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Influence of Shrimp Waste Protein Isolate (*Litopenaeus vannamei*) on Physicochemical, Textural, Sensory, and Microbiological Quality of Ready-to-Cook Balls During Frozen Storage

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Abstract

Shrimp processing generates substantial by-products, including heads and shells, accounting for 40–60% of total shrimp weight and serving as rich sources of protein, minerals, chitin, and carotenoids. This study explored the valorisation of whiteleg shrimp (*Litopenaeus vannamei*) waste by extracting shrimp protein isolate powder and incorporating it into ready-to-cook (RTC) snack products, specifically shrimp balls, to improve their nutritional profile. Shrimp protein isolate powder (SPI) was obtained using a pH-shift extraction method, yielding high protein (79.52 ± 1.09%) with low moisture (10.76 ± 0.29%), minimal fat (1.57 ± 0.83%), and retained minerals (ash 6.57 ± 0.13%). SPI was incorporated into shrimp balls at levels of 0–50% and evaluated for proximate composition, biochemical and physical properties, sensory attributes, and microbial quality over 90 days of frozen storage. Shrimp balls showed significantly higher protein content (up to 29.64 ± 0.02%) with minor changes in moisture, fat, and ash. Biochemical parameters, including pH, free fatty acids, and peroxide value, indicated minimal lipid oxidation. Cooking yield, colour, and hardness were influenced by SPI supplementation, with the least SPI incorporation levels in shrimp balls showing improved textural properties. Sensory evaluation revealed that moderate supplementation maintained desirable appearance, flavour, and acceptability, while higher levels reduced scores. Microbiological analysis indicated acceptable microbial quality, with no *Staphylococcus aureus or Escherichia coli* detected. The study demonstrates that shrimp waste can be converted into functional protein isolates for RTC products, enhancing nutritional quality while maintaining sensory and microbial stability.

Keywords: Shrimp protein isolate powder (SPI), food by-product valorisation, microbial quality, nutritional enrichment

Introduction

Shrimp processing generates substantial quantities of waste, and its proper disposal is essential for minimising environmental pollution. Shrimp head and shell waste, comprising about 40-45 percentage of the total weight of the shrimp, is the most significant waste generated by the processing industries (Subasinghe, 1999) or even sometimes more than 50–60% of the catch volume, depending on the level of processing and final product (Senphan & Benjakul, 2012). Shrimp processing waste comprises mainly head and shell, which is an excellent source of nutrients and contains a large quantity of nutritional, bioactive, and functional compounds, including protein, lipids, minerals, chitin and carotenoids. Shrimp cephalothorax is a rich source of PUFA, protein, specifically essential amino acids, macro and micro minerals (Abuzar et al., 2023). According to Castillo, Nègre-Sadargues, and Lenel (1982), shrimp contains many biomolecules, such as astaxanthin, β-carotene, cryptoxanthin, iso-zeaxanthin, isocryptoxanthin, canthaxanthin, pheoenicoxanthin, zeaxanthin, and echinenone. Indian shrimp industries produce more

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than 1 lakh tonnes of shrimp by-products, which contain protein (35-40%), minerals (10-15%), chitin (10-15%) and carotenoids (Sachindra & Bhaskar, 2008).

According to the WHO in 2022, one of the biggest nutritional challenges worldwide is protein-energy malnutrition (PEM), and nearly 1 billion people do not get enough protein in their diets, leading to stunted growth and development (Wu et al., 2014). Alternative protein sources have been explored for decades amid growing interest in reducing PEM. The increasing demand for protein-enriched foods is also associated with growing health and fitness awareness. Due to rapid urbanisation and changing lifestyles, cooking methods have been highly affected (Mendonca et al., 2023). Further, due to busy lifestyles, people prefer to eat foods that are easy to cook and require very little time, leading to increased demand for ready-to-cook products (Temgire, Borah, Kumthekar, & Idate, 2021). Hence, the market of 'Ready-to-Cook (RTC)' products is expanding and is expected to rise by USD 582.2 million at a CAGR of 7.83% between 2023 and 2028. The market demand for Ready-to-Cook food is increasing rapidly for suitable food options among working people (Technavio, 2023). The novelty of the present study lies in the valorisation of shrimp processing waste to produce SPI and its incorporation into edible RTC products, such as shrimp balls.

In this regard, trials were conducted in this study to extract shrimp protein isolate powder from shrimp head waste and further, to explore the possibilities of adding the extracted SPI to snack food, i.e., shrimp balls. The developed shrimp balls were supplemented with shrimp protein isolate powder (SPI) extracted from whiteleg shrimp (*L. vannamei*) to improve their nutritional profile of balls as well as to aid the utilisation of these extracted proteins.

Materials and Methods

Whiteleg shrimp (*L. vannamei*) were procured from Thind Farm, Abohar, India. Extraction of shrimp protein isolate powder was performed using the pH shift method with minor alterations as described by Córdova-Murueta, García-Carreño, and Navarrete-del-Toro (2013). The shrimp head meat was homogenised into a thick paste with the help of a grinder (Sujata Dynamix mixer grinder, India), and 2 M NaOH was added to adjust the pH to 11.0, followed by centrifugation (Thermo Scientific Sorvall ST 16R) at 5000rpm for 30 minutes. The supernatant was re-adjusted to pH 5.5 using 2 M HCl, followed by centrifugation to recover the SPI. The obtained isolate was dried at 65 °C in a laboratory oven, followed by pulverisation to convert it into a fine powder (Huda, Abdullah, Santana, & Yang, 2012).

Shrimp Balls were prepared following the methodology described by Canti, Wirawan, and Lestari (2024), with slight modifications, using the ingredients listed in Table 1. All ingredients were blended according to the specified formulation to obtain a homogeneous dough. Portions of approximately 10 g were then weighed and manually shaped into spherical units. The formed balls were steam-cooked for 20–25 minutes, allowed to cool to ambient temperature, and subsequently packaged and stored at –18 °C until further analysis. Balls were prepared using 0-50% (A-F) composition of SPI as mentioned in Table 1 and packed in batches of 3-4 balls in each package. The storage study was conducted for 90 days, where samples were analyzed at 15-day intervals during frozen storage.

The proximate composition of SPI and products, i.e., shrimp balls, which include moisture, protein, fat, ash, and carbohydrate content, was analysed using the official methodology described in AOAC International (2022). Biochemical parameters, including pH, were measured by the methodology given by Trout et al. (1988), whereas FFA and PV were measured using the standard methodology of Koniecko (1979).

Cooking yield was determined following the procedure described by Murphy, Criner, and Grey (1975). Briefly, the weight of each sample was recorded before cooking and immediately again after cooking. Cooking yield was expressed as the percentage ratio of the cooked weight to the raw weight.

Hardness (g force) was determined using a Texture Analyzer (TA-XT2 Plus, Stable Microsystems, Surrey, UK) equipped with a blade cutter attachment at a pre-test speed of 2 mm/s and compression distance of 20 mm (Surasani, Singh, Gupta, & Sharma, 2019). Colour in terms of L^*, a^*, b^* and overall whiteness of SPI, as well as shrimp balls, were evaluated using a Konica Minolta Colour meter (CR-400, Tokyo, Japan) as per the method described by Surasani et al. (2019). For shrimp balls, the colour on the surface was analysed.

