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Crustacean waste biorefinery as a sustainable cost-effective business model

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ABSTRACT

Marine-derived food wastes mainly include seafood, fish and feed production resources. From the crustaceans traded annually, 6 to 8 million tonnes of valuable shrimp, lobster and/or crab shells waste are produced worldwide. In this systemic work, the researchers with complementary technical expertise, covering the fields of chemical engineering design, chemistry, materials, predictive environmental sciences and economy, worked together to develop a sustainable multiproduct pipeline for the biorefinery of unwanted by-products. All process bio-products from the shells waste were recovered, separated, and purified. Only harmless solvents, namely water, the protonating acetic acid under mild functional conditions and buffers, conjugated with solid–liquid extraction, centrifugation, and membrane ultrafiltration technologies were applied. Here, a success business model is shown after its standardised evaluation in terms of purification performance, economic impact, and life cycle assessment has been performed, driving this sector towards a sustainable ocean-based economy.

1. Introduction

Although biorefinery as a business model is not a new concept, the marine biorefinery concept is. In 2017, the OECD prepared the official report "Biorefineries Models and Policy, through the working party on Biotechnology, Nanotechnology and Converging Technologies" [1], in which the types of biorefineries and the public policies supporting them were exhaustively reviewed. Food waste is, among all types of waste, considered as one of the most concerning issues to overcome worldwide, since the solution of this global problem will significantly impact all the Sustainable Development Goals (SDGs) as stated in the European Biorefinery 2030 vision [2]. Nevertheless, and in our opinion, the development of successful biorefinery business models will impact towards the 17 SDGs (Figure S1 in Supporting Information). According to the Food and Agriculture Organization [3], food waste minimization/mitigation and new solutions to improve food products and services reusing food residues will be the key to achieve the European Biorefinery 2030 vision [2]: "requiring future biorefineries to be better integrated, flexible, and operating more sustainably".

The recognition that huge amounts of pre- and post-produced food are wasted is not recent, but the solutions to surpass this problem are still scarce. The most recent numbers indicate that, around $^1/_3$ of the food produced for human consumption is globally lost or wasted, which represents more than 1.3 billion tons *per* year [3]. Marine-derived food wastes are a more specific type of residues, but no less problematic. These include mainly fish and seafood wastes. The last numbers reported indicate that, from the fish caught, around 70 % is industrially processed [4], thus resulting in the production of large amounts (approximately 20–80 %) of wastes [5]. Seafood, by its turn being the most traded food commodity worldwide [6], reached a global market of USD 164.1 billion in 2018, and is projected to reach USD 194 billion by 2027 [7]. From the seafood traded annually, 6 to 8 million tons of crab, shrimp and lobster shells waste are globally produced [8]. Contrarily to what happens in developing countries, where the shells waste is simply dumped in landfill, in the developed countries, their disposal can be costly [8].

Crustacean shells are composed mainly of 20–30 % of proteins, 30–40 % of calcium carbonate ($CaCO_3$), 20–30 % of chitin and a smallest amount of astaxanthin [9]. There is a high commercial value for these natural compounds, namely considering their main uses. Briefly,

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a) Proteins. The scarcity of natural and non-mammal animal' proteins is becoming an urgent matter, which conjugated with the quality of seafood proteins, allows their application in human food and animal feed (reaching a market value up to $100 million) [8]
b) Calcium carbonate. It has a market price around USD 60–66 or USD 14,000 *per* ton sold as coarse or ultrafine particles, respectively [8]. It has applications in the pharmaceutical, agricultural, construction and paper sectors, being recognized as more biocompatible when compared with the same compound obtained from geological sources.
c) Chitin. It is the second most abundant biopolymer in nature, with a market value of USD 42.29 billion in 2020, which is projected to reach USD 69.3 billion in 2028, while growing at a CAGR of 5.07 % from 2021 to 2028 [10]. This biopolymer has potential applications in medicine, food & beverages, cosmetics, agriculture, and healthcare, due to its low toxicity, high biocompatibility, biodegradability, bioactivity, antibacterial and wound-healing properties [11–13].
d) Astaxanthin. It is a xanthophyll with an hydroxyl and keto endings on each ionone ring [14], with a market value up to USD 1.0 billion and expected to reach USD 3.4 billion by 2027 [15], mostly due to the increasing awareness of its benefits to a healthier lifestyle [15]. Astaxanthin is used in animal feed supplements [16], human food [17], nutraceutical formulations [18] and cosmetics [19].

Despite the last projections [8], considering the biorefinery of crustaceans (and shrimp in particular) a multimillion-dollar business, with no surprise, is still not a reality. The development of sustainable, efficient, and scalable downstream pipelines, allowing the recovery of each compound present in these residues is still a challenge. There have been several attempts to develop new technologies for the recovery of chitin [20–24], astaxanthin [25,26], minerals [27], and/or proteins [23] from these residues. Nevertheless, the economic viability of the seafood shells (or any) biorefinery business will only be guaranteed by the appropriate design of a multi-product pipeline. Meanwhile, this will require innovation and researchers with complementary expertise working together, covering the fields of Chemical Engineering, Chemistry, Economy, Materials and Environmental Sciences.

In this work, a sustainable biorefinery of shrimp shells waste is reported. The main products composing the shrimp shells, namely chitin, proteins, calcium carbonate and astaxanthin, were recovered and purified. This biorefinery process was developed to be sustainable, by using harmless solvents and technologies. Here, acetic acid under mild conditions, water and buffer conjugated with solid–liquid extraction, centrifugation, and ultrafiltration were considered, all recognized by their straightforward scale-up. The economic and life cycle assessment (LCA) analyses were carried and this biorefinery' business model evaluated.

2. Materials and methods

2.1. Materials

Acetic acid (glacial), dimethyl sulfoxide (DMSO, 99.9% purity) and n-hexane (99% purity) were acquired at Merck. Ethanol absolute anhydrous (99.9% purity) was obtained from Carlo Erba. Acetone (99.5% purity) and ethyl acetate (99.5% purity) were purchased from Honeywell. Ammonium sulfate (99.5% purity) was acquired at Fluka. Trichloroacetic acid (TCA, 99% purity) and astaxanthin were purchased at Sigma-Aldrich. Argentine red shrimp shell wastes were obtained from a local market in Ljubljana, Slovenia, between June 2020 and June 2021.

2.2. Biomass characterization

Consecutive cycles of solid–liquid extraction (SLE) were performed to determine the total amount of astaxanthin and proteins present in the biomass. In this sense, acetic acid and McIlvaine buffer pH 7.0 were used to extract astaxanthin and proteins, respectively, using a SLR of 0.2, 20 min of extraction time and 25 °C. Samples were analysed in triplicate, being the average presented.

Chitin content was determined according to Black and Schwartz [28]. 0.2–0.4 g of the dried raw was placed in a beaker with 50 mL of 1 M HCl and heated for 1 h at 100 °C. The sample was washed with distilled water and filtered. The residue was placed back into a beaker with 100 mL of 5 wt/v % NaOH solution and heated for 1 h at 100 °C. Shrimp shells were washed twice with distilled water and twice with 15 mL of acetone and then filtered. Samples were dried at 110 °C to constant weight and later incinerated in a furnace at 600 °C for 6 h. The weight loss represented the chitin content in the sample, and the result shown represents the average of 5 samples.

To determine the amount of minerals, and specifically $CaCO_3$, samples were incinerated in a furnace at 600 °C for 6 h with their weight measured before and after incineration. The ash content in each sample represents the inorganic compound content, and the result shown corresponds to the average of 5 samples.

2.3. Solid-liquid extraction

Shrimp shells were firstly grounded in a mortar, while frozen with liquid nitrogen. Then, different organic solvents (acetic acid, ethanol, acetone, DMSO, ethyl acetate and hexane) were added in a solid–liquid ratio (SLR) of 1:10 and the astaxanthin extraction proceeded in small reactors using a Carousel^{TM} apparatus from Radleys Tech, at 25 °C and 250 rpm for 1 h. The performance of each system was evaluated through UV–vis spectroscopy with emphasis at 482 nm that corresponds to astaxanthin absorbance peak, using a Synergy H1 Hybrid Multi-Mode Reader from BioTek.

Once the best solvent has been selected, a response surface methodology was used to perform the simultaneous optimization of different parameters. Herein, a 2^2 factorial planning was carried out to evaluate the influence of the SLR and time of extraction (independent variables) on the solvent ability to extract astaxanthin (dependent variable). Detailed data is given in Table S1 in Supporting Information (SI) for the coded and decoded matrices. The results obtained were statistically analysed considering a confidence level of 95 %. The software Statsoft Statistica 10.0© was used in the statistical analysis and preparation of the response surface plots.

Further studies, namely influence of the acetic acid concentration and consecutive extraction cycles, were carried out using the same solid–liquid extraction protocol but by adjusting the SLR, extraction time and, later, the acetic acid concentration according to the optimized conditions.

