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

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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)

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 1423.4 ± 59.6, 43.3 ± 1.2 to 1485.4 ± 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.

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.

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

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important role in the hydrolysis (Annadurai, Sadeeshkumar, Vijayalaksmi, & Pirithiviraj, 2012; Aspmo, Horn, & 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 (1000 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 powder (100 g/L in water) was hydrolysed for15 h at constant stirring, under controlled conditions of temperature and pH (60 °C at pH 6). The reaction was terminated by heating the mixture at 95 °C for 20 min in a water bath. Each protease used was added so all mixtures had the same enzymatic activity per gram of sample. The conditions of pH, temperature and time of reaction, as well as the enzymes and their combinations were selected based on the combination of those parameters that resulted in the higher concentration of free amino acids in a preliminary experiment (data not shown). The resulting slurries were centrifuged at 8000 × *g* for 20 min and aliquots were analysed to determine the degree of hydrolysis (DH) and amounts of free amino acids.

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

2.3. Analyses

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

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

The fatty acid composition was analysed by GC-FID after transesterification to methyl esters (FAMEs) with a mixture BF_3 methanol at 20 °C according to the IUPAC standard method (IUPAC, 1992; Peinado, Girón, Koutsidis, & Ames, 2014; Yaich et al., 2011). Analysis of FAMEs was carried out with a DANI Master GC equipped with an auto sampler, a DANI FID detector (DANI Instruments S.p.A, Italy) and an Agilent DB-23 (60 m × 0.25 mm, 0.25 μm) capillary column (Agilent Technologies, Cheshire, UK). The oven temperature was programmed from 90 °C to 240 °C at 4 °C/min and the injector and detector temperatures were set at 250 °C. The carrier gas was helium at 1.0 mL/min constant flow (split ratio 10:1). Data analysis, identification and quantification of FAMEs was accomplished by comparing the retention times and areas of the peaks with those of pure standards (Supelco® 37 Component FAMEMix, Sigma–Aldrich, Poole, UK) and analysed under the same conditions. The results were expressed as a g of each fatty acid/100 g of the lipid fraction.

2.3.2. Degree of hydrolysis, DH

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

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

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

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

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).