-2), 85-95.

Refsgaard, H.H.F., Brockhoff, P.B. & Jensen, B. (1998). Sensory and chemical changes in farmed Atlantic salmon (*Salmo salar*) during frozen storage. *Journal of Agricultural and Food Chemistry*, 46, 3473-3479.

Triqui, R. & Zouine, K. (1999). Sensory and instrumental assessments of the ripening process of anchovy (*Engraulis encrasicholus*). *Lebensmittel-Wiss. U.-Technology*, 32, 203-207.

Grigorakis, K., Taylor, K.D.A. & Alexis, M.N. (2003). Organoleptic and volatile aroma compounds comparison of wild and cultured gilthead sea bream (*Sparus aurata*) : sensory differences and possible chemical basis. *Aquaculture*, 225, 109-119.

Morita, K., Kubota, K. & Aishima, T. (2001). Sensory characteristics and volatile components in aromas of boiled prawns prepared according to experimental designs. *Food Research International*, 34, 473-481.

Rosenfeld, H.J., Baardseth, P. & Skrede, G. (1997). Evaluation of carrot varieties for production of deep fried carrot chips – IV. The influence of growing environment on carrot raw material. *Food Research International*, 30(8), 611-618.

Tamine, A.Y., Muir, D.D., Barclay, M.N.I., Khaskheli, M. & McNulty, D. (1997). Laboratory-made Kishk from wheat, oat, and barley : 2. Compositionnal quality and sensory properties. *Food Research International*, 30(5), 319-326.

King, B.M. & Duineveld, C.A.A. (1999). Changes in bitterness as beer ages naturally. *Food Quality and Preference*, 10, 315-324.

Mielnik, M.B., Aaby, K.A., Rolfsen, K., Ellekjaer, M.R. & Nilsson, A. (2002). Quality of comminuted sausages formulated from mechanically deboned poultry meat. *Meat Science*, 61, 73-84.

Varlet, V., Sérot, T. & Prost, C. (2007a). New procedure for the study of odour representativeness of aromatic extracts from smoked salmon. *Food Chemistry*, 100, 820-829.

Varlet, V., Sérot, T., Cardinal, M., Knockaert, C. & Prost, C. (2007b). Olfactometric determination of the potent odor-active compounds in salmon muscle (*Salmo salar*) by using four smoke generation techniques. *Journal of Agricultural and Food Chemistry*, 55, 4518-4525.

Stone, H. & Sidel J. (1998). Quantitative descriptive analysis : Developments, applications and the future. *Food Technology*, 52: 48-52, 1998.

Varlet, V., Sérot, T., Knockaert, C., Cornet, J., Cardinal, M., Monteau, F., Le Bizec, B. & Prost, C. (2007c). Organoleptic characterisation and PAH content of salmon (*Salmo salar*) fillets smoked according to four industrial smoking techniques. *Journal of Science and Food Agriculture*, 87, 847-854.

Knockaert, C. (1990). Le fumage du poisson. *Service de la documentation et des publications*, IFREMER, Brest.

Sérot, T., Baron, R., Knockaert, C. & Vallet, J.L. (2004). Effect of smoking processes on the contents of 10 major phenolic compounds in smoked fillets of herring (*Cuplea harengus*). *Food Chemistry*, 85(1), 111-120.

Chan, W.S. & Toledo, R.T. (1975). Effect of smokehouse temperature, humidity and air flow on smoke penetration into fish muscle. *Journal Food Science*, 40, 240-243.

Schlich, P. (1998). What are the sensory differences among coffees ? Multi-panel analysis of variance and FLASH analysis. *Food Quality Preference*, 9(3), 103-106.

Marchand, S., De Revel, G. & Bertrand, A. (2000). Approaches to wine aroma: release of aroma compounds from reactions between cysteine and carbonyl compounds in wine. *Journal of Agricultural and Food Chemistry*, 48, 4890-4895.

Pripis-Nicolau, L., De Revel, G., Bertrand, A. & Maujean, A. (2000). Formation of flavor components by the reaction of amino acid and carbonyl compounds in mild conditions. *Journal of Agricultural and Food Chemistry*, 48, 3761-3766.

Alén, R., Kuoppala, E. & Oesch, P. (1996). Formation of the main degradation compound groups from wood and its components during pyrolysis. *Journal of Analytical and Applied Pyrolysis*, 36, 137-148.

Nonier M.F., Vivas, N., Vivas de Gaulejac, N., Absalon, C., Soulié, P. & Fouquet, E. (2005). Pyrolysis-gas chromatography/mass spectrometry of *Quercus* sp. wood. Application to structural elucidation of macromolecules and aromatic profiles of different species. *Journal of Analytical and Applied Pyrolysis*, 75(2), 181-193.

Simon, R., De la Calle, B., Palme, S., Meier, D. & Anklam, E. (2005). Composition and analysis of liquid smoke flavouring primary products. *Journal of Separation Science*, 28, 871-882.

Fiddler, W., Wasserman, A.E. & Doerr, R.C. (1970). « Smoke » flavour fraction of a liquid smoke solution. *Journal of Agricultural and Food Chemistry*, 18, 934-936.

Shahidi, F. & Naczk, M. (2003). Contribution of phenolic compounds to the flavor and color characteristics of foods. In : *Phenolics in food and nutraceuticals*, CRC Press Llc (pp 443-481). Boca Raton, Florida, USA.

Swiegers, J.H., Bartowshy, E.J., Henschke, P.A. & Pretorius, I.S. (2005). Yeast and Bacterial Modulation of Wine Aroma and Flavour: Part 4. The Australian Journal of Grape and Wine Research, 11, 139-173.

Campo, E., Ferreira, V., Escudero, A., Marqués, J.C. & Cacho, J. (2006). Quantitative gas chromatography-olfactometry and chemical quantitative study of the aroma of four Madeira wines. Analytica Chimica Acta, 563 (1-2), 180-187.

Bilan

L'hypothèse formulée sur les différences des caractéristiques sensorielles engendrées par des teneurs et des compositions de composés volatils odorants différentes au cours des procédés de fumage ou au cours du process d'obtention de la fumée liquide vaporisée ultérieurement sur les filets a été confirmée. Les analyses olfactométriques ont montré la distinction entre les produits fumés par générateur externe de ceux traités par fumée liquide, en ce qui concerne leur composition en composés volatils odorants (nature et/ou concentrations).

En effet, les produits fumés par générateur externe présentaient des concentrations en composés volatils odorants furaniques, phénoliques et dérivés d'enolones importantes qui augmentaient graduellement avec la température de pyrolyse atteinte par le générateur de fumée et les paramètres du fumage (temps de fumage, température du fumoir). Ces trois catégories de composés volatils odorants sont connues pour provenir de la fumée. La température de pyrolyse semble être le paramètre le plus important permettant d'expliquer la différence de composition chimique des fumées générées avec les différents générateurs, et donc d'expliquer les différences de composés volatils odorants observées entre les filets de saumon fumé. Les saumons fumés avec la fumée produite à la plus faible température de pyrolyse (friction, 380°C) présentent des concentrations en composés volatils odorants carbonylés importantes et en composés phénoliques et furaniques moindres par rapport à celles obtenues avec de fortes températures de pyrolyse. Des travaux antérieurs avaient déjà montré une plus grande quantité de composés carbonylés dans la phase vapeur de la fumée obtenue par friction par rapport à l'autocombustion (Husaini, S.A. & Cooper, G.E., 1957). Au fur et à mesure que la température de pyrolyse augmente (450°C en autocombustion jusqu'à 500°C pour les plaques thermostatées), les saumons fumés présentent des quantités de composés phénoliques, furaniques très importantes et des quantités de composés carbonylés relativement moindres. Les paramètres du fumage (temps d'exposition à la fumée et température du fumoir) peuvent également jouer un rôle important dans la dégradation des constituants de la matrice, notamment des lipides, et par conséquent dans la génération de composés carbonylés aliphatiques. Pour de faibles temps d'exposition à la fumée et de faibles températures de fumoir, les saumons présentent des quantités de composés carbonylés aliphatiques les plus importantes. Cependant, avec l'augmentation des paramètres du fumage, le dépôt des composés volatils odorants de la fumée est plus important que la dégradation des lipides éventuelle et les odeurs des composés volatils odorants de la fumée masquent celles des composés carbonylés aliphatiques.

Les filets de saumon traités par fumée liquide présentaient des teneurs et des compositions de composés volatils odorants différentes des filets fumés par les autres méthodes de fumage. Ainsi, nous avons pu noter la présence de dérivés de pyridine et de produits d'oxydation lipidique. Ces composés ne sont pas présents dans les saumons fumés par générateurs externes et semblent donc provenir de la fumée liquide elle-même. Concernant les composés phénoliques, les concentrations sont généralement moins importantes que dans les saumons fumés par friction, autocombustion ou plaques thermostatées sauf pour le syringol et les isomères de crésol.

Au cours d'une deuxième phase de l'étude, nous avons tenté d'établir une relation entre ces compositions en composés volatils odorants déterminés par GC-MS/O et les caractéristiques odorantes définies par analyse sensorielle. Pour cela, nous avons utilisé un traitement statistique de régression PLS2. Cette étude a donc non seulement permis d'établir le rôle des paramètres du fumage (temps d'exposition à la fumée et température du fumoir) dans l'évolution de l'odeur globale et des concentrations en composés volatils mais également de relier les concentrations en composés volatils odorants avec les odeurs globales. Ainsi, aux paramètres de fumage les plus bas, les saumons fumés par friction, autocombustion ou plaques thermostatées présentaient de faibles concentrations en composés volatils odorants de la fumée qui ne masquaient pas les caractéristiques odorantes de poisson (odeurs de saumon, odeurs grasses, odeurs de hareng) plutôt causées par les composés carbonylés du poisson. Avec l'augmentation de la température du fumoir et du temps d'exposition à la fumée, les saumons fumés par friction, autocombustion et plaques thermostatées présentaient des quantités de composés phénoliques et furaniques plus importantes et étaient caractérisés par des odeurs de « fumée feu de bois ». Ce résultat est en adéquation avec les odeurs individuelles des composés phénoliques identifiés dans le saumon fumé avec des descripteurs « fumés » voire « brûlés » et celles des composés furaniques détectés avec des odeurs « cuites ». De même, pour les paramètres de fumage les plus bas (1 heure, 22°C), les saumons traités par fumée liquide étaient caractérisés en olfactométrie par une perception plus importante des produits d'oxydation lipidique et de dérivés de pyridine détectés avec des odeurs « vertes » et « chimiques ». Ces caractéristiques odorantes sont également caractéristiques de l'odeur végétale avec lesquelles ont été décrits ces mêmes saumons en analyse sensorielle. Avec l'augmentation des paramètres de fumage, les saumons traités par fumée liquide présentaient des concentrations en syringol et p-crésol plus importantes et étaient caractérisés par des odeurs de fumée froide. Ainsi, l'implication du syringol et du p-crésol dans l'odeur de fumée froide est en adéquation avec les odeurs individuelles de ces composés perçus en olfactométrie avec des descripteurs « brûlé » ou « caoutchouc brûlé ».

Nous avons donc rempli les deux objectifs de cette étude puisque d'une part nous avons identifié les composés volatils odorants responsables de la perception de l'odeur de poisson fumé et que nous avons caractérisé leurs contributions dans l'odeur globale. D'autre part, grâce à cette étude, nous pouvons également fournir des indications aux industriels sur le rôle des paramètres de fumage et des générateurs de fumée pour la production de produits fumés aux odeurs requises.
Cependant, ces informations sont à pondérer par le fait que d'autres facteurs que ceux étudiés tels que la nature, la granulométrie ou l'humidité du bois employé peuvent également intervenir. Pour des questions de temps, nous avons décidé de ne pas approfondir le rôle de ces autres facteurs c'est pourquoi nous nous étions placés dans des conditions de pratiques industrielles en utilisant le même matériel (bois, granulométrie, …) que celui employé dans l'industrie pour produire des saumons de qualité commerciale. Nos résultats sont donc uniquement valables dans le cas d'un fumage de saumon à froid avec du hêtre, mais ce, quel que soit le générateur externe utilisé. Concernant nos résultats sur le saumon traité par fumée liquide, nous n'avons caractérisé qu'un seul type de condensats de fumée. Des travaux supplémentaires seraient donc à effectuer sur le rôle d'autres types de condensats ainsi que le rôle du bois (granulométrie, humidité), de l'air (vitesse sur le foyer, humidité) et sur d'autres aliments (autres poissons, produits carnés). Cette étude constitue néanmoins un des premiers travaux permettant d'expliquer les caractéristiques odorantes de saumons fumés par leurs compositions en composés volatils odorants.

PARTIE 4 : Evaluation du rôle des composés volatils odorants dans la perception de l'odeur de fumée du poisson fumé

Partie 4 : Evaluation du rôle des composés volatils odorants dans la perception de l'odeur de fumée du poisson fumé

Dans les travaux présentés dans la partie 3, nous avons pu montrer qu'il existait bien une relation entre les concentrations de certains composés volatils odorants et l'odeur globale des saumons fumés décrite par analyse sensorielle. Cependant, une odeur ne peut se résumer à l'addition des odeurs individuelles des composés volatils odorants dans laquelle ils sont impliqués. En effet, ces composés peuvent avoir un rôle dans la perception odorante générale de par leur odeur individuelle mais également par les modifications de perception qu'ils peuvent entraîner en mélange avec d'autres composés volatils, odorants ou non, ou encore avec les constituants de la matrice. Il faut donc distinguer les interactions odorantes de masque ou synergiques entre les composés volatils odorants et les interactions physico-chimiques entre les composés volatils odorants et les constituants de la matrice de redéposition. En effet, nous avons pu avoir une idée de l'importance des interactions entre les composés volatils odorants et la matrice lors des tests de représentativité. La redéposition des extraits sur matrice avait apporté un gain de représentativité de 10 % par rapport à une redéposition sur mouillettes de carton. Ainsi, pour connaître l'influence aussi bien de l'odeur individuelle d'un composé volatil odorant mais également de son effet sur la perception odorante, nous avons mis en place une stratégie d'omission sélective des composés volatils odorants puis de remise en mélange en sortie de colonne du chromatographe. Pour cela, nous avions déjà développé un prototype au laboratoire : le GC-GOOD (Gas Chromatography- Global Odorant Omission Detection) (Hallier, A. et al., 2004). Le prototype GC-GOOD permettait la sélection manuelle de composés volatils en sortie de colonne GC puis la récupération des composés à l'état gazeux dans un sac ou une seringue (Selli, S. et al., 2006). Pour cela, l'effluent gazeux est divisé en deux par un diviseur d'éluat. Une partie de l'effluent est donc dirigée vers un détecteur FID ce qui permet la visualisation simultanée de l'élution tandis que l'autre partie de l'effluent est dirigée sur une vanne trois-voies qui, par sa commutation, permet soit l'élimination soit la récupération des composés volatils. Cependant, ce système comportait certains inconvénients qu'il a fallu résoudre. En effet, comme nous voulons prendre en compte l'effet matrice, il faut soustraire les composés sélectionnés de l'odeur globale et récupérer le reste de l'extrait à l'état liquide pour le redéposer sur une matrice réelle non fumée afin de rétablir les éventuelles interactions entre les autres composés volatils odorants et les constituants de la matrice d'étude. De plus, la commutation de la vanne trois-voies nécessite la présence constante d'un opérateur. Ainsi, le système de sélection chromatographique et la récupération à l'état liquide de l'effluent gazeux constituaient nos points

critiques. Pour les résoudre, nous avons développé le système GC-COOL (Gas Chromatography-Concentration Omission of Odorants at Liquid state). La sélection chromatographique n'est plus manuelle mais automatisée et gérée depuis l'ordinateur d'acquisition des signaux FID par un logiciel informatique que nous avons développé. Puis la récupération à l'état liquide de l'effluent gazeux se fait par une étape de cryocondensation des composés dans un piège porté à températures négatives (< 0°C), suivie d'une étape d'élution du piège par un solvant neutre.

Pour évaluer le rôle des composés volatils odorants dans la perception de l'odeur de fumée, un développement méthodologique a été mis en place pour valider d'une part, l'efficacité de la récupération (expérimentations menées sur standards), et d'autre part, la représentativité odorante d'extraits aromatiques (expérimentations menées sur fumée liquide) récupérés par ce système.

Puis, une fois le prototype GC-COOL caractérisé, nous avons conçu une stratégie d'omission pour déterminer la contribution des différents composés volatils odorants de la fumée liquide dans l'odeur globale de fumée. Cependant, nous ne pouvions pas réaliser toutes les combinaisons d'omissions individuelles étant donné le grand nombre de composés odorants initiaux. Parmi les 27 composés volatils odorants retrouvés dans le saumon fumé traité par fumée liquide, seuls 17 ont été identifiés dans la fumée liquide. Par conséquent, 10 composés ont été générés par des réactions entre les constituants de la chair du saumon, ou avec la fumée liquide sous les conditions du fumage. Nous nous sommes donc intéressés à l'influence des 17 composés volatils de la fumée liquide sur les caractéristiques odorantes globales du saumon traité par fumée liquide. Le nombre de combinaisons totales pour étudier l'influence de chaque composé seul ou en mélanges est donné par la formule suivante :

$$\sum_{p=0}^{n} C_n^p = \frac{n\,!}{p\,!\,(n-p)\,!}$$

Dans le cas de 17 composés volatils odorants (n = 17), cela correspond à 131 070 combinaisons possibles. Nous avons donc fait le choix de regrouper les composés volatils odorants de la fumée liquide en familles sur des critères de structure chimique, d'odeurs et de temps de rétention ainsi que selon la capacité du système GC-COOL à les individualiser chromatographiquement étant donné la complexité du chromatogramme de l'extrait aromatique de fumée liquide. Nous avons donc réalisé 6 familles constituées de 4 à 6 composés volatils odorants. Ainsi, nous avons pu étudier l'influence des omissions individuelles de ces 6 familles sur les caractéristiques odorantes globales du saumon fumé par atomisation de fumée liquide. Les éventuels effets de ces

omissions seront donc directement imputables aux odeurs individuelles des composés présents dans chaque famille ainsi que de la perception odorante résultant de leur mélange. Cependant, nous n'avons pas accès à l'interaction odorante entre deux composés d'une même famille deux à deux par exemple, interaction qui peut se révéler très différente de l'interaction odorante globale. Ainsi, nous avons pu établir les effets des omissions de certaines familles de composés notamment les composés furaniques et carbonylés. Puis nous nous sommes intéressés à l'identification du rôle individuel dans l'odeur globale de certains composés volatils odorants clés très étudiés tels que les crésols, le guaiacol et le syringol. Les résultats de l'étude PLS2 présentés en partie 3 montrent bien que ces trois composés semblent jouer un rôle important dans les caractéristiques de l'odeur globale.

Ainsi, la présentation et l'évaluation de l'efficacité du système GC-COOL ainsi que l'évaluation du rôle des composés volatils odorants dans la perception de l'odeur de fumée a été l'objet d'une publication en cours de rédaction prévue pour être soumise dans *Journal of Chromatography A*.

INNOVATIVE GAS CHROMATOGRAPHY-OLFACTOMETRIC METHOD : GAS CHROMATOGRAPHY – CONCENTRATION / OMISSION OF ODORANTS AT LIQUID STATE (GC-COOL). CONTRIBUTION OF ODORANT VOLATILE COMPOUNDS OF LIQUID SMOKE IN THE OVERALL SMOKE ODOUR OF SMOKED SALMON.

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Abstract :

A new gas chromatography-olfactometric method (GC-COOL : Gas Chromatography-Concentration/Omission of Odorants at Liquid state) has been optimised in order to study the role of the most potent odorant volatile compounds in the odour of smoked salmon treated by liquid smoke. This method allows to carry out a preliminar chromatographic sorting of volatile compounds and to concentrate or to omit one or several of them after their elution of the capillary column of the gas chromatograph. The selected compounds are then condensed in a cold trap and the elution of the trap by ethanol allowed to recover the selected compounds at liquid state. GC-COOL device was applied to investigate the role of odorant volatile compounds of liquid smoke in the overall smoke odour of salmon treated by liquid smoke. The GC-COOL extracts recovered in ethanol were redeposited on unsmoked salmon in order to evaluate the effect of the omission of a potent odorant on the overall smoked salmon aroma. Thus, this method has allowed to identify the odorant contributions of several groups of odour-active compounds. Thanks to GC-COOL, we have shown that furannic and phenolic compounds influenced the overall odour due to their individual odour but also by odorant interactions with other components. The role of guaiacol, syringol and cresol isomers was particularly studied and odorant interactions not only implying smoky odours were put in evidence by similarity and odorant intensity tests.

INTRODUCTION

Smoked food consumption is more and more increasing especially for the typical smoky organoleptic chracteristics that this food process confers to the food. Smoke flavourings as liquid smoke in smoking processes are more and more used because they offer better repeatability of products quality and provides better controled contaminants levels as polycyclic aromatic hydrocarbons (PAH) (Hattula et al., 2001). Therefore, the use of smoke flavourings appears as a suitable alternative to the traditional smoking. However, products treated by liquid smoke were commonly perceived with « cold smoke » or « vegetal » aromatic notes (Cardinal et al., 1997). These characteristics show that the liquid smoke process is not enough optimised in order to have a similar organoleptic quality of traditionally smoked products perceived with a "wood fire smoke" odour. Therefore, to improve the control of smoking process by atomisation of liquid smoke, it is necessary to identify the role of odorant volatile compounds on the overall odour of smoked products (Varlet et al., 2006 and 2007a). A previous study has aimed to investigate the relationships between the odorant volatile compounds concentration and the sensory characteristics of smoked salmon (Varlet et al., 2007d). Several compounds seemed particularly implied in certain odours of smoked salmon but the model could not take into account odorant interactions between the odorant volatile compounds themselves or/and other components of the matrix. Moreover, the knowledge about the compounds responsible for the flavor of liquid smoke and products treated by liquid smoke is poor. In 1970-1980's, scientists were agree on the flavor properties of phenol fractions distilled from liquid smoke : the low-boiling fraction (60-90°C) composed primarily of phenol, cresols, guaiacol, methylguaiacol and ethylguaiacol had a hot and bitter taste ; the medium fraction that distilled over at 91-132 °C and contained (Z)- and (E)-isoeugenol, syringol and methylsyringol had a pure and characteristic smoke flavor ; the high-boiling phenol fraction (133-200°C) had an acid, chemical property that was judged of poor quality (Maga, 1987). Since these works, no more studies were carried out about odorant compounds of liquid smoke except hypotheses about the carbonyles role in smoke flavor (Olsen, 1976). Besides, these conclusions were recently criticized especially about the odorant role of syringol and derivatives and the tasty role of guaiacol (Kostyra et al., 2005). As smoke flavourings are going to be more and more used, the improvement of knowledge about liquid smoke aroma compounds and their contribution to the overall odour of a product treated with liquid smoke is necessary.

