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Shelf-Stability of Kiln- and Liquid-Smoked Inulin-Fortified Emulsion-Type *Pangasius* Mince Sausage at Refrigerated Temperature

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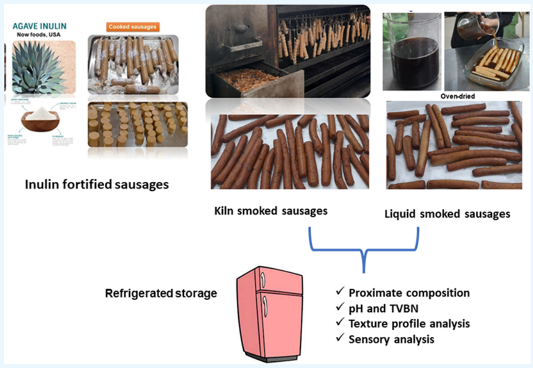

ABSTRACT: The objective of this study was to investigate the effects of the kiln (SK-S) and liquid smoking (LS-S) processes on the quality of inulin-fortified emulsion-type *Pangasius* mince sausages. The moisture content during the storage significantly ($p < 0.05$) decreased in C-S (control) sausages and increased ($p < 0.05$) in SK-S and LS-S sausages. The protein content decreased ($p < 0.05$) in C-S, SK-S, and LS-S throughout the storage period. Initially, among the three processed sausages, LS-S showed a lower pH value, and as the days of storage progressed, all the treatments exhibited a declining trend ($p < 0.05$). A significant ($p < 0.05$) increase in the PV was observed in all the sausages during the storage days at 5 ± 1 °C, but the intensity of the increase was lower in SK-S and LS-S. The total viable count of C-S and SK-S sausages reached the limit of acceptability (6 $\log_{10}$cfu g^{-1}) on the 20th day and on the 24th day of storage. The electrophoretic protein pattern of LS-S samples exhibited retention of all bands, indicating the lower proteolysis of MHC, actin, and troponin T in comparison with other treatments. The hardness ($p < 0.05$) and cohesiveness ($p > 0.05$) values of both SK-S and LS-S reduced as the storage days progressed. The present study indicates that the emulsion-type *Pangasius* sausages incorporated with inulin powder (2%) exposed to kiln smoking and commercial liquid smoking retained good-to-better sensory attributes up to day 16 (C-S) and day 20 (SK-S and LS-S) under refrigerated storage at 5 ± 1 °C in low-density vacuum polyethylene (LDPE) pouches.

1. INTRODUCTION

Food fortification is the practice of enhancing commonly consumed foods with bioactive substances to improve their nutritional value. The technique is a cost-effective, sustainable, high-impact solution and the accepted method for improving the diet as well as the prevention and control of health risks of consumers.[1] Chadare et al.[2] stated that an optimum fortificant must be adopted, factoring both the target population's eating patterns and the appropriate processes and fortification ranges from 1 to 50%, as per the affinity between the fortifying ingredient and the vehicle foods. For the development of new innovative fish products, minced fish (MF) is one of the good material(s).[3] Among the various value-added products made from fish, sausages are considered popular because of their great taste and quality.[4] Sausages are made by stuffing additives and minced fish meat into casings to produce the finished product.

Pangasianodon hypophthalmus meat possesses high excellent nutritional and sensory properties and can be filleted easily due to the absence of the intramuscular pin bone with a good yield of minced meat. Fish is an excellent source of protein, fat, minerals, and vitamins but lacks dietary fiber content. Additionally, the consumption of dietary fiber-rich food (soluble and insoluble) is related to reducing the risks associated with health hazards. Water-soluble fiber is more beneficial than insoluble fiber and also helps in lowering cholesterol and glucose levels in the blood. In addition to improving the nutritional quality of meat products, fiber significantly improves the functional properties of protein and extends the product's shelf-life due to its bioactive properties (antioxidant and antimicrobial). 'Soluble fibers' based on viscosity are classified into (i) viscous: glucan, gum, pectin, psyllium, and galactomannan and (ii) nonviscous: inulin-resistant starch, resistant dextrins, fructo-oligosaccharides, and polydextrose).[5] Inulin is one of several soluble fibers that is currently gaining prominence as a fortifier and texture modifier in meat-based products.

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Various combinations of processing techniques, such as drying, smoking, and additives, have recently been used to retain the quality and enhance the stability of sausages (extend the shelf-life). The smoked sausage market has lately surged in comparison with cooked sausage due to its typical flavor and odor. Smoking is typically done via the conventional method, which involves exposure to smoke from burning wood.[6] The intensive uncontrolled smoking and unsanitary handling practices used during the smoking process deposit the carcinogenic polycyclic aromatic hydrocarbon (PAH)[7] on the fish. In smoked foods, several technical approaches have been implemented to reduce PAH contamination. The most effective method of smoking is to use liquid smoke or indirect smoking. Liquid smoke contains organic components with antibacterial and antioxidative properties such as phenols and acetic acid that impede the spoilage-causing microbes and lengthen the shelf-life of the product.[8] The shelf-life of food relies on factors such as (i) its components, (ii) processing method, (iii) sort of packaging used, and (iv) the storage conditions. To achieve the desired shelf-life, it is essential to comprehend the interactions between the four factors and optimizing each one.[9] Temperature (pasteurization, sterilization, refrigeration, chilling, or freezing) is a key factor in increasing the length of storage of the processed products.[10] Therefore, the current study was aimed to develop inulin-fortified kiln- and liquid-smoked sausage and further investigate its stability at refrigerated temperatures (5 ± 1 °C).

2. MATERIALS AND METHODS

2.1. Materials. *Pangasius* fish (*Pangasionodon hypophthalmus*) with the average length and weight of 46 ± 2 cm and 1100 ± 85 g, respectively, were bought from the Agartala fish market. The fish were washed, filleted, and minced under hygienic conditions. The minced meat was packed in LDPE pouches and kept in ice condition until the sausage was prepared. Commercial inulin (derived from blue agave, Now foods, Bloomingdale, USA) and hickory-flavored liquid smoke (Colgin, Dallas) were purchased. The ingredients for the preparation of sausage were purchased locally, such as ginger powder (Zizira, Meghalaya); wheat flour (Ashirwad brand); garlic powder, soya bean flour, annatto seeds, and herb mix (urban platter); vegetable oil (sunflower; Freedom brand), and Himalayan pink salt (Chef Urbano). All the reagents utilized were of analytical grade.

