Croatian Journal of Food Science and Technology
www.ptfos.unios.hr/cjfst/
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Evaluation of the effects of vacuum packaging on the shelf life of ready-to-cook (RTC) marine fish sausages at frozen storage conditions

Md Faruk Abdulla, Subho Murmu, Mst Tahsin Farzana, Md Rashedul Kabir Mondol, Md Tariqul Islam*
University of Rajshahi, Department of Fisheries, Motihar, Rajshahi-6205, Bangladesh

ARTICLE INFO

TYPE: Original scientific paper
https://doi.org/10.17508/CJFST.2025.17.2.01

***CORRESPONDENCE**
Tariqul Islam
tariqul.fish@ru.ac.bd

ARTICLE HISTORY
Received: March 31, 2025
Accepted: May 26, 2025

CITATION
Abdulla MF, Murmu S, Farzana MT, Mondol MRK, Islam MT. Evaluation of the effects of vacuum packaging on the shelf life of ready-to-cook (RTC) marine fish sausages at frozen storage conditions. Croatian Journal of Food Science and Technology (2025) 17 (2) 1 - 17

KEYWORDS
vacuum packaging; fish sausage; ready-to-cook; shelf life; frozen storage

KEY CONTRIBUTION

Croaker fish sausage was lighter in colour than the tuna and mackerel sausages. Mackerel fish sausage had the highest hardness value compared to tuna and croaker sausages. Vacuum packaging extended the shelf life of the marine fish sausage by two months compared to air packaging under frozen storage conditions. Vacuum packaging showed slightly better results compared to air packaging in extending shelf life of fish sausage.

ABSTRACT

Vacuum packaging combined with low storage temperatures is an effective technique for extending the shelf life of fishery products. Ready-to-cook (RTC) fish sausages from marine fish (tuna, mackerel, and croaker) were produced by optimizing ingredients and processing conditions. Shelf life was assessed by analysing moisture, pH, free fatty acid (FFA), thiobarbituric acid reactive substances (TBARS), and aerobic plate count (APC) at monthly intervals during frozen storage under packaging in a multilayer plastic pouch. The protein content of fish sausages was 10.92-13.44%, with the highest protein found in tuna sausage and the lowest in mackerel sausage. Croaker sausage was the lightest in comparison to the tuna and the mackerel sausages. The hardness value of mackerel sausage was higher than that of tuna and croaker sausages. The moisture content ranged from 57.23-58.99%, where with the highest value found in a croaker sausage and the lowest in a tuna sausage. Significantly ($p<0.05$) lower moisture content was observed in the vacuum pack sample, particularly in the mackerel, compared to the control sample during the storage period. During the frozen storage period, almost all samples showed a decreasing trend in pH and an increasing trend in moisture, FFA, TBARS, and APC levels. No significant differences ($p>0.05$) were observed in FFA values between the air-packed and vacuum-packed samples during the frozen storage period. However, moisture, pH, FFA, and TBARS values were within the acceptable limits during the frozen storage period. The APC exceeded the acceptable limit (7 log CFU/g), at approximately 6 months for air-packed and 7 months for vacuum-packed tuna sausage; 5 months for air-packed and 7 months for vacuum-packed mackerel sausage, and 4 months for air-packed and 6 months for vacuum-packed croaker sausage. Vacuum packaging showed slightly better results compared to air packaging in extending shelf life, which could be utilised by small-scale entrepreneurs for fish sausage production.

Introduction

Food preferences have been shifting alongside economic and social development. Due to current social and cultural changes, there is a high demand for value-added fish products and RTC convenient goods (Rathod and Pagarkar, 2013). Value-added fish products such as fish balls, fish crackers, fish marinades, fish sticks, fish fingers, fish burgers, etc., are well known for their distinctive taste and flavour (Thi et al., 2013). Another way to enhance the value of fish is by producing various value-added products made from it (Viji et al., 2015). Nowadays, many urban residents, particularly working mothers and homemakers, choose ready-to-cook (RTC) or prepared foods over raw materials due to their busy lifestyles. To meet the growing demand for convenient food options, food companies are increasingly developing RTC products (Adetunji and Odetokun, 2012). In Bangladesh, the production of value-added fish products from various types of fish mince could significantly contribute to resource utilization, and food security (Hoque et al., 2021). There are not many value-added fishery products available in the market, predominantly in the superstores of Bangladesh. Although a few companies have recently introduced some value-added meat products under frozen conditions, the presence of similar fish-based items remains scarce. In many Asian countries, particularly in Bangladesh, fish sausages, fish fingers, fish cutlets, fish rolls, fish balls, fish samosas, fish pakoras, fish papadas, chillichilli fish, fish-soup powder, fish flakes, fish curry, etc., are not yet widely available.

Among these RTC fishery products, fish sausage has recently gained widespread recognition as a popular value-added product. Consumers today prefer sausages due to their convenience, variety, affordability, and high nutritional value (Huda et al., 2012). According to Raju et al. (2003), fish sausage is produced by combining fish meat with additives, stuffing the mixture into appropriate casings, and then heating the mixture. However, due to its short shelf life, fish sausage has limited transportability, often resulting in inconsistent product quality for consumers (Raju et al., 2003; Aubourg et al., 2004). Fish sausages are better than chicken sausages because of their high nutritive value, but are less favoured due to their weak texture (Dinçer and Çakli, 2015). During frozen storage, fish and fishery products undergo a number of chemical and physical changes, which can negatively impact the quality and stability of the final product.

A significant portion of food experiences quality degradation and pathogen contamination due to inadequate packaging and storage conditions. According to Tørngrena et al. (2018), the role of food packaging has changed from simple passive preservation techniques to sophisticated active techniques that go beyond conventional applications. Some retail establishments have begun selling fish products in standard polythene (PE) pouches; however, this type of packaging does not guarantee the preservation of product quality or extend shelf life. Innovative packaging technologies are being adopted by the seafood packaging sector in response to consumer demand and the need to keep the quality and stability of frozen fish products. Therefore, vacuum packing is considered a sophisticated method for packaging and storing fishery products, and it plays a vital role in supporting the retail marketing of fresh fishery products (Patil et al., 2020). To extend the shelf life of fishery goods and prevent deterioration, vacuum packaging can be combined with ice or refrigeration (Shalini et al., 2000). The food industry widely uses vacuum packaging because it effectively reduces oxidative reactions in products at a relatively low cost. According to Kumar and Ganguly (2014), vacuum packages are transparent, chemical and oil-resistant. It has been researched whether pretreatment prior to vacuum packaging can further extend the shelf life of fresh fish goods.

