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DETERMINATION OF SHELF LIFE FOR SAUSAGES PRODUCED FROM SOME FRESHWATER FISH USING TWO DIFFERENT SMOKING METHODS

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ABSTRACT

In the present study, microbiological, chemical and sensory changes of sausage samples produced from fillets of *Chondrostoma regium*, *Luciobarbus mystaceus* and *Capoeta trutta* implemented with traditional and liquid smoke were investigated during a storage period of 70 days at 4 ± 1C. The results of microbiological, physicochemical, chemical and sensory analyses showed that there were no differences between sausages smoked with either liquid smoke or traditional smoke ($P > 0.05$). As a result, each of the three fish species can be used in the production of sausage; there was no significant difference in terms of implementation of traditional or liquid smoke in product quality, but liquid smoke is more practical for implementation. The quality of the vacuum-packed and stored sausage samples decreased during storage, but the sausages were of satisfactory eating quality until approximately 56 days of storage.

PRACTICAL APPLICATIONS

With this study, we determined that fish species without economic importance could be used for industrial targets by using those species as a meat resource for sausage production. We also found that liquid smoke was easy to use for sausage production and it did not have any negative effects in terms of sensory, microbiological and chemical quality of fish sausage.

INTRODUCTION

Fish meat has played an important role in the human diet because it contains about 18% high-quality nutritious protein, vitamins, minerals, carbohydrates, low saturated fat and omega fatty acids, and water-soluble components. However, the dietary qualities are reduced when fish is processed into fishmeal and oil (Chung and Lee 1990; Rustad 2003; Yapar *et al.* 2006; Boran *et al.* 2008).

Fish meat has been preserved for centuries by smoking as smoking is the oldest meat and seafood preservation method. Smoked fish has a characteristic flavor and color, and smoking also has antimicrobial and antioxidant characteristics for the food. Two smoking methods are utilized. These are hot and cold smoking. Over the past three decades, liquid smoke flavors have been used increasingly as an alternative form of smoking. Liquid smoke has some advantages over traditional smoking such as easy application, lower cost, environmental friendliness and easier control of smoke contaminants like polycyclic aromatic hydrocarbons, and carcinogenic and mutagenic molecules produced during pyrolysis of wood (Doe 1998; Koral *et al.* 2009; Alcicek and Atar 2010; Alcicek *et al.* 2010).

The aquatic food industry has, by processing and mincing, developed a variety of fish products. Different attempts have been made to produce new food products from various minced fish. Since traditional techniques like canning and freezing can reduce quality in the processing of underutilized fish species, alternative techniques, such as production of fish ball from croaker, fish meat, fish cake and fish sausages, are expected to increase the usage of these species. Sausages from fish can be produced by using methods similar to those used to produce meat sausage. Fish sausages have a short shelf life at refrigerated

temperatures in the absence of preservatives. Sausages made from oily fish can rapidly develop hydrolytic rancidity or oxidative rancidity flavors under chilled or frozen storage conditions (Rustad 2003; Yapar *et al.* 2006; Oksuz *et al.* 2008).

Chondrostoma regium, *Luciobarbus mystaceus* and *Capoeta trutta* are fish species living in the Euphrates–Tigris River System. They are low in the food chain and have not been utilized widely due to the presence of plentiful intramuscular bones. However, these species have been marketed fresh or frozen in the past years (Duman and Duman 1996; Geldiay and Balik 1996).

The aim of this study was to investigate the microbiological, chemical and sensory changes in sausages produced from fillets of *C. regium*, *L. mystaceus* and *C. trutta*, and implemented with traditional or liquid smoke, during storage at 4 ± 1C. As a result of the study, we aim to increase the economical use of these species and promote liquid smoke implementation to the benefit of fish sausage production.

