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Corn starch, dietary oat fibre, and cod liver oil fortified rohu fish (*Labeo rohita*) mince sausages: Effects on physicochemical, textural, sensorial, and microbial quality during refrigerated storage

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Abstract

The present work aimed to enhance the utilisation of *Labeo rohita* (rohu) by developing fortified fish sausages, and evaluate their storage stability under refrigerated conditions. The sausages were formulated using rohu mince, with optimised concentrations of corn starch (8%), cod liver oil (8%), and dietary oat fibre (2.5%). Changes in physicochemical properties were observed over time, with hardness increasing until day 28, and cohesiveness decreasing by the end of storage. Sausage colour was influenced by muscle characteristics, ingredient composition, and their interactions. Biochemical markers such as total volatile basic nitrogen (TVB-N), peroxide value (PV), free fatty acids (FFA), and thiobarbituric acid reactive substances (TBARS), along with microbiological parameters (total plate count and psychrophilic count), showed a steady increase during storage but remained within acceptable limits for up to 49 days, ensuring good consumer quality. The present work provided key insights for food processors, demonstrating the development of value-added fish products with enhanced nutritional profiles and extended storage stability. The findings would offer a sustainable strategy for improving fish processing practices, contributing to the growing demand for nutritious and stable seafood products.

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Introduction

Fish is one of the most widely consumed animal-based foods globally, contributing 17% of the total animal protein intake for the global population (FAO, 2020). In recent years, the development of fish mince and mince-based products, such as surimi and sausages, has expanded opportunities for creating fish products enriched with functional ingredients (Chattopadhyay *et al*., 2023; Sharma *et al*., 2024a; 2024b). These innovations aim to improve product characteristics while offering health benefits to consumers. A prominent example of a value-added product is fish sausage, which involves blending fish mince with salt, spices, seasonings, starch, fat, and other ingredients (Chattopadhyay *et al*., 2023). This mixture is finely ground into a paste, stuffed into natural or synthetic casings, and then cooked (Nithin *et al*., 2015). Researchers have investigated various functional ingredients, including carrageenan, chitosan, oat bran, chicory root inulin, pea fibre, and fish oil, for their ability to enhance fish-based products, including sausages (Cardoso *et al*., 2007; 2008; 2010; Chattopadhyay *et al*., 2020). Extensive research supports the positive health effects of these functional ingredients within human nutrition (Mann and Cummings, 2009; Cena and Calder, 2020; Van Dael, 2021).

Starch plays a multifaceted role in food products, serving as an adhesion, binding, gelling, and moisture retention agent (Pietrasik, 1999; Aktaş and Genccelep, 2006). It contributes to maintaining the juiciness and tenderness of meat products, and can also partially replace the more expensive fish or meat components in a product to achieve the desired gel characteristics. For quite some time, potato starch has been the preferred non-meat ingredient to enhance the gelling properties of sausage-like products (Yoo,

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2011). However, given corn starch's increasing production, demand, and cost-effectiveness, it has emerged as a compelling alternative to potato starch.

The recent surge in interest in incorporating dietary fibres into food has captured public attention, primarily because of their potential to promote health and nutrition. Soluble fibres are known for their capacity to lower blood lipid levels, while insoluble fibres exhibit laxative properties (Elleuch *et al.*, 2011). Among dietary fibres, oats have garnered a reputation as a healthy food, particularly for their cholesterol-lowering properties, making them beneficial in the context of coronary heart diseases. The presence of beta-1,3 linkages or beta-1,4 linkages in the beta-D-glucan molecule within oats classifies them as soluble or insoluble oat fibre (Xu, 2012). Beyond their positive health effects, incorporating oat fibres in fish and fish products can enhance various functional properties such as water-holding capacity, oil-binding capacity, and emulsification in food. Currently, soluble fibres are the more commonly utilised option for technological purposes.

Another crucial functional ingredient associated with supporting human health, particularly in preventing cardiovascular diseases, is fish oil (Liao *et al.*, 2022). Marine fish, particularly fatty fish rich in *n*-3 polyunsaturated fatty acids (PUFA) containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are recognised as *n*-3 supplements or a source of food that offers various health benefits (Siriwardhana *et al.*, 2012). Therefore, to enhance fat quality and consequently human health, optimising the level of *n*-3 enrichment in the diet is essential. Interestingly, there is limited research available regarding the incorporation of fish oil into fish and fishery products, such as sausages and frankfurters (Chuapoehuk *et al.*, 2001; Panpipat and Yongsawatdigul, 2008).

Given the global decline in marine fish production, it has become increasingly important to utilise readily available resources in a sustainable manner. Numerous efforts have been dedicated to developing healthy dietary options to meet the demands of today's world. In light of these considerations, the present work was designed to create fish mince-based sausages using freshwater Indian major carp (*Labeo rohita*) or rohu, incorporating corn starch, insoluble dietary oat fibre, and cod liver oil (CLO), and evaluate their physicochemical, textural, sensorial, and microbial characteristics under refrigerated storage.

Materials and methods

Materials

Rohu was obtained from Dadar Fish Market, Mumbai, India. The fish were packed in a polyurethane insulated container (fish: ice ratio of 1:2, w/w), and transported to the Department of Post-Harvest Technology, ICAR-CIFE, Mumbai, where they were stored in ice at 4°C until processing. All other reagents used were of analytical grade, and sourced from Merck, Hi-Media, and Qualigens.

Processing of fish

Rohu sample was processed into mince using a deboning machine (Baader 694, Lubeck, Germany), which utilised a counter-rotating belt and drum mechanism. The dressed fish were introduced into the drum sieve with 5 mm diameter holes to create the mince. The temperature was carefully controlled, remaining below 10°C throughout the process. The resulting mince was then packaged in polythene pouches made of low-density polyethylene (LDPE), and stored in a refrigerator until it was ready for sausage preparation.

