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Chitin production from crustacean biomass: Sustainability assessment of chemical and enzymatic processes

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ABSTRACT

Several compounds with enhanced functional properties of interest in the nutraceutical and medical sectors can be recovered by using the biomass currently wasted in fishing extractive and processing activities, promoting the sustainability of this sector and leading to its development under a bio-economical framework. In particular, it has been observed that crustaceans are an important fraction of the total biomass discarded by fisheries, mainly in those metiers involving coastal waters. Crustacean biomass can be destined to the production of chitin/chitosan (in combination with food use of muscle or protein hydrolysates production) since their exoskeletons are one of the most important sources of this polysaccharide available for commercial use. In this work, the sustainability of both the chemical and enzymatic process to obtain chitin at pilot scale was analysed. The three sustainability dimensions were evaluated and integrated by hierarchical methods to provide a consistent comparison baseline between processes. The results indicated that the enzymatic process could be an adequate alternative that should be considered for chitin extraction, especially if water recovery is employed.

1. Introduction

Considerable amounts (around 7.3 million tonnes) of valuable biomass have been wasted every year with traditional fishing practices during the last decades (Kelleher, 2005; FAO, 2014). However, according to the recent reform of the Common Fisheries Policy, vessels must keep on board and land both target and those non-target species subject to quota regulations (EC, 2013). Consequently, a significant quantity of low commercial-value marine biomass has to be managed adequately, through different valorisation processes (Alonso et al., 2010). Therefore, through the sustainable production of bio-products from traditionally dumped marine biomass, CFP is also oriented to promote and support bio-economy, like other strategies developed in the European Commission (Lewandowski, 2015). Apart from a more efficient biomass consumption, the reduction in the number of vessel trips per catch as the new scenario after CFP implementation, could result in important reductions of fossil emissions (Huisingh et al., 2015), another key dimension integrating the bio-economy framework (Ingrao et al., 2016). Also, other fish species not subject to the TAC/quota system are being captured and commercialized for human consumption. Recently, one small crustacean species (*Munida* spp.) has been identified as potentially interesting for consumption, considering its excellent organoleptic characteristics. Therefore, its capture and subsequent landing has also been planned to develop its commercialization (specifically the tails of this species). Diverse potential valorisation processes to achieve valuable bio-compounds (enzymes, glycosaminoglycans, astaxanthin, proteins, poly-unsaturated fatty acids, hydroxyapatite, chondroitin sulphate, hyaluronic acid, collagen, gelatine, chitin, chitosan, etc.) from marine biomass, can be found in literature (Kumar, 2000; Kim and Mendis, 2006; Ferraro et al., 2010; Vázquez et al., 2013; Younes and Rinaudo, 2015). The bio-compound chitin (or either the deacetylated product chitosan) can be recovered, among other sources, from marine crustacean shells, which contain high quantities of this polysaccharide (Synowiecki and Al-Khateeb, 2003; Hajji et al., 2014). The biological and physicochemical properties of chitin and chitosan make them interesting polymers for several applications, including biotechnology, food, medicine, cosmetics, wastewater treatments, etc. (Shahidi et al., 1999; Kumar, 2000; Prashanth and

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Tharanathan, 2007; Muzzarelli, 2009; Dash et al., 2011).

Almost a 7% of discarded marine biomass in the Northern Spanish and Portuguese coastal bottom otter trawl fleets corresponds to crustacean species (mainly *Polybius henslowii* and *Munida* spp.), according to the work developed in the LIFE + Project FAROS (Pérez et al., 2011; Ordóñez-Del Pazo et al., 2014). Therefore, the production of chitin/chitosan as well as fish protein hydrolysates (FPH) and pigments, seems to be a suitable manner of making the best use of this available resource. The conventional preparation of chitin (deproteinization step) from marine waste material involves the use of strong acids and bases at high temperature (100-110 °C), requiring high energy consumption and generating effluents that must be neutralized by an adequate treatment (Percot et al., 2003; De Holanda and Netto, 2006; Trung and Stevens, 2013; Pachapur et al., 2015).

For all these reasons, other technologies for the deproteinization step of chitin production, like microbial treatment (by lactic acid and miscellaneous fermentation) and enzymatic processes using crude extracts and/or isolated enzymes, have been proposed (Healy et al., 1994; Gildberg and Stenberg, 2001; Rao and Stevens, 2005; Duan et al., 2012; Hajji et al., 2014; Paul et al., 2015). The use of enzymes for deproteinization of crustacean shells, avoiding the necessity of strong alkaline treatments during this stage, has been considered since they are specific, fast in action and, most of times, reduce the use of energy, chemicals and/or water, when compared with conventional processes (Jegannathan and Nielsen, 2013). The enzymatic method allows the recovery of high-added value products besides chitin, like FPH, pigments or peptides (Lee et al., 1999; Armenta-López et al., 2002; Cahú et al., 2012). Besides, the chitin obtained through enzymatic processes presents a higher quality, since chemical treatments cause depolymerisation and deacetylation of the polysaccharide (Vázquez et al., 2013). At present, most of these studies were performed at laboratory and pilot conditions and although these experiences are the key step before large-scale applications, some modifications of operational process conditions are usually necessary (Zhang et al., 2012; Kaur and Dhillon, 2015).

Although the advantages of these bioprocesses over the chemical extraction of chitin have been claimed and qualitatively reported (Healy et al., 1994; Beaney et al., 2005; Jegannathan and Nielsen, 2013), a quantification of both the environmental and economic aspects of these advantages is not available yet. For instance, production of both enzymes and chemical reagents, even if used in small quantities, might require more energy and raw materials than it saves (Nielsen et al., 2007; Kim et al., 2009). Impact on food safety derived from the potential presence of pollutants is an important issue that should also be considered. In this sense, the evaluation of the risk of adverse effects on human health needs particular attention.

The objective of this work is to compare the sustainability of the chemical and enzymatic processes for chitin production at pilot scale. With that aim, the study includes not only a techno-economic and environmental impact evaluation, but also the analysis of the product safety itself by a human health risk assessment. The results obtained were integrated by a hierarchical method in order to provide a straight and comprehensive comparison between both processes.

2. Methodology

In this section, a description of both processes, including the inventory of relevant data, is presented. Furthermore, the employed methodologies to perform the whole assessment are also described. The Ecological Footprint (EF) was the selected method to account for main environmental impacts, while Risk Assessment (RA) methodology was employed to account for the presence and levels of pollutants (heavy metals) in the chitin produced. Analytical methods for heavy metals quantification were also described. To integrate the different criteria considered, a hierarchical method was selected.

