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Bioprocessing of Shrimp Waste Using Novel Industrial By-Products: Effects on Nutrients and Lipophilic Antioxidants

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Abstract: The production of marine foods is on the rise, and shrimp is one of the most widely consumed. As a result, a considerable amount of shrimp waste is generated, becoming a hazardous problem. Shrimp waste is a rich source of added-value components such as proteins, lipids, chitin, minerals, and carotenoids; however, new bioprocesses are needed to obtain these components. This work aimed to characterize the chemical and nutraceutical constituents from the liquor of shrimp waste recovered during a lactic acid fermentation process using the novel substrate sources whey and molasses. Our results showed that the lyophilized liquor is a rich source of proteins (25.40 ± 0.67%), carbohydrates (38.92 ± 0.19%), minerals (calcium and potassium), saturated fatty acids (palmitic, stearic, myristic and lauric acids), unsaturated fatty acids (oleic acid, linoleic, and palmitoleic acids), and astaxanthin (0.50 ± 0.02 μg astaxanthin/g). Moreover, fermentation is a bioprocess that allowed us to obtain antioxidants such as carotenoids with an antioxidant capacity of 154.43 ± 4.73 μM Trolox equivalent/g evaluated by the ABTS method. Our study showed that liquor from shrimp waste fermentation could be a source of nutraceutical constituents with pharmaceutical applications. However, further studies are needed to separate these added-value components from the liquor matrix.

Keywords: astaxanthin; shrimp waste; carotenoids; fermentation

1. Introduction

Marine foods production reached 178.5 million tons in 2018, of which 11.4% corresponds to crustaceans [1]. Shrimps are the most economically important crustaceans; in 2018, México was the seventh-largest producer of shrimp [2]. During shrimp consumption around 40–50% (*w*/*w*) is considered waste; this causes significant biomass, which is a source of pollution as it is often thrown into the sea [3]. In addition, shrimp waste is a source of added-value components such as protein (35–50%) [4], chitin (15–20%), minerals (10–15%), lipids, flavor compounds, and pigments such as astaxanthin [5–7].

In this sense, researchers have focused on extraction methods that involve the use of organic chemicals, which are hazardous [4,8,9]. On this subject, lactic fermentation has been reported as a safe, technologically flexible, economical, and eco-friendly method to recover bioactive compounds from shrimp waste [4,7,10]. Lactic fermentation of shrimp waste produces the release of lipophilic compounds, as well as the denaturation of proteins,

and solubilization of $CaCO_3$ in the form of calcium lactate due to the decrease in pH by the production of lactic acid from lactic acid bacteria, in addition of the proteolytic enzymes of the microorganisms used [7,10]. In this sense, there are several works in the literature where different lactic acid bacteria such as *Bacillus cereus*, *Enterococcus faecalis*, *Lactobacillus brevis*, *Lactobacillus casei*, and *Lactobacillus paracasei* were used, as well as carbon sources (glucose and sucrose) to carry out the lactic fermentation of shrimp waste at 10–50 °C and different fermentation times ranging from hours to weeks [7,10,11]. Moreover, other industry by-products like whey (predominantly contains lactobacilli and streptococci) and molasses (contain sucrose, glucose, and fructose) have been study as lactic acid bacteria and carbon sources, respectively [12,13]. At the end of the lactic fermentation process, two phases are obtained: a solid one (which consists of partially purified chitin) and a liquid one (composed of proteins, lipids, carbohydrates, and carotenoids); the latter also called liquor [7,8].

The liquor phase resulting from fermentation is rich in lipophilic pigments with antioxidant capacity such as astaxanthin, which possesses antioxidant activity up to 100–500-fold higher than other antioxidants like α-tocopherol and β-carotene [3,14]. Likewise, astaxanthin has been related to anti-inflammatory, antidiabetic, antiproliferative, and UV-protective effects [3,15–17]. Nonetheless, the studies regarding the valorization of bioactive compounds of liquor from shrimp waste fermentation are scarce. Moreover, as far we know, industrial by-products like whey and molasses have not been used for lactic fermentation of shrimp exoskeleton to recover bioactive compounds. Thus, this study aimed to evaluate the chemical and nutraceutical characteristics of a liquor produced from fermented shrimp waste using novel substrates as whey and molasses.

2. Materials and Methods

2.1. Reagents and Chemicals

The total dietary fiber kit consisted of a thermostable α-amylase (20 mL, 3000 U/mL; 10,000 U/mL on soluble starch) (Megazyme. E-BLAAM), a purified protease (20 mL, 50 mg/mL; ~350 tyrosine U/mL) (Megazyme E-BSPRT), and a purified amyloglucosidase (20 mL, 3300 U/mL on soluble starch) (Megazyme E-AMGDF). Fatty Acid Methyl Esters (FAME) mix C4-C24 external standard (Sigma 18919). ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate)) (Sigma A1888), TROLOX (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) (Sigma 238813). The commercial mixture of lactic bacteria (*Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactococcus lactis*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, and *Leuconostoc mesenteroides*) for cheese (Bioprox, Ika-lac, DF, México). The rest of the reagents mentioned in the manuscript were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Biological Material

The shrimp exoskeleton (*Litopenaeus vannamei*) was purchased from a local market in Culiacán, Sinaloa, México. Shrimp (*L. vannamei*) waste was washed and ground to start the fermentation process. Molasses was obtained from a sugar cane processing facility from Eldorado, Sinaloa, México, then placed in containers and stored at room temperature until use. The commercial mixture of lactic bacteria was inoculated with 3785 mL of commercial milk, whey was separated from cheese and stored at room temperature until use.