The microbiological quality of the shrimp balls was evaluated by determining the Total Plate Count (TPC), *S. aureus*, and *E. coli* using the spread plate technique, following the standard procedures outlined by APHA (1992). Sensory analysis of the shrimp balls incorporated with SPI at different levels was done as described by Popper, Rosenstock, Schraidt, and Kroll (2004). A semi-trained panel comprising 11 scientists and faculty members evaluated the sensory attributes. Sensory attributes were evaluated using a nine-point hedonic scale, such as Like extremely – 9, Like Very Much – 8, Like Moderately – 7, Like Slightly – 6, Neither Like nor Dislike – 5, Dislike Slightly – 4, Dislike Moderately – 3, Dislike Very Much – 2, and Dislike Extremely – 1.

All analyses were performed in triplicate, and the obtained data were statistically analysed using two-way ANOVA SPSS version 26 followed by Duncan's multiple range test (DMRT). Results were expressed as Mean ± Standard Error (SE), and differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Shrimp protein isolate powder was obtained through a pH shift method, incorporating minor adjustments to the procedure outlined by Córdova-Murueta et al. (2013). Specific details regarding the yield of SPI are presented in Table 2. Protein recovery from various sources frequently employs protein hydrolysis as a key technique.

The proximate composition analysis of shrimp protein isolate powder revealed a low moisture content (10.76 ± 0.29%), indicating effective dehydration and enhanced shelf stability (Table 3). Protein constituted the major fraction (79.52 ± 1.09%), demonstrating the efficiency of the isolation process in producing a highly concentrated protein product. Fat content was minimal (1.57 ± 0.83%), reflecting effective lipid removal during processing, while ash content was 6.57 ± 0.13%, representing the mineral constituents retained in the final isolate.

The proximate composition of shrimp balls during frozen storage is presented in Table 3. Protein

Table 1. Composition of ingredients used for the preparation of shrimp balls

Ingredients	Composition of ingredients (g)					
	A(0%)	B(10%)	C(20%)	D(30%)	E(40%)	F(50%)
Potato Starch	58.0	49.2	40.4	31.6	22.8	14.0
Soybean flour	15.0	15.0	15.0	15.0	15.0	15.0
Bread crumbs	15.8	15.8	15.8	15.8	15.8	15.8
Shrimp Protein Isolate powder (SPI)	0.0	8.8	17.6	26.4	35.2	44.0
Common Salt*	1.5	1.5	1.5	1.5	1.5	1.5
Black pepper	1.0	1.0	1.0	1.0	1.0	1.0
Cumin powder	1.0	1.0	1.0	1.0	1.0	1.0
Red chilli powder	0.5	0.5	0.5	0.5	0.5	0.5
Ginger powder	2.0	2.0	2.0	2.0	2.0	2.0
Garlic powder	2.0	2.0	2.0	2.0	2.0	2.0
Citric acid	0.1	0.1	0.1	0.1	0.1	0.1
Baking powder	1.2	1.2	1.2	1.2	1.2	1.2
Monosodium glutamate	0.2	0.2	0.2	0.2	0.2	0.2
Cinnamon powder	1.0	1.0	1.0	1.0	1.0	1.0
CMC Powder	0.7	0.7	0.7	0.7	0.7	0.7
Oil (mL)	25.0	25.0	25.0	25.0	25.0	25.0
Water (mL)	75.0	75.0	75.0	75.0	75.0	75.0

SPI- Shrimp protein isolate powder; A- Balls with 0% powder, B- Balls with 10% powder, C- Balls with 20% powder, D- Balls with 30% powder, E- Balls with 40% powder, and F- Balls with 50% powder

Table 2. Recovery (%) of shrimp protein isolate powder from whiteleg shrimp (*Litopenaeus vannamei*) waste

Sl. No.	Recovery yield of shrimp protein isolate powder based on total shrimp weight (%)	Recovery yield of shrimp protein cephalothorax meat weight (%)	Recovery yield of shrimp protein isolate powder against cephalothorax meat weight (%)	Moisture content in shrimp protein isolate (%)
1.	6.76	73.87	9.01	87.88

content ranged from 8.12 ± 0.02 to 29.64 ± 0.02% because of different % incorporated in the balls. Moisture content varied between 41.69 ± 0.48 and 44.06 ± 0.90%, while fat and ash content showed slight increase over the storage period. Salman et al. (2022) reported a decreasing trend in moisture content in silver carp (*Hypophthalmichthys molitrix*) cutlets over 120 days of frozen storage, with reductions in moisture and protein attributed to alterations in protein structure, decreased water-holding capacity, and leaching of water-soluble nitrogen. Similarly, Surasani, Raju, Singh, and Joshi (2022) observed moisture contents of 67.78–68.02% in sausages prepared with rohu protein isolate, indicating that variations in proximate composition are largely influenced by the inherent properties of the raw material, consistent with observations by Surasani et al. (2019). Mhatre et al. (2024) reported protein degradation in Bombay duck fish balls stored for 60 days, with treatments T1–T4 showing protein contents decreasing from 12.61 to 11.06%, 10.25 to 9.26%, 9.6 to 8.28%, and 12.81 to 11.02%, respectively, reflecting leaching of water-soluble nitrogen compounds. Ninan, Bindu, and Joseph (2008) documented an increase in fat content from 14.50 to 15.20% in the same sample, which could be due to deep frying of the product, a trend similarly noted in the present study. Ash content changes have been reported by Kolekar and Pagarkar (2013), ranging from 3.18 to 3.85% in Catla fish balls over a 12-day chilled storage period, while Singh et al. (2024) recorded carbohydrate values of 14.90–16.44% in rohu fish balls over 28 days.

The biochemical composition of SPI revealed a near-neutral pH of 6.50 ± 0.03, a low free fatty acid (FFA) content of 0.90 ± 0.14% (as oleic acid), and a peroxide value (PV) of 0.84 ± 0.19 meq/kg, indicating minimal lipid hydrolysis and primary oxidation, and confirming the powder's biochemical stability for incorporation into value-added seafood products (Table 4). During frozen storage, the pH of shrimp balls ranged from 5.85 ± 0.01 to 6.62 ± 0.01, while FFA and PV values ranged from 0.57 ± 0.10 to 3.08 ± 0.12% and 0.49 ± 0.12 to 0.94 ± 0.06 meq/kg, respectively (Table 4). Decreases in pH during freezing can be attributed to the concentration of ions and solutes in the unfrozen phase, precipitating salts and producing a more acidic environment, particularly under slower freezing conditions (Zhang, Kim, Puolanne, & Ertbjerg, 2022). Dileep, Shamasundar, Binsi, Badii, and Howell (2005) reported a pH of 6.92 for ribbon fish (*Trichiurus* spp.) meat, while Wang, Potoroko, and Tsirulnichenko (2021) observed an initial pH of 7.55 in fish balls that decreased over nine days due to environmental and microbial carbon dioxide, followed by increases from protein breakdown and microbial activity. pH is an important freshness indicator, with reductions linked to energy depletion, lactate formation, glycogen degradation, or stress (Emire & Gebremariam, 2010). FFA increased during storage, consistent with Pawar, Pagarkar, Rathod, Patil, and Mahakal (2013), who observed a rise from 0.95 to 2.12 mg/100 g in cutlets over 105 days, reflecting enzymatic lipid hydrolysis in frozen fish (Tokur, Polat, Beklevik, & Özkütük, 2004). Similarly, FFA in Tilapia burgers stored at –18 °C increased from 2.73 to 5.92 over eight months. PV, indicative of primary