2.1. Description of the processes

In the present study, both alkali and enzymatic hydrolysis were designed to be applied to the crustacean *Munida* spp. Both processes are quite similar in terms of operational units needed and were scaled-up based on pilot plant experiences. A common process flow diagram (PFD) is presented in Fig. 1 for the production of chitin. The main differences between both processes are the concentrations and volumes of alkali and acid reagents, the use of enzymes, the temperature (T), the processing time and the chitin yield. A range of values for chitin yield, between 5% and 22%, including the ones obtained in this work (Manni et al., 2010; Valdez-Peña et al., 2010; Younes et al., 2012) was considered for the enzymatic hydrolysis (with and without water recovery), whereas for chemical hydrolysis the chitin yield varied between 20 and 35% (Kurita, 2006; Manni et al., 2010; Antelo et al., 2015). Therefore, increasing the efficiency and quality of the enzymatic process is one of the most important challenges. Among enzymes, various commercial proteases to remove protein from crustacean shell have been assayed (Rao et al., 2000; Xu et al., 2008; Valdez-Peña et al., 2010), being Alcalase the most effective and employed enzyme to that aim (Baek and Cadwallader, 1995; Synowiecki and Al-Khateeb, 2000; De Holanda and Netto, 2006; Valdez-Peña et al., 2010; Lopes et al., 2016).

Briefly, triturated entire specimens of *Munida* spp. are fed to a stainless steel hydrolysis reactor (with a volume of 10 m^3), with a solid/water ratio of 1/5 (w/v), where the removal of proteins from crustacean raw material is carried out at a controlled temperature (55 °C and 110 °C for enzymatic and chemical hydrolysis, respectively) and pH (only in the enzymatic process, in which it is set to 8). In the enzymatic process, the hydrolysis occurs by the addition of Alcalase, being the use of NaOH 1 M only to control pH value; whereas in the chemical one, the hydrolysis takes place by the consumption of NaOH 4 M. After rinsed with water, the wet cake produced after hydrolysis is sent to an acid-resistant stirred tank (with a volume of 4 m^3) in order to remove salts still associated with chitin, mainly calcium carbonate and pseudohydroxyapatite (Trung and Stevens, 2013). In the present study, this demineralization stage consists in washing the cake with diluted hydrochloric acid (HCl) 1 M and 4 M, for enzymatic and chemical processes, respectively, with a solid/liquid ratio 1/5 (w/v) at room temperature. The efficiency of the demineralization step is lower for the enzymatic process (values around 19% were obtained in previous experiments). Furthermore, the enzymatic process cannot remove all the proteins, since around 5%–10% of residual protein is usually still linked with the isolated chitin (Younes and Rinaudo, 2015). Therefore, in the third step, common for both processes, the final isolated chitin is treated with an extra volume of NaOH (1 M and 4 M for enzymatic and chemical processes, respectively) (Oh et al., 2007; Pérez-Martín, 2010) to increase its purity and to maintain the structure of chitin (Beaney et al., 2005; Younes and Rinaudo, 2015). This last stage of the process is carried out in a stainless steel stirred tank (with a volume of 3 m^3) at controlled temperature (40-60 °C). In order to remove traces of pigments, chitin was then treated by a solution of NaClO 15% w/v at a 1:2 (solid to NaClO ratio) (Pérez-Martín, 2010), as presented in Fig. 1.

Therefore, the main difference between the two processes lies in the first deproteinization step. The alkaline treatment at high temperature is a more efficient procedure to remove protein from the shells, but it also causes depolymerisation and deacetylation of

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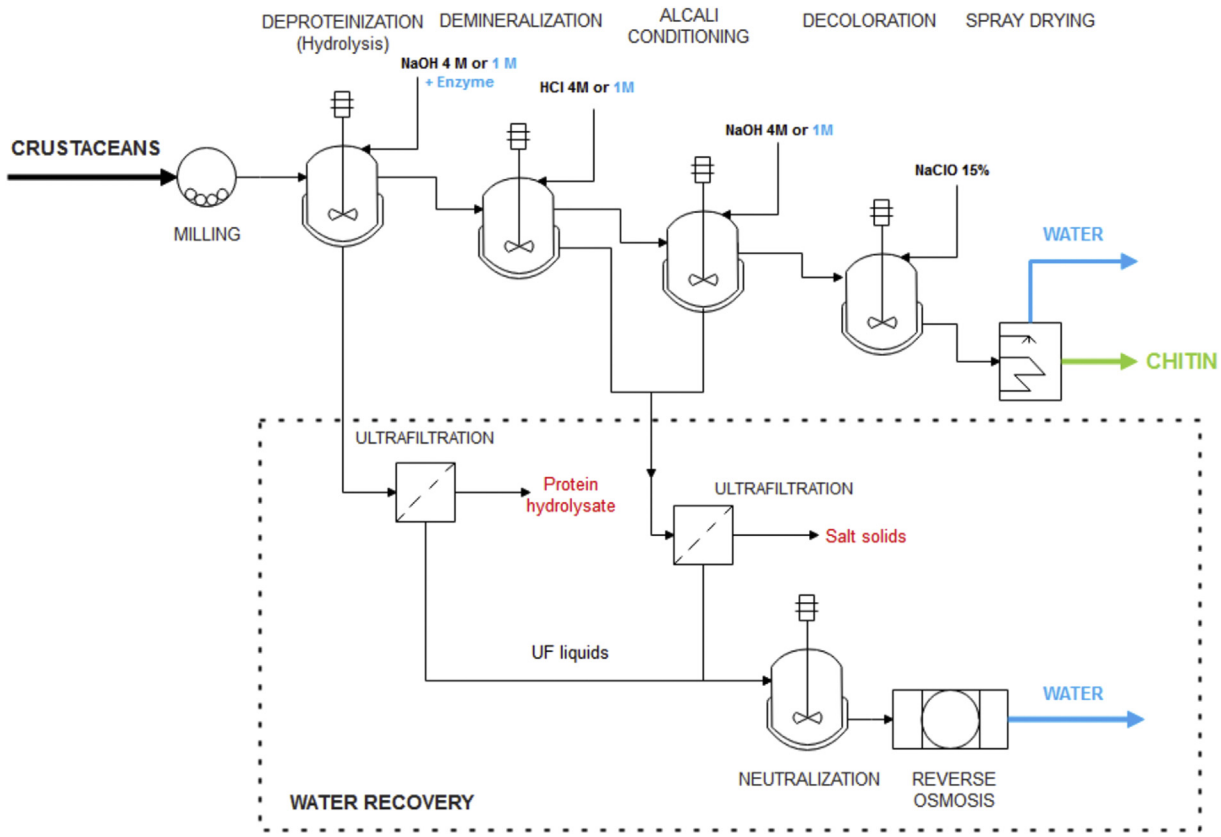

Fig. 1. Process flow diagram (PFD) of the extraction of chitin.

chitin. On small-scale and semi-large scale laboratories, degradation of chitin can be largely prevented by the application of mild conditions (diluted NaOH and 30 °C), but long deproteinization times are required (20 h) (Trung and Stevens, 2013). At pilot and industrial scale, where large masses of by-products have to be treated in a short time, high concentrations of NaOH and temperatures in the range of 100 °C or above are usually applied to accelerate the process.

