

Northumbria Research Link

Citation: Peinado Pardo, Irene, Koutsidis, Georgios and Ames, Jenny (2015) Production of seafood flavour formulations from enzymatic hydrolysates of fish by-products. *LWT - Food Science and Technology*, 66 (1). pp. 444-452. ISSN 0023-6438

Published by: Elsevier

URL: <http://dx.doi.org/10.1016/j.lwt.2015.09.025>
<<http://dx.doi.org/10.1016/j.lwt.2015.09.025>>

This version was downloaded from Northumbria Research Link:
<http://nrl.northumbria.ac.uk/id/eprint/24148/>

Northumbria University has developed Northumbria Research Link (NRL) to enable users to access the University's research output. Copyright © and moral rights for items on NRL are retained by the individual author(s) and/or other copyright owners. Single copies of full items can be reproduced, displayed or performed, and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided the authors, title and full bibliographic details are given, as well as a hyperlink and/or URL to the original metadata page. The content must not be changed in any way. Full items must not be sold commercially in any format or medium without formal permission of the copyright holder. The full policy is available online: <http://nrl.northumbria.ac.uk/policies.html>

This document may differ from the final, published version of the research and has been made available online in accordance with publisher policies. To read and/or cite from the published version of the research, please visit the publisher's website (a subscription may be required.)



**Northumbria
University**
NEWCASTLE



UniversityLibrary

Accepted Manuscript

Production of seafood flavour formulations from enzymatic hydrolysates of fish by-products

I. Peinado, G. Koutsidis, J. Ames



PII: S0023-6438(15)30196-1

DOI: [10.1016/j.lwt.2015.09.025](https://doi.org/10.1016/j.lwt.2015.09.025)

Reference: YFSTL 4973

To appear in: *LWT - Food Science and Technology*

Received Date: 14 June 2015

Revised Date: 7 September 2015

Accepted Date: 13 September 2015

Please cite this article as: Peinado, I., Koutsidis, G., Ames, J., Production of seafood flavour formulations from enzymatic hydrolysates of fish by-products, *LWT - Food Science and Technology* (2015), doi: 10.1016/j.lwt.2015.09.025.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

21 Conversion of low value fish derived materials such as fish powder, into more valuable
22 products such as flavour precursors and subsequently flavour compounds might be a
23 commercially viable proposition for the fish industry.

24

25

26 **Chemical compounds studied in this article:**

27 Glutamic acid (Pubchem CID 611); Aspartic acid (Pubchem CID: 424); Leucine (Pubchem
28 CID: 6106); Lysine (Pubchem CID: 5962); 1-Octen-3-ol (Pubchem CID: 18827); 2,4-
29 Heptadienal (Pubchem CID: 20307); 4-Heptenal (Pubchem CID:71590); 1-Hepten-4-ol
30 (Pubchem CID: 19040).

31

32

33 Key words: enzymatic hydrolysis, amino acids, volatiles, fish by-products, seafood flavour.

34

35 1. Introduction

36 Traditionally, waste from the fish industry such as small catch, flesh, viscera etc. are either
37 disposed of or utilised as fishmeal for animal feeding. Nevertheless, over the last few
38 decades, raised awareness on the environmental impact of products and processes has led to
39 retailers and consumers making concerted efforts to make the best use of all resources.
40 Nowadays, there is growing interest in using food wastes as sources of materials or
41 ingredients that are capable of providing added value to consumer products including uses in
42 foods. Some examples of this are the extraction and recovery of different compounds of
43 interest such as amino acids, peptides, collagen or omega fatty acids from fish wastes
44 (Guerard, Dufosse, Broise, & Binet, 2001). Development of novel means of processing is
45 required to convert the wastes and by-products into forms that are safe, marketable and
46 acceptable to the consumer.

47 Fish wastes have also been utilised for the production of fish powders or fish protein
48 hydrolysates, used as nitrogen source for microbial growth and enzyme production. Autolytic
49 process, which depends only on endogenous enzymes, is considered to be economically
50 advantageous; however, exogenous commercial enzymes are sometimes preferred since they
51 allow controlled hydrolysis, hence control over the properties of the resulting products. Many
52 enzymes have attracted interest for the hydrolysis of fish proteins (e.g., papain, alcalase,
53 neutrase, Flavourzyme[®], Protamex[®]). Characteristics of the final hydrolysate will depend on
54 the enzyme(s) added, but also on the substrate, which plays an important role in the
55 hydrolysis (Annadurai, Sadeeshkumar, Vijayalaksmi, & Pirithiviraj, 2012; Aspino, Horn, &
56 H. Eijsink, 2005; Ghorbel et al., 2005; Souissi, Bougatef, Triki-ellouz, & Nasri, 2007).

57 Flavour is an important factor to determine the quality of fish and fish derived products as
58 well as consumer acceptance. Fishy flavour often makes products derived from fish less
59 acceptable (Ganeko et al., 2008). This characteristic aroma is influenced by the species but

60 also by the conditions used for its post-harvest handling, storage and cooking. Some fish such
61 as salmon or trout, have a strong flavour while might have a relatively mild smell before
62 cooking that becomes strong and pleasant after heating (Ganeko et al., 2008; Whitfield,
63 Freeman, Last, Bannister, & Kennett, 1982; Whitfield, Last, Shaw, & Tindale, 1988).
64 Important aroma compounds, characteristic of fresh fish, are lipid derived volatile
65 compounds generated mainly by oxidative enzymatic reactions and autoxidation of lipids
66 such as aldehydes and ketones. However, compounds derived from Maillard reaction such as
67 pyrazines and furans, also make important contributions to the flavour and aroma of fish
68 products after frying or grilling (Giri, Osako, & Ohshima, 2010).
69 The aim of this study was to demonstrate the use of by-products of the fish industry (fish
70 powder) for the generation of fish flavour formulations after protease biocatalysis and
71 subsequent heating in the presence of glucose and/or fish oil.

72

73 **2. Material and Methods**

74 **2.1. Chemicals**

75 Proteases (Biocatalysts Ltd, UK), fish oil and fish powder (Croda International plc, UK), as
76 well as glucose and glycerol, (Sigma-Aldrich Company Ltd, Poole, UK) used to produce the
77 model systems were all food grade. Chemicals used for analytical determinations: disodium
78 tetraborate decahydrate, sodium dodecyl sulphate (SDS), *o*-phthaldialdehyde (OPA),
79 dithiothreitol (DTT), serine, hydrochloric acid, *iso*-octane, C7 - C30 saturated alkanes
80 (1,000 µg/mL each component in hexane) were all analytical grade purchased from Sigma-
81 Aldrich.

82 **2.2. Hydrolysis and formation of aromas**

83 Table 1 summarizes the characteristics of the commercial proteases as well as the
84 composition of the fish powder used as starting materials to produce fish-like aromas. Fish

85 powder (100 g/L in water) was hydrolysed for 15 h at constant stirring, under controlled
86 conditions of temperature and pH (60°C at pH 6). The reaction was terminated by heating the
87 mixture at 95°C for 20 min in a water bath. Each protease used was added so all mixtures had
88 the same enzymatic activity per gram of sample. The conditions of pH, temperature and time
89 of reaction, as well as the enzymes and their combinations were selected based on the
90 combination of those parameters that resulted in the higher concentration of free amino acids
91 in a preliminary experiment (data not shown). The resulting slurries were centrifuged at 8,000
92 x g for 20 min and aliquots were analysed to determine the degree of hydrolysis (DH) and
93 amounts of free amino acids.

94 Subsequent reactions to generate aroma compounds were carried out with selected slurries of
95 the fish powder hydrolysates (FPHs) based on the degree of hydrolysis and free amino acid
96 content. Aliquots of FPHs (0.2 mL) were mixed, homogenised with a glucose solution (0.05
97 mL, 80 µmol/mL) in glass reaction vials and freeze-dried. Glycerol (500 µL) was added to
98 each freeze-dried sample to facilitate homogenisation while fish oil (1.5 g/100 g) was added
99 to some of the samples according to the experimental design (Table 2). All samples in closed
100 vials were then homogenised at 60°C for 10 min and subsequently heated at 110°C for 30 min
101 to promote flavour formation. Fish powder hydrolysates without addition of fish oil and
102 before heating were used as control. All samples were prepared and analysed in triplicate.

103 **2.3. Analyses**

104 2.3.1. Chemical analyses. Composition of fish powder and fish oil.

