Journal of Cleaner Production 246 (2020) 119027

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro

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

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ARTICLE INFO

Article history:
Received 7 February 2019
Received in revised form
12 September 2019
Accepted 24 October 2019
Available online 25 October 2019

Handling Editor. Jun Bi

Keywords:
Fish discards
Valorisation
Fish protein hydrolysates
Fish peptones
Pediococcus accidilactici
Sustainability

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.

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

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https://doi.org/10.1016/j.jclepro.2019.119027
0959-6526/

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 (Blanco et al., 2015) and bioactive compounds (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 (Chalamaiah et al., 2012), enzymes (Halim et al., 2016), or hydrolysis conditions (Vázquez et al., 2017) have been extensively studied. 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 diets (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 operating in ICES areas VIIIc and IXa, 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*). As reflected in Ordóñez-Del Pazo et al. (2014) and Fernandes et al. (2015), discard rates of considered fishing trawling fleets are highly variable, depending on the fishing ground, the time of the year and the target specie/s. In general, discards rates are low in some specific fisheries, but moderate to high in most trawling trips, ranging between 15% and 75% (kg discards/kg total capture). These figures suppose hundreds of kilograms of biomass that must be properly handled and valorised somehow in-land to avoid the waste of a valuable resource by throwing it back to the sea.

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 −18 °C 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 evaluatedFor 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):

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

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); ν_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.

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

Lab-scale hydrolysis were carried out in a controlled pH-Stat system with a 5 L glass-reactor (suspending 1 kg of milled discards in 2 L of distilled water, (S:L) ratio of 1:2 w/v) using 5M NaOH as alkaline reagent for pH-control. Optimal conditions obtained in previous section for BW were applied for all fish discards: 60 °C, pH8.65, agitation of 200 rpm and 1% (v/w) of Alcalase 2.4L. At the end of the hydrolysis (4 h), the content of the reactors was filtered (100 μm) to remove bones, the liquid hydrolysates were

centrifuged (15000 g/20 min) to recover oils (adding a step of decantation for 5 min) and final FPHs were quickly heated (90 °C/15 min) for enzyme deactivation. In Fig. 1, a schematic flowchart of FPH sprocessing from fish discards is shown. After the sterilisation (121 °C/15 min) and centrifugation (15000 g/20 min) of FPHs, the recovered liquid phases were denominated as fish peptones .

Pilot plant trials were performed in a stainless reactor of 500 L equipped with control of temperature, agitation, reagent addition and pH (pH-Stat system). Hydrolysis was executed following the same experimental conditions as at lab scale but with initial loads of fish discards of 50–150 kg. Me, AM, H, BW and Bo discards were chosen for pilot plant productions.

