Title: Hydrolysis as a valorization strategy for unused marine food biomass: boarfish and small-spotted catshark discards and byproducts.

Running title: Hydrolysis as a fish valorization strategy

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Abstract

Discarded fish represents a serious concern for the sustainability of fisheries; the main outcome of the analyses of the impact of discarding has been the implementation of a zero-discard policy in EU waters. One problem to implement this policy is to find alternatives to help fishers to alleviate the costs of non-discarding. Boarfish and small-spotted catshark are highly discarded in NW coast of Iberian Peninsula. Boarfish protein hydrolysates (BPH) were prepared using a pancreas crude extract (PCE) from small-spotted catshark and compared with commercial proteases. Results showed that BPH prepared with PCE and commercial trypsin produced greater amount of protein than Alcalase hydrolysates, however the latter showed greater solubility. Emulsifying and foaming properties were higher when using bovine trypsin, followed by PCE and Alcalase. Antioxidant activities were similar in all hydrolysates. Valuable compounds usually wasted could be employed for processing fish difficult to handle and with low flesh yield.

Practical Application

This manuscript addresses the problem of underutilized marine biomass: discards and byproducts from boarfish (*Capros aper*) and small-spotted catshark (*Scyliorhinus canicula*). These species are highly discarded or used for low value processes. One of the main challenges which faces the objective of by-catch and discard reduction is finding alternatives which help fishery industry to overcome the costs associated with landing captures. Fish protein hydrolysates can have many different potential applications, in this manuscript some of them are suggested, such as utilization as a food or feed ingredient.

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46 **Keywords:** fish discards, fish upgrading, protein hydrolysates, antioxidant activity,
47 bioactive peptides, functional properties.

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1. Introduction

Unsustainable fishing practices have been recognized as a problem for decades. Discards and fish byproducts are key factors contributing to the problem, not only due to their impact on economic and ecological questions but also because it promotes a significant waste of potential food resources. With the aim of promoting sustainable fishing practices within European fishing fleets, the European Commission is dealing with the wasteful practice of discarding by including in the new Common Fishery Policy (CFP) reform, to be implemented in 2015-2016, the obligation of landing all species under TAC's regulation in European waters. This reform also stipulates the prohibition of using those catches of species below the minimum conservation reference size, for direct human consumption. Furthermore, in spite of politics and government efforts to reduce by-catch, it is foreseen that the existence of discards of species without a TAC control will continue, essentially due to strategic or commercial reasons. For the reasons above mentioned, it will be necessary to have tools to convert discards into landed products developing new markets and processing techniques for these materials. This is in line with the *Blue Growth* initiative of European Horizon 2020, which main objective is ensuring and supporting the sustainable growth of scarce natural marine resources. The success of this *blue strategy* it would be achieved by the development of a *blue technology* focused on the exploration of bioactive compounds obtained from marine organisms (including discards and byproducts) with potential interest in the food/feed/pharmaceutical industries. In line with this current policy framework, this paper addresses the problem of wasted biomass through the study of valorization of two species highly discarded in NW coast of the Iberian Peninsula: Boarfish (*Capros aper*) and Small-spotted catshark (*Scyliorhinus canicula*).

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89 Boarfish has been an unwelcome by-catch in mixed demersal, pelagic, and
90 crustacean trawl-fisheries in some European countries (Borges *et al.* 2008, Enever *et al.*
91 2007), leading to a high rate of discards. The lack of previous commercial interest in
92 boarfish fishery could be related with the scarce presence in Northeast Atlantic fisheries
93 (Farrell *et al.* 2012), however in the last decades there has been an increment in its
94 abundance (O'Donnell *et al.* 2012) existing now a TAC control system. Landings have
95 increased significantly in last years, essentially due to Ireland, Danish and Scottish
96 fleets which have started to target this species commercially for fishmeal production
97 (White *et al.* 2011). However, boarfish still represents a problem of discarded biomass
98 due to the 7% of discards still taking place in those fisheries (ICES 2013) and also
99 Spanish trawling fleets, catch and discard large quantities of boarfish (up to 4600
100 t/year) due to no quota availability (Ordoñez del Pazo *et al.* 2014). The above
101 mentioned reform of the CFP affect to boarfish captures as this species has been
102 proposed to be included in the list of species not allowed to be discarded.

