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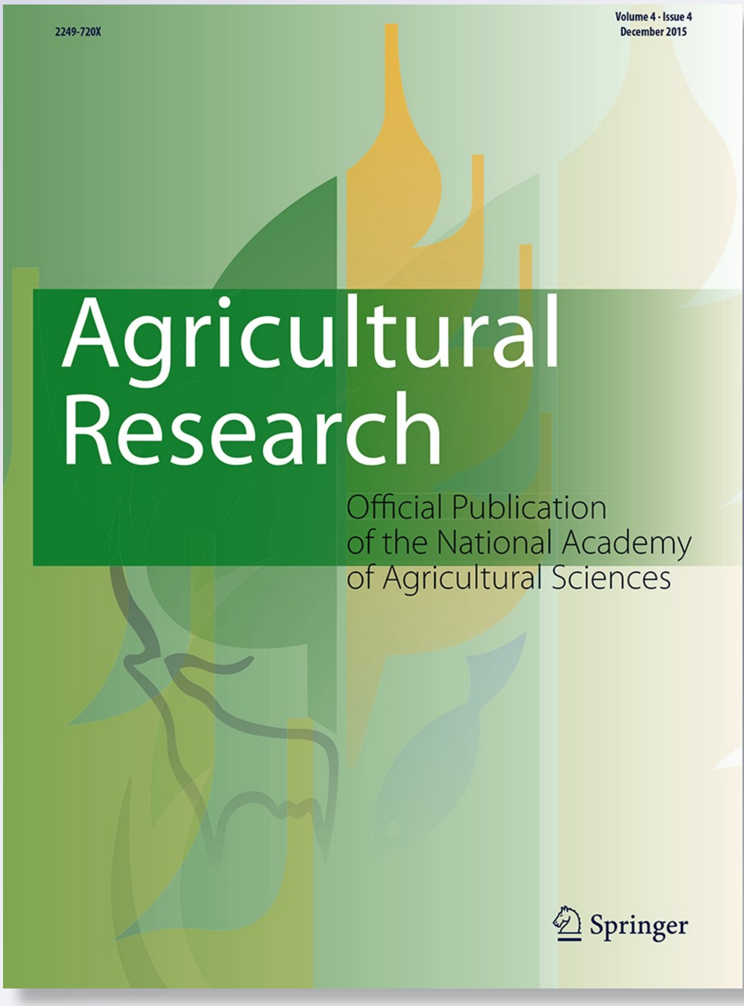

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FULL-LENGTH RESEARCH ARTICLE

Physico-chemical Changes in Liquid Smoke Flavoured Yellowfin Tuna (*Thunnus albacares*) Sausage During Chilled Storage

C. T. Nithin[1] · T. R. Ananthanarayanan[1] · R. Yathavamoorthi[2] · J. Bindu[1] · C. G. Joshy[1] · T. K. Srinivasa Gopal[1]

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Abstract A liquid smoke flavoured sausages were prepared from yellowfin tuna using a commercial liquid smoke flavouring. Physico-chemical changes in the sausages during chilled storage at 3 ± 1 °C were analysed and compared with that of a control. Total plate count was estimated at every 15th day of storage. Liquid-smoked sausages were were found to have higher moisture content but recorded lower values for total volatile base-nitrogen, trimethylamine and thiobarbituric acid ($P < 0.01$). Free fatty acid value in both types of sausages did not show any significant difference ($P > 0.01$). Instrumental colour measurements showed lower L* (lightness), a* (redness) and b* (yellowness) values for liquid smoke flavoured sausages ($P < 0.01$). The liquid-smoked sausages showed lower hardness values ($P < 0.01$). Both liquid-smoked and control sausages were found to be sensory wise acceptable up to 55th days of storage and subsequently rejected on the 60th day. The microbial count in both the types of sausages was within the acceptable limit during this period.