Preparation of sausages

Optimised concentrations of corn starch (8%), CLO (8%), and dietary oat fibre (2.5%) were utilised as functional ingredients in the formulation of sausages made from rohu. These concentrations were derived from previous studies conducted by our group (Gore *et al.*, 2021; 2022; 2024). A schematic illustration of the preparation process of sausage using rohu is shown in Figure 1. The sausages were prepared following the method described by Lee (1984), where the minced fish was ground with 2.5% NaCl for 3 min using a pre-cooled silent cutter. The mixture was initially comminuted using a food grade stainless steel silent cutter (Stephan UMC 5 Electronic cutter, Germany) for 3 min, followed by gradual addition of optimised concentration (8%) of CLO for 3 min, and then corn starch (8%) was added, and the mixing was continued for another 3 min. Finally, optimised oat fibre powder (2.5%) was gradually added, and mixing was continued for an additional 3 min. Throughout the mixing process, the temperature was maintained below 15°C by adding ice. The prepared mince was then filled manually using a stainless-steel sausage stuffer (Kitchener 5-Lb, China) into a krehalon casing of 2.5 cm diameter, taking care to eliminate the trapped air as much as

possible. The ends of the tubes were tied and pre-incubated at 40°C for 30 min, followed by heating at 90°C for 20 min in a thermostatically controlled water bath (Strike 300, Steroglass, Perugia, Italy). The resulting sausages were immediately cooled in ice, and then stored in a refrigerator at 4°C overnight for further analysis.

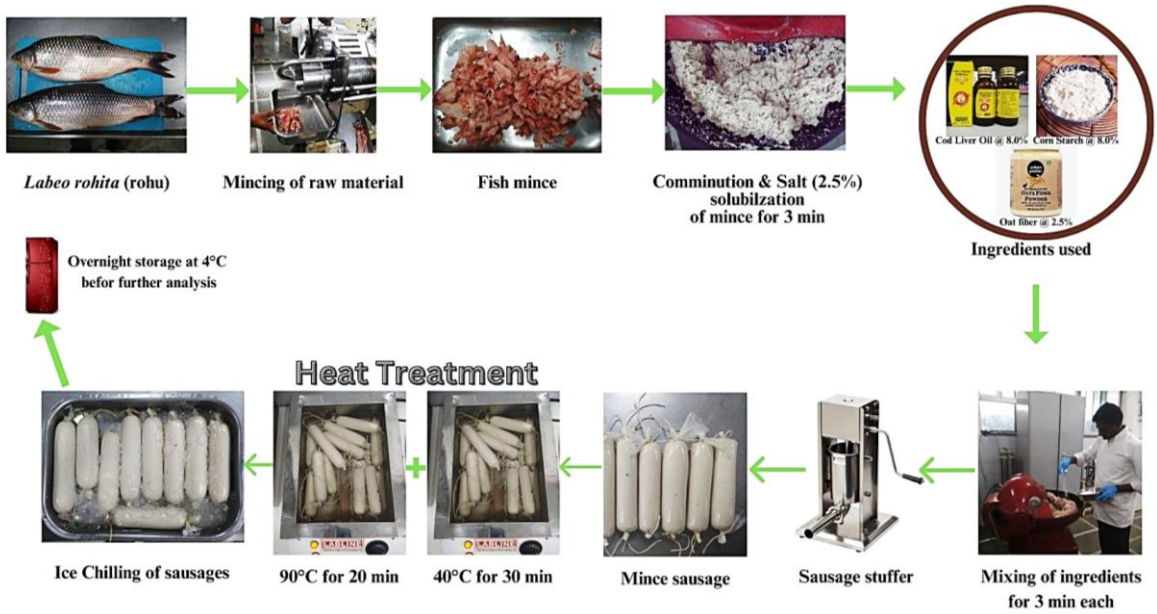

Figure 1. Schematic illustration of preparation process of fortified sausage using rohu fish.

Proximate composition

The sample's proximate composition was assessed following standard methods outlined in AOAC (2005). Moisture content was determined through the oven drying method at 100 ± 2°C for 16 - 18 h. Total nitrogen content was analysed *via* the Kjeldahl method using a nitrogen analyser (Kelplus-KES12L VAI, Pelican, India). Fat content was determined using the Soxhlet method, while ash content was established by gravimetric incineration of the sample at 550°C in a microwave furnace (Phoenix SEM, USA) for up to 6 h. The results were reported as g/100 g (wet weight basis).

Measurement of pH

10 g sausage samples for each treatment groups before and after cooking were mixed separately with 50 mL of distilled water in a homogeniser (Polytron system PT 2100, Germany) for 30 seconds and pH value of the homogenates were measured with a digital pH meter (Eutech tutor pH/ºC meter, Eutech Instruments, Singapore) previously standardised by buffers of pH 4.8 and 9.2.

Determination of gel strength

The gel strength of the sausages was assessed following the protocol established in our previous study (Gore *et al.*, 2024). Prior to analysis, the sausages were allowed to equilibrate at room temperature (25°C) for approximately 2 h. The sausages were removed from their synthetic casings, and sliced into cylindrical samples, each 2.5 cm in length. These samples were subjected to a puncture test using a Rheo Tex apparatus (Type SD-700, Sun Scientific Co. Ltd., Setagaya-KU, Japan), fitted with a 5 mm diameter round-ended metal probe, operating at 60 mm/min with a 2 kg load cell. The apparatus recorded the breaking force (in g) and the deformation (in mm) at the point when the gel samples lost structural integrity and ruptured. Each test was performed in triplicate, with gel strength expressed in g.cm as shown in Eq. 1:

$$Gel\ strength\ (g.cm) = Breaking\ force\ (g) \times Deformation\ (cm) \quad \text{(Eq. 1)}$$

Determination of water holding capacity (WHC)

The WHC of the sausage sample was assessed following the procedure outlined by Verbeken *et al.* (2005). Briefly, 10 g of the sausage sample was centrifuged at 12,000 *g* for 30 min at 4°C. The WHC was determined as the percentage of retained water, calculated using Eq. 2:

$$WHC(\%) = \frac{W_2}{W_1} \times 100 \quad \text{(Eq. 2)}$$

where, W_2 = weight (g) of the sausage sample after centrifugation, and W_1 = weight (g) of the sausage sample before centrifugation.

Determination of folding score

The folding test for sausage samples from each treatment group was performed on three round gel slices (30 mm diameter × 3 mm thick) using a 5-point scale (Lanier, 1992). The folding score sheet included characteristics with scores based on the following scale: 5 = gel that does not break when folded in quadrants; 4 = no breakage occurs when folded in half; 3 = gel gradually breaks at the first fold; 2 = break occurs at the first fold; and 1 = gel disrupts when gently pressed with finger.