MATERIALS AND METHODS

Fish Meat and Ingredients

Fresh *C. regium*, *L. mystaceus* and *C. trutta* species collected from Keban Dam Lake (Elaziğ, Turkey) were transported on ice to the Firat University Laboratories. The fish were cut, gutted, skinned and filleted. Fish fillets were minced with a mechanical mincer (hole size: 3 mm in diameter). The sausage formula used for the present study was modified from the beef sausage formula of the Turkish Standard Institute (TSI 1990) and fish sausage formulas of Dincer (2008) and Dincer and Caklı (2010). Unlike TSI (1990), for the present study, fish mince was used instead of beef. Salt, red pepper, black pepper, sugar, pimento, coriander, ginger, soybean flour, potato starch and fat (50% beef fat + 50% sunflower oil) were purchased from local markets in Elaziğ, Turkey. Ice was obtained from ice machines. Ascorbic acid, sodium nitrite and sodium polyphosphate were purchased from food additive firms. Commercial liquid smoke (oak wood-based smoke) was bought from a food trade company (Zesti Eurosmoke, Istanbul, Turkey).

Preparation of Sausage

The formula used for the production of sausages is given in Table 1. Sausages of each fish species were divided into two different groups that were implemented using traditional or liquid smoke and a total of six different sausage groups were obtained. Fish meat and beef fat were minced and weighed separately. The appropriate quantities of the various ingredients were weighed. Sausage batter was prepared in sequential steps as follows: First step, minced fish fillets were mixed with sodium polyphosphate ice-water (1/3 of the total) for 3 min with an industrial bowl cutter. In the second step, ice-water (1/3 of the total), salt, red pepper, black pepper, sugar, pimento, coriander, ginger, soybean flour and potato starch were added with additional mixing at a temperature of 3C. The last step involved the addition of the preservatives (ascorbic acid, sodium nitrite) and ice-water (1/3 of the total) which were mixed at 13C. After that, sausage batter was divided into two groups. The first group was smoked in an oven, while the second group was added liquid smoke. Liquid smoke was added at 0.2% of the batter weight and the batter was mixed for the last time.

TABLE 1. FORMULATION OF FISH SAUSAGE

Ingredients	%
Minced fish meat	66.00
Soybean flour	2.54
Potato starch	2.54
Red pepper	0.11
Black pepper	0.20
Pimento	0.05
Coriander	0.13
Ginger	0.05
Sugar	0.15
Salt	2.00
Sodium nitrite (E 250)	0.01
Ascorbic acid (E 300)	0.02
Sodium polyphosphate (E 452i)	0.20
Ice	18.00
Fat (50% sunflower oil + 50% beef fat)	8.00

The comminuted batter was stuffed into No. 18-19 salting small intestine by using a manual stuffer. Traditional smoking groups were smoked at 75 ± 3C in a convection oven with 71 ± 3C internal temperature for 30 min (smoke was produced from oak with combustion). All groups of sausages were cooked at 85C in a convection boiler with 75C internal temperature for 10 min. After cooking, the sausages were immediately cooled in cold water (1:1, 6–7C).

Packing

Two sausage groups of each fish species implemented using traditional and liquid smoke were created and both groups were vacuum-packed (high barrier nylon polyethylene bags) and stored at 4 ± 1C until analysis on days 1, 7, 14, 21, 28, 42, 56 and 70. The obtained sausage groups from *C. regium* with traditional smoke and liquid smoke, groups from *L. mystaceus* with traditional and liquid smoke, and groups from *C. trutta* with traditional and liquid smoke were named A, B, C, D, E, F, respectively.

Analysis

Physicochemical Analysis. The pH value of fish meat and sausage samples were measured according to AOAC (1990). Ten grams of samples was homogenized with 90 mL of deionized water and the pH was measured with a digital pH meter (EDT.GP 353, U.K.). The water activity (a_w) was determined by using a digital water activity meter (TESTO-400) (Doe *et al*. 1983).

