Valorisation of fish discards assisted by enzymatic hydrolysis and microbial bioconversion: Lab and Pilot plant studies and preliminary sustainability evaluation.

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Abstract

The new EU fishing policies (Landing Obligation) are aimed at preventing the elimination of fishing discards overboard. These new biomasses that have to be landed from 2019 force to stablish valorisation protocols since, in most cases, they cannot be used directly for human consumption. In this context, the aim of this work was to develop an integral process based on enzyme proteolysis that permitted jointly the production and recovery of fish protein hydrolysates (FPHs), oils, bioactive peptides and fish peptones. This procedure was initially applied to ten fish discards to lab scale. FPHs of high quality in terms of soluble protein and amino acid contents, digestibility and bioactivities were obtained. The growth and metabolites productions by *Pediococcus acidilactici* on peptones from FPHs was also evaluated with excellent results. Pilot plant trials confirmed the results of FPHs production obtained at lab scale. Finally, a comparison with the nowadays most common use of fish biomass (fish meal production) has been made as a preliminary sustainability assessment of the proposed FPHs valorisation chain.

Keywords: fish discards; valorisation; fish protein hydrolysates; fish peptones; *Pediococcus accidilactici*; sustainability.

1. Introduction

The Common Fisheries Policy (CFP) of the European Commission introduced in 2013 (EC, 2013) a discard mitigation strategy which states that all catches of species subjected to catch quotas and/or Minimum Conservation Reference Size (MCRS) will have to be landed and will be counted against quota. This so-called Landing Obligation (LO) was being gradually implemented, since 2015 to 2019 when all EU fisheries are required to land all catches except a set of *de minimis* percentage of catches that are yearly set based on the scientific data of catches acquired from onboard observers and landing notes together with survival studies for different species, like rays or the Norwegian lobster. These expected increases in landings of previously discarded captures, from around 100 kg up to 3 tons per trip and vessel, might produce additional environmental impacts on land. Therefore, a quick elimination in appropriate conditions is necessary to avoid adverse effects, caused by poor hygienic and sanitary conditions.

Important amounts of individuals of size below the minimum legal size of various species subject to TAC are going to be landed and cannot be destined for direct human consumption, so they must be properly managed following a different commercialization and management route than usual. For this fraction that will define as FNHC, together with those specimens above MCRS that lack of quality enough to be sold, a wide range of available technological alternatives exist (Mango and Catchpole, 2014; Iñarra et al., 2019) but not all of them may be equally feasible.

Fish meal obtained after a thermal process of fish by-products, to coagulate the protein and separate the oil, is the most common and extended process but the biomass undergoes a low valorisation level (generally obtaining low quality products), the fish wastes must be transported from fishing ports to meal plants and the environmental impacts (air pollution, odours, high water consumptions, etc.) of those plants is huge. Thus, alternatives for a best use of these new biomasses from LO maximising the obtaining of compounds of high commercial interest in diverse sectors of application must be studied.

Valorisation processes directed by enzymatic hydrolysis to produce fish protein hydrolysates (FPHs) including the recovery of essential nutrients and bioactive compounds (Blanco et al., 2015, Halim et al., 2016) could be an excellent and viable practice to efficiently upgrade this new FHNC biomass. The preparation and characterization of FPHs covering different species, enzymes, or hydrolysis conditions have been extensively studied (Chalamaiah et al., 2012; Halim, et al., 2016; Vázquez et al., 2017). FPHs have demonstrated excellent functional properties as antioxidants against free radicals (Batista et al., 2010; Nasri et al., 2013), antihypertensive pharmacological agents, specifically, as inhibitors of the angiotensin-I converting enzyme (Ghassem et al., 2011; Nasri et al., 2013) and antimicrobial properties (Jiang et al., 2014; Wang et al., 2018).

On the other hand, since FPHs are rich in soluble proteins and with high digestibility, they can be also employed as ingredient of aquaculture and pet-food diets (Ospina-Salazar, 2016; Swanepoel and Goosen, 2018) with very promising results. Finally, it must be mentioned that FPHs could be also used

as substrate to obtain peptones (mixture of polypeptides and free amino acids) useful as ingredient of culture media for microbial growths (Pleissner and Venus, 2016). A great percentage of microbial bioproduction costs are due to the price of peptones (Djellouli et al., 2017; Shi et al., 2018) being the search of new protein fractions from food wastes an essential research issue (Pleissner and Venus, 2016).

In this work, we present an effective valorisation strategy based on enzymatic hydrolysis studying lab and pilot plant productions. Initially, the optimal conditions of enzymatic hydrolysis were studied for blue whiting as representative species. Using obtained values, FPHs from ten fish discards species were then produced together with the recovery of fish oil. Chemical and functional properties of FPHs were also determined. Additionally, fish peptones were produced from FPHs and succesfully applied in the culture of *Pediococcus acidilactici* and the production of lactic acid and pediocin. The production of several FPHs at pilot plant scale was performed confirming the industrial viability of the proposed process. Finally, a preliminary analysis of the most relevant environmental and socio-economic impacts of the proposed FPHs production has been made based on the comparison (mainly focused in the energy consumptions) with the nowadays most extended option to add value to the fish biomass (fish meal production), putting into the light the main advantages of the present valorisation strategy.

2. Material and methods

2.1. Fish material processing

All fish species, classified as discards by Galician fishing fleets, were captured in the North Atlantic Ocean: Blue whiting (BW, *Micromesistius poutassou*), Mackerel (M, *Scomber scombrus*), Red scorpionfish (RS, *Scorpaena scrofa*), Pouting (P, *Trisoreptus luscus*) and Gurnard (Gu, *Trigla* spp.), Grenadier (G, *Macrourus sp.*), Megrim (Me, *Lepidorhombus boscii*), European hake (H, *Merluccius merluccius*), Boarfish (Bo, *Capros aper*) and Atlantic horse mackerel (HM, *Trachurus trachurus*). They were separated from commercial species on board and the death specimens were directly preserved in ice. Once landed in the port, discards were immediately homogenised by grinding and stored at -18ºC until use. Proximal composition was determined in both raw materials and hydrolysates: 1) water, ash and organic matter content (AOAC, 1997), 2) total nitrogen (AOAC, 1997) and total protein as total nitrogen x 6.25 and 3) total lipids (Bligh and Dyer, 1959).

2.2. Optimisation of enzyme hydrolysis of BW discards

First, the combined effect of *pH* and temperature (*T*) on the digestion of blue whiting grinding individuals by Alcalase 2.4L (2,4 AnsonUnit/g, AU/g enzyme, Novozymes, Nordisk, Denmark) was evaluated. For this purpose, rotatable second order designs of two variables were carried out (with 5 replicas in the center of the experimental domain) (Box et al., 2005), whose designs are shown in Table S1 (supplementary material). The rest of the experimental conditions remained constant: agitation, (S:L) ratio and enzyme concentration (Table S1, supplementary material). These experiments were carried out in a pH-Stat system equipped with a 100 mL enzyme reactor with temperature and agitation control.

Secondly, the individual effect of enzyme concentration was studied using the same experimental equipment and maintaining constant (in the optimal values obtained in the previous factorial plans), the rest of experimental conditions. In the same way, the individual effect of (S:L) ratio on BW hydrolysis was also finally tested. In all optimisation experiments, after hydrolysis (4 h) the mini reactors were centrifuged (15000 g/20 min) and the sediments (mainly bones) and supernatants quantified.

Additionally, the degree of hydrolysis (*H*, as %) was determined in all hydrolysis kinetics by the pH-Stat method (Adler-Nissen, 1986) employing the mathematical models previously reported (Vázquez et al., 2017). The kinetic data of *H* were adjusted to Weibull equation (Vázquez et al., 2016):

$$H = H_m \left\{ 1 - \exp\left[-\ln 2 \left(\frac{t}{\tau} \right)^{\beta} \right] \right\} \quad \text{with} \quad v_m = \frac{\beta H_m \ln 2}{2\tau} \qquad (1)$$

where, *H* is the degree of hydrolysis (%); *t* the time of hydrolysis (min); H_m the maximum degree of hydrolysis (%); β a parameter related with the maximum slope of muscle hydrolysis (dimensionless); v_m the maximum rate of hydrolysis (% min^{-1}) and τ the time required to achieve the semi-maximum degree of hydrolysis (min). On the other hand, the ratio of digestion/liquefaction (V_{dig}) of raw material to liquid phase was calculated as the percentage of liquid FPH produced in relation of the sum of solid raw material and the water and alkali added for hydrolysis process.

200 *2.3. Production of fish protein hydrolysates (FPHs) at lab and pilot plant scale*

201 Lab-scale hydrolysis were carried out in a controlled pH-Stat system with a 5 L
202 glass-reactor (suspending 1 kg of milled discards in 2 L of distilled water, (S:L)
203 ratio of 1:2 w/v) using 5M NaOH as alkaline reagent for pH-control. Optimal
204 conditions obtained in previous section for BW were applied for all fish discards:
205 60ºC, pH8.65, agitation of 200 rpm and 1% (v/w) of Alcalase 2.4L. At the end of
206 the hydrolysis (4 h), the content of the reactors was filtered (100 μm) to remove
207 bones, the liquid hydrolysates were centrifuged (15000 g/20 min) to recover oils
208 (adding a step of decantation for 5 min) and final FPHs were quickly heated
209 (90ºC/15 min) for enzyme deactivation. In Figure 1, a schematic flowchart of
210 FPH sprocessing from fish discards is shown. After the sterilisation (121ºC/15
211 min) and centrifugation (15000 g/20 min) of FPHs, the recovered liquid phases
212 were denominated as fish peptones.
213
214 Pilot plant trials were performed in a stainless reactor of 500 L equipped with
215 control of temperature, agitation, reagent addition and pH (pH-Stat system).
216 Hydrolysis was executed following the same experimental conditions as at lab
217 scale but with initial loads of fish discards of 50-150 kg. Me, AM, H, BW and Bo
218 discards were chosen for pilot plant productions.
219
220 2.4. *Chemical analyses and determination of bioactivities*
221 The profile of fatty acids from fish oil was analysed by GC-chromatography after
222 chemical methylation (Lepage and Roy, 1986). FPHs were stored at -18ºC until
223 analysis. The basic analyses of FPH were: 1) total soluble protein (Lowry et al.,
224 1951); 2) total sugars (Dubois et al., 1956); 3) total protein as total nitrogen x

225 6.25 (AOAC, 1997); 4) proximal composition (as previously cited), 5) amino
226 acids content (quantified by ninhydrin reaction, using an amino acid analyzer
227 (Biochrom 30 series, Biochrom Ltd., Cambridge, UK), according to the method
228 of Moore et al. (1958); 5) *in vitro* digestibility (pepsin method: AOAC Official
229 Method 971.09 following the modifications reported by Miller et al., (2002).
230 Molecular weights of FPH were determined by Gel Permeation Chromatography
231 (GPC). The system used was an Agilent 1260 HPLC consisting of quaternary
232 pump (G1311B), injector (G1329B), column oven (G1316A), refractive index
233 (G1362A), diode array (G1315C) and dual-angle static light scattering (G7800A)
234 detectors. Standard and samples were eluted with a 0.15M ammonium acetate /
235 0.2M acetic acid buffer at pH 4.5 pumped at 1 mL/min through four columns
236 (PSS, Germany): Proteema precolumn (5 µm, 8 x 50 mm), Proteema 30Å (5
237 µm, 8 x 300 mm), Proteema 100Å (5 µm, 8 x 300 mm) and Proteema 1000Å (5
238 µm, 8 x 300 mm) after a 100 µL injection. Column oven and light scattering
239 detector were kept at 30ºC and refractive index detector was maintained at
240 40ºC. Detectors were calibrated with a polyethylene oxide standard (PSS,
241 Germany) of 106 kDa (Mw) and polydispersity index 1.05. Absolute molecular
242 weights were estimated with refractive index increments (dn/dc) of 0.185.
243
244 Antihypertensive and antioxidant (AO) activities were also determined in final
245 FPH samples obtained at the end of hydrolysis. Briefly, *in vitro* Angiotensin I-
246 converting enzyme (ACE) inhibitory activity (I_{ACE}) was based on the protocol
247 defined by Estévez et al. (2012) and IC_{50} values (protein-hydrolysate
248 concentration that generates a 50% of I_{ACE}) were calculated according to dose-
249 response modelling as previously reported (Amado et al., 2013). The

antioxidant capacity of FPH were analysed by three methods: a) 1,1-Diphenyl-2-picryhydrazyl (DPPH) radical-scavenging ability following the microplate protocol developed by Prieto et al. (2015a); b) ABTS (2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid) bleaching method according the microplate protocol recently published (Prieto et al., 2015a); c) Crocin bleaching assay using an opimised microplate report (Prieto et al., 2015b). All antihypertensive and AO determinations were done in triplicate employing FPH samples at concentration of 1 g/L of soluble protein.

