The effect of different fermentation intervals on the quality characteristics of heat-treated and traditional sucuk

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A B S T R A C T
Fermentation time has an influence on the quality characteristics of fermented meat products. The effect of heat treatment on physicochemical, sensory and microbiological properties of sucuk was determined during fermentation and after heat application, and the properties of heat-treated sucuk samples were compared with those of traditional sucuk. Optimum fermentation period was determined for sucuk samples with desirable characteristics. Heat-treated sucuk were fermented at different fermentation intervals (0, 1, 2, 3, 4 and 5 days). Nine days of fermentation was included for traditional sucuk. All process parameters were applied under industrial conditions. Heat treatment increased the pH values, dry matter contents (protein, fat and salt), thioctic acid reactive substances of sucuk while decreasing the moisture content, free fatty acidity, and all bacterial counts (total viable and lactic acid bacteria, Staphylococcus/Micrococcus and Enterobacteriaceae) (P < 0.05). Significant differences in the instrumental color properties of heat-treated and traditional sucuk were found (P < 0.05). In terms of physicochemical, sensory and microbial properties, fermentation for three or more days before heat treatment resulted in sucuk samples with better acceptability, and produced sucuk samples with quality characteristics similar to those of traditional sucuk.

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1. Introduction
Sucuk is a spicy, dry, fermented sausage which is popular in Turkey and can also be found in countries located in Balkans, Middle East and Caucasus. Sucuk was mentioned in the “Divan-i Lugat-i Türk” (Compendium of the languages of the Turks, originally written by Mahmud of Kashgar in 1072) (Atalay, 1991). Sucuk is a sausage produced from a mixture of meat (beef, sheep and/or water buffalo meat), fat (beef fat and sheep tail fat), salt, sugar, garlic, spices and seasonings, and this mixture is stuffed into a casing where fermentation is carried out until a semi or dry product is obtained (TSE, 2002).

The production of sucuk relies on traditional techniques, and processing is greatly affected by the habits of artisans, raw materials and regions. However, the quality and particularly the safety of such products are related with the microflora of the fermented product. It is difficult to obtain fermented sucuks of a standard and high quality (İnal, 1964; Ockerman & Gökalp, 1987), mostly due to variations in technological, hygienic and microbiologic conditions and processing technology (Kılıç, 2009).

Heat treatment is the application of choice to enhance the safety and quality characteristics of meat products in comparison to non-thermal technologies like irradiation and high pressure processing (Thippareddi & Sanchez, 2006). During the last three decades, the manufacturing technology of traditional sucuk has been modified, and heat treatment has become a part of production although thermal processing is not a part of traditional sucuk processing.

Heat treatment of sucuk is used to eliminate pathogens, to extend shelf life, to shorten production time and to decrease production costs. In most modern food processing plants, this treatment is generally applied in a sealed oven by steam injection to raise temperature and relative humidity, and sucuk are held under these conditions for a time that is sufficient to eliminate all pathogens. However, in small-scale enterprises, heat treatment under different conditions is generally applied based on the decisions of artisans. The description of “pasteurized sucuk” is solely made by the manufacturers, and heat-treatment conditions vary between manufactures. The Turkish Standards Institute (TSE (Turkish Standard Institution), 2007) and the Official Notice on Fresh Meat, Prepared Meat and Prepared Meat Preparations in Turkish Food Codex (Turkish Food Codex, 2000) have recently described this type of heat-treated products as “sucuk-like meat product” rather than “pasteurized sucuks”.

There are a number of studies on traditional fermented sucuks related with various aspects including physical, chemical and microbiological properties (Karasoys, 1959; Kolsarici, Ertaş, & Şahin, ...
1986; Onuk, 1940), changes in these properties during processing (Ertas & Göğüş, 1980; Gökalp & Ockerman, 1985; Gürakan, Bozoğlu, & Wiess, 1995; Sançoban, Karakaya, & Caner, 2006; Yıldırım, Ülgen, & Özeren, 1978), the effect of ingredients (Aksu & Kaya, 2004; Bozkurt & Erkmen, 2004; Kayarð & Gök, 2004), biogenic amine contents (Aytan, 2004; Arthur, & ÇALIM, 2009), and proteolytic changes (Dalmıs, Ardıç, & ÇALIM, 2009), biogenic amine formation (Kurt & Zorba, 2004; Turantas, & Öneren, 1978), the effect of different fermentation intervals in conjunction with the mixing stage. The starter culture was a mixture of Staphylococcus carnosus and Lactobacillus plantarum was obtained from Chr. Hansen Laboratory (Chr. Hansens Lab., Hørsholm, Denmark). Hunter color measurements were taken immediately after slicing the samples to prevent color degradation because of light and oxygen. Measurements were taken in accordance with the recommendations on color determination by the American Meat Science Association (Hunt et al., 1991). Hunter L’, a’, b’ values and reflectance values were measured by a Minolta 508d colorimeter (Chuo-Ku, Osaka, Japan) using the D65 illuminant. Percent reflectance values of sucul samples at 500, 525, 560, 570 and 650 nm were also determined by the same instrument. The samples were sliced and wrapped with a single layer of stretch film by slight pressure application to obtain a uniform, bubble-free surface. It was ensured that there was no gap between the sample surface and the film, and the readings were carried out on the surface of the wrapped samples by the colorimeter. Six readings were taken and averaged out for each replication. Pigment nitrosation (NI), and pigment discoloration (RSI) values were calculated from percent reflectance values according to Hunt (1987) and Hunter and Harold (1987). Turkish sausage characteristics (TSC) values were calculated according to Üren and Babayigit (1996): Total color difference (TCD), hue angle, chroma (saturation index) and browning index (BI) were calculated using Hunter L’, a’, and b’ values.

