



## Biotechnological Valorisation of Shrimp Waste: Physicochemical and Microbial Quality

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### ABSTRACT

Only about twenty of the hundreds of shrimp species known worldwide are of significant commercial and medicinal value. The production and consumption of shrimp generate enormous amounts of waste, mostly shells, which poses environmental and public health risks. This study aimed to determine the chemical composition and microbiological quality of shrimp waste in order to assess its biotechnological value. Shrimp wastes were collected from Kenitra, Northwest Morocco. The physicochemical properties of the wastes were determined following standard methods. Microbiological analysis of the shrimp wastes and their fermented by-product were performed according to standard procedures. The results showed that the shrimp wastes contain high amount of dry matter (24.91%), organic matter (16.76%), chitin (16.57%), ashes (8.15%), total protein (13.54%), and a relatively low amount of fat (2.52%). The mineral contents were predominantly calcium (5.57 mg/L), iron (0.92 mg/L), and phosphorus (0.72 mg/L). A total of nine microbial strains were identified in the fermented samples, with *Escherichia coli* (54.73%), *Proteus mirabilis* (9.47%), *Klebsiella pneumoniae* (8.42%), and *Enterobacter* sp. (7.36%) being the most common. *Salmonella enteritidis* was absent in the fermented wastes. Lactic acid bacteria and yeasts were significantly superior in the fermented wastes, while total aerobic mesophilic flora, molds, total coliforms, faecal coliforms, and *Clostridium sulphur* reducers were significantly superior in non-fermented samples. These results have demonstrated that shrimp wastes are rich in minerals, organic matter, and hygienic microbial flora, and devoid of pathogenic strains. These findings could therefore encourage the use of shrimp wastes for agronomic, nutritional, and pharmaceutical purposes.

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**Keywords:** By-Products, Shrimp Waste, Physicochemical, Fermentation, Valorization.

### Introduction

Shrimp, a common crustacean species that belongs to the class Malacostraca and the order Decapoda, is rich in protein, minerals, amino acids, astaxanthin, fatty acids, vitamins, and antioxidants.<sup>1</sup> Approximately 20 of the hundreds of shrimp species found worldwide are significant for trade, biotransformation, and industrial application.<sup>2,3</sup> In 2020, 5 million tonnes of shrimp were produced worldwide, accounting for roughly 5.35 percent of the 93.4 million tonnes of fish produced worldwide.<sup>4-6</sup> In terms of value, shrimp production, including catches and shrimp farming represent the most significant fish production globally.<sup>7</sup> Although, shrimp harvests have reached new records lately, the world's aquaculture production of crustaceans in 2018 was 9.4 million tons, representing 11.4% of the world's total aquaculture production.<sup>2,8</sup> Shrimp is the most valuable fish product export and shrimp farming is a significant source of jobs in several tropical developing nations.<sup>2,9</sup>

In the last decade, China supplied 38% of the world's shrimp, making it the largest producer of shrimp in the world.<sup>10</sup> India came second with 13%, followed by Indonesia with 11%, Vietnam with 10%, and Ecuador with 10%.<sup>11,12</sup> Other important producers of shrimp are Thailand (7%) and Mexico (3%).<sup>13</sup> However, the increased production of shrimps and their by-products are suggested to impact the environment if they are not treated properly.

Due to the rising demand for high-quality seafood in the 1980s, shrimp aquaculture production increased significantly, especially in Southeast Asia.<sup>9,14</sup> The economic potential of shrimp farming also contributed to the region's increased agricultural production.<sup>9</sup> Currently, China is the leading producer and exporter of shrimp worldwide, with 20,809,393 tonnes produced in 2020, followed by Vietnam and Thailand.<sup>15,16</sup> The production of shrimp in South Asia generates a substantial quantity of waste that have great environmental impact.<sup>17,18</sup> The most dominant waste product of shrimp production and consumption is the shells.<sup>17</sup> To minimize their environmental impacts, many countries and research organizations have explored different ways to manage shrimp shells.<sup>19,20</sup>

Morocco makes a small but excellent contribution to the global shrimp-producing industry. Due to its geographical location, ideal temperature, and bathymetry, *Parapenaeus longirostris*, commonly called the deep-water rose shrimp is the most prevalent species on Morocco's Atlantic Ocean.<sup>21,22</sup> According to the Maritime Fisheries Department,<sup>4</sup> the projected yearly production of shrimp is 5,991 tonnes. Shrimp holds a significant position in the Moroccan economy. In 2013, Morocco exported 7.78 tonnes of shrimp, valued at 158.9 million U.S. dollars. In 2018, shrimp exports contributed up to 584 million tons or 4% of all fisheries product exports, valued at roughly 2.2 billion U.S. dollars.<sup>4</sup> However, the farming, consumption, and processing of shrimps in Morocco are suggested to produce a huge quantity of waste, such as

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shells.<sup>23,24</sup> Therefore, few researchers addressed the valorization of shrimp shells in small-scale projects such as the use of micro-particles of shrimp shells to treat wastewater via infiltration-percolation procedure in Agadir (South Morocco),<sup>23</sup> and use of shrimp shell powder in carbon paste electrode for the electrochemical detection of dopamine and paracetamol.<sup>25</sup>

Shrimp is the most valuable product on the global market, and the seafood processing industry is expanding as a result of shifting customer preferences and rising demand.<sup>26</sup> The edible portion of shrimp, which is only meant for human consumption and makes up around 60% of its total weight,<sup>27</sup> is processed after it has been shelled and beheaded; an industrial process that produces a lot of garbage (heads, shells, and tails).<sup>28</sup> However, without prior treatment, this waste is often dumped in public landfills, having a detrimental effect on the environment and public health.<sup>29</sup>