Determination of colour

The colour of sausage samples in each treatment group was assessed using a colorimeter (Hunter LabScan XE, USA). Prior to colour measurement, the sausage samples were allowed to equilibrate to room temperature for 30 min. Sausage samples, cut to dimensions of 2.5 cm in diameter and 3 cm in height, were used for colour property determination. *L**, *a**, and *b** values were measured, and whiteness and chroma were calculated following the method described by Park (1994) using Eqs. 3 and 4:

$$WI = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2} \quad \text{(Eq. 3)}$$

$$C^* = (a^{*2} + b^{*2})^{0.5} \quad \text{(Eq. 4)}$$

Texture profile analysis (TPA)

The TPA of sausage samples for each treatment group was conducted using a Texture Analyser TX-700 (Lamy Rheology, France). The load cell employed was a cylindrical probe with a diameter of 50 mm, and equipped with a 50 kg sensor. The samples underwent compression twice with the following parameters: 40% height at a deformation rate of 1 mm/s; down speed of 1 mm/s; force to start, 0.5 N; wait position, 5 mm; delay, 5 s; and up speed, 1 mm/s. A force-time curve was recorded, and peak force, time difference, and area of peaks were calculated from the recorded data.

Determination of biochemical parameters of sausage

The total volatile base nitrogen (TVB-N) was determined following the official European steam-distillation method (EU, 2008). The peroxide value (PV) and free fatty acid (FFA) content of the sausage were measured according to AOAC (2005). Thiobarbituric acid reactive substances (TBARS) were determined according to Tarladgis *et al.* (1960).

Determination of microbiological parameters of sausage

Microbial quality, such as total plate count (TPC) and total psychrophilic count (PC) of sausages stored at refrigerated temperature (4 ± 1°C) were enumerated and reported according to BAM (2004).

Sensory parameters of sausage

The sensory attributes of the sausage samples from each treatment group were assessed by a 10-member trained panel from the Department of Post-harvest Technology, ICAR-CIFE, Mumbai. The panel, comprised of staff and students familiar with sausage consumption, evaluated the samples. The sausages were sliced into thin portions and presented to the panellists on fibre plates. Evaluation criteria included colour, odour, taste, texture, flavour, appearance, and overall acceptability, using a 9-point hedonic scale as described by Mailgaad *et al*. (1999).

Statistical analysis

The data were obtained in triplicate, and subjected to a One-way analysis of variance (ANOVA). The results were expressed as mean ± standard deviation. Duncans' Multiple Range Test (DMRT) was conducted to test the significance difference ($p < 0.05$).

Results and discussion

Changes in proximate composition and pH of sausages

The changes in proximate composition and pH of the sausages during refrigerated storage are presented in Table 1. All proximate parameters exhibited significant differences ($p < 0.05$) throughout the storage period. Moisture content initially decreased from 67.11 to 65.85% by day 35, then increased to 67.67% by day 49. Protein content decreased from 15.82 to 13.81% by day 28, followed by an increase to 14.61% on day 49. Fat content decreased from 7.26 to 6.33% by day 21, with minor fluctuations thereafter. Carbohydrate content increased from 4.53 to 8.81% by day 28, while ash

Table 1. Changes in proximate composition and pH of sausages during refrigerated storage.

	Storage day							
	0th Day	**7th Day**	**14th Day**	**21st Day**	**28th Day**	**35th Day**	**42nd Day**	**49th Day**
Moisture (%)	67.11 ± 0.06^{c}	68.01 ± 0.25^{f}	67.72 ± 0.01^{e}	66.93 ± 0.05^{c}	64.91 ± 0.24^{a}	66.17 ± 0.20^{b}	67.39 ± 0.09^{d}	67.67 ± 0.00^{e}
Protein (%)	15.82 ± 0.03^{b}	14.46 ± 0.77^{ab}	14.60 ± 0.81^{ab}	13.98 ± 0.84^{a}	13.81 ± 1.49^{a}	14.80 ± 0.09^{ab}	14.74 ± 0.78^{ab}	14.61 ± 0.82^{ab}
Fat (%)	7.26 ± 0.22^{cd}	7.14 ± 0.07^{cd}	7.01 ± 0.06^{c}	6.69 ± 0.13^{b}	7.34 ± 0.06^{d}	6.60 ± 0.35^{ab}	6.33 ± 0.10^{a}	6.98 ± 0.07^{c}
Ash (%)	2.81 ± 0.04^{c}	2.74 ± 0.04^{b}	2.74 ± 0.01^{b}	2.74 ± 0.02^{b}	2.73 ± 0.01^{b}	2.76 ± 0.03^{bc}	2.67 ± 0.03^{a}	2.72 ± 0.02^{b}
Carbohydrate (%)	4.53 ± 0.16^{a}	5.11 ± 1.08^{ab}	5.31 ± 0.93^{ab}	6.86 ± 0.03^{abc}	8.81 ± 2.17^{c}	7.17 ± 0.44^{bc}	6.25 ± 1.16^{ab}	6.26 ± 0.27^{ab}
Crude fibre (%)	2.23 ± 0.10^{b}	2.14 ± 0.07^{ab}	2.13 ± 0.05^{ab}	2.12 ± 0.01^{ab}	2.10 ± 0.01^{ab}	2.09 ± 0.01^{ab}	2.04 ± 0.05^{a}	2.03 ± 0.08^{a}
pH	6.39 ± 0.00^{b}	6.39 ± 0.01^{b}	6.38 ± 0.01^{ab}	6.34 ± 0.01^{ab}	6.35 ± 0.00^{ab}	6.39 ± 0.00^{b}	6.33 ± 0.08^{a}	6.33 ± 0.01^{a}

Data are mean ± SD of triplicates (n = 3). Different lowercase superscripts in similar row indicate statistical difference ($p < 0.05$).

content remained between 2.67 and 2.81%. Fibre content showed a slight decrease from 2.23 to 2.03% over the storage period.

The inclusion of corn starch, dietary oat fibre, and CLO influenced the moisture content of the sausages. Similarly, Zapata and Pava (2018) found that adding quinoa flour increased protein, ash, and fibre contents while reducing moisture in sausages made from tilapia fillet waste. Sini *et al*. (2008) noted a decrease in protein content in rohu sausages due to the inclusion of corn flour, while hydrogenated oil increased fat content. Rahmanifarah *et al.* (2015) reported stable moisture levels in fish sausages from silver carp during refrigerated storage. Oksuz *et al.* (2008) observed a decrease in moisture in dry African catfish sausages stored at 4°C, with protein content increasing from 20.71 to 42.5% after 70 days due to moisture reduction. In mortadella, an inverse relationship between protein and fish oil was noted (Cáceres *et al*., 2008), and a decrease in moisture content was observed in lantern fish sausages prepared with fish protein isolate (Moosavi Nasab *et al*., 2018).