2.2. Preparation of Inulin-Fortified Emulsion-Type *Pangasius* Mince Sausage. The sausages were prepared by the method described by Sherpa et al.,[11] with mince to other ingredients in the ratio of 85:15. In a stainless steel silent cutter (Table Fine Cutter; India), 850 g of pangasius mince was adequately amalgamated with 25 g of salt prior to being minced for 3 min. Minced pangasius meat was combined for 2 min with 10 mL of vegetable refined oil that had previously been infused with annatto seeds. Then, 10 g sugar, 20 g soya flour, 15 g wheat flour, 10 g ginger powder, 10 g garlic powder, and 10 g of herb mix containing oregano, rosemary, basil, and parsley were incorporated to the batter mix along with 40 g of ice cubes. To this batter, a 20 g of blue agave inulin powder was added and blended well for another 2 min. Employing a hand-stuffer (Model no. 12009, Fantes Sausage Maker with Suction Base; HIC Harold Import Co., Lakewood, NJ, USA), the sausage batter was pumped into cellulose casings (2.5 cm diameter). The stuffed sausages were cooked for 45 min at 90 ± 2 °C in a temperature-controlled water bath (VS-1205N-T, Vision Scientific Ltd., Korea). The sausages were cooled, and the casing was peeled off, packed, and stored in LDPE at a refrigerated temperature till the smoking operation was performed.

2.3. Total Phenolic Content and Antioxidant Activity of Liquid Smoke. The total phenolic content of liquid smoke was measured using the Folin−Ciocalteau reagent (FCR) following the method described by Slinkard and Singleton[12] with a few modification(s). A 100 μL of liquid smoke was mixed with 0.75 mL of FCR, which was prediluted in distilled water 10-fold. After 5 min, 0.75 mL of sodium carbonate solution (6% v/v) was added to the mixture. After mixing, the solution was allowed to stand at room temperature for 1 h. Using a spectrophotometer, the absorbance at 760 nm was read (Thermo Fisher Scientific, Finland). Tannic acid (Merck, India) standard solutions ranging from 0 to 600 ppm were used to create the standard curve. The total phenolic content in liquid smoke was expressed as mg tannic acid equivalents per mL.[13]

The DPPH radical scavenging activity of liquid smoke was analyzed by the method described by Dhanabalan et al.[14] The 2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt radical scavenging activity of the liquid smoke was measured by the method of Arnao et al.[15]

2.4. Preparation of Kiln- and Liquid-Smoked Inulin-Fortified Emulsion-Type Sausage. Both kiln- and liquid-smoked sausages are processed according to the method of Filho et al.[16] For the preparation of kiln-smoked sausages, the inulin-fortified emulsion type sausage was hung on hooks inside a masonry smoker (Smoking kiln, Suan, Kolkata, India) and maintained for 30 min/60 °C. It was further subjected to 3 h at 80−90 °C and cooled down to a temperature of 6 °C. Sausages were vacuum-packed using a vacuum machine (KoneteollisuusOy, Finland) under a refrigeration temperature of 5 ± 1 °C.

To prepare liquid-smoked sausages, inulin-fortified emulsion-type sausages are submitted to forced air circulation in an oven (30 min/60 °C) (Hot air dryer-Suan, Kolkata, India) and are immersed in 5% (15 min) hickory liquid smoke (optimized based on sensory evaluation). Again, the sausages were submitted to forced air circulation in the oven (3 h for 80 °C), cooled at 6 °C temperature, and stored at 5 ± 1 °C in a vacuum pack.

2.5. pH, Total Volatile Base Nitrogen, and Lipid Oxidation. The pH was analyzed using a digital pH meter (LMMP-30, Labman, India) equipped with an insertion electrode. The methods used to measure TVBN, peroxide value, free fatty acids, and thiobarbituric acid-reactive substance were steam distillation,[17] iodometric assay,[18,19] and spectrophotometric method.[20]

2.6. Total Viable Count. The total viable count of bacteria in the sausage samples was measured by the spread plate technique[21] using nutrient agar.

2.7. Textural Parameters. Texture profile parameters were analyzed by a texture profile analyzer (TA, XT plus Stable Micro System, Surrey, England) as per the settings described by Buda et al.[22]

2.8. Protein Patterns. Protein patterns in different storage days of smoked fish sausages were displayed by SDS-PAGE. 15 μL of protein was mixed with the same amount of sample buffer and was loaded onto polyacrylamide gels. The method followed was according to Priyadarshini et al.[23]

2.9. Sensory Scores. Sensory evaluation of smoked sausages for acceptability on a 9-point hedonic scale[24] was rated. The panelists were students at the College of Fisheries, who were well acquainted with the sausage products.

2.10. Statistical Analysis. The data obtained from the current study were subjected to one-way ANOVA using SPSS statistical tools (version 22.0). To determine the significant differences between the means at the 5% probability level ($p < 0.05$), Duncan's new multiple range test (at the 95% level) was conducted. All experiments were run in triplicate.

3. RESULTS AND DISCUSSION

3.1. Total Phenolic Content of the Liquid Smoke. The total phenolic content (TPC) of liquid smoke is presented in Figure 1. The liquid smoke is a result from the process of smoldering, carbonization, and fast pyrolysis of biomass as a raw material which generally contains high contents of lignin, cellulose, and hemicelluloses.[25,26] Liquid smoke contains many functional compounds such as phenolics, flavonoids, acids, furans, and ketones, giving the treated food a unique and pleasant woody and smoky flavor and aroma.[27] Total phenolic content (TPC) is an important indicator of the antioxidant capacity, antimicrobial ability, and flavoring intensity of liquid smoke.[28] In the present study, the TPC using the Folin–Ciocalteu assay increased (219.80, 369.37, 420.31, and 512.92 mg GAE mL^{-1}) concomitantly with an increase in the percentage (%) of liquid smoke (Hickory) at 5, 10, 15, and 20%, which could be due to the increased concentration of the phenolic content.[29] Mistry[30] screened and quantified commercial Colgin hickory-flavored liquid smoke (the same brand liquid smoke was utilized in the current study) by electrospray ionization mass spectrometry (ESI-MS). According to their study, the contents of catechol, guaiacol, and syringol were found to be 3444.20, 23.03, and 91.42 mg L^{-1} respectively.

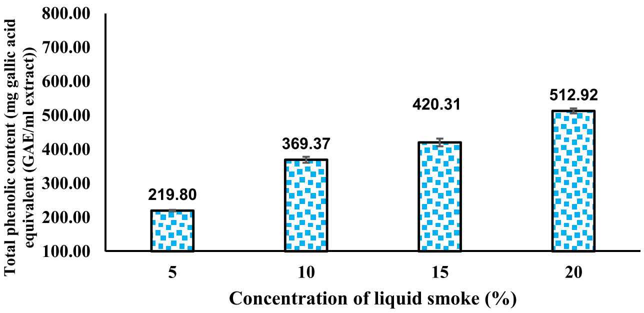

Figure 1. Total phenolic content of liquid smoke at different concentrations. The values represent the mean, and error bar represents standard deviation.