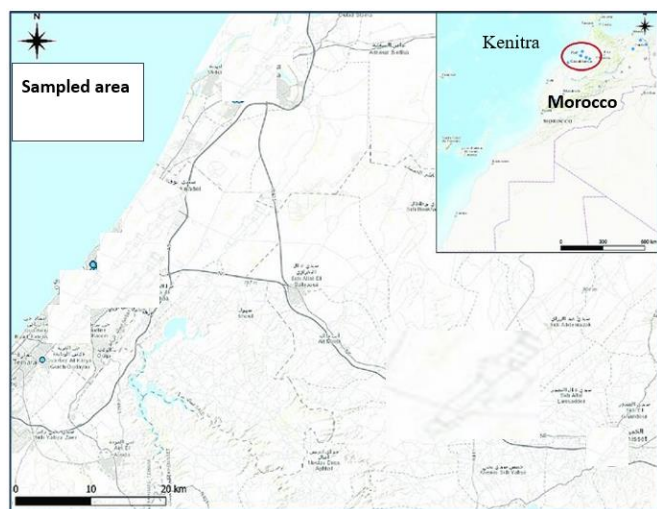
Shrimp waste has many components that are worth recovering due to their nature as renewable and exploitable by-products.<sup>30</sup> The most significant components of shrimp waste include minerals, proteins, amino acids, fats, chitin, astaxanthin, and lipids.<sup>31,32</sup> As stated by Mechri *et al.*<sup>33</sup> the value-addition of shrimp by-products signals not just environmental interest but also a strong biotechnological interest. Subsequent research revealed that shrimp by-products can be utilized as intermediate or raw materials for the production of additional products meant for pharmaceutical, nutraceutical, animal and human nutritional, and cosmetic use.<sup>1,34</sup> Shrimp waste is currently being used for novel purposes, such as fertilizers, and plant bio-stimulants and elicitors.<sup>35,36</sup>

The present study aimed to investigate the physical, chemical, and microbiological properties of shrimp by-products (raw and fermented) sampled from Northwest Morocco. The findings from this study is expected to provide a preliminary information on the composition and nutritional value of shrimp by products. The results obtained could also be harnessed for biotechnological application, especially in the food, pharmaceutical, and agricultural industries.

## Materials and Methods

### Sample collection

Samples of shrimp waste, including heads, shells, and tails, were collected from various sales outlets in the city of Kenitra (Figure 1). The samples were packed and transported to the laboratory for qualitative and quantitative characterization. All laboratory analyses were conducted in the Plant and Animal Production and Agro-Industry Laboratory, Ibn Tofail University, Faculty of Science, Kenitra, Morocco.



**Figure 1:** Map of the study area (Kenitra, Northwest Morocco)

### Sample preparation

The shrimp samples were crushed under aseptic condition at ambient temperature using an electric mill (Moulinex XXL Picadora, France).

### Physicochemical analyses

#### pH measurement

The pH of the crushed shrimp sample was measured daily using digital pH meter (Thermo Scientific Orion Star) at an ambient temperature of 20°C.

#### Measurement of total acidity

The total acidity of crushed shrimp sample was measured by titrating 10 mL filtrate (obtained from the crushed shrimp waste) with a standard alkaline solution (N/9 NaOH) using phenolphthalein as a colour indicator. Acidity was calculated using the following equation 1, and expressed as percentage (%).

$$\text{Acidity (\%)} = \frac{\text{Volume of NaOH (mL)} \times \text{Normality of NaOH} \times \text{Volume of sample taken (mL)}}{\text{Sample mass (g)}} \times 100 \quad \dots \text{(Eq. 1)}$$

#### Measurement of humidity level and dry matter

The shrimp waste sample (1 g) was weighed in raw form and dehydrated at 105°C for 24 hours. The dried samples were cooled in a desiccator. The humidity of the sample was calculated as the difference between the weight of the raw sample (wet waste) and that of the dry sample (waste after elimination of water) (equation 2).<sup>37</sup> The dry matter was calculated according to the formula shown in equation 3.

$$\text{Humidity (\%)} = \frac{(P - P_s)}{(P - P_0)} \times 100 \quad \dots \text{(Eq. 2)}$$

$$\text{Dry matter (\%)} = 100 - \text{Humidity (\%)} \quad \dots \text{(Eq. 3)}$$

Where;

P: weight of the crucible + sample before drying.

Ps: weight of the crucible + sample after drying.

P0: weight of the empty crucible.

#### Determination of total ash, organic matter, and mineral contents

The dried shrimp sample (1 g) was incinerated in a muffle furnace at 550°C for 5 hours. After cooling, the percentage ash content was calculated using the formula below (Equation 4). The organic matter content was determined as the difference between the initial dry sample weight and the weight of the resulting ash after incineration, and expressed as a percentage.

$$\text{Total ash (\%)} = \frac{M_{\text{ash}} \times 100}{M_{\text{(sample)}}} \quad \dots \text{(Eq. 4)}$$

Where M refers to mass.

The mineral contents were determined by spectrophotometric method according to the French International Standard (AFNOR T90-014) NF V18-106.<sup>38,39</sup>

#### Determination of total nitrogen, orthophosphate (PO<sub>4</sub>), total phosphorus, and chlorides

Total nitrogen was determined using the Kjeldahl method.<sup>40,41</sup> In this method, total nitrogen was determined by the mineralization of organic nitrogen in the sample with sulfuric acid in the presence of potassium sulphate and selenium as catalysts. The ammoniacal nitrogen formed was then determined by acidimetry after distillation, in the presence of a methyl red and methylene blue as colour indicators.<sup>42</sup>

Orthophosphate (PO<sub>4</sub>) content of the shrimp waste was determined using colorimetric method.<sup>43</sup> In this assay, a phosphomolybdic complex was formed in an acidic medium, which was then reduced by ascorbic acid to a blue colour complex. The intensity of the complex was measured spectrophotometrically at 700 nm.<sup>44</sup>

Total phosphorus was obtained after the mineralization of the shrimp waste in an acidic medium, in the presence of sodium persulfate at 150°C for 2 h. The organic and combined phosphorus were transformed into soluble orthophosphates. The total phosphorus content was estimated as orthophosphates.

