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Research article

Biochemical properties and shelf life of value-added fish cube and powder developed from hilsa shad (*Tenualosa ilisha*)

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ABSTRACT

Hilsa shad (*Tenualosa ilisha*) is one of the most popular and tastiest fishes in Southeast Asia because of its unique soft texture, mouth-watering flavor, high nutritive value with high protein, and very high lipid, attractive body shape, and highly appealing shiny appearance. Recently increased productions of hilsa, in association with high demand but very high price, make it to be a good candidate for value-addition. The major challenge of hilsa value addition lies in its high lipid content, while lipids are the reasons for its unique tastiness. Under a national initiative, the present study therefore developed novel methods to prepare hilsa cube and hilsa powder by stabilizing lipids and proteins, which contained the original taste and flavor of hilsa, and had good storability in freezing and room temperature conditions for 6 months. The pre-spawning, moderate size (600–700g) female hilsa was used for product formulation. Proximate composition, biochemical qualities (TVB-N, TMA-N, pH, PV, TBA value, histamine content) of the hilsa cube and powder were analyzed following standard methods and sensory properties were analyzed using a 5-point hedonic scale. The biochemical parameters and sensory properties of the products varied based on ingredients used and the final product states. Both the products were in good quality for 6 months of storage, since cryoprotectants protected the protein quality both during freezing and high-temperature processing. Carotenoid extract from fresh carrots was found to be effective in reducing lipid oxidation in hilsa. Biochemical attributes in all products gradually decreased with storage time ($p < 0.05$). Almost similar sensory attributes were observed in both hilsa powder and hilsa cube, which were gradually decreased ($p > 0.05$) with the progress of the storage period. Both the products were within the acceptable quality limit during all storage periods and conditions. The results suggest that hilsa cube and powder have excellent quality standards, storage stability, and the possibility for fortifying ready-to-eat value-added hilsa products for the consumers at home and aboard.

1. Introduction

Hilsa shad (*Tenualosa ilisha* Hamilton, 1822), locally called '*Ilish*' (Fm: Clupeidae, Sub-Fm: Alosinae), belongs to a diverse assemblage of fishes that exploits a expansive choice of habitats in the Indian Ocean belt from the Indonesian coasts to the Persian Gulf and the Gulf of Aden (Rahman, 2006). Currently, more than 75% of the global hilsa production has been recorded from Bangladesh waters, while 15% comes from Myanmar, 5% from India, and 5% from the rest hilsa producing countries, viz., Thailand, Malaysia, Indonesia, Iran, Kuwait, etc (Miah, 2015). Though the hilsa fish is distributed throughout the Indian Ocean coasts, due to its exclusive production in Bangladesh waters, it has internationally been recognized as a unique product of Bangladesh and, according to World Intellectual Property Organization, has been registered as the geographical indication (GI) Tag (DC, 2017). Due to Bangladesh government initiatives for hilsa fishery conservation, hilsa production has been increased from 1.99 lakh MT in 2003-04 to 5.33 lakh MT in 2018-19, which is about a 3-fold increment (DoF, 2020). Presently, hilsa production contributes 1% to the GDP and 12% to the total fish production of Bangladesh (DoF, 2020). Recently increased production of

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hilsa also demands its suitable processing and value-addition, retaining high nutritional composition and original taste and flavor during extended storage time.

Hilsa is an anadromous fish that frequently migrates from seawater to estuarine and/or riverine waters and *vice-versa* for spawning and maturation purposes (Rahman et al., 2000; Hossain et al., 2018). Biochemically, hilsa is a dark-fleshed fish. It simultaneously contains a high level of protein and very high level of lipid (Nowsad et al., 2019). Generally, high lipid fishes are relatively less suitable for long term freezing. Because, high lipid fish contains a high percentage of dark muscles, which are frequently degraded and its polyunsaturated fatty acids (PUFAs) are oxidized to organic peroxides (Hultin, 1988; Mohanty et al., 2012). Icing is considered to be a widely practiced short-term but effective preservation method for fatty fish like hilsa. On the other hand, because of oxidation of lipids and development of rancidity, sun-drying of hilsa is not possible (Nowsad, 2007). Considering its nutritional values, characteristic texture and flavour development and rural community preference, salting is practiced as the most preferred preservation of hilsa (Hosen et al., 2018; Nowsad, 2014). However, now-a-days, hilsa salting has also been facing various problems related to processing conditions, raw material quality and market price (Nowsad, 2014). On the other hand, hilsa contains enormous number of very minute and sharp branched pin bones, which often restrict or do not allow different segments of consumers to eat this tasty fish, although they like hilsa very much (Debashish, 2016). Despite all these problems, hilsa is pondered to be a delicious fish in this region owing to noticeably lenient-oily texture, fabulous mouth-feel, mouth-watering delicate smell, moderate size, high nutritive value, attractive body shape and highly appealing shiny appearance. It was observed that female hilsa matures faster and grows larger than the male of the same age group (Pillay, 1958; Pillay and Rao, 1962; Rahman, 2006). Female hilsa acquires very high level of lipid including PUFAs during pre-spawning, as high as 18–22% and becomes tastier (Rao et al., 2012; Nath and Banerjee, 2012). Hilsa contains higher amount of essential amino acids, essential fatty acids - especially PUFAs, macro- and micro-minerals and vitamins (Mohanty et al., 2011). Many authors found hilsa to be highly beneficial to the consumers' health, because the PUFAs contain distinctly higher level of high-density lipoproteins (HDL) and lower level of low-density lipoproteins (LDL) (Mohanty et al., 2011; Moniruzzaman et al., 2014; Nowsad et al., 2019). This ratio of HDL and LDL in the lipid may lessen the menace of cancer, cardiovascular disease, diabetes, and other health hazards in consumer body (Rao et al., 2012). Hilsa is tastier during pre-spawning than post-spawning or maturing stages (Moniruzzaman et al., 2014). Hilsa taken from the sweet water, especially from the river and river-mouth to sea are favored to that taken from the marine sources (Rao et al., 2012; Wahab et al., 2019). The distinctive taste and flavor of hilsa has been ascribed to the transformation of saturated fatty acids into mono- and poly-unsaturated fatty acids, *viz*. oleic, lenoleic, lenolenic, arachidonic, eicosapentaenoeic and docosahexaenoeic acids (Mohanty et al., 2011; Nath and Banerjee, 2012; Nowsad et al., 2019; Rao et al., 2012). Considering huge consumer attractions, health benefits, nutritional and nutraceutical values and the recent production increment of hilsa, the fish would need to be value-added. Therefore, initiatives were taken by the Ministry of Fisheries and Livestock and the Department of Fisheries to formulate ready-to-cook or ready-to-eat value-added products like hilsa cube and powder, the present study being the part of that national initiatives (DoF, 2016). On the other hand, due to high lipid content, the challenge of such kind of new products may include stability of lipid during storage for keeping original taste and flavor, owing to the taste of hilsa derives mainly from its unsaturated fatty acid contents (Nowsad et al., 2019; Rao et al., 2012). Unsaturated fatty acids should not be eliminated but to be kept with the products and need to be stabilized to retain original taste and flavor. Therefore, the present study aimed to develop hilsa cube and hilsa powder, which were boneless and stable against oxidation of unsaturated fatty acids at long storage conditions, having an original taste, flavor and nutrients intact, as like as fresh hilsa. The study also aimed to assess the biochemical composition and shelf life of the products under various storage conditions.