Mass balance (MB, %) was determined for each step following Equation (1):

$$MB(\%) = \frac{[compound]_{eachstep}}{[compound]_{initial}} \times 100 \quad (1)$$

where $[compound]_{each\ step}$ and $[compound]_{initial}$ correspond to each compound concentration at each step of the process and the initial compound concentration in the biomass, respectively.

The polishing protocols applied as well as the methods used for the multi-product characterization can be found in Supporting Information.

2.4. Environmental evaluation: LCA

An environmental evaluation of the biorefinery process proposed (without considering solvents reuse) was performed by applying LCA based on the ReCiPe 2016 Midpoint impact assessment method at the Hierarchist perspective [29]. The impacts from the production of liquid nitrogen, acetic acid, McIlvaine buffer, sodium hydroxide, ultrapure water, and electricity were calculated based on the amounts consumed

(Table S2 in Supporting Information) and respective impact factors taken from Ecoinvent 3.7 database [30].

2.5. Economic evaluation

An economic evaluation was performed in this work to determine the production costs and the potential profit, or Return, from processing shrimp shells and obtaining 4 products from it: astaxanthin, proteins, calcium carbonate and chitin. After generating that process design, the economic evaluation considered its unit operations, operation parameters, necessary materials, labour, and others (such as utilities and waste disposal) at an initial case study of 1 kg of shrimp shells. To calculate the production costs, Equations (2) to (6) were used:[31,32].

$$\frac{ProductionCost}{UnitMassofProcessedShells} = \frac{ProductionCost}{Batch} \tilde{A}\cdot \frac{ShellsProcessed}{Batch} \quad (2)$$

$$\frac{ProductionCost}{Batch} = \frac{Capital + MaterialsandConsumables + Labor + Others}{Batch} \quad (3)$$

$$\frac{Capital}{Batch} = \frac{Capital}{year} \tilde{A}\cdot \frac{Batches}{year} \quad (4)$$

$$\frac{MaterialsandConsumbles}{Batch} = \sum_{i=1}^{n} \left(\frac{Useofmaterial_i}{batch} \times \frac{Priceofmaterial_i}{Unitofmaterial_i} \right) \quad (5)$$

$$\frac{ProductionCost}{UnitMassofProduct} = \frac{ProductionCost}{UnitMassofProcessedShells} \tilde{A}\cdot \frac{Product}{UnitMassofProcessedShells} \quad (6)$$

Equation (2) provides an integrated production cost that comprises the cost of generating astaxanthin, proteins, calcium carbonate and chitin and it calculates the cost *per* unit mass of processed shrimp shells. Equations 3–5 show how to populate the required values for Equation (2). Furthermore, it is possible to obtain individual production costs for each product using Equation (6). The value of annual capital (used in Equation (4)) is calculated by treating the sum of all equipment costs as a fixed-term loan with an annual interest rate of 12 % and a term of 10 years [33,34]. Additionally, the contribution of "Labour" and "Others" (which comprise utilities and waste management) was fixed at 15 % and 4 % of the final production cost, respectively.[33,35,36] During the construction of this first model (using a base calculation of 1 kg of shrimp shells), every piece of equipment was considered (even if some are duplicated for similar unit operations) and materials costs were obtained from Sigma-Aldrich at their largest presentation. Additionally, all process times were fixed at 1 h as an initial approach, except for the cold storage described in a later section (duration of 96 h). All data used to populate the economic model is included in Table S3 in Supporting Information.

Additionally, it is possible to calculate the potential profit return, or simply the Return. This was calculated using Equation (7), which relates the potential gain of selling the product at a specific price and the amount of product obtained from the processed shrimp shells with the production cost calculated with Equation (2).

$$Return = \frac{Product}{UnitMassofProcessedShells} \times \frac{SellingPrice}{UnitMassofProduct} - \frac{ProductionCost}{UnitMassofProcessedShells} \quad (7)$$

First, an analysis of the impact of changing the production scale was performed. For this, the analysis started at 1 kg of processed shells while performing increments by ten times to end with 1,000 kg. This allowed collecting data on how the integrated production cost (Equation (2)) changes when the operation scale is modified. Additionally, the Return data was also collected.

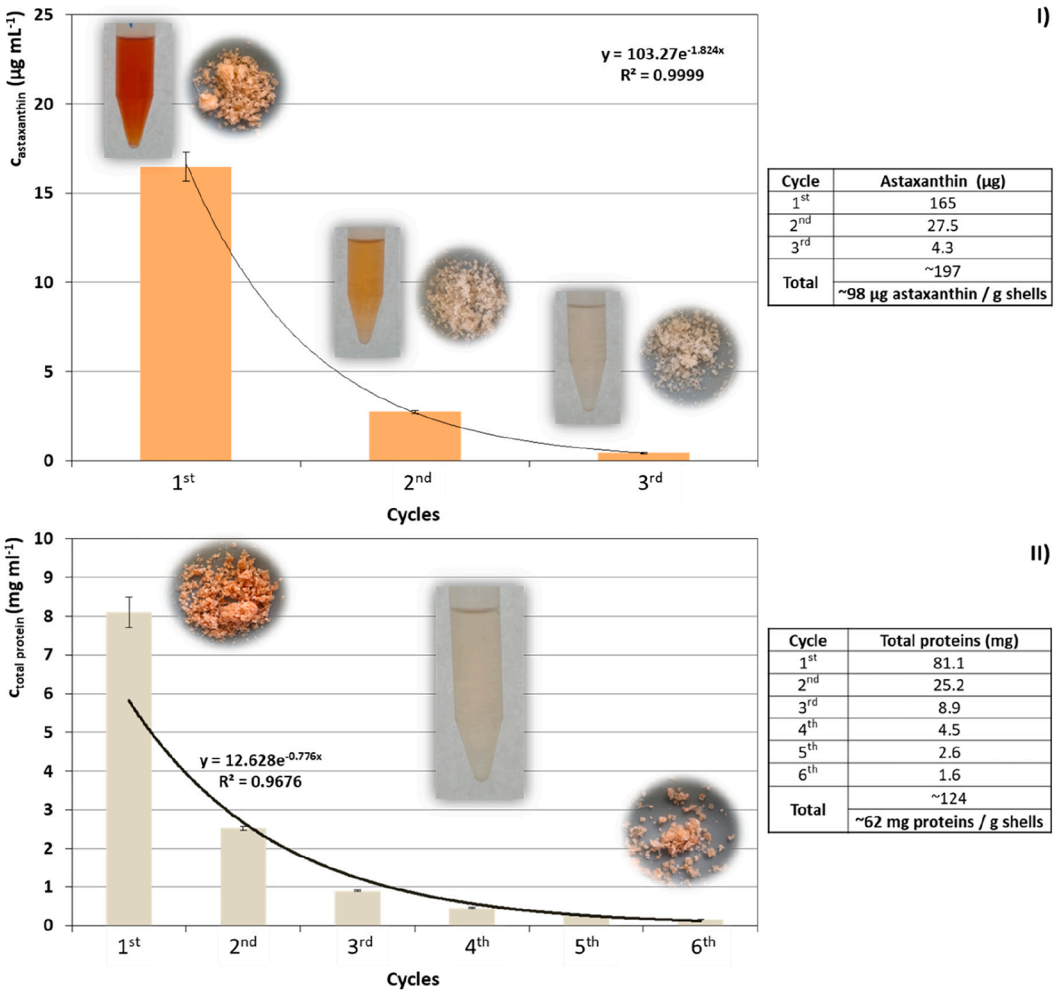
As the main objective of the multi-product process developed in this work is to be applied at large scales, subsequent analyses were performed at the 1,000 kg scale. At this stage, the model was modified to accommodate a more realistic approach for large-scale production. This was meant to modify the process time for centrifugation and ultrafiltration to 8 h (except for the last two centrifugation steps to 16 h) to accommodate the full potential work shift patterns. Duplicated equipment was eliminated, and only unique models were preserved (*i.e.*, if two tanks of similar volume are needed then one was removed). Materials costs were obtained from Alibaba for bulk prices. Lastly, ultrafiltration membranes were set-up to be replaced every ten cycles.

After modifying the model to accommodate larger scales, two analyses were performed. The first was to determine the production cost for each of the four products, while considering only the unit operations required to obtain each product. This allowed us to obtain four slightly different processes focused on a single product. Moreover, it allowed to contrast if focusing on a single-product scenario could be more relevant than focusing in the (four) multi-product process. The second analysis done at large-scale was the determination of its local robustness (sensitivity analysis) for three additional scenarios (scenarios 1 to 3 description in Table S4 in Supporting Information) when improving the recovery yields of each product (+10 %, +15 %, and + 20 %), process time (0.75x, 0.50x, and 0.25x), materials costs (0.75x, 0.50x, and 0.25x), capital required (0.75x, 0.50x, and 0.25x), solid–liquid ratio for the extractions (0.25, 0.30, and 0.35), and the product selling price (2x, 5x, and 10x). For the selling price of each product, an average was considered between small-scale product (Sigma-Aldrich prices) and large-scale product (Alibaba).