The chloride content of the shrimp waste sample was determined according to standard method (AFNOR T90-014).<sup>45</sup> In this method, chlorides were measured by the Mohr titrimetric method with silver nitrate and potassium chromate.

#### *Sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>) and calcium (Ca<sup>2+</sup>) contents*

Minerals, including sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), and calcium (Ca<sup>2+</sup>) in shrimp waste sample were determined by flame photometry (Jenway PFP7). The colour intensity was measured spectrophotometrically at 395 nm.

#### *Determination of total protein content*

The total protein content was estimated from the total nitrogen content of the test sample according to the following formula:

$$\text{Total protein} = 6.25N (\%)$$

#### *Determination of total fat content*

The total fat of the sample was extracted with ethanol by Soxhlet extraction method.

#### *Determination of chitin content*

The chitin content in the shrimp waste was evaluated using the enzymatic method.<sup>46-48</sup> Briefly, the shrimp waste was first hydrolysed with chitinase to remove salts and proteins. The hydrolysed sample was dried, weighed, then decalcified by treatment with 0.5 N HCL. The samples were washed and treated with 0.5 N NaOH at 100°C for 3 to 6 hours to release the chitin from its glycoprotein complexes. Thereafter, the residual material was treated with chitinase (1 mg/mL) for 3 hours until complete hydrolysis. The hydrolysate was incubated at pH 5.2 at 37°C in the presence of N-acetyl glucosaminidase, and the released acetyl glucosamine was measured colorimetrically.<sup>49,50</sup>

#### *Microbiological analysis*

##### *Flora of hygienic interest*

##### *Total aerobic mesophilic flora (TAMF)*

Shrimp waste samples were incubated on a plate count agar (PCA). Briefly, 1 mL of the sample in dilutions ranging from 10<sup>-1</sup> to 10<sup>-7</sup> were incubated at 30°C for 48 hours. Thereafter, the total aerobic mesophilic flora (TAMF) was estimated.

##### *Total and faecal coliforms*

The total coliforms of shrimp waste was assessed on MacConkey medium to visualize the presence of coliforms as an indicator of hygiene.<sup>51,52</sup> In our case, prepared samples were incubated at 37°C for 48 hours. Further, the fecal coliforms are incubated on the same medium at 44°C. Then, microbial results were read for each type of microorganism.

##### *Assessment of pathogenic flora*

##### *Staphylococci and faecal streptococci*

The enumeration of *Staphylococci* was carried out on selective Chapman medium or Mannitol Salt Agar medium containing 75% NaCl.<sup>53,54</sup> The cultures were incubated at 37°C for 24 hours. On the other hand, faecal streptococci were counted on sodium azide selective medium after their incubation at 37°C for 24 hours.

##### *Salmonella, shigella, and sulphite-reducing clostridiums*

To evaluate *Salmonella* and *Shigella* spp., the shrimp waste sample was incubated in 1 mL of pre-enrichment medium to which 9 mL of a selective medium of tetrathionate broth or selenite cysteine broth (SCB) was added. After homogenization, the tubes were incubated at 44°C for 24 hours for the first phase and then re-incubated at 37°C for 24 hours for the second phase. To evaluate the sulphite-reducing *clostridiums*, a reinforced clostridium agar (RCA) medium containing cysteine as a reducing agent was used. In this method, the sulphur produced from the degradation of the medium by the *clostridium* combines with iron to impart a black colour to the *clostridium* colonies. After 48 hours of

incubation at 37°C, the black colonies at the bottom of the tubes were counted.

#### *Spoilage bacteria*

##### *Proteolytic and lipolytic flora*

To evaluate proteolytic flora, solid casein nutrients were used. Casein hydrolysis was studied on a medium composed of plate count agar supplemented with 50% skimmed milk. The medium was poured into petri dishes, and inoculated with 1 mL of the shrimp sample in different dilutions ranging from 10<sup>-1</sup> to 10<sup>-5</sup>. After three days of incubation at 30°C, the hydrolyzed casein showed a clear halo around the colony, which facilitated their observation and counting. On the other hand, lipolytic flora was evaluated via the modified RATH method (Standard 41.1966). This approach uses butterfat associated with Victoria blue (M.G.B.B.V.). The test is considered positive when the colonies exhibit a true-blue colour.

#### *Yeasts and molds*

Yeasts and molds were studied on Sabouraud's dextrose agar (SDA) and Potato dextrose agar (PDA) supplemented with chloramphenicol (0.1 mL). The agar were inoculated with the shrimp sample and incubated at 30°C for 48 hours, after which the fungal colonies were observed and then counted.

#### *Biotransformation*

##### *Selection of fermentation substrate*

Suitable fermentation substrates were selected from different biotopes, including milk, and sugar cane press juice. Then fermented juice of shrimp waste were prepared from the selected biotopes (milk, and sugar cane press juice). Briefly, 8 kg of the shrimp waste, supplemented with a 20% carbon source, were placed in a barrel and inoculated with a starter culture of selected strains (LH 4, LH 10, and LH 20) at a concentration of 0.6 g/L, and allowed to ferment at ambient temperature and controlled pH for 6 days.

#### *Isolation of acidifying bacteria*

Dilutions of 10<sup>-1</sup> up to 10<sup>-6</sup> of the fermented shrimp waste were carried out under sterile conditions. The fermented samples were inoculated on De Man–Rogosa–Sharpe agar medium (MRS) using the last three dilutions (10<sup>-4</sup>, 10<sup>-5</sup>, and 10<sup>-6</sup>) in triplicate. After incubation at 30°C for 48 hours, the acidifying bacterial colonies were isolated. The choice of the sourdough was based on four criteria including biomass production, fermentation potential, acidifying power, and production of bacteriocin.