In this context, multilayer polymers, such as a combination of polyamide (a moisture barrier) and polythene (a gas barrier), can help protect food from oxygen and moisture, potentially extending its

shelf life (Dixon, 2011). According to Kaiser et al. (2018), this innovative technology combines the unique properties of different polymers to create a packaging that offers enhanced durability and protection. Compared to conventional single-layer films, multi-layer films provide superior resistance to water vapour and gases such as carbon dioxide, oxygen, and aromatic compounds. Additionally, multi-layer films offer strong sealing properties, high mechanical strength, and low-temperature resistance (Tartakowski, 2010). To maximize the effectiveness of the packaging material, various polymers are layered within a single foil or sheet in multilayered packaging systems. PET/PE, polyamide (PA)/PE, and PET/PE/ethylene vinyl alcohol (EVOH)/PE are common combinations found in multilayered foils and sheets (Wagner Jr., 2016).
Many studies have investigated the shelf life of fish sausage under frozen storage conditions. However, marine fish sausages suitable for storage in such conditions have not yet been developed in Bangladesh. The purpose of the present study was to develop and evaluate the quality of vacuum-packed fish sausages in comparison to air-packed ones under frozen storage conditions.

Materials and methods

Sample collection

After being purchased from the fish landing centre in Cox's Bazar, Skipjack Tuna (*Katsuwonus pelamis*), Indian Mackerel (*Rastrelliger kanagurta*), and Hooghly Croaker (*Panna heterolepis*) were transported under icing conditions to the Quality Control Laboratory at the Department of Fisheries, University of Rajshahi, Bangladesh.

Preparation of marine fish sausage

Upon arrival, the fish were washed, skinned, and cut into pieces to make mince using a meat mincer (MK-G1800P, Panasonic Corporation, Japan). Initially, 650 g of fish mince was placed in a dough maker bowl and then different ingredients were added, including 140 g corn powder, 24 g salt, 15 g sugar, 2.5 g sodium tripolyphosphate ($Na_5P_3O_{10}$), 2.5 g potassium sorbate ($C_6H_7KO_2$), 2 g pepper, 5 g chilli powder, 3 g coriander, 2 g ginger paste, 2 g garlic paste, 50 g vegetable oil and 100 ml chilled water. The ingredients were slowly mixed until a unique mixture was obtained. The prepared mixture was then transferred into the sausage stuffer to prepare fish sausages. Then the cylinder-shaped sausages (around 6-8 cm) were boiled in hot water until they floated. The fish sausages were then ready for packaging and preservation.

Packaging and storage

A transparent, multilayered (PE/PA/PE) pouch composed of polyethylene and polyamide, with a thickness of 70 µm was used as the packaging material. Two types of packaging were employed: air packing, which served as the control, and vacuum packing, which was treated. Air packing was conducted using a household heat sealer (Kingstar, China), while vacuum packing was performed using a vacuum packaging machine (C-100, Multivac, Germany). All packaged samples were stored in a household deep freezer (-18 °C). At one-month intervals, the samples were analysed both biochemically and microbiologically in triplicate in the laboratory.

Proximate composition

After preparing fish sausages, proximate analysis, including moisture, ash, lipid, and crude protein, was carried out following the AOAC method (AOAC, 1980), with certain modifications. Carbohydrate content was calculated indirectly by subtracting the total amount of protein, lipid, ash and moisture content from 100. All determinations were performed in triplicate, and the mean values were reported.

Colour

The colour of the fish sausages was measured using a colourimeter (NH310, 3nh, China). Prior to measurement, calibration was performed using a white colour standard. After calibration with white colour, the colourimeter was used to assess the samples. Then, five samples of each product were subjected to a colour test in terms of lightness (L*), redness (a*), and yellowness (b*). Each individual value reported was the average of five readings.

Hardness

A penetration test (Shears test) was conducted using a texture analyser (FRTS-100, Japan) to determine the hardness of the fish sausages. The test parameters were as follows: pre-test speed of 2.0 mm/s, post-test speed of 5.0 mm/s, distance of 5.0 mm, test time of 5.0 s, trigger type set to auto, and trigger force of 10 g. During the test, sausage slices were positioned on a support rig and penetrated using disc probes. A penetration test was performed on this setup to evaluate the toughness of the sausage flesh. Hardness values were given in terms of Newton (N/mm).

Biochemical analysis for shelf life study

The shelf life of sausages stored at frozen temperatures was evaluated by analysing several factors, including moisture content, pH, free fatty acid (FFA), thiobarbituric acid reactive substances (TBARS), and aerobic plate count (APC). The details are shown below:

pH value:

Ten (10) grams of fish sausage sample from each packaging type was homogenized with 50 mL of distilled water. Then the pH of the homogenate was measured using a glass electrode pH meter (HI2002-edge, Hanna Institute, USA).

Free fatty acids (FFA):

The free fatty acids (FFA) in marine fish sausages were estimated following the method described by Karim et al. (2017). Approximately, a 2.0±0.1 g of fish sausage was homogenized with 40 mL of chloroform using a homogenizer (IKA T18 digital ULTRA TURRAX, Germany), and then the mixture was filtered through Whatman No. 541 filter paper, and the final 40 mL volume was prepared using chloroform. Chloroform extract (25 mL) was mixed with 25 mL of neutral ethanol, and 1 mL of 1% phenolphthalein solution. The mixture was titrated against a standard sodium hydroxide (NaOH) solution until a persistent pink colour appeared lasting for 15 seconds. The following equation was used to determine the FFA value:

$$FFA\ (\%\ oleic\ acid) = \frac{V\ (mL)\ x\ N\ x\ 0.282\ x\ 100}{W\ (g)}$$

where, V = Titration (mL) of NaOH, N = Normality of NaOH, and W = fish muscle (g) per 25 mL subsample.

