Highlights in BioScience
ISSN:2682-4043
DOI:10.36462/H.BioSci.202502

Research Article

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Shrimp waste utilization: Exploring composition, bioactive potential, and safety treatments

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Received: February 21, 2025
Accepted: May 22, 2025
Published: June 15, 2025

Citation: Omar IAS, Farag MAM, Taha AR, GabAlla AA, Rayan MA. Shrimp waste utilization: Exploring composition, bioactive potential, and safety treatments. 2025 June 15;8:bs202502

Data Availability Statement: All relevant data are within the paper and supplementary materials.
Funding: The authors have no support or funding to report.
Competing interests: The authors declare that they have no competing interests.

Abstract

The global focus on waste management and recycling is increasing, driven by the need to protect the environment from the harmful effects of waste accumulation. This study evaluated the chemical composition, bioactive potential, and microbial quality of shrimp waste, revealing significant variations across treatments and locations. Ghalyoun shrimp heads exhibited the highest protein (41.6%) and fat (16.9%) contents, while shells and tails from Port Said and head from Ghalyoun shrimp were rich in calcium (76.55 mg/g, 75.9 mg/g), respectively. Phenolic content was highest in Jumbo Suez heads (77.06 mg GAE/g DM), exhibiting maximum antioxidant activity of 65.8% (DPPH assay) in the same sample. Furthermore, HPLC analysis showed pyrogallol dominance in Ghalyoun shells (32,974 ppm) and Ismalawy heads (32,907 ppm), while Jumbo Suez heads had the highest gallic acid (4,508 ppm) and catechol (8,514 ppm). Ellagic acid peaked in Jumbo Suez (10,643 ppm), confirming strong bioactive potential. Heavy metals, including Pb and Ni, were highest in heads (1.3 mg/g and 1.34 mg/g, respectively), but effective chelation treatments, particularly T6 (acetic acid 12.5% + citric acid 5% + Sodium chloride 5%), significantly reduced these levels and microbial contamination, lowering total microbial counts to 3.57 log CFU/g. The findings suggest shrimp waste, particularly heads, as a valuable resource for antioxidants, proteins, and minerals, with appropriate treatments ensuring safety for industrial applications.

Keywords: Shrimp waste valorization, bioactive compounds, protein and mineral recovery, heavy metal and microbial reduction, antioxidant activity

Introduction

Shrimps are highly valued crustaceans enjoyed worldwide as a premium food source and an integral component of a balanced diet. The global shrimp market has experienced significant growth over the years, highlighting their importance as a rich nutritional source for human populations [1]. Shrimps are an excellent source of high-quality protein, vitamins, and astaxanthin, along with vital minerals such as phosphorus (P), calcium (Ca), selenium (Se), copper (Cu), zinc (Zn), and iodine (I_2) [2]. In 2023, global shrimp production totaled 5.6 million tons and it is expected to increase to 7.28 million tons by 2025, growing at an Annual Growth Rate of 6.1% between 2020 and 2025 [3; 4]. Consequently, shrimp waste production is estimated at around 3.8 million tons annually, constituting 50-60% of the total catch volume [5]. According to the FAO, a steady upward trend in shrimp consumption has been observed worldwide, indicating its growing dietary relevance and consumer demand [6]. Shrimp processing industries generate substantial amounts of waste, including shrimp heads, shells, and tails, which account for approximately 50% of the raw material. The increasing disposal of shrimp waste into the environment significantly contributes to pollution and poses serious health risks. As a result, effectively processing and utilizing shrimp shell waste is crucial for reducing environmental harm and advancing sustainability efforts [7; 8].

This waste is not merely a byproduct; it is a resource rich in valuable bioactive compounds, including polysaccharides, proteins, carotenoids, and fatty acids. These compounds have demonstrated diverse bioactivities, such as antioxidant, antimicrobial, anti-inflammatory, and antitumor effects, making them highly promising for applications in the pharmaceutical, nutraceutical, and cosmeceutical industries [9]. Harnessing these bioactive components aligns with sustainable

resource management and circular economy principles, emphasizing waste valorization to minimize environmental impact and maximize economic value. We would like to emphasize that one of the innovative aspects of this study is its focus on the utilization of Egyptian shrimp waste, a locally abundant raw material that has not been sufficiently exploited previously. Additionally, the study takes into account the specific physical and chemical characteristics of marine waste in the Egyptian environment, which may differ from those in other regions due to climatic and environmental variations. This study hypothesizes that shrimp waste, when processed and treated effectively, can serve as a valuable resource for high-value compounds while mitigating environmental hazards. Therefore, the main aim of this study is to investigate the chemical composition of shrimp waste from various locations, assess its protein content and bioactive potential, and evaluate methods to reduce heavy metals and microbial contamination. Ultimately, this research seeks to provide insights into sustainable utilization strategies for shrimp waste, offering a blueprint for industries, researchers, and policymakers to adopt more environmentally friendly and economically viable waste management practices.

Materials and Methods

Raw shrimp wastes (head, shell, and tail) were collected from local markets located in Ismailia, Port Said, Suez City, and Kafr El-Sheikh cities. Shrimp wastes were transported to the laboratory in an icebox. The ingredients, including salt, spices, garlic, and starch, were sourced from local markets in Ismailia Governorate, Egypt. All chemicals utilized in this study were of analytical grade. The shrimp wastes were separated into two groups: the first one was left unwashed, while the second one was thoroughly washed multiple times with tap water. Each group was further separated into two categories: one comprising shrimp heads and the other consisting of shell waste, including tails. All samples were analyzed to determine their chemical composition, microbiological load, mineral and heavy metal content, antioxidant activity (DPPH assay), and total phenolic content. It is noteworthy to mention that the untreated samples were analyzed in parallel across all tests to accurately assess the treatment effects.