Chemical Analysis. Thiobarbituric acid reactive substances (TBARS) were determined by a selective third-order derivative spectrophotometric method (Tarladgis *et al*. 1960). TBARS content was expressed as mg of malondialdehyde (MDA)/kg for fish meat and sausage samples. Determination of total volatile basic nitrogen (TVB-N) was based on the method of Varlık *et al*. (1993).

Microbiological Analysis. Ten grams of each fish species and meat sausage were sampled aseptically, transferred to a stomacher bag and added 90 mL of 0.1% sterilized peptone water (buffer peptone water, LAB M, Limited, Lancashire, U.K.). The mixture was homogenized for 90 s with a stomacher (Stomacher 400, Lab. Blender, London, U.K.).

The concentrations of the various groups of bacteria as well as fungi were determined as colony forming units (cfu/g) on agar media, with the exception of the concentration of coliforms that was assessed by a Most Probable Number (MPN) method. Total mesophilic anaerobic bacteria were determined on Brewer anaerobic agar (Merck 1.05410, Merck KgaA, Darmstadt, Germany) incubated at 30C for 3 days in an anaerobic jar. Total psychrophilic and mesophilic aerobic bacteria were determined on plate count agar (Merck 1.05463) after incubation at 7C for 10 days and 30C for 3 days. Lactic acid bacteria (LAB) were determined on MRS agar ((Merck 1.10661) Merck KgaA, Darmstadt, Germany) after incubation at 28C for 2 days. Coliforms were assessed by an MPN technique using lactose broth (Merck 1.10266) at 37C for 1 day, and confirmed with brilliant green bile lactose broth (Merck 1.05454) at 37C for 1 day. *Escherichia coli* was determined on Chromocult TBX agar (Merck 1.16122) by incubation at 44C for 1 day, which was performed after an incubation period at 30C for 4 h. *Staphylococcus aureus* was determined on Baird–Parker agar (Merck 1.05406) after 37C for 1 day (after incubation, catalase test was carried out). Yeasts and molds were enumerated on potato dextrose agar (Merck 1.10130) acidified with 10% tartaric acid after incubation at 21C for 5 days (Halkman 2005).

Sensory Analysis. Ten experienced panelists (6 male and 4 female, aged 25–55 years) from Firat University, who were familiar with the sensory assessment of seafood products, evaluated the sensory quality. Sensory analysis was performed using the methods of Kurtcan and Gonul (1987) and Fernández-Fernández *et al*. (2002). The panelists were asked to evaluate sample taste, odor and texturing on a 5-point hedonic scale ranging from very poor (1) to very good (5).

Statistical Analysis. All analytical determinations were on days 1, 7, 14, 21, 28, 42, 56 and 70. Experiments were replicated twice on different occasions with different fish samples. Each sample was analyzed three times and the mean was calculated. Data were subjected to analysis of variance. The Tukey's honest significant difference procedure was used to test for differences between means ($P < 0.05$) using SAS 6.1 (SAS Institute, Inc., Cary, NC) (SAS 1999).