3. Results

3.1. Biomass characterization

To characterize the biomass, consecutive cycles of SLE were performed to extract astaxanthin and proteins. This was accomplished with distinct solvents that are more suitable to maintain the structure of the compounds intact. Therefore, acetic acid was used to extract the natural colorant whereas McIlvaine buffer pH 7.0 was applied to isolate total proteins as it is known that proteins are more sensitive to the solvent used as well as its pH. These results are shown in Fig. 1, evidencing that



Cycle	Astaxanthin (µg)
1st	165
2nd	27.5
3rd	4.3
Total	~197
	~98 µg astaxanthin / g shells

Cycle	Total proteins (mg)
1st	81.1
2nd	25.2
3rd	8.9
4th	4.5
5th	2.6
6th	1.6
Total	~124
	~62 mg proteins / g shells

Fig. 1. Determination of the total amount of astaxanthin (I) and proteins (II) present in the shrimp shells. The biomass used in these experiments was collected during summertime.

within 3 consecutive cycles of SLE, it was possible to completely extract astaxanthin from the shrimp shells, as represented by the almost colourless supernatant and biomass. This data shows that the biomass used presents *circa* of 98 µg of astaxanthin *per* gram of shells (standard deviation < 10 %). In contrast, to fully remove total proteins from the shells, it was required 6 consecutive extraction cycles, leading to the quantification of approximately 62 mg of proteins *per* gram of shells (standard deviation < 10 %). Considering the minerals and chitin content, the incineration results showed that the biomass used presents (323 ± 45) mg and (110 ± 7) mg of minerals and chitin *per* gram of shells, respectively.

3.2. Biomass fractionation

After the full characterization of the shrimp shells waste, the design of the multi-product pipeline applied to shrimp shell wastes started by the study of the extraction and purification of astaxanthin as the top priority due to its high economic value and its great sensitivity to heat, intense light, and oxidative conditions. Afterwards, the fractionation of the remaining compounds was carried out, namely chitin, proteins, and the remaining $CaCO_3$ present after its use in the jellification process to obtain the colorant (more details later in this section). In this sense, preliminary studies were performed to optimize the extraction and purification of the colorant. Typically, astaxanthin is extracted from crustacean wastes using a multi-step approach while applying moderate to high temperature and pressure. Contrarily to what is being applied elsewhere [37–42], in this work, we report only the use of industrially approved solvents and technologies of easy scale-up. A SLE was carried out at room temperature and ambient pressure while using more sustainable organic solvents with moderate to low toxicity [43], namely *n*-hexane, DMSO, acetic acid, ethyl acetate, acetone and ethanol. The extraction performance of these solvents is displayed in Fig. 2, alongside the macroscopic view of the supernatant resultant from the solid–liquid extraction. It is clear from this figure that all solvents under study were able to extract this colorant from the shrimp shell wastes, though with different performances. The solvent extraction performance followed the trend: DMSO < *n*-hexane < ethyl acetate < acetone ≈ ethanol < acetic acid. A plausible explanation for this tendence lies on the composition of the shells. Crustacean exoskeleton is known to be very rigid as it is made out of a 3-layered cuticle composed mostly of chitin with trapped proteins and minerals [24]. Herein, the extensive intra- and intermolecular hydrogen bonding within the chitin chains is crucial to confer the rigidity of the shells protecting the animals, while also being responsible for trapping minerals, proteins and the minor components present in the shells, such as astaxanthin. In this context, the performance of these solvents follows an increasing polarity trend, with acetic acetic being able to more easily disturb the shells' hydrogen bond network and allow the release of the different compounds composing the crustacean exoskeleton. Here, acetic acid was the best solvent as it not only allowed the extraction of a higher amount of astaxanthin (in other words, it is responsible for the highest extraction yield) but also kept the vibrant orange coloration. It is also interesting to see that, depending on

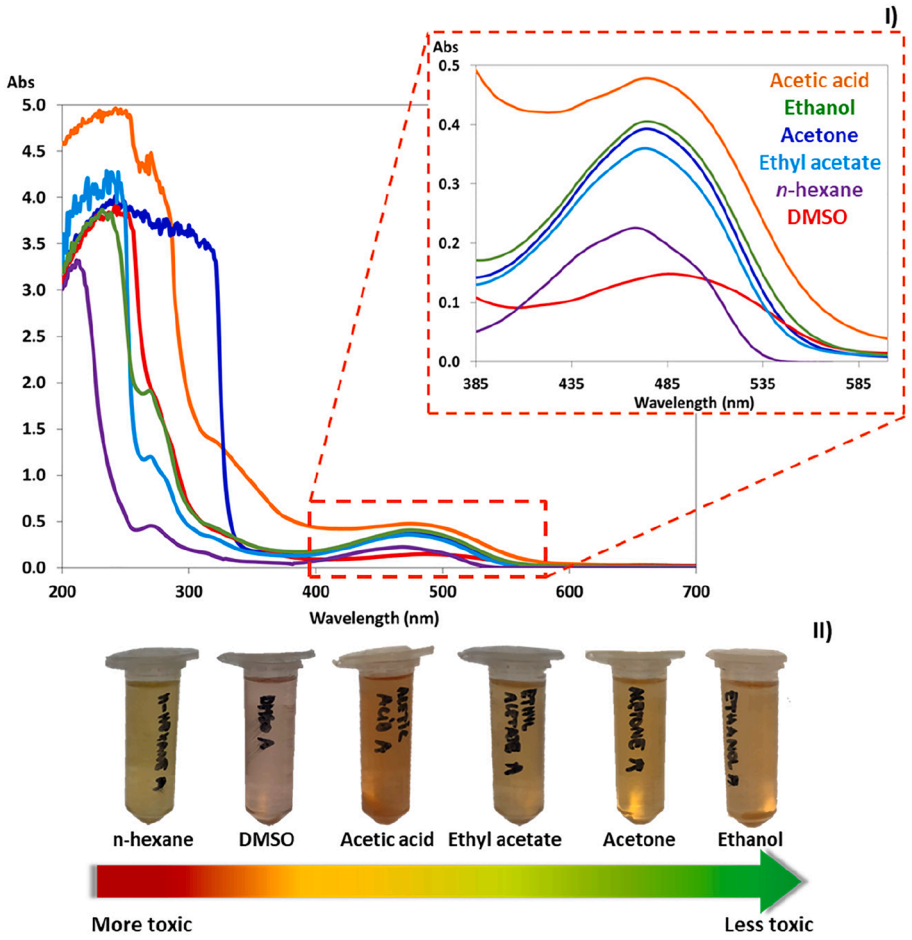


Fig. 2. I) Screening of different organic solvents upon their ability to extract astaxanthin from shrimp shell wastes: –, acetic acid; –, ethanol; –, acetone; –, DMSO; –, ethyl acetate and –, n-hexane. II) Supernatant of the solid–liquid extractions after 10 min of centrifugation.

the solvent, this natural colorant displays different shades of orange. DMSO presents a lighter coloration, as a result of the lowest amount of astaxanthin being extracted, but also leads to a solution with a darker orange colour with a maximum towards the red light. In contrast, *n*-hexane is able to extract almost the double amount of astaxanthin when compared with DMSO, although a light orange/yellowish colour of the extract is obtained. Ethyl acetate, acetone and ethanol resemble each other in both the extractive performance and the coloration of the supernatant, displaying a light orange colour. Therefore, acetic acid was the solvent selected to further optimize the SLE of astaxanthin. This solvent presents a usable classification according to the Pfizer solvent selection guide for medicinal chemistry [43], and a global demand of approximately 15 million tons *per* year with several applications in the chemical and food industries [44]. Acetic acid can be produced using chemical and biological (fermentation) methods, though the chemical manufacturing processes are still the predominant option due to the lower cost and the current high demand. Nevertheless, it is exactly this high demand allied with the global warm concerns that triggered researchers to focus more on the development of a sustainable and simple process to produce acetic acid [44]. In fact, chitin has been extracted from shrimp shells and converted into acetic acid [45], putting in practice two of the current crucial concepts: Biorefinery and Circular Economy. Furthermore, acetic acid is currently being used in food additives and food preservation, as an antimicrobial and artificial food ripening agent, acidulant, flavour and taste enhancer, not to mention its application in edible packaging materials [44]. Hence, acetic acid is a greener and attractive option for the extraction of astaxanthin from shrimp shell wastes.

