Fish by-products as a source of proteolytic enzymes and bioactive peptides

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Introduction

The increasing of fish production, at an average annual rate of 3.2%, and the growth in per capita fish consumption from 9.9 kg in 1960 to 19.7 in 2013 have generated large amounts of organic wastes. These wastes include viscera, carcass, head, skin and bones, that are discarded without recovery efforts and which represent for more than 60% of biomass [1]. This situation creates a burdensome disposal problem and environmental concerns as they are highly perishable. So, marine bioprocess industry has a prominent potential to reuse and apply more by-products as valuable products.

Proteases are one of the most important enzymes used worldwide, accounting for approximately 50% of the industrial enzymes market. These enzymes have several industrial applications in food, feed, agriculture, cosmetics and pharmaceutics [2]. Fish internal organs constitute approximately 20% of the marine biomass, being fish viscera a rich source of proteases, i.e., different digestive enzymes (pepsin, trypsin, chymotrypsin, elastase).

Enzymatic hydrolysis has gained more attention to obtain hydrolysates with improved and more defined nutritional, functional and bioactive properties. Enzymatic processes have been implemented in a broad range of industries in recent decades because they are specific, fast in action and often save raw materials, energy, chemicals and/or water [3].

In this context, enzymes from fish viscera can be employed to produce protein hydrolysates, enhancing the value of processing by-products. For this reason, the use of fish viscera become the use of the enzymatic hydrolysis economically more appealing and more sustainable.

Currently, some research studies have been carried out with commercial enzymes in order to obtain bioactive peptides from different animal matrices. Bioactive peptides are special proteins segments that have numerous potential physiological functions within the body, namely antioxidant and antihypertensive or angiotensin converting enzyme (ACE) inhibition.

Therefore, the present study aims to use a commercial enzyme (alcalase) and an enzymatic extract from fish viscera to generate bioactive peptides from fish by-products and evaluate their potential antioxidant and antihypertensive activity.

Methods

Homogenization and addition of an extraction buffer to the viscera

Centrifugation

Collection of supernatant that comprises the viscera enzymes

Hydrolysis of fish with
Viscera enzymatic extract (70 U/mL)
or
Alcalase (2600 U/mL)

Fish Protein Hydrolysates (freeze-dried)

- Degree of hydrolysis
- FPLC
- Antioxidant activity (ABTS, DPPH and ORAC)
- Antihypertensive activity (ACE inhibition)

Results

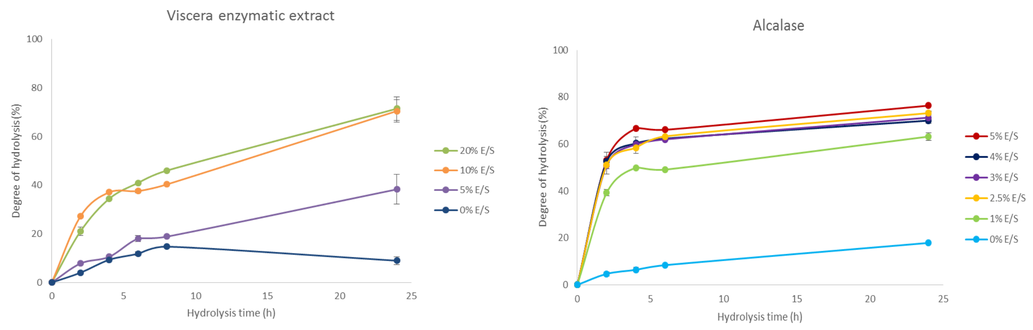

Figure 1 - Degree of hydrolysis obtained for fish during hydrolysis with viscera enzymatic extract and alcalase

Viscera enzymatic extract: 10% E/S, 6h
Alcalase: 3% E/S, 2h

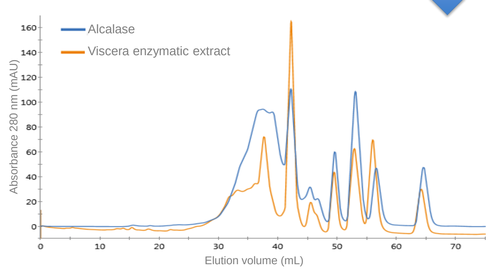

Figure 2 – Peptide profile of fish protein hydrolysates

Table 1 – Characterization of fish protein hydrolysates

	Degree of hydrolysis (%)	Protein content (%)
Viscera enzymatic extract	32.5 ± 2.8	78.8 ± 3.5
Alcalase	52.1 ± 2.7	83.6 ± 2.8

Table 2 – Bioactivity of fish protein hydrolysates

Bioactivity	**Antioxidant activity**			**Antihypertensive activity**
	ABTS (mg ascorbic acid equivalent/ g hydrolysate)	DPPH (mg Trolox equivalent/ g hydrolysate)	ORAC (mg Trolox equivalent/ g hydrolysate)	IC_{50} (µg of protein/mL)
Viscera enzymatic extract	10.4 ± 0.9	4.0 ± 0.1	142.0 ± 4.2	554.4
Alcalase	11.0 ± 0.5	4.0 ± 0.4	153.2 ± 29.4	101.1

Conclusions

Fish viscera are usually discarded as waste or low-value by-products. This research work describes a process to extract the enzymes of fish viscera and apply them to hydrolyze fish by-products. The selected conditions for the enzymatic hydrolysis was 10% (E/S) during 6 h using the viscera enzymatic extract and 3% (E/S) during 2 h using alcalase. Fish protein hydrolysates, in addition to their high protein content, demonstrated to contain small peptides. All protein hydrolysates proved to have a notable antioxidant capacity, similar results were achieved for hydrolysates obtained with viscera enzymatic extract and alcalase. Fish protein hydrolysates were also able to inhibit the ACE, however, hydrolysates obtained with alcalase revealed a higher antihypertensive activity. Therefore, the viscera enzymatic extract showed a great potential to hydrolyze fish by-products demonstrating an added value because, in this way, the enzymes and the hydrolysates are obtained using the same by-products, being aligned with the concept of a circular economy. In this way, it will be possible to use the animal by-products based on a sustainable approach and to promote added value compounds for animal and human nutrition.

References

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