2. Materials and methods

2.1. Preparation of hilsa cube

Premium quality fresh female hilsa (*Tenualosa ilisha* Hamilton, 1822) were purchased from the Daulat Khan Ghat of Bhola district, Bangladesh, which were already dead but freshly landed from the adjacent Meghna River. Collected hilsa were transported to the Fish Processing and Quality Control Laboratory of Bangladesh Agricultural University, Mymensingh in iced condition within 24 h of harvest. The sampling was done following the ethical guidelines of the Department of Fisheries

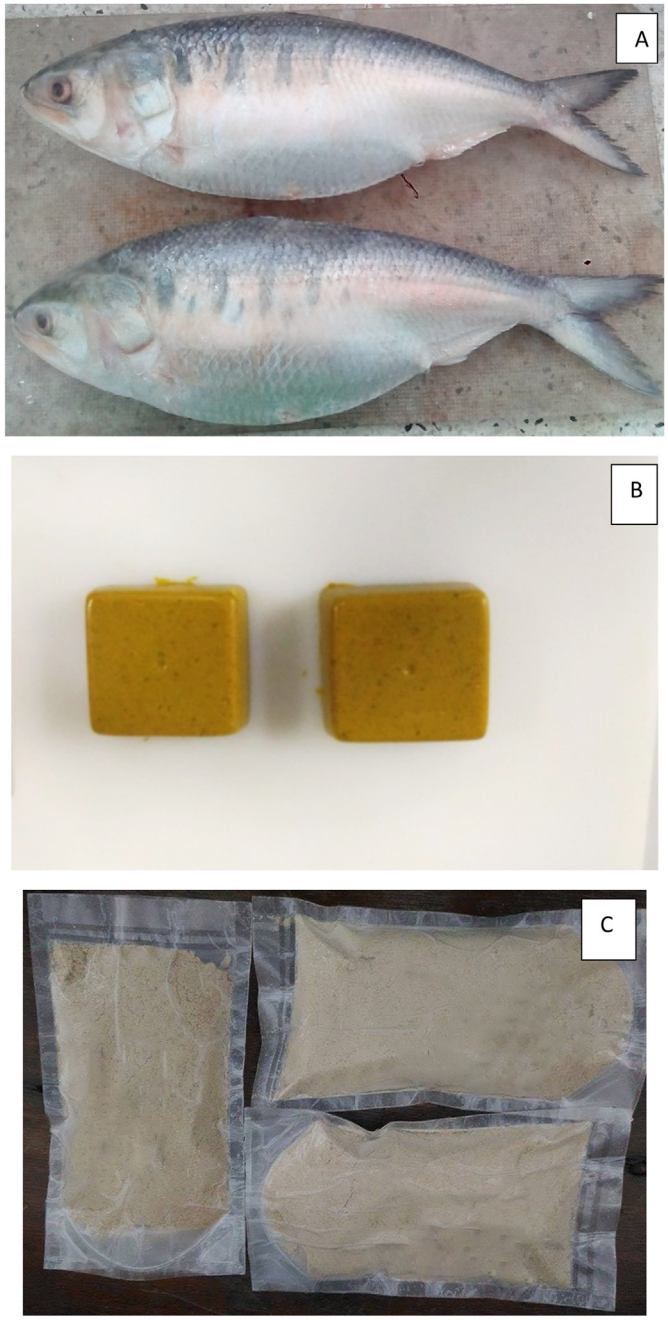

Figure 1. Fresh hilsa (A); hilsa cube (B); and hilsa powder (C).

Technology of Bangladesh Agricultural University. All fish had almost uniform medium-sized weight, ranging from 600-700g, with pre-spawning gonad (Mohanty et al., 2011). Raw hilsa (Figure 1A) were cleaned, gutted, washed, minced, and deboned by a mechanical meat grinder (Panasonic, Model: MK/MG-1300, Malaysia). Hilsa cube was prepared following the methods as described by Khanapur et al. (2017) with some modification due to high lipid content in raw materials hilsa. The method for the preparation of hilsa cube was standardized by using different proportions of cryoprotectants, ingredients, herbal antioxidants and spices for a different time and temperature schedules, and the final formulation thus obtained has been presented in Table 1. A series of herbal antioxidants from vegetable origin were searched and finally, carotenoid extracted from carrot was found to be most effective to stabilize the hilsa mince against lipid oxidation at room (26±4 °C) and frozen storage (20±2 °C) temperatures. Further, in order to standardize the effective proportion of carrot slurry or extract to be used, several trial and error experiments were made. Final contents determined for fresh carrot slurry and powder carotenoid extract were 15% and 4%, for hilsa cube and hilsa powder respectively (Table 1). Analytical and food-grade chemicals were purchased from Merck and BDH. Agar-agar was purchased from Hi-Media, India Ltd. The spices powder and other ingredients were purchased from the local markets. In case of unavailability of spices in powder form, raw spices were purchased and dried in the hot air oven (Binder, E56, Germany) at 60 °C for 24 h. The dried spices were ground with a mechanical grinder to make powder and sieved by a metallic sieve. Carrot slurry was prepared by grinding fresh, cleaned, and peeled carrot with two-volume of water and then added to the mixer. Following the ratio of ingredients mentioned in Table 1, manual mixing was done for 2–3 min. The mixer was cooked for 3 min with a gas oven at 90–100 °C. Finally, agar-agar was added to the hot mixer to facilitate gelatinization of the ingredients. We used this short time cooking to facilitate stabilization of protein by removing water and gelatinization of fish mince, ingredients and spice mixture through agar-agar. Upon fan-blown cooling to room temperature (28 ± 2 °C), the mixer was weighed and stuffed into aluminum moulds to obtain final hilsa cube (2 × 2 × 2 cm^3, 12.0 g), as described by Khanapur et al. (2017). Hilsa cube (Figure 1B) was wrapped by aluminum foil, frozen at -35 °C for 24 h under quick freezing to stabilize frozen block and then kept in frozen-storage (−20±2 °C) for biochemical and shelf-life study for 6 months. Samples were taken in triplicate for any analysis.

2.2. Preparation of hilsa powder

Although the basic principles and methods of hilsa powder preparation were the same as those described by Huda et al. (2012) and reviewed by Shaviklo (2015), due to high lipid content in hilsa, some modifications were made. The most important one was the incorporation of natural antioxidant, carotenoid powder from fresh carrot. In addition, similar food-grade chemicals, as used in the case of hilsa cube (sucrose, sorbitol and sodium tri-polyphosphate), were also used in hilsa powder as dry-oprotectants to stabilize protein against high-temperature drying, as shown in Table 1. Same as hilsa cube, the method for the formulation of hilsa powder was standardized through trialing with different proportions of dryoprotectants and carrot powder under different time and temperature schedules, the selected final formulation is presented in the lower section of Table 1. Earlier, carrot powder was prepared by drying and grinding fresh carrot slices and stored vacuum-packed in the refrigerator (4±1 °C). An effective concentration of carrot powder for hilsa powder preparation was determined to be 4.0 %.