2.5. Microbial bioconversion of fish peptones from FPHs

Pediococcus acidilactici NRRL B-5627 was used in the evaluation of fish peptones as nitrogen source and *Carnobacterium piscicola* CECT 4020 (Spanish Type Culture Collection) was the indicator microorganism for bacteriocin (Pediocin SA-1) bioassays. Stock cultures were stored at −80ºC on Man, Rogosa and Sharpe medium (MRS) with 25% glycerol. Inocula (0.5%, v/v) consisted of cellular suspensions from 16 h aged in MRS (incubated at 30ºC) and adjusted to an optical density-OD (700 nm) of 0.900.

The composition of the cost-effective culture media based on fish peptones are shown in Table S4 (supplementary material) employing MRS commercial medium (Pronadisa, Spain) as control. In all cases, initial pH was adjusted to 7.0 with 5M NaOH and solutions sterilised at 121ºC for 15 min. Micro-organisms were grown, by duplicate, in 300 mL Erlenmeyer flasks with 180 mL of medium at 30ºC and orbital agitation of 200 rpm. At pre-established times, each culture sample was divided into two aliquots: 1) The first one was processed for the

determination of biomass (as dry weight), productions of lactic and acetic acid by HPLC and the consumption of soluble proteins and reducing sugars (Lowry et al., 1951; Bernfeld, 1951) according to Vázquez et al. (2018); 2) The second one was used to extract and determine antimicrobial activity using *C. piscicola* as indicator (Murado et al., 2002). All determinations were carried out in duplicate. Growth and metabolite productions were simulated by the logistic equation (Vázquez and Murado, 2008):

$$P = \frac{P_m}{1 + \exp\left[2 + \frac{4v_P}{P_m}\left(\lambda_P - t\right)\right]} \qquad (2)$$

where, P is the concentration of the corresponding bioproduction (X: biomass, La: lactic acid, A: acetic acid or B: bacteriocin) (in g/L for X, La, A; and BU/mL for B); t is the time of culture (h); P_m is the maximum concentration of each bioproduction in the asymptotic phase (g/L or BU/mL); v_P is the maximum bioproduction rate (g L^{-1} h^{-1} or BU mL^{-1} h^{-1}); and λ_P is the lag phase of the bioproductions (h).

2.6. Numerical and statistical analyses

Data fitting procedures and parametric estimations were carried out by minimisation of the sum of quadratic differences between observed and model-predicted values, using the non-linear least-squares (quasi-Newton) method provided by the macro 'Solver' of the Microsoft Excel spreadsheet. Confidence intervals from the parametric estimates (Student's t test) and consistence of mathematical models (Fisher's F test) were evaluated by "SolverAid" macro.

3. Results and discussion

3.1. Optimisation of enzyme hydrolysis of BW

Initially, the optimal conditions of hydrolysis for BW were studied. BW was the chosen species to carry out the factorial experiments because it is the species most discarded by the fishing fleets that work in the North Atlantic Ocean (Egerton et al., 2018; Uhlmann et al., 2019). Figure 1 shows the graphical results of the different studies of optimisation. The degrees of correlation between the experimental data and predicted by the equations (degree of explicability of the equations) were 0.822 and 0.814 for H_m and V_{dig} responses, respectively (Table 1). Both equations were also statistically robust since F-Fisher tests were satisfied (data not shown). The optimal values that maximise the process of hydrolysis were calculated by numerical derivation: T_{opt}= 59.5ºC and pH_{opt}= 8.61 for H_m and T_{opt}= 60.5ºC and pH_{opt}= 8.69 for V_{dig}.

Then, and using the average values (60ºC, pH8.65), the individual effects of Alcalase concentration and S:L ratio on the hydrolysis process were evaluated (Figure 1C-F). The difference between the concentrations of Alcalase 1% and 2% (39.5±1.6% and 40.9±1.8% for H_m and 94.3±2.0% and 95.0±3.0% for V_{dig}, respectively) were not statistically significant ($p > 0.05$) but they were higher for H_m response and equal for V_{dig} response than employing 0.1% and 0.5% of Alcalase. Taking into account V_{dig} as dependent variable, the effect of increasing (S:L) ratios was not significant ($p>0.05$). For H_m, 1:2 and 1:3 ratios led to higher degrees of hydrolysis than 1:1 and 1:1.5 ratios.

3.2. Production of FPHs at lab-scale

All fish discards were hydrolysed based on the conditions defined in the previous section. In Table 2 the material balances of recovered products after substrates hydrolysis are shown. The inorganic parts, basically bones almost completely free of organic matter, were separated by filtration and were between 6% and 17% of the initial weight of the raw material. RS, G and mainly Bo were the species with the largest amount of skeleton and M the lowest. The yields of digestion (values of V_{dig}) of initial fish discards by commercial protease varied for each species ranging 82% for Gu to 94% for Me. No oil was extracted after proteolysis for H, P and G samples and Gu and RS (5.5% and 4.1% v/w, respectively) revealing the best options for oil recovery. Inexplicably once the hydrolysis of M and AM (well-known fatty species) was carried out, the volumes of oil separated were low (less than 1.5% v/w) perhaps because in these cases the applied process was ineffective along with the fact that these discards were captured in winter when their content in oil is lower (García-Moreno et al., 2013a).

The composition of fatty acids in the recovered oil samples is summarised in Table S2 (supplementary material). The predominant fatty acids were, in all cases, oleic acid, palmitic acid, DHA and EPA. The presence of DHA was superior to 7.6% (reaching up to 20% in BW) and the sum of EPA and DHA higher than 11.3% (around 28% in BW). The joint value of DHA and EPA for M oil (23.7%) was pretty similar to oil samples extracted from fillets of AM (19.5-22%) but the content of palmitic and oleic acids was significantly lower in the published references ($p<0.05$) (Romotowska et al., 2016a). Other authors

working with mackerel caught in the Mediterranean Sea and extracting lipids by preheating and pressing achieved a percentage of DHA+EPA of 27.9% (García-Moreno et al., 2013a). In the same article, the sum of fatty acids in horse mackerel samples was statistically equal (22.05±0.30%) to our result of AM (21.28±0.72%) ($p>0.05$). The ω-3/ ω-6 ratio was greater than 2.9 (9.4 in M). This last figure was lower than that found by García-Moreno & co-authors (2013b) (13.6), but their fatty acid profile did not include C18:3n6, C20:2n6, C20:3n6 and C22:2n6 data. In any case, ratio values< 0.5 are defined as harmful being our oils therefore advisable as ingredient for healthy human foods (Simonopoulos and DiNicolantonio, 2017).

The total proteins of FPHs were in the range 36-54 g/L for Prs, 38-55 g/L for Pr-tN and 39-55 g/L for the total sum of amino acids (Tables 2 and S3, supplementary material). FPHs from Me, BW, AM and G produced the largest concentration of protein and FPHs obtained from M, H and RS the lowest ones. Overall, the *in vitro* digestibilities (Dig) of hydrolysates were almost total (higher than 92%), achieving up to 97% in FPH from BW.

The content of amino acids is a very important aspect to be determined in fish protein hydrolysates (Karoud et al., 2018). As it can be observed (Table S2, supplementary material), the most abundant amino acids are, in all cases, glutamic and aspartic acids followed by leucine and lysine. Furthermore, the presence of glycine was very significant in FPHs of Gu, Bo and Me. Several studies have reported the same predominance of glutamic and aspartic acid in fish hydrolysates of several fish species (Ghassem et al., 2011; Klompong et

al., 2009; Pires et al., 2015). Essential amino acids (Ile, Leu, Val, Lys, Met, Phe, Thr, His and Arg) are also significantly present in our FPHs. These levels of amino acids together with the high digestibility of FPHs reveal its extraordinary nutritional value as potential ingredient of: 1) healthy food supplements (Nikoo et al., 2016; Shahidi and Ambigaipalan, 2015), 2) aquaculture feed and pet food diets (Martínez-Álvarez et al., 2015; Swanepoel and Goosen, 2018) and 3) microbial culture media (Vázquez et al., 2016).

In Figure 3, experimental and predicted data for the hydrolysis of the ten species of discards are displayed. As it is shown, the ability of equation (1) for describing the kinetic profiles was, in all cases, corroborated due to that (Table 3): a) the parameters were statistically significant, b) the values of R^2 were superior to 0.992 and c) the consistency of the equation was statistically demonstrated for each fit ($p<0.005$). Although, similar hyperbolic patterns of FPHs time-courses were reported for other fish discards (García-Moreno et al., 2013b, García-Moreno et al., 2017; Blanco et al., 2015), no mathematical approaches were used to simulate the corresponding experimental data. In FPHs from Me, G and BW the maximum degrees of hydrolysis (H_m= 47% in Me) were significantly higher than the other ones ($p<0.05$). This degree of hydrolysis in Bo was however half (H_m= 23%). Blanco et al. (2015) reported equal degree of hydrolysis (23%) for a Bo hydrolysate generated by Alcalase for 2 h and S:L ratio of 1:5 (w/v). However, lower values of hydrolysis were observed for Bo-FPH generated after 24 h of Papain (17%) and Alcalase (12.5%) digestion (Hayes et al., 2016) and for FPH obtained from Mediterranean BW (13-16%) using Alcalase + Trypsin (Pérez-Gálvez et al., 2015).

399 The values of τ were also lower in FPH (Bo, M and AM) with inferior H_m values
400 (Table 3). Nevertheless, Alcalase hydrolysis was faster (higher value of v_m) in
401 those FPHs mentioned. Because the conditions of hydrolysis were identical in
402 all proteolysis and the content of amino acids in final FPH were very similar, the
403 difference of hydrolysis degrees can only be explained by the difference in the
404 profile, type and configuration of protein and peptides present in each fish
405 substrate.
406
407 *3.3. In vitro bioactivities of FPHs*
408 In general, AO results were not especially remarkable, DPPH scavenging
409 activities were always lower than 50% being FPHs from G and Me the best and
410 worst hydrolysates, respectively (Table 4). These relative values between FPHs
411 are similar to data obtained from Crocin and ABTS protocols. Altough our BW
412 (19.8% of DPPH, 8.3 μg of Trolox/mL and 3.9 μg of BHT/mL) and RS (34.7% of
413 DPPH, 14.9 μg of Trolox/mL and 6.6 μg of BHT/mL) antioxidant activities were
414 low they are in concordance with values reported for BW Alcalase-FPH and RS
415 head Trypsin-FPH (Egerton et al., 2018; Aissaoui et al., 2015). In a similar way,
416 hydrolysates from Pacific hake generated by different proteases (Bromelain,
417 Alcalase, etc.) led to identical DPPH percentages (among 18-30%) to those
418 found in the present work for H-FPH (30.7%) (Cheung et al., 2012). However,
419 FPH from Cape hake showed quite similar DPPH inhibition values (around
420 40%) but higher ABTS activity (2.5 mg/mL as Trolox equivalent) than FPH of M
421 here produced (Teixeira et al., 2016).
422

423 The data of I_{ACE} (%) varied from 22% to 78% being FPHs of G and Gu (>70%)
424 the most bioactive samples (Table 4). In this context, peptides from BW fillet-
425 FPH also achieved 75% of ACE inhibition (Geirsdottir et al., 2011). Our levels
426 for M and AM (46% in both substrates) were lower than those generated by
427 combining Subtilisin and Trypsin (65-67%) on individuals captured in
428 Mediterranean Sea (García-Moreno et al., 2013b). The joint effect of those
429 proteases and the previous extraction of oils to the proteolysis step could be the
430 reason of such difference. Subsequently, the samples that were higher than
431 50% of I_{ACE} were selected for the dose-response bioassays in order to obtain
432 the values of IC_{50}. Following the same order of I_{ACE} activities, FPHs from Gu
433 and G showed the statistically significant lowest IC_{50} values ($p<0.05$), that is,
434 they are the most bioactive samples (165 μg/mL and 185 μg/mL, respectively).
435 The values of IC_{50} for the remaining FPHs (P, RS, Bo and H) were ranging
436 between 254-330 μg/mL. This last figure of H (330 μg/mL) was quite similar to
437 that observed by Savinase applied for 2 h on H heads (260 μg/mL) (Karoud et
438 al., 2018). In general our FPHs were more bioactive than those obtained from
439 head and muscle of RS (Aissaoui et al., 2015; Aissaoui et al 2017) and fillets of
440 BW (Geirsdottir et al., 2011). Underutilised Bo captures hydrolysed by Papain
441 and Alcalase for 24 h led to inhibitions of 45% and 67%, respectively (Hayes et
442 al., 2016). The difference with our data (56%) may be due to the longer time of
443 hydrolysis (24 h) or the larger concentration of enzyme employed.
444
445 Average molecular weights (Mw) of FPHs ranged from 743 Da in P to 1380 Da
446 in HM (Table 5), with no apparent relationships found between Mw and either
447 antioxidant or antihypertensive activities. GPC profiles (Figure 4) were