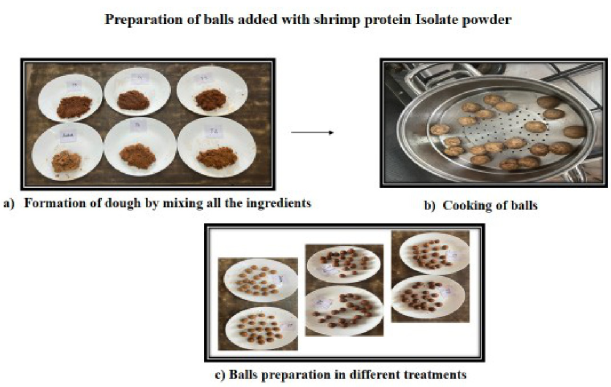

Fig. 1. Pictorial representation of the preparation of shrimp balls supplemented with shrimp protein isolate powder (SPI)

Table 3. Proximate composition % (Mean ± SE) of shrimp balls during frozen storage

Storage period	Attributes	A	B	C	D	E	F
0 Day	Moisture (%)	43.65±0.66^{1a}	43.81±0.62^{1a}	43.94±0.90^{1a}	43.52±0.11^{1a}	44.06±0.90^{1a}	43.70±0.61^{1a}
	Protein (%)	8.54±0.01^{1f}	13.20±0.02^{1e}	17.55±0.01^{1d}	21.93±0.01^{1c}	26.02±0.01^{1b}	29.64±0.02^{1a}
	Fat (%)	12.10±0.64^{1a}	11.95±0.59^{1a}	11.81±0.87^{1a}	12.27±1.21^{1a}	11.70±0.90^{1a}	12.13±0.60^{1a}
	Ash (%)	2.02±0.51^{1b}	2.12±0.12^{1b}	2.17±0.40^{1b}	2.29±0.13^{1b}	2.73±0.20^{1b}	3.77±0.18^{1a}
	Carbohydrate (%)	33.70±0.51^{1a}	28.95±0.11^{1b}	24.53±0.39^{1c}	19.99±0.10^{1d}	15.49±0.21^{1e}	10.76±0.20^{1f}
15 days	Moisture (%)	43.26±0.56^{1a}	43.26±0.44^{1a}	42.38±1.02^{12a}	42.58±0.33^{1a}	43.70±0.23^{12a}	43.14±0.23^{12a}
	Protein (%)	8.39±0.15^{2f}	13.12±0.01^{1e}	17.50±0.01^{12d}	21.90±0.01^{12c}	26.02±0.15^{1b}	29.60±0.03^{12a}
	Fat (%)	12.15±0.39^{1ab}	11.96±0.20^{1ab}	11.99±0.05^{1ab}	12.28±0.44^{1a}	11.78±0.16^{1b}	12.17±0.16^{1ab}
	Ash (%)	2.02±0.44^{1b}	2.12±0.04^{1b}	2.19±0.14^{1b}	2.30±0.34^{1b}	2.75±0.54^{1b}	3.78±0.21^{1a}
	Carbohydrate (%)	34.18±0.34^{1a}	29.54±0.34^{1b}	25.94±0.90^{1c}	20.94±0.21^{1d}	15.75±0.30^{1e}	11.31±0.20^{1f}
30 days	Moisture (%)	43.10±0.20^{1a}	43.14±0.41^{1a}	41.79±0.59^{12a}	42.51±0.27^{1a}	43.58±0.23^{12a}	43.06±0.04^{12a}
	Protein (%)	8.31±0.01^{3f}	13.12±0.01^{1e}	17.47±0.02^{23d}	21.88±0.01^{23c}	25.97±0.01^{12b}	29.58±0.03^{123a}
	Fat (%)	12.22±0.09^{1a}	11.96±0.08^{1a}	12.14±0.28^{1a}	12.33±0.17^{1a}	11.99±0.06^{1a}	12.23±0.22^{1b}
	Ash (%)	2.02±0.95^{1b}	2.14±0.15^{1b}	2.19±0.43^{1b}	2.31±0.45^{1ab}	2.75±0.13^{1ab}	3.78±0.25^{1a}
	Carbohydrate (%)	34.40±1.19^{1a}	29.63±0.77^{1b}	25.96±0.51^{1c}	20.96±0.57^{1d}	16.43±0.42^{1e}	11.35±0.47^{1f}
45 days	Moisture (%)	42.61±0.50^{1ab}	43.11±0.41^{1a}	41.72±0.36^{2b}	42.45±0.28^{1ab}	42.85±0.36^{12ab}	42.86±0.21^{12ab}
	Protein (%)	8.27±0.01^{34f}	13.09±0.03^{1e}	17.45±0.02^{34d}	21.85±0.02^{34c}	25.93±0.02^{2b}	29.54±0.01^{23a}
	Fat (%)	12.23±0.20^{1a}	12.02±0.04^{1a}	12.14±0.17^{1a}	12.34±0.12^{1a}	11.99±0.10^{1a}	12.24±0.24^{1a}
	Ash (%)	2.05±0.28^{1b}	2.14±0.29 ^{1b}	2.19±0.20^{1b}	2.31±0.32^{1b}	2.76±0.42^{1ab}	3.79±0.84^{1a}
	Carbohydrate (%)	34.84±0.80^{1a}	29.64±0.66^{1b}	26.51±0.25^{1c}	21.03±0.21^{1d}	16.47±0.26^{1e}	11.56±0.91^{1f}
60 days	Moisture (%)	42.56±0.55^{1a}	43.04±0.41^{1a}	41.72±0.49^{2a}	42.37±0.39^{1a}	42.74±0.35^{12a}	42.79±0.20^{12a}
	Protein (%)	8.23±0.02^{4f}	13.02±0.01^{1e}	17.39±0.02^{4d}	21.82±0.02^{4c}	25.89±0.02^{23b}	29.51±0.02^{3a}
	Fat (%)	12.28±0.11^{1a}	12.05±0.07^{1a}	12.15±0.13^{1a}	12.34±0.10^{1a}	12.06±0.07^{1a}	12.29±0.08^{1a}
	Ash (%)	2.06±0.27^{1b}	2.16±0.31^{1b}	2.23±0.52^{1b}	2.31±0.50^{1ab}	2.77±0.35^{1ab}	3.80±0.70^{1a}
	Carbohydrate (%)	34.86±0.44^{1a}	29.73±0.30^{1b}	26.52±1.02^{1c}	21.15±0.18^{1d}	16.54±0.63^{1e}	11.60±0.45^{1f}
75 days	Moisture (%)	42.55±0.56^{1a}	43.03±0.41 ^{1a}	41.71±0.35^{12a}	42.35±0.36^{1a}	42.70±0.36^{12a}	42.75±0.20^{12a}
	Protein (%)	8.17±0.04^{5f}	12.98±0.02^{1e}	17.33±0.02^{5d}	21.77±0.02^{5c}	25.85±0.02^{34b}	29.50±0.01^{3a}
	Fat (%)	12.31±0.19^{1a}	12.08±0.08^{1a}	12.19±0.24^{1a}	12.35±0.16^{1a}	12.06±0.08^{1a}	12.31±0.07^{1a}
	Ash (%)	2.07±0.58^{1b}	2.17±0.69^{1a}	2.25±0.17^{1a}	2.32±1.01^{1a}	2.79±0.70^{1a}	3.81±0.30^{1a}
	Carbohydrate (%)	34.90±0.91^{1a}	29.74±0.48^{1b}	26.57±0.65^{1c}	21.20±1.37^{1d}	16.60±0.78^{1e}	11.62±0.44^{1f}
90 days	Moisture (%)	42.54±0.51^{1a}	42.94±0.42^{1a}	41.69±0.48^{2a}	42.33±0.39^{1a}	42.66±0.34^{2a}	42.70±0.20^{2a}
	Protein (%)	8.12±0.02^{5f}	12.90±0.02^{1e}	17.26±0.02^{6d}	21.73±0.02^{6c}	25.79±0.05^{5b}	29.42±0.02^{4a}
	Fat (%)	12.35±0.03^{1a}	12.11±0.08^{1a}	12.27±0.22^{1a}	12.37±0.14^{1a}	12.13±0.07^{1a}	12.32±0.17^{1a}
	Ash (%)	2.09±0.08^{1b}	2.18±0.36^{1b}	2.26±0.14^{1b}	2.32±0.25^{1b}	2.79±0.32^{1b}	3.83±0.24^{1a}
	Carbohydrate (%)	34.91±0.51^{1a}	29.87±0.70^{1b}	26.64±0.41^{1c}	21.26±0.38^{1d}	16.63±0.53^{1e}	11.73±0.53^{1f}