For the preparation of hilsa powder, the collected fresh hilsa from the Meghna River were initially washed, gutted, cleaned and then washed again, and cooked at 95°-100 °C in 3 volumes of water for 5 min to partially remove lipid and to facilitate muscle-bone disintegration. The cooked fish was taken out from the pan and fanned to cool down to room temperature (28±2 °C). The bones were removed and meat mince was taken through a mechanical meat grinder (Panasonic, Model: MK/MG 1300, Malaysia). Now, hilsa mince was added with dryoprotectants and carrot powder as in Table 1, thoroughly mixed and then kept in a hot-air oven (Binder, Model: E56, Germany) at 60 °C for 20 h in order to dry the mince. The dried mixture was pulverized and screened using a metallic sieve with a cutoff mesh size of 600 microns. Hilsa powder thus prepared was vacuum packaged (Hualian Machinery Group, single chamber vacuum packing machine, Model: HVC-510F/2A-G 20 m^3, China) in poly-nylon vacuum pouches. The pouched hilsa powder (Figure 1C) was stored at room temperature (26±4 °C) for 6 months for quality analysis.

Table 1. Stabilizers used in hilsa cube and hilsa powder preparations.

Products	Cryoprotectants/spices	% Value (Based on mince weight)
Hilsa cube	Sucrose	4.0%
	D-sorbitol	4.0%
	Sodium tri-polyphosphate	0.3%
	Carrot slurry from fresh carrot	15.0%
	Agar agar	4.0%
	Red pepper	0.1%
	Onion powder	0.2%
	Bay leaf powder	0.1%
	Ginger powder	0.1%
Hilsa powder	Sucrose	4.0%
	D-sorbitol	4.0%
	Sodium tri-polyphosphate	0.3%
	Carotenoid (as powder extracted from fresh carrot)	4.0%

2.3. Determination of proximate composition

Proximate compositions (moisture, crude protein, crude lipid, ash and crude fiber as carbohydrate) of fresh hilsa, hilsa cube and hilsa powder were analyzed according to the methods described in the Association of Official Analytical Chemists (AOAC, 2005) with certain modifications. The crude fiber was analyzed according to the AOAC Official Method (Weende Method), taken from Lamata (2012), with certain modifications. The triplicate samples were analyzed each time. Proximate composition of fresh hilsa and hilsa cube were analyzed on wet-weight (W/W), while that of hilsa powder was analyzed on dry-weight (D/W) basis.

2.4. Biochemical quality analysis and shelf-life study during storage

Total volatile base nitrogen (TVB-N) and tri-methyl amine nitrogen (TMA-N) were determined according to the methods given in AMC (1979). The method of Egan et al. (1981) for the determination of peroxide value (PV) was used, as adopted from Woods and Aurand (1977). Thiobarbituric acid (TBA) reactive substance value was measured according to Tarladgis et al. (1960), while histamine content was determined according to AOAC (1990). The pH was measured using a pH meter (Corning pH Meter, Model 250, Denver Instrument Company, U.S.A) after homogenizing 2.0 g of hilsa cube or powder samples with 10 ml distilled water in an electric blender (WBL-15GC40, Walton, Bangladesh).

2.5. Sensory quality attributes evaluation

The developed hilsa powder and cube were evaluated by a 9- member trained sensory test panel in every month using a 5-point hedonic scales for taste, flavor, color, consistency and solubility, as suggested by many authors (Abbey et al., 2019; Sigh et al., 2018). A panel test score sheet with hedonic characteristics and scores for sensory evaluation was developed considering all critical elements of sensory attributes and their hedonic characteristics, particularly suitable for oily hilsa products (Table 2). Earlier, a 9-member test panel was formed from the Department of Fisheries Technology according to Stone and Sidel (2004), who

Table 2. Hedonic characteristics and scores for sensory evaluation of hilsa cube and hilsa powder.

Attributes	Hedonic characteristics	Hedonic choice	Hedonic scores	Give tick (✓) mark
Taste	Excellent fresh hilsa taste	Excellent	5	
	Very good fresh hilsa taste	Very good	4	
	Good fresh hilsa taste	Good	3	
	Low hilsa taste	Low	2	
	No hilsa taste	Very low	1	
Flavor	Excellent fresh hilsa flavor	Excellent	5	
	Very good fresh hilsa flavor	Very good	4	
	Good fresh hilsa flavor	Good	3	
	Low hilsa flavor, slightly rancid flavor	Low	2	
	No hilsa flavor, rancid flavor	Very low	1	
Color	Orangey-yellow	Excellent	5	
	Light orangey-light yellow	Very good	4	
	Grayish yellow	Good	3	
	Gray	Low	2	
	Faint	Very low	1	
Solubility (homogenized in 5 vol. of water)	80–90% soluble, no visible trace	Excellent	5	
	60–70% soluble, slight sign of trace	Very good	4	
	40–50% soluble, moderate sign of trace	Good	3	
	20–30% soluble, visible trace	Low	2	
	<20% soluble, granular trace visible	Very low	1	
Consistency (for cube)	Highly compact at 10 min of thawing, no sign of water loosing	Excellent	5	
	Compact, lose slight water at 10 min of thawing	Very good	4	
	Lose moderate water, slightly disintegrate at 10 min of thawing	Good	3	
	Lose water, moderately disintegrate at 10 min of thawing	Low	2	
	Disintegrate at 10 min of thawing	Very low	1	
Consistency (for powder)	Fine dust/not oily	Excellent	5	
	Mostly fine dust/slight oily	Very good	4	
	Moderately fine dust/ moderately oily	Good	3	
	Mostly coarse dust/ slightly sticky	Low	2	
	Coarse dust and sticky	Very low	1	

have been the faculties, regular staffs and graduate students, involved in the test panels of seafood products in the department before but did not involve in the current research program or formulation process. Selected panelists were from 30 to 63 years of age, and with one male and eight females where the consents were taken from all patients in the experiment. During pre-testing, if any members showed extreme liking/disliking or allergies to new hilsa products were carefully excluded. As per the guidelines of IS0 3972 (2011), basic training was conducted with the panelists for familiarizing them with details of sensory analysis. Then they were further trained and refreshed with the current panel test procedure, hedonic characteristics and scales and scoring procedures with dummy samples. They were made skilled and prepared to understand the true hilsa taste and flavour, with any minute deviation of these from the original. Most importantly, the test panelists were made available on each month at the fixed date and time of sensory analysis and thus, full-house participation was ensured in each session. On the day of panel test, the assessors were informed at least 30 min before the panel session starts that they would not consume any meals or drinks, smoke or use gums or mints and also instructed not to talk to other panelists during testing, as suggested by Kulawik et al. (2016). Panel sessions were carried out in the Post-harvest Fish Loss Reduction Laboratory of the Department of Fisheries Technology at Bangladesh Agricultural University. A piece of score sheet (Table 2) was given to each of the individual panelist for evaluation of the products separately. The panelists put numerical values in respect to each sensory attribute assessed. Same panelists, attributes, hedonic characteristics, choice and scores (Table 2) were used separately in different date for assessing hilsa cube and hilsa powder.

2.6. Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) and the mean comparisons were carried out by Duncan's Multiple Range Test using SPSS package software (SPSS 16.00 for windows, SPSS Inc., Chicago, IL, USA). For sensory quality analyses, 9 panelists ($n = 9$) and for proximate and biochemical analysis, triplicate ($n = 3$) samples were considered for statistical analysis. A significant difference was defined at $p < 0.05$.