Since water consumption was identified as a significant environmental impact in previous work (Antelo et al., 2015), water recovery employing ultrafiltration (UF) and reverse osmosis (RO), was proposed as an additional measure to drastically reduce the consumption of this resource in the enzymatic extraction of chitin in the proposed design (Fig. 1). One UF unit was included after the deproteinization reactor to concentrate the FPH, while other UF unit was placed to treat the liquid flows coming from the demineralization and mild chemical deproteinization steps. The filtrated flows from the two UF units were fed to a stirred tank where a neutralization reaction between $HCl_{(aq)}$ and $NaOH_{(aq)}$ was produced. NaCl solute contained in the resulting flow (11.7 g L^{-1}, approximately) was removed from water through a RO membrane unit. The water recovered with this process configuration can save more than 18,000 m^3 per year (Table 1), although it has associated other disadvantages, like additional electricity consumption (3.5 and 0.1 $kWh \cdot m^{-3}$ for UF and RO, respectively) (IDAE, 2010) and membranes replacement (0.0089 and 0.031 $€ \cdot m^{-3}$, respectively) (Knops et al., 2007). These flows were accounted in Tables 1 and 2, where environmental and economic inventories are presented, respectively. UF provides an optimum and necessary pretreatment to RO membranes, since the RO cleaning frequency will be greatly diminished, due to both reductions of RO fouling and the aggression attack of chemicals consumed in the conventional pretreatments (Knops et al., 2007). The design efficiencies of UF and RO units were around 95% and 85%, respectively. Although NaCl concentration arriving to the RO unit (25.1 g L^{-1}, approximately) would be higher than that obtained on the enzymatic process, but lower than seawater, water reclamation could be feasible. However, it was not proposed in the chemical process since the objective was to compare the "worst" and typical industrial implementation of this alternative with other novel ones.

2.2. Assessment methodologies

The different flows of materials and energy were compiled per year and they can be seen in Table 1 for the three considered process alternatives (enzymatic process with and without water reclamation, and chemical process), grouped according to the different categories: i) energy; ii) water and other resources consumption; iii) products and; iv) emissions generated. The flows corresponded to the processing of 1523.1 tonnes per year of *Munida* spp. biomass, which is the availability of raw material in the location of the study (Ordóñez-Del Pazo et al., 2014). Inventory was based on pilot scale experiences for the extraction of chitin, developed under the framework of this work from different types of crustacean biomass (Antelo et al., 2007; Pérez-Martín, 2010). For the calculation of EF, flows in Table 1 were converted into bioproductive area (ha) by specific equivalence factors (energy intensity and natural and/or energy productivity, depending on the case) for the land use types available from the National Footprint Account (GFN, 2010). These values are specific for each subcategory, and they are compiled from several studies reported in Table 3. However, when the same category was not found, the most similar

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Table 1
Inventory data of the enzymatic (EnzH), with and without water recovery, and chemical hydrolysis (CheH) processes (per year).

	Units	EnzH	EnzH (with water recovery)	CheH
Inputs				
Waste to be treated				
Crustacean biomass	t	1523.1	1523.1	1523.1
Resources consumed				
Electricity	kWh	991,960.0	1,058,001.3	5,123,708.4
Water	m^3	19,791	1791	30,462.0
NaOH	t	93.8	93.8	304.6
HCl	t	694.8	694.8	971.5
NaClO	t	171.6	171.6	148.6
Enzymes (Alcalase)	t	7.6	7.6	–
Outputs				
Products				
Chitin	t	205.6	205.6	418.9
Protein hydrolisate	t	428.4	428.4	–
Air emissions				
CO_2	kg	148,397.22	158,277.0	766,506.78
Wastes				
Wastewater	m^3	3460	393.5	9139

Table 2
Cost of raw materials, reactive and utilities, and sales price of products for enzymatic (EnzH) and chemical hydrolysis (CheH) processes (euros per year).

	Price units	Purchase/sale price	EnzH (€/y)	EnzH (with water recovery) (€/y)	CheH (€/y)
Inputs					
Waste to be treated					
Crustacean biomass	€/t	800	1,218,480	1,218,480	1,218,480
Resources consumed					
Electricity	€/kWh	0.116[a]	115,067	122,728	594,350
Water	€/m^3	1.81[b]	35,822	3242	55,136
NaOH	€/t	378.0[c]	35,456	35,456	115,138.8
HCl	€/t	75.2[c]	52,249	52,249	73,056.8
NaClO	€/t	25.7[c]	4410	4410	3819.02
Enzymes (Alcalase)	€/t	25,000[d]	190,000	190,000	–
Membranes replacement (UF − RO)	€/m^3	0.0089–0.031[e]	–	758.6	–
Outputs					
Products					
Chitin	€/t	15,000[f]	3,084,000	3,084,000	6,283,500
Protein hydrolyzate	€/t	2,800[g]	959,616	959,616	–
Wastes					
Wastewater	€/m^3	0.0917[h]	317.3	36.1	838.0
Gross profit	€		2,391,814	2,416,256	4,222,681

[a] Eurostat, 2011.
[b] AEAS, 2010.
[c] ICIS, 2015.
[d] Aspmo et al., 2005.
[e] Knops et al., 2007.
[f] Ferraro et al., 2010.
[g] He et al., 2015.
[h] RD 606/2003.

Table 3
Values of energy intensity and productivity natural and energy, used in the EF calculation.

Categories	Energy intensity (GJ/units)	Productivity	
		Natural (Units/ha/y)	Energy (GJ/ha/y)
Water	–	1500[a]	–
NaOH	40[a]		71[b]
HCl	40[a]	–	71[b]
NaClO	50[a]	–	71[b]
Enzyme (Alcalase)	1.64[c]	28.05[a]	71[b]

[a] Doménech Quesada (2010).
[b] Wackernagel (1998).
[c] Agostinho et al. (2015).

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one was used. The different types of area considered in the present study were: i) fossil energy; ii) arable land; iii) pasture and; iv) forest area. Sea area and built-up land type were not considered. Regarding sea area, although crustacean biomass will directly affect this land type, it is considered as a by-product of the fishing extractive activity (discarded and returned to sea). For that reason, it was not accounted as a resource in this assessment.