103 Small-spotted catshark is one of the most abundant elasmobranchs in the
104 Northeast Atlantic. Although commercial landings are made for human consumption
105 and despite its uses as a bait or fishmeal productions, generally has a low commercial
106 value and is taken as a bycatch in many European fishing fleets. For that reason,
107 historically, *S.canicula* landings have been reported under a generic commercial
108 category of “sharks”. As a consequence of the lack of species-specific landings data for
109 this species, ICES is not in a position to set an individual TAC for their stocks. Thus,
110 Spanish landings have increased significantly in recent years reaching 396 t in 2012
111 (ICES 2013), however the percentage of discards by the commercial fishery is also very
112 high (90%) (Enever *et al.* 2007,Perez *et al.* 2011).

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3 113 Fish protein hydrolysates have been previously proposed as an alternative to use
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5 114 underutilized fish species (Batista *et al.* 2010). Fish enzymatic hydrolysis provides,
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7 115 under controlled conditions a valuable source of peptides and amino-acids with
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9 116 advantages in functional and structural properties compared with the intact protein, such
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11 117 as potential applications in food/feed/pharmaceutical products (Klompong *et al.*
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13 118 2007, Bougatef *et al.* 2010, Amiza *et al.* 2012). Hydrolysis might constitute therefore a
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15 119 feasible alternative to upgrade wasted biomass of boarfish and small-spotted catshark.
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19 120 Only few studies have compared the effect of different enzymes in fish
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21 121 hydrolysates properties (Klompong *et al.* 2007, Bougatef *et al.* 2010) or used fish
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23 122 viscera enzymes to obtain fish protein hydrolysates (Bougatef *et al.* 2010), although
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25 123 viscera are known to represent an important byproduct of the fish industry (Blanco *et al.*
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27 124 2013). Up to our knowledge, this is the first time comparing between different enzymes
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29 125 including the utilization of a crude pancreas extract from viscera by-products.
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31 126 Utilization of these viscera, considered as a byproduct provides both economic benefits
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33 127 in terms of supplying a cheap resource of enzymes and also reduces the potential
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35 128 environmental pollution that these materials might cause.
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40 129 Therefore, this study was aimed to produce BPH using a pancreas crude extract
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42 130 (PCE) obtained from viscera of small-spotted catshark and to compare it, in terms of
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44 131 molecular weight distribution, functional properties and antioxidative activities, with
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46 132 BPH obtained with commercial proteases, Alcalase (ALC) and bovine trypsin (BT).
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49 133 **2. Materials and Methods**

50 134 **2.1. Biological samples**

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56 135 Fresh boarfish specimens were obtained by local fishing fleet and immediately
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58 136 frozen. Samples were transported to the laboratory and maintained at -18°C until used.
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3 137 Approximately 4 kg of boarfish were thawed, degutted, skinned and fillets were
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5 138 obtained. Muscle tissue was ground, mixed thoroughly, separated in different batches
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7 139 and stored in sealed plastic bags at -20°C. Three samples of boarfish were prepared to
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9 140 determine muscle yield. Each sample consisted in three batches of ten individuals.