Keywords Yellowfin tuna sausage · Liquid smoke · Chilled storage · Physico-chemical changes

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Introduction

Sausage is one of the prominent value added product, which has gained wide popularity during recent years. Fish sausage can be defined as fish mince mixed with salt, spices, seasonings, starch, fat, etc., and ground well to get a fine pasty mass that is stuffed and sealed in natural or synthetic casings and cooked thereafter. Smoked sausages are a delicacy especially in China, Germany [11], Brazil [31] and Spain [18]. Smoking imparts a characteristic flavour and colour to the product and increases its shelf-life as a consequence of the antimicrobial activity of smoke components. However, smoking of food possesses the chances of its contamination by carcinogens like polycyclic aromatic hydrocarbons (PAH) [2, 32, 35]. PAH are compounds consisting of two or more condensed aromatic carbon rings and are formed during the incomplete combustion of organic material [34]. About 660 different compounds have been identified under PAH group [30]. Apart from carcinogenicity, PAHs are also found to cause hemato, cardio, renal, neuro, immuno, reproductive and developmental toxicities in humans and laboratory animals

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[28]. Due to the health concern associated with PAH, European Union [10] has set a maximum limit of 30 ppb for the sum content of four PAHs (benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene) and separate maximum of 5 ppb for benzo(a)pyrene alone in smoked fish and fishery products. Quite a lot of studies have focused on the factors affecting and ways of minimising PAH contamination in smoked foods [27, 35]. Various authors have reported that use of "liquid smoke" can considerably reduce the PAH content in foods [24, 32]. Liquid smoking possesses additional advantages like applicability in a wide range of foods, minimising environmental pollution, closer control over the flavour and diverse modes of application (spraying on the surface, dipping and blending with the food, etc.) [21]. Studies pertaining to the application of liquid smoke have mainly focused on its antimicrobial [8, 36] and antioxidant activity in food [14, 25]. Limited information is available on their effects on the physico-chemical and textural characteristics of fish and fishery products. The aim of this study was to understand the effect of incorporation of liquid smoke on the physico-chemical changes and sensory properties of sausage developed from yellowfin tuna during chilled storage. The choice of the species was on account of the tremendous potential for yellowfin tuna in India. The sausages were developed in two batches, one with the liquid smoke flavour and the other with no flavour added (Control).

Material and Methods

Preparation of Tuna Sausage

Fresh yellowfin tuna were purchased from nearby market and transported to the lab in iced condition with a fish to ice ratio of 1:1 (w/w). The fishes were then washed in potable water, and the loins were separated manually. Black meat was separated off, and the temperature of the meat was kept low by icing. The meat was then comminuted in a bowl chopper (Maschinenfabrik Dornhan, MTK, 661). After proper mixing, other ingredients were added in the proportion as given in Table 1. This mixture was separated into two equal portions; first portion was directly stuffed into polyamide casings (35 mm dia) to a length of 100 mm by using a hand operated sausage stuffer which was taken as control. To the second portion, 0.2 % (Quantity fixed by trial preparations and subsequent sensory analysis) of a commercial liquid smoke (SMOKEZ ENVIRO 24 PB, Red arrow international, GRAS certified by FDA and USDA) was added and mixed again in the bowl chopper and stuffed in the casings. After stuffing, both ends of the sausages were tied tightly by using a twine. Sausages were cooked at 90 °C in an automated water bath for 40 min followed by sudden cooling by dipping in chilled water at a temperature around 4–5 °C. Both cooking and cooling were carried out separately for the two batches to avoid flavour contamination. The sausages were then iced in an insulated box and kept in a chill room maintained at 2–4 °C for further analysis.

Table 1 Ingredients of yellowfin tuna sausage

Ingredients	Percentage
Yellowfin tuna meat	87
Corn flour	4.5
Hydrogenated vegetable fat	3
Salt	2.5
Sugar	2
Sodium tripolyphosphate	0.12
Ginger (juice)	0.5
Garlic (paste)	0.5

Proximate Composition and Biochemical Analysis

Proximate composition of the sausages was determined according to AOAC [3] except for carbohydrate content (by difference). Measurement of total volatile base-nitrogen (TVB-N) and trimethylamine (TMA-N) as Conway [6] and expressed as mg nitrogen/100 g. Free fatty acid content was determined according to AOCS [4] and expressed as mg% oleic acid. Thiobarbituric acid (TBA) value determined by the method of Tarladgis, Watts & Younathan [37] and expressed as milligram malonaldehyde/kg of sample.