Thiobarbituric acid reactive substance (TBARS) value:

The TBARS value was determined using the method of Wang and Xiong (2005), with slight modifications. Two (2) grams of sample were mixed with 1.5 mL of 1% thiobarbituric acid (TBA) solution and 8.5 mL of

2.5% trichloroacetic acid-hydrochloric acid solution (TCA-HCl). The mixture was homogenized using a homogenizer (IKA T18 digital ULTRA TURRAX, Germany), and then placed in a water bath at 100 °C for 30 minutes. After cooling at room temperature, 5 mL of a mixture (without fish) was combined with 5 mL of chloroform and centrifuged for 10 minutes at 3500 rpm. A UV spectrophotometer (1601 PC, Shimadzu, Japan) was then used to measure the absorbance of the supernatant (coloured) at 532 nm. The TBARS value was expressed as milligrams of malonaldehyde (MDA) per kilogram of fish.

$$TBARS\ (mg\ MDA/kg) = (\text{A}\lambda 532/W)\ x\ 9.48$$

where Aλ532= absorbance at 532 nm, w= weight of the sample (g).

Aerobic plate count (APC):

The aerobic plate count (APC) was determined using plate count agar (Sigma-Aldrich, USA) following the standard pour plate method and the decimal dilution techniques by APHA (1992). The results were expressed as log colony-forming units (log CFU/g). Inoculated plates were incubated at 35 °C for 48 hours in an incubator (Poleko, Poland), after which visible colonies were counted.

$$APC\ (CFU/g) = \frac{\sum C}{[(1\ x\ n_1) + (0.1\ x\ n_2)]\ x\ (d)}$$

The letters stand for: C denotes the total number of colonies counted in all plates, n_1 = number of plates counted in the first dilution, n_2 = number of plates counted in the second dilution, d = dilution from which the first counts were obtained.

Statistical analysis

Each experiment was performed in triplicate. The mean (±) standard deviation was used to express the value. One-way ANOVA followed by Tukey’s post hoc test in SPSS version 20 was used for colour and texture analysis, The student's t-test was used for shelf life study. In both cases, differences between treatments were considered statistically significant at the p<0.05 level.

Results

The biochemical and microbiological study was used to determine the quality and shelf life of marine fish sausage, including measurements of pH, FFA, TBARS and APC under both air-packed and vacuum-packed conditions during frozen storage.

Proximate composition

The protein content in marine fish sausages ranged from 10.92 to 13.44%, with the highest value observed in tuna sausage and the lowest in mackerel sausage. Moisture content ranged from 57.23 to 58.99%, with croaker sausage having the highest moisture and tuna sausage the lowest. In contrast, carbohydrate content ranged from 14.49 to 15.83%, with the highest value recorded in mackerel sausage and the lowest in tuna sausage (Table 1).

Table 1. Proximate composition of three marine fish sausages

Products	Moisture (%)	Crude Lipid (%)	Crude Protein (%)	Ash (%)	Crude fibre (%)	Carbohydrates (%)
Tuna sausage	57.23±0.27	7.90±0.30	13.44±0.26	3.04±0.28	3.92±0.09	14.49±0.12
Mackerel sausage	57.89±0.11	7.74±0.17	10.92±0.07	3.29±0.28	4.33±0.13	15.83±0.76
Croaker sausage	58.99±0.59	5.22±0.07	13.14±0.13	3.64±0.10	4.47±0.08	14.54±0.72

Colour

In this study, the highest lightness (L*) value was observed, in croaker fish sausage (64.38), and the lowest (53.35) in mackerel fish sausage. Significant differences ($p<0.05$) in lightness were found among the fish sausage types. The highest redness (a*) value, 9.74, was found in mackerel fish sausage and the lowest (8.64) in croaker fish sausage. No significant difference in redness was observed between tuna and mackerel sausages ($p>0.05$); however, both were significantly ($p<0.05$) higher than that of croaker sausage. The highest yellowness (b*) value was 25.28, found in croaker fish sausage, while the lowest was 21.25 in mackerel fish sausage. In the case of yellowness, significant differences ($p<0.05$) were found among the different fish sausages (Figure 1).

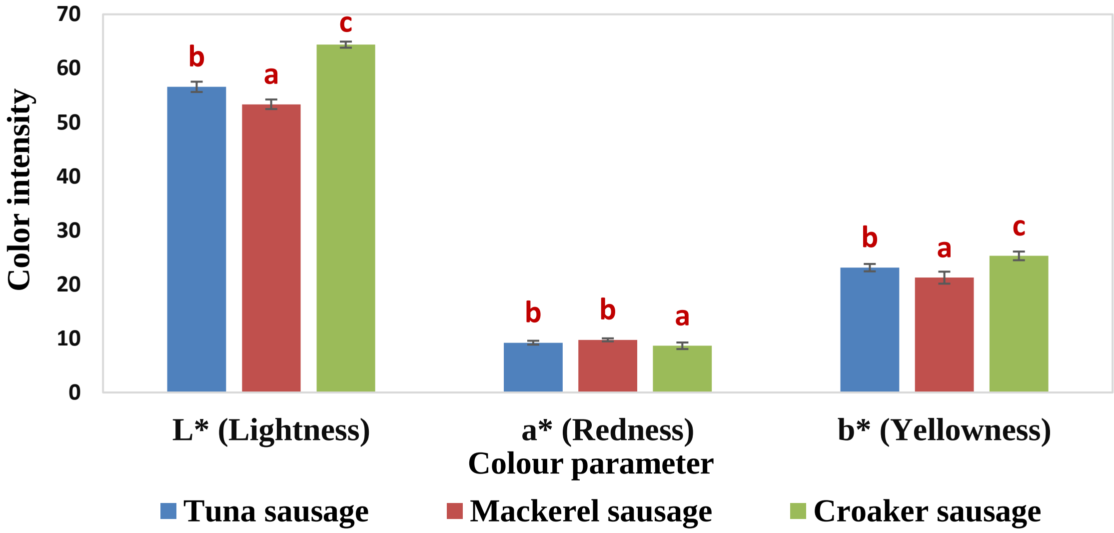

Figure 1. Colour measurement of three marine fish sausages. Different letters on the top of each parameter's bars denote a significant difference among the three fish sausages ($p<0.05$)

Hardness

Hardness is one of the main factors influencing the quality and consumer acceptability of fish-based products such as fish sausages. As shown in Figure 2, the hardness of the fish sausages ranged between 3.63 to 7.65 N/mm. The highest hardness value (7.65 N/mm) was found in mackerel fish sausage, while the lowest (3.63 N/mm) was observed in tuna fish sausage. There were no significant differences ($p>0.05$) in hardness between tuna and croaker fish sausages. However, significantly ($p<0.05$) higher hardness was observed in mackerel sausage compared to that of others (Figure 2).

