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

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.

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

Peinado, I. [1*]; Koutsidis, G. [1] & Ames, J.[2]

[1]Department of Biology, Food and Nutrition, Health and Life Sciences. Northumbria University. Newcastle City Campus, Ellison Place, Newcastle Upon Tyne, NE1 8ST, UK.

[2]Centre for Research in Biosciences, Faculty of Health and Applied Sciences, The University of the West of England, Frenchay Campus, Bristol, BS16 1QY, UK

[*] Corresponding author: Irene Peinado-Pardo

E-mail address: irene.peinadopardo@unibz.it

Abstract.

Amino acid-rich extracts derived from fish by-products were utilised to generate flavour model systems with added glucose and/or fish oil.

Combination of endo and exo peptidases resulted in the most marked increased in free amino acids, particularly for leucine, lysine and glutamic acid (48.3 ± 3.4 to 1,423.4 ± 59.6, 43.3 ± 1.2 to 1,485.4 ± 25.6 and 143.6 ± 21.7 to 980.9 ± 63.6 µg/g respectively).

Main volatile products formed after heating the systems were 4-heptenal, 2,4-heptadienal, and some pyrazines. Increased concentrations of 1-octen-3-ol or 1-hepten-4-ol were also observed in the heated systems compared to the controls. All of these volatile compounds have been identified among the volatile profile of cooked seafood.

[*] Corresponding author permanent address:

Irene Peinado-Pardo
Faculty of Science and Technology
Piazza Università, 5
I - 39100 Bozen-Bolzano
email: irene.peinadopardo@unibz.it / irpeipar@gmail.com

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

Chemical compounds studied in this article:

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

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

1. Introduction

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

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

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

also by the conditions used for its post-harvest handling, storage and cooking. Some fish such as salmon or trout, have a strong flavour while might have a relatively mild smell before cooking that becomes strong and pleasant after heating (Ganeko et al., 2008; Whitfield, Freeman, Last, Bannister, & Kennett, 1982; Whitfield, Last, Shaw, & Tindale, 1988). Important aroma compounds, characteristic of fresh fish, are lipid derived volatile compounds generated mainly by oxidative enzymatic reactions and autoxidation of lipids such as aldehydes and ketones. However, compounds derived from Maillard reaction such as pyrazines and furans, also make important contributions to the flavour and aroma of fish products after frying or grilling (Giri, Osako, & Ohshima, 2010).

The aim of this study was to demonstrate the use of by-products of the fish industry (fish powder) for the generation of fish flavour formulations after protease biocatalysis and subsequent heating in the presence of glucose and/or fish oil.

2. Material and Methods

2.1. Chemicals

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

2.2. Hydrolysis and formation of aromas

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

85 powder (100 g/L in water) was hydrolysed for15 h at constant stirring, under controlled
86 conditions of temperature and pH (60ºC at pH 6). The reaction was terminated by heating the
87 mixture at 95ºC 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 60ºC for 10 min and subsequently heated at 110ºC 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 BF_3 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 4ºC/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 $$DH = \frac{h}{h_{tot}} \cdot 100\% \qquad \textit{(Equation 1)}$$

$$h = \frac{(serine-NH_2)-b}{a} \quad \textit{(Equation 2)}$$

$$Serine - NH_2 = \frac{Abs_{sample}-Abs_{blank}}{Abs_{standard}-Abs_{blank}} \cdot 0.9516 \cdot 0.1 \cdot \frac{100}{X} \cdot P \quad \textit{(Equation 3)}$$

Where h_{tot} depends on the specific raw material, and for the present study was estimated as h_{tot} = 8.6; h = meqv serine / g protein; serine-NH_2= meqv serine-NH_2 / g protein; *a* and *b* depend on the specific raw material, and for the present study they were estimated as *a* = 1.00, *b* = 0.4; X = g sample; P = protein % in the sample; 0.1 is the sample volume (L) (Nielsen et al., 2001).

2.3.3. Free amino acids

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

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

2.3.4. Volatiles analysis

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

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

2.4. Sensory Evaluation

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

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

Significant differences between the samples were established by the statistical function T-Friedman and compared with the tabulated X^2=7.81 (α = 0.05) with (t−1) degrees of freedom (Meilgaard, Civille, & Carr, 1999).