Texture Profile Analysis

Texture profile analysis was performed in a food texture analyser (Lloyd instruments, UK, Model LRX plus). Data obtained from the instrument were recorded and compiled with the help of software "Nexygen". The polyamide casings of the sausage were removed, and the sausage was cut into uniform portions of 3 cm length. The samples were axially compressed to 40 % of the original height with a cylindrical plunger of 50-mm diameter, at a crosshead speed of 12 mm/second through a 2-cycle sequence. The following texture parameters were measured from force deformation curve: Hardness 1 (peak force during the first compression cycle), Hardness 2 (peak force during the second compression cycle), Springiness (height that the sample recovers during the time that elapses between the end of the first compression and the start of the second expressed in mm) and Cohesiveness (extent to which the sample could be deformed before rupture).

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Instrumental Colour Measurement

Objective colour was measured by crushing the sausage and spreading it as a uniform layer in the sample holder. The L* (lightness), a* (redness) and b* (yellowness) values were obtained by using the Hunter Lab MiniScan® XP Plus spectrocolourimeter, model No D/8-S (Hunter Associates Laboratory Inc., Reston, VA, USA) with geometry of diffuse 80 (Sphere-8 mm view) and an illuminant of D 65 optical sensor and 100 standard observer. The instrument was standardized with a white tile and a light trap. Means of the readings were determined on three locations in each sample.

Sensory Evaluation

Sensory analysis of the sausages was done by a five member expert panel using a 9-point hedonic scale prescribed by Meilgaard, Civille & Carr [26]. The score of 9 denoted the quality description "likes extremely" and 1 denoted "dislikes extremely". A score of 4 was considered as the margin of acceptance. Sausages were cut into uniform thin slices and warmed in a microwave oven for 1.5 min and presented in coded plates. Panellists were asked to score for appearance, colour, odour, flavour, taste and texture of the samples. Overall acceptability was calculated by adding the scores for all attributes and dividing by the total number of attributes.

Total Plate Count

Samples were analysed at every 15th day of storage. 5 g of the sample was aseptically cut and then blended for 2 min in 0.85 % normal saline. Serial dilutions of the samples were made using normal saline. Appropriate dilutions were plated on plate count agar. Plates were incubated for 48 h at 37 °C. Values are reported as log cfu/g of the sample ± standard error.

Statistical Analysis

All the samples were analysed in triplicates, and data were reported as means ± standard error (SE). Statistical analysis was carried using SAS 9.3. Data except that of sensory analysis were analysed by Two-way analysis of variance to see the effect of treatment, storage days and their interaction. Means were compared using Tukey's test and *t*-test at 1 % level of significance. Sensory values were compared using Kruskal–Wallis one-way analysis of variance for each treatment at different days of storage. Mann–Whitney nonparametric test was performed to see the significant difference between treatment sensory scores during each storage day.