The economic criterion was easily calculated as the sales price minus the operation costs. The investment costs were not considered, since the process flow diagram and the equipment required is very similar for the considered processes and quite simple. However, the maintenance costs of the equipment for the enzymatic process will be significantly lower due to both the less amount of chemicals used and the lower operation temperature. On the other hand, the process with water recovery would pose an additional cost due to acquisition of the UF and RO equipment, which could pose 80% of the equipment budget. Operation costs involve the consumption of raw materials, reactive and utilities (water, electricity and fuel). Detailed values used to estimate the net benefits of the processes are shown in Table 2.

On the other hand, crustacean biomass can contain different levels of pollutants. Among them, heavy metals are the most commonly monitored (Blasco et al., 2002; Cheung and Wong, 2006; Habte et al., 2015). The significant biosorption capacity of chitin is well known as a way of eliminating metals from water (Onsøyen and Skaugrud, 1990; Zhou et al., 2004), indicating the tendency of this biopolymer to retain these pollutants. Therefore, it is necessary to analyse the fate of these compounds along the process stages, and to evaluate the potential risk due to the presence of metals in the product, in order to include risk as an additional criterion in the sustainability assessment, if values of concern are obtained. Risk indexes were calculated according to U.S. EPA guidelines (U.S. EPA, 1989). The relevant exposure scenario in this case would be the direct ingestion of chitin or chitin-derived products. Metal levels (As, Cd, Cr, Hg and Pb) in the raw material (*Munida* spp.), chitin and effluents from the deproteinization and demineralization steps were analysed in this study at lab scale. Different techniques were employed according to the metal. As, Cd and Pb were analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS), while Cr and Hg were measured by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and Cold Vapour Atomic Absorption Spectrometry (CV-AAS), respectively. Limits of Quantification (LOQ) for the different metals were $\geq$0.25, 0.03, 0.03, 0.05 and 2.5 mg kg^{-1} (dw), for As, Cd, Pb, Hg and Cr, respectively. All measurements were done in triplicate, being all determinations carried out in a homologated laboratory accredited by the Spanish entity of National Accreditation (ENAC) in accordance with regulation UNE-EN ISO/IEC 17025. Samples digestion was carried out in a closed PTFE (polytetrafluoroethylene) vessel microwave (Savillex®) with nitric acid and hydrogen peroxide. Afterwards, the samples were introduced in a muffle furnace at 120 °C, during at least all night, in order to complete the digestion process. After that, the digested samples were carried to a known volume with milliQ water.

Exposures by the considered pathway were calculated under a conservative approach, employing a simple model. The dose by chitin ingestion is estimated by Eq. (1):

$$DD_i = C_i \cdot IngR \cdot BW^{-1} \quad (1)$$

where *DDi* is the daily dose intake for the metal i (mg of metal/kg/d), C_i is the concentration of the metal in chitin (mg/kg dw), *IngR* is the ingestion rate of chitin (3.0E-03 kg/d) and *BW* is the body weight (70 kg). From the *DDi*, risk can be characterised by the calculation of hazard quotients (*HQ*) and a global hazard index (*HI*) (Eq. (2)).

$$HQ_i = DD_i/RfD_i,\ HI = \Sigma_i HQ_i \quad (2)$$

where HQ_i and RfD_i are the hazard quotient (unitless) and the reference dose (mg/kg/d)· for metal i. *RfDs* are specific for each pollutant and are defined as the dose below which no adverse effects are produced (U.S. EPA, 2004) (Table 4). C_i was considered in this study as the metal content in chitin (As, Cd, Cr, Hg and Pb).

Finally, mathematical multicriteria decision-making based on the Analytical Hierarchy Process (AHP) methodology was employed in the current work (Saaty, 2008). This method is based in the structure of matrices and the corresponding eigenvector to create the accurate weights by using pairwise comparisons. The size of the comparison matrices depends on the number of alternatives and/or criteria. A consistency index (*CI*) is obtained through the calculation of the maximum eigenvector (λ_{max}) of each matrix of order n, being n the number of criteria. (Eq. (3)).

$$CI = (\lambda_{max} - n)/(n - 1) \quad (3)$$

For ranking the different alternatives, the preference score calculation is based on the following equation:

$$S_j = \sum_i w_i \cdot r_{ij} \quad (4)$$

where S_j, is the score of the alternative j, w_i is the ponderation of each criterion i and r_{ij} is the rating of the alternative *j* as a function of the criterion *i*.

Two criteria groups were considered in the AHP analysis: in the first one, the water and reagents consumption, the products obtained, the GHG emission and the economic benefit were considered; the second group was based on the EF impacts derived from the resources and energy consumption, produced waste and the economic benefit. Subsequently, the three available process alternatives – the enzymatic hydrolysis, with and without water recovery, and the chemical extraction of chitin – were ranked employing the different criteria considered in each group. The values of the different criteria were normalized to the same unifying decision scale, set at a length of 8 (Tables 5 and 6). Data were uniformly distributed according to an index between 1 and 8, including the range of values corresponding to the three alternatives. The scale starts with the lowest value for those criteria to minimise (i.e. water and reagents consumption or any other impact) and begins with the highest value for the criteria to maximize (products and the economic benefit). Besides the indexed data, values sets were also required. They account for the preferences given to the different indicators, which are obtained by the pairwise comparisons between them. The value of the final index obtained indicated the preferred option, in which the lowest index value implies a better score.

3. Results and discussion

3.1. Environmental impact and gross profit

Considering the flows presented in Table 1, total EF values of 1785.3 ha (2.8 ha/t), 1782.4 ha (2.8 ha/t) and 2220.8 ha (5.3 ha/t) were obtained for the enzymatic (without and with water recovery) and chemical extraction of chitin, respectively. In this footprint indicator, the impact is converted in more hectares of land needed to produce the resources consumed and assimilate the emissions generated. Therefore, it is clear that the chemical process presents a larger global impact on the different footprint land categories,

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Table 4
Metal content in the Munida spp. biomass, protein hydrolysate, demineralization and deproteinization effluents and chitin in mg·kg^{-1} (dw), RfDs in mg·kg·d^{-1} and HQ (unitless).

	As	Cd	Cr	Hg	Pb
Source					
Munida spp	42.7 ± 4.4	0.49 ± 0.06	26.1 ± 10.7	0.16 ± 0.07	0.44 ± 0.17
Protein hydrolisate	2.01 ± 0.03	0.01 ± 0.001	1.19 ± 0.82	0.1 ± 0	0.04 ± 0
Demineralization effluent	2.0 ± 0	0.01 ± 0.002	0.80 ± 0.18	0.1 ± 0	0.04 ± 0
Deproteinization effluent	2.0 ± 0	0.01 ± 0	0.58 ± 0.16	0.1 ± 0	0.04 ± 0
Chitin	4.7 ± 0.16	0.02 ± 0.001	6.2 ± 1.2	0.24 ± 0.01	0.13 ± 0.04
RfD	0.0003	0.001	1.5	0.0001	0.0036[a]
HQ (Chitin)	0.67	0.001	0.0001	0.10	0.001
HQ (*Munida* spp)	61	0.021	0.0007	0.068	0.005

[a] Baars et al., 2001.