uk dough was stuffed into a 38 mm fibrous casing in the shape of sausage stick with a vacuum stuffer. Fermentation started at 20 °C, 1 m/s air velocity and 90% relative humidity (RH), and RH was lowered to 3% every day to obtain 75% RH at the 5th day. Sucuks were fermented for 0, 1, 2, 3, 4 and 5 day and heat-treated in a steam oven. Heat treatment was started at 50 °C until the core temperature reached 40 °C, then oven temperature was increased to 80 °C until the core temperature reached 68 °C, and cooled immediately to 10 °C with cold water shower. Sucuks were hang-dried for 6 h at ambient temperature (20–25 °C), and samples were immediately analyzed. Traditional sucul, on the other hand, was produced in a 9-day fermentation period. The fermentation conditions were the same with heat-treated suculs until the 5th day, and after that, the conditions were stabilized at 20 °C, 1 m/s air velocity and 75% RH.

2.3. Sampling and sample preparation

From two replications, sucul samples were taken before and after heat treatment on the 0, 1, 2, 3, 4 and 5 days of fermentation. For traditional suculs, samples were analyzed after 9 days of fermentation in addition to 0, 1, 2, 3, 4 and 5 days. pH, moisture, salt, ash, fat, protein, residual nitrite, free fatty acidity, 2-thiobarbituric acid and instrumental color analyses, and microbial counts were determined in suculs. Sensory analyses were determined only for heat-treated and traditional products. All analyses were carried out in duplicates.

2.4. Chemical analyses

Ten grams of sample was homogenized in 100 mL of distilled water, and pH of this mixture was determined by a pH meter (Cole Parmer, Model 5996-50, USA). Moisture, salt, ash, protein, fat and residual nitrite contents measurements were determined according to the methods described by AOAC (1990). The lipid fraction for free fatty acids (FFA) analyses was extracted from the product according to Bligh and Dyer (1959). FFA analysis was done according to the alkaline titration method and the FFA was calculated as mg KOH/g fat. Thiobarbituric acid reactive substances (TBARS) test was performed according to Tarladgis, Watts, Younathan, and Dugan (1960).

2.5. Determination of instrumental color

Instrumental color measurements were taken immediately after slicing the samples to prevent color degradation because of light and oxygen. Measurements were taken in accordance with the recommendations on color determination by the American Meat Science Association (Hunt et al., 1991). Hunter L’, a’, b’ values and reflectance values were measured by a Minolta 508d colorimeter (Chuo-Ku, Osaka, Japan) using the D65 illuminant. Percent reflectance values of sucul samples at 500, 525, 560, 570 and 650 nm were also determined by the same instrument. The samples were sliced and wrapped with a single layer of stretch film by slight pressure application to obtain a uniform, bubble-free surface. It was ensured that there was no gap between the sample surface and the film, and the readings were carried out on the surface of the wrapped samples by the colorimeter. Six readings were taken and averaged out for each replication. Pigment nitrosation (NI), and pigment discoloration (RSI) values were calculated from percent reflectance values according to Hunt (1987) and Hunter and Harold (1987). Turkish sausage characteristics (TSC) values were calculated according to Üren and Babayigit (1996): Total color difference (TCD), hue angle, chroma (saturation index) and browning index (BI) were calculated using Hunter L’, a’, and b’ values.

