Liquid by-products from fish canning industry as sustainable sources of ω3 lipids

Ana Monteiro [a], Diogo Paquincha [a], Florinda Martins [a], Rui P. Queirós [b], Jorge A. Saraiva [b], Jaroslava Švarc-Gajić [c], Nataša Nastić [c], Cristina Delerue-Matos [a], Ana P. Carvalho [a]

[a] REQUIMTE/LAQV, Instituto Superior de Engenharia, Porto Polytechnic Institute, Rua Dr. António Bernardino de Almeida, 431, 4249-015 Porto, Portugal
[b] QOPNA, Departamento de Química, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
[c] Department of Biochemical and Pharmaceutical Engineering, Faculty of Technology, University of Novi Sad, Serbia

A B S T R A C T

Fish canning industry generates large amounts of liquid wastes, which are discarded, after proper treatment to remove the organic load. However, alternative treatment processes may also be designed in order to target the recovery of valuable compounds; with this procedure, these wastewaters are converted into liquid by-products, becoming an additional source of revenue for the company.

This study evaluated green and economically sustainable methodologies for the extraction of ω3 lipids from fish canning liquid by-products. Lipids were extracted by processes combining physical and chemical parameters (conventional and pressurized extraction processes), as well as chemical and biological parameters. Furthermore, LCA was applied to evaluate the environmental performance and costs indicators for each process. Results indicated that extraction with high hydrostatic pressure provides the highest amounts of ω3 polyunsaturated fatty acids (3331,5 mg L^{-1} effluent), apart from presenting the lowest environmental impact and costs. The studied procedures allow to obtain alternative, sustainable and traceable sources of ω3 lipids for further applications in food, pharmaceutical and cosmetic industries. Additionally, such approach contributes towards the organic depuration of canning liquid effluents, therefore reducing the overall waste treatment costs.

Keywords:
Lipid extraction
Omega-3 polyunsaturated fatty acids
Waste valorization
Liquid by-products
Functional ingredients
LCA

1. Introduction

The growing environmental awareness encourages the use of sustainable feedstocks and minimization of wastes during the production of consumer goods. Although the term "waste" refers to any substance that the holder discards, this term does not exclude substances with potential economic re-utilization. Therefore, a substance resulting from a production process whose primary objective is not its own production may be regarded as a by-product (instead of a waste), as long as a certain number of conditions are verified (Directive, 2008/98/EC).

Food industry generates considerable amounts of processing by-products, which have been identified as particularly appealing for reuse due to the high volumes produced and the interesting variety of chemical compounds presented. The reutilization of such by-products presents the additional advantage of being subjected to traceability, a key parameter for consumer safety and acceptance regarding products from alternative sources.

Among food industry, fish canning is an important economic sector in Galicia (NW Spain) and the North of Portugal, processing mainly oily fish, such as tuna, sardine, sardine-type species and mackerel (Bugallo et al., 2013; Ferraro et al., 2013). One of the major concerns of this industry is related with the wastes generated: both solid and liquid wastes may create a serious environmental problem if discarded without proper treatment, due to their very rich organic load (mainly proteins and lipids). Therefore, the removal of these compounds is crucial, in order to decrease their environmental hazard. On the other hand, instead of being merely removed and discarded, these nutritional (and bioactive) compounds may be recovered, thus becoming a relevant source of revenue to the companies, counterbalancing the (obligatory) costs related to the treatment of wastes before discard, and even allow the creation of new jobs (Blanco et al., 2007).

Although several studies have been undertaken in order to

predict potential new and more profitable applications for fish processing by-products, very few value-added compounds were able to reach the market and be sold in large quantities. In fact, solid fractions from fish canning are usually sold (at very low prices) to produce animal feed, fishmeal or flour (Ferraro et al., 2013; Penven et al., 2013), while liquid fractions are discarded. Possible explanations rely on overestimation of market possibilities, too small amounts of high quality by-products being available on a regular basis, and very high costs to isolate specific components (Olsen et al., 2014). Nevertheless, some of these limitations may be overcome, e.g. by focusing on the recovery of bioactive compounds with an already existent (and high-valued) market, such as lipids rich in ω3 polyunsaturated fatty acids (PUFA), known to prevent cardiovascular and inflammatory autoimmune diseases, as well as having important roles on brain and retina (Ágh et al., 2014). These bioactive lipids are mainly found in fish oil, reaching ca. 30% of the total fatty acid content, although this value is dependent on environmental and genetic parameters.

Fish canning processing may be briefly summarized through the following sequential steps: the preliminary operations (fish reception, washing, brining and cutting), processing (cooking, canning and trimming), and final operations (sealing and sterilization). Auxiliary operations also take place, such as solid waste management and wastewater treatment processes (Bugallo et al., 2013). One of the factors with large environmental impact in the process is the use of water: this natural resource is used in brining, cooking, in distinct washing steps and even in cases where steam generation is required (Table 1). Therefore, liquid effluents are extremely variable in qualitative and quantitative composition. Studies concerning valorisation of liquid effluents from fish processing are extremely scarce, and strongly focused on the wastewater treatment (Cristóvão et al., 2015; Rio et al., 2018). In this paper we exploited the use of alternative physical-chemical and biological-chemical methods for the extraction of ω3-rich lipids from liquid by-products, as they link the efficient utilization of (food grade) organic solvents with temperature, pressure or enzymes, in an improved and more environmentally-friendly solution. Furthermore, the alternatives proposed for lipid extraction were evaluated through a Life Cycle Assessment (LCA), in order to assess their environmental and economic impacts. The selected procedures may simultaneously provide an alternative source of functional ingredients (ω3-PUFA) to incorporate in food, pharmaceutical and cosmetic products, and substantially reduce costs in the management of the remaining liquid waste streams.

2. Material and methods

2.1. Samples

The raw material used in this study was the liquid effluent resulting from the cooking step of mackerel (*Scomber japonicus*), kindly provided by La Gondola company (Matosinhos, Portugal). The abovementioned processing step is performed in steam driers, after brining and evisceration of fish. The raw fish was firstly disposed in large metal trays, which are then placed inside the steam-cooking equipment, operated at 100 °C for a few minutes. Afterwards, the trays were removed from the steam drier, the cooked fish continued its processing along the process chain, and the liquid by-product (condensate) generated during the steam processing was collected from the bottom of the metal trays. Then, the liquid was filtered with cotton cheesecloth (in order to remove eventual solid particles in suspension) and stored at −20 °C until use.