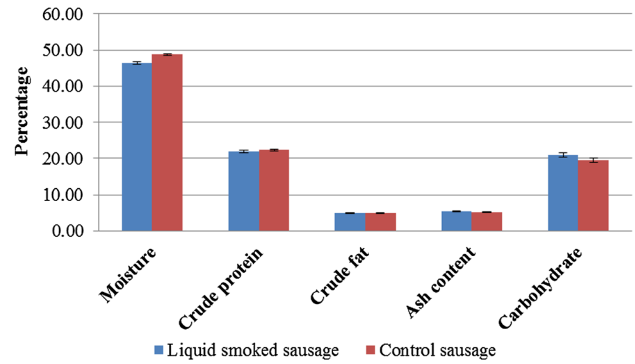

Fig. 1 Comparison of proximate composition of control and liquid-smoked sausage

Results and Discussion

Proximate Composition

Result of proximate composition analysis of the sausages is given in Fig. 1. Significant difference was observed between two sausages in terms of moisture content ($P < 0.01$). Liquid-smoked sausages showed higher moisture content (48.73 ± 0.39 %) than control (46.45 ± 0.27 %). However, no significant difference was observed in crude protein, crude lipid and ash content ($P > 0.01$). Protein content in control sausage was 22.00 ± 0.21 %, while that of liquid-smoked sausage was 22.31 ± 0.35 %. Fat content of liquid-smoked and control sausage was 4.98 ± 0.10 % and 4.83 ± 0.14 %, respectively. Ash content of sausage with liquid smoke was 5.21 ± 0.14 % and that of control was 5.43 ± 0.12 %. Sausage with liquid smoke was having a carbohydrate content of 19.43 ± 0.57 %, carbohydrate content in control sausage was 20.62 ± 0.64 %. A reduction in crude protein, lipid and ash content of mud eel (*Monopterus albus*) after smoking was reported by Vishwanath, Lilabati, and Bijen [40]. Reduction in lipid content may be due to the loss of volatile short-chain fatty acids and methyl esters due to the elevated temperature associated with smoking [5]. Loss of water-soluble minerals as drip during smoking can result in lowering the ash content [40]. Similar reduction in crude protein content was also observed in *Clarias batrachus* [20]. However, such losses were not observed in liquid-smoked sausages.

Total Volatile Base-Nitrogen (TVB-N) & Trimethylamine Nitrogen (TMA-N)

TVB-N content quantifies the basic volatile compounds such as trimethyl amine, dimethyl amine, methyl amine, ammonia, etc., formed as a result of microbial action and thermal breakdown of endogenous compounds. Changes in

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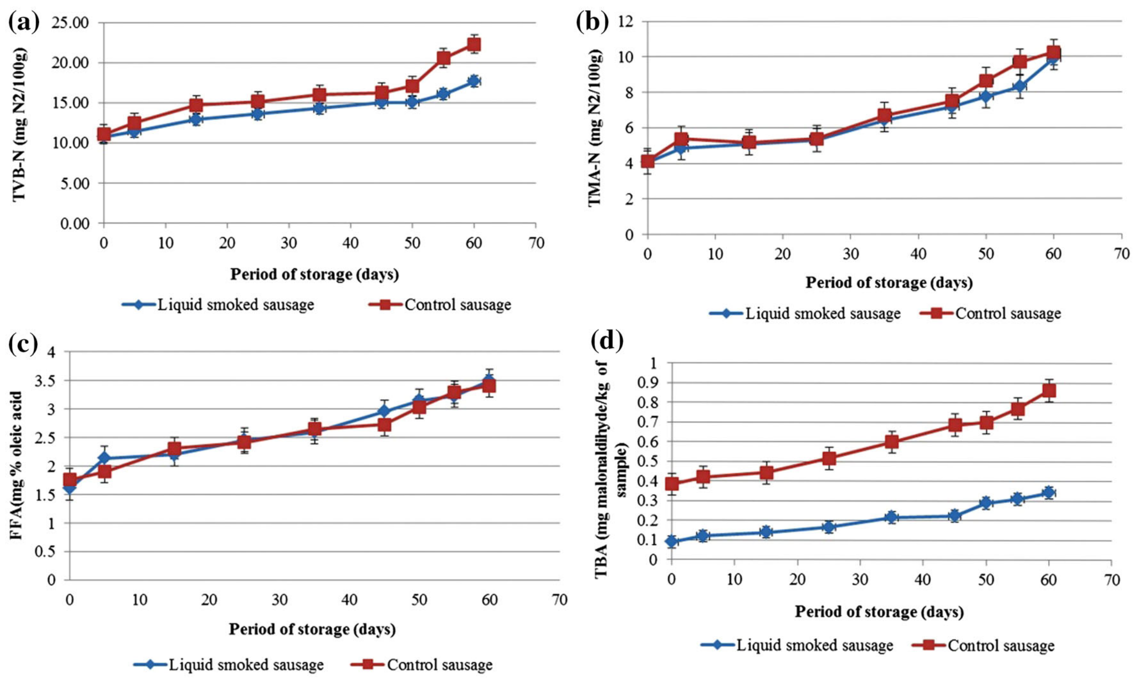

Fig. 2 Changes in chemical spoilage indices of control and liquid-smoked sausage during chilled storage (a = TVB-N, b = TMA-N, c = FFA, d = TBA)

TVB-N content of liquid smoke flavoured and control sausages are shown in Fig. 2a. From the initial day, liquid-smoked sausage and control showed significant difference in TVB-N content ($P < 0.01$). Higher TVB-N values were observed in control.

