

# Food Quality and Shelf-Life Assessment of Ready-To-Heat Breaded Product Prepared from Swordtip Squid (*Uroteuthis duvaucelii*)

Md Ahosanul Haque, Laboni Rani Sinha, Tahamina Alam Tama,  
Md. Mohibbullah, Asif Wares Newaz

Department of Fishing and Post Harvest Technology, Sher-e-Bangla Agricultural University, Dhaka-1207,  
Bangladesh

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## Abstract:

The current study was carried out to develop an optimized recipe-based ready-to-heat breaded product from sword tip squid (*Uroteuthis duvaucelii*) and to evaluate the nutritional quality as well as shelf-life of the developed product under frozen storage. Value-added, ready-to-heat seafood has become more popular globally in recent years due to its ease of processing and consumption. The study was carried out during the period from July 2024 to June 2025. The proximate composition and major minerals were examined. Total volatile basic nitrogen (TVBN), non-protein nitrogen (NPN), free fatty acids (FFAs), peroxide value (PV) and total plate count (TPC) were assessed at 30-day intervals for up to 90 days to assess the product's shelf life. For raw squid and prepared breaded product, the moisture content of  $88.08 \pm 1.73$  and  $29.47 \pm 4.52\%$ , crude protein content  $9.75 \pm 1.41$  and  $12.35 \pm 2.85\%$ , fat  $0.56 \pm 0.04$  and  $23.22 \pm 4.28\%$ , carbohydrates  $0.94 \pm 0.14$  and  $31.88 \pm 4.35\%$ , and ash  $1.08 \pm 0.22$  and  $3.08 \pm 1.1\%$  were recorded, respectively. Among major minerals, Sodium (Na), Calcium (Ca), Phosphorus (P), Iron (Fe) and Zinc (Zn) contents were found at a level of  $41 \pm 1.01$ ,  $34 \pm 2.74$ ,  $20 \pm 1.98$ ,  $3.76 \pm 0.03$  and  $1.19 \pm 0.08\%$ , respectively. After 90 days of frozen storage, TVBN, NPN, FFA, and PV values increased from  $7.2 \pm 0.42$  to  $20.6 \pm 1.27$  mg/100g, from  $151 \pm 11.31$  to  $284 \pm 9.89$  mg N/100g, from  $0.34 \pm 0.04$  to  $1.07 \pm 0.13\%$  oleic acid, and from  $1.83 \pm 0.53$  to  $9.95 \pm 1.06$  meq. of O /kg oil, respectively. At 0, 30, 60, and 90 days of storage, the total plate count of prepared battered and breaded squid rings was 4.1, 1.3, 0.9, and 1.7 log cfu/g, respectively. The sensory evaluation of the prepared end product showed that the overall acceptability score decreased slightly when the storage duration increased, but the product had good acceptance over the storage period. The findings suggest that battered and breaded squid rings made from *U. duvaucelii* have potential for commercial application as convenient, nutritious seafood items with good shelf stability.

**Keywords:** Proximate composition, Mineral content, Squid ring, Sensory evaluation, Shelf life.

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## I. Introduction

Seafood consumption is increasing gradually owing to its high nutritional attributes, causing its augmenting demand throughout the globe. Over the last few decades, consumption of seafood products per capita has doubled and reached over 20 kg/year (Guillen et al., 2018). In many countries, seafood proteins are a vital source of nourishment, particularly in developing and underdeveloped nations where protein intake is inadequate. Among a large number of aquatic species, cephalopods constitute an important part of the marine resource and are quite suitable for human consumption (Jeyasekaran et al., 2010). Cephalopods are members of the phylum Mollusca's class Cephalopoda, which is a small group of highly developed, well-organized, and solely marine organisms. It represents a highly interesting biological group due to their nutritional value for human health and for their commercial significance (Torrinha et al., 2014; Singh et al., 2022). Squids are vital members of the cephalopod group, and due to their nutritional value and global demand, squid fisheries have transitioned from bycatch to main fishing. There are about 290 species of squids found worldwide, and about 30-40 species have commercial importance, which are distributed mainly in the regions of the Arabian Sea, Indo-Pacific, and along the coastline from the African coast to the Red Sea (Arkhipkin et al., 2015).

Value-added squid products are very popular all over the world. Squid form a major food resource and are used in cuisines around the world, notably in Japan, where it is eaten as 'ika-somen', sliced into vermicelli-like strips; as sashimi; and as tempura (Davidson, 2014). Besides, in European and North American markets, ready-to-heat squid products have gained popularity in recent years due to their crisp texture and golden-brown

appearance as well as their nutritional value (Bandre et al., 2018). Moreover, Thailand is the largest exporter of squid and cuttlefish in Asia.

Squid meat is a popular seafood all over the world due to its cheap, versatile, and tasty applications. With about 76% edible portion, squid meat contains a high amount of protein (18-20%) and low levels of lipid and carbohydrate (Chavan et al., 2020).

The high tidal amplitude, sufficient tidal current, fewer pollutants and high phytoplankton abundance offer an ideal environment for cephalopods around the coastal islands of Bangladesh (Kamal et al., 2022). Out of seven types of squids found in the various coastal areas of Bangladesh, two species, *Photololigo duvaucelii* and *Uroteuthis duvaucelii*, are considered edible and most widely distributed (Quddus & Safi, 1983). *U. duvaucelii*, frequently found in the coastal region of the Indian subcontinent, is found to be particularly rich in protein, essential amino acids (leucine and tryptophan) and minerals like sodium, calcium, magnesium and potassium (Loppies et al., 2023; Remyakumari et al., 2018). Significant content of omega-3 polyunsaturated fatty acids, including EPA and DHA, found in squids is considered essential for growth and maintenance of the body (Ozyurt et al., 2006). Therefore, considering the nutritional potential and prospects of developing novel food products, this species is chosen for the current research.

There is great global demand for seafood-based products, especially value-added battered and breaded seafood products, including those made from squids and cuttlefish. The ever-increasing speedy lifestyle serves as one of the most prominent driving forces for gaining popularity of these ready-to-heat 'convenience foods'. In Bangladesh, consumption of squid or squid products has not been popularised yet, with few exceptions among foreigners and high-end hotels. But there is wide scope to utilise the food value of squid by developing value-added products. Crumbed foods, including those coated with breading and battering, are highly favoured these days, and their use has grown over the last couple of decades (Bandre et al., 2018; Varela et al., 2011). Batters have become more sophisticated complex systems in which the nature of the ingredients is very wide-ranging and their interaction affects the finished product (Fizman, 2003). The processes of battering and breading provide special functions in food products, including improving the appearance of the products, increasing the texture, reducing the oil uptake during the frying process and increasing the shelf life of the coated products (Varela et al., 2011). Foods that have been battered or breaded are often deep-fried or shallow-fried. As a result, these items have a distinct flavour and texture (Venugopal, 2006). It can also significantly enhance the sensory quality of meat products and could be an effective method of value addition with better consumer acceptability (Ahamed et al., 2007). Crispiness is another aspect that has been reported as the most critical property determining their consumer acceptance (Primo-Martin and van Deventer, 2011).