2.2. Extraction processes

2.2.1. Physical-chemical methods

2.2.1.1. Conventional extraction. Conventional extraction was hereby described as the process employing solvents and temperature, with mechanical agitation. Lipids from liquid effluent were extracted according to the procedure described by Hara and Radin (1978), with modifications (Alonso et al., 2003), using a mixture of isopropanol and hexane at 50 °C, 70 °C and 90 °C, under continuous mixing at 1500 rpm in a heating magnetic stirrer (AREC.X, Velp Scientifica), for 30 min. Phase separation was achieved by centrifugation at 12000 rpm (Heraus Megafuge 16R, Thermo Scientific). Next, the organic phase was collected and the solvent was evaporated under low pressure (Buchi Rotavapor R-200, with Vacuum Controller V-850), for gravimetric quantification of total lipids. Assays were performed in triplicate. Due to a problem occurred during storage, it was not possible to perform further analysis with these extracts.

2.2.1.2. Pressurized liquid extraction

2.2.1.2.1. High hydrostatic pressure extraction (HHPE). Assays were conducted in a Hydrostatic press (FPG7100, Stanstead Fluid Power, Stanstead, United Kingdom), equipped with a pressure vessel of 100 mm inner diameter and 250 mm height, and surrounded by an external jacket to control the temperature (kept at 20 °C). Since different solvents and proportions were used, the polarity index (PI) was employed to enable a simplification of data.

Table 1
Main process steps involving the generation of wastewaters during fish canning processing.

Process step	Purpose	% of total process wastewater	Compounds present in wastewater
Brining	provide flavour	10	Blood, salt, scales
Washing during heading & eviscerating	avoid the presence of non-desired solids	35	Blood, salt
Cooking (the prepared fish is placed on perforated trays that facilitate the spillage of oil and water, followed by a thermal treatment with steam at approximately 100 °C and atmospheric pressure)	(i) eliminate part of the water in the meat, so that it is not liberated inside the can during the sterilization; (ii) remove part of the oil/grease. which may provide strong flavours to the final product; (iii) coagulate proteins, facilitating the later removal of the skin and spine; (iv) provide colour, texture and flavour characteristics to the product.	15	Oil, protein
Can washing before sterilization	remove residues from the process	40	oil

PI values were calculated as weighted values of the individual PI for each solvent and for water (the main component of effluent samples). Each sample received a code consisting on a letter followed by a number. Numbers were coded as 1 for samples without organic solvents (PI= 10.2), 2 for mixtures of ethanol and by-product (PI = 7.8) and 3 for mixtures of by-product, isopropanol and hexane (PI = 5.2). Letters concerned the experimental conditions under study: A for a pressure of 150 MPa, B for 300 MPa and C for 450 MPa, all during 10 min; samples coded as D concerned experiments carried out at 300 MPa and 20 min (Table S1).

Extractions with water (X1, with X referring to the letters A-D, according to Table S1) and ethanol (X2) originated final mixtures with only one phase, probably due to the low amount of the lipid fraction, thus preventing the recovery of an organic phase. Therefore, it was necessary to add an extra separation step, in which the previously treated sample was mixed with hexane, placed 1 min in the vortex and separated by centrifugation (Adam et al., 2012). After centrifugation, the upper organic layer was recovered, dried and weighted (for lipid determination). For those extracts with isopropanol and hexane (X3), lipids were recovered by phase separation and centrifugation, as described for the conventional extraction. All the experimental assays were performed in triplicate, and all the lipid fractions were subsequently analyzed in terms of fatty acid profile, whereas aqueous phases were analyzed for protein content.

2.2.1.2.2. Subcritical water extraction (SWE) Subcritical extraction was performed in a home-made subcritical extractor/reactor (Fig. S1). Total capacity of high-pressure stainless steel vessel was 1.7L and pressurization of the vessel was performed with 99.999% nitrogen (Messer, Germany) through a valve. Nitrogen was used in order to prevent oxidation at high temperatures. The operating pressure in the vessel was monitored by in-built manometer (Inol, Slovenija, model IM 811A12). The process temperature was measured by a thermocouple Pt100 and regulated by a temperature controller (Nigos, Serbia, model 1011P). Vibrating platform was used in order to increase mass transfer and prevent local overheating in contact with heater.

Extractions were performed with samples of effluent under different conditions of temperature (90, 130 and 190 °C), time (1 or 2.5 h) and solvent (pure water, water with sodium dodecyl sulfate 0.5% or water with butanol (1:1)) (Table S2); a pressure of 5 MPa and agitation rate of 3 Hz were kept constant for all the assays. After the subcritical step, the process vessel was immediately cooled in a flow-through water-bath at 20 ± 2 °C, and depressurization was achieved by valve opening and purging nitrogen through a valve. Again, experimental assays were performed in triplicate. Lipid amounts were quantified gravimetrically and analyzed in terms of fatty acid profile.

2.2.2. Biological-chemical methods

Enzymatic hydrolysis was carried out as a pre-extraction step, under controlled conditions (pH 8 and 50 °C) and different amounts of Alcalase® 2.4L (Sigma-Aldrich): 0.1%, 0.6% and 1.1% (v/v) in 20 mL of effluent. After 3 h of hydrolysis, enzymes were inactivated by heating at 95 °C for 15 min. The resulting (triplicate) aqueous solutions were subsequently submitted to an extraction step with isopropanol and hexane at 1500 rpm for 30 min, in order to allow lipids to migrate and be collected in the organic phase. Finally, determination of total lipids and fatty acid profile occurred in the organic phases, while the aqueous phases were also collected for protein quantification. Hydrolysis degree was calculated according to the pH-stat method (Dumay et al., 2009).

2.3. Analytical assays

2.3.1. Biochemical composition

Total lipids were determined gravimetrically by the method of Hara and Radin (1978) with modifications (Alonso et al., 2003), as detailed in the previous subsections. Total proteins were assayed by the Lowry method (Lowry et al., 1951).

2.3.2. Fatty acid profile

Fatty acid methyl esters were obtained by transesterification of triplicate lipid samples, according to the acidic method described by Lepage and Roy (1984), with modifications (Cohen et al., 1988), using heptadecanoic acid as internal standard and acetyl chloride as catalyst. The analysis of those esters was carried out with a Shimadzu GC-2010 gas chromatograph with AOC-20i Auto Injector, equipped with a FID and a polar, 60 m long capillary column of fused silica (CP-Sil 88, Agillent). The injector and detector temperatures were 250 and 270 °C, respectively, and the column temperature was placed at 100 °C for 5 min and subsequently increased until 215 °C at a rate of 1 °C min^{-1}. Pure standards (Sigma) were used for fatty acid identification, which was based on comparison of peak retention times of samples and standards. Peak areas were quantified and calculations were performed according to the AOCS Official Method Ce 1b-89 (Firestone, 1994).

2.4. Statistical analysis

Analysis of variance and Tukey (HSD) or Games-Howell post hoc tests were employed to statistically analyze the results, using IBM® SPSS® 22 Statistics software for Windows (SPSS Inc., Chicago, IL, USA). Differences were considered significant when $p < 0.05$.

