Production of bioactive peptides from salmon processing side-streams

B. Iñarra, J. Ibarruri, C. Bald, M. Gutierrez, D. San Martin, N. Luengo and J. Zufía

AZTI, Food Research, Basque Research and Technology Alliance (BRTA). Parque Tecnológico de Bizkaia, Astondo Bidea, Edificio 609, 48160 Derio - Bizkaia, Spain
Keywords: by-products, valorisation, hydrolysis, bioactive molecules.
Presenting author email: binarra@azti.es

Introduction

The seafood value chain is of paramount importance in a world with an exponential population growth. In 2013, fish accounted for about 17 % of the global population's intake of animal protein and the total food fish consumption has risen a 122 % from 1990 to 2018. However, fish value chain side-streams account to up to 70 % of the raw material, due the combination of policies (discard landings), processes inefficiencies and the inedible part of fishes, such as backbones or heads.

The objective of the WaSeaBi project [1] is to unravel challenges that forestalls more sound exploitation of the aquatic resources. This can be obtained by developing, sorting technologies, storage solutions and decision tools that may secure an efficient, sustainable supply system for by-catches, additionally as for solid and liquid side-streams from aquaculture, fisheries and therefore the aquatic processing industries to biorefining operations. This can lead to valorisation of those raw materials into marketable products.

Backbones of salmon (*Salmo salar*) were use as model of fish transforming industry side-stream to produce hydrolysates. These by-products, along with heads and guts, where previously used as raw material for the production of fishmeal and fish oil. An improved handling, with the separation of fractions, allowed their use as a food grade fraction unveiling their potential for more valuable uses.

In this work, the use of this fraction as a source of peptides with bioactive properties was evaluated.

Material and methods

The hydrolysis processes were performed at laboratory scale using a Symphony 7100 Bathless Dissolution Distek equipment (Distek Inc., North Brunswick, NJ, USA), controlling and monitoring temperature, time and stir speed. The pH of each run of the experimental design was controlled manually and adjusted with NaOH 1 M in a final volume of 500 mL (250 g of side-stream + 250 g water). All the processes were carried out with 1 % enzyme (protein based), for 3 h at 250 rpm and at the optimum pH and temperature of each enzyme or enzyme combinations. After the hydrolysis process enzymes were inactivated by heat treatment at 95 ºC during 15 min. Then, the content of the reactor was sieved to separate the bones, and centrifugated (2650 g; 15 min; ambient temperature) to separate 4 different layers, from the top to the bottom 1) the oil fraction, 2) an oil-water emulsion, 3) a water based fraction (the protein hydrolysate) and 4) a solid pellet.

Six enzymes, with different enzymatic activity, where tested to produce protein hydrolysates: a broad-spectrum endo-proteases (P), an endo-protease of the serine type (A), a trypsin specific protease (T), a chymotrypsin like protease (C), a blend of endo- and exo-peptidases (F) and a glutamic acid specific protease (G).

The protein hydrolysates were then freeze dried for their evaluation in the bioactivity test. Antioxidant activity of the samples were assessed by ABTS method, the antihypertensive capacity was evaluated by the angiotensin converting enzyme (ACE) inhibition method. The antimicrobial properties were assessed in a two-step approach, a first screening with the agar diffusion method (ADM), and then the minimum inhibitory concentration (MIC) analysis of promising samples. Antimicrobial activity was evaluated against the growth of: *Salmonella enterica* (CECT 4156), *Escherichia coli* (CECT 516), *Bacillus subtilis* (CECT 39), *Bacillus cereus* (CECT 131), *Staphylococcus aureus* (CECT 435), *Aeromonas salmonicida* (CECT 5173) and *Vibrio vulnificus* (CECT 529).

Analytical characteristics of the protein hydrolysates were also evaluated: protein concentration (Kjeldahl method), solid content (dry mater at 105 ºC until constant weight), mineral content (solids matter at 700 ºC until constant weight) and molecular weight protein fingerprint (SEC-HPLC).

Results and discussion

Hydrolysis processes with the different enzymes yielded 7-10 % oil, 0.5-2.4 % emulsion or interphase, 55-70 % liquid or protein hydrolysate (water based fraction), 10-23 % solid pellet and 11-14 % bone fraction (Figure 1). The protein hydrolysate contained 6.1-7.5 % protein that implies a 40-65 % protein yield.

Salmon backbone hydrolysates were tested in ADM and six positive results were detected, whit *Aeromonas Salmonicida* the hydrolysates with A, P+F and P+G showed an inhibition halo, while with *Bacilus Cereus*, *Staphylococcus Aureus* and *Bacilus Subtilis*, only the hydrolysates with F presented positive results.