TMA-N content (Fig. 2b) varied significantly for both the sausages ($P < 0.01$). Liquid-smoked sausage showed lower values compared to control. TMA-N, a volatile amine, represents the majority of TVB-N and is associated with the ''fishy'' odour of a fish muscle. Generally, it is produced by bacterial enzyme action, reducing the trimethylamine oxide to trimethylamine. Studies conducted on the effect of thermal processing on the TMA-N values reported that the TMA-N content increased significantly from the raw material after heat treatment due to the breakdown of trimethylamine oxide [13]. A part of the initial TMA-N content can be attributed to this reason. Studies on the antimicrobial activity of smoke condensates have shown significant but variable effects on the growth of microorganism [36]. Phenolic compounds in wood smoke have been reported to show antimicrobial activity against a wide range of spoilage and pathogenic bacteria. The smoke flavouring used in this study contained a total phenolic content of 15.0–20.0 mg/ml. Exact mechanism of inactivation of microbes due to phenolic compounds is still unknown. Some of the studies suggest that these compounds penetrate the cell wall and interfere with the cellular metabolism [7]. Other pathways include inhibition of active sites of enzymes [15]. Carbonyl compounds such as formaldehyde and acrolein in smoke condensates are also known to exhibit antibacterial properties by similar mechanism of penetrating the cell wall and subsequent inactivation of enzymes on the cytoplasmic membrane and cytoplasm. Another mechanism of bacterial inactivation due to carbonyl compounds is through interfering nutrient uptake by cells [38].

Free Fatty Acid Value

Changes in free fatty acid value of the sausages are given in Fig. 2c. Free fatty acid value in both sausage did not show any significant difference ($P > 0.01$). Free fatty acid formation which is a measure of hydrolytic rancidity in the product can be due to the enzymatic hydrolysis of fat or due to the action of bacterial lipases on fat [1]. Further, Gallardo, Aubourg & Perez-Martın [12] reported that during thermal treatment, breakdown of triglycerides and phospholipids might occur, which can also lead to free fatty acid formation. The initial FFA formation can be attributed to the combined action of all these causes; however, free fatty acid formation in the sausages during the storage can only be attributed to lipid degradation due to bacterial action. The chances for degeneration of fat by lipases are meagre as most of them are heat labile and would have undergone denaturation during heat treatment.