2.5. LCA

LCA study was based on primary data given by assays carried out and secondary data given by proper LCI data sets, available on Ecoinvent. The methodology of impact assessment employed was the IMPACT 2002+. Processes under study consisted on: process 1 (conventional lipid extraction with temperature and solvents – 2.2.1.1.), process 2 (lipid extraction with HHP and solvents – 2.2.1.2.1), and process 3 (lipid extraction with biological-chemical methods – 2.2.2.). Within each process, the experimental conditions used in LCA calculations were those that provided the highest lipid amount. A sensitivity analysis was also performed for each process, considering the range defined by one standard deviation above the mean and one standard deviation below it for each process. Subcritical extraction process was not studied due to the low recovery values obtained.

3. Results and discussion

Fish canning processing generates large amounts of liquid effluents, arising from the various steps of the process that use water, either in liquid or steam form. Since those effluents have a potential polluting impact on environment, they must comply with the Emission Limit Values before being able to be discarded, either into the municipal collectors or directly to the environment. Therefore, they need to be submitted to treatment processes so as to decrease the levels of pollutants, namely those from organic source, as proteins and lipids. The sustainable recovery of economically valuable compounds from these effluents may provide a way to simultaneously reduce their polluting impact and economically compensate the treatment costs involved.

To the best of our knowledge, previous studies on the extraction of ω3-lipids from liquid effluents from fish processing are

extremely scarce. Some of the few partially related studies found were the characterization of stickwater from fish processing by-products (resulting liquid phase after oil removal), which reported 2–18% of lipids after freeze-drying (Bechtel, 2005), and the use of tuna canning wastewater to produce microcapsules rich in ω3 (Suriani and Taulu, 2015).

The present study selected only those liquid by-products from steam-cooking process (the richest in valuable organic compounds), instead of a mixture with the whole effluents produced (from different sources), since, according to Antelo et al. (2015), the valorization of specific parts, rather than the use of the whole amount, is more optimal.

3.1. Effect of temperature and solvents (conventional extraction)

Among the effects influencing the extraction processes, temperature is one of the most described, due to its recognized influence on the final extraction yields. Additionally, organic solvents are described as being able to rupture cell walls or disrupting interaction forces between lipids and tissue matrix, thus enhancing lipid extraction (Adeoti and Hawboldt, 2014). In order to avoid the use of environmentally hazardous solvents, alternative methods using isopropanol and hexane (Hara and Radin, 1978) have been selected. Although these solvents are less effective than chloroform and methanol for lipid extraction, they are classified as food grade, and this was considered a critical issue so that the extraction process hereby studied could be later scaled-up for food industry.

The amount of lipids recovery from mackerel by-product samples at 50°, 70° and 90 °C were 3.2, 3.6 and 2.4 g L^{-1} effluent, respectively. Another study from Garcia-Sanda et al. (2003) on the recovery of lipids in tuna cooking effluents at 70 °C, reports amounts of 2 g L^{-1} in total lipids. From the comparative analysis of lipids and temperature, it can be observed that there is an increase in total lipids extracted when temperature shifted from 50 to 70 °C, but such tendency disappears when temperature is further increased to 90 °C. This result indicates that temperatures higher than 70 °C during extraction process may reduce lipid extraction. In fact, a study by Civit et al. (1982) concerning the effect of pH and temperature on the recovery of protein and oil from fishery bloodwater waste, also refers that temperatures above 75-80 °C do not improve the recovery.

3.2. Pressurized liquid extraction

3.2.1. High hydrostatic pressure extraction (HHPE)

HHP, conventionally used for pasteurization processes, can also be employed for extraction of bioactive compounds. Under HHP, the differential pressure between the inside and the outside of cell membranes is extremely large. This leads to rapid permeation due to cell deformation and wall damage, and faster equilibrium concentration between both sides of the membrane, while increased solubility can also occur for several compounds. Furthermore, the application of high pressure maintains the solvent below its boiling point, thereby allowing a high penetration into the sample. These conditions reduce the solvent volumes needed and shorten the extraction times. This procedure can be performed with low temperatures, allowing its use in thermo-sensitive compounds such as fatty acids (Santos et al., 2013).

Results regarding the effect of pressure at constant time (A) and the effect of time at constant pressure (B) on the amount of lipids, proteins and ω3 extracted with different mixtures of solvents tested are presented in Fig. 1. Analysis of results regarding lipid content allow us to conclude that: (i) for a constant extraction time of 10 min, an increase in pressure from 150 to 300 MPa promotes a statistically significant enhancement in yield for isopropanol/hexane extracts (PI = 5.2), whereas an increase in pressure from 300 to 450 MPa is only significant for ethanol extracts (PI = 7.8); (ii) for a constant pressure of 300 MPa, the amount of lipids extracted increases with the extraction time and decreases with the PI; (iii) the higher lipid yield (19.84 g lipid L^{-1} effluent) was obtained for the extraction at 300 MPa for 20 min and lower PI (i.e., with isopropanol and hexane). Therefore, it would be expectable to obtain even higher yields when using increased extraction periods of time. The direct link between lipid yield and solvent PI is not unexpected, since lipids are compounds with a low polarity and, thus, easily soluble in low polarity solvents. In fact, matching polarity of the targeted compounds increases the relative strength of interactions between solvent and lipid molecules, thus enhancing the extraction process (Adeoti and Hawboldt, 2014).

The amount of protein in fish by-products was maximal (17.6 g L^{-1} effluent) for the lower PI extracts obtained at higher pressure (450 MPa) and 10 min, although such value was statistically equivalent to the one obtained during extraction with the same solvent mixture PI at 300 MPa during 20 min. In fact, similar to the amount of extracted lipids, also the amount of protein increases with decreasing PI. These results may be tentatively explained because of the strong interaction between the extraction solvents and lipids, which may weaken the bonds between lipid and protein molecules, thus allowing an increased lipid extraction yield in the organic phase, and enhancing free-bound protein content in the aqueous phase. With regard to the effect of pressure and time on protein extraction, the amount of protein decreases with increasing time and pressure in case of PI 10.2 and 7.8, whereas those variations are not linear in the case of PI 5.2. The amount of protein extracted from solid mackerel by-products was reported to be 184 g kg^{-1} by García-Moreno et al. (2013), but solid matrices are always more concentrated in both proteins and lipids than liquid by-products.

The highest amount of total ω3 PUFA (3331.5 mg L^{-1} effluent) was obtained for the assay with the lowest PI (5.2), under 300 MPa and 20 min.

Table 2 presents the fatty acid profile for the experimental conditions of HHPE tested. From analysis of this table, it can be observed that the main fatty acids identified were palmitic (C16:0), oleic (C18:1 ω9), eicosapentaenoic (C20:5 ω3) and docosahexaenoic (C22:6 ω3) acids. These results are in line with previous studies on fatty acid profile of lipids extracted from mackerel (García-Moreno et al., 2013). Experimental conditions A3 (mixture of isopropanol and hexane with a PI of 5.2, extracted at 150 MPa during 10 min) provided the highest amounts of eicosapentaenoic (99.8 g kg^{-1} lipid) and docosahexaenoic (192.8 g kg^{-1} lipid) acids, the most relevant ω3-PUFA from nutritional and functional points of view.