Although squid products have a promising export market, due to lack of expertise and proper policy, squids found in Bangladesh's marine environment cannot be utilized properly. The goal of the study was to efficiently utilize the marine resources by formulating a value-added breaded product from Indian sword tip squid (*Uroteuthis duvaucelii*). This research was focused on these issues and emphasized developing improved processing techniques. Therefore, the current research was aimed to meet the protein demand of the masses, to increase export potential, and to diversify the seafood industry through formulating a value-added crumbed product from sword tip squid (*Uroteuthis duvaucelii*).

## **II. Material And Methods**

### **Experimental Site and Period**

The study was conducted at the Fishing and Post-Harvest Technology laboratory, Sher-e-Bangla Agricultural University, Dhaka 1207, during the period of July 2024 to June 2025.

### **Collection of Raw Material**

For the present experiment, a properly chilled swordtip squid (*Uroteuthis duvaucelii*) specimen was collected from the Dhaka North City Corporation (DNCC) market of Dhaka city (Figure 1). Approximately 3.0 kg of samples were collected. Collected squids were transported to the Fishing and Post-harvest Technology laboratory, Sher-e-Bangla Agricultural University, Dhaka, in chilled condition. Specimens were weighed on a random basis, and the average individual weight recorded was  $68.46 \pm 1.22$  grams. A certain number of specimens were immediately used for analysis. The rest of the samples were systematically packaged in an LDPE pouch and stored at  $-18^{\circ}\text{C}$  in a refrigerator for further use.

### **Analysis of Proximate Composition of Squid**

After collection, raw squid samples were immediately sent to the laboratory of the Animal Nutrition and Feed Section of the Department of Livestock Services, Farmgate, Dhaka, for analysis. Proximate compositions of squid were determined by the conventional method of AOAC (Association of Official Analytical Chemists) on a weight basis (AOAC, 2005).

**a) Estimation of Moisture Content**

The method described in AOAC (2005) was followed for moisture determination. At first, the initial weight of the samples was taken. Then samples were dried in an oven at about 105°C for about 24 hours and cooled in a desiccator, weighed again and recorded as final weight. The percentage of moisture content was then calculated by using following equation:

$$\text{Percentage (\%)} \text{ of moisture} = (\text{Final weight of sample} - \text{Initial weight} / \text{Initial weight of sample}) \times 100$$



**Figure 1:** Collection of fresh squid (*U. duvaucelii*) specimen for experimental purpose

**b) Estimation of Protein Content**

The protein content of the squid was determined by the micro-Kjeldahl method (AOAC, 2005). It involves conversion of organic nitrogen to ammonium sulphate by digestion (sample 0.5 g) with concentrated sulphuric acid in a micro-Kjeldahl flask for 45 minutes. The digest was diluted, made alkaline with sodium hydroxide and distilled. The liberated ammonia was collected in a boric acid solution and was titrated with 0.1 N H<sub>2</sub>SO<sub>4</sub>. The percentage of protein in the sample was calculated by the following equation:

$$\text{Percentage (\%)} \text{ of protein} = (c - b) \times 14 \times d \times 6.25 / a \times 1000 \times 100$$

Where, a = Sample weight (g); b = Volume of NaOH required for back titration and neutralize 25ml of 0.1N H<sub>2</sub>SO<sub>4</sub> (for sample); c = Volume of NaOH required for back titration and neutralization 25ml of 0.1N H<sub>2</sub>SO<sub>4</sub> (for blank); d = Normality of NaOH used for titration; 6.25 = Conversion factor of N to protein; 14 = Atomic weight of N.

**c) Estimation of Fat Content**

The crude fat content was estimated by the AOAC (2005) method with slight modifications. Continuously extracting fat using petroleum ether as a non-polar solvent. About 2 g (W<sub>1</sub>) of the sample was taken into a thimble, and the mouth of the thimble was closed with a cotton plug. Extraction was carried out for 8 hours using petroleum ether in a Soxhlet apparatus. After cooling the apparatus, the solvent containing extracted fat was filtered into a previously weighed conical flask (W<sub>2</sub>). Little amounts of ether were used to wash the apparatus's flask, and the washings were then added to the flask. The solvent was removed by evaporation, and the fat was dried at 80 to 100 °C, cooled in a desiccator, and weighed (W<sub>3</sub>).

$$\text{Fat content (\%)} \text{ of fat} = (W_3 - W_1 / W_2 - W_1) \times 100$$

**d) Estimation of Total Ash Content**

The ash content was determined by heating sample for 6 h in a furnace at 600 °C (AOAC, 2005). Results were expressed as percentage of wet weight.

$$\text{Ash content (\%)} \text{ of fat} = (W_3 - W_1 / W_2 - W_1) \times 100$$

**e) Estimation of Carbohydrate Content**

Carbohydrate content was determined by calculating the difference between 100% and the sum of values of moisture, protein, fibre, lipid, and ash.

**f) Estimation of Specific Mineral Content**

An atomic absorption spectrophotometer (Varian AA 240 FS) was used to assess the minerals after the ash (obtained in ash determination) was dissolved in diluted HCl (6 N). For flame and hollow cathode lamps, a fixed ratio of acetylene to air is given. The light wavelengths (nm) used to analyse various minerals are as follows: 178.8 for phosphorus, 213.9 for zinc, 422.7 for calcium, 248.3 for iron, and 589.0 for sodium.

### **Preparation of Breaded Product from Squid**

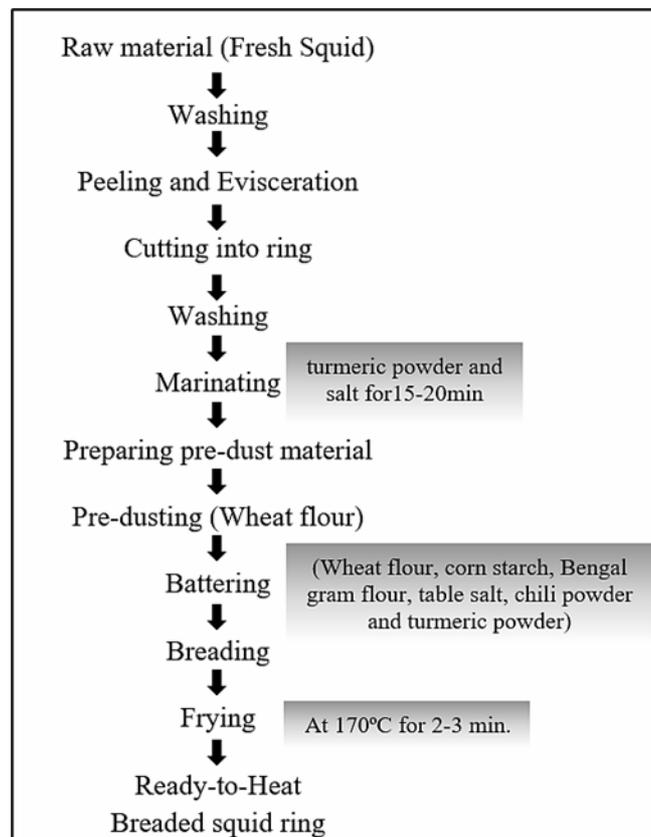
The entire steps of breaded ring preparation from fresh swordtip squid are presented in Figure 2.