3. Results

3.1. Proximate composition

The proximate composition of fresh hilsa meat (W/W), hilsa cube (W/W) and hilsa powder (D/W) have been shown in Table 3. The moisture, protein, lipid, ash and fiber contents were 65.26, 18.52, 15.13, 1.35 and 0.7% in fresh hilsa; 49.37, 22.71, 16.80, 3.35 and 9.50% in hilsa cube, and 4.21, 55.89, 27.35, 2.65 and 10.05% in hilsa powder, respectively. Proximate compositions were found to vary based on different ingredients used, and the processing conditions and measurement procedures applied, like wet weight (W/W) or dry weight (D/W) basis. The crude protein contents of fresh hilsa (18.52%) and hilsa cube (22.71%) measured on W/W basis was found to increase in hilsa powder (55.98%), because it was measured in D/W basis. Likewise, the crude lipid contents in fresh hilsa (15.13%) and hilsa cube (16.80%) measured on W/W basis was also increased in hilsa powder (27.35%), because of analyzing on D/W basis. Ash content was higher in hilsa cube (3.35%) than hilsa powder (2.65%) and fresh hilsa (1.35%). Crude fiber content was less than 1.0% in fresh hilsa, but very high in cube (9.50%) and powder (10.05%).

Table 3. Proximate composition of fresh hilsa, hilsa cube and hilsa powder.

Samples	Moisture	Crude protein	Crude lipid	Crude ash	Crude fiber
Fresh hilsa fish (% W/W)	65.26 ± 0.07	18.52 ± 0.79	15.13 ± 0.67	1.35 ± 0.05	0.7 ± 0.1
Hilsa cube (% W/W)	49.37 ± 0.49	22.71 ± 0.70	16.80 ± 0.20	3.35 ± 0.27	9.50 ± 0.87
Hilsa powder (% D/W)	4.21 ± 1.04	55.89 ± 0.48	27.35 ± 1.34	2.65 ± 0.15	10.05 ± 0.07

Data are the mean ± SD ($n = 3$). 'W/W'- wet weight basis; 'D/W' - dry weight basis.

3.2. Biochemical quality and shelf-life analysis

Biochemical properties (TVB-N, TMA-N, PV, TBA, pH, histamine) of hilsa cube and powder were investigated each month during the 6 months of storage and the results are presented in Table 4.

3.2.1. TVB-N

For both hilsa cube and powder, TVB-N values were gradually increased throughout the storage period ($p < 0.05$) (Table 4). Initially, at 0 month, the TVB-N value in the frozen hilsa cube was 1.11 ± 0.14 mg/100g, which was increased to 9.80 ± 0.26 mg/100g after 6 months of storage time ($p < 0.05$). Then again, TVB-N values in hilsa powder were 1.27 ± 0.01 and 15.97 ± 0.21 at 0 month and 6 months, respectively ($p < 0.05$). But although increased, cumulative contents of all these nitrogenous bases were within the acceptable limit, as will be discussed later.

3.2.2. TMA-N

For both the hilsa products (Table 4), increased trends of TMA-N values were also observed throughout the storage period. TMA-N values in frozen hilsa cube and dried hilsa powder were ranged from 1.03 to 3.87mg/100g and 0.85–4.04 mg/100g, during the 6 months of frozen storage (−20±2 °C) and room temperature storage (26±4 °C), respectively ($p < 0.05$). Throughout the present study (Table 4), TMA-N values in both the hilsa cube and hilsa powder remained in acceptable condition up to 6 months of storage.

3.2.3. Peroxide value

In hilsa cube and hilsa powder, peroxide values (PV) were 1.80–9.30 mEq/kg of oil and 3.40 to 17.40 mEq/kg of oil during 0 and 6 months of storage, respectively ($p < 0.05$) (Table 4). Peroxide values were gradually increased in both hilsa cube and hilsa powder during the frozen and room temperature storages for 6 months but did not reach the detectable level of rancidity, as also observed during the sensory quality analysis (Table 5). Between the products, hilsa powder had higher PV than hilsa cube ($p < 0.05$). In the case of hilsa powder, a sudden increase of PV was recorded in the 5th month (12.0 mEq/kg oil) and 6th month (17.40 mEq/kg oil) in room temperature storage, indicating an increment of oxidation, but still the values were within the acceptable limit, as no detectable level of rancidity was noticed.

3.2.4. TBA value

TBA values were also increased in both hilsa cube and hilsa powder in respective storage conditions, ranging from 0.12 to 0.93 mg malondialdehyde/kg weight and 0.2–1.53 mg malondialdehyde/kg weight during 0 and 6 months of frozen storage and room temperature storage, respectively ($p > 0.05$). Although shown an upward trend with the progress of the storage time, the TBA values were found much lower in hilsa products than the threshold limit of lipid deterioration, despite hilsa being a high-lipid species. This indicated the efficacy of carotenoid from fresh carrot in reducing lipid oxidation in hilsa. In the case of hilsa powder, in the 5th and 6th months of storage, a sudden increase of TBA values (1.09–1.53 mg malondialdehyde/kg weight) was however recorded, but these values were much lower than the threshold limit of rancidity.

3.2.5. pH

The pH value was within the neutral range but slightly increased gradually from 6.58 to 7.17 and 6.73 to 7.56 in cube and powder, respectively during 0–6 months of storage ($p > 0.05$). The pH values although initially increased but remained within the neutral range all along with the storage. A clear relation between the deviations in pH and biochemical properties of the fish and products was observed. In the case of hilsa powder stored at the 5th month (7.41) and 6th month (7.56) in room temperature, however, a slight increment of pH compared to neutral values was recorded. But this slight pH increment could not show any negative quality implications in the stored products.

3.2.6. Histamine

Histamine value in frozen hilsa cube was 0.56 ± 0.01 mg/100 g at 0 month, which reached to 8.46 ± 0.05 mg/100g after 6 months of

Table 4. Changes in TVB-N, TMA-N, peroxide value, TBA, pH and histamine contents in hilsa cube and hilsa powder during different storage conditions.

