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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-cyclopentenone	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-methylbenzene	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]

compounds	LRI (DB5)	means of identification[b]	odorant attributes given by the judges[c]	friction			liquid smoke		
				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 μg /100 g for smoldering to 653 μg/100 g for thermostated plates). The fact that guaiacol is perceived similarly even at different concentrations could indicate that the concentration 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 μg 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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