Treatment of shrimp waste

The following treatments were carried out to reduce the heavy metals content and microbiological load: acetic acid 25% for 30 min (Treatment 1), citric acid 10% for 30 min (Treatment 2), sodium chloride 10% for 30 min (Treatment 3), combination of 12.5% acetic acid + 5% citric acid (Treatment 4), combination of 12.5% acetic acid + 5% sodium chloride (Treatment 5), and combination of 12.5% acetic acid + 5% citric acid + 5% sodium chloride (Treatment 6) [10].

Shrimp shell powder (SSP) preparation

Shrimp shell waste, including heads, shells, and tails, was separated and dried in a hot air oven at 60°C for 24 h. The dried shrimp wastes were finely ground using a blender, with each batch processed three times for 2–3 minutes. The ground shrimp wastes were then sieved to obtain a fine SSP. The powder was carefully packed in airtight glass bottles to preserve its quality and stored in a refrigerator at 4°C until it was required for further use [11]. **Figure 1** explains the treated and processed shrimp wastes from the different locations.

Figure 1. The treated and processed shrimp wastes from the different locations. **A1**: Heads only (Jumbo Suez shrimp), **A2**: Shrimp shell and tail (Jumbo Suez shrimp), **B1**: Heads only (Port Said shrimp), **B2**: Shrimp shell and tail (Port Said shrimp), **C1**: Heads only (Ismalawy shrimp), **C2**: Shrimp shell and tail (Ismalawy shrimp), **D1**: Heads only (Ghlayoun farmed shrimp), **D2**: Shrimp shell and tail (Ghlayoun farmed shrimp).

Determination of chemical composition

The shrimp waste samples were analyzed for ash, protein, and fat content using the Official Methods of Analysis. The samples were digested with a nitric acid and perchloric acid mixture (4:1, v/v), heated to 70-90°C for 10 minutes, cooled, and analyzed for cadmium (Cd), lead (Pb), nickel (Ni), cobalt (Co), and zinc (Zn) using an atomic absorption spectrophotometer (Thermo Electron Corp., S series, China) [12].

Antioxidants determination

Shrimp shells were dehydrated at 41°C for 48 h and ground into fine particles. For preliminary extraction, 5 g of shrimp shell was mixed with 50 mL of absolute methanol and stirred for 2 h at room temperature [1]. The mixture was filtered using filter paper, and the filtrate was evaporated at 40°C under vacuum conditions using a rotary evaporator. Light exposure was minimized throughout the process to protect the extract from degradation.

The antioxidant capacity was evaluated using the DPPH assay, following the procedure outlined by [13]. The extract (500 μg/mL) was diluted to concentrations of 50, 100, 150, and

200 µg/mL. One milliliter of each sample was combined with 1 mL of 0.4 mM DPPH solution and 2 mL of methanol in a sealed glass container. After 30 min of reaction at 37°C, the mixture was transferred to a disposable polystyrene cuvette, and the UV-visible spectrum was recorded. A blank sample, prepared with 2 mL of methanol and 1 mL of DPPH solution, was used as a control. The inhibition percentage, representing the decrease in absorbance of the sample compared to the initial absorbance of the blank, was measured at 515 nm using a spectrophotometer. Each sample was prepared and analyzed in triplicate, with all samples protected from light to prevent DPPH degradation. The inhibition of the DPPH radical was estimated through the following equation:

$$\%\text{Inhibition} = \left(\frac{\text{ACO} - \text{AAT}}{\text{ACO}}\right) \times 100\% \quad (1)$$

Where ACO represents the absorbance of the control at time $t = 0$, and AAT represents the absorbance of the samples at time $t = 30$ minutes.

Total polyphenol content (TPC)

To determine the TPC, 10 mg of methanolic extracts were dissolved in distilled water to give a concentration of 10 mg/mL [14]. This stock solution was then diluted to prepare concentrations of 0.5, 1, and 2 mg/mL. The TPC was estimated using the Folin-Ciocalteu reagent method, as described by [15], with gallic acid serving as the standard. Briefly, 0.5 mL of the extract was added to test tubes, followed by 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate (Na_2CO_3). The mixture was thoroughly mixed using a shaker and allowed to stand for 30 minutes. Absorbance was measured at 765 nm, and the results were expressed as mg gallic acid equivalent per gram dry matter as mg GAE/g DM.

HPLC analysis of phenolic compounds

Extraction, separation, and quantification were carried out following the procedure [16]. Methanol was added to 5 grams of each sample, and the mixture was centrifuged for 10 minutes at 10,000 rpm. After filtering the supernatant through a 0.2 µm Millipore membrane, 1-3 mL was collected in a vial for injection into an HPLC (Hewlett-Packard, series 1200) equipped with a quaternary HP pump (series 1100), solvent degasser, UV detector set at 280 nm, and auto-sampling injector. The temperature of the C18 reverse-phase column was maintained at 35°C. Methanol and acetonitrile were used as the mobile phase at a flow rate of 1.0 mL/min with gradient separation. After dissolving Sigma Co. phenolic standards in the mobile phase, they were injected into the HPLC. Using Hewlett-Packard software for data analysis, the concentration of phenolic compounds was determined based on retention time and peak area.

Total aerobic plate count (TAPC)

Samples of fresh and treated shrimp's shell and head were bacteriologically analyzed for total aerobic bacterial counts and total coliforms at 37 °C, following the method described below: The agar plate method was used to determine total aerobic bacterial counts on nutrient agar, according to [17]. A 10 g sample was blended in a high-speed blender under sterile conditions for 3 minutes in 90 mL of buffered peptone water. Decimal dilutions were prepared for the determination of various bacterial groups. The plates were incubated at 37 ± 2 °C for 48 hours.

