



# Characterization of Biscuits Fortified with Shrimp (*Litopenaeus vannamei*) Waste Protein Concentrate

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**Abstract:** Shrimp has accounted for a substantial share of crustacean utilization in recent years and processing results in the accumulation of solid trash, such as the head, shell, and tail parts. The accumulation of bio wastes without proper use has led to waste of materials, waste disposal issues, and environmental contamination. In this sequence, the present study was carried out with an aim to prepare biscuits supplemented with protein concentrate from shrimp head and shell waste to enhance their protein content. The prepared biscuits along with shrimp waste were examined for various physicochemical and biochemical parameters along with organoleptic evaluation. Mineral profiling was also carried out to assess the presence of major minerals in the shrimp waste as well as biscuits prepared from it. The shrimp waste has significantly enhanced the protein as well as mineral content of biscuits, however, also enhanced TMA and TVB-N value which affects the shelf life of biscuits. All the biochemical findings were confirmed with the FT-IR analysis. Prepared biscuits were also subjected to a storage study of 150 days at ambient temperature; and a significant variation was observed in physicochemical and biochemical composition as well as in the organoleptic properties of the biscuits within due course of time. At the end of the storage time, biscuits were quite safe. Therefore, the present investigation can revolutionize shrimp farmers as well as shrimp processors for efficient utilization of shrimp head and shell waste into value-added products and also to grasp more economic benefits.

**Keywords:** White shrimp, *Litopenaeus vannamei*, Shrimp waste, Protein-enriched biscuits, FT-IR spectroscopy, Waste management

The world's largest industry for producing seafood, crustacean aquaculture, provides a food source high in protein. Shrimp and shrimp products are widely consumed all over the world and their demand is increasing yearly owing to their delicacy and nutritional value. The amount of shrimp produced globally was 5.03 million tons in 2020, and by 2025, it is predicted to reach 7.28 million tons with a compound annual growth rate of 6.1% from 2020 to 2025. More than 80 percent of the world's shrimp produced in Asia and plays significant role in the shrimp farming industry (Mao et al., 2017). In Asia, Thailand is the leading exporter of farmed shrimp exporting to the USA, Europe, Canada, Japan, and South Korea. Out of all the shrimp species, *Litopenaeus vannamei*, or Pacific white shrimp, is the most widely farmed species in Thailand. Shrimp are usually frozen, either with or without a shell, and are exported or kept based on the need from the market. About 50–60% of the solid waste is produced during processing as by-products, which include the head, viscera, shell, and other components. Additionally, the washing and cooking process produces pollution and wastewater (per ton of cooked shrimp, about one gallon is

created). These by-products make up to 45–50% of the catch and, because of their uncontrolled discharge, they pollute the environment and provide disposal challenges. Shrimp waste is discarded in enormous numbers, which results in the loss of important and bioactive components, even though a limited amount is used as animal feed and an element in aquaculture feed formulation.

The economics of shrimp producers as well as processors would benefit from the recovery of these valuable components from waste and their subsequent use in profitable goods. Due to the decrease in the disposal of shrimp waste, this would also help in lowering environmental contamination. Shrimp waste possesses valuable components, i.e., protein/peptides, chitin/chitosan, pigments, enzymes, lipids, minerals and vitamins. However, the sources and processing conditions affect the levels of every component. Shrimp biscuit, also known as "hae bee hiam" in Singapore, is a popular snack that originated from Southeast Asia. The biscuits are frequently consumed around the world and well preferred by all age groups. Therefore, it is very logical to opt for biscuits as a base

material to develop such functional foods. Keeping these things in view, the present study was designed to utilize shrimp head and shell waste to prepare protein-supplemented functional biscuits.

### MATERIAL AND METHODS

**Procurement and processing of shrimp:** Pacific white shrimp (*Litopenaeus vannamei*) was procured from a local Shrimp farm of Fatehabad, Haryana during the season 2022-2023. The shrimps were transported to the laboratory, washed twice, and the head and shell were separated manually. The waste material was washed and dried using a lab scale oven (Equitron stream 7051-091) at 50°C for 4 to 6 hr to a moisture content of 10 %. Then, the dried material was ground into a fine powder using a mixer (Havells vitonika, 500w) and sieved (particle size < 1 mm). The prepared powders were stored in a cool and dry place till further use.