#### **a) Thawing of Raw Materials**

Thawing is a critical step that greatly influences the quality of the final product. Frozen squid was thawed gradually to minimise moisture loss, prevent protein denaturation, and avoid microbial growth. To speed up the process, the frozen squid was placed in running room-temperature water, allowing it to thaw rapidly over 30-60 minutes. This method helps maintain the texture and structural integrity of the squid.

#### **b) Washing and Evisceration**

After thawing, the sample was washed properly with potable water several times to remove slime and dirt. Then, the tentacles were removed. The gut content and gladius of each specimen were removed. The fin from the body and skin from the mantle were peeled off carefully. Care should be taken during the removal of the ink sac to avoid ink contamination in the edible parts.



**Figure 2:** Flowchart showing chronological steps of squid ring preparation

#### **c) Squid Ring Preparation**

After evisceration and removing tentacles, the mantle was washed again and placed on a cutting board. Then, pressing the tubular mantle against the board, it was cut by a sharp knife into uniform ring-shaped pieces with the width of about 2 cm. Uniformity in size ensures even cooking (Figure 3). After cutting into rings, these were washed with potable water, and excess water was soaked up using paper towels. Removing excess moisture is crucial because it allows the coating to adhere properly and prevents dangerous oil splatter during frying. Then the sample was marinated in turmeric powder, pepper and salt and kept for 15-20 minutes in a large clean bowl.

#### **d) Batter Preparation**

All the ingredients and spices used for batter preparation are listed below in Table 1. For spices, commercially available spice powder was used. For onion paste, fresh onion was ground in a blender. All the ingredients were then hand-mixed thoroughly, and gradually potable water was added until a smooth, slightly runny batter was formed. For getting a batter with optimum viscosity, water was used at a ratio of 1:2 (dry

batter: water). The batter was then chilled to reduce oil absorption during frying, which ensured a lighter, healthy fried product.



**Figure 3:** Preparation of squid ring from the eviscerated and cleaned mantle

**e) Coating Procedure**

Before dipping into the batter, the squid rings were lightly floured. Vegetable oil was used for frying. For the coating purpose, squid rings were soaked uniformly in prepared batter and coated with commercially available breadcrumbs (Figures 4a-c). Coated unfried products were separately arranged in a tray prior to frying or deep freezing for further analysis.

**Table 1** Percentage-wise ingredients used for batter preparation

Ingredients	Amount (%)
Wheat flour	20.0
Corn Starch	12.0
Water	60.0
Sugar	0.5
Table salt	2.0
Onion	1.5
Black pepper	0.5
Monosodium glutamate	0.5
Coriander	1.0
Cumin	0.5
Ginger	1.0
Chili powder	0.5

**f) Frying of Squid Rings**

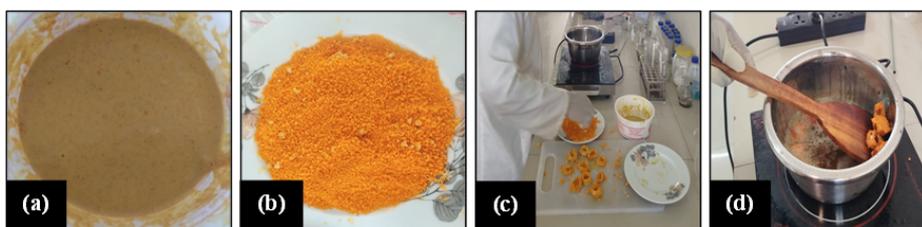
The frying of battered and breaded squid rings is a critical step that determines the final texture, colour, flavour, and overall quality of the dish. In the present study, primarily the battered and breaded squid rings were grouped into two; one-fourth (about 10/12) of the total rings were deep-fried at 170°C for 3 minutes. Fried rings were taken out of the frying pan until the characteristic golden-brown colour was achieved (Figure 4d). Fried rings from fresh squids were used for initial biochemical and sensory analysis. Remaining coated products were only oil blanched for a minute at the same temperature. This process made the coating and breadcrumbs attach firmly to the ring and also helps to reduce microbial load. After blanching, they were kept on food-grade paper towel to soak up excessive oil from the surface of the squid ring. These samples were then kept in a refrigerator at -18°C and used for further studies.

**g) Packaging & Storage**

The coated product may turn rancid, dehydrate, and discolour during freezing storage. By using the right packaging materials, these issues may be avoided, and the shelf life can be increased. Zipper bags were used to package the ready-to-cook breaded product. The packaged products were kept at -18°C in cold storage. Biochemical Analyses

**Total Volatile Base Nitrogen (TVB-N)**

TVB-N content was determined by the procedure given by AOAC (2005). Using Conway microdiffusion units, results were expressed in terms of mg nitrogen /100 g.



**Figure 4:** Different steps of coating squid rings: **(a)** preparation of batter; **(b)** preparation of bread crumb; **(c)** coating process of squid ring; **(d)** Deep-frying process

#### **Free Fatty Acids (FFAs)**

Free fatty acids (FFA) were determined according to the acid base titration method and calculated as mg NaOH/g fat (AOAC, 2005). Acid-base titration measurements with 5 grams of cooking oil sample were weighed at each stage and incorporated into a 250 mL Erlenmeyer which had been known to be empty weight. Added 50 mL of 96% ethanol and heated at 400°C. After the oil was already cold, 2 mL of phenolphthalein indicator was added. It is titrated with a standard solution of 0.1 M NaOH to form a pink solution and is not lost for 30 seconds. The volume of NaOH used is noted.

#### **Peroxide Value (PV)**

Peroxide value (PV) was determined by a titrimetric method (AOAC, 2005). A 5 g sample was taken into a dry 250 mL glass-stoppered Erlenmeyer flask, and 30 mL of acetic acid–chloroform solution (3:2 v/v) was added. Then, swirl to dissolve the sample and add 0.5 mL of saturated KI solution and swirl for 1 minute. 30 mL of distilled water was taken and mixed thoroughly. Then, titrated with 0.01 N sodium thiosulphate solution with constant swirling until the yellow colour almost disappears and added 0.5 mL of 1% starch solution. The solution will turn blue. Titration was carried out until the blue colour just disappears. Finally, PV was calculated by using this formula.

$$PV \text{ (meq/kg)} = \frac{(S - B) \times N \times 1000}{W}$$

Where, S = volume of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> for sample (mL); B = volume of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> for blank (mL); N = normality of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>; W = weight of sample (g).