105 The moisture, ash and extractable fat content of the fish powder were calculated according to
106 the Association of Official Analytical Chemists (AOAC, 2000). Total protein was determined
107 by the Kjeldahl method using a nitrogen conversion factor of 6.25 (Ortiz et al., 2006; Yaich
108 et al., 2011).

109 The fatty acid composition was analysed by GC-FID after transesterification to methyl esters
110 (FAMEs) with a mixture BF₃ methanol at 20°C according to the IUPAC standard method
111 (IUPAC, 1992; Peinado, Girón, Koutsidis, & Ames, 2014; Yaich et al., 2011). Analysis of
112 FAMEs was carried out with a DANI Master GC equipped with an auto sampler, a DANI
113 FID detector (DANI Instruments S.p.A, Italy) and an Agilent DB-23 (60 m × 0.25 mm, 0.25
114 µm) capillary column (Agilent Technologies, Cheshire, UK). The oven temperature was
115 programmed from 90°C to 240°C at 4°C/min and the injector and detector temperatures were
116 set at 250°C. The carrier gas was helium at 1.0 mL/min constant flow (split ratio 10:1). Data
117 analysis, identification and quantification of FAMEs was accomplished by comparing the
118 retention times and areas of the peaks with those of pure standards (Supelco® 37 Component
119 FAMEMix, Sigma-Aldrich, Poole, UK) and analysed under the same conditions. The results
120 were expressed as a g of each fatty acid/100 g of the lipid fraction.

121 2.3.2. Degree of hydrolysis, DH

122 The Degree of Hydrolysis (DH) was estimated following a modified OPA spectrophotometric
123 method using aqueous serine, (0.1 g/L) as the reference standard (Church, Porter, Catignani,
124 & Swaisgood, 1985; Nielsen, Petersen, & Dambmann, 2001). For the OPA reagent, disodium
125 tetraborate decahydrate (7.620 g) and sodium dodecyl sulphate (SDS; 200 mg) were
126 dissolved in 150 mL deionized water followed by the addition of 4 mL of o-phthaldialdehyde
127 (160 mg) in ethanol and dithiothreitol (176 mg, 99 %, DTT). The final solution was made up
128 to 200 mL with deionized water. For the analysis, aliquots of FPH or serine standard solution
129 (50 µL) were placed in the wells of a 98-well micro-plate containing 150 µL of OPA-reagent
130 and the absorbance was read at 340 nm. The DH was calculated using equations 1,2 and 3
131 (Church et al., 1985; Nielsen et al., 2001).

$$132 \quad DH = \frac{h}{h_{tot}} \cdot 100\% \quad (\text{Equation 1})$$

$$133 \quad h = \frac{(\text{serine-NH}_2) - b}{a} \quad (\text{Equation 2})$$

$$134 \quad \text{Serine} - \text{NH}_2 = \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}}{\text{Abs}_{\text{standard}} - \text{Abs}_{\text{blank}}} \cdot 0.9516 \cdot 0.1 \cdot \frac{100}{X} \cdot P \quad (\text{Equation 3})$$

135 Where h_{tot} depends on the specific raw material, and for the present study was estimated as
 136 $h_{\text{tot}} = 8.6$; $h = \text{meqv serine} / \text{g protein}$; $\text{serine-NH}_2 = \text{meqv serine-NH}_2 / \text{g protein}$; a and b
 137 depend on the specific raw material, and for the present study they were estimated as $a =$
 138 1.00 , $b = 0.4$; $X = \text{g sample}$; $P = \text{protein \% in the sample}$; 0.1 is the sample volume (L)
 139 (Nielsen et al., 2001).

140 2.3.3. Free amino acids

141 The free amino acid content was calculated following the same method as Elmore, Koutsidis,
 142 Dodson, Mottram, & Wedzicha, (2005). Aliquots of the FPHs (500 μL) were mixed with HCl
 143 (500 μL , 0.01 mol/L) and centrifuged at 7,200 \times g for 15 min. Centrifuged supernatant (100
 144 μL) was derivatized using the EZ-Faast amino acid kit (Phenomenex, Cheshire, UK), and
 145 analysed by (GC-MS). The derivatized amino acids were extracted into *iso*-octane (100 μL)
 146 and analysed in electronic ionization mode at 70 eV using a 6890 GC coupled to a 5973 MSD
 147 instrument (Agilent, Palo Alto, CA). Derivatized amino acid solution (1 μL) was injected at
 148 250 $^{\circ}\text{C}$ in split mode (10:1) onto a 10 m \times 0.25 mm \times 0.25 μm Zebron ZB-AAA capillary
 149 column (film composition 50% phenyl 50% dimethyl polysiloxane) (Phenomenex, Cheshire,
 150 UK). The oven temperature was 110 $^{\circ}\text{C}$ for 1 min, then increased at 30 $^{\circ}\text{C}/\text{min}$ to 320 $^{\circ}\text{C}$, and
 151 held at 320 $^{\circ}\text{C}$ for 2 min. The transfer line was held at 320 $^{\circ}\text{C}$, and the carrier gas was helium
 152 at a constant flow rate of 1.1 mL/min. The ion source was maintained at 320 $^{\circ}\text{C}$. Standard mix
 153 stock solution (200 $\mu\text{mol}/\text{L}$ each) of 15 non-basic amino acids (Ala, Asp, Glu, Gly, His, Ile,
 154 Leu, Lys, Met, Orn, Phe, Pro, Ser, Thr, Val) in hydrochloric acid (0.1 mol/L) and 2 basic
 155 amino acids (Asn, and Gln,) in water were prepared; different dilutions (10 to 150 $\mu\text{mol} / \text{L}$)
 156 were derivatized, and calibration curves were plotted for each amino acid (effect of food

157 matrix composition was studied by spiking samples). Norvaline (100 μ L (0.2 mmol/L)) was
158 used as the internal standard.

159 2.3.4. Volatiles analysis

160 GC/MS analyses were performed using an Agilent 7890A gas chromatograph equipped with
161 a DB-WAX capillary column (60m x 0.25mm i.d. x 0.25 μ m FT) and coupled to a BenchToF
162 Time of Flight Mass Spectrometer (Markes International Ltd, Llantrisant UK) and a CTC
163 CombiPal autosampler (CTC Analytics AG, Zwingen, Switzerland). HS-SPME was
164 performed on aqueous extracts (200 μ L) in 2 mL of saturated NaCl solution. Samples were
165 incubated at 40°C for 40 min followed by a 1 min extraction using a CAR/PDMS/DVB
166 SPME fibre (Supelco, Sigma-Aldrich Company Ltd, UK) and desorption at 260°C for 5 min.
167 The oven temperature was 40°C (held for 5min), 40–200°C at 4°C/min, then to 250°C at
168 8°C/min, held for 5 min. Helium was used as the carrier gas at a flow rate of 1 mL/min.

169 The volatile compounds were identified by comparing their mass spectra with spectral data
170 from the National Institute of Standards and Technology 2008 library as well as retention
171 indices published in the literature (Ganeko et al., 2008; Giri et al., 2010; pherobase. org).
172 Relative retention indices were determined by injection into the column of a solution
173 containing a series of *n*-alkanes (C7–C30, saturated alkanes (1,000 μ g/mL in hexane) Sigma-
174 Aldrich Company Ltd, UK) in the same temperature programmed run as described above.
175 Quantification of selected compounds was carried out using external calibration curves.

176 **2.4. Sensory Evaluation**

177 Consumers' preferences were assessed by the Friedman Pairwise ranking analysis (Escriche,
178 Fernández-Segovia, Serra, Andrés, & Barat, 2001; González-Tomás, Carbonell, & Costell,
179 2004; Peinado, Rosa, Heredia, Escriche, & Andrés, 2012). This test is used with a non-trained
180 panel, to evaluate sets of three to six samples, considering a single attribute each time.
181 Twenty-seven subjects constituted the panel. The samples selected were presented to each of

182 the subjects in all possible paired combinations. The selection of the sensory attributes was
183 based on the characteristic criteria of the samples as well as some previous experiments on
184 similar products (Ganeko et al., 2008; Giri et al., 2010). Panellists were asked which of the
185 two samples presented they would assess as: “stale” (smell of fish cooked for too long),
186 “fried” (smell of fish cooked in fat or oil, usually over direct heat), “grilled” (typical fish
187 cooked in a grill), and their overall preference.