2. Materials and methods

2.1. Materials

Two batches of sucul were produced under industrial conditions in a meat processing plant, one of the most prominent sucul establishment located in İzmir, Turkey. Fresh boneless beef, beef fat, spices, salt, sugar and nitrite were obtained from the plant facility. The beef and subcutaneous beef fat were taken from chest and round regions of two East Anatolian Red bullock carcasses from 2-year-old animals. A lyophilized starter culture mixtures commonly used by sausage manufacturers were mainly followed. Sucuk dough was prepared from beef and beef fat, salt, sugar, clean dry garlic, spices, nitrite, and starter culture according to the following recipe; 90 kg beef (about 20% fat), 10 kg subcutaneous fat, 2 kg salt, 0.4 kg sucrose, 1.1 kg garlic, 0.9 kg red bell pepper, 1 kg cumin, 0.7 kg black pepper, 0.25 kg pimento and 5 g NaN3O2. Additives and spices were added during mincing of the meat using a 1.3 cm plate mincer. Starter culture was added during the mixing stage. The starter culture was a mixture of L. plantarum and S. carnosus, and each was added at a dose of 102–106 cfu/kg of sausage dough. The mixture was held at 4 °C for 12 h and then re-minced using a 3 mm plate while frozen fat was added slowly. Sucuks from 2-year-old animals. A lyophilized starter culture mixtures commonly used by sausage manufacturers were mainly followed. Sucuk dough was prepared from beef and beef fat, salt, sugar, clean dry garlic, spices, nitrite, and starter culture according to the following recipe; 90 kg beef (about 20% fat), 10 kg subcutaneous fat, 2 kg salt, 0.4 kg sucrose, 1.1 kg garlic, 0.9 kg red bell pepper, 1 kg cumin, 0.7 kg black pepper, 0.25 kg pimento and 5 g NaN3O2. Additives and spices were added during mincing of the meat using a 1.3 cm plate mincer. Starter culture was added during the mixing stage. The starter culture was a mixture of L. plantarum and S. carnosus, and each was added at a dose of 102–106 cfu/kg of sausage dough. The mixture was held at 4 °C for 12 h and then re-minced using a 3 mm plate while frozen fat was added slowly. Sucuks from 2-year-old animals. A lyophilized starter culture mixtures commonly used by sausage manufacturers were mainly followed. Sucuk dough was prepared from beef and beef fat, salt, sugar, clean dry garlic, spices, nitrite, and starter culture according to the following recipe; 90 kg beef (about 20% fat), 10 kg subcutaneous fat, 2 kg salt, 0.4 kg sucrose, 1.1 kg garlic, 0.9 kg red bell pepper, 1 kg cumin, 0.7 kg black pepper, 0.25 kg pimento and 5 g NaN3O2. Additives and spices were added during mincing of the meat using a 1.3 cm plate mincer. Starter culture was added during the mixing stage. The starter culture was a mixture of L. plantarum and S. carnosus, and each was added at a dose of 102–106 cfu/kg of sausage dough. The mixture was held at 4 °C for 12 h and then re-minced using a 3 mm plate while frozen fat was added slowly.
2.6. Microbiological enumeration

Two sucuk samples were taken for each fermentation interval before and after heat treatment. Duplicate 10 g samples were aseptically cut from the central part of each sucuk stick (100 g) after the aseptic removal of casing and homogenized in 90 mL of 0.1% (w/v) sterile peptone water (PW) (Merck Co., Darmstadt, Germany) for 2 min by means of Colworth Stomacher 400 (Seward, London, UK). Homogenate was serially diluted in PW, and appropriate dilutions were cultured in duplicates as follows. Total mesophilic aerobic bacterial (TMB) counts were determined by spreading on plate count agar (PCA, Merck). Plates were incubated at 30 °C for up to 96 h. Total Enterobacteriaceae (TE) were enumerated by pouring plate method in violet red bile dextrose (VRBD) agar (Merck) after overlaying with the same medium and incubated at 37 °C for 24 h. Staphylococcus and Micrococcus (SM) were determined by the spread-plate method on Baird Parker (BP) agar (Merck) supplemented with egg yolk and potassium telluride. Petri plates were incubated at 37 °C for 72 h. Lactic acid bacteria (LAB) were enumerated on de Man Rogosa Sharpe (MRS) agar (Merck) overlayed with the same medium after incubation at 30 °C for 72 h. Bacterial counts were expressed as colony-forming units per gram of sample (cfu/g) and were transformed into logarithms.

2.7. Sensory evaluation

Sensory evaluation of sucuk samples was carried out for both heat-treated and traditional sucuks. Ten experienced panelists were trained on the sensory characteristics of different types of sucuks for three hours in three sessions. Panel was repeated twice, and in each session four randomized samples were served to the panel members. Evaluations were performed in individual booths under white fluorescent lighting. The panelists were provided unsalted bread and water at room temperature to clean the palate in between samples. Values were scored on a 10-point hedonic scale (1 = dislike extremely – 10 = like extremely). Products with sensory scores above 5 were assumed to be acceptable.

2.8. Statistical analyses

Data were analyzed by the analysis of variance (ANOVA). Student’s t test was used to compare the results for sucuks before and after heat-treatment within each fermentation interval. Turkey’s multiple comparison test was used as a post ANOVA technique to determine significant differences among the means. Minitab (Minitab, State College, PA) software (ver. 13.0 for Windows) was used for statistical analyses.