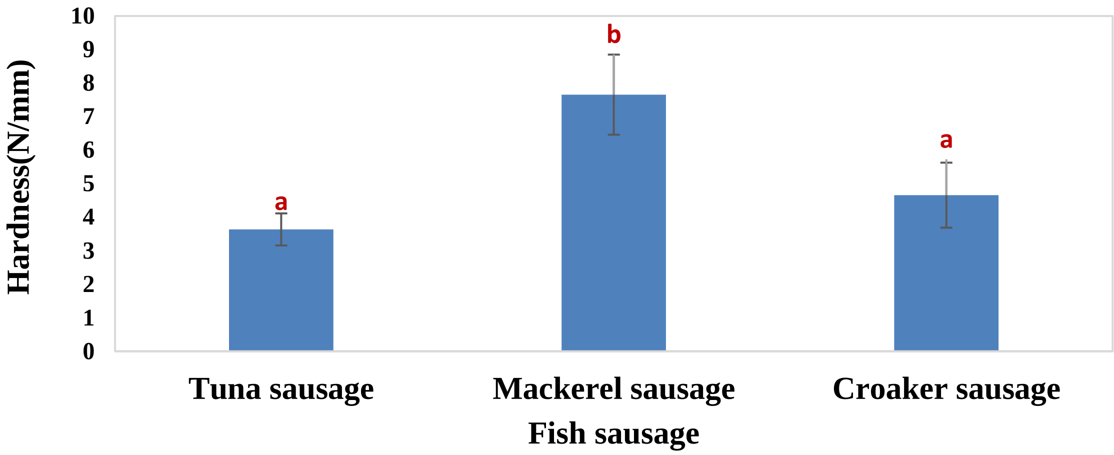

Figure 2. Hardness (N/mm) of three marine fish sausages. Different letters on the top of each parameter's bars denote a significant difference among the three fish sausages (p<0.05)

Moisture

In the present study, the initial moisture content was 61.72% in tuna sausage. Moisture content gradually increased until the 3rd month of storage, then decreased in the 4th month and remained stable for the rest of the storage period. From the 2nd month onward, significantly (p<0.05) higher moisture content was observed in vacuum-packed samples compared to the control sample (Table 2). For mackerel sausage, the initial moisture content was 56.11%. Moisture content increased gradually up to the 2nd month, followed by a decreasing trend until the 4th month, with fluctuations observed thereafter. Although moisture content was higher in the vacuum-packed samples from the 2nd month onward, statistically significant difference (p<0.05) between the air-packed and vacuum-packed samples was only observed in the 5th month of storage (Table 2). In croaker sausage, the initial moisture was 56.11%. Moisture content increased gradually up to the 1st month, and then a decreasing trend with some fluctuations was observed. Generally, higher moisture content was observed in vacuum-packed samples from the 1st month onward compared to the air-packed samples. However, a significant difference (p<0.05) between the air- packed and vacuum-packed samples was found only in the 4th and 5th months of storage (Table 2).

Table 2. Moisture content (%) of three marine fish sausages at frozen storage conditions

Fishes	Treatments	Storage period						
		0M	1M	2M	3M	4M	5M	6M
Tuna	Air pack (control)	61.72±1.10	65.61±0.36	64.78±0.13	66.11±0.35	63.97±0.44	63.95±0.55	64.14±0.99
	Vacuum pack	61.72±1.10	64.19±1.78	66.72±0.63	69.75±1.37	66.01±0.04	66.68±0.18	67.19±0.31
	p-value	0.50	0.19	0.02*	0.03*	0.01*	0.01*	0.03*
Mackerel	Air pack (control)	56.11±0.53	58.18±3.16	59.43±0.64	57.61±0.35	56.93±0.27	57.21±0.33	62.06±1.17
	Vacuum pack	56.11±0.53	58.16±0.01	59.25±0.37	58.24±0.23	57.17±0.12	59.74±0.70	60.39±1.19
	p-value	0.50	0.46	0.38	0.08	0.12	0.02*	0.15
Croaker	Air pack (control)	58.90±0.60	63.76±0.71	63.56±1.68	62.83±2.17	60.54±0.32	61.39±0.23	62.78±0.66
	Vacuum pack	58.90±0.60	63.77±0.07	64.82±0.46	63.75±1.28	63.49±0.87	62.73±0.52	63.84±0.57
	p-value	0.50	0.49	0.21	0.33	0.02*	0.04*	0.11

*p<0.05 indicated the significant difference between air-packed and vacuum-packed samples in each month of storage.

pH

The initial pH value of tuna sausage was 6.81. The pH value decreased gradually up to the 3rd month of storage in both air-packed and vacuum-packed samples, and then fluctuated for the rest of the storage period. However, a significantly lower pH value was observed in the vacuum-packed sample compared to the control sample during the 3rd, 4th, and 5th months of storage (Table 3). The initial pH value in mackerel fish sausage was 6.72. The pH value in mackerel sausage gradually increased up to the 3rd month and then followed a decreasing trend, with fluctuations observed in the rest of the storage period. During the entire storage period, there were no significant differences (p>0.05) in pH values between air-packed and vacuum-packed samples (Table 3). For croaker sausage, the initial pH value was 7.16. The pH value gradually decreased up to the 5th month, followed by a slight increase in the final month of storage. There were no significant differences (p>0.05) between the two samples throughout the frozen storage periods (Table 3).