188 Significant differences between the samples were established by the statistical function T-
189 Friedman and compared with the tabulated $\chi^2=7.81$ ($\alpha = 0.05$) with $(t-1)$ degrees of freedom
190 (Meilgaard, Civille, & Carr, 1999).

191 Afterwards, Tukey’s honestly significant difference (HSD) was calculated to establish
192 between which samples these differences lay (equation 4), (Meilgaard et al., 1999):

$$193 \quad HSD = q_{\alpha,t,\infty} \left(p \cdot \frac{t}{4} \right)^{\frac{1}{2}} \quad (\text{Equation 4})$$

194 where $q_{\alpha,t,\infty}$ is a tabulated value, p is the number of panellists and t the number of samples
195 ($t=4$), (Meilgaard et al., 1999).

196 **2.5. Statistics**

197 Analysis of variance (ANOVA) and the Friedman test (p -value < 0.05) were carried out using
198 SPSS to estimate the differences in amino acid composition of the FPHs. Principal
199 Component Analysis, PCA, (SPSS) was applied to differentiate the FPHs based on their
200 volatile compound.

201 Furthermore, a correspondence analysis was performed to establish whether the selected
202 samples and the evaluated sensory attributes map. This tool establishes the association
203 between categorical variables (Beh, Lombardo, & Simonetti, 2011; Guerrero et al., 2010).

204

205 **3. Results and discussion**206 **3.1. Hydrolysis of fish powder**

207 **Degree of hydrolysis (DH):** the OPA method to determine the DH is based on the specific
208 reaction between the OPA-reagent and primary amino groups, in the presence of a thiol to
209 form 1-alkylthio-2-alkyl-substituted isoindoles that can be quantified spectrophotometrically
210 at 340 nm (Medina-Hernández et al, 1990). The DH is presented in table 2; All proteases
211 produced a high DH compared to the control FP. Individual proteases, “A” (endo and exo
212 peptidase activities) and “B” (exopeptidase activity) showed high degrees of hydrolysis (30.5
213 $\pm 1.2\%$ and $46.0 \pm 0.7\%$ respectively). The fact that the DH was higher with enzyme “B”
214 indicates that having dual enzymatic activity within one enzyme does not necessarily increase
215 the DH. The same conclusion could be achieved when enzyme “B” was combined with
216 enzymes “C” or “D” (endopeptidases). However, the combination “B+E” produced the
217 highest DH ($57.4 \pm 0.9\%$). It is not easy to compare the hydrolysates prepared using the
218 different proteases because they have optimal working conditions and specificities.

219 **The individual free amino acid** content of the FPHs is illustrated in table 2 together with the
220 changes in the concentrations for the amino acids in the FPHs compared to the control (ΔC
221 %). 17 amino acids were identified and quantified in the different FPHs. Lysine, leucine,
222 glutamic acid and alanine, were the most abundant in most of the FPHs (235 - $1,484 \mu\text{g/g}$),
223 reaching their highest concentrations for the combination “B+C” (Lys [$1,484 \pm 43 \mu\text{g/g}$], Leu
224 [$1,423 \pm 48 \mu\text{g/g}$], Glu [$981 \pm 142 \mu\text{g/g}$] and Ala [$939 \pm 135 \mu\text{g/g}$]). His, Ile, Phe, Ser and
225 Thr, were in the range of 178 - $742 \mu\text{g/g}$, also with their highest concentrations for the
226 combination “B+C”; while Gly, Pro, Asp, Met, His, Tyr and Trp, were found in smaller
227 concentrations. Depending on the enzymes/combination of enzymes, there were significant
228 differences in the concentration of the amino acids within the FPHs; some amino acids, such

229 as Ala, Gly or Pro, increased their concentration, up to 3-6 fold compared to the control-FPH
230 (regardless of their initial concentration) while some others such as Lys, Met or Leu
231 increased their concentrations up to 23-35 fold compared to the control-FPH (Table 2).

232 *3.2. Development of aromas*

233 A total of 32 volatile compounds were identified in the heated fish powder hydrolysates (H-
234 FPHs) (Table 3). Most of the compounds identified in the control sample (heated without the
235 addition of external enzymes), were also identified in the H-FPHs heated with glucose with
236 or without fish oil (Table 4).

237 **Aldehydes** significantly contribute to the overall aroma of cooked fish/seafood due to their
238 low threshold values (Table 3). In the present study, the concentration of aldehydes increased
239 in the H-FPHs, being higher in those samples containing fish oil (Table 4). This increase in
240 samples containing oil might be expected, as some aldehydes might be generated from lipid
241 oxidation, e.g., hexanal, present in much higher concentrations in the H-FPHs containing fish
242 oil, derives mainly from the oxidation of linoleic acid. Moreover, some other aldehydes, such
243 as 2-methylpropanal, 4-heptenal and 2,4-heptadienal, not found in the control, were abundant
244 in the H-FPHs. 2,4-Heptadienal, which is a degradation product of linolenic acid (Decker,
245 Elias, & McClements, 2010), was only found in samples containing fish oil (Table 4). Some
246 branched short chain aldehydes could result from deamination of amino acids. The major
247 aldehyde in the H-FPHs, regardless of the incorporation of fish oil, was 3-methyl-butanal,
248 which presence was attributed to the high concentration of leucine in the FPHs. While in
249 some other cases aldehydes can originate from the Strecker degradation of amino acids, for
250 instance, 2-methylbutanal, which was also in considerable concentrations in the H-FPHs, may
251 be derived from isoleucine. Due to their low threshold values, the Strecker aldehydes
252 including 2-methylpropanal, 2-methylbutanal and 3-methylbutanal, might impart nutty/malty
253 nuances to the product while, some others aldehydes such as heptanal, octanal or nonanal

254 might impart a more characteristic fishy flavour (Caprino et al., 2008; Giri et al., 2010; Selli
255 & Cayhan, 2009).

256 **Alcohols** are mainly formed by an enzymic peroxidation of the n-3 and n-6 polyunsaturated
257 fatty acids, present in fish tissue. 1-Penten-3-ol, significantly increased in samples containing
258 fish oil (Table 4). Although not all alcohols are likely to have an important contribution to
259 odour, due to their relatively high odour threshold values (Table 3), unsaturated alcohols such
260 as 1-octen-3-ol, with generally much lower threshold values than the saturated counterparts,
261 might have a greater impact on the overall aroma (Kawai & Sakaguchi, 1996; Selli &
262 Cayhan, 2009) .

263 Amongst the **ketones** identified, 2-heptanone, 2-octanone, 2-nonanone and undecanone,
264 slightly increased in the H-FPHs, regardless the addition of fish oil. However, 1-penten-3-
265 one, not present in the control, appeared in all H-FPHs, with a significant increase in those H-
266 FPHs containing fish oil. Due to its low odour threshold value (Table 3), this compound,
267 which might result as a degradation product of linolenic acid, is likely to contribute pungent
268 and fish-like notes to the aroma (Decker et al., 2010; Giri et al., 2010). Ketones are mainly
269 produced a result of lipid-oxidation and/or amino acid degradation due to the Strecker
270 reaction, and are associated with off-flavour (Selli & Cayhan, 2009)

271 **Acids** such as acetic acid, propanoic acid, 2-methyl propanoic acid, butanoic acid and 3-
272 methyl butanoic acid with relatively low threshold values (Table 3), have been reported to
273 result from fermentation in several fish products (Giri et al., 2010). In the present study acetic
274 acid was identified but its concentration did not differ significantly when compared to the
275 control. These acids can derive either from lipolysis or from amino acid metabolism
276 (deamination) (Montel, Masson, & Talon, 1998).

277 **Sulphur-containing compounds** dimethyl disulphide, (cooked cabbage aroma), and
278 dimethyl trisulphide, (meaty and cooked onion aroma), increased considerably. These

279 compounds, usually associated with deterioration of seafood, have a very strong effect on the
280 overall food aroma even at low concentrations because of their low threshold values (Table 3)
281 (Le Guen, Prost, & Demaimay, 2001; Selli & Cayhan, 2009). They are known to originate
282 from the free, peptidic and proteinic sulphur amino acids, such as methionine, which
283 concentration increased considerably after enzymatic hydrolysis (Table 2).

