VER
Vascular & Endovascular Review

Study of The Affecting Factors To Preparation Protein Isolate From Local Shrimp Waste And Some Of Its Functional Properties

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ABSTRACT

The protein isolate of local shrimp residues was prepared by the base dissolution and acid precipitation method. The affecting factors of isolate yield were studied, including the mixing ratio, time, temperature, and the best pH for solubility and precipitation. The amino acid content and some functional properties were studied. The result showed that the protein content of Fresh and dried shrimp shells were (10.91%, 13.02%) higher than of fresh and dried shrimp heads (6.45%, 7.89%). The best mixing ratio to obtain the highest protein percentage was 1:10, temperature 60c_o and extraction time was 60 min. The best pH for protein solubility was 10, and the pH 4.5 was the best for protein recovery. The protein isolated from shrimp waste have a high content of polar amino acids. The protein isolate has a high water and fat binding capacity, in addition to its emulsifying and foaming properties.

KEYWORDS: Shrimp waste, Protein Isolate, Factors affecting the isolate ratio, Functional properties.

How to Cite: Murtadha A. Hanoon, Dhuha D. Salman, (2025) Study of The Affecting Factors To Preparation Protein Isolate From Local Shrimp Waste And Some Of Its Functional Properties, Vascular and Endovascular Review, Vol.8, No.11s, 454-464.

INTRODUCTION

The biodiversity of crustacean invertebrates in Iraq, specifically in the Shatt al-Arab region is good despite of the environmental conditions to which the region has been exposed. *Penaeus indicus* is one of the major commercial species of the world (1) and it is a type of shrimp that is abundant in fish markets in Basra . Every year, approximately 6–8 million tons of crustacean shell waste are discarded by the seafood industry, thus simultaneously wasting resources and polluting the environment (2). Shrimp processing entails removing the head and hard carapace, which make up nearly 40–60% of the entire animal and generate a lot of waste (1). Only 65% of the shrimp is edible and the remainder is discarded as inedible waste (cephalothorax and exoskeleton). Over the years, techniques have been developed for the exploitation and recovery of these by-products from valuable biopolymers (3). Total world shrimp production, which reached 5.03 million tons in 2020, is expected to increase to 7.28 million tons by 2025 For this reason,

recovering these wastes generated during processing will be beneficial for the shrimp processors and the economy of the country (4). The proportion of shrimp heads and shells is estimated to be between 30-40% from the shrimp weight (5). The main components of shrimp waste are protein (35–50%), chitin (15–25%), minerals (10–15%), and astaxanthin, Shrimp and other crustacean processing waste are rich in essential by-products such as carotenoids, proteins, minerals, flavor compounds, and chitin. In crustaceans, carotenoids are found in the form of carotenoproteins, which are stable carotenoid complexes attached to high-density lipoproteins (6) The protein itself comprises of 60-75% collagen, 4-5% elastine and 20-35% keratine (7). Shrimp proteins are Carotenoprotein occurs as carotenolipoproteins in their ovaries and eggs and as chitinocarotenoids and crustacyanins in their exo skeletons (8).Carotenoproteins have been identified as natural colorants (9).with bioactivities, including antioxidative properties (8)Therefore, the production of carotenoprotein from shrimp shells and head waste can be used as a functional additive in foods and beverages (10).

A base extraction is usually employed for deproteinization of shells during isolation of the chitin from shrimp. Protein concentrate can be obtained by base solubilization followed by isoelectric precipitation , This process is applied to raw materials such as muscle or by-products of shrimp, which are sources of animal protein (11). when proteins have net charges, positive or negative, they initiate an electrostatic protein–water interaction that decreases protein–protein hydrophobic interactions (12), Consequently, the protein molecules become more polar, with an increase in the associated water content, rendering them water soluble (13). The economy ofindustrial processing of crustaceans can be improved by the full utilization of chitin and proteins included on a dry basis of the shell waste in amounts ranging from 14 to 32% and from 18 to 42%, respectively (14). The aim of the present study was to obtain local shrimp wastes (head and shell) from *Penaeus indicus* shrimp and preparation isolate protein, studying amino acid composition, protein solubility and some functional

properties such as water and oil absorption capacity, emulsifying and foaming properties.

MATERIALS AND METHODS

Sample collection

shrimp waste was collection from (Penaeus indicus) which obtained from a local market in Baghdad. The heads and shells were separated and washed and cleaned well from meat, blood and impurities. The waste was stored in sealable bags and frozen at 18°C until used.

Proximate analysis

The chemical composition of shrimp head and shrimp shell was determined according to the methods described by AOAC (2016) (15). Moisture was determined by oven drying at 105c° for 24 h, ash by burn at 600°C until white or grey ash was obtained. Total lipids were determined by extraction with hexane using the soxhlet apparatus and total proteins by the kjeldhal method.

Preparation of the isolated protein

The isolate protein from head and shell shrimp was prepared as mentioned (16) with some modification ,The heads and shells of the shrimp were chopped separately and then mixed with distilled water at a ratio of 1:10 for (60 min) . The pH was adjusted to 10 using 1M sodium hydroxide. The mixture was left on the magnetic mixer for an hour, then separated by centrifugation at 10,000 x g for 20 minutes. The filtrate was then separated from the precipitate. The pH of the filtrate was reduced to 4.5 using 1M hydrochloric acid. The precipitate was taken and the pH was adjusted to 7. The sample was lyophilized using a freeze dryer and stored at a temperature of 18-C until use.