Table 5
Range of the decision criteria values used for indexing.

Criteria	Units	Index							
		1	2	3	4	5	6	7	8
Water consumption	m^3	1500.0	5571.4	9642.9	13,714.3	17,785.7	21,857.1	25,928.6	30,000.0
Reagents consumption	t	90.0	220.0	350.0	480.0	610.0	740.0	870.0	1000.0
Products obtained	t	800.0	742.9	685.7	628.6	571.4	514.3	457.1	400.0
GHG emitted	t	120.0	214.3	308.6	402.9	497.1	591.4	685.7	780.0
Economic benefit	€	4,000,000.0	3,857,142.9	3,714,285.7	3,571,428.6	3,428,571.4	3,285,714.3	3,142,857.1	3,000,000.0

Table 6
Range of the decision criteria values used for indexing.

Criteria	Units	Index							
		1	2	3	4	5	6	7	8
Resources consumption	ha	500.0	685.7	871.4	1057.1	1242.9	1428.6	1614.3	1800.0
Energy	ha	100.0	228.6	357.1	485.7	614.3	742.9	871.4	1000.0
Wastes	ha	25.0	64.3	103.6	142.9	182.1	221.4	260.7	300.0
Economic benefit	€	4,000,000	3,857,142.9	3,714,285.7	3,571,428.6	3,428,571.4	3,285,714.3	3,142,857.1	3,000,000.0

compared to the enzymatic methods. This results is expected (De Holanda and Netto, 2006; Pachapur et al., 2015) considering the higher resources and energy necessary by the chemical process (Table 1). It has to be mentioned that the relative EF value would be higher for the enzymatic hydrolysis (higher than 8 ha/t) if chitin was considered as the only product obtained, due to the lower yield of this process when compared to the chemical one. The contribution of the different categories (resources, energy and residues) can be observed in Fig. 2. For both enzymatic processes, the category of resources was the main contributor to the total EF value, representing around 86.7% and 87.4%, respectively, of the total impact on footprint, followed by energy (10.9% and 10.2%, respectively) and finally by the residues generated (2.4% for both processes). Similar contributions from different categories in both processes reveal that water consumption impact is not well captured by EF, when compared with the impact associated with energy consumption and land-consuming products (Hoekstra, 2009). In the case of the chemical process, both resources and energy are equally important for the total EF value, contributing with 47.9% and 42.4%, respectively. Residues contributed with only 9.8% to the total EF value.

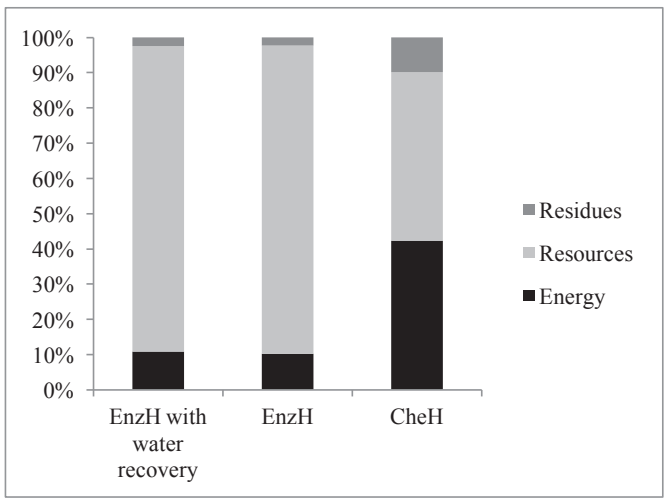

Fig. 2. Contribution of the different categories (energy, resources and residues) to the global EF value of both processes, enzymatic and chemical hydrolysis.

Regarding land categories (Fig. 3), all the considered alternatives showed the highest pressure on energy land (values around 60% and 90% for the enzymatic and the chemical processes, respectively). This was due not only to the energy consumption of the process itself, but also to the energy used in the transformation of the utilities that cannot be obtained directly from nature. The next most affected land category was the arable land, being more evident for the enzymatic processes. The important footprint on this type of land is associated to the enzyme production. This can be explained analysing the resources category corresponding to the enzymatic process, with and without water recovery (Fig. 4a). In this figure, it is possible to observe that the amount of enzyme (around 53.5%), followed by HCl (close to 32%) were the major

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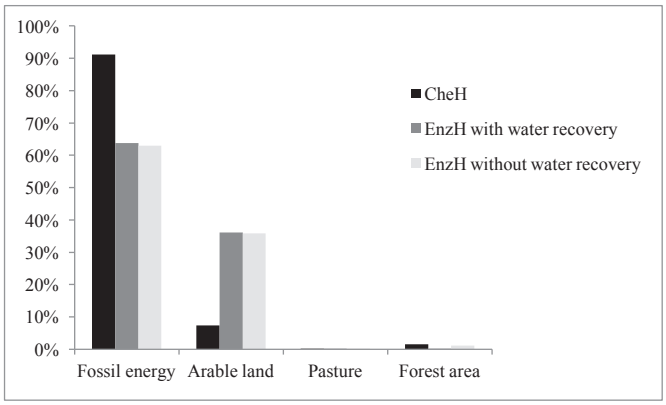
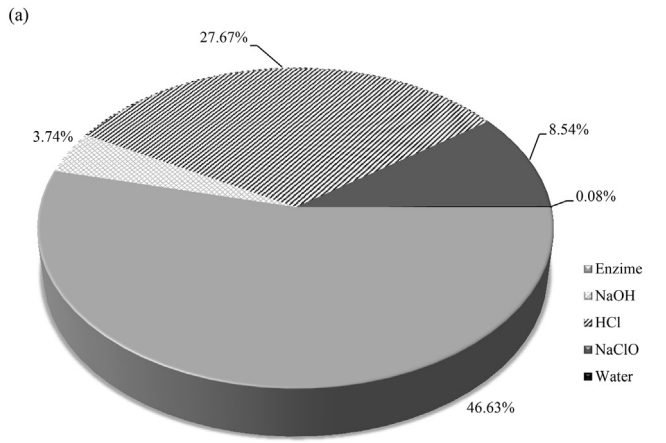


Fig. 3. Pressure of the chitin extraction, by the three considered processes, on the different footprint land-components.



Note: in the case of the enzymatic hydrolysis without water recovery, the impact on land, associated with water consumption, was around 0.9%.