284 **Furans:** Amongst the heterocyclic compounds identified, furans, which possess low odour
285 threshold values, were present in much higher concentrations in the H-FPHs containing fish
286 oil. They can be formed from amino acids by the Amadori rearrangement pathway, but also
287 by the oxidation of fatty acids, i.e. the formation of 2-pentylfuran, which is one of the
288 resulting products from the oxidation of linoleic acid (Giri et al., 2010; Taylor & Mottram,
289 1990; Whistler & Daniel, 1985).

290 **Pyrazines,** characteristic compounds derived from the Maillard reaction imparting amongst
291 other roasted and nutty flavour (Fox & Wallace, 1997; Giri et al., 2010), importantly
292 increased in the H-FPHs. However, the fact that there were no significant differences in their
293 concentration in the model systems with added fish oil compared to those without fish oil
294 demonstrates that the addition of lipo-oxidation products did not contribute to the pool of
295 carbohydrates. This might have been due to carbohydrates being in excess in the model
296 systems (i.e. added glucose).

297 Figure 1 illustrates the PCA conducted to evaluate the differences in the volatile composition
298 of the different samples. The first three components explain 88.1% of the total variability.
299 The first two principal components (PC1: 39.9% and PC2: 31.6%) differentiate between the
300 H-FPHs containing additional fish oil from those without it. In the same way some of the
301 volatile compounds such as hexanal, heptanal, 4-heptanal, 2,4,-heptadienal, 1-penten-3-ol or
302 1-octen-3-ol, derived from fatty acids such as linoleic and linolenic acids, are located on the
303 right side of the plot together with the H-FPHs containing fish oil. The two H-FPHs controls

304 (with and without fish oil) are separated from the compounds that illustrated a higher increase
305 as a result of the addition of enzymes. These compounds include 2-methylbutanal, 3-
306 methylbutanal, 1-hepten-4-ol and the sulphur compounds, which have also been found in the
307 volatile profile of cooked fish or meals containing seafood (Ganeko et al., 2008; Giri et al.,
308 2010; Selli & Cayhan, 2009), The addition of fish oil, however, did not have a significant
309 impact on the formation of these compounds or pyrazines. The use of enzymes did produce a
310 high DH with different concentrations of the free amino acids in the FPHs that would have
311 been expected to have a high impact on the formation of the volatile compounds. However,
312 the differences due to the use of these various enzymes were not significant in terms of
313 concentrations of the Maillard reaction products including pyrazines, sulphur compounds and
314 some aldehydes.

315 ***3.3 Sensory evaluation***

316 Only enzyme B with increased amounts of fish oil was selected to carry out the sensory
317 evaluation (Figure 2). The selection of enzyme B was based on its high release of free amino
318 acids. Different concentrations of fish oil (0, 1.5 and 3 g/100g) were investigated to establish
319 the role of fish oil on the formation of aroma, as well as its influence on sensory perception.

320 Panellists evaluated a total of six pairs of samples, corresponding to all the possible
321 combinations. The statistic of Friedman test for each sample was compared with the statistic of
322 chi-square (X^2) with 3 degrees of freedom (7.82, $\alpha = 0.05$). A significant difference was
323 observed for all the attributes in the samples evaluated.

324 Friedman test was followed by specific comparisons using Tukey's Honestly Significant
325 Difference (HSD) multiple comparison post-hoc statistical test (Meilgaard et al., 1999). The
326 value of q tabulated for 3 degrees of freedom ($\alpha = 0.05$), was 3.63 and the HSD value
327 obtained by equation 4 was 18.85. The rank sums (addition of twice the sum of the
328 frequencies of the columns to the sum of the frequencies of the rows for each sample

329 (Peinado et al., 2012)) were calculated, a table of rank sum differences was prepared and the
330 differences were compared with the value of HSD being significant when this value was
331 exceeded (Figure 2).

332 Panellist did not find significant differences between the H-FPHs samples regardless the
333 concentration of added fish oil (0, 1.5 and 3 g/100g) for all the attributes. However, panellists
334 found significant differences for “stale” when “100% FO” was compared to H-FPHs without
335 addition of fish oil. For “fried” aroma, significant differences were found when “100% FO”
336 was compared with H-FPHS with 0 and 1.5 g/100g of fish oil. Finally for “grilled” aroma,
337 panellists found significant differences between “100% FO” and all the H-FPHs regardless
338 the addition of fish oil. For the global preference the three H-FPHs had similar scores.
339 Furthermore, figure 2 illustrates the two-dimensional plot of the sample scores and compound
340 loadings obtained by the correspondence analysis. The first two dimensions explained
341 99.99% of the total variance (dimension 1, 97.4%; dimension 2, 2.6%). H-FPHs with
342 different concentrations of fish oil were preferred by the panellists. “Fried” and “grilled”
343 contributed the most to the global preference while “stale” contributed negatively to the global
344 preference of the product. There were no differences between the three H-FPHs in terms
345 global preference.

346

347 **4. Conclusions**

348 Heating FPHs (as a source of amino acids), a source of sugar and fish oil successfully
349 produced volatiles at a laboratory scale. Enzyme “B” (exopeptidase) on its own or in
350 combination with endopeptidases is suggested as the starting point to liberate amino acids
351 from fish protein while the dual activity enzyme “A” produced a lower amount of free amino
352 acids.

353 The use of various enzymes produced different amounts of amino acids in the FPHs with
354 important amounts of lysine, leucine, glutamic acid and alanine being released. These
355 increased on free amino acids will have an influence on the characteristic compounds derived
356 from the Maillard reaction, such as pyrazines, sulphur compounds or some aldehydes. Fish
357 oil had a great impact on the volatile compounds associated with fish aroma; its addition
358 enhanced the concentration of some lipid oxidation products such as hexanal, heptanal, 4-
359 heptanal, 2,4-hexadienal, 1-penten-3-ol or 1-octen-3-ol, characteristic impact compounds in
360 seafood, that have been previously identified in the volatile profile of cooked fish or meals
361 containing seafood. “Grilled” and “fried” aromas, characteristics of FPHs heated with fish
362 oil, were preferred by panellists, while fish oil on its own produced unpleasant aromas.
363 Future work involving different types and concentrations of fish oil together with sensory
364 evaluation is suggested to investigate the acceptability of seafood-derived fish-like flavouring
365 formulations based on such approaches.

366

367 **Acknowledgments**

368 The authors would like to thank Frutarom (UK) Ltd, Biocatalysts (UK) and the Technology
369 Strategy Board (TSB), (UK) for the financial support given to this investigation.

370

371 **References**

372

373 Annadurai, D., Sadeeshkumar, R., Vijayalaksmi, M., & Pirithiviraj, N. (2012). Studies on
374 Growth of Marine Bacteria Using Marine Fish Waste Medium. *International Journal*
375 *of Pharmaceutical & Biological Archives* 3(4), 910–913. doi:ISSN: 0976 – 3333

376 Aspino, S. I., Horn, S. J., & H. Eijsink, V. G. (2005). Enzymatic hydrolysis of Atlantic cod
377 (*Gadus morhua* L.) viscera. *Process Biochemistry*, 40(5), 1957–1966.
378 doi:10.1016/j.procbio.2004.07.011

379 Beh, E. J., Lombardo, R., & Simonetti, B. (2011). A European perception of food using two
380 methods of correspondence analysis. *Food Quality and Preference*, 22(2), 226–231.
381 doi:10.1016/j.foodqual.2010.10.001

382 Caprino, F., Moretti, V. M., Bellagamba, F., Turchini, G. M., Busetto, M. L., Giani, I.,
383 Pazzaglia, M. (2008). Fatty acid composition and volatile compounds of caviar from
384 farmed white sturgeon (*Acipenser transmontanus*). *Analytica Chimica Acta*, 617(1-2),
385 139–47. doi:10.1016/j.aca.2008.02.005

386 Church, F. C., Porter, D. H., Catignani, G. L., & Swaisgood, H. E. (1985). An o-
387 phthalaldehyde spectrophotometric assay for proteinases. *Analytical Biochemistry*,
388 146(2), 343–8. doi:10.1016/0003-2697(85)90549-4