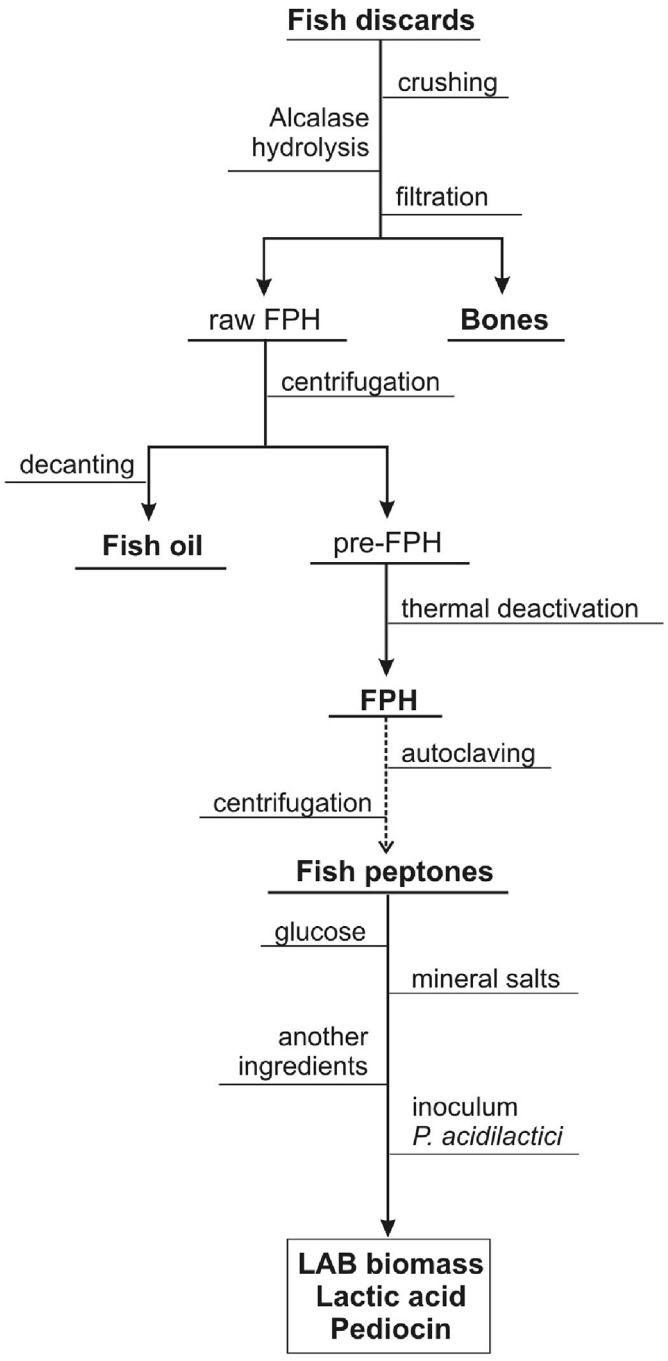

Fig. 1. Schematic flowchart of fish discards processed by enzymatic hydrolysis and subsequent microbial bioconversion. LAB: Lactic acid bacterium.

2.4. Chemical analyses and determination of bioactivities

The profile of fatty acids from fish oil was analysed by GC-chromatography after chemical methylation (Lepage and Roy, 1986). FPHs were stored at −18 °C until analysis. The basic analyses of FPH were: 1) total soluble protein (Lowry et al., 1951); 2) total sugars (Dubois et al., 1956); 3) total protein as total nitrogen x 6.25 (AOAC, 1997); 4) proximal composition (as previously cited), 5) amino acids content (quantified by ninhydrin reaction, using an amino acid analyzer (Biochrom 30 series, Biochrom Ltd., Cambridge, UK), according to the method of Moore et al. (1958); 5) *in vitro* digestibility (pepsin method: AOAC Official Method 971.09 following the modifications reported by Miller et al. (2002). Molecular weights of FPH were determined by Gel Permeation Chromatography (GPC). The system used was an Agilent 1260 HPLC consisting of quaternary pump (G1311B), injector (G1329B), column oven (G1316A), refractive index (G1362A), diode array (G1315C) and dual-angle static light scattering (G7800A) detectors. Standard and samples were eluted with a 0.15M ammonium acetate/0.2M acetic acid buffer at pH 4.5 pumped at 1 mL/min through four columns (PSS, Germany): Proteema precolumn (5 μm, 8 × 50 mm), Proteema 30 Å (5 μm, 8 × 300 mm), Proteema 100 Å (5 μm, 8 × 300 mm) and Proteema 1000 Å (5 μm, 8 × 300 mm) after a 100 μL injection. Column oven and light scattering detector were kept at 30 °C and refractive index detector was maintained at 40 °C. Detectors were calibrated with a polyethylene oxide standard (PSS, Germany) of 106 kDa (Mw) and polydispersity index 1.05. Absolute molecular weights were estimated with refractive index increments (dn/dc) of 0.185.

Antihypertensive and antioxidant (AO) activities were also determined in final FPH samples obtained at the end of hydrolysis. Briefly, *in vitro* Angiotensin I-converting enzyme (ACE) inhibitory activity (I_{ACE}) was based on the protocol defined by Estévez et al. (2012) and IC_{50} values (protein-hydrolysate concentration that generates a 50% of I_{ACE}) were calculated according to dose-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 −80 °C 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 30 °C) 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 S2 (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 121 °C for 15 min. Micro-organisms were grown, by duplicate, in 300 mL Erlenmeyer flasks with 180 mL of medium at 30 °C 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):

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

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). Fig. 2 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: $T_{opt} = 59.5\,^{\circ}C$ and $pH_{opt} = 8.61$ for H_m and $T_{opt} = 60.5\,^{\circ}C$ and $pH_{opt} = 8.69$ for V_{dig}.

Then, and using the average values (60 °C, pH8.65), the individual effects of Alcalase concentration and S:L ratio on the hydrolysis process were evaluated (Fig. 2C–F). The difference between the concentrations of Alcalase 1% and 2% ($39.5 \pm 1.6\%$ and $40.9 \pm 1.8\%$ for H_m and $94.3 \pm 2.0\%$ and $95.0 \pm 3.0\%$ 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., 2013).