The most potent odorant volatile compounds of smoked salmon and salmon treated by liquid smoke have been already identified by gas chromatography coupled to olfactometry (GC/O)

(Varlet et al., 2007a). Gas chromatography coupled to olfactometry is a good tool to characterize aromatic extracts and identify odorant volatile compounds. Olfactometry allows to determine the individual odours which could be responsible for the final overall odour of a matrix. However, the final odour is not only constituted by the sum of individual odours. Indeed, mask or synergic effects can occur between the odorant volatile compounds or with the matrix components Gómez-Míguez et al., 2007). Therefore, after having identified the odorant volatile compounds by GC/O, it is essential to know how they act in the overall aroma. As result, to know the influence of certain odorant volatile compounds on the overall odour, the strategy is to omit them from the rest of the extract. A previous strategy was developed in our laboratory and allows to recover at gaseous state the sorted extract. The Gas Chromatography-Global Omission Odorant Detection (GC-GOOD) approach has allowed to understand the role of certain compounds on the overall odour of fish *Silurus glanis* (Hallier et al., 2004). However, the recovery of the sorted extract was at gaseous state and was not suitable for us. Indeed, we aim to take into account the physical/chemical interactions between the odorant volatile compounds and the matrix by a redeposition of the extract on a solid matrix. Indeed, the GC-GOOD extracts were recovered in PFTE bags which were not very useful for storage (Mehinagic et al., 2003). An alternative to PFTE bags was glass gas syringes (Rannou et al., 2005). It allows a better storage but it has not resolved the problems caused by the gaseous state of the aromatic sorted extracts (Selli et al., 2006). Moreover, only few judges could assess one extract due to its gaseous state and the extract can not be re-used after its sensory evaluation. Finally, the GC-GOOD device requires the presence of an operator due to the manual action of the switch. Then, the strategy developed in this study has integrated the GC-GOOD theory and has improved it by an automatisation of the omission of the compounds and a cryocondensation of the compounds which allows a recovery at liquid state after an elution of the trap instead of at gaseous state. The improved GC-GOOD was then named Gas Chromatography-Concentration Omission of Odorants at Liquid state (GC-COOL).

The objectives of this paper were firstly to present the GC-COOL device and potentialities.

The efficiency of the GC-COOL system has to be validated from an analytical point of view because it must be checked that the method used is quantitative and not selective towards certain compounds. This analytical validation was performed on aromatic standards solutions commonly found in smoked products. Secondly, the odorant characteristics of the recovered GC-COOL extracts were compared to the initial aromatic extract in order to assess the eventual odorant defects generated by the device.

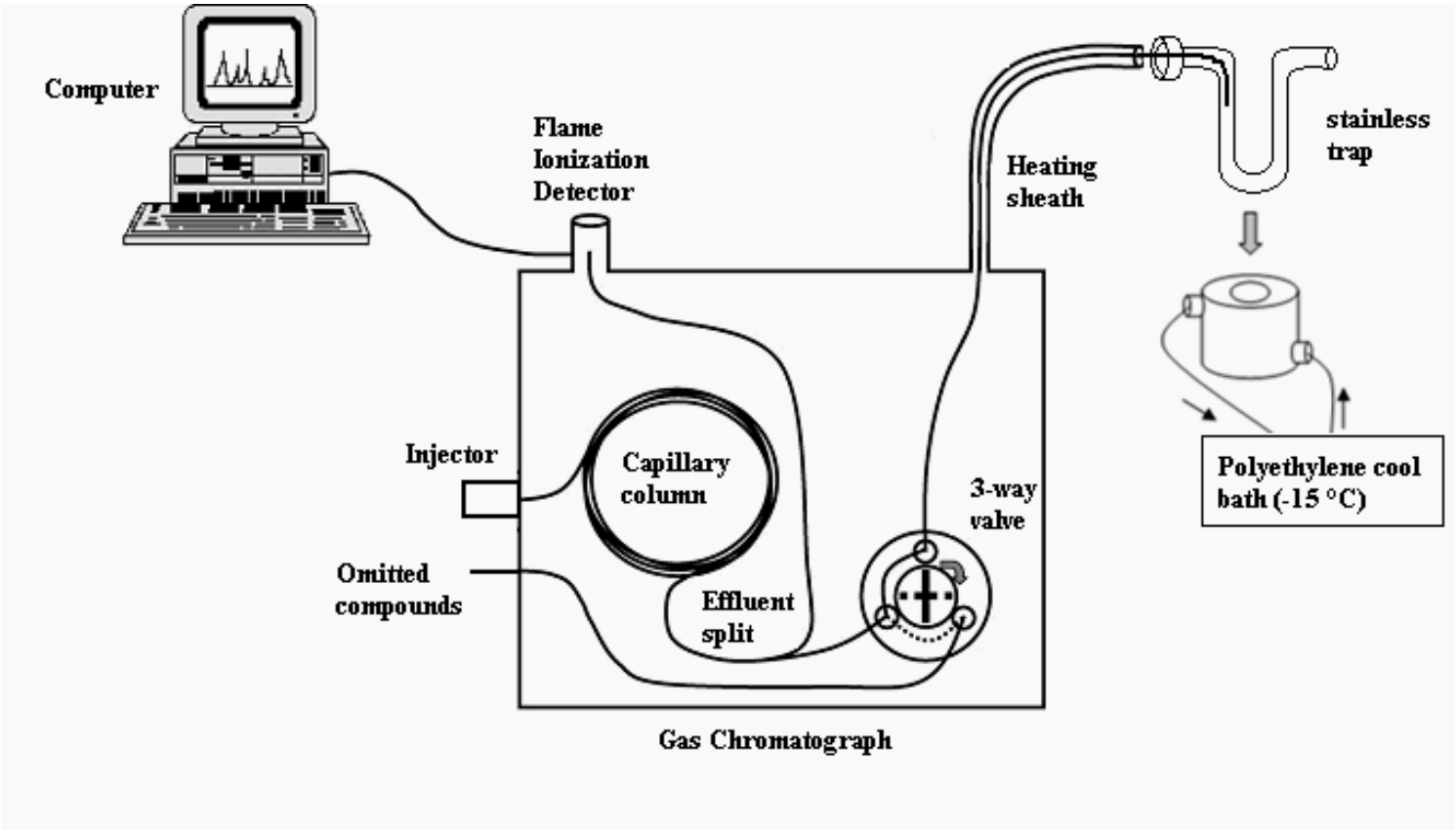


Fig. 1. Diagram of the GC-COOL system

Finally, the GC-COOL strategy was applied to the liquid smoke in order to identify the compounds or groups of compounds responsible for the typical smoke odours of salmon treated by liquid smoke.

MATERIAL AND METHODS

1. Chemical and reagents

The following solvents were used: diethyl ether (purity: 99.5%) from Panreac (Barcelona, Spain), ethanol (purity: 95%) from VWR (Fontenay-sous-bois, France). All water was purified by a MilliQ_ system (Millipore, France).

2. GC-COOL Method

2.1. GC-COOL system

The development of the system can be divided in two steps: the chromatographic omission of volatiles and the recovery of the selected cold trapped volatiles. The GC-COOL device must be able to select one or several compounds in a chromatogram and recover the selected compounds. The device was installed on a gas chromatograph (Varian Star 3900, Palo Alto, CA, USA).

2.1.1. Volatile compounds omission system

The chromatographic omission of volatile compounds was performed by an automatised version of the GC-GOOD system already developed in our laboratory (Hallier et al., 2004). The GC-GOOD system allowed to remove one or several volatile compound from the global odour of the aromatic extract. The GC effluent was split 1:1 between the FID and a three-way valve (Fig. 1). This valve enabled the volatile compounds to be directed to a collector or to be omitted. The control of the omission was assumed by the FID bound to a computer which permitted the simultaneous visualization of the chromatography. In the previous GC-GOOD system, the three-way valve was manually switched according to the chromatographic signal. Then, a software has been optimised by our laboratory to enable the three-way valve to be automatically switched. It allows more reliable omissions, a better repeatability and does not need the presence of an operator during the elution. The software permits the omission of compounds as more as

required and can be coupled with an autosampler. The GC effluent of the omitted compounds was then directed in the oven (bin) whereas the selected compounds were directed to a collector. To prevent the condensation of the volatile compounds between the oven and the collector, a heating transfer line set between 180 and 200°C was installed.

2.1.2. Recovery of the selected volatiles: collection system

The collector was constituted by a stainless tube, screwed with the transfer line. This tube was plunged in a cold bath of ethanol maintained between -10 and -15°C thanks the circulation of polyethylene glycol in an iron ring (Fig. 1). At the end of the collection, the tube was therefore unscrewed and eluted by 200 µL of ethanol at ambient temperature (between 20 and 25 °C). Then, the condensed volatile compounds were obtained at liquid state in ethanol.

2.2. Analytical validation of GC-COOL

2.2.1. Standard solutions

Standard flavour compounds were obtained from Sigma Aldrich (Steinheim, Germany) (Table 1). These standards were odorant volatile compounds belonging to the four main family of chemical structure of the molecules commonly found in liquid smoke that is to say phenolic compounds, enolones derivatives, aldehydes and furannic compounds (Kostyra et al., 2005 ; Nonier et al., 2005). We have performed solutions of these standards at 1 mg/mL in order to assess the efficiency of the device.

2.2.2. GC analysis

Aromatic extracts were introduced in the gas chromatograph in a split/splitless injector heated at 270°C. The volatile compounds were separated on a DB5-MS capillary column (J&W Scientific, Folsom, CA, USA) 30 m × 0.32 mm, 0.5 µm thickness. The detector used was a flame ionization detector heated at 280°C. The oven temperature of the oven was programmed according to the following steps: from 70°C to 85°C (1 min) at 5°C.min^{-1}, then to 165°C at 3°C.min^{-1} and, finally, to 280°C (3 min) at 10°C.min^{-1}.

2.3. Sensory validation of GC-COOL

2.3.1. Raw material

Salmons (*Salmo salar*), reared in Norway, were purchased from seafood wholesaler. The liquid smoke used came from a national manufacturer (France).

2.3.2. Isolation of volatiles

A Likens-Nickerson apparatus was used for the preparation of the SDE liquid smoke extracts (Varlet et al., 2007b). This extraction method led to a recovery yield of 75 % and a similarity mark of aromatic extract of 72 % by comparison with the initial matrix. A 500 mL round-bottomed flask was used as the sample flask to contain: 150 mL of purified water, 100 µg of dodecane (internal standard) and 30 mL of liquid smoke. A 30 mL round-bottomed flask containing 30 mL of diethyl ether was linked to the upper arm of the SDE apparatus because the density of diethyl ether is lower than the density of water. The steam was cooled thanks to the circulation of polyethylene glycol at – 5°C. The contents in the sample and solvent flasks were heated to boiling. The temperature of the diethyl ether flask was maintained at 50°C by a water bath. The distillation-extraction was continued for 3 hr. The volume of the extract was reduced to 5 mL by evaporating the solvent using a Kuderna Danish apparatus and to 1 mL under a gentle cold stream of nitrogen.
The SDE liquid smoke extracts recovered in the GC-COOL trap in 200 µL of ethanol after their elution in the gas chromatograph were named GC-COOL extracts.

2.3.3. Sensory analysis of the GC-COOL extracts

The panel was composed of twelve judges (males and females, between 23 and 50 years old) which were trained in smoke odour recognition and more specifically in liquid smoke odour. The samples were 3 g cubes of salmon on which liquid smoke, SDE liquid smoke extracts or GC-COOL aromatic extracts were redeposited by gently sprinkling them on the cubes, respecting the iso-intensity conditions, that is to say, in the same concentrations than those found in fishes treated by liquid smoke (Varlet et al., 2007a). They were left at rpoom temperature during one hour before the sensory analysis sessions. These sessions took place in a sensory room, in separated booths, under natural light and in room temperature of 20°C.

2.3.4. Generation of descriptors

The preliminary training sessions have allowed to generate the main descriptors of fishes treated by liquid smoke. The panellists had to comment on the aroma characteristics using their own odorant attributes and among a list of 19 odorant attributes already generated in other works dealing with smoke flavourings and phenolic compounds odours (Ojeda et al., 2002 ; Kostyra et al., 2006). The eight attributes the most frequently cited were retained for the descriptive and similarity tests. They were: burnt, sharp, fatty (butter), salmon-like, herring-like, wood fire smoke, cold smoke (soot), spicy/aromatic herb.

2.3.5. Evaluation of the GC-COOL device influence on the odorant perception of the GC-COOL extracts

To assess that the GC-COOL device allows to obtain an extract with the same odorant characteristics than the extract injected in the GC-COOL, we have compared the aroma profiles of: SDE aromatic extract of liquid smoke and the GC-COOL extract corresponding to the SDE extract injected after its elution in the GC-COOL. These two kinds of aromatic extracts were redeposited on cubes of unsmoked salmon in iso-intensity conditions that is to say in the same quantities than those found in fishes treated by liquid smoke. The representativeness of the SDE aromatic extracts of smoked salmon obtained by the extraction method has been already published (Varlet et al., 2007b). SDE aromatic extracts were found to be similar at more than 70 % of the initial smoked salmon matrix. Following this methodology, the odorant characteristics of SDE liquid smoke aromatic extract and of GC-COOL extract were also compared to those of unsmoked salmon on which liquid smoke was redeposited in iso-intensity conditions. Therefore, the aroma profiles of three references were built: the smoked salmon reference (3 g of salmon + 23 µL of liquid smoke + 200 µL of ethanol), the SDE reference (3 g of salmon + 1 µL of SDE liquid smoke extract + 200 µL of ethanol) and the GC-COOL reference (3 g of salmon + 5 × 2 µL of SDE liquid smoke extract + 200 µL of ethanol of the eluted trap without omission of compounds). The intensity of each descriptor was assessed by giving a mark on an unstructured scale from « 0 » to « 10 ». The average of the different marks was used to carry out the aroma profiles of the references.

3. Application of the GC-COOL device for the identification of the contribution of odorant volatile compounds of liquid smoke in the smoke odour of salmon treated by liquid smoke

3.1. Omission families of compounds

Among the 27 odorant volatile compounds found in salmon treated by liquid smoke (Varlet et al., 2007a), 17 were identified in aromatic extract of liquid smoke, which means that 10 compounds were generated during the process between the fish flesh and the liquid smoke. We have chosen to study the influence of the 17 odorant volatile compounds of liquid smoke on the overall odour of smoked salmon. Therefore, these compounds were ranked in six families of compounds (from Sol 1 to Sol 6) whose omission has been planned in order to know their influence on smoked salmon odour. They were presented in Table 2. The omission families were carried out taking into account the chemical structure and the retention time of odorant volatile compounds. In the family Sol 1, the furannic compounds have been omitted from the global extract. In the family Sol 2, the enolones derivatives have been taken out. In the family Sol 6, the alkyls aryls ethers and alkans were omitted. Finally, all the phenolic compounds have been sorted in three families, from Sol 3 to Sol 5, according to their retention time and the ability of the GC-COOL device to select the chromatographic aera because the chromatogram of liquid smoke SDE extract was complex. Sol 7 corresponds to GC-COOL extract in which none of compounds was omitted. Therefore, Sol 7 constitutes a GC-COOL reference.

3.2. Similarity tests for the omission strategy

In similarity tests, the GC-COOL extracts were presented to the judges who were asked to assess the proximity of the aromatic extract to the reference (Sol 7) by noting the extract on an unstructured 100 mm scale, anchored at the left end with « odour far from the reference » and at the right end with « odour identical to the reference ». There was only one scale per odorant attribute. GC-COOL reference and GC-COOL extracts (GC-COOL reference minus the groups of odorant compounds omitted) similarities were assessed after the redeposition of the extracts on 3 g cubes of salmon.

3.3. Odorant intensity tests for the omission strategy

In odorant intensity tests, the samples were presented to the judges and they had to assess the proximity of the aromatic extract to the reference (GC-COOL reference) by noting the extract on an unstructured 100 mm scale. The reference was placed in the middle of the scale which was

Table 1. Recovery yields of standards compounds with the GC-COOL device

Chemical structure of compounds	Standard compounds	Molecular weight	Recovery yield (%) (Mean of three injections)	Standard deviation (%)
Enolones	2-methylcyclopentenolone	96.13	24	14
Furannic compounds	furfuryl alcohol	98.10	31	4
	2-acetylfuran	110.11	22	7
	5-methylfurfural	110.11	25	3
Phenolic compounds	phenol	94.11	21	9
	o-crésol	108.14	26	10
	m-crésol	108.14	23	5
	guaiacol	124.14	25	11
	4-methylguaiacol	138.16	22	4
	4-ethylguaiacol	152.19	18	2
	syringol	154.16	32	7
	eugénol	164.20	19	2
	4-propylguaiacol	166.22	19	2
	isoeugénol	164.20	23	4
Aldehydes	decadienal	152.23	28	7

Table 2. Composition of the odorant volatile compounds families in the omission strategy

Name of the family	Composition	Odorant compounds omitted
Sol 1	(Global extract) – (furannic compounds)	Furfural, furfuryl alcohol, 2-acetylfuran, 5-methylfurfural
Sol 2	(Global extract) – (enolones derivatives)	2-hydroxy-3-methyl-2-cyclopenten-1-one, 2,3-dimethyl-2-cyclopenten-1-one, 2,3,4-trimethyl-2-cyclopenten-1-one, 2-hydroxy-3-ethyl-2-cyclopenten-1-one
Sol 3	(Global extract) – (phenolic compounds)	Phenol, o-cresol, p-cresol, guaiacol, 2,4 and 3,5-dimethylphenols
Sol 4	(Global extract) – (phenolic compounds)	4-methylguaiacol, 4-ethylguaiacol, 4-vinylguaiacol, 4-allylsyringol
Sol 5	(Global extract) – (phenolic compounds)	Syringol, eugenol, 4-propylguaiacol, (E) and (Z)-isoeugenols
Sol 6	(Global extract) – (hydrocarbons)	2,3-dimethoxytoluene, indanone, 1,2,3-trimethoxy-5-methylbenzene, 2,3,5-trimethoxytoluene
Sol 7	Global extract	None of compounds was omitted

anchored at the left end with « lower intensity than the reference » and at the right end with « higher intensity than the reference ». There was only one scale per odorant attribute.

3.4. Statistical treatments

In order to assess significant differences between the scores of the judges, one-way analysis of variances (ANOVA) were carried out. ANOVA analyses were performed using Statgraphics Plus 5.1 software (Sigma Plus, Paris, France).

RESULTS AND DISCUSSION

1. Efficiency of the GC-COOL system

1.1. Assessment of recovery yields : analytical validation

Table 1 shows the average recovery yields of the 15 standards used. For each injection, according to the compound, from 18 % to 32 % of the quantity injected is recovered at liquid state after the elution of the trap by 200 µL of ethanol. The recovery seems to be relatively independant of the nature and the molecular weight of the compounds and leads to a mean recovery of 23 % whatever the compounds. However, it can be said that in each chemical structure class of compounds, the best recovery yields are reached for the lightest compounds but with important standard deviations.

The important standard deviations are the consequence of the high volatility of the lightest compounds. Indeed, the trapping of the most volatile compounds is difficult because it requires more negative temperatures in the trap. However, the difference of temperature between the trapping step and the elution step causes an aerosol effect which is more and more important when the difference of temperature increases. Then, working at very low temperatures increases the quantity of condensed compounds in the trap but increases also the risk of evaporation as soon as the trap will be brought at ambient temperature. Moreover, the manual elution of the trap is a critical point for the recovery of this type of compounds. The elution must be done with ethanol at room temperature in order to solubilize the heaviest condensed compounds but it favorizes also the volatility of the lightest compounds hence the important standard deviations.

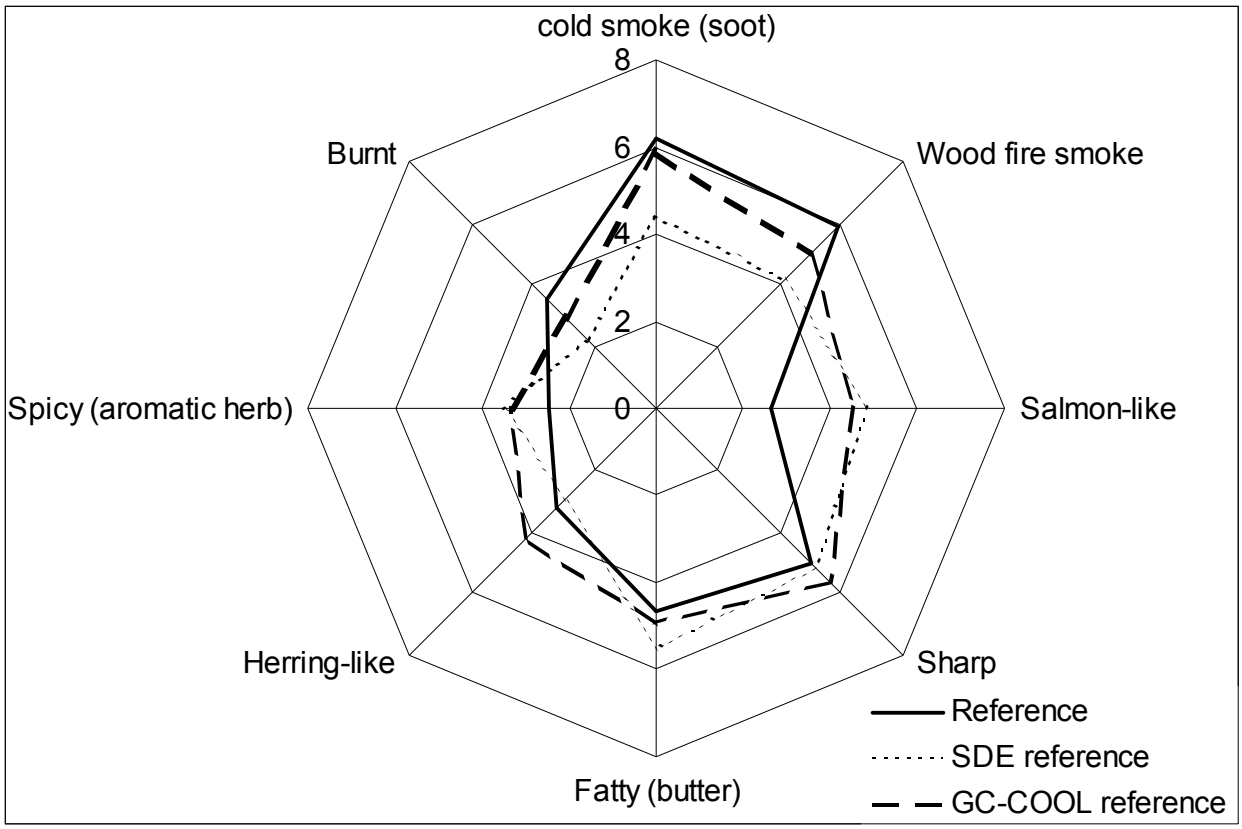


Figure. 2. Aroma profiles in iso-intensity conditions of smoked salmon references. Reference : 3 g of salmon + 23 µL of liquid smoke + 200 µL of ethanol, SDE reference : 3 g of salmon + 1 µL of SDE liquid smoke extract + 200 µL of ethanol, GC-COOL reference : 3 g of salmon + 5 × 2 µL of SDE liquid smoke extract + 200 µL of ethanol of the eluted trap.

1.2. Assessment of representativeness of the injected extracts: odorant validation of SDE extraction and GC-COOL collection

Fig. 2 shows the aroma profiles of the three references resulting from the descriptive tests. The three references have a similar aroma profile and similar intensity marks whatever the odorant attribute. The odorant volatile compounds recovered in the trap at liquid state and redeposited in iso-intensity conditions on unsmoked real matrix are quite representative of the SDE extract and of the original liquid smoke. An one-way analysis of variance (ANOVA) was performed in order to assess differences between the three references. As the *P*-values were always more than 5 %, it can be concluded that there is no effect of the reference type on the notes given by the judges, whatever the odorant attributes.

Then, GC-COOL strategy has been validated thanks to the good similarities of odorant attributes intensity of the aromatic extract compared with the original reference

2. Efficiency of the GC-GOOD automatisation of the GC-COOL device

To assess the repeatability of the GC-COOL device, we have performed several injections in order to concentrate some compounds of the SDE extract. The repeatability has been evaluated by the concentration of syringol from SDE aromatic extract of liquid smoke. The chromatograms of Fig. 3 show the syringol peak in the SDE extract chromatogram (1 µL injected) and the syringol peak in the GC- COOL extract after having trapped only the syringol. An equivalent of 30 µL of SDE extract was injected in GC-COOL and about 3 µL of the SDE extract were recovered in the trap (due to the average recovery yield of 20 % and the split of 50 % between the valve and the FID). We can notice that the syringol abundance on SDE extract chromatogram divided by 200 (corresponding to the dilution in 200 µL of ethanol in the trap) corresponds to the third of the abundance of syringol in the GC-COOL extract. Syringol has well been concentrated by a factor 3 in GC-COOL extract.

