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1 **Use of random forest methodology to link aroma profiles to volatile compounds:**
2 **application to enzymatic hydrolysis of Atlantic salmon (*Salmo salar*) by-products**
3 **combined with Maillard reactions**

4
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16

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18 random forest, hydrolysate, Maillard reactions

19

20

21 1. Introduction

22 Today, using available resources has become a matter of major concern in all the sectors of
23 activity. This is particularly true in the context of the fishing industry, which produces
24 considerable quantities of by-products such as heads, viscera, skin, backbones, cutoffs and
25 blood. The waste may represent 65 % of the initial material in the case of the tuna canning
26 industry and a similar situation can be observed with farmed salmon. Although using major
27 waste as fishmeal (Refstie, Olli, & Standal , 2004; Nguyen, Pérez-Gálvez, & Bergé, 2012) is a

28 widespread practice, other applications can play a part in reducing this waste while offering
29 higher added value. Applications include recovery of long-chain polyunsaturated fatty acids (de
30 Oliveira et al. 2017), using bioactive compounds that are beneficial for human health
31 (Charoenphun, Youravong, & Cheirsilp, 2013), and developing cosmetic products
32 (Venkatesan, Anil, Kim, & Shim, 2017).

33 According to the FAO (2014), the need to gain approval from the regulatory authorities for the
34 specific health claims of nutraceuticals and health supplements may be a serious obstacle to
35 their development and they therefore consider that using the by-products from fish processing
36 directly as food, or indirectly as food by producing feed ingredients, is a more realistic solution.
37 Enzymatic hydrolysis has been studied extensively for over 30 years (Ravallec-Ple, Gilmartin,
38 Van Wormhoudt, & Le Gak, 2001; Halim, Yusof, & Sarbon, 2016) and appears to be an
39 efficient means of recovering valuable components, such as proteins, from marine biomass
40 (Sathivel *et al.*, 2003; Nguyen *et al.* 2011). In addition, developing cost efficient industrial food
41 grade protease has made it possible to produce new kinds of protein hydrolysate for different
42 applications (Aspevik, Egede-Nissen, & Oterhals, 2016). In the case of fish protein
43 hydrolysates (FPH), while their functional properties and nutritional value have been
44 recognized as good, their use as food ingredients can be limited by the fish flavor that persists
45 even after processing (Sylla, Bergé, Prost, Musabyemariya, & Seydi, 2009).

46 To reduce or mask the natural fish odor in the products, one of the solutions could be to promote
47 the Maillard reaction (MR) during production of the hydrolysate by adding sugar to the by-
48 product (Kouakou et al., 2014; Zhao, Shen, Guo, Wu, & Dai, 2016). The MR is a complex
49 series of chemical interactions that occurs during the processing between the lysine amino
50 group in peptides or proteins and the carbonyl group of reducing sugars. This reaction leads to
51 a variety of intermediates and brown products such as melanoidins, which play an important
52 role in the aroma, taste and color of processed foods (Machiels & Istasse, 2002). The MR can

53 add a pleasant flavor to the food through the development of roasted notes and therefore play a
54 role in consumer acceptability. Temperature, time, pH, and water activity are all factors strongly
55 that are involved in MR (Ajandouz, Tchiakpe, Dalle Ore, Benajiba, & Puigserver, 2006), but
56 are also known to influence hydrolysate characteristics (Molla & Hovannisyan, 2011; Prabha,
57 Narikimelli, Infanshia Sajini, & Vincent, 2013). Producing fish hydrolysates with aromatic
58 notes such as a caramelized odor for human food applications is therefore challenging.
59 The main purpose of this work was to better understand the relationships between volatile
60 compounds and the odor properties of hydrolysates in order to identify the main compounds
61 potentially involved in sensory perceptions. To achieve this aim, an experimental design
62 methodology was used to create a range of samples thanks to variation in four factors:
63 enzyme/substrate ratio, hydrolysis time, quantity of sugar and cooking time. These parameters
64 were chosen as being representative of the main parameters involved in hydrolysis conditions
65 and controlled at the industrial scale. Parameter levels were set according to previous results
66 (Kouakou et al., 2014). After an hydrolysis step associated with Maillard reactions, the
67 hydrolysates were submitted to a panel for sensory description and gas chromatography was
68 used to quantify the volatile compounds. In line with the work carried out by Vigneau,
69 Courcoux & Symoneaux (2018), we assumed that the random forest methodology could be
70 applied to both link an entire sensory profile to volatile compounds, and identify the importance
71 of these compounds in sample sensory characteristics. This study was oriented towards the
72 relationships between volatile compounds and sensory profiles and will not include other results
73 on the chemical characteristics of hydrolysates.

74

75 2. Materials and methods

76 2.1. Raw material and additives

77 Salmon by-products (backbones from the filleting process) were provided by the company
78 Copalis (Boulogne/Mer, France) from fish processing plants. One hundred and fifty kg of by-
79 products were roughly ground and frozen at -20°C by Copalis and transferred to the laboratory
80 by refrigerated transportation. On arrival, the raw material was divided into four kg samples
81 and stored in plastic bags at -20°C until hydrolysis processing.

82 The enzymes used for the hydrolysis was provided in liquid form by Novozymes AS
83 (Bagsvaerd, Denmark). Novozym[®] F.M.2.4 L (EC number: 3.4.21.62) is a bacterial serine
84 endopeptidase (subtilisin) prepared from a strain of *Bacillus licheniformis*. This enzyme was
85 developed to hydrolyze food proteins. It also satisfies the purity requirements for food-grade
86 enzymes, as set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and
87 the Food Chemicals Codex (FCC). The optimal working conditions for Novozym[®] F.M.2.4 L
88 are reported to be a pH between 7 and 9 and a temperature between 30 and 65°C. Novozym[®]
89 F.M.2.4 L has a declared activity of 2.4 Anson Units (AU) g⁻¹ (Novo Nordisk AS). To protect
90 against oxidation of the hydrolysates, a commercial mixture of natural antioxidants, Naturox
91 (tocopherols and rosemary) from the company Jan Dekker International, was used at a level of
92 250 mg per kg of raw material. After preliminary experiments (Kouakou, 2012), this level was
93 chosen as the minimum content needed to significantly reduce lipid oxidation in hydrolysates.

94 Of all the sugars available for promoting flavor generation (Ames, Guy, & Kipping, 2001), D-
95 xylose was chosen for the good reactivity of pentose, as well as for economic reasons. Xylose
96 was provided by Danisco (Denmark).

97 The standards used to identify the volatile compounds were purchased on Sigma-Aldrich. The
98 following purity was specified: pentane (≥99.0%), hexane (≥97.0%), dodecane (99.0%),
99 methylbenzene (99.9%), ethylbenzene (≥99.0%), styrene (≥99.5%), benzaldehyde (≥99.0%),
100 acetaldehyde (≥99.5%), propanal (97%), 2-methylpropanal (99.0%), butanal (≥99.0%), 2-
101 methylbutanal (95.0%), 3-methylbutanal (97.0%), hexanal (98.0%), heptanal (95.0%), ethanol

102 ($\geq 99.8\%$), 1-propanol ($\geq 99.9\%$), 1-penten-3-ol (99.0%), (E)-2-penten-1-ol (95.0%), (Z)-2-
103 penten-1-ol (95.0%), 2-butanone (99.0%), 2,3-butanedione (97.0%), 1-hydroxy-2-propanone
104 (90.0%), ethyl acetate (99.5%), acetic acid ($\geq 99.9\%$), 3-methylbutanoic acid (99.0%), 2-
105 methylfuran (99.0%), furfural (99.0%), 2-methylpyrazine (99.0%), 2,5-dimethylpyrazine
106 ($\geq 99.9\%$), dimethyl disulfide (98.0%), methional ($\geq 97.0\%$), 3-methyl-1-butanol ($\geq 99.9\%$), γ -
107 butyrolactone (99.0%), 2-acetylthiazole (99.0%) and 2-furanmethanol (99.0%). Two other
108 standards were used: 2-methyl-1-propanol (Merck, 99.0%) and 2-propanone (Riedel de Haën,
109 $\geq 99.9\%$).

110

111

112 2.2. Experimental design

113 Four processing variables were investigated using the response surface methodology (RSM)
114 and a randomized three level-four factor Composite Draper-Lin design (Statgraphics Centurion
115 XV.II, Statpoint, Herndon, USA). The three levels chosen for the selected factors were
116 enzyme/substrate ratio (E/S) (0.1, 0.25, and 0.4 %) (w/w), hydrolysis time (HT) (10, 50, 90
117 min), sugar (xylose) concentration (X) (2, 6, 10 g.kg⁻¹) and cooking time (CT) (30, 60, 90 min).
118 A total of nineteen experiments was required. Results from a previous study (Kouakou et al.,
119 2014) have shown that adding 10g of sugar to 1 kg of by-product was enough to develop roasted
120 notes during enzymatic hydrolysis. This level was thus set as the high level in the experimental
121 design in order to limit any possible residual sugar in the hydrolysate. A sample produced
122 without added sugar and in hydrolysis conditions set at the highest level for each factor (E/S,
123 0.4; hydrolysis time, 90 min; cooking time, 90 min) was introduced as a supplementary sample
124 to illustrate a non-Maillard reaction sample (Table 1). Each sample was the result of one
125 production. The central point of the experimental design was repeated three times (samples 5,
126 10, and 17) in order to test the repeatability of the productions.