448 characterized by one broad light scattering signal, a main peak with a shoulder
449 in the refractive index signal and several peaks not completely resolved in UV.
450 While the profiles were similar for all FPHs, the proportions of each peak varied
451 amongst species, reflecting differences in Mw. These are generally within the
452 range of molecular weights previously reported for FPH: a) In BW, Mw from 40
453 Da to 20 kDa (Cudennec, 2008) or greater than 900 Da for 75-90% of FPHs
454 mass (García-Moreno et al., 2017) are comparable to Mw of 907 Da reported
455 here; b) in M, Mw of 840 Da found in the present study are within the
456 distribution limits of 27 Da and 2794 Da previously reported (Beaulieu, 2009); c)
457 for H, we estimate a Mw of 937 Da, slightly lower than the lower limit of the
458 1216 to 3492 Da range found in hake heads (Karoud, 2018).
459
460 *3.4. P. accidilactici culture in peptones from FPHs*
461 In Figure 5, bacterial cultivations on MRS (control medium) and on fish peptone
462 media are displayed. In all low-cost media the growths of *P. acidilactici* were at
463 least equal or higher than those found in MRS (Table 6). Sigmoid experimental
464 data of growth, lactic acid and pediocin were perfectly fitted (R^2=0.973-0.997,
465 p<0.001) by logistic equation (2).
466
467 High maximum growths (as value of H_m) were found in cultures using peptones
468 from Bo and BW followed by M and AM. All of them were significantly greater
469 than MRS and the rest of peptones (p<0.05). However, both maximum growth
470 rates (v_x) and the lag phase of growths (λ_x) did not show significant difference
471 between media (p>0.05). Only the value of v_x for BoP was significantly higher
472 than MRS and GP led to the lowest growth rate. BoP, MP and BWP showed the

473 best efficiencies to produce biomass in terms of the growth yields regarding
474 nutrient uptake ($Y_{X/RS}$ and $Y_{X/Pr}$). On the other hand, the maximum
475 concentrations and rates of lactic acid productions as well as lag phases (λ_{La})
476 were statistically equal for all peptones tested ($p>0.05$). The values of $Y_{La/RS}$
477 were very similar in all cultures and GuP, MeP and BoP demonstrated its higher
478 efficiency in the release of lactic acid in relation to the consumption of protein
479 substrates. Finally, the maximum production of pediocin was obtained in MRS
480 followed very closely by BWP, BoP and RSP. No significant differences of
481 pediocin rates and lag phases were observed among media ($p>0.05$). MRS was
482 also the most productive and effective nutrient formulation and BWP, BoP and
483 RSP the best options from alternative media for pediocin.
484
485 These findings are in line with the results reported for nitrogen sources derived
486 from enzymatic and alkaline effluents generated in the isolation of chitin from
487 squid pens (Vázquez et al., 2018). Other marine and fish peptones, obtained
488 from viscera of tuna, trout, salmon, swordfish and effluents from cephalopod
489 thermal processing also demonstrated to be an adequate ingredient of culture
490 broth to produce lactic acid bacteria and bacteriocins (Aspmo et al., 2005;
491 Vázquez and Murado., 2008). The application of FPH from fish discards species
492 to microbiological productions is almost unexplored. Hydrolysates from filleting
493 wastes of hake appeared of sufficient nutritional value to support growth of
494 several bacteria (Martone et al., 2005). In addition, our alternative peptones
495 revealed a valuable reduction of the costs from each microbial production: 3-5
496 folds, 3 folds and 2-3 folds for biomass, lactic acid and pediocin productions,
497 respectively. These highlighties were obtained taking into account the market

cost of the peptones commonly included in MRS medium and comparing the obtained productions (values of X_m, L_m and BT_m) among media.

3.5. Production of FPHs at pilot plant

Five fish discard species were selected to carry out productions of enzyme hydrolysates at scale of 50-150 kg of raw materials in a 500 L-reactor under the conditions optimised above. After Alcalase hydrolysis, rests of bones were collected in the filter mesh present inside the reactor and FPH were passed through a discontinuous Tricanter Veronesi SAT 140. The yields of oils recovered after centrifugation was much lower and, in some cases almost null, than those obtained at lab scale (data not shown) due to the fact that most of the oil is emulsified in FPH phase and the centrifugation speed of the Tricanter, less than 6000 g, is not enough to separate oil phase from emulsion.

However, the other parameters analysed in pilot plant FPH were in agreement with results obtained at lab scale (Table 7). The final hydrolysis (H_f) of FPH calculated using the total volumen of 5M NaOH added to maintain constant pH in the enzymatic reaction for 4h of processing, was statistically similar ($p>0.05$) to the H_m-values presented in Table 2 ($p<0.05$). The chemical composition of FPH in terms of protein content (Prs and Pr-tN) was also identical for BW, Bo and H and slightly but significantly lower for Me and AM ($p<0.05$). The results of *in vitro* digestibilities were also similar in BW, Bo and AM and a little lower in Me and H. Finally, the data of amino acids in FPH generated to large volume were statistically equal to those at 5L-scale (data not shown).

3.6. Preliminary sustainability assessment

As previously mentioned in this work, the different level of impacts that the new landed biomass due to the LO compliance could cause calls for the need of developing *on-demand* management and valorisation solutions for each type of port by using the best available techniques in terms of valorisation and making the best use of landed biomass. Nowadays, these alternatives at real scale represent the fish oil and meal companies or, directly the waste treatment alternatives.

In recent years, the trend is to concentrate the fish meal productionl in big plants decentralised from ports/fish processors, resulting in higher logistics costs due to the need of an optimal transport network that could reduce the margin that fish meal companies will pay for fish discards to fishermen. Moreover, it could happen that these companies could charge fishermen or port authorities with a cost to manage the big amounts of biomass landed by LO, representing an extra impact that risks the future sustainability of fishing activity.

The main environmental impacts associated to fish meal plants are high water and energy consumption and the discharge of effluents with high organic content. In addition to GHG emissions, particulate matter and air born chemicals, fishmeal processing plants also generate considerable odours from the storage of putrescible materials and the cooking and drying processes. Therefore, populations living near fishmeal plants are exposed to air, soil and water pollution.

548 In this work, we present a sustainable, effective and viable valorisation chain
549 model based on enzymatic hydrolysis to obtain FPHs that tries to overcome the
550 above exposed main issues related to fish meal plants. The proposed
551 valorisation strategy to obtain FPHs would allow to the fishing sector to process
552 *in situ* high amounts of biomass landed in the ports without highly complex,
553 costly equipment, being a scalable, flexible and easy-to-implement technology
554 while generating and retaining more value (the average market price of FPHs is
555 between 4–10 €/kg) than in the case where the fish biomass is directly sold to
556 the fish meal companies (that pay around 0.03–0.05 €/kg). The fish meals
557 generated from fish by-products can achieve market values of 0.3–1.2 €/kg
558 depending on the chemical composition and characteristics of the final product.
559 Even more, if marine peptones are produced for microbial culture media
560 purposes, the revenues can be exponentially increased since market prices for
561 similar non-marine products are in the range of 75 € to 100 € for 0.5 kg of
562 bactopeptone and beef extract, respectively. By considering that the proposed
563 process previously described (Figure 1) obtains alternative peptones with a
564 valuable reduction of the costs from each biological production, the potential of
565 the FPHs valorisation chain is very important.
566
567 From an environmental point of view, *in situ* FPHs plants eliminate the transport
568 logistics to centralized points like fish meal factories, eliminating both the
569 associated economic costs and the related environmental impacts due to
570 transport (Lopes et al., 2015). Moreover, it will also avoid to the populations
571 living near the fishmeal processing plants the exposition to air, soil and water
572 pollution together with noises and odours that hydrolysis minimise.

Now, regarding the fact that heat treatments present in the fish meal production that are very high energy demanding (that results on higher environmental impacts related to this power consumption), FAO determined that a small fish meal plant with a processing capacity of 10 to 60 tons of raw material (from now on denoted as *rm*) per day and an evaporation section consumes in average 55 kg/t raw material of fuel oil and up to 35 kwh/t rm of electric power (FAO, 1986). By using energy conversion factors from *Government Emission Conversion Factors for GHG Company Reporting 2018*, we can calculate the electric power:

E_{fuel} = 55 kg fuel/t rm x 11.9 kWh/kg fuel = 654.5 kWh/t rm.

And the total electric consumption of the fish meal plant will be:

$E_{total} = E_{fuel} + E_{elec}$ = 689.5 kWh/t rm

These data can be translated to GHG emissions by using the adequate factors but the values of energy consumption are enough to compare between proposed FPHs and fish meal approach. For FPHs, we selected energy data from a small plant with similar facilities to nowadays installed in the Port of Marin (Galicia) called iDVP (Integral Discards Valorisation Point). It has been developed in the framework of the LIFE iSEAs project (Iñarra et al., 2019). The main energy demanding processes of FPH manufacture are hydrolysis, deactivation and drying steps, being the last one the most energy consumer (Figure 1). Drying at industrial scale is mostly utilized by spray dryers and, thus, their performance must be evaluated for making a conclusion on energy status of the operation. The average energy consumption for industrial spray dryers is in the range between 3,500 and 11,500 kJ/kg evaporated water (Petrova et al.,

2018). We will consider an average energy consumption of 4,880 kJ/kg of evaporated water (Petrova et al., 2018). If we consider a calculation base of 1,000 kg of fish biomass and by following the flowchart depicted in Figure 1 (obtaining FPHs with 10% of final humidity), we can estimate the energy required to eliminate all the required water to obtain the final product:

E_{drying} = 1.098·10^{7} kJ/t rm = 3,050 kWh/ t rm

This value is much higher than the fish meal production due to the fact that spray drying equipment, which allows to obtain the solid FPH in the required conditions, needs much higher temperature of processing in a shorter time of drying than for the case of fish meal. Meanwhile, for the other heat demanding processes of FPH production (heating of the raw material up to 60ºC and deactivation enzymatic phase), heating loads are far lower. Assuming that the heat capacity of the mixture fish and water (at ratio 1:2 w/v) will be the water one (4.18 kJ/kg K) and considering an initial temperature of tap water of 15ºC, the approximate electric power consumption for enzymatic hydrolysis is calculated in 156.8 kWh and 104.5 kWh for heating + hydrolysis and termination of hydrolysis, respectively. Adding both values (261.3 kWh) it suposses less than 10% of the total energy consumption in the FPHs plant.

The aim of our future research will be the comparison at real scale of these two processes (fish meal and FPHs) by using the same type of biomass input to both of them, recording real data of energy consumptions to eliminate uncertainties and inaccuracies in the established mass and energy balances. In addition, impacts associated with waste management and valorisation steps will

be assessed by different methodologies like ecological footprint (EF) and Life Cycle Assessment (LCA). To solve electric power consumption of drying step, integration of FPHs technology on the production of a higher fish processing factory (e.g. canning industry) with an efficient energy system will be very valuable and will minimize its important environmental impacts. Additionally, it will give them a strategical advantage against possible competitor in the sector while leading the fight for a greener and sustainable fishing sector.

4. Conclusions

In this study, an enzymatic process was optimized for the hydrolysis of BW captured by North Atlantic fishing fleets and discarded for commercialisation and human consumption. This optimal procedure was applied to ten fish discarded species for the selective recovery and production of oils, FPH, bioactive peptides and fish peptones. Microbial bioconversion of those fish peptones was evaluated by the production of *P. accidilactici* biomass, lactic acid and a potent bacteriocin (pediocin SA-1). Regarding preliminary sustainability assessment, despite the high energy consumption of FPHs drying we can ensure that is a most efficient, flexible and scalable solution to overcome the main impacts caused by the biomass landed due to LO since it provides fishing sector agents a viable alternative to manage and valorise *in-situ* high amounts of biomass, generating valuable products as the FPHs and reducing environmental impacts associated to fish meal plants.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgements

The authors thank to Ana Durán, Margarita Nogueira and Araceli Menduíña for their excellent technical assistance. This research was funded by the projects LIFE iSEAS (LIFE+Programme, LIFE13 ENV/ES/000131), CVMar+i (0302_CVMAR_I_1_P, POCTEP 2015), GAIN (H2020 grant agreement N° 773330) and Xunta de Galicia (*Grupos de Potencial Crecimiento*, IN607B 2018/19).

References

Adler-Nissen, J., 1986. Enzymic hydrolysis of food proteins, Elsevier Applied Science Publishers.

Aissaoui, N., Abidi, F., Marzouki, M.N., 2015. ACE inhibitory and antioxidant activities of red scorpionfish (*Scorpaena notata*) protein hydrolysates. J. Food Sci. Technol. 52, 7092–7102.

Aissaoui, N., Abidi, F., Hardouin, J., Abdelkafi, Z., Marrakchi, N., Jouenne, T., Marzouki, M.N., 2017. Two novel peptides with angiotensin I converting enzyme inhibitory and antioxidative activities from *Scorpaena notata* muscle protein hydrolysate. Biotech. Appl. Biochem. 64, 201–210.

Amado, I.R., Vázquez, J.A., González, M.P., Murado, M.A., 2013. Production of antihypertensive and antioxidant activities by enzymatic hydrolysis of protein concentrates recovered by ultrafiltration from cuttlefish processing wastewaters. Biochem. Eng. J. 76, 43–54.

AOAC, 1997. Association of Official Analytical Chemistry. Methods of Analysis., 15th ed., Washington DC, USA.

Aspmo, S.I., Horn, S.J., Eijsink, V.G.H., 2005. Hydrolysates from Atlantic cod (*Gadus morhua* L.) viscera as components of microbial growth media. Proc. Biochem. 40, 3714-3722.