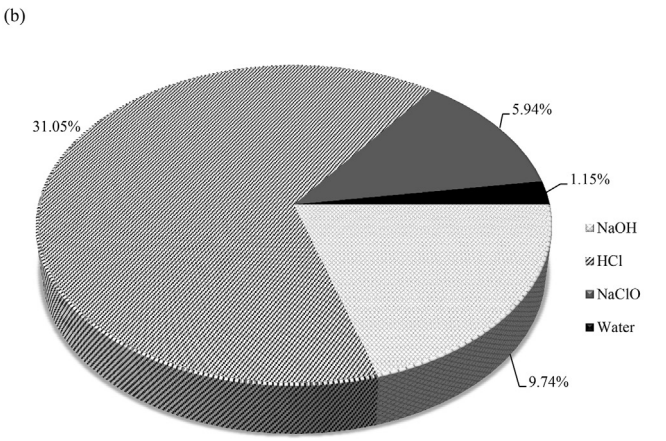


Fig. 4. Contribution of the resources to the total EF value in the, (a) enzymatic and (b) in the chemical, processes.

contributors to the total EF value, presenting NaClO (almost 10%) and NaOH (4.3% for both processes) a much lower influence. The significant effect of enzyme consumption in the resources category is due on the one hand, to the low natural productivity of the raw material employed in the enzyme production and, on the other, to the energy intensity required for the process. Enzyme production is based on raw materials derived from agriculture, such as corn, potato and sugar cane (Nielsen et al., 2007). Therefore, the value of natural productivity used (31.3 t/ha) to account for the consumption of raw materials was estimated as the average of the natural productivity of these agriculture resources typically used in enzyme production. The energy intensity value (1.64 GJ/kg) was estimated from the embodied energy related to the industrial production of cellulase enzyme (Agostinho et al., 2015). Despite the important footprint associated with enzyme production itself, the enzymatic process seems to be, globally, 20% more favorable to the environment compared to the chemical one. In the chemical process, the main flow contributing to the resources category was HCl (64.9%), followed by NaOH (20.3%), NaClO (12.4%) and water (2.4%) (Fig. 4b). As in the enzymatic process, the impact derived from the consumption of the chemicals was directly related with the amounts required by the process, since a very similar equivalence factor was employed for the three chemical reagents (Table 3). Finally, in the residues category, the main contributor to the total EF value were CO_2 emissions derived from electricity consumption (values above 90%).

Although important quantities of water are consumed in the enzymatic and chemical processes (Table 1), the high natural productivity associated to this resource in EF methodology implied an impact which does not considerably affect the total value of affected area. In fact, it has been observed that the contribution to the impact on land due to the typical water-consuming products or water-polluting activities is not so evident with EF methodology, when compared with the impact on the necessary land to absorb the emissions resulted from the use of energy and the use of arable land (Hoekstra, 2009). In this sense, the water footprint (WF), usually represented by virtual water flows (blue, green and gray) could result in an improvement of the water management for a particular scenario. Nevertheless, in the case of industrial WF assessments, not all the water components (green and gray) are considered, being this methodology better applied to other sectors (Gu et al., 2014; Pellegrini et al., 2016). Therefore, in order to have an oriented evaluation regarding the different footprints methods, the inventory and the characterization aspects are important issues that needs to be considered in order to have a standardization of the footprint interpretation (Fang and Heijungs, 2015).

On the other hand, although the use of UF and RO systems were proposed in the present work, new methods to reduce water consumption proposed the use of seawater for chemical washing instead of freshwater, considering that most crustacean processing industries are located near the sea (Pachapur et al., 2015). Besides reducing the consumption of water, which can be a vital resource depending on the location, the total production cost could also be reduced 10–13% if seawater was used instead of freshwater (Pachapur et al., 2015).

Regarding the benefits, the results indicated that the chemical extraction of chitin was more advantageous, with a gross profit around 4,200,000 €, whereas the enzymatic processes presented almost half this value (2,400,000 € approximately) (Table 2). Despite the fact that the chemical process presented higher production costs, including waste management, the benefit associated with the higher yield of chitin increases greatly the profit of the process. Therefore, in order to have a complete comparison between both processes, an integration of the different environmental indicators with the economical one, by a hierarchical method, will elucidate the most sustainable option for producing chitin at a semi-large scale.

3.2. Fate of metals in the enzymatic process and potential risks

Levels of metals in the raw material, intermediate and final

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Table 7
Global weights associated with the criteria considered in the first hierarchy.

Criteria	Normalized weights				
	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5
	Equal	Environment	Economic	Water	CO_2 emissions
Water	0.20	0.54	0.08	0.69	0.08
Reagents	0.20	0.12	0.08	0.08	0.08
Products	0.20	0.03	0.08	0.08	0.08
CO_2	0.20	0.28	0.08	0.08	0.69
Economic benefit	0.20	0.03	0.69	0.08	0.08

Table 8
Global weights associated with the criteria considered in the second hierarchy.

Criteria	Normalized weights			
	Scenario 6	Scenario 7	Scenario 8	Scenario9
	Resources	Equal	Economic	Environment
Resources	0.72	0.25	0.08	0.32
Energy	0.12	0.25	0.08	0.32
Wastes	0.12	0.25	0.08	0.32
Economic benefit	0.04	0.25	0.75	0.04

products can be seen in Table 4. As and Cr presented higher concentrations in *Munida* spp. than the other metals. In general, As levels found in crustacean biomass (muscle in most cases) in different locations around the world are generally higher than other hazardous metals which present legislation limits in marine organisms (Cd, Pb or Hg), being in the order of magnitude found in this study (Alonso-Hernández et al., 2012; Olmedo et al., 2013; Iamiceli et al., 2015). It can be observed that an important reduction of metal content is produced in the obtained chitin, except for Hg. This was due to the elimination of water-soluble metal species (including toxic species, like for example inorganic As) in the liquid flows produced during the washing and deproteinization steps of the extraction process. On the contrary, Hg levels in chitin were a 60% higher than in the raw material, indicating the tendency of this metal to remain bounded to this polysaccharide. Although it can be thought that a similar metal fate would be obtained in the chemical process, since washing stages are also included, more alkali and acid are also employed, something which could favour a higher retention of metals.