Product name	Storage period (month)	TVB-N (mg/100g)	TMA-N (mg/100g)	Peroxide value (mEq./kg of oil)	TBA (mg malondialdehyde/kg)	pH value	Histamine content (mg/100g)
Hilsa cube (-20±2 °C)	0	1.11 ± 0.14^{f}	1.03 ± 0.00^{f}	1.80 ± 0.10^{f}	0.12 ± 0.01^{d}	6.58 ± 0.03^{e}	0.56 ± 0.02^{g}
	1st	1.28 ± 0.01^{f}	1.55 ± 0.26^{ef}	3.80 ± 0.20^{e}	0.15 ± 0.03^{d}	6.65 ± 0.07^{e}	1.37 ± 0.1^{f}
	2nd	2.32 ± 0.51^{e}	1.81 ± 0.41^{de}	4.20 ± 0.52^{de}	0.32 ± 0.02^{c}	6.78 ± 0.03^{d}	1.97 ± 0.12^{e}
	3rd	4.22 ± 0.26^{d}	2.32 ± 0.51^{cd}	4.60 ± 0.20^{d}	0.40 ± 0.02^{c}	6.95 ± 0.01^{c}	4.83 ± 0.06^{d}
	4th	6.40 ± 0.37^{c}	2.83 ± 0.2^{bc}	5.40 ± 0.10^{c}	0.51 ± 0.07^{b}	7.03 ± 0.05^{bc}	6.96 ± 0.06^{c}
	5th	8.38 ± 0.36^{b}	3.35 ± 0.35^{ab}	6.20 ± 0.10^{b}	0.85 ± 0.11^{a}	7.11 ± 0.09^{ab}	7.51 ± 0.03^{b}
	6th	9.80 ± 0.26^{a}	3.87 ± 0.52^{a}	9.30 ± 0.26^{a}	0.93 ± 0.05^{a}	7.17 ± 0.05^{a}	8.46 ± 0.14^{a}
Hilsa powder (26±4 °C)	0	1.19 ± 0.13^{g}	0.85 ± 0.0^{f}	3.40 ± 0.20^{g}	0.21 ± 0.03^{f}	6.73 ± 0.07^{e}	0.87 ± 0.07^{g}
	1st	3.19 ± 0.00^{f}	1.49 ± 0.26^{e}	4.20 ± 0.20^{f}	0.35 ± 0.07^{e}	6.81 ± 0.03^{e}	1.96 ± 0.14^{f}
	2nd	6.84 ± 0.05^{e}	1.92 ± 0.01^{d}	4.80 ± 0.10^{e}	0.48 ± 0.13^{e}	6.98 ± 0.08^{d}	2.31 ± 0.2^{e}
	3rd	8.30 ± 0.14^{d}	2.13 ± 0.36^{d}	5.40 ± 0.40^{d}	0.65 ± 0.05^{d}	7.13 ± 0.01^{cd}	4.23 ± 0.07^{d}
	4th	10.43 ± 0.16^{c}	2.84 ± 0.29^{c}	8.80 ± 0.08^{c}	0.81 ± 0.01^{c}	7.27 ± 0.11^{bc}	6.02 ± 0.12c
	5th	13.20 ± 0.00^{b}	3.41 ± 0.26^{b}	12.00 ± 0.40^{b}	1.09 ± 0.03^{b}	7.41 ± 0.01^{ab}	8.19 ± 0.21^{b}
	6th	15.97 ± 0.21^{a}	4.04 ± 0.0^{a}	17.40 ± 0.46^{a}	1.53 ± 0.12^{a}	7.56 ± 0.17^{a}	9.91 ± 0.14^{a}

Data are the mean ± SD (n = 3).
Different superscripts in the same column within the same product differ significantly ($p < 0.05$).

Table 5. Sensory characteristics of hilsa cube and powder during storage period.

Products	Attributes	Month						
		0	1st	2nd	3rd	4th	5th	6th
Hilsa cube (-20±2 °C)	Taste	5 ± 0.00^{a}	5 ± 0.00^{a}	5 ± 0.00^{a}	5.0 ± 0.00^{a}	4.88 ± 0.07^{b}	4.5 ± 0.20^{c}	4.5 ± 0.20^{c}
	Flavor	5 ± 0.00^{a}	5 ± 0.00^{a}	5 ± 0.00^{a}	5.0 ± 0.00^{a}	4.8 ± 0.10^{b}	4.5 ± 0.18^{c}	4.5 ± 0.18^{c}
	Color	5 ± 0.00^{a}	5 ± 0.00^{a}	5 ± 0.00^{a}	5.0 ± 0.00^{a}	4.8 ± 0.17^{b}	4.5 ± 0.18^{c}	4.5 ± 0.16^{c}
	Solubility	5 ± 0.00^{a}	5 ± 0.00^{a}	5 ± 0.00^{a}	5.0 ± 0.00^{a}	4.7 ± 0.17^{b}	4.6 ± 0.18^{b}	4.6 ± 0.18^{b}
	Consistency	5 ± 0.00^{a}	5 ± 0.00^{b}	5 ± 0.00^{c}	5.0 ± 0.06^{a}	4.6 ± 0.20^{c}	4.5 ± 0.16^{c}	4.5 ± 0.18^{c}
Hilsa powder (26±4 °C)	Taste	5 ± 0.00^{a}	5 ± 0.00^{a}	5 ± 0.10^{a}	4.8 ± 0.07^{b}	4.7 ± 0.14^{b}	4.5 ± 0.12^{c}	4.0 ± 0.18^{d}
	Flavor	5 ± 0.00^{a}	5 ± 0.00^{a}	5 ± 0.00^{a}	4.8 ± 0.14^{b}	4.8 ± 0.18^{b}	4.5 ± 0.14^{c}	4.0 ± 0.18^{d}
	Color	5 ± 0.00^{a}	5 ± 0.00^{a}	5 ± 0.00^{a}	4.7 ± 0.20^{b}	4.6 ± 0.18^{bc}	4.5 ± 0.13^{c}	4.3 ± 0.22^{d}
	Solubility	5 ± 0.00^{a}	5 ± 0.00^{a}	5 ± 0.10^{a}	4.8 ± 0.09^{b}	4.7 ± 0.17^{bc}	4.6 ± 0.18^{c}	4.0 ± 0.20^{d}
	Consistency	5 ± 0.00^{a}	5 ± 0.00^{a}	5 ± 0.00^{a}	4.8 ± 0.17^{b}	4.7 ± 0.18^{bc}	4.6 ± 0.16^{cd}	4.5 ± 0.13^{e}

Data are the mean ± SD (n = 9).
Different superscripts in the same row within the same product differ significantly ($p < 0.05$).

storage ($p < 0.05$). In hilsa powder, histamine content was varied from 0.87 ± 0.03 to 9.91 ± 0.12 mg/100g during 0–6 months of storage ($p < 0.05$) (Table 4). Although slightly increased in the 4th to 6th month in both the products, the histamine contents in the hilsa cube and powder were found to be much lower than the values reported as poisonous threshold limit, indicating that the new hilsa products could meet the international food safety standards.

3.3. Sensory quality attributes analysis

Sensory quality characteristics (taste, flavor, color, solubility, and consistency) of hilsa cube and powder during 6 months of storage are shown in Table 5. During the storage period, the sensory hedonic scores given by the panelists for both hilsa powder and cube remained within the range of 4–5 points, indicating the attributes being confined within hedonic characteristics of 'very good' to 'excellent'. Similar 'very good' to 'excellent' sensory qualities were observed in hilsa cube till the 3rd month and in hilsa powder till the 2nd month ($p > 0.05$). Significant changes towards decrement in all sensory attributes were observed for hilsa cube and powder at 4th and 3rd month, respectively ($p < 0.05$). During the further extension of storage till the 6th month, the respective sensory attributes were gradually decreased with the progress of the storage period ($p < 0.05$). But these decreasing trends in sensory attributes were found to be more prominent in hilsa powder than hilsa cube. For both the products, however, sensory hedonic scores were still within the acceptable limit and closer to those of fresh samples of 0-month storage.