The composition of fatty acids in the recovered oil samples is summarised in Table S3 (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., 2016). 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., 2013). In the same article, the sum of fatty acids in horse mackerel samples was statistically equal ($22.05 \pm 0.30\%$) to our result of AM ($21.28 \pm 0.72\%$) ($p > 0.05$). The ω-3/ω-6 ratio was greater than 2.9 (9.4 in M). In any case, ratio values below 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 S4, 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 most abundant amino acids are, in all cases, glutamic and aspartic acids followed by leucine and lysine (Table S4, supplementary material). 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), 2) aquaculture feed and pet food diets (Martínez-Álvarez et al., 2015) and 3) microbial culture media (Vázquez et al., 2016).

In Fig. 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 (Blanco et al., 2015; García-Moreno et al., 2017), 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

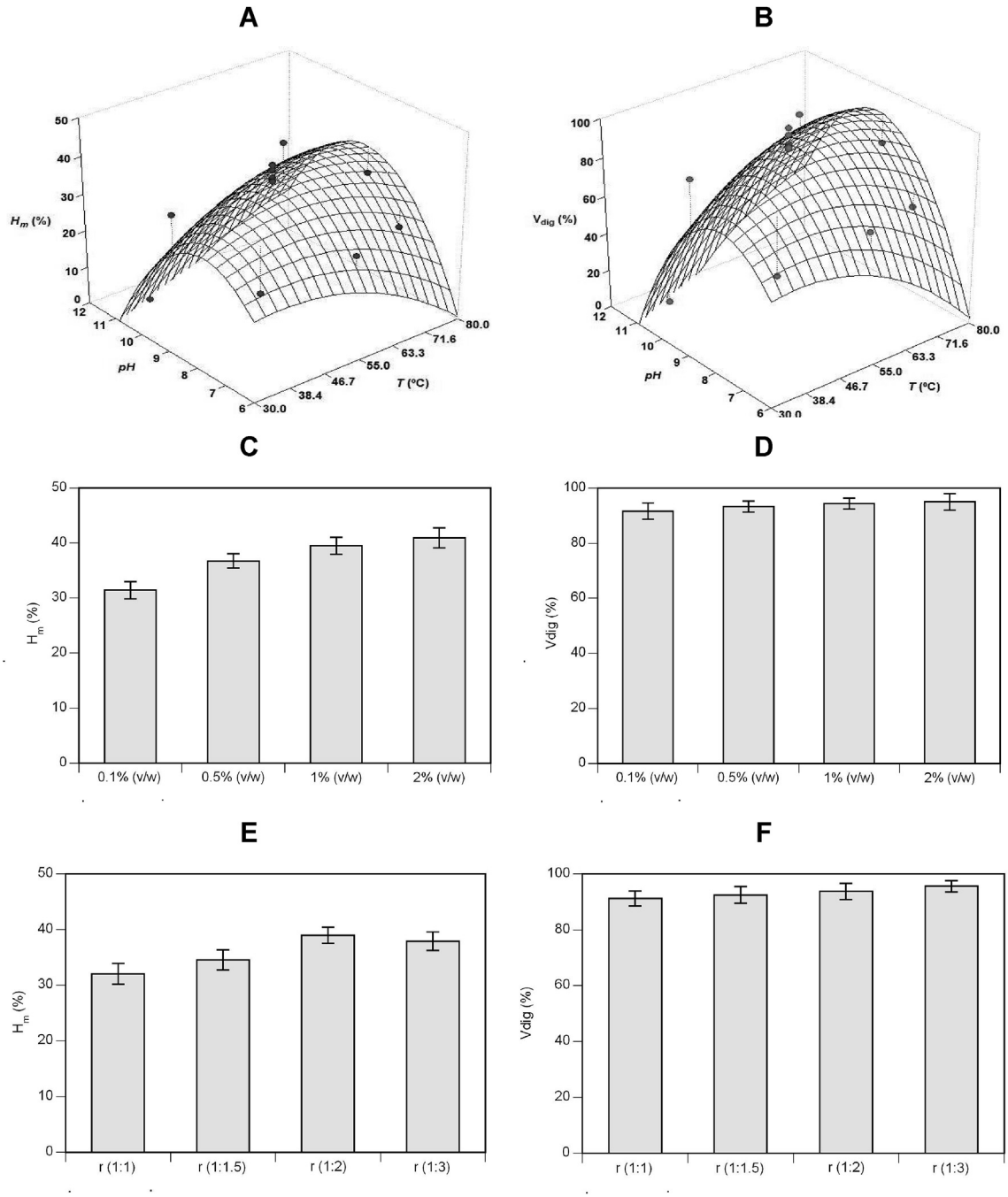

Fig. 2. Optimisation studies of Alcalase hydrolysis of blue whiting discards. A: Experimental and predicted response surfaces describing the simultaneous effect of pH and T on H_m response. B: Experimental and predicted response surfaces describing the simultaneous effect of pH and T on V_{dig} response. 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 $\alpha = 0.05$.