2.3. Lactic Fermentation

The lactic fermentation process was carried out as previously published Cabanillas-Bojórquez, et al. [18]. Briefly, ground shrimp waste (400 g) was mixed with the recovered liquid fraction from the grinding; then, molasses and whey were added at a ratio of 1:5 w/v to shrimp waste. The volumes of whey and molasses were obtained by material balance (Equations (1) and (2)) to adjust 8.4 °Brix for a final volume of 2 L in a Batch reactor (BioFlo 120 Eppendorf, HH, DE) at 20 °C without agitation in anaerobic conditions, and 108 h fermentation time. It is important to highlight that this modified method is under a

patenting process (RGP-DDAJ-27446). After the fermentation process, liquor was lyophilized at −50 °C and 0.070 mPa for 7 d in a freeze dryer (LABCONCO FreeZone 18 with Bulk tray, MO, USA). The lyophilized solid was stored under darkness at −20 °C (Figure 1).

$$2000 = W + M \quad (1)$$

$$16{,}800 = Bw * W + Bm * M \quad (2)$$

where: 2000 is the total volume used in mL; W is referred to whey volume in mL; M is molasses volume; 16,800 is total volume (2000 mL) multiplied by 8.4 °Brix; Bw is total soluble solids of whey in °Brix; and Bm is total soluble solids of molasses in °Brix.

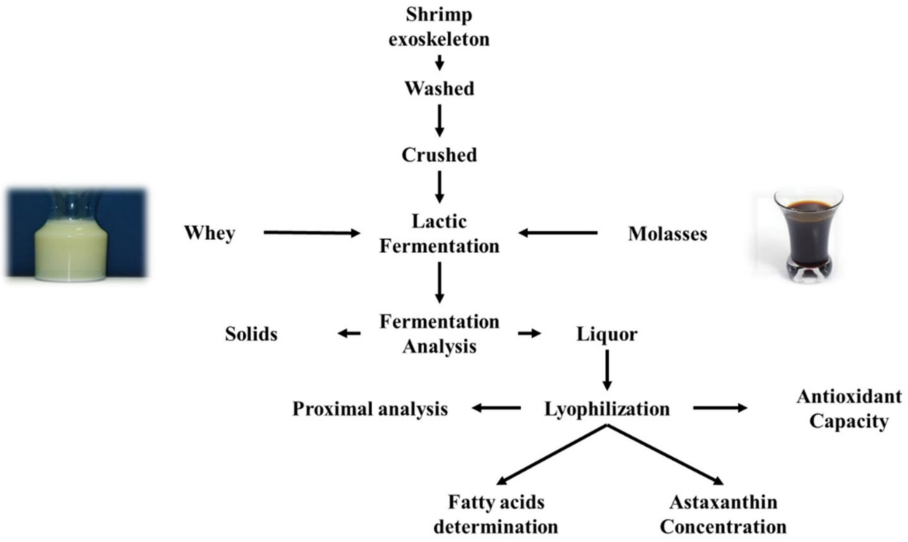

Figure 1. Process diagram to produce the lyophilized liquor from fermented shrimp waste.

2.4. Analysis of Fermentation Liquor

The liquor pH was measured using a potentiometer (Apera Instruments, PH700, Columbus, OH, USA). Liquor samples of 10 mL were centrifuged (Thermo Scientific, Legend XTR, Waltham, MA, USA) at 3000× *g* for 15 min at 25 °C. The supernatant was collected and diluted (1/10 *v*/*v*) [11,19]. Total soluble solids of supernatant liquor samples were determined following the official method 22.014 [19] by direct measuring in a digital refractometer (Mettler Toledo, RE40D, CDMX, México). The results were expressed as degree Brix. Likewise, total titratable acidity was determined following the official method 942.15 [19]; the supernatant liquor samples were potentiometric titration with 0.1 N NaOH until a pH of 8.4 using a potentiometer (Apera Instruments, PH700, Columbus, OH, USA) [11]; the results were expressed as grams of lactic acid equivalent per liter (g lactic acid/L). Total sugars were determined following the methodology of Devindra [20] with slight modifications. A total of 200 µL supernatant liquor samples were made up to 500 µL with distilled water and mixed with anthrone reagent; the mixture was placed in a boiling water bath for 8 min and cooled rapidly. The absorbance at 620 nm was measured in a spectrophotometer (JENWAY 7305, Staffordshire, UK). A glucose curve was constructed as a standard, and the results were expressed as milligrams of glucose per milliliter (mg/mL).

On the other hand, an isolation study of the lactic acid bacteria from the fermented liquor was carried out at the end of the process following the methodology of Velázquez-López, et al. [21]. One mL of supernatant liquor was used for serial dilutions (1:10, 1:100, 1:1000, 1:10,000, and 1:100,000) with sterile water, and 100 µL were inoculated in potato dextrose agar (PDA) plates. All inoculated Petri dishes were incubated at 37 °C for 72 h. The results were expressed as colony-forming units (CFU)/mL. Furthermore, different morphological colonies were selected for subculture by cross streaking on PDA plates, and the Gram stain technique was performed on the isolated colonies.