In the case where a compound is in a very important quantity and could be the subject of coelutions, the GC-COOL device is helpful to identify the eventual coeluted compounds. Indeed, syringol is one of the most abundant compounds in SDE extract. Thanks to GC-COOL concentration, the identification of eugenol was possible in the GC-COOL extract whereas it was more difficult in SDE extract (Fig. 3).

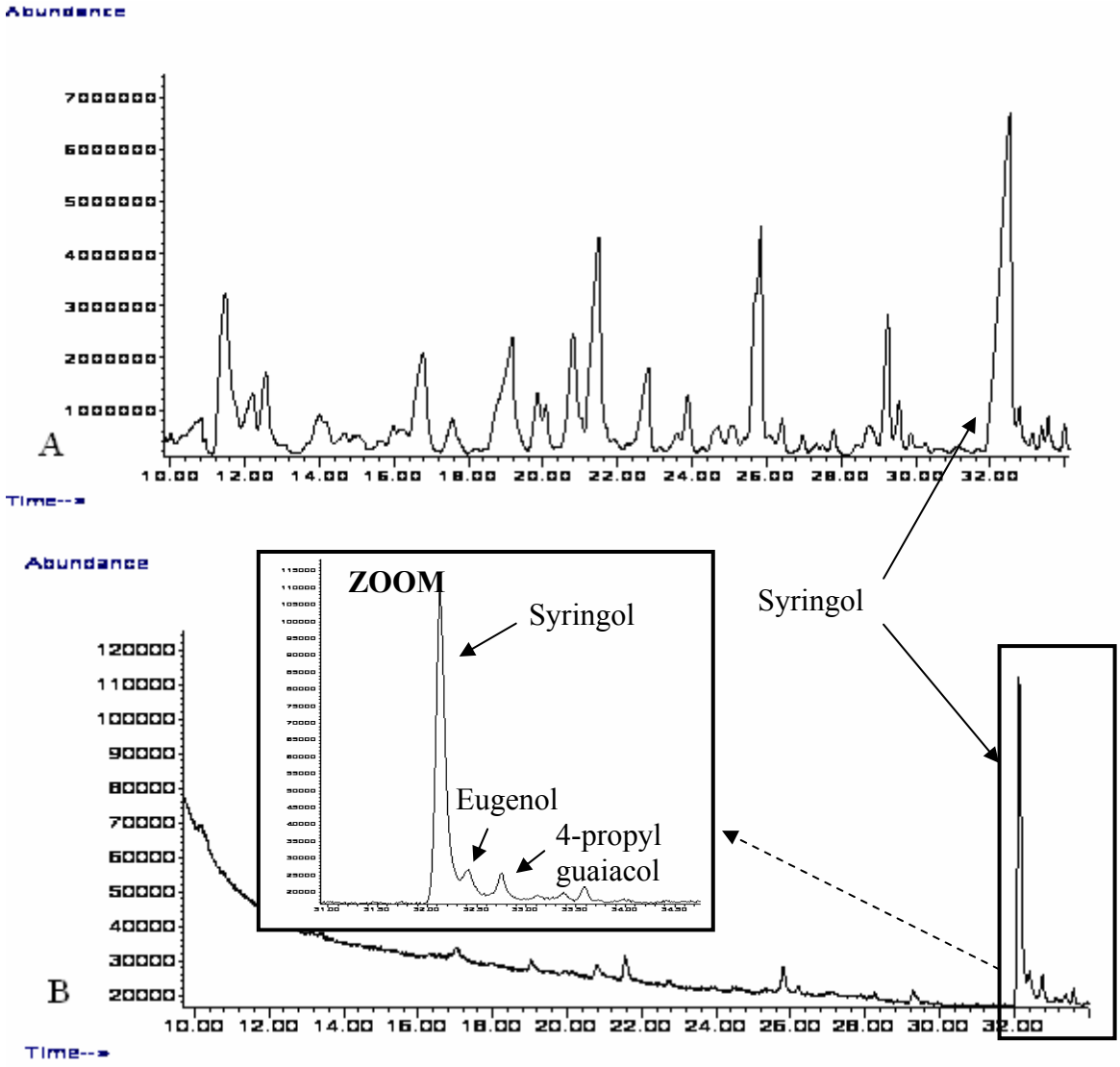


Figure 3. Chromatogram of SDE liquid smoke extract (A) and GC-COOL extract in concentration mode performed on syringol (B).

Therefore, the GC-COOL apparatus is able to concentrate several chromatographic zones or individual selected compounds whatever their retention time.
The second mode of the GC-COOL is the omission mode. In the case of odorant compounds, it allows to evaluate the influence of the omitted compounds on the overall odour of a product. In order to validate this mode, we have applied the omission strategy of the GC-COOL device on SDE aromatic extracts of liquid smoke which have been further redeposited on unsmoked salmon.

3. Application of the GC-COOL device for the identification of the contribution of odorant volatile compounds of liquid smoke in the smoke odour of salmon treated by liquid smoke

3.1. Roles of different families of odorant volatile compounds on the perception of smoke odour

In order to assess the influences of the omissions of groups of odorant volatile compounds, an analysis of variance (ANOVA) was performed on the similarity notes of the attributes of the extracts for the factors products and judges (Table 3).
A judge effect is noticeable for the scores given to salmon-like, herring-like and spicy odorant attributes. Concerning fish-like odours, the judge effect is only due to one or two judges out of the twelve, who have assessed the extract very differently. The other judges were consensual for these attributes. Concerning the spicy odour, the judges were not consensual and the scores were very different hence a judge effect. This result could be due to the subjectivity of this attribute and a lack of consensuality about spicy aromatic note despite of the indication « aromatic herb ».
Significant product effects on the odorant attributes have been therefore investigated by a LSD test performed on similarity scores (Table 4). This table applied a multiple comparison procedure to determine which means was significantly different from which others. Within each line, the levels which have common lines form a group of means within there is no statistically significant differences. Within each group of lines, the top lines are linked to high means and the bottom lines to lower means of similarity marks.
Contribution of odorant volatile compounds in the perception of the overall odour (global feeling). The omission of the compounds of Sol 4 and Sol 5 (phenolic compounds) did not have effect. However, the omissions of the compounds of Sol 1 (furannic compounds) but especially of Sol 3 (phenol, cresol isomers, guaiacol and dimethyls phenols) seem to be responsible for a decrease of similarity but not significant. Therefore, the odorant attributes for which the omissions of the compounds of Sol 1 and Sol 3 are influent could contribute the most to the

overall odour quality. As the omissions of Sol 1 and Sol 3 are influent for wood fire smoke, herring-like and burnt odorant attributes, it can be hypothesized that these three attributes are the main components of the overall odour quality.

Contribution of odorant volatile compounds in the perception of odorant wood fire smoke attribute. The ANOVA has shown a significant product effect on the odorant wood fire smoke attribute at a level 5 %. The LSD test has shown that the omission of furannic compounds but especially phenol, guaiacol and cresol isomers causes a significant decrease of similarity. The omission of the other phenolic compounds (Sol 4 and Sol 5) causes a decrease but not significant. They seem to play a role in the similarity perception of this attribute but they are not so implied than furannic and Sol 3 odorant phenolic compounds.

Finally, the omissions of enolone derivatives (Sol 2) and alkyls aryls ethers (Sol 6) have no effect on the similarity perception of wood fire smoke odorant attribute.

Contribution of odorant volatile compounds in the perception of odorant fatty attribute. The ANOVA has shown a significant product effect on the odorant herring-like attribute at a level 11 %. The LSD test has shown that the omission of alkyls aryls ethers has no effect on the perception of this odour. The omissions of enolones derivatives, furannic and phenolic compounds except those of Sol 4 seem to cause a decrease but not significant. Conversely, the omission of the phenolic compounds of Sol 4 group (4-methylguaiacol, 4-ethylguaiacol, 4-vinylguaiacol and 4-allylsyringol) has a significant effect and is responsible for a decrease of similarity for the fatty attribute.

Contribution of odorant volatile compounds in the perception of odorant herring-like attribute. The ANOVA has shown a significant product effect on the odorant herring-like attribute at a level 5 %. The LSD test has shown that the omissions of furannic compounds, enolones derivatives and alkyls aryls ethers cause a decrease of similarity but not significant. Indeed, only the phenolic compounds omissions as those of Sol 5 and Sol 3 have caused a significant decrease in the similarity perception of this odour.

Contribution of odorant volatile compounds in the perception of odorant spicy attribute. None of omissions of compounds causes a significant decrease of similarity.

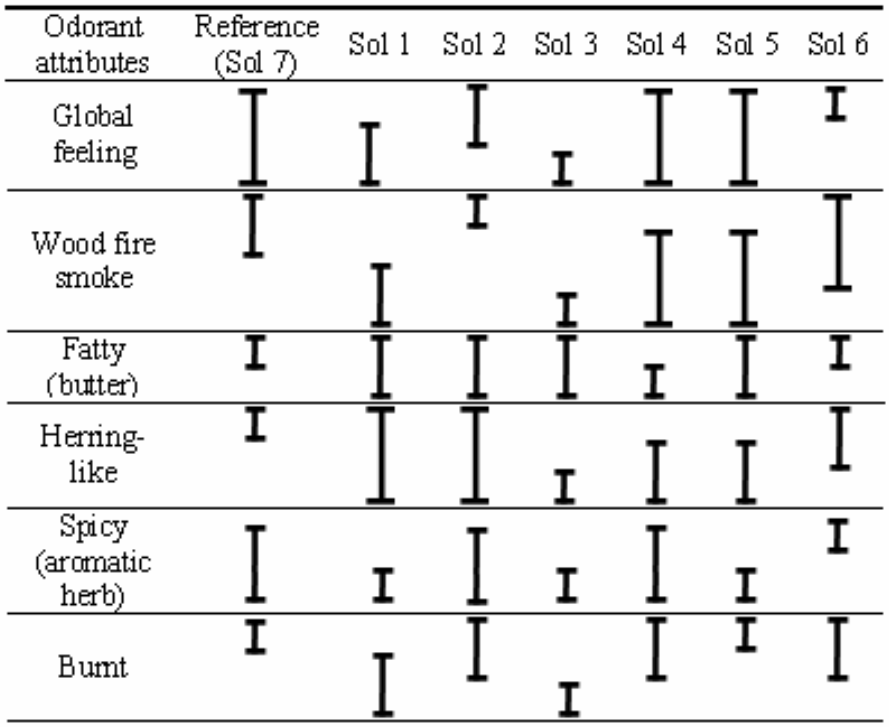
Contribution of odorant volatile compounds in the perception of odorant burnt attribute. The ANOVA has shown a significant product effect on the odorant herring-like attribute at a level 5 %. The LSD test has shown that the omission of syringol, eugenol, 4-propylguaiacol and isoeugenol isomers has no effect and seems not to play a role in the similarity perception of this burnt odour. Only omissions of furannic compounds but especially phenolic compounds of Sol 3 have caused a significant decrease of similarity.

Table 3. P-values results of the analysis of variance for the similarity notes of the odorant attributes of the extracts for the factors judges and products (from Sol 1 to Sol 6).

Odorant attributes	Factors	
	Judges	Products
Global feeling	0.4749	0.0666
Cold smoke (soot)	0.6325	0.6633
Wood fire smoke	0.3762	**0.0017**
Salmon-like	**0.0475**	0.4492
Sharp	0.1720	0.7590
Fatty (butter)	**0.0432**	0.1068
Herring-like	0.8064	**0.0500**
Spicy (aromatic herb)	**0.0091**	0.0681
Burnt	0.1788	**0.0085**

In bold : significant p-values at a 5 % level.

Table 4. Results of the LSD test performed on similarity scores for the different families of odorant volatile compounds omissions according to each significantly affected odorant attribute.



The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5,0% risk of calling each pair of means significantly different when the actual difference equals 0.

The effects of omissions of these families have led to conclusions only compliant to these families of compounds because there are a lot of possibilities of odorant interactions.

The contribution of each group of odorant volatile compounds in the overall odour composition can be linked to their individual odours. For example, the compounds of Sol 1 and Sol 3 were already described with « smoky » aromatic notes in olfactometric studies (Varlet et al., 2006 and 2007a). These odorant attributes could be related to the wood fire smoke odour in which they are implied. Moreover, the important role of furannic compounds and phenol, guaiacol, cresols isomers and dimethyls phenols in the wood fire smoke and burnt odorant attributes has already been noticed (Varlet et al., 2007d). More precisely, cresols isomers were found to be implied in cold smoke odour and the other compounds in wood fire smoke odours. Thus, the absence of product effect for cold smoke attribute seems a little surprising because food treated by liquid smoke and liquid flavourings themselves have been reported with cold smoke/soot odours (Kostyra et al., 2005 ; Varlet et al., 2007c). However, the previous studies about odours of products treated by liquid smoke have only assessed on cold smoke odorant attribute. Our results show that the judges seem to be more sensitive to burnt than cold smoke and when they assess the cold smoke odours, they could assess in reality a mixture of cold smoke and burnt odours. Therefore, we have to investigate the contribution of cresols isomers in the burnt perception because even if they are the compounds the most related to cold smoke odours, they are not sufficient to explain the cold smoke odour. Then, to explain the absence of products effects of common liquid smoke odorant attributes as cold smoke and sharp on similarity scores, we can formulate two hypotheses. Firstly, these odours could be composed by odorant volatile compounds generated during the smoking process between liquid smoke and fish flesh under the parameters of smoking. Secondly, these odours could be the results of odorant interactions which means that the selection of families of compounds was not optimum and that omissions of others combinations of families should be investigated. Concerning the absence of product effect for salmon-like odorant attribute, it could be due to odorant volatile compounds of the fish flesh that is the reason why omission of odorant volatile compounds of liquid smoke has no effect on the overall odours of smoked salmon. However, this conclusion would mean that there is not odorant interactions responsible for salmon-like odour, between the odorant volatile compounds of liquid smoke and the matrix whereas it is the case for herring-like odorant attribute. Indeed, the omission of phenolic compounds of Sol 5 (syringol, eugenol, 4-propylguaiacol and isoeugenol isomers) or this of Sol 3 (phenol, cresols isomers, guaiacol and dimethyls phenols) or Sol 4 (4-methylguaiacol, 4-ethylguaiacol, 4-vinylguaiacol and 4-allylsyringol) has caused a significant decrease of similarity in the herring-like odorant perception. However, the individual odours of

phenolic compounds are usually described by smoke, roasty notes. Then, the herring-like odour of smoked salmon could be composed by odorant interactions implying these phenolic compounds.

Another case of odorant interaction between phenolic compounds can be noticed with the compounds of Sol 4 implied in the fatty odour of smoked salmon. Indeed, 4-methylguaiacol and 4-ethylguaiacol were described by sugared odours of vanailla and candy and 4-vinylguaiacol and 4-allylsyringol were described by green and smoky aromatic notes (Varlet et al., 2007a). None of these individual odours can explain the decrease of similarity for fatty attribute caused by the simultaneous omission of these compounds. Therefore, odorant interactions could be responsible for this effect.

As result, the investigations carried out on the omissions of families of odorant volatile compounds lead to the following conclusions:

1. Earlier authors (Kim et al., 1974 ; Radecki et al., 1977) considered furannic compounds as to contribute to soften the heavy smoky aromas. These conclusions are in adequation with their individual odours described by roasty, warm, caramel-like odours (Maga, 1987 ; Varlet et al., 2007a). However, wood fire smoke odorant attribute has been previously related to high contents of furannic compounds (Varlet et al., 2007d). These compounds seem to be responsible for smoky aromas by odorant interactions mechanism because they are influent on an odour different from their individual odours. In our study, decreases of similarity for the wood fire smoke and burnt odorant perception consecutive to the simultaneous omission of furfural, furfuryl alcohol, 5-methylfurfural and 2-acetylfuran have been established. Therefore, indivual odorant intensity test for each furannic compound could enlight on the real role of these compounds concerning the smoky odour.

2. A distinction between furannic compounds and enolones derivatives must be carried out. These compounds are often ranked as carbonyl compounds due to their chemical structure. However, it has been shown that furannic compounds could play a main role in smoky odorant perception while enolone derivatives seem to be minor contributors.

3. Carbonyl compounds such as enolones derivatives have been identified as contributors of the wood smoke and liquid smoke flavors (Fiddler et al., 1970 ; Kim et al., 1974). Among them, cycloten and 3-methylocyclopenten were found the most important odorant volatile carbonyles (Kostyra et al., 2005). The odorant attributes of enolone derivatives are not very consensual. They were identified as « grassy » (Maga, 1987) or cooked/spicy in smoked salmon (Varlet et al., 2007a). Due to their « grassy » odours, Maga attributed to these compounds a minor role in the smoke flavor (Maga, 1987). The absence of significant effect caused by their simultaneous

omission should confirm this conclusion. However, a relation between wood fire smoke and high contents of enolone derivatives has been recently put in evidence in smoked salmon (Varlet et al., 2007d). Therefore, individual investigations must be planned. Enolone derivatives could be implied in odorant interactions with others components such as other odorant volatile compounds. Indeed, furannic and phenolic compounds can not explain alone the totality of the smoke flavor (Fujimaki et al., 1974 ; Olsen et al., 1976).

4. The odorant volatile hydrocarbons seem not to contribute to the overall smoked salmon odour. From our knowledge, no information is available concerning their role except olfactometric study which has shown them exhibiting cooked/green/roasty odours. The simultaneous omission of 2,3-dimethoxytoluene, indanone, 1,2,3-trimethoxy-5-methylbenzene and one of the main volatile compounds of liquid smoke aromatic extract, 2,3,5-trimethoxytoluene, did not cause changes in the overall odour. Further investigations by GC-COOL will allow to assess if they interact with other volatile compounds on the overall smoked salmon odour.

5. Concerning the smoky odour, the phenolic compounds of Sol 3 (phenol, cresols isomers, guaiacol and dimethylphenols) seem to be the main odorant volatile compounds implied in smoked salmon odour quality. In contrast to earlier opinions and in adequation with recent conclusions (Kostyra et al., 2005), syringol and derivatives seem not to be major contributors to the smoky odour. Their omission, simultaneously with 4-propylguaiacol, did not cause significant decreases of similarity for smoky odorant attributes. Kostyra et al. have observed that among guaiacol derivatives, some contribution of guaiacol and 4-methylguaiacol was possible but not of other derivatives, including eugenol and its isomers (Kostyra et al., 2005). Therefore, the absence of effects of the Sol 5 omission except in herring-like odour, the presence of guaiacol in Sol 3 whose omission is very important seem to confirm these two conclusions and should be checked by individual omission tests. Then, we have chosen to investigate more precisely the individual contributions of several key phenolic compounds in order to explain the results of omissions of certain families. The roles of cresols isomers, guaiacol and syringol were then individually studied.

3.2. Role of several odorant volatile compounds in the overall smoke odour of salmon treated by liquid smoke

The influence of cresols isomers, guaiacol and syringol on the overall odour was assessed individually by similarity and odorant intensity tests.

The influences of the omissions of these odorant volatile compounds have been studied by an analysis of variance (ANOVA) on the similarity and odorant intensity notes of the attributes of the extracts for the factors solutions (GC-COOL extracts minus guaiacol, syringol or cresol isomers) and judges, respectively presented in Table 5 and Table 6. A judge effect is noticeable for the scores of similarity given to wood fire smoke and salmon-like odorant attributes and for the scores of odorant intensity given to fatty. The judge effect on the similarity scores for wood fire smoke and salmon-like is only due to one or two judges out of the eleven selected for these tests, who have assessed the extract very differently. The judge effect noticeable on the odorant intensity notes for fatty is due to a lack of consensuality of the judges. The judge effect can be understood by the difficulty to assess the odorant intensity of this attribute because odorant saturation can be rapidly reached.

Table 5. *P*-value results of the analysis of variance for the similarity notes of the attributes of the extracts for the factors judges and solutions (GC-COOL extracts minus guaiacol, syringol or cresols)

Odorant attributes	Factors	
	Judges	Solutions
Global feeling	0.3719	**0.0000**
Cold smoke (soot)	0.2326	**0.0000**
Wood fire smoke	**0.0026**	**0.0000**
Salmon-like	**0.0237**	**0.0000**
Sharp	0.3472	**0.0000**
Fatty (butter)	0.4152	**0.0012**
Herring-like	0.8522	**0.0015**
Spicy (aromatic herb)	0.0666	**0.0000**
Burnt	0.1203	**0.0000**

In bold : significant p-values at a 5 % level.

Table 6. *P*-value results of the analysis of variance for the odorant intensity notes of the attributes of the extracts for the factors judges and solutions (GC-COOL extracts minus guaiacol, syringol or cresols)

Odorant attributes	Factors	
	Judges	Solutions
Cold smoke (soot)	0.8091	0.6618
Wood fire smoke	0.4964	0.3333
Salmon-like	0.0945	0.5543
Sharp	0.5495	**0.0258**
Fatty (butter)	**0.0362**	**0.0072**
Herring-like	0.1795	**0.0010**
Spicy (aromatic herb)	0.1722	0.5523
Burnt	0.7431	0.3889

In bold : significant p-values at a 5 % level.

Omissions of cresol isomers, syringol and guaiacol caused significant similarity differences by comparison with the reference for all odorant attributes. The same omissions caused significant odorant intensity differences by comparison with the reference for the sharp, fatty and herring-like odorant attributes. Significant product effects for the similarity and odorant intensity scores for each odorant attributes have been therefore investigated by LSD tests and respectively given in Tables 7 and 8. The LSD test on similarity scores has allowed to notice that guaiacol omission leads to the most important differences and cresols omissions leads to the least whatever the odorant attribute. Syringol omission leads to intermediate significant differences of similarities. Omissions of compounds cause close significant similarity differences by comparison with the reference only for wood fire smoke, sharp, fatty and herring-like.

Concerning the influence of omission of compounds on the odorant intensity scores, the LSD test has allowed to notice that only the omission of cresol isomers has caused a significant difference by a weaker perception of the sharp odorant intensity. For herring-like, only the guaiacol omission causes a significant difference by a higher perception of the odorant intensity of this attribute. Finally, for fatty, the omission of cresol isomers seems to be responsible for a higher perception of odorant intensity but not significant. Conversely, omissions of syringol and especially guaiacol cause a significant higher perception of odorant intensity.

Guaiacol, syringol and cresol isomers are odorant volatile compounds very important in the smoked salmon odour because their omissions cause significant differences of similarity. The volatile compounds omitted were previously identified in salmon treated by liquid smoke with smoky/burnt odours (Varlet et al., 2007a). Guaiacol was described by smoke, vanilla and ink aromatic notes. Syringol was described by burnt rubber and spicy odour and cresol isomers were detected with chemical/animal, spicy and burnt aromatic notes. Cresol isomers are implied in the overall odour of salmon treated by liquid smoke due to their individual odorant role and due to a contribution in odorant interactions responsible for the overall odour. Their individual role is noticeable in the similarity because their omission causes differences for odorant attributes in adequation with their individual smoky/burnt odours. Their individual role is also remarkable in the odorant intensity because their omission is responsible for a decrease of perception of sharp intensity and several works have already attributed bitter and pungent odours to phenolic compounds with low ebullition point as cresol isomers (Baltes et al., 1979 ; Shahidi et al., 2003). However, cresol isomers are also implied in odorant interactions. Indeed, their omission causes differences of similarities for odorant descriptors like fishy or fatty which are not in adequation with their individual smoky/burnt odours odours. The odorant interactions implying cresol isomers could be also responsible for the decrease of perception of sharp intensity because this

Table 7. Significant results of the LSD test for the similarity scores of the different products (GC-COOL extracts minus guaiacol, syringol or cresols) according to each odorant attribute.