Amiza and Ng (2015) reported a significant interaction between the surimi-to-silver catfish mince ratio and the level of potato starch (PS) on fat and ash contents in fish sausages. Higher fat content was observed in sausages with 0/100 (surimi-silver catfish mince), 3% PS; 40/60, 3% PS; and 0/100, 7% PS. Cardoso *et al*. (2010) found a non-significant increase in carbohydrate content in minced fish products fortified with dietary fibre and ω3 fatty acids stored at 2 and 10°C.

A significant difference ($p < 0.05$) in the pH of rohu sausages was observed during refrigerated storage at 4°C. Initially, the pH was 6.39, which decreased to 6.33 by day 49, likely due to acid generation from biochemical reactions. This was consistent with Oliveira Filho *et al*. (2010), who reported a pH drop in minced Nile tilapia sausages during storage. Cardoso *et al.* (2010) also observed pH changes in fibre-enriched fish products, attributing them to biochemical reactions. In contrast, Chattopadhyay *et al*. (2019) found a slight pH increase in chitosan-containing sausages, likely due to protein degradation and ammonia formation. Other studies, such as Majumdar *et al.* (2015), Dinçer *et al.* (2017), and Maheshwara *et al.* (2017), similarly reported pH changes in fish sausages influenced by ingredients and storage conditions. Factors like fish species, diet, and stress levels also affect pH (Periago *et al*., 2005).

Changes in physical and functional properties of sausages

Table 2 summarises the changes in the physical and functional properties of sausages, including gel strength, WHC, and folding characteristics during refrigerated storage. Figure 2 illustrates gel strength, which significantly varied ($p < 0.05$) throughout storage. Gel strength ranged from 191.28 to 214.18 g·cm, increasing from an initial 191.28 to 214.18 g·cm by day 42, then decreasing to 208.34 g·cm on day 49. Notably, the lowest gel strength (193.45 g·cm) after the initial increase was observed on day 21. The enhanced gel strength may be attributed to the inclusion of starch and dietary fibre. Hake mince frankfurter sausages with 5.2% chicory root inulin and additional hake mince (without pork fat) showed a significant increase ($p < 0.05$) in gel strength over 80 days at 2 ± 1°C, compared to those with 7.8% pork fat (Cardoso *et al*., 2008). This increase indicates greater firmness during storage. Conversely, Maheshwara *et al.* (2017) reported a decrease in gel strength from 325 to 190 g·cm in Bull's Eye fish sausages over 25 days.

The WHC declined during refrigerated storage, ranging from 92.04 to 85.79%. The highest WHC (92.04%) was recorded on the first day, decreasing to 86.15% by day 49, with the lowest value (85.79%) observed on day 21. This decrease may be attributed to moisture loss, as no additional water was added during formulation. Fibres from cereals typically exhibit a lower capacity for retaining water and oil due to their chemical properties (Elleuch *et al*., 2011). Balange and Benjakul (2009) reported that mince had lower WHC compared to surimi. In contrast, Desmond *et al.* (1998) found that adding tapioca starch and oat fibre to low-fat beef burgers improved WHC, which increased with higher concentrations of these ingredients. Hughes *et al*. (1997) also noted that carrageenan and oat fibre enhanced WHC in frankfurters; however, decreasing fat content from 30 to 5% decreased WHC, negatively impacting product characteristics. Similarly, Maheshwara *et al*. (2017) reported an increase in expressible moisture content from 5.01 to 14.16% in Bull's Eye fish sausages after 25 days of refrigeration, potentially due to reduced gel strength.

Table 2. Changes in physical and functional properties of sausages during refrigerated storage.

Functional property	Storage day							
	0th Day	**7th Day**	**14th Day**	**21st Day**	**28th Day**	**35th Day**	**42nd Day**	**49th Day**
Gel strength (g.cm)	191.28 ± 5.28^{a}	195.40 ± 14.19^{ab}	199.49 ± 10.50^{abc}	193.45 ± 15.41^{ab}	202.03 ± 9.79^{abc}	211.18 ± 7.31^{c}	214.18 ± 9.26^{c}	208.34 ± 8.42^{bc}
WHC (%)	92.04 ± 0.83^{d}	90.11 ± 0.64^{cd}	89.21 ± 1.68^{bc}	85.79 ± 2.11^{a}	87.01 ± 1.59^{ab}	87.82 ± 0.54^{ab}	87.27 ± 0.84^{ab}	86.15 ± 0.79^{a}
Folding score	2.67 ± 0.58^{a}	2.67 ± 0.58^{a}	2.00 ± 0.00^{a}	2.33 ± 0.58^{a}	2.00 ± 0.00^{a}	2.33 ± 0.58^{a}	2.00 ± 0.00^{a}	2.00 ± 0.00^{a}

Data are mean ± SD of triplicates (n = 3). Different lowercase superscripts in similar row indicate statistical difference ($p < 0.05$).

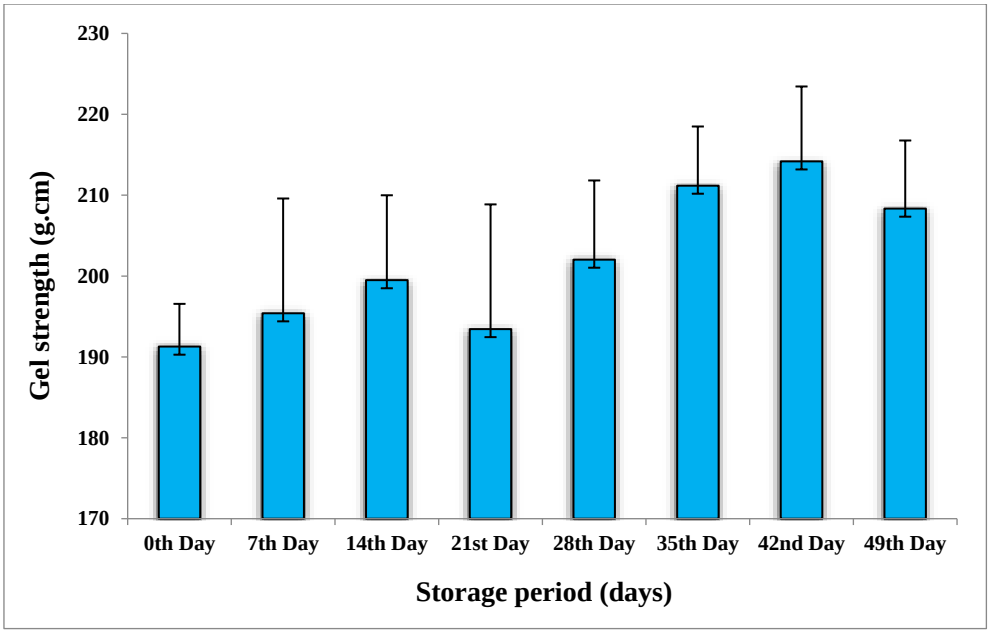

Figure 2. Changes in gel strength (g.cm) of sausages during refrigerated storage. Bars indicate mean ± SD. Means with different lowercase letters are significantly different ($p < 0.05$).