**Preparation of shrimp head and shell waste protein concentrate:** The protein concentrate was prepared. The shrimp head and shell waste powder was mixed with distilled water (1:10 (w/v)) and stirred while maintaining a pH of 10-11 using 0.1 N sodium hydroxide (NaOH). Then, the prepared mixture was heated at 80-90 °C for 1 to 2 h to facilitate protein extraction. The mixture was allowed to cool and filtered using filter paper to separate the liquid containing extracted proteins. Subsequently, the filtrates were acidified to pH of 4-5 using 0.1 N hydrochloric acid (HCl) that allows the precipitation as well as the separation of proteins. The precipitates were filtered and dried in an oven (Equitron stream 7051-091) at a temperature of 50-60°C up to a moisture content of 10 %. Finally, the extracted protein concentrates were stored in a PET container.

**Preparation of functional biscuits:** Biscuits were prepared by the modified method of Khan et al. (2014). The ingredients used for the preparation of biscuits i.e., shrimp waste protein concentrate (50g) (approximately 15 % of the total ingredients), wheat flour (150g) (approximately 50 % of the total ingredient), hydrogenated fat (40g), sugar (12g), ground sugar (12g), milk (50ml), baking powder (4g), and salt (0.010g), cardamom powder (10 g as a flavoring agent) were finely mixed and the dough was prepared using a lab scale planetary mixture (Hobart N-50). The dough so prepared was pressed into a flat sheet and were cut with help of die to give appropriate shape. Then, the biscuits were baked at 230 °C for 15-20 min, cooled for 3-5 min, and packed in LDPE pouches. The functional biscuits were evaluated up to 150 days at a 30-day interval for their physicochemical, biochemical, and organoleptic properties

**Physicochemical composition:** Various physicochemical parameters viz., moisture, crude protein, crude fat, pH, and

ash content of shrimp head and shell waste, shrimp waste protein concentrate, and shrimp biscuits were determined by standard methods, on dry weight basis .

Moisture content was estimated using following formula:

$$\text{Moisture content (\%)} = \frac{W1 - W2}{W2} \times 100$$

Where, W1= Weight of sample before drying; W2= Weight of sample after drying in oven

To assess the pH of samples, 5 g of sample was ground with 50 ml distilled water using a pestle and mortar, samples were filtered and using a digital pH meter, the pH value was recorded. Crude protein determination was carried out using the micro Kjeldahl method.

$$\text{Crude protein (\%)} = \frac{N \times 6.25}{W}$$

Where, N= Nitrogen (%); W=Weight of sample; 6.25=conversion factor for protein

The Soxhlet extraction apparatus was used for the determination of crude fat content of samples. To estimate crude fiber, the fat free sample obtained after the estimation of fat was used.

$$\text{Fibre (\%)} = \frac{W2 - W1}{W} \times 100$$

Where, W=weight of the sample; W1=weight of the crucible with sample after muffle furnace; W2= weight of the crucible with sample after oven drying

Total carbohydrate content was calculated by subtracting the total of moisture, protein, crude fat, ash and crude fiber from 100. The total energy content was calculated by multiplying protein, crude fat and carbohydrate values obtained from analysis by their respective calorific value i.e., 4, 9 and 4 respectively; and expressed as Kcal/100g. To estimate ash content, one gram of the sample was collected and burned on a hot plate in a pre-weighed silica crucible. The crucible was placed in muffle furnace at 650 °C for 5 h. Using the crucible's weight differential, the ash content was calculated and represented as a percentage.