3. Results and discussion

The pH values of sucuks before heat treatment decreased significantly (P < 0.05) from an initial value of 6.06–4.87 during 5-days fermentation (Table 1). The production of lactic acid from carbohydrates by lactic acid bacteria is responsible for this reduction (Lücke, 2000). Heat treatment increased the pH values (P < 0.05) (Table 1), by 0.12 and 0.38 pH units for day 0 and 5, respectively, due to protein denaturation (Wardlaw, Shelley, & Acton, 1973). The pH of traditional sucuk was 5.01, and this value was significantly higher than those at day 4 and 5 (P < 0.05). The standard for traditional sucuk (TSE, 2002) states that ripened sausages must have a pH value between 4.7 and 5.4, the 9-day fermented traditional sucuk samples were within this range. Standard for

### Table 1

| pH values, proximate compositions, FFA and TBARS values of the sucuk samples. |
|---------------------------------|-----|-----|-----|-----|-----|-----|
|                                | 0   | 1   | 2   | 3   | 4   | 5   |
| pH BHe 6.06 ± 0.06aA           | 5.85 ± 0.03ab  | 5.58 ± 0.01bc  | 5.23 ± 0.04bd  | 4.95 ± 0.01e  | 4.87 ± 0.05ef  | 5.01 ± 0.01G   |
| AH 6.18 ± 0.02aA               | 5.85 ± 0.01ab  | 5.64 ± 0.05bc  | 5.41 ± 0.01bd  | 5.25 ± 0.01c  |               |               |
| Moisture (%)                   | BHe 75.58 ± 0.03aA | 53.95 ± 0.28ab  | 50.88 ± 0.27ac  | 47.40 ± 0.28bc  | 43.55 ± 0.14cd  | 39.63 ± 0.31de  | 32.48 ± 0.46F   |
| AH 51.30 ± 0.14bA              | 49.45 ± 0.31ab  | 47.20 ± 0.40ac  | 45.03 ± 0.31bd  | 41.93 ± 0.13c  | 39.33 ± 0.24d  |               |
| Salt (%) CHF 5.26 ± 0.02aA     | 2.97 ± 0.01ab  | 3.23 ± 0.01ab  | 3.36 ± 0.02ab  | 3.66 ± 0.01ac  |               |               |
| AH 2.96 ± 0.01aA               | 3.05 ± 0.01ab  | 3.18 ± 0.02ab  | 3.33 ± 0.01ac  |               |               |               |
| Ash (%) CHF 3.60 ± 0.01aA      | 3.90 ± 0.02ab  | 4.16 ± 0.01ab  | 4.47 ± 0.03ab  | 4.64 ± 0.01bc  | 4.91 ± 0.01c  | 5.17 ± 0.02d  |
| AH 4.16 ± 0.01aA               |               |               |               |               |               |               |
| Fat (%) CHF 25.66 ± 0.04aA     | 27.59 ± 0.43ab  | 21.81 ± 0.05ac  | 20.42 ± 0.02bc  | 19.25 ± 0.01cd  | 18.37 ± 0.02de  | 17.29 ± 0.03F  |
| AH 29.84 ± 0.11aA              | 30.72 ± 0.71ab  | 31.86 ± 0.30ac  | 33.15 ± 0.18bc  | 35.33 ± 0.31d  | 36.98 ± 0.21e  |               |
| Protein (%) BHe 12.26 ± 0.09aA | 14.32 ± 0.12ab  | 15.49 ± 0.06ab  | 16.57 ± 0.30ac  | 17.28 ± 0.28bc  | 18.77 ± 0.21cd  | 20.97 ± 0.02E  |
| AH 15.19 ± 0.18bA              | 15.75 ± 0.21ab  | 16.45 ± 0.09ab  | 17.09 ± 0.35ac  | 18.04 ± 0.10bc  | 18.79 ± 0.22c  |               |
| FFA (mg KOH/g fat) CHF 1.68 ± 0.02aA | 1.82 ± 0.05ab  | 2.04 ± 0.02bc  | 2.18 ± 0.02bd  | 2.44 ± 0.02e  | 2.65 ± 0.02ef  | 2.82 ± 0.05C  |
| AH 1.53 ± 0.02aA               | 1.57 ± 0.03ab  | 1.62 ± 0.02ac  | 1.66 ± 0.02bd  | 1.78 ± 0.02de  | 1.88 ± 0.02e  |               |
| TBARS (mg ma/kg product) BHe 0.253 ± 0.007aA | 0.276 ± 0.008ab  | 0.293 ± 0.010ab  | 0.316 ± 0.010ac  | 0.347 ± 0.011bd  | 0.362 ± 0.006c  | 0.709 ± 0.011E  |
| AH 0.620 ± 0.012aA             | 0.642 ± 0.002bc  | 0.649 ± 0.009cd  | 0.658 ± 0.010wed  | 0.658 ± 0.011d  |               |               |
| TBARS (mg ma/kg fat) CHF 0.987 ± 0.031aA | 0.999 ± 0.019ab  | 0.988 ± 0.012ab  | 0.972 ± 0.013bc  | 1.036 ± 0.029d  | 0.990 ± 0.005ab  | 1.729 ± 0.031H  |
| AH 2.078 ± 0.004aA             | 2.058 ± 0.047ab  | 2.003 ± 0.013bc  | 1.926 ± 0.007cd  | 1.837 ± 0.016d  |               |               |