RESULTS AND DISCUSSION

Microbiological Changes during Storage

The microflora, total mesophilic anaerobic bacteria (TMAB), total mesophilic aerobic bacteria (TMA), total psychrophilic aerobic bacteria (TPC), LAB, coliform count (CC), *E. coli* (EC), *S. aureus* (SA), yeast and mold count (TMYC) of the fish meat prior to processing, as well as changes during processing and the subsequent storage of sausages smoked in an oven and sausages added liquid smoke were determined. In the fish fillets prior to processing, the concentrations (expressed as $\log_{10}$ cfu/g) were as follows: TMBA: 4.1 ± 1.5–4.6 ± 1.8, TMA: 4.4 ± 0.6–4.6 ± 0.7, TPC: 4.4 ± 0.8–4.9 ± 0.6, LAB: 2.6 ± 0.8–2.9 ± 0.9, SA: 1.4 ± 0.9–2.1 ± 0.2 and TMYC: 2.4 ± 1.2–2.9 ± 1.3. The concentration of CC was $2.2 \pm 0.9 - 2.8 \pm 1.0$ MPN/g. *E. coli* was not detected. Processing of fish meat into sausages decreased the concentration of microorganisms in both traditionally smoked sausages and sausages implemented with liquid smoke. The concentrations of TMA, TMAB, TPC and LAB decreased significantly ($P < 0.05$) (Fig. 1). The concentration of these groups of bacteria increased again during the subsequent storage of the sausages. There were no differences in bacterial growth between traditionally smoked sausages and sausages implemented with liquid smoke. The changes in the concentration of coliforms and *S. aureus* during processing of fish meat into sausages and subsequent storage of the sausages were not significant. However, the concentrations of coliforms and *S. aureus* (results not shown) were low in all samples. *E. coli* was not detected in any sample (<0.03 MPN/g). The decrease in the concentration of yeast and molds (TMYC) during processing of the fish fillets into sausages was significant, but there was no significant increase during the subsequent storage period. A concentration of up to 10^6 cfu/g of TMA has been reported to be acceptable for sausages (Adams *et al*. 1987; Goktan