Odorant attributes	Reference	- guaiacol	- syringol	- cresol isomers
Global feeling	I	I	I	I
Cold smoke (soot)	I	I	I	I
Wood fire smoke	I	I	I	I
Salmon-like	I	I	I	I
Sharp	I	I	I	I
Fatty (butter)	I	I	I	I
Herring-like	I	I	I	I
Spicy (aromatic herb)	I	I	I	I
Burnt	I	I	I	I

The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5,0% risk of calling each pair of means significantly different when the actual difference equals 0.

Table 8. Significant results of the LSD test for the odorant intensity scores of the different products (GC-COOL extracts minus guaiacol, syringol or cresols) according to each odorant attribute.

Odorant attributes	Reference	- guaiacol	- syringol	- cresol isomers
Sharp	I	I	I	I
Fatty	I	I	I	I
Herring-like	I	I	I	I

The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5,0% risk of calling each pair of means significantly different when the actual difference equals 0.

odour is not only due to cresol isomers individual odours. Then, the decrease of perception of sharp intensity could partly explain the differences of the perception of similarity by comparison with the reference.

Syringol has also an individual odorant role and a role in odorant interactions which compose the overall odour of salmon smoked by atomisation of liquid smoke. Its individual role is only noticeable in the similarity because its omission causes differences for odorant attributes in adequation with their individual smoky/burnt odours. Moreover, its individual role seems to be more important than this of cresol isomers due to more significant differences by comparison with the reference. Syringol is not individually implied in the odorant intensity because none of the odorant attributes in adequation with its individual odour is significantly different of the reference. However, this compound is involved in odorant interactions because its omission causes differences of similarities for odorant attributes which are not in adequation with its individual odours. Indeed, syringol is implied in the odorant intensity of fatty because its omission causes a higher perception of the intensity of this attribute. Then, the increase of the perception of fatty intensity could partly explain the differences of the perception of similarity by comparison with the reference.

Guaiacol has also an individual odorant role and a role in odorant interactions which compose the overall odour of salmon smoked by atomisation of liquid smoke. Its individual role is only noticeable in similarity because its omission causes differences for odorant attributes in adequation with their individual smoky/burnt odours. Moreover, its individual role seems to be more important than these of cresol isomers and syringol due to more significant differences by comparison with the reference. However, this compound is involved in odorant interactions because its omission causes differences of similarities for odorant attributes which are not in adequation with its individual odours. Moreover, guaiacol is implied in the odorant intensity of raw fish odours as herring-like and fatty because its omission causes a higher perception of the intensity of these attributes. Then, the increase of the perception of the intensity of these odours could partly explain the differences of the perception of similarity by comparison with the reference.

Guaiacol, syringol and cresol isomers are majoritory compounds in liquid smoke and salmon smoked by atomisation of liquid smoke. Moreover, they make part of the most potent odour-active compounds in these matrices. However, their individual omissions did not cause decrease of cold smoke, burnt or wood fire smoke odorant intensities. Therefore, it seems that the intensity of « smoke » odours of salmon treated by liquid smoke is composed by odorant interactions of other aroma compounds. These compounds are nevertheless very important in the

quality of the « smoke » odours because their omissions modify the odorant similarity. Moreover, the odorant interactions in which they are implied can rule certain odorant attributes intensities and have an impact on similarity.

These results are in accordance with conclusions recently formulated about the roles of volatile compounds in the odour of smoke flavourings.

1. Among phenolic compounds, phenol and cresol isomers were thought to play the most important role in the smoke flavouring odour (Kostyra et al., 2005). Besides, among phenolic compounds, high contents of p-cresol has been previously shown as related to cold smoke odour in salmon treated by liquid smoke (Varlet et al., 2007d). Redeposited on real food matrix, we have checked that cresol isomers were also very important for the overall odour similarity and that they were responsible for a part of the sharp odorant intensity of smoked salmon.

2. Phenolic compounds are thought to be mainly responsible for the strength of smoke flavouring odours and a contribution of guaiacol was possible (Kostyra et al., 2005). When liquid smoke is vapourised on salmon, we have observed that neither guaiacol nor syringol had an impact on the strength of « smoke » odours. They had only a role in odorant interactions with other components which decrease the odorant intensity of the fish attributes as herring-like or fatty, especially for guaiacol omission. They had also an important role in the quality of the overall odour of smoked salmon.

3. In contrast to earlier opinions of other authors (Kim et al., 1974 ; Hamm, 1976), syringol and its derivatives are thought not to be main contributors of the odour identified as typical for freshly smoked product even if syringol is a main compound in liquid smoke. They were perceived with weak odour which did not resemble the odour of smoke-cured product at all (Kostyra et al., 2005). Syringol was also found as a majoritory compound in the liquid smoke used in this study (Varlet et al., 2007a). Our works were only focused on the individual role of syringol and not these of its derivatives. We have found that syringol is not important in the smoke odour intensity as reported in other works (Cardinal et al., 2006). In adequation with Kostyra et al. (Kostyra et al., 2005), we found that omission of syringol and its derivatives (Sol 5) did not cause significant decrease in « wood fire smoke » similarity. However, the unique omission of syringol has caused a significant decrease of similarity whatever the attribute of the overall odour. Therefore, this compound is important in the smoke odour quality. Moreover, syringol is implied in odorant interactions with other components because its omission causes higher perception of fatty intensity. These results seem not to be in adequation with the individual odour of syringol described with burnt rubber, spicy aromatic notes and its implication in the cold smoke odour detected in previous study (Varlet et al., 2007d). Thus, syringol is not

individually responsible for cold smoke odour intensity but could be implied in odorant interactions which could rule directly the cold smoke quality or indirectly by modifying the intensity of other odorant attributes as fatty. The role of the concentrations of odorant volatile compounds in odorant interactions could be also very important. Indeed, according to its quantity, an odorant volatile compound could influence very differently the attributes of the overall odour, by its own odour, or by odorant interactions.

CONCLUSION

The GC–COOL system has enabled the selection of natural volatile compounds present in an odor extract (other extraction methods could be used) and the assessment of their global odor when they were mixed in their own extracted relative concentrations. For the first time to our knowledge, this strategy takes into account eventual matrix effects by a redeposition of the recovered aromatic extract on real matrix. Among the new perspectives offered by the GC-COOL device, the investigation of the influence of odorant volatile compounds on the overall odour composition of a food matrix has been tested on smoked salmon. By the omission of certain odorant volatile compounds, it becomes possible to know the roles that an odour-active compound plays in the overall odour and it allows to put in evidence odorant interactions. The omissions of families of compounds have confirmed the predominant role of individual odours of phenolic compounds but also of furannic compounds in the smoky flavor. The roles played by guaiacol, syringol and cresol isomers have been better elucidated. The complexity of the influence of their omissions has underlined the difficulty to study odorant interactions and has confirmed the fact that an overall odour is not only composed by the sum of individual odours. Therefore, odorant interactions were also put in evidence by the implication in the overall odour characteristics of these three key compounds whose individual odours were very different of the odours in which they are involved. These interactions can also influence the odorant intensity and the similarity. The relationships between these two characteristics are narrow and more investigation must be developped in order to better identify the effects of omissions of odorant volatile compounds, alone or in mixture, on the overall odour of a matrix. This method was applied to salmon treated by liquid smoke but it could be extended to other foodstuffs in order to know the roles that play their odour-active compounds on their overall odours. The GC-COOL device allows to recover individual or several. According to the quantities injected, the concentration can be increased in order to obtain standard compounds. It opens new opportunities to recover expensive internal standards like labelled standards from an extract or to

purify molecules of therapeutic interest. It permits also to concentrate an aera with an odorant interest on a chromatogram of an aromatic extract in order to better analyze it by NMR for example. Then, the concentration mode of the GC-COOL can be seen as a preparative GC but allows to recover the selected compounds at liquid state. Therefore, GC-COOL efficiency should be checked on its ability to purify molecules or internal labelled standard. Moreover, more investigation will be led in order to improve the efficiency of the cryotrapping.

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REFERENCES

Baltes, W., Sochtig, I. (1979). Low molecular weight ingredients of smoke flavor preparations. Z. Lebensm. Unters. Forsch., 169, 9-16.

Cardinal, M., Berdagué, J.L., Dinel, V., Knockaert, C., Vallet, J.L. (1997). Effet de différentes techniques de fumage sur la nature des composés volatils et les caractéristiques sensorielles de la chair du saumon. Sciences des Aliments, 17, 679-696 (In French).

Cardinal, M., Cornet, J., Sérot, T., Baron, R. (2006). Effects of the smoking process on odour characteristics of smoked herring (*Cuplea harengus*) and relationships with phenolic compound content. Food Chem., 96, 137-146.

Fiddler, W., Wasserman, A.E., Doerr, R.C. (1970). A « smoke » flavor fraction of a liquid smoke solution. J. Agric. Food Chem., 18(5), 934-936.

Fujimaki, M., Kim, K., Kurata, T. (1974). Analysis and comparison of flavor constituents in aqueous smoke condensates from various woods. Agric. Biol. Chem., 38(1), 45-52

Gómez-Míguez, M.J., Cacho, J.F., Fereeira, V., Vicario, I.M., Heredia, F.J. (2007).Volatile components of Zalema white wines. Food Chemistry, 100(4), 1464-1473.

Kim, K., Kurata, T., Fujimaki, M. (1974). Identification of flavour constituents in carbonyl, non-carbonyl neutral and basic fractions of aqueous smoke condensates. Agric. Biol. Chem., 38, 53-63.

Kostyra, E., Baryłko-Pikielna, N. (2005). Volatiles composition and flavour profile identity of smoke flavourings. Food Qual. Pref., 17(1-2), 85-95.

Hamm, R. (1976). Analysis of smoke and smoked food. In IUFoST-IUPAC symposium advances in smoking of foods (1655-1665). 8-10th September, Warsaw, Poland.

Hattula, T., Elfving, K., Mroueh, U.M., Luoma, T. (2001). Use of liquid smoke flavouring as an alternative to traditional flue gas smoking of rainbow trout fillets (*Oncorhyncus mykiss*). Lebensmittel-Wissenschaft Und Technologie, 34, 521-525.

Maga, J.A. (1987). The flavor chemistry of wood smoke. Food Rev. Int., 3(1&2), 139-183.

Mehinagic, E., Prost, C., Demaimay, M. (2003). Representativeness of Apple Aroma Extracts Obtained by Vacuum Hydrodistillation. Workk presented at 5th Pangborn Sensory Science Symposium, July 20-24, Boston, USA.

Nonier, M.F., Vivas, N., Vivas de Gaulejac, N., Absalon, C., Soulié, P., Fouquet, E. (2005). Pyrolysis-gas chromatography/mass spectrometry of *Quercus* sp. wood. Application to structural elucidation of macromolecules and aromatic profiles of different species. J. Anal. Appl. Pyrolysis, 75(2), 181-193.

Ojeda, M., Bárcenas, P., Pérez-Elortondo, F.J., Albisu, M., Guillén, M.D. (2002). Chemical references in sensory analysis of smoke flavourings. Food Chem., 78(4), 433-442.

Olsen, C.Z. (1976). Chemical composition and application of smoke flavor. Proc. Eur. Meet. Meat Res. Workers, N°22, F 7:1, F 7:8.

Radecki, A., Grzybowski, J., Halkiewicz, J., Lamparczyk, H. (1977). Isolation and indentification of some components of the lower-boiling fraction of commercial smoke flavourings. Acta Aliment. Pol., 3, 203-207.

Rannou, C., VenZeveren, E., Le Bail, A., Prost, C. (2005). Représentativité odorante de l'arôme du pain. Work presented at 56èmes journées techniques des Industries Céréalières, october 20-21, Paris, France (In French).

Selli, S., Rannou, C., Prost, C., Robin, J., Serot, T. (2006). Characterization of aroma-active compounds in Rainbow trout (Oncorhyncus mykiss) eliciting an off-odor. J. Agric. Food Chem., 54(25), 9496-9502.

Shahidi, F.; Naczk, M. Contribution of phenolic compounds to the flavor and color characteristics of foods. In *Phenolics in food and nutraceuticals*, CRC Press Llc ; Boca Raton, Florida, USA, 2003, 443-481.

Varlet, V., Knockaert, C., Prost, C., Sérot, T. (2006). Comparison of odor-active volatile compounds of fresh and smoked salmon. J. Agric. Food Chem., 54, 3391-3401.

Varlet, V., Sérot, T., Cardinal, M., Knockaert, C., Prost, C. (2007a). Olfactometric determination of the most potent odor-active compounds in salmon muscle (*Salmo salar*) smoked by using four smoke generation techniques. J. Agric. Food Chem., 55, 4518-4525.

Varlet, V., Prost, C., Sérot, T. (2007b). New procedure for the study of odour representativeness of aromatic extracts from smoked salmon. Food Chem., 100, 820-829.

Varlet, V., Sérot, T., Knockaert, C., Cornet, J., Cardinal, M., Monteau, F., Le Bizec, B., Prost, C. (2007c). Organoleptic characterization and PAH content of salmon (*Salmo salar*) smoked according to four industrial smoking techniques. J. Sci. Food Agric., 87(5), 847-854.

Varlet , V., Sérot, T., Cardinal, M., Courcoux, P., Cornet, J., Knockaert, C., Prost, C. (2007d). Relationships between sensory characteristics and the most odorant volatile compounds on salmon smoked by four industrial smoking techniques. Food Chem., submitted.

Bilan

Un prototype innovant de tri chromatographique a été élaboré. Des portions d'un chromatogramme ou des composés individuels peuvent ainsi être sélectionnés, isolés et récupérés sous forme liquide. Par rapport au GC-GOOD, le GC-COOL a subi des améliorations conséquentes. Tout d'abord, au niveau de la sélection des composés par le système GC-GOOD, la commutation de la vanne trois-voies a été automatisée et est gérée par le développement d'un logiciel permettant la commutation automatique programmée de la vanne. Le logiciel peut également supporter le couplage avec un injecteur automatique.

Après avoir optimisé le mode de sélection chromatographique, il a fallu développer un système de concentration des composés choisis (ou omis) afin de les obtenir à l'état liquide dans un solvant neutre du point de vue toxique et odorant. L'opimisation du système de cryoconcentration a nécessité des étapes d'évaluation de l'efficacité du piégeage et de la reprise par un solvant et d'évaluation de la représentativité de l'extrait récupéré. Le GC-COOL permet ainsi de récupérer à l'état liquide les composés retenus avec un rendement moyen légèrement supérieur à 20 % indépendemment des propriétés physico-chimiques des composés. De plus, du fait de sa non sélectivité, la stratégie GC-COOL a conduit à des notes de représentativité odorante très satisfaisantes puisqu'aucune différence significative n'a été décelée entre la fumée liquide, l'extrait aromatique et l'extrait aromatique injecté et récupéré au GC-COOL redéposés sur des cubes de saumon. De plus, ces redépositions ont été réalisées dans des conditions d'iso-intensité, c'est-à-dire dans les mêmes concentrations que celles quantifiées dans la matrice réelle.

En ce qui concerne l'étude de l'influence des composés volatils odorants de la fumée liquide sur l'odeur globale « fumée », le système GC-COOL a permis de formuler des premières conclusions sur le rôle de ces composés. Ainsi, l'omission de certaines familles de composés volatils odorants a causé des baisses significatives de similarité de certains descripteurs par rapport à la référence. Par exemple, les omissions simultanées des composés de la famille 3 (phénol, o-crésol, p-crésol, guaiacol, 2,4 et 3,5-dimethylphénol) entraînent une différence significative de similarité des descripteurs « fumée feu de bois », « brûlé » et « hareng » par rapport à la référence. De même, les omissions simultanées des composés du groupe 1 (furfural, alcool furfurylique, 2-acétylfurane et 5-méthylfurfural) causent une différence significative de similarité des descripteurs « fumée feu de bois » et « brûlé » par rapport à la référence. Les omissions simultanées des composés du groupe 4 (4-méthylguaiacol, 4-éthylguaiacol, 4-vinylguaiacol et 4-allylsyringol) sont responsables d'une différence significative de similarité

des descripteurs « gras » et « hareng » par rapport à la référence. Enfin, les omissions simultanées des composés du groupe 5 (syringol, eugénol, 4-propylguaiacol, (E)-isoeugénol et (Z)-isoeugénol) entraînent une différence significative de similarité du descripteur « hareng » par rapport à la référence.

Parfois, les effets sur ces descripteurs étaient en adéquation avec l'odeur individuelle des composés volatils odorants détectés par olfactométrie. Dans d'autres cas, l'existence d'interactions odorantes a été mise en évidence du fait de l'inadéquation entre les odeurs individuelles de composés volatils odorants et les descripteurs odorants de l'odeur globale dans lesquels leurs omissions provoquaient une baisse significative de similarité. Par exemple, les omissions des composés du groupe 5, c'est-à-dire de composés volatils odorants dont les perceptions en olfactométrie étaient plutôt qualifiées par des descripteurs « fumé » ou « épicé », n'entraînent pas de différence de similarité sur ces descripteurs mais sur le descripteur « hareng ».

L'omission des composés furaniques et de certains composés phénoliques a confirmé leur rôle dans l'odeur « fumée feu de bois » déjà mis en évidence dans les travaux statistiques PLS2 sur l'étude des relations entre les descripteurs odorants de l'odeur globale et les concentrations en composés volatils odorants. L'étude plus spécifique des crésols, du guaiacol et du syringol a permis de mieux cerner le rôle individuel de ces composés dans l'odeur globale du saumon fumé et de relativiser certaines conclusions formulées depuis les années 1970 – 1980 sur les composés phénoliques responsables des propriétés organoleptiques « fumées » de tels produits. En effet, des travaux anciens avaient permis d'effectuer une classification des composés phénoliques en trois groupes : les composés phénoliques à faible point d'ébullition (60-90°C) comprenant le phénol, les crésols, le guaiacol, le 4-méthylguaiacol et le 4-éthylguaiacol avaient un goût chaud et amer ; les composés phénoliques à point d'ébullition intermédiaire (91-132°C) comprenant le syringol, le méthylsyringol, le (E) et le (Z)-isoeugénol avaient une flaveur caractéristique de la fumée ; les composés phénoliques à haut point d'ébullition (133-200°C) avaient des propriétés chimiques et acides jugées de qualité médiocre (Maga, J.A., 1987 ; Toth, L. et al., 1984). En accord avec les conclusions d'autres travaux récents (Kostyra, E. et al., 2005), nous avons montré que cette classification était à relativiser. En effet, parmi les composés phénoliques, le o-crésol et le p-crésol étaient impliqués dans l'odeur globale de la fumée. Leurs effets sur la flaveur des produits fumés ne peuvent se résumer à une contribution au goût chaud et amer. De même, en adéquation avec d'autres études sur le hareng fumé (Cardinal, M. et al., 2006), le syringol qui fait pourtant partie des composés phénoliques présentant une flaveur caractéristique de fumée ne semble pas être un composé majoritaire dans l'intensité de l'odeur « fumée ». De

même, le guaiacol ne semble pas avoir un fort impact sur l'intensité de l'odeur « fumée » mais semble quand même jouer un rôle, comme pressenti par d'autres auteurs (Kostyra, E. et al., 2005), notamment dans des interactions odorantes car son omission cause une baisse de l'intensité odorante des descripteurs « gras » et « hareng ».

Les analyses par omission au GC-COOL doivent être considérées comme complémentaires des résultats obtenus par PLS2. En effet, le traitement PLS a permis d'identifier la relative implication du syringol dans l'odeur « fumée froide ». Grâce à l'étude GC-COOL, nous avons pu déterminer la nature de cette implication. Par exemple, même s'il semble logique que le syringol joue un rôle dans le descripteur « fumée froide » du fait de son odeur individuelle, nous nous sommes aperçus que ce n'était pas l'odeur individuelle du syringol qui était responsable de son implication dans la perception de l'intensité de l'odeur « fumée froide » mais que cette implication était dûe à des interactions odorantes entre le syringol et d'autres composants volatils ou de la matrice. Ainsi, à l'unique issue des résultats PLS2, nous aurions conclu trop rapidement à l'implication individuelle du syringol dans l'odeur « fumée froide ». Dans le cas où cette odeur serait préférée par les consommateurs, nous aurions recommandé des paramètres de fumage favorisant la présence de ce composé, sans toutefois se préoccuper des autres composés, ce qui aurait conduit à des odeurs différentes de celle attendue puisque ce sont les interactions entre le syringol et d'autres composants à une certaine concentration qui constituent la majeure partie de l'odeur « fumée froide ». Ainsi, la stratégie d'omission des composés volatils doit être considérée non seulement comme une vérification des conclusions du traitement PLS2 mais comme un complément indispensable aux résultats individuels obtenus par olfactométrie.

Ainsi, l'omission individuelle du syringol, du guaiacol et du p-crésol a permis de vérifier les implications individuelles de ces composés dans la composition de l'odeur globale du saumon fumé par atomisation de fumée liquide. Le guaiacol apparaît comme un des composés les plus importants car son omission cause les différences les plus significatives avec la référence. Viennent ensuite le syringol et le p-crésol. Cependant, il semblerait que l'influence de ces composés dans l'odeur globale s'exerce plus par leurs interactions avec la matrice et les autres composés volatils que par leur odeur individuelle. Ce constat illustre bien la complexité de l'odeur des produits fumés.

Des analyses plus exhaustives avec un plan expérimental d'omissions devront être mises en place pour analyser plus en profondeur le rôle individuel et en mélanges de certains composés volatils odorants. La contribution odorante individuelle des composés furaniques et des dérivés d'enolones dans l'odeur globale « fumée » devra être étudiée. En effet, il a été démontré que ni

l'essentiel des composés phénoliques, ni la totalité des composés phénoliques ne restituaient la complète flaveur de la fumée (Maga, J.A., 1987).

Le GC-COOL offre ainsi de nouvelles perspectives en chimie analytique utilisant la chromatographie en phase gazeuse. L'omission de composés durant l'élution offre de nouvelles pistes d'études sur l'influence en mélanges des composés volatils odorants. Son application à l'odeur du saumon fumé par atomisation de fumée liquide a permis d'étudier la contribution de certaines familles de composés odorants sur l'odeur globale de la fumée liquide. De plus, l'étude de l'odeur d'autres matrices, alimentaires ou non alimentaires, peut être envisagée du moment que l'extrait aromatique résultant reste injectable dans un chromatographe en phase gazeuse. L'isolement et la concentration d'une zone chromatographique sélectionnée peuvent conduire à l'obtention de standards aromatiques, au recyclage d'étalons internes marqués coûteux, à la levée d'incertitudes concernant l'identification de composés ou encore à la purification de molécules volatiles d'intérêt thérapeutique (thymol, carvacrol, camphre, …).

Nous devons toutefois formuler quelques réserves quant au système GC-COOL. Aujourd'hui, nous sommes limités dans la quantité injectée compte tenu du système capillaire de la chromatographie. Dans le but de réduire le nombre d'injections chromatographiques, des travaux avec des injecteurs grand volume et des colonnes capillaires de diamètre plus importants pourraient également être envisagés. Une autre façon de réaliser moins d'injections serait d'améliorer le rendement en évitant de favoriser une récupération sélective de certains composés. Cependant, les problèmes d'effet aérosol dû à de trop grandes différences entre la température de cryoconcentration et la température ambiante rendent ce développement assez difficile.