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12 141 A pancreas crude extract was prepared with 60 individuals of small-spotted
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14 142 catshark (*S. canicula*), from local market (Vigo, Spain), as follows: specimens were
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16 143 kept in ice all the time, were transported to the laboratory, where they were eviscerated.
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18 144 Pancreas used as a source of trypsin, were removed, cleaned with deionised water and
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20 145 stored separately in sealed plastic bags at -20°C. Pancreas were cut into small pieces,
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22 146 frozen in liquid nitrogen, and then powdered using a Waring blender. The powder was
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24 147 suspended (1:9, w/v) in Extraction buffer (0.05 M Tris-HCl, pH 8.2, containing 0.02 M
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26 148 CaCl₂). The suspension was stirred continuously at 4°C for 3 hours and centrifuged for
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28 149 30 min at 4°C at 10.000 x g. The supernatant was collected, referred as PCE and stored
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30 150 at -20°C until used for hydrolysis.

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36 151 **2.2. Enzymes**

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38 152 Besides the PCE, two commercial enzymes were also used for preparation of
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40 153 BPH, the food-grade enzyme Alcalase (ALC), a bacterial serine endopeptidase provided
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42 154 by Novo Nordisk (Denmark), and the Bovine Trypsin (BT) which is also a serine
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44 155 protease that was purchased in Sigma Aldrich. In order to use the same units of trypsin
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46 156 activity to hydrolyze the muscle of boarfish, we firstly assayed the trypsin amidase
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48 157 activity in the PCE using DL-BAPNA as substrate, following the methodology of
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50 158 Blanco *et al.* (2013).

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55 159 **2.3. Enzymatic hydrolysis**
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Hydrolysates were prepared in triplicate using ALC, bovine trypsin BT or PCE in a stirred and thermostated 2L reactor connected to a pH electrode and a temperature probe. Raw material (300 g) was thawed and suspended in water (1:5 w/v). Optimum temperature, depending on the enzyme, was maintained constant throughout the hydrolysis reaction (55°C for ALC and 37°C for PCE and BT). The pH was adjusted to its optimum by adding 1 M NaOH (pH 8.0 for ALC and pH 7.5 for PCE and BT). pH was maintained constant during the hydrolysis reaction by continuous addition of 1M NaOH to the reaction mixture. Enzyme concentration was adjusted to attain the same activity units in all cases, and the hydrolysis reaction was allowed to continue for up to 2 hours under constant stirring. After hydrolysis enzyme was inactivated by heating at 90°C for 10 minutes. The resulting slurry was centrifuged, freeze-dried and kept frozen at -20°C until used.

2.4. Chemical analysis

Muscle and hydrolysates were analyzed for crude protein (N x 6.25) content by Kjeldhal method (AOAC 1997) in a DigiPREP digester (SCP Science) and a TitroLine Easy Unit (SCHOTT Instruments). Lipid content was determined by the methodology of Bligh and Dyer (1959). Moisture was determined after heating the sample overnight at 105°C and ash content was determined after heating the sample overnight at 550°C (AOAC, 1997).

2.5. Characterization of hydrolysates

2.5.1. Degree of hydrolysis: OPA method

The extent of enzymatic hydrolysis was determined using the o-phthaldialdehyde (OPA) reagent following the method described by Nielsen *et al.* (1997). Degree of hydrolysis (DH) is obtained according to the following expression:

$$DH = \left[\frac{Abs\ sample - Abs\ zero}{Abs\ control - Abs\ zero} * \frac{0.9516 * 10}{Sample\ (g) * \% N * 6.25 - Abs\ zero} \right] - \left[0.4 * \frac{100}{8.6} \right]$$

184 N: Percentage of nitrogen content in sample.

185 **2.5.2. Amino-acid composition**

186 Amino-acids were determined in the freeze-dried hydrolysate. For this, an acid
187 hydrolysis was necessary with 6 N hydrochloric acid, containing 0.1 % phenol under
188 inert atmosphere and heating up to 110°C for 24 hours. Then, HCl was removed from
189 the hydrolysate with vacuum. The hydrolysate was resuspended in 20 to 50 l of 0.2 M
190 sodium citrate buffer pH 2.2, to which a known amount of norleucine was added as an
191 internal standard and applied to an automated amino-acid analyser (Biochrom30
192 Amino-Acid Analyser). Differences in the amino-acid content among hydrolysates were
193 tested by one-way analysis of variance (ANOVA). Significance levels were set at
194 P<0.05.