3.3. Optimization of the solid-liqud ratio and extraction time

Once acetic acid has been selected as the solvent with the best performance, two other parameters affecting the SLE were studied using a 2^2 factorial planning, namely the solid–liquid ratio (SLR) and the extraction time on the acetic acid aptitude to recover astaxanthin (response variable). However, since this work aims at a first glance for the extraction and purification of this natural colorant (due to its highest value/cost), the proteins content being extracted was also analysed since these are simultaneously the main contaminants owing to their abundance. These results are shown in Fig. 3. Tables S5 and S6 show the experimental and theoretical results. In general, these results show a negligible difference between the predicted and experimental data. The regression coefficients, standard deviation, *t*-student, and *p*-values were also calculated and reported in Tables S7 and S8. As astaxanthin is the target compound, it is desirable to achieve the highest extraction yield possible, while extracting the lowest amount of proteins. In this sense, the most performant condition should be present in the red region in Fig. 3.I and II and, simultaneously, in the yellow to green region in Fig. 3.III and IV. A careful analysis of Fig. 3.II and IV indicates that, while the SLR is the most significant condition under study (also shown by the Pareto chart, Figure S2 in Supporting Information), both graphics present a mirror plane near 50 min of solid–liquid extraction. In other words, this means that, for a fixed SLR, the performance of the system will be approximately the same considering 30 min (mirror plane/50 min-20) or 70 min (mirror plane/50 min + 20), for example. Hence, from an economical and sustainable points of view, it is recommended the selection of a lower extraction time. Considering then the goal of this

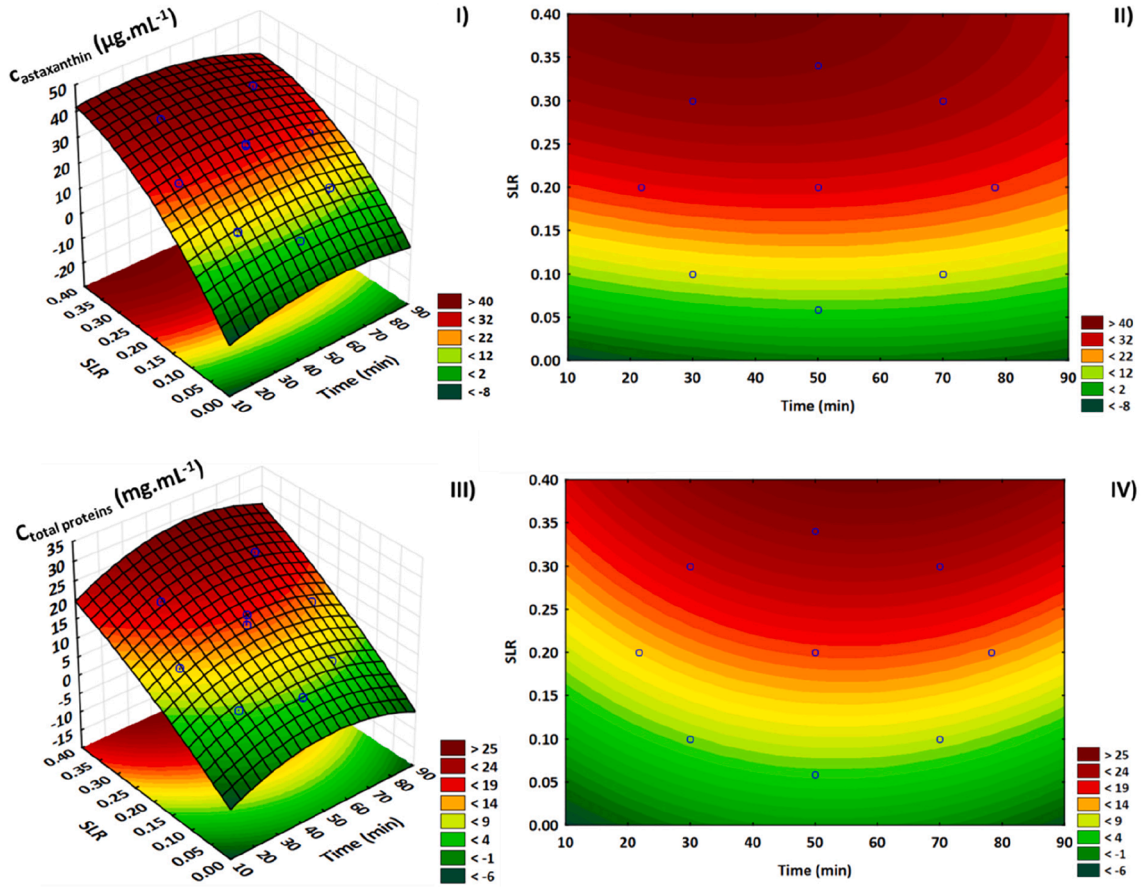


Fig. 3. Response surface plots (left) and contour plots (right) of the concentration of astaxanthin (I and II)) and proteins (III and IV) after the solid–liquid extraction with acetic acid. The biomass used in these experiments was collected during summertime.

work, the selection of a SLR of 0.20 and 20 min of extraction time seems to be the most promising approach, thus being used in further experiments.

3.4. Effect of different concentrations of acetic acid

It is well-known that aqueous solutions of a compound sometimes performs better than the pure solvent [46]. Therefore, a complete study was performed from 0 to 100 wt% of acetic acid. Once again, the concentrations of both astaxanthin and proteins were considered and presented in Fig. 4. Herein, it is also shown the colour of the biomass after the extraction as well as the supernatant obtained. According to Fig. 4.I, the amount of astaxanthin being extracted increases with the acetic acid concentration. The same behaviour is visible for proteins, though this effect is not as pronounced, reaching an equilibrium at 70 wt% of acetic acid. The extractive performance of astaxanthin is evident through the colour of the supernatant, where it is visible that, up to 50 wt% of acetic acid, there is almost no colorant being extracted and then, the colour becomes more intense and vibrant with the increase in the acid concentration. However, for the systems with 90 and 100 wt% of acetic acid there is no colour difference at naked eye, being the small difference only detected upon the absorbance analysis. Considering these results, the pure solvent using a SLR of 0.2 for 20 min at 25 °C was shown to be more efficient, thus being selected for further studies.

3.5. Astaxanthin polishing

Then, the biorefinery process was envisioned, considering the recovery of the remaining compounds constituting the shrimp shells waste. As previously discussed, the first compound to be isolated should be astaxanthin due to its higher sensitivity to intense light and oxidative conditions. However, to isolate and purify it, an extra step was planned to remove proteins from the extract as these are present in a content three orders of magnitude higher than the colorant. Therefore, three different methodologies were applied, namely precipitation, ultrafiltration and consecutive cycles of solid–liquid extraction. The proteins' precipitation was attempted using three conventional protocols*: i)* different concentrations of ammonium sulphate (30 and 40 wt%), *ii)* cold acetone and *iii*) trichloroacetic acid (TCA). However, none of these protocols worked, probably due to the absence of water in the proteins' sample. Then, Amicons with a cut-off of 3 and 10 kDa were used to perform an ultrafiltration by retaining the proteins and recovering astaxanthin in the permeate due to the considerable difference in molecular weight. Yet, this procedure did not work once again as the Amicon's membrane was not resistant to such high concentrations of acetic acid. Finally, consecutive solid–liquid extraction cycles were performed attempting first the proteins removal while using water as the solvent, since astaxanthin is almost insoluble in water owing to its hydrophobic character. The last cycle was carried out with pure acetic acid, as previously described. A schematic representation of the process is displayed in Fig. 5.I showing also the coloration of the supernatant and shells resultant from the solid–liquid extraction. It should be here highlighted that the shells obtained after the treatment with acetic acid present a much light coloration than what was shown in Fig. 4.II.

This is a result of a new batch of shrimp shells being used, obtained at a different period. It clearly evidences that the astaxanthin amount present in the biomass varies significantly according to the season. Hence, this cycle study was performed in November (2020) and March (2021), being the results shown in Fig. 5.II. Independently of the season, both astaxanthin and total proteins follow the same trend, namely