**Trimethylamine (TMA) and total volatile base nitrogen (TVB-N):** To determine TMA content, 10g sample was extracted with trichloroacetic acid (TCA) (10 %) and filtered using Whatman No.1. Thereafter, 1 mL of the obtained supernatant was carefully pipetted into the outer compartment of the Conway micro-diffusion unit. In the inner chamber, 1ml of 0.01N H<sub>2</sub>SO<sub>4</sub> was added, followed by 1ml saturated potassium carbonate and 0.5 ml of formaldehyde solution in the outer chamber. The unit was set aside at room temperature for the night. In the inner chamber, titration was performed with 0.01N NaOH and blank in the same way without sample was also run. The results were expressed as mg of N/100 g of sample. In order to determine the TVB-N

content, 10 ml of TCA (20%) was used to homogenize 5 g of material. The homogenate was then filtered through Whatman No. 1 filter paper and brought to a 100 ml volume using distilled water. The filtrate was used for further analysis. The micro-Conway diffusion unit's edges were coated with petroleum jelly, and the inner chamber was filled with 1 ml of 0.01 N H<sub>2</sub>SO<sub>4</sub>. Additionally, the outer chamber was filled with 0.5 ml of normal potassium carbonate and 1 ml of TCA extract. Overnight, the unit was left untouched. Tashiro's indicator was used to titrate against standard 0.01 percent NaOH in order to calculate the amount of unreach acid in the inner chamber. Blank was simultaneously prepared with 1 ml of 2 % TCA solution. TVB-N was expressed as mg of N/100g.

**Organoleptic evaluation:** The samples of biscuits were evaluated for organoleptic quality on the basis of appearance, taste, texture, smell, overall acceptability on a 9-point hedonic scale by the panels of semi-trained judges consisting 10 members, where, 9-Like extremely, 8-Like very much, 7-Like moderately, 6-Like slightly, 5-Neither like nor dislike, 4-Dislike, 3-Dislike moderately, 2-Dislike very much, 1-Dislike extremely. The results of judges were recorded on a Performa and samples with highest average scores were marked as best.

**Mineral analysis:** Using inductively coupled atomic absorption spectrometry (ICP-AAS), the minerals in the raw materials and shrimp biscuits were calculated. Exactly 250 mg of dried sample was digested with 10 ml of diacid mixture (nitric acid and hydrogen peroxide in the ratio of 4:1) in a microwave digestion unit (Anton Paar multiwave 7501). The digested samples were dissolved in doubled distilled water and then filtered using Whatman filter paper no. 1. The volume of the filtrate was then made up to 25 ml with double distilled water and was used for the determination of minerals.

**FT-IR (Fourier-transform infrared spectroscopy):** Using an FT-IR spectrometer (Nicolet-6700, Thermo Electron Scientific Instruments Corporation, USA), the raw material and produced shrimp biscuits were qualitatively assessed. The spectra were recorded in the wave number range of 4000-400/cm with a maximum resolution of 0.85/cm, and all samples were evaluated at an ambient temperature of 29±3°C. The measured spectra for the samples were analyzed in accordance with the guidelines given by.

**Statistical analysis:** Statistical analysis was carried out using standard procedures with 16.0 (IBM, SPSS Inc., USA) software. Additionally, post hoc tests (Tukay's Multiple Range Test) were used to assess the data.

## RESULTS AND DISCUSSION

**Physicochemical and biochemical composition of shrimp head/shell waste and protein concentrate:** The

percent crude protein, crude fat, ash, moisture, crude fiber, carbohydrate and energy content of shrimp head and shell waste were 42.34, 7.31, 21.11, 13.94, 9.20, 6.10, and 259.55 kcal/100g, respectively. Protein concentrate possessed protein, ash, moisture, total carbohydrate, and total crude fat content of 76.79, 11.17, 9.11, 2.16, and 0.77, percent whereas, the energy was 322.73 kcal/100g also observed similar trend. These findings provide insights into the nutritional composition of the extracted protein concentrate from shrimp head and shell waste. The relatively low levels of crude fat and carbohydrate content endorses the suitability of protein concentrate for certain dietary needs, such as low-crude fat or low-carbohydrate diets.