195 **2.5.3. Size-exclusion chromatography**

196 The molecular weight distribution of hydrolysates was estimated by gel filtration
197 chromatography in an Agilent 1260 Infinity System (Agilent Technologies), equipped
198 with a Superdex Peptide 10/300 GL column, with 0.1% TFA in 30% acetonitrile as
199 eluent. The samples of freeze-dried hydrolysates were dissolved in eluent (0.1% TFA in
200 30% ACN) (10 mg/mL), filtered through 0,45 µm and ultrafiltrated using a 10 kDa
201 molecular weight cut-off Amicon Ultra Device. Aliquots of 10 µL were injected, eluted
202 at 0.4 mL/min and monitored at 220 nm. The column was calibrated with the following
203 standard proteins (Sigma): Cytochrome C (12400 Da), aprotinin (6500 Da), Angiotensin
204 II (1046 Da), Leucine-Enkephalin (555 Da), Val-Tyr-Val (379 Da) and Gly-Tyr (238
205 Da).

2.5.4. Functional properties

2.5.4.1. Nitrogen solubility index (NSI)

The solubility of freeze-dried hydrolysates was measured following the methodology described by Gbogouri *et al.* (2004). All determinations were done in triplicate. The NSI was calculated by the following equation:

$$NSI (\%) = \frac{\text{Supernatant nitrogen concentration}}{\text{Sample nitrogen concentration}} * 100$$

2.5.4.2. Foaming capacity and stability

Foaming capacity (FC) and stability (FS) were measured according to the methodology described by Diniz and Martin (1997). Determinations were calculated by the following equations:

$$FC = \frac{V_{t0} - V_{t30}}{V_i} * 100$$

$$FS = \frac{V_{t0} - V_{t30}}{V_{t0}} * 100$$

Where V_{t0} is the total volume after stirring, V_i is the initial volume of the solution and V_{t30} is volume after 30 minutes. Experiments were done in triplicate.

2.5.4.3. Emulsifying capacity and stability

Emulsifying capacity and stability were measured following the procedure described by Gbogouri *et al.* (2004). Each determination was performed in triplicate. Emulsifying stability was calculated by the following equation:

$$\% ES = \frac{V_t - V_a}{V_t} * 100$$

221 Where V_t is the total volume of the emulsion and V_a is the volume of the
222 aqueous phase.

223 **2.5.4.4. Scavenging effect on DPPH free radical**

224 The ability of the hydrolysates to scavenge α,α -diphenyl- β -picrylhydrazyl
225 (DPPH) free radicals was assessed spectrophotometrically by the method of Shimada
226 *et al.* (1992) with some modifications. A volume of 1 mL of hydrolysate (20 mg/mL)
227 was added to 1 mL of 0.1 mM DPPH in 95% ethanol. The mixture was shaken and left
228 for 30 minutes at ambient temperature in the dark. After centrifugation, the absorbance
229 of samples was recorded at 517 nm. Control and blank containing water and ethanol,
230 and water and DPPH respectively, were also prepared and measured accordingly.

231 The percentage inhibition was calculated using the following equation:

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$$\text{Percentage inhibition} = \frac{(\text{Abs control} - \text{Abs sample}) \times 100}{\text{Abs control}}$$

233 **2.5.4.5. Theoretical identification of antioxidant peptides**

234 Uniprot database was employed to obtain the amino-acid sequence of cardiac
235 muscle myosin heavy chain (T1T9H4) from *Capros aper*. This sequence was analyzed
236 using BIOEDIT (Hall, 1999) to search for peptides with a previously reported
237 antioxidant activity. The presence of previously reported antioxidant sequences within
238 the structure of *Capros aper* myosin would provide information about the peptides that
239 could be responsible for the antioxidant activity of the hydrolysates produced in this
240 study.