Batista, I., Ramos, C., Coutinho, J., Bandarra, N.M., Nunes, M.L., 2010. Characterization of protein hydrolysates and lipids obtained from black scabbardfish (*Aphanopus carbo*) byproducts and antioxidative activity of the hydrolysates produced. Proc. Biochem. 45, 18- 24.

Beaulieu, L., Thibodeau, J., Bryl, P., Carbonneau, M.-É., 2009. Proteolytic processing of Atlantic mackerel (*Scomber scombrus*) and biochemical characterisation of hydrolysates. Int. J. Food Sci. Tech. 44(8), 1609-1618.

Bernfeld, P., 1951. Enzymes of starch degradation and synthesis. Adv. Enzymol. 12, 379-427.

Blanco, M., Sotelo, C.G., Pérez-Martín, R.I., 2015. Hydrolysis as a valorization strategy for unused marine food biomass: Boarfish and small-spotted catshark discards and by-products. J. Food Biochem. 39, 368–376.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911-917.

Box, G.E., Hunter, J.S., Hunter, W.G., 2005. Statistics for experimenters: design, innovation, and discovery. Wiley-Interscience New York.

Chalamaiah, M., Dinesh Kumar, B., Hemalatha, R., Jyothirmayi, T., 2012. Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. Food Chem. 135, 3020–3038.

Cheung, I.W.Y., Cheung, L.K.Y., Tan, N.Y., Li-Chan, E.C.Y., 2012. The role of molecular size in antioxidant activity of peptide fractions from Pacific hake (*Merluccius productus*) hydrolysates. Food Chem. 134, 1297–1306.

Cudennec, B., Ravallec-Plé, R., Courois, E., Fouchereau-Peron, M., 2008. Peptides from fish and crustacean by-products hydrolysates stimulate cholecystokinin release in STC-1 cells. Food Chem. 111, 970–975

Djellouli, M., Martínez-Álvarez, O., Arancibia, M.Y., Florez-Cuadrado, D., Ugarte-Ruíz, M., Domínguez, L., Zadi-Karam, H., Karam, N., Roudj, S., López-Caballero, M.E., 2017. Effect of seafood peptones on biomass and metabolic activity by *Enterococcus faecalis* DM19. LWT Food Sci. Technol. 81, 94-100.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350-356.

Egerton, S., Culloty, S., Whooley, J., Stantone, C., Ross, R.P., 2018. Characterization of protein hydrolysates from blue whiting (*Micromesistius poutassou*) and their application in beverage fortification. Food Chem. 245, 698–706.

Estévez, N., Fuciños, P., Sobrosa, A.C., Pastrana, L., Pérez, N., Rúa, M.L., 2012. Modeling the angiotensin converting enzyme inhibitory activity of peptide mixtures obtained from cheese whey hydrolysates using concentration-response curves. Biotechnol. Prog. 28, 1197–1206.

European Commission (2013). Regulation (EU) No 1380/2013 of the European Parliament and the Council of 11 December 2013 on the Common Fisheries Policy, amending Council Regulations (EC) No 1954/2003 and (EC) No 1224/2009 and repealing Council Regulations (EC) No 2371/2002 and (EC) No 639/2004 and Council Decision 2004/585/EC.

FAO, 1986. The production of fish meal and oil. FAO Fisheries Technical Paper, 142. Rome.

García-Moreno, P.J., Pérez-Gálvez, R., Espejo-Carpio, F.J., Muñío, M.M., Guadix, A., Guadix, E.M., 2013a. Lipid characterization and properties of protein hydrolysates obtained from discarded Mediterranean fish species. J. Sci. Food Agric. 93, 3777–3784.

García-Moreno, P.J., Pérez-Gálvez, R., Morales-Medina, R., Guadix, A., Guadix, E.M., 2013b. Discarded species in the west Mediterranean Sea as sources of omega-3 PUFA. Eur. J. Lipid Sci. Technol. 115, 982–989.

García-Moreno, P.J., Pérez-Gálvez, R., Espejo-Carpio, F.J., Ruiz-Quesada, C., Pérez-Morilla, A.I., Martínez-Agustín, O., Guadix, A., Guadix, E.M., 2017. Functional, bioactive and antigenicity properties of blue whiting protein hydrolysates: effect of enzymatic treatment and degree of hydrolysis. J. Sci. Food Agric. 97(1), 299-308.

Geirsdottir, M., Sigurgisladottir, S., Hamaguchi, P.Y., Thorkelsson, G., Johannsson, R., Kristinsson, H.G., Kristjansson, M.M., 2011. Enzymatic hydrolysis of blue whiting (*Micromesistius poutassou*); functional and bioactive properties. J. Food Sci. 76, C14-C20.

Ghassem, M., Arihara, K., Babji, A.S., Said, M., Ibrahim, S., 2011. Purification and identification of ACE inhibitory peptides from Haruan (*Channa striatus*) myofibrillar protein hydrolysate using HPLC-ESI-TOF MS/MS. Food Chem. 129, 1770-1777.

Halim, N.R.A., Yusof, H.M., Sarbon, N.M., 2016. Functional and bioactive properties of fish protein hydolysates and peptides: A comprehensive review, Trends Food Sci. Technol. 51, 24-33.

Hayes, M., Mora, L., Hussey, K., Aluko, R.E., 2016. Boarfish protein recovery using the pH-shift process and generation of protein hydrolysates with ACE-I and antihypertensive bioactivities in spontaneously hypertensive rats. Innov. Food Sci. Emerg. Technol. 37, 253–260.

Iñarra, B., Bald, C., Cebrián, M., Antelo, L.T., Franco-Uría, A., Vázquez, J.A., Pérez-Martín, R.I., Zufía, J. 2019. What to Do with Unwanted Catches: Valorisation Options and Selection Strategies. In: Uhlmann S., Ulrich C., Kennelly S. (eds) The European Landing Obligation. SpringerOpen, Chapter 17, pps 333-359.

Jiang, L., Wang, B., Li, B., Wang, C., Luo, Y., 2014. Preparation and identification of peptides and their zinc complexes with antimicrobial activities from silver carp (*Hypophthalmichthys molitrix*) protein hydrolysates. Food Res. Int. 64, 91-98.

789 Karoud, W., Sila, A., Krichen, F., Martinez-Alvarez, O., Bougatef, A., 2018.
790 Characterization, surface properties and biological activities of protein
791 hydrolysates obtained from hake (*Merluccius merluccius*) heads. Waste
792 Biomass Valor. DOI 10.1007/s12649-017-0069-9
793
794 Klompong, V., Benjakul, S., Yachai, M., Visessanguan, W., Shahidi, F., Hayes,
795 K.D., 2009. Aminoacid composition and antioxidative peptides from protein
796 hydrolysates of yellow stripe trevally (*Selaroides leptolepis*). J. Food Sci. 74,
797 C126–C133.
798
799 Mango, S., Catchpole, T., 2014. Using discards not destined for human
800 consumption. Envir. Conserv. 41, 290-301.
801
802 Martínez-Alvarez, O., Chamorro, S., Brenes, A., 2015. Protein hydrolysates
803 from animal processing by-products as a source of bioactive molecules with
804 interest in animal feeding: A review. Food Res. Int. 73, 204-212.
805
806 Martone, C.B., Borla, O.P., Sánchez, J.J., 2005. Fishery by-product as a
807 nutrient source for bacteria and archaea growth media. Bior. Technol. 96, 383–
808 387.
809
810 Miller, E.L., Bimbo, A.P., Walters, D.E., Barlow, S.M., Sheridan, B., 2002.
811 Determination of nitrogen solubility in dilute pepsin hydrochloric acid solution of
812 fishmeal: interlaboratory study. J. AOAC Int. 85, 1374-1381.
813
814 Moore, S., Spackman, D.H., Stein, W.H., 1958. Chromatography of amino acids
815 on sulfonated polystyrene resins. An improved system. Anal. Chem. 30, 1185-
816 1190.
817
818 Murado, M.A., González, M.P., Vázquez, J.A., 2002. Dose–response
819 relationships: an overview, a generative model and its application to the
820 verification of descriptive models. Enz. Microb. Technol. 31, 439-455.
821
822 Nasri, R., Younes, I., Jridi, M., Trigui, M., Boutagef, A., Nedjar-Arroume, N.,
823 Dhulster, P., Nasri, M., Karra-Chaabouni, M., 2013. ACE-inhibitory and
824 antioxidative activities of Goby (*Zosterissessor ophiocephalus*) (FPH): effect on
825 meat lipid oxidation. Food Res. Int. 54, 552-561.
826
827 Nikoo, M., Benjakul, S., Rahmanifarah, K., 2016. Hydrolysates from marine
828 sources as cryoprotective substances in seafoods and seafood products.
829 Trends Food Sci. Technol. 57, 40–51.
830
831 Lepage, G., Roy, C.C., 1986. Direct transesterification of all classes of lipids in
832 a one-step reaction. J. Lipid Res. 27, 114-120.
833
834 Lopes, C., Antelo, L.T., Franco-Uría, A., Alonso, A.A., Pérez-Martín, R., 2015.
835 Valorisation of fish by-products against waste management treatments-
836 Comparison of environmental impacts. Waste Manag. 46, 103-112.
837

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265-275.

Ospina-Salazar, G.H., Ríos-Durán, M.G., Toledo-Cuevas, E.M., Martínez-Palacios, C.A., 2016. The effects of fish hydrolysate and soy protein isolate on the growth performance, body composition and digestibility of juvenile pike silverside, *Chirostoma estor*. Animal Feed Sci. Technol. 220, 168-179.

Petrova, I., Tolstorebrov, I., Eikevik, T.M., 2018. Production of fish protein hydrolysates step by step: technological aspects, equipment used, major energy costs and methods of their minimizing. Int. Aquatic Res. 10, 223.

Pires, C., Teixeira, B., Cardoso, C., Mendes, R., Nunes, M.L., Batista, I., 2015. Cape hake protein hydrolysates prepared from alkaline solubilised proteins pre-treated with citric acid and calcium ions: Functional properties and ACE inhibitory activity. Proc. Biochem. 50, 1006–1015.

Pleissner, D., Venus, J., 2016. Utilization of protein-rich residues in biotechnological processes. Appl. Microbiol. Biotechnol. 100, 2133–2140.

Prieto, M.A., Curran, T., Gowen, A., Vázquez, J.A., 2015a. An efficient methodology for quantification of synergy and antagonism in single electron transfer antioxidant assays. Food Res. Int. 67, 284–298.

Prieto, M.A., Vázquez, J.A., Murado, M.A., 2015b. Crocin bleaching antioxidant assay revisited. Application to microplate to analyse antioxidant and prooxidant activities. Food Chem. 167, 299–310.

Romotowska, P.E., Karlsdóttir, M.G., Gudjónsdóttir, M., Kristinsson, H.G., Arason, S., 2016a. Seasonal and geographical variation in chemical composition and lipid stability of Atlantic mackerel (*Scomber scombrus*) caught in Icelandic waters. J. Food Comp. Anal. 49, 9–18.

Shahidi, F., Ambigaipalan, P., 2015. Novel functional food ingredients from marine sources. Curr. Opin. Food Sci. 2, 123–129.

Shi, S., Li, J., Blersch, D. M., 2018. Utilization of solid catfish manure waste as carbon and nutrient source for lactic acid production. Appl. Microbiol. Biotechnol. 102, 4765-4772.

Simopoulos, A.P., DiNicolantonio, J.J., 2017. Mediterranean diet: ω-6 and ω-3 fatty acids and diabetes. Am. J. Clin. Nutr. 106, 953-954.

Swanepoel, J.C., Goosen, N.J., 2018. Evaluation of fish protein hydrolysates in juvenile African catfish (*Clarias gariepinus*) diets. Aquacult. 496, 262-269.

Teixeira, B., Pires, C., Nunes, M.L., Batista, I., 2016. Effect of in vitro gastrointestinal digestion on the antioxidant activity of protein hydrolysates prepared from Cape hake by-products. Int. J. Food Sci. Technol. 51, 2528–2536.

Uhlmann, S.S., Ulrich, C., Kennelly, S.J., 2019. The European landing obligation reducing discards in complex, multi-Species and multi-jurisdictional fisheries. Springer Open ISBN 978-3-030-03307-1.

Vázquez, J.A., Murado, M.A., 2008. Mathematical tools for objective comparison of microbial cultures. Application to evaluation of 15 peptones for lactic acid bacteria productions. Biochem. Eng. J. 39, 276–287.

Vázquez, J.A., Caprioni, R., Nogueira, M., Menduíña, A., Ramos, P., Pérez-Martín, R.I., 2016. Valorisation of effluents obtained from chemical and enzymatic chitin production of *Illex argentinus* pen by-products as nutrient supplements for various bacterial fermentations. Biochem. Eng. J. 116, 34–44.

Vázquez, J.A., Blanco, M., Massa, A.E., Amado, I.R., Pérez-Martín, R.I., 2017. Production of fish protein hydrolysates from *Scyliorhinus canicula* discards with antihypertensive and antioxidant activities by enzymatic hydrolysis and mathematical optimization using response surface methodology. Mar. Drugs 15, 306.