#### *Purification and characterization*

##### *Macroscopic examination*

The regular milky white or brown colonies on the MRS medium were marked at the base of the dish and retained for purification. Purification was carried out on MRS medium. After four successive subcultures, colonies were presumed pure. Cultures were conserved on the same MRS medium incubated at +4°C in the dark.

##### *Gram test*

The bacterial strains under investigation were inoculated on a solid MRS medium, incubated at 30°C and 45°C for three days. The classic Gram staining technique using crystal violet, Lugol, acetone alcohol, and fuchsin was selected, and microscopic observations were made.

##### *Catalase test*

Catalase test was carried out by emulsifying a bacterial culture in a drop of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) at 30 g/L placed on a slide. The release of gas bubbles (O<sub>2</sub>) indicates a positive test for catalase.

##### *Gas (CO<sub>2</sub>) production*

Gas production was tested using hemolysis tubes containing 5% semi-solid MRS medium. The medium was liquefied by placing on a water bath at 45°C, then seeded deeply with a bacterial suspension. The culture medium was subsequently covered with a layer of agar (20 g/L). The elevation of the agar layer indicates the presence of gas pockets which is a positive gas production test.

### Fermentation of sugars

Sugar fermentation was assessed by cultivation of the isolated bacterial strain in M1 medium. First, the sugars (glucose, maltose, galactose, raffinose, lactose, and arabinose) were sterilized by heating at 80°C for 2 min, and added to the medium at a final concentration of 20 mg/L. After incubation at 30°C for 24 hours in liquid MRS medium, the cells were suspended in sterile physiological water and used as inoculum. A change in colour of the indicator from purple to yellow indicates sugar fermentation.

### Identification

Lactic acid bacteria from Bio-Mérieux were used as standard for the identification of lactic acid bacteria. A young culture of the isolated bacterial strain was seeded in a well plate at 0.1 mL per well. The plate was incubated at 30°C for 24 hours, after which the identification was performed automatically with the aid of a computer.

### Acidifying power

After cultivating the lactic acid bacteria in the MRS medium at 30°C for 48 hours at an initial pH of 5.88, the acidifying power was ascertained by comparing the initial and final pH values. Acidity was measured titrimetrically. In this case, 10 mL of the culture filtrate was titrated with a 0.1 N sodium hydroxide solution using 1% methanolic phenolphthalein solution as colour indicator. Acidity was expressed as milligram lactic acid per 100 milliliters of culture.

### Statistical analysis

Data were presented as mean  $\pm$  standard deviation (SD). Differences between mean values were compared using one-way analysis of variance (ANOVA), and paired sample T-test. P-value < 0.05 was regarded as significant. Statistical analysis was done using SPSS software (version 25).

## Results and Discussion

### Physicochemical properties of shrimp waste

The physicochemical properties of shrimp waste are presented in Table 1. The average temperature was estimated at 21°C, while the average pH was estimated at 6.88, and the humidity was around 75%. The shrimp waste was found to be rich in organic matter, estimated at 16.76%, and dry matter representing 24.91% of the total weight. Total ash was estimated at 8.15%, total protein content was 13.54%, chitin content was 16.57%, and a total fat content was found to be 2.52%. Minerals and trace elements were found in variable quantities, with calcium being the most dominant with concentration of 5.57 mg/L, followed by iron at 0.92 mg/L, phosphorus at 0.72 mg/L, and potassium at 0.69 mg/L. Others include magnesium and zinc recorded at 0.425 and 0.21 mg/L, respectively.

**Table 1:** Physicochemical properties of shrimp wastes before treatment.

Parameter	Unit	Quantity
pH	-	6.88
Dry matter	(%)	24.91
Ashes	(%)	8.15
Organic material	(%)	16.76
Total nitrogen	(%)	3.61
Total protein	(%)	13.54
Fat	(%)	2.52
Chitin	(%)	16.57
Total phosphorus	(%)	0.72
Total potassium	(%)	0.69
Calcium	(mg/L)	5.57
Magnesium	(mg/L)	0.425
Iron	(mg/L)	0.22
Zinc	(mg/L)	0.21

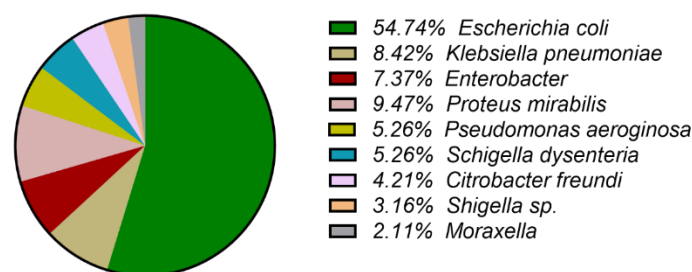
### Microbial load before fermentation

The microbial load of the shrimp waste prior to fermentation are shown in Table 2. The shrimp waste had a significant load of total aerobic mesophilic flora (TAMF) fluctuating from  $1.1 \times 10^8$  to  $4.2 \times 10^7$  cfu/g and an average of  $3.7 \times 10^7$  cfu/g. In terms of hygiene flora, total coliforms counts were  $4.7 \times 10^5$  cfu/g, and the streptococci counts ranged from 89 to 145 cfu/g with a mean count of 124 cfu/g.

In addition, there were variable toxigenic flora with majority of them being *Staphylococci* which varied from  $1.5 \times 10^4$  to  $5.2 \times 10^4$  cfu/g with an average count of  $3 \times 10^4$  cfu/g. The results showed that 10 out of the 12 strains identified were catalase+, coagulase+, DNase+, and phosphatase+. These strains were identified as *Staphylococcus aureus*. All the samples analyzed showed a significant load of *Clostridium*s, which varied between 213 and 720 cfu/g, with an average count of 424.3 cfu/g.