#### **Non-Protein Nitrogenous Compounds (NPN)**

NPN content was determined by the TCA method (Velankar and Govindan, 1958) with necessary modification. About 5 g of sample was ground with 10 ml of 15% TCA for 5 min using a mortar and pestle. This facilitates separating NPN (filtrate) from true protein. NPN was determined by the micro-Kjeldahl distillation method, where 10 mL of conc. H<sub>2</sub>SO<sub>4</sub> was added to the filtrate for digestion purposes. The mixture was digested until the contents were clear. Distillate was collected in boric acid solution and titrated with .01 HCL. Results were expressed as mg NPN/100 g sample.

#### **Microbiological Study**

To determine the initial microbiological quality of raw materials and changes in microbial load of prepared products under storage conditions. Total plate count (TPC) was determined according to the method as described in Zhang et al. (2025) with necessary modifications. 10 grams of the sample were aseptically transferred into a sterile flask containing 90 mL of sterile peptone water (0.1%) and homogenised in a blender at 1400 rpm for 1-2 minutes to provide a homogenate of 1/10 dilution. One mL of the homogenate was transferred with a sterile pipette to another sterile test tube containing 9 mL of sterile buffered peptone water (0.1%) and mixed well to make the next dilution, from which further decimal serial dilutions were prepared. Then, 0.1 mL samples of serial dilutions (1:10, diluent, and 0.1% peptone water) of the homogenates were spread on the surface of solidified plate count agar media. 2.35 gm of agar with 100 ml of water was used to prepare the agar plate. The colonies were counted for total plate count, having plates with 30-300 colonies after incubation at 37°C for 48 hours. All counts were converted to logarithms of the colony-forming units per gram (log CFU/g). The microbial load of the sample was determined after bringing them into the laboratory, whereas periodic determination of TPC of the stored breaded product was carried out at 0, 30, 60 and 90 days of storage.

#### **Sensory Analysis**

Ten experienced members (aged between 25 and 45 years) were selected from the teachers, students and staff of the Faculty of Fisheries and Marine Science of Sher-e-Bangla Agricultural University, Dhaka. The sample was presented to the panellists randomly. For the '0 day' sensory evaluation, freshly prepared, fried, coated squid rings were presented to the panel members. Remaining sensory evaluations were carried out following the same procedure on the 30th, 60th and 90th days, when oil-blanching frozen squid rings were taken out from the refrigerator, thawed, fried and presented to the testing panel members. They were then allowed to score against each of the sensory parameters, viz., appearance, colour, odour, taste, texture and overall acceptability (Roy et al., 2014). Scoring was done based on a 9-point Hedonic scale where 9 = Like Extremely, 8 = Like Very Much, 7 = Like Moderately, 6 = Like Slightly, 5 = Neither Like nor Dislike, 4 = Dislike Slightly, 3 = Dislike Moderately, 2 = Dislike Very Much, and 1 = Dislike Extremely (Islam et al., 2022). For each sensory parameter, the mean value was used for calculation.

#### **Statistical analysis**

Data analysis was done by MS Excel 2019 and the SPSS 25.0 statistical software package. The expressed results represent the average of three measurements, and the data were displayed as mean ± standard deviation.

### III. Result

In the present study, ready-to-heat (RTH) product of squid ring from squid have been developed based on optimized recipe. Up on frying the final product turned golden brown in appearance (Figure 5). Further, the quality of the products during estimating the proximate, biochemical, microbiological and organoleptic parameters.



Figure 5: Fried ring prepared from squid *U. duvaucelii*

#### Proximate Composition of Fresh Squid (*U. duvaucelii*)

Moisture, protein, crude fat and ash content of squid were presented in Figure 6a. The proximate composition of *U. duvaucelii* showed a content of 88.08±1.73% for moisture, 9.75±1.41% for protein, 0.56±0.04% for fat, 1.08±0.22% for ash and 0.94±0.14% for carbohydrate. Relatively high moisture content was observed in *U. duvaucelii* as compared to its total fat content and also revealed better content of protein and ash. High moisture content indicates freshness but also implies shorter shelf life and higher perishability. Protein was the second most abundant component found in squid after water.

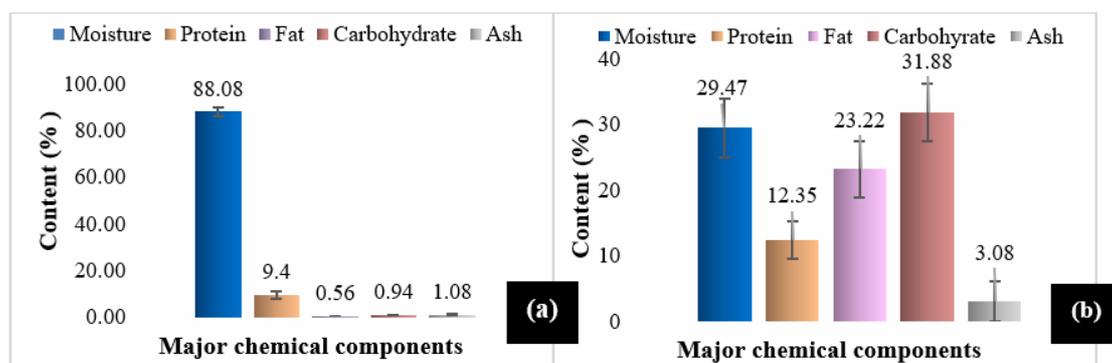


Figure 6: Proximate composition of (a) raw squid (*U. duvaucelii*) and (b) fried breaded squid ring (on % DM basis)

#### Mineral Composition of Raw Squid (*U. duvaucelii*)

Minerals are an essential part of the diet of any living organism, as they are required by the body to carry out various important metabolic functions (Ahmed et al., 2024). Indian squid contains different vital minerals, including sodium, calcium, phosphorus, iron and zinc. The selected mineral content in Indian squid was analyzed, and results are presented in Table 2. Among the analyzed minerals, iron, zinc, and sodium are mainly derived from its muscle tissues and haemocyanin-rich blood. Calcium and phosphorus in Indian squid mainly originate from mantle muscle, connective tissues, intracellular fluids, and minor contributions from residual cartilage structures. Iron is linked to oxygen-binding pigments, zinc to enzyme-rich mantle tissues, and sodium to body fluids and intracellular electrolytes regulating osmotic balance and nerve–muscle function (Soetan et al., 2010; Ha and Bhagavan, 2011).