*($p < 0.05$) significance level. Values are represented as Mean ± S.E.

**Superscript with a different alphabet shows significant ($p < 0.05$) variation within the row

***Superscript with different numbers shows significant ($p < 0.05$) variation within the column

A: Balls without shrimp protein isolate powder; B: Balls with 10% shrimp protein isolate powder; C: Balls with 20% shrimp protein isolate powder; D: Balls with 30% shrimp protein isolate powder; E: Balls with 40% shrimp protein isolate powder; F: Balls with 50% shrimp protein isolate powder

Table 4. Biochemical quality analysis of shrimp balls during frozen storage

Storage period	Attributes	A	B	C	D	E	F
0 Day	pH	6.62±0.01^{1a}	6.30±0.01^{1d}	6.54±0.01^{1c}	6.31±0.01^{1d}	6.59±0.02^{1b}	6.56±0.01^{1bc}
	FFA (% Oleic acid)	0.58±0.02^{4a}	0.58±0.02^{3a}	0.60±0.04^{4a}	0.57±0.04^{4a}	0.66±0.16^{4a}	0.65±0.09^{4a}
	PV (meq/kg)	0.49±0.12^{1a}	0.57±0.10^{2a}	0.56±0.15^{1a}	0.49±0.04^{4a}	0.66±0.13^{12a}	0.53±0.06^{4a}
15 days	pH	6.55±0.10^{1a}	6.28±0.02^{1b}	6.53±0.01^{1a}	6.31±0.01^{1b}	6.56±0.02^{1a}	6.51±0.04^{1a}
	FFA (% Oleic acid)	0.72±0.18^{4a}	0.75±0.19^{3a}	0.86±0.18^{34a}	0.66±0.09^{4a}	0.94±0.09^{4a}	1.03±0.09^{34a}
	PV (meq/kg)	0.52±0.11^{1a}	0.56±0.08^{2a}	0.69±0.03^{1a}	0.60±0.06^{4a}	0.64±0.07^{2a}	0.63±0.05^{34a}
30 days	pH	5.90±0.01^{2c}	5.89±0.01^{2c}	6.21±0.00^{2b}	6.17±0.02^{12b}	6.50±0.01^{1a}	6.43±0.03^{1a}
	FFA (% Oleic acid)	1.13±0.33^{34a}	1.32±0.49^{23a}	1.50±0.18^{23a}	1.32±0.18^{3a}	1.50±0.18^{3a}	1.50±0.1^{23a}
	PV (meq/kg)	0.65±0.11^{1a}	0.59±0.06^{2a}	0.64±0.07^{1a}	0.64±0.02^{3a}	0.69±0.03^{12a}	0.69±0.07^{234a}
45 days	pH	5.89±0.01^{2c}	5.89±0.01^{2c}	6.18±0.02^{23b}	6.16±0.02^{12b}	6.38±0.01^{1a}	6.38±0.01^{1a}
	FFA (% Oleic acid)	1.63±0.31^{23a}	1.87±0.18^{12a}	2.25±0.32^{12a}	1.88±0.19^{2a}	2.05±0.19^{2a}	1.82±0.18^{2a}
	PV (meq/kg)	0.71±0.10^{1a}	0.65±0.04^{12a}	0.68±0.06^{1a}	0.64±0.00^{3a}	0.69±0.01^{12a}	0.71±0.06^{23a}
60 days	pH	5.89±0.01^{2c}	5.88±0.01^{2c}	6.11±0.01^{3b}	6.10±0.00^{12b}	6.36±0.01^{1a}	6.37±0.01^{1a}
	FFA (% Oleic acid)	2.10±0.18^{12b}	2.25±0.32^{1ab}	2.06±0.19^{12b}	2.62±0.19^{1ab}	2.81±0.00^{1a}	2.63±0.18^{1ab}
	PV (meq/kg)	0.75±0.06^{1a}	0.66±0.01^{12a}	0.76±0.05^{1a}	0.69±0.01^{23a}	0.73±0.01^{12a}	0.74±0.03^{23a}
75 days	pH	5.88±0.01^{2a}	5.87±0.01^{2a}	6.02±0.04^{4a}	6.02±0.02^{12a}	6.17±0.33^{1a}	6.23±0.01^{1a}
	FFA (% Oleic acid)	2.40±0.49^{12a}	2.63±0.19^{1a}	2.75±0.32^{1a}	2.95±0.18^{1a}	2.94±0.18^{1a}	2.99±0.19^{1a}
	PV (meq/kg)	0.76±0.07^{1a}	0.68±0.09^{12a}	0.80±0.04^{1a}	0.81±0.07^{12a}	0.85±0.02^{12a}	0.85±0.02^{12a}
90 days	pH	5.87±0.01^{2b}	5.85±0.26^{2a}	6.02±0.04^{4a}	5.87±0.02^{2a}	6.04±0.29^{1a}	6.12±0.01^{1a}
	FFA (% Oleic acid)	2.64±0.10^{1a}	2.70±0.10^{1a}	2.78±0.34^{1a}	3.00±0.25^{1a}	3.02±0.12^{1a}	3.08±0.46^{1a}
	PV (meq/kg)	0.78±0.02^{1a}	0.85±0.06^{1a}	0.82±0.13^{1a}	0.89±0.04^{1a}	0.87±0.05^{1a}	0.94±0.06^{1a}

*($p < 0.05$) significance level. Values are represented as Mean ± S.E.