De même, pour améliorer le rendement de récupération des composés, nous pourrions thermostater l'éthanol à différentes températures afin d'améliorer l'élution et la reprise des composés condensés, mais il semblerait que ce soit plus le caractère manuel de l'élution qui soit essentiel.

SYNTHÈSE ET PERSPECTIVES

Conclusion

Le fumage industriel tel qu'il est pratiqué aujourd'hui va être amené à évoluer sous la pression de contraintes sanitaires, environnementales et organoleptiques. Il est donc primordial pour les professionnels d'anticiper ces développements par une meilleure connaissance du procédé.

Les études entreprises dans ce manuscrit ont permis d'observer que le fumage du saumon tel qu'il est pratiqué dans l'industrie actuellement est en conformité avec la législation européenne. Les taux de HAP et notamment de benzo[a]pyrène sont en effet largement inférieurs à ceux imposés. Cependant, en fonction des pays, les codes d'usage ne sont pas les mêmes et les teneurs finales en HAP sont très variables. Ainsi, le dosage des HAP dans les aliments fumés doit être réalisé de manière quasi-systématique afin de garantir la sécurité sanitaire des aliments fabriqués.

Les techniques de fumage sont responsables du relargage de la fumée dans l'atmosphère du fait de son temps de séjour limité dans le fumoir. Il en résulte une pollution environnementale. Afin de réduire et de contrôler cette contamination, l'utilisation de condensats de fumée liquide va être amenée à se généraliser comme aux Etats-Unis. Nos travaux ont montré que la revaporisation de fumée liquide en enceinte de fumage se caractérise par une absence de rejets atmosphériques lors du traitement des denrées. De plus, la contamination finale en HAP du produit traité par fumée liquide est similaire à celle obtenue avec d'autres modes industriels de génération de fumée. Cependant, nous avons pu mettre en évidence un problème sur le statut du fumage par fumée liquide car ce procédé peut être assimilé à une technique de fumage comme en France dans la filière carnée, conduisant dans notre cas à des produits conformes (< 5 µg de benzo[a]pyrène / kg). Néanmoins, il peut aussi être considéré comme une technique d'aromatisation ce qui conduit dans notre cas à des produits non-conformes puisque la teneur en benzo[a]pyrène de ces produits dépasse celle autorisée (0,03 µg de benzo[a]pyrène / kg). Une autre critique peut être formulée à l'égard de la fumée liquide concernant la différence observée entre les caractéristiques organoleptiques du saumon fumé par les techniques traditionnelles et celles du saumon traité par fumée liquide. Ainsi, le choix et l'amélioration des condensats de fumée reste une étape primordiale pour l'obtention de propriétés organoleptiques finales souhaitées.

Nos travaux ont également été conduits dans le but de comprendre les différences organoleptiques en fonction des procédés de fumage. Nous avons donc caractérisé les principales techniques industrielles de génération de fumée, y compris la revaporisation de fumée liquide, et étudié leurs effets sur l'odeur finale du produit. Nous avons mis en évidence trois catégories de

composés : ceux propres à la chair de poisson (dégradation des lipides, des acides aminés, …), ceux de la fumée (dégradation de la cellulose, de l'hémicellulose et surtout de la lignine) et ceux provenant de réactions entre les composés de la fumée et les constituants du poisson sous l'effet des paramètres de fumage. Ainsi, nous avons pu distinguer trois sortes de molécules parmi les composés de la fumée qui se retrouvaient dans la composition des composés volatils odorants du saumon fumé : les composés phénoliques, qui constituent le squelette odorant « fumé » du poisson fumé, les composés furaniques et les dérivés d'enolones dont le rôle pourrait être très important dans l'odeur globale. Le rôle des paramètres du fumage (température de pyrolyse, temps de fumage, …) ont été identifiés dans la génération des composés volatils odorants et reliés aux précurseurs de la chair de poisson et de la fumée pour les quatre types de générateurs de fumée. La température de pyrolyse est un paramètre essentiel pour comprendre la composition chimique de la fumée. Une température du foyer élevée favorise la déshydratation des constituants du bois ce qui conduit à l'augmentation des quantités de composés phénoliques et furaniques. Une température plus basse conduit à une déshydratation moins forte et moins rapide qui conduit à des quantités de composés phénoliques et furaniques moins importantes ce qui augmente la part des dérivés d'enolones parmi les composés volatils odorants du poisson fumé. De plus, grâce à l'étude des relations entre les teneurs en composés volatils odorants et la composition de l'odeur globale et grâce au développement d'un système de chromatographie préparative, nous avons pu mettre en évidence l'influence individuelle et en mélange de certains composés volatils les plus odorants sur l'odeur globale.

Ainsi, aujourd'hui, grâce aux résultats obtenus, nous sommes en mesure de proposer des recommandations aux industriels afin de fabriquer des produits fumés aux odeurs préalablement requises en jouant sur les paramètres du fumage ayant été identifiés comme à l'origine de la création de composés volatils odorants responsables de ces odeurs tout en limitant la présence des HAP.

Perspectives

Les surveillances sanitaires et organoleptiques des produits fumés et la préoccupation environnementale du procédé de fumage sont à l'origine du développement des générateurs de fumée externes. Ceux-ci doivent assurer la production d'une fumée homogène et répétable d'une campagne de fumage à une autre afin de garantir la qualité organoleptique « fumé » des aliments. L'externalisation des générateurs a permis de produire une fumée moins nocive du point de vue

des contaminants du fumage puisque la distance à parcourir avant d'atteindre l'aliment est plus longue et que parfois des étapes de filtration et de purification peuvent avoir lieu entre le générateur et la cellule de fumage.

Cependant la fumée a un temps de séjour limitée dans la cellule de fumage avant d'être rejetée dans l'atmosphère ce qui est un inconvénient environnemental majeur. La fumée liquide se présente comme une alternative séduisante au fumage. Cependant, compte-tenu des différences organoleptiques entre les produits traités par fumée liquide et ceux fumés par les générateurs externes, compte-tenu des législations différentes entre le fumage et l'aromatisation, une synthèse sur les différents modes de génération de fumée se devait d'être réalisée.

Cependant, toutes nos conclusions (notamment organoleptiques) concernant les résultats obtenus sur les produits traités par fumée liquide sont à pondérer par le fait de l'utilisation d'un unique type de produit. Des analyses supplémentaires devraient être réalisées afin d'obtenir des conclusions plus solides concernant les caractéristiques organoleptiques et sanitaires que confèrent les condensats de fumée (fumées liquides aqueuses, huiles de fumée ou encore poudres de fumée solides) aux aliments. En effet, la technologie des condensats de fumée apparaît comme une solution d'avenir pour le fumage. Aux Etats-Unis, 70 % des aliments fumés le sont par fumée liquide. Les condensats de fumée apportent de nombreux avantages industriels et sanitaires. Au niveau industriel, l'utilisation de tels produits permet de réduire les risques d'accident matériel et humain puisqu'il n'y a plus de pyrolyse in situ, le stockage est plus aisé et plus sûr que le stockage de bois, la conservation est plus facile, le nettoyage des appareils également. L'utilisation de fumée liquide permet l'élimination de rejets dans l'atmosphère de fumée résiduelle et l'obtention de caractéristiques organoleptiques plus homogènes et répétables. Au niveau sanitaire, des étapes de purification et de filtration permettent une réduction efficace et contrôlée des HAP et de garantir une qualité sanitaire répétable des produits tout au long de l'année. Cependant, l'utilisation de condensats de fumée exige une traçabilité du procédé d'obtention des condensats de fumée et une harmonisation des législations et de l'étiquetage pour éviter toute confusion de la part du consommateur. En effet, si l'on peut filtrer et débarrasser les condensats d'élements nocifs tels que les HAP, on peut également rajouter des composés. Ainsi, de nouvelles législations devront être mises en place pour statuer sur les possibilités de reformulation. En effet, il existe déjà des condensats de fumée liquide dans lesquels sont rajoutés des émulsifiants afin d'homogénéiser la fumée liquide. A l'heure actuelle, en France, les produits traités par de tels condensats jouissent du même étiquetage que des produits traités par fumée liquide sans adjuvant. Ainsi, une communication et une plus grande transparence sur de tels procédés devront être mise en place. En effet, certaines catégories de

consommateurs (groupes religieux, allergies, …) exigent plus de clarté dans l'étiquetage des produits fumés face à la diversité des condensats de fumée. Dans certains groupes religieux, la nourriture doit subir un rite avant d'être ingérée et provenir de source sûre. Ces consommateurs seraient alors trompés par l'utilisation d'adjuvants non autorisés par leur conviction religieuse. Dans le cas des allergies alimentaires, l'utilisation de certains adjuvants dans les condensats de fumée peut conduire à des intoxications plus ou moins graves. Il est donc nécessaire d'augmenter la traçabilité sur de tels produits.

De même, des analyses hédoniques supplémentaires seront nécessaires pour connaître les réactions des consommateurs vis-à-vis des descripteurs organoleptiques afin d'orienter les procédés de fumage vers ces préférences tout en tenant compte de la conformité des produits vis-à-vis des normes sanitaires et microbiologiques que les changements de paramètres de fumage induiront. En effet, les différents produits ont été discriminés selon leurs intensités odorantes en fonction de certains descripteurs mais nous n'avons pas d'informations sur les descripteurs préférés des consommateurs. Ce genre de renseignements pourrait nous aider dans l'établissement de recommandations aux industriels car pour l'instant, il paraît impossible de favoriser simultanément des odeurs beurrées et des odeurs de fumée « feu de bois » obtenues pour des intensités différentes de fumage. Il faudra donc anticiper ces cas de figures en ciblant au mieux les rôles des paramètres de fumage dans la création de composés volatils odorants responsables de la perception de ces odeurs globales.
Une validation des résultats pourrait être programmée afin de vérifier la réciprocité des conclusions. En effet, nous avons pu discriminer les produits selon leur mode de production de fumée et les paramètres de fumage mais sommes-nous réellement en mesure de réaliser la traçabilité d'un produit fumé ? Pouvons-nous certifier du mode de génération de fumée et des paramètres de fumage avec un dosage des composés volatils odorants et d'HAP et avec des résultats d'analyse sensorielle ? Les recommandations que nous pouvons formuler aux industriels ne sont établies que dans le sens de la caractérisation mais la performance de cette caractérisation devra être évaluée pour renseigner sur la pertinence de ces recommandations.

D'autres travaux devront être également menés avec la stratégie GC-COOL afin d'identifier le rôle individuel et en mélange des composés volatils odorants et leur contribution dans les odeurs du saumon fumé. En effet, l'originalité de la méthode « descendante » d'omission des composés volatils odorants de l'extrait aromatique ensuite redéposé sur matrice réelle permet de prendre en compte les éventuelles interactions avec les autres composés volatils odorants ou non et avec les

constituants de la matrice. Cette stratégie permet de partir de l'extrait initial et d'en omettre les constituants alors que les démarches « ascendantes » réalisées antérieurement par certaines équipes partaient de standards aromatiques pour se rapprocher autant que possible de l'odeur initiale. Ainsi ces études ne prennaient en compte que les odeurs individuelles des composés volatils odorants dans leurs formulations de fumées. Or, l'odeur globale n'est pas constituée uniquement de la somme des odeurs individuelles de composés odorants mais doit également prendre en compte les interactions odorantes de masque, de synergie … Ainsi, le manque d'adéquation entre les composés phénoliques odorants suspectés de constituer le squelette « fumé » de produits fumés et l'odeur « fumée » de tels produits avait interprété par la nécessité de prendre en compte les composés carbonylés odorants. Certes, les composés carbonylés odorants interviennent dans l'odeur globale « fumée » au même titre que les composés phénoliques odorants. Cependant, nos travaux ont montré l'égale nécessité de prendre en compte les interactions odorantes dont les effets peuvent parfois être surprenants. Le système GC-COOL constitue l'un des premiers appareils permettant de réaliser des études de l'influence des composés volatils odorants sur l'odeur globale d'une matrice et de prendre en compte ces interactions. Cependant, la définition d'un cadre d'étude précis ainsi que des analyses préliminaires sont nécessaires pour optimiser son utilisation. Nos travaux antérieurs ont ainsi permis d'obtenir des résultats olfactométriques d'une part, et sensoriels d'autre part, afin d'identifier les composés volatils odorants les plus impliqués dans un type de descripteurs odorants de l'odeur globale. Le traitement statistique Partial Least Squares s'avère un outil précieux pour orienter l'utilisation du GC-COOL. En effet, étant encore au début du développement de ce système d'omission, étant donné le coût opérationnel qu'entraîne son emploi, il est important d'optimiser les injections et d'utiliser le GC-COOL en tant que moyen de vérification car son utilisation en routine reste encore à mettre au point.

De même, les nouvelles perspectives de recherches offertes par le GC-COOL avec notamment la possibilité d'isoler et de concentrer les composés d'une certaine zone chromatographique doivent être concrètement entreprises pour définitivement asseoir son efficacité. L'obtention de standards aromatiques, le recyclage d'étalons internes marqués coûteux ou encore la purification de molécules volatiles d'intérêt thérapeutique (thymol, camphre, …) doivent être réalisés ce qui ouvre de nouvelles possibilités de collaborations autres que dans le milieu de la recherche alimentaire. L'utilisation de molécules marquées en tant qu'étalons internes est souvent effectuée dans le secteur de la recherche environnementale tandis que la purification de molécules à potentialités thérapeutiques intéresserait le secteur de la recherche clinique ou plus largement le

milieu de la santé. Ainsi, il serait intéressant de confronter la performance d'un tel système dans un cadre de recherches dans lequel il n'a pas été conçu au préalable.

En ce qui concerne les études sur les HAP, ces travaux ont également servi et pourront continuer à alimenter la réflexion autour du procédé de fumage (Proposed draft code of practice for the reduction of contamination of food with polycyclic aromatic hydrocarbons (PAH) from smoking and direct drying processes (FAO-WHO CX/CF 07/1/16 (E))) et du statut de la fumée liquide. En effet, l'atomisation de fumée correspond à une méthode d'aromatisation de surface (par opposition à une aromatisation de formulation, qui a lieu durant la fabrication de l'aliment) de l'aliment et doit se conformer aux taux imposés pour des méthodes d'aromatisation. Cependant, en France, ce procédé a déjà été assimilé à une méthode de fumaison classique par la DGCCRF, tout en devant respecter les taux imposés pour des méthodes d'aromatisation. Cependant, la différence entre les valeurs maximales autorisées dans les aliments fumés (5 µg/kg) et ceux traités par fumée liquide (0.03 µg/kg) montre bien que ces procédés ne peuvent être considérés équivalents. La faible concentration maximale autorisée de benzo[a]pyrène dans les produits traités par fumée liquide peut se comprendre étant donné l'importance des valeurs maximales autorisées en benzo[a]pyrène et benzo[a]anthracène (10 µg/kg et 20 µg/kg respectivement). Ces fortes valeurs peuvent s'expliquer par l'utilisation d'une moindre quantité de condensats de fumée liquide dans le cas d'aromatisation d'aliments par de la fumée liquide. Or, l'atomisation de fumée liquide dans une cellule de fumage emploie des quantités de fumée liquide bien supérieures à celles employées en aromatisation. Ainsi, une plus grande contamination en HAP serait attendue, ce qui n'est pas le cas car la concentration de HAP dans les fumées liquides est aujourd'hui largement en-dessous des valeurs autorisées (10 µg de benzo[a]pyrène / kg et 20 µg de benzo[a]anthracène / kg) qui ne semblent plus adaptées.

De ces constats ressortent trois points :

_ Si l'on veut que l'atomisation de fumée liquide soit assimilable à un fumage classique, alors il faut harmoniser les taux maximaux autorisés et ramener celui des produits traités par fumée liquide dans une cellule de fumage à ceux de la fumaison classique. La vaporisation de fumée liquide s'avérerait alors la méthode de fumage la moins vectrice d'HAP.

_ Si l'atomisation de fumée liquide ne peut être considérée comme un fumage classique, alors le taux maximal autorisé devra être celui des techniques d'aromatisation ce qui serait très difficile à obtenir compte-tenu de sa faiblesse (0.03 µg/kg) au regard des grandes quantités de fumée liquide vaporisées dans le fumoir. Ces produits seraient alors non conformes.

_ Enfin, dans tous les cas, les teneurs maximales de HAP dans les fumées liquides seraient à revoir. En effet, le dernier règlement européen en vigueur (2065/2003/EC) fixe des teneurs maximales en benzo[a]pyrène et benzo[a]anthracène de respectivement 10 et 20 µg/kg. Un grand pas a été réalisé depuis les règlements nationaux non harmonisés puisqu'en 1992, en Italie par exemple, les taux maximaux de benzo[a]pyrène et de benzo[a]anthracène étaient respectivement de 10 et 20 mg/kg. Cependant, aujourd'hui, les industriels arrivent à commercialiser des fumées liquides dont les concentrations en benzo[a]pyrène et benzo[a]anthracène sont largement en dessous du µg/kg ce qui explique qu'on puisse les utiliser en grandes quantités en revaporisation dans une cellule de fumage. Ainsi, les valeurs définies dans le règlement paru en 2003 paraissent déjà obsolètes et à redéfinir, surtout si l'atomisation de fumée liquide n'est pas considérée comme une méthode de fumage.

Ces travaux ont également permis de souligner la nécessité de l'harmonisation de la législation européenne sur la contamination maximale autorisée en HAP. En effet, le dernier règlement en vigueur (1881/2006/EC) fixe à 5 µg/kg le taux maximal de benzo[a]pyrène autorisé dans les aliments fumés. Or, ce taux peut sembler constituer une récession pour l'Allemagne ou la Slovaquie qui avait fixé une valeur nationale à 1 µg/kg (Wenzl, T., et al., 2006). Pour d'autres pays comme la France où il n'existait pas de valeur autorisée mais uniquement des recommandations, cette harmonisation est au contraire perçue comme bénéfique, surtout parce que la France fait partie des premiers producteurs mondiaux de saumon fumé. De plus, cette harmonisation garantit une sécurité sanitaire du point de vue des HAP de ce type d'aliments au niveau européen pour éviter de retrouver des produits d'importation dont les taux de HAP sont supérieurs à ceux autorisés dans le pays de consommation alors qu'ils avaient été fabriqués en respect des taux autorisés dans le pays de production.

Enfin, concernant les HAP, à la lumière de ces études, on peut s'interroger sur la nécessité de garder le benzo[a]pyrène comme marqueur de la contamination en HAP. En effet, d'autres HAP ont été montrés comme aussi sinon plus toxiques que le benzo[a]pyrène mais l'on dispose de beaucoup moins de recul et d'études sur ces composés comme les dibenzopyrènes. En effet, les récentes réglementations européennes ont recommandé le suivi de ces HAP lourds (1881/2006/EC). Ainsi, on ne peut se limiter au dosage unique du benzo[a]pyrène dans les aliments fumés. Les futurs règlements devront intégrer des taux maximaux en autres HAP. Une autre alternative serait d'établir une approche consensuelle de la contamination d'aliments par un mélange de HAP de concentrations et de toxicités différentes. Celle-ci devrait prendre en compte aussi bien les petites quantités d'HAP de haut poids moléculaires, toxiques et formés pendant les procédés thermiques industriels que les grandes quantités d'HAP de faible poids

moléculaire, peu toxiques et constituant l'essentiel de la contamination environnementale courante et pouvant également être formés durant les procédés thermiques industriels. L'approche par Facteurs d'Equivalence Toxique (TEF) conférant un potentiel de toxicité par rapport au benzo[a]pyrène pris comme référence aux différentes quantités d'HAP retrouvées dans un aliment, et additionnées pour donner une Quantité d'Equivalence Toxique (TEQ), paraît la plus intuitive et reste utilisée pour les dioxines. L'avantage est que la contamination en HAP est illustrée par une seule valeur mais de nombreux inconvénients sont également soulevés. En effet, cette méthode utilisée par l'AFSSA et l'INERIS, prend le benzo[a]pyrène comme référence pour établir les TEF mais le benzo[a]pyrène est-il vraiment le plus toxique des HAP ? De plus, cette approche reste empirique puisqu'elle suppose l'additivité des toxicités des HAP dans un aliment alors que des effets antagonistes ou synergiques peuvent avoir lieu et fausser le TEQ théorique.

Ainsi, l'optimisation des procédés de fumage, aussi bien dans la filière halieutique que dans la filière carnée devra aller de paire avec la surveillance constante des taux de HAP générés.

BIBLIOGRAPHIE

A

Abbott, N., Etiévant, P.X., Langlois, D., Lesschaeve, I., Issanchou, S. (1993). Evaluation of the representativeness of the odor of beer extracts prior to analysis by GC eluate sniffing. J. Agric. Food Chem., 41, 777-780.

Acree, T.E., Barnard, J.C., Cunningham, D.G. (1984). A procedure for the sensory analysis of gas chromatographic effluents. Food Chem.,14, 1821-1822.

Acree, T.E., Barnard, J.C. (1994). Gas chromatography-olfactometry and CharmAnalysis™. In : *trends in flavour research*. Eds H. Maarse et D. G. Van Der Heij, Elsevier Science BV, Amsterdam, 211-220.

AFSSA (2003). Avis de l'Agence française de sécurité sanitaire des aliments relatif à une demande d'avis sur l'évaluation des risques présentés par le benzo[a]pyrène (B(a)P) et par d'autres hydrocarbures aromatiques polycycliques (HAP), présents dans diverses denrées ou dans certaines huiles végétales, ainsi que sur les niveaux de concentration en HAP dans les denrées au-delà desquels des problèmes de santé risquent de se poser. Saisine n°2000-SA-0005.

Alasalvar, C., Anthony Taylor, K.D., Shahidi, F. (2005). Comparison of volatiles of cultured and wild sea bream (*Sparus aurata*) during storage in ice by dynamic headspace analysis/gas chromatography – mass spectrometry. J. Agric. Food Chem., 53, 2616-2622.

Alén, R., Oesch, P., Kuoppala, E. (1995). Py-GC/AED studies on the thermochemical behavior of softwood. J. Anal. Appl. Pyrolysis, 35, 259-265.

Alén, R., Kuoppala, E., Oesch, P. (1996). Formation of the main degradation compound groups from wood and its components during pyrolysis. J. Anal. Appl. Pyrolysis, 36, 137-148.

Andral, B., Stanisiere, J.Y., Sauzade, D., Damier, E., Thebault, H., Galgani, F., Boissery, P. (2004). Monitoring chemical contamination levels in the Mediterranean based on the use of mussel caging. Marine Pollution Bulletin, 49, 704-712.

Andreoli, R., Manini, P., Bergamaschi, E., Mutti, A., Franchini, I., Niessen, W.M.A. (1999). Determiantion of naphthalene metabolites in human urine by liquid chromatography – mass spectrometry with electrospray ionization. J. chromatogr. A, 847, 9-17.

Ansorena, D., Gimeno, O., Astiasarán, I., Bello, J. (2001). Analysis of volatile compounds by GC-MS of a dry fermented sausage : chorizo de Pamplona. Food Res. Int., 34, 67-75.

Aro, T., Brede, C., Manninen, P., Kallio, H. (2002). Determination of semivolatile compounds in Baltic herring (*Cuplea harengus membras*) by supercritical fluid extraction – supercritical fluid chromatography – gas chromatography – mass spectrometry. J. Agric. Food Chem., 50, 1970-1975.

Aursand, M., Bleivik, B., Rainuzzo, J.R., Jørgensen, L., Mohr, V. (1994). Lipid distribution and composition of commercially farmed Atlantic salmon (*Salmo salar*). J. Sci. Food Agric., 64, 239-248.

B

Baek, H.H., Cadwallader, K.R. (1997). Character-impact aroma compounds of crustaceans. In : Flavor and lipid chemistry of seafoods (Shahidi, F. & Cadwallader, K.R., Eds). Washington, D.C., American Chemical Society, 85-94.