3.2. Total Antioxidant Capacity of the Liquid Smoke. The antioxidant activity assays, i.e., 2,2-diphenylpicrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, are depicted in Figure 2. The DPPH activity of liquid smoke at 5, 10, 15, and 20% was found to be 88.84, 88.70, 86.09, and 86.35%, respectively. The ABTS activity was found at 81.32, 86.45, 86.36, and 83.61% respectively. The DPPH activity slightly reduced at 15 and 20%, which might be due to the reduced dilution of high-pigmented and hydrophilic antioxidants.[31,32] The ABTS activity increased with concentration, which is due to the content of phenolic compounds and flavonoids present in the sample.[33] The quantitative screening of commercial hickory liquid smoke (Colgin brand) revealed that catechol was present in substantial amounts along with higher levels of the methoxy-substituted phenols, guaiacol and syringol, which are known to possess potential antioxidant activity.[34]

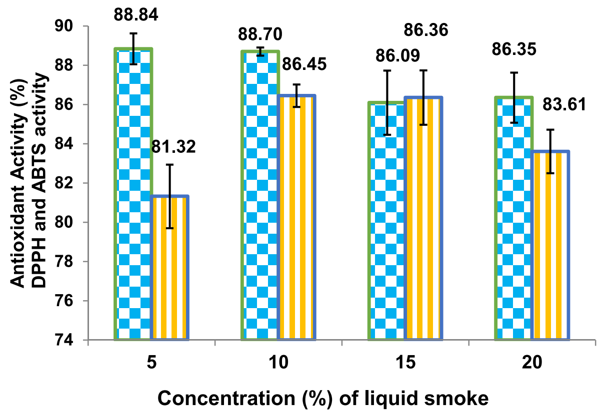

Figure 2. Total antioxidant activity (DPPH and ABTS activity) of liquid smoke at different concentrations. [checkered blue legend] represents DPPH and [striped yellow legend] represents ABTS activity. The values represent mean and error bar the standard deviation.

3.3. Proximate Composition. The proximate compositions of inulin-fortified control (C-S), kiln-smoked (SK-S), and liquid-smoked (LS-S) sausages stored at 5 ± 1 °C are represented in Table 1. In the current study, the protein, fat, and ash content increased after smoking and oven-drying (LS-S) on 0th day, which is attributed to moisture loss.[35] During the course of 20 days of storage, a considerable decline in the moisture content of C-S, from 76.47 to 72.08%, was noted. Priyadarshini et al.[36] observed a similar trend in shrimp analogue made from tilapia surimi during 20 days of refrigerated storage, which was contributed by the moisture vapor migration from the product surfaces due to the different water vapor pressures from the cold air around them.[37] The moisture content in SK-S and LS-S samples increased sharply ($p < 0.05$) from 65.7 to 68.37% and 64.76 to 67.31%, respectively, over the course of 24 days' storage period. The moisture loss might have been prevented due to the formation of phenol–protein complex by the deposition of smoke compounds, forming a barrier on sausage causing moisture retention.[38] As the storage period increased, the protein content decreased significantly ($p < 0.05$) in C-S, SK-S, and LS-S from 12.31, 12.99, and 16.52% to 10.37% (20th day), 11.99% (24th day), and 15.85% (24th day), respectively. The result is consistent with the observations of Filho et al.[16] on catfish (*Sciadesherz bergii*) sausage stored at refrigerated temperature for 30 days, who hypothesized that the decrease in the protein content might be due to the conversion of proteins to volatile nitrogenous bases or nonprotein nitrogen. As the storage period progressed, the fat and ash contents showed a significant increase in the C-S values, respectively, which was due to the reduced moisture content that might have increased the dry matter of the product.[39] In SK-S and LS-S, fluctuations were observed, which could be due to the changes in the moisture content of the sausages.

3.4. pH and Total Volatile Base Nitrogen. The pH and TVB-N content of inulin-fortified control (C-S), kiln smoke (SK-S)-, and liquid smoke (LS-S)-treated sausages during the refrigerated storage period are depicted in Figure 3. The initial pH values ($n = 3$) of C-S, SK-S, and LS-S were 6.6 ± 0.01, 6.55

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Table 1. Changes in Proximate Composition of the Refrigerated Smoked Inulin-Fortified Sausages[a]

		storage days						
parameters	treatment	0	4	8	12	16	20	24
moisture (%)	C-S	76.47 ± 1.23^{d}	74.67 ± 0.27^{c}	73.53 ± 0.45^{bc}	72.38 ± 0.91^{ab}	72.13 ± 0.65^{a}	72.08 ± 0.88^{a}	R
	SK-S	65.7 ± 0.13^{a}	66.49 ± 0.44^{ab}	67.01 ± 0.64^{b}	67.95 ± 0.61^{cd}	67.34 ± 0.35^{bc}	67.99 ± 0.71^{cd}	68.37 ± 0.41^{d}
	LS-S	64.76 ± 0.05^{a}	65.47 ± 0.22^{b}	65.38 ± 0.18^{ab}	66.27 ± 0.21^{c}	66.7 ± 0.34^{cd}	66.94 ± 0.8^{d}	67.31 ± 0.19^{d}
protein (%)	C-S	12.31 ± 0.16^{c}	12.16 ± 0.9b^{c}	12.26 ± 0.47^{c}	11.42 ± 0.19^{b}	11.51 ± 0.24^{bc}	10.37 ± 0.37^{a}	R
	SK-S	12.99 ± 0.48^{ab}	13.6 ± 0.21^{b}	13.56 ± 0.5^{b}	12.43 ± 0.59^{a}	12.43 ± 0.22^{a}	12.65 ± 0.98^{ab}	11.99 ± 0.28^{a}
	LS-S	16.52 ± 0.93^{ab}	17.39 ± 0.51^{b}	17.81 ± 0.89^{b}	17.04 ± 0.5^{ab}	17.2 ± 0.6^{b}	16.57 ± 0.58^{ab}	15.85 ± 0.72^{a}
fat (%)	C-S	4.57 ± 0.36^{ab}	4.44 ± 0.65^{a}	5.08 ± 0.24^{bc}	5.58 ± 0.4^{c}	5.56 ± 0.15^{c}	5.45 ± 0.24^{c}	R
	SK-S	9.09 ± 0.47^{c}	8.06 ± 0.89^{b}	6.16 ± 0.6^{a}	6.13 ± 0.51^{a}	5.7 ± 0.67^{a}	5.38 ± 0.33^{a}	5.71 ± 0.1^{a}
	LS-S	5.37 ± 0.77^{a}	5.61 ± 0.55^{a}	6.11 ± 0.74^{a}	6.23 ± 0.63^{a}	5.99 ± 1.18^{a}	5.7 ± 0.67a	5.67 ± 0.13^{a}
ash (%)	C-S	1.83 ± 0.88^{ab}	1.32 ± 0.29^{a}	1.63 ± 0.34^{a}	2.46 ± 0.29^{b}	2.64 ± 0.31^{b}	2.6 ± 0.48^{b}	R
	SK-S	4.86 ± 0.74^{a}	5.7 ± 0.16^{b}	5.5 ± 0.33^{b}	5.72 ± 0.11^{b}	5.42 ± 0.02^{ab}	5.14 ± 0.11^{ab}	5.61 ± 0.29^{b}
	LS-S	5.35 ± 0.23^{a}	5.52 ± 0.22^{a}	5.62 ± 0.18^{a}	5.46 ± 0.26^{a}	5.5 ± 0.33^{a}	5.69 ± 0.19^{a}	5.48 ± 0.23^{a}

[a]Values of proximate composition of each treatment group for different storage days are presented as mean ± SD of three independent replications (n = 3). A one-way ANOVA ($p < 0.05$) and Duncan multiple range test (homogeneous subsets indicated by alphabets) were performed to determine the statistical significance within (lower case alphabets). C-S: inulin-fortified sausage without smoking; SK-S: kiln-smoked inulin-fortified sausage; and LS-S: liquid-smoked inulin-fortified sausage. *R refers to reject.