a A Means in the same column with different lowercase superscripts are significantly different (P < 0.05).
A B Means in the same row with different capital superscripts are significantly different (P < 0.05).
c FFA: free fatty acids; TBARS: Thiobarbituric acid reactive substances.
Values are given as mean ± S.D. from duplicate determinations.
Traditional: 9 days fermented.
“heat-treated sucuk-like meat product” (TSE, 2007) and Turkish Food Codex (2000) state that they must have a pH value below 5.8. In this study, the pH values of 4- and 5-day fermented samples was at threshold and below the pH limit (5.4), respectively (Table 1).

Moisture content during the first 5 days of fermentation decreased significantly from an initial value of 57.58–39.63% (P < 0.05), the traditional sucuk had a moisture content of 32.48%, below the critical limit of the Turkish Standard (TSE, 2002) (Table 1). Heat treatment decreased the moisture content of the sucuk samples in the range of 6.3–0.3% for zero and 5-day fermentation, respectively. The Turkish Standard for heat-treated sucuk (TSE, 2007) states that samples must have moisture contents below 45%. The salt, ash, fat, and protein contents increased gradually throughout fermentation as a result of dry (Table 1). For each fermentation interval, heat treatment caused significant increases in salt, ash, fat and protein contents (P < 0.05).

The FFA values of sucuks increased during the fermentation (Table 1). Initial value was 1.68 and the fifth day value was 2.65 mg KOH/g fat. The FFA value of traditional sucuk was 2.82 mg KOH/g fat. These results were in good agreement with other studies on fermented sausages (Fernández, de la Hoz, Diaz, Cambero, & Ordonez, 1995; Stahnke, 1995; Toldra, 1998). Heat treatment decreased the FFA values of sucuk samples significantly for all fermentation intervals (P < 0.05) probably due to degradation of unsaturated FFA during heating.

Lipid oxidation in sucuk samples were expressed as the TBARS values in kg of product. The TBARS values of the sucuk samples before and after heat treatment increased significantly from day 0 to 5-days of fermentation (P < 0.05) (Table 1). Increase in TBARS values of heat-treated sucuss was significant for all fermentation intervals (P < 0.05), indicating that oxidative reactions increased during heat treatment. The most frequently used chemical tests for the quality assessment of lipid oxidation in animal product is TBARS. The significant increases of TBARS values both before and after heat treatment might be a result of lipid increase in the samples during drying. The fat content of the succuks during production and before and after heat treatment were significantly different (P < 0.05). Therefore, TBARS values on a fat basis were calculated. As seen in Table 1, for the fermentation intervals, no significant differences between day 0 and day 2 of fermentation were observed on a fat basis (P > 0.05); however, significant differences were observed in TBARS values between day 3 and day 5 of fermentation before heat treatments (P < 0.05). Heat treatment significantly decreased fat based TBARS values for each fermentation interval, and TBARS values of heat-treated sucuk fermented for 5 days were similar to that of traditional sucuk (P < 0.05). Evaporation and/or decomposition of thiobarbituric reactive substances during heat treatment might be a reason for this reduction. Another reason might be the relative increase of fat amount during fermentation and drying. Ilikkan, Ercoskun, Vural, and Sahin (2009) and Yildiz-Turp and Serdaroglu (2008) substituted animal fat with hazelnut oil in sucuk, and observed similar increases in the amount of fat and fresh weight-based TBARS values in control samples. When the TBARS values of these studies were re-calculated as TBARS values on a fat basis, it was found that the results were similar to those in the present study.

The lightness (L*) values of non-heat-treated sucuk samples decreased (P < 0.05) during fermentation (Table 2). The decrease in L* values of traditional sucsuks were also observed by Bozkurt and Bayram (2006) and Kayaardı and Gök (2004). The decrease in L* value represent darkening due to drying (Üren & Babayigit, 1996). However, heat treatment increased (P < 0.05) the L* values of sucsuks. The denaturation of myoglobin may result in a light color (Chasco, Lizaso, & Beriain, 1996). Compared to the L* values of heat-treated samples at different fermentation intervals, traditional sucuk had a lower L* value (P < 0.05).

Redness (a*) value increased significantly (P < 0.05) from an initial value of 14.53–15.97 during 5 days of fermentation. The increase in redness during the first day of fermentation might be due to the formation of nitrosomyoglobin, related to the characteristic red color of this type of meat product (Wirth, 1986). However, the redness values of the sucsuks before heat treatment between day 1 and day 5 of fermentation were similar (P > 0.05) while the redness values of the heat-treated sucsuks fluctuated. Heat treatment decreased a* values of all sucsuks (P < 0.05).