127

128 2.3 Enzymatic hydrolysis

129 Frozen minced by-products were thawed at 4°C for 15 hours. For each design experiment, 4 kg
130 of salmon by-products were ground with antioxidant at a knife rotation speed of 1000 rpm, in
131 a Roboqbo Qb8-3 reactor (capacity of 8 liters) (Bentivoglio, Italy). The reactor had a double
132 jacket to make thermal exchanges (heating or cooling) possible, and thus reached the optimal
133 temperature of 40°C for the enzyme within 5 minutes. Hydrolysis was started at a speed of 300
134 rpm by adding the enzyme. pH was not controlled in order to stay close to industrial conditions.
135 Once the hydrolysis time had elapsed, xylose was added just before stopping the hydrolysis
136 reaction by heating the product to 95°C for 30 to 90 min, depending on the experimental design
137 (cooking time factor). The choice of cooking conditions (time and temperature) was defined in
138 order to favor the Maillard reactions while at the same time providing sufficient inactivation
139 time for enzyme activity in agreement with regulatory obligation. Once this step had been
140 completed, the temperature of the reactor was adjusted down to a temperature of 40°C and the
141 hydrolyzed product was removed through a sieve to eliminate the bones. This product was then
142 centrifuged at 8200 g for 30 min at 20°C in a Beckman coulter to separate and collect the
143 aqueous fraction. In this paper, the word hydrolysate will refer to this fraction. All the samples
144 were stored at -80°C for further sensory and biochemical analyses.

145

146

147

148 2.4. Sensory evaluation

149 The sensory analysis was carried out with sixteen panelists (12 females, 4 males, between 32
150 and 65 years old) from an internal panel at IFREMER. They already had experience in salmon
151 hydrolysate evaluation and had received training in the quantification of descriptors for 1h twice

152 a week over a three-month period (Cardinal, Baron, Kouakou, Prost, & Courcoux, 2014), but
153 received further training before starting this experiment. During preliminary screening on
154 process parameters, the following steps were proposed: - a sorting task on odor perception with
155 21 hydrolysates, - a discussion session with the whole panel in order to find a consensus on the
156 main discriminative odors; this discussion was based on the results of the sorting as well as the
157 list of descriptors previously used, -a scoring session where panelists were invited to test 6
158 samples illustrating the main characteristics of the hydrolysates in order to share a consensual
159 intensity level for each attribute, - two profiling sessions to check the panel's discriminative
160 power and the agreement between panelists and the whole panel. From the initial twenty-one
161 panelists, sixteen were selected for their ability to recognize the selected odors, and for the good
162 correlation between their individual scores and mean panel sensory scores. They were invited
163 then to carry out a quantitative descriptive analysis (Stone & Sidel, 2004) on the sensory
164 characteristics of salmon hydrolysates from the experimental design. The hydrolysates were
165 presented in plastic flasks wrapped in aluminum foil in accordance with the conditions
166 described by Kouakou et al. (2014). Using a continuous scale from 0 to 10, the panelists had to
167 score the six following odor descriptors: fatty fish, pickled (like pickled anchovies), roasted,
168 burnt, rancid and mud (sulfur notes). Twenty samples were scored in two sessions. Sample
169 presentation was balanced according to factor levels in order to have the range of variation for
170 each processing factor within each session. The tests were performed in individual booths
171 equipped with computers using data acquisition software (Fizz, Biosystems, Couternon,
172 France) under white lighting and at ambient temperature (20°C).

173

174 2.5. Volatile compounds

175 The procedure for analyzing the volatile compounds was adapted from Kouakou et al. (2014).

176 2.5.1. Extraction of the volatile compounds by Headspace Solid Phase MicroExtraction
177 (HS-SPME).

178 Five ml of hydrolysate were placed in a 20-mL glass vial closed with a screw top and equipped
179 with a Teflon septum. The sample was equilibrated for 60 min at 40°C. The extraction of the
180 volatile compounds was performed using a Carboxen/PDMS fibre (85 µm, 1 cm,
181 Carboxen/PDMS StableFlex, Supelco, Sigma-Aldrich Chimie, Lyon, France) for 15 min at
182 40°C. Analyses were performed in triplicate on each hydrolysate.

183 2.5.2. Gas chromatography / Mass spectrometry / FID

184 The apparatus used was a gas chromatograph (Agilent 7890A, Wilmington, DE, USA)
185 equipped with a flame ionization detector (FID) and coupled to a mass spectrometer (electronic
186 impact source, Agilent 5975CNetwork, Wilmington, DE, USA). The inlet temperature was
187 260°C, the FID detector temperature 250°C and the MS detector temperature 280°C. The carrier
188 gas was helium and the pressure was 150 kPa. The splitless mode was used for the injection,
189 and the desorption time was 7 min. The capillary column was a DB-WAX (30 m, 0.25 mm, 0.5
190 µm, J&W Scientific, Folsom, CA). The program used was 40°C for 10 min, ramped up to 240°C
191 at 7°C/min then equilibrium at 240°C for 3 min. Effluent from the end of the GC was split 1/1
192 between the MS and FID. Peaks were integrated with MSD Chemstation software (Agilent
193 Technologies). Mass spectra were recorded in electron impact mode (70 eV) between 33 and
194 300 m/z mass range at a scan rate of 2.7 scan.s⁻¹.

195 The volatile compounds were identified according to 3 criteria: comparison with the literature
196 of their Kovats retention index, comparison of their mass spectra with those of the Wiley 6
197 library, and comparison of their retention index with those of the corresponding standards when
198 the standard was available. The semi-quantified results were obtained from the FID
199 chromatogram and expressed as a peak area. The results obtained are only semi-quantitative in
200 order to compare the samples, but do not reflect the exact quantity of each volatile compound

201 present in the hydrolysate. Analyses were performed in triplicate on each hydrolysate which
202 means that for each volatile compound and each hydrolysate, the mean relative peak area is
203 obtained from 3 values.

204

205 2.6. Statistical analysis

206 A standardized Principal Component Analysis (PCA) was performed on the mean of the panel
207 score for each product and each sensory descriptor to highlight the main odor characteristics of
208 the products. The link between volatile organic compounds and sensory perception of the
209 products was investigated using regression trees and random forest methodologies. Regression
210 trees (RT) belong to recursive partitioning techniques and their aim is to predict a quantitative
211 response from a set of quantitative predictors (Breiman, Friedman, Olshen, & Stone, 1984). In
212 our case, the response was a sensory attribute; the panel mean score and predictors were the
213 volatile compounds. A regression tree can be considered as a set of decision rules created by
214 recursively splitting the whole set of products into subsets by maximizing the homogeneity of
215 the two resulting nodes. Random forests (RF) were introduced by Breiman (2001) and consist
216 in a large number of regression trees, randomly generated by resampling the training dataset in
217 order to improve the predictive accuracy of individual trees. Random forests make it possible
218 to compute the Variable Importance measure (VI) which quantifies the role played by each
219 variable in predicting the response. The confidence intervals of these importance measures were
220 obtained by repeating the RF on the same learning set. This technique is a simple tool for
221 selecting predictors with a significant effect on the response. The regression tree based on this
222 selection of compounds can be considered to be more robust than the one built on the complete
223 set of predictors.

224 **One of the main features of the random forest methodology is the robustness of the predictions,**
225 **obtained thanks to the construction principle of the forests: bagging (bootstrap aggregating).**

226 Each decision tree for a random forest is created from the training set by a doubly randomized
227 process: the bootstrapping of the individuals (random resampling with replacement of products
228 in our case) and the random selection of variables at each node of the trees (at each node, the
229 best volatile compound is chosen among a third of all the compounds). One single decision tree
230 has a tendency to overfit and the bagging process leads to an improvement in the predictive
231 performance. The samples that are not selected for a given tree (the Out-of-Bag or OOB
232 samples) may be used as a validation step or the solution. The computation of the Variable
233 Importance is based on the mean decrease in accuracy among all trees for the Out-of-Bag
234 samples when the values of the given variable are randomly permuted. Out-of-Bag samples
235 play the role of validation set without having to divide the data-set into calibration and
236 validation sets. In addition, the length of a decision tree (the number of leaves) is obtained by
237 minimizing the error of prediction generally obtained by LOO (leave one out) cross-validation
238 step.

239 This type of machine learning techniques has recently been used in many fields, including
240 sensory studies (Gomez-Meire, Campos, Falqué, Díaz, & Fdez-Riverola, 2014; Brillante et al.,
241 2015, Vigneau et al. 2018), demonstrating its accuracy and robustness even in the case of non-
242 linear relationships, interactions between predictors or high correlations among a set of
243 predictors. In addition, regression trees may be considered as a technique for supervised
244 clustering, providing decision rules and giving a simple interpretation of the link between
245 response and predictors.

246 As the sensory profile of products is composed of several sensory attributes, we considered a
247 multivariate generalization of the RT and RF methodologies. Introduced by De'ath (2002) in
248 the field of ecology, multivariate regression trees and random forests have been developed for
249 predicting a multivariate response. In this case, the splitting rule was based on the minimization

250 of the inertia in the child nodes. In our study, each node in the multivariate regression tree was
251 described by means of the sensory profile of the individuals belonging to this node.