Table 2: Calcium and phosphorous content of whole raw squid sample

Mineral	Content (mg/100g)
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Sodium (Na)	41±1.01
Calcium (Ca)	34±2.74
Phosphorus (P)	20±1.98
Iron (Fe)	3.76±0.03
Zinc (Zn)	1.19±0.08

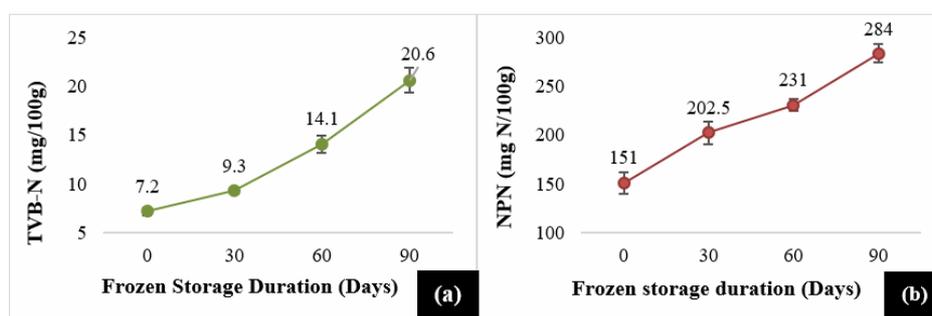
### Proximate Composition of Breaded Squid Ring

The proximate composition of breaded squid rings showed a content of 29.47±4.52% for moisture, 12.35±2.85% for protein, 23.22±4.28% for fat, 31.88±4.35% for carbohydrate and 3.08±1.1% for total ash. (Figure 6b). Evaporation during short frying removes substantial water from both the squid and its coating, causing muscle fibers to compact as water exits (Llorca et al., 2001). The frying and cooking procedures are responsible for the fish product decreased moisture content (Rahman et al., 2019).

### Biochemical Evaluation

#### Total Volatile Base Nitrogen (TVB-N) Value

Figure 7a shows that at zero days of storage, the TVB-N value was 7.2±0.42 mg/100g. Over the course of the freezing-temperature storage period, it increased to 9.3±0.28, 14.1±0.85, and 20.6±1.27 mg/100g after 30-, 60-, and 90-days storage respectively. During frozen storage, TVB-N content in squid rings increased slowly with the increase of storage period. A level of 30 mg muscle TVB-N/100 g was considered by Gökodlu et al. (1998) as the upper limit above which some fishing products are considered spoiled and unfit for human consumption. Giménez et al. (2002) also proposed a level of more than 25 mg N/100 g as an unacceptable value in fish and fishery products.



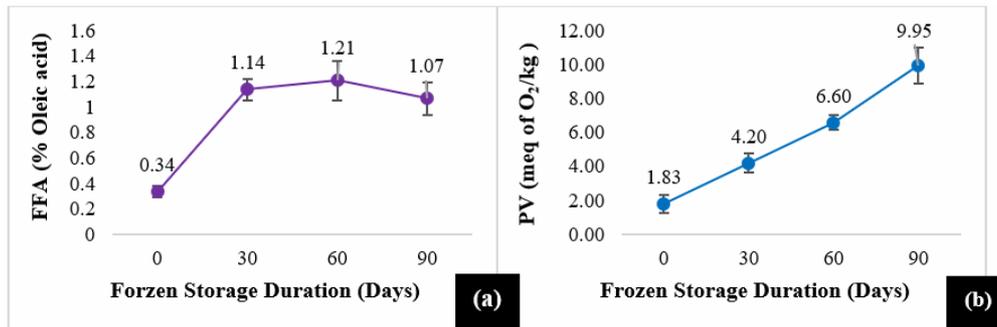
**Figure 7:** (a) Changes in total volatile base nitrogen (TVB-N) value and (b) NPN value of prepared breaded squid ring over 90 days of frozen storage

#### Non-Protein Nitrogenous Compounds (NPN) Value

In this study, the initial NPN value at day zero of frozen storage was 151±11.31 mg N/100 g. Over the course of the freezing-temperature storage period, it increased to 202.5±12.02, 231±5.65, and 284±9.89 mg N/100 g after 30, 60, and 90 days of storage, respectively (Figure 7b). The gradual increase in NPN contents was observed in coated squid rings during refrigerated storage. Non-protein nitrogenous (NPN) compounds are a diverse group of nitrogen-containing compounds that play a crucial role in the chemistry and nutritional composition of seafood. High NPN values (>300 mg N/100g) often indicate microbial spoilage or excessive enzymatic activity (Dhruve et al., 2023).

#### Free Fatty Acids (FFAs) Value

At zero days of storage, the FFA value was 0.34±0.04%. Over the course of the freezing-temperature storage period, it increased to 1.14±0.08% and 1.21±0.16% of KOH after 30 and 60 days of storage, respectively, and at 90 days of storage, the value decreased to 1.07±0.13% (Figure 8a).



**Figure 8:** (a) Changes in Free fatty acid value (left) and (b) Peroxide value of prepared breaded squid ring over 90 days of frozen storage

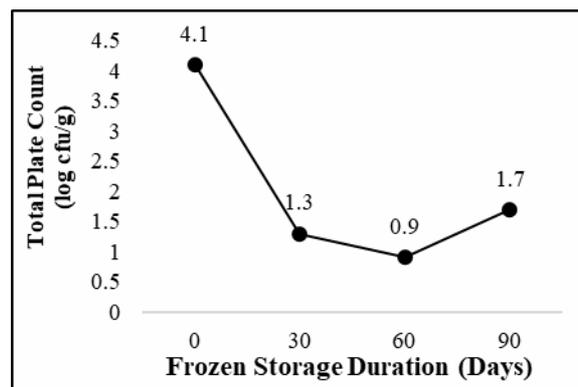
According to the above data, the FFA value of the prepared breaded product ( $1.07 \pm 0.13\%$ ) merely crossed the optimum value (equal to or less than 1%), which has not any impact on the quality of the prepared product to consume up to 90 days after freezing. The current results were also close to those for deep-fried nuggets studied by Oppong et al. (2021), where the FFA content ranged from 1.02% to 1.46%.

### Peroxide Value (PV)

In this study at day zero of storage, the initial PV of the developed breaded and battered coated squid rings was  $1.83 \pm 0.53$  meq of O<sub>2</sub>/kg. Peroxide values increased from  $4.2 \pm 0.56$ ,  $6.6 \pm 0.42$ , and  $9.95 \pm 1.06$  meq of O<sub>2</sub>/kg oil for 30-, 60-, and 90-day frozen storage, respectively, which were well within the maximum limits (Figure 8b). Peroxide value (PV) is a measure of the degree of oxidation in the fat. It is an important index quality parameter that establishes the shelf life of food items.

### Microbial Evaluation

Estimation of microbial load is an integral part of the quality determination of preserved food products and provides an important indication of the product's shelf life under certain storage conditions. TPCs of prepared end products were recorded as 4.1, 1.3, 0.9 and 1.7 log CFU/g at 0, 30, 60 and 90 days of frozen storage, respectively (Figure 12).



**Figure 9:** Total plate count (TPC) of prepared breaded squid rings up to 90 days of frozen storage

### Sensory Evaluation

The shelf life of the prepared coated squid items was investigated using sensory assessment. Appearance, colour, taste, texture, odour, and overall acceptability were evaluated during the storage period. The sensory characteristics of coated squid rings that were kept under frozen storage ( $-18^{\circ}\text{C}$ ) showed notable variations (Figure 10). At the beginning of the study the scores of 8.1, 8.0, 7.9, 8.1, 8.4 and 8.5 were recorded against appearance, colour, odour, taste, texture and overall acceptability, respectively. At the end of the 90-day period, these scores were reduced to 7.2, 7.0, 6.1, 6.4, 7.1 and 6.3, respectively, for the same set of parameters.