The Hazard Quotient for each metal analysed in the chitin can be seen in Table 4. At first glance, the HI obtained (0.78) can be considered of concern, close to the safety limit of 1. This was not expected taking into account the very low daily intake of chitin, which is mainly ingested as a health supplement in the form of chitosan. Although the obtained value was lower than 1, only one of the several exposure pathways to metals affecting human health was considered, which means that only an incremental risk is being evaluated. Regarding metal contribution, the influence of Cr, Cd and Pb to total risk was negligible, with As and Hg representing 87% and 13% of the HI. Besides, if this crustacean (*Munida* spp.) was destined to human consumption, (assuming an ingestion rate of 30 g per day), the HQ for As would exceed the threshold value 60 times. The main contribution of this metal is due to both its high content in crustacean species and its low reference dose, which reflects its toxic character. Under a screening level risk assessment, the estimation of the HQ is developed assuming that all the metal is absorbed by the body. However, it is well known that bioavailability plays an important role in metal exposure. Another key factor that has to be taken into account is metal speciation, since only some metal species are toxic. In this sense, there are recent studies that quantify risk by ingestion of commercial fish and seafood species including the key aspect of bioavailability or oral bioaccessibility (Cano-Sancho et al., 2015). Considering the results obtained in the present study, the attention must be focused on arsenic content, for which a refinement in the risk assessment process was developed. It is considered that only inorganic forms of As are toxic to human health (Hughes, 2002), while the remaining organic forms are easily excreted from the body without causing almost any adverse effects (Johnson and Farmer, 1991). In fact, both the U.S. EPA and the European Food Safety Authority (EFSA) have only proposed reference or benchmark doses for inorganic As. Regarding its carcinogenic effects, it is recognised by the IARC (1987) and the U.S. EPA (2004) as a known carcinogen. However, very low percentages of toxic inorganic As (maximum of 10% as a conservative fraction) were reported in works related with As speciation in marine food (mainly in crustacean species) (Storelli and Marcotrigiano, 2001; Lewis et al., 2012; Ruttens et al., 2012; Olmedo et al., 2013; Zhang et al., 2013). On the other hand, the oral bioaccessibility of inorganic As presented high values (around 90%) for cooked crustacean species (Maulvault et al., 2011; Cano-Sancho et al., 2015). Therefore, and considering both bioavailability and the inorganic fraction for this metal, the refined HQ due to As exposure was reduced from 61 to 5.5 for direct ingestion of *Munida* and from 0.67 to 0.06 for chitin ingestion. Again, a significant reduction on the HQ of the remaining metals could be produced once the bioavailability and speciation was considered. That is the case of mercury, for example, where MeHg is the most toxic species and can be found in different fractions and bioavailabilities (generally low) depending on the species (Cano-Sancho et al., 2015). Although this can be relevant for the HI due to the ingestion of chitin, it almost will not affect the HI of *Munida* ingestion, which still was very high. Further research to elucidate the distribution and speciation of metals (especially for As) in this crustacean biomass must be performed, since direct consumption of *Munida* as seafood is proposed as one of the alternatives to make the best use of this discarded resource. Besides, the HI due to the chitin produced by the chemical process should be considered higher than that produced by enzymatic methods. This would not only be due to the metal fate, but also to the intrinsic risk derived from much harder operational conditions, involving a higher quantity of alkali and acid reagents.

3.3. Assessment of sustainability criteria

The hierarchy was implemented considering different preferences towards the selected criteria, by using several weights combinations in order to cover all the relevant scenarios. Risk indexes were not included in this integral evaluation due to the low values obtained from the resulting chitin metal concentrations. Besides, the metal removal effect of the washing steps was present in both the chemical and enzymatic process and therefore, a low concentration can be also expected in the chemical extracted chitin. Thus, no relevant decision weight was associated with the risk criterion. Two decision hierarchies were defined: one used “raw”

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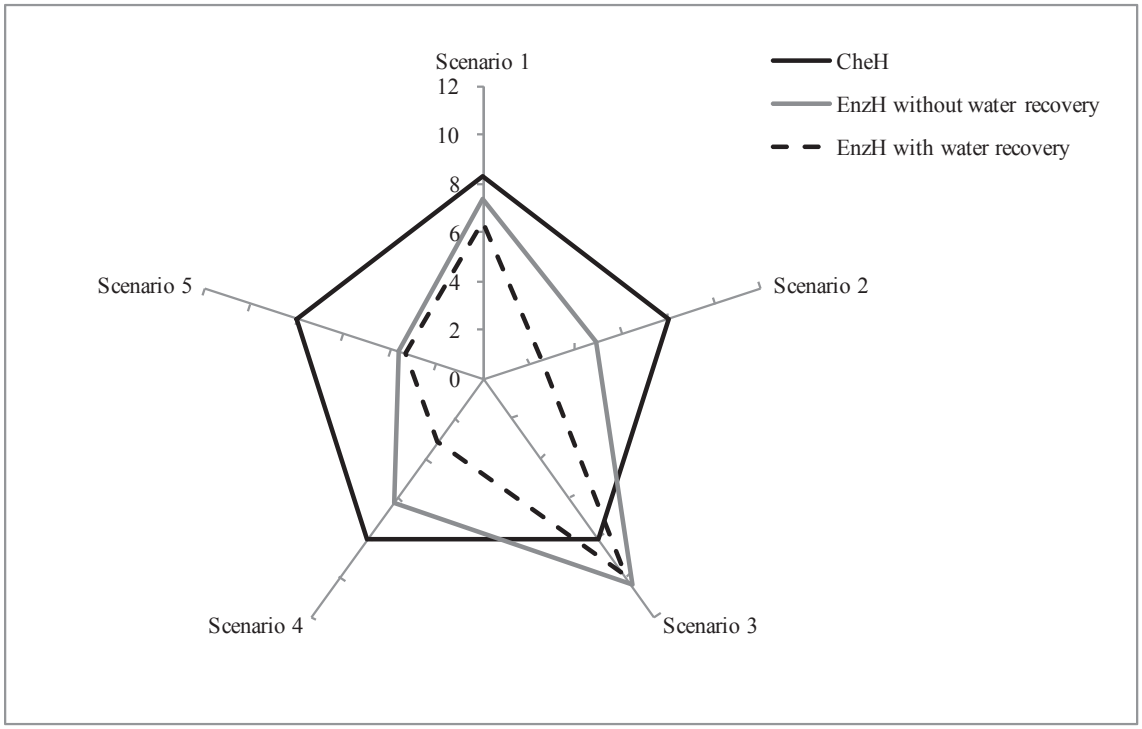
(a)



(b)