Factors affecting the yield and percentage of isolates protein

The protein isolate was prepared as mentioned by (17, 18) with some modifications.

Determination of the best mixing ratio:

The best mixing ratio of shrimp waste with water using mixing ratios (1:5) (1:10) (1:15) w/v.

Determination of the best time extraction:

Determine the best time for extracting the protein isolate of shrimp waste mixing time were (30,60,120) minutes

Determination of the best extraction Temperature:

Determine the best temperature for the extraction of isolate protein of shrimp waste and different temperatures were (25,40,60,80)c°

Determination of the best pH for protein solubility:

according to the method reported by (AOAC, 2003) with some modification 100 mg each of protein samples were dispersed in 20 ml of distilled water and the pH of the suspension was adjusted to 2–10 with 0.5 M NaOH or 0.5 M HCl. The suspensions were stirred with a magnetic stirrer for 30 min at room temperature before centrifuging at 10000 × g for 20 min. The protein content in the supernatant was determined using Biuret method.

Determination of the best pH for precipitation:

The best pH for protein precipitation of shrimp waste was determined (16). The filtrate with pH 10 was taken and distributed into 20 ml test tubes pre-weighed and then reduced to pH 5.5,5,4.5,4,3.5 by HCL (1M), followed by chilled centrifugation at 10000 x g for 20 minutes, the filtrate was discarded and the precipitate and tube were weighed to determine the maximum yield of isolate protein indicative of the optimum pH for protein precipitation.

Amino acid analysis

The amino acid composition was analyzed using HPLC, A 0.2% sample was taken, and 12 ml of 6M (HCl) was added. The mixture was then placed in an oven at 110°C for 24 hours. Filtered and washed twice with distilled water, the mixture was then rotary at 50°C. After drying, 10 ml of distilled water was added, and the rotary was repeated until dry. 3.5 ml of 0.02M (HCl) was then added. The acidity was adjusted by adding a base, and the mixture was injected into an amino acid detector after adding the OPA or FMOC reagent to the sample to detect amino acids.

Functional properties:

Water absorption capacity:

The water absorption capacity of the protein isolated from both shrimp heads and shrimp shells was estimated as previously reported (19) with some modifications, where 100 mg of the sample was weighed and placed in a 10 ml test tube, then 1000 µl of distilled water were added to it gradually with stirring using a magnetic mixer and left for 30 minutes at room temperature 25°C, then centrifuged at a speed of 2000 rpm for 20 minutes and the filtrate was carefully removed, then the

tube was weighed with the sample, Albumin and casein were used as standard proteins for comparison.
The percentage of water associated with the following equation was calculated as

$$Water\ absorption\ capacity = \frac{weight\ of\ sampie - weight\ of\ precipitate}{weight\ of\ sample}$$

Oil absorption capacity:
The method used by (19) was adopted with some modifications to estimate the oil absorption capacity of protein isolated from both shrimp heads and shells., Albumin and casein were used as
standard proteins for comparison, The oil was weighed by 100 mg of the sample in a 10 ml tube and the sample was mixed with 1000 µl of sunflower oil and the mixture was left at Room temperature 25 °C for 30 minutes, then centrifugation at a speed of 6000 rpm for 30 minutes. After that, the filtrate was carefully removed, then the tube with the sample was weighed and the oil absorption capacity of the samples was estimated according to the following equation:

$$Water\ absorption\ capacity = \frac{weight\ of\ sampie - weight\ of\ precipitate}{weight\ of\ sample}$$

Emulsification properties
The method referred to by (20) was adopted with some modifications.. The emulsification property (EA) and its stability were determined by preparing 5ml of protein isolate solution of shrimp heads and shells (10 mg/ml) and homogenising with 5ml of oil for 1 minute. Centrifugation was done at 2000 × g for 5 min. The height of the emulsified layer and the height of the total contents in the tube were measured. Emulsifying activity (EA) was calculated as:

$$EA(\%) = \frac{height\ of\ emulsified\ layer\ in\ the\ tube\ \times 100}{height\ of\ the\ total\ contents\ in\ the\ tube}$$

Protein solution (10 mg/ml) was prepared at different pH from 2 to 10 to study the effect of pH.The stability of the emulsions (ES) was determined at different temperatures (30,50,70)°C for 30 minutes before centrifugation at 2000 x g 5 minutes.

$$ES(\%) = \frac{height\ of\ emulsified\ layer\ after\ heating\ \times 100}{height\ of\ the\ total\ contents\ in\ the\ tube}$$