241 **3. Results and Discussion**

242 **3.1 Characterization of biological samples**

3.1.1. Boarfish

Boarfish is a small fish (no longer than 16 cm), with a head representing a significant portion of the fish (figure 1a). Muscle yield was determined showing that the average content of muscle is $22.49\% \pm 2.12$. Since edible fish present higher yields (40-60%) (21), the low yield of boarfish explains why this species has not been targeted for human consumption and probably why it is highly discarded. Valorization of fish species can include the obtention of high value compounds. Mechanically deboning and skinning, yields ground muscle and bones and skin separated (figure 1b). Bones and skin can be valorized by obtaining collagen, gelatins and fish oils, whereas muscle can be used to produce protein hydrolysates.

3.1.2. Small-spotted catshark

Shark viscera represent an important environmental problem, large quantities of discards and by-products from this species can be generated at sea and landings points (approximately 11,000 kg/year of viscera in the port of Vigo, own data). Previous unpublished data in our laboratory indicate that in small-spotted catshark, viscera represents 3.7–26.9% of entire body and pancreas the 0.48–3.62% of viscera, being relatively easy the separation of the pancreas from the rest of the viscera (figure 1c). Fish stomach and pancreas represent an interesting source of enzymes, in particular proteases, and the potential recover of these enzymes from fish viscera can be an option if the performance is similar or better to commercial ones.

3.2 Enzymatic hydrolysis

The hydrolysis exhibited typical curves (figure 2), similar to others found in literature (Souissi *et al.* 2007). ALC was the most efficient hydrolyzing boarfish muscle protein (23% DH), while BT and PCE showed lower values (11% and 8% DH respectively). A higher proteolytic activity of Alcalase, compared with other enzymes

has also been previously reported by Klompong *et al.* (2007). These results might be explained because Alcalase is able to hydrolyze proteins with broader specificity than other enzymes such as trypsin, which only catalyzes the hydrolysis of peptide bonds of the carboxyl groups in the basic amino-acids arginine or lysine (Adler-Nissen, 1986). Nielsen *et al.* (1997) have also attributed variations in hydrolysis degree to differences in several factors such as type of substrate, enzyme employed, temperature, pH, etc.

3.3 Chemical composition of hydrolysates

The protein content in freeze-dried hydrolysates was similar ranged from 72% to 79% (Table 1), which agrees with previous results reported by other authors (Souissi *et al.* 2007). This high protein content shows a potential for the use of these hydrolysates as a protein supplement for aquaculture or other animal feed applications. The lipid content showed little variation among three hydrolysates, ranging from 6.47% to 7.3%, similar to those previously reported by Olsen *et al.* (2004). Ash content in hydrolysates varies from 8.6-12.85%. These values are in agreement with Amiza *et al.* (2012), who found variations in ash content as degree of hydrolysis increases. However, the higher ash content in Alcalase hydrolysate might be also attributed to the addition of alkali required for adjustment of pH to 8.0. The content of moisture ranged from 4.64% to 5.31 %, values which are similar to other studies (Olsen *et al.* 2004).

3.4 Peptide molecular range of hydrolysates

The molecular size distribution of hydrolysates presented some differences, although more similitudes were found between PCE and BT. Those similitudes are due to the presence of the highest percentage of 3.19 to 0.66 kDa peptides in PCE and BT hydrolysates (74.23% and 45.26% respectively). In the other hand, hydrolysates prepared with ALC showed the highest percentage of 0.66 to 0.13 kDa peptides (99.81%) (Table 2). These results are in concordance with DH data demonstrating that

ALC was more efficient in the hydrolysis of boarfish muscle proteins. Additionally, BT hydrolysates showed some peptides with molecular weights ranging from 15- 3 kDa, that were not present in the other two hydrolysates. Also, PCE hydrolysate presented the highest percentage of peptides with a molecular weight range lower than 130 Da (9.82%), which might correspond mainly to free amino-acids. As expected, PCE and BT hydrolysates showed very similar peptide molecular range distribution due to same enzyme specificity. Pancreas extract contains high amounts of trypsin (Blanco *et al.* 2013) which has a well-documented specificity on protein substrates (Adler-Nissen 1986, Simpson 2000). In contrast, since Alcalase has different substrate specificity (Nielsen *et al.* 2001) a different molecular weight distribution pattern was obtained.