252 Multivariate regression trees and random forests were carried out using language R 3.5.1 (R
253 Core Team, 2018) and the R packages mvpart (De'ath, 2014) and randomForestSRC (Ishwaran
254 & Kogalur , 2019).

255

256 3. Results and discussion

257 3.1. Odor characteristics of the hydrolysates

258 The first plane of the principal component analysis (PCA) with standardization performed on
259 the means of the sensory scores of each hydrolysate and each descriptor, accounted for 73.2%
260 of the total variance (Fig. 1a). The first axis (54.9% of total variance) was mainly created by
261 the roasted, pickled, rancid and fat criteria (Fig. 1b) and made a clear separation possible
262 between one group of samples associated with a roasted and pickled odor and three samples:
263 15, 20 and 16. These samples were distributed according to their main odor characteristic, mud
264 for sample 15, fat fish and rancid for numbers 16 and 20. The medium position of samples 8
265 and 12 on this first axis reflected intermediate sensory characteristics. The second axis (18.3%
266 of total variance) added specific information through the 'burnt' descriptor that particularly
267 differentiated samples 4 and 11. The three replicated samples, 5, 10 and 17, presented similar
268 profiles and were close on this sensory map; a clustering analysis performed on the principal
269 components of PCA confirmed that these samples were grouped in the same class of products
270 (not shown).

271 A first general approach suggested that the sample separation could not be explained only by
272 the level of sugar added to the by-product, but also by the specific process conditions associated.
273 While most hydrolysates on the right side of the sensory map were produced with the lowest
274 level of sugar or without sugar, an exception can be seen with sample 16. In this case, all the

275 factor conditions, including the sugar factor, were set at the high level except the cooking time
276 (CT) set at the lowest level. Sample 20, the only sample with no sugar added, presented similar
277 characteristics to sample 16, the highest scores for fatty fish and rancid odors. Although
278 hydrolysis conditions, such as a long hydrolysis time associated with a high enzyme/substrate
279 ratio, seemed favorable for producing small peptides and therefore for making reactions
280 possible between amino groups in peptides or proteins and reducing sugar, the results showed
281 that the hydrolysates were mainly characterized by odors illustrating an oxidation reaction. The
282 absence of sugar (sample 20) or a too short cooking time (sample 16) could explain these results.
283 Sample 15, characterized by a mud odor, was processed at the lowest level for each of the four
284 factors. Samples 8 and 12 had similar characteristics but at a lower intensity than sample 15.
285 The same level of xylose (2 g.kg^{-1}) in the three samples could suggest either the need to add a
286 sufficient quantity of sugar in the reaction mixture to favor a roasted aroma, and/or the
287 importance of combining other factors such as E/S, HT and CT at a required level for each of
288 them to prevent or mask the formation of sulfur notes (Farmer, Mottram & Whitfield, 1989). It
289 was likely that these hydrolysis conditions were not conducive to developing Maillard reactions
290 and their related aroma. In the case of the two samples separated on the second axis, samples 4
291 and 11, the only common processing condition for these two samples produced with medium
292 or high levels of sugar was the low level of E/S (0.1%). This low level could result in lower
293 enzyme activity and therefore a lower production of peptides with different sizes. Li, Zhong,
294 Yokoyama, Shoemaker, Zhu, & Xia (2013) mentioned in their study that rice protein
295 hydrolysates with a higher degree of hydrolysis were found to have more pyrazines such as 2,5-
296 dimethyl-pyrazine or methyl-pyrazine. The formation of these compounds from α -amino acids,
297 along with reducing sugars such as xylose could therefore be reduced as the hydrolysis
298 conditions were not favorable for small peptide production.
299

300 3.2. Volatile compounds in the hydrolysates

301 • Identification of volatile compounds

302 A total of 44 volatile compounds was identified in the hydrolysates (Table 2). The chemical
303 compounds belonged to various chemical classes such as aldehydes (7), ketones (7), alcohols
304 (6), benzene compounds (4), alkanes (3), sulfur compounds (3) and others (14). Most of the
305 compounds were identified in the 20 hydrolysates, with variation only in their quantity (Table
306 3).

307 Carbonyl compounds, **aldehydes** and **ketones** were the most abundant volatile compounds in
308 the hydrolysates. Aldehydes are generated via two main formation pathways: lipid oxidation
309 and Maillard reaction. Aliphatic aldehydes, such as hexanal, heptanal or nonanal, are mainly
310 derived from the lipid oxidation occurring in fish flesh (Varlet, Prost, & Sérot, 2007).. The
311 second pathway for producing aldehydes is through Strecker degradation, which occurs during
312 the Maillard reaction (Varlet et al., 2007; Xu et al., 2018).. Aldehydes are one of the most
313 important odor-active compounds because of their low odor threshold values (Peinado,
314 Koutsidis & Ames, 2016a). They may produce desirable aromas (roasty, malty, cocoa, nutty)
315 and undesirable aromas (green, rancid, oxidized) (Giri, Osako, Okamoto, & Ohshima, 2010).
316 Like aldehydes, ketones can be formed through lipid oxidation and the Maillard reaction
317 (Peinado, Miles, & Koutsidis, 2016b). Most of the ketones identified are associated with buttery
318 or creamy aromas on the one hand or ethereal, solvent aromas on the other.

319

320 **Alcohols** were the second most abundant compounds. Alcohols can be formed by secondary
321 decomposition of the hydroperoxides in fatty acids, or by enzymatic peroxidation of the n-3
322 and n-6 polyunsaturated fatty acids present in fish flesh (Peinado et al., 2016b). Alcohols have
323 various odor thresholds, meaning that they contribute in different ways to the overall aroma.

324 Alcohols are associated with alcoholic and green odors. The amount of 1-penten-3-ol seems to
325 be related to the amount of oil in the product (Peinado et al., 2016a, 2016b).

326 **Benzene compounds.** Benzene compounds are not significant potent odorants. Only
327 benzaldehyde has a relatively low odor threshold (350-3500ppb in water, (Leffingwell, 2019).
328 Most probably, benzaldehyde could be produced through the Maillard reaction, but it could also
329 be generated by oxidation or photochemical degradation of toluene, or other hydrocarbons
330 (Varlet et al., 2007).

331 **Sulfur compounds,** such as dimethyl disulfide and dimethyl trisulfide, are generally associated
332 with a deterioration of the material because of their strong unpleasant odor and low detection
333 threshold (Peinado et al., 2016a). These compounds may originate in the raw material or be
334 generated during the fermentation process from the free, peptidic and proteinic sulfur amino
335 acids in fish flesh (Peinado et al., 2016a).

336 **Furans and pyrazines** are generated through the Maillard reaction. Their odor is associated
337 with empyreumatic aromas such as toasty, cocoa, nutty, chocolate and caramel. These
338 compounds are formed mainly when hydrolysates are heated.

339 • Semi-quantification of the volatile compounds

340 Quantitatively, significant amounts of carbonyl compounds (aldehydes and ketones) and
341 alcohols were present (expressed in relative peak area/g of product). Carbonyl compounds are
342 generally odor-active compounds contributing to the overall odor of the food product (Varlet et
343 al., 2007). Alcohols have slightly lower odor thresholds than carbonyl compounds, depending
344 on their nature and quantity. In comparison, furans, pyrazines and sulfur compounds are present
345 in relatively low quantities, but generally have low odor thresholds. These compounds are thus
346 particularly important for the overall aroma of the product.

347 The hydrolysate containing the highest quantity of volatile compounds was sample 18. This
348 hydrolysate was obtained by applying the highest level of xylose concentration, hydrolysis time

349 and cooking time. These parameters seemed to have a particular impact on the production of
350 volatile compounds, especially those generated during the Maillard reaction. Sample 18
351 contained the highest quantity of furans and pyrazines. In the literature, these compounds are
352 known to be odor-active and responsible for roasted and burnt odors. On the contrary, sample
353 15 was the one with the lowest total quantity of volatile compounds. This sample was obtained
354 with the lowest level of all the parameters involving the production of few volatile compounds.
355 A direct relationship between the nature and quantity of volatile compounds produced, and the
356 process parameters applied was observed.

357 In more detail, the most represented volatile compounds in all the hydrolysates were 3-
358 methylbutanal, ethanol, 2-propanone + 2-methylpropanal and 1-penten-3-ol. 3-methylbutanal
359 is associated with malty, ethereal, aldehydic, chocolate and fatty odors
360 (www.thegoodscentcompany.com). 2-propanone + 2-methylpropanal are described
361 respectively as ethereal, solvent, apple and aldehydic, floral, and green
362 (www.thegoodscentcompany.com). Considering the two alcohols, 1-penten-3-ol is described
363 as green, vegetable, tropical and fruity whereas ethanol is perceived as alcoholic, ethereal and
364 medical (www.thegoodscentcompany.com).