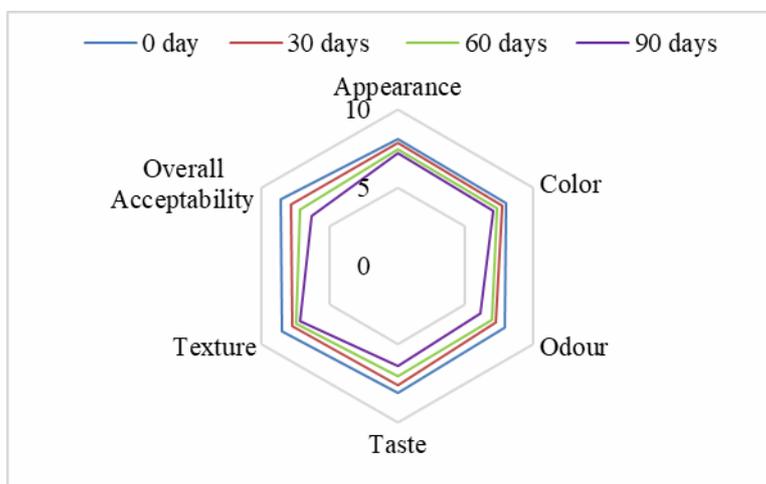


Figure 10: Sensorial quality changes of breaded squid ring during frozen storage

#### IV. Discussion

##### Value added product development

In the present piece of research work, the initial focus was given to developing a value-added product from squid with seasoned batter and breadcrumbs. To develop a product with enhanced flavor, texture, and superior market appeal, several trials have been conducted to optimize the recipe. Different ingredients and cooking parameters play a pivotal role in achieving the desired quality of the product. In the coated product the breading layer improves crispiness and protects the fish muscle from excessive moisture loss during frying. Cooking parameters—especially time and temperature—play a critical role in determining sensory attributes such as color, aroma, flavor, texture, and overall acceptability (Kilinc et al., 2023). For frying the coated squid ring, a 170°C temperature was standard due to its ability to offer superior product yield, better coating adhesion, and higher moisture retention compared to lower (150°C) or higher (190°C) temperatures (Zhang et al., 2020). Deep frying at 170°C for 3–5 minutes was reported ideal for coated fish products (Oke et al., 2018). Deep frying also ensures crispness with lower fat uptake through uniform heating. During this process rapid heat transfer promotes starch gelatinization and protein denaturation, producing a golden-brown crust through Maillard reactions (Oppong et al., 2021). Insufficient temperature or short cooking time results in a pale, soggy coating and undercooked interior, while excessive heat or prolonged frying causes excessive moisture loss, dark coloration, oil absorption, and a dry, tough texture (Llorca et al., 2001). Therefore, a battered and breaded product with desirable crispness, a juicy interior texture, and better consumer acceptance from squid has been developed through optimizing the recipe and time–temperature combinations.

##### Nutritional aspects of raw squid and

The proximate composition of raw squid varies owing to various factors, including location of habitat, season, abundance of food, post-harvest care, etc. In addition, these variations affect sensory quality as well as the quality of raw squid or processed product to a great extent. Moisture content is one aspect that affects immensely shelf-life and muscle texture. Indian squid (*Uroteuthis duvauceli*) generally exhibits high moisture content typical of cephalopod muscle tissues. One study reported about 80.47% moisture in *U. duvauceli*, along with high protein and low fat and ash contents, indicating its lean, water-rich nature (Remyakumari et al., 2018). Another proximate analysis showed moisture around 79.36%, similar to other research findings for fresh Indian squid (Ahmed et al., 2024). Comparative work on cephalopods in the Bay of Bengal (including *U. duvauceli*) also underscores elevated moisture relative to protein and lipid fractions. Generally, squid moisture content is higher than fat, consistent with cephalopod physiology, where water is the principal component (Bhandari et al., 2025). These moisture levels are crucial for processing, storage, and quality assessment of squid products. The tender texture and palatability of squid meat can be attributed to the high-water content (Chavan et al., 2020).

*U. duvauceli* has also been recognised as a good source of high-quality protein typical of cephalopods. Studies report protein content generally ranging from about 15–18% in fresh squid muscle (Remyakumari et al., 2018). According to Thanonkaew et al. (2006), differences in squid protein levels could be caused by differences in habitat, age, food, metabolic rate and rate of movement. In a previous biochemical analysis of swordtip squid, 15.04% protein was recorded, where the mantle portion was found to contain even higher protein content of about 22% (Raman and Mathew, 2014). In the present study a lower protein content of about 9% could be due to the drip of highly water-soluble myofibrillar protein during post-harvest ice storage. Low fat

content and high protein concentrations in cephalopods make them appropriate for human consumption. This protein is nutritionally valuable because it contains essential amino acids and highly soluble myofibrillar proteins, making Indian squid an important lean seafood for human nutrition, especially for the elderly population (Remyakumari et al., 2018; Roper et al., 1984).

With lipid content typically ranging from 0.8% to 2.0%, Indian squid is considered a low-fat seafood. A lipid value recorded in the present study was in agreement with results of Thanonkaew et al. (2006), who reported protein content of 0.52 and 0.47% for the head and body portions, respectively. Fat content is also influenced by species, body size, sex, environmental temperature, water depth, salinity, spawning season, and food (Mukholik, 1995; Nair et al., 1985). The mantle generally contains fewer lipids than the viscera (Okuzumi and Fujii, 2000). Squid lipids are nutritionally important because they include phospholipids and omega-3 fatty acids, particularly EPA and DHA, which contribute to cardiovascular health (Murthy et al., 2012). The low total fat but high proportion of essential fatty acids makes Indian squid a lean and healthy protein source, widely recommended in balanced seafood diets.

Being active marine predators, cephalopods deposit a decent amount of minerals in their bodies, incorporated through their diet and direct absorption from saline water through their body surface and gills (Lall, 2002). This study suggests that *Uroteuthis duvaucelii* contains a moderate amount of essential ash. According to FAO data for *Loligo vulgaris* (a common squid species), the total ash content is about 1.3% of wet weight, which is close to the ash content of this study (*Uroteuthis duvaucelii*), 1.08% of wet weight. The ash content contributes to the nutritional quality and can influence the shelf life and processing behaviour of the squid. Islam et al. (2020) found that *U. duvaucelii* from Bangladeshi waters had a lower ash content of 0.38%, whereas Mediterranean species often ranged between 0.45% and 0.73% (Ozogul et al., 2008). These differences may reflect environmental variations, seasonal factors, nourishment sources, species-specific physiology, or methodological differences in analysis (Fallah et al., 2009).