**Superscript with a different alphabet shows significant ($p < 0.05$) variation within the row

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A: Balls without shrimp protein isolate powder; B: Balls with 10% shrimp protein isolate powder; C: Balls with 20% shrimp protein isolate powder; D: Balls with 30% shrimp protein isolate powder; E: Balls with 40% shrimp protein isolate powder; F: Balls with 50% shrimp protein isolate powder

FFA: Free Fatty Acids as % Oleic acid; PV: Peroxide Value as meq/kg

lipid oxidation, was influenced by protein supplementation, as reported by Moosavi-Nasab, Mohammadi, and Oliyaei (2018) in lantern fish sausages containing 2–4% protein isolate and a control, with PV values ranging from 1.42–4.82 meq/kg over 60 days at 4 °C. These findings highlight the effects of frozen storage and protein isolate incorporation on the biochemical stability of shrimp balls.

The cooking yield is influenced by factors including the cooking method, rate of cooking, and composition of the product. The cooking yield of the A balls was 98.58 ± 1.89%, while balls B, C, D, E, and F recorded cooking yields of 95.55 ± 0.34%, 98.31 ± 0.35%, 98.25 ± 0.69%, 98.73 ± 0.13%, and 98.88 ± 0.55%, respectively. No significant ($p > 0.05$) differences were observed among most treatments, indicating that supplementation with shrimp protein isolate had a minimal impact on cooking yield. Comparatively, Bainy, Bertan, Corazza, and Lenzi (2015) analysed the cooking yield of baked and grilled fish burgers prepared from *Tilapia* species and reported values of 88.56% and 85.30%, respectively. Surasani, Raju, Sofi, and Shafiq (2021) studied pangas mince emulsions incorporated with rohu protein isolate and observed cooking yields ranging from 93.34 to 80.54% over three months of frozen storage. Khushboo, Kaushik, Widell, Slizyte, and Kumari (2023) emphasized that cooking yield is an important parameter for product commercialisation, reflecting the extent of weight loss during cooking,

Table 5. Texture and Colour (Mean ± SE) values of shrimp balls during frozen storage

Storage period	Attributes	A	B	C	D	E	F
0 Day	Hardness (g. force)	1238.81±0.73^{7a}	1177.06±1.15^{7b}	1087±1.15^{7c}	926.30±1.19^{7d}	856.39±1.66^{7e}	708.93±1.08^{7f}
	L*	26.86±0.51^{1a}	27.24±0.51^{1a}	27.37±0.38^{1a}	27.37±0.32^{1a}	27.46±0.23^{1a}	27.69±0.33^{1a}
	a*	10.91±0.64^{1b}	11.59±0.82^{1ab}	12.16±0.52^{1ab}	12.22±1.04^{1ab}	12.37±1.31^{1ab}	14.10±0.51^{1a}
	b*	21.64±0.23^{3a}	20.79±0.20^{4b}	20.76±0.28^{4b}	20.41±0.18^{3b}	20.39±0.17^{6b}	20.32±0.46^{3b}
15 days	Hardness (g. force)	1250.32±0.58^{6a}	1200.32±0.34^{6b}	1099.71±0.69^{6c}	951.33±0.61^{6d}	865.68±0.37^{6e}	728.41±1.20^{6f}
	L*	26.07±0.53^{12a}	26.80±0.74^{1a}	26.42±0.35^{12a}	26.76±0.32^{12a}	26.92±0.90^{2a}	26.95±0.51^{12a}
	a*	9.51±0.76^{2c}	10.33±0.61^{12bc}	10.87±0.59^{2bc}	11.76±0.15^{12ab}	11.87±0.40^{12ab}	13.05±0.50^{1a}
	b*	21.79±0.17^{3a}	21.00±0.08^{4a}	20.95±0.03^{4b}	20.65±0.33^{3b}	20.65±0.10^{6a}	20.57±0.22^{23b}
30 days	Hardness (g. force)	1271.90±0.65^{5a}	1230.37±0.61^{5b}	1131.25±0.49^{5c}	971.71±0.77^{5d}	894.13±0.58^{5e}	738.29±1.27^{5f}
	L*	25.75±0.59^{12a}	25.95±0.53^{12a}	26.02±0.43^{23a}	26.14±0.27^{23a}	26.15±0.12^{3a}	26.22±0.46^{23a}
	a*	8.46±0.53^{23c}	9.12±0.38^{23bc}	10.02±0.48^{23abc}	10.48±0.21^{23ab}	10.84±0.18^{123a}	10.73±0.96^{2ab}
	b*	21.96±0.82^{23a}	21.34±0.12^{3a}	21.37±0.11^{3b}	21.32±0.29^{2b}	21.06±0.14^{5b}	21.02±0.28^{123b}
45 days	Hardness (g. force)	1294.47±0.58^{4a}	1251.31±0.61^{4b}	1159.41±0.73^{4c}	991.36±0.66^{4d}	914.19±0.59^{4e}	757.75±0.73^{4f}
	L*	25.02±0.57^{23a}	25.16±0.15^{23a}	25.27±0.37^{34a}	25.30±0.23^{3a}	25.31±0.10^{4a}	25.62±0.32^{34a}
	a*	8.07±0.62^{23c}	8.64±0.20^{3bc}	9.07±0.43^{34b}	10.07±0.07^{3a}	10.27±0.14^{23a}	10.46±0.23^{2a}
	b*	22.05±0.06^{23a}	21.64±0.09^{23ab}	21.58±0.09^{23b}	21.52±0.15^{12b}	21.26±0.10^{34b}	21.24±0.08^{12b}
60 days	Hardness (g. force)	1302.47±0.57^{3a}	1275.62±0.72^{3b}	1204.55±0.82^{3c}	1015.41±0.54^{3d}	943.03±0.30^{3e}	802.48±0.58^{3f}
	L*	23.93±0.43^{34a}	24.00±0.01^{34a}	24.29±0.37^{45a}	24.32±0.09^{4a}	24.47±0.07^{5a}	24.71±0.31^{45a}
	a*	7.53±0.26^{3c}	8.14±0.11^{3bc}	8.74±0.21^{4ab}	9.37±0.49^{3a}	9.55±0.51^{3a}	9.83±0.23^{2a}
	b*	22.19±0.12^{12a}	21.82±0.10^{12ab}	21.70±0.07^{123b}	21.60±0.12^{12b}	21.46±0.05^{23b}	21.43±0.28^{1b}
75 days	Hardness (g. force)	1311.42±0.78^{2a}	1299.78±0.68^{2b}	1221.69±0.90^{2c}	1027.85±0.66^{2d}	968.37±0.67^{2e}	827.47±0.67^{2f}
	L*	23.09±0.44^{5a}	23.22±0.11^{4a}	23.34±0.33^{5a}	23.67±0.38^{4a}	23.73±0.87^{6a}	23.83±0.13^{5a}
	a*	6.06±0.29^{4b}	6.49±0.26^{4ab}	7.04±0.16^{5ab}	7.09±0.06^{4ab}	7.30±0.14^{4b}	7.42±0.16^{3a}
	b*	22.41±0.13^{12a}	21.95±0.02^{12b}	21.83±0.04^{12b}	21.75±0.10^{12b}	21.67±0.03^{12b}	21.65±0.26^{1b}
90 days	Hardness (g. force)	1326.35±0.58^{1a}	1310.36±0.59^{1b}	1251.18±0.70^{1c}	1054.41±0.58^{1d}	988.56±0.51^{1e}	865.15±0.58^{1f}
	L*	20.63±0.57^{5a}	20.70±0.27^{5a}	20.82±0.19^{6a}	20.83±0.39^{5a}	21.06±0.03^{7a}	21.16±0.02^{6a}
	a*	4.24±0.19^{5b}	4.29±0.32^{5b}	4.51±0.16^{6ab}	4.97±0.05^{5ab}	5.32±0.34^{5a}	5.36±0.36^{4a}
	b*	22.60±0.11^{1a}	22.11±0.05^{1b}	22.06±0.03^{1b}	22.02±0.02^{1b}	21.93±0.03^{1b}	21.78±0.22^{1b}