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

$$HSD = q_{\alpha,t,\infty}\left(p \cdot {}^{t}/_{4}\right)^{\frac{1}{2}} \qquad \textit{(Equation 4)}$$

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

2.5. Statistics

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

Furthermore, a correspondence analysis was performed to establish whether the selected samples and the evaluated sensory attributes map. This tool establishes the association 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 ± 1.2% and 46.0 ± 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 ± 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 µg/g),
223 reaching their highest concentrations for the combination "B+C" (Lys [1,484 ± 43 µg/g], Leu
224 [1,423 ± 48 µg/g], Glu [981 ± 142 µg/g] and Ala [939 ± 135 µg/g]). His, Ile, Phe, Ser and
225 Thr, were in the range of 178-742 µg/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

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

3.2. Development of aromas

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

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

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

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

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

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

Sulphur-containing compounds dimethyl disulphide, (cooked cabbage aroma), and 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%) differenciate 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

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

3.3 Sensory evaluation

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

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

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

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

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

4. Conclusions

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

The use of various enzymes produced different amounts of amino acids in the FPHs with important amounts of lysine, leucine, glutamic acid and alanine being released. These increased on free amino acids will have an influence on the characteristic compounds derived from the Maillard reaction, such as pyrazines, sulphur compounds or some aldehydes. Fish oil had a great impact on the volatile compounds associated with fish aroma; its addition enhanced the concentration of some lipid oxidation products such as hexanal, heptanal, 4-heptanal, 2,4,-hetadienal, 1-penten-3-ol or 1-octen-3-ol, characteristic impact compounds in seafood, that have been previously identified in the volatile profile of cooked fish or meals containing seafood. “Grilled” and “fried” aromas, characteristics of FPHs heated with fish oil, were preferred by panellists, while fish oil on its own produced unpleasant aromas.

Future work involving different types and concentrations of fish oil together with sensory evaluation is suggested to investigate the acceptability of seafood-derived fish-like flavouring formulations based on such approaches.

Acknowledgments

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

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 Aspmo, 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-
392 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

systems. *Journal of Agricultural and Food Chemistry*, *53*(4), 1286–93. doi:10.1021/jf048557b

Escriche, I., Fernández-Segovia, I., Serra, J. A., Andrés, A., & Barat, J. M. (2001). Evaluación sensorial del bacalao desalado, pretratado térmicamente listo para cocinar. *Alimentaria: Revista de Tecnología E Higiene de Los Alimentos*, *322*, 51–54. doi:ISSN 0300-5755

Fox, P. F., & Wallace, J. M. (1997). Formation of Flavor Compounds in Cheese. In *Advances in Applied Microbiology* (Vol. 45, pp. 17–85). doi:10.1016/S0065-2164(08)70261-2

Ganeko, N., Shoda, M., Hirohara, I., Bhadra, A., Ishida, T., Matsuda, H., … Matoba, T. (2008a). Analysis of volatile flavor compounds of sardine (*Sardinops melanostica*) by solid phase microextraction. *Journal of Food Science*, *73*(1), S83–8. doi:10.1111/j.1750-3841.2007.00608.x

Ghorbel, S., Souissi, N., Triki-Ellouz, Y., Dufosse, L., Guerard, F., & Nasri, M. (2005). Preparation and testing of Sardinella protein hydrolysates as nitrogen source for extracellular lipase production by *Rhizopus oryzae*. *World Journal of Microbiology and Biotechnology*, *21*(1), 33–38. doi:10.1007/s11274-004-1556-2

Giri, A., Osako, K., & Ohshima, T. (2010). Identification and characterisation of headspace volatiles of fish miso, a Japanese fish meat based fermented paste, with special emphasis on effect of fish species and meat washing. *Food Chemistry*, *120*(2), 621–631. doi:10.1016/j.foodchem.2009.10.036

González-Tomás, L., Carbonell, I., & Costell, E. (2004). Influence of type, concentration and flow behaviour of hydrocolloid solutions on aroma perception. *European Food 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 Represetativeness 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

and sand-lobsters. *Australian Journal of Chemistry*, *35*(2), 373–383. doi:10.1071/CH9820373

Whitfield, F. B., Last, J. H., Shaw, K. J., & Tindale, C. R. (1988). 2,6-Dibromophenol: The cause of an iodoform-like off-flavour in some Australian crustacea. *Journal of the Science of Food and Agriculture*, *46*(1), 29–42. doi:10.1002/jsfa.2740460104

Yaich, H., Garna, H., Besbes, S., Paquot, M., Blecker, C., & Attia, H. (2011). Chemical composition and functional properties of *Ulva lactuca* seaweed collected in Tunisia. *Food Chemistry*, *128*(4), 895–901. doi:10.1016/j.foodchem.2011.03.114