3.2.2. Subcritical water extraction (SWE)

Water is the greenest solvent that can be used in extraction processes and, although hydrophobic compounds such as lipids are poorly soluble in it, the use of co-solvents can substantially improve the extraction yields (Herrero et al., 2013).

Lipid amounts obtained following extraction under subcritical conditions (temperature of 90, 130 or 190 °C, pressure of 5 MPa, and water, either itself or combined with sodium dodecyl sulfate - SDS - or butanol as co-solvents) are depicted in Fig. 2. Results were combined in four different graphs, each one presenting two assays with only one experimental variable among them. As can be observed, only the assays with water and butanol (at 90 °C, during 1 h) showed statistically significant differences between each other. Furthermore, the assay with butanol was the one providing the highest amount of extracted lipids (13.9 mg L^{-1} effluent), far above the average value obtained with the remaining subcritical assays

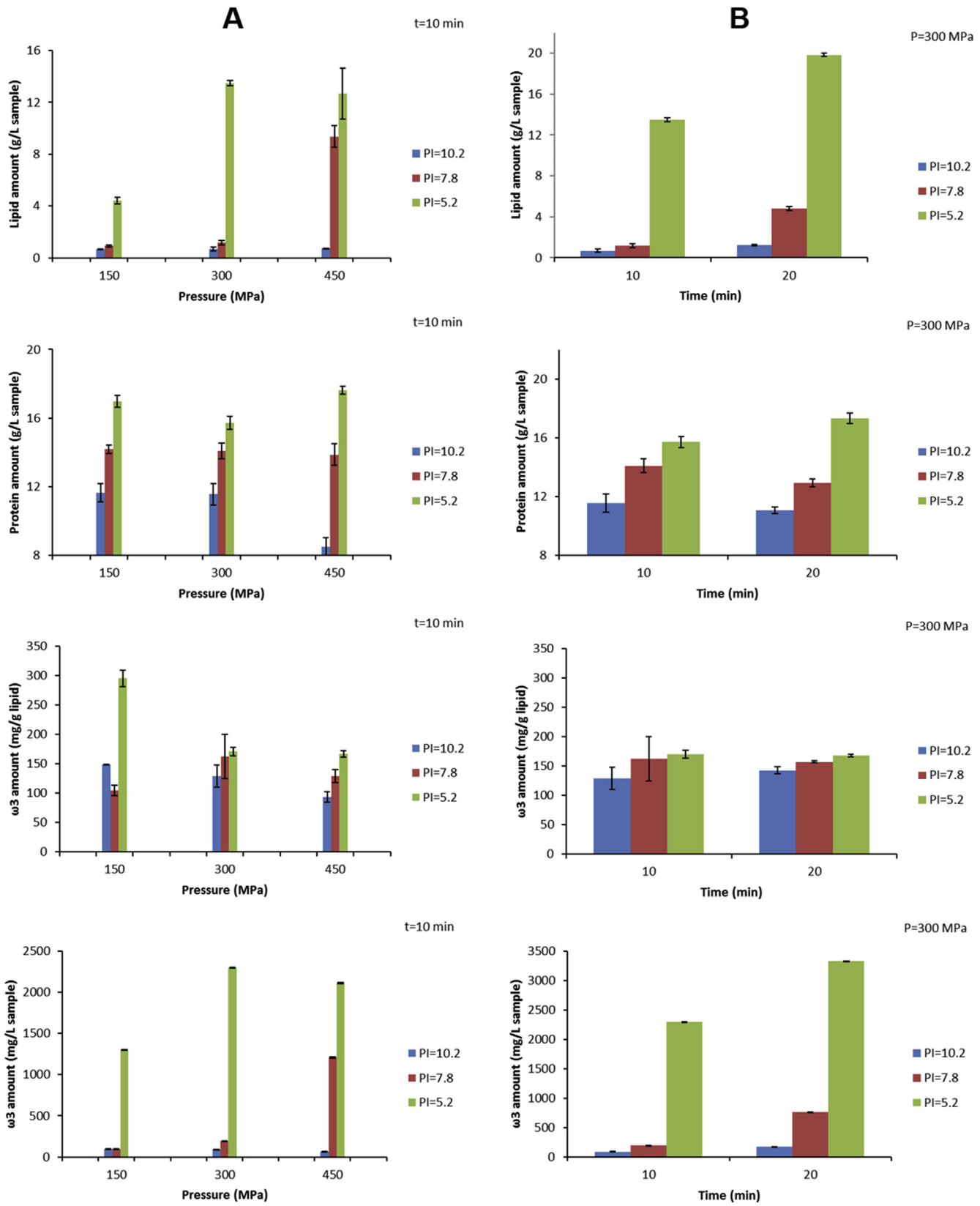

Fig. 1. Lipid, protein and ω3 amounts in mackerel cooking effluent extracted with high hydrostatic pressure (HHP): trials were carried out during 10 min under different pressures (A), and during different times at 300 MPa (B).

Table 2
Fatty acid profile in mackerel effluent samples following different high-pressure extraction procedures (mg g $^{-1}$ lipid).