*($p < 0.05$) significance level. Values are represented as Mean ± S.E.

**Superscript with a different alphabet shows significant ($p < 0.05$) variation within the row

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A: Balls without shrimp protein isolate powder; B: Balls with 10% shrimp protein isolate powder; C: Balls with 20% shrimp protein isolate powder; D: Balls with 30% shrimp protein isolate powder; E: Balls with 40% shrimp protein isolate powder; F: Balls with 50% shrimp protein isolate powder

L*-Lightness; a*- Redness/Greenness; b*- Blueness/Yellowness

which primarily depends on the product's ability to retain fat. In their study, balls with varying percentages of fish gelatin exhibited cooking yields of 70.9% in the control, 75.6% in T1 (3% gelatin), 78.8% in T2 (4% gelatin), 81.8% in T3 (5% gelatin), and 85.6% in T4 (6% gelatin). Surasani et al. (2022) reported cooking yields in patties prepared from GIFT Tilapia protein isolate with fish powder incorporation at 5, 10, and 15%, recording yields of 92.13, 91.88, and 88.40%, respectively. Gall, Otwell, Koburgier, and Appledorf (1983) reviewed cooking yields of four fillet species (Grouper, Red Snapper, Florida Pompano, and Spanish Mackerel) prepared using different cooking methods such as baking, broiling, deep frying, and microwaving. The highest yields were observed in microwave-cooked fillets, while deep-fried fillets exhibited the lowest yields.

Table 6. Sensory analysis (Mean ± SE) of shrimp balls during frozen storage

Storage period	Attributes	A	B	C	D	E	F
0 Day	Appearance	$9.00±0.01^{1a}$	$8.90±0.04^{1b}$	$8.72±0.01^{1d}$	$8.64±0.01^{1e}$	$8.82±0.01^{1c}$	$8.90±0.01^{1b}$
	Texture	$8.45±0.01^{1b}$	$8.45±0.01^{1b}$	$8.18±0.02^{1d}$	$8.64±0.01^{1a}$	$8.45±0.01^{1b}$	$8.40±0.02^{1c}$
	Flavour	$8.72±0.01^{1a}$	$7.36±0.01^{1d}$	$7.64±0.01^{1c}$	$7.18±0.03^{1e}$	$7.00±0.01^{1f}$	$8.00±0.01^{1b}$
	Odour	$8.45±0.01^{1a}$	$7.00±0.01^{1c}$	$6.91±0.01^{1d}$	$6.90±0.02^{1d}$	$7.00±0.01^{1c}$	$7.27±0.02^{1b}$
	Overall Acceptability	$8.59±0.03^{1a}$	$7.64±0.01^{1d}$	$8.00±0.01^{1c}$	$7.68±0.02^{1d}$	$7.95±0.01^{1c}$	$8.18±0.02^{1b}$
15 days	Appearance	$8.72±0.03^{2a}$	$8.71±0.01^{2b}$	$8.51±0.01^{2d}$	$8.55±0.01^{2a}$	$8.69±0.01^{2a}$	$8.71±0.01^{2a}$
	Texture	$8.43±0.01^{1a}$	$8.24±0.03^{2d}$	$8.15±0.01^{1e}$	$8.35±0.01^{2c}$	$8.41±0.02^{12ab}$	$8.37±0.01^{1bc}$
	Flavour	$8.51±0.01^{2a}$	$7.19±0.02^{2d}$	$7.49±0.01^{2c}$	$7.13±0.02^{1e}$	$6.87±0.01^{2f}$	$7.94±0.02^{2b}$
	Odour	$8.32±0.01^{2a}$	$6.91±0.01^{2cd}$	$6.85±0.03^{2f}$	$6.86±0.02^{12de}$	$6.92±0.01^{2c}$	$7.16±0.01^{2b}$
	Overall Acceptability	$8.55±0.02^{1a}$	$7.59±0.01^{12d}$	$7.71±0.01^{2c}$	$7.59±0.02^{2d}$	$7.67±0.01^{2c}$	$8.09±0.01^{2b}$
30 days	Appearance	$8.18±0.01^{3e}$	$8.23±0.01^{3d}$	$8.27±0.01^{3d}$	$8.46±0.01^{3b}$	$8.36±0.01^{3c}$	$8.54±0.01^{3a}$
	Texture	$8.41±0.01^{1a}$	$8.00±0.01^{3d}$	$8.09±0.01^{2b}$	$8.05±0.02^{3bc}$	$8.36±0.03^{2a}$	$8.37±0.01^{1a}$
	Flavour	$8.09±0.02^{3a}$	$7.00±0.01^{3d}$	$7.32±0.01^{3c}$	$7.00±0.01^{2d}$	$6.75±0.02^{3e}$	$7.91±0.02^{2b}$
	Odour	$8.20±0.01^{3a}$	$6.82±0.01^{3c}$	$6.73±0.01^{3d}$	$6.83±0.04^{23c}$	$6.68±0.02^{3c}$	$7.09±0.01^{2b}$
	Overall Acceptability	$8.54±0.01^{1a}$	$7.54±0.03^{23d}$	$7.64±0.02^{3c}$	$7.50±0.03^{3de}$	$7.45±0.03^{3e}$	$8.00±0.02^{3b}$
45 days	Appearance	$8.12±0.01^{4c}$	$8.22±0.01^{4a}$	$8.21±0.01^{4a}$	$7.98±0.01^{4e}$	$8.01±0.01^{4d}$	$8.15±0.01^{4b}$
	Texture	$8.18±0.01^{2a}$	$7.83±0.01^{4d}$	$7.97±0.01^{3b}$	$7.85±0.01^{4d}$	$7.82±0.01^{3d}$	$7.93±0.01^{2c}$
	Flavour	$8.05±0.01^{3a}$	$6.57±0.01^{4d}$	$6.99±0.03^{4b}$	$6.97±0.01^{2b}$	$6.71±0.02^{4c}$	$6.97±0.02^{3b}$
	Odour	$7.59±0.02^{4a}$	$6.52±0.05^{4d}$	$6.66±0.01^{4e}$	$6.79±0.01^{4c}$	$6.61±0.02^{4d}$	$7.01±0.01^{3b}$
	Overall Acceptability	$8.21±0.01^{2a}$	$7.51±0.02^{34d}$	$7.59±0.02^{34c}$	$7.39±0.02^{4e}$	$7.29±0.03^{4f}$	$7.81±0.02^{4b}$
60 days	Appearance	$8.09±0.01^{4b}$	$8.20±0.01^{4a}$	$8.20±0.01^{4a}$	$7.82±0.01^{5c}$	$7.72±0.01^{5d}$	$8.09±0.01^{5b}$
	Texture	$8.08±0.03^{3a}$	$7.73±0.01^{5c}$	$7.82±0.01^{4b}$	$7.73±0.01^{5c}$	$7.64±0.01^{4d}$	$7.64±0.02^{3d}$
	Flavour	$8.00±0.01^{4a}$	$6.49±0.01^{5e}$	$6.85±0.01^{5c}$	$6.82±0.02^{3c}$	$6.55±0.01^{5d}$	$6.91±0.02^{4b}$
	Odour	$7.27±0.01^{5a}$	$6.23±0.01^{5d}$	$6.13±0.02^{5e}$	$6.77±0.01^{4b}$	$6.59±0.01^{4c}$	$6.05±0.02^{4f}$
	Overall Acceptability	$8.00±0.03^{3a}$	$7.45±0.03^{4c}$	$7.54±0.02^{4b}$	$7.27±0.01^{5e}$	$7.14±0.01^{5f}$	$7.36±0.02^{5d}$
75 days	Appearance	$7.95±0.01^{5b}$	$7.57±0.01^{5e}$	$7.81±0.01^{5c}$	$7.56±0.01^{6e}$	$7.68±0.01^{6d}$	$8.01±0.003^{6a}$
	Texture	$7.99±0.01^{4a}$	$7.51±0.01^{6c}$	$7.57±0.02^{5b}$	$7.49±0.02^{6c}$	$7.39±0.02^{5d}$	$7.39±0.02^{4d}$
	Flavour	$7.59±0.02^{5a}$	$5.99±0.01^{6b}$	$5.97±0.01^{6b}$	$5.48±0.02^{4c}$	$5.12±0.02^{6d}$	$5.98±0.02^{5b}$
	Odour	$6.53±0.01^{6a}$	$5.97±0.01^{6b}$	$5.84±0.01^{6c}$	$5.97±0.01^{5b}$	$6.01±0.01^{5b}$	$6.01±0.02^{4b}$
	Overall Acceptability	$7.59±0.01^{4a}$	$6.99±0.01^{5bc}$	$7.01±0.02^{5b}$	$6.96±0.02^{6bc}$	$6.89±0.05^{6d}$	$6.91±0.05^{6bc}$
90 days	Appearance	$7.73±0.01^{6a}$	$7.27±0.01^{6e}$	$7.45±0.01^{6c}$	$7.36±0.02^{7d}$	$7.63±0.02^{7b}$	$7.47±0.03^{7c}$
	Texture	$7.27±0.01^{5b}$	$7.36±0.02^{7a}$	$7.15±0.02^{6c}$	$7.13±0.01^{7cd}$	$7.05±0.02^{6d}$	$6.95±0.01^{5e}$
	Shrimp Flavour	$7.27±0.02^{6a}$	$5.45±0.01^{7b}$	$5.18±0.01^{7d}$	$5.00±0.01^{5e}$	$4.91±0.01^{7f}$	$5.21±0.01^{6c}$
	Odour	$5.45±0.02^{7a}$	$5.00±0.01^{7b}$	$4.64±0.02^{7c}$	$4.45±0.03^{6f}$	$4.54±0.02^{6e}$	$4.63±0.04^{5d}$
	Overall Acceptability	$7.20±0.03^{5a}$	$6.72±0.02^{6b}$	$6.36±0.01^{6c}$	$6.00±0.01^{7e}$	$6.09±0.01^{7d}$	$6.32±0.02^{7c}$