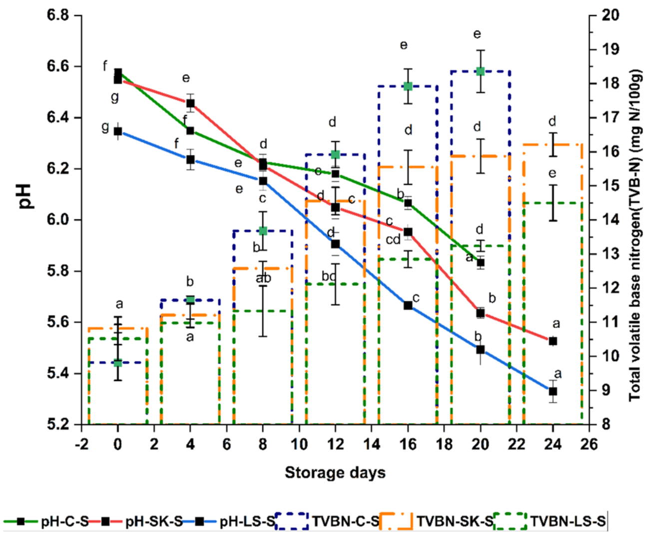

Figure 3. Changes in the pH and TVB-N of sausages during refrigerated storage. Mean ± SD, n = 3, p < 0.05; C-S, control; SK-S, kiln-smoked sausages; LS-S, liquid-smoked sausages.

± 0.01, and 6.35 ± 0.03, respectively. The SK-S sausage samples revealed a pH value of 6.55 ± 0.01, lower than the control sausage. Silins and Liepins[40] stated that the reduced values might be because of the production of different organic acids during the smoking process. The pH of the LS-S samples was 6.35 ± 0.03 on 0th day, which was lower than that of the other two treatments because of the presence of acidic and phenolic compounds in the liquid smoke and dehydration due to oven-drying.[41] Similarly, Araújo et al.[42] reported pH values of 6.81 and 6.71 for traditional-smoked and liquid-smoked sausages prepared from catfish (*Sciadesherz bergii*). The pH reduced significantly ($p < 0.05$) from 6.6 ± 0.01 to 5.83 ± 0.025, respectively, as the storage progressed in C-S. According to Kim et al.[43] and Choi et al.,[44] the reduction in pH from 0th to 16th day can be attributed to the formulation's ingredients and electrostatic interactions between polysaccharides and polar and nonpolar groups of proteins. Nonetheless, 20th day onward, the C-S sausages exhibited low pH of 5.83 ± 0.03 with the appearance of sliminess and stickiness on the sausage, which was ascribed to the growth of lactic acid bacteria (LAB).[45] In the current study, in both SK-S and LS-S, on 24th

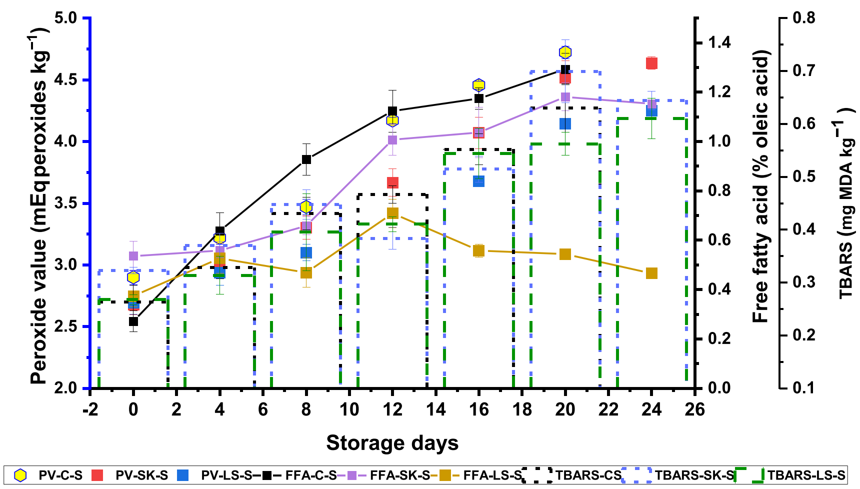

Figure 4. Changes in the peroxide value, free fatty acids, and TBARS of sausages during refrigerated storage. Mean ± SD, n = 3, p < 0.05; C-S, control; SK-S, kiln-smoked sausages; LS-S, liquid-smoked sausages.

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Table 2. Changes in the Total Viable Count (TVC) of the Refrigerated Smoked Inulin-Fortified Sausages[a]

parameters	treatment	D-1	D-4	D-8	D-12	D-16	D-20	D-24
TVC ($\log_{10}$ cfu g^{-1})	C-S	2.91 ± 0.04^a	3.22 ± 0.04^b	4.30 ± 0.05^c	4.85 ± 0.06^d	5.20 ± 0.08^e	5.98 ± 0.06^f	R
	SK-S	2.82 ± 0.04^a	3.14 ± 0.04^b	4.41 ± 0.03^c	4.89 ± 0.06^d	5.31 ± 0.04^e	5.65 ± 0.10^f	6.22 ± 0.08^g
	LS-S	$<2^a$	2.65 ± 0.07^b	2.89 ± 0.04^c	3.16 ± 0.02^d	3.50 ± 0.06^e	4.20 ± 0.02^f	4.75 ± 0.07^g

[a]Values of total viable count (TVC) of each treatment group for different storage days are presented as mean ± SD of three independent replications ($n = 3$). A one-way ANOVA ($p < 0.05$) and Duncan multiple range test (homogeneous subsets indicated by alphabets) were performed to determine the statistical significance within storage days (lower case alphabets).C-S: inulin-fortified sausage without smoking; SK-S: kiln-smoked inulin-fortified sausage; and LS-S: liquid-smoked inulin-fortified sausage. *R refers to reject.

day of storage, the pH decreased significantly as the storage progressed, reaching 5.5 ± 0.02 and 5.33 ± 0.04, respectively. The presence of LAB may have contributed to the pH values plummeting in the sausages packed in refrigerated conditions.[46]