	A1	A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	D3
SFA												
C14:0	27.6± 0.9	19.1± 2.0	54.8± 9.0 ^{a}	28.3± 2.3	28.0± 9.4	26.2± 0.9	24.4± 4.4	20.5± 1.5	26.1± 0.3	33.4± 0.6	25.5± 0.07	26.0± 0.3
C16:0	111.6± 4.0	79.9± 7.9	217.5± 37 ^{a}	110.9± 6.3	112.9± 34	98.5± 2.4	100.5± 17	78.3± 5.2	99.3± 0.9	131.8± 1.4	98.7± 0.2	99.4± 1.1
C18:0	30.7± 1.0 ^{ab}	24.3± 1.4 ^{ab}	57.3± 10 ^{c}	29.0± 0.2 ^{ab}	30.9± 6.8 ^{ab}	24.3± 1.2 ^{ab}	28.2± 4.5 ^{ab}	19.0± 1.1 ^{a}	24.2± 0.2 ^{ab}	34.3± 0.3 ^{b}	24.3± 0.1 ^{ab}	24.3± 0.4 ^{ab}
C24:0	0.3± 0.2	0.2± 0.0	0.8± 0.1	0.3± 0.01	0.3± 0.09	0.3± 0.00	0.4± 0.04	0.1± 0.01	0.02± 0.01	0.4± 0.00	0.2± 0.00	0.05± 0.02
MUFA												
C16:1 ω7	24.8± 0.7	16.9± 1.8	48.8± 8 ^{a}	25.4± 2.3	25.4± 8.3	24.7± 0.8	20.7± 3.7	20.2± 1.7	24.6± 0.4	29.6± 0.4	24.6± 0.2	24.8± 0.2
C18:1 ω9	62.9± 1.3	43.4± 5.5	124.9± 20 ^{a}	64.7± 5.7	65.3± 22.7	59.9± 2.9	55.6± 9.2	55.5± 10	59.5± 1.3	76.1± 1.0	59.4± 0.02	59.6± 0.7
C18:1 ω7	16.1± 0.4 ^{a}	11.2± 1.4 ^{ab}	32.0± 5.7 ^{c}	16.3± 1.2 ^{a}	16.6± 5.5 ^{a}	14.5± 0.8 ^{ab}	14.1± 2.9 ^{ab}	4.2± 4.9 ^{b}	14.8± 0.2 ^{ab}	19.1± 0.3 ^{a}	14.5± 0.03 ^{ab}	14.4± 0.3 ^{ab}
PUFA												
C18:2 ω6	7.6± 0.1	5.5± 1.0	15.0± 2.2 ^{a}	6.7± 0.7	8.1± 2.6	7.4± 0.3	6.0± 0.8	5.9± 0.5	7.4± 0.2	8.6± 0.03	7.4± 0.05	7.4± 0.06
C18:3 ω3	6.3± 0.1	4.5± 0.7	12.6± 2.1 ^{a}	5.4± 0.8	6.5± 2.2	6.6± 0.3	4.3± 0.9	5.2± 0.4	6.5± 0.1	6.7± 0.2	6.3± 0.06	6.6± 0.07
C20:3 ω6	0.6± 0.0 ^{a}	0.3± 0.0 ^{b}	0.8± 0.2 ^{c}	0.4± 0.01 ^{abd}	0.5± 0.05 ^{ad}	0.4± 0.02 ^{abd}	0.3± 0.00 ^{b}	0.3± 0.02 ^{b}	0.3± 0.01 ^{bd}	0.4± 0.06 ^{abd}	0.4± 0.01 ^{abd}	0.4± 0.01 ^{abd}
C20:4 ω6	17.7± 0.7 ^{ab}	0.4± 0.1 ^{c}	16.5± 4.3 ^{abd}	17.3± 0.9 ^{ab}	11.5± 1.8 ^{adef}	4.8± 0.6 ^{ce}	14.9± 2.1 ^{abdf}	5.0± 2.7 ^{ce}	9.6± 0.9 ^{ef}	18.9± 0.3 ^{b}	8.6± 0.01 ^{ef}	10.3± 0.01 ^{def}
C20:5 ω3	46.5± 0.1	33.3± 3.6	99.8± 16.2 ^{a}	40.3± 5.7	52.8± 14.9	52.9± 2.1	29.6± 3.2	40.5± 3.7	51.6± 1.5	45.2± 2.2	49.2± 0.5	52.3± 0.7
C22:5 ω3	9.1± 0.7 ^{a}	5.9± 0.6 ^{a}	14.0± 3.2 ^{b}	7.6± 1.0 ^{a}	9.3± 3.1 ^{ab}	9.5± 0.3 ^{ab}	6.3± 0.4 ^{a}	7.3± 0.5 ^{a}	9.5± 0.6 ^{ab}	8.4± 0.4 ^{a}	8.9± 0.1 ^{a}	9.5± 0.1 ^{ab}
C22:6 ω3	86.7± 0.5	60.9± 7.9	192.8± 32.9 ^{a}	75.5± 11.1	93.6± 33.1	101.5± 4.1	53.1± 4.9	75.8± 6.5	99.0± 2.9	82.6± 3.8	92.9± 1.3	99.6± 1.3

Values are means± standard deviation.
Values with different letters are significantly different.

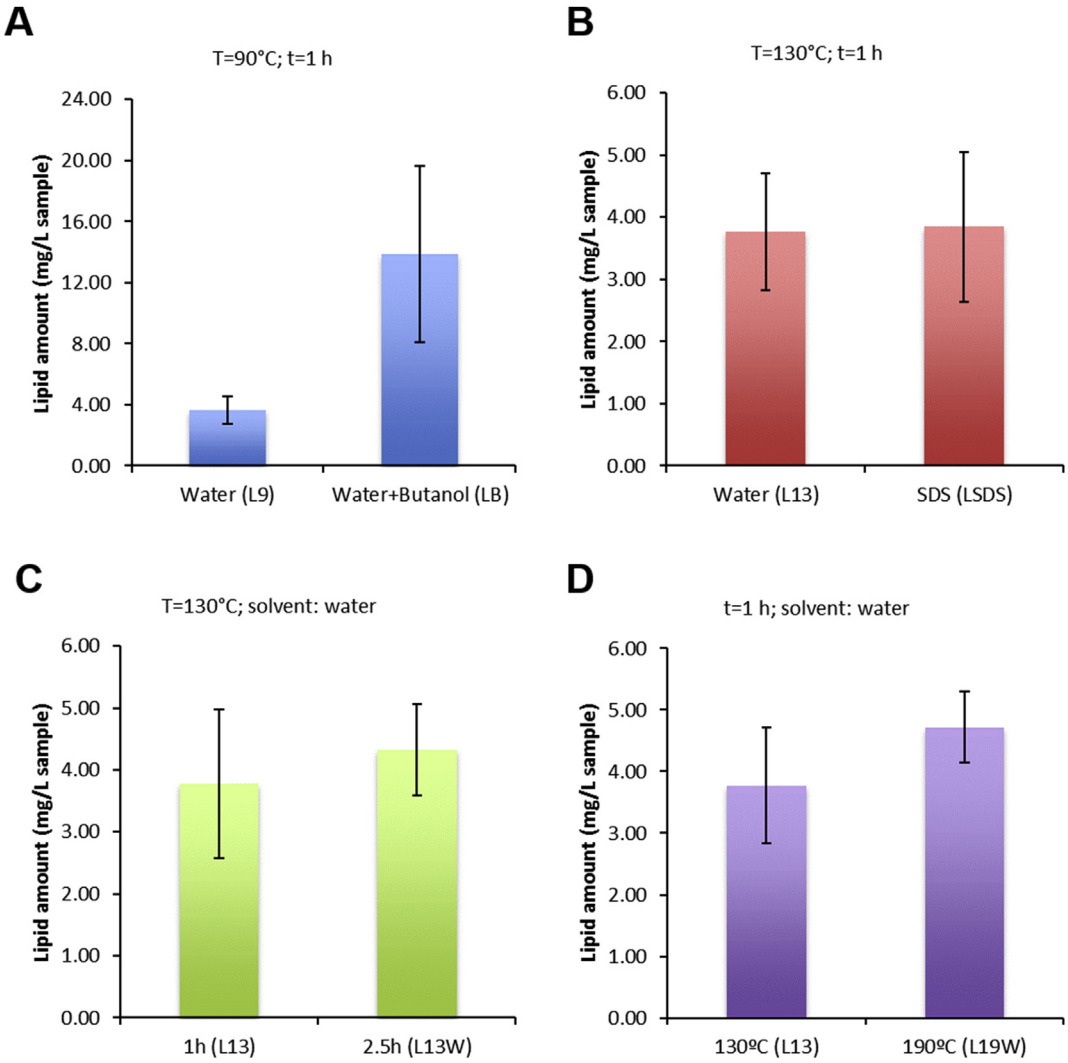

Fig. 2. Lipid amounts in mackerel effluent extracted under subcritical conditions; results are depicted in comparative modes, where the identical experimental parameters are indicated in the top of each graph, and the parameter under study is described in X axis (assay codes are also indicated in X axis, under brackets).