365

366 3.3. Predicting sensory characteristics from volatile compounds

367 The importance of volatile compounds as predictors of the main odor characteristics of
368 enzymatic hydrolysates is presented in Fig.2. The importance measure quantifies the
369 contribution of each volatile compound to the prediction of the sensory profile. The confidence
370 interval for each importance value was obtained by repeating 50 random forests. A compound
371 was therefore significantly more important if the lower limit of its confidence interval was
372 greater than zero. Of all the volatile compounds identified, eleven contributed significantly to
373 the sensory profile prediction: methanethiol, 2,5-dimethylpyrazine, 1-hydroxy-2-propanone,

374 propanone, 2-methyl-1-propanol, furfural, 2-methylfuran, 2,3-pentanedione, hexanal, dodecane
375 and 3-hydroxy-2-pentanone. The odor description of these compounds ranged from cabbage
376 and garlic for methanethiol, to green, herbal and fatty for hexanal and included roasted, caramel,
377 butter, wood, truffle or ethereal notes for the other compounds. These eleven compounds were
378 selected to build the optimal regression tree for predicting hydrolysate sensory profiles (Fig. 3).
379 A regression tree is built by recursively splitting the set of products into two groups by choosing,
380 at each node, the most discriminant predictor (a volatile compound) and the appropriate
381 threshold. This technique leads to a supervised clustering of the whole set of products.
382 Therefore, the optimal tree is the best clustering of samples for predicting the sensory profile
383 from the volatile composition.

384 Specific odors produced during Maillard reactions, and especially roasted odors, have been
385 identified as potentially interesting notes for food applications. The first compound which
386 played a part in splitting the initial 20 samples into 2 groups was 2,5- dimethylpyrazine at a
387 threshold value of 56×10^3 peak area/g of product. Five hydrolysates with a 2,5-
388 dimethylpyrazine value below this threshold were grouped together. A mud odor was the
389 characteristic for three of them when methanethiol level was higher than 29.9×10^3 peak area/g
390 of product, and the two samples left had fat and rancid notes for a level of methanethiol below
391 this threshold. Methanethiol was not identified among the highly abundant volatile compounds,
392 but was selected in the random forest procedure as a discriminative compound for sensory
393 prediction. The low odor threshold (0.02 ppb) of this compound originated from the breakdown
394 of sulfur-containing amino acids such as cysteine or methionine (Varlet & Fernandez, 2010),
395 which could explain its importance on the sensory characteristics of the hydrolysates. 2,5-
396 dimethylpyrazine was described as cocoa, roasted nuts, roast beef, woody, grass, medical. This
397 compound was known to be produced through the Maillard reaction. Its odor threshold is

398 relatively high (800-1 800 ppb). Both these compounds were identified as odor-active
399 compounds possibly impacting the roasted odor of a food product.

400 The fifteen remaining samples, with a level of 2, 5- dimethylpyrazine higher than the 56.2×10^3
401 peak area/g of product, were first separated according to the level of the compound 1-hydroxy-
402 2-propanone. One sub-set of seven samples with no specific characteristics was identified when
403 the level of this compound was less than 494×10^3 peak area/g of product, and a group of eight
404 samples when the level was greater. This latter group was finally divided into two sub-sets
405 depending on their 3-hydroxy-2-pentanone content. A group of three samples, with a burnt
406 odor, appeared when the level of this compound was higher than 60×10^3 peak area/g of product.
407 When the level of 3-hydroxy-2-pentanone was below 60×10^3 , the five samples left presented
408 specific roasted and pickled notes.

409 Three compounds: 2,5-dimethylpyrazine, 1-hydroxy-2-propanone and 3-hydroxy-2-pentanone
410 were identified as playing a part in empyreumatic aromas. Considering the formation pathway
411 of 2,5-dimethylpyrazine, as well as its odor description, it is hardly surprising that a higher
412 amount of this compound will enhance the Maillard notes. But the relative ratio between the
413 three compounds may have an influence on the nature of the sensory characteristics, either
414 roasted, burnt or neutral. The main groups of products identified through the regression tree
415 were in line with previous sensory results with a few slight variations. The two groups with
416 specific notes, either mud or fatty and rancid were clearly separate from the others. The odor
417 activity of the volatile compounds selected in the regression tree was confirmed in a second
418 step through olfactometry measurements. Regarding the three replicated samples (5, 10 and
419 17), they were distributed into three different groups. All these groups had a common threshold
420 for 2,5-dimethylpyrazine, greater than 56.2×10^3 and only small level differences on 1-hydroxy-
421 2-propanone and 3-hydroxy-2-pentanone were detected. It is therefore likely that the variability
422 in sensory measurements, and especially the pickle odor, could explain this result.

423 To mask potential fishy odors through the production of roasted notes, the results suggest
424 finding processing conditions that make it possible to combine the presence of 2,5-
425 dimethylpyrazine and 1-hydroxy-2-propanone, while limiting the level of 3-hydroxy-2-
426 pentanone to avoid the burnt characteristic. Sensory results have shown that perception of
427 roasted notes increased with the cooking time and sugar level, thus confirming that the Maillard
428 reaction setting was driven by sugar content and a sufficient period at high temperature.
429 However, controlling these factors did not seem to be enough. The low level of the E/S ratio
430 (0.1), combined with a too short hydrolysis time, could lead to burnt or mud odors, depending
431 on the cooking time used rather than a roasted odor, even when there was a high sugar content.
432 A low E/S ratio or a short hydrolysis time may affect the hydrolysis reaction by reducing the
433 number of peptide bonds broken and by therefore reducing the potential generation of certain
434 Maillard reaction compounds. We can suppose that the cooking time used can then control the
435 nature of the compounds formed, either for caramelization products with a long cooking time,
436 or sulfur compounds with a short cooking time.
437 Moreover, for further application of these results, a complementary study will be needed to
438 investigate taste perception and the possible effects on bitterness or other characteristics of
439 process parameters such as a long heating time at 95°C.

440

441 Conclusion

442 This study based on experimental design methodology confirmed previous results on the
443 advantages of coupling Maillard reactions and enzymatic hydrolysis as a way of producing
444 hydrolysates with a range of aromatic properties making it possible to mask initial fish odors.
445 Results suggest some appropriate process conditions such as level of sugar, E/S ratio combined
446 with hydrolysis time for obtaining a typical roasted note. One of the main conclusions of the
447 study concerns the use of RT and RF methodologies to predict, for one of a first times, a whole

448 odor profile from volatile compounds. The results show that four main volatile compounds
449 contribute to separate hydrolysates into five groups according to their specific sensory
450 characteristics. Three of them, 2,5-dimethylpyrazine, 1-hydroxy-2-propanone and 3-hydroxy-
451 2-pentanone are mainly involved in the perception of roasted notes while methanethiol is
452 associated with a mud odor. The distribution of the three replicates in different sensory groups
453 in the final regression tree probably reflects higher variability in sensory measurements
454 compared to instrumental analysis, and reminds us of the importance of the choice of sensory
455 descriptors used in profiling. In order to consolidate the results obtained, it may be necessary
456 to add to the RF analysis replicated samples obtained from the same production batch, as well
457 as new samples produced from salmon by-products of other origin (plant, country), or samples
458 hydrolyzed with different enzymes that have an influence on the volatile compounds of the
459 hydrolysates. However, once these considerations have been integrated, the results obtained in
460 this study, which follow up on the works of Vigneau et al. (2018), suggest that a multivariate
461 version of regression trees and random forest methodologies may be a useful tool in practice
462 for establishing the main relationships between sensory perception and major volatile
463 compounds.

464

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468

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583

Figure Caption

Fig 1. (a) Representation of salmon hydrolysates on the first two dimensions of Principal Component Analysis (PCA) from profiling data.

(b) Projection of sensory descriptors in the first plane of PCA

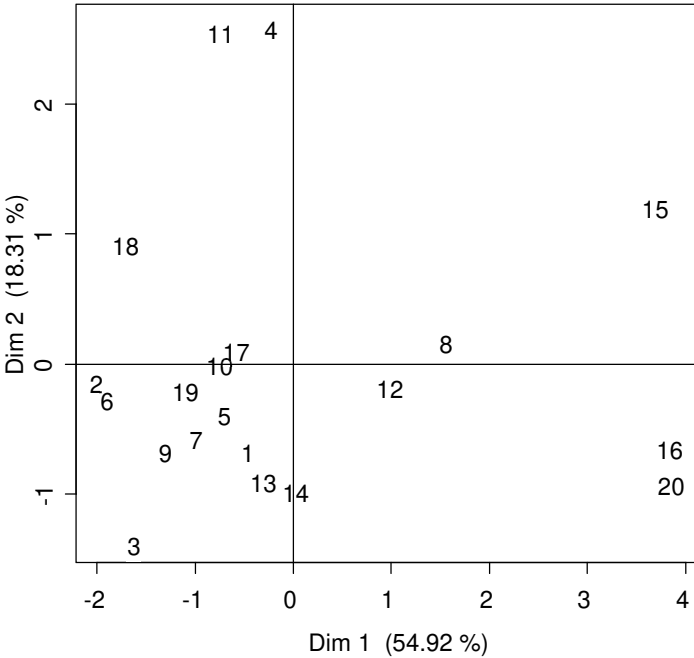
Fig.2 Variable importance of the 44 volatile compounds in sensory descriptors of odor. Confidence intervals (95%) of the importance of compounds were obtained with 50 random forests of 1000 trees.

Fig.3 Regression tree for prediction of all sensory descriptors from volatile compounds

Legend: Number (n) of samples for each group defined by a specific sensory profile with sample reference number

Fig 1.