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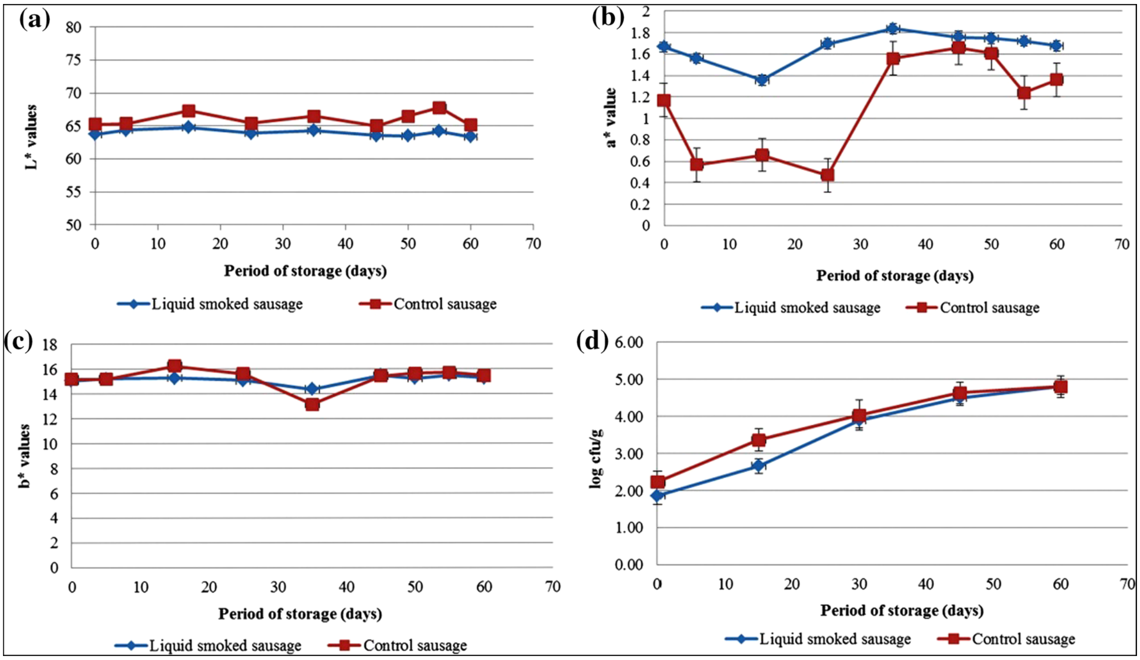

Fig. 3 Changes in colour values (a = L*, b = a*, c = b* values) and *d* = Total plate count of the sausages during chilled storage

Thiobarbituric Acid Value (TBA)

Changes in thiobarbituric acid value of the sausage during storage is given in Fig. 2d. TBA value showed a significant difference between liquid-smoked sausage and control ($P < 0.01$). In sausage with liquid smoke flavouring, initial TBA value was 0.09 ± 0.006 which gradually increased to 0.33 ± 0.007 on 60th day. Control sausage showed a steady increase in TBA value from 0.39 ± 0.012 at the initial day to 0.85 ± 0.009 on the day of rejection. Thiobarbituric acid value has been used to measure the concentration of relatively polar secondary reaction products, especially aldehydes. The increase in TBA value can be an indicator of the formation of secondary lipid oxidation products [22]. Investigations in the field of smoke and smoked foods reveal that phenols in general and methoxyphenols in particular are responsible for the antibiotic and antioxidant properties in smoked foods [9, 14, 36]. Phenols and phenolic esters are produced during the pyrolysis of lignin. They include guaiacol (2-methoxyphenol) and syringol (2, 6-dimethoxyphenol), along with their homologous derivatives, a variety of associated compounds with methyl, ethyl, propyl, vinyl, allyl and propenyl side chains are also evolved [21]. The major phenolic compounds identified in wood smoke include phenol, p-cresol, o-cresol, guaiacol, 4-methyl guaiacol, 4-ethyl guaiacol, syringol, eugenol, 4-propyl guaiacol and isoeugenol [39]. The major mechanisms of antioxidant activity of phenolic compounds include metal scavenging and metal chelation [17]. In addition to this, certain neoformed compounds produced as result of maillard reaction between amino acids and carbonyl groups are also reported to possess considerable antioxidant activities [23].

L*, a*, b* Colour Values

Changes in colour of products can be attributed to various factors such as processing conditions, temperature of storage and other biochemical reactions taking place in the product. Characteristic colour of smoked foods is a result of maillard reaction, in which the carbonyl groups present in the smoke react with the amino groups present on the food surface to produce a brown colouration [21]. The instrumental colour measurements shown that L* (Fig. 3a) and b* (Fig. 3c) values for the liquid-smoked sausage were significantly lower than that of control sausage ($P < 0.01$). Lower yellowness and lightness values were also observed by Schwert, Verlindo, Cichoski, Oliveira, and Valduga [31] in liquid-smoked Brazilian calabrese sausage. While considering the a* values (Fig. 3b), sausage with liquid smoke was having higher values than control. The smoke flavouring used in this study contained a total carbonyl content of 22–30 %.