Table 3. pH of three marine fish sausages at frozen storage conditions

Fishes	Treatments	Storage period						
		0M	**1M**	**2M**	**3M**	**4M**	**5M**	**6M**
Tuna	Air pack (control)	6.81±0.23	6.53±0.01	6.48±0.02	6.19±0.01	6.49±0.01	6.40±0.08	6.47±0.11
	Vacuum pack	6.81±0.23	6.56±0.02	6.49±0.03	6.06±0.01	6.45±0.01	6.13±0.04	6.45±0.09
	p-value	0.50	0.10	0.30	0.04*	0.03*	0.03*	0.42
Mackerel	Air pack (control)	6.72±0.06	6.79±0.01	6.87±0.06	7.03±0.02	6.75±0.03	6.62±0.15	6.82±0.07
	Vacuum pack	6.72±0.06	6.80±0.04	6.89±0.02	6.98±0.03	6.79±0.01	6.58±0.25	6.74±0.09
	p-value	0.50	0.43	0.38	0.11	0.12	0.43	0.20
Croaker	Air pack (control)	7.16±0.06	7.03±0.07	7.01±0.01	6.91±0.08	6.93±0.03	6.69±0.13	6.82±0.21
	Vacuum pack	7.16±0.06	7.01±0.05	7.01±0.01	7.07±0.03	6.97±0.01	6.76±0.18	6.86±0.13
	p-value	0.50	0.31	0.15	0.10	0.35	0.34	0.43

*p<0.05 indicated the significant difference between air-packed and vacuum-packed samples in each month of storage.

Free fatty acid (FFA)

The initial FFA value of tuna sausage was 0.39% oleic acid. In air-packed samples, the FFA value decreased up to the 2nd month, while in vacuum-packed samples it declined until the 3rd month. Thereafter, an increasing trend was observed in both samples for the rest of the storage period. There were no significant differences in FFA values observed between air- and vacuum-packed samples (Table 4). For mackerel sausage, the initial value of FFA was also 0.39% oleic acid (Table 4). The FFA values gradually increased in both samples over the entire storage period. No significant differences (p>0.05) were found between the air- and vacuum-packed samples. For croaker sausage, the initial FFA value was 0.39% oleic acid. A slow, gradual increase in FFA was observed with minor fluctuations during storage. However, there were no significant differences (p>0.05) between the air-packed and vacuum-packed samples throughout the frozen storage period (Table 4).

Table 4. Free fatty acid (5% oleic acid) values of three marine fish sausages at frozen storage conditions

Fishes	Treatments	Storage period						
		0M	**1M**	**2M**	**3M**	**4M**	**5M**	**6M**
Tuna	Air pack (control)	0.39±0.05	0.49±0.00	0.21±0.00	0.32±0.05	0.67±0.05	0.70±0.06	0.79±0.04
	Vacuum pack	0.39±0.05	0.46±0.05	0.35±0.10	0.28±0.10	0.63±0.10	0.60±0.05	0.88±0.15
	p-value	0.50	0.21	0.09	0.35	0.35	0.12	0.23
Mackerel	Air pack (control)	0.39±0.05	0.42±0.10	0.39±0.05	0.39±0.05	0.49±0.00	0.53±0.05	0.78±0.10
	Vacuum pack	0.39±0.05	0.42±0.00	0.35±0.00	0.39±0.05	0.60±0.05	0.53±0.05	0.67±0.05
	p-value	0.50	0.50	0.21	0.50	0.21	0.50	0.16
Croaker	Air pack (control	0.39±0.05	0.32±0.05	0.42±0.00	0.25±0.05	0.67±0.05	0.53±0.05	0.67±0.05
	Vacuum pack	0.39±0.05	0.32±0.05	0.42±0.00	0.35±0.00	0.56±0.00	0.60±0.05	0.60±0.05
	p-value	0.5	0.5	0.0	0.0	0.0	0.1	0.1

*p<0.05 indicated the significant difference between air-packed and vacuum-packed samples in each month of storage.

Thiobarbituric acid reactive substances (TBARS)

In tuna sausages, the initial TBARS value was 7.42 mg MDA/kg flesh. TBARS values increased gradually during the storage period in both samples. Comparatively lower TBARS values were found in the vacuum-packed samples than in the control sample. However, significant differences (p<0.05) between two samples were only observed in the 5th and 6th months of storage. For mackerel sausage, the initial TBARS value was 6.67 mg MDA/kg flesh. An increasing trend in TBARS values was observed up to the 2nd month of storage in both packaging conditions, followed by a gradual decline for the remainder of the storage period. Vacuum-packed samples generally exhibited lower TBARS values than control samples for almost the entire storage period (Table 5). There were no statistically significant (p>0.05) differences between the two samples. In croaker sausage, the initial TBARS value was 7.88 mg MDA/kg. TBARS values gradually increased over the entire storage period in both samples. Significantly (p<0.05) lower TBARS were observed in the vacuum-packed samples compared to the control sample in the 4th, 5th, and 6th months of storage (Table 5).

Table 5. Thiobarbituric acid reactive substances (mg malonaldehyde/kg fish) of three marine fish sausages at frozen storage conditions

Fishes	Treatments	Storage period						
		0M	**1M**	**2M**	**3M**	**4M**	**5M**	**6M**
Tuna	Air pack (control)	7.42±.13	7.06±0.09	7.50±0.60	8.44±0.01	8.22±0.33	8.56±0.16	8.62±0.08
	Vacuum pack	7.42±.13	7.52±0.42	7.45±0.49	8.40±0.25	8.08±0.36	7.84±0.14	8.31±0.11
	p-value	0.50	0.14	0.47	0.42	0.37	0.02*	0.04*
Mackerel	Air pack (control)	6.67±.03	8.75±0.50	10.41±.01	10.37±.60	8.96±0.20	9.18±0.08	9.28±0.25
	Vacuum pack	6.67±.03	7.88±0.66	9.95±0.10	9.89±0.49	8.80±0.50	9.23±0.15	8.82±0.36
	p-value	0.50	0.14	0.01*	0.24	0.36	0.38	0.14
Croaker	Air pack (control)	7.88±.57	9.57±0.11	9.67±0.50	11.23±.47	9.88±0.09	10.02±.20	10.54±.37
	Vacuum pack	7.88±.57	10.28±0.08	9.81±0.55	10.75±.08	9.27±0.12	9.32±0.18	9.64±0.04
	p-value	0.50	0.01*	0.41	0.14	0.02*	0.03*	0.04*

*p<0.05 indicated the significant difference between air-packed and vacuum-packed samples in each month of storage.