3.5 Amino-acid composition of hydrolysates

All hydrolysates presented very similar amino-acid composition (Table 3) in which is notable the higher percentage of Glutamic, Asparagine and Alanine accounting for 13%, 10% and 9% respectively. Several authors have described similar amino-acid composition for fish protein hydrolysates (Amiza *et al.* 2012). It is also noteworthy the higher content of 8 amino-acids in PCE hydrolysate compared to the other hydrolysates ($P \leq 0.05$). 2 of these 8 amino-acids in PCE hydrolysates are hydrophobic (Alanine, Phenylalanine), this is important because the effects of hydrophobicity on functional properties (Klompong *et al.* 2007).

3.6. Functional properties

3.6.1. Nitrogen solubility index (NSI)

Protein solubility is related with some functional properties such as emulsion and foaming capacity (Klompong *et al.* 2007). The solubility was measured in the pH range of 2 to 9, showing a positive correlation between hydrolysis degree and nitrogen solubility. Thus, ALC hydrolysates, which showed the highest DH, exhibited superior

percentage of NSI over the pH range studied (84-95%). This could be explained because the smaller peptides are expected to have proportionally more polar residues, with the ability to form hydrogen bonds with water and increase solubility (Shimada *et al.* 1992). Other authors have previously reported high soluble protein values in ALC hydrolysates (Shimada *et al.* 1992, Amiza *et al.* 2012). In the other hand, PCE hydrolysates and BT hydrolysates showed lower values, 49-59% and 60-71% respectively, as it was expected due to their lower hydrolysis degree. The slightly lower solubility values found in PCE hydrolysates might be also related with its amino-acid composition, with a higher proportion of hydrophobic residues compared with the other two hydrolysates. Other authors have also reported high solubility in peptides with low molecular weight and low hydrophobic residues (Walsh *et al.* 2003).

3.6.2. Emulsifying capacity and stability

Emulsifying capacity (EC) and emulsion stability (ES) were higher in BT hydrolysate and PCE, respectively (figure 3a). Hydrolysis degrees in both cases were lower than ALC. Since hydrolysates are surface-active materials due to their hydrophilic and hydrophobic groups, they promote oil-in water emulsions. Small peptides, although diffuse rapidly and adsorb at the water-oil interface, may have a negative effect in the stabilization of the emulsion because they cannot unfold and reorient at the interface (Shimada *et al.* 1992). Therefore, hydrolysates with high DH would have poorer emulsion properties due to the presence of a higher amount of small size peptides. Other authors have also found a reduction in emulsification properties in hydrolysates with small peptides and free amino- acids (Amiza *et al.* 2012).

3.6.3. Foaming capacity and stability

The higher foaming capacity (FC) and foaming stability (FS) values were found in BT (52.91 % and 34.5 %) followed by PCE (21.25 % and 16.79%) and ALC

hydrolysates (11.45 % and 7.26 %) (figure 3b). This is in agreement with previous studies reporting high foaming capacity in low hydrolysed proteins (Klompong *et al.* 2007, Diniz and Martin, 1997). According to literature, foaming capacity is not only governed by the length of the polypeptide chain, but also by hydrophobicity and surface tension at the water-air interface (Diniz and Martin, 1997, Amiza *et al.* 2012). Hydrophobicity of unfolded proteins or high molecular weight peptides is generally positively related to foam properties (Klompong *et al.* 2007). Foaming capacity of protein is known to improve by exposing more hydrophobic residues and by increasing capacity to decrease surface tension (Dickinson 1989, Mutilangi *et al.* 1996). The better foaming properties found in BT hydrolysate, showing an intermediate value of DH, and a proportion of 15.4-3.19 kDa, suggest that a limited hydrolysis allows the formation of a wide range of polypeptide molecular weight polypeptide chains, with the ability of increasing the surface activity of the hydrolysate, without the presence of undesirable very small peptides or free amino-acid.