2.5. Proximal Analysis of Liquor

Moisture, ash, protein, lipids, total dietary fiber, and carbohydrates contents were determined by the official methods 925.098, 942.05, 988.05, 920.39, and 32-05.01 of the AOAC [19], respectively. All the results were expressed as mean percentage ± standard deviation.

2.6. Mineral Composition

Lyophilized liquor ash was used for mineral composition by atomic absorption spectrometry (AA FS flame AA 280FS + SIPS 20, Agilent Technologies, CA, USA) following the official method 985.35 of the AOAC [19]. The result was expressed as milligram per kilogram (mg/kg).

2.7. Determination of Fatty Acids

According to the methodology reported by Pretorius and Schönfeldt [22], the fatty acids from lyophilized liquor were extracted with slight modifications. A sample of 0.15 g was converted to methyl ester by transesterification. The fatty acid methyl esters were identified using an Agilent 7890 Gas Chromatograph with a capillary column Agilent DB-23 (30 m length, 0.25 mm internal diameter, and 0.25 um film thickness) and flame ionization detector. Helium, at 40 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Fatty acid methyl esters were identified by comparing the retention times of the peaks from samples with those of standards obtained from the FAME mix (18,919, Sigma-Aldrich, St. Louis, MO, USA). Fatty acids were expressed as a percentage of each individual fatty acid of the sample.

2.8. Astaxanthin Concentration

The astaxanthin concentration of lyophilized fermented liquor supercritical was analyzed according to Hu, et al. [23] with slight modifications. A sample of 2.5 g was mixed with 10 mL of ethyl acetate and homogenized; then the samples were agitated and centrifuged at 7500× *g* for 15 min at 4 °C, and the supernatant was collected to carry out the assay. The extract was filtered using a membrane filter (0.45 µm) and injected in a Varian HPLC system (CA, USA), including a Varian 9012 solvent delivery instrument, a Varian 9050 variable wavelength U.V.–VIS detector, and a Rheodyne 7161 manual injector of a 20 µL loop sample. In addition, Star chromatography workstation version 6.0 was used to analyze the data. A chromatographic Phenomenex Kinetex C18 column (250 mm × 4.6 mm, 5 µm) (Phenomenex, CA, USA) was used for compound separation. The flow rate, detection wavelength, temperature, and injection volume were 0.8 mL/min, 474 nm, 25 °C, and 20 µL, respectively. The mobile phase was a solution of acetonitrile/methanol/dichloromethane in an 80:15:5 (*v*/*v*/*v*) ratio. Astaxanthin identification was performed by comparing the retention time of the sample with the astaxanthin standard. A standard curve of astaxanthin (R2 = 0.992) was obtained for the injection of six concentrations (0.22, 0.55, 1.10, 4.97, 9.95, and 22.11 µg/mL). The astaxanthin concentration in fermented liquor extract was performed for quintupled and calculated using the integrated area of HPLC peak areas and expressed as micrograms of astaxanthin per gram (µg/g) of lyophilized liquor.

2.9. Antioxidant Capacity

The antioxidant capacity of the samples was measured using the ABTS method Re, et al. [24]. A sample of 2.5 g was mixed with 10 mL of ethyl acetate and homogenized; then, samples were agitated and centrifuged at 7500× *g* for 15 min at 4 °C, and the supernatant was collected to carry out the assay. An aliquot of 150 µL of the extract was mixed with 2850 µL of ABTS solution (7.4 mM ABTS, 2.6 mM K2S2O8, and 80% ethanol), and incubated for 2 h at room temperature. Finally, the absorbance at 734 nm was measured in a spectrophotometer (JENWAY 7305, Staffordshire, UK). The results are expressed as Trolox equivalent micromoles for lyophilized fermented liquor gram (µM TE/g).

2.10. Statistical Analysis

Data were reported by descriptive statistics using the statistical package Minitab 17 (Minitab Inc., State College, PA, USA). Each experiment was performed in triplicate or otherwise specified. Data were reported as mean ± standard deviations.

3. Results

3.1. Analysis of Fermentation Liquor

The results of pH, total soluble solids, total titratable acidity (T.T.A.), and total sugars of the fermented liquor from shrimp waste are shown in Figure 2.