(a)



(b)

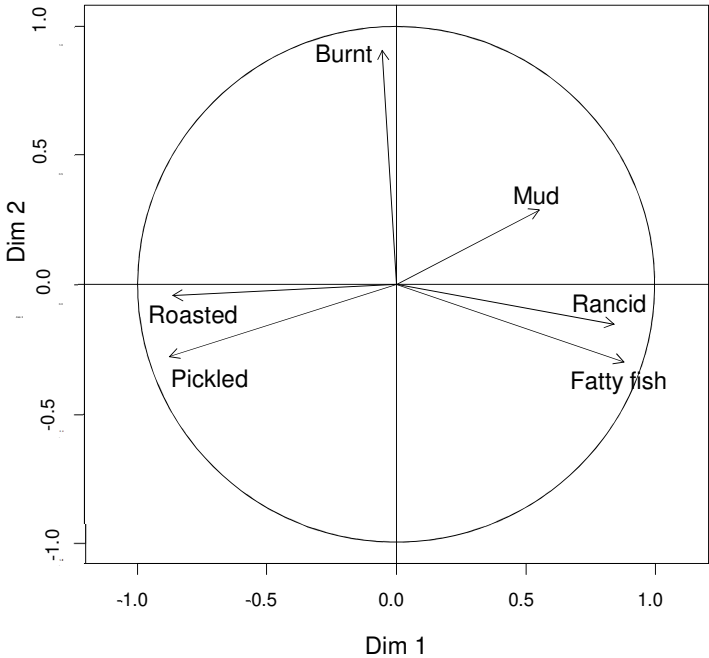


Fig.2

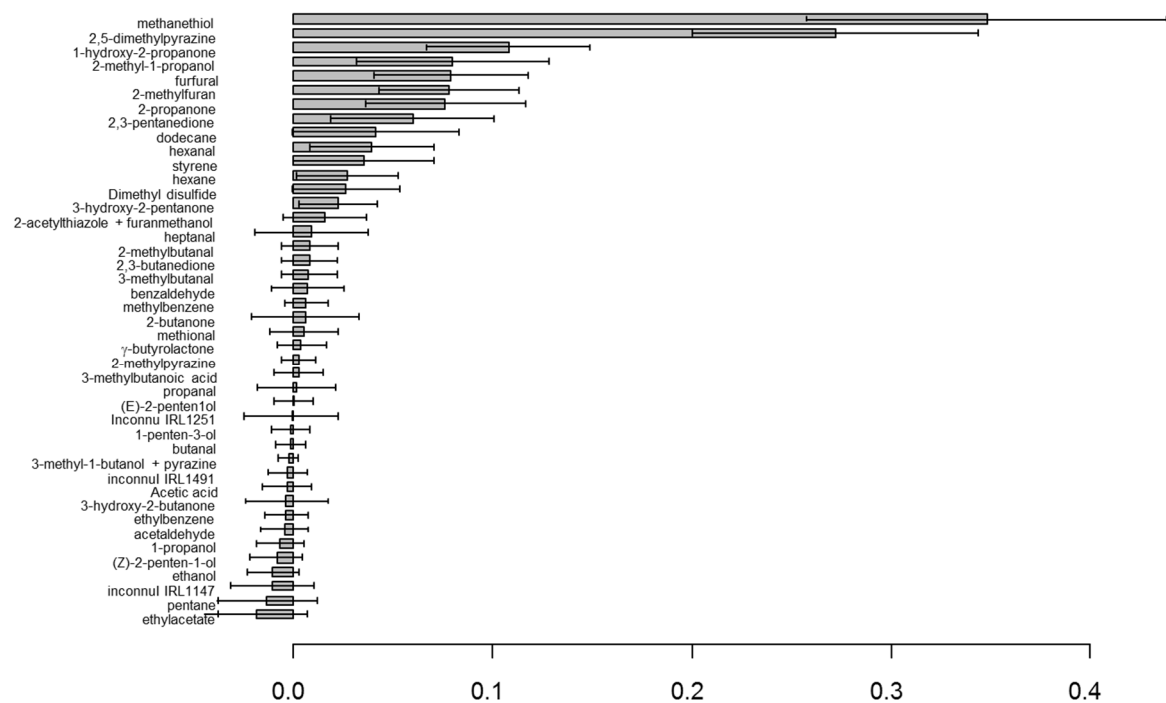


Fig.3

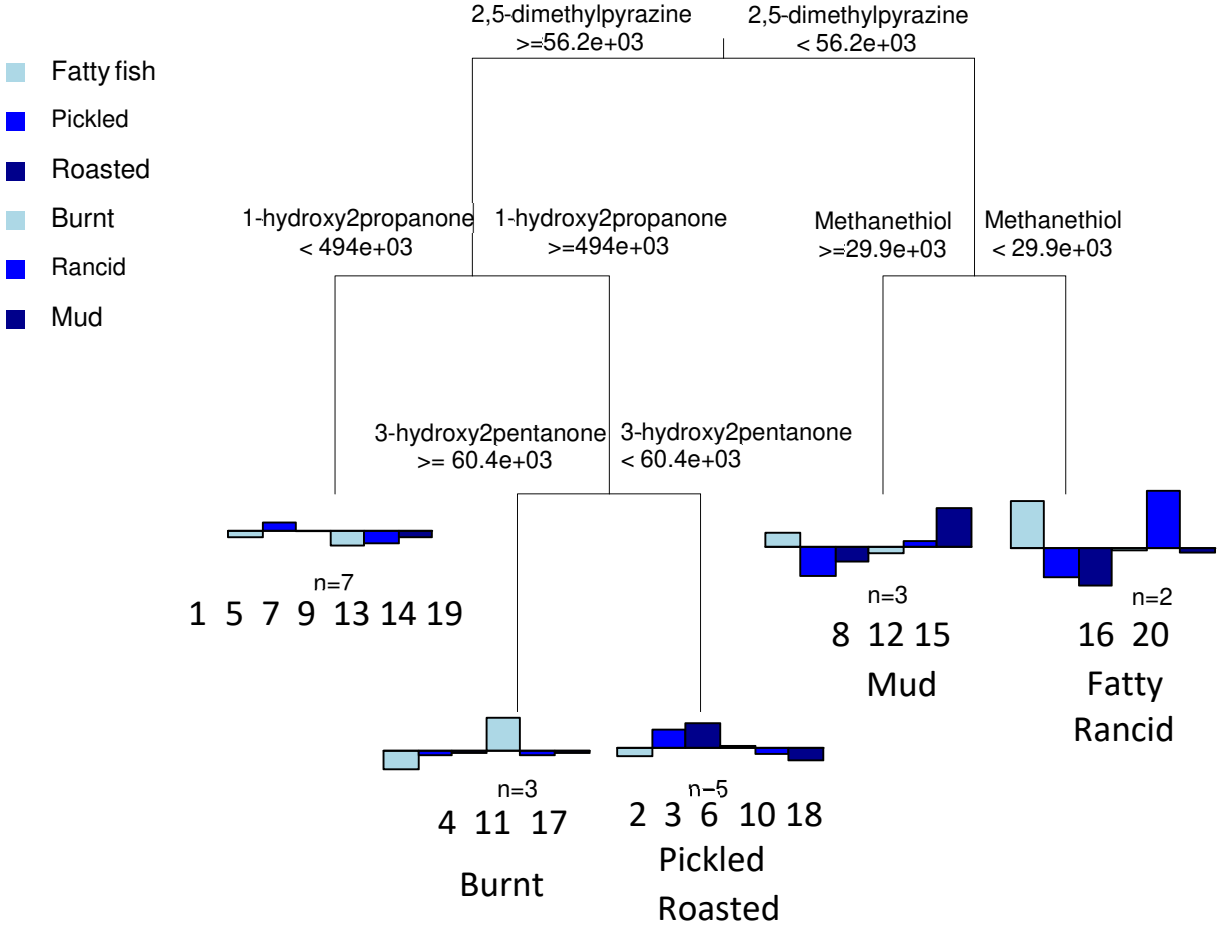


Table 1. Factor levels for the experimental design

Independent factors				
Run	E/S	X	HT	CT
1	0.4	2	90	90
2	0.25	10	50	60
3	0.25	6	90	60
4	0.1	10	10	90
5	0.25	6	50	60
6	0.25	6	50	90
7	0.25	6	10	60
8	0.1	2	90	30
9	0.4	6	50	60
10	0.25	6	50	60
11	0.1	6	50	60
12	0.25	2	50	60
13	0.25	6	50	30
14	0.4	10	10	30
15	0.1	2	10	30
16	0.4	10	90	30
17	0.25	6	50	60
18	0.1	10	90	90
19	0.4	2	10	90
20	0.4	0	90	90

(extra sample)

Independent factors E/S, X, HT, CT represent the Enzyme/Substrate ratio ($\text{g}\cdot 100\text{g}^{-1}$), Xylose concentration ($\text{g}\cdot \text{kg}^{-1}$), Hydrolysis Time at 40°C (min) and Cooking Time at 95°C (min) respectively

Table 2: Volatile compounds identified in the hydrolysates.