($p < 0.05$). 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

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 summarised.

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

from Mediterranean BW (13–16%) using Alcalase + Trypsin (Pérez-Gálvez et al., 2015).

The values of τ were also lower in FPH (Bo, M and AM) with inferior H_m values (Table 3). Nevertheless, Alcalase hydrolysis was faster (higher value of v_m) in those FPHs mentioned. Because the conditions of hydrolysis were identical in all proteolysis and the content of amino acids in final FPH were very similar, the difference of hydrolysis degrees can only be explained by the difference in the profile, type and configuration of protein and peptides present in each fish substrate.

3.3. In vitro bioactivities of FPHs

In general, AO results were not especially remarkable, DPPH scavenging activities were always lower than 50% being FPHs from G and Me the best and worst hydrolysates, respectively (Table 4). These relative values between FPHs are similar to data obtained from Crocin and ABTS protocols. Altough our BW (19.8% of DPPH, 8.3 μg of Trolox/mL and 3.9 μg of BHT/mL) and RS (34.7% of DPPH, 14.9 μg of Trolox/mL and 6.6 μg of BHT/mL) antioxidant activities were low they are in concordance with values reported for BW Alcalase-FPH (Egerton et al., 2018) and RS head Trypsin-FPH (Aissaoui et al., 2015). In a similar way, hydrolysates from Pacific hake generated by different proteases (Bromelain, Alcalase, etc.) led to identical DPPH percentages (among 18–30%) to those found in the present work for H-FPH (30.7%) (Cheung et al., 2012).

The data of I_{ACE} (%) varied from 22% to 78% being FPHs of G and Gu (>70%) the most bioactive samples (Table 4). In this context, peptides from BW fillet-FPH also achieved 75% of ACE inhibition (Geirsdottir et al., 2011). Subsequently, the samples that were higher than 50% of I_{ACE} were selected for the dose-response bioassays in order to obtain the values of IC_{50}. Following the same order of I_{ACE} activities, FPHs from Gu and G showed the statistically significant lowest IC_{50} values ($p < 0.05$), that is, they are the most bioactive samples (165 μg/mL and 185 μg/mL, respectively). The values of IC_{50} for the remaining FPHs (P, RS, Bo and H) were ranging between 254 and 330 μg/mL. This last figure of H (330 μg/mL) was quite similar to that observed by Savinase applied for 2 h on H heads (260 μg/mL) (Karoud et al., 2018). In general our FPHs were more bioactive than those obtained from head of RS (Aissaoui et al., 2015), muscle of RS (Aissaoui et al., 2017) and fillets of BW (Geirsdottir et al., 2011). Underutilised Bo captures hydrolysed by Papain and Alcalase for 24 h led to inhibitions of 45% and 67%, respectively (Hayes et al., 2016). The difference with our data (56%) may be due to the longer time of hydrolysis (24 h) or the larger concentration of enzyme employed.

Average molecular weights (Mw) of FPHs ranged from 743 Da in P to 1380 Da in HM (Table 5), with no apparent relationships found between Mw and either antioxidant or antihypertensive activities. GPC profiles (Fig. 4) were characterized by one broad light scattering signal, a main peak with a shoulder in the refractive index signal and several peaks not completely resolved in UV. While the profiles were similar for all FPHs, the proportions of each peak varied amongst species, reflecting differences in Mw. These are generally within the range of molecular weights previously reported for FPH: a) In BW, Mw from 40 Da to 20 kDa (Cudennec, 2008) or greater than 900 Da for 75–90% of FPHs mass (García-Moreno et al., 2017) are comparable to Mw of 907 Da reported here; b) in M, Mw of 840 Da found in the present study are within the distribution limits of 27 Da and 2794 Da previously reported (Beaulieu, 2009); c) for H, we estimate a Mw of 937 Da, slightly lower than the lower limit of the 1216–3492 Da range found in hake heads (Karoud, 2018).

3.4. P. accidilactici culture in peptones from FPHs

In Fig. 5, bacterial cultivations on MRS (control medium) and on fish peptone media are displayed. In all low-cost media the growths of *P. acidilactici* were at least equal or higher than those found in MRS (Table 6). Sigmoid experimental data of growth, lactic acid and pediocin were perfectly fitted ($R^2 = 0.973–0.997$, $p < 0.001$) by logistic equation (2).