389 Decker, E., Elias, R., & McClements, D. J. (2010). *Oxidation in Foods and Beverages and*
390 *Antioxidant Applications: Management in Different Industry Sectors*. Woodhead
391 Publishing. Retrieved from [https://www.elsevier.com/books/oxidation-in-foods-and-](https://www.elsevier.com/books/oxidation-in-foods-and-beverages-and-antioxidant-applications/decker/978-1-84569-983-3)
392 [beverages-and-antioxidant-applications/decker/978-1-84569-983-3](https://www.elsevier.com/books/oxidation-in-foods-and-beverages-and-antioxidant-applications/decker/978-1-84569-983-3)

393 Elmore, J. S., Koutsidis, G., Dodson, A. T., Mottram, D. S., & Wedzicha, B. L. (2005).
394 Measurement of acrylamide and its precursors in potato, wheat, and rye model

- 395 systems. *Journal of Agricultural and Food Chemistry*, 53(4), 1286–93.
396 doi:10.1021/jf048557b
- 397 Escriche, I., Fernández-Segovia, I., Serra, J. A., Andrés, A., & Barat, J. M. (2001).
398 Evaluación sensorial del bacalao desalado, pretratado térmicamente listo para cocinar.
399 *Alimentaria: Revista de Tecnología E Higiene de Los Alimentos*, 322, 51–54.
400 doi:ISSN 0300-5755
- 401 Fox, P. F., & Wallace, J. M. (1997). Formation of Flavor Compounds in Cheese. In *Advances*
402 *in Applied Microbiology* (Vol. 45, pp. 17–85). doi:10.1016/S0065-2164(08)70261-2
- 403 Ganeko, N., Shoda, M., Hirohara, I., Bhadra, A., Ishida, T., Matsuda, H., ... Matoba, T.
404 (2008a). Analysis of volatile flavor compounds of sardine (*Sardinops melanostica*) by
405 solid phase microextraction. *Journal of Food Science*, 73(1), S83–8.
406 doi:10.1111/j.1750-3841.2007.00608.x
- 407 Ghorbel, S., Souissi, N., Triki-Ellouz, Y., Dufosse, L., Guerard, F., & Nasri, M. (2005).
408 Preparation and testing of Sardinella protein hydrolysates as nitrogen source for
409 extracellular lipase production by *Rhizopus oryzae*. *World Journal of Microbiology*
410 *and Biotechnology*, 21(1), 33–38. doi:10.1007/s11274-004-1556-2
- 411 Giri, A., Osako, K., & Ohshima, T. (2010). Identification and characterisation of headspace
412 volatiles of fish miso, a Japanese fish meat based fermented paste, with special
413 emphasis on effect of fish species and meat washing. *Food Chemistry*, 120(2), 621–
414 631. doi:10.1016/j.foodchem.2009.10.036
- 415 González-Tomás, L., Carbonell, I., & Costell, E. (2004). Influence of type, concentration and
416 flow behaviour of hydrocolloid solutions on aroma perception. *European Food*
417 *Research and Technology*, 218(3), 248–252. doi:10.1007/s00217-003-0852-7

- 418 Guerard, F., Dufosse, L., Broise, D. D. La, & Binet, A. (2001). Enzymatic hydrolysis of
419 proteins from yellowfin tuna (*Thunnus albacares*) wastes using Alcalase. *Journal of*
420 *Molecular Catalysis B: Enzymatic* 11, 1051–1059. doi:1381-1177/01
- 421 Guerrero, L., Claret, A., Verbeke, W., Enderli, G., Zakowska-Biemans, S., Vanhonacker, F.,
422 ... Hersleth, M. (2010). Perception of traditional food products in six European
423 regions using free word association. *Food Quality and Preference*, 21(2), 225–233.
424 doi:10.1016/j.foodqual.2009.06.003
- 425 Kawai, T., & Sakaguchi, M. (1996). Fish flavour. *Critical Reviews in Food Science and*
426 *Nutrition*, 36(3), 257–298. doi:10.1080/10408399609527725
- 427 Leffingwell.com: <http://www.leffingwell.com/odorthre.htm> (last accessed: 12/01/2015)
- 428 Le Guen, S., Prost, C., & Demaimay, M. (2001). Evaluation of the Representativeness of the
429 Odoe of Cooked Mussel Extracts and the Relationship between Sensory Descriptors
430 and Potent Odorants. *J. Agric. Food Chem.*, 49, 1321–1327. doi:10.1021/jf000781n
- 431 Medina Hernández, M. J., Villanueva Camañas, R. M., Monfort Cuenca, E., & García
432 Alvarez-Coque, M. C. (1990). Determination of the protein and free amino acid
433 content in a sample using o-phthalaldehyde and N-acetyl-L-cysteine. *The Analyst*,
434 115(8), 1125–8. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/2256555>
- 435 Meilgaard, M., Civille, G. V., & Carr, B. T. (1999). Attribute difference test III. Pairwise
436 ranking test: Friedman analysis. In *Sensory evaluation techniques, 3rd ed* (3rd ed., pp.
437 103–106). CRC Press, Taylor & Francis Group. Retrieved from <http://goo.gl/wvAjSK>
- 438 Montel, M. C., Masson, F., & Talon, R. (1998). Bacterial role in flavour development. *Meat*
439 *Science*, 49, S111–S123. doi:10.1016/S0309-1740(98)90042-0
- 440 Nielsen, P. M., Petersen, D., & Dambmann, C. (2001). Improved Method for Determining
441 Food Protein Degree of Hydrolysis. *Food Chemistry and Toxicology*, 66(5), 642–646.
442 doi:10.1111/j.1365-2621.2001.tb04614.x

- 443 Ortiz, J., Romero, N., Robert, P., Araya, J., López-Hernández, J., Bozzo, C., ... Rios, A.
444 (2006). Dietary fiber , amino acid , fatty acid and tocopherol contents of the edible
445 seaweeds *Ulva lactuca* and *Durvillaea antarctica*. *Food Chemistry*, 99, 98–104.
446 doi:10.1016/j.foodchem.2005.07.027
- 447 pherobase.com: <http://goo.gl/DjyL0B> (last accessed: 10/02/2015)
- 448 Peinado, I., Girón, J., Koutsidis, G., & Ames, J. (2014). Chemical composition , antioxidant
449 activity and sensory evaluation of five different species of brown edible seaweeds.
450 *Food Research International*, 66, 36–44. doi:10.1016/j.foodres.2014.08.035
- 451 Peinado, I., Rosa, E., Heredia, A., Escriche, I., & Andrés, A. (2012). Optical, Mechanical and
452 Sensorial Properties of Strawberry Spreadable Products Formulated with
453 Isomaltulose. *Food and Bioprocess Technology*, 6(9), 2353–2364.
454 doi:10.1007/s11947-012-0970-y
- 455 Selli, S., & Cayhan, G. G. (2009). Analysis of volatile compounds of wild gilthead sea bream
456 (*Sparus aurata*) by simultaneous distillation–extraction (SDE) and GC–MS.
457 *Microchemical Journal*, 93(2), 232–235. doi:10.1016/j.microc.2009.07.010
- 458 Souissi, N., Bougatef, A., Triki-ellouz, Y., & Nasri, M. (2007). Biochemical and Functional
459 Properties of Sardinella (*Sardinella aurita*) By-Product Hydrolysates, 45(2), 187–194.
460 doi:ISSN: 1330-9862
- 461 Taylor, A. J., & Mottram, D. S. (1990). Composition and odour of volatiles from autoxidised
462 methyl arachidonate. *Journal of the Science of Food and Agriculture*, 50(3), 407–417.
463 doi:10.1002/jsfa.2740500313
- 464 Whistler, R. L., & Daniel, J. R. (1985). Carbohydrates. In *Food Chemistry* (pp. 248–339). O.
465 Fennema (Ed.). doi:10.1007/978-3-540-69934-7
- 466 Whitfield, F. B., Freeman, D. J., Last, J. H., Bannister, P. A., & Kennett, B. H. (1982). Oct-1-
467 en-3-ol and (5Z)-Octa-1,5-dien-3-ol, compounds important in the flavour of prawns

468 and sand-lobsters. *Australian Journal of Chemistry*, 35(2), 373–383.

469 doi:10.1071/CH9820373

470 Whitfield, F. B., Last, J. H., Shaw, K. J., & Tindale, C. R. (1988). 2,6-Dibromophenol: The

471 cause of an iodoform-like off-flavour in some Australian crustacea. *Journal of the*

472 *Science of Food and Agriculture*, 46(1), 29–42. doi:10.1002/jsfa.2740460104

473 Yaich, H., Garna, H., Besbes, S., Paquot, M., Blecker, C., & Attia, H. (2011). Chemical

474 composition and functional properties of *Ulva lactuca* seaweed collected in Tunisia.