Aerobic plate count (APC)

The initial aerobic plate count of tuna sausage was 3.64 logs CFU/g on plate count agar. APC values gradually increased over the entire storage period in both samples. Although lower APC values were found in the vacuum-packed sample, no significant differences (p>0.05) were observed between air- and vacuum-packed samples throughout the storage period (Table 6). In mackerel sausage, the initial aerobic plate count was 3.61 logs CFU/g on plate count agar. The values gradually increased with some fluctuations during the entire storage period. From the 3rd month onward, vacuum-packed samples showed comparatively lower APC values than the control sample (Table 6). There was a significant difference (p<0.05) only in the 6th month of storage. In croaker sausage, the initial APC value was 3.98 logs CFU/g. Then the values increased in the 1st month for vacuum-packed and in the 2nd month for the control samples, followed by a decline up to the 3rd month. A subsequent increasing trend was observed in both samples. From the 3rd month onward, vacuum-packed samples consistently showed lower APC values than the control sample (Table 6). There were significant differences (p<0.05) between air- and vacuum-packed samples only in the 4th and 5th months of the storage period (Table 6).

Table 6. Aerobic plate count (log CFU/g) values of three marine fish sausages at frozen storage conditions

Fishes	**Treatments**	**Storage period**							
		0M	**1M**	**2M**	**3M**	**4M**	**5M**	**6M**	**7M**
Tuna	Air pack (control)	3.64±0.09	4.16±0.54	4.88±0.11	5.20±0.11	5.29±0.23	6.74±0.21	7.01±0.18	
	Vacuum pack	3.64±0.09	3.40±0.10	4.51±0.20	5.02±0.15	5.11±0.27	6.46±0.17	6.59±0.22	6.95±0.24
	p-value	0.50	0.09	0.07	0.35	0.15	0.14	0.08	
Mackerel	Air pack (control)	3.61±0.13	5.34±0.19	5.76±0.08	5.03±0.11	5.93±0.03	6.70±0.27	7.82±0.07	
	Vacuum pack	3.61±0.13	5.74±0.02	5.54±0.34	5.02±0.09	5.68±0.32	6.29±0.12	6.38±0.24	6.84±0.07
	p-value	0.50	0.05	0.24	0.46	0.19	0.09	0.01*	
Croaker	Air pack (control)	3.98±0.14	5.75±0.03	5.54±0.34	5.15±0.15	7.12±0.02	7.28±0.09	7.51±0.33	
	Vacuum pack	3.98±0.14	5.69±0.59	5.69±0.59	4.50±0.54	5.94±0.01	6.56±0.15	7.32±0.16	
	p-value	0.50	0.45	0.12	0.22	0.01*	0.01*	0.27	

*p<0.05 indicated the significant difference between air-packed and vacuum-packed samples in each month of storage.

Discussion

The initial quality of fish sausages was determined based on proximate composition, colour and hardness., Shelf life of fish sausages was determined through various biochemical and microbiological analyses of both air-packed and vacuum-packed samples during frozen storage conditions.

Proximate composition is very important in evaluating the nutritional quality of a product. Fish sausages contain a very low amount of carbohydrates (approximately 15%), primarily due to the limited use of corn powder. On the other hand, the protein content in fish sausages was around 11-13%. Therefore, these products could be used as protein-rich fishery products in the market. Amano (1965) a slightly higher protein content of 14–15% in fish sausages, while Chuapoehuk et al. (2001) found 13.73% protein in catfish sausages, both slightly exceeding the values obtained in this study. The RTC fish sausages developed here contained approximately 5-8% fat, which is comparable to Amano's findings (1965), where fish sausage contained 5-6% lipids. However, Chuapoehuk et al. (2001) reported a lower level of lipid content (3.16%). The difference in fat content of fish sausages mainly depends on the type of fish used.

One of the key quality factors influencing the acceptability and commercialization of fish mince products is colour (Sachindra and Mahendrakar, 2010). The colour of fish sausage is a very important parameter affecting consumer acceptance. The findings of the present study demonstrate that changes in colour characteristics are significantly influenced by the type of meat used in sausage formulation. The results revealed high lightness values in all samples, which suggests that the sausages were lighter in colour, particularly the croaker sausage, where the highest L* value was observed. This may be attributed to smaller particle sizes in the sausage matrix, which reflect more light (Poyato et al., 2014). In this study, L*, a*, and b* values ranged from 56.58 to 64.39, 8.65 to 9.75, and 21.26 to 25.29, respectively. In comparison, Gimeno (2001) reported colour values for traditional meat sausages as follows; L* = 56.14, a*= 16.85, and b*=10.63. These findings indicate that fish sausages in the current study had lower redness (a*), a higher yellowness (b*) and a comparable level of lightness (L*). Fish sausages were determined to have higher yellowness and less lightness and redness. Similar colour trends were reported by Koizumi and Nonaka (1980) and Cardoso et al. (2008), who investigated low-fat, healthy fish sausage formulations. Dinçer et al*.* (2017) recorded even higher lightness levels (67.95-72.02) in fish sausage which exceeded the values found in the current research.

Hardness is one of the main factors influencing the cellular structure and textual acceptability of food. Foods with higher hardness values are not preferred by consumers. In the present study, mackerel fish sausages exhibited the highest hardness value (7.65 N/mm), indicating a firmer texture. According to Herrero et al. (2008), ingredients in sausage formulation are crucial in determining the product's firmness. A study by Murphy et al. (2004), found that increasing the levels of surimi and fat in a meat surimi sausage mixture led to a drop in hardness force values, from 28.7 to 15.1 N, which were notably higher than the values in the present study. Similarly, Cardoso et al. (2008) reported higher values for South African hake sausages, with the control group showing the highest hardness value (35.3 N), and the lowest at 27.7 N. Among the fish sausages, the tuna sausages were softer than the others, having a very low value of hardness.

Moisture content is a crucial factor in assessing both the quality and shelf life of a product. In this study, the moisture content ranged from 56.11 to 66.11% and from 56.11 to 69.75% in air-packed and vacuum-packed samples, respectively. According to Huda et al. (2012), the moisture content of five samples of commercial Malaysian fish sausages ranged from 66.5 to 73.3%. Amano (1965) reported that fish sausages contained between 67% and 68% water during periodic inspections. In addition, Chuapoehuk et al. (2001) found 74.50% water in catfish sausages. The findings of the current study are nearly similar to those conducted earlier.