Vázquez, J.A., Ramos, P., Valcarcel, J., Antelo, L.T., Novoa-Carballal, R., Reis, R.L., Pérez-Martín, R.I., 2018. An integral and sustainable valorisation strategy of squid pen byproducts. J. Clean. Prod. 201, 207-218.

Wang, L., Sun, J., Ding, S., Qi, B., 2018. Isolation and identification of novel antioxidant and antimicrobial oligopeptides from enzymatically hydrolyzed anchovy fish meal. Proc. Biochem. 74, 148-155.

TABLE CAPTIONS

Table 1. Second order models describing the joint effect of temperature (*T*) and *pH* on Alcalase hydrolysis of blue whiting. Optimal values of the two variables (T_{opt}, pH_{opt}) to reach the maximum responses (Y_{max}) from the empirical equations are also summarized.

Table 2. Mass balances and proximal analysis of the products obtained from alcalase hydrolysates of whole body of fish discards. Showed errors are the confidence intervals for n=2 and α=0.05. m_b: percentage of the bones recovered; V_{oil}: percentage of the oil recovered; V_{dig}: percentage of the digestion/liquefaction of the solid by-products to the liquid phase; Prs: Total soluble protein determined by Lowry; TS: Total sugars; Dig: Digestibility; Pr-tN: Total protein determined as total nitrogen x 6.25; H: Humidity; Ash: Ashes; OM: Organic matter.

Table 3. Kinetic parameters and confidence intervals obtained from Weibull equation [3] modeling the time course of the hydrolysis degree (H) of fish discard by-products mediated by alcalase. Determinaton coefficients (R^2) and p-values are also shown.

Table 4. Bioactivites (antioxidant and antihypertensive) of FPH obtained from fish discards. Showed errors are the confidence intervals for n=2 and α=0.05.

Table 5. Molecular weight of FPH from fish discards. Mn: number average molecular weight; Mw: weight average molecular weight; PDI: polydispersity index.

Table 6. Numerical values and confidence intervals for parameters derived from logistic equation applied for *P. acidilactici* productions. R^2 is the determination coefficient among experimental and predicted data. The production yields ($Y_{P/Rs}$ and $Y_{P/Pr}$) are also calculated. NS: not significant.

Table 7. Chemical characteristics of FPH produced at pilot plant scale. H_f: final degree of hydrolysis calculated at the end of Alcalase actuation. Showed errors are the confidence intervals for n=2 and α=0.05.

FIGURE CAPTIONS

Figure 1. Schematic flowchart of fish discards processed by enzymatic hydrolysis and subsequent microbial bioconversion. LAB: Lactic acid bacterium.

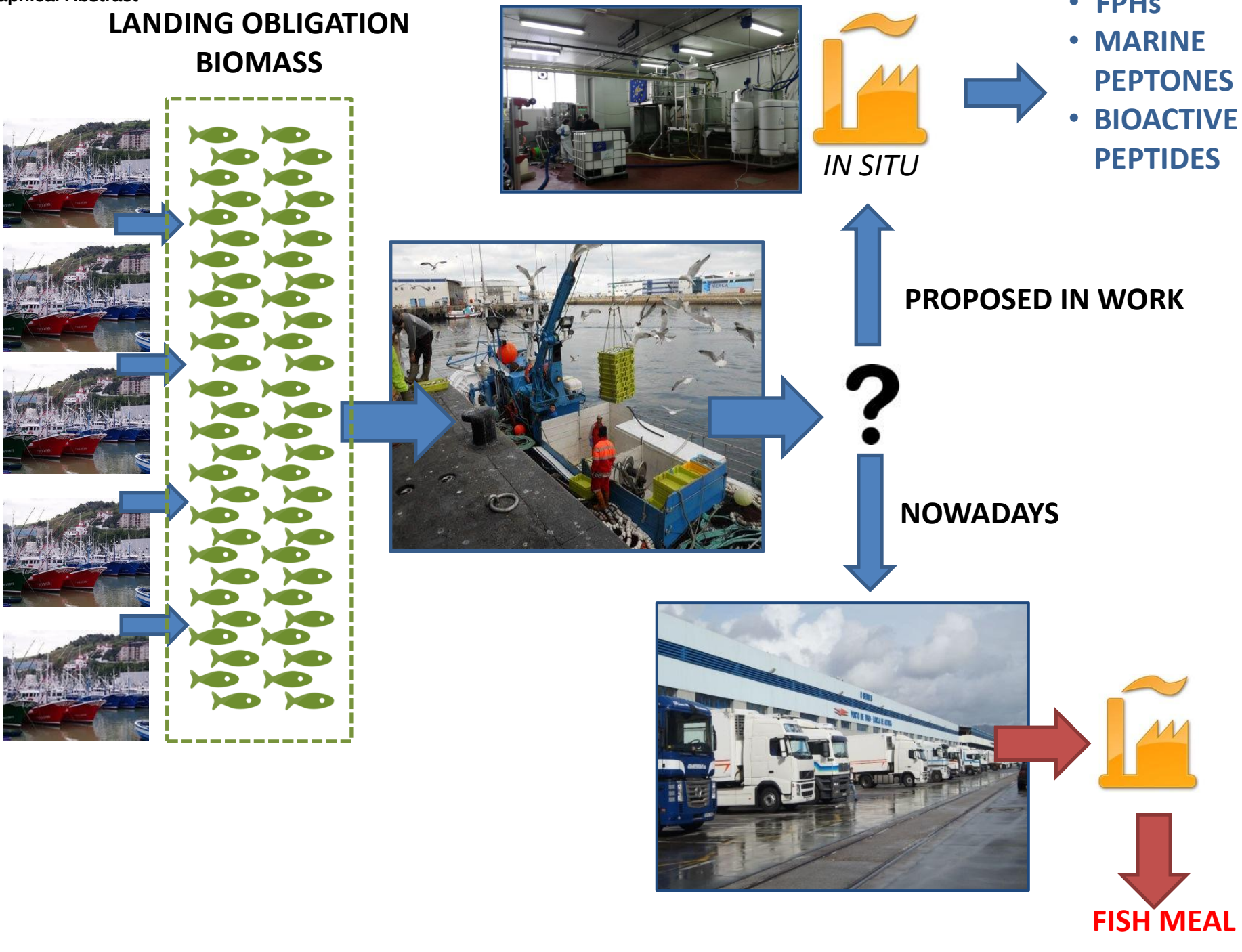
Figure 2. Optimisation studies of Alcalase hydrolysis of blue whiting discards. A: Experimental and predicted response surfaces describing the simultaneous effect of pH and T on H_m response. B: Experimental and predicted response surfaces describing the simultaneous effect of pH and T on V_{dig} response. C: Individual effect of Alcalase concentration over H_m. D: Individual effect of Alcalase concentration over V_{dig}. E: Individual effect of S:L ratio over H_m. F: Individual effect of S:L ratio over V_{dig} . Error bars are the confidence intervals for n=2 and α=0.05.

Figure 3. Alcalase hydrolysis of whole bodies (WB) from fish discards. Experimental data of kinetics (symbols) were fitted to the Weibull Equation (1) (continuous line). Error bars are the confidence intervals for n=2 and α=0.05.

Figure 4. Distribution of molecular weights of FPH analysed by GPC. Red: Right angle light scattering detector; Black: refractive index detector; Blue: UV detector (280nm).

Figure 5. Culture kinetics of *P. acidilactici* grown on different media formulated with peptones obtained from WB 1 (left) and WB 2 (right) of fish discards. MRS medium was used as control in both cases. From WB 1, ●: MeP, ●: BoP; ●: MP; ●: BWP; ●: AMP; ●: MRS 1. From WB 2, ●: GP, ●: HP; ●: PP; ●: RSP; ●: GuP; ●: MRS 2.
Experimental data of biomass (X), lactic acid (La), acetic acid (Aa) and pediocin (BT) were fitted to the Eq. (2). Reducing sugars (Rs) and proteins (Pr) uptakes were also shown. The confidence intervals of experimental data (for two replicates) were in all cases less than 10% of the experimental mean value and omitted for clarity.

Graphical Abstract



- Fish discards were valorised and a whole of added-value products were obtained.
- Fish oils, fish protein hydrolysates (FPHs), bioactives and peptones were produced.
- Media with fish peptones were successfully evaluated for *P. accidilactici* growth.
- *In-situ* scalable, flexible and efficient FPHs valorization processes are defined.
- Preliminary assessment shows that FPHs technology minimizes impacts when compared with operating fish meal plants.

Table 1. Second order models describing the joint effect of temperature (*T*) and *pH* on Alcalase hydrolysis of blue whiting. Optimal values of the two variables (T_{opt}, pH_{opt}) to reach the maximum responses (Y_{max}) from the empirical equations are also summarized.

Second order models	R^2_{adj}	T_{opt} (ºC)	pH_{opt}	Y_{max} (%)
H_m (%) = 37.04 + 2.88 T – 6.35 pH + 6.64 T pH – 3.22 T^2 – 12.56 pH^2	0.822	59.5	8.61	38.0%
V_{dig} (%) = 92.6 + 7.44 T – 13.8 pH + 16.7 T pH – 8.06 T^2 – 29.3 pH^2	0.814	60.5	8.69	94.7%

Table 2. Mass balances and proximal analysis of the products obtained from alcalase hydrolysates of whole body of fish discards. Showed errors are the confidence intervals for n=2 and α=0.05. m_b: percentage of the bones recovered; V_{oil}: percentage of the oil recovered; V_{dig}: percentage of the digestion/liquefaction of the solid by-products to the liquid phase; Prs: Total soluble protein determined by Lowry; TS: Total sugars; Dig: Digestibility; Pr-tN: Total protein determined as total nitrogen x 6.25; H: Humidity; Ash: Ashes; OM: Organic matter.

FPH	m_b (%)	V_{oil} (%)	V_{dig} (%)	Prs (g/L)	Pr-tN (g/L)	TS (g/L)	Dig (%)	H (%)	Ash (%)	OM (%)
BW	6.7±0.3	0.95±0.07	93.4±0.8	47.8±4.8	49.9±1.7	1.20±0.07	97.2±0.4	92.3±1.2	1.1±0.1	6.4±0.9
Me	9.1±0.4	1.21±0.24	94.2±2.2	53.9±5.1	55.4±2.9	1.06±0.25	95.7±0.9	93.1±0.4	1.0±0.1	6.0±0.3
Bo	17.4±0.4	0.61±0.04	93.1±0.0	39.3±1.9	40.7±2.2	1.31±0.37	94.0±0.7	93.5±0.2	0.8±0.0	5.7±0.2
AM	8.5±0.7	1.40±0.20	88.9±1.1	47.6±3.2	48.8±0.2	1.40±0.23	94.3±2.9	92.7±0.2	1.0±0.0	6.3±0.2
M	6.1±1.1	0.80±0.20	92.8±9.0	36.4±0.7	38.6±3.8	0.74±0.31	93.5±3.2	92.5±1.0	1.1±0.0	6.4±0.9
H	10.3±1.5	-	91.0±4.2	36.5±1.7	38.8±1.7	0.72±0.08	95.1±1.1	94.5±0.8	0.8±0.1	4.8±0.2
P	7.4±0.6	-	92.2±0.3	44.3±2.3	45.2±0.8	0.79±0.05	93.2±0.4	93.7±1.4	1.0±0.3	5.4±1.1
RS	11.2±0.4	4.10±0.20	87.5±0.2	36.8±1.6	38.1±0.2	0.60±0.02	94.6±2.5	92.8±0.3	1.1±0.1	6.2±0.4
G	11.0±0.4	-	90.2±0.5	47.1±1.1	48.8±0.8	0.50±0.02	91.9±0.3	93.2±0.1	0.9±0.1	5.9±0.1
Gu	10.1±1.3	5.50±1.57	82.2±1.2	41.1±5.4	42.5±1.2	0.92±0.00	94.4±0.7	93.5±0.1	0.9±0.2	5.6±0.1

Table 3. Kinetic parameters and confidence intervals obtained from Weibull equation [3] modeling the time course of the hydrolysis degree (H) of fish discard by-products mediated by alcalase. Determinaton coefficients (R^2) and p-values are also shown.

FPH	H_m (%)	α (dimensionless)	τ (min)	v_m (% min^{-1})	R^2	p-values
M	28.85±0.06	0.617±0.010	6.12±0.13	1.007±0.015	0.996	<0.005
AM	33.29±0.09	0.587±0.011	6.39±0.16	1.059±0.019	0.995	<0.005
BW	42.13±0.33	0.639±0.017	16.65±0.44	0.561±0.016	0.992	<0.005
Me	47.36±0.18	0.677±0.007	23.05±0.23	0.482±0.006	0.999	<0.005
G	45.52±0.17	0.705±0.010	18.56±0.25	0.599±0.009	0.998	<0.005
H	33.53±0.10	0.773±0.014	13.66±0.25	0.658±0.010	0.996	<0.005
Gu	31.59±0.31	0.588±0.013	21.50±0.56	0.299±0.009	0.996	<0.005
RS	36.96±0.12	0.673±0.008	18.81±0.20	0.459±0.006	0.999	<0.005
P	27.62±0.08	0.741±0.016	10.25±0.24	0.693±0.012	0.994	<0.005
Bo	23.00±0.05	0.612±0.010	6.09±0.14	0.802±0.012	0.996	<0.005

Table 4. Bioactivites (antioxidant and antihypertensive) of FPH obtained from fish discards. Showed errors are the confidence intervals for n=2 and α=0.05.