475 *Food Chemistry*, 128(4), 895–901. doi:10.1016/j.foodchem.2011.03.114

476

477 **Figure Caption**

478 **Figure 1.** Biplot for the different heated fish powder hydrolysates generated with the
479 different enzymes (Control: fish powder heated without addition of enzymes; A: H-FPH-
480 Flavopro Umami 852; B: H-FPH-Flavopro 750; B+C: H-FPH- Flavopro 750+Promod439;
481 B+D: H-FPH-Flavopro 750+Promod671; B+E: H-FPH-Flavopro 750+Promod144; _O stands
482 for addition of fish oil (1.5 g/100g)) and the volatile compounds obtained by the PCA. (PC1:
483 39.9 %, PC2: 31.6 %)

484

485 **Figure 2:** Two-dimensional correspondence plot (99.9 % of the total variance: dimension 1,
486 97.4 %; dimension 2, 2.6 %) obtained from performing the correspondence analysis for the
487 four selected samples considering the fish powder hydrolysate obtained with enzyme A and
488 increasing concentrations of fish oil (0, 1.5, 3 g/100g and pure fish oil heated under the same
489 conditions). Rank sum for the different attributes obtained by Friedman test. a, b and c Values
490 in the same row with significant differences (95 %).

491

Table 1: Description of commercial enzymes used for the fish powder hydrolysis. Characterization of fish powder (ash, moisture, fat, protein, carbohydrates (g/100g)). Composition of fish oil (n=3).

Enzymes characteristics*

	Enzyme	Activity	Optimum pH	Optimum T^a
A	Flavopro Umami F825MDP	Leucine aminopeptidase	5.5-7.5	45-55
B	Flavopro 750P	Casein peptidase	5.5-7.5	45-55
C	Promod 144	Papain	5.0-7.6	50-70
D	Promod 439	Casein Protease	6.0-9.0	45-60
E	Promod 671	Casein Protease	5.5-8.0	30-50

Fish powder composition (%)

x^w	ash	protein	fat^a	carbohydrates
4.67 ± 0.16	22.4 ± 0.3	60.3 ± 0.6	1.5 ± 0.4	11.1 ± 0.70

Fat composition (g/100g total fat)

	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C20:5	C22:6
Fish powder fat ^a	4.3 ± 0.5	47.0 ± 1.3	16.3 ± 0.5	15.3 ± 0.6	0.40 ± 0.03	-	0.67 ± 0.09	0.55 ± 0.02
Fish oil	10.4 ± 0.3	29.1 ± 0.8	16.7 ± 1.8	8.2 ± 0.5	2.8 ± 0.3	3.8 ± 0.6	11.2 ± 0.7	6.5 ± 0.3

*Biocatalysts, Ltd.

Table 2. Fish powder hydrolysates obtained with individual enzymes or combination of enzymes. Degree of Hydrolysis (DH %) and concentration of individual free amino acids ($\mu\text{g} / \text{g}$). Changes in the concentration of the individual free amino acids compared to the control (ΔC)*. (n=3).

		Fish powder	FPH				
		Control	A	B	B+C	B+D	B+E
DH	(%)	7 ± 2	31 ± 1	46 ± 1	23 ± 1	21 ± 6	57 ± 5
Alanine	$\mu\text{g} / \text{g}$	235 ± 8	365 ± 4	829 ± 40	938 ± 33	655 ± 34	677 ± 19
	ΔC (%)		55 ± 2	252 ± 17	299 ± 14	178 ± 14	188 ± 8
Glycine	$\mu\text{g} / \text{g}$	155 ± 5	114 ± 3	322 ± 10	315 ± 10	371 ± 26	247 ± 6
	ΔC (%)		-27 ± 2	107 ± 6	103 ± 7	139 ± 17	59 ± 4
Valine	$\mu\text{g} / \text{g}$	47 ± 3	224 ± 15	661 ± 50	726 ± 59	448 ± 47	541 ± 16
	ΔC (%)		374 ± 31	$1,297 \pm 106$	$1,435 \pm 123$	848 ± 97	$1,045 \pm 33$
Leucine	$\mu\text{g} / \text{g}$	48 ± 3	675 ± 15	$1,113 \pm 51$	$1,423 \pm 59$	929 ± 47	$1,025 \pm 16$
	ΔC (%)		$12,989 \pm 31$	$2,205 \pm 105$	$2,848 \pm 123$	$1,825 \pm 98$	$2,022 \pm 33$
Isoleucine	$\mu\text{g} / \text{g}$	29 ± 3	227 ± 5	613 ± 50	683 ± 14	412 ± 8	522 ± 9
	ΔC (%)		670 ± 16	$1,981 \pm 170$	$2,218 \pm 47$	$1,299 \pm 27$	$1,674 \pm 32$
Threonine	$\mu\text{g} / \text{g}$	54 ± 6	194 ± 21	604 ± 29	742 ± 48	473 ± 18	541 ± 72
	ΔC (%)		260 ± 39	$1,023 \pm 53$	$1,280 \pm 88$	778 ± 33	906 ± 135
Serine	$\mu\text{g} / \text{g}$	60 ± 8	178 ± 42	616 ± 20	652 ± 34	429 ± 51	454 ± 99
	ΔC (%)		198 ± 70	927 ± 33	988 ± 57	616 ± 85	658 ± 166
Proline	$\mu\text{g} / \text{g}$	50 ± 2	60 ± 3	174 ± 2	154 ± 6	116 ± 5	130 ± 14
	ΔC (%)		19 ± 6	247 ± 5	207 ± 13	130 ± 10	158 ± 27
Aspartic acid	$\mu\text{g} / \text{g}$	51 ± 6	84 ± 2	260 ± 33	274 ± 36	181 ± 6	160 ± 13
	ΔC (%)		68 ± 4	421 ± 66	450 ± 72	262 ± 12	222 ± 25
Methionine	$\mu\text{g} / \text{g}$	11 ± 3	125 ± 8	298 ± 26	346 ± 23	239 ± 17	263 ± 6
	ΔC (%)		997 ± 63	$2,511 \pm 224$	$2,932 \pm 204$	$1,997 \pm 147$	$2,202 \pm 54$
Glutamic acid	$\mu\text{g} / \text{g}$	144 ± 23	470 ± 31	880 ± 7	981 ± 64	573 ± 86	431 ± 25
	ΔC (%)		227 ± 21	513 ± 5	583 ± 44	299 ± 60	200 ± 17
Phenylalanine	$\mu\text{g} / \text{g}$	27 ± 1	255 ± 31	360 ± 43	451 ± 15	292 ± 15	352 ± 2
	ΔC (%)		860 ± 115	$1,256 \pm 162$	$1,597 \pm 55$	997 ± 55	$1,227 \pm 6$
Lysine	$\mu\text{g} / \text{g}$	43 ± 1	369 ± 80	818 ± 157	$1,485 \pm 22$	887 ± 101	836 ± 66
	ΔC (%)		751 ± 186	$1,789 \pm 363$	$3,332 \pm 50$	$1,949 \pm 233$	$1,830 \pm 152$
Other	$\mu\text{g} / \text{g}$	23 ± 2	138 ± 3	187 ± 14	286 ± 3	167 ± 5	229 ± 3
	ΔC (%)		687 ± 120	831 ± 206	$1,273 \pm 271$	760 ± 209	$1,094 \pm 169$

Production of Fish powder hydrolysates (FPH): fish powder (100 g/L in water) + commercial enzymes ([A, B, C, D, E], Table 1), heated overnight (15 h) at constant stirring (pH 6, and 60 °C, enzyme (10-20 g/L).

* ΔC (%) = $100 \cdot [\text{concentration of each free amino acid in the fish powder hydrolysates} - \text{concentration of each free amino acid in the control}] / \text{concentration of each free amino acid in the control}$

Table 3. Retention time, retention index and odour descriptors of volatile compounds found in the different fish powder hydrolysates after heating them with or without fish oil (H-FPHs) (n=3).