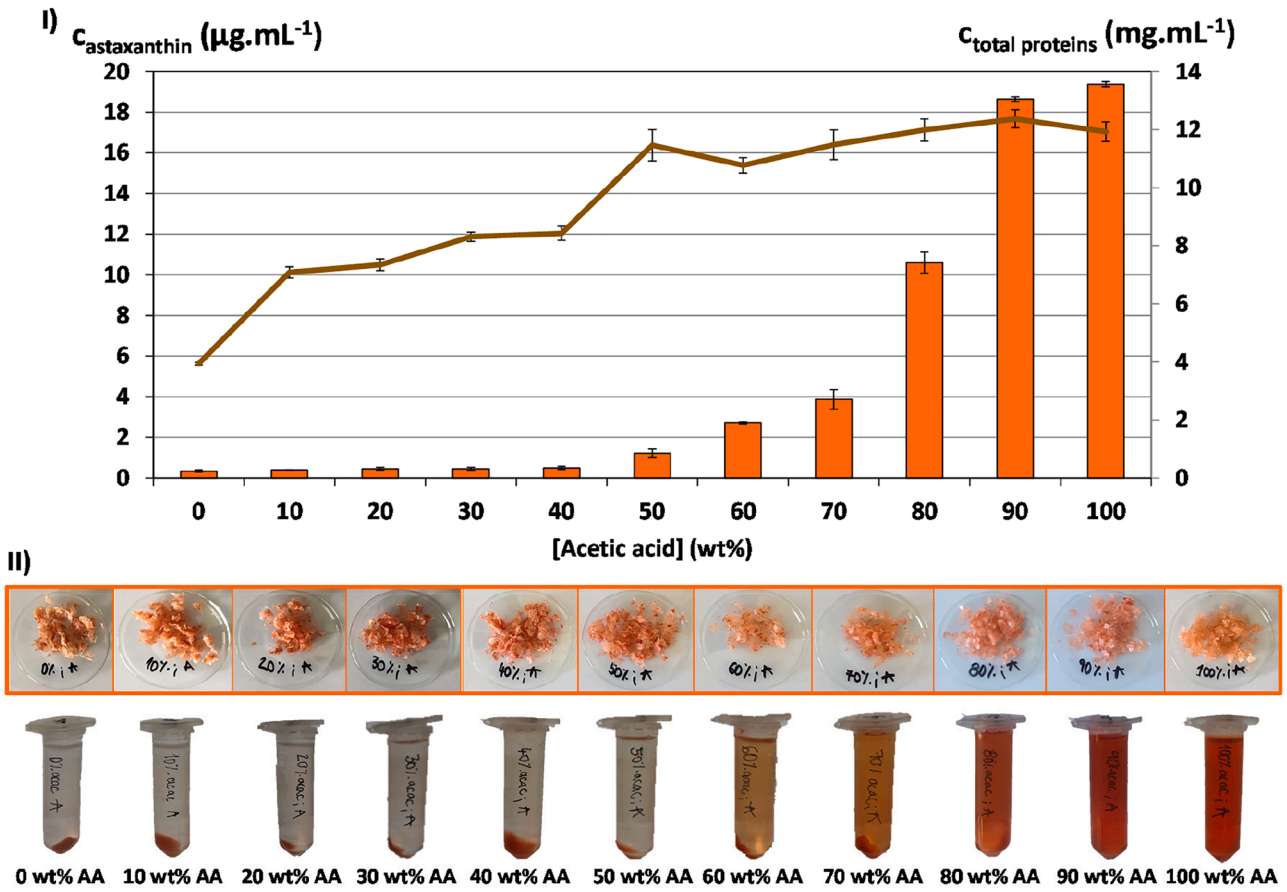


Fig. 4. I) Influence of the concentration of acetic acid (AA) upon the amount of astaxanthin (bars) and total proteins (line) being extracted during the solid–liquid extraction. II) Coloration of the biomass after the solid–liquid extraction (top) and the macroscopic view of the amount of astaxanthin being extracted from the shrimp shell waste (bottom). Biomass used was collected during summertime. SLR used was 0.2 and the extraxction lasted 20 min.

presenting the lowest concentration after the second cycle with water and the highest concentration when extracted with acetic acid. The main difference is that with November biomass, water always removed a negligible amount of astaxanthin, namely 0.49 ± 0.01 µg mL^{-1} and 0.5 ± 0.1 µg mL^{-1} in the first and second cycles, respectively. In contrast, the biomass from March allowed the extraction of 3.2 ± 0.2 µg mL^{-1} and 0.16 ± 0.01 µg mL^{-1} of astaxanthin in the first and second cycles, respectively. Nevertheless, astaxanthin was mostly extracted when acetic acid was used with a concentration of 7.4 ± 0.9 µg mL^{-1} and 9.3 ± 0.8 µg mL^{-1} using the November and March biomasses, respectively. These results evidence that the amount of astaxanthin present in the biomass in colder months is considerably lower (more than half) than the amount accumulated during summer time (*cf.* Fig. 4). According to Rødde *et al.* [9] astaxanthin content in shrimp shells varies from 14 to 39 mg kg^{-1} in wet samples during the year. On the other hand, total proteins seem not to be affected by seasonality as the overall amount being extracted is approximately the same in warmer and colder months (*cf.* Figs. 4 and 5). This data is in accordance to literature that show low variability during the year (33–40 % of the dry biomass weight) [9]. When the colder months are compared (Fig. 5), it can be observed that the amount of proteins being extracted during the 1st cycle with water in November is quite similar to amount extracted with acetic acid, *i.e.* ~ 5 mg mL^{-1}. In contrast, in March this amount triples when astaxanthin is extracted with acetic acid instead of water. This might not only be due to seasonality but also by the shrimps feed, as it is well known that organisms accumulate this carotenoid along the food hierarchy chain [47]. Overall, these results show a great variability in the amount of astaxanthin present in shrimps shells, which would already be expected with biological samples. Furthermore, these results also allow the conclusion that the 2nd extraction cycle with water should be avoided as it consumes energy to only retrieve $^{1}/_{10}$ of the total amount of proteins being extracted during the entire process. It is also evident that, even with various extraction cycles, it is not possible to obtain a protein-free astaxanthin extraction.

From the experimental data collected, it was apparent that, after a few hours at room temperature, or in some cases after sample storage in the fridge for a day, a side reaction was taking place leading to sample jellification. Borić *et al.* [48] have reported the use of aqueous solutions of acetic acid to perform the demineralization of shrimp shells. Hence, it was concluded that during the extraction of astaxanthin with acetic acid, we were also extracting $CaCO_3$. After some time (minutes to hours depending on the biomass batch), the solvent reacts with $CaCO_3$, resulting in a jellified sample. In fact, it was found that this jellification process was crucial to obtain an astaxanthin protein-free sample. However, it should be stressed that if the $CaCO_3$ content in the shells are lower, it takes longer time for the jellyfication process and the sample should be stored at 4 °C until it does. At this stage, if the astaxanthin extract is diluted four times with water and centrifuged at moderate to high speed, it leads to astaxanthin precipitation, while keeping proteins dissolved in water. These results are present in Table S9 in Supporting Information. Firstly, it was considered one extraction cycle with water and then, a second cycle with acetic acid. Afterwards, the jellified astaxanthin-rich extract was precipitated with water and centrifuged, being the pellet resuspended in three different solvents, namely acetic acid, ethanol and ethyl acetate. These results show that the water supernatant barely presents any astaxanthin but presents almost all the proteins remaining intact after storage at 4 °C. Moreover, when acetic acid, ethanol and ethyl acetate were used to resuspend the astaxanthin

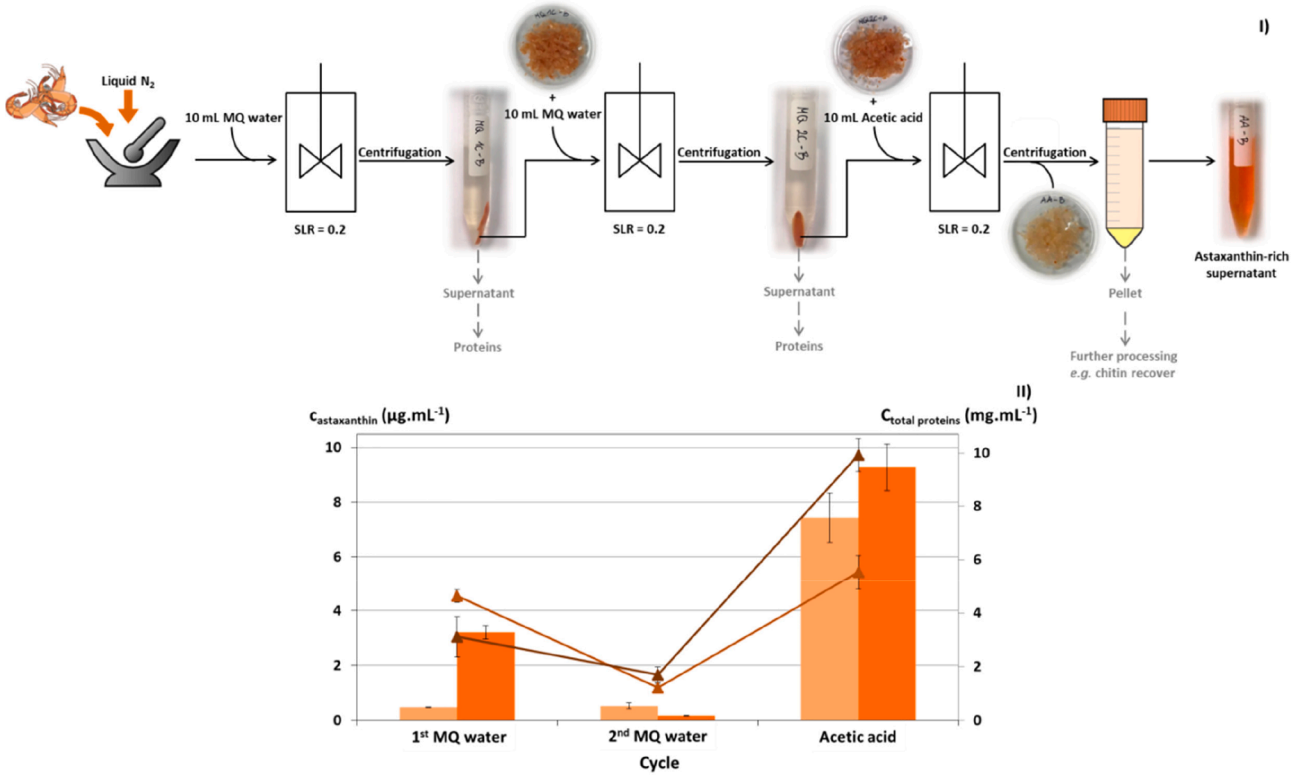


Fig. 5. I) Schematic representation of the consecutive solid–liquid extraction cycles to firstly remove the proteins' content from the extract and isolate astaxanthin at the end. II) Concentration analysis of astaxanthin (bars) and total proteins (line) after each cycle. Light colors correspond to the biomass collected in November 2020 whereas darker colours represent the shrimp shells from March 2021.