Figure Caption

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

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

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 (µg / 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	µg / 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	µg / 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	µg / g	47 ± 3	224 ± 15	661 ± 50	726 ± 59	448 ± 47	541 ± 16
	ΔC (%)		374 ± 31	1,297 ± 106	1,435 ± 123	848 ± 97	1,045 ± 33
Leucine	µg / g	48 ± 3	675 ± 15	1.113 ± 51	1.423 ± 59	929 ± 47	1.025 ± 16
	ΔC (%)		12,989 ± 31	2,205 ± 105	2,848 ± 123	1,825 ± 98	2,022 ± 33
Isoleucine	µg / g	29 ± 3	227 ± 5	613 ± 50	683 ± 14	412 ± 8	522 ± 9
	ΔC (%)		670 ± 16	1,981 ± 170	2,218 ± 47	1,299 ± 27	1,674 ± 32
Threonine	µg / g	54 ± 6	194 ± 21	604 ± 29	742 ± 48	473 ± 18	541 ± 72
	ΔC (%)		260 ± 39	1,023 ± 53	1,280 ± 88	778 ± 33	906 ± 135
Serine	µg / 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	µg / 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	µg / 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	µg / g	11 ± 3	125 ± 8	298 ± 26	346 ± 23	239 ± 17	263 ± 6
	ΔC (%)		997 ± 63	2,511 ± 224	2,932 ± 204	1,997 ± 147	2,202 ± 54
Glutamic acid	µg / 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	µg / g	27 ± 1	255 ± 31	360 ± 43	451 ± 15	292 ± 15	352 ± 2
	ΔC (%)		860 ± 115	1,256 ± 162	1,597 ± 55	997 ± 55	1,227 ± 6
Lysine	µg / g	43 ± 1	369 ± 80	818 ± 157	1.485 ± 22	887 ± 101	836 ± 66
	ΔC (%)		751 ± 186	1,789 ± 363	3,332 ± 50	1,949 ± 233	1,830 ± 152
Other	µg / g	23 ± 2	138 ± 3	187 ± 14	286 ± 3	167 ± 5	229 ± 3
	ΔC (%)		687 ± 120	831 ± 206	1,273 ± 271	760 ± 209	1,094 ± 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 ºC, enzyme (10-20 g/L).

*ΔC (%) = 100•[concentration of each free amino acid in the fish powder hydrolysates – concentration of each free amino acid in the control]/ 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 hydrolisates after heating them with or without fish oil (H-FPHs) (n=3).

	RT	RI	Odour threshold	Identification	Odour description
Aldehydes					
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]
Alcohols					
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]
Ketones					

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 esther	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-pentyil 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]

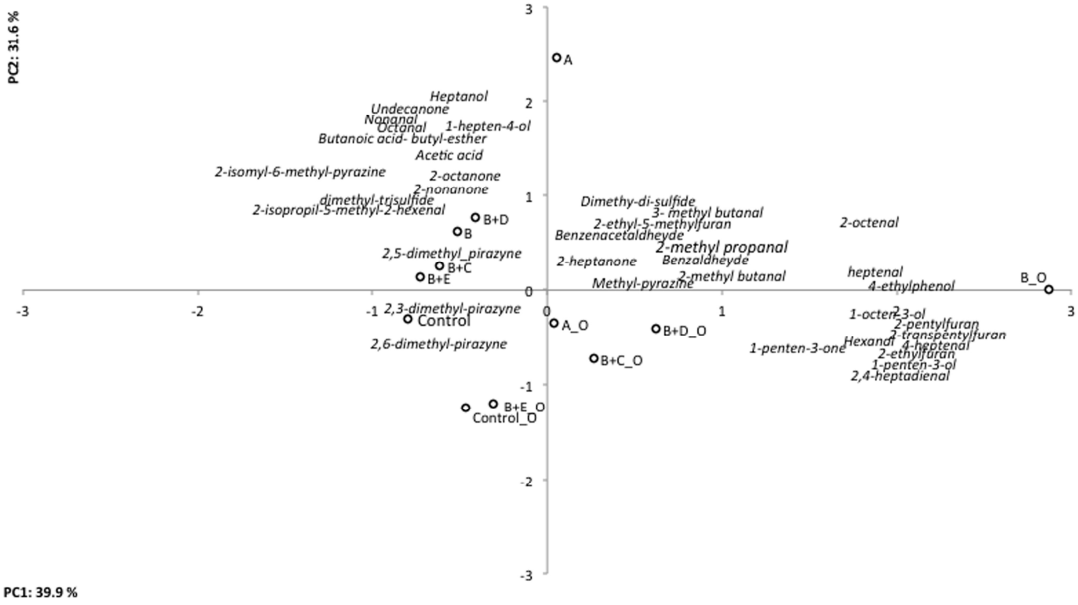
[A] Giri et al., 2010; [B] Ganeko et al., 2008; [C] pherobase.org; [D] http://www.leffingwell.com/odorthre.htm