Volatile compound	CAS number	RI ^a	Identification ^b	Odour threshold ^c	Compound origin ^d	Odour description ^e
<i>Alkanes</i>						
Pentane	109-66-0	500	MS, RI, Std			
Hexane	110-54-3	600	MS, RI, Std			
Dodecane	112-40-3	1198	MS, RI, Std			
<i>Benzene compounds</i>						
Methylbenzene	108-88-3	977	MS, RI, Std		MR ⁵	sweet
Ethylbenzene	100-41-4	1138	MS, RI, Std			
Styrene	100-42-5	1268	MS, RI, Std	730		sweet balsam floral plastic
Benzaldehyde	100-52-7	1541	MS, RI, Std	350-3 500	MR ⁵	strong sharp sweet bitter almond cherry
<i>Aldehydes</i>						
Acetaldehyde	75-07-0	702	MS, RI, Std	15-120	MR	pungent ethereal aldehydic fruity
Propanal	123-38-6	794	MS, RI, Std	9.5-37	LO	earthy alcohol wine whiskey cocoa nutty
2-methylpropanal	78-84-2	794	MS, RI, Std	0.1-2.3	MR ¹	fresh aldehydic floral green
butanal	123-72-8	869	MS, RI, Std	9-37.3		pungent cocoa musty green malty bready
2-methylbutanal	96-17-3	909	MS, RI, Std	1	MR ¹	musty cocoa coffee nutty
3-methylbutanal	590-86-3	913	MS, RI, Std	0.2-2	MR ¹	ethereal aldehydic chocolate peach fatty
Hexanal	66-25-1	1095	MS, RI, Std	4.5-5	LO ¹	fresh green fatty aldehydic grass leafy fruity sweaty
Heptanal	111-71-7	1196	MS, RI, Std	3	LO ²	fresh aldehydic fatty green herbal wine-lee ozone
<i>Alcohols</i>						
ethanol	64-17-5	935	MS, RI, Std	100 000	F ³ , LO ³	strong alcoholic ethereal medical
1-propanol	71-23-8	1060	MS, RI, Std	9 000		alcoholic fermented fusel musty
2-methyl-1-propanol	78-83-1	1121	MS, RI, Std	7 000		ethereal winey
1-penten-3-ol	616-25-1	1180	MS, RI, Std	400	LO ⁴	pungent horseradish green vegetable tropical fruity
(E)-2-penten-1-ol	1576-96-1	1326	MS, RI, Std			mushroom
(Z)-2-penten-1-ol	1576-95-0	1334	MS, RI, Std			green plastic ethereal fruity
<i>Ketones</i>						
2-propanone	67-64-1	814	MS, RI, Std	500 000		solvent ethereal apple pear
2-butanone	78-93-3	900	MS, RI, Std	50 000		acetone-like ethereal fruity camphor
2,3-butanedione	431-03-8	977	MS, RI, Std	2.3-6.5		strong butter sweet creamy pungent caramel
2,3-pentanedione	600-14-6	1076	MS, RI, Std			pungent sweet butter creamy caramel nutty cheese
3-hydroxy-2-butanone	513-86-0	1297	MS, RI	800		sweet buttery creamy dairy milky fatty

Volatile compound	CAS number	RI ^a	Identification ^b	Odour threshold ^c	Compound origin ^d	Odour description ^e
1-hydroxy-2-propanone	116-09-6	1312	MS, RI, Std			pungent sweet caramellic ethereal
3-hydroxy-2-pentanone	3142-66-3	1355	MS, RI			herbal truffle
<i>Acids and esters</i>						
Ethyl acetate	141-78-6	883	MS, RI, Std	5-5 000		ethereal fruity sweet weedy green
Acetic acid	64-19-7	1452	MS, RI, Std		F ¹	sharp pungent sour vinegar
3-methylbutanoic acid	503-74-2	1682	MS, RI, Std	120-700	F ¹	sour stinky feet sweaty cheese tropical
<i>Furans</i>						
2-methylfuran	534-22-5	863	MS, RI, Std		MR	ethereal acetone chocolate
Furfural	98-01-1	1471	MS, RI, Std	3 000-23 000	MR ³	sweet woody almond fragrant baked bread
<i>Pyrazines</i>						
2-methylpyrazine	109-08-0	1281	MS, RI, Std	60-105 000	MR	nutty cocoa roasted chocolate peanut green
2,5-dimethylpyrazine	123-32-0	1339	MS, RI, Std	800-1 800	MR ⁴	cocoa roasted nuts roast beef woody grass medical
<i>Sulfur compounds</i>						
Methanethiol	74-93-1	676	MS, RI	0.02		decomposing cabbage garlic
Dimethyl disulfide	624-92-0	1085	MS, RI, Std	0.16-12	M ³ , F ³	sulfurous vegetable cabbage onion
Methional	3268-49-3	1465	MS, RI, Std	0.2	MR ^{2,3}	musty potato tomato earthy vegetable creamy
<i>Others</i>						
Unknown LRI 1147						
3-methyl-1-butanol + pyrazine	123-51-3	1228	MS, RI, Std MS, RI	250-300 +	F ³	fusel oil alcoholic whiskey fruity banana pungent sweet corn like roasted hazelnut barley
Unknown LRI 1251						
Unknown LRI 1491						
g-butyrolactone	96-48-0	1655	MS, RI, Std			creamy oily fatty caramel
2-acetylthiazole + 2-furanmethanol	24295-03-2 98-00-0	1670	MS, RI, Std MS, RI, Std			nutty popcorn roasted peanuts hazelnut alcoholic chemical musty sweet caramel bread coffee

^aRI: Retention Index (RI) calculated on a DB-WAX column

^bMethods of identification of the volatile compounds : RI: Comparison of the retention index calculated with the literature, MS: comparison of the mass spectra of the compound with a database, Std : comparison of the retention index of the volatile compound with that of the corresponding standard

^cOdour threshold expressed in parts per billion (<http://www.leffingwell.com/odorthre.htm>)

^dCompound origin :LO: lipid oxidation, MR: Maillard reaction, F: fermentation, M: marine, O: other

¹Peinado et al. (2016) LWT 66:444-452, ²Varlet et al. (2007) Food Chemistry 1536-1556, ³Giri et al., Food Res Int 43:1027-1040, ⁴Peinado et al. (2016b) Food Chem 212:612-619, ⁵Chung et al. (2002)

^d www.thegoodscentscompany.com

Table 3: Relative quantity of the volatile compounds of the fish hydrolysates expressed in relative peak area per gram of product. Means are obtained from 3 measures. Standard deviation (SD) is specified for all the samples.