4. Discussion

4.1. Proximate composition

Proximate composition study represents hilsa as an ideal fish with unique characteristics, while many authors also found its uniqueness as having high protein and very high lipid (Kaisar et al., 2017; Mohanty et al., 2011; Moniruzzaman et al., 2014; Nowsad, 2014; Nowsad et al., 2019; Rao et al., 2012). In the new hilsa products, proximate compositions were found to vary according to ingredients used (cryoprotectants, spices, antioxidants), the processing conditions applied (freezing and drying) and measurement procedures used (W/W or D/W). So, the variation in moisture content was related to the differences in the final form of products, where the cube was wet-frozen product but the powder was dry at ambient temperature. Comparable interpretations were also made by Jahan et al. (2017) and Rahman et al. (2012) while analyzing the compositions of fish powder products. Free water in the wet hilsa cube was crystalized to frozen solid state during quick freezing (−35 °C) and crystallization continued during subsequent frozen storage -20 °C), but ultimate moisture content in the cube was reduced slightly due to incorporation of dry ingredients and spices. Although hilsa cube and powder were prepared from the same initial hilsa meat, removal of water from hilsa powder by oven drying resulted in very low moisture content (4.21%), with consequent higher values of other proximate compositions in the hilsa powder than hilsa cube. Several studies reported similar increased trends in protein and lipid content in fish powder than its parent raw product (Chattopadhyay et al., 2004; Chowdhury et al., 2017; Kasozi et al., 2018; Rahman et al., 2012). On the other hand, for the fresh hilsa meat, similar results were found by many authors where moisture content of fresh hilsa was varied over a range from 65.33 to 78.92% (Azam et al., 2004) and from 62.31 to 69.29% (Wahab et al., 2019). Hossain et al. (2018) obtained muscle moisture of hilsa to be 60.37–67.89%, which was also within the range of present findings and in agreement with the study. Besides, the moisture content in hilsa powder (4.21%) obtained during the present study was found to be similar with the moisture level (4.8%) in a stable powder prepared from tuna fish trimmings, as observed by Abbey et al. (2016), or with the moisture content ranging from 5.14% - 5.82% in much good quality freeze-dried and spray dried fish and surimi powders as reviewed by Shaviklo (2015). Therefore, the moisture content obtained in the present hilsa powder suggests its ability to remain stable against disintegration and/or contamination in room temperature conditions, if adequate packaging is maintained.

The protein content of fresh hilsa in this study (18.52%) was similar with the protein contents of 17.61%–18.55% in adult and juvenile hilsa found by Dewan et al. (2015) or 17.02%–19.44% in young and gravid hilsa found by Moniruzzaman et al. (2014), the fish being obtained from the same Meghna river of Bangladesh, as same as the present fish sources. However, the hilsa powder had lower protein content (55.89%) than tuna trimming powder (80.71%) obtained by Abbey et al. (2016) or tilapia surimi powder (62.05%) obtained by Ramirez et al. (1999).

The fat content found in fresh hilsa in this study (15.13%) was within the range of fat content of 14.0%–25.0% in fresh hilsa studied from Indian waters at different times of the year (Majumdar et al., 2005) and 13.84%–16.94% in hilsa obtained from the Pyra, Kirtonkhola and Meghna rivers of Bangladesh (Moniruzzaman et al., 2014). However, the present study found higher fat content than that of Indian marine hilsa (12.4%) and river hilsa (8.78–14.51%) observed by Rao et al. (2012), estuarine hilsa (1.66–13.11%) from West Bengal (Nath and Banerjee 2014), and different size group of hilsa (5.68–26.87%) observed by De et al. (2019). In our study, hilsa cube was found to have similar lipid content (18.8%) as that of fresh hilsa, while hilsa powder had reasonably higher lipid content (27.35%) than fresh hilsa (Table 3).

Differences in ash content between the products might be resulted from the differences in the preparation of products and use of ingredients, as explained earlier (Table 1). The increased ash content in

hilsa cube might be governed by higher levels of polysaccharides, carrot slurry and spices, which were lesser in proportion in case of hilsa powder and absent in fresh hilsa. Kaisar et al. (2017) studied three different hilsa stocks and found ash contents within the range of 1.23%–1.31%, which was similar to present fresh hilsa, but much lower than that of hilsa cube and powder. Present ash content in fresh hilsa (1.35%) was also similar to that of spray-dried saithe surimi powder (1.41%), found by Shaviklo (2015).

The higher crude fiber contents in the newly developed hilsa cube (9.5%) and hilsa powder (10.05%) might be due to the addition of different carbohydrates like agar-agar, fresh carrot, and sucrose during the preparation of the products. Fiber contents in hilsa powder and cube were slightly higher (Table 3) than that in tuna fish frame powder (7.53 %), as observed by Abbey et al. (2016).

Proximate composition analysis showed that nutritionally hilsa is very rich, having both the high levels of protein and high lipid in the pre-spawning matured fish. At least 50% of these lipids are unsaturated, of which about 30–35% belong to PUFAs (Mohanty et al., 2011 & 2012; Moniruzzaman et al., 2014; Nowsad, 2014; Nowsad et al., 2019). Only female hilsa were used in this new product formulation research, owing to its higher content of PUFAs, higher taste appeal, and a higher level of flavor components compared to the male of similar size and same age group (Mohanty et al., 2012; Nowsad et al., 2019). It has now been understood that the superb taste and unique flavor in hilsa meat have been derived from its lipids, mainly PUFA components (Mohanty et al., 2012; Nowsad et al., 2019). The fish lipids are highly unstable at room or refrigeration temperatures, as they readily undergo aerial oxidation. So the lipids need to be removed by washing to make any stable consumer fish product (Abbey et al. 2016, 2019; Shaviklo, 2015). On the other hand, without PUFAs, the developed hilsa product will be taste and flavorless. In order to keep hilsa taste and flavor intact in the products as much as possible, stabilization of PUFAs by antioxidants is essentially required. Since synthetic antioxidants are not permitted in food, we searched and detected an effective antioxidant from vegetable origin, carotenoid from fresh carrot slurry or carrot powder, to use in hilsa cube and hilsa powder. In hilsa cube formulation, we have kept almost all lipids in the product; while in case of powder, we partially removed lipids by draining out semi-boiled water and allowed a good portion to retain with the boiled mince, in order to impart hilsa taste and flavor in the powder product as well. These have been reflected in the proximate composition analysis, where both hilsa cube and powder showed a substantial quantity of crude lipid. On the other hand, with the full content of hilsa lipid in powder product, it would be sticky and would be very difficult to dry. Therefore, the present product formulation protocols were finalized through series of trial and error experiments.

In order to protect the protein from denaturation in frozen storage in case of hilsa cube and in high-temperature processing in case of hilsa powder, we used cryoprotectants like sucrose, sorbitol and sodium tripolyphosphate in cube and the same chemicals as dryoprotectants in powder, as used by Suzuki (1981) in frozen surimi processing and by Huda et al. (2012) in dried fish powders, respectively. This group of chemicals seemed to be performed well in protecting proteins in both cases of cube and powder, as have been reflected in the protein contents of the products (Table 3).

4.2. TVB-N

Initial lower TVB-N values in the fish and products indicated their acceptable quality characteristics. Many authors observed that low TVB-N values of the products are the indications of fresh and premium quality fish and increased TVB-N with the increment of storage time are the indications of quality degradation (Jeyasekaran and Saralaya, 1991; Saritha et al., 2014). According to Nowsad (2007), fresh fish contains a TVB-N content of 35–40 mg/100g of fish muscle, while 50–70 mg/100g is considered as the maximum safe level, based on species. TVB-N value was found to be 3.5 mg/100g in horse mackerel surimi at 150 days of frozen storage by Köse and Uzuncan (1999), which also supported the results of the present study. The increased trends in TVB-N values with the increased chilled and frozen storage time were also reported for many value-added fishery products (Khanipour et al., 2014; Kolekar and Pagarkar, 2013). The present study (Table 4) also showed similar trends where the TVB-N values were increased in both the hilsa products with the progress of storage time, but the values remained within the acceptable limit. The degradation of proteins and non-protein nitrogenous compounds might be linked with this rapid increment of TVB-N during storage (Nowsad, 2007). Therefore, the present study could conclude that both the hilsa cube and powder remained acceptable up to 6 months in frozen and room temperature storage respectively, where degradation of proteins was not so occurred.