Furthermore, the analyzed shrimp waste showed a significant load of proteolytic bacteria which varied from  $2 \times 10^5$  cfu/g to  $3.6 \times 10^6$  cfu/g with an average load of  $2.8 \times 10^6$  cfu/g, while the average load of lipolytic bacteria were  $2.3 \times 10^6$  cfu/g.

The percentages of identified Enterobacteriaceae species isolated from the shrimp by-products are presented in Figure 2. In total, 9 microbial strains were identified in the shrimp wastes, with *Escherichia coli* being the most dominant (54.73%), followed by *Proteus mirabilis* (9.47%),



**Figure 2:** Enterobacteriaceae species isolated from shrimp by-products

*Klebsiella pneumoniae* (8.42%), and *Enterobacter* (7.36%). In contrast, *Moraxella* and *Shigella spp.* were the least abundant, with percentage occurrences of 2.10 and 3.15%, respectively. On the other hand, *Salmonella enteritidis* was absent in all the shrimp waste samples.

There are several studies on shrimp waste and their valorization in different fields. These studies have demonstrated that shrimp waste is rich in organic and mineral ingredients. However, the composition of the shrimp waste differed depending on several factors, including species, geographical location, parts of the shrimps, extraction methods, and processing techniques. For example, Gulzar *et al.*<sup>55</sup> investigated the chemical composition of shrimp processing by-products, including oils and pigments, and their applications. The results obtained showed that shrimp wastes are rich in protein, fatty acids, and minerals. The total lipid content varied between 1.0 and 8.0%, depending on the species. The total fatty acid content was around 37.5%, saturated fatty acids was 30.4%, and monounsaturated fatty acids was 22.25%. In another study, Ali Said Al Hoqani *et al.*<sup>56</sup> investigated the mineral composition of Omani shrimp shells using Inductively Coupled Plasma-Optical Emission Spectroscopy. Results showed that the content of calcium and phosphorus were  $117.6 \pm 0.01$  and  $119.12 \pm 0.09$  ppm, respectively. According to Younes *et al.*,<sup>57</sup> shrimp shells are the main marine source of chitin, and these shells have a rich content of protein (20–40%), calcium carbonate (20–50%), and chitin (15–40%), and a considerable amount of pigments, and lipids.<sup>58</sup> The high calcium carbonate content of the shell shields the delicate bodily tissue of the shrimp from harsh environmental conditions.<sup>59</sup> Crustacean shell waste, such as that of shrimps, possesses commercially important carotenoids, including lutein, astaxanthin, astacene, and zeaxanthin.<sup>60</sup> Shrimp shells have a high mineral content as well as valuable proteins that have been used as dietary supplements and as a preservative.<sup>61,62</sup>



**Table 2:** Microbial load of shrimp wastes

Sample	Microbial Load (cfu/g)						
	TAMF	Colif.	Staphy.	Strep F	Clost. c	Lipoly.	Proteo.
1	1.7.10 <sup>8</sup>	2.10 <sup>5</sup>	4.10 <sup>4</sup>	120	720	3.10 <sup>6</sup>	2.10 <sup>6</sup>
2	1.5.10 <sup>7</sup>	2.1.10 <sup>5</sup>	2.2.10 <sup>4</sup>	130	580	2.10 <sup>6</sup>	2.10 <sup>5</sup>
3	1.2.10 <sup>7</sup>	1.1.10 <sup>6</sup>	5.2.10 <sup>4</sup>	110	450	1.3.10 <sup>6</sup>	2.6.10 <sup>6</sup>
4	2.5.10 <sup>8</sup>	3.2.10 <sup>4</sup>	2.3.10 <sup>4</sup>	135	520	2.5.10 <sup>6</sup>	3.2.10 <sup>6</sup>
5	3.1.10 <sup>7</sup>	3.5.10 <sup>4</sup>	3.1.10 <sup>4</sup>	89	423	1.2.10 <sup>5</sup>	2.10 <sup>6</sup>
6	2.5.10 <sup>7</sup>	3.2.10 <sup>5</sup>	4.3.10 <sup>4</sup>	122	325	2.2.10 <sup>6</sup>	2.2.10 <sup>6</sup>
7	2.5.10 <sup>7</sup>	2.7.10 <sup>5</sup>	2.7.10 <sup>4</sup>	140	213	1.8.10 <sup>6</sup>	5.3.10 <sup>5</sup>
8	1.2.10 <sup>7</sup>	2.1.10 <sup>5</sup>	3.5.10 <sup>4</sup>	113	450	3.2.10 <sup>6</sup>	3.5.10 <sup>6</sup>
9	4.2.10 <sup>7</sup>	5.2.10 <sup>5</sup>	3.2.10 <sup>4</sup>	140	347	3.12.10 <sup>6</sup>	3.6.10 <sup>6</sup>
10	1.1.10 <sup>8</sup>	2.1.10 <sup>5</sup>	1.5.10 <sup>4</sup>	126	320	2.3.10 <sup>6</sup>	2.10 <sup>5</sup>
11	2.5.10 <sup>7</sup>	2.2.10 <sup>5</sup>	2.3.10 <sup>4</sup>	145	332	2.10 <sup>6</sup>	3.2.10 <sup>6</sup>
12	3.2.10 <sup>7</sup>	4.2.10 <sup>5</sup>	1.8.10 <sup>4</sup>	118	412	2.9.10 <sup>6</sup>	2.6.10 <sup>6</sup>
Average	<b>6.2.10<sup>7</sup></b>	<b>3.1.10<sup>5</sup></b>	<b>3.10<sup>4</sup></b>	<b>124</b>	<b>424.3</b>	<b>2.3.10<sup>6</sup></b>	<b>2.1.10<sup>6</sup></b>

**TAMF:** Total aerobic mesophilic flora; **Colif:** Total coliforms; **Clost:** *Clostridia*; **Staph:** *Staphylococcus*; **Strep F:** *Streptococcus fecalis*; **Lipoly:** Lipolitics; **Proteo:** Proteolytics.