High maximum growths (as value of H_m) were found in cultures using peptones from Bo and BW followed by M and AM. All of them were significantly greater than MRS and the rest of peptones ($p < 0.05$). However, both maximum growth rates (v_x) and the lag phase of growths (λ_x) did not show significant difference between media ($p > 0.05$), except BoP (higher than MRS) and GP (lower). BoP, MP and BWP showed the best efficiencies to produce biomass in terms of the growth yields regarding nutrient uptake ($Y_{X/RS}$ and $Y_{X/Pr}$). On the other hand, the maximum concentrations and rates of lactic acid productions as well as lag phases (λ_{La}) were statistically equal for all peptones tested ($p > 0.05$). The values of $Y_{La/RS}$ were very similar in all cultures and GuP, MeP and BoP demonstrated its higher efficiency in the release of lactic acid in relation to the consumption of protein substrates. Finally, the maximum production of pediocin was obtained in MRS followed very closely by BWP, BoP and RSP. MRS was also the most productive and effective nutrient formulation and BWP, BoP and RSP the best options from alternative media for pediocin.

These findings are in line with the results reported for nitrogen sources derived from enzymatic and alkaline effluents generated in the isolation of chitin from squid pens (Vázquez et al., 2018). Peptones from salmon viscera (Aspmo et al., 2005) and from tuna, trout and swordfish viscera (Vázquez and Murado., 2008) also demonstrated to be an adequate ingredient of culture broth to produce