Prior to heat treatment, yellowness (b*) value of sucsuks increased significantly (P < 0.05) by the first day of fermentation.
and then decreased until the 5th day of fermentation ($P < 0.05$). These results were in good agreement with results reported by Bozkurt and Bayram (2006) and Kayaradi and Gök (2004). Heat treatment increased $b$' color values of all the sucuks significantly ($P < 0.05$). These changes in $b$' color values during fermentation are probably due to the oxygen consumption by microorganisms during their exponential growth phase leading to a decrease in oxymyoglobin which contributes to the value of this color coordinate system (Üren & Babayigıt, 1996; Úren & Babayigıt, 1997).

Total color difference (TCD) was calculated from Hunter $L^*$, $a^*$, and $b^*$ values (Homco-Ryan et al., 2004; Maskan, 2001). The TCD values decreased significantly on day 4 ($P < 0.05$), and increased insignificantly ($P > 0.05$) on the 5th day of fermentation in the sucuks before heat treatment. Heat treatment increased ($P < 0.05$) TCD values of sucuks from all fermentation intervals.

Hue angle for non-heat-treated sucuks decreased significantly ($P < 0.05$) during fermentation (Table 2). Heat treatment increased hue angle value of the sucuks significantly ($P < 0.05$). Bozkurt and Bayram (2006) reported a decrease in hue angle for traditional sucuks produced without nitrite during fermentation. Heat treatment decreased the chroma and browning index (BI) values ($P < 0.05$); however, inconsistent results were obtained for the sucuks before and after heat treatment during the fermentation.

Results for residual nitrite levels showed significant differences ($P < 0.05$) during fermentation and for the traditional sucuk samples (Table 3). The nitrite level of the sucuks decreased from an initial value of 27.75–10.01 during 5 days fermentation, and level of the nitrite in the traditional sucuk was 7.65 ppm. Heat treatment decreased residual nitrite contents of the sucuks ($P < 0.05$) at all fermentation intervals. The rapid decrease in nitrite level observed in dry sausages is well documented (Alley, Cours, & Demeyer, 1992). Sodium nitrite used in cured meat products interacts with various constituents in the meat’s complex biological systems. Thus at the end of the manufacturing process only about 10–20% of the nitrite added can be detected. Residual nitrite levels can drop even further during storage and distribution, and again during preparation and consumption (Cassens, 1995; Cassens, 1996; Cassens, 1997).

The nitrosation values (NI) were calculated from R560/R500. R560 is an estimate of nitrosomyoglobin and R500 estimates Fe II native pigments (myoglobin and oxymyoglobin) since myoglobin and oxymyoglobin have similar molar absorbance coefficients at 500 nm, and nitrosomyoglobin has an absorbance maximum at 560 nm. Therefore, small NI values indicate high conversion of myoglobin and oxymyoglobin to nitrosomyoglobin (Üren & Babayigıt 1996; Wong, 1989). The NI value of the sucuks before heat treatment decreased from an initial value of 1.11–0.95 during 5 days of fermentation, and the NI value of the traditional sucuk was 1.08. The sucuk samples after heat treatment had similar NI values to traditional sucuk ($P > 0.05$). Üren and Babayigıt (1997) reported that the NI value of most appreciated traditional sucuks by panelists was 1.59, and the NI values of 11 sucuks ranked between 0.97 and 2.15. In our study, the NI values of the sucuks were lower than the findings of Üren and Babayigıt (1997), indicating higher formation of nitrosomyoglobin.

The RSI value is an estimate of Fe II/Fe III ratio and is defined as R570/R650. R570 is related to Fe II pigments (myoglobin, oxymyoglobin and nitrosomyoglobin) and R650 indicates the amount of the Fe III pigment, metmyoglobin. Metmyoglobin has an absorption maximum at 627 nm and Fe II pigments have similar molar absorbance coefficients at 570 nm. Small RSI values indicate low levels of brown metmyoglobin. This means that the nitrosomyoglobin content of sausages must be high and metmyoglobin content must be low (Üren & Babayigıt, 1996; Wong, 1989). There were insignificant differences in pigment discoloration (RSI) values in the sucuks during 5 days of fermentation; however, heat treatment increased the RSI value significantly for all samples ($P < 0.05$) except for the 5th day of fermentation. Üren and Babayigıt (1997) reported that the RSI value of most appreciated traditional sucuks by panelists was 0.689, and the RSI values of 11 sucuks ranged between 0.406 and 0.689. In our study, the RSI values of the sucuks were similar to the results of Üren and Babayigıt (1997).