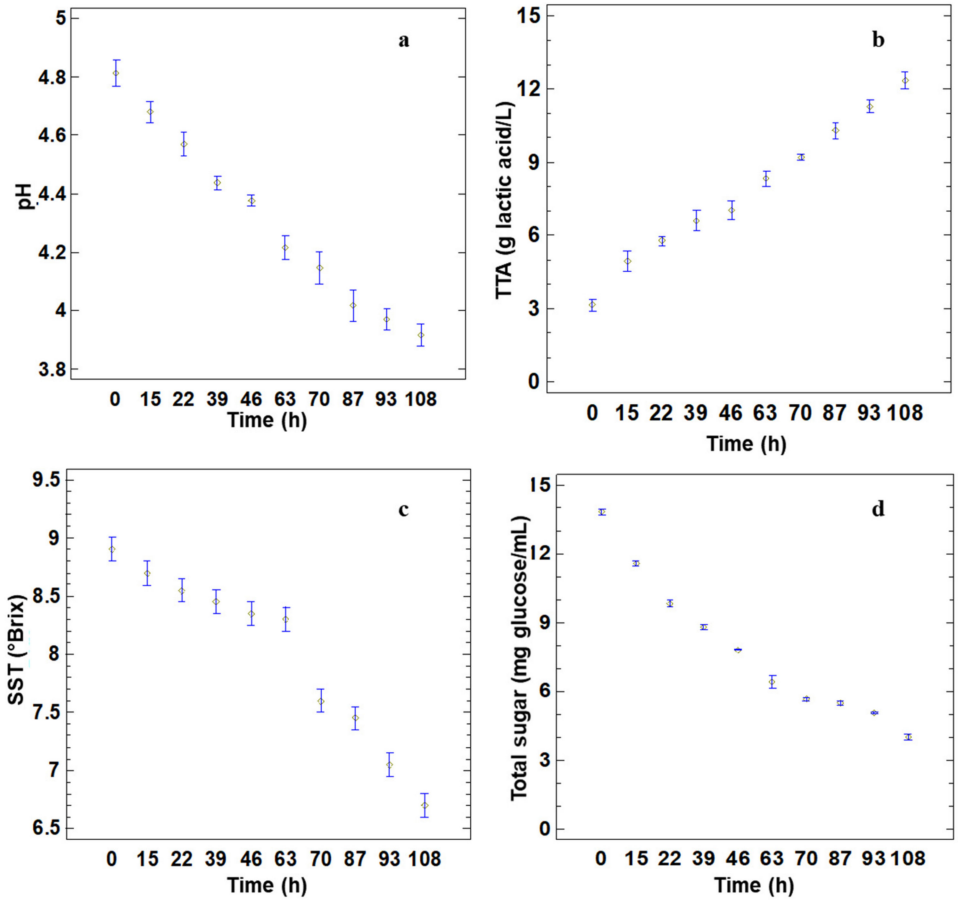

Figure 2. Analysis of pH (**a**), total soluble solids (**b**), total titratable acidity (**c**) and total sugar of fermented liquor from shrimp waste (**d**).

Fermented liquor from shrimp waste showed decreased pH (from 4.9 to 3.86) during the lactic fermentation process until the end of the process (144 h). In addition, in contrast to pH, total titratable acidity increased from 2.78 to 12.78 g of lactic acid equivalent/L, while the total soluble solids and total sugar were reduced from 9 to 7.8 °Brix and 13.9 to 4 mg of glucose/mL, respectively. On the other hand, fermented liquor at the end of the process has $6.46 \pm 0.141 \times 10^6$ CFU/mL, and Gram stain (Figure 3) showed that isolated bacteria from fermented liquor were mostly Gram-positive bacteria from the whey.

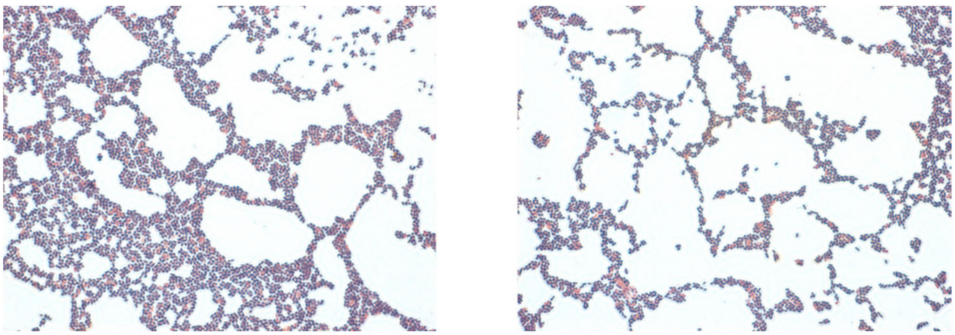

Figure 3. Gram stain of fermented liquor from shrimp waste at final process, observed at 100×.

3.2. Proximal Analysis of Liquor

The proximate composition of the lyophilized fermented liquor from shrimp waste is shown in Table 1. Lyophilized fermented liquor from shrimp waste showed a moisture content of 2.85 ± 0.27%. In addition, the ash content in lyophilized fermented liquor was 19.32 ± 0.57%. Our results showed a high protein content in fermented liquor from shrimp waste with values of 25.40 ± 0.67% and nitrogen content of 6.26 ± 0.15%. Likewise, our results showed that the lipid content in the fermented liquor was 6.29 ± 0.51%. On the other hand, the total dietary fiber content in our samples was 7.20 ± 0.58%, and the total carbohydrate content in lyophilized fermented liquor from shrimp waste was 38.92 ± 0.19%. In addition, the lyophilized fermented liquor showed a potential source of minerals like calcium, potassium, and sodium (Table 1).

Table 1. Proximate analysis and mineral composition of the lyophilized liquor produced from fermented shrimp waste.