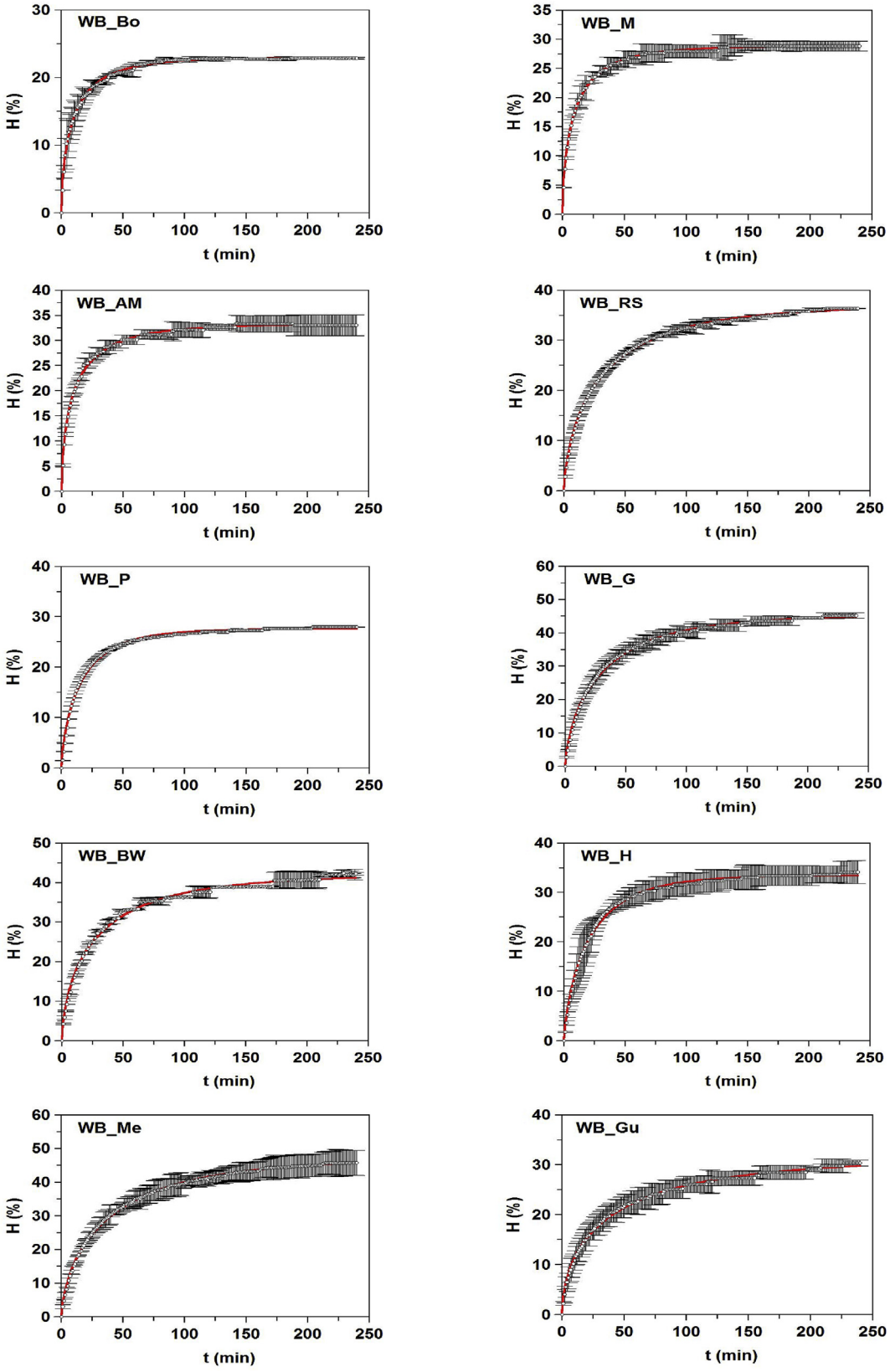

Fig. 3. Alcalase hydrolysis of whole bodies (WB) from fish discards. Experimental data of kinetics (symbols) were fitted to the Weibull Equation (1) (continuous line). Error bars are the confidence intervals for $n = 2$ and $\alpha = 0.05$.

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.

FPH	ANTIOXIDANT			ANTIHYPERTENSIVE	
	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

lactic acid bacteria and bacteriocins, respectively. The application of FPH from fish discards species to microbiological productions is almost unexplored. In addition, our alternative peptones revealed a valuable reduction of the costs from each microbial production: 3–5 folds, 3 folds and 2–3 folds for biomass, lactic acid and pediocin productions, 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 4 h 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. To follow the waste framework Directive of the European Parliament Council (2008) that establishes the hierarchy of valorisation options for any food waste or by-product, maintaining the discards in the food chain through the commercialization of by-catches (subjected to legislation), the production of food ingredients or the obtaining of valuable bio-molecules is considered as the priority option in this work. Other options of less value can be also foreseen and evaluated such as products for industrial uses, the production of energy, composting or incineration, mainly for those fractions of the landed biomass of poor quality or conservation level or than cannot be introduced in the food/feed