TSC is a ratio of oxymyoglobin and metmyoglobin and formulated as (R570–R525)/(R650–R525). TSC values are a measure of both pigment nitrosation and pigment discoloration and TSC can be a suitable parameter to measure the color of cured meat products (Üren & Babayigıt, 1996). During fermentation, TSC values fluctuated, and heat treatment increased them significantly ($P < 0.05$) with an exception of the samples from day 1 and day 5. The amount of oxymyoglobin may decrease during the heat treatment, leading to an increase in TSC values. Üren and Babayigıt (1996) reported that the TSC value of most appreciated traditional sucuks by panelists was 0.02, and the TSC values of 11 sucuks ranged between 0.02 and 0.26, as found in the present study.

### Table 3

| Residual nitrite contents; NI, RSI and TSC values of the sucuk samples. |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                             | 0                           | 1                           | 2                           | 3                           | 4                           | 5                           |
| Fermentation time (day)     |                             |                             |                             |                             |                             |                             |
|                             | Traditionalf               |                             |                             |                             |                             |                             |
| Residual nitrite (ppm)      |                             |                             |                             |                             |                             |                             |
| BH                         | 27.75 ± 0.09<sup>a</sup>    | 21.72 ± 0.02<sup>b</sup>    | 16.84 ± 0.27<sup>c</sup>    | 13.69 ± 0.12<sup>d</sup>    | 11.06 ± 0.07<sup>e</sup>    | 10.01 ± 0.21<sup>f</sup>    |
| AH                         | 9.82 ± 0.03<sup>a</sup>     | 8.30 ± 0.03<sup>b</sup>     | 7.21 ± 0.23<sup>c</sup>     | 5.75 ± 0.42<sup>d</sup>     | 3.97 ± 0.23<sup>e</sup>     | 2.74 ± 0.04<sup>f</sup>     |
| NI                         | 1.11 ± 0.01<sup>a</sup>     | 1.09 ± 0.02<sup>b</sup>     | 1.12 ± 0.01<sup>c</sup>     | 1.15 ± 0.01<sup>d</sup>     | 1.13 ± 0.01<sup>e</sup>     | 0.95 ± 0.01<sup>f</sup>     |
| AH                         | 1.13 ± 0.01<sup>a</sup>     | 1.10 ± 0.01<sup>b</sup>     | 1.17 ± 0.05<sup>c</sup>     | 1.12 ± 0.01<sup>d</sup>     | 1.12 ± 0.01<sup>e</sup>     | 1.07 ± 0.01<sup>f</sup>     |
| RSI                        | 0.38 ± 0.01<sup>a</sup>     | 0.40 ± 0.01<sup>b</sup>     | 0.41 ± 0.02<sup>c</sup>     | 0.40 ± 0.01<sup>d</sup>     | 0.40 ± 0.02<sup>e</sup>     | 0.44 ± 0.01<sup>f</sup>     |
| AH                         | 0.47 ± 0.01<sup>a</sup>     | 0.45 ± 0.01<sup>b</sup>     | 0.46 ± 0.01<sup>c</sup>     | 0.43 ± 0.01<sup>d</sup>     | 0.43 ± 0.01<sup>e</sup>     | 0.44 ± 0.02<sup>f</sup>     |
| TSC<sup>g</sup>             | 0.03 ± 0.001<sup>a</sup>    | 0.03 ± 0.003<sup>b</sup>    | 0.02 ± 0.003<sup>c</sup>    | 0.02 ± 0.001<sup>d</sup>    | 0.02 ± 0.001<sup>e</sup>    | 0.04 ± 0.001<sup>f</sup>    |
| AH                         | 0.07 ± 0.001<sup>a</sup>    | 0.07 ± 0.001<sup>b</sup>    | 0.09 ± 0.023<sup>c</sup>    | 0.05 ± 0.001<sup>d</sup>    | 0.08 ± 0.001<sup>e</sup>    | 0.03 ± 0.001<sup>f</sup>    |

<sup>a</sup> Means in the same column with different lowercase superscripts are significantly different ($P < 0.05$).
<sup>b</sup> Means in the same row with different capital superscripts are significantly different ($P < 0.05$).
<sup>c</sup> NI: pigment nitrosation; RSI: pigment discoloration; TSC: Turkish sausage characteristic value.
<sup>d</sup> BH: before heat treatment; AH: after heat treatment.
<sup>e</sup> Traditional: 9 days fermented.
<sup>f</sup> The TSC is described as a ratio of oxymyoglobin and metmyoglobin and formulated as (R570–R525)/(R650–R525). TSC value is a measure of both pigment nitrosation and pigment discoloration and can be a suitable parameter to measure the color of cured meat products (Üren & Babayigıt, 1996).
3.2. Microbial counts