*($p < 0.05$) significance level. Values are represented as Mean ± S.E.
**Superscript with different alphabet shows significant ($p < 0.05$) variation within the row
***Superscript with different numerical shows significant ($p < 0.05$) variation within the column
A: Balls without shrimp protein isolate powder; B: Balls with 10% shrimp protein isolate powder; C: Balls with 20% shrimp protein isolate powder; D: Balls with 30% shrimp protein isolate powder; E: Balls with 40% shrimp protein isolate powder; F: Balls with 50% shrimp protein isolate powder

The SPI exhibited a lightness (L*) of 26.33 ± 0.90, redness (a*) of 6.79 ± 0.17, and yellowness (b*) of 29.4 ± 10.35, indicating a moderately light appearance with slight red and pronounced yellow hues (Table 5). For shrimp balls, L*, a*, and b* ranged from 20.63 ± 0.57 to 27.69 ± 0.33, 4.24 ± 0.19 to 14.10 ± 0.51, and 20.32 ± 0.46 to 22.60 ± 0.11, respectively, while hardness (texture) varied from 708.93 ± 1.08 to 1326.35 ± 0.58 g, reflecting variations in texture and colour due to supplementation and frozen storage. Huda, Shen, Huey, and Dewi (2010) reported hardness values of 1.71–3.01 kg in market fish balls, highlighting variability in textural properties. L* indicates brightness, a* the green–red spectrum, and b* the blue–yellow spectrum (Akram, Butt, Shukat, & Zia, 2020). Li et al. (2022) observed decreases in L* during frozen storage in shrimp and fried/baked nuggets (Oppong, Panpipat, Cheong, & Chaijan, 2021). It is considered that changes in L* values during frozen storage may be associated with pigment oxidation, dehydration, and structural changes in the product matrix. In the present study, aerobic packaging and the absence of colour preservatives may have accelerated L* reduction. The a* value decreased over time, indicating loss of redness, consistent with sardine and mackerel studies (Chaijan, Benjakul, Visessanguan, & Faustman, 2005). Conversely, in the present study, minor variations were observed in b* during storage, reflecting accumulation of yellow pigments linked to lipid oxidation (Zhu, Yan, Dai, & Zhang, 2022). Overall, storage duration and lack of protective measures significantly influenced the colour and texture of shrimp balls. Increase in hardness during frozen storage may be associated with moisture loss and ice crystal formation which leads to microstructural damage, disruption of muscle fibres, and protein denaturation. Also, with the storage time period, the ice crystals were expanded, which further indicated the elevation of hardness in the product (Zhu et al., 2022). Serdaroğlu, Yıldız-Turp, and Abrodímov (2005) described that the quantity of starch added is also an important factor for the hardness of the product. Huda et al. (2010) analysed the hardness of six different fish ball samples, which were collected from the local market in Malaysia, and recorded the values for the hardness of six samples as 1.76, 3.01, 1.84, 1.71, 2.46 and 1.77 kg. All the fish ball samples had different hardness and shear force. The highest shear force applied to check the hardness was for the 2nd fish ball sample.