Analysis	Results
Moisture	2.85 ± 0.27%
Ash	19.32 ± 0.57%
Protein	25.40 ± 0.67%
Lipids	6.29 ± 0.51%
Carbohydrates	38.92 ± 0.19%
Total dietary fiber	7.20 ± 0.58%
Sodium (Na)	160 mg/kg
Calcium (Ca)	950 mg/kg
Potassium (K)	592 mg/kg
Magnesium (Mg)	83 mg/kg
Iron (Fe)	1.27 mg/kg
Zinc (Zn)	1.43 mg/kg
Copper (Cu)	0.17 mg/kg
Manganese (Mn)	0.37 mg/kg

3.3. Lipophilic Antioxidants

Lyophilized fermented liquor was rich in saturated fatty acids such as caproic, caprylic, capric, lauric, myristic, palmitic, stearic, arachidic, and behenic acids. In addition, lyophilized fermented liquor has unsaturated fatty acids such as palmitoleic, oleic, and linoleic acids (Table 2).

On the other hand, astaxanthin has been reported as the predominant carotenoid in shrimp waste [18,23], lyophilized fermented liquor from shrimp waste showing an astaxanthin concentration of 0.50 ± 0.02 µg astaxanthin/g of lyophilized liquor (Table 3). Likewise, as evaluated by the ABTS method, the antioxidant capacity of lyophilized fermented liquor was 154.43 ± 4.73 µg TE/g (Table 3).

Table 2. Lipids composition of the lyophilized liquor produced from fermented shrimp waste.

Compounds	Results
Caproic acid (C6:0)	1.20%
Caprylic acid (C8:0)	0.70%
Capric acid (C10:0)	1.56%
Lauric acid (C12:0)	2.05%
Myristic acid (C14:0)	7.79%
Palmitic acid (C16:0)	31.94%
Palmitoleic acid (C16:1)	1.50%
Stearic acid (C18:0)	13.17%
Oleic acid (C18:1)	28.51%
Linoleic acid (C18:2)	3.40%
Arachidic acid (C20:0)	0.31%
Behenic acid (C22:0)	0.26%

Table 3. Lipophilic antioxidants of the lyophilized liquor produced from fermented shrimp waste.

Analysis	Results
Astaxanthin concentration	0.50 ± 0.02 µg Astaxanthin/g of lyophilized fermented liquor
Antioxidant activity	154.43 ± 4.73 µM Trolox equivalent/g of lyophilized fermented liquor

4. Discussion

During the lactic fermentation process of shrimp waste, it has been reported that pH decreases due to acid production such as lactic acid by bacteria [11,25]. In contrast, the total titratable acidity increases during the lactic fermentation process; this could be due to the acid accumulation [4,25]. On the other hand, total soluble solids are an indirect parameter of sugars and other compounds; therefore, a decrease during the lactic fermentation process of shrimp waste could be due to the consumption of the carbon source (provided mainly by molasses) [4,11]. In addition, in this work, we found that total sugar was decreased during the lactic fermentation process, which has been reported by previous studies [4,25]; this could be due to bacteria utilized the carbon source for growth and lactic acid production [4,11,25].

The proximal composition of fermented liquor has lower moisture than reported by Gimeno et al. [10], who showed that the fermented solid fraction of the shrimp waste lyophilizate. In addition, Narayan et al. [7] reported a higher moisture content than our results in fermented lyophilized liquor, and they obtained the liquor under optimized lactic fermentation of shrimp waste. These differences may be attributed to the different shrimp species (*Penaeus monodon*) and the specific lactic acid bacteria (*Pediococcus acidolactici* CFR2182).

On the other hand, fermented liquor has high ash content, which could affect the potential liquor applications for the food industry [26]. In addition, Gimeno et al. [10] reported a lower ash content in the fermented freeze-dried shrimp waste. Similar behavior was reported by Bueno-Solano, et al. [27] in liquid hydrolysate from fermented shrimp waste. Therefore, mineral removal from solid fraction occurs during the fermentation process and can be found in the liquid fraction, namely liquor [28]. The lower values, as compared to ours, reported by the authors can be attributed to the different degrees of mineral separation due to the pH change during the fermentation process using the specific lactic acid bacteria (*Lactobacillus plantarum*) and sucrose.

Lactic acid fermentation is an efficient bioprocess to recover proteins from shrimp waste due to the joint action of microbial proteases and the decreased pH caused by lactic acid production [6,11,28]. In this sense, fermented liquor has a protein content similar to the reported by Gimeno et al. [10], which found a decreased protein content from 33.8% to 8.27% in shrimp waste during fermentation with *Lactobacillus plantarum*. In contrast, our results are lower than Bueno-Solano et al. [27], which reported a higher protein content

in a fermented liquor. These differences could be attributed to the type of recuperation process, the shrimp species (*Penaeus* spp.), the lactic fermentation conditions (10% cane sugar, 5% *w*/*v* commercial inoculum, 36 h and 30 °C), among other factors. On the other hand, during the fermentation process, the protein can produce hydrolysates, which have been reported that have biological potential against chronic degenerative diseases and may also be found in the fermented liquor [4,11].

The lipid content of fermented liquor was lower than Narayan et al. [7], which found a higher lipid content in lyophilized fermented liquor of shrimp waste under optimized lactic fermentation conditions (5% *v*/*w Pediococcus acidolactici* CFR2182 inoculum, 15% *w*/*w* glucose, and 72 h of fermentation time at 37 °C). Likewise, it has been reported that the lipid content in shrimp waste depends on the shrimp species and origin and can range from 0.76 to 4.9%, which is lower than we observed; also, during the lactic fermentation, we used whey as inoculate, which could increase the lipid content [6,11,15,29].