Shrimp shell have been shown to have total protein content of 44% (by dry weight), and a high concentration of essential amino acids.<sup>63,64</sup> Shrimp head waste in particular, have been found to have high content of protein (50 – 65% dry weight), making it a good source of essential amino acids for aquaculture animals and poultry.<sup>64</sup>

In Morocco, few studies have investigated the mineral and organic constituents of shrimp shell. For example, Abali *et al.*<sup>23</sup> investigated shrimp-shells waste for application in the treatment of wastewater in Agadir city, Morocco. The results revealed that the primary components of shrimp shell are chitin and calcium bicarbonates. In another study, the mineral content of the pink shrimp shell was estimated at 21.50%, which was higher than that of the gray shrimp shell (12.95%).<sup>21</sup>

These results agree with the findings from the present study, and therefore confirm that Moroccan shrimp is rich in minerals and organic constituents. Therefore, waste from seafoods shells are valorized in various domains, including food, pharmaceutical, and agronomic fields.<sup>65</sup>

#### Microbial parameters

##### Isolated microbial strains

Lactic acid bacteria were isolated from different biotopes based on three criteria: acidifying power, fermentation ability, and antibacterial potency (Table 3). From the results, twenty strains of lactic acid bacteria were isolated, and were divided into two groups; (i) A group of eight lactic acid bacteria (LH2, LH3, LH4, LH7, LH10, LH11, LH13, and LH20) characterized by strong saccharolytic, acidifying, and antibacterial activity, and (2) A group of twelve lactic acid bacteria (LH1, LH5, LH6, LH8, LH9, LH12, LH14, LH15, LH16, LH17, LH18, and LH19) characterized by medium saccharolytic, acidifying, and antibacterial properties. Three isolates, including LH4, LH10, and LH20, showed strong fermentation and acidifying power on the MRS medium. The final pH values were 3.72 for the LH 4 strain, 3.74 for LH10, and 3.79 for LH 20, with a high acidity of 0.91, 0.95, and 1.05%,

respectively. These three strains were selected for carrying out the fermentation of shrimp by-products.

##### Selected fermentation starter

Table 4 summarizes the results obtained from the fermentation of the shrimp wastes. In terms of pH, the final values were estimated at 3.72, 3.74, and 3.79 for LH 4, LH 10, and LH 20 strains, respectively. The acidity of the strains was estimated at 0.91, 0.95, and 1.05%, respectively.

##### Acidity of isolated strains

The selected acidifying strains were tested to determine the best strain(s) suited for the fermentation of the shrimp wastes. The fermentation was followed by the determination of pH and acidity. The results obtained (Figure 3) showed that the pH was gradually decreased during the fermentation of the shrimp wastes for the three selected strains. These tests also demonstrated that the LH4 strain had a strong acidifying power, which facilitated the process of shrimp waste fermentation. The pH decreased to the lowest value of 3.20 for the LH4 strains. Similarly, the pH of the other two fermentations inoculated with the sourdough, that is LH 10 and LH 20, gradually decreased to a stable value of 3.28 and 3.50, respectively.

The acidity increased gradually to a value of around 0.13 - 1.28 after 10 days of fermentation, and a value of 1.30 after 12 days of fermentation. Acidification levels measured were slightly normal and always below the threshold for high acidification potential (Figure 4). The products resulting from the biological fermentation masked the odour of the shrimp waste, and gave the finished product an acidic and fresh smell. On the other hand, the three selected strains adapted to the prevailing temperature conditions during fermentation, making it possible to reduce the pH and increase the acidity of the fermentation medium. The LH4 bacteria produced the best acidification of the medium. This strain was selected as the shrimp wastes fermentation starter.

**Table 3:** Selected acidifying lactic acid bacteria (LAB) on MRS medium at 30°C and pH 5 for 24 hours of incubation

Biotope	LAB strain	pH <sub>i</sub>	pH <sub>f</sub>	Final acidity	Catalase	Gram
Cow milk	LH 1	5.52	4.05	0.90	-	+
	LH 2	5.71	3.85	0.96	-	+
	LH 3	5.73	3.90	0.93	-	+
	<b>LH 4</b>	5.54	<b>3.72</b>	1.04	-	+
Sugar cane press juice	LH 5	5.70	4.15	0.94	-	+
	LH 6	5.70	4.02	0.92	-	+
	LH 7	5.58	3.82	0.89	-	+
	LH 8	5.63	4.20	0.88	-	+
Fermented vegetable juice	LH 9	5.63	4.20	0.88	-	+
	<b>LH 10</b>	5.73	<b>3.74</b>	0.93	-	+
	LH 11	5.75	4.30	0.89	-	+
	LH 12	5.60	4.02	0.95	-	+
Fermented fruit juice	LH 13	5.65	3.90	0.75	-	+
	LH 14	5.72	4.50	0.81	-	+
	LH 15	5.74	4.42	0.74	-	+
	LH 16	5.85	4.56	0.78	-	+
	LH 17	5.69	4.35	0.79	-	+
	LH 18	5.64	4.58	0.77	-	+
	LH 19	5.74	4.54	0.86	-	+
	<b>LH 20</b>	5.82	<b>3.79</b>	0.89	-	+