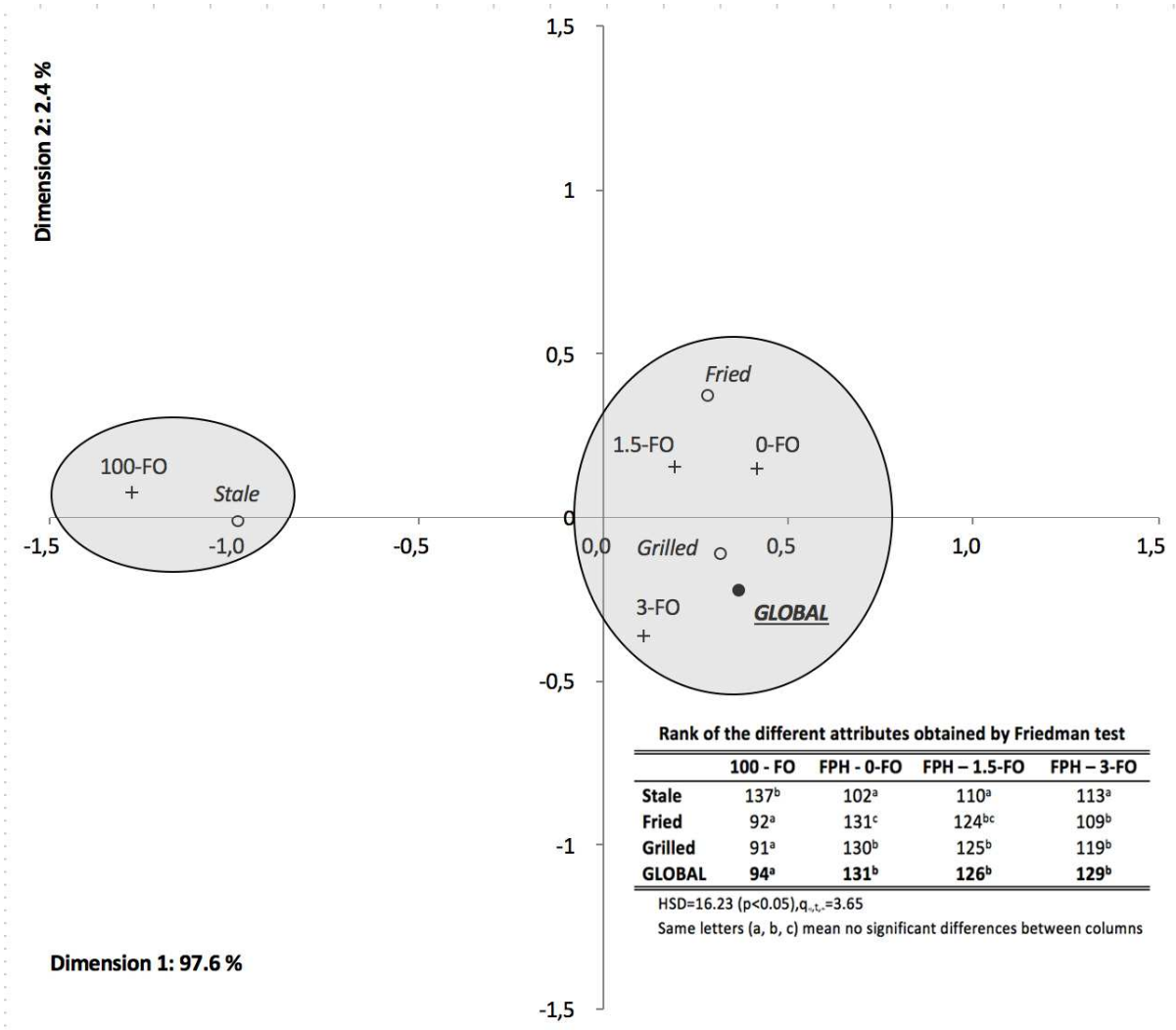
Odour tresholds in water (µg/L)

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**
Aldehydes												
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
Alcohols												
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
Pyrazines												
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 ºC for 10 minutes, followed by heating at 110 ºC for 30 minutes.





Rank of the different attributes obtained by Friedman test

	100 - FO	FPH - 0-FO	FPH – 1.5-FO	FPH – 3-FO
Stale	137^{b}	102^{a}	110^{a}	113^{a}
Fried	92^{a}	131^{c}	124^{bc}	109^{b}
Grilled	91^{a}	130^{b}	125^{b}	119^{b}
GLOBAL	**94^{a}**	**131^{b}**	**126^{b}**	**129^{b}**

HSD=16.23 ($p<0.05$), $q_{\alpha,t,\infty}=3.65$

Same letters (a, b, c) mean no significant differences between columns

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.