These promising samples were analysed with the MIC method to quantify their bioactivity resulting that between 100 and 300 mg/ml were needed for the total inhibition of bacteria growth. These values represent a first promising approach to a valuable ingredient taking into account that raw hydrolysates were tested, and usually results reported in literature are referred to fractionate hydrolysate or even single peptides [2].

Regarding antioxidant activity, all the produced hydrolysates showed high antioxidant capacity with 350 to 550 µmol Trolox /g protein, being the most active those produce whit A+F and P+F (Figure 2). The inclusion of the exopeptidase F clearly increases the antioxidant activity of the hydrolysate that is related with the higher obtained degree of hydrolysis and the presences of lower molecular weight peptides [3].

Finally, the hydrolysates with positive results in the antimicrobial tests were also evaluated for their antihypertensive activity. All the hydrolysates presented activity with an IC50 value (concentration that inhibits 50 % of the ACE activity) at about 1.4-1.5 mg/ml that are values similar to those reported in literature when talking about crude hydrolysates [4].

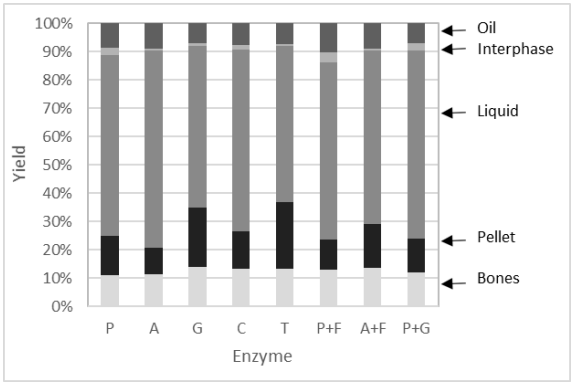

Figure 1: Hydrolysis yields

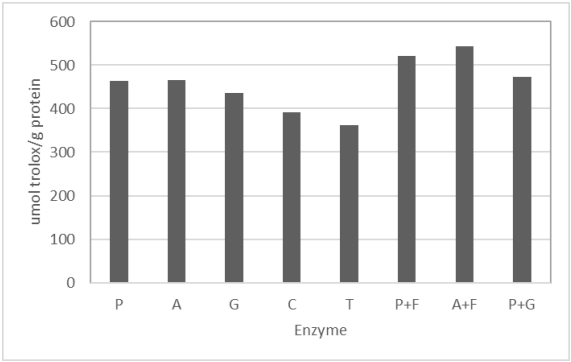

Figure 2: Antioxidant capacity results (ABTS)

Conclusions

A large number of hydrolysates, using different kinds of enzymes with different specific activities, were produced and several positive results were obtained for tested bioactivities, however, the best values for each bioactivity were obtained with different enzymes and enzyme combinations.

Taking into account that bioactivity tests were performed with raw hydrolysates, obtained results are of great interest and will be further investigated within the WaSeaBi project in several ways, on the one hand by the fractionation of the hydrolysates and, on the other hand, by scaling up the processes to better analyse yields and characteristics.

Acknowledgements

The WaSeaBi project has received funding from the Bio Based Industries Joint Undertaking (JU) under grant agreement No 837726. The JU receives support from the European Union's Horizon 2020 research and innovation programme and the Bio Based Industries Consortium. This communication reflects only the author's view and that the JU is not responsible for any use that may be made of the information it contains.

References

[1] WaSeaBi: Optimal utilization of seafood side-streams through the design of new holistic process lines project. BBI-JU funded project No 837726. https://www.waseabi.eu/

[2] Välimaa, A.-L., Mäkinen, S., Mattila, P., Marnila, P., Pihlanto, A., Mäki, M., Hiidenhovi, J.: Fish and fish side streams are valuable sources of high-value components. Food Quality and Safety 3(4), 209-226 (2019). doi:10.1093/fqsafe/fyz024

[3] Idowu, AT, Benjakul, S, Sinthusamran, S, Sookchoo, P, Kishimura, H. Protein hydrolysate from salmon frames: Production, characteristics and antioxidative activity. J Food Biochem. 2019; 43:e12734. https://doi.org/10.1111/jfbc.12734

[4] Ewart, H.S., Dennis, D., Potvin, M., Tiller, C., Fang, L.-h., Zhang, R., Zhu, X.-m., Curtis, J.M., Cloutier, S., Du, G., Barrow, C.J.: Development of a salmon protein hydrolysate that lowers blood pressure. European Food Research and Technology 229(4), 561-569 (2009). doi:10.1007/s00217-009-1083-3