(3.6–4.7 mg L^{-1}). Therefore, it can be concluded that, under the range of experimental conditions tested and for this particular type of sample, the use of pure water or water with 0.5% SDS (Fig. 2 - B), the increase of time from 1 to 2.5 h (Fig. 2 - C) or temperature from 130 to 190 °C (Fig. 2 - D) do not significantly interfere with the extracted lipids. Such conclusion is reinforced by the fatty acid profile obtained for the assays performed under the various experimental conditions (Table S3), where it can be seen that the ω3-PUFA were only quantified for the assay employing water and butanol. A possible explanation for the results obtained may rely in the occurrence of coagulation of proteins, due to the high temperatures used, causing entrapment of the lipid compounds and therefore reducing their extraction yield, as suggested by Dong et al. (2016)

3.3. Biological-chemical processes

Biological processes are widely used in the concentration of proteins, also providing high amounts of oil due to increasing lipid release. These processes are essentially characterized by the use of enzymatic or bacterial activity (Adeoti and Hawboldt, 2014). For lipid extraction, the use of enzymes may help to release bound lipids, thus enhancing extraction yields; however, an additional step with organic solvent extraction is mandatory in order to recover the lipid fraction; therefore, the enzymatic step is considered as a pre-extraction step.

The hydrolysis degree reached during incubation with Alcalase ranged between 77.8 and 79.6%, and there were no significant differences among the different amounts of enzyme used. The highest amount of lipids was achieved with 0.1% of enzyme, and these numbers decrease with increasing enzyme concentration (Fig. 3-A). Such decrease in total lipid content with increasing amounts of enzyme may be tentatively explained by lipid oxidation occurred during hydrolysis, since this reaction was performed at 50 °C during 3 h. Protein amounts are equivalent for all the enzyme concentrations tested (protein quantification for 0.1% of enzyme was not performed) (Fig. 3-B). These results are not surprising, since the amount of protein in the effluent sample was not very high (ca. 18–24 g L^{-1}) and thus, the lowest amount of enzyme tested was enough to promote an almost complete hydrolysis of protein and concomitant release of bound lipids (Dumay et al., 2009). Regarding ω3 contents, the highest results (170 mg g^{-1} lipid, corresponding to 500 mg L^{-1} effluent) were obtained for the hydrolysis carried out with the lowest amount of enzyme (Fig. 3-C,D). Fatty acid profiles (Table S4) of the extracts from the three different experimental conditions present similar amounts of the various fatty acids for the extracts obtained with 0.6 and 1.1% of enzyme, which are statistically different from those obtained in the extracts with 0.1% of enzyme. The later extracts were the ones with the highest amounts of the various fatty acids under scrutiny, probably due to the same reasoning previously presented, regarding the amounts of total lipids.

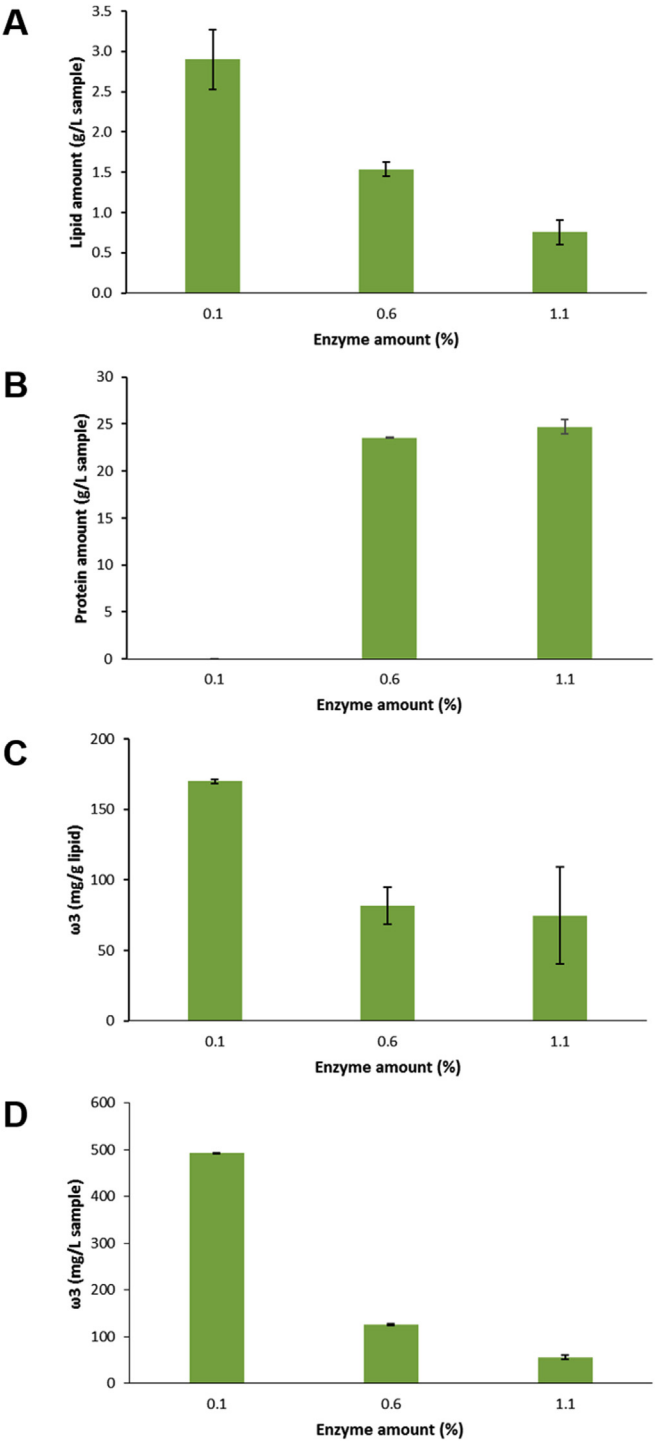

Fig. 3. Lipid, protein and ω3 amounts in effluent samples extracted after biological hydrolysis.