Folding scores ranged from 2.0 to 2.33, and remained consistent over 49 days of refrigerated storage, with no significant differences ($p > 0.05$) observed in sausages stored at these temperatures. This stability may be attributed to the use of powdered starch and oat fibres in the sausage formulation. In contrast, Maheshwara *et al.* (2017) reported a decrease in folding scores from AA to C in Bull's Eye fish sausages after 25 days of refrigerated storage.

Changes in colour attributes of sausages

Colour is a key quality attribute influencing consumer acceptance of fish products. The instrumental colour parameters of sausages, including whiteness, *L**, *a**, *b**, and chroma, are presented in Table 3, with significant differences ($p < 0.05$) observed over 49 days of refrigerated storage. The highest whiteness (72.55) and lightness (76.18) values occurred on day 35, while the lowest values for both (68.96 and 72.54, respectively) were noted on day 49. No consistent trend in whiteness was observed, which could be due to the white starch and yellowish oat fibre masking the colour of CLO, contributing to elevated whiteness and lightness (Muthia *et al.*, 2010). The enhanced whiteness may also be attributed to an increase in lightness (*L** values) due to light dispersion caused by the presence of emulsified oil droplets (Pérez-Mateos *et al.*, 2004). However, the decrease in these values by day 49 may be linked to bacterial or enzymatic activity.

The *a** values of sausages ranged from 0.61 to 1.13, with redness increasing over time, but decreasing by day 49. The red coloration may result from pigment leaching from minced meat and CLO. Chroma values, primarily influenced by *b** values, ranged from 13.57 to 14.46, with *b** increasing until day 28, then decreasing until day 42, and peaking on day 49. Yellowness followed a similar pattern, increasing until day 28, decreasing, and then increasing again by day 49. This variation may be due to the oat fibre's colour, with inconsistent mixing leading to fluctuating yellowness. Starch content also affected yellowness, with higher starch levels reducing yellowness (Hughes *et al.*, 1997; Amiza and Ng, 2015).

Changes in textural characteristics of sausages

Texture profile analysis (TPA) is a key measure of the structural integrity of the emulsion matrix and its alignment with consumer preferences for fish products (Souissi *et al.*, 2016). Table 4 presents the changes in the textural properties of sausages during refrigerated storage. The TPA results showed a significant difference ($p < 0.05$) in hardness, while cohesiveness, adhesiveness, and elasticity remained unchanged ($p > 0.05$) over 49 days. No consistent pattern in hardness was observed, though the increase may be due to water loss and fat retention, leading to a denser emulsion matrix (Chattopadhyay *et al.*, 2019). Zapata and Pava (2018) found a similar increase in hardness with quinoa flour

Table 3. Changes in colour attributes of sausages during refrigerated storage.

Colour attribute	Storage day							
	0th Day	**7th Day**	**14th Day**	**21st Day**	**28th Day**	**35th Day**	**42nd Day**	**49th Day**
Whiteness	71.24 ± 0.17^{b}	71.83 ± 0.45^{bcd}	71.55 ± 0.95^{bc}	72.14 ± 0.42^{cd}	71.08 ± 0.48^{b}	72.55 ± 0.13^{d}	72.06 ± 0.36^{cd}	68.96 ± 0.85^{a}
*L**	74.80 ± 0.15^{b}	75.56 ± 0.46^{bcd}	75.19 ± 1.14^{bc}	75.96 ± 0.46^{cd}	74.77 ± 0.63^{b}	76.18 ± 0.13^{d}	75.57 ± 0.46^{bcd}	72.54 ± 1.06^{a}
*a**	0.61 ± 0.12^{a}	0.63 ± 0.07^{a}	0.72 ± 0.09^{abc}	0.69 ± 0.14^{ab}	0.82 ± 0.23^{bc}	0.88 ± 0.05^{c}	1.13 ± 0.08^{d}	0.74 ± 0.16^{abc}
*b**	13.85 ± 0.42^{ab}	13.99 ± 0.29^{abc}	13.90 ± 0.41^{ab}	14.05 ± 0.24^{bc}	14.10 ± 0.53^{bc}	13.60 ± 0.10^{ab}	13.52 ± 0.33^{a}	14.45 ± 0.42^{c}
Chroma	13.86 ± 0.42^{ab}	14.00 ± 0.29^{abc}	13.92 ± 0.41^{ab}	14.07 ± 0.24^{abc}	14.13 ± 0.54^{bc}	13.64 ± 0.10^{ab}	13.57 ± 0.34^{a}	14.46 ± 0.42^{c}

Data are mean ± SD of five replicates (n = 5). Different lowercase superscripts in similar row indicate statistical difference ($p < 0.05$).

Table 4. Changes in textural characteristics of sausages during refrigerated storage.

Textural characteristic	Storage day							
	0th Day	**7th Day**	**14th Day**	**21st Day**	**28th Day**	**35th Day**	**42nd Day**	**49th Day**
Hardness (N)	8.17 ± 0.21^{a}	11.06 ± 0.88^{cde}	10.88 ± 0.68^{cd}	10.55 ± 0.35^{c}	12.04 ± 0.45^{de}	9.01 ± 0.76^{ab}	9.78 ± 1.28^{bc}	12.36 ± 0.82^{e}
Cohesiveness	0.83 ± 0.05^{a}	0.82 ± 0.08^{a}	0.82 ± 0.02^{a}	0.82 ± 0.05^{a}	0.78 ± 0.05^{a}	0.78 ± 0.05^{a}	0.74 ± 0.05^{a}	0.77 ± 0.09^{a}
Adhesiveness (J/m^3)	1.97 ± 1.76^{a}	0.63 ± 0.20^{a}	1.43 ± 0.65^{a}	0.53 ± 0.75^{a}	1.63 ± 1.56^{a}	3.67 ± 3.80^{a}	2.50 ± 1.73^{a}	1.23 ± 1.63^{a}
Elasticity (mm)	1.04 ± 0.04^{a}	1.07 ± 0.03^{a}	1.06 ± 0.02^{a}	1.58 ± 0.03^{a}	1.07 ± 0.04^{a}	1.04 ± 0.05^{a}	1.06 ± 0.06^{a}	1.06 ± 0.06^{a}

Data are mean ± SD of triplicates (n = 3). Different lowercase superscripts in similar row indicate statistical difference ($p < 0.05$).

in frankfurter sausages. In mortadella, higher fish oil content led to increased hardness, cohesiveness, and adhesiveness due to pre-emulsification with caseinates (Cáceres *et al*., 2008). The gradual increase in hardness during refrigeration may also result from protein denaturation (Sini *et al*., 2008).