	ANTIOXIDANT			ANTIHYPERTENSIVE	
FPH	**DPPH (%)**	**ABTS (μg/mL)**	**Crocin (μg/mL)**	**I_{ACE} (%)**	**IC_{50} (μg/mL)**
M	26.75 ± 1.09	13.35 ± 0.77	5.49 ± 0.68	46.08 ± 4.02	-
AM	30.61 ± 5.01	16.30 ± 6.01	7.46 ± 2.01	46.06 ± 5.93	-
BW	19.81 ± 0.52	8.29 ± 0.85	3.89 ± 0.45	39.55 ± 1.85	-
Me	15.13 ± 3.25	5.99 ± 1.89	3.04 ± 0.09	21.53 ± 10.21	-
G	48.45 ± 2.13	19.07 ± 0.87	10.27 ± 0.96	77.48 ± 7.09	185.2 ± 35.8
H	30.67 ± 1.96	13.00 ± 0.75	5.96 ± 0.15	56.24 ± 7.52	330.4 ± 41.2
Gu	29.32 ± 3.00	15.32 ± 1.12	8.51 ± 0.39	70.01 ± 4.11	165.1 ± 28.1
RS	34.69 ± 3.00	14.94 ± 1.05	6.61 ± 1.00	59.86 ± 5.12	272.1 ± 30.2
P	38.32 ± 2.41	14.68 ± 0.91	9.08 ± 0.15	63.54 ± 3.06	253.5 ± 19.5
Bo	33.42 ± 4.15	18.79 ± 2.11	7.99 ± 0.26	56.32 ± 8.85	325.2 ± 35.8

Table 5. Molecular weight of FPH from fish discards. Mn: number average molecular weight; Mw: weight average molecular weight; PDI: polydispersity index.

FPH	Mn (Da)	Mw (Da)	PDI
AM	402	1380	3.43
Gu	396	1328	3.35
Bo	438	1276	2.91
Me	428	1157	2.70
RS	423	1026	2.43
H	274	937	3.42
BW	289	907	3.14
M	369	840	2.28
G	356	758	2.13
P	325	743	2.29

Table 6. Numerical values and confidence intervals for parameters derived from logistic equation applied for *P. acidilactici* productions. R^2 is the determination coefficient among experimental and predicted data. The production yields ($Y_{P/Rs}$ and $Y_{P/Pr}$) are also calculated. NS: not significant.

Parameters	MeP	BoP	MP	BWP	AMP	GP	HP	PP	RSP	GuP	MRS 1	MRS 2
						Biomass (*X*)						
X_m (g/L)	1.04±0.07	1.48±0.08	1.25±0.05	1.42±0.09	1.10±0.06	0.90±0.04	0.91±0.04	0.91±0.05	0.97±0.04	0.91±0.07	0.97±0.08	0.96±0.05
v_x (g L^{-1} h^{-1})	0.14±0.05	0.15±0.03	0.12±0.03	0.12±0.04	0.12±0.04	0.06±0.01	0.07±0.02	0.07±0.02	0.08±0.02	0.08±0.03	0.06±0.03	0.09±0.02
λ_x (h)	5.42±2.14	4.15±3.00	2.70±1.09	3.27±2.08	4.36±1.57	4.28±1.64	4.09±1.67	4.15±2.04	4.03±1.21	4.32±2.41	2.98±2.33	4.20±1.69
$Y_{X/Rs}$ (gX/gRs)	0.105	0.150	0.136	0.148	0.138	0.103	0.097	0.097	0.100	0.105	0.131	0.129
$Y_{X/Pr}$ (gX/gPr)	0.664	0.956	0.714	0.884	0.671	0.568	0.513	0.598	0.585	0.735	0.658	0.677
R^2	0.991	0.994	0.986	0.990	0.993	0.995	0.995	0.993	0.997	0.987	0.982	0.993
						Lactic acid *(La)*						
La_m (g/L)	8.01±0.48	7.98±0.49	7.34±0.51	7.35±0.36	7.21±0.83	7.54±0.45	7.87±0.42	7.94±0.53	7.98±0.49	7.55±0.60	7.67±0.40	7.75±0.43
v_{La} (g L^{-1} h^{-1})	0.52±0.28	0.58±0.24	0.49±0.33	0.62±0.22	0.74±0.23	0.67±0.22	0.61±0.16	0.55±0.17	0.57±0.16	0.48±0.17	0.90±0.25	0.63±0.18
λ_{La} (h)	2.79±2.40	3.13±2.62	2.81±1.98	3.02±1.62	3.19 (NS)	3.55±2.03	3.74±1.87	3.01±2.43	3.04±2.24	2.73 (NS)	4.18±1.75	4.00±1.91
$Y_{La/Rs}$ (gLa/gRs)	0.875	0.825	0.848	0.746	0.863	0.858	0.846	0.841	0.833	0.858	0.841	0.824
$Y_{La/Pr}$ (gLa/gPr)	5.51	5.25	4.46	4.46	4.21	4.75	4.46	5.18	4.85	6.00	4.24	4.32
R^2	0.986	0.991	0.983	0.973	0.982	0.991	0.993	0.990	0.991	0.985	0.990	0.993
						Acetic acid *(Aa)*						
Aa_m (g/L)	0.32±0.05	22.0±0.08	0.17±0.08	0.16 (NS)	1.02±0.15	0.25±0.03	0.18±0.04	0.29±0.01	16.37 (NS)	0.17±0.03	0.30±0.09	0.29±0.09
v_{Aa} (g L^{-1} h^{-1})	0.03±0.01	0.17 (NS)	0.09±0.03	0.16 (NS)	0.01 (NS)	0.04±0.03	0.01±0.01	0.04±0.01	0.11 (NS)	0.12 (NS)	0.02 (NS)	0.02±0.01
λ_{Aa} (h)	7.49 (NS)	130.9 (NS)	6.38 (NS)	7.70 (NS)	24.86 (NS)	7.88±3.40	7.10±6.72	7.89±1.24	134.8 (NS)	8.27 (NS)	6.21 (NS)	6.52±3.02
$Y_{Aa/Rs}$ (gAa/gRs)	0.040	0.025	0.023	0.005	0.030	0.028	0.024	0.029	0.022	0.028	0.039	0.032
$Y_{Aa/Pr}$ (gAa/gPr)	0.255	0.160	0.123	0.028	0.145	0.156	0.124	0.181	0.130	0.197	0.196	0.169
R^2	0.965	0.722	0.874	0.476	0.743	0.966	0.930	0.996	0.693	0.874	0.944	0.983
						Pediocin *(BT)*						
BT_m (BU/mL)	148.2±12.9	190.4±8.9	184.1±14.3	202.5±9.3	149.3±16.4	132.4±16.6	166.3±14.9	183.7±16.4	190.3±10.0	174.8±12.0	220.7±13.6	214.0±16.6
v_{BT} (BU mL^{-1} h^{-1})	10.5±4.0	11.0±2.0	10.8±3.2	12.2±2.2	7.11±2.50	9.19±4.92	9.00±2.89	8.70±2.40	11.2±2.3	9.52±2.32	12.2±2.9	10.0±2.6
λ_{BT} (h)	9.87±2.72	7.58±1.57	8.82±2.51	7.86±1.53	6.81±3.66	9.79±3.93	7.70±2.97	7.93±2.88	7.93±1.74	8.24±2.25	6.07±2.14	4.58±2.78
$Y_{BT/Rs}$ (BU/mgRs)	14.94	19.10	21.55	21.67	17.43	15.65	17.94	19.56	19.79	18.74	23.63	22.46
$Y_{BT/Pr}$ (BU/mgPr)	94.1	121.47	113.28	129.58	85.01	86.63	94.56	120.41	115.23	131.15	119.04	117.66
R^2	0.989	0.997	0.992	0.997	0.986	0.977	0.989	0.991	0.996	0.994	0.994	0.992

Table 7. Chemical characteristics of FPH produced at pilot plant scale. H_f: final degree of hydrolysis calculated at the end of Alcalase actuation. Showed errors are the confidence intervals for n=2 and α=0.05.

FPH	H_f (%)	V_{dig} (%)	Prs (g/L)	Pr-tN (g/L)	Dig (%)
BW	39.9±2.2	93.4±0.8	46.3±3.1	48.5±2.2	95.8±0.9
Me	43.8±2.5	92.6±1.3	45.1±0.9	47.1±1.8	93.0±1.1
Bo	24.2±1.3	91.3±1.7	37.2±1.5	38.5±1.6	92.0±1.8
AM	31.8±1.6	90.4±1.8	42.2±1.4	43.1±0.9	91.1±2.0
H	32.1±1.2	93.1±2.5	35.9±0.8	37.5±1.2	92.3±1.0

Figures

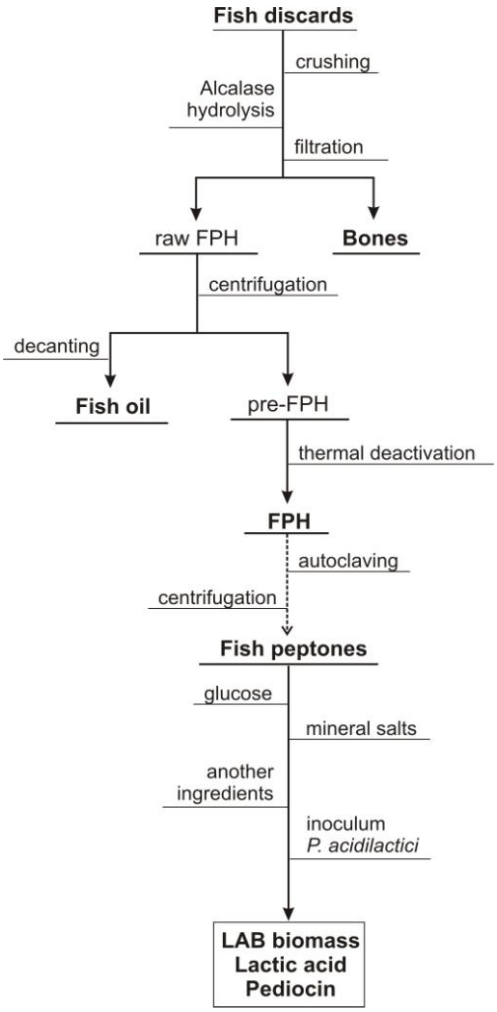


Figure 1. Schematic flowchart of fish discards processed by enzymatic hydrolysis and subsequent microbial bioconversion. LAB: Lactic acid bacterium.

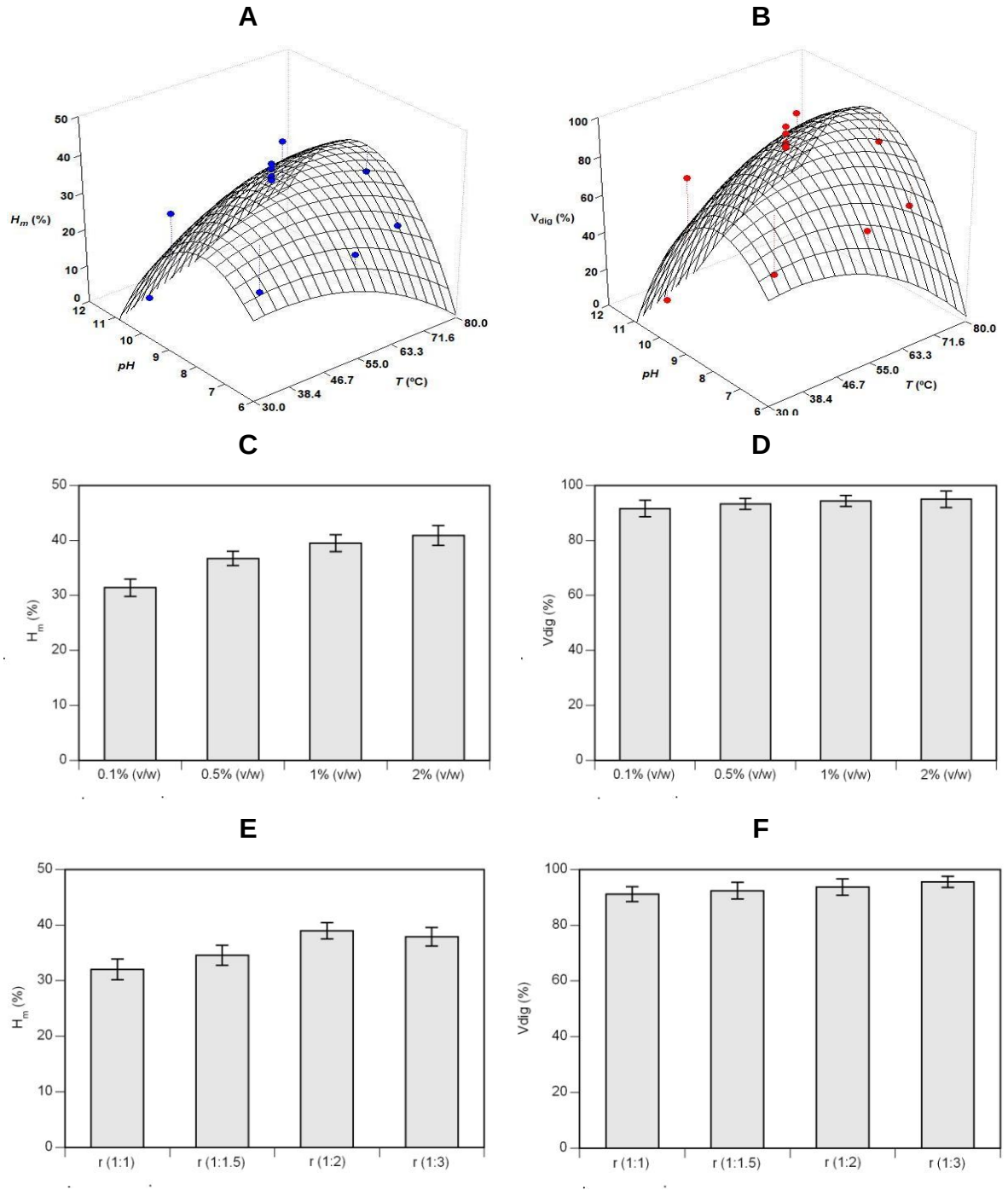


Figure 2. Optimisation studies of Alcalase hydrolysis of blue whiting discards. A: Experimental and predicted response surfaces describing the simultaneous effect of pH and T on H_m response. B: Experimental and predicted response surfaces describing the simultaneous effect of pH and T on V_{dig} response. C: Individual effect of Alcalase concentration over H_m. D: Individual effect of Alcalase concentration over V_{dig}. E: Individual effect of S:L ratio over H_m. F: Individual effect of S:L ratio over V_{dig} . Error bars are the confidence intervals for n=2 and α=0.05.