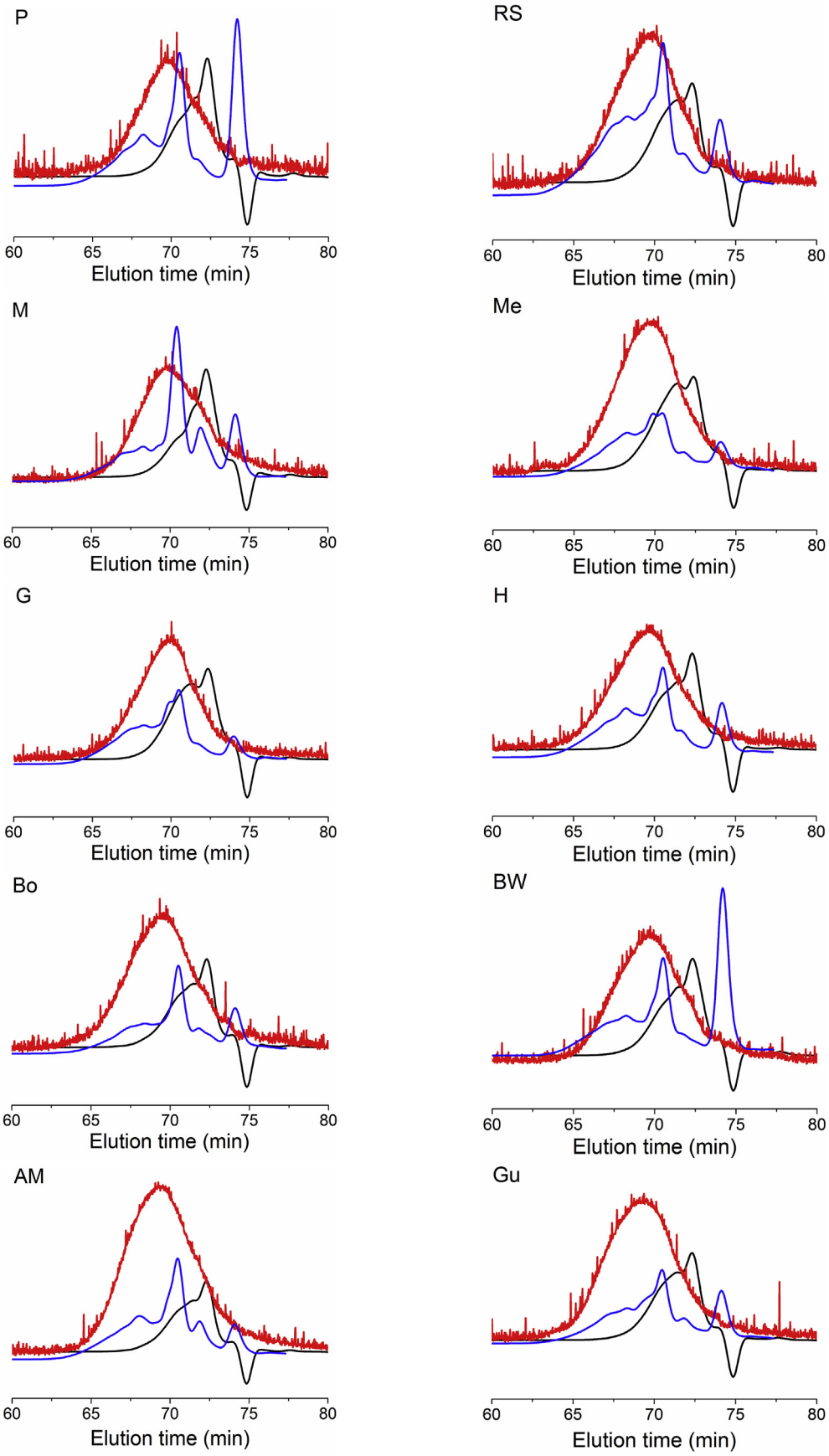

Fig. 4. Distribution of molecular weights of FPH analysed by GPC. Red: Right angle light scattering detector; Black: refractive index detector; Blue: UV detector (280 nm). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