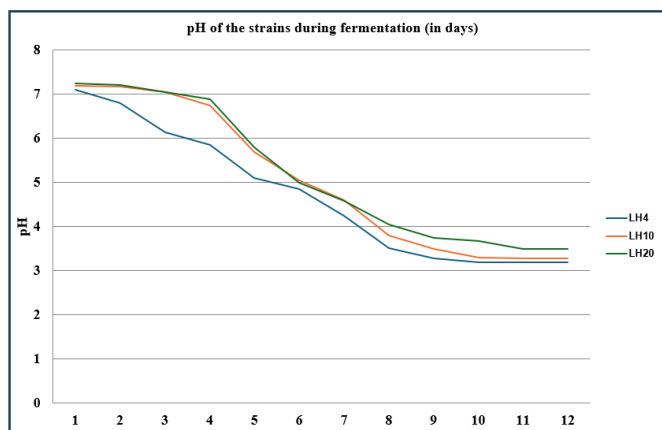
pH<sub>i</sub>: initial pH; pH<sub>f</sub>: final pH.**Table 4:** Evolution of pH and acidity after six days of fermentation of shrimp wastes at room temperature

Strain	pH <sub>initial</sub>	pH <sub>final</sub>	Initial acidity (%)	Final acidity (%)
LH 4	6.08	3.72	0.20	0.91
LH 10	6.02	3.74	0.18	0.95
LH 20	6.14	3.79	0.17	1.05

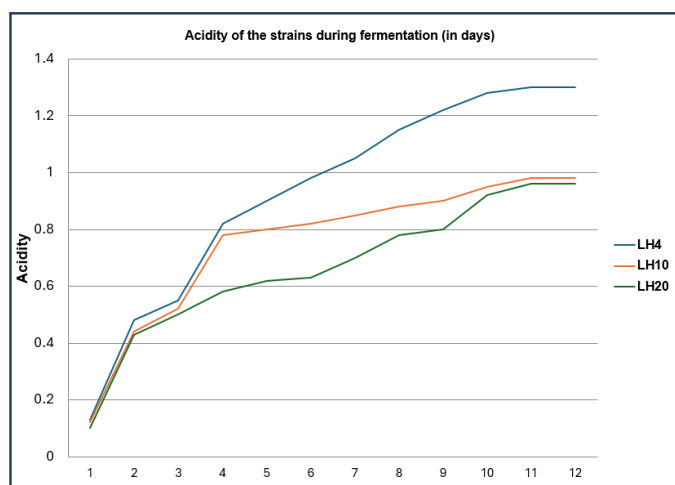
*Fermentation of shrimp wastes*

The processing of shrimp wastes by biological means was carried out in several steps. Fermentation was controlled and monitored by measuring pH until the product was stabilized. The results obtained are presented in Figure 5. The results revealed that the pH was initially estimated at 7.3 and gradually decreased during fermentation to a stable value of 3.7.

The inoculum reduced the lag phase and accelerated the fermentation process, which under normal conditions can take two days or more. This decrease in pH in the fermentation product demonstrated good fermentation conditions, which resulted in an increase in acidity and a decrease in pH. Consequently, these demonstrated a good stability and preservation of the fermented product. The stable product obtained had the following characteristics: Disappearance of the unpleasant odour characteristic of shellfish by-products, and improvement in hygienic quality.

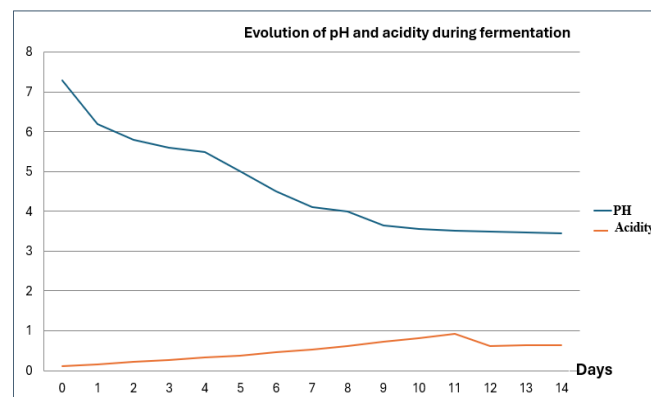


**Figure 3:** pH variation over time during fermentation of shrimp wastes inoculated with pure cultures of lactic acid bacteria



**Figure 4:** Acidity variation over time during fermentation of shrimp wastes inoculated with pure cultures of lactic acid bacteria

Currently, different studies have investigated the microbial properties of Shrimp wastes.<sup>1,66</sup> These studies focused on the beneficial and pathogenic microorganisms. For example, Khairina *et al.* (2016)<sup>67</sup> investigated the microbiological properties of “Ronto” a Traditional Fermented Shrimp from South Borneo, Indonesia. Nine samples of “Ronto” were collected from different districts in South Borneo. The results showed that the total bacteria were estimated at 3.01-log 5.36, and total lactic acid bacteria were estimated at log 0.77-log 3.38. Furthermore, proteolytic bacteria and halophilic bacteria in all the samples were log 2.7- log 4.79 and log 3.24 - log 5.3, respectively. Using a CaCO<sub>3</sub>-MRS agar, about 27 lactic acid bacteria were identified during culture. Two solitary “Ronto” were chosen, examined, and categorized based on their morphological, physiological, and biochemical traits. The lactic acid bacteria that were involved in this fermentation were identified as *Pediococcus dextrinicus* (Mees 1934) and *Pediococcus halophilus* [(Mees 1934). In another study, Liñan-Vidriales *et al.* (2021),<sup>68</sup> investigated the microbial properties of rice bran fermented with *Bacillus* and *Lysinibacillus* species of Pacific white shrimp (*Penaeus vannamei*). A total of twenty-two bacterial strains with various morphological traits were discovered in terms of probiotic strains. All of the isolates were found to be rod-shaped, spore-forming, non-motile, positive for catalase and oxidase, negative for arginine dihydrolase and nitrate reduction, and positive for oxidase and catalase.