The studies of total dietary fiber and carbohydrates of fermented liquor are scarce; therefore, a proper comparison of our results is difficult. However, we found that higher carbohydrates could be due to the whey and molasses incorporation in the lactic fermentation, which could exceed the bacteria consumption and accumulate with the by-products of the process [12,27,28], therefore, this may result in an overestimation of the total carbohydrate content.

Fermented liquor obtained in this work is a source of calcium, potassium, and sodium; this could be due to during the lactic acid fermentation process, sugars are consumed by lactic acid bacteria, which produces lactic acid, and this acid solubilizes calcium carbonate and other minerals from shrimp waste [26]. In addition, it has been reported that during lactic fermentation of shrimp waste, calcium carbonate is converted to calcium lactate by lactic acid interaction [29]. Likewise, calcium lactate is an important mineral used in the food industry [4,29,30]. Therefore, lyophilized fermented liquor obtained by industrial by-products (whey and molasses) is a source of compounds with industrial applications.

The fermented liquor lipid composition obtained in this work is in concordance with Gomez-Estaca et al. [31], which showed that shrimp waste is a source of saturated and unsaturated fatty acids. In this sense, it has been reported that unsaturated fatty acids could have healthy effects since they reduce the risk of incidence of cardiovascular diseases and cancer [3,29]. In addition, lipids composition on fermented liquor increase by fermented process due to the lipid release from matrix complex by pH decrease and protein denaturation [5,32,33]. Likewise, lipids could protect the carotenoids such as astaxanthin because it has been reported that astaxanthin could be esterified and increase its stability [5,8,15,17,32]. Therefore, fermented liquor obtained by whey and molasses could be a promissory bioprocess with industrial applications; but further studies are needed for its safe use in humans.

It has been reported that the ideal growth temperature of lactic acid bacteria is between 10–50 °C and that lactic fermentation is a feasible process to extract bioactive compounds such as astaxanthin [10,13]. Astaxanthin from fermented liquor was lower than Gimeno et al. [10] and Pacheco, et al. [34]. They reported a higher astaxanthin extraction from the solid fraction of shrimp waste after lactic fermentation with *Lactobacillus plantarum*. Even though we used similar temperature conditions, differences between the astaxanthin content could be attributed to the matrix used in the studies (solid fractions of the fermented shrimp waste vs. fermented liquor), as well as the solvents used for astaxanthin quantification. In addition, during lactic fermentation of shrimp waste, astaxanthin from fermented liquor is found in free or esterified form and thus is more susceptible to chemical degradation due to pH and the enzymes present in the fermentation process [18].

Moreover, Aranday-García et al. [11] showed that astaxanthin can be obtained in its free or trans-isomer forms from shrimp waste from a fermentation process using successive inoculation of *Lactobacillus brevis* and *Rhizopus oligosporus*. In addition, lactic acid fermentation is also a bioprocess to obtain free and trans-isomer forms of astaxanthin as shown by

Gimeno et al. [10] and Pacheco, et al. [34]. Thus, we hypothesize that the astaxanthin in our samples is in these forms, but further characterization is needed.

In this work we found that lyophilized fermented liquor from shrimp waste using whey and molasses is a potential method to obtain astaxanthin. It could be due to the possible synergism of the lactic acid bacteria from whey to separate lipophilic compounds, like astaxanthin from shrimp waste, by the protein denaturation of the matrix due to the decrease in pH by lactic acid production of bacteria and the proteolytic enzymes as bacteria used in previous reports [10,11,26]. In addition, the use of whey and molasses could be a feasible and economical option to obtain a liquor rich in astaxanthin.

In the same way, the antioxidant capacity of fermented liquor from shrimp waste has been attributed to lipophilic antioxidants such as astaxanthin [4,15]. In this sense, the antioxidant capacity of fermented liquor was similar to Sowmya and Sachindra [14] and Lira, et al. [35], which reported the antioxidant capacity of a fermented liquor of shrimp waste using the ABTS assay. Lactic fermentation can be an efficient method for extracting pigments with high antioxidant capacity. Likewise, fermented liquor could contain antioxidant pigments such as astaxanthin [3,8,36]. Previous reports have related the antioxidant activity of shrimp waste to their total carotenoid and astaxanthin content. Furthermore, peptide hydrolysates from the fermented liquor could also play an important role in the measured antioxidant capacity. However, further studies are needed in this subject [11,26,36]. Therefore, the mixture of lactic acid bacteria in lactic fermentation can be an efficient process that could separate and extract potential bioactive compounds from shrimp waste [4,6].

5. Conclusions

Lactic fermentation is a biotechnological process to extract the bioactive compounds from shrimp waste, such as lipophilic compounds. Our results show that the liquid fraction—namely liquor—of lactic fermentation of shrimp waste using novel substrates sources as whey and molasses (which in turn are by-products from other industrial processes), is a promissory by-product due to the rich composition of proteins, lipids, and carotenoids as astaxanthin, among other metabolites. Further studies are needed to reduce the content of undesirable components in fermented liquor such as ash, carbohydrates, fiber, as well as to describe its microorganisms composition.