4.3. TMA-N

Like TVB-N, TMA-N values were also increased with the increment of storage time in both the products in respective storage conditions. Farag (2013) found similar results for salted oily sardine (*Sardinella* spp.), where the initial TMA-N value of 3.84 mg/100g was increased to 32.5 mg/100g after 120 days at 30 °C and to 4.78 mg/100g after 8 weeks of storage at 0 °C. Fish and products with TMA-N value up to 4 mg/100 g is considered to be acceptable (Kaya and Basturk, 2015). TMA-N values were also found to be in increasing trend in frozen hilsa; from 1.68 mg/100 g at 0 day to 10 mg/100 g at 75 days of storage, as examined by Kamal et al. (1996). In the present study (Table 4), both the hilsa cube and hilsa powder remained in acceptable condition up to 6 months of storage with the lower or acceptable range of TMA-N values. This might due be to the use of premium quality fresh fish, removal of gut and skin during the pre-processing of hilsa cube and powder, and use of dryoprotectants and cryoprotectants for stabilizing both protein and lipid, as will be discussed with their roles in stabilizing the products later.

4.4. Peroxide value (PV)

In the present study, both the products (Table 4) showed PV within the acceptable limit after 6 months of storage, but between the products, hilsa powder had higher PV than hilsa cube ($p < 0.05$). This might have resulted from the higher content of lipid in powder, high-temperature hot-air oven drying (60 °C for 20 h) during powder preparation and storing the product at room temperature (26±4 °C). All these might facilitate comparatively higher oxidations in powder products. On the other hand, as many authors agreed upon, limited oxidation products might be produced, although very slowly, during the frozen storage (−20 °C) of hilsa cube in the present study. Clucas and Ward (1996) mentioned that during freezing and frozen storage, oxidation also takes place but very slowly in high lipid fish. Saeed and Howel (2002) reported that lipid oxidation in fish was dependent on initial lipid content, storage temperature and length of storage period. Present findings are also in agreement with these statements. Several studies reported increased PV during the storage, e.g., in a new product from kilka (*Clupeonella cultriventris*) with tempura batter during frozen storage at -18 °C (Khanipour et al., 2014). Al-Bulushi et al. (2005) found burger of 3 months frozen storage did not reach detectable levels of rancidity, while Nath and Singh (2019) found similar results in *Pangasianodon hypophthalmus* dry surimi powder at refrigeration conditions. Although the PV of present hilsa products increased with the progress of storage time in both conditions, they were acceptable or much below the threshold limit of lipid rancidity. Connell (1990) reported the acceptable limit of PV to be 10–20 ml.Eq/kg of oil. The lower PV found in the present hilsa products might be due to carotenoids imparted from carrot slurry or carrot powder used in the products. Carrot contains natural antioxidants and has got strong anti-oxidative properties in fish muscles (Mousumi et al., 2021). In addition, carrots could also act as a filler ingredient to bind protein and lipid in the muscle gel matrix (Mousumi et al., 2021). Many researchers

found that colored vegetables like carrots contain carotenoids, polyphenols, vitamins, and phenolic antioxidants (Silva Dias, 2014).

4.5. TBA value

TBA values were increased in both hilsa cube and powder in respective storage conditions. Orak and Kayısoglu (2008) studied TBA values of whole, gutted and filleted whiting (*Gadus euxinus*), gray mullet (*Mugil cephalus*), and anchovy (*Engraulis encrasicholus*) during frozen storage for 9 months at –26 °C, where fatty pelagic fish anchovy had higher TBA values of 6.80, 4.41 and 4.75 mg malondialdehyde/kg than other two species having 0.54, 0.45 and 0.61 mg/kg, and 0.79, 0.34 and 0.16 mg/kg, respectively. Hoque et al. (2011) observed increasing trends in TBA values during storage of cuttlefish (*Sepia pharaonis*) skin gelatin-based film, stored with chicken meat powder without and with cover for 21 days. In the present study, although shown an increasing trend with the length of storage, the TBA values were much lower in hilsa products compared to the above studies, in spite of hilsa being a high-lipid species. TBA value signifies the degree of rancidity in the products and fish, the value higher than 8.0 mg/kg is considered as spoiled or deteriorated (Ukekpe et al., 2014). Therefore, the TBA values of hilsa cube and hilsa powder, as have been observed to remain within the acceptable range, indicated that no detectable rancidity of lipid or no rancidity-based quality deterioration occurred in new hilsa products. These TBA results also support the findings of peroxide values that carrot slurry and carrot powder used in hilsa cube and hilsa powder have strong anti-oxidative properties in stabilizing fish lipid against the lipid oxidation.

4.6. pH

In our study, the pH values although initially increased but remained within the neutral range. A good relationship between changes in pH and sensory qualities of the fish samples was observed, where the sensory attributes were slightly decreased with the increase in pH values in both hilsa powder and hilsa cube. Kyrana et al. (1997) reported that the formation of lactic acid during anaerobic glycolysis was the reason for the initial lower pH in the fish meat or its products. In addition, with the progress of fish storage, an increase in pH values reflected the production of alkaline bacterial metabolites in spoiling fish and coincided with the increase in total volatile basic nitrogen (Kyrana et al., 1997). Gradual increase of pH in *Pangasianodon hypophthalmus* dry surimi powder was reported by Nath and Singh (2019), which was also similar to the results of the present study. Bremner (2002) reported a pH of 7.0 or neutral in fresh fish and post mortem pH of fish varied from 6.0 to 7.1, which were found to be acceptable organoleptically (Erkan and Ozden, 2008). These results were mostly similar to those of the present study. Therefore, the little change in pH values during the storage of hilsa products in the present study might not have any impact on product quality.

4.7. Histamine

Histamine is a sign of quality deterioration of wet fish and products produced from histidine of the peptide chain. Commonly, proteins of oily pelagic fish contain a higher amount of histidine as both free and bound state. Upon the death of fish, bacterial enzymes assist the transformation of histidine into histamine (Connell, 1990). Improper handling and processing of freshly caught marine fish may lead to formation of histamine after 40–50 h of death (Nowsad, 2007). Being hilsa an oily pelagic fish, histamine could be a good indicator to understand the product qualities. Chong et al. (2011) reported a value of 10 mg/kg could be a sign of histamine poisoning. In the present study, histamine content was very low and gradually increased with the increment of storage period and processing conditions. Many authors reported a very high level of histamine in fish with prolonged storage at higher ambient temperatures. Farag (2013) reported an increased histamine content from 1 to 760 mg/kg in salted sardine (*Sardinella* spp.) during 4 months of storage at 30 ± 5 °C, but 1–8 mg/kg in frozen sardine during 8 weeks of storage at 0 °C. Kanjana and Pornpimol (2016) reported the average histamine contents in marine and freshwater fish to be 7.41–19.07 mg/kg and 7.88–10.76 mg/kg, respectively, while specifically 11.30 mg/kg in skipjack tuna (*Katsuwonus pelamis*). Different international food safety standards set different permissible limits for histamine, i.e. 10 mg/100g by the EU Regulation No. 2073/2005 (EU, 2005), 20 mg/100g in fish sauce by the Canadian Food Inspection Agency (CFIA, 2003) and 20 mg/100g by the Australian Food Standards Code (AFSC, 2001). Histamine contents in the present hilsa cube and powder (Table 4) were found to be much lower than the values reported as poisonous threshold limit in the above studies and thus, our new hilsa products have been found to meet the international food safety standards.