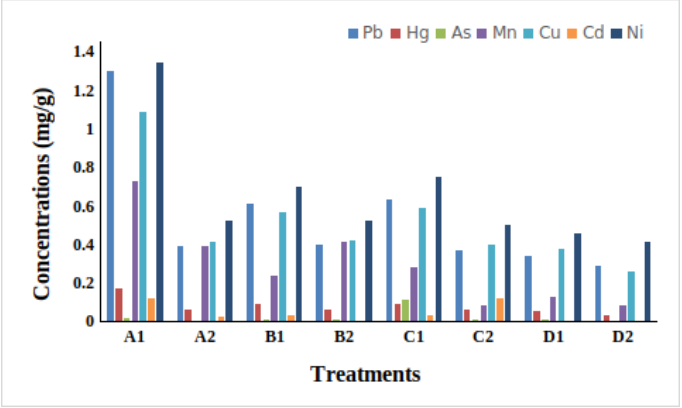

Figure 2. Minerals concentration (mg/g) in shrimp wastes samples. **A1**: Heads only (Jumbo Suez shrimp), **A2**: Shrimp shell and tail (Jumbo Suez shrimp), **B1**: Heads only (Port Said shrimp), **B2**: Shrimp shell and tail (Port Said shrimp), **C1**: Heads only (Ismalawy shrimp), **C2**: Shrimp shell and tail (Ismalawy shrimp), **D1**: Heads only (Ghlayoun farmed shrimp), **D2**: Shrimp shell and tail (Ghlayoun farmed shrimp).

Coliform group counts

Violet red bile (VRB) agar was used as the medium. Plates were incubated at 35°C for 18-24 hours, and purple, round colonies were counted. For fecal coliform and *E. coli*, purple colonies were confirmed on eosin methylene blue (EMB) agar, with blue-black, metallic-sheen colonies streaked onto slant agar. Results were reported as log CFU/g [18].

Statistical analysis

The data were evaluated using Analysis of Variance (ANOVA) test, conducted with SPSS software (version 16.0 for Windows, SPSS Inc., Chicago). Duncan's multiple range tests were employed to determine significant differences among treatment means at $P < 0.05$.

Results and Discussions

Proximate composition

The chemical composition of shrimp waste samples revealed significant variations across treatments and locations. Moisture content was highest in heads from Ismalawy and Jumbo Suez shrimp (C1: 30.5%, A1: 29.4%) and lowest in shells and tails from Jumbo Suez shrimp (A2: 25.9%). Protein content was notably highest in Ghalyoun farmed shrimp (D1: 41.6%, D2: 40.8%), reflecting their superior nutritional value, while Port

Said shrimp exhibited the lowest protein levels (B2: 35.9%, B1: 36.3%). Ash content was highest in shells and tails, particularly in Ismalawy and Jumbo Suez shrimp (C2: 31.39%, A2: 29.50%), likely due to the mineral-rich exoskeleton, while Ghalyoun shrimp heads had the lowest ash levels (D1: 13.2%). Fat content was highest in Ghalyoun shrimp heads (D1: 16.9%), indicating their lipid-rich potential, and lowest in Ismalawy and Jumbo Suez shells and tails (C2: 5.11%, A2: 5.95%) **Table 1**.

Table 1. Chemical composition of shrimp waste samples

Treatments	Chemical composition (%) on dry weight basis			
	Moisture	Protein	Ash	Fat
A1	29.41 ± 1.04^{a}	39.8 ± 0.98^{ab}	15.9 ± 0.57^{f}	14.9 ± 0.98^{b}
A2	25.9 ± 0.87^{c}	38.7 ± 1.30^{b}	29.5 ± 0.87^{b}	5.95 ± 0.87^{f}
B1	27.05 ± 0.75^{b}	36.3 ± 1.70^{c}	23.75 ± 1.03^{c}	12.9 ± 0.76^{c}
B2	26.7 ± 0.54^{bc}	35.6 ± 0.66^{c}	18.3 ± 1.06^{b}	8.97 ± 0.55^{e}
C1	30.5 ± 0.63^{a}	38.4 ± 0.58^{b}	20.03 ± 0.98^{d}	11.07 ± 1.2^{d}
C2	26.9 ± 0.86^{bc}	36.0 ± 0.93^{c}	31.39 ± 0.88^{a}	5.11 ± 0.64^{f}
D1	28.3 ± 0.32^{a}	41.6 ± 1.20^{a}	13.2 ± 0.79^{g}	16.9 ± 1.40^{a}
D2	26.8 ± 0.55^{bc}	38.1 ± 1.20^{a}	17.8 ± 0.56^{e}	14.6 ± 1.50^{b}

Results are expressed as average ± standard deviation of triplicate determinations. **A1** refers to heads only (Jumbo Suez shrimp), **A2** to shrimp shell and tail (Jumbo Suez shrimp), **B1** to heads only (Port Said shrimp), **B2** to shrimp shell and tail (Port Said shrimp), **C1** to heads only (Ismalawy shrimp), **C2** to shrimp shell and tail (Ismalawy shrimp), **D1** to heads only (Ghlayoun farmed shrimp), and **D2** to shrimp shell and tail (Ghlayoun farmed shrimp). Superscript letters a-f in the same columns indicate significant differences within the same strain ($P < 0.05$).

These results suggest that heads are nutritionally denser, with Ghalyoun farmed shrimp standing out as the most valuable resource for protein and fat extraction, while shells and tails are ideal for mineral recovery. It was found that no significant difference existed in crude protein content among shrimp shells, tails, and heads of the different five species of shrimp byproducts [19]. However, crude fat varied significantly across species and parts, with heads having the highest content (2.17-6.88%). Ash content (8.18-13.45%) was highest in shells/tails, reflecting mineral richness, while crude fiber (mainly chitin) peaked in shells/tails (7.86-10.78%) and exhibited antimicrobial potential. These differences might be attributed to species, age, and environmental factors.