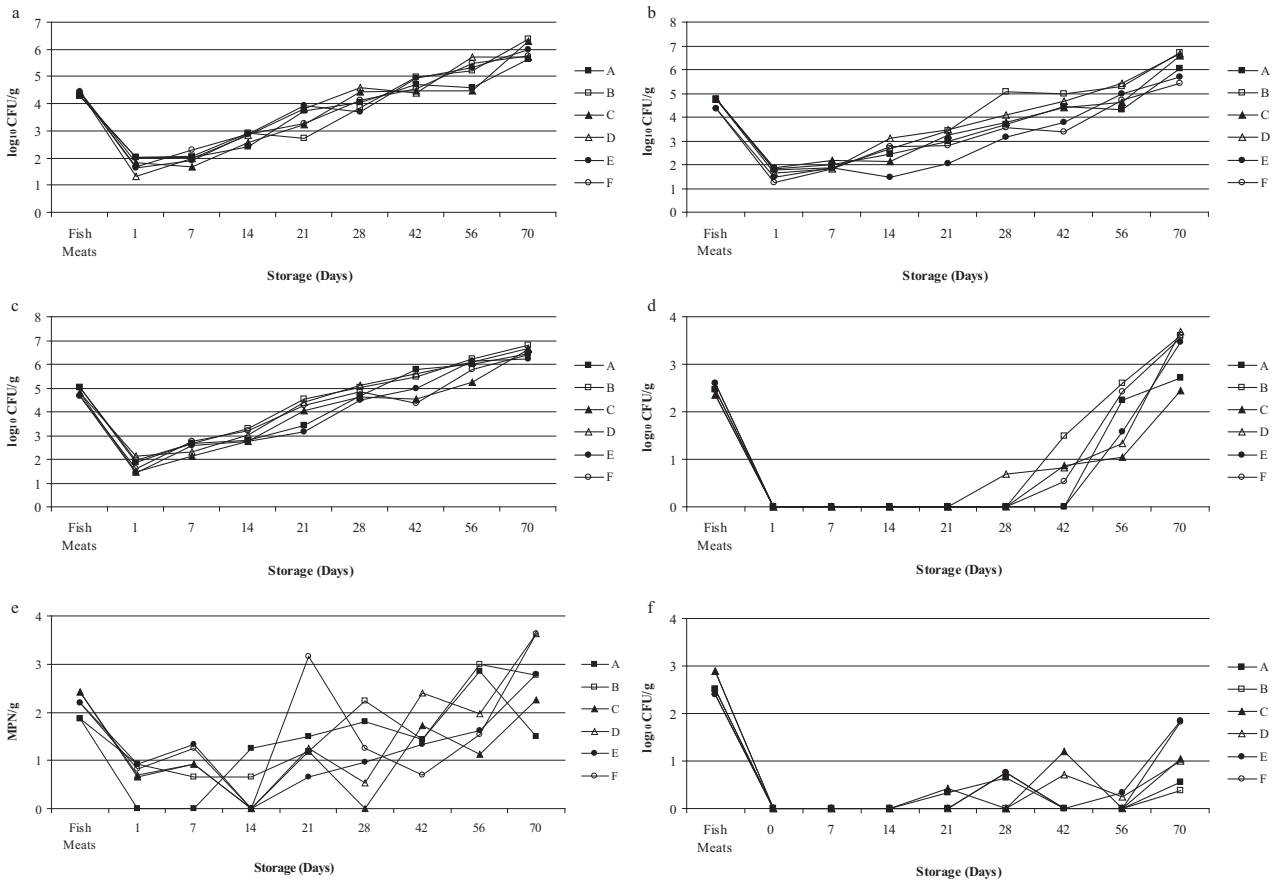

FIG. 1. CONCENTRATION OF (A) TOTAL MESOPHILIC ANAEROBIC BACTERIA (TMAB; LOG_{10} CFU/G); (B) TOTAL MESOPHILIC AEROBIC BACTERIA (TMA; LOG_{10} CFU/G); (C) TOTAL PSYCHROPHILE (LOG_{10} CFU/G) TPC; (D) LACTIC ACID BACTERIA (LAB; LOG_{10} CFU/G); (E) COLIFORM COUNT (CC; MPN/G); (F) YEAST AND MOLD (TMYC; LOG_{10} CFU/G) IN THE FISH MEATS PRIOR TO PROCESSING INTO SAUSAGES AND IN THE FISH SAUSAGES DURING STORAGE AT 4 ± 1C

1990; Sekin and Karagozlu 2004). In the present study, this limit was exceeded on day 56. There is no reported acceptable limit for the concentration of TMAB and TPC, but according to Baumgart (1990), the acceptable limit for LAB is 10^6 cfu/g. In this study, LAB did not exceed this limit during the storage period. It has been reported that the acceptable limit for coliforms in food is 9 MPN/g (Halkman 2005). In this study, the concentration of coliforms did not exceed this limit. According to the Turkish Food Codex (2009), the maximum acceptable concentration of SA is 10^3 cfu/g. SA did not exceed this limit during the study. *E. coli* must not be evident in any food (TSI 2002) and was not detected in this study. The concentration of yeast and molds must not exceed 10^3 cfu/g in food that has received heat treatment such as sausages (Turkish Food Codex 2009; TSI 2002). The concentration of yeast and molds did not exceed this limit during the study. No difference was found between liquid and traditional smoking techniques in terms of microbial growth. Similar results were reported by different authors from the previous studies (Kolsarıcı and Guven 1998; Alcicek 2010).

Physicochemical Changes during Storage

The pH values of *C. regium*, *L. mystaceus* and *C. trutta* fillets were in the range of 6.6–6.8. After the sausage-making process, the pH values were 6.38, 6.46, 6.47, 6.46, 6.29 and 6.27 for A, B, C, D, E and F, respectively. The pH declined significantly for all sausage groups during the storage period ($P < 0.05$) (Fig. 2), probably due to bacterial growth. A pH decrease during storage has been observed also in the previous studies of fish sausages (Raju *et al.* 2003; Filho *et al.* 2010). As in the previous studies (Goulas and Kontaminas 2005; Alcicek and Atar 2010), it was found that there were no differences between traditional and liquid smoke on the pH values of the sausage samples. The a_w values of the fish fillets were in the range of 0.93–0.95. The a_w values of the sausage groups were in the range