The changes in the TVB-N (mgN 100 g^{-1}) values of C-S, SK-S, and LS-S in the present study exhibited an increasing trend with the progression of the storage days (Figure 3). TVB-N is one of the conventional chemical indices used to express the level of contamination in meat and their products. TVB-N is produced through the decarboxylation of amino acids and food degradation brought about by microbial infection.[47] The values in the current study ranged from 9.82 ± 0.52 to 18.36 ± 0.62 mgN 100 g^{-1} for C-S ($p < 0.05$), 10.82 ± 0.12 to 15.87 ± 0.5 mgN 100 g^{-1} ($p < 0.05$) for SK-S, and 10.52 ± 0.64 to 13.24 ± 0.16 mgN 100 g^{-1} for LS-S on the 20th day of refrigerated storage, respectively. On 0th day, the SK-S samples showed higher TVBN values, followed by LS-S sausages, which could be inferred to the acceleration of protein breakdown as a result of higher heating temperature.[48] On the 24th day of storage, the TVBN levels of SK-S and LS-S were 16.21 ± 0.34 and 14.5 ± 0.52 mgN 100 g^{-1}, respectively. The increase in LS-S at the quiescent rate could be due to the presence of high concentrations of antioxidant and antimicrobial compounds, such as carbonyl compounds, phenols, and organic acids, which are capable of potently thwarting the growth of the spoilage enteric bacteria.[49] Fadiloglu and Coban[50] reported the TVB-N content of 31.06 mgN per 100 g on the 28th day in liquid smoke carp sausages treated with goji berry extract stored at 2 ± 1 °C which crossed the limit of acceptability. In the present study, the values are within the acceptable limit of 25 mgN per 100 g for human consumption.[51]

3.5. Lipid Oxidative Products. The changes in the peroxide value (PV) of C-S, SK-S, and LS-S are depicted in Figure 4. The peroxide values at the starting day of the storage (0th day) were observed as 2.90 ± 0.06, 2.68 ± 0.08, and 2.69 ± 0.09 meq peroxides kg^{-1} in C-S, SK-S, and LS-S, respectively. The findings contemplated with the results of Kumar et al.,[52] where it was observed that as the drying temperature of *Pangasius* fillets increased, the PV reduced. Wu and Mao[53] explained that drying operations that are associated with high temperatures might hasten the breakdown of peroxides into their carbonyl components, maintaining a low PV. In the present study, the higher smoking and drying temperatures could be the reasons for lower PV in SK-S and LS-S. A significant ($p < 0.05$) increase in the PV was observed in all the sausages during the storage days at 5 ± 1 °C. The intensity of increase in the PV of sausages was lower in SK-S (4.51 ± 0.13 meq peroxides kg^{-1}) and LS-S (4.14 ± 0.04 meq peroxides kg^{-1}) compared to the control (4.72 ± 0.05 meq peroxides kg^{-1}) on 20th day. The reason might be owed to the deposition of phenolic compounds from the smoking process, liquid smoke, and the presence of spices such as ginger, garlic, and herbs in the sausages. A PV of 5 meq peroxides kg^{-1} is considered the maximum acceptable level for muscle foods,[54] and all the sausages were within the limit till the end of the storage period.

The free fatty acid (FFA) content of *Pangasius* emulsion-type sausages of C-S, SK-S, and LS-S during the refrigerated storage period is presented in Figure 4. Lipid hydrolysis is one of the primary developments that take place in fish muscle during storage, and endogenous enzymes further catalyze the process that generates FFAs. Triglycerides and phospholipids would break down as a result of the thermal treatment, yielding excess free fatty acids.[55] During the storage, there were statistical differences between the treatments and the storage days. At the beginning of the storage, the FFA levels for C-S, SK-S, and LS-S were 0.27 ± 0.04, 0.54 ± 0.06, and 0.36 ± 0.04% oleic acid, respectively. The lower FFA values in C-S might be attributed to the added inulin and polyphenol-rich spices which reduced the oxidative spoilage of the sausages.[56] The higher initial (0th day) FFA value in SK-S is possibly due to the enzymatic and thermolytic hydrolysis along with the exposure to surrounding air during the process of kiln smoking.[57] As the storage days advanced, the FFA values of C-S (1.29 ± 0.06%) and SK-S (1.18 ± 0.05%) sausages increased significantly ($p < 0.05$). The LS-S (0.54 ± 0.02%) sausages exhibited very low FFA value, which could be due to the concentrated deposits of phenolic compounds on the product. Nithin et al.[58] reported a similar trend in liquid smoke-flavored yellowfin tuna (*Thunnus albacares*) sausage during chilled storage. According to FAO,[54] a good-quality meat product should have the FFA content (as % oleic acid) less than 1.2%. In the present study, C-S exceeded the acceptable limit on the 20th day of storage.

Malonaldehyde is a breakdown product of the lipid hydroperoxides and peroxides that are formed during lipid oxidation and is measured in terms of TBARS content[59] (Figure 4). The findings showed that the TBARS values for C-S (20th day), SK-S (24th day), and LS-S (24th day) steadily increased from an initial value of 0.26 ± 0.03 to 0.63 ± 0.05 mg MDA kg^{-1}, 0.32 ± 0.01 to 0.64 ± 0.02 mg MDA kg^{-1}, and 0.27 ± 0.01 to 0.61 ± 0.04 mg MDA kg^{-1}, respectively. The high temperatures (90 °C) in a smoking kiln combined with drying procedures could hasten the breakdown of peroxides into their carbonyl components, which might be the reason for the increased TBARS value in SK-S.[60] In all the sausages, regardless of the treatment, TBARS showed a significantly ($p < 0.05$) accelerating trend with an increase in the storage period. The partial drying of the sausages and the enhanced oxidation of unsaturated fatty acids might be responsible for the increase in TBARS.[61] In SK-S and LS-S, the increase in TBARS was steady, owing to the antioxidant property of the phenolic

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Table 3. Changes in the Texture Profile Analysis (TPA) of the Refrigerated Smoked Inulin-Fortified Sausages[a]