Foam capacity and stability:
Foam capacity and foam stability were estimated by the method mentioned (19) for the protein isolate of shrimp heads and shells by homogenize 0.25 g of the sample with 25 ml of deionised distilled water by a laboratory homogenizer equipped by the German company (Heidolph) at 6000 r/min for 2 minutes and recording the foam volume before and after homogenizing, then the samples were left after the foam capacity test for time periods (5,15,30) minutes and then the foam stability was estimated at each time according to the following equation:

$$Foam\ capacity(FC)\% = \frac{Volume\ after\ whipping\ - Volume\ befor\ whipping(ml)}{Volume\ befor\ whipping(ml)} \times 100$$

$$Foam\ stability(FC)\% = \frac{Volume\ after\ standing\ - Volume\ befor\ whipping(ml)}{Volume\ befor\ whipping(ml)} \times 100$$

RESULTS AND DISCUSSION

Proximate analysis
The chemical composition of shrimp shell and shrimp head were illustrated in (table 1) The results showed a higher protein content in fresh and dried shrimp shells compared to shrimp head reaching 10.91, 13.02, 6.45 and 7.89, respectively. The low protein in shrimp shell and head is possibly caused by differences in the handling of waste. (21) reported that the protein content of shrimp shell flour produced was lower due to the process of soaking the shrimp shells which impacted a reduction in protein content .. The research (22, 23) reported that exoskeletons consist of approximately 20–40% proteins, 30–60% minerals (pre dominantly calcium carbonate), 20–30% polysaccharides, and 0–14% other compounds such as pigments (e.g., astaxanthin) and lipids (muscle residues and carotenoids)

Determination of the best mixing ratio:

The results indicate that there is a significant effect (P>0.05) in the mixing ratios of shrimp heads and shells with the water used in extraction and the protein extraction rate. It was noted from Figure (1) for shrimp heads that the use of ratios (1:5, 1:10, 1:15) led to a protein extraction rate of (71.1, 83, 84.4)%, respectively, while protein rate from shrimp shells was reached to (69.6, 74.9, 75.9)%, respectively (Figure 2) . The results indicated that there was no significant difference in the percentage of protein extracted at mixing ratios of 1.10 and 1.15, so the mixing ratio of 1.10 was adopted.

(16) found that a mixing ratio of 1:10 was the best for achieving the best protein content from lupin seed meal. (24) also showed that the best mixing ratio for producing isolated sesame protein from sesame seed meal was at a mixing ratio of 1:30 when using different mixing ratios (1:10, 1:20, 1:30, 1:40). (25) (26) found that the extracted protein ratios were not significantly affected by the mixing ratio of peanut powder to water when using mixing ratios (1:10, 1:20, 1:30).

Table 1. Chemical Composition of fresh and dried Shrimp Shell and Shrimp Head.

Sample	Moisture%	Ash%	Protein%	Fiber%	lipid%
Shell-Fresh	62.92	2.93	10.91	9.21	10.70
Shell-Dried	10.49	1.62	13.02	21.17	8.18
Head-Fresh	70.47	2.50	6.45	4.91	12.22
Head-Dried	11.89	2.70	7.89	13.78	9.25

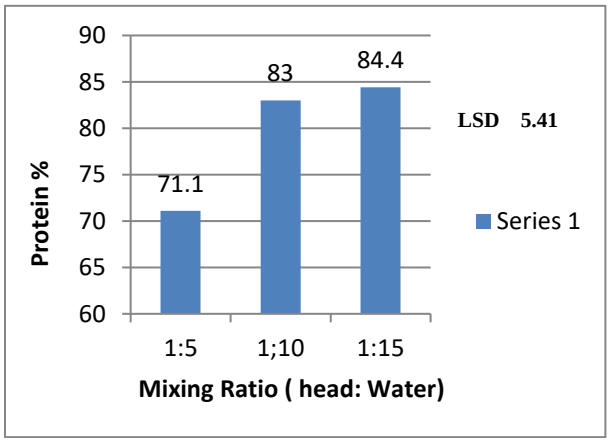


Fig. 1 – Effect of Mixing Ratio (head: water) on the Protein percent isolated from shrimp Head, at 25°C, pH10 and the Extraction Time 1 hour.

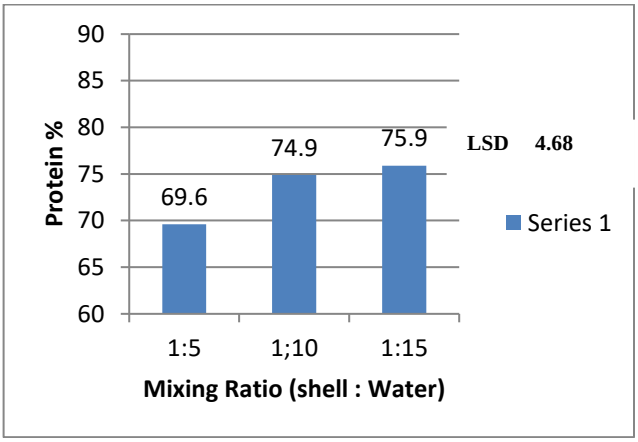


Fig. 2 – Effect of Mixing Ratio (shell: water) on the Protein percent isolated from shrimp Shell with temperature 25°C, pH10 and the Extraction Time was 1 hour

Determination of the best extraction Temperature:

The effect of different temperatures (25, 40, 60, 80) °C on the efficiency of isolated protein extraction from shrimp heads and shells was studied. The results indicate that there is a significant effect (P>0.05) on the protein percent extraction at deferent temperature. The protein extraction efficiency for heads at the above temperatures was (76.9, 57, 80.5, 48.9)%, respectively, and for shells it was (80, 67.5, 77.2, 36)%, respectively. No significant difference was observed between temperatures of 25 and 60 °C for both shells and heads. Therefore, a temperature of 60 °C was adopted in preparing isolated protein from shrimp waste in this study (Figures 3 and 4). The decrease in the percentage of protein extracted at 40 c° may be attributed to the enzyme activity at this temperature.