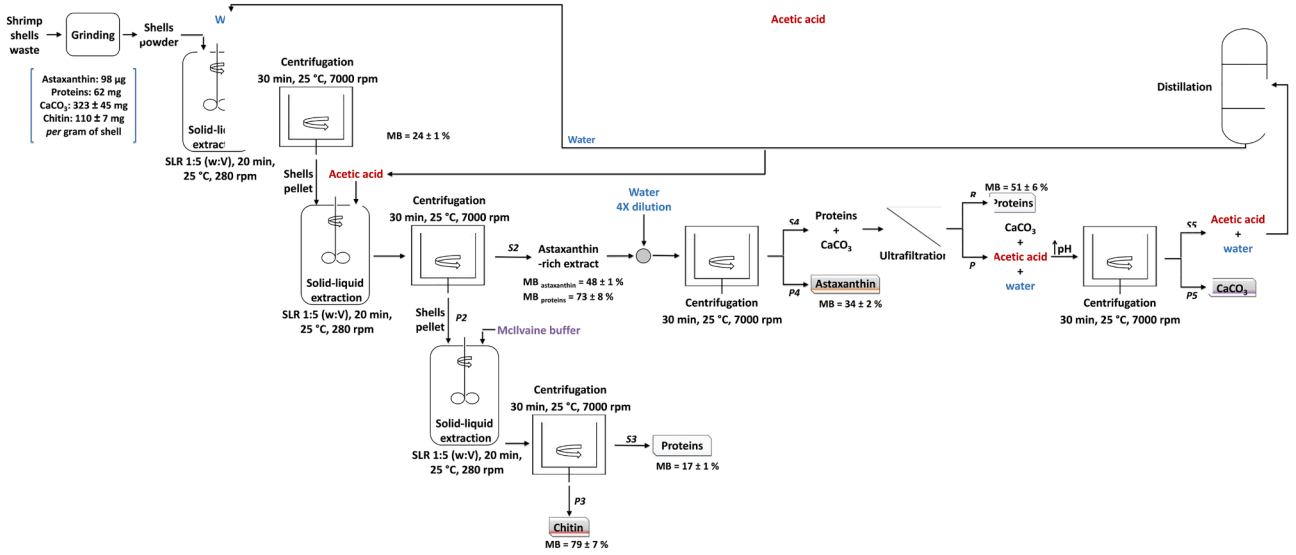


Fig. 6. Proposed integrated platform representing the multi-product pipeline biorefinery of shrimp shells waste, with solvents recycling. MB means mass balance.

Table 1
Mass balance of astaxanthin, proteins and chitin in the different steps of the downstream process. The mass balance of $CaCO_3$ is not shown since it is part of the step to extract astaxanthin.

	Process step	Astaxanthin	Total proteins	Chitin
Mass Balance (%)	(S_1 + S_3 + R)	—	92 ± 6	—
	P_3	—	—	79 ± 7
	P_4	34 ± 2	—	—

pellet, it is evident that acetic acid is again the best solvent. It should also be noted that after astaxanthin resuspension, there was still present a very small amount of a white pellet, which can be some residual content of proteins precipitated during the resuspension of the colorant or some $CaCO_3$ precipitation. Despite the efforts made to resuspend this white pellet, none of the solvents used were found to be appropriate.

In the end, the best results achieved (March) were also analysed with HPLC-DAD for a more accurate quantification of astaxanthin. As shown in Table S9 in Supporting Information, the amount of astaxanthin detected is approximately half of the amount detected using UV–Vis. A plausible explanation for this resides in the fact that the colorant quantification was performed using a calibration curve with free astaxanthin, which presents a retention time around 4 min, as can be seen in Figure S3 in Supporting information. However, all the chromatograms obtained after astaxanthin extraction from shrimp shells show two other peaks at ~ 11 and 12 min, which correspond to astaxanthin in a mono- and diester forms, respectively, as previously discussed [49,50].

A final integrated process was proposed as shown in Fig. 6, with the principal results depicted in Table 1. Herein, simple, and easily scalable techniques were integrated to guarantee the extraction and separation of the target compounds, namely by using SLE, centrifugation, precipitation, and ultrafiltration. The biomass follows a linear extraction process of three consecutive cycles of SLE and centrifugation steps to recover the pellet and allow the fractionation of the different compounds, leaving chitin as the final pellet. This allowed the isolation of 79 ± 7 % of the chitin present in the shrimp shells. In the first cycle of SLE, the extraction is performed with water to allow solely the extraction of proteins (fraction S_1), while the pellet moves towards the second SLE with acetic acid. This supernatant (fraction S_2) is rich in astaxanthin, proteins and $CaCO_3$ (*cf.* Table S10 in Supporting Information). Indeed, it is the reaction between the $CaCO_3$ and acetic acid that leads to the jellification of the supernatant (after a few hours up to 4 days, depending on the shell composition in $CaCO_3$), which represents the critical step in the process. Once the supernatant is a gel (composed of calcium acetate [$Ca(CH_3COO)_2$] produced after the reaction between $CaCO_3$ and acetic acid), this can be diluted 4 times and centrifuged, allowing the complete isolation of more than 34 ± 2 % of astaxanthin in a pellet form (P_4). We highlight however, that most of the $CaCO_3$ (*circa* of 99%) is used in this step. This means a loss in its content, although this is not problematic considering the comparison of market prices between $CaCO_3$ and astaxanthin. The corresponding supernatant (fraction S_4) is composed of proteins and just a small content in $CaCO_3$ at *circa* of 4.0 ± 0.6 mg. g_{shells}^{-1} (value determined through Total Reflection X-ray fluorescence – TXRF; *cf.* Experimental section), which are further separated using an ultrafiltration, allowing to separate more 51 ± 6 % of proteins. Afterwards, by simply alkalizing the medium overnight, the remaining $CaCO_3$ present in fraction P was precipitated (fraction P_5). This fraction was further analysed using X-ray diffraction (XRD) and identified as amorphous $CaCO_3$, as represented in the XRD pattern found in Figure S4 in Supplementary Information. Once the supernatant has been collected, the solvents (acetic acid and water) can simply be separated by distillation and reused in new extraction cycles.

Finally, by performing a third cycle of SLE with McIlvaine buffer at pH 7.0, it is possible to recover the remaining part of proteins present in the biomass (fraction S_3). Therefore, 92 ± 6 % of proteins were obtained in the process (sum of fractions S_1, S_3 and R), and stored in a McIlvaine buffer solution by performing a simple ultrafiltration to easily maintain their structure intact.

To the best of our knowledge, this is the first time a full biorefinery approach has been envisioned for the waste management of crustacean shells, in particular, for shrimp shells wastes. Therefore, it is not possible to directly compare our results with literature. Until now, crustacean shells have only been industrially explored for the extraction and isolation of chitin. However, this has been carried out using a conventional and harsh protocol with high concentrations of acid and base at elevated temperatures to perform demineralization and deproteination, being followed by the decoloration of chitin with hazardous organic solvents [51]. The alternative often employed is the use of enzymatic methods, yet these are costly and with relatively low yields [51]. Regarding the extraction of astaxanthin, it is commonly performed using microalgae while applying organic solvents (acetone, methanol, ethanol, DMSO, ethyl acetate), acids and edible oils, and/or by microwave-assisted, ultrasound-assisted and enzymatic methods [47]. However, it is not always easy to achieve an efficient, biocompatible and cost-effective approach to disrupt these rigid cell walls, which are completely different from the crustacean shells. When crustacean shells waste is used for the isolation of astaxanthin, a few reports using neoteric solvents such as ionic liquids [52] and deep eutectic solvents [53,54] have been described. Nevertheless, these extraction protocols were combined with an ultrasound-assisted approach, which is not simple to apply at an industrial scale. Overall, this biorefinery platform seems to be one of the most efficient, biocompatible, sustainable and cost-effective technologies with a feasible scale-up up to date. Hence, being a realistic business model to contribute the current bioeconomy approach, as proved below.