3.4. LCA and sensitivity analysis

LCA evaluates environmental impacts caused by a product or service throughout its life cycle, being performed in four phases: (i) purpose and scope, (ii) inventory, (iii) impact assessment and (iv) interpretation. Thus, the objective of this LCA study was to evaluate the environmental performance influence of the extraction methods targeting the recovery of ω3-rich lipids. In the inventory phase, data concerning inputs and outputs was gathered. This study was based on primary data given by assays executed or available in bibliography, and secondary data given by proper LCI data sets, existing on Ecoinvent. The recovery of 95% of hexane used was assumed to be possible. Besides, the amount of enzyme used in the biological-chemical method was not considered, due to its very low mass (<1%). In the impact assessment phase, the potential environmental impacts associated with the use of raw materials, energy, emissions and waste were evaluated. In the last phase of LCA (interpretation), conclusions were drawn considering the established goal. The methodology of impact assessment used was the "IMPACT 2002+", which proposes a feasible implementation of a midpoint in a combined approach to damage. Four main damage categories

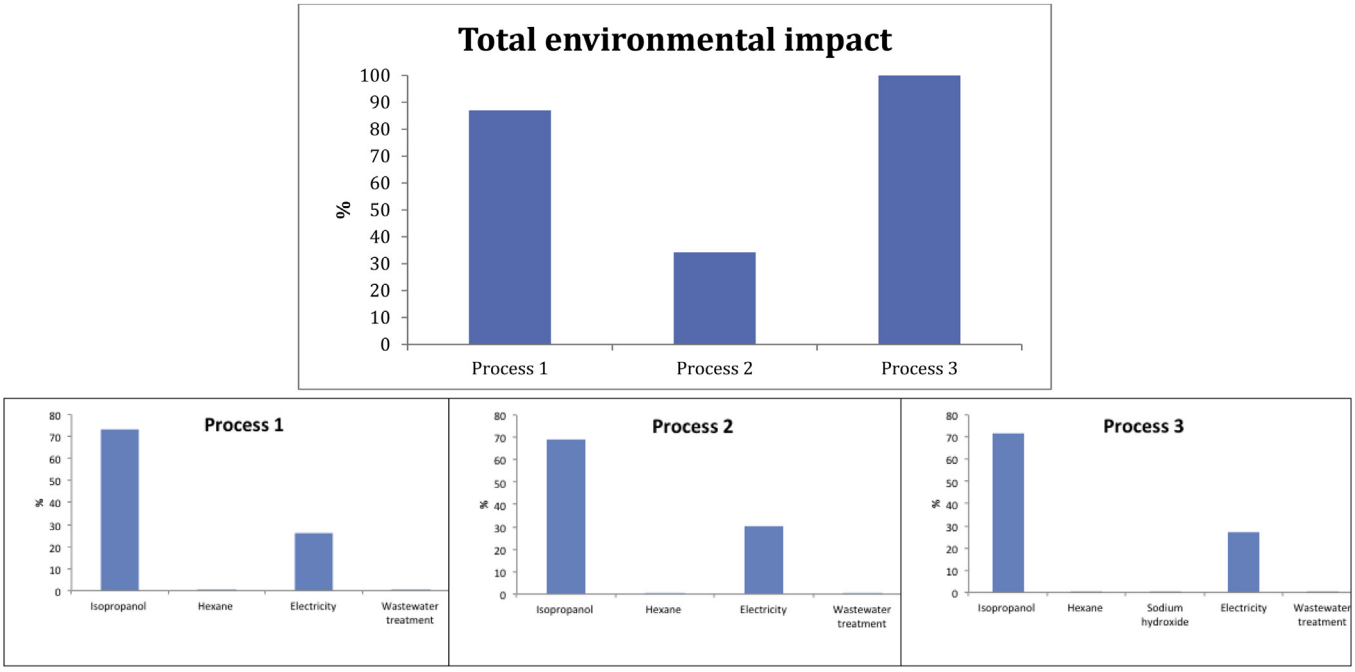

Fig. 4. Global environmental impact for the recovery of ω3-rich lipids with three different extraction methods, and percentual impact of the different processing steps among each method: Process 1 (conventional lipid extraction with temperature and solvents) – extraction includes electricity, isopropanol and hexane; Process 2 (lipid extraction with HHP and solvents) – extraction includes electricity, isopropanol and hexane; Process 3 (lipid extraction with biological-chemical methods) – hydrolysis includes the use of enzyme, sodium hydroxide and electricity, whereas extraction includes the use of isopropanol and hexane.

aggregated were considered: climate change, ecosystem quality, human health and resources. In this method, results are expressed in points, due to the normalization at damage, which facilitates calculations. To calculate the global indicator, a default weighting of 1 was considered, meaning that all categories have the same weight. LCA study was performed with a "cradle to gate" approach: thus, system boundaries end at the recovery process, as detailed in Fig. S2.

Fig. 4 presents the environmental impact, expressed in percentage, resulting from the three extraction methods studied: Process 1 – conventional extraction under the effect of temperature and solvents – *2.2.1.1*; Process 2 – extraction under the effect of high hydrostatic pressure and solvents – *2.2.1.2*.1; Process 3 – extraction under the effect of enzymes and solvents – *2.2.2*. As observed, the process that presents the lowest impact is Process 2. Regarding the percentual contribution of each item within the global environmental impact, the use of isopropanol and electricity consumption were the major contributors, with isopropanol as the most important. The percentual impact in each damage category (human health, ecosystem quality, climate change and resources) for each process is very similar (Fig. S3), with resources as the main contributor, followed by climate changes and human health, in equivalent proportions. It was also possible to conclude that the process presenting the lowest impact per kg of lipids obtained (44.3 mP kg^{-1}) is the one using high hydrostatic pressure process (Process 2). On the contrary, the biological-chemical process (Process 3) presents the highest impact per kg of lipids (277 mP kg^{-1}). The impact value for Process 1 (regarding the effect of temperature and solvents) is similar to the one obtained for the biological-chemical process (219 mP kg^{-1}). A LCA sensitivity analysis was also performed considering the ranges defined by one standard deviation above the mean value of lipids obtained, and one standard deviation below it, for each process. Analyzing the results obtained it is possible to conclude that processes 1, 2 and 3 present environmental impact values ranging between 207 and 231, 43.9–44.7 and 246–318, respectively (Table S5). It is worthwhile to mention there is no overlapping in the values of environmental impact for the different processes when these ranges are considered. Concerning the results for the economic analysis, they were similar to the ones from environmental evaluation: the process with lower costs per kg of lipids extracted is Process 2 (0.091 € g^{-1}), whereas the biological process presents the highest costs (1.256 € g^{-1}), and Process 1 the intermediate cost values (0.465 € g^{-1}).