A decrease in protein percent was also observed at temperature 80, which may be a result of protein denaturation. These results were close to what (24) found that the best temperature for protein extraction was 50°C and the protein extraction efficiency was 94.62% when preparing sesame seed protein isolate (27) (28) concluded that the best extraction temperature was 45°C in his study on the preparation of white shrimp (litopenaeus vannamei) protein isolate , at which the protein extraction efficiency reached 83.3%.

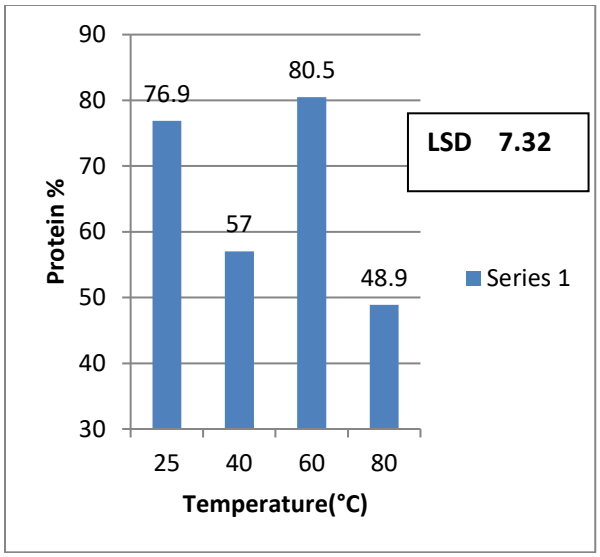


Fig. 3 **– Effect of the temperature on the Protein percent isolated from shrimp Head with Mixing Ratio** 1:10**, pH10 and Extraction Time 1 hour.**

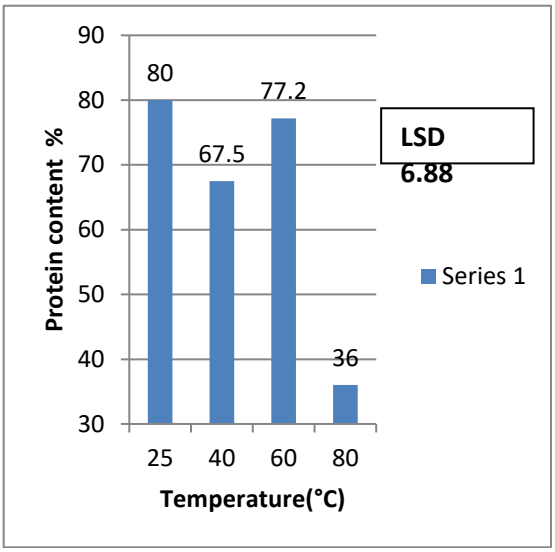


Fig. 4 – Effect of the temperature on the Protein percent isolated from shrimp Shell with Mixing Ratio 1:10, pH10 and the Extraction Time was 1 hour.

Figure (5-6) shows the effect of the extraction Time on the percentage of isolated protein
The extraction time was (30,60,120)min , and the extraction percentage for the heads, was (72.2, 85, and 82)%, respectively, while the extraction percentage for the shells, was (66, 81, and 83)%, respectively. Statistical analysis results at the significant level (P>0.05) showed a non-significant difference in the percentage of protein isolated from shrimp shells and heads at extraction times of 60 and 120.

Accordingly, 60 minutes was adopted as the standard extraction time for the production of the studied protein isolate for both the heads and shells. (24) indicated that the best extraction time was 60 minutes when using extraction times of (30, 45, 60, 75, 90) minutes for the isolated protein production of sesame seed powder. (29) reported that the optimum extraction time for apple seed protein isolate was 60 minutes with different extraction times ranging from 10 to 80 minutes. (30) (28) found that the best time to extract protein from white shrimp (litopenaeus vannamei) is 30 minutes.

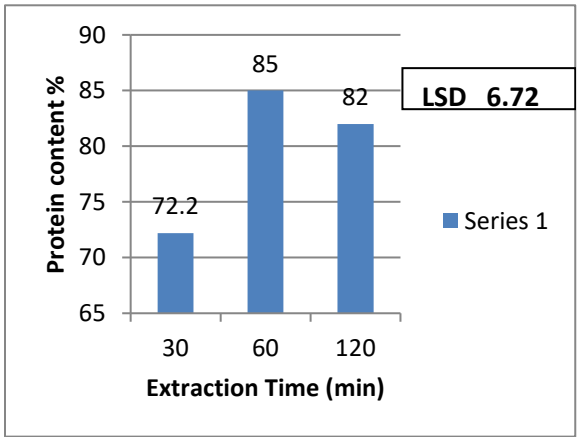


Fig. 5 – Effect of the extraction times on the protein percent isolated from shrimp head with Mixing Ratio 1:10**, pH10 and 25°C.**