Cohesiveness remained consistent throughout storage with no significant differences ($p > 0.05$), aligning with the findings of Dinçer *et al.* (2017). Likewise, springiness exhibited no significant variation over time. In the present work, cohesiveness may be linked to the CLO content in the sausages. However, Pietrasik (1999) and Prabpree and Pongsawatmanit (2011) reported that starch levels did not affect sausage springiness, and both starch and fat contents had no impact on springiness (Amiza and Ng, 2015).

Changes in biochemical parameters of sausages

The variations in biochemical parameters, including TVB-N, PV, FFA, and TBARS in sausages stored under refrigerated conditions are shown in Figure 3. Significant differences ($p < 0.05$) were observed in all biochemical parameters over the 49-day storage period.

TVB-N, an indicator of fish quality deterioration, increased significantly from 2.51 mgN/100 g on day 0 to 35.11 mgN/100 g by day 49, though the values remained within the acceptable limit of 35 - 40 mgN/100 g. Similar trends were reported by Çoban *et al.* (2019) in common carp sausages treated with propolis, and by Nithin *et al.* (2015) who observed higher TVB-N values in control yellowfin sausages compared to those with liquid smoke flavouring. Maheshwara *et al.* (2017) also found increasing TVB-N levels in Bull's-Eye fish sausages during refrigerated storage due to protein hydrolysis. Sausages made from Japanese threadfin bream fish treated with nisin (25 or 50 ppm) showed the least TVB-N increase during storage (Raju *et al*., 2003).

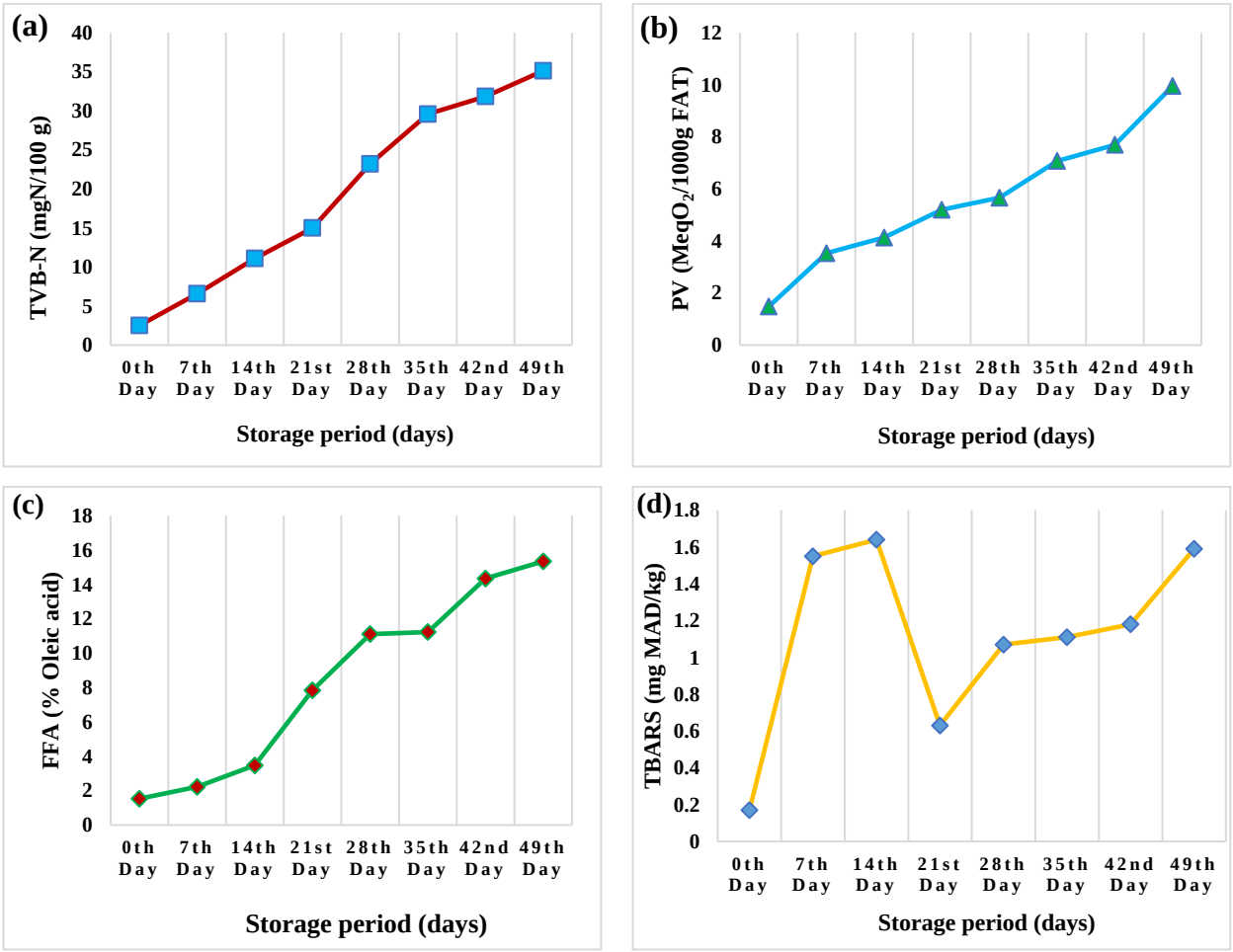

Figure 3. Changes in **(a)** TVB-N, **(b)** PV, **(c)** FFA, and **(d)** TBARS contents of sausages during refrigerated storage.