Overall, the data underscore the potential of shrimp waste for sustainable valorization, with location and waste type playing key roles in determining their suitability for specific applications. Several studies have reported variable protein levels in shrimp waste, ranging from 39–70% [20; 21]. The protein content of shrimp waste from *Penaeus merguensis* was reported to be 24.03% [22]. It was found that raw shrimp head waste of *Penaeus semisulcatus* contained 65.76% protein [23]. Shrimp wastes were also observed to contain 35–40% protein [24], while the protein content of shrimp biowaste was reported to be 28.3% [25]. The variations in protein content of raw shrimp waste may be attributed to differences in shrimp species and the sources of raw materials. Confirming its potential, shrimp waste powder was found to nutritionally enrich food products while preserving their sensory qualities and safety, establishing a practical pathway for sustainable seafood byproduct utilization [26].

Minerals concentration in shrimp waste

The trace mineral analysis of shrimp waste samples revealed notable differences across treatments and locations, highlighting the nutrient diversity in shrimp heads and shells **Figure 2**. Magnesium (Mg) content was highest in heads from Port Said (B1: 88.4 mg/g) and Ismalawy (C1: 87.9 mg/g), indicating their potential as rich Mg sources, while the lowest levels were found in shells and tails from Port Said (B2: 69.4 mg/g). Sodium (Na) content peaked in heads from Port Said and Ghalyoun shrimp (B1 and D1: 23.06 mg/g), whereas shells and tails from Ghalyoun (D2: 9.19 mg/g) had the lowest levels. Iron (Fe) concentrations were generally low across all samples, with the highest values in Port Said heads (B1: 0.18 mg/g) and the lowest in Ismalawy and Ghalyoun shells and tails (C2, D2: 0.05 mg/g), suggesting limited Fe availability. Fe is predominantly stored as ferritin in the liver, spleen, and bone marrow, while in human blood, it binds to hemoglobinan iron-containing protein responsible for oxygen binding and transport [19].

Phosphorus (P) content ranged from 9.46 mg/g (C2) to 13.4 mg/g (D1), with Ghalyoun heads containing the highest levels, reflecting their potential as a phosphorus source. Calcium (Ca) was notably high in shells and tails, particularly from Port Said (B2: 76.55 mg/g) and Ghalyoun (D1: 75.9 mg/g), underscoring the mineral richness of exoskeleton-based waste. Selenium (Se) levels varied, with the highest in Ismalawy shells and tails (C2: 0.67 mg/g), indicating a promising source of this essential trace element. Potassium (K) content was highest in Ghalyoun heads (D1: 4.78 mg/g), followed by shells and tails from Jumbo Suez (A2: 4.14 mg/g), showcasing their potential for potassium recovery. These results are in agreement with those obtained by [27], who observed that calcium (155 ± 4.00 mg/g), iron (42.11 ± 2.00 mg/g), copper (40 ± 2.00 mg/g), and manganese (12.6 ± 2.2 mg/g) were prevalent in shrimp waste. It was reported that calcium was abundantly present in prawn shells, with a concentration 17 times higher than that of magnesium [28]. Furthermore, it was found that the concentrations of minerals such as Na, K, P, Ca, Mg, Fe, and Mn in shrimp waste powder from *Penaeus* spp. were 53.2, 47.5, 21.8, 89.1, 27.1, 39.4, and 17.4 mg/g, respectively [29].

Overall, the findings from this study demonstrate that shrimp heads typically contain higher concentrations of essential minerals such as magnesium, sodium, and potassium when compared to other shrimp by-products. In contrast, the shells and tails of shrimp were found to be particularly rich in calcium and selenium. These distinct mineral distribution patterns highlight the unique nutritional composition associated with each type of shrimp waste. Moreover, the variation in mineral content based on both waste type and collection location presents valu-

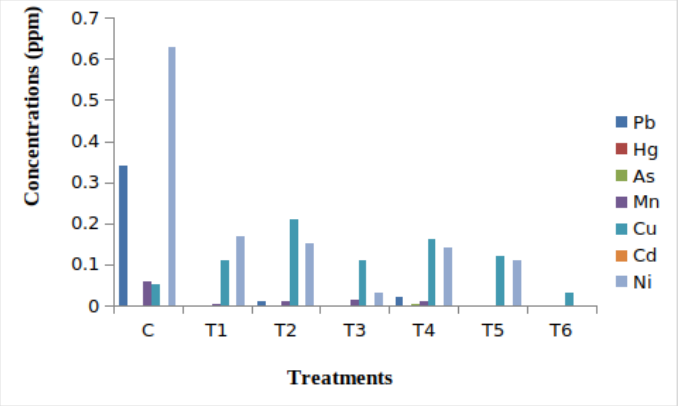

Figure 3. Heavy metals elements (mg/g) of shrimp wastes. **A1**: Heads only (Jumbo Suez shrimp), **A2**: Shrimp shell and tail (Jumbo Suez shrimp), **B1**: Heads only (Port Said shrimp), **B2**: Shrimp shell and tail (Port Said shrimp), **C1**: Heads only (Ismalawy shrimp), **C2**: Shrimp shell and tail (Ismalawy shrimp), **D1**: Heads only (Ghlayoun farmed shrimp), **D2**: Shrimp shell and tail (Ghlayoun farmed shrimp).