Volatile compound	Mean relative peak area / g of product ($\times 10^3$) \pm SD									
	1	2	3	4	5	6	7	8	9	10
<i>Alkanes</i>										
Pentane	154 \pm 24	111 \pm 10	122 \pm 19	68 \pm 10	96 \pm 6	68 \pm 12	43 \pm 1	57 \pm 5	177 \pm 49	84 \pm 21
Hexane	45 \pm 5	28 \pm 6	24 \pm 2	19 \pm 1	23 \pm 6	25 \pm 6	20 \pm 4	15 \pm 2	30 \pm 8	17 \pm 3
Dodecane	64 \pm 2	62 \pm 21	50 \pm 9	79 \pm 15	71 \pm 3	53 \pm 9	89 \pm 6	85 \pm 12	52 \pm 10	101 \pm 5
<i>Benzene compounds</i>										
Methylbenzene	85 \pm 19	-	89 \pm 8	16 \pm 2	18 \pm 1	21 \pm 6	52 \pm 8	71 \pm 3	32 \pm 3	23 \pm 2
Ethylbenzene	122 \pm 1	17 \pm 9	71 \pm 5	45 \pm 6	40 \pm 4	39 \pm 8	82 \pm 6	97 \pm 4	73 \pm 3	68 \pm 6
Styrene	252 \pm 38	198 \pm 41	180 \pm 5	199 \pm 13	223 \pm 11	164 \pm 16	172 \pm 2	205 \pm 20	240 \pm 50	216 \pm 12
Benzaldehyde	57 \pm 5	67 \pm 4	78 \pm 10	60 \pm 8	57 \pm 3	71 \pm 5	58 \pm 10	54 \pm 3	59 \pm 3	60 \pm 6
<i>Aldehydes</i>										
Acetaldehyde	299 \pm 9	346 \pm 36	307 \pm 9	359 \pm 15	336 \pm 3	305 \pm 19	442 \pm 15	244 \pm 2	335 \pm 13	374 \pm 23
Propanal	1107 \pm 169	440 \pm 22	563 \pm 23	425 \pm 19	602 \pm 15	370 \pm 23	911 \pm 52	1095 \pm 31	633 \pm 23	864 \pm 37
Butanal	342 \pm 39	172 \pm 38	188 \pm 25	200 \pm 47	180 \pm 25	169 \pm 15	260 \pm 20	238 \pm 3	241 \pm 9	225 \pm 23
2-methylbutanal	1040 \pm 30	1249 \pm 172	1487 \pm 183	886 \pm 102	1013 \pm 75	1471 \pm 64	648 \pm 73	802 \pm 13	999 \pm 72	1011 \pm 72
3-methylbutanal	6935 \pm 410	6551 \pm 109	7877 \pm 329	2288 \pm 158	5549 \pm 106	6220 \pm 161	2929 \pm 195	5355 \pm 23	5938 \pm 214	5461 \pm 86
Hexanal	397 \pm 32	152 \pm 5	214 \pm 23	104 \pm 14	223 \pm 20	149 \pm 6	229 \pm 32	375 \pm 21	251 \pm 19	252 \pm 1
Heptanal	55 \pm 5	23 \pm 4	27 \pm 2	24 \pm 4	29 \pm 4	23 \pm 3	39 \pm 5	36 \pm 5	33 \pm 3	35 \pm 7
<i>Alcohols</i>										
Ethanol	6512 \pm 393	6511 \pm 387	6183 \pm 268	6311 \pm 198	7935 \pm 78	7553 \pm 462	7178 \pm 750	7338 \pm 484	7455 \pm 463	6033 \pm 349
1-propanol	259 \pm 38	152 \pm 7	181 \pm 17	185 \pm 21	189 \pm 22	164 \pm 43	186 \pm 24	187 \pm 21	172 \pm 21	194 \pm 15
2-methyl-1-propanol	47 \pm 4	35 \pm 4	32 \pm 6	39 \pm 1	37 \pm 4	34 \pm 6	54 \pm 2	36 \pm 2	48 \pm 9	37 \pm 6
1-penten-3-ol	3636 \pm 316	2021 \pm 46	1873 \pm 53	2580 \pm 53	2269 \pm 108	1424 \pm 46	3387 \pm 115	2681 \pm 125	2385 \pm 87	3059 \pm 75
(E)-2-penten-1-ol	151 \pm 12	78 \pm 6	54 \pm 2	105 \pm 3	82 \pm 4	45 \pm 2	149 \pm 15	99 \pm 6	96 \pm 11	123 \pm 9
(Z)-2-penten-1-ol	193 \pm 28	101 \pm 4	121 \pm 10	142 \pm 11	122 \pm 12	86 \pm 6	146 \pm 6	232 \pm 16	126 \pm 15	169 \pm 10
<i>Ketones</i>										
2-propanone + 2-methylpropanal	6041 \pm 799	7095 \pm 398	5341 \pm 334	8189 \pm 559	5474 \pm 117	5700 \pm 402	7413 \pm 608	2619 \pm 304	6147 \pm 353	6107 \pm 322
2-butanone	691 \pm 49	577 \pm 30	703 \pm 29	638 \pm 29	602 \pm 66	476 \pm 11	638 \pm 21	408 \pm 34	604 \pm 17	609 \pm 28
2,3-butanedione	1103 \pm 26	733 \pm 63	852 \pm 80	654 \pm 66	767 \pm 18	860 \pm 60	836 \pm 113	801 \pm 58	879 \pm 167	721 \pm 9
2,3-pentanedione	95 \pm 7	76 \pm 18	95 \pm 16	57 \pm 7	97 \pm 17	76 \pm 11	64 \pm 4	212 \pm 35	89 \pm 12	115 \pm 8
3-hydroxy-2-butanone	1674 \pm 198	1638 \pm 118	1169 \pm 63	2151 \pm 54	1824 \pm 57	2086 \pm 51	1280 \pm 28	1584 \pm 3	1500 \pm 68	1757 \pm 60
1-hydroxy-2-propanone	348 \pm 35	630 \pm 42	553 \pm 47	1305 \pm 45	494 \pm 25	882 \pm 29	470 \pm 11	111 \pm 9	424 \pm 31	494 \pm 32
3-hydroxy-2-pentanone	56 \pm 2	44 \pm 3	49 \pm 2	61 \pm 3	50 \pm 1	43 \pm 3	57 \pm 3	59 \pm 2	37 \pm 3	54 \pm 5

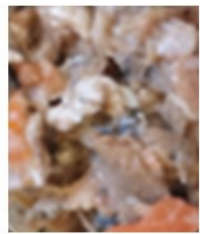
Volatile compound	Mean relative peak area / g of product (x10 ³) ± SD									
	1	2	3	4	5	6	7	8	9	10
<i>Acids and esters</i>										
Ethyl acetate	122 ± 26	91 ± 21	80 ± 2	102 ± 17	108 ± 10	96 ± 15	120 ± 7	102 ± 1	101 ± 2	99 ± 5
Acetic acid	599 ± 160	422 ± 19	494 ± 129	758 ± 194	601 ± 75	778 ± 304	574 ± 71	463 ± 123	464 ± 112	693 ± 83
3-methylbutanoic acid	46 ± 7	41 ± 11	43 ± 1	20 ± 3	35 ± 6	44 ± 3	33 ± 2	40 ± 4	38 ± 2	41 ± 3
<i>Furans</i>										
2-methylfuran	46 ± 27	71 ± 10	72 ± 7	162 ± 19	59 ± 12	94 ± 6	64 ± 27	-	68 ± 8	52 ± 9
Furfural	103 ± 6	111 ± 12	94 ± 1	107 ± 9	87 ± 1	83 ± 3	83 ± 3	39 ± 3	91 ± 4	91 ± 4
<i>Pyrazines</i>										
2-methylpyrazine	26 ± 5	54 ± 13	34 ± 4	69 ± 2	32 ± 7	60 ± 11	25 ± 3	32 ± 6	32 ± 5	29 ± 6
2,5-dimethylpyrazine	75 ± 24	85 ± 9	133 ± 7	85 ± 8	91 ± 20	152 ± 34	78 ± 6	-	77 ± 4	93 ± 10
<i>Sulfur compounds</i>										
Methanethiol	41 ± 6	43 ± 8	39 ± 4	40 ± 3	43 ± 2	46 ± 1	44 ± 1	33 ± 2	47 ± 4	53 ± 8
Dimethyl disulfide	40 ± 6	118 ± 18	88 ± 9	124 ± 2	77 ± 10	88 ± 3	72 ± 12	33 ± 5	110 ± 36	71 ± 3
Methional	46 ± 1	55 ± 3	54 ± 6	48 ± 4	48 ± 2	45 ± 1	42 ± 1	41 ± 3	43 ± 1	48 ± 3
<i>Others</i>										
Unknown LRI 1147	32 ± 2	27 ± 3	23 ± 3	16 ± 5	17 ± 1	14 ± 3	18 ± 2	16 ± 2	15 ± 3	21 ± 2
3-methyl-1-butanol + pyrazine	96 ± 4	122 ± 24	86 ± 3	128 ± 14	135 ± 11	142 ± 16	94 ± 4	99 ± 5	108 ± 6	96 ± 2
Unknown LRI 1251	71 ± 2	36 ± 1	36 ± 5	44 ± 7	55 ± 12	58 ± 11	30 ± 4	27 ± 5	47 ± 5	67 ± 12
Unknown LRI 1491	93 ± 4	67 ± 9	67 ± 14	70 ± 9	75 ± 9	87 ± 12	79 ± 12	94 ± 7	72 ± 11	80 ± 8
g-butyrolactone	28 ± 3	27 ± 4	22 ± 4	28 ± 5	22 ± 2	27 ± 5	25 ± 5	21 ± 4	22 ± 3	23 ± 2
2-acetylthiazole + 2-furanmethanol	39 ± 4	37 ± 1	31 ± 4	42 ± 1	26 ± 5	49 ± 3	19 ± 1	12 ± 1	27 ± 4	26 ± 2