Baird, W.M., Hooven, L.A., Mahadevan, B. (2005). Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action. Environ. Mol. Mutagen., 45, 106-114.

Bakker, M.I., Casado, B., Koerselman, J.W., Tolls, J., kollöffel, C. (2000). Polycyclic aromatic hydrocarbons in soil and plant samples from the vicinity of an oil refinery. The Science of the Total Environment, 263, 91-100.

Ballesteros, E., García-Sánchez, A., Ramos Martos, N. (2006). Simultaneous multidetermination of residues of pesticides and polycyclic aromatic hydrocarbons in olive and olive-pomace oils by gas chromatography/tandem mass spectrometry. J. Chromatogr. A., 1111, 89-96.

Barranco, A., Alonso-Salces, R.M., Bakkali, A., Berrueta, L.A., Gallo, B., Vicente, F., Sarobe, M. (2003). Solid-pahse clean-up in the liquid chromatographic determination of polycyclic aromatic hydrocarbons in edible oils. J. Chromatogr. A, 988, 33-40.

Barrefors, G., Petersson, G. (1995a). Volatile hydrocarbons from domestic wood burning. Chemosphere, 30(8), 1551-1556.

Barrefors, G., Petersson, G. (1995b). Assessment by gas chromatography and gas chromatography-mass spectrometry of volatile hydrocarbons from biomass burning. J. Chromatogr. A, 710, 71-77.

Barrefors, G., Björkqvist, S., Ramnäs, O., Petersson, G. (1996). Gas chromatographic separation of volatile furans from birchwood smoke. J. Chromatogr. A, 735, 151-155.

Bencze Røra, A.M., Birkeland, S., Hultmann, L., Rustad, T., Skåra, T., Bjerkeng, B. (2005). Quality characteristics of farmed Atlantic salmon (*Salmo salar*) fed diets high in soybean or fish oil as affected by cold-smoking temperature. Lebensm.-Wiss. U.-Technol., 38, 201-211.

Berdagué, J.L., Tournayre, P. (2004).Dispositif d'analyse par chromatographie en phase gazeuse olfaction et procédé. Brevet FR0350257.

Berdagué, J.L., Tournayre, P. (2005). Gas chromatography-olfaction analyses device and method. Patent WO/2005/001470 A2.

Bernet, C., Dirninger, N., Etiévant, P., Schaeffer, A. (1999). Evaluation de la représentativité aromatique d'extraits de vins de gewurtztraminer. Sciences des Aliments, 19, 701-709.

Berrill, I.K., Porter, M.J.R., Bromage, N.R. (2004). The influence of dietary lipid inclusion and daily ration on growth and smoltification in 1+ Atlantic salmon (*Salmo salar*) parr. Aquaculture, 242, 513-528.

Berset, J.D., Ejem, M., Holzer, R., Lischer, P. (1999). Comparison of different drying, extrcation and detection techniques for the determination of priority polycyclic aromatic hydrocarbons in background contaminated soil samples. Anal. Chim. Acta, 383, 263-275.

Birkeland, S., Bencze Røra, A.M., Skåra, T., Bjerkeng, B. (2004). Effects of cold smoking procedures and raw material characteristics on product yield and quality parameters of cold smoked Atlantic salmon (*Salmo salar* L.) fillets. Food Res. Int**.,** 37(3), 273-286.

Birkholz, D. A., Coutts, R.T., Hrudey, S.E. (1988). Determination of polycyclic aromatic compounds in fish tissue. J. Chromatogr., 449, 251-260.

Bramanti, E., D'Ulivo, L., Lomonte, C., Onor, M., Zamboni, R., Raspi, G., D'Ulivo, A. (2006). Determination of hydrogen sulfide and volatile thiols in air samples by mercury probe derivatization coupled with liquid chromatography–atomic fluorescence spectrometry. Anal. Chem. Acta, 579(1-2), 38-46.

Buttery, R.G., Turnbaugh, J.G., Ling, L.C. (1988). Contribution of volatiles to rice aroma. J. Agric. Food Chem., 36(5), 1006-1009.

Buttery, R.G., Teranishi, R., Ling, L.C., Turnbaugh, J.G. (1990). Quantitative and sensory studies on tomato paste volatiles. J. Agric. Food Chem., 38, 336-340.

C

Cadwallader, K.R., Tan, Q., Chen, F., Meyers, S.P. (1995). Evaluation of the aroma of cooked spiny lobster tail meat by aroma extract dilution analysis. J. Agric. Food Chem., 43, 2432-2437.

Campo, E., Ferreira, V., Escudero, A., Marqués, J.C., Cacho, J. (2005). Quantitative gas chromatography-olfactometry and chemical quantitative study of the aroma of four Madeira wines. Anal. Chem. Acta, in press.

Cardinal, M., Berdagué, J.L., Dinel, V., Knockaert, C., Vallet, J.L. (1997). Effet de différentes techniques de fumage sur la nature des composés volatils et les caractéristiques sensorielles de la chair de saumon. Sci. Alim., 17, 679-696.

Cardinal, M., Gunnlaugsdottir, H., Bjoernevik, M., Ouisse, A., Vallet, J.L., Leroi, F. (2004). Sensory characteristics of cold-smoked Atlantic salmon (*Salmo salar*) from European market and relationships with chemical, physical and microbiological measurements. Food Res. Int., 37, 181-193.

Cardinal, M., Cornet, J., Sérot, T., Baron, R. (2006). Effects of the smoking process on odour characteristics of smoked herring (*Cuplea harengus*) and relationships with phenolic compound content. Food Chem., 96, 137-146.

Carrapiso, A .I., Ventanas, J., García, C. (2002). Characterization of the Most Odour-Active Compounds of Iberian Ham Headspace. J. Agric. Food Chem. 50, 1996-2000.

Cha, Y.J., Cadwallader, K.R. (1995). Volatile Components in Salt-Fermented Fish and Shrimp Pastes. J. Food Sci. 60, 19-24.

Cha, Y.J., Cadwallader, K.R. (1998). Aroma-active compounds in skipjack tuna sauce. J. Agric. Food Chem., 46, 1123-1128.

Chan, W.S., Toledo, R.T. (1975). Effect of smokehouse temperature, humidity and air flow on smoke penetration into fish muscle. J. food Sci., 40, 240-243.

Chandrasekhar, T.C., Kaveriappa, K.M. (1985). A process for reduction of benzo(a)pyrene content in smoked oil sardine. In A. Reilley (Ed.), *Spoilage of tropical fish and product development*. FAO Fisheries Rep. No. 317, Rome, 262-266..

Charles, M., Martin, B., Ginies, C., Etiévant, P.X., Coste, G., Guichard, E. (2000). Potent Aroma Compounds of Two Red Wine Vinegars. J. Agric. Food Chem., 48, 70-77.

Chen, B.H., Wang, C.Y., Chiu, C.P. (1996). Evaluation of analysis of polycyclic aromatic hydrocarbons in meat products by liquid chromatography. J. Agric. Food Chem., 44, 2244-2251.

Chen, Y.C., Chen, B.H. (2003). Determination of polycyclic aromatic hydrocarbons in fumes from fried chicken legs. J. Agric. Food Chem., 51(14), 4162-4167.

Chiu, C.P., Lin, Y.S., Chen, B.H. (1997). Comparison of GC-MS and HPLC for overcoming matrix interferences in the analysis of PAHs in smoked food. Chromatographia, 44(9/10), 497-504.

Chung, H.Y., Cadwallader, K.R. (1994). Aroma extract dilution analysis of blue crab claw meat volatiles. J. Agric. Food Chem., 42, 2867-2870.

Chung, H.Y., Chen, F., Cadwallader, K.R. (1995). Cooked blue crab claw meat aroma compared with lump meat. J. food Sci., 60(2), 289-291.

Chung, H.Y. (1999) Volatile components in crabmeats of *Charybdis feriatus*. J. Agric. Food Chem., 47, 2280-2287.

Chung, H.Y., Yung, I.K.S., Kim, J.S. (2001) Comparison of volatile components in dried scallops (*Chlamys farreri* and *Patinopecten yessoensis*) prepared by boiling and steaming methods. J. Agric. Food Chem., 49, 192-202.

Chung, H.Y., Yung, I.K.S., Ma, W.C.J., Kim, J.S. (2002). Analysis of volatile components in frozen and dried scallops (*Patinopecten yessoensis*) by gas chromatography/mass spectrometry. Food Res. Int., 35, 43-53

Chung, H.Y., Yeung, C.W., Kim, J.S., Chen, F. (2006). Static headspace analysis-olfactometry (SHA-O) of odor impact components in salted-dried white herring (*Ilisha elongata*). Food Chem., in press.

Clifford, M.N., Tang, S.L., Eyo, A.A. (1980). Smoking of foods. Process Biochemistry, June/July, 8-11.

Cornu, M., Beaufort, A., Rudelle, S., Laloux, L., Bergis, H., Miconnet, N., Sérot, T., Delignette-Muller, M.L. (2006). Effet of temperature, water-phase salt and phenolic contents on *Listeria monocytogenes* growth rates on cold-smoked salmon and evaluation of secondary models. Int. J. Food Microbiol., 106(2), 159-168.

Cros, S., Lignot, B., Bourseau, P., Jaouen, P., Prost, C. (2004). Desalination of mussel cooking juices by electrodialysis : effect on the aroma profile. J. Food Eng., 69(4), 425-436.

Curioni, P.M.G., Bosset, J.O. (2002). Key odorants in various cheese types as determined by gas chromatography-olfactometry. Int. Dairy J. 12, 959-984.

D

Da Porto, C., Moret, S., Soldera, S. (2005). A study on the composition of distillates obtained from smoked marc. Anal. Chim. Acta, 563(1), 396-400.

Daun, H. (1979). Interaction of wood smoke components and foods. Food Technol., 33, 66-71.

Davis, S.C., Makarov, A.A., Hughes, J.D. (1999). Supersonic molecular beam – hyperthermal surface ionisation coupled with time-of-flight mass spectrometry applied to trace level detection of polynuclear aromatic hydrocarbons in drinking water for reduced sample preparation and analysis time. Rapid. Commun. Mass Spectrom., 13, 247-250.

Debonneville, C., Orsier, B., Flament, I., Chaintreau, A. (2002). Improved Hardware and Software for Quick Gas Chromatography-Olfactometry Using CHARM and GC-« SNIF » Analysis. Anal. Chem., 74, 2345-2351.

Dos Santos Barbosa, J.M., Ré-Poppi, N., Santiago-Silva, M. (2006). Polycyclic aromatic hydrocarbons from wood pyrolysis in charcoal production furnaces. Environ. Res., 101, 304-311.

Duedahl-Olesen, L., White, S., Binderup, M.L. (2006). Polycyclic aromatic hydrocarbons (PAH) in Danish smoked fish and meat products. Polycyclic Aromatic Compounds, 26, 163-184.

Dufour, C. (1991). Le fumage du poisson : étude bibliographique. Thèse pour le doctorat vétérinaire, ENV d'Alfort.

Dugay, A., Herrenknecht, C., Czok, M., Guyon, F., Pages, N. (2002). New procedure for selective extraction of polycyclic aromatic hydrocarbons in plants for gas chromatographic – mass spectrometric analysis. J. Chromatogr. A, 958, 1-7.

E

Easley, D.M., Kleopfer, R.D., Carasea, A.M.. (1981). Gas chromatographic-mass spectrometric determination of volatile organic compounds in fish. J. Assoc. Off. Anal. Chem., 64(3), 653-656.

Easton, M.D.L., Luszniak, D., Von der Geest, E. (2002). Preliminary examination of contaminant loadings in farmed salmon, wild salmon and commercial salmon feed. Chemosphere, 46, 1053-1074.

EC 88/388. (1988). Council Directive of 22 June 1988 on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production. Official Journal of the European Union, L. 184, 1-10.

EC 2065/2003. (2003). Regulation (EC) No 2065/2003 of the european Parliament and of the Council of 10 November 2003 on smoke flavourings used or intended for use in or on foods. Official Journal of the European Union, L. 309, 1-8.

EC 208/2005. (2005a). Commission Regulation (EC) No 208/2005 of 4 February 2005 amending Regulation (EC) No 466/2001 as regards polycyclic aromatic hydrocarbons. Official Journal of the European Union L 34 : 3-5.

EC 2005/108. (2005b). Commission Recommendation of 4 February 2005 on the further investigation into the levels of polycyclic aromatic hydrocarbons in certain foods. Official Journal of the European Union L 34 : 43-45.

EC 10/2005. (2005c).Commission Directive 2005/10/EC of 4 February 2005 laying down the sampling methods and the methods of analysis for the official control of the levels of benzo(a)pyrene in foodstuffs. Official Journal of the European Union L 34 : 15-20.

EC 1881/2006. (2006). Commission Regulation of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L 364 : 5-24.

Echarte, M., Angeles Zulet, M., Astiasaran, I. (2001). Oxidation process affecting fatty acids and cholesterol in fried and roasted salmon. J. Agric. Food Chem., 49, 5662-5667.

Espe, M., Nortverdt, R., Lie, Ø., Hafsteinsson, H. (2002). Atlantic salmon (*Salmo salar* L.) as raw material for the smoking industry. II : Effect of different smoking methods on losses of nutrients and the oxidation of lipids. Food Chem., 77, 41-46.

Espe, M., Kiesssling, A., Lunestad, B.T., Torrissen, O.J., Bencze Røra, A.M. (2004). Quality of cold smoked salmon collected in one French hypermarket during a period of 1 year. Lebensm.-Wiss. u.-Technol., 37, 627-638.

Etiévant, P.X., Moio, L., Guichard, E., Langlois, D., Lesschaeve, I., Schlich, P., Chambellant, E. (1994). Aroma extract dilution analysis (AEDA) and the representativeness of the odour of food extracts in : Trends and flavour research (Eds : H. Maarse and D.G. Van Der Heij, Elsevier Science B.V.), 179-190.

Etiévant, P.X., Callement, G., Langlois, D., Issanchou, S., Coquibus, N. (1999). Odor intensity evaluation in gas chromatography-olfactometry by finger span method. J. Agric. Food Chem., 47, 1673-1680.

F

Fazzalari, F.A.(1978). Compilation of odor and taste threshold values data. ASTM Data Series DS 48A. American Society for Testing and Materials, Philadelphia, U.S.A.

Ferrari, S., Mandel, F., Berset, J.D. (2002). Quantitative determiantion of 1-hydroxypyrene in bovine urine samples using high-performance liquid chromatography with fluorescence and mass spectrometric detection. Chemosphere, 47, 173-182.

Ferreira, V., Pet'Ka, J., Aznar, M. (2002a). Aroma extract dilution analysis. Precision and optimal experimental design. J. Agric. Food Chem., 50, 1508-1514.

Ferreira, V., Ortín, N., Escudero, A., López, R., Cacho, J. (2002b). Chemical characterization of the aroma of grenache rosé wines : aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. J. Agric. Food Chem., 50, 4048-4054.

Fiddler, W., Wasserman, A.E., Doerr, R.C. (1970). A “smoke” flavor fraction of a liquid smoke solution. J. Agric. Food Chem., 18(5), 934-936.

Flament, I. (1990). Analysis of boiled crab meat flavour : identification of new alcoholic constituents. Lebensm.-Wiss. u.-Technol., 23, 274-275.

Forehand, J.B., Dooly, G.L., Moldoveanu, S.C. (2000). Analysis of polycyclic aromatic hydrocarbons, phenols and aromatic amines in particulate phase cigarette smoke using simultaneous distillation and extraction as a sole sample clean-up step. J. Chromatogr. A, 898, 111-124.

Foster, W.W., Simpson, T.H., Campbell, D. (1961). Studies of the smoking process for foods. The role of smoke particles. J. Sci. Food Agric., 9, 635-644.

Frank, D.C., Owen, C.M., Patterson, J. (2004). Solid phase microextraction (SPME) combined with gas-chromatography and olfactometry-mass spectrometry for characterization of cheese aroma compounds. Lebensm.-Wiss. U.-Technol., 37, 139-154.

Frankel, E.N. (1983). Volatile lipid oxidation products. Prod. Lipid Res., 22(1), 1-33.

Friedrich, J.E., Acree, T.E. (1998). Gas chromatography olfactometry (GC/O) of dairy products. Int. Dairy Journal, 8, 235-241.

Fukami, K., Ishiyama, S., Yaguramaki, H., Masuzawa, T., Nabeta, Y., Endo, K., Shimoda, M. (2002). Identification of distinctive volatile compounds in fish sauce. J. Agric. Food Chem., 50, 5412-5416.

G

Gallart-Jornet, L., Barat, J.M., Rustad, T., Erikson, U., Escriche, I., Fito, P. (2007). A comparative study of brine salting of Atlantic cod (Gadus morhua) and Atlantic salmon (Salmo salar). J. Food Eng., 79(1), 261-270.

García-Falcón, M.S., Simal-Gándara, J. (2005). Polyclic aromatic hydrocarbons in smoke from different woods and their transfer during traditional smoking into chorizo sausages with collagen and tripe casings. Food Addit. Contamin., 22(1), 1-8.

Giessing, A.M.B., Mayer, L.M., Forbes, T.L. (2003). Synchronous fluorescence spectrometry of 1-hydroxypyrene : a rapid screening method for identification of PAH exposure in tissue from marine polychaetes. Marine Environ. Research, 56, 599-615.

Girard, J.P., Talon, R., Sirami, J. (1982). Le fumage électrostatique : son efficacité relativement à deux paramètres technologiques. Sci. Alim., 2, 1-15.

Girard, J.P. (1988). La fumaison, Technologie de la viande et des produits carnés.171-214.

Girard, B., Nakai, S. (1991). Static headspace gas chromatographic method for volatiles in canned salmon. J. Food Sci., 56(5), 1271-1274

Girard, B., Nakai, S. (1994a). Nonparametric discriminant analysis of static headspace volatiles for grading fresh Pacific salmon. J. Food Qual., 17(5), 409-421.

Girard, B., Nakai, S. (1994b). Grade classification of canned pink salmon with static headspace volatile patterns. J. Food Sci., 59(3), 507512.

Girard, B., Durance, T. (2000). Headspace volatiles of sockeye and pink salmon as affected by retort process. J. Food Sci., 65(1), 34-39.

Gómez-Míguez, M.J., Cacho, J.F., Fereeira, V., Vicario, I.M., Heredia, F.J. (2007).Volatile components of Zalema white wines. Food Chemistry, 100(4), 1464-1473.

González-Mancebo, S., García-Santos, M.P., Hernáadez-Bonito, J., Calle, E., Casado, J. (1999). Nitrosation of phenolic compounds : inhibition and enhancenements. J. Agric. Food Chem., 47(6), 2235-2240.

Gordon Bell, J., McEvoy, J., Webster, J.L., McGhee, F., Millar, R.M., Sargent, J.R. (1998). Flesh lipid and carotenoid composition of Scottish farmed Atlantic salmon (*Salmo salar*). J. Agric. Food Chem., 46, 119-127.

Goulas, A.E., Kontominas, M.G. (2005). Effect of salting and smoking-method on the keeping quality of chub mackerel (*Scomber japonicus*) : biochemical and sensory attrobutes. Food Chem., 93, 511-520.

Grigorakis, K., Taylor, K.D.A., Alexis, M.N. (2003). Organoleptic and volatile aroma compounds comparison of wild and cultured gilthead sea bream (*Sparus aurata*) : sensory differences and possible chemical basis. Aquaculture, 225, 109-119.

Grosch, W. (1987). Reactions of hydroperoxides – Products of low molecular weight. In : Autoxidation of unsaturated lipids (Ed Chan, H.W.S.), Academic Press : NewYork, 95-109.

Grosch, W. (1994). Determination of potent odourants in foods by aroma extract dilution analysis (AEDA) and calculation of odour activity values (OAVs). Flavour Fragrance J., 9,147-158.

Guillén, M.D., Manzanos, M.J., Zabala, L. (1995). Study of a commercial liquid smoke flavoring by means of gas chromatography / mass spectrometry and Fourier transform infrared spectroscopy. J. Agric. Food Chem., 43, 463-468.

Guillén, M.D., Manzanos, M.J. (1996a). Study of the components of a solid smoke flavouring preparation. Food Chem., 55(3), 251-257.

Guillén, M.D., Ibargoitia, M.L. (1996b). Relationships between the maximum temperature reached in the smoke generation processes from *vitis vinifera* L. shoot sawdust and composition of the aqueous smoke flavoring preparations obtained. J. Agric. Food Chem., 44, 1302-1307.

Guillén, M.D., Ibargoitia, M.L. (1996c). Volatile components of aqueous liquid smokes from *Vitis vinifera* L shoots and *Fagus sylvatica* L wood. J. Sci. Food Agric., 72, 104-110.

Guillén, M.D., Manzanos, M.J. (1997). Characterization of the components of a salty smoke flavouring preparation. Food Chem., 58(1/2), 97-102.

Guillén, M.D., Ibargoitia, M.L. (1998). New components with potential antioxidant and organoleptic properties, detected for the first time in liquid smoke flavoring preparations. J. Agric. Food Chem., 46, 1276-1285.

Guillén, M.D., Ibargoitia, M.L. (1999a). Influence of the moisture content on the composition of the liquid moke produced in the pyrolysis process of *Fagus sylvatica* L. wood. J. Agric. Food, Chem., 47, 4126-4136.

Guillén, M.D., Manzanos, M.J. (1999b). Extractable components of the aerial parts of *salvia lavandulifolia* and composition of the liquid smoke flavoring obtained from them. J. Agric. Food Chem., 47, 3016-3027.

Guillén, M.D., Ibargoitia, M.L. (1999c). GC/MS analysis of lignin monomers, dimers and trimers in liquid smoke flavourings. J. Sci. Food Agric., 79, 1889-1903.

Guillén, M.D., Manzanos, M.J. (1999d). Smoke and liquid smoke. Study of an aqueous smoke flavouring from the aromatic plant *Thymus vulgaris* L. J. Sci. Food Agric., 79, 1267-1274.

Guillén, M.D., Sopelana, P., Partearroyo, M.A. (2000a). Polycyclic aromatic hydrocarbons in liquid smoke flavorings obtained from different types of wood. Effect of storage in polyethylene flasks on their concentrations. J. Agric. Food Chem., 48, 5083-5087.

Guillén, M.D., Sopelana, P., Partearroyo, M.A. (2000b). Determination of polycyclic aromatic hydrocarbons in commercial liquid smoke flavorings of different compositions by gas chromatography – mass spectrometry. J. Agric. Food Chem., 48, 126-131.

Guillén, M.D., Sopelana, P., Partearroyo, M.A. (2000c). Study of several aspects of a general method for the determination of polycyclic aromatic hydrocarbons in liquid smoke flavourings by gas chromatography-mass spectrometry. Food Addit. Contamin., 17(1), 27-44.

Guillén, M.D., Manzanos, M.J., Ibargoitia, M.L. (2001). Carbohydrate and nitrogenated compounds in liquid smoke flavorings. J. Agric. Food Chem., 49, 2395-2403.

Guillén, M.D., Manzanos, M.J. (2002a). Study of the volatile composition of an aqueous oak smoke preparation. Food Chem., 79, 283-292.

Guillén, M.D., Errecalde, M.C. (2002b). Volatile components of raw and smoked black bream (*Brama raii*) and rainbow trout (*Oncorrhyncus mykiss*) studied by means of solid phase microextraction and gas chromatography/mass spectrometry. J. Sci. Food Agric., 82, 945-952.

Guillén, M.D., Manzanos, M.J. (2005a). Characteristics of smoke flavourings obtained from mixtures of oak (*Quercus* sp.) wood and aromatic plants (*Thymus vulgaris*. L. and *Salvia lavandulifolia* Vahl.). Flavour Fragr. J., 20, 673-685.