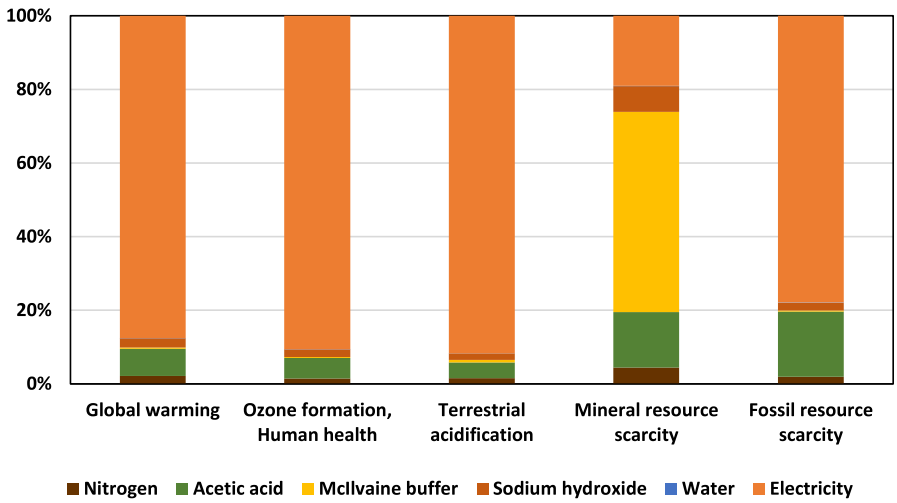


Fig. 7. LCA results for the biorefinery process to obtain astaxanthin, total proteins and chitin *per* gram of shrimp shells.

3.6. Environmental analysis

The Life Cycle Assessment (LCA) considers the different steps of the biorefinery required to obtain the different products from the biomass, including the protein-free astaxanthin in a powder form. Fig. 7 and Table S11 in Supporting Information present the LCA data for five impact categories, showing that most (78–92 %) of the impacts related with global warming (*i.e.*, carbon footprint), ozone formation, terrestrial acidification and fossil resource scarcity are due to electricity consumption. McIlvaine buffer production dominates the impacts related with mineral resource scarcity mainly because of phosphorus reserves depletion.

It should be noted that the amount of electricity consumed represents the worst-case scenario as it was estimated based on the nominal power of the equipment over the full period of utilization. Lower electricity consumption values would be expected at the industrial scale, which means that there is still a high potential to decrease the environmental impacts of the process. Besides, the use of cleaner energy sources could improve even more the environmental performance of the process. For instance, at laboratorial scale, the carbon footprint of the process would be reduced by 90% if photovoltaic electricity was used instead of the electricity production mix considered, which relies on a 55% contribution from fossil fuels (mainly coal and natural gas).

3.7. Economic analysis

Part of the data required to assess the shrimp shells waste biorefinery, as a business model, is the downstream process economic evaluation. The data used are a collection of values for the integrated production cost, individual production costs and potential Return. It is worth mentioning that the analysis included $CaCO_3$ contribution to the integrated process cost, but not studies as an individual product since at the end of the process, only 1% is recovered due to its reaction in the jellification process, which is crucial for astaxanthin isolation. The first analysis performed comprised the evaluation of the impact that causes a change in the production scale (1, 10, 100, and 1,000 kg of shrimp shells) in the base model created, as the scale-up process is not a simple linear process [55]. The corresponding results are shown in Fig. 8. Fig. 8.I includes the results for the integrated production cost and the potential Return, while Fig. 8.II shows the individual production cost for each product (astaxanthin, proteins, and chitin) considering the multi-product process. Considering the base model constructed, the integrated production costs are high enough, at all scales, to provide any positive Return. Considering past reports, the production cost stabilizes as the scale increases mainly because the cost of equipment tends to stabilize and the amount of final products increases, thus increasing the financial Return of the process [34,56]. Moreover, this is emphasized by the increase in the percentage that the contribution of the capital represents in the total production cost (Fig. 8.III).

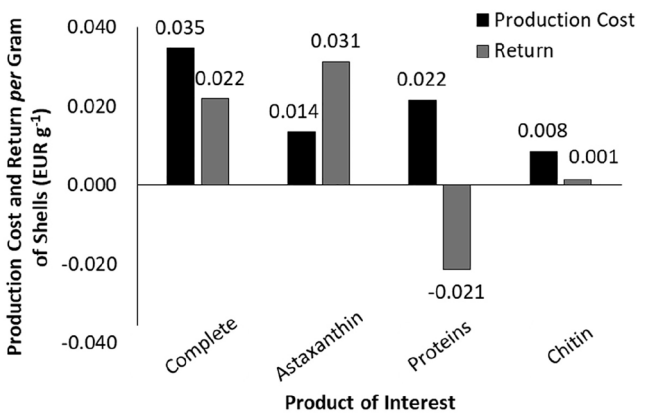


Fig. 9. Contrast between production cost and return considering the bioprocess developed to obtain all 4 bioproducts ("complete") or the bioprocess to generate a single product.

As mentioned in the model construction section, the details used for the base model considered that every single unit operation contained a unique equipment, but in practice some unit operations require equipment that can be shared. Thus, to account for some large-scale consideration, the model was modified to accommodate the equipment removal when duplicated. Moreover, the model was also updated to include decreased costs for materials aiming to mimic bulk acquisition using prices available from Alibaba. Moreover, process time was also modified to be extended for centrifuge processes to 8 h (except for the final two which were extended to 16 h), this allows to mimic the processing behaviour during work shift patterns. Furthermore, the increase in production time also impacts the size requirement for centrifuges and ultrafiltration as it gives more time to process larger volumes by

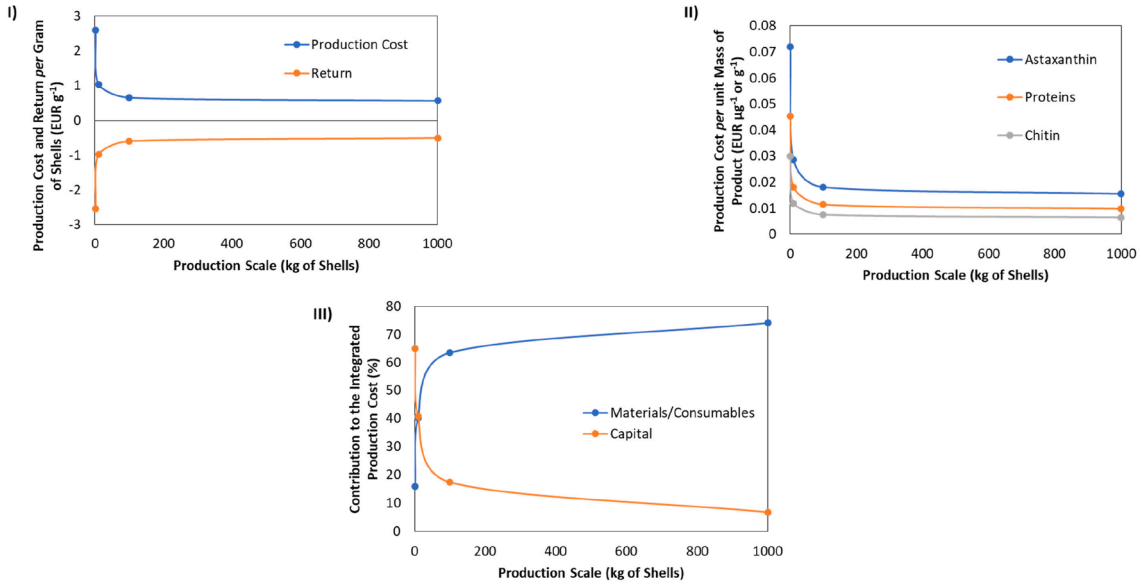


Fig. 8. Results for the scale analysis. I) shows results for the entire production cost and potential return *per* gram of shells processed and II) shows the production cost *per* unit mass for each product of interest. The contribution of the capital and materials/consumables is shown in III).

decreasing flow rates and membrane flux (and membrane area), respectively.