Fish and fishery products are evaluated for shelf life using pH measurement. Generally, the acceptable post-mortem pH range is 6.8–7.0 (Mohamed et al., 2018). During storage, the pH values of sausages packed in air and vacuum showed variations. However, the pH values of fish sausages in this study ranged from 6.01 to 7.16 and 6.06 to 7.16 in the air-packed and vacuum-packed, respectively. These values were slightly higher than the acceptable range. This could be attributed to the use of sodium tripolyphosphate and potassium sorbate. Stamatis and Arkoudelos (2007) reported that pH levels of vacuum-packed *Scomber colias japonicus* fluctuated throughout a 15-day storage period. The accumulation of amines and other volatile chemicals due to autolytic and microbial activity on proteins and other molecules may contribute to an increasing trend in pH during the later stages of storage (Binsi et al., 2007). These findings are mostly consistent with the results of the current study.

Free fatty acids (FFA) have been shown to negatively affect the protein characteristics; as a result, FFA accumulation has been associated with reduced product acceptability and faster oxidization compared to higher molecular weight lipid groups (Sikorski et al., 1994). According to Bimbo (1998), a maximum permissible value of 5% oleic acid FFA is recommended based on quality criteria for crude fish oil. In this study, the final FFA values of all three fish sausages remained within the acceptable limit. Reinitz and Yu (1981) found that the FFA levels in tuna fish increased steadily up to the fourth day in white muscle and up to the twelfth day in dark muscle. However, for frozen white cheek sharks in frozen storage, a similar declining trend in FFA was noticed in the third month following an initial increase (Nazemroaya et al., 2009). The oxidation of FFA and substrate depletion may be the cause of this decline (Namulema et al., 1999).

Thiobarbituric Acid Reactive Substances (TBARS) measure the amount of malonaldehyde, a secondary product formed during the oxidation of polyunsaturated fatty acids (Ashton, 2002). This occurs during the second phase of lipid auto-oxidation, in which peroxides are decomposed into reactive compounds such as ketones and aldehydes (Feliciano et al., 2010). Increased rates of oxidation and proteolysis can enhance the production of these secondary products, including malondialdehyde and biogenic amines (Kurt and Zorba, 2009). According to Nunes et al. (1992), the acceptable TBA value for fish products ranged from 5 to 15 mg MDA/kg flesh. Similarly, Bozkurt and Erkmen's (2004) reported a strong correlation between extended storage time for and higher TBA values in *sucuk* (Turkish dry-fermented sausage). Although TBA values increased gradually over time in both air- and vacuum-packed sausages, they remained within acceptable limits throughout the frozen storage period. In muscle systems, lipid oxidation often starts in the intracellular phospholipid fractions at the membrane level (Gray et al., 1996). The relatively low fat and oil content used in this study may have contributed to the lower TBARS values, as lipid oxidation susceptibility is closely related to fat concentration (Murphy et al., 2004). In the current study, using lower fat and oil values results in lower TBA readings during storage. The TBA test on the sausage revealed no signs of spoiling at the end of the storage. In the current study, TBARS values of the marine fish sausages were within the acceptable limit.

The Aerobic Plate Count (APC) is a commonly used microbiological method for determining the number of viable microorganisms present in foods (Biyani et al., 2018). The microbiological activities in foods are influenced by both intrinsic factors, such as water content, nutrients, pH, and chemical composition, and extrinsic factors as storage temperature and exposure to air (Gram et al., 2002). The acceptable limit APCs for ready-to-cook fish and fishery products is 7 log CFU/g (Carroll and Paulson, 1977).

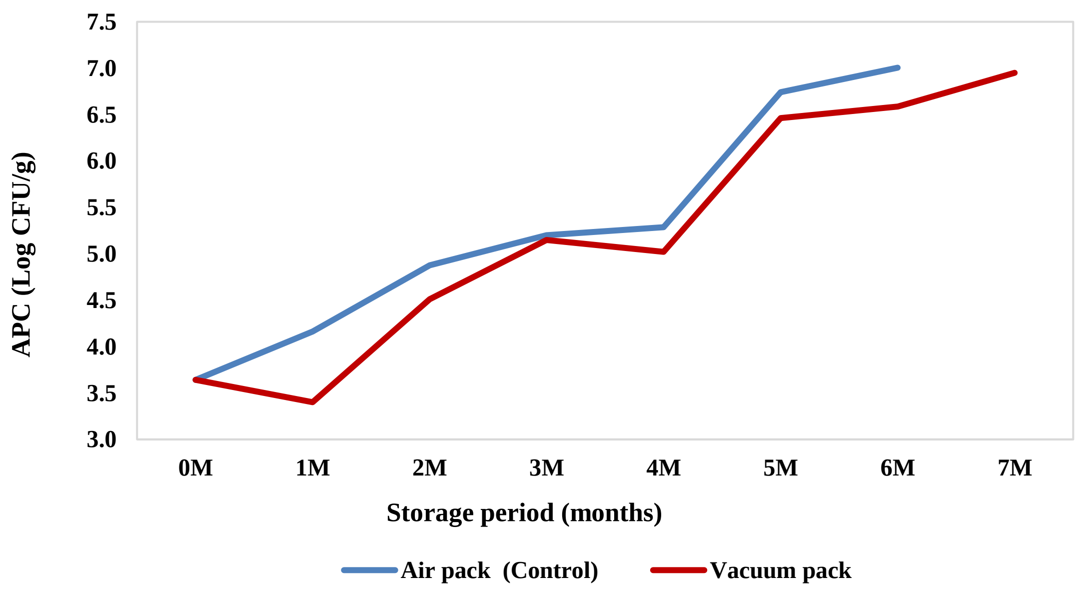

Figure 3. Changes in APC values (Log CFU/g) of tuna fish sausage at frozen storage conditions