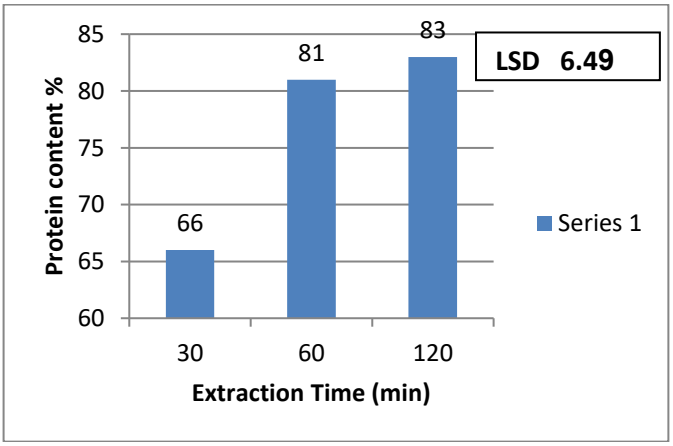


Fig. 6 – Effect of the extraction times on the protein percent isolated from shrimp Shell with Mixing Ratio 1:10**, pH10 and 25°C.**

Determination of the best pH for protein solubility:

The results of the protein solubility of shrimp heads and shells were shown in Figure (7-8). the statistical analysis showed that there is a significant difference at the level of (P>0.05) and that protein solubility is high in acidic media at pH 2 and reached 30% for heads and 40.5% for shells. Then the of protein solubility began to decrease with the increase in pH to reach the lowest at pH 4 and reached 13.6% for heads and 26.1% for shells. This may be due to the fact that the protein is at its isoelectric point at this pH. (4). Then the solubility increased with the increase in pH to reach 60.7% at pH6 for heads and 42.1%for shells . It was noted that the best pH for heads was 10, as it reached 85.5%, while the best pH for shells was found at 12, as it reached 76%. However, pH 10 was adopted to avoid the effect of the basic pH on the protein. (24) concluded that pH 10 was the best for sesame seed protein solubility, which achieved the highest protein solubility for the production of sesame protein isolate from sesame seed meal. (31) reported that the solubility of sunflower seed protein isolate increases with increasing pH after the isoelectric point and reaches its highest value at pH 10. It is not preferable to raise the pH to more than 10 to avoid undesirable changes such as denaturation and color change, which may affect the functional properties and sensory qualities of the resulting protein isolate.

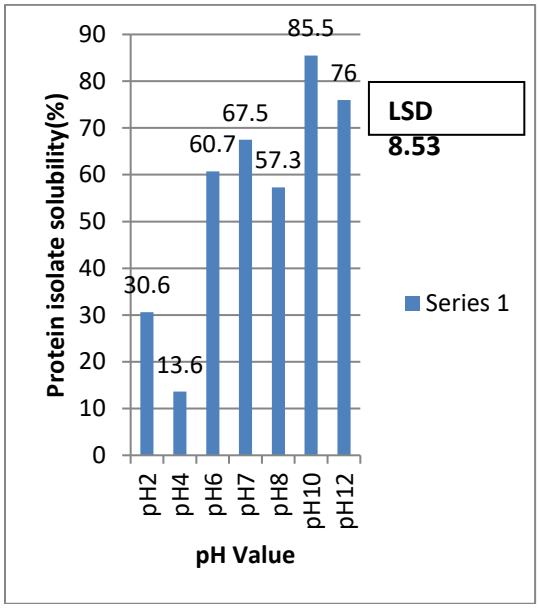


Fig. 7 – Effect of pH on the isolate protein solubility from shrimp Head, at 25 c°, The protein percentt in the supernatant was determined using Biuret method

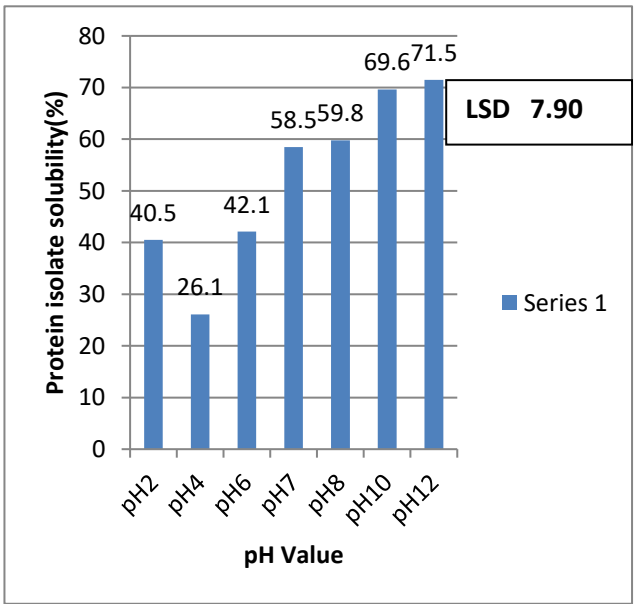


Fig. 8 – Effect of pH on the isolate protein solubility from shrimp Shell, at 25c°, The protein percent in the supernatant was determined using Biuret method