**Figure 5:** Evolution of pH and acidity of shrimp wastes during fermentation

Additionally, the ability of each isolate to grow at various NaCl concentrations was examined, and it was shown that they could all grow at 2.5% NaCl. Eight isolates showed antagonistic activity against at least one *Vibrio* species, with inhibition zones ranging from 14 to 15 mm. Furthermore, it was shown by the 16S rRNA gene sequence analysis that one strain of *Bacillus toyonensis* BCT-7112T and seven isolates were closely related to *Lysinibacillus fusiformis* NBRC 15717T. The families Rhodobacteraceae, Vibrionaceae, and Flavobacteriaceae were the most predominant.

#### Microbiological analysis of shrimp wastes after fermentation Total aerobic mesophilic flora (TAMF)

The total aerobic mesophilic flora was monitored during fermentation, and the results are presented in Table 5. Before fermentation, the TAMF count was around 1.5.107 cfu/g, which was reduced to 5.3.105 cfu/g after fermentation and stabilization of the wastes. This was essentially the result of a decrease in pH and increased production of organic acids.

#### Microflora of hygienic interest

The evolution of microbial populations in the shrimp wastes before and after fermentation is presented in Table 5. The results showed that the studied microbial populations were significantly variable between fermented and non-fermented Shrimp wastes. Lactic acid bacteria and yeast were significantly superior in fermented wastes ( $p < 0.05$ ). In contrast, TAMF, molds, total coliforms, *Faecal coliforms*, and *Clostridium* sulphur reducers were significantly superior in non-fermented wastes. On the other hand, *Salmonella* strains were absent in both fermented and non-fermented shrimp wastes ( $p < 0.05$ ).

In the present study, nine different species, including total aerobic mesophilic flora and total coliforms; *Clostridia*, *Staphylococcus*, *Streptococcus*, *Lipolitics*, *Proteolytics*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Enterobacter*, were the most dominant. However, the microbial communities were significantly variable between non-fermented and fermented Shrimp wastes. Lactic acid bacteria and Yeast were significantly superior in fermented wastes. In contrast, TAMF, molds, total coliforms, *Faecal coliforms*, and *Clostridium* sulphur reducers were significantly superior in non-fermented shrimp wastes. On the other hand, *Salmonella* strains were absent in both fermented and non-fermented shrimp by-products. The highest load of Total coliforms, *Faecal coliforms*, and *Clostridium* sulphur reducers in non-fermented shrimp by-products is thought to be related to the sampling areas or the processing method of the shrimp by-products. Similar results have been reported by many authors on waste and edible parts of Shrimps.<sup>69,70</sup> Moreover, these results demonstrated that the fermentation process permitted the elimination of a wide range of pathogenic microorganisms, including Total coliforms, *Faecal coliforms*, *Clostridium* sulphur reducers, and Molds. The effectiveness of the fermentation process of the shrimp wastes is reflected in the results obtained, which showed the absence of any trace of toxicity and pathogenicity.

**Table 5:** Evolution of microbial populations in the shrimp wastes before and after fermentation

Microorganism	Load (cfu/g)	
	Before fermentation	Fermented product
Lactic acid bacteria	2.1 x 10 <sup>5</sup>	3.7 x 10 <sup>9</sup> *
TAMF	1.5 x 10 <sup>7</sup> *	5.3 x 10 <sup>5</sup>
Total coliforms	3.9 x 10 <sup>5</sup> *	0
Faecal coliforms	1.3 x 10 <sup>3</sup> *	0
<i>Clostridium Sulphur reducers</i>	7.8 x 10 <sup>2</sup> *	0
<i>Salmonella</i>	0	0
Yeasts	1.5 x 10 <sup>3</sup>	1.3 x 10 <sup>4</sup> *
Molds	95*	0

“\*” denotes statistically significant difference at  $p \leq 0.05$ ). TAMF: Total aerobic mesophilic flora

This could be attributed to the lactic acid bacteria, which have a high acidifying power, thus preventing the proliferation of pathogenic bacteria. The absence of pathogenic bacteria is suggested to promote the use of Shrimp by-products in food, pharmaceutical, and agronomic industries.

## Conclusion

The processing of marine products is the most active segment of the agri-food business. The most valuable and traded fisheries product worldwide is shrimp. Since about 60% of shrimp's weight is meant for human consumption, the shelling and heading processes produces a large amount of wastes. Without being treated, shrimp wastes are regularly dumped into public landfills, endangering the environment and human health. In this study, the chemical composition, and microbial quality of by-products prepared from shrimp shells were evaluated. Physicochemical parameters evaluated included temperature, pH, minerals, and organic compounds. The results obtained showed that the average temperature was estimated at 21°C, the average pH was estimated at 6.8, and the humidity was around 75%. The shrimp wastes were rich in dry matter, organic matter, chitin, ashes, and total protein, while fat was present only in small amount. The most predominant mineral was calcium, followed by iron, phosphorus, and potassium. In contrast, magnesium and zinc were present in the lowest amount in Shrimp by-products. In total, nine microbial strains were identified in Shrimp waste samples. *Escherichia coli* was the most dominant, followed by *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Enterobacter*. In contrast, *Moraxella* and *Shigella* species were present in the lowest amount at 2.10 and 3.15%, respectively. *Salmonella enteritidis* was absent in all shrimp waste samples. Lactic acid bacteria and yeast were significantly superior in fermented shrimp wastes, while, TAMF, molds, total coliforms, faecal coliforms, and *Clostridium sulphur reducers* were significantly superior in non-fermented shrimp wastes. Neither fermented nor non-fermented shrimp wastes contained *Salmonella* strains. It is proposed that the absence of harmful bacteria in shrimp wastes could encourage their use in agronomic, pharmaceutical, and food industries.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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