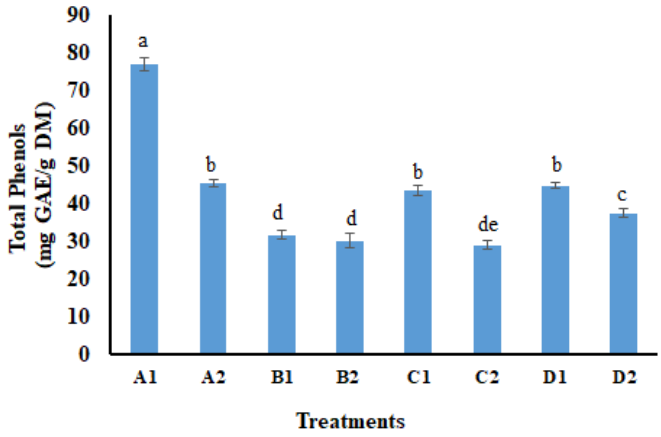

Figure 4. Total phenolic content of shrimp waste samples: **A1** (heads only, Jumbo Suez shrimp), **A2** (shrimp shell and tail, Jumbo Suez shrimp), **B1** (heads only, Port Said shrimp), **B2** (shrimp shell and tail, Port Said shrimp), **C1** (heads only, Ismalawy shrimp), **C2** (shrimp shell and tail, Ismalawy shrimp), **D1** (heads only, Ghlayoun farmed shrimp), and **D2** (shrimp shell and tail, Ghlayoun farmed shrimp). Error bars indicate ± standard deviation. Values with different letters are significantly different ($P < 0.05$).

able opportunities for selective resource recovery. Such targeted extraction and utilization strategies can contribute significantly to sustainable practices in various sectors, including agriculture, pharmaceutical development, and the production of dietary supplements, thereby promoting environmental sustainability and economic efficiency.

Heavy metals in shrimp waste samples

Shrimp heads are prone to heavy metal accumulation, leading to diminished nutritional quality. This phenomenon occurs because aquatic organisms can bioaccumulate heavy metals from various environmental sources, such as sediments, atmospheric deposition, and wastewater discharge [30]. The hepatopancreas, a key detoxification and metal storage organ in crustaceans, plays a significant role in this process. Additionally, the gills, due to their direct exposure to water, further facilitate the rapid uptake of heavy metals [31]. The analysis of heavy metal concentrations in shrimp waste samples revealed significant variations depending on the sample type (heads vs. shells and tails) and location **Figure 3**. Lead (Pb) levels were highest in Jumbo Suez heads (A1: 1.3 mg/g), while the lowest levels were observed in Ghalyoun shells and tails (D2: 0.29 mg/g). Mercury (Hg) concentrations were relatively low across all samples, with A1 (0.17 mg/g) showing the highest level and D2 (0.032 mg/g) the lowest. Arsenic (As) content peaked in Ismalawy heads (C1: 0.11 mg/g) but was minimal in most other samples, with A2 and D2 both at 0.005 and 0.006 mg/g, respectively.

Manganese (Mn) levels were highest in A1 (0.73 mg/g) and lowest in C2 and D2 (0.13 mg/g), reflecting the nutritional richness of heads compared to shells and tails. Copper (Cu) was most abundant in A1 (1.09 mg/g) and lowest in D2 (0.26 mg/g). Cadmium (Cd) concentrations were generally low, with the highest levels detected in A1 and C2 (0.118 mg/g each), while several samples, such as B2, D1, and D2, showed no detectable Cd. Nickel (Ni) was highest in A1 (1.34 mg/g) and lowest in D2 (0.41 mg/g). Zinc (Zn), an essential trace metal, showed the highest levels in Ghalyoun heads (D1: 60.6 mg/g) and the lowest in Ismalawy shells and tails (C2: 21.7 mg/g). In general, these results are in agreement with those obtained by [32]. The FAO/WHO established maximum permissible limits of 0.50 μg/g for Pb, Cd, As, and Hg. Accordingly, the contents of Pb, Cd, As, and Hg in the different investigated samples are higher than the limited values [31].

Overall, shrimp heads consistently exhibited higher concentrations of heavy metals compared to shells and tails, with Jumbo Suez and Ghalyoun shrimp showing the most notable levels. While heads are nutrient-rich, the elevated heavy metal concentrations necessitate appropriate treatment and monitoring to ensure safe and sustainable utilization of shrimp waste.

Bioactive compounds of shrimp waste

Total phenols content of shrimp waste samples (TP)

The total phenolic content of shrimp waste samples demonstrated significant variability across treatments and locations ($P < 0.05$) **Figure 4**. The highest total phenol content was observed in the heads from Jumbo Suez shrimp (A1: 77.06 mg GAE/g DM), indicating their superior potential as a source of antioxidant compounds. This was followed by the shell and tail from Jumbo Suez shrimp (A2: 45.45 mg GAE/g DM), heads from Ghalyoun farmed shrimp (D1: 44.74 mg GAE/g DM), and Ismalawy shrimp (C1: 43.64 mg GAE/g DM). Shells and tails generally exhibited lower phenol content, with Jumbo Suez

Table 2. HPLC analyses of phenolic compounds (ppm) in shrimp waste samples

Phenolic compound	A1	A2	B1	B2	C1	C2	D1	D2
Pyrogallol	29677.6	16574.9	20061.1	25282.1	32906.8	23698.2	24694.7	32974.3
Gallic	4507.5	2521.6	1669.64	908.5	1078.3	2374.4	1218.1	0
3-OH Tyrosol	3560.2	1764.9	2203.06	655.1	2589.5	509.3	3679.2	209.6
Catechol	8514.0	5500.8	1786.36	172.5	406.8	111.1	3512.0	459.0
4-Amino benzoic	480.4	258.2	43.25	20.1	149.8	211.8	211.8	27.42
Catechein	5109.7	4577.9	2441.58	700.2	872.5	303.5	1084.5	490.5
Chlorogenic	2686.1	1672.4	702.04	91.9	1441.9	267.4	1537.7	221.4
P-OH-benzoic	1389.2	647.8	161.35	180.6	163.4	114.9	564.9	181.9
Benzoic	1832.6	896.7	1091.2	242.5	1320.4	153.4	758.8	54.2
Caffeic	2059.3	1341.5	257.02	65.1	679.4	154.9	613.4	112.5
Vanillic	1605.3	405.1	377.6	269.9	305.6	164.7	805.2	182.7
Caffeine	1721.5	558.2	1177.96	223.6	1058.3	186.2	508.6	0
Oleuropein	3142.6	337.7	3304.3	283.1	913.5	515.5	524.1	822.8
Ferulic	928.5	394.8	745.98	224.1	832.5	179.6	951.6	74.6
Ellagic	10643.3	7854.7	1950.2	1689.2	1622.8	1260.2	578.6	967.2
Coumarin	582.6	180.4	41.29	29.3	44.4	37.20	75.16	38.82