Sensory analysis of SPI and shrimp balls during frozen storage is presented in Table 6. Sensory scores for appearance, texture, flavour, odour, and overall acceptability gradually decreased during storage, irrespective of treatment. Nurhayati et al. (2024) reported that the incorporation of composite powder in fish balls improved appearance scores, with 1.5% supplementation showing the highest acceptability compared to control and commercial fish balls. In the present study, appearance scores ranged from 7.27 ± 0.02 to 9.00 ± 0.01 during storage, with

Table 7: Changes in total plate count (CFU/g x 10^2) (Mean ± SE) of shrimp balls during frozen storage

Days	A	B	C	D	E	F
0	15.75±0.55^{1ab}	15.06±0.93^{1b}	16.15±0.45^{1a}	15.87±0.17^{1ab}	15.92±0.60^{1ab}	16.10±0.15^{1a}
15	11.10±0.57^{2c}	13.21±0.15^{2b}	15.37±0.21^{1a}	14.93±0.05^{2a}	14.99±0.78^{2a}	15.05±0.15^{2a}
30	10.95±0.34^{2d}	12.93±0.05^{3abc}	13.54±0.41^{2a}	12.19±0.10^{3c}	12.46±0.23^{3bc}	13.17±0.21^{3ab}
45	9.57±0.28^{3c}	10.84±0.03^{4b}	11.11±0.43^{3b}	11.25±0.19^{4ab}	10.98±0.10^{4b}	11.94±0.06^{4a}
60	9.02±0.59^{34bc}	9.87±0.02^{5a}	9.98±0.09^{4a}	9.79±0.13^{5ab}	8.91±0.14^{5c}	10.02±0.02^{5a}
75	7.98±0.06^{45a}	8.05±0.03^{6a}	8.21±0.20^{5a}	8.10±0.46^{6a}	8.16±0.23^{6a}	8.34±0.16^{6a}
90	7.03±0.02^{5a}	7.25±0.12^{7a}	7.46±0.30^{5a}	7.27±0.20^{7a}	7.12±0.15^{7a}	7.06±0.05^{7a}

*($p < 0.05$) significance level

**Superscript with different alphabets shows significant ($p < 0.05$) variation within the row

***Superscript with different numerical shows significant ($p < 0.05$) variation within the column

A: Balls without shrimp protein isolate powder; B: Balls with 10% shrimp protein isolate powder; C: Balls with 20% shrimp protein isolate powder; D: Balls with 30% shrimp protein isolate powder; E: Balls with 40% shrimp protein isolate powder; F: Balls with 50% shrimp protein isolate powder

treatment A receiving the highest scores initially, while lower scores were observed in treatments with higher levels of shrimp protein isolate incorporation. Oriakpono et al. (2011) reported that variations in organoleptic properties and colour are mainly associated with spoilage activity and biochemical changes occurring during storage, which can affect freshness and consumer acceptability. Texture scores ranged from 6.95 ± 0.01 to 8.64 ± 0.01 throughout frozen storage. Ibrahim (2015) observed that the incorporation of fish protein isolate may produce a slightly grainy texture, thereby influencing the panel's preferences. In the present study, moderate incorporation levels, particularly treatment B (10% shrimp protein isolate powder), showed comparatively better texture acceptability by the end of the storage study than higher incorporation levels.

Flavour scores decreased progressively during storage and ranged from 4.91 ± 0.01 to 8.72 ± 0.01. Similar findings were reported by Canti et al. (2024), where for fish balls containing 0% (F1), 15% (F2), and 30% (F3) protein isolate, highest flavour preference was shown for F1 and the lowest for F3. A comparable trend was observed in the present study, where treatment A obtained the highest flavour scores, while treatment E showed the lowest flavour acceptability. Odour scores also declined during storage, ranging from 4.45 ± 0.03 to 8.45 ± 0.01. Nurhayati et al. (2024) demonstrated that the addition of composite powder in tuna fish balls reduced odour intensity. Similarly, incorporation of shrimp protein isolate influenced odour perception in the present study, particularly at higher supplementation levels. Alkuraieef, Alsuhaibani, Alshawi, and Aljahani (2020) evaluated the sensory quality of mackerel-based fish balls and fish fingers and reported overall acceptability scores ranging from 8.36 to 8.45 for fish balls and 8.45 to 8.65 for fish fingers during six months of frozen storage, indicating the stability of sensory attributes under frozen conditions. In the present study, overall acceptability scores ranged from 6.00 ± 0.01 to 8.59 ± 0.03, with lower to moderate supplementation levels showing comparatively higher acceptability throughout storage.

The microbiological quality of SPI and shrimp balls was evaluated by analysing total plate count (TPC), *S. aureus*, and *E. coli* counts. TPC values remained within acceptable limits and showed slight reductions during frozen storage, possibly due to freezing stress, whereas no growth of *E. coli* or *S. aureus* was observed throughout 90 days of storage (Table 7). Microbial growth is influenced by extrinsic factors such as temperature and atmosphere, and intrinsic factors like pH, water activity, and nutrient composition (Ratrinia, Hasibuan, & Zein, 2021). Shrimp, being highly perishable, requires microbial counts within acceptable limits to ensure safety and quality (Amin et al., 2024). Previous studies have shown reductions in TPC during storage due to freezing and food additives in the product (Liston, 1980; Ninan et al., 2008; Vanitha, Dhanapal, Sravani, & Reddy, 2013). Similarly, Jeyasanta, Allwin, and Patterson (2017) and Alkuraieef et al. (2020) reported minimal microbial contamination in shrimp-based products under controlled storage. Salmonella, *E. coli*, and *S. aureus* are key food safety indicators (Compaore et al., 2022), and TPC provides an estimate of overall viable microbial load and spoilage-associated microorganisms (Yusuf et al., 2023). Overall, the sensory attributes and microbiological stability of the shrimp balls indicate that supplementation with shrimp protein isolate powder and frozen storage maintain product quality over time.

The present study demonstrated the effective utilisation of shrimp processing waste to produce shrimp protein isolate (SPI) powder and its incorporation into value-added shrimp balls. Supplementation with SPI significantly improved the nutritional quality of the product, particularly through enhancement of protein content, while also influencing physicochemical, textural, and sensory characteristics during frozen storage. Among the different incorporation levels, the 10% SPI-enriched shrimp balls exhibited the most desirable balance between nutritional improvement and consumer acceptability. Frozen storage for 90 days resulted in gradual changes in proximate composition, biochemical parameters, texture, colour, and sensory attributes; however, the products remained microbiologically safe throughout the storage period. The absence of *S. aureus* and *E. coli* during storage further indicated satisfactory hygienic handling and product safety. Overall, the findings suggest that shrimp protein isolate recovered from shrimp waste can be successfully incorporated into ready-to-cook seafood products at an acceptable level without adversely affecting product quality. Further studies focused on formulation optimisation and the incorporation of natural antimicrobial and antioxidant agents may help improve sensory stability and extend the storage life of SPI-supplemented products.

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