These minerals, when utilized through value-added products prepared from squid, could contribute substantially to meeting human mineral requirements. Although required in minute amounts, deficiency of these minerals disrupts the basic function of the body, expresses deficiency syndromes, hinders growth and activity, and even poses the risk of life-threatening issues in an individual. Minerals have undoubted roles in coordinating healthy metabolic systems by ensuring a strong immune system and keeping the vital organs active and well nourished. Calcium is an essential micronutrient for healthy bones and teeth, as well as for nerve transmission from the brain to every part of the body and muscular contraction. (Lall, 2002). According to the National Institute of Health (NIH), depending on the age, the dietary need for calcium ranges from 1000 to 1300 mg/day in mature human beings. On the other hand, phosphorus is another essential nutrient element that plays a role as the structural component of bones, teeth, DNA, RNA, cell membranes, and ATP. In addition, phosphorus plays key roles in the regulation of gene transcription, activation of enzymes, maintenance of normal pH in extracellular fluid, and intracellular energy storage (Lall, 2002). Along with other vital minerals, iron supports oxygen transport and zinc enhances immunity and growth, while sodium helps maintain fluid balance, nerve function, and proper muscle contraction.

The proximate analysis of this study has shown a promising value for most of the nutritional parameters that are supported by the research carried out by Zlatanov et al. (2006). Overall, it can be coined that the low fat and high protein content of the Indian squid plays a beneficial role in the formulation of protein-rich and mineral-value-added products for targeted populations.

### **Nutritional Quality of the developed value-added product**

Substantial changes in some of the nutritional parameters of breaded products have been found when compared to raw squid. The most obvious modifications were the addition of breading and the absorption of oil during frying, which result in an increase in energy and fat content. Breading and cooking drastically change the nutritional value of squid rings. Among the major chemical constituents, the most conspicuous changes took place in moisture content, where the fried value-added product lost almost two-thirds of its original moisture. Due to the high-temperature frying process, moisture evaporates from the food surface, and the food becomes more concentrated in fat, protein, and carbohydrate due to water loss (Pinthus et al., 1995). Lower moisture content in the developed product than the raw material was also reported in earlier studies (Ihm et al., 1992; Tagkaya et al., 2003; Ejaz et al., 2013). Vanitha et al. (2013) also observed decreased moisture content of fish fillets produced from Mrigal (*Cirrhinus mrigala*) and fish burgers produced from Catla (*Catla catla*) during frozen storage. Repetitive frying was also found to affect the physicochemical composition, sensory attributes, and textural properties of fried fish and fish products, especially moisture content, protein content, color, and fat content (Manral et al., 2008; Tadesse et al., 2020).

In the present study, another noticeable change was observed in fat content, where the crude fat content of fried squid rings rose to around 23.22% from a mere 0.56%. Vegetable oil used in the frying process could be a major source of these elevated fat levels, which was also reported by Aktaruzzaman et al. (2022), who

conducted research on fried coated fish products. Bognár (1998) stated that frying can raise the fat content by 10–20%, depending on cooking time, temperature, and the type of oil used. A similar factor behind the increased lipid content in the fried product in vegetable oil was reported by Sehgal et al. (2008) and Tokur et al. (2006).

Protein is considered the most crucial among major nutrients during the formation of any value-added product. In the present study, protein content was found to increase slightly (12.35%) in the coated product in comparison to raw squid (9.75%). This increase was supposed to be due to the contribution from different coating ingredients like wheat flour and corn starch. A similar trend was also reported in fish balls after the cooking process as compared to the raw fish (Duman and Peksezer, 2016). The carbohydrate content of raw squid was minimal, while the carbohydrate content of ready-to-heat end products led to a substantially larger amount (31.57%) due to the addition of breadcrumbs, wheat flour, and cornstarch. A significant rise in the total ash content was recorded in cooked breaded squid rings (2.48%) in comparison to raw squid (1.08%).

The preparation process also led to a slightly higher ash content in the final product than the fresh squid sample. This can be attributed to the addition of the species and other ingredients, including NaCl (Praneetha et al., 2012). From the findings of the present study, it is evident that changes in the proximate compositions of breaded squid rings were in agreement with the findings of Vanitha et al. (2013) and Aktaruzzaman et al. (2022).

### **Evaluation shelf-life parameters**

TVB-N is a measure of decomposed protein and non-protein nitrogenous compounds (Huss, 1995). All samples increased with storage time, which was probably caused by bacterial and endogenous proteolytic enzymatic actions (Hernandez-Herrero et al., 1999). In the present study, the TVB-N value rose from  $7.2 \pm 0.42$  to  $20.6 \pm 1.27$  mg/100 g over 90 days of frozen storage. Chomnawang et al. (2007) stated that the increase of TVB-N value through breakdown of TMAO and some amino acids during frozen storage is related to bacterial spoilage, activity of endogenous enzymes, and degradation of tissue proteins. The current results were also similar to the report of Ninan et al. (2008), where the TVB-N value in tilapia fish cutlets varied between 12.4 and 20.2 mg/100 g over a 180-day period. Arslan et al. (1997) also studied carp flesh during an 11-month storage period and found 19.68–24.4 mg/100 g TVBN. TVB-N is widely used as an index to assess the keeping quality and shelf life of seafood products, and a value less than 25 mg/100 g is considered acceptable for a good-quality frozen fish product. (Bouletis et al., 2017). Therefore, the prepared breaded squid product in the present study remained well within the acceptable limit after 90 days of frozen storage and was deemed to be safe for consumption.

Non-protein nitrogenous compounds (NPNs) also contribute significantly to their overall nutritional value and sensory characteristics. The flavor of seafood depends on the species, the fat content, and the presence as well as the type of non-protein nitrogenous compounds, as reported by Venugopal & Shahidi (1996). The increase of NPN content during the storage can be a consequence of endogenous or microbial proteolytic enzymatic activity. (Lapa-Guimarães et al., 2005). A previous study by Ghoneim et al. (2022) revealed that NPN values increased as time of frozen storage increased, which were 237, 258, 306, and 345 mg N/100 g sample in tuna fish burgers and 182, 223, 291, and 328 mg N/100 g sample in carp fish burgers at zero, 2, 4, and 6 months of frozen storage, respectively, which were correlated with the findings of this research.

Free fatty acids (FFAs) are formed as a result of enzymatic hydrolysis (i.e., lipase and phospholipase) of triglycerides and phospholipids, and they serve as an important index for future oxidative spoilage (Rostamzad et al., 2011). Concentration of FFA in coated fish products is a crucial quality factor that affects lipid breakdown, shelf life, rancidity, and nutritional value. High contents of FFA are responsible for undesirable tastes and off-flavors of fish products (Rostamzad et al., 2011). In this study, the amount of FFA goes up for around 60 days, and then it shows a slight decreasing trend that continues till the end of the experimental period of 90 days. This rise in FFAs might be because of incomplete inhibition of lipases/phospholipases during frozen storage and the thawing of squid rings (Duman et al., 2018). These endogenous lipases/phospholipases acted on triglycerides (TAG) and phospholipids (PL) and caused an initial rise in FFA levels by the hydrolyzing process. Thermal degradation of lipids during frying and baking could also serve as a synergistic factor for an elevated FFA level (Aubourg et al., 2021). The decreasing trend of FFA after 60 days could be explained by the oxidative breakdown of highly exposed and reactive FFA compared to intact lipids (Atayeter and Ercoşkun, 2011). In this study, the FFA value of frozen stored breaded squid rings reached 1.07%, which can be considered fairly good for consumption. Earlier scientific studies report that the acceptable FFA limit for battered, breaded, and pre-fired fish products is around 3%, and those with lower value (<1% oleic acid) can be considered of quite good quality (Pawar et al., 2019). Therefore, the developed valued product in this research remained consumable up to 90 days of frozen storage when judged based on FFA value.