A1 refers to heads only (Jumbo Suez shrimp), **A2** to shrimp shell and tail (Jumbo Suez shrimp), **B1** to heads only (Port Said shrimp), **B2** to shrimp shell and tail (Port Said shrimp), **C1** to heads only (Ismalawy shrimp), **C2** to shrimp shell and tail (Ismalawy shrimp), **D1** to heads only (Ghlayoun farmed shrimp), and **D2** to shrimp shell and tail (Ghlayoun farmed shrimp).

shrimp shells (A2: 45.45 mg GAE/g DM) being an exception, showcasing relatively high phenol levels compared to other shell samples. The lowest phenol content was recorded in shells and tails from Ismalawy shrimp (C2: 29.06 mg GAE/g DM) and Port Said shrimp (B2: 30.23 mg GAE/g DM), as well as heads from Port Said shrimp (B1: 31.78 mg GAE/g DM). It was reported that the total phenolic content in shrimp shell waste from *Palaemon serratus* and *Palaemon varians*, collected along the Portuguese coast, ranged from 4.7 to 10.4 mg GAE/g DM [1]. The differences may be attributed to variations in species, environmental conditions, and processing methods, which influence the phenolic compound levels in shrimp waste [33].

Overall, shrimp heads consistently exhibited higher total phenolic content compared to shells and tails, with Jumbo Suez shrimp heads being the richest source. These findings suggest that shrimp heads, particularly from Jumbo Suez and Ghalyoun shrimp, could be prioritized for antioxidant extraction in nutraceutical and pharmaceutical applications, supporting sustainable valorization strategies.

HPLC analyses of phenolic compounds

The HPLC analysis revealed variability in the phenolic compound profiles of shrimp waste samples across different treatments and locations **Table 2**. Pyrogallol was the dominant phenolic compound, with the highest levels observed in Ghalyoun shells (D2: 32,974.3 ppm) and Ismalawy heads (C1: 32,906.8 ppm), while gallic acid and catechol were most abundant in Jumbo Suez heads (A1: 4,507.5 ppm and 8,514.0 ppm, respectively). Ellagic acid was prominent in Jumbo Suez samples (A1: 10,643.3 ppm; A2: 7,854.7 ppm), underscoring their strong bioactive potential. Heads consistently exhibited higher phenolic concentrations than shells and tails, particularly in samples from Jumbo Suez and Ghalyoun, which demonstrated superior phenolic diversity and content. In contrast, Port Said samples showed the lowest phenolic levels, with shells and tails generally being less phenol-rich than heads.

These findings highlight the significant bioactive potential of shrimp heads, particularly from Jumbo Suez and Ghalyoun, for use in nutraceutical, pharmaceutical, and functional food industries, supporting the sustainable valorization of shrimp waste. The results also suggest that treatment conditions play a crucial role in the preservation or degradation of phenolic compounds in shrimp waste, with significant differences across the groups. The variability in phenolic content aligns with previous studies highlighting the sensitivity of phenolic compounds to environmental factors and processing conditions, which can significantly influence their bioavailability and antioxidant properties [34; 35].

Antioxidant activity (DPPH %) of shrimp waste samples

The antioxidant activity was assessed using DPPH (2,2-diphenyl-1-picrylhydrazyl), a stable free radical compound with maximum absorbance at 517 nm. When DPPH radicals interact with proton-donating substances, such as antioxidants, the radicals are neutralized, leading to a reduction in absorbance [36].

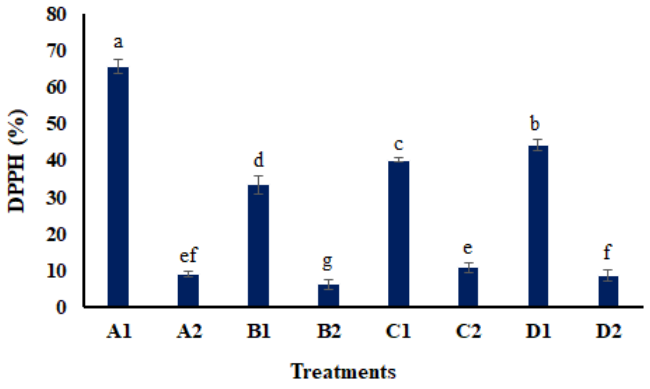

Figure 5. Antioxidant activity (DPPH %) of shrimp waste samples: **A1** (heads only, Jumbo Suez shrimp), **A2** (shrimp shell and tail, Jumbo Suez shrimp), **B1** (heads only, Port Said shrimp), **B2** (shrimp shell and tail, Port Said shrimp), **C1** (heads only, Ismalawy shrimp), **C2** (shrimp shell and tail, Ismalawy shrimp), **D1** (heads only, Ghlayoun farmed shrimp), and **D2** (shrimp shell and tail, Ghlayoun farmed shrimp). Error bars indicate ± standard deviation. Values with different letters are significantly different ($P < 0.05$).