		storage days						
parameters	treatment	0	4	8	12	16	20	24
hardness	C-S	3579.47 ± 355.85^{b}	3509.92 ± 243.13^{b}	2434.96 ± 412.48^{a}	2054.46 ± 45.56^{a}	1994.86 ± 248.24^{a}	1911.85 ± 389.91^{a}	R
	SK-S	3318.37 ± 648.97^{ab}	3514.36 ± 455.88^{c}	3291.75 ± 209.58^{ab}	3057.17 ± 340.69^{abc}	2816.16 ± 646.61^{abc}	2487.18 ± 329.8^{ab}	2279.62 ± 134.24^{a}
	LS-S	4240.8 ± 295.2^{e}	3920.41 ± 407.24^{de}	3460.14 ± 307.75^{cd}	3278.51 ± 16.43^{c}	3153.1 ± 273.12^{bc}	2582.35 ± 131.66^{ab}	2417.03 ± 557.47^{a}
cohesiveness	C-S	0.77 ± 0.04^{b}	0.71 ± 0.02^{a}	0.76 ± 0.02^{b}	0.74 ± 0.02^{ab}	0.77 ± 0.02^{b}	0.73 ± 0.03^{ab}	R
	SK-S	0.74 ± 0.01^{a}	0.76 ± 0.03^{a}	0.74 ± 0.05^{a}	0.74 ± 0.01^{a}	0.75 ± 0.02^{a}	0.72 ± 0.05^{a}	0.72 ± 0.01^{a}
	LS-S	0.75 ± 0.01^{a}	0.74 ± 0.02^{a}	0.71 ± 0.04^{a}	0.74 ± 0.03^{a}	0.75 ± 0.02^{a}	0.76 ± 0.04^{a}	0.73 ± 0.03^{a}
springiness	C-S	0.89 ± 0.02^{bc}	0.9 ± 0^{bc}	0.85 ± 0.05^{ab}	0.88 ± 0.02^{bc}	0.91 ± 0.01^{c}	0.83 ± 0.02^{a}	R
	SK-S	0.9 ± 0.02^{b}	0.92 ± 0.01^{b}	0.92 ± 0.02^{b}	0.9 ± 0.02^{b}	0.9 ± 0.01^{b}	0.89 ± 0.02^{ab}	0.86 ± 0.04^{a}
	LS-S	0.92 ± 0.02^{a}	0.91 ± 0.01^{a}	0.93 ± 0.01^{a}	0.92 ± 0.01^{a}	0.91 ± 0.01^{a}	0.93 ± 0.02^{a}	0.91 ± 0.01^{a}
gumminess	C-S	4765.72 ± 6113.83^{b}	1660.34 ± 176.7^{ab}	1467.44 ± 147.09^{ab}	1587.83 ± 9.88^{ab}	1158.91 ± 130.33^{ab}	1392.14 ± 259.72^{ab}	R
	SK-S	2464.5 ± 480.73^{c}	2675.98 ± 359.15^{c}	2288.88 ± 419.5^{bc}	2447.66 ± 174.4^{c}	2108.42 ± 542.91^{abc}	1646.66 ± 201.28^{ab}	1528.62 ± 95.64^{a}
	LS-S	3193.94 ± 173.78^{d}	2925.78 ± 383.95^{cd}	2580.2 ± 318.01^{bc}	2322.29 ± 157.43^{ab}	2378.86 ± 250.13^{abc}	1856.86 ± 504.73^{a}	1883.96 ± 160.97^{a}
chewiness	C-S	1033.82 ± 120.37^{ab}	1288.59 ± 97.05^{bc}	1442.37 ± 7.68^{c}	1155.04 ± 221.3^{abc}	982.51 ± 15.36^{ab}	855.92 ± 432.32^{a}	R
	SK-S	2235.82 ± 484.74^{c}	2455.09 ± 327.86c	2097.99 ± 403.69^{bc}	2208.17 ± 180.55^{c}	1904.9 ± 479.25^{bc}	1474.86 ± 204.08^{ab}	1272.65 ± 59.97^{a}
	LS-S	2929.08 ± 127.67^{c}	2676 ± 394.48^{bc}	2151.97 ± 151.94^{ab}	2384.07 ± 314.75^{bc}	2154.91 ± 252.87^{ab}	1727.93 ± 489.14^{a}	1707.1 ± 152.87^{a}
resilence	C-S	0.34 ± 0.02^{b}	0.3 ± 0.01^{a}	0.35 ± 0.03^{b}	0.32 ± 0.02^{ab}	0.34 ± 0.01^{b}	0.32 ± 0.03^{ab}	R
	SK-S	0.31 ± 0.01^{a}	0.33 ± 0.03^{a}	0.32 ± 0.05^{a}	0.31 ± 0.01^{a}	0.32 ± 0.02^{a}	0.3 ± 0.02^{a}	0.3 ± 0.01^{a}
	LS-S	0.32 ± 0.02^{ab}	0.33 ± 0.02^{ab}	0.3 ± 0.03^{a}	0.32 ± 0.02^{ab}	0.31 ± 0.01^{ab}	0.35 ± 0.03^{b}	0.3 ± 0.02^{a}

[a]Values of texture profile parameters of each treatment group for different storage days are presented as mean ± SD of three independent replications ($n = 3$). A one-way ANOVA ($p < 0.05$) and Duncan multiple range test (homogeneous subsets indicated by alphabets) were performed to determine the statistical significance within (lower case alphabets). C-S: inulin-fortified sausage without smoking; SK-S: kiln-smoked inulin-fortified sausage; and LS-S: liquid-smoked inulin-fortified sausage. *R refers to reject.

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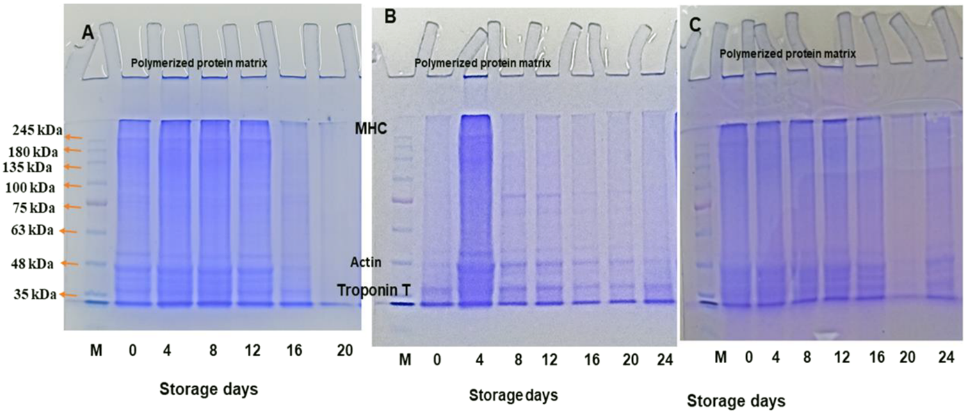

Figure 5. Changes in protein pattern on SDS-PAGE of the refrigerated inulin-fortified sausages with different treatments. (A) C-S––inulin-fortified sausage without smoking; B: SK-S––traditional smoked inulin-fortified sausage; and C: LS-S––liquid-smoked inulin-fortified sausage.

compounds present in the deposited smoke and liquid smoke along with the different spices used in the formulation of the sausage and also packaging.

3.6. Total Viable Count. The changes in the total viable count of C-S, SK-S, and LS-S during storage are illustrated in Table 2. The total viable counts are regularly used to predict and determine the shelf-life of food products. Initially, the TVC values of C-S, SK-S, and LS-S were found to be 2.91, 2.82, and less than 2 $\log_{10}$cfu g^{-1}, respectively. On 20th day C-S, SK-S, and LS-S exhibited 5.98, 5.65, and 4.20 $\log_{10}$cfu g^{-1}, respectively. In the case of SK-S and LS-S, the TVC reached 6.22 $\log_{10}$cfu g^{-1} (24th day) and 4.75 $\log_{10}$cfu g^{-1} (24th day), respectively. In SK-S sausages, the type of wood, temperature, and exposure of the product play important roles in the deposition of antimicrobial and antioxidant compounds onto the product, and in the current study, the wood smoke was not sufficient to provide the microbial safety of the sausage. The microbial count of C-S sausage samples reached the legal limit of acceptability (6 $\log_{10}$ cfu g^{-1}) of microbial standards in cooked meat products, as reported by Jay et al.[62] on 20th day, and in SK-S on 24th day of storage. The liquid smoke and drying conditions of the LS-S sausages might have shown the negative impact on the growth of microorganisms in the present study.