Determination of the best pH for precipitation:

Figure (9-10) shows the effect of pH on protein recovery when preparing protein isolate. The pH values (3, 3.5, 4, 4.5, 5, 5.5) were used for each of the shrimp heads and shells.), and the statistical analysis showed that there is a significant difference at the level of (P>0.05) , The efficiency of protein recovery for shrimp heads was (1.2, 2.8, 3.1, 3.3, 3.1, 1.1)%, respectively, while for the shells it was (2.4, 2.6, 2.7, 3.2, 2.9, 0.4)%, respectively. The pH of 4.5 was superior to the rest of the pH values in preparing protein isolate for shrimp heads and shells. Therefore, it was used in preparing the isolate in this study. These results were consistent with what (32) found when preparing sesame seed protein isolate from de-oiled sesame seed powder. The reason was attributed to the fact that the net charge on the surface of the protein at pH 4.5 is as low as possible, so the protein tends to aggregate and precipitate at the bottom of the solution. While (27) (28), (24) used pH 4 and (33) adopted pH 4.9 in the preparation of sesame seed protein isolate.

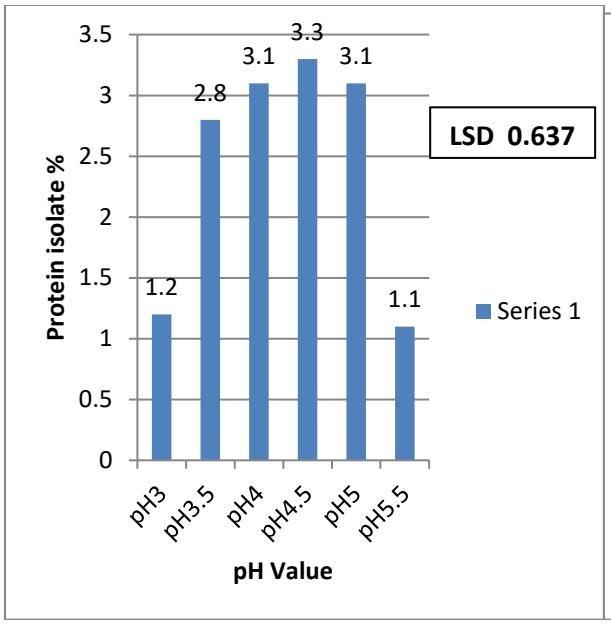


Fig. 9 – Effect of pH on the isolate protien precipitation from shrimp Head extract, at 25c° and pH10

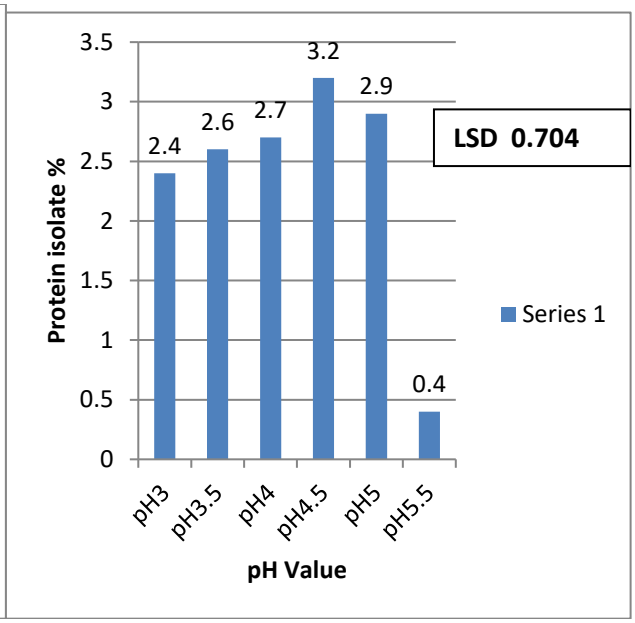


Fig. 10 – Effect of pH on the isolate protein precipitation from shrimp Shell extract ,at 25c°, and pH 10 The protein percent in the supernatant was determined using Biuret method

Amino acid content: Table(2,3,4) shows that the shrimp head protein isolate contains the polar amino acids (Aspartic acid , glutamic acid ,Serine, Histidine , Glysine , Arginine, Cystine, Tyrosine, Lysine) at a concentration of 7125.166 ppm and non-polar amino acid (Alanine, Valine, Methionine ,Phenylalanine, Isoleucine, Leucine) at a concentration of 2786.715 ppm.Thus, the percentage of polar amino acids is higher than non-polar amino acids in shrimp heads. While the concentration of polar amino acids in shrimp shells reached 3035.441ppm and included Aspartic acid, glutamic acid ,Serine ,Histidine, Glysine, Arginine, Cystine Tyrosine, Lysine, and non –polar amino acid concentration was 2514.180ppm include Valine , Methionine Isoleucine ,Tyrosine ,Leucine , Phenylalanine , Lysine. Polar and nonpolar amino acids play a role in determining the physicochemical and functional properties of proteins. One of the most important properties affected by increased polarity is solubility, which increases with the increase in the percentage of polar amino acids.

Table 2 Amino acids contents of isoleted shrimp Head protein (ppm).