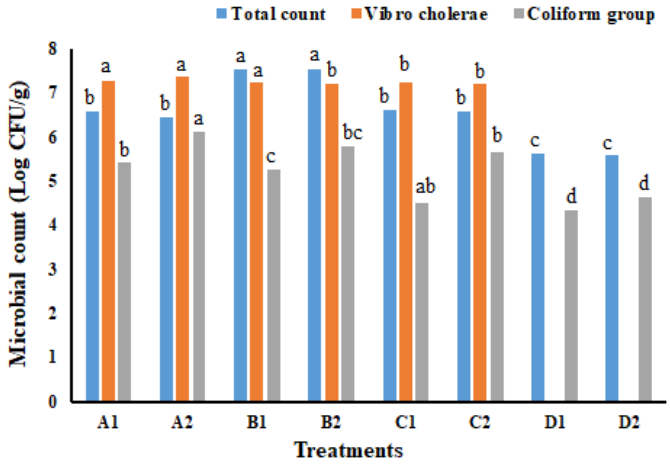

Figure 6. Microbial analysis of shrimp waste samples. **A1**: Heads only (Jumbo Suez shrimp), **A2**: Shrimp shell and tail (Jumbo Suez shrimp), **B1**: Heads only (Port Said shrimp), **B2**: Shrimp shell and tail (Port Said shrimp), **C1**: Heads only (Ismalawy shrimp), **C2**: Shrimp shell and tail (Ismalawy shrimp), **D1**: Heads only (Ghlayoun farmed shrimp), **D2**: Shrimp shell and tail (Ghlayoun farmed shrimp).

Figure 5 illustrates the DPPH radical-scavenging activity of various shrimp waste samples at a concentration of 1 mg/mL. The antioxidant activity (DPPH %) of shrimp waste samples showed significant variation across treatments and locations ($P < 0.05$). The highest antioxidant activity was observed in heads from Jumbo Suez shrimp (A1: 65.793%), indicating their superior free radical scavenging capacity. Ghalyoun heads (D1: 44.337%) and Ismalawy heads (C1: 40.18%) also exhibited notable antioxidant activity, underscoring the potential of shrimp heads as rich sources of antioxidants.

In contrast, shells and tails consistently showed lower activity, with the lowest values recorded in Port Said shells and tails (B2: 6.382%) and Ghalyoun shells and tails (D2: 8.864%). Among shell and tail samples, Jumbo Suez (A2: 9.083%) and Ismalawy (C2: 10.965%) demonstrated slightly higher activity. These results highlight that shrimp heads, particularly from Jumbo Suez and Ghalyoun shrimp, are a valuable source of antioxidants, while shells and tails exhibit significantly lower activity, making heads the primary target for antioxidant recovery in valorization strategies. The results indicate that the samples likely contained peptides or chitooligosaccharides, which act as electron donors. These compounds can react with free radicals, stabilizing them and terminating the radical chain reaction [37; 38; 39].

Microbial quality of shrimp's waste samples

Bacterial growth is a primary factor in the spoilage of fish and fish products. Therefore, bacterial count is recommended as a key indicator for assessing the quality of food products [40]. The microbial analysis of shrimp waste samples revealed significant differences in total microbial count, *Vibrio cholerae*, and coliform group levels across treatments ($P < 0.05$) **Figure 6**. The highest total microbial count was observed in Port Said shrimp, with both heads (B1: 7.52 log CFU/g) and shells/tails (B2: 7.54 log CFU/g) showing significantly higher values compared to samples from other locations. Jumbo Suez shrimp heads (A1: 6.58 log CFU/g) and Ismalawy heads (C1: 6.62 log CFU/g) exhibited moderate microbial loads, while Ghalyoun shrimp (D1: 5.62 log CFU/g, D2: 5.58 log CFU/g) had the lowest counts, suggesting better microbial quality.

For *Vibrio cholerae*, Jumbo Suez shrimp (A1: 7.26 log CFU/g, A2: 7.37 log CFU/g) showed the highest contamination, highlighting a potential safety concern. Port Said samples (B1: 7.25 log CFU/g, B2: 7.19 log CFU/g) and Ismalawy samples (C1: 7.22 log CFU/g, C2: 7.21 log CFU/g) followed closely, while *Vibrio cholerae* was not detected in Ghalyoun shrimp (D1 and D2). Coliform group levels were highest in Jumbo Suez shells and tails (A2: 6.11 log CFU/g) and moderate in Port Said samples (B2: 5.77 log CFU/g). Ghalyoun shrimp (D1: 4.33 log CFU/g, D2: 4.64 log CFU/g) consistently showed the lowest coliform levels, further emphasizing their microbial safety. Ismalawy shrimp (C1: 4.49 log CFU/g) also had low coliform counts in the heads, aligning with better overall microbial quality.

In summary, shrimp samples collected from Port Said exhibited the highest levels of microbial contamination, with particularly elevated values observed in the total microbial count. On the other hand, shrimp waste originating from Ghalyoun farms consistently demonstrated the lowest microbial loads across all evaluated parameters. These results underscore the significant influence of geographic location and specific waste type on the microbial safety of shrimp by-products. The notably lower contamination levels found in Ghalyoun shrimp suggest that this

source possesses superior microbial quality, making it a more suitable candidate for various potential applications, including those in agriculture, food processing, and biotechnological industries where microbial safety is a critical consideration.

Table 3. Effect of some treatments on reducing microbial count of shrimp wastes.

Treatments	Microbial count (Log CFU/g)		
	Total count	*Vibrio cholerae*	Coliform group
C	5.32[a]	ND	4.75[a]
T1	4.65[c]	ND	4.15[b]
T2	5.12[b]	ND	3.24[c]
T3	4.36[c]	ND	4.13[b]
T4	4.46[c]	ND	3.19[c]
T5	4.59[c]	ND	4.13[b]
T6	3.57[d]	ND	2.23[d]

C: Control, **T1**: Acetic acid 25% /30 min, **T2**: Citric acid 10% /30 min, **T3**: Sodium chloride 10% /30 min, **T4**: Acetic acid 12.5% + Citric acid 5% /30 min, **T5**: Acetic acid 12.5% + Sodium chloride 5% /30 min, **T6**: Acetic acid 12.5% + Citric acid 5% + Sodium chloride 5% /30 min. a–d values in the same columns with different superscript letters within a same strain are significantly different (P<0.05). ND: Not detected.