The progression of oxidative rancidity in fish is evaluated by PV, which indicates the extent of lipid oxidation. This rancidity leads to off-flavours and off-odours, degrading the quality and shelf life of fish products. In the present work, PV values ranged from 1.48 to 9.95 meqO_2/1000 g fat. A significant increase ($p < 0.05$) was observed, with PV increasing from 1.48 meqO_2/1000 g fat on day 0 to 9.95 meqO_2/1000 g fat on day 49, though it remained within the acceptable limit of 10 meqO_2/1000 g fat. Similar results were reported by Çoban *et al.* (2019), who found PV values reaching 11.30 meqO_2/1000 g fat after nine weeks of refrigerated storage in common carp sausages. Maheshwara *et al.* (2017) also noted higher PV values (14.5 mmol O_2/kg fat) in Bull's-Eye sausages stored for 25 days. In lantern fish sausages treated with fish protein isolate (FPI), PV increased during 60 days at 4°C (Moosavi Nasab *et al*., 2018). Raju *et al.* (2003) observed a slower increase in PV in Japanese threadfin bream sausages treated with 50 ppm nisin compared to control samples during refrigeration.

The formation of FFA in sausages during cooking primarily results from the breakdown of triglycerides and phospholipids (Gallardo *et al*., 1989). Additionally, the activity of bacterial lipases or enzymatic hydrolysis can contribute to FFA generation, indicating hydrolytic rancidity (Rodríguez *et al*., 2008). The initial FFA levels in the product reflect a combination of these factors. In the present work, FFA values ranged from 1.53 to 15.34% oleic acid during refrigerated storage, with the maximum FFA value of 15.34% oleic acid reached by the end of the 49-day storage period. Çoban *et al.* (2019) reported a significant increase in FFA values for control carp sausages, increasing from 2.36 to 14.90% oleic acid, while sausages with 1 and 2% propolis showed increases from 2.35 to 8.85% and 2.33 to 5.67% oleic acid, respectively, during refrigeration. Higher FFA levels were also found in control tilapia sausages, attributed to the enzymatic hydrolysis of phospholipids and triglycerides (Basamma *et al*., 2015). However, Nithin *et al*. (2015) suggested that FFA formation during storage is primarily due to lipid degradation from bacterial action, as most lipases are heat-labile and denature during heat treatment. Similar findings were noted by Moosavi-Nasab *et al.* (2018). Raju *et al.* (2003) observed increased FFA values in Japanese threadfin bream fish sausages treated with nisin, with a slower rate of increase in the 50 ppm nisin-treated samples compared to the control during refrigeration.

TBARS are widely recognised as indicators of oxidative rancidity in fish muscle, with increasing values signalling the formation of secondary lipid oxidation products, particularly aldehydes. In the present work, TBARS values ranged from 0.17 to 1.64 mg malondialdehyde (MDA)/kg of sausage, exhibiting significant differences ($p < 0.05$) during refrigerated storage. A notable increase in TBARS was recorded on the 7th day, followed by a sharp decrease on the 21st day, after which levels stabilised. This variation may be attributed to the high levels of ω-3 PUFA in CLO, which are more susceptible to oxidation (Pérez-Mateos *et al*., 2004). Cardoso *et al.* (2010) similarly observed fluctuating MDA content in minced hake products containing CLO during refrigeration at 2°C. The elevated oxidation levels early in CLO product preparation likely stem from the cooking process (90°C for 1 h), as ω-3 PUFA are more prone to thermal degradation than vegetable oils. Importantly, TBARS values remained below 0.5 mg MDA/kg, well within the acceptable limit of 1 - 2 µmol malondialdehyde/g fat proposed by Connell (1995) for fish products. Furthermore, fluctuations in TBARS indicated low lipid oxidation rates, likely linked to increased MDA degradation through reactions with muscle proteins (Kristinsson *et al*., 2006). Panpipat and Yongsawatdigul (2008) also reported elevated TBARS values associated with substituting vegetable oil with tuna oil in vacuum-packaged sausages.

Changes in microbial quality of sausages

In the present work, microbial quality changes were assessed through total plate count (TPC) and psychrophilic count (PC), revealing a consistent increase in both counts (Table 5). The PC remained undetected until the 7th day of refrigerated storage, after which it increased from 3.30 to 5.24 log CFU/g by the 49th day. Importantly, both TPC and PC levels remained well below the maximum acceptable limits of 7 and 6 log CFU/g for fish, respectively (ICMSF, 2002). These low bacterial counts can be attributed to high-quality raw materials, hygienic heat processing, and optimal storage conditions, with heat treatment likely inactivating vegetative microbial cells. However, a gradual increase in bacterial counts was noted throughout the storage period.

Table 5. Changes in microbial quality of sausages during refrigerated storage.

Microbial quality	Storage day							
	0th Day	**7th Day**	**14th Day**	**21st Day**	**28th Day**	**35th Day**	**42nd Day**	**49th Day**
TPC (log CFU/g)	3.35 ± 0.01[a]	3.88 ± 0.01[b]	3.94 ± 0.02[b]	4.16 ± 0.06[c]	4.34 ± 0.03[d]	5.20 ± 0.04[e]	5.24 ± 0.01[e]	6.02 ± 0.02[f]
PC (log CFU/g)	ND	ND	3.30 ± 0.00[a]	3.53 ± 0.01[b]	3.90 ± 0.01[c]	4.02 ± 0.02[d]	4.31 ± 0.01[e]	5.24 ± 0.02[f]

Data are mean ± SD of triplicates (n = 3). Different lowercase superscripts in similar row indicate statistical difference ($p < 0.05$). ND: not detected.

Similar findings were reported by Hegde *et al.* (1990), who observed an increase in TPC from 1.4 × 10^2 to 5.2 × 10^4 CFU/g in croaker fish sausage containing potato starch during 56 days of storage at 0 - 5°C. Maheshwara *et al*. (2017) noted that sausages remained acceptable until the 25th day, with counts ranging from 2.6 × 10^2 to 3.7 × 10^5 CFU/g, likely due to the combined effects of heat processing and storage temperature. Cardoso *et al.* (2008) found lower bacterial counts in frankfurters containing dietary fibre, which could be attributed to heat treatment (10 min at 90°C), vacuum packaging, chill storage, and salt addition. Additionally, lower total viable and psychrophilic counts were observed in chitosan-treated pangasius sausages during 60 days of refrigerated storage (Chattopadhyay *et al*., 2019).