	RT	RI	Odour threshold	Identification	Odour description
<i>Aldehydes</i>					
2-methyl propanal	6.03	647	0.1-2.3 ^D	MS, RI Std	Green, Pungent, Burnt, Malty, Toasted, Fruity ^C
2-methyl butanal	8.42	912	1 ^D	MS, RI Std	Green, Almond, Strong burnt, Malty, Cocoa ^C
3-methyl butanal	8.53	914	0.2-2 ^D	MS, RI Std	Cashew, apple ^A , almond-like, toasted, malty, green ^C Herbaceous
hexanal	16.54	1079	4.5-5 ^D	MS, RI Std	Fishy, grass ^{A,B,C}
heptanal	21.60	1170	3 ^D	MS, RI Std	Citrus like ^A , dry fish ^B green, fatty, solvent, smoky, Rancid ^C
4-heptenal	24.14	1226	0.8-10 ^D	MS, RI Std	Boiled potato, creamy, sweet, biscuit-like ^{A,B,C}
octanal	26.06	1286	0.7 ^D	MS, RI Std	Lemon, stew-like, boiled meat-like, rancid, soapy, citrus, green, flower, fruit, orange ^{A,B,C}
nonanal	30.03	1405	1 ^D	MS, RI Std	Gravy, green, fruity, gas, chlorine, floral, waxy, sweet, melon, soapy, fatty, citrus fruit ^{A,B,C}
2-octenal	31.30	1512	3 ^D	MS, RI	Aromatic, oxidized oil-like ^A , Green ^C
benzaldehyde	33.014	1539	350-3,500 ^D	MS, RI	Bitter almond ^{A,C,C} , Burnt sugar, Woody ^C
2,4-heptadienal	33.52	1548	15-95 ^A	MS, RI Std	Fatty, fishy ^{A,C} , aromatic, oxidized oil-like ^B
<i>Alcohols</i>					
1-penten-3-ol	20.321	1145	350-400 ^{A,D}	MS, RI Std	Burnt, meaty ^A , paint like chemical like ^B grassy-green ^C
4-ethyl phenol	23.70	1213	140 ^D	MS, RI	Shoe polish, phenolic, leather, smoky ^{A,B,C}
1-octen-3-ol	31.79	1519	1-1.5 ^{A,D}	MS, RI Std	Fishy, grassy ^A , sweet earthy ^C
1-heptanol	31.96	1522	3-5.4 ^{A,D}	MS, RI, Std	Fresh, light green, nutty ^{A,B,C}
4-hepten-1-ol	33.57	1597	-	MS, RI Std	Fishy ^C
<i>Ketones</i>					

1-penten-3-one	13.45	1020	1-1.3 ^D	MS, RI, Std	Pungent, fish-like, rotten, fruity, plastic, leather ^{A,B,C}
2-heptanone	21.43	1167	140-3,000 ^D	MS, RI Std	Cured ham-like, toasted, nutty, gas, gravy, soapy, Fruity ^C
2-octanone	25.86	1280	50 ^{A,D}	MS, RI Std	Gas, stewed, fatty, green, fruity, cheese-apple ^C
2-nonanone	29.83	1395	5-200 ^D	MS, RI Std	Fruity, soapy, fatty, green, earthy, baked ^C
undecanone	36.79	1601	5-7 ^{A,D}	MS, RI Std	Tallow, musty ^A Fruity, musty, dusty, green ^C
Acids					
butanoic acid butyl ester	23.09	1196	100 ^D	MS, RI	Fresh, Sweet, Fruity ^C
acetic acid	32.13	1525	30-150 ^D	MS, RI	Sour, Vinegar, Pungent ^C
Sulfur compounds					
dimethyl disulfide	16.01	1069	0.16-12 ^{A,D}	MS, RI	Sulfur, Cabbage, Ripened cheese, Putrid ^{A,C}
Dimethyl trisulfide	29.66	1390	0.005-0.01 ^D	MS, RI, Std	Rotten food, Sulfury, Fishy, Cauliflower, Cabbage, Onion ^{A,C}
Furans					
2-ethyl furan	10.16	950	8 ^{A,D}	MS, RI	Rubber, Pungent, Acid, Sweet ^C
2-ethyl-5-methyl furan	14.02	1031	-	MS, RI	
2-pentyl furan	23.70	1213	6 ^{A,D}	MS, RI, Std	Buttery, Green bean-like ^{A,C}
Pyrazines					
Methyl pyrazine	25.01	1253	60-105,000 ^D	MS, RI, Std	Nutty, Roasty, Cocoa, Chocolate ^C
2,5-dimethyl pyrazine	27.24	1321	800-1,800 ^D	MS, RI, Std	Cocoa, Roasted nut, Roastbeef, Woody ^C
2,6-dimethyl pyrazine	24.47	1327	200-9,000 ^D	MS, RI, Std	Baked potato, Nutty, Fruity ^C
2,3-dimethyl pyrazine	28.18	1348	2,500-35,000 ^D	MS, RI, Std	Nutty, musty ^C

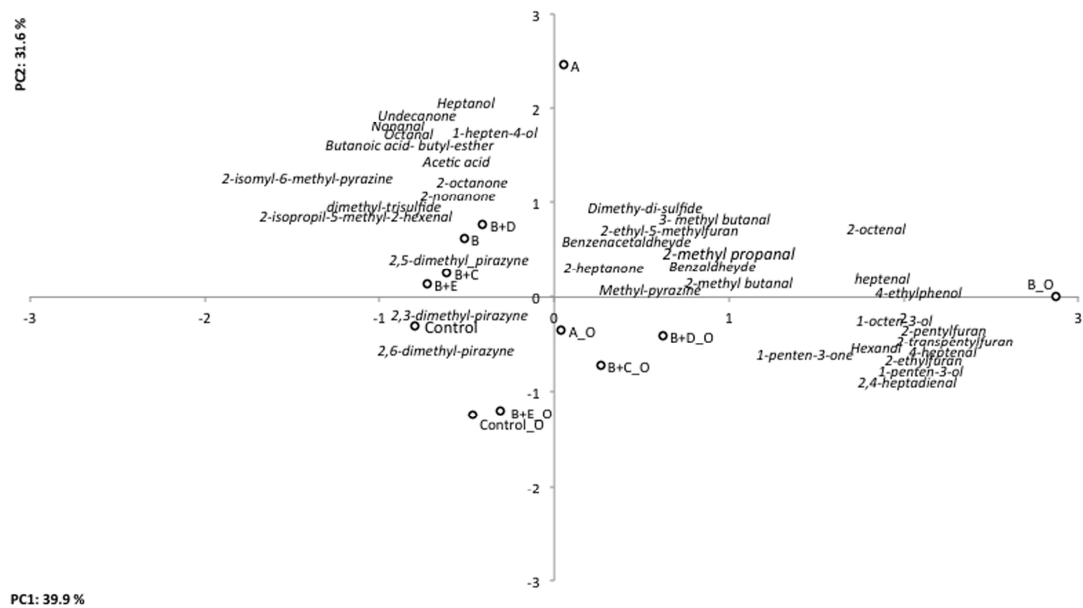
^A Giri et al., 2010; ^B Ganeko et al., 2008; ^C pherobase.org; ^D <http://www.leffingwell.com/odorthre.htm>