Amino acid	Head protein (ppm)	Shell protein (ppm)
Aspartic acid	14.078	12.058
glutamic acid	370.136	307.491
Serine	2144.999	523.953
Histidine	1173.605	1018.024
Glysine	885.449	643.202
Arginine	510.813	204.255
Alanine	169.355	124.641
Cystine	1014.341	884.591
Valine	274.883	198.292
Methionine	556.924	226.499
Tyrosine	561.779	149.340
Phenylalanine	255.347	172.060
Isoleucine	296.467	127.118
Leucine	348.290	137.777
Lysine	1335.415	820.320
Total	9911.881	5549.621

Table (4) Concentration of amino acids in protein isolates .

Sample	polar	Non-polar
Head	7125.166	2786.715
Shell	3035.441	2514.180

Functional properties:

Water absorption capacity:

Table (5) shows the water absorption capacity of protein isolate from shrimp shells and heads, Significant differences were found at the significance level (P>0.05), the highest absorption capacity of the head protein isolate was 5.03 ml/g, while the shells showed lower results of 3.962 ml/g, which could be due to the high percentage of hydrophilic amino acids in the head protein isolate, which led to an increase in water absorption capacity, as shown by the results of amino acid analysis (HPLC). The ability of the protein to bind water is due to the ability of the protein to form hydrogen bonds between water molecules and the polar groups of peptide chains in the protein and this leads to an increase in the ability of the protein to bind water due to its content of hydrophilic polar amino acids that form hydrogen bonds with water.

In the light of the results, it was observed that the head protein isolate was superior in water absorption capacity to both albumin and casein standard proteins (0.8 ml/g and 2.4ml/g, respectively), which may be due to the lower concentration of polar amino acids leading to a decrease in the water carrying capacity of the standard proteins (34).

(35) founds that the water absorption capacity of shrimp boiled and peeled water protein was 2.5ml/g. (25) . It was observed from the results that the water absorbability of the head protein isolate was similarly to (36) who found it to be 2.31 ml/g.

Oil absorption capacity:

Table (5) shows that there are significant differences at the significant level (P>0.05) and the highest lipid absorption capacity was for the protein isolate of the peels and amounted to 3.136 ml/g, while it was 1.8 ml/g in the heads, which are lower than the absorption capacity of the protein isolate of water for heads and peels. It is noted that the protein isolate of the heads outperformed the standard protein albumin, which reached 1.7ml/g in lipid absorption capacity, while the protein isolate of the peels outperformed both the standard proteins albumin and casein.

The mechanism of lipid binding is due to the nonpolar end groups of the protein that bind to the hydrocarbon chains and thus contribute to lipid absorption. The binding to lipids is due to the presence of hydrophobic groups within the amino acid chain structure that help form bonds with lipids and increase the amount of bound lipids, and the lipid absorption capacity is affected by several factors such as protein chain length, molecular surface area, electrical charges and polarity (34).

The results indicate that the protein isolate of shrimp heads and shells outperformed the chickpea grain isolate in lipid absorption capacity of 1.44 ml/g according to (37). (28) also found that the lipid absorption capacity of white shrimp proteins was 3.5 ml/g.. (25)

Foam capacity and stability:

Table (6) shows the foam formation and stability of protein isolate compared to both shrimp shells and heads. Statistical analysis results at (P>0.05) showed significant differences between heads and shells, with the foam percentage reaching 36.4% for heads and 31.6% for shells. This is due to the dependence of foam formation on the properties of the surface protein membrane, such as elasticity, viscosity, cohesion, and air impermeability, while foam stability depends on properties such as mechanical strength, protein-protein interactions, and other factors. The decrease continued after 10 minutes by 26.4% and 20.8% for the heads and peels, respectively, and after 30 minutes by 12.8% and 11.2%, respectively. A significant difference was found between 5, 10, and 30 minutes, while no significant difference was observed at 30 minutes between the heads and peels. The stability of the foam in the samples is attributed to its increased ability to rise to the surface, which helps bind to water and form strong interfacial membranes that enclose air bubbles (38).

(39) found that the foaming capacity of Moringa oleifera leaf powder protein isolate was 10%, while the foaming capacity of the plant cover was 2%. They attribute this to the limited availability of proteins that diffuse across the air-water interface to form foam.

The foaming capacity and stability were lower than the results obtained by (28) (25) for white shrimp protein isolate, which were 53.5%, and the foam stability was 41.2%. The foaming capacity of the peel and head protein isolate was lower than the foaming capacity of the sugar beet leaf isolate obtained by (40) (60%).

Table 5 Water and Oil absorption capacity of protein isolate from shrimp waste(head , shell) and control protein casein and albumin.