3.7. Texture Profile Analysis. The changes in the texture profile analysis of C-S, SK-S, and LS-S during refrigerated storage are provided in Table 3. The hardness of C-S reduced significantly ($p < 0.05$) from 3579.47 to 1911.85 g, in SK-S from 3318.37 to 2279.62 g ($p < 0.05$), and in LS-S from 4240.8 to 2417.03 g ($p < 0.05$), respectively, during the progression of storage. On the 0th day, it can be observed that LS-S exhibited a higher hardness value, which can be ascribed to the reduced moisture content on subjecting to the oven-drying process. According to Berizi et al.,[63] water retention and fat reduction were used as the strategies to overcome texture hardening since inulin can replace fat only when its macromolecules are rehydrated and swollen. The hardness value of both SK-S and LS-S reduced significantly as the storage days progressed. The result of the present study is in agreement with that of Nithin et al.[58] who observed a similar trend in liquid smoke-flavored yellowfin tuna (*Thunnus albacares*) sausage during chilled storage. Foods that include phenolic components can generate hydrogen bonds with water to promote moisture retention. This increasing moisture content causes the hardness to decrease. The lower hardness of the sausage resulted from the smoke depositing on its surface and the smoke ingredients cross-linking with the meat constituents.[64]

Cohesiveness is a crucial factor while handling sausages, especially when slicing these products, because overly cohesive or adhesive items are difficult to cut and have an unappealingly sticky texture.[65] The cohesiveness of C-S reduced significantly ($p < 0.05$) from 0.77 to 0.73, in SK-S from 0.74 to 0.72 ($p > 0.05$), and in LS-S from 0.75 to 0.73 ($p > 0.05$), respectively. The slight variations in the values during the progression of storage were contributed by the slight increase in the moisture content.[66]

Springiness (height recovery from the end of the first bite to the beginning of the second bite)[67] values were higher for the LS-S (0.92 mm) samples compared to SK-S (0.9 mm) and C-S (0.89 mm). A similar observation was reported by Nithin et al.[58] The springiness values for C-S, SK-S, and LS-S reduced to 0.83 mm (20th day), 0.89 mm (24th day), and 0.93 mm (24th day), respectively, at the end of the storage period. A decreasing trend in springiness was observed in all the sausages, which is attributed to the decreasing pH and water-holding capacity[68] during the progression of the storage period.

Gumminess and chewiness time dependence are mostly the result of the increase in hardness since cohesiveness and springiness remained constant.[69] On the initial day, C-S exhibited a higher gumminess value. The reason could be that more moisture was available for absorption from the dietary fiber (inulin) powder.[70] In the present study, gumminess decreased from 4765.72 to 1392.14 g for C-S, 2464.5 to 1528.62 g for SK-S, and 3193.94 to 1883.96 g respectively for LS-S during refrigerated storage. Andres et al.[69] stated that harder, gummy, and more cohesive products with higher chewiness, lower springiness, and resilience characteristics were produced as the product fat content increased.

Chewiness is a secondary parameter that depends on hardness, which can reflect the results with respect to hardness.[71] In the present study, chewiness decreased from 1033.82 to 855.92 g for C-S, 2235.82 to 1272.65 g for TS-S, and 2929.08 to 1707.1 g for LS-S during refrigerated storage. Irrespective of the treatment, a decrease in chewiness was observed, indicating that less energy was needed during the mastication process.[72] The results indicated that softening of texture occurred more prominently in C-S which was probably due to the proteolytic action promoted by muscle

Table 4. Changes in the Sensory Scores of the Refrigerated Smoked Inulin-Fortified Sausages[a]

		storage days						
parameters	treatment	0	4	8	12	16	20	24
general appearance	C-S	8.0 ± 0.67^{d}	8.0 ± 0.72^{d}	7.09 ± 0.83^{d}	6.75 ± 0.64^{bc}	6.41 ± 0.68^{b}	5.75 ± 0.90^{a}	NP
	SK-S	8.58 ± 0.51^{d}	8.51 ± 0.50^{d}	8.42 ± 0.51^{d}	7.80 ± 0.72^{c}	7.66 ± 0.62^{c}	6.83 ± 0.57^{b}	5.67 ± 0.74^{a}
	LS-S	8.50 ± 0.43^{e}	7.70 ± 0.59^{d}	7.76 ± 0.85^{bc}	7.19 ± 0.51^{c}	7.08 ± 0.61^{b}	6.80 ± 0.60^{d}	6.35 ± 0.43^{a}
color	C-S	7.76 ± 0.90^{c}	7.54 ± 0.61^{c}	7.58 ± 0.57^{c}	7.49 ± 0.67^{c}	6.80 ± 0.77^{b}	6.36 ± 0.44^{a}	NP
	SK-S	8.45 ± 0.58^{c}	8.20 ± 0.77^{c}	8.05 ± 0.72^{bc}	7.70 ± 0.70^{b}	7.77 ± 0.50^{b}	6.55 ± 0.66^{a}	6.31 ± 0.71^{a}
	LS-S	8.61 ± 0.54^{d}	8.04 ± 0.72^{c}	7.91 ± 0.88^{c}	7.55 ± 0.77^{bc}	7.55 ± 0.81^{bc}	7.17 ± 0.78^{ab}	6.88 ± 0.9^{a}
texture	C-S	8.53 ± 0.57^{c}	8.00 ± 0.72^{b}	7.84 ± 0.90^{b}	7.56 ± 0.93	7.51 ± 0.84^{b}	5.8 ± 1.01^{a}	NP
	SK-S	8.50 ± 0.47^{d}	8.26 ± 0.69^{cd}	7.72 ± 0.84^{bc}	7.50 ± 0.97^{b}	7.34 ± 0.98^{b}	7.27 ± 0.95^{b}	6.50 ± 1.24^{a}
	LS-S	8.20 ± 0.65^{c}	8.2 ± 0.70^{c}	8.11 ± 0.79^{c}	7.52 ± 1.01^{b}	7.34 ± 1.02^{b}	7.19 ± 1.16^{b}	6.29 ± 1.11^{a}
odor	C-S	8.09 ± 0.64^{c}	8.09 ± 0.68^{c}	8.02 ± 0.89^{c}	7.52 ± 1.08^{bc}	7.18 ± 1.05^{bc}	5.58 ± 1.06^{a}	NP
	SK-S	8.45 ± 0.69^{d}	8.26 ± 0.82^{cd}	8.25 ± 0.82^{cd}	7.85 ± 0.84^{cd}	7.58 ± 0.72^{b}	6.51 ± 1.03^{a}	6.22 ± 0.86^{a}
	LS-S	8.44 ± 0.67^{d}	8.2 ± 0.58^{bc}	8.01 ± 0.86^{cd}	7.89 ± 0.92^{bcd}	7.55 ± 0.96^{b}	6.62 ± 0.9^{a}	6.53 ± 0.77^{a}
overall acceptability	C-S	8.35 ± 0.68^{e}	7.74 ± 0.80^{d}	6.92 ± 1.15^{c}	6.68 ± 0.70^{c}	6.29 ± 0.83^{b}	5.56 ± 0.97^{a}	NP
	SK-S	8.46 ± 0.72^{a}	8.26 ± 0.80^{a}	8.07 ± 0.91^{bc}	7.89 ± 0.79^{bc}	7.64 ± 1.06^{b}	7.52 ± 0.90^{b}	6.02 ± 1.00^{a}
	LS-S	8.50 ± 0.60^{e}	8.10 ± 0.90^{cd}	7.80 ± 0.70^{de}	7.70 ± 1.10^{cd}	7.40 ± 0.90^{c}	6.70 ± 1.10^{b}	6.00 ± 0.70^{a}