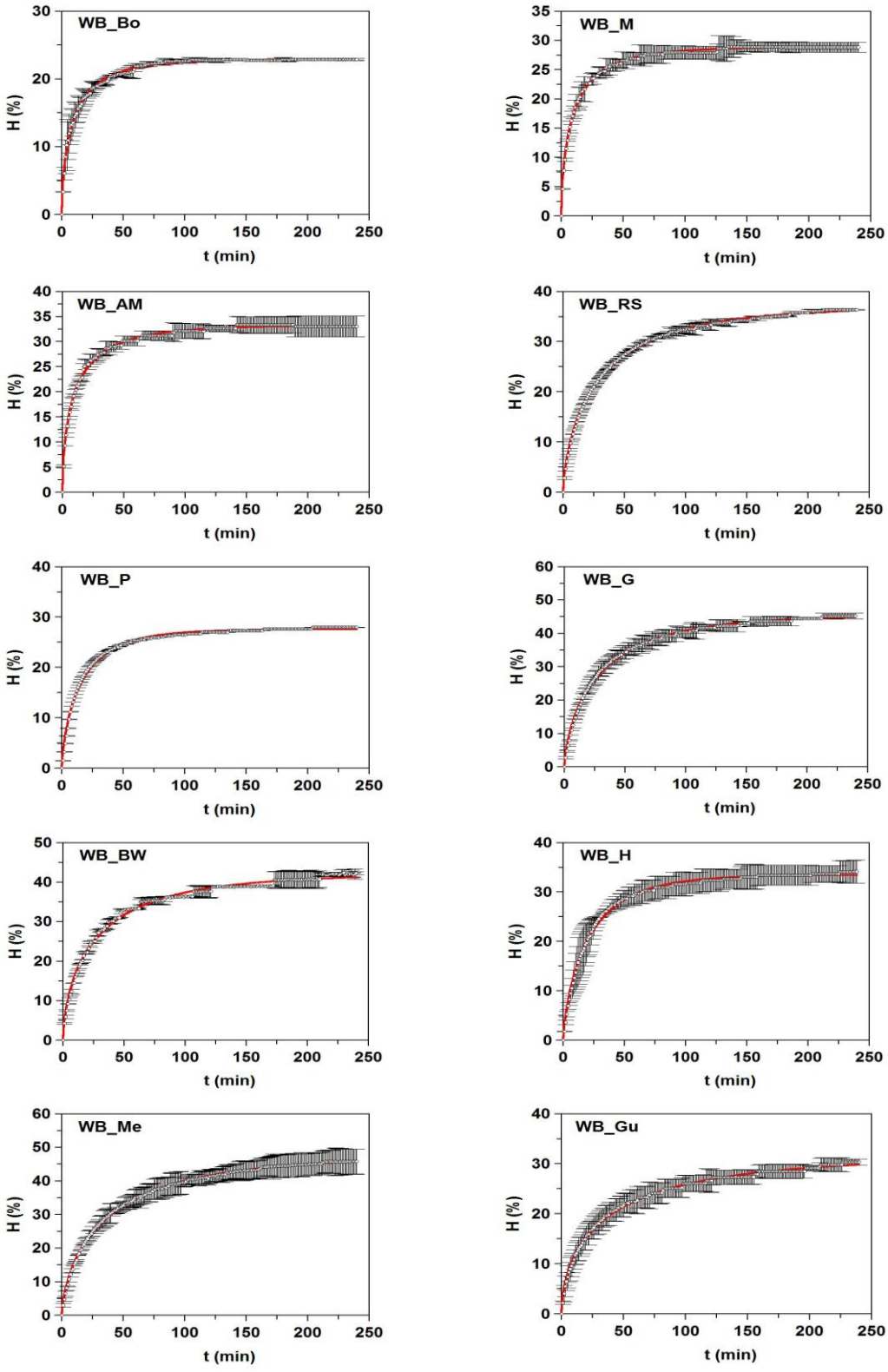


Figure 3. Alcalase hydrolysis of whole bodies (WB) from fish discards. Experimental data of kinetics (symbols) were fitted to the Weibull Equation (1) (continuous line). Error bars are the confidence intervals for n=2 and α=0.05.

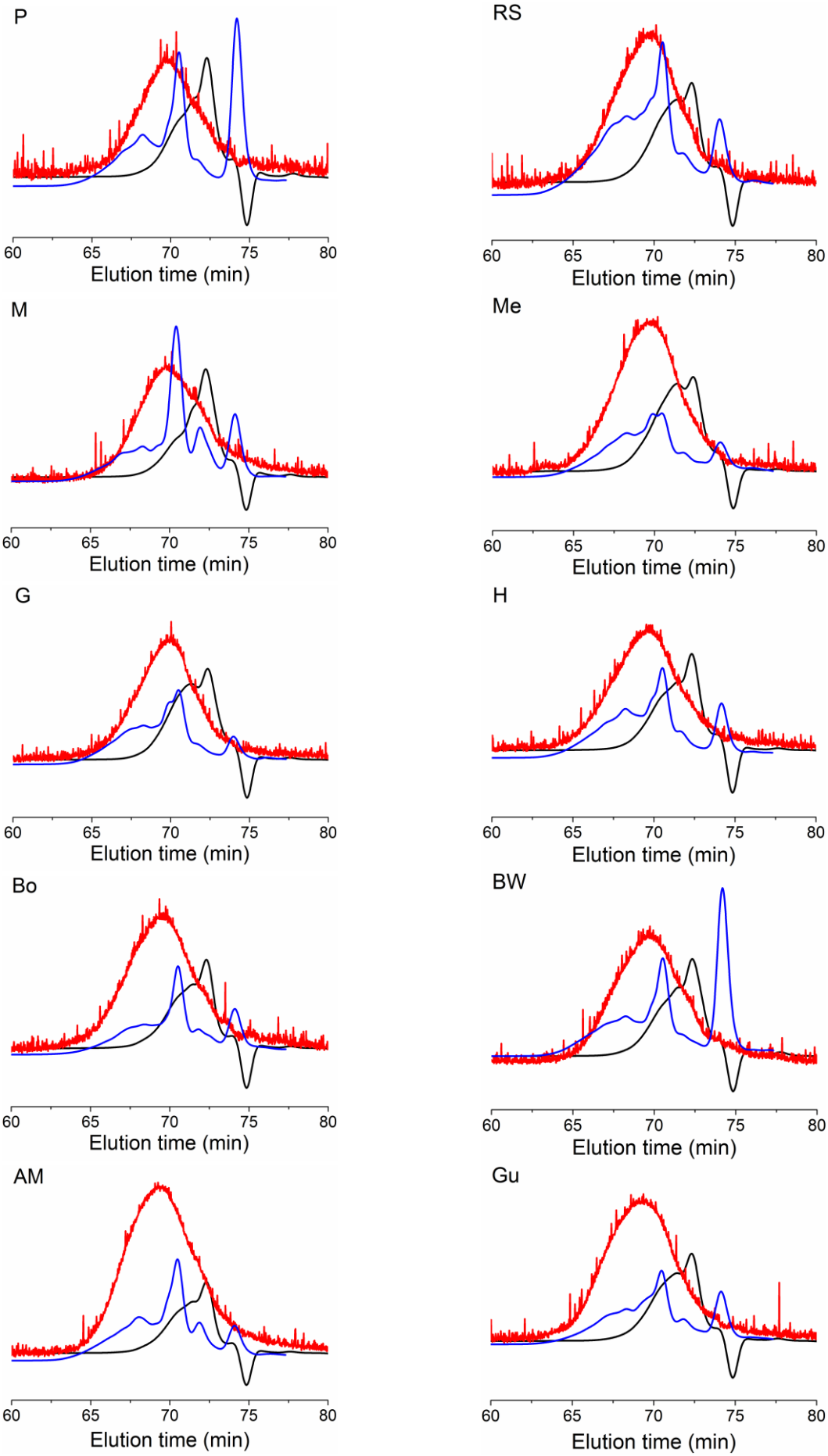


Figure 4. Distribution of molecular weights of FPH analysed by GPC. Red: Right angle light scattering detector; Black: refractive index detector; Blue: UV detector (280nm).

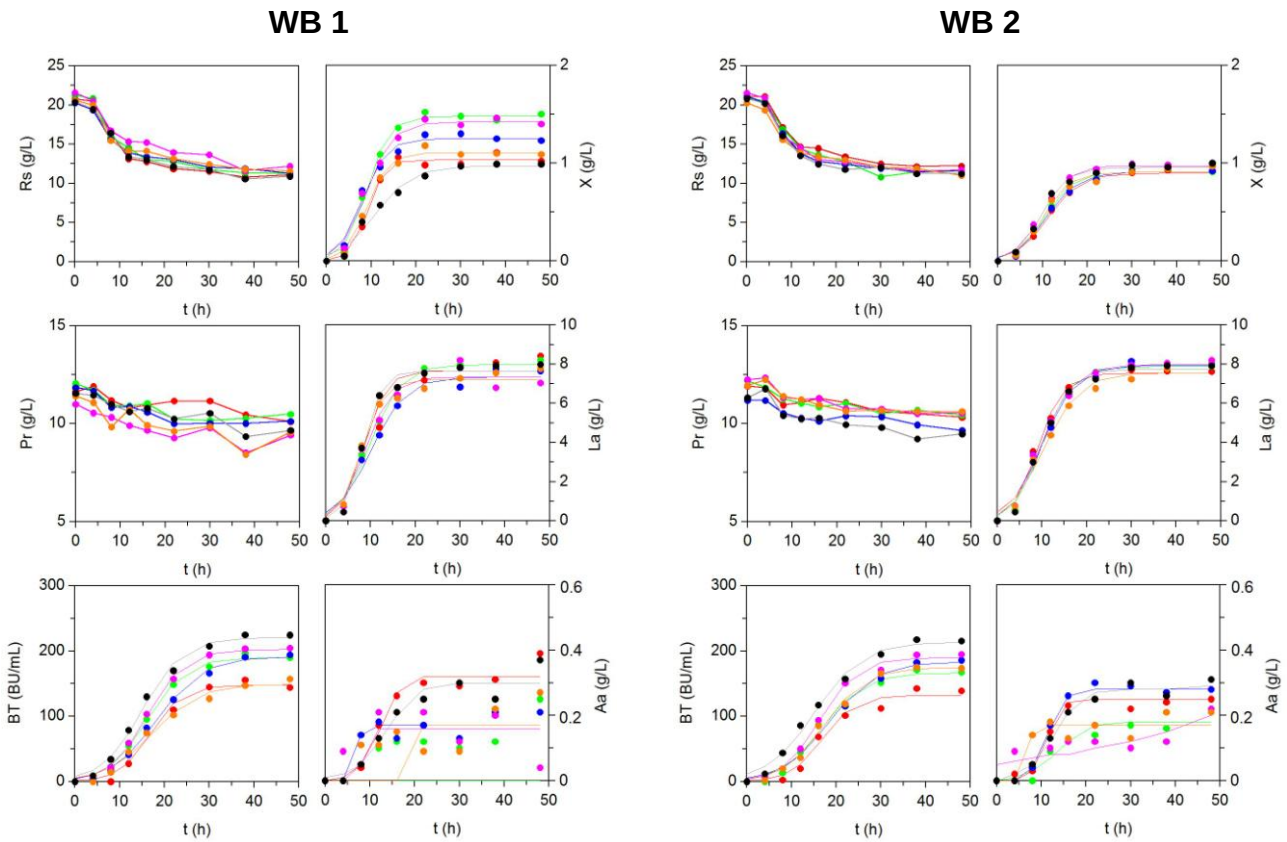


Figure 5. Culture kinetics of *P. acidilactici* grown on different media formulated with peptones obtained from WB 1 (left) and WB 2 (right) of fish discards. MRS medium was used as control in both cases. From WB 1, ●: MeP, ●: BoP; ●: MP; ●: BWP; ●: AMP; ●: MRS 1. From WB 2, ●: GP, ●: HP; ●: PP; ●: RSP; ●: GuP; ●: MRS 2.
Experimental data of biomass (X), lactic acid (La), acetic acid (Aa) and pediocin (BT) were fitted to the Eq. (2). Reducing sugars (Rs) and proteins (Pr) uptakes were also shown. The confidence intervals of experimental data (for two replicates) were in all cases less than 10% of the experimental mean value and omitted for clarity.