Sample	Water Absorption Capacity (ml/g)	Fat Absorption Capacity (ml/g)	L.S.D.
Head IP	5.03	1.8	1.004 *
Shell IP	3.962	3.136	0.722 *
Albumin	0.8	1.7	0.569 *
Casein	2.4	2.4	0.278 NS
L.S.D.	1.037 *	0.885 *	---

IP:isolated protein

Table 6 : Foam capacity and stability of protein isolate from shrimp waste (head ,shell)

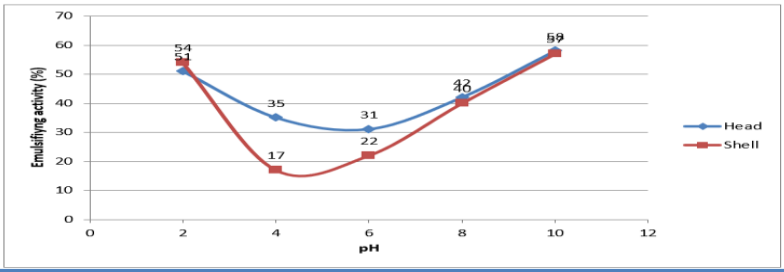
Sample	Foam capacity(FC)%	Foam stability(FS)%			L.S.D
		5min	10min	30min	
Head IP	36.4	32.8	26.4	12.8	6.73 *
Shell IP	31.6	27.3	20.8	11.2	5.29 *
L.S.D.	3.82 *	4.05 *	3.79 *	2.07 NS	---

IP:isolated protein

Emulsification properties:

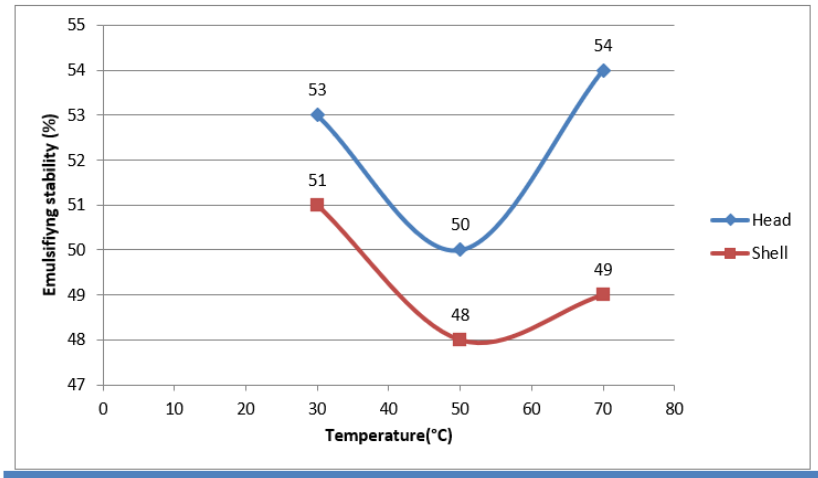
Figure (10) shows that protein isolate from shrimp shells and heads possesses emulsifying properties and a high emulsifying stability. Comparing Table (3) with Table (4), it was observed that protein isolate exceeds the water and fat absorption capacity, which in turn positively impacts emulsifying properties. HPLC results also showed that protein isolate from shrimp remains contains both polar and nonpolar amino acids, thus acting as an emulsifying agent. Statistical analysis results revealed significant differences at the (P>0.05) level between all pH values of shrimp heads and shells. No significant differences were found between shells and heads at pH values (2, 8, 10).

Figure (10) illustrates the effect of pH on the emulsifying activity of protein isolate from shrimp heads and shells. The emulsifying activity values at pH 2 were observed to be 51% and 54% for heads and Shell , respectively. This value then decreased with increasing pH, reaching its lowest value at pH 4, i.e., the point of electrical equilibrium, where it reached 35% and 17% for heads and peels, respectively. These values then began to increase with increasing pH until they reached 58% and 57% at pH 10, respectively, outperforming all pH values. The high emulsifying activity at the maximum pH values is due to high protein solubility, The reason for the decrease in emulsifying activity at pH 4 is due to the low protein solubility , which hinders its transport and diffusion around the water and oil surfaces. In contrast, the high protein solubility leads to rapid diffusion around the water and oil surfaces, thus increasing the emulsifying capacity (41). Stability showed no significant difference between temperatures of 30, 50, and 70 for both shells and heads. However, a significant difference was found between heads and shells at pH 7. Figure (11) shows the effect of temperature on emulsion stability at pH 10. It was observed that the emulsion stability value for protein isolation from shrimp heads and shells at 30°C was 53% and 51%, respectively. The emulsion stability value decreased with increasing temperature at 50°C, reaching 50% and 48%, respectively, and at 70°C, reaching 54% and 49%, respectively. (42) indicated that protein molecule will unfold in acidic and basic media and when it moves away from the neutral point which exposes the lipophilic functional groups and this leads to improved emulsifying properties. The low emulsifying activity value at the neutral point is due to the neutralization of the electrical charges of the protein molecule and hence the absence of electrostatic forces of attraction which aids the diffusion of the protein molecule in the medium.



sample	2	4	6	8	10	L.S.D.
Head IP	51	35	31	42	58	6.47 *
Shell IP	54	17	22	40	57	7.45 *
L.S.D.	4.31 NS	5.77 *	5.08 *	3.63 NS	2.68 NS	---

Fig. 10 – Effect of pH on the emulsifying activity of the isolate protein from shrimp waste .



sample	30c	50c	70c	L.S.D.
Head	53	50	54	2.86 NS
Shell	51	48	49	3.04 NS
L.S.D.	2.47 NS	2.75 NS	3.61 *	---

Fig. 11 – Effect of temperature on the emulsifying stability of the isolate protein from shrimp waste.

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