Guillén, M.D., Errecalde, M.C., Salmerón, J., Casas, C. (2005b). Headspace volatile components of smoked swordfish (*Xiphias gladius*) and cod (*Gadus morhua*) detected by means of solid phase microextraction and gas chromatography-mass spectrometry. Food Chem., 94(1), 151-156.

H

Hallier, A., Courcoux, P., Sérot, T., Prost, C. (2004). New gas chromatography-olfactometric investigative method, an dits application to cooked *Silurus glanis* (european catfish) odor characterization. J. Chromatogr. A, 1056(1-2), 201-208.

Hamm, R. (1977). Analysis of smoke and wood smoke. Pure & Appl. Chem, 49, 1655-1666.

Hanaoka, K., Vallet, N., Giampaoli, P., Heyd, B., MacLeod, P. (2001). Possible influence of breathing on detection frequency and intensity rating in gas chromatography-olfactometry. Food Chem., 72, 97-103.

Hattula, T., Elfving, K., Mroueh, U.M., Luoma, T. (2001). Use of liquid smoke flavouring as an alternative to traditional flue gas smoking of rainbow trout fillets (Oncorhyncus mykiss). Lebensm.-Wiss. U.-Technol., 34, 521-525.

Hedberg, E., Kristensson, A., Ohlsson, M., Johansson, C., Johansson, P.A., Swietlicki, E., Vesely, V., Wideqvist, U., Westerholm, R. (2002). Chemical and physical characterization of emissions from birch wood combustion in a wood stove. Atm. Environ., 36, 4823-4837.

Hierro, E., De La Hoz, L., Ordóñez, J.A. (2004). Headspace volatile compounds from salted and occasionally smoked dried meats (cecinas) as affected by animal species. Food Chem., 85, 649-657.

Hsieh, R.J., Kinsella, J.E. (1989). Oxidation of polyunsaturated fatty acids : mechanisms, products, and inhibition with emphasis on fish. Adv. Food Nutr. Res., 233-341.

Hultin, H.O. (1994). Lipid oxidation in fish muscle. In : *Advances in seafood biochemistry, composition and quality* (eds G. J. Flick, JR. And R.Y. Martin), Technomic Publishing company, Lancaster, Pennsylvania, 99-102.

Husaini, S.A., Cooper, G.E. (1957). Fractionation of wood smoke and the comparison of chemical composition of sawdust and friction smokes. Food Technol., 11, 499-502.

Huss, H.H. (1999). La qualité et son évolution dans le poisson frais. FAO Document technique sur les pêches. N°348, Rome. FAO – Organisation des Nations Unies pour l'alimentation et l'agriculture.

I

INERIS. Doornaert, B, Pichard, A, Gillet, C. (2003). Hydrocarbures aromatiques polycycliques (HAPs). Evaluation de la relation dose-réponse pour des effets cancérigènes : Approche substance par substance (facteurs d'équivalence toxique – FET) et approche par mélanges. Evaluation de la relation dose-réponse pour des effets non cancérigènes : Valeurs Toxicologiques de Référence (VTR). INERIS-DRC-03-47026-ETSC-BDo-N°03DR177.

J

Jacob, V., Denis, I., Foster, P. (1998). La métrologie des composés organiques volatils (COV) : méthodes et difficultés. Analusis magazine, 26(9), 33-37.

Janoszka, B., Warzecha, L., Błaszczyk, U., Bodzek, D. (2004). Organic compounds formed in thermally treated high protein food Part I: Polycyclic aromatic hydrocarbons. Acta Chromatographica, 14, 115-128.

Järvenpää, E., Huopalahti, R., Tapanainen, P. (1996). Use of supercritical fluid extraction-high performance chromatography in the determination of polynuclear aromatic hydrocarbons from smoked and broiled fish. J. Liq. Chrom. & Rel. Technol., 19(9), 1473-1482.

Jira, W. (2004a). Chemical reactions of smoking. Fleischwirtschaft International, 4 , 21-25

Jira, W. (2004b). A GC/MS method for the determination of carcinogenic polycyclic aromatic hydrocarbons (PAH) in smoked meat products and liquid smokes. Eur. Food Res. Technol., 218, 208-212.

Joffraud, J., Leroi, F., Roy, C., Berdagué, J.L. (2001). Characterisation of volatile compounds produced by bacteria isolated from the spoilage flora of cold-smoked salmon.

Jonsdottir, R., Olafsdottir, G., Martinsdottir, E., Stefansson, G. (2004). Flavor characterization of ripened cod roe by gas chromatography, sensory analysis, and electronic nose. J. Agric. Food Chem., 52, 6250-6256.

Jørgensen, L.V., Huss, H.H., Dalgaard, P. (2001). Significance of volatile compounds produced by spoilage bacteria in vacuum-packed cold-smoked salmon (*Salmo salar*) analyzed by GC/MS and multivariate regression. J. Agric. Food Chem., 49, 2376-2381.

Josephson, D.B., Lindsay, R.C., Stuiber, D.A.(1984). Variations in the occurences of enzymically derived volatile aroma compounds in salt- and freshwater fish. J. Agric. Food Chem., 32, 1344-1347.

Josephson, D.B., Lindsay, R.C. (1987). Retro-aldol degradations of unsaturated aldehydes: Role in the formation of*c*4-heptenal from *t*2, *c*6-nonadienal in fish, oyster and other flavors J. Am. Oil Chem. Soc., 64, 132-138

Josephson, D.B., Lindsay, R.C., Stuiber, D.A. (1991a). Influence of maturity on the volatile aroma compounds from fresh Pacific and Great Lakes salmon. J. Food Sci., 56(6), 1576-1579.

Josephson, D.B., Lindsay, R.C., Stuiber, D.A. (1991b).Volatile Carotenoid-related Oxidation Compounds Contributing to Cooked Salmon Flavour. Lebensm-Wiss. Technol. 24, 424-432.

K

Kacker, T., Haupt, E.T., Garms, C., Francke, W., Steinhart, H. (2002). Structural characterisation of humic acid-bound PAH residues in soil by 13C-CPMAS-NMR-spectroscopy : evidence of covalent bonds. Chemosphere, 48(1), 117-131.

Kangsadalampai, K., Butryee, C., Manoonphol, K. (1996). Direct mutagenicity of the polycyclic aromatic hydrocarbon-containing fraction of smoked and charcoal-broiled foods treated with nitrite in acid solution. Food Chem. Toxicol., 35, 213-218.

Kann, C.A., Meijer, G.A.L. (2007). The risk of contamination of food with toxic substances present in animal feed. Animal Feed Science and Technology, 133, 84-108.

Kannapan, S., Indra Jasmine, G., Jeyachandran, P., Tamil Selvi, A. (2000). Polyaromatic hydrocarbons in processed fishery products. J. Food Sci. Technol., 37(6), 596-601.

Karl, H., Leinemann, M. (1996). Determination of PAH in smoked fishery products from different smoking kilns. Zeitschrift fur Lebensmittel Unterschung und Forschung, 202, 459-464.

Karl, H. (1997). Influence of the smoking technology on the quality of smoked fish regarding undesirable compounds. In : Seafood from producer to consumer, integrated approach to quality, Luten, J.B., Børresen, T. and Oehlenschläger, J., Eds. Elsevier Science B.V., 633-639.

Kawai, T., Ishida, Y., Kakiuchi, H., Ikeda, N., Higashida, T., Nakamura, S. (1991). Flavor components of dried squid. J. agric. Food Chem., 39, 770-777.

Kazerouni, N., Sinha, R., Hsu, C.H., Greenberg, A., Rothman, N. (2001). Analysis of 200 food items for benzo(a)pyrene and estimation of its intake in an epidemiologic study. Food Chem. Toxicol., 39, 423-436.

Kennaway, E.L. (1924). On cancer producing tars and tar-fractions. Journal of Industrial Hygiene, 5(12), 462-488.

Kennaway, E.L. (1930). Further experiments on cancer-producing substances. Biochemical Journal, 24, 497-504.

King, A.J., Readman, W., Zhou, J.L. (2004). Determination of polycyclic aromatic hydrocarbons in water by solid-phase microextraction–gas chromatography–mass spectrometry. Anal. Chim. Acta, 523(2), 259-267.

Kishikawa, N., Wada, M., Kuroda, N., Akiyama, S., Nakashima, K. (2003). Determination of polycyclic aromatic hydrocarbons in milk samples by high-performance liquid chromatography with fluorescence detection. J. Chromatogr. B, 789, 257-264.

Kiss, G., Varga-Puchony, Z., Hlavay, J. (1996). Determination of polycyclic aromatic hydrocarbons in precipitation using solid-phase extraction and column liquid chromatography. J. Chromatogr. A, 725, 261-272.

Kjällstrand, J., Ramnäs, O., Petersson, G. (1998). Gas chromatographic and mass spectrometric analysis of 36 lignin-related methoxyphenols from uncontrolled combustion of wood. J. Chromatogr. A, 824, 205-210.

Kjällstrand, J., Petersson, G. (2001a). Phenolic antioxidants in alder smoke during industrial meat curing. Food Chem., 74, 85-89.

Kjällstrand, J., Petersson, G. (2001b). Phenolic antioxidants in wood smoke. Sci. Total Environ., 277, 69-75.

Knockaert, C. (1990). Le fumage du poisson. Service de la documentation et des publications, Ifremer, Brest.

Koffi Houessou, J., Benac, C., Delteil, C., Camel, V. (2005). Determination of polycyclic aromatic hydrocarbons in coffee brew using solid-phase extraction. J. Agric. Food Chem., 53, 871-879.

Kołodziejska, I., Niecikowska, C., Sikorski, Z.E., Kołakowska, A. (2004). Lipid oxidation and lysine availability in Atlantic mackerel hot smoked in mild conditions. Bull. Sea Fish. Institute, 161(1), 15-27.

Kostyra, E., Baryłko-Pikielna, N. (2005). Volatiles composition and flavour profile identity of smoke flavourings. Food Qual. Pref., 17(1-2), 85-95.

Kubota, K., Shijiyama, H., Kobayashi, A. (1986). Volatile components of roasted shrimp. Agric. Biol. Chem., 50(11), 2867-2873.

L

Lacoste, F., Raoux, R., Dubois, D., Soulet, B. (2003). Problématiques des hydrocarbures aromatiques polycycliques dans les corps gras. Oléagineux, corps gras, lipides, 10(4), 287-295.

Lafleur, A.L., Taghizadeh, K., Howard, J.B., Anacleto, J.F., Quilliam, M.A. (1996). Characterization of flame-generated C_{10} to C_{160} polycyclic aromatic hydrocarbons by atmospheric-pressure chemical ionization mass spectrometry with liquid introduction via heated nebulizer interface. J. Am. Soc. Mass Spectrom., 7, 276-286.

Lage Yusty, M.A., Cortizo Daviña, J.L. (2005). Supercritical fluid extraction and high-performance liquid performance liquid chromatography – fluorescence detection method for polycyclic aromatic hydrocarbons investigation in vegetable oil. Food Control, 16, 59-64.

Lantz, A.W., Vaisey, M. (1970). Flavor effects of different woods on whitefish smoked in a kiln with controlled temperature, humidity, and air velocity. Journal Fisheries Research Board of Canada, 27(7), 1201-1207.

Larráyoz, P., Ibáñez, F.C., Ordóñez, A.I., Torre, P., Barcina, Y. (2000). Evaluation of supercritical fluid extraction as sample preparation method for the study of Roncal cheese aroma. Int. Dairy J., 10, 755-759

Larsen, J.C., Larsen, P.B. (1998). Chemical carcinogens. Air Pollution and Health. Hester, R.E., Harrison, R.M. Eds. Cambridge, UK, 33-56.

Lee, G.H., Suriyaphan, O., Cadwallader, K.R. (2001). Aroma components of cooked tail meat of American lobster (*Homarus americanus*). J. Agric. Food Chem., 49, 4324-4332.

Lee, J.H., Schlautman, M.A., Carraway, E.R., Yim, S., Herbert, B.E. (2004). Quantifying ground-state complexation between Ag^+ and polycyclic aromatic hydrocarbons in dilute aqueous solution via fluorescence quenching. J. Photochemistry Photobiology A : Chemistry, 163, 165-170.

Le Guen, S., Prost, C., Demaimay, M. (2000a). Characterization of odorant compounds of mussels (*Mytilus edulis*) according to their origin using gas chromatography-olfactometry and gas chromatography-mass spectrometry. J. Chromatogr. A, 896, 361-371.

Le Guen, S., Prost, C., Demaimay, M. (2000b). Critical Comparison of Three Olfactometric Methods for the Identification of the Most Potent Odorants in Cooked Mussels (*Mytilus edulis*). J. Agric. Food Chem., 48(4), 1307-1314.

Le Guen, S., Prost, C., Demaimay, M. (2001). Evaluation of the representativeness of the odor of cooked mussel extracts and the relationship between sensory descriptors and potent odorants. J. Agric. Food Chem., 49, 1321-1327.

Le Guen, S. (2001). Caractérisation de l'arôme des moules (*Mytilus edulis*) par analyse sensorielle, chromatographique et olfactométrique. Thèse de doctorat, Université de Nantes, Faculté des sciences et des techniques.

Leis, J.R., Ríos, A., Rodríguez-Sánchez, L. (1998). Reactivity of phenolic nuclepohiles towards nitroso compounds. Part 2. Reaction with alkyl nitrites (*O*-nitroso compounds). J. Chem. Soc., Perkin Trans. 2, 2729-2733.

Lenges, J., Luks, D., Vo Thi, N.B. (1976). Dosage du 3,4-benzopyrène dans les produits de viande et de poissons fumés. Revue des Fermentations et des Industries Alimentaires, 31, 20-22.

Le Pape, M.A., Grua-Priol, J., Prost, C., Demaimay, M. (2004). Optimization of dynamic headspace extraction of the edible red algae *Palmaria palmata* and identification of the volatile components. J. Agric. Food Chem., 52, 550-556.

Leroi, F., Joffraud, J.J., Chevalier, F., Cardinal, M. (1998). Study of the microbiological ecology of cold-smoked salmon during storage at 8 °C. Int. J. Food Microbiol., 39, 111-121.

Li, S., Olegario, R.M., Banyasz, J.L., Shafer, K.H. (2003). Gas chromatography – mass spectrometry analysis of polycyclic aromatic hydrocarbons in a single puff of cigarette smoke. J. Anal. Appl. Pyrolysis, 66, 155-163.

Lindsay, R.C. (1994). Flavour of fish. In Seafoods : *chemistry, processing technology, and quality* (Eds Shahidi, F. and Botta, J.R.), Blackie academic and professional Press, NewYork 75-84.

Lintelmann, J., Fischer, K., Matuschek, G. (2006). Determination of oxygenated polycyclic aromatic hydrocarbons in particulate matter using high-performance liquid chromatography–tandem mass spectrometry. J. Chromatogr. A, 1133(1-2), 241-247.

López, R., Ezpeleta, E., Sánchez, I., Cacho, J., Ferreira, V. (2004). Analysis of the aroma intensities of volatile compounds released from mild acid hydrolysates of odourless precursors extracted from Trempanillo and Grenache grapes using gas chromatography-olfactometry. Food Chem., 88, 95-103.

Lundstrom, R.C., Racicot, L.C. (1983). Gas chromatographic determination of dimethylamine and trimethylamine in seafoods. J. Assoc. Off. Anal. Chem., 66(5), 1158-1163.

Lustre, A.O., Isseberg, P. (1969). Volatile components of hardwood sawdust smoke: components of phenolic fraction. J. Agr. Food Chem., 17(6), 1387-1393.

M

Maga, J.A. (1987). The flavor chemistry of wood smoke. Food Reviews Int., 3(1&2), 139-183.

Mansur, M.A., Bhadra, A., Takamura, H., Matoba, T. (2003). Volatile flavor compounds of some sea fish and prawn species. Fisheries Science, 69, 864-866.

Marcé, R.M., Borrull, F. (2000). Solid-phase extraction of polycyclic aromatic compounds. J. Chromatogr. A, 885, 273-290.

Marsili, L., Caruso, A., Fossi, M.C., Zanardelli, M., Politi, E., Focardi, S. (2001). Polycyclic aromatic hydrocarbons (PAHs) in subcutaneous biopsies of Mediterranean cetaceans. Chemosphere, 44, 147-154.

Marvin, C.H., Smith, R.W., Bryant, D.W., McCarry, B.E. (1999). Analysis of high-molecular-mass polycyclic aromatic hydrocarbons in environmental samples using liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J. Chromatogr. A, 863, 13-24.

McDaniel, M.R., Miranda-Lopez, R., Watson, B.T., Michaels, N.J., Libbey, L.M. (1989). Pinot noir: a sensory/gas chromatographic approach. In *Flavors and off-flavors*; Charalambous,G., Ed.; Elsevier Science B.V.: Amsterdam.

McGill, A.S., Hardy, R., Burt, J.R., Gunstone, F.D. (1974). Hept-cis-4-enal and its contribution to the off-flavour in cold stored cod. J. Sci. Food Agric., 25, 1477-1489.

Means, J.C. (1998). Compound-specific gas chromatographic / mass spectrometric analysis of alkylated and parent polycyclic aromatic hydrocarbons in waters, sediments, and aquatic organisms. Journal of AOAC International, 81(3), 657-672.

Mehinagic, E., Prost, C., Demaimay, M. (2003). Representativeness of apple aroma extract obtained by vacuum hydrodistillation : comparison of two concentration techniques. J. Food Sci., 68(8), 2411-2415.

Metz, B., Kersten, G. F. A., Hoogerhout, P., Brugghe, H. F., Timmermans, H. A., de Jong, A., Meiring, H., ten Hove, J., Hennink, W. E., Crommelin, D. J., and Jiskoot, W. (2004). Identification of formaldehyde-induced modifications in proteins: reactions with model peptides. J. Biol. Chem., 279, 6235-6243.

Miler, K.B.M., Sikorski, Z.E. (1990). Smoking in : Seafood : Resources, nutritional composition, and preservation, 163-180.

Milo, C., Grosch, W. (1995). Detection of odor defects in boiled cod and trout by gas chromatography-olfactometry of headspace samples. J. Agric. Food Chem., 43, 459-462.

Milo, C., Grosch, W. (1996). Changes in the odorants of boiled salmon and cod as affected by the storage of the raw material. J. Agric. Food Chem., 44, 2366-2371.

Moio, L., Chambellant, E., Lesschaeve, I., Issanchou, S., Schlich, P., Etiévant, P.X. (1995). Production of reprentative wine extracts for chemical and olfactory analysis. Ital. J. Food Chem., 3, 265-278.

Moret, S., Conte, L., Dean, D. (1999). Assessment of polycyclic aromatic hydrocarbon content of smoked fish by means of a fast HPLC/HPLC method. J. Agric. Food Chem., 47, 1367-1371.

Moret, S., Conte, L.S. (2000). Polycyclic aromatic hydrocarbons in edible fats and oils : occurence and analytical methods. J. Chromatogr. A, 882, 245-253.

Morita, K., Kubota, K., Aishima, T. (2001). Sensory characteristics and volatile components in aromas of boiled prawns prepared according to experimental designs. Food Res. Int., 34, 473-481.

Mottier, P., Parisod, V., Turesky, R.J. (2000). Quantitative determination of polycyclic aromatic hydrocarbons in barbecued meat sausages by gas chromatography coupled to mass spectrometry. J. Agric. Food Chem., 48, 1160-1166.

Mottram, D. S. (1992). Meat flavour, the chemistry of meat flavour. Meat Focus International, 1, 87-93.

Müller, W.D. (1991). Curing and smoking. Fleischwirtsch, 71(1), 61-65.

Munoz, D., Doumenq, P., Guiliano, M., Jacquot, F., Scherrer, P., Mille, G. (1997). New approach to study of spilled crude oils using high resolution GC-MS (SIM) and metastable reaction monitoring GC-MS-MS. Talanta, 45, 1-12.

N

Niedziela, J.C., MacRae, M., Ogden, I.D., Nesvadba, P. (1998). Control of Listeria monocytogenes in Salmon ; Antimicrobialeffect of salting, smoking and specific smoke compounds. Lebensm.-Wiss. u.-Technol., 31(2), 156-161.

Nirmaier, H.P., Fischer, E., Meyer, A., Henze, G. (1996). Determination of polycyclic aromatic hydrocarbons in water samples using high-performance liquid chromatography with amperometric detection. J. Chromatogr. A, 730, 169-175.

Nonier, M.F., Vivas, N., Vivas de Gaulejac, N., Absalon, C., Soulié, P., Fouquet, E. (2005). Pyrolysis-gas chromatography/mass spectrometry of *Quercus* sp. wood. Application to structural elucidation of macromolecules and aromatic profiles of different species. J. Anal. Appl. Pyrolysis, 75(2), 181-193.

Nordgarden, U., Hemre, G.I., Hansen, T. (2002). Growth and body composition of Atlantic salmon (*Salmo salar* L.) parr and smolt fed diets varying in protein and lipid contents. Aquaculture, 207, 65-78.

Nyman, P.J., Perfetti, G.A., Joe JR, F.L., Diachenko, G.W. (1993). Comparison of two clean-up methodologies for the gas chromatographic / ma ss spectrometric determination of low nanogram / gram levels of polynuclear aromatic hydrocarbons in seafood. Food Addit. Contam., 10(5), 489-501.

O

Ojeda, M., Bárcenas, P., Pérez-Elortondo, F.J., Albisu, M., Guillén, M.D. (2002). Chemical references in sensory analysis of smoke flavourings. Food Chem., 78(4), 433-442.

P

Pagliuca, G., Gazzoti, T., Zironi, E., Serrazanetti, G.P., Mollica, D., Rosmini, R. (2003). Determination of high molecular mass polycyclic aromatic hydrocarbons in a typical italian smoked cheese by HPLC-FL. J. Agric. Food CHem., 51, 5111-5115.

Pennarun, A.L., Prost, C., Demaimay, M. (2002). Identification and origin of the character-impact compounds of raw oyster *Crassostrea gigas*. J. Sci. Food Agric., 82, 1652-1660.

Pet'Ka, J., Ferreira, V., Cacho, J. (2005. Posterior evaluation of odour intensity in gas chromatography-olfactometry: comparison of methods for calculation of panel intensity and their consequences. Flavour Fragrance J., 20, 278-287.

Philips, D.H. (1999). Polycyclic aromatic hydrocarbons in the diet. Mutat. Res., 443, 139-147.

Piveteau, F., Le Guen, S., Gandemer, G., Baud, J.P., Prost, C., Demaimay, M. (2000). Aroma of fresh oysters *Crassostrea gigas* : Composition and Aroma Notes. J. Agric. Food Chem. 48, 4851-4857.

Pointet, K., Milliet, A. (2000). PAHs analysis of fish whole gall bladders and livers from the Natural Reserve of Camargue by GC/MS. Chemosphere, 40, 293-299.

Polligné, I., Collignan, A., Trystram, G. (2001). Characterization of traditional processing of pork meat into *boucané*. Meat Sci., 59, 377-389.

Pollien, P., Ott, A., Montignon, F., Baumgartner, M., Muñoz-Box, R., Chaintreau, A. (1997). Hyphenated Headspace-Gas Chromatography-Sniffing Technique : Screening of Impact Odorants and Quantitative Aromagram Comparisons. J. Agric. Food Chem., 45, 2630-2637.

Popp, P., Bauer, C., Möder, M., Paschke, A. (2000). Determination of polycyclic aromatic hydrocarbons in waste water by off-line coupling of solid-phase microextraction with column liquid chromatography. J. Chromatogr. A, 897, 1-2, 153-159.