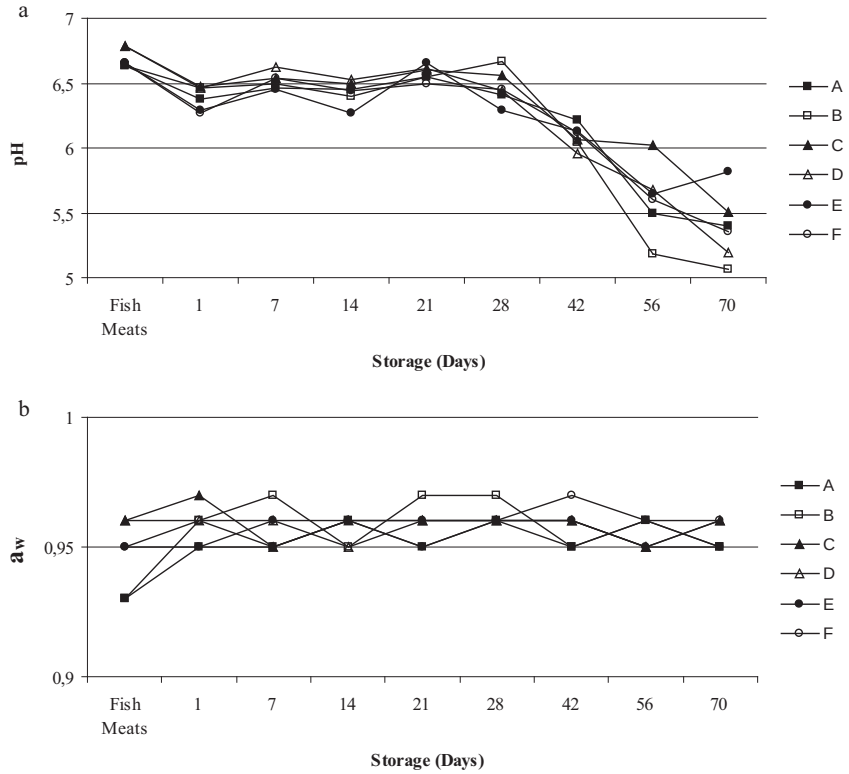

FIG. 2. PH (A) AND A_W (B) IN THE FISH MEATS PRIOR TO PROCESSING INTO SAUSAGES AND IN THE FISH SAUSAGES DURING STORAGE AT 4 ± 1C

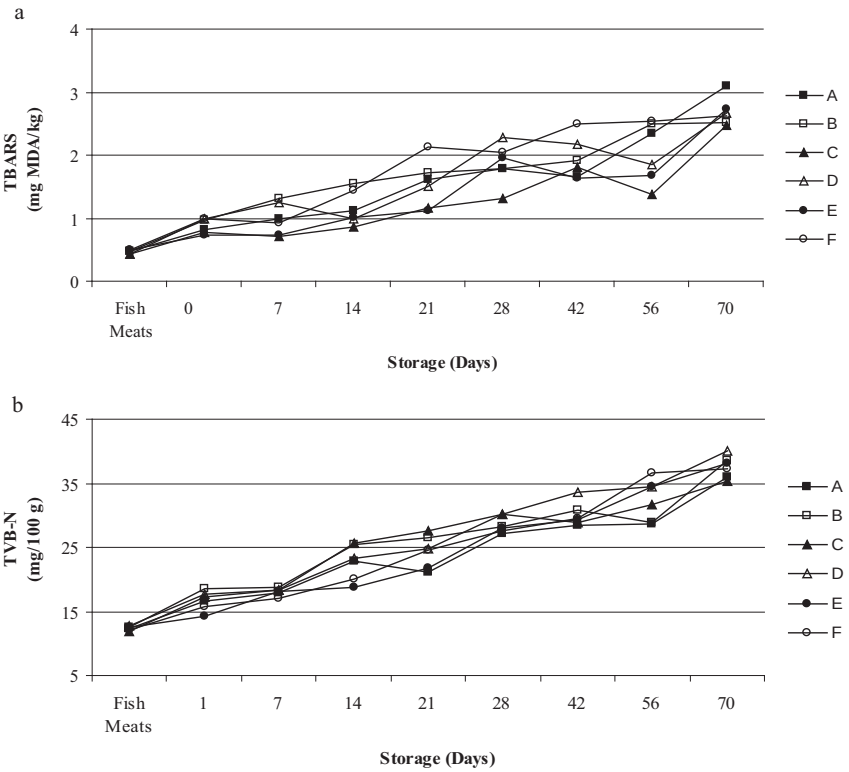

FIG. 3. THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS) (A) AND TOTAL VOLATILE BASIC NITROGEN (TVB-N) (B) IN THE FISH MEATS PRIOR TO PROCESSING INTO SAUSAGES AND IN THE FISH SAUSAGES DURING STORAGE AT 4 ± 1C