Texture Profile Analysis

Texture is an important sensory characteristic that determines quality or acceptability of fishery products. Hardness, springiness and cohesiveness are primary mechanical parameters which can be used to characterize the texture properties of smoked sausages [29]. Result of texture profile analysis of the product is shown in Table 2. Control

Table 2 Changes in texture profile during chilled storage (mean ± SE)

Day	Hardness 1(N)		Hardness 2(N)		Cohesiveness		Springiness (mm)	
	Liquid smoked	Control	Liquid smoked	Control	Liquid smoked	Control	Liquid smoked	Control
0	36.375 ± 0.040^{xe}	36.527 ± 0.045^{xf}	26.057 ± 0.208^{xb}	27.843 ± 0.074^{ye}	0.243 ± 0.002^{xc}	0.253 ± 0.001^{xcb}	8.893 ± 0.032^{xa}	9.346 ± 0.122^{ya}
5	45.497 ± 0.029^{xa}	38.347 ± 0.133^{ye}	23.541 ± 0.160^{xe}	27.262 ± 0.126^{yf}	0.206 ± 0.001^{xe}	0.238 ± 0.001^{ycd}	7.477 ± 0.026^{xc}	6.963 ± 0.207^{yd}
15	41.148 ± 0.094^{xb}	44.347 ± 0.113^{ya}	21.352 ± 0.137^{xf}	26.316 ± 0.096^{yg}	0.195 ± 0.000^{xf}	0.216 ± 0.001^{yfe}	8.002 ± 0.100^{xb}	8.081 ± 0.042^{xb}
25	32.773 ± 0.049^{xg}	42.773 ± 0.058^{yb}	20.462 ± 0.091^{xg}	30.651 ± 0.092^{yd}	0.227 ± 0.004^{xd}	0.262 ± 0.001^{yb}	7.555 ± 0.045^{xc}	6.747 ± 0.059^{yd}
35	38.677 ± 0.855^{xc}	40.557 ± 0.069^{yd}	27.010 ± 0.118^{xa}	30.376 ± 0.120^{yd}	0.238 ± 0.004^{xc}	0.281 ± 0.001^{ya}	7.247 ± 0.070^{xc}	7.457 ± 0.063^{xc}
45	37.983 ± 0.090^{xd}	43.018 ± 0.128^{yb}	20.188 ± 0.028^{xg}	35.512 ± 0.169^{ya}	0.266 ± 0.005^{xab}	0.208 ± 0.002^{yf}	7.252 ± 0.162^{xc}	7.374 ± 0.058^{xc}
50	36.762 ± 0.157^{xe}	41.551 ± 0.043^{yc}	24.551 ± 0.092^{xd}	30.992 ± 0.048^{yc}	0.236 ± 0.001^{xc}	0.225 ± 0.006^{xe}	7.230 ± 0.025^{xc}	7.433 ± 0.019^{xc}
55	34.699 ± 0.076^{xf}	40.823 ± 0.102^{yd}	25.447 ± 0.131^{xc}	31.346 ± 0.076^{yb}	0.268 ± 0.001^{xa}	0.226 ± 0.006^{yde}	7.707 ± 0.097^{xc}	7.360 ± 0.014^{yc}
60	31.502 ± 0.087^{xh}	42.061 ± 0.108^{yc}	27.006 ± 0.112^{xa}	27.234 ± 0.062^{xe}	0.256 ± 0.001^{xb}	0.204 ± 0.005^{yf}	7.759 ± 0.061^{xbc}	7.320 ± 0.026^{yc}

Different superscripts (a, b, c…) in the same column indicate significant differences between means ($P < 0.01$)