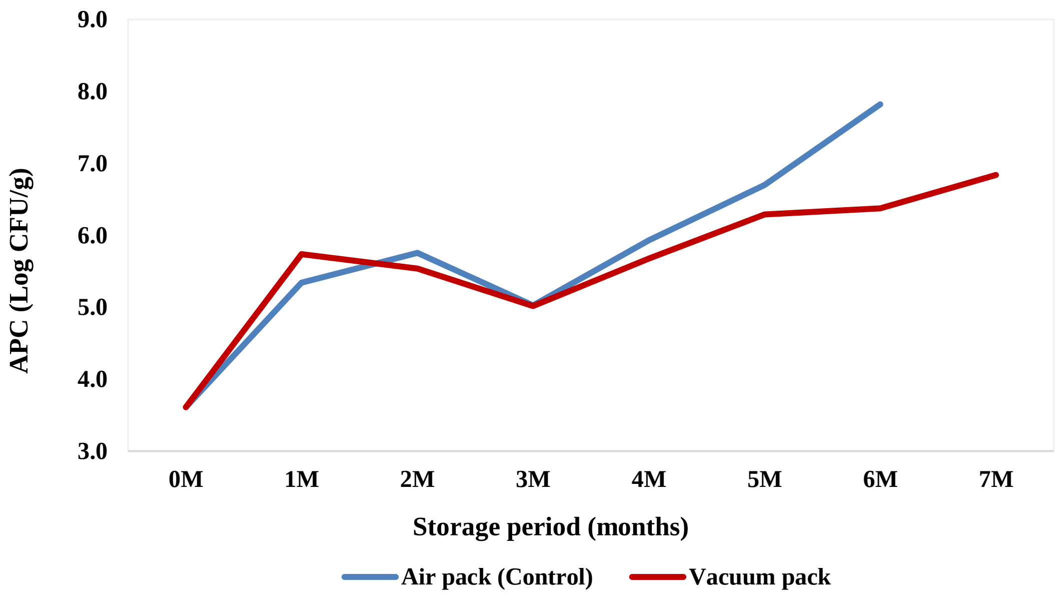

Figure 4. Changes in APC values (Log CFU/g) of mackerel fish sausage at frozen storage conditions

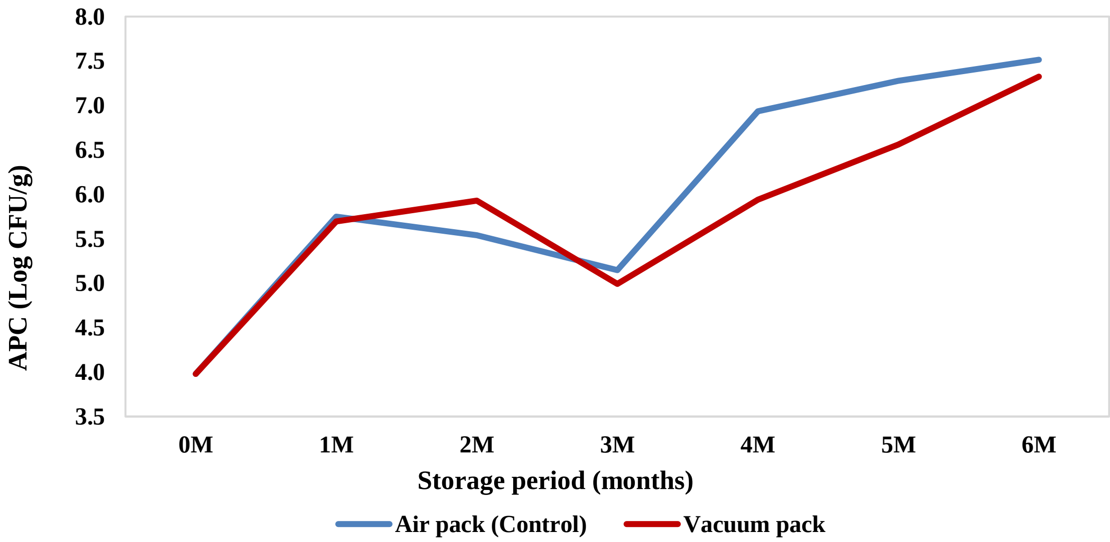

Figure 5. Changes in APC values (Log CFU/g) of croaker fish sausage at frozen storage conditions

In the case of tuna sausage, the APCs exceeded the upper acceptable limit of 7 log CFU/g, defined for the ready-to-cook products, after approximately 6 months in air-packed and 7 months in vacuum-packed samples (Figure 3). In the case of mackerel sausage, the APCs exceeded 7 log CFU/g at around 5 months for the air-packed and 7 months for the vacuum-packed (Figure 4). Similarly, in the croaker sausage, the APCs exceeded the acceptable limit at approximately 7 log CFU/g at approximately 4 months for the air pack and 6 months for the vacuum pack sample (Figure 5). Sachindra et al. (2005), reported that the total plate count (TPC) for sausage samples started at 4.09 log CFU/g, and under air-packed and vacuum-packed conditions, the TPC crossed the acceptable limit by days 16 and 32, respectively. Maheshwara et al. (2017) reported that fish sausage stored under refrigerated conditions remained microbiologically acceptable up to the 25th day, with counts ranging from 2.6×10^2 CFU/g to 3.7×10^5 CFU/g. It has been revealed from the current study that frozen storage extends shelf life of fish sausages in terms of microbial stability, particularly when vacuum packaging is employed.

Conclusion

Fish sausages prepared from three marine fish species were packaged in multilayer plastic pouches and stored under frozen conditions. These sausages contained, around 12.5% crude protein, classifying them as a protein-rich foods. In the present study, tuna sausages were found to be softer in texture (in terms of hardness) than the other two marine fish sausages. Throughout the storage period, the samples' moisture content, pH, FFA, and TBARS values in all packing conditions remained within permissible limits. However, in the case of bacterial counts, the upper acceptable limit, 7 log CFU/g, was exceeded by the APCs for ready-to-cook products at approximately 6 months for air-packed and 7 months for vacuum-packed samples of tuna sausages; 5 months for air-packed and 7 months for vacuum-packed sample of mackerel sausages; and 4 months for air-packed and 6 months for vacuum-packed croaker sausages. These findings suggest that vacuum packaging is slightly more effective than the air-packing in extending the shelf life of fish sausages. This improvement in shelf life could help make fish sausages more widely available. Therefore, it is recommendable that, small-scale entrepreneurs in Bangladesh receive training in the preparation of fish sausages. Promoting this product could serve as a healthier, high-protein alternative to traditional meat-based sausages for consumers.

Author Contributions: Conceptualization, M.T.I. and M.R.K.M.; methodology and investigation, M.F.A., S.M., and T.F.; writing—original draft preparation, M.F.A., and M.T.I.; writing—review and editing, M.T.I. and M.R.K.M.; supervision, M.T.I. and M.R.K.M. All authors have read and agreed to the published version of the manuscript.

Funding: The authors are thankful to the Sustainable Coastal and Marine Fisheries Project (SCMFP), Window 1 Grant under the Department of Fisheries, Ministry of Livestock and Fisheries, GoB (LoI-39) for funding the present study.

***Conflicts of Interest*:** The authors declare no conflict of interest.

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