Changes in sensory quality of sausages

Sensory evaluation of sausages, assessing attributes such as appearance, colour, odour, taste, flavour, texture, and overall acceptability, was conducted using a 9-point hedonic scale during 49 days of refrigerated storage at 4°C (Figure 4). Scores decreased for several attributes: appearance (from

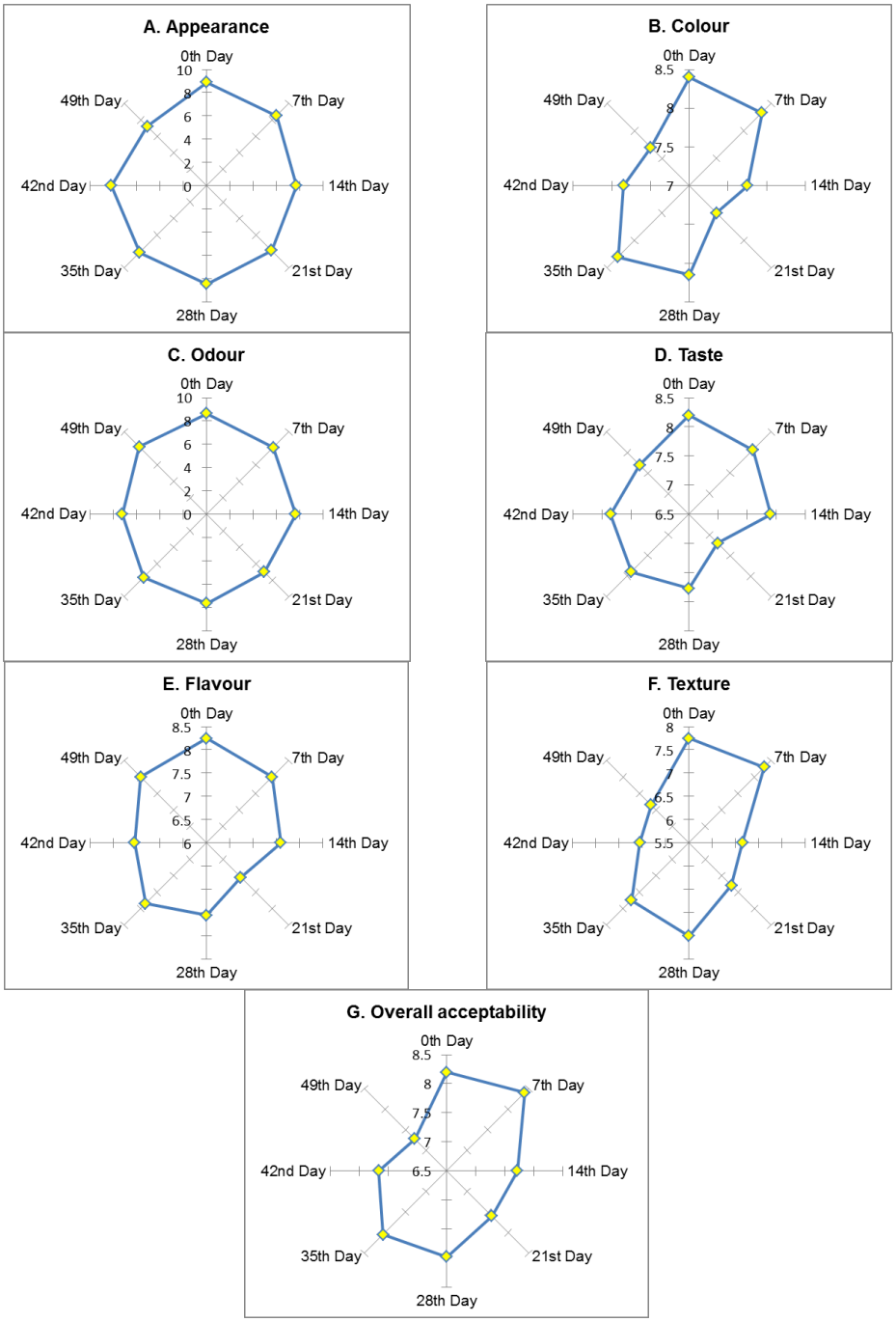

Figure 4. Changes in sensory scores of sausages during refrigerated storage: **(A)** appearance, **(B)** colour, **(C)** odour, **(D)** taste, **(E)** flavour, **(F)** texture, and **(G)** overall acceptability.

8.90 to 7.20), colour (from 8.40 to 7.70), odour (from 8.65 to 8.20), taste (from 8.20 to 7.70), flavour (from 8.25 to 8.00), texture (from 7.75 to 6.65), and overall acceptability (from 8.20 to 7.28). Sausages were formulated from freshwater fish. Panellists generally preferred marine fish, which may have influenced their sensory evaluations of these freshwater fish sausages. Despite this variability, the sausages received a "moderately liked" rating overall after 49 days. No significant darkening, off-odours, or off-flavours were reported, except for slight saltiness, indicating that CLO and oat fibre effectively masked the natural fishy odour. These findings were consistent with those of Cardoso *et al.* (2010), who noted that the addition of CLO to minced hake products resulted in a darker colour perception, while other sensory attributes remained unchanged during storage at 2 and 10°C. Pérez-Mateos *et al.* (2004) reported that the incorporation of refined menhaden and purified marine oils caused minimal changes in the sensory attributes of surimi seafood products, including crab analogs, during two months of refrigerated storage. Similarly, Hughes *et al.* (1997) found that reducing fat content in frankfurters from 30 to 5% significantly affected flavour characteristics, whereas the addition of oat fibre or carrageenan had no effect, and no interaction was observed between fat levels and other ingredients in the sensory analysis of frankfurters.

Conclusion

Rohu fish mince sausages, formulated with optimised concentrations of corn starch, CLO, and dietary oat fibre, demonstrated changes in various physicochemical and textural properties during refrigerated storage, reflecting the dynamic nature of sausage quality during storage. The incorporation of carbohydrates and fats influenced moisture content, while fibre content decreased over time. Despite changes in gel strength and WHC, the sausages maintained acceptable quality parameters throughout the 49-day refrigerated storage period. Importantly, the biochemical and microbiological quality parameters, including TVB-N, PV, FFA, TBARS, total plate count, and psychrophilic count, remained within acceptable limits, indicating the overall safety and quality of the fortified sausages. Since the study specifically used rohu fish to prepare the sausages, there may be some differences in observed changes when using other fish varieties due to the distinct compositions of different fishes. The present work contributed valuable insights into developing value-added fish products, offering improved nutritional value and extended storage stability. It also provided an understanding of formulation, storage, and quality maintenance strategies for fish-based sausage products, aiding the development of products with extended shelf life and consumer acceptance. The findings of the present work could guide food processors in enhancing the utilisation of rohu fish through the production of fortified fish sausages, meeting both nutritional and quality expectations with a good shelf life.

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