The biochemical composition was assessed based on the TVB-N, TMA, and pH values of the shrimp head and shell waste. These parameters are often used as indicators of freshness and the absence of significant changes in these values suggests that the waste material is suitable for further processing. The TVB-N, TMA, and pH of the shrimp head and shell waste were 8.42, 4.20, and 7.81mg/100gm, respectively. The TVB-N, TMA and pH values for protein concentrate were 7.23, 3.72, and 7.68 mg/100g, respectively (Table 1). The TVB-N was much lower than acceptable limit (30-40 mg N/100) reported that TMA value of the thawed shrimp head were of 17.76 mg/100g in fresh head of *L. vannamei*. Lower TMA values indicate that the raw material was fresh. Besides, pH is also an important parameter that affects the shelf life and quality of seafood products. The observed pH value also confirms that the shrimp waste is in favourable condition for utilization.

**Mineral composition of shrimp waste and shrimp biscuits:** The phosphorous (P), potassium (K), sodium (Na), iron (Fe), copper (Cu), zinc (Zn), magnesium (Mg) and manganese (Mn) content of shrimp waste were 1094.35, 610, 693, 19.74, 7.49, 4.38, 32.11, and 26.01mg/100g, respectively. The resulting shrimp biscuits still contain 674.61, 400 and 1603.8mg/100g phosphorus, potassium and sodium. Additionally, 30.65, 29.31, 21, 1.99 and 3.09mg/100g magnesium, iron, copper, and zinc were also enhanced due to addition of protein concentrate, showcasing the potential to create value-added food products from shrimp waste. Similar observations were reported by in the preparation of tapioca cookies by incorporation of fish carcass flour of different species.

**Shelf-life evaluation of the Biscuits incorporated with shrimp head and shell waste protein concentrate**

**Effect of storage on physicochemical and biochemical composition of biscuits:** Concerning physicochemical composition, the results showed a significant alteration in moisture, crude protein, crude fat, crude fiber, ash,

carbohydrate content, and energy value; highlighting the impact of storage duration on the product. As the biscuits containing shrimp head and shell waste protein concentrate were stored, the moisture content gradually increased, suggesting that the packing material may have had a permeability error. The crude protein decreased gradually over storage period of 150 days. This gradual decrease in crude protein of shrimp waste biscuits here shown could be associated with the decrease in the available amino acids. The crude fat, crude fiber, and ash content of the biscuit exhibited negligible changes over time. These findings

indicate that the biscuit maintained its dietary fiber and mineral content. The carbohydrate content demonstrated non-significant increase. Observed that in biscuits produced from breadfruit and wheat flours enriched with edible fish meal. The energy content of the biscuit remained consistent throughout the storage period.

The biochemical composition, the analysis focused on TVB-N, TMA, and pH revealed significant changes in the biochemical parameters of the biscuits over time. The TVB-N content, an indicator of protein degradation and spoilage, increased gradually throughout the storage period might be

**Table 1.** Physicochemical, biochemical, and mineral composition of shrimp head and shell waste, protein concentrate, wheat flour, and functional biscuits

Parameters	Shrimp head and shell waste powder	Shrimp waste protein concentrate	Shrimp biscuits	Wheat flour
Physicochemical composition				
Protein (%)	42.34	76.79	14.16	10.23
Crude fat (%)	7.31	0.77	16.14	1.33
Ash (%)	21.11	11.17	7.01	1.00
Moisture (%)	13.94	9.11	5.54	3.33
Crude fiber (%)	9.20	-	4.51	0.51
Carbohydrate (%)	6.10	2.16	52.64	83.6
Energy (kcal/100g)	259.55	322.73	331.76	364
Biochemical composition				
TVB-N (mg/100g)	8.42	7.23	4.29	-
TMA (mg/100g)	4.20	3.72	2.19	-
pH	7.81	7.68	6.50	-
Mineral composition (mg/100g)				
Phosphorous (P)	1094.35	-	674.61	108
Potassium (K)	610	-	400	107
Sodium (Na)	693	-	1603.8	2.00
Iron (Fe)	19.74	-	21.6	2.39
Copper (Cu)	7.49	-	1.99	0.22
Zinc (Zn)	4.38	-	3.09	2.22
Magnesium (Mg)	32.11	-	29.31	120.0
Manganese (Mn)	26.01	-	30.65	0.82