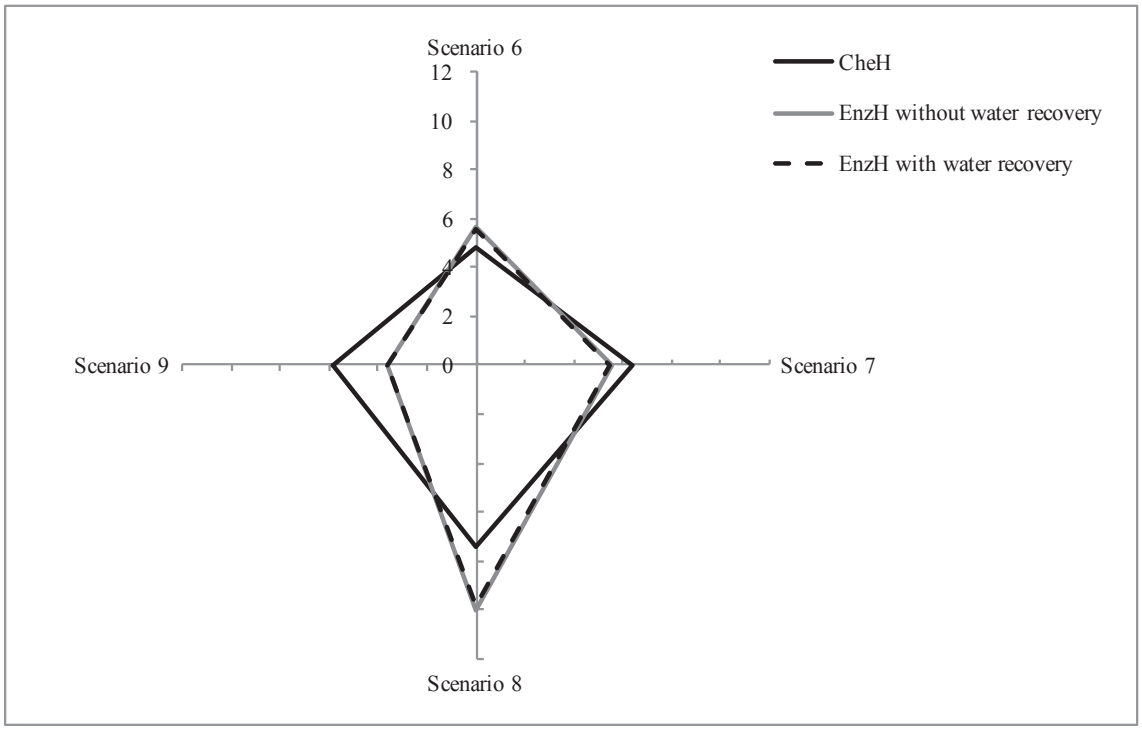


Fig. 5. Global index obtained for the different alternatives by considering two set of criteria: (a) values of water consumption, reagents consumption, final products, GHG and economic benefit; (b) values of the EF related with resources consumption, energy, wastes produced as well as the economic benefit obtained.

flows (water and reagents consumption, total production, CO_2 emissions and benefits), while the other one considered as criteria EF impact categories (resources, energy and waste) together with the economic gross benefit. Subsequently, different classifications

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were evaluated in both hierarchies, being the preferences and weights of the different criteria within each classification data set detailed in Tables 7 and 8, for the first and second hierarchy, respectively. The objective of defining two decision structures was to identify if significant differences in the final score for the three process alternatives were obtained through similar classifications. In the first hierarchy, the results indicated that, as expected, only the economic classification (scenario 3) was the one selecting the chemical process as the best alternative, being the enzymatic hydrolysis with water recovery the preferred option within the remaining classifications (Fig. 5a). The difference between the chemical process and the enzymatic hydrolysis without water recovery varied between 38% (scenario 4) to 94% (scenario 5). Comparing both enzymatic hydrolysis, it is clear that the process with water recovery is a better alternative. The difference between both processes was less evident in the scenario 5 (in which CO_2 emissions were the most important criteria considered), since the enzymatic hydrolysis with water recovery consumes more electricity and consequently, CO_2 emissions are higher than in the other process.

In the second hierarchy (Fig. 5b), the chemical process was selected as the best alternative in the scenarios 6 and 8, in contrast with the enzymatic hydrolysis without water recovery, which resulted to be the worst. These scenarios corresponded to the cases in which more weight was given to the impact of resources consumption (scenario 6) and economic benefit (scenario 8), respectively. Regarding scenario 6, despite the higher consumption of water and chemicals associated to the chemical process, both enzymatic processes presented a higher impact on land footprint due to the enzyme consumption. Scenario 8 ranked the chemical process in first place for the same reasons already explained in the first hierarchy. For the remaining cases, the chemical process was the last option to choose, varying between 96% (scenario 7) and 99% for both the enzymatic processes (scenario 9). The enzymatic hydrolysis with water recovery seems to be the optimal solution in the scenario 7 (equal weights among criteria), whereas in the scenario 9 (in which a less weight was given to the economic criteria and, equal to the remaining ones), both enzymatic hydrolysis processes resulted equally better.

The results indicated that, depending on the criteria employed, different classifications can be obtained for the same alternatives. It is clear that the use of an aggregate indicator to account for the environmental impact of the processes provides a more in depth assessment than employing raw input-output fluxes. While this latter simplified approach identified only the economic advantage of the chemical process, the assessment under EF criteria pointed out the additional advantage of the chemical process related to resources consumption.

4. Concluding remarks

The results obtained indicated that the advantages of the enzymatic extraction of chitin over the chemical extraction are not so evident, especially if the enzymatic process does not include specific units for water reuse. However, the costs associated the acquisition of this equipment could compromise process viability. On the other hand, the enzymatic process has a wide research field to propose new advances which can result in significant advantages in the years to come. However, advances to improve the environmental impacts usually associated to the chemical process were also reported, like a “soft” alkaline treatment (Lertsutthiwong et al., 2002; Percot et al., 2003; Trung and Stevens, 2013), with a much lower chemicals consumption, and the possibility of NaOH, water and FPH recovery (Zhao and Xia, 2009; Zhao et al., 2010, 2011). The major disadvantage of this process would be the long operation time, which can be overcome by including additional processing lines, being the evaluation of the immobilized costs needed to elucidate process viability. Although the enzymatic process is claimed to be more sustainable than the chemical method (Oh et al., 2007; Sini et al., 2007; Manni et al., 2010; Valdez-Peña et al., 2010), two main improvements in these processes are needed to fulfil this statement. The first one is to increase the yield of the produced chitin up to values normally obtained in the chemical process, which has already been achieved with different substrates (Vázquez et al., 2016). The fact that *Munida* presents a “harder” shell than other crustacean species like shrimp have to be considered when analysing the economic benefit derived from the enzymatic process, which could be comparable to the chemical one. The second is to mitigate the environmental impacts and the costs associated to the enzyme production itself. Besides the use of FPH as an ingredient for animal feed, a possibility would be to employ the FPH obtained in the enzymatic hydrolysis of the crustacean biomass as peptones for bacterial growth (Vázquez et al., 2016) that could produce the proper enzymes (proteases) employed in the process, closing the resources reutilization cycle and minimising costs. Therefore, the higher potential of integrating mass and energy flows in bio-based processes (Lopes, 2015) can strengthen the contribution of the fisheries sector to global bio-economy.

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