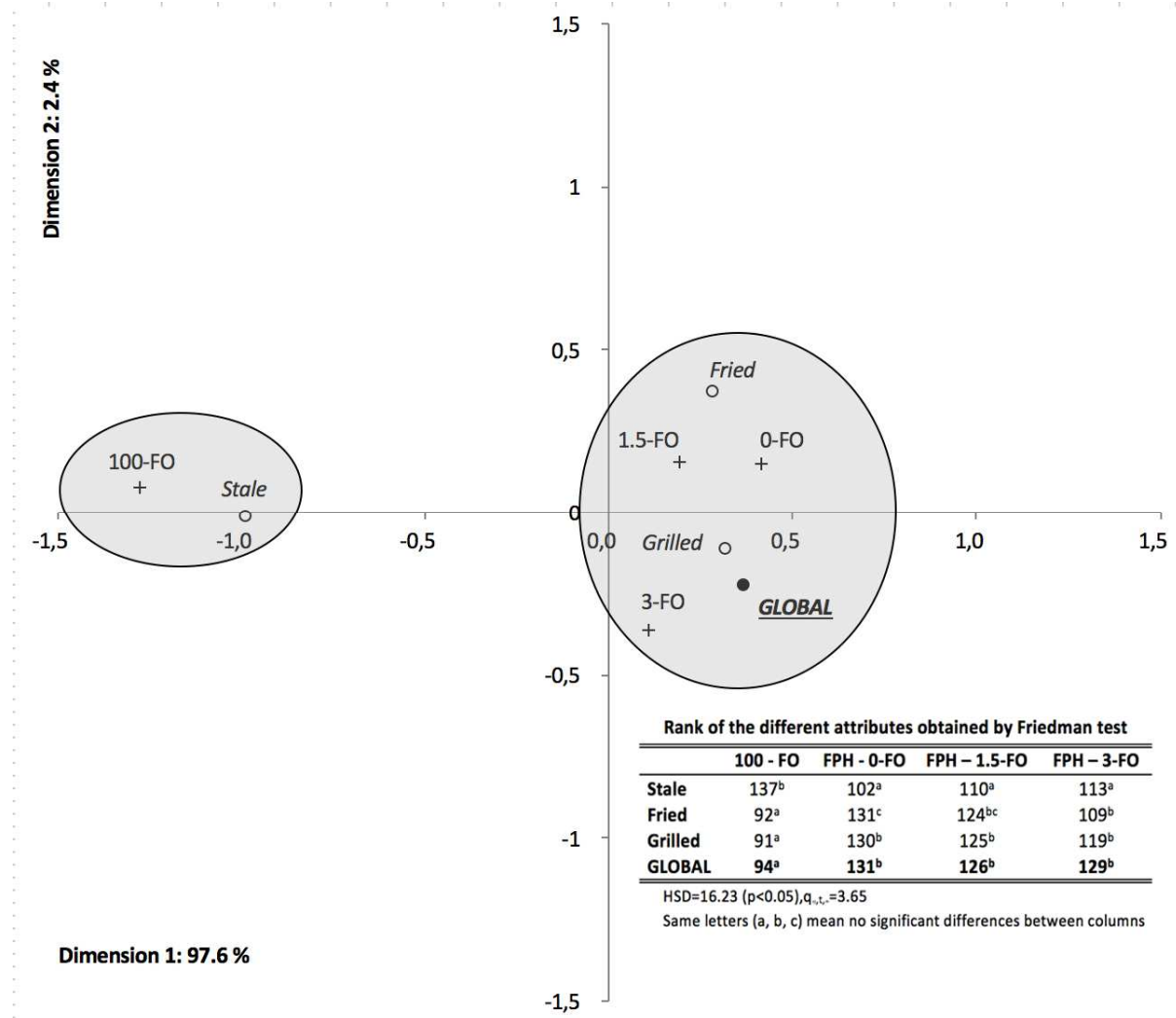
Odour thresholds in water (µg/L)

Table 4. Fish powder hydrolysates obtained with the enzymes or combination of enzymes. Volatile compounds associated with fish-like aroma in the different heated fish powder hydrolysates (H-FPHs) with or without addition of fish oil (1.5 g/100g) expressed as ug / mL. (n=3).

	Control		A + H-FPH		B+ H-FPH		(B+C) + H-FPH		(B+D) + H-FPH		(B+E) + H-FPH	
	No-FO	1.5 % FO	No-FO	1.5 % FO	No-FO	1.5 % FO	No-FO	1.5 % FO	No-FO	1.5 % FO	No-FO	1.5 % FO
<i>Aldehydes</i>												
2-methyl butanal	4 ± 2	35 ± 5	312 ± 6	270 ± 18	1,361 ± 78	955 ± 120	588 ± 134	681 ± 99	436 ± 171	528 ± 230	317 ± 1	618 ± 13
3-methyl butanal	9 ± 6	65 ± 18	1,073 ± 131	1,139 ± 116	1,475 ± 68	1,295 ± 94	1,275 ± 221	1,113 ± 160	1,046 ± 272	881 ± 229	769 ± 20	1,026 ± 172
hexanal	0.323 ± 0.007	1.200 ± 0.651	0.524 ± 0.119	1.688 ± 0.641	0.441 ± 0.079	5 ± 1	0.358 ± 0.109	1.609 ± 0.159	0.361 ± 0.055	1.571 ± 0.128	0.285 ± 0.062	1.758 ± 0.785
heptanal	0.174 ± 0.029	0.262 ± 0.065	0.392 ± 0.018	0.406 ± 0.002	0.329 ± 0.035	0.572 ± 0.067	0.278 ± 0.049	0.349 ± 0.109	0.181 ± 0.019	0.279 ± 0.012	0.153 ± 0.002	0.316 ± 0.016
4-heptenal	-	0.103 ± 0.077	0.033 ± 0.012	0.239 ± 0.027	0.032 ± 0.003	0.508 ± 0.159	0.047 ± 0.028	0.211 ± 0.194	0.040 ± 0.002	0.161 ± 0.015	0.010 ± 0.005	0.240 ± 0.135
octanal	0.017 ± 0.002	0.008 ± 0.002	0.054 ± 0.007	0.011 ± 0.005	0.038 ± 0.014	0.006 ± 0.002	0.027 ± 0.002	0.013 ± 0.009	0.024 ± 0.004	0.011 ± 0.007	0.013 ± 0.002	0.006 ± 0.001
nonanal	3.605 ± 0.710	0.692 ± 0.140	12 ± 2	3 ± 1	8 ± 2	0.915 ± 0.088	5.192 ± 0.219	2.131 ± 1.067	3.969 ± 0.832	1.572 ± 0.999	3.371 ± 0.617	0.737 ± 0.008
2,4-heptadienal	0	0.212 ± 0.015	0	0.215 ± 0.197	0	2 ± 1	0	0.417 ± 0.056	0	0.455 ± 0.044	0	0.501 ± 0.042
<i>Alcohols</i>												
1-penten-3-ol	0.052 ± 0.007	4 ± 1	0.139 ± 0.036	17 ± 3	0.729 ± 0.152	44 ± 3	0.797 ± 0.132	26 ± 3	1.010 ± 0.329	17 ± 2	0.365 ± 0.006	23 ± 2
1-octen-3-ol	0.063 ± 0.002	0.136 ± 0.051	0.120 ± 0.004	0.367 ± 0.022	0.108 ± 0.014	0.787 ± 0.260	0.101 ± 0.005	0.289 ± 0.025	0.106 ± 0.009	0.345 ± 0.266	0.102 ± 0.014	0.420 ± 0.032
4-hepten-1-ol	0.939 ± 0.048	0.618 ± 0.177	1.353 ± 0.061	1.005 ± 0.026	1.206 ± 0.084	1.015 ± 0.051	1.094 ± 0.118	0.965 ± 0.092	1.199 ± 0.027	0.925 ± 0.316	1.132 ± 0.125	0.881 ± 0.002
<i>Pyrazines</i>												
Methyl pyrazine	1.137 ± 0.896	2.295 ± 0.340	6 ± 1	9.802 ± 0.228	8.609 ± 0.023	7.958 ± 0.356	9 ± 2	9 ± 3	7 ± 1	7.326 ± 0.614	11 ± 2	11 ± 1
2,5-dimethyl pyrazine	3 ± 1	5 ± 1	40 ± 6	49 ± 3	27 ± 1	21 ± 1	48 ± 3	43 ± 8	46 ± 4	42.625 ± 0.216	55 ± 9	61 ± 6
2,6-dimethyl pyrazine	3 ± 1	4 ± 1	8 ± 2	12 ± 2	7.453 ± 0.015	7.104 ± 0.252	14 ± 3	15 ± 4	36 ± 4	39 ± 3	41 ± 4	69 ± 3
2,3-dimethyl pyrazine	0.136 ± 0.057	0.419 ± 0.112	0.582 ± 0.140	0.944 ± 0.016	1.096 ± 0.051	0.755 ± 0.043	1.133 ± 0.088	0.965 ± 0.247	3.931 ± 0.555	3 ± 1	4 ± 2	4 ± 1

Development of aroma: 1. Aliquots of FPHs (0.2 mL) mixed with a dextrose solution (0.05 mL (80 µmol/mL)) and glycerol (500 µL); 2. Addition of fish oil (1.5 g/100g); 3. Samples homogenised at 60 °C for 10 minutes, followed by heating at 110 °C for 30 minutes.





Highlights.

Proteases were used to derive amino-acid-rich ingredients from by-products.

Combinations of peptidases lead to the highest concentration in free amino acids.

4-heptenal and 2, 4-heptadienal were the main volatile generated.

Low-value fish materials as an alternative for the fish industry.