of 0.95–0.97. The a_w did not change significantly during the storage period.

Chemical Changes during Storage

The TBARS test is widely used to measure lipid oxidation in food products (Yu *et al.* 2002). Acceptable limit value of TBARS content is between 7 and 8 mg MDA/kg (Sinnuber and Yu 1958). TBARS values for fish fillets were in the range of 0.44–0.49 mg MDA/kg. The initial TBARS values for the different sausage groups were in the range of 0.73–0.98 mg MDA/kg (Fig. 3). TBARS increased over time during storage as in other studies (López-Caballero *et al.* 2005; Dincer 2008), and in the present study, after the 42nd day, the difference between the initial content and the content of the stored sausages was significant ($P < 0.05$)

Although the value of TBARS increased for all sausage groups, it was lower than the limit value during the storage period. The TVB-N is built up by ammonia, trimethylamine and dimethylamine originating from the breakdown of nucleotides and from the deamination of amino acids by microorganisms (Contreras-Guzman 2002; Filho *et al.* 2010). According to Huss (1995), the acceptable TVB-N level is 35–40 mg N/100g. The TVB-N values of the fish meats and the produced sausages prior to storage were below 18 mg/100 mg. The values of TVB-N increased

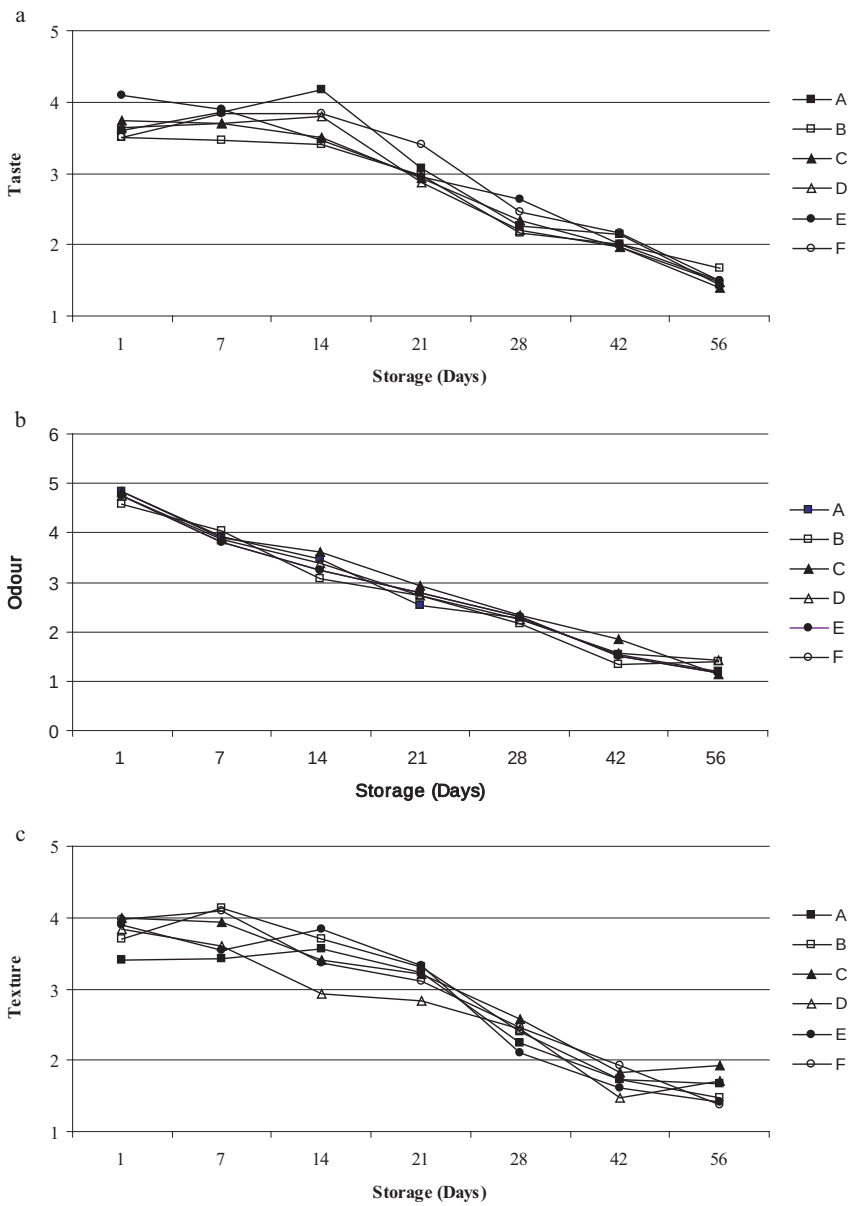

FIG. 4. CHANGES IN TASTE (A), ODOR (B) AND TEXTURE (C) OF FISH SAUSAGES DURING STORAGE AT 4 ± 1C

during the storage period and exceeded the acceptable limit on the 70th day for all sausage groups (Fig. 3). Similar results for TVB-N have been seen in the previous studies (Hu *et al.* 2007; Filho *et al.* 2010). Traditional and liquid smoke processes similarly influenced the TBV-N level for all sausage groups. This result was similar to Alcicek and Atar (2010).

Sensory Changes during Storage

Figure 4 shows the results of the sensory evaluation (taste, odor and texture) of different smoked sausages. The spoilage patterns were described by the panelists as follows: softening of texture before off-odors developed and the presence of bitter and rancid off-flavors. The limits of acceptability for taste, odor and texture were reached after 56 days for all groups of samples. However, the acceptable scores for texture, odor and taste were close the upper limit (1); all the sausage groups were still eatable on the 56th day.