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References

1. FAOSTAT. *Fishery and Aquaculture Statistics Yearbook 2018*; FAOSTAT: Rome, Italy, 2018.
2. SAGARPA. *Camarón, Producción en Crecimiento*; Secretaría de Agricultura y Desarrollo Rural, Pesca y Alimentación , Pesca, A., Eds.; SAGARPA: Mexico City, Mexico, 2018; Volume 2018.
3. Saini, R.K.; Moon, S.H.; Keum, Y.-S. An updated review on use of tomato pomace and crustacean processing waste to recover commercially vital carotenoids. *Food Res. Int.* **2018**, *108*, 516–529. [CrossRef]
4. Ximenes, J.C.M.; Hissa, D.C.; Ribeiro, L.H.; Rocha, M.V.P.; Oliveira, E.G.; Melo, V.M.M. Sustainable recovery of protein-rich liquor from shrimp farming waste by lactic acid fermentation for application in tilapia feed. *Braz. J. Microbiol.* **2019**, *50*, 195–203. [CrossRef]
5. Li, J.; Sun, W.; Ramaswamy, H.S.; Yu, Y.; Zhu, S.; Wang, J.; Li, H. High Pressure Extraction of Astaxanthin from Shrimp Waste (Penaeus Vannamei Boone): Effect on Yield and Antioxidant Activity. *J. Food Process Eng.* **2016**, *40*, e12353. [CrossRef]
6. Martí-Quijal, F.J.; Khubber, S.; Remize, F.; Tomasevic, I.; Roselló-Soto, E.; Barba, F.J. Obtaining Antioxidants and Natural Preservatives from Food By-Products through Fermentation: A Review. *Fermentation* **2021**, *7*, 106. [CrossRef]
7. Narayan, B.; Velappan, S.P.; Zituji, S.P.; Manjabhatta, S.N.; Gowda, L.R. Yield and chemical composition of fractions from fermented shrimp biowaste. *Waste Manag. Res.* **2010**, *28*, 64–70. [CrossRef] [PubMed]
8. Mao, X.; Guo, N.; Sun, J.; Xue, C. Comprehensive utilization of shrimp waste based on biotechnological methods: A review. *J. Clean. Prod.* **2017**, *143*, 814–823. [CrossRef]
9. Razi Parjikolaei, B.; Errico, M.; El-Houri, R.B.; Mantell, C.; Fretté, X.C.; Christensen, K.V. Process design and economic evaluation of green extraction methods for recovery of astaxanthin from shrimp waste. *Chem. Eng. Res. Des.* **2017**, *117*, 73–82. [CrossRef]
10. Gimeno, M.; Ramírez-Hernández, J.Y.; Mártinez-Ibarra, C.; Pacheco, N.; García-Arrazola, R.; Bárzana, E.; Shirai, K. One-solvent extraction of astaxanthin from lactic acid fermented shrimp wastes. *J. Agric. Food Chem.* **2007**, *55*, 10345–10350. [CrossRef]
11. Aranday-García, R.; Román Guerrero, A.; Ifuku, S.; Shirai, K. Successive inoculation of Lactobacillus brevis and Rhizopus oligosporus on shrimp wastes for recovery of chitin and added-value products. *Process Biochem.* **2017**, *58*, 17–24. [CrossRef]
12. Flores-Albino, B.; Arias, L.; Gómez, J.; Castillo, A.; Gimeno, M.; Shirai, K. Chitin and L(+)-lactic acid production from crab (Callinectes bellicosus) wastes by fermentation of Lactobacillus sp. B2 using sugar cane molasses as carbon source. *Bioprocess Biosyst. Eng.* **2012**, *35*, 1193–1200. [CrossRef]
13. Marcia, E.; Malespín, J.; Sánchez, M.; Benavente, M. Estudio de la fermentación láctica para la extracción de quitina a partir de desechos de crustáceos. *Nexo Rev. Cient.* **2011**, *24*, 33–42. [CrossRef]
14. Sowmya, R.; Sachindra, N.M. Evaluation of antioxidant activity of carotenoid extract from shrimp processing byproducts by in vitro assays and in membrane model system. *Food Chem.* **2012**, *134*, 308–314. [CrossRef]
15. Cao, Y.R.; Yang, L.; Qiao, X.; Xue, C.H.; Xu, J. Dietary astaxanthin: An excellent carotenoid with multiple health benefits. *Crit. Rev. Food Sci. Nutr.* **2021**, *59*, 1880–1902. [CrossRef] [PubMed]
16. Messina, C.M.; Manuguerra, S.; Renda, G.; Santulli, A. Biotechnological Applications for the Sustainable Use of Marine By-products: In Vitro Antioxidant and Pro-apoptotic Effects of Astaxanthin Extracted with Supercritical CO_2 from Parapeneus longirostris. *Mar. Biotechnol.* **2019**, *21*, 565–576. [CrossRef]
17. Stachowiak, B.; Szulc, P. Astaxanthin for the Food Industry. *Molecules* **2021**, *26*, 2666. [CrossRef] [PubMed]
18. Cabanillas-Bojórquez, L.A.; Gutiérrez-Grijalva, E.P.; González-Aguilar, G.A.; López-Martinez, L.X.; Castillo-López, R.I.; Bastidas-Bastidas, P.D.J.; Heredia, J.B. Valorization of Fermented Shrimp Waste with Supercritical CO_2 Conditions: Extraction of Astaxanthin and Effect of Simulated Gastrointestinal Digestion on Its Antioxidant Capacity. *Molecules* **2021**, *26*, 4465. [CrossRef]
19. AOAC. *Official Methods of Analysis*; AOAC: Rockville, MD, USA, 2012.
20. Devindra, S. Estimation of glycemic carbohydrates from commonly consumed foods using modified anthrone method. *Indian J. Appl. Res.* **2015**, *5*, 45–47.
21. Velázquez-López, A.; Covatzin-Jirón, D.; Toledo-Meza, M.D.; Vela-Gutiérrez, G. Bebida fermentada elaborada con bacterias ácido lácticas aisladas del pozol tradicional chiapaneco. *CienciaUAT* **2018**, *13*, 165–178. [CrossRef]
22. Pretorius, B.; Schönfeldt, H.C. Cholesterol, fatty acids profile and the indices of atherogenicity and thrombogenicity of raw lamb and mutton offal. *Food Chem.* **2021**, *345*, 128868. [CrossRef]
23. Hu, J.; Lu, W.; Lv, M.; Wang, Y.; Ding, R.; Wang, L. Extraction and purification of astaxanthin from shrimp shells and the effects of different treatments on its content. *Rev. Bras. Farmacogn.* **2019**, *29*, 24–29. [CrossRef]
24. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Rad. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
25. Shirai, K.; Guerrero, I.; Huerta, S.; Saucedo, G.; Castillo, A.; Obdulia Gonzalez, R.; Hall, G.M. Effect of initial glucose concentration and inoculation level of lactic acid bacteria in shrimp waste ensilation. *Enzyme Microb. Technol.* **2001**, *28*, 446–452. [CrossRef]
26. Sedaghat, F.; Yousefzadi, M.; Toiserkani, H.; Najafipour, S. Bioconversion of shrimp waste Penaeus merguiensis using lactic acid fermentation: An alternative procedure for chemical extraction of chitin and chitosan. *Int. J. Biol. Macromol.* **2017**, *104*, 883–888. [CrossRef]
27. Bueno-Solano, C.; López-Cervantes, J.; Campas-Baypoli, O.N.; Lauterio-García, R.; Adan-Bante, N.P.; Sánchez-Machado, D.I. Chemical and biological characteristics of protein hydrolysates from fermented shrimp by-products. *Food Chem.* **2009**, *112*, 671–675. [CrossRef]