In terms of industrial approach, a SME will generate ca. 220 m^3 of cooking wastewater per month; therefore, the implementation of Process 2 (HHP processing) for lipid recovery and extraction would provide a monthly recovery of 4364.8 kg of lipid extracts rich in ω3-PUFA.

4. Conclusions

Liquid effluents from fish canning industry are considered as a waste and are consequently discarded, after a proper (and costly) treatment. In order to provide the industry with a payback for these costs, the recovery of specific and high-valuable compounds (ω3 PUFA) from those effluents was hereby studied. Experimental results from extraction procedures were validated through LCA in terms of environmental impact and economic analysis.

From the various extraction procedures studies, it was concluded that HHPE was the one providing the highest ω3 PUFA yields. LCA validated this process as the most adequate one due to its minor environmental impact and lowest production cost.

Acknowledgements

REQUIMTE received financial support from FEDER (NORTE-07-0124-FEDER-000069 and Norte-01-0145-FEDER-000011-RL1–QUALIFOOD), PEst-C/EQB/LA0006/2013, and from FCT (UID/QUI/50006/2013). Authors acknowledge La Gondola for supplying the raw material used. QOPNA acknowledges FCT/MEC (FCT UID/QUI/00062/2013), and FEDER (PT2020 Partnership Agreement).

References

Adam, F., Abert-Vian, M., Peltier, G., Chemat, F., 2012. "Solvent-free" ultrasound-assisted extraction of lipids from fresh microalgae cells: a green, clean and scalable process. Bioresour. Technol. 114, 457–465.

Adeoti, I.A., Hawboldt, K., 2014. A review of lipid extraction from fish processing by-product for use as a biofuel. Biomass Bioenergy 63, 330–340.

Agh, N., Jasour, M.S., Noori, F., 2014. Potential development of value-added fishery products in underutilized and commercial fish species: comparative study of lipid quality indicators. J. AOCS 91, 1171–1177.

Alonso, L., Cuesta, E.P., Gilliland, S.E., 2003. Production of free conjugated linoleic acid by Lactobacillus acidophilus and Lactobacillus casei of human intestinal origin. J. Dairy Sci. 86, 1941–1946.

Antelo, L.T., De Hijas-Liste, G.M., Franco-Uría, A., Alonso, A.A., Pérez-Martín, R.I., 2015. Optimisation of processing routes for a marine biorefinery. J. Clean. Prod. 104, 489–501.

Bechtel, P.J., 2005. Properties of stickwater from fish processing byproducts. J. Aquat. Food Prod. Technol. 14, 25–38.

Blanco, M., Sotelo, C.G., Chapela, M.J., Pérez-Martín, R.I., 2007. Towards sustainable and efficient use of fishery resources: present and future trends. Trends Food Sci. Technol. 18, 29–36.

Bugallo, P.M.B., Cristóbal Andrade, L., Magán Iglesias, A., Torres López, R., 2013. Integrated environmental permit through Best Available Techniques: evaluation of the fish and seafood canning industry. J. Clean. Prod. 47, 253–264.

Civit, E.M., Parin, M.A., Lupin, H.M., 1982. Recovery of protein and oil from fishery bloodwater waste. Effect of pH and temperature. Water Res. 16, 809–814.

Cohen, Z., Vonshak, A., Richmond, A., 1988. Effect of environmental conditions on fatty acid composition of the red alga *Porphyridium cruentum*: correlation to growth rate. J. Phycol. 24, 328–332.

Cristóvão, R.O., Botelho, C.M., Martins, R.J.E., Loureiro, J.M., Boaventura, R.A.R., 2015. Fish canning industry wastewater treatment for water reuse – a case study. J. Clean. Prod. 87, 603–612 .

DIRECTIVE 2008/98/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 19 November 2008
on waste and repealing certain Directives (accessed at Official Journal of the European Union).

Dong, T., Knoshaug, E.P., Pienkos, P.T., Laurens, L.M.L., 2016. Lipid recovery from wet oleaginous microbial biomass for biofuel production: a critical review. Appl. Energy 177, 879–895.

Dumay, J., Allery, M., Donnay-Moreno, C., Barnathan, G., Jaouen, P., Carbonneau, M.E., Bergé, J.P., 2009. Optimization of hydrolysis of sardine (Sardina pilchardus) heads with Protamex: enhancement of lipid and phospholipid extraction. J. Sci. Food Agric. 89, 1599–1606.

Ferraro, V., Carvalho, A.P., Piccirillo, C., Santos, M.M., Castro, P.M., Pintado, M.E., 2013. Extraction of high added value biological compounds from sardine, sardine-type fish and mackerel canning residues - a review. Mater. Sci. Eng. C 33, 3111–3120.

Firestone, D., 1994. Method Ce 1b-89. In: The Official Methods and Recommended Practices of the American Oil Chemists' Society. AOCS, Champaign, IL.

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

Garcia-Sanda, E., Omil, F., Lema, J.M., 2003. Clean production in fish canning industries: recovery and reuse of selected wastes. Clean. Technol. Environ. Policy 5, 289–294.

Hara, A., Radin, N.S., 1978. Lipid extraction of tissues with a low toxicity solvent. Anal. Biochem. 90, 420–426.

Herrero, M., Castro-Puyana, M., Mendiola, J.A., Elena Ibañez, E., 2013. Compressed fluids for the extraction of bioactive compounds. Trends Anal. Chem. 43, 67–83.

Lepage, G., Roy, C.C., 1984. Improved recovery of fatty acid through direct trans-esterification without prior extraction or purification. J. Lipid Res. 25, 1391–1396.

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

Olsen, R.L., Toppe, J., Karunasagar, I., 2014. Challenges and realistic opportunities in the use of by-products from processing of fish and shellfish. Trends Food Sci. Technol. 36, 144–151.

Penven, A., Perez-Galvez, R., Berge, J.P., 2013. By-products from fish processing: focus on French industry. In: Perez-Galvez, R., Berge, J.P. (Eds.), Utilization of Fish Waste. CRC Press, pp. 1–25 .

Rio, A.V., Pichel, A., Fernandez-Gonzalez, N., Pedrouso, A., Fra-Vázquez, A., Morales, N., Mendez, R., Campos, J.L., Mosquera-Corral, A., 2018. Performance and microbial features of the partial nitritation-anammox process treating fish canning wastewater with variable salt concentrations. J. Environ. Manag. 208, 112–121.

Santos, M.C., Salvador, Â.C., Domingues, F.M., Cruz, J.M., Saraiva, J.A., 2013. Use of high hydrostatic pressure to increase the content of xanthohumol in beer wort. Food Bioprocess Technol. 6, 2478–2485.

Suriani, N.W., Taulu, M.L.S., 2015. The characteristics of omega-3 fatty acids concentrated microcapsules from wastewater byproduct of tuna canning (*Thunnus* sp.). Int. J. ChemTech Res. 8, 235–243.