After completing the large-scale model modifications, the model was modified to provide individual production costs for each product but only considering the specific unit operations needed for each product. The first analysis performed contrasted the production cost and Return of the integrated process and the individual processes (Fig. 9). These results show that the highest production cost is obtained for the integrated process, which was expected as it is the longest process and, therefore, includes more unit operations, more equipment, which in turn is translated to more capital; utilizes more materials and has a longer duration. The least expensive process is the solely isolation of chitin, as it encompasses the least amount of unit operations. Together with the potential of chitin sold as a commercial product is enough to provide a low positive return. The production of proteins is similar to the complete process as the output streams are connected to chitin and astaxanthin, while it has almost the same process length as with calcium carbonate. This provided a high production cost and negative return. Alternatively, astaxanthin provides a large production cost, but its high selling price (1.21 EUR.mg^{-1}) allows it to generate a large positive Return. Contrasting the single-product and integrated processes, generating only astaxanthin is a more attractive option from an economic perspective, but an integrated process will have the advantage of reducing wastes that could potentially further reduce production costs, although this is beyond the scope of this study. Moreover, the integrated process could explore other venues for exploitation of proteins. The small amount of $CaCO_3$ could also be used to increase the Return. Hence, by selling these products, *i.e.* proteins and $CaCO_3$, not as a final product but as part of circular economy and resource efficiency strategies by reducing the cost of aquaculture through the modification of water hardness and alkalinity, this approach is considered as part of the integrated process cost and Return [57,58].

This parameter allowed for a reduction of the production cost (and increase of Return), but after the 2nd scenario evaluated, its contribution became the opposite. This behaviour can be explained by the contribution of the process time to the design of the size of the ultrafiltration operation. At small process times, each unit operation will need to process material faster, which for the ultrafiltration step means the need for a higher membrane area, which is an indication of the dominance of this consumable towards the production cost. When the process time becomes larger, the membrane area required decreases and the process gets more profitable. However, there is a specific point, where the extension of the process time will not impact anymore the available batch *per* year, which will in turn generates a lower amount of product, allowing the production costs increase, and the consequent profit decrease.

After, a sensitivity analysis in the large-scale model was performed to determine the impact of improving the process and commercial parameters (Fig. 10). This analysis contrasted again the production cost *per* unit mass of processed shells waste (Fig. 10.I) and the potential improved Return (Fig. 10.II and 10.III – zoom-in of low Return section). The incorporation of the selling price into this analysis allowed to determine how a potential increase in the commercial parameter can influence the process. As expected, an increase in commercial price of any product will not impact the production costs, but it will greatly improve the Return. Another interesting result was obtained when reducing the process time.

Finally, the results show that, for the process we developed, it is essential to increase the scale to obtain the full effect of economy of scale and purchase discounted materials in bulk. Moreover, it is critical to maintain a reduced amount of protein contaminants to increase the commercial price for future product being sold.

3.8. Shrimp shells' biorefinery pipeline as a business

Even though it is true that the waste biorefineries have been steadily increasing over the past years (mostly due to the adoption of the 2030 Agenda for Sustainable Development that obliges individual countries to take actions aimed at achieving the 17 goals of sustainable development) the same trend is not observed yet for the specific case of marine waste biorefineries [59].

To move from the concept of a marine waste biorefinery into a business model, several parameters need to be considered, namely the *i)* choice of location for the biorefinery implementation, *ii)* feedstock, *iii)*

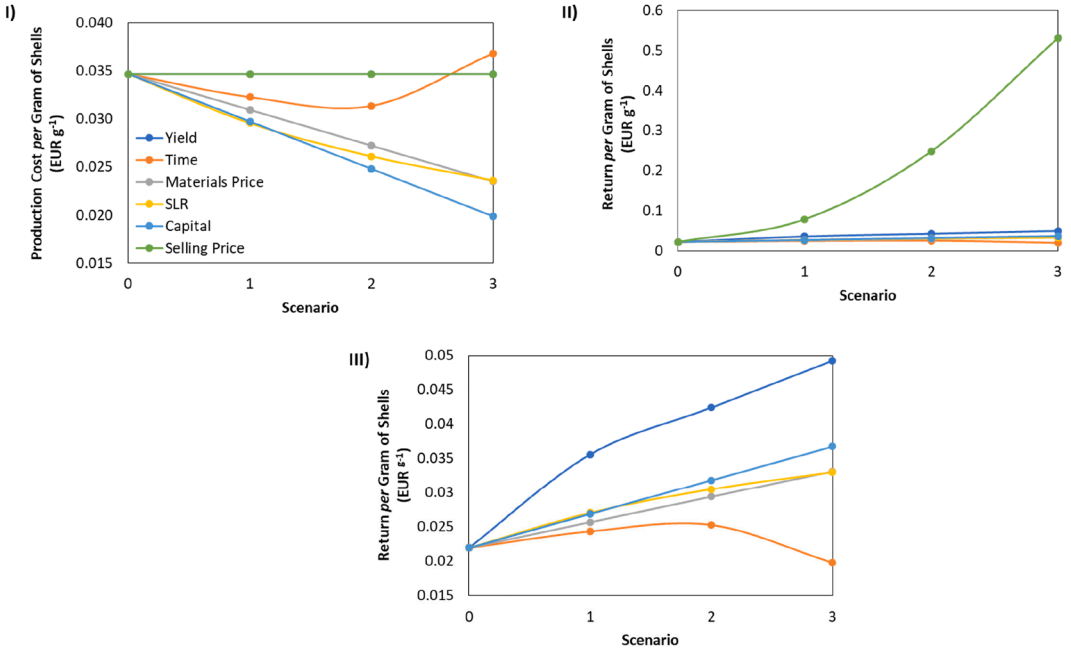


Fig. 10. Production costs and return results for the sensitivity analysis considering the large-scale (1,000 kg) base scenario (complete bioprocess for the 4 products). Sensitivity analysis included variations in the variables: Recovery yield, process time, price for materials, solid–liquid ratio for extraction operations, capital input and the products selling price. I) shows production costs *per* gram of processed shells, II) shows the return *per* gram of processed shells, while III) shows the same results as II) but removing the impact of selling price to be able to zoom-in into the impact of the process variables.

conversion/downstream process, and *iv)* type and number of final products. Considering the feedstocks, not only the price variation but also their abundance are key factors for the success of a biorefinery, which in this work is an advantage considering that the biorefinery is fed with seafood wastes, with very low commercial value and for which price fluctuations are not expected. Abundance is also guaranteed being our raw material a residue with an annual production of 750 000 tons in Europe but ranging from 6 to 8 million tons of waste worldwide. In terms of conversion/downstream technology, we proved the efficiency of our process. It is simple, which allows less residues and losses during the process, and with low environmental impact (as shown by the LCA data). Furthermore, it permits to obtain 3 products with high commercial interest (for example in food, feed, pharma, cosmetics and nutraceuticals fields), being this the key for the success of this business model. From the analysis done, we proved a positive Return when chitin, proteins, and astaxanthin are considered as final products, being the process scale-up mandatory for the expected Return to be higher. Finally, but no less important, is the location selected for the implementation of the biorefinery business. It should be balanced considering factors like the proximity to seafood companies, the price of human resources, electricity and taxes, the presence of good transport networks, just to mention a few. To have a broader idea on the potential of our multi-product biorefinery as a successful business model, other factors were analysed and detailed on the CANVAS represented in Figure S5. With this work, we show the significant potential of shrimp shells waste biorefinery and for that we have defined our vision for the business by detailing the key partners, activities, resources & costumers, the principal costs, and revenues.

As an attempt to demonstrate the commercial potential of this biorefinery model, we have done a simple calculation considering the United Kingdom (UK) as an example. According to information collected by Seafish (Sea Fish Industry Authority was established by the Government in 1981 and is a non-departmental public body created to support the seafood industrial sector in the UK), in 2021 there were *circa of* 344 seafood processing sites across UK [60]. From the last reports, the amount of crustaceans' wastes produced annually by the UK is in average around the 15 000 tons (which include shells waste from crab and Nephrops) [61] and spend around 70 EUR *per* ton (this value represents £60 and was calculated according to the exchange rate in November 2021) to discard these wastes [61]. Considering both, the biorefinery process we developed and the average market price of astaxanthin (1.2 $EUR.mg^{-1}$), proteins (2.12 $EUR.kg^{-1}$) and chitin (0.113 $EUR.g^{-1}$) (for more details on how we came up with these prices please consult Table S3 in Supporting information), we did a simple calculation to understand better the profit generated from this biorefinery process as a business. From the last data reported, the UK has 344 industries producing crustaceans wastes and an average of 15 000 tons *per* year are produced. In this sense, and to simplify the calculations, we considered that, on average, each company produces around 44 tons of shells waste *per* year. Instead of being discarded, these 44 tons of residues may be sold as feedstocks, which implies saving money, not only because their discard is no longer needed (with approximated costs around 3 080 EUR *per* year), but also because by selling the shells as feedstock, some economic revenue is obtained. By implementing this biorefinery process as a business model, and considering the data obtained in the present work from the biomass characterization in terms of the average amount of each product obtained and the average price of each product in the market (Table S3 in Supporting Information), we can predict the annual profit obtained from the multi-product process (Equation (7)). If processing the 44 tons, the annual profit achieved is around 1 337 902.9 EUR, while by processing the annual amount of wastes produced in UK (15 000 tons), a total profit around 469 852 404.0 EUR is envisioned, which clearly demonstrates the economic benefits of this biorefinery process as a cost-effective business, driven our future to a sustainable ocean-based bioeconomy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2022.135937.

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