There were no statistically differences ($P < 0.05$) between traditional smoke and liquid smoke treatment (Fig. 4). Similar results have been reported by Goulas and Kontaminas (2005). According to Munker and Meyer (1994), even though food implemented with traditional smoke gets higher rating from the panelists than food implemented with liquid smoke, food implemented with liquid smoke still obtain a higher rating than the minimum required for commercial consumer levels. It has been reported that the mode of administration, pot life and intensity of liquid smoke affected the sensory quality of food (Kolsarıcı and Guven 1998).

Consequently, neither species differences nor smoking technique had different effects on fish sausages. The liquid smoke, which contains smoke components of traditional smoke, had the same effects on the sensory quality in fish sausages as the traditional smoke technique.

CONCLUSION

The quality of vacuum-packed fish sausages stored at 4 ± 1C decreased with increasing storage time and reached the limit for eatable quality after approximately 56 days. Both smoking processes (traditional and liquid smoke) influenced the sausage samples similarly. However, liquid smoke could be preferred for fish sausages rather than traditional smoke due to advantages like easy implementation, easier control, lower cost, lower content of harmful smoke components like polycyclic aromatic hydrocarbons, and carcinogenic and mutagenic molecules produced during pyrolysis of wood and environmental friendliness. Dincer and Cakli (2010) stated that fish sausage is regarded as a wholesome food for children and the elderly. Because there is no such information, the knowledge about difference between the effects of traditional and liquid smoke techniques on the shelf life of fish sausages was tried to increase by this study. The next step of this study will be to expand our studies to more fish species and more smoking methods. Our overall aim is to increase the economic value of noncommercial fish species and develop alternative products for consumers.

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