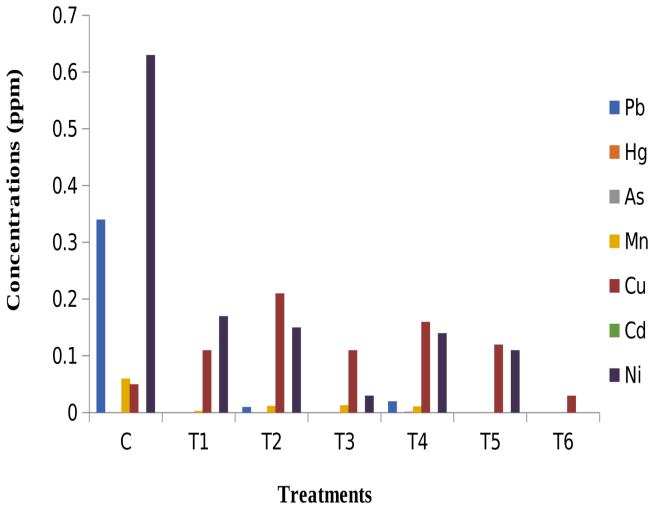

Figure 7. Effect of some treatment on reduce the heavy metals of shrimp wastes. All treatments were applied for 30 minutes. The specific treatments were: C: Control, T1: Acetic acid 10 %, T2: Citric acid 10 %, T3: Sodium chloride 10 %, T4: Acetic acid 12.5 % + Citric acid 5 %, T5: Acetic acid 12.5 % + Sodium chloride 5 %, T6: Acetic acid 12.5 % + Citric acid 5 % + Sodium chloride 5 %. All treatments were applied for 30 minutes.

Treatments to reduce heavy metals in shrimp waste

The chelation method was identified as a promising technique for effectively removing heavy metals from shrimp waste samples. The formulations included: T1 (10% acetic acid), T2 (10% citric acid), T3 (10% sodium chloride), T4 (12.5% acetic acid + 5% citric acid), T5 (12.5% acetic acid + 5% sodium chloride), and T6 (12.5% acetic acid + 5% citric acid + 5% sodium chloride), each applied for 30 minutes. Interestingly, these treatments were able to chelate the heavy metals present in the shrimp waste. The concentrations of acid and salt were selected based on previous studies that demonstrated their effectiveness under similar conditions [10]. Regarding the use of 25% acetic acid in treatment T1 compared to 12.5% in treatments T4–T6, this was intentionally designed to evaluate the impact of high versus low concentrations on product quality and microbial inhibition. It is worth noting that the lower concentration (12.5%) was combined with other substances to enhance its efficacy, whereas the higher concentration (25%) was applied alone without additional components, to assess its standalone effectiveness.

T6 (12.5% acetic acid + 5% citric acid + 5% sodium chloride for 30 minutes) resulted in the lowest levels of heavy metals (Pb, Hg, As, Mn, Cu, Cd, Ni, and Zn) in shrimp waste **Figure 7**. It was demonstrated that acetic acid effectively chelated heavy metals in green mussels [10]. Chelating organic acids can remove exchangeable, carbonate, and reducible fractions of heavy metals through washing processes [41; 42]. Sodium acetate has also been shown to chelate heavy metals such as arsenic (As), lead (Pb), cadmium (Cd), and nickel (Ni) in green mussels, reducing their levels to those deemed safe for human consumption [32].

Effect of some treatments on reducing microbial content in shrimp waste

The results demonstrate that various treatments effectively reduced the microbial content of shrimp wastes, with all treatments successfully eliminating *Vibrio cholerae* **Table 3**. The control sample (C) had the highest microbial counts, emphasizing the need for intervention. Among the treatments, T6 (12.5% acetic acid + 5% citric acid + 5% sodium chloride) was the most effective, achieving the lowest total microbial count (3.57 log CFU/g) and coliform count (2.23 log CFU/g), highlighting the synergistic effect of combining acids and salt. Single-agent treatments, such as T1 (25% acetic acid) and T2 (10% citric acid), were less effective, with higher microbial counts compared to the combined treatments. This study underscores the superior antimicrobial efficacy of combination treatments like T6, making them highly suitable for enhancing the microbial safety and quality of shrimp wastes.

Economic feasibility

Repurposing shrimp waste safeguards the environment and promotes the circular economy through sustainable resource utilization. The proposed treatments are amenable to scale-up, as they utilize locally available and low-cost chemicals such as acetic acid, sodium chloride, and citric acid. The procedures for preparing shrimp shell powder are straightforward and do

not require complex industrial techniques, thereby facilitating large-scale application. Moreover, the resulting shell powder exhibits promising properties that qualify it for direct use in the food industry as an ingredient in nutraceutical products.

Conclusion

This study highlights the significant potential of shrimp waste as a resource for sustainable valorization, with variations in chemical composition, mineral content, heavy metals, bioactive compounds, and microbial quality across different treatments and locations. Shrimp heads, particularly from Ghalyoun and Jumbo Suez regions, emerged as the most nutritionally dense components, with high protein, fat, and phenolic compound levels, making them ideal for antioxidant extraction and functional food applications. Shells and tails, on the other hand, were rich in calcium and selenium, suitable for mineral recovery. Heavy metal analysis showed that heads generally had higher concentrations, necessitating effective treatments like chelation to ensure safety. The combined treatment of acetic acid, citric acid, and sodium chloride (T6) proved to be the most effective in reducing heavy metal levels and microbial contamination, including *Vibrio cholerae*, showcasing its efficacy as a decontamination strategy. Antioxidant activity further confirmed the bioactive potential of shrimp heads, with Jumbo Suez samples demonstrating superior free radical scavenging capacity.

Overall, our work uniquely targets Egyptian shrimp waste, leveraging its distinct regional traits to fill a research gap and advance localized circular economy strategies. By emphasizing location-specific treatments, this study maximizes waste valorization while ensuring safety, enabling applications in agriculture, pharmaceuticals, and nutraceuticals to uphold circular economy principles.

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