Volatile compound	Mean relative peak area / g of product (x10 ³) ± SD									
	11	12	13	14	15	16	17	18	19	20
<i>Alkanes</i>										
Pentane	66 ± 11	73 ± 5	166 ± 12	141 ± 28	373 ± 53	107 ± 8	194 ± 38	89 ± 24	124 ± 17	187 ± 30
Hexane	17 ± 1	28 ± 7	34 ± 4	35 ± 8	99 ± 16	42 ± 7	41 ± 9	17 ± 4	16 ± 2	27 ± 6
Dodecane	87 ± 6	100 ± 23	42 ± 8	97 ± 24	50 ± 5	123 ± 15	86 ± 10	92 ± 15	93 ± 8	118 ± 18
<i>Benzene compounds</i>										
Methylbenzene	22 ± 3	41 ± 12	27 ± 4	45 ± 7	35 ± 2	45 ± 4	17 ± 1	25 ± 2	28 ± 3	31 ± 6
Ethylbenzene	64 ± 7	91 ± 4	73 ± 6	153 ± 20	42 ± 5	80 ± 6	36 ± 2	98 ± 8	88 ± 4	80 ± 6
Styrene	215 ± 1	263 ± 37	115 ± 10	197 ± 45	210 ± 3	284 ± 32	190 ± 11	183 ± 51	259 ± 27	295 ± 17
Benzaldehyde	58 ± 7	56 ± 5	57 ± 10	60 ± 7	50 ± 3	63 ± 2	55 ± 5	73 ± 7	55 ± 8	56 ± 3
<i>Aldehydes</i>										
Acetaldehyde	414 ± 13	305 ± 7	296 ± 37	458 ± 22	409 ± 15	377 ± 3	374 ± 14	399 ± 8	406 ± 10	334 ± 22
Propanal	1106 ± 45	1070 ± 76	955 ± 84	1461 ± 44	1250 ± 24	941 ± 26	1022 ± 85	510 ± 26	1126 ± 84	1508 ± 215
Butanal	307 ± 11	269 ± 21	230 ± 30	309 ± 6	232 ± 25	233 ± 5	272 ± 21	238 ± 18	342 ± 11	375 ± 7
2-methylbutanal	992 ± 91	653 ± 46	554 ± 20	555 ± 55	381 ± 27	998 ± 46	970 ± 20	2459 ± 69	523 ± 35	647 ± 11
3-methylbutanal	4720 ± 143	4662 ± 135	4054 ± 626	3174 ± 87	2220 ± 245	5922 ± 492	5453 ± 221	7391 ± 206	2960 ± 97	5599 ± 123
Hexanal	297 ± 37	377 ± 36	331 ± 22	316 ± 43	351 ± 26	329 ± 12	315 ± 18	130 ± 9	325 ± 32	738 ± 57
Heptanal	38 ± 6	41 ± 4	43 ± 6	38 ± 6	50 ± 5	47 ± 2	40 ± 6	32 ± 7	57 ± 3	81 ± 8
<i>Alcohols</i>										
Ethanol	7824 ± 586	7497 ± 386	6772 ± 608	7139 ± 367	6595 ± 405	6524 ± 425	6085 ± 755	6464 ± 308	6817 ± 538	6295 ± 415
1-propanol	234 ± 41	199 ± 16	210 ± 22	213 ± 29	149 ± 11	203 ± 22	240 ± 12	218 ± 24	211 ± 29	207 ± 59
2-methyl-1-propanol	45 ± 6	45 ± 8	28 ± 5	47 ± 2	53 ± 8	64 ± 4	48 ± 5	45 ± 2	40 ± 1	73 ± 8
1-penten-3-ol	3551 ± 386	2821 ± 197	2353 ± 41	3185 ± 92	2202 ± 81	2408 ± 56	3275 ± 171	3305 ± 122	4231 ± 21	4432 ± 141
(E)-2-penten-1-ol	137 ± 14	111 ± 11	81 ± 4	129 ± 4	94 ± 4	85 ± 5	132 ± 8	120 ± 7	199 ± 2	188 ± 16
(Z)-2-penten-1-ol	180 ± 8	154 ± 33	167 ± 7	176 ± 14	112 ± 10	143 ± 9	197 ± 10	206 ± 13	198 ± 5	319 ± 17
<i>Ketones</i>										
2-propanone + 2-methylpropanal	5866 ± 479	3832 ± 87	2896 ± 260	5027 ± 123	3021 ± 81	4473 ± 429	5817 ± 518	11064 ± 208	5998 ± 273	2638 ± 203
2-butanone	609 ± 44	458 ± 17	415 ± 27	542 ± 25	282 ± 38	560 ± 28	623 ± 62	838 ± 7	597 ± 5	468 ± 37
2,3-butanedione	786 ± 137	1027 ± 62	1051 ± 83	822 ± 14	903 ± 78	925 ± 125	710 ± 25	716 ± 79	828 ± 66	843 ± 16
2,3-pentanedione	113 ± 21	146 ± 22	170 ± 32	157 ± 29	123 ± 13	180 ± 25	111 ± 13	76 ± 14	68 ± 12	124 ± 24
3-hydroxy-2-butanone	1630 ± 81	2107 ± 64	2042 ± 49	1755 ± 62	1849 ± 37	2190 ± 74	2316 ± 139	1323 ± 63	1717 ± 19	1717 ± 89
1-hydroxy-2-propanone	502 ± 32	211 ± 8	212 ± 4	273 ± 15	135 ± 5	314 ± 12	552 ± 35	1123 ± 77	295 ± 9	113 ± 6
3-hydroxy-2-pentanone	85 ± 12	55 ± 5	76 ± 4	64 ± 4	56 ± 1	55 ± 2	67 ± 3	60 ± 5	55 ± 2	61 ± 2

Volatile compound	Mean relative peak area / g of product (x10 ³) ± SD									
	11	12	13	14	15	16	17	18	19	20
<i>Acids and esters</i>										
Ethyl acetate	131 ± 4	103 ± 3	90 ± 13	126 ± 6	86 ± 29	118 ± 21	105 ± 15	110 ± 8	125 ± 7	76 ± 15
Acetic acid	587 ± 330	371 ± 86	404 ± 45	449 ± 87	671 ± 128	477 ± 84	636 ± 134	907 ± 116	455 ± 23	787 ± 200
3-methylbutanoic acid	38 ± 7	30 ± 4	28 ± 3	29 ± 2	23 ± 6	39 ± 4	33 ± 2	45 ± 1	23 ± 1	32 ± 1
<i>Furans</i>										
2-methylfuran	67 ± 7	-	50 ± 9	54 ± 7	-	66 ± 8	71 ± 12	192 ± 32	61 ± 10	-
Furfural	82 ± 8	62 ± 2	51 ± 5	83 ± 9	39 ± 2	64 ± 3	96 ± 10	150 ± 5	82 ± 3	48 ± 5
<i>Pyrazines</i>										
2-methylpyrazine	43 ± 7	33 ± 13	74 ± 4	26 ± 3	29 ± 7	51 ± 3	31 ± 2	61 ± 9	47 ± 8	23 ± 3
2,5-dimethylpyrazine	85 ± 13	45 ± 12	58 ± 9	62 ± 10	24 ± 3	55 ± 8	110 ± 5	159 ± 17	71 ± 12	19 ± 2
<i>Sulfur compounds</i>										
Methanethiol	42 ± 5	32 ± 1	40 ± 10	45 ± 9	33 ± 1	27 ± 5	44 ± 2	40 ± 2	42 ± 3	19 ± 1
Dimethyl disulfide	66 ± 10	31 ± 6	40 ± 7	67 ± 1	33 ± 5	71 ± 12	62 ± 8	140 ± 22	48 ± 5	19 ± 5
Methional	49 ± 3	37 ± 2	41 ± 2	38 ± 4	32 ± 2	51 ± 1	49 ± 2	68 ± 4	36 ± 1	43 ± 5
<i>Others</i>										
Unknown LRI 1147	41 ± 4	15 ± 3	14 ± 3	30 ± 9	14 ± 2	16 ± 1	21 ± 3	27 ± 4	21 ± 3	30 ± 2
3-methyl-1-butanol + pyrazine	108 ± 10	115 ± 10	93 ± 7	101 ± 10	104 ± 1	99 ± 5	106 ± 5	89 ± 4	90 ± 3	100 ± 4
Unknown LRI 1251	45 ± 5	67 ± 5	151 ± 48	48 ± 8	42 ± 5	143 ± 12	77 ± 17	64 ± 6	63 ± 8	97 ± 9
Unknown LRI 1491	81 ± 14	89 ± 8	90 ± 23	78 ± 12	84 ± 15	75 ± 6	94 ± 8	83 ± 10	82 ± 7	96 ± 7
g-butyrolactone	26 ± 3	26 ± 3	22 ± 1	22 ± 3	21 ± 2	25 ± 2	24 ± 1	30 ± 1	23 ± 3	27 ± 6
2-acetylthizaole + 2-furanmethanol	27 ± 4	18 ± 1	14 ± 3	13 ± 1	8 ± 2	23 ± 4	26 ± 2	68 ± 3	27 ± 4	42 ± 4

Salmon by-product Processing

Raw Material



Enzymatic hydrolysis & Maillard Reaction

Material after hydrolysis



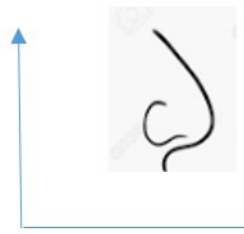
Centrifugation (8200 g for 30 min at 20°C)



Hydrolysate

Experimental design (20 samples)

Analyses

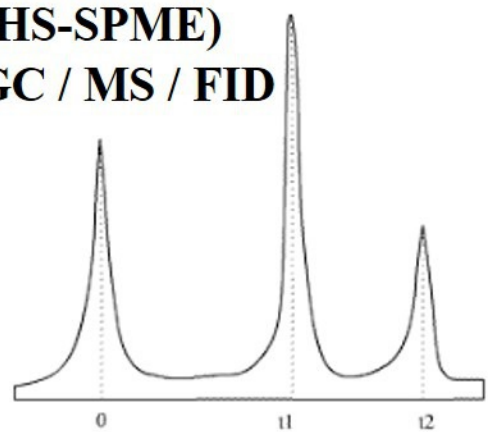


- Fatty fish
- Pickled
- Roasted
- Burnt
- Rancid
- Mud

Odor profile

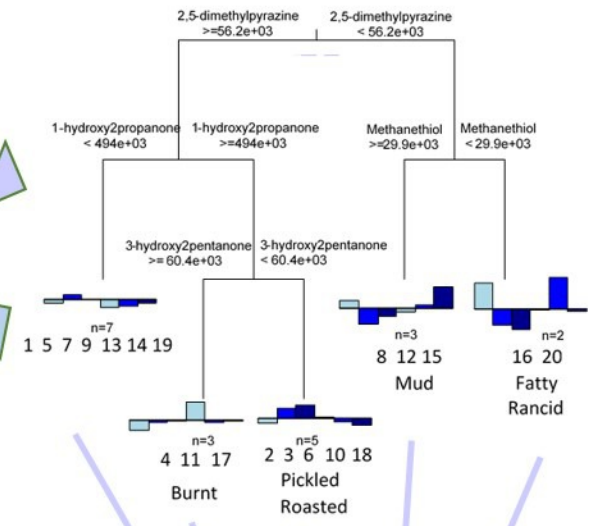
Volatile compounds

(HS-SPME)
GC / MS / FID



Prediction of odour profile from volatile compounds

Regression trees and Random Forest



5 groups of hydrolysates with specific odor profiles