Peroxide value (PV) is a measure of primary products of lipid oxidations like fatty acids and hydroperoxides and one of the crucial indicators of oxidative spoilage (Ozogul et al., 2008). Due to lipid

oxidation, many substances are produced, some of which give unpleasant flavor and odor to meat (Fernandez et al., 2013). The cause of the elevated level of initial PV might be attributed to the oxidation caused by the reaction between the sample's oxygen and frying medium. Although the rate of thermal oxidation is found to be higher than that of autoxidation, frying oil's ability to dissolve oxidation products often results in lower peroxide values (Oke et al. 2018; Secci and Parisi, 2016). The quality of fish meat products is considered to be decreased by lipid peroxidation, which leads to reduction in nutritional quality and severe health problems. In this study, PV of fried breaded squid rings gradually increased over time and reached  $9.95 \pm 1.06$  meq. of O<sub>2</sub>/kg oil at the end of 90 days of frozen storage. Generally, up to 10 meq/kg of oil is considered the acceptable limit of PV for fish products (Jeon et al., 2002). Lajolina et al. (1983) even proposed a higher value of 30 meq. of O<sub>2</sub>/kg of fat for fisheries products up to which they may remain fit for consumption. According to Singh et al. (2023), battering and breading serve as oxygen barriers and prevent the production of peroxide in the product. The peroxide value of fish balls produced from *Labeo rohita* was measured by Singh et al. (2023) on the twenty-first day of storage and ranged from 1.52 to 3.72 meq/kg oil. A similar increase in the PV content was observed by Tokur et al. (2004) during the frozen storage of fish burgers produced from tilapia. Opong et al. (2021) revealed that the PV of cooked samples increased with storage time, reaching 4.30 meq/kg fat in oven-baked nuggets and 4.03 meq/kg fat in deep-fried nuggets after 90 days. Findings from Moosavi-Nasab et al. (2019) also demonstrated an increase in PV in fried fish nuggets, ranging from 1.54 to 4.34 meq/kg fat as the frozen storage period increased up to 90 days.

### **Microbiological quality and shelf life**

Total plate count (TPC) is a useful approach for assessing the microbiological quality of any food product, including raw fish and value-added fishery products (Duman and Özpolat, 2012). In the present study, a mixed pattern of microbial growth was observed for prepared breaded squid rings during frozen storage. A steep downward trend in microbial load was recorded for the first 30 days, where TPC dropped from 4.1 to 1.3 log CFU/g. In the next 30 days, this rate of decrease slowed down remarkably, but after 60 days, TPC increased slightly from 0.9 to 1.3 log CFU/g. This sort of pattern in changes of microbial load of food products under frozen storage is affected by various factors. While freezing is an effective way to slow microbial activity, it does not kill all microorganisms. Instead, it creates a stressful environment that injures, rather than kills, many bacteria, allowing for potential recovery later. (Yamamoto et al., 2001). The initial decrease and subsequent slight increase in microbial counts during frozen storage are primarily due to initial cold shock and injury followed by long-term adaptation and the activity of psychrotrophic microorganisms (Myhrvold et al., 2015). Mahmoudzadeh et al. (2010) reported similar findings for fish mince and burger, where the maximum decrease was found during the first month of the storage period. These findings are also in agreement with the findings of Ejaz et al. (2013). The maximum acceptable microbial load for fresh and fishery products is 7 log CFU/g, whereas products having less than 5.7 log CFU/g are considered good quality products by the International Commission of Microbiological Standards for Foods (ICMSF, 1986). Therefore, it can be stated that, according to the above data, prepared breaded squid rings were safe for consumption up to 90 days of frozen storage.

### **Sensory attributes**

The sensory quality of fried squid rings was evaluated using a 9-point hedonic scale (0–9), where higher scores indicate greater acceptability. At the beginning of the storage period, the product showed excellent sensory characteristics. Scores for appearance (8.1), color (8.0), odor (7.9), taste (8.1), texture (8.4), and overall acceptability (8.5) indicated that the squid rings were “liked very much” by panelists. Appearance and color received relatively high scores, reflecting the attractive golden-brown coating developed during frying, which is desirable for consumer appeal (Carvalho and Ruiz-Carrascal, 2018). Aroma scores suggested that the frying process produced a pleasant, characteristic seafood flavor without any off-odors, indicating good raw material quality and proper processing conditions. Texture was rated favorably, demonstrating a desirable combination of crispy outer coating and tender inner flesh, which is considered an important quality parameter for battered and breaded seafood products. Taste scores were also high, confirming that seasoning, coating, and frying conditions contributed to a balanced and palatable flavor profile (Baykal et al., 2023). However, with frozen storage, a gradual decline in sensory scores was observed, which is in agreement with the study of Ejaz et al. (2013). Appearance and color decreased to 7.2 and 7.0, respectively, possibly due to slight dehydration and minor pigment changes during storage. Odor and taste showed more noticeable reductions, reaching 6.1 and 6.4, which may be linked to slow oxidative changes and flavor deterioration over time. Despite these reductions, which are supported by the findings of Pawar et al. (2019), all sensory attributes remained above the acceptable limit on the hedonic scale. The overall acceptability score of 6.3 indicates that the product was still “liked slightly,” demonstrating good sensory stability during frozen storage for up to 90 days.

## V. Conclusion

Rising global population and socioeconomic changes have increased consumer demand for convenient ready-to-eat foods. Value-added seafood products such as fish sticks, fish balls, fish burgers, oyster balls, and calamari help meet this demand by providing nutrition, convenience, and cost savings. Battering and breading play an important role in enhancing the value, taste, texture, and acceptability of fish, shellfish, and mollusks, making products like fish fingers, cutlets, nuggets, and balls highly popular worldwide. This study demonstrates that low-cost and underutilized squid species can be effectively used to produce breaded squid rings, ensuring better resource utilization throughout the year. The developed product was found to be nutritious and a good source of energy, protein, and minerals. Quality evaluation showed that all parameters remained well within acceptable limits, indicating good shelf life under domestic freezer storage without significant deterioration. Owing to the availability of the raw material and necessary ingredients, commercial production of frozen breaded squid rings offers strong potential for small- and large-scale entrepreneurship, market expansion, and economic benefits. Overall, the study highlights the nutritional, economic, and industrial importance of value-added seafood products while providing useful information for future research in this field.

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## Competing Interests

Authors have declared that no competing interests exist.

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