4.8. Sensory quality attributes analysis

Hilsa has got highly classified mouth-watering smell and characteristic taste, for which the fish is so attractive, lovable, and popular to many people around the globe (Nowsad, 2014; Nowsad et al., 2019). The smell of hilsa is so distinctive that it is recognizable from a far distance when it is cooked. Hilsa lovers and consumers can easily recognize the true hilsa taste and flavor. When the fish like hilsa is cooked, the PUFAs and proteins are broken down into very strong volatile flavor-active metabolites that readily spread around very quickly and trigger the human olfactory nerves to recognize characteristic hilsa flavor (Nowsad et al., 2019). During conducting present sensory tests, the panelists were trained adequately to recognize true hilsa taste and flavor and understand a slight deviation of taste and flavor from the original that might happen due to processing conditions or storage.

During the storage period, the hedonic scales in both hilsa powder and cube remained within the range of 4–5 points, indicating the attributes being confined within 'very good' to 'excellent'. During the extended storage till the 6th month, the respective sensory attributes were slightly decreased with the progress of the storage period ($p < 0.05$). These decreasing trends of sensory attributes were in good agreement with other authors in other fish foods, who found decreased sensory attributed in heckle (*Alburnus mossulensis*) fish balls during -18 °C frozen storage (Duman and Peksezer, 2016). In our study, decreasing trends in sensory attributes were found to be more prominent in hilsa powder than hilsa cube and this might be due to differences in storage temperature and fat content in the products. The result also supported the comparative higher oxidative changes (PV and TBA values) taken place in hilsa powder than hilsa cube (Table 4). For both the products, however, sensory hedonic scores were within the acceptable limit and closer to those of fresh samples. Premium quality of raw material hilsa and their appropriate handling, enhanced hygiene and sanitation practices, incorporation of stabilizers and antioxidants, and better packaging and storage conditions could significantly extend the shelf-life and storage stability of both the newly developed hilsa products. The analyzed sensory attributes of this study suggested that hilsa cube was more stable than hilsa powder, although both the products were within the acceptable quality limit under 6 months of storage.

Very good consistency of hilsa cube with no sign of drip loss was recorded upon 10 min of thawing, as observed by the panelists. The hedonic scores of this attribute were found almost similar throughout the 6 months of frozen storage. This might be happened due to water bound protein-carbohydrate-fat matrix was formed soon after cooking at the cooling of the cube slurry and during subsequent freezing. We used agar-agar for gelatinization of the water-protein-fat complex. Freezing of cube at -35 °C helped to crystalize water molecules very quickly as and where it remained inside the gel matrix, to retain the cube as a reformed solid state. Therefore, it helped reducing drip loss when thawed and kept the cube intact, because of such a functional water-bound protein-carbohydrate-fat matrix.

Sensory quality results reflected well with those of biochemical properties of the products during the storage. Such acceptable and stable biochemical qualities (Table 4) and sensory attributes (Table 5) have justified the application of stabilizers-cryoprotectants or dryoprotectants, in both products. Incorporation of commercial cryoprotectants (4% sucrose, 4% sorbitol and 0.2% sodium tri-polyphosphates, as in Table 1) in both the products might prevent myofibrillar protein denaturation during freezing and long-frozen storage (hilsa cube) and high-temperature drying and room temperature storage (hilsa powder). In consequence, these additives maintained the functional properties of protein and increased the shelf life of the products. In the freezing preservation of muscle-based foods, use of sugars and polyols to protect proteins as cryoprotectants is a common phenomenon, but these chemicals can also be used during fish or fishery product drying as dryoprotectants Suzuki (1981); Huda et al. (2012). Carjaval et al. (2005) reported that the drying process could lead to denaturation of proteins due to its aggregation when water is removed from the protein matrix. During drying, denaturation of protein in muscle based foods mainly occurs due to the high drying temperature and subsequent water loss (Huda et al., 1998). Fish protein losses its functional properties at high temperature (Santana et al., 2012*)*. However, dryoprotectants could charge for water replacement to protect protein denaturation during drying (Carpenter et al., 2004). Therefore, in this study cryoprotectants were used in hilsa cube in order to prevent protein denaturation during frozen storage, and the same chemicals were used as dryoprotectants in hilsa powder to prevent protein denaturation during drying. The results also supported the findings of Huda et al. (2012) who reported strong dryoprotective effects of various polyols like sucrose, sorbitol, polydextrose, palatinose and trehalose, as these chemicals protected the proteins of surimi powder during drying and subsequently improved their functional properties.

We have detected and used a new natural antioxidant for oily fish product preservation (Mousumi et al., 2021), carotenoid from fresh carrot, which was found to have a good anti-oxidative properties and come up as an effective stabilizer against lipid oxidation in hilsa cube and powder. Since the fish lipid, mainly the PUFAs were protected from oxidation by this herbal antioxidant, true hilsa taste and flavor were remained mostly intact in the products during the entire storage period tested. This has been reflected by the sensory panel scores of the panelists as well.

The use of spices was also useful as antioxidants that protected the oxidative changes and other chemical damages during storage of the hilsa products. In addition, vacuum packaging conditions could further contribute to increasing the shelf-life of the products, as also observed by Chong et al. (2011).

In this study, we used a moderate size (600–700 g) pre-spawning female hilsa for product formulation. Our earlier research (Moniruzzaman et al., 2014; Nowsad et al., 2019) and many other studies (Mohanty et al., 2011 & 2012; Nath and Banerjee, 2014; Rao et al., 2012) have supported that moderate size hilsa of around 600–900 g could obtain higher levels of essential fatty acids and PUFAs. Pre-spawning female hilsa has been found to be tastier because of more PUFAs than the male of similar size, as explained earlier. Therefore in the present study, pre-spawning female hilsa of adequate size range might have contributed further in retaining original taste and flavor in the hilsa cube and hilsa powder.

5. Conclusion

Value-addition of hilsa shad (*Tenualosa ilisha*) could be attained through the formulation of bone-less and stabilized hilsa cube and hilsa powder. This study is the first report on stabilized value-added hilsa products formulation, its nutritional and bio-chemical quality and storage stability. Pre-spawning moderate size female hilsa was used and stabilizations against protein denaturation and lipid oxidation in hilsa meat were done by the cryoprotectants and carotenoid from fresh carrot, respectively. Developed hilsa cube and powder contained high nutritional quality, acceptable and stable biochemical and sensory qualities, and intact hilsa taste and flavor for 6 months of storage. The study suggests that both the products could be introduced as ready-to-cook convenient products and be used to fortify hilsa taste and flavor in the final consuming products like soup, noodles, curry, snack or school-feeding bakery or milk-based food-stuffs for the hilsa lovers at home and aboard.

Declarations

Author contribution statement

Alam AKM Nowsad: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Al-Shahriar: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Md. Sazedul Hoque: Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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