**Table 2.** Changes in physicochemical composition of biscuits during storage

Storage period (days)	Moisture (%)	Protein (%)	Crude fat (%)	Crude fiber (%)	Ash (%)	Carbohydrate (%)	Energy (kcal/100g)*
0	5.54	14.16	16.14	4.51	7.01	52.64	331.76
30	5.89	13.96	16.016	4.46	6.99	52.68	330.64
60	6.24	13.71	15.88	4.38	6.95	52.82	329.67
90	6.64	13.41	15.73	4.29	6.90	53.01	328.65
120	7.07	13.04	15.55	4.20	6.83	53.30	327.57
150	7.52	12.61	15.35	4.11	6.76	53.65	326.44

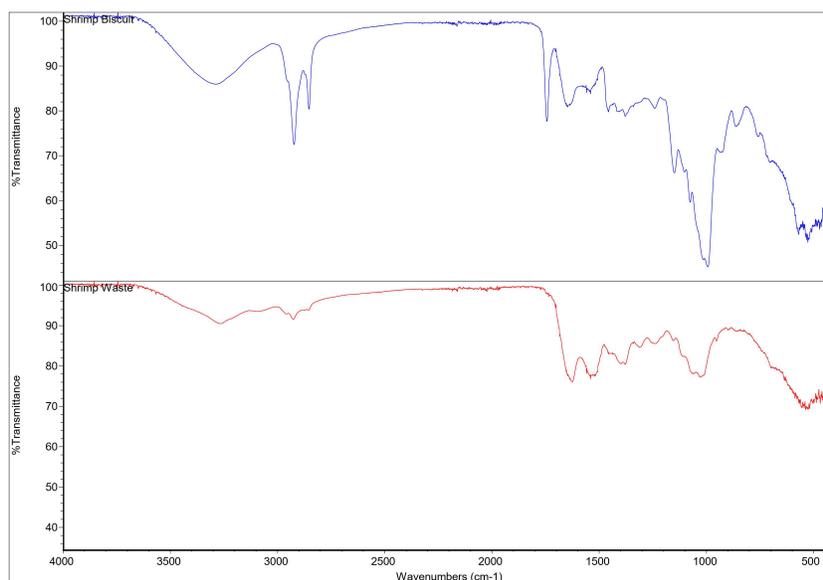
due to the increased microbial activity, the breakdown of large amounts of amino acids and an accelerated rate of deamination. Similarly, TMA is responsible for the characteristic "fishy" odor, also exhibited an upward trend during storage which was caused by degradation of protein and non-protein nitrogenous compound. The pH of the biscuits gradually decreased which may be due to the production of volatile compounds continuously after the baking process.

**Table 3.** Changes in biochemical composition of biscuits during storage

Storage period (Days)	TVB-N(mg/100g)	TMA (mg/100g)	pH
0	4.29	2.19	6.50
30	5.11	2.67	6.10
60	5.88	3.18	5.81
90	6.47	3.71	5.53
120	7.56	4.27	5.25
150	9.22	4.93	5.14

**Changes in organoleptic scores of biscuits during storage:** Over the course of the storage period, a significant decline was noted in every organoleptic characteristic of the biscuits. However, the overall acceptability of the biscuits remained relatively stable throughout the storage period. This finding suggests although the sensory qualities of the biscuits deteriorated during storage, consumers still were moderately acceptable. Similar results for organoleptic characteristics well observed in Biscuits fortified with Fish protein concentrate.

**FTIR studies of raw material and shrimp biscuits:** Fourier Transform Infrared Spectrometry (FTIR) was used to identify functional groups as well as other fingerprint groups present in raw material and produced biscuits. Bands ranging from 3675.16 to 3270.47/cm showed intramolecular bonded O-H stretching, indicating the presence of alcohol and hydroxyl groups in the sample. The bands around the 2925.55 to 2852.61/cm indicate the presence of C-H stretching which indicates the presence of alkanes. The range of 2160.69 to 2023.43/cm is known as C-C Stretching, and it indicates the