SUPPLEMENTARY MATERIAL

Table S1. Experimental domain and coding of the independent variables in the factorial design executed to study the joint effect of pH and temperature on the Alcalase hydrolysis of blue whiting discards.

	Natural values	
Coded values	**pH**	**T (ºC)**
-1.41	6.0	30.0
-1	6.9	37.3
0	9.0	55.0
+1	11.1	72.7
+1.41	12.0	80.0

Codification: $Vc = (Vn-V_0)/\Delta Vn$
Decodification: $Vn = V_0 + (\Delta Vn \times Vc)$
Vn = natural value of the variable to codify
ΔVn = increment of Vn for unit of Vc
V_0 = natural value in the centre of the domain
Vc = codified value of the variable

Constant conditions: Agitation= 200 rpm; r (S:L)= 1:2; [Alcalase]= 1% (v/w) or 24 AU/kg of BW.

Table S2. Fatty acids (as %) recovered after enzyme proteolysis of fish discards. The sum of DHA+EPA and the ratio ω–3/ ω–6 were also calculated. Errors are the confidence intervals for n=2 and α=0.05.

Formula	Fatty acids	M	Bo	RS	AM	Me	BW
C6:0	Caproic acid	-	0.02±0.01	-	-	-	-
C8:0	Caprylic acid	-	-	-	-	-	0.01±0.01
C10:0	Capric acid	0.07±0.01	0.05±0.01	0.04±0.02	0.04±0.03	0.05±0.00	0.04±0.03
C12:0	Lauric acid	0.15±0.02	0.15±0.02	0.14±0.04	0.12±0.06	0.18±0.04	0.10±0.02
C13:0	Tridecanoic acid	0.05±0.01	0.05±0.01	0.06±0.02	0.06±0.01	0.05±0.01	0.06±0.01
C14:0	Myristic acid	5.14±0.20	2.33±0.21	2.02±0.31	5.56±0.69	2.93±0.22	2.69±0.47
C14:1	Myristoleic acid	0.33±0.03	0.23±0.06	0.08±0.02	0.07±0.02	0.17±0.02	0.06±0.05
C15:0	Pentadecanoic acid	0.93±0.06	0.26±0.08	0.27±0.03	0.43±0.05	0.37±0.03	0.53±0.12
C15:1	Pentadecenoic acid	2.03±0.29	6.96±0.47	6.63±0.78	10.14±0.62	6.73±0.39	9.15±0.67
C16:0	Palmitic acid	17.54±0.76	10.67±0.52	9.89±0.70	15.19±0.91	9.94±0.72	13.71±0.51
C16:1n7c	Palmitoleic acid	8.14±0.53	11.70±0.65	7.56±0.33	10.94±0.40	14.04±0.49	5.55±0.19
C17:0	Heptadecanoic acid	0.26±0.05	0.24±0.04	0.24±0.06	0.36±0.02	0.27±0.07	0.41±0.08
C17:1	Heptadecanoleic acid	0.40±0.09	0.23±0.02	0.25±0.04	0.37±0.07	0.38±0.14	0.44±0.03
C18:0	Stearic acid	3.52±0.41	2.31±0.32	2.32±0.21	3.11±0.24	1.65±0.19	3.01±0.25
C18:1n9c.t	Oleic acid	20.90±0.57	27.74±1.02	30.63±0.79	19.86±0.51	17.11±0.42	24.57±0.92
C18:2n6c.t	Linoleic acid	1.32±0.21	1.46±0.12	1.27±0.16	1.61±0.21	1.70±0.33	2.06±0.12
C20:0	Arachidic acid	0.35±0.06	0.17±0.00	0.17±0.03	0.26±0.04	0.18±0.02	0.22±0.02
C18:3n6	γ-Linolenic acid	-	0.49±0.05	0.54±0.07	0.12±0.01	0.08±0.01	1.35±0.09
C18:3n3	Linolenic acid	0.94±0.17	0.35±0.02	0.38±0.04	0.65±0.08	0.43±0.02	1.02±0.10
C20:1n9	Eicosenoic acid	3.32±0.81	1.18±0.20	1.02±0.24	4.09±0.61	1.38±0.30	3.13±0.20
C21:0	Henicosanoic acid	-	0.29±0.04	0.04±0.04	0.07±0.00	0.06±0.02	0.07±0.01
C20:2n6	Eicosadienoic acid	0.22±0.01	0.01±0.00	-	0.08±0.01	0.06±0.01	0.24±0.02
C22:0	Docosanoic acid	2.21±0.17	0.11±0.02	0.13±0.02	1.71±0.21	0.14±0.11	0.13±0.03
C20:3n6	Dihomo-linolenic acid (DGLA)	-	0.29±0.03	0.11±0.01	0.09±0.01	0.32±0.09	0.34±0.07
C20:4n6	Arachidonic acid	3.75±0.65	0.63±0.07	0.64±0.04	0.68±0.06	0.87±0.08	0.73±0.05
C23:0	Tricosanoic acid	0.32±0.00	0.09±0.02	0.10±0.03	0.14±0.01	0.09±0.00	0.09±0.01
C21:4n3	Heneicosatetraenoic acid	1.25±0.14	1.01±0.10	1.25±0.15	1.14±0.08	1.30±0.12	1.21±0.08
C22:2n6	Docosadienoic acid	-	0.17±0.08	1.95±0.16	0.31±0.10	0.23±0.14	0.32±0.06
C20:5n3	Eicosapentaenoic acid (EPA)	10.29±0.83	5.27±0.17	3.71±0.29	8.94±0.37	7.02±0.19	7.58±0.31
C24:0	Lignoceric acid	0.75±0.09	0.38±0.05	0.33±0.11	0.63±0.03	0.58±0.05	0.53±0.05
C24:1n9	Nervonic acid	2.31±0.29	15.36±0.41	20.60±0.95	0.90±0.07	18.50±0.41	0.68±0.08
C22:6n3	Docosahexaenoic acid (DHA)	13.39±0.86	9.82±0.21	7.61±0.53	12.34±0.61	13.19±1.02	19.99±0.77
	DHA+EPA (%)	23.68±1.03	15.09±0.19	11.32±0.41	21.28±0.72	20.21±0.89	27.57±0.82
	r: ω-3 / ω-6	9.37±0.52	5.41±0.09	2.86±0.15	7.98±0.18	6.73±0.17	5.92±0.09

Table S3. Amino acids content of FPH (% or g/100 g total amino acids) from fish discards. OHPro: hydroxyproline. Pr: protein concentration calculated. in g/L. as the total sum of amino acids present in FPH. Showed errors are the confidence intervals for n=2 and α=0.05.

Amino acids	BW	M	RS	P	Gu	H	AM	Bo	G	Me
Asp	10.39±0.10	9.86±0.10	10.23±0.07	10.02±0.02	9.38±0.52	11.48±0.27	10.01±0.36	9.69±0.22	10.52±0.04	11.21±0.21
Thr	4.40±0.16	4.73±0.13	4.99±0.07	4.45±0.17	4.62±0.20	4.24±0.33	4.49±0.82	4.74±0.11	4.73±0.15	4.05±0.27
Ser	5.13±0.13	4.82±0.18	5.02±0.02	4.77±0.06	4.31±0.19	5.15±0.08	4.96±0.24	4.95±0.08	4.58±0.15	5.45±0.01
Glu	14.90±0.27	13.39±0.49	15.81±0.37	13.87±0.10	13.74±0.52	16.80±0.06	13.84±0.51	13.82±0.41	14.77±0.24	15.63±0.10
Gly	5.96±0.18	5.78±0.15	5.92±0.05	6.17±0.02	8.63±1.92	4.75±0.07	6.27±0.11	7.22±0.03	4.81±0.03	7.02±0.07
Ala	7.26±0.14	6.98±0.05	7.25±0.06	7.17±0.12	6.96±0.34	6.81±0.22	6.92±0.59	6.52±0.15	6.79±0.11	7.45±0.19
Cys	0.54±0.02	0.61±0.04	0.41±0.02	0.55±0.03	0.85±0.17	0.56±0.10	0.53±0.04	0.70±0.06	0.76±0.07	0.50±0.03
Val	4.52±0.02	5.25±0.07	4.00±0.08	4.50±0.11	4.16±0.10	3.65±0.11	4.22±0.87	4.76±0.16	4.75±0.07	3.51±0.06
Met	3.63±0.15	3.57±0.17	2.61±0.07	3.54±0.19	3.06±0.25	3.89±0.15	3.21±0.56	3.31±0.09	3.71±0.18	3.77±0.22
Ile	3.72±0.07	4.32±0.04	3.71±0.11	3.75±0.14	4.01±0.16	2.94±0.31	3.45±0.98	4.09±0.16	4.10±0.04	2.68±0.18
Leu	8.36±0.07	8.34±0.07	7.89±0.06	7.97±0.03	7.60±0.38	8.01±0.19	7.65±0.27	7.56±0.01	8.57±0.04	7.53±0.09
Tyr	3.57±0.16	3.47±0.06	2.94±0.03	3.56±0.07	3.42±0.41	3.84±0.10	3.38±0.05	3.61±0.10	3.69±0.06	3.53±0.10
Phe	4.79±0.19	4.58±0.23	4.12±0.06	4.66±0.15	4.55±0.23	4.69±0.27	4.49±0.37	4.56±0.23	4.94±0.19	4.76±0.21
His	2.02±0.07	4.34±0.13	2.62±0.02	3.23±0.10	2.39±0.29	2.46±0.23	2.99±0.02	2.37±0.02	2.37±0.08	2.51±0.17
Lys	8.52±0.11	7.72±0.13	8.86±0.05	8.39±0.08	7.19±0.62	8.91±0.45	8.68±0.16	7.68±0.07	8.28±0.20	7.70±0.29
Arg	6.00±0.08	5.41±0.10	6.37±0.06	6.14±0.08	6.71±0.32	5.58±0.34	6.29±0.23	6.21±0.06	5.46±0.07	5.76±0.21
OHPro	3.04±0.52	3.14±0.25	2.94±0.29	3.31±0.28	2.97±0.21	2.81±0.48	3.68±1.23	3.41±0.11	3.74±0.07	2.96±0.24
Pro	3.72±0.28	3.68±0.22	4.48±0.04	4.07±0.26	5.63±0.77	3.45±2.21	4.19±0.42	4.74±0.05	3.48±0.10	4.04±0.23
Pr (Σaa) (g/L)	49.85±2.74	38.67±4.71	38.52±1.99	45.42±0.82	43.01±3.75	39.51±2.93	49.72±3.25	41.01±0.98	47.12±2.59	54.59±4.03

Table S4. Composition of the culture media (in g/L) used for the fermentation of *P. acidilactici*. MPe: culture medium formulated with megrim peptone. BoP: culture medium formulated with boarfish peptone. MP: culture medium formulated with mackerel peptone. BWP: culture medium formulated with blue whithing peptone. AMP: culture medium formulated with Atlantic mackerel peptone. GP: culture medium formulated with complete grenadier peptone. HP: culture medium formulated with hake peptone. PP: culture medium formulated with pouting peptone. RSP: culture medium formulated with red scorpionfish peptone. GuP: culture medium formulated with gurnard peptone.

	MeP	BoP	MP	BWP	AMP	GP	HP	PP	RSP	GuP	MRS
Glucose	20	20	20	20	20	20	20	20	20	20	20
Yeast extract	4	4	4	4	4	4	4	4	4	4	4
Sodium acetate	5	5	5	5	5	5	5	5	5	5	5
Ammonium citrate	2	2	2	2	2	2	2	2	2	2	2
K_2HPO_4	2	2	2	2	2	2	2	2	2	2	2
$MgSO_4$	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
$MnSO_4$	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Tween 80	1	1	1	1	1	1	1	1	1	1	1
Meat extract	-	-	-	-	-	-	-	-	-	-	8
Bactopeptone	-	-	-	-	-	-	-	-	-	-	10
Fish Peptone*	10	10	10	10	10	10	10	10	10	10	-

*Soluble proteins (as Lowry-method) at 10 g/L in the final media.