3.6.4. Scavenging effect on DPPH free radical

Antioxidant activities of PCE, BT and ALC hydrolysates were 27.3 %, 25.23% and 23.25 % respectively (figure 4). These results are in agreement with Klompong *et al.* (Klompong *et al.* 2007) showing a decrease in antioxidant activity as the degree of hydrolysis increases. Besides, superior antioxidant activities found in PCE hydrolysates might be related with its higher Glycine and Proline concentration, since Gimenez *et al.* (Gimenez *et al.* 2009) have shown the positive correlation between the concentration of these two amino-acids and antioxidant capacity. Antioxidant results for muscle hydrolysates are higher than those obtained by other authors, who found scavenging effect of only 15, 4% (Wu *et al.* 2003) and 10,55% (Bougatéf *et al.* 2010).

These results reveals that BPH contains peptides with the ability to react with free radicals to convert them to more stable products ant terminate the radical chain reaction.

3.6.5. Theoretical identification of antioxidant peptides

Four peptides (KD, EL, PR, VKV) with previously reported antioxidant activity were identified within the boarfish myosin amino-acid sequence (Tang *et al.* 2010, Wang *et al.* 2008, Suetsuna and Ukeda, 1999). These results are in concordance with the low molecular weight peptides (0.13-0.005 kDa) found in BPH prepared with ALC (0.19% area), BT (4.37% area) and PCE (9.82% area).

Conclusion

A pancreas crude extract and two commercial enzymes were able to hydrolyze muscle proteins of boarfish. Notably, PCE extracts behave very similar to BT hydrolysates and exhibited the best antioxidant capacity. These results highlight the potential of unused marine resources to produce compounds with interest in the food/feed/pharmaceutical industries, while improving environmental protection and promoting the integral use of the total catches biomass.

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530 **Tables**

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Sample/Hydrolysate	Composition (%)			
	Moisture	Protein	Lipids	Ash
Minced muscle	78.63 ± 0.487	85,65 ± 1,7	5,38 ± 1,00	6,69 ± 0,10
Alcalase hydrolysate	5.31 ± 1.008	72,43 ± 2,42	7,3 ± 0.28	12.85 ± 0.9
Pancreas hydrolysate	5.26 ± 3.062	78,43 ± 1,41	6.47 ± 0.31	9.81 ± 0.22
TB hydrolysate	4.64 ± 0.784	79,12 ± 1,21	7,10 ± 0.21	8.6 ± 0.28

533 TABLE 1. CHEMICAL COMPOSITION OF *CAPROS APER* HYDROLYSATES.
 534 VALUES, EXPRESSED IN A WET BASIS, ARE MEANS OF TRIPLICATES
 535 DETERMINATIONS

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TABLE 2. MOLECULAR WEIGHT DISTRIBUTION OF BOARFISH HYDROLYSATES PREPARED WITH ALCALASE (ALC), PANCREAS CRUDE EXTRACT (PCE) AND BOVINE TRYPSIN (BT).VALUES ARE EXPRESSED AS PERCENTAGE OF THE AREAS IN CHROMATOGRAMS.