[a]Values of sensory scores of each treatment group for different storage days are presented as mean ± SD of three independent replications (n = 3). A one-way ANOVA ($p < 0.05$) and Duncan multiple range test (homogeneous subsets indicated by alphabets) were performed to determine the statistical significance within (lower case alphabets). C-S: inulin-fortified sausage without smoking; SK-S: kiln-smoked inulin-fortified sausage; and LS-S: liquid-smoked inulin-fortified sausage; NP: not performed.

endopeptidases (calpains I and II and cathepsins B, D, H, and L).[73]

Resilience reflects the meat elasticity.[74] In the present study, there is no significant difference observed in all the sausages in resilience values during the storage period, which indicates that oxidative damage did not occur during storage[75] because of vacuum packaging and phenolic compounds present in the spices and smoke.

3.8. Changes in the Protein Pattern. Changes in the protein pattern of C-S, SK-S, and LS-S during refrigerated storage are presented in Figure 5. The *Pangasius* mince sausage protein contains a myosin heavy chain (245 kilodaltons, kDa), actin (52 kDa), and troponin (35 kDa). In Figure 5A, polymerized protein in the wells of the SDS-PAGE gel was observed. High-molecular-weight (HMW) bands and MHC were observed from 0 to 12 days. From the 16th day onward, disappearance of the MHC and a decrease of the band intensities, actin, and troponin T were noticed, which were due to degradation caused by the protease activity.[76] Maqsood et al.[77] stated that the oxidation of proteins and the generation of endoproteinases by microorganisms that are responsible for meat tenderization might be the causes of the degradation of various protein bands in the meat during chilled storage. In SK-S samples, on 0th day, immediately after the completion of the smoking process, the protein bands appeared lighter. On day 4, HMW, MHC, actin, and troponin T were observed. From Figure 5, it can be clearly seen that the disappearance of the MHC occurred from the 8th day onward. Baylan et al.[78] reported the disappearance of the myosin heavy chain (MHC) band in the 9th week of refrigerated storage of smoking rainbow trout. The lower band intensities of the MHC and actin in SK-S samples could be due to the formation of polyphenol−protein complexes with low solubility (insoluble aggregates).[79] The LS-S samples had the retention of all bands even after subjecting to oven-drying, suggesting that inulin and phenolic compounds from liquid smoke prevented the proteolysis of MHC, actin, and troponin. Li et al.[80] reported that inulin can form complexation with polyphenol (e.g., epicatechin and its oligomers) which enhances the antioxidant activity. In the present study, the combination of inulin with liquid smoke was found to be efficient for maintaining the stability of the *Pangasius* mince sausage.

3.9. Sensory Scores. The changes in the sensory scores of *Pangasius* sausage (C-S, SK-S, and LS-S) during the storage period are represented in Table 4. During the initial days of storage, the sensory quality (texture, odor, general appearance, color, and overall acceptability) scored high points in all the treatment groups including control throughout the storage period; however, the attributes gradually declined with due course of storage time. The sensory qualities of meat and meat-based products are important aspects for consumers and affect the experienced quality, which ultimately influences the consumer attention to buy the same product at a posterior moment.[81] All sensory metrics, including general appearance, color, texture, odor, and overall acceptability, exhibited a downward trend with the increasing refrigerated storage days. The general appearance and color sensory scores reduced as the days progressed. The decrease in color during storage might be due to the changes in the meat pigment myoglobin.[82] The LS-S samples exhibited better odor scores compared to other treatments, which suggest that the vacuum packaging, inulin dietary fiber, and phenolic compounds of the liquid smoke controlled the lipid oxidative products, which are commonly responsible for the development of odoriferous flavors. Similar observations were reported by Madane et al.[83] when using *Moringa oleifera* flower as an antioxidant dietary fiber in chicken meat nuggets. In C-S samples, the texture loss was higher compared to that of SK-S and LS-S. It could be due to the loss of moisture content in products while being stored. Further lowering of pH and denaturation of proteins were observed at such low pH after the bacterial degradation of muscle fiber proteins.[84] Sensory shelf-life can alternatively be calculated as the time required for the product's overall acceptability scores to fall below 6.0 on a 9-point structured hedonic scale.[85] Afshari et al.[86] found the reduction in the intensity of odor and textural scores with the incorporation of inulin and β-glucan. The overall scores are the cumulative scores of all the sensory attributes, and C-S sausages received

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lower scores. The findings of the study are in agreement with the reports of Madane et al.[87] The observations in the present study indicated that *Pangasius* sausages incorporated with inulin powder treated with kiln smoking and commercial liquid smoke retained good-to-better sensory attributes up to day 16 (C-S) and day 20 (SK-S and LS-S) under refrigerated storage at 5 ± 1 °C in vacuum-packed low-density polyethylene (LDPE) pouches.

4. CONCLUSIONS

In conclusion, the current study shows comparable effects of kiln smoking and liquid-smoked oven drying on the quality of inulin-fortified *Pangasius* sausages during refrigerated storage. The study also proved that liquid smoke could be an easy way to develop smoke-flavored sausages. *Pangasius hypophthalmus*, the catfish, which is considered as underutilized and low-priced could be efficiently utilized for the development of emulsion-type sausage utilizing the mince. Inulin, a valuable byproduct of agro-industry could be used to enhance the quality of minced meat, and in the present research, its incorporation complimented the kiln- and liquid-smoking processes. From the present study, it is evident that both the SK-S and LS-S sausages can be stored for 20 days under refrigerated condition at 5 ± 1 °C in vacuum-packed low-density polyethylene (LDPE) pouches. However, the LS-S samples displayed much better-quality parameters in comparison with the SK-S samples, suggesting that sausages can be developed utilizing commercial liquid smoke flavorings without the risk of deposition of benzopyrene.

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Notes

The authors declare no competing financial interest.

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