**Fig. 1.** FT-IR analysis of (a) shrimp biscuit and (b) shrimp head and shell waste

**Table 4.** Changes in organoleptic evaluation of biscuit during storage

Storage period (days)	Appearance	Taste/Flavour	Texture	Smell	Overall acceptability
0	8.49	7.50	6.52	7.50	7.50
30	7.80	7.31	6.02	7.31	7.11
60	7.50	6.81	5.73	7.01	6.76
90	7.31	6.52	5.53	6.52	6.47
120	6.91	6.12	5.33	6.42	6.20
150	6.52	5.53	5.04	5.83	5.73

**Table 5.** FT-IR spectral table depicting absorption band, functional groups and compound class concerning shrimp waste and shrimp biscuits

Frequency range	Absorption (cm <sup>-1</sup> )		Functional groups	Compound class
	Shrimp waste	Shrimp biscuits		
4000-3000	3675.16	-	O-H stretching	Alcohol
	3270.47	3288.86	O-H stretching	Alcohol
3000-2500	2925.55	2922.42	C-H stretching	Alkane
	-	2852.61	C-H stretching	Alkane
2400-2000	2160.69	-	C≡C stretching	Alkyne
	2023.43	-	C≡C stretching	Alkyne
2000-1650	1972.07	-	C-H bending	Aromatic compound
	1702.41	1743.48	C=O stretching	Conjugated acid, esters
1670-1600	1623.35	1647.31	C=C stretching	α,β-unsaturated ketone, alkane, conjugated alkane
1600-1300	1558.70	1539.50	N-O stretching	Nitro compound
	1539.62	1455.53	N-O stretching	Nitro compound
	1378.98	1377.96	C-H bending	Aldehyde
1400-1000	1307.96	-	O-H bending	Phenol
	1234.95	1238.91	C-N stretching	Amine
	-	1149.03	C-N stretching	Amine
	1025.17	1075.02	C-F stretching	Fluoro compound
1000-650	952.29	992.59	C=C bending	Alkene
	-	860.73	C-Cl stretching	Halo compound
	634.10	-	C-Br stretching	Halo compound

presence of alkynes. The bands ranging 1972.07/cm represents the C-H bending and indicates presence of aromatic compounds. The spectrum between 1702.41-1743.48/cm represents C=O stretching which indicates the presence of conjugated acids and esters respectively. The range of 1623.35 to 1647.31 cm in the spectrum reveals C=C stretching, indicating α and β-unsaturated ketone, alkane, and conjugated alkane, in that sequence. The bands between 1558.70 to 1455.53/cm and 1378.98-1377.96/cm indicate the N-O stretching and C-H bending respectively which represents nitro compounds and aldehyde, respectively. The band area representing N-O stretching has increased in biscuits in comparison to shrimp head and shell waste which indicates the enrichment of various proteins as well as amino acids in the functional biscuits. The spectrum 1307.96/cm indicates O-H bending which represents phenol and spectrum ranging from 1234.95 to 1149.03/cm indicates C-N stretching which represents Amines in sample. The spectrum 1025.17-1075.02/cm indicates C-F stretching claims the presence of fluoro compounds and peaks 952.29-992.59/cm, 860.73/cm and 634.10/cm indicates C=C bending, C-Cl stretching and C-Br stretching which represents alkene and halo compounds respectively.

## CONCLUSION

The present study successfully demonstrated the potential of utilizing shrimp (*Litopenaeus vannamei*) head and shell waste as a valuable ingredient in the development of protein-enriched functional biscuits. The physicochemical analysis of Pacific white shrimp (*Litopenaeus vannamei*) head and shell waste revealed high protein, ash, and mineral content, including phosphorus, sodium and iron. The shrimp protein concentrate showed an even higher protein content with reduced fat and moisture levels. Biscuits enriched with this shrimp waste demonstrated improved protein and fat levels compared to wheat flour along with enhanced mineral content. During 150 days of storage, the biscuits showed a significant decrease in protein and an increase in moisture, TVB-N and TMA. Despite these changes, sensory scores for overall acceptability remained within acceptable limits until day 120. FT-IR analysis confirmed the presence of functional groups such as O-H, C-H, C=O, and N-O, indicating the retention of key bioactive compounds in both waste and biscuits. These findings demonstrate the nutritional enhancement and storage stability of biscuits enriched with shrimp processing by products.

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