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AMINO ACID	HYDROLYSATES		
	PCE	ALC	BT
Asparagine*	108.6 ± 0.07	107 ± 0.04	104.5 ± 0.06
Threonine	47.3 ± 0.12	52.1 ± 0.06	52.4 ± 0.12
Serine*	59.4 ± 0.04	58.4 ± 0.03	58.4 ± 0.04
Glutamic acid*	140.6 ± 0.09	138 ± 0.06	136 ± 0.09
Glycine*	85.5 ± 0.06	80.8 ± 0.04	81.4 ± 0.06
Alanine*	102.3 ± 0.10	93 ± 0.07	95.5 ± 0.10
Cysteine	4.2 ± 0.03	5.3 ± 0.04	7.2 ± 0.04
Valine	41.9 ± 0.04	45.4 ± 0.07	47.5 ± 0.12
Methionine	28.1 ± 0.06	29.6 ± 0.06	29.6 ± 0.07
Ileucine	32.4 ± 0.04	37.5 ± 0.07	37.7 ± 0.15
Leucine	74.6 ± 0.09	78.4 ± 0.06	78.3 ± 0.12
Tyrosine*	26.6 ± 0.02	26.4 ± 0.02	26.1 ± 0.02
Phenylalanine*	37.4 ± 0.02	35.5 ± 0.01	35.1 ± 0.02
Hydroxyllysine	2 ± 0.02	2.4 ± 0.09	0.9 ± 0.02
Histidine	18.2 ± 0.03	19.7 ± 0.04	20.2 ± 0.04
Lysine	78.3 ± 0.07	80.1 ± 0.1	83.8 ± 0.12
Arginine	43.9 ± 0.08	43.9 ± 0.05	43.7 ± 1.42
Hydroxyproline	1.7	1 ± 0.20	2.1 ± 0.12
Proline*	39.2 ± 0.05	38 ± 0.03	38.5 ± 0.05

TABLE 3. AMINO ACID COMPOSITION (RESIDUES/1000 RESIDUES) OF BOARFISH HYDROLYSATES PREPARED WITH ALCALASE (ALC), PANCREAS CRUDE EXTRACT (PCE) AND BOVINE TRYPSIN (BT). RESULTS ARE MEAN VALUES OF THREE REPLICATES ± STANDARD DEVIATION. ANOVA ANALYSIS WAS PERFORMED FOR 8 AMINO ACIDS TO TEST DIFFERENCES AMONG HYDROLYSATES. ASTERISK (*) INDICATES STATISTICAL DIFFERENCE AMONG MEANS (P<0,05).

FIGURE CAPTIONS

FIGURE 1. BIOLOGICAL SAMPLES USED FOR THE STUDY: SPECIMEN OF *CAPROS APER* (FIGURE 1A), GROUNDED BOARFISH MUSCLE (FIGURE 1B) AND SPECIMEN OF *SCYLIORHINUS CANICULA* AND ITS VISCERA (FIGURE 1C).

FIGURE 2. HYDROLYSIS CURVES FOR BOARFISH HYDROLYSATES WITH DIFFERENT ENZYMES, ALCALASE (ALC), PANCREAS CRUDE EXTRACT (PCE), AND BOVINE TRYPSIN (BT). VALUES PRESENTED ARE MEAN OF TRIPLICATES ANALYSIS. ERROR BARS REPRESENT STANDARD DEVIATION.

FIGURE 3. EMULSIFYING CAPACITY (GR OIL/ GR PROTEIN) AND STABILITY (%) (A); FOAMING CAPACITY AND STABILITY (B) OF BOARFISH HYDROLYSATES OBTAINED WITH THE ENZYMES: PANCREAS CRUDE EXTRACT (PCE), BOVINE TRYPSIN (BT) AND ALCALASE (ALC). ERROR BARS REPRESENT STANDARD DEVIATION.

FIGURE 4. DPPH-RADICAL SCAVENING ACTIVITY OF BOARFISH HYDROLYSATES EXPRESSED AS % INHIBITION. THE CONTROL ASCORBIC ACID WAS USED FOR COMPARISON. DATA ARE PRESENTED AS MEAN VALUES OF TRIPLICATES. ERROR BARS REPRESENT STANDARD DEVIATION.

FIGURE 1.

FIGURE 2.

FIGURE 3.

FIGURE 4.

254x190mm (96 x 96 DPI)