Different superscripts (x & y) in same row indicate significant difference between treatment means ($P < 0.01$)

sausage showed higher Hardness 1 values than liquid-smoked sausage ($P < 0.01$). Martinez, Salmeron, Guillen, and Casas [24], in one of their studies, found that application of a phenol-rich liquid smoke considerably reduced the hardness, fructurability, cohesiveness, gumminess and chewiness in bacons. Sink, and Hsu [33], in their study on Frankfurters, reported that all palatability properties decreased with increasing phenol content of the product. Maga [21] reported that phenolic compounds present in food can form hydrogen bonds with water which can result in increased water retention. This increased moisture content results in lowering hardness [29]. Levine, Reznick, and Packer [19] have reported that carbonyl compounds such as aldehydes and ketones also can cause substantial changes in the structure of proteins. Storage days showed a significant effect on the hardness 1 values ($P < 0.01$). In liquid-smoked sausage, hardness1 value during the initial day was 36.375 ± 0.040, which decreased to 31.502 ± 0.087 on the 60th day of storage. In the case of control, the initial hardness value was 36.527 ± 0.045 which increased to 42.061 ± 0.108 on the day of rejection. However, there were variations from the trend in both the sausage which can be attributed to the difference in individual samples due to the result of non-uniform mechanical stuffing. A significant difference was observed between two samples in terms of hardness 2 ($P < 0.01$). As observed in the case of hardness 1, control samples showed higher hardness than liquid-smoked samples. During storage the springiness values showed a decreasing trend in both sausages. Liquid-smoked samples showed higher springiness than control ($P < 0.01$). This can be attributed to the increased moisture content. Cohesiveness in both types of sausages were homogenous in nature ($P > 0.01$).

Total Plate Count

Total plate count of liquid-smoked and control sausages is shown in Fig. 3d. Liquid-smoked sausages showed lower microbial load than control ($P < 0.01$). The microbial count in both sausages was within the acceptable range during the 60 days of chilled storage. Sunen, Fernandez-Galian, and Aristimuno [36] reported that certain liquid smoke flavourings were effective in retarding the growth of *Aeromonas hydrophila*, *Yersinia enterocolitica* and *Listeria monocytogenes*. Guaiacol, one of the major smoke-derived phenols, has shown antibacterial activity against *Bacillus subtilis*. [16].

Sensory Acceptance

Overall acceptability score obtained by both the sausages is given in Table 3. During the initial days of storage, liquid-smoked sausages received higher score for the

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Table 3 Changes in overall acceptability during chilled storage (mean ± SE)

Day	Liquid smoked	Control
0	8.5 ± 0.033^{xa}	7.3 ± 0.115^{ya}
10	8.0 ± 0.058^{xa}	7.0 ± 0.088^{ya}
20	8.0 ± 0.058^{xa}	7.0 ± 0.058^{ya}
35	7.7 ± 0.173^{xa}	7.0 ± 0.173^{ya}
50	6.5 ± 0.173^{xa}	5.5 ± 0.173^{ya}
55	5.1 ± 0.058^{xa}	6.5 ± 0.058^{ya}
60	4.0 ± 0.058^{xb}	5.0 ± 0.058^{yb}

Different letters (a, b, c…) in the same column indicate significant differences between means ($P < 0.01$)

Different letters (x & y) in same row indicate significant difference between treatment means ($P < 0.01$)

attributes; odour, flavour, texture and taste from the sensory panel. Phenolic compounds in smoke and smoke flavouring have been unanimously accepted as the major compounds contributing the smoke aroma [21]. After 50th day, a significant change in sensory properties was observed in both the sausages. Sensory panel found control samples to be more acceptable than the one with liquid smoke flavour. This change in the sensory preference could be due to the changes in phenols during storage. Maga [21] reported that the amount of individual phenols can dramatically decrease during storage. Towards the end of storage, both the sausages were reported to be having mild sour taste. Both liquid-smoked and control sausages were found to be sensory-wise acceptable up to 60th day of storage.

Conclusions

Liquid smoking can significantly influence the physico-chemical properties of minced fish products by imparting antioxidant and antimicrobial properties and by influencing the textural attributes. Liquid-smoked sausages showed lower damages due to lipid oxidation. No significant difference was observed in the case of free fatty acid value. Liquid-smoked sausages showed lower hardness when compared to control sausages. Springiness values were higher in liquid-smoked sausages. Liquid-smoked sausages showed higher sensory acceptance during the initial days of storage; toward the end, a major shift in this trend was observed. Total plate count of liquid-smoked sausages was lower than control, which was also reflected in TVB-N and TMA-N contents which indicated lower values.

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