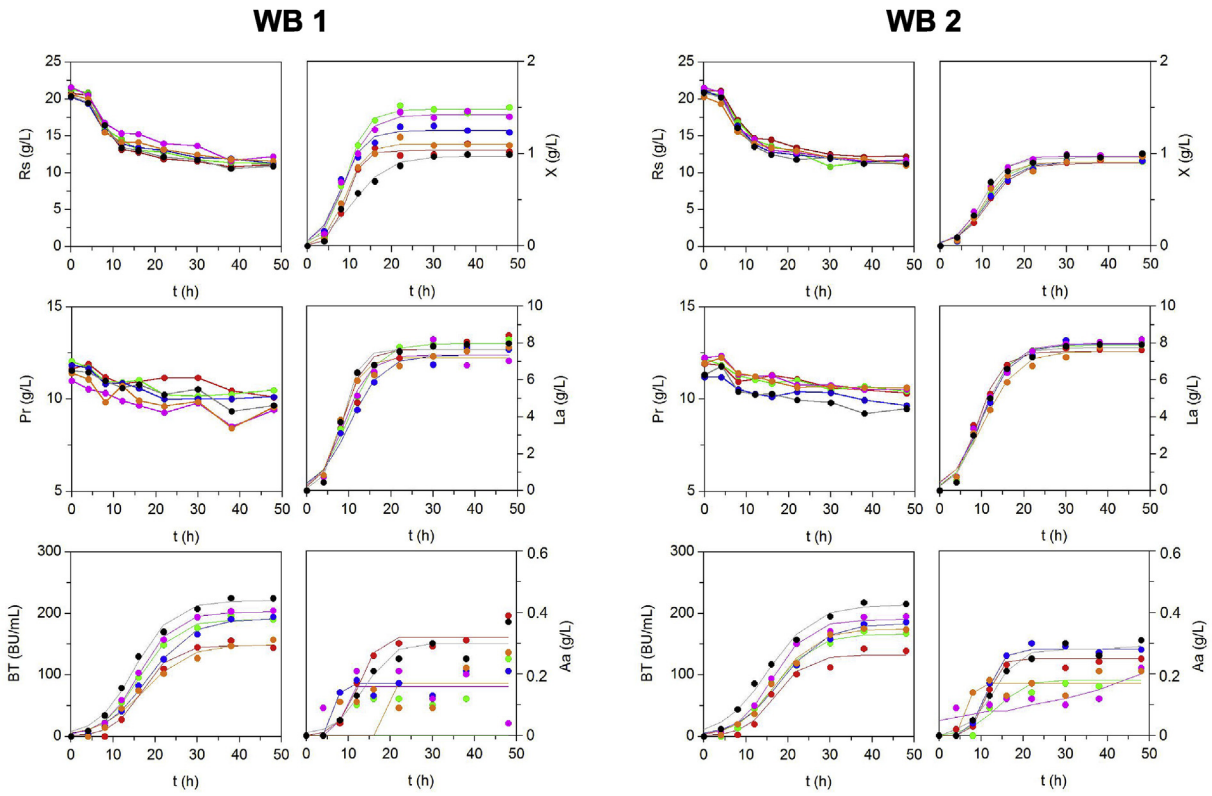

Fig. 5. Culture kinetics of *P. acidilactici* grown on different media formulated with peptones obtained from WB 1 (left) and WB 2 (right) of fish discards. MRS medium was used as control in both cases. From WB 1, ●: MeP, ●: BoP; ●: MP; ●: BWP; ●: AMP; ●: MRS 1. From WB 2, ●: GP, ●: HP; ●: PP; ●: RSP; ●: 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.

valorisation chains. Finally, landfilling the previously discarded biomass is the last option and cannot be considered as a valorisation option.

As presented in Antelo et al. (2015) and Iñarra et al. (2019), for the selection of the most suitable valorisation solution in a specific scenario, there are many different criteria that must be considered and weighed at the same time, which makes this task very complicated without an adequate systematic methodology. All the aspects that may condition the feasibility of a valorisation option in a concrete scenario can be classified into three main categories: i) **Technical parameters** (related with the technical feasibility of a solution); ii) **Market and environmental aspects** and; iii) **Economic aspects** (factors that affect the economic feasibility of the solution).

Nowadays, these valorisation 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.

In this work, we present a sustainable, effective and viable valorisation chain model based on enzymatic hydrolysis to obtain FPHs that tries to overcome the above exposed main issues related to fish meal plants. The proposed valorisation strategy to obtain FPHs would allow to the fishing sector to process *in situ* high amounts of biomass landed in the ports without highly complex, costly equipment, being a scalable, flexible and easy-to-implement technology while generating and retaining more value (the average market price of FPHs is between 4 and 10 €/kg) than in the case where the fish biomass is directly sold to the fish meal companies (that pay around 0.03–0.05 €/kg). The fish meals generated from fish by-products can achieve market values of 0.3–1.2 €/kg depending on the chemical composition and characteristics of the final product. Even more, if marine peptones are produced for microbial culture media purposes, the revenues can be exponentially increased since market prices for similar non-marine products are in the range of 75 € to 100 € for 0.5 kg of bactopeptone and beef extract, respectively. By considering that the proposed process previously described (Fig. 1) obtains alternative peptones with a valuable reduction of the costs from each biological production, the potential of the FPHs valorisation chain is very important.

From an environmental point of view, *in situ* FPHs plants eliminate the transport logistics to centralized points like fish meal factories, eliminating both the associated economic costs and the related environmental impacts due to transport (Lopes et al., 2015). Moreover, it will also avoid to the populations living near the fishmeal processing plants the exposition to air, soil and water

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

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 10–60 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:

$$E_{fuel} = 55 \text{ kg fuel/t rm x } 11.9 \text{ kWh/kg fuel} = 654.5 \text{ kWh/t rm}$$

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

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

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 (Fig. 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 Fig. 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:

$$E_{drying} = 1.098 \cdot 10^7 \text{ kJ/t rm} = 3{,}050 \text{ kWh/ t rm}$$

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 60 °C 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 15 °C, 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 supposses 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 minimise 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 optimised for the hydrolysis of BW captured by North Atlantic fishing fleets and discarded for commercialization and human consumption. This optimal digestion procedure (60 °C, pH8.65, agitation of 200 rpm, S:L ratio of 1:2 and 1% (v/w) of Alcalase 2.4L) was applied to ten fish discarded species for the selective recovery and production of oils, FPH, bioactive peptides and fish peptones. The largest fish oil recovered was Gu (5.5% v/w) and the best omega-3/omega-6 ratio was found in M (9.3). In all cases, the concentration of proteins and the profile of amino acids (including essentials) was remarkable. Hydrolysates of G and Gu showed the highest antioxidant and antihypertensive properties, respectively. Five species were chosen to scale-up the production of hydrolysates (using a 500 L-reactor) confirming the good results obtained at 5 L-reactor. Microbial bioconversion of fish discard peptones was evaluated by the production of *P. accidilactici* biomass, lactic acid and a potent bacteriocin (pediocin SA-1) revealing its excellent capacity to support the mentioned bioproductions in comparison to the commercial medium. Regarding preliminary sustainability assessment, despite the high energy consumption of FPHs drying it can be ensured 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.

Declaration of competing 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).

Appendix A. Supplementary data

Supplementary data to this article can be found online at

https://doi.org/10.1016/j.jclepro.2019.119027.

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