Porter, P.J., Kramer, D.E., Kennish, J.M. (1992). Lipid composition of light and dark flesh from sockeye salmon. Int. J. Food Sci. Technol., 27, 365-369.
Priser, C., Etiévant, P.X., Nicklaus, S., Brun, O. (1997). Representative champagne wine extracts for gas chromatography olfactometry analysis. J. Agric. Food Chem., 45, 3511-3514.

Priser, C. (1997). Contribution à la caractérisation objective de l'arôme de vin de Champagne par des méthodes sensorielles et physico-chimiques. Dissertation ENSBANA : Dijon, France.

Prost, C., Sérot, T., Demaimay, M. (1998). Identification of the Most Potent Odorants in Wild and Farmed Cooked Turbot (*Scophtalamus maximus* L.). J. Agri. Food. Chem. 46, 3214-3219.

Prost, C., Hallier, A., Cardinal, M., Serot, T., Courcoux, P. (2004). Effect of storage time on raw sardine (*Sardina pilchardus*) flavor and aroma quality. J. Food Sci., 69(5), 198-204.

Pupin, A.M., Toledo, M.C.F. (1996). Benzo(a)pyrene in olive oils on the Brazilian market. Food Chem., 55(2), 185-188.

R

Rauber, C.D.S., Guterres, S.S., Schapoval, E.E.S. (2005). LC determination of citral in Cymbopogon citratus volatile oil. J. Pharmaceutical and Biomedical Analysis, 37(9), 597-601.

Refsgaard, H.H.F., Brockhoff, P.B., Jensen, B. (1998). Biological variation of lipid constituents and distribution of tocopherols and astaxanthin in farmed Atlantic salmon (Salmo *salar*). J. Agric. Food Chem., 46, 808-812.

Refsgaard, H.H.F., Haaar, A.M., Jensen, B. (1999). Isolation and quantification of volatiles in fish by dynamic headspace sampling and mass spectrometry. J. Agric. Food Chem., 47, 1114-1118.

Reports on Tasks for Scientific Cooperation (2004). Collection of occurrence data on polycyclic aromatic hydrocarbons in food. Repor of experts participating in Task 3.2.12 October 2004, Directorate-General Health and Consumer Protection.

Richter, H., Howard, J.B. (2000). Formation of polycyclic aromatic hydrocarbons and their growth to soot – a review of chemical reaction pathways. Progress in Energy and Combustion Science, 26, 565-608.

Rivera, L., Curto, M.J.C., Pais, P., Galceran, M.T., Puignou, L. (1996). Solid-phase extraction for the selective isolation of polycyclic aromatic hydrocarbons, azaarenes and heterocyclic aromatic amines in charcoal-grilled meat. J. Chromatogr. A, 731, 85-94.

Robb, D.H.F., Kestin, S.C., Warriss, P.D., Nute, G.R. (2002). Muscle lipid content determines the eating quality of smoked and cooked Atlantic salmon. Aquaculture, 205, 345-358.

Roda, A., Simoni, P., Ferri, E.N., Girotti, S., Lus, A., Rauch, P., Poplstein, M., Pospisil, M., Pipek, P., Hochel, I., Fukal, L. (1999). Determination of PAHs in various smoked meat products and different samples by enzyme immunoassay. J. Sci. Food Agric., 79, 58-62.

Rojo Camargo, M.C., Toledo, M.C.F. (2003). Polycyclic aromatic hydrocarbons in Brazilian vegetables and fruits. Food Control, 14, 49-53.

Rørvik, L.M. (2000). *Listeria monocytogenes* in the smoked salmon industry. Int. J. Food Microbiol., 62, 183-190.

Rusz, J., Miler, K.B.M. (1977). Physical and chemical processes involved in the production and application of smoke. Pure & Appl. Chem., 49, 1639-1654.

S

Sadhra, S., Wheatley, A.D. (2007). Polycyclic aromatic hydrocarbon emissions from clinical waste incineration. Chemosphere, 66(11), 2177-2184.

Sainclivier, M. (1985). L'industrie alimentaire halieutique. Des techniques ancestrales à leurs réalisations contemporaines : salage, séchage, fumage, marinage, hydrolysats. Bulletin scientifique et technique de l'Ecole Nationale Supérieure Agronomique Centre de Recherches de Rennes, 219-285.

Sampels, S., Pickova, J., Wiklund, E. (2004). Fatty acids, antioxidants and oxidation stability of processed reindeer meat. Meat Sci., 67, 523-532.

Sanders, E.B., Goldsmith, A.I., Seeman, J.I. (2003). A model that distinguishes the pyrolysis of D-glucose, D-fructose, and sucrose from that of cellulose. Application to the understanding of cigarette smoke formation. J. Anal. Appl. Pyrolysis, 66, 29-50.

Santana Rodríguez, J.J., Padrón Sanz, C. (2000). Fluorescence techniques for the determination of polycyclic aromatic hydrocarbons in marine environment : an overview. Analusis, 28(8), 710-717.

Sarrazin, C., Le Quéré, J.L., Gretsch, C., Liardon, R. (2000). Representativeness of coffee aroma extracts : a comparison of different extraction methods. Food Chem., 70, 99-106.

Schaller, E., J. O. Bosset, Escher, F. (1998). Electronic noses and their application to food. Lebens.-Wiss u.-Technol.,31, 305-316.

Sekiwa, Y., Kubota, K., Kobayashi, A. (1997). Characterisitic flavor components in the brew of cooked clam (Meretrix lusoria) and the effect of storage on flavor formation. J. Agric. Food Chem., 43(5), 826-830.

Selli, S., Rannou, C., Prost, C., Robin, J., Sérot, T. (2006). Characterization of aroma-active compounds in Rainbow trout (*Oncorhyncus mykiss*) eliciting an off-odour. J. Agric. Food Chem., 54(25), 9496-9502.

Senger-Emonnot, P., Rochard, S., Pellegrin, F., George, G., Fernandez, X., Lizzani-Cuvelier, L. (2006). Odour active aroma compounds of sea fig (*Microcosmus sulcatus*). Food Chem., 97, 465-471.

Sérot, T., Regost, C., Prost, C., Robin, J., Arzel, J. (2001a). Effect of dietary lipid sources on odour-active compounds in muscle of turbot (*Psetta maxima*). J. Sci. Food Agric., 81, 1339-1346.

Sérot, T., Prost, C., Visan, L., Burcea, M. (2001b). Identification of the main odor-active compounds in musts from French and Romanian hybrids by three olfactometric methods. J. Agric. Food Chem., 49, 1909-1914.

Sérot, T., Regost, C., Arzel, J. (2002). Identification of odour-active compounds in muscle of brown trout (*Salmo trutta*) as affected by dietary lipid sources. J. sci. Food Agric., 82, 636-643.

Sérot, T., Baron, R., Knockaert, C., Vallet, J.L. (2004). Effect of smoking processes on the contents of 10 major phenolic compounds in smoked fillets of herring (*Cuplea harengus*). Food Chem., 85, 111-120.

Shahidi, F., Naczk, M. (2003). Contribution of phenolic compounds to the flavor and color characteristics of foods. In : *Phenolics in food and nutraceuticals*, CRC Press Llc ; Boca Raton, Florida, USA.

Sheehan, E.M., O'Connor, T.P., Sheehy, P.J.A., Buckley, D.J., Fitzgerald, R. (1996). Effect of dietary fat intake on the quality of raw and smoked salmon. Irish Journal of Agricultural and Food Res., 35, 37–41.

Shimmo, M., Adler, H., Hyötyläinen, T., Hartonen, K., Kulmala, M., Riekkola, M.L. (2002). Analysis of particulate polycyclic aromatic hydrocarbons by on-line coupled supercritical fluid

extraction–liquid chromatography–gas chromatography–mass spectrometry. Atmospheric Environment, 36(18), 2985-2995.

Shimoda, M., Shiratsuchi, H., Minegishi, Y., Osajima, Y. (1993). Flavor deterioration of nonfermented coarse-cut sausage during storage. Flavor as a factor of quality for nonfermented sausage. 2. J. Agric. Food Chem., 41, 946-950.

Sigurgisladottir, S., Sigurdardottir, M.S., Torrissen, O.J., Vallet, JL.L., Hasteinsson, H. (2000). Effects of different salting and smoking processes on the microstructure and the texture of Atlantic salmon (*Salmo salar*) fillets. Food Res. Int., 33, 847-855.

Sikorski, Z.E. (1989). Smoking of fish and carcinogens. In : Fish smoking and drying (Burt, J.R., Eds). Elsevier Applied Science, London, UK, 73-83.

Silva Elipe, M.V., Huskey, S.E.W., Zhu, B. (2003). Application of LC–NMR for the study of the volatile metabolite of MK-0869, a substance P receptor antagonist**.** Journal of Pharmaceutical and Biomedical Analysis, 30(5), 1431-1440.

Sim, P.G., Boyd, R.K., Gershey, R.M., Guevremont, R., Jamieson, W.D, Quillian, M.A., Gergely, R.J. (1987). A comparison of chromatographic and chromatographic/mass spectrometric techniques for the determination of polycylic aromatic hydrocarbons in marine sediments. Biom. Environ. Mass Spectr., 14, 375-381.

Šimko, P. (1991). Changes of benzo(a)pyrene contents in smoked fish during storage. Food Chem., 40, 293-300.

Šimko, P. (2002). Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavourings additives. J. Chromatogr. B., 770, 3-18.

Šimko, P. (2005). Factors affecting elimination of polycyclic aromatic hydrocarbons from smoked meat foods and liquid smoke flavourings. Mol. Nutr. Food Res., 49, 637-647.

Simon, P., Lafontaine, M., Delsaut, P., Morele, Y., Nicot, T. (2000). Trace determination of urinary 3-hydroxybenzo(a)pyrene by automated column-switching high-performance liquid chromatography. J. Chromatogr. B, 748, 337-348.

Simon, R., De La Calle, B., Palme, S., Meier, D., Anklam, E. (2006). Composition and analysis of liquid smoke flavouring primary products. J. Sep. Sci., 28, 871-882.

Simon, R., Palme, S., Anklam, E. (2007). Validation (in-house and collaborative) of a method based on liquid chromatography for the quantitation of 15 European-priority polycyclic aromatic hydrocarbons in smoke flavourings : HPLC-method validation for 15 EU priority PAH in smoke condensates. Food Chem., in press.

Simoneit, B.R.T., Rogge, W.F., Lang, Q., Jaffé, R. (2000). Molecular characterization of smoke from campfire burning of pine wood (*Pinus elliottii*). Chemosphere : Global Change Science, 2, 107-122.

Siskos, I., Zotos, A., Taylor, A.K.D. (2005). The effect of drying, pressure and processing time on the quality of liquid-smoked trout (Salmo gairdnerii) fillets. J. Sci. Food Agric., 85(12), 2054-2060.

Speer, K., Steeg, E., Horstmann, P., Kühn, T., Montag, A. (1990). Determination and distribution of polycyclic aromatic hydrocarbons in native vegetable oils, smoked fidh products, mussels and oysters, and bream from the river Elbe. J. High Resolution Chrom., 13, 104-111.

Stephan, A., Bücking, M., Steinhart, H. (2000). Novel analytical tools for food flavours- a review. Food Res. Int., 33, 199-209.

Stołyhwo, A., Sikorski, Z.E. (2005). Polycyclic aromatic hydrocarbons in smoked fish – a critical review. Food Chem., 91(2), 303-311.

Storelli, M.M., Stuffler, R.G., Marcotrigiano, G.O. (2003). Polycyclic aromatic hydrocarbons, polychlorinated biphenyls, chlorinated pesticides (DDTs), hexachlorocyclohexane, and hexachlorobenzene residues in smoked seafood. J. Food Prot., 66(6), 1095-1099.

Suñen, E. (1998) Minimum inhibitory concentration of smoke wood extracts against spoilage and pathogenic micro-organisms associated with foods. Letters in Applied Microbiology, 27, 45-48.

Suñen, E., Aristimuño, C., Fernandez-Galian, B. (2003). Activity of smoke wood condensates against *Aeromonas hydrophila* and *Listeria monocytogenes* in vacuum-packaged, cold-smoked rainbow trout stored at 4 °C. Food Res. Int., 36, 111-116.

Swan, J.S., Burtles, S.M. (1978). The development of flavour in potable spirits. Chem. Soc. Rev., 7(2), 201-211.

T

Takino, M., Daishima, S., Yamaguchi, K., Nakahara, T. (2001). Determination of polycyclic aromatic hydrocarbons by liquid chromatography-electrospray ionization mass spectrometry using silver nitrate as a post-column reagent. J. Chromatogr. A, 928 , 53-61.

Tamakawa, K. (2004). Polycyclic aromatic hydrocarbons (PAHs). In : Pesticide, veterinary and other residues in food (Watson, D., Eds). Woodhead Publishing Limited, Cambridge, UK.

Tanchotikul, U., Hsieh, T.C.Y. (1989). Volatile flavor components in crayfish watse. J. Food Sci., 54(6), 1515-1520.

Tanchotikul, U., Hsieh, T.C.Y. (1991). Analysis of volatile flavor components in steamed rangia clam by dynamic headspace sampling and simultaneous distillation and extraction. J. Food Sci., 56(2), 327-331.

Tandon, K.S., Baldwin, E.A., Shewfelt, R.L. (2000). Aroma perception of individual volatile compounds in fresh tomatoes (*Lycopersicon esculentum*, Mill.) as affected by the medium of evaluation. Postharvest Biol. Technol., 20(3)261-268.

Tilgner, D.J. (1977). The phenomena of quality in the smoke curing process. Pure & Appl. Chem., 49, 1629-1638.

Timm, M., Jørgensen, B.M. (2002). Simultaneous determination of ammonia, dimethylamine, trimethylamine and trimethylamine-N-oxide in fish extracts by capillary electrophoresis with indirect UV-detection. Food Chemistry, 76, 509-518.

Timón, M.L., Carrapiso, A.I., Jurado, A., Van De Lagemaat, J. (2004). A study of the aroma of fried bacon and fried pork loin. J. Sci. Food Agric., 84, 825-831.

Tinkougdou Kabré, A., Diarra, D., Traoré, A. (2003). Le fumage du poisson au Burkina Faso : comparaison des caractéristiques et de la rentabilité de trois types de fumoirs améliorés. Cahiers d'études et de recherches francophones / Agricultures, 12(6), 409-417.

Toth, L., Potthast, K. (1984). Chemical aspects of the smoking of meat and meat products. Adv. Food Res., 29, 87-158.

Triqui, R., Reineccius, G.A. (1995a). Changes in flavor profiles with ripening of anchovy (*Engraulis encrasicholus*). J. Agric. Food chem., 43, 1883-1889.

Triqui, R., Reineccius, G.A. (1995b). Flavor development in the ripening of anchovy (*Engraulis encrasicholus* L.). J. Agric. Food chem., 43, 453-458.

Triqui, R., Zouine, K. (1999). Sensory and instrumental assessments of the ripening process of anchovy (*Engraulis encrasicholus*). Lebensm.-Wiss. U.-technol., 32, 203-207.

Triqui, R., Bouchriti, N. (2003). Freshness assessments of Moroccan sardine (*Sardina pilchardus*): comparison of overall sensory changes to instrumentally determined volatiles. J. Agric. Food Chem., 51(26), 7540-7546.

Triqui, R. (2006). Sensory and flavor profiles as a means of assessing freshness of hake (*Merluccius merluccius*) during ice storage. Eur. Food Res. Technol., 222, 41-47.

U

Ullrich, F., Grosch, W. (1987). Identification of most intense volatile flavour compounds formed during autooxidation of linoleic acid. Z.Lebensm.Unters.Forsch., 184, 277-282.

V

Van Gemert, L.J. (1981). Coordination of sensory and instrumental analysis. In : Isolation, separation and identification of volatile compounds in aroma research. Eds H. Maarse et R. Belz, Akademie-Verlag, Berlin, 240-258.

Van Ruth, S.M. (2001). Methods for gas chromatography-olfactometry : a review. Biomolecular Engineering, 17, 121-128.

Van Ruth, S.M. (2004). Evaluation of two gas chromatography-olfactometry methods : the detection frequency and perceived intensity method. J. Chromatogr. A, 1054(1-2), 33-37.

Van Stijn, F., Kerkhoff, M.A.T., Vandeginste, B.G.M. (1996). Determiantion of polycyclic aromatic hydrocarbons in edible oils and fats by on-line donor-acceptor complex chromatography and high-performance liquid chromatography with fluorescence detection. J. Chromatogr. A, 750, 263-273.

Vasiliadou, S., Ambrosiadis, I., Vareltzis, K., Fleroutis, D., Gavriilidou, I. (2005). Effect of smoking on quality parameters of farmed gilthead sea bream (*Sparus aurata* L.) and sensory attributes of the smoked product. Eur. Food Res. Technol., 2217, 232-236.

Vejaphan, W., Hsieh, T.C.Y., Williams, S.S. (1988). Volatile flavor components from boiled crayfish (Procambarus clarkii) tail meat. J. Food Sci., 53(6), 1666-1670.

Veyrand, B., Brosseaud, A., Sarcher, L., Varlet, V., Monteau, F., Marchand, P., Andre, F., Le Bizec, B. (2007). Innovative method for determination of 19 polycyclic aromatic hydrocarbons in food and oil samples using gas chromatography coupled to tandem mass spectrometry based on an isotope dilution approach. J. Chromatogr. A, 1149(2), 333-344.

Visciano, P., Perugini, M., Amorena, M., Ianieri, A. (2006). Polycyclic aromatic hydrocarbons in fresh and cold-smoked Atlantic salmon fillets. J. Food Prot., 69(5), 1134-1138.

Vives, I., Grimalt, J.O. (2002). Method for integrated analysis of polycyclic aromatic hydrocarbons and organochlorine compounds in fish liver. J. Chromatogr. B, 768, 247-254.

W

Wang, G., Lee, A.S., Lewis, M., Kamath, B., Archer, R.K. (1999). Accelerated solvent extraction and gas chromatography/mass spectrometry for determination of polycyclic aromatic hydrocarbons in smoked food samples. J. Agric. Food Chem., 47, 1062-1066.

Weisshoff, H., Preiss, A., Nehls, I., Win, T., Mugge, C. (2002). Development of an HPLC-NMR method for the determination of PAHs in soil samples – a comparison with conventional methods. Anal. Bioanal. Chem., 373(8), 810-819.

Wenzl, T., Simon, R., Kleiner, J., Anklam, E. (2006). Analytical methods for polycyclic aromatic hydrocarbons (PAHs) in food and the environment needed for new food legislation in the European Union. Trends in Analytical Chemistry, 25(7) , 716-725.

Wilkes, J.G., Conte, E.D., Kim, Y., Holcomb, M., Sutherland, J.B., Miller, D.W. (2000). Sample preparation for the analysis of flavours and off-flavours in foods. J. Chromatogr. A, 880, 3-33.

Wu, J., Wong, M.K., Lee, H.K., Shi, C.Y., Ong, C.N. (1997). Determination of polycyclic aromatic hydrocarbons in *Rougan*, a traditional chinese barbecued food, by capillary gas chromatography. Environmental Monitoring and Assessment, 44, 577-585.

Y

Yamagiwa, K., Ichikawa, K. (1915). Über die künstliche erzeugung von papillom. Verh. Jap. Pathol. Ges., 5, 606-616.

Yaylayan, A.V., Keyhani, A. (2001). Elucidation of the mechanism of pyrrole formation during thermal degradation of ^{13}C-labeled L-serines. Food Chem., 74, 1-9.

Yeakub Ali, M., Cole, R.B. (2001). SFE-plus-C_{18} cleanup and selective extraction method for GC/MS quantitation of polycyclic aromatic hydrocarbons in smoked meat. J. Agric. Food Chem., 49, 4192-4198.

Young, O.A., Berdagué, J.L., Viallon, C., Rousset-Akrim, S., Theriez, M. (1997). Fat-borne volatiles and sheepmeat odour. Meat Sci., 45(2), 183-200.

Ytrestøyl, T., Coral-Hinostroza, G., Hatlen, B., Robb, D.H.F., Bjerkeng, B. (2004). Carotenoid and lipid content in muscle of Atlantic salmon, *Salmo salar*, transferred to seawater as 0 + or 1+ smolts. Comparative Biochemistry and Physiology Part B, 138, 29-40.

Yurchenko, S., Mölder, U. (2005). The determination of polycyclic aromatic hydrocarbons in smoked fish by gas chromatography mass spectrometry with positive-ion chemical ionization. J. Food Composition and Anal., 18, 857-869.

Z

Zabik, M.E., Booren, A., Zabik, M.J., Welch, R., Humphrey, H. (1996). Pesticide residues, PCBs and PAHs in baked charbroiled, salt boiled and smoked Great Lakes lake trout. Food Chem., 55(3), 231-239.

Zamperlini, G.C.M., Santiago-Sivla, M., Vilegas, W. (2000). Solid-phase extraction of sugar cane soot extract for analysis by gas chromatography with flame ionisation and mass spectrometric detection. J. chromatogr. A, 889, 281-286.

RÉSUMÉ

Les travaux décrits dans ce manuscrit ont pour objectif la caractérisation des composés volatils responsables des qualités odorantes du saumon fumé ainsi que l'évaluation des contaminants chimiques apportés par le fumage. L'évaluation organoleptique a nécessité un développement méthodologique de caractérisation simultanée de l'odeur globale et des composés volatils odorants du saumon fumé. L'odeur globale a ainsi été évaluée par analyse sensorielle et la caractérisation des composés volatils odorants a nécessité la mise au point d'une méthode d'extraction quantitative et représentative de l'arôme de saumon fumé et d'une méthode d'analyse par chromatographie en phase gazeuse couplée à l'olfactométrie et la spectrométrie de masse. Cette double caractérisation a permis d'identifier les principaux composés volatils odorants de l'arôme du saumon fumé et d'en étudier l'influence sur la perception odorante globale du saumon fumé. L'évaluation sanitaire a nécessité un développement méthodologique pour la détermination des Hydrocarbures Aromatiques Polycycliques, contaminants du fumage, analysés par chromatographie en phase gazeuse couplée à la spectrométrie de masse en tandem.
Les méthodes développées ont été validées par leurs capacités à discriminer des saumons fumés par quatre techniques de fumage industriel aboutissant à l'obtention de produits aux qualités sensorielles, à des profils aromatiques et sanitaires différents.

TITLE

Characterisation of volatile compounds responsible for the odorant qualities of smoked salmon (*Salmo salar*) and assessment of contaminants of smoking process (Polycyclic Aromatic Hydrocarbons)

ABSTRACT
The works described in this manuscript aim to characterise the volatile compounds responsible for the odorant qualities of smoked salmon and to evaluate the chemical smoking contaminants occurence. The organoleptic evaluation required a methodological development of simultaneous characterisation of the overall odour and odorant volatile compounds of smoked salmon. Therefore overall odour was assessed by sensory analysis and the characterisation of odorant volatile compounds required the optimisation of a representative and quantitative extraction method of the smoked salmon aroma and an analysis method by gas chromatography coupled to olfactometry and mass spectrometry. This double characterisation allowed to identify the main odorant volatile compounds and to study their influence on the overall odorant perception of smoked salmon. The sanitary evaluation required a methodological development for the determination of Polycyclic Aromatic Hydrocarbons, contaminants of smoking process, by means of gas chromatography coupled to tandem mass spectrometry.
The methods used were validated by their ability to discriminate smoked salmons processed by four industrial smoking techniques leading to products with differences for sensory qualities, aroma and PAH profiles.

Mots clés : saumon fumé, olfactométrie, hydrocarbures aromatiques polycycliques, composés volatils odorants.

Discipline : Sciences de l'aliment

Spécialité : Chimie des arômes

N° :