28. Bhaskar, N.; Suresh, P.V.; Sakhare, P.Z.; Sachindra, N.M. Shrimp biowaste fermentation with Pediococcus acidolactici CFR2182: Optimization of fermentation conditions by response surface methodology and effect of optimized conditions on deproteination/demineralization and carotenoid recovery. *Enzyme Microb. Technol.* **2007**, *40*, 1427–1434. [CrossRef]
29. Simopoulos, A.P. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed. Pharmacother.* **2002**, *56*, 365–379. [CrossRef]
30. Sánchez-Camargo, A.P.; Almeida Meireles, M.Â.; Lopes, B.L.F.; Cabral, F.A. Proximate composition and extraction of carotenoids and lipids from Brazilian redspotted shrimp waste (Farfantepenaeus paulensis). *J. Food Eng.* **2011**, *102*, 87–93. [CrossRef]
31. Gómez-Estaca, J.; Calvo, M.M.; Álvarez-Acero, I.; Montero, P.; Gómez-Guillén, M.C. Characterization and storage stability of astaxanthin esters, fatty acid profile and α-tocopherol of lipid extract from shrimp (L. vannamei) waste with potential applications as food ingredient. *Food Chem.* **2017**, *216*, 37–44. [CrossRef]
32. Higuera-Ciapara, I.; Felix-Valenzuela, L.; Goycoolea, F.M. Astaxanthin: A review of its chemistry and applications. *Crit. Rev. Food Sci. Nutr.* **2006**, *46*, 185–196. [CrossRef] [PubMed]
33. Vázquez, J.; Ramos, P.; Mirón, J.; Valcarcel, J.; Sotelo, C.; Pérez-Martín, R. Production of Chitin from Penaeus vannamei By-Products to Pilot Plant Scale Using a Combination of Enzymatic and Chemical Processes and Subsequent Optimization of the Chemical Production of Chitosan by Response Surface Methodology. *Mar. Drugs* **2017**, *15*, 180. [CrossRef]
34. Pacheco, N.; Garnica-González, M.; Ramírez-Hernández, J.Y.; Flores-Albino, B.; Gimeno, M.; Bárzana, E.; Shirai, K. Effect of temperature on chitin and astaxanthin recoveries from shrimp waste using lactic acid bacteria. *Bioresour. Technol.* **2009**, *100*, 2849–2854. [CrossRef] [PubMed]
35. Lira, G.M.; Lopez, A.M.Q.; Firmino, G.O.; Santos, S.D.; Bezerra, R.D.S. Total carotenoids and antioxidant activity of fillets and shells (in natura or cooked) of "Vila Franca" shrimp (Litopenaeus Schmitti) in different intervals of storage under freezing. *Cienc. Agrotec.* **2017**, *41*, 94–103. [CrossRef]
36. Weeratunge, W.K.O.V.; Perera, B.G.K. Formulation of a fish feed for goldfish with natural astaxanthin extracted from shrimp waste. *Chem. Cent. J.* **2016**, *10*, 44. [CrossRef] [PubMed]