Before the heat treatment, significant changes were found in bacterial loads during fermentation \((P < 0.05)\). Microbial counts for TMAB and LAB showed that the bacterial load generally remained constant during the first 2–3 days of fermentation and then declined significantly \((P < 0.05)\) as shown in Table 4. The TMAB count was initially 7.91 log cfu/g and did not change \((P > 0.05)\) during the first 2 days of the fermentation period. Then, it decreased to 6.51 log cfu/g on the 5th day, and 5.23 log cfu/g at the 9th day \((P > 0.05)\) (traditional sucuk). Nazlı (1998) investigated the effect of starter culture on the fermentation of sucuk and reported that a TMAB count of 7.40 on the 1st day of fermentation and 5.7 and 6.48 log cfu/g for the 6th and 9th days of fermentation, respectively. On the other hand, higher TMAB counts, which were initially 7.54 log cfu/g increased to 8.0 log cfu/g after 8 days of ripening in sucuk with a starter culture, were reported by Bozkurt and Erkmen (2002). Gökalp and Ockerman (1985) found TMAB counts were around 8 log cfu/g on the 1st day, increased to about 9 log cfu/g and then decreased to about 8.5 log cfu/g on the 9th day of fermentation in sucuk ripened at 16–18 °C with different starter culture preparations.

LAB counts were similar to TMAB counts and showed a slight increase from an initial value of 7.60–7.93 log cfu/g \((P > 0.05)\) on the 2nd day of fermentation, remained constant until 3rd day, and then decreased to 6.59 log cfu/g on the 5th day, and finally 5.45 log cfu/g in the traditional sucuk. Similar trends were reported for LAB counts by other authors (Ayhan et al., 1999; Dalmıs & Soyer, 2008; Gökalp & Ockerman, 1985). However, LAB levels were generally lower when compared to these studies, probably due to the use of different starter cultures and processing conditions.

SM counts decreased significantly \((P < 0.05)\) until 3rd day and stabilized during the 4th and 5th days of fermentation. The SM counts were in the range reported by other investigators (Dalmıs & Soyer, 2008; Nazlı, 1998) showing a decrease after the beginning of fermentation. On the other hand, with increasing fermentation time TE showed a sharp decline during the 5 days of fermentation, and the TE count was below the detection level \(<1 \text{ log cfu/g}\) in traditional sucuk, mainly related to the pH decrease and dehydration (Lücke, 2000). Generally, Enterobacteriaceae, which have the ability to form biogenic amines, are outcompeted and suppressed by the fermentation microflora (Bauer, 2004). Ayhan et al. (1999) showed that Enterobacteriaceae counts of sucuk made with starter culture decreased from an initial value of 3.20 log cfu/g to 2.25 log cfu/g on the 8th day of fermentation. Another study (Samelis, Metaxopoulos, Vlassi, & Pappa, 1998) showed that Enterobacteriaceae were progressively eliminated from the ripened dry sausages after 3 or 7 days of fermentation for different batches of sausages. Nazlı (1998) investigated the effect of starter culture on the ripening of sucuk and reported that counts for SM and counts for coliform bacteria decreased from initial values of 7.2 log cfu/g and 5.54–4.79 and 2.84 log cfu/g after 9 day of fermentation, respectively.

While these trends for bacterial counts during 5 day intervals of fermentation and traditional sucuk could be explained by lactic acid production by LAB and pH decline (Ince, 1992; Lücke, 2000), differences in terms of higher or lower levels of bacterial counts reported in comparable studies can be attributed to the use of different starter cultures, composition of the product, different level of initial contamination of the meat, and different processing conditions.

The heat treatment of sucuks caused a significant reduction \((P < 0.05)\) in bacterial loads for all samples fermented between zero and 5 days (Table 4). Reductions in TMAB and LAB level were 4–5 log cycles \((P < 0.05)\) after heat treatment. Wardlaw et al. (1973) reported 4.5 log cycle reduction in LAB level after heat treatment \((71°C \text{ internal temperature})\) of summer sausage fermented at 38 °C for 36 h. In another study, Dalmıs and Soyer (2008) stated that heat treatment caused 1 log cycle, 2 log cycle and 2.5 log cycle decreases for TMAB, LAB and SM levels, respectively, in sucuk fermented at 20 °C for 3 days using a starter culture mixture. In the present study, reduction of these bacteria for fermented sucuk was higher.

Heat treatment of sucuk reduced the counts for SM by 5.20, 4.90 and 4.17 log cfu/g for day 0, 1 and 2, respectively, while the SM level was below the detection limit (2 log cfu/g) for the rest of fermentation intervals. Following heat treatment at each fermentation interval, TE levels were below the detection limit \(<1 \text{ log cfu/g}\). Heat treatment caused microbial reduction from >4.60 log cfu/g to >2.46 log cfu/g for samples from day 0 to day 5.

By the 5th day of fermentation the magnitude of log reduction for the samples from each fermentation interval did not increase. Slightly lower log reductions than those for zero day fermentation was observed towards the end of fermentation. As a result, increasing fermentation time did not increase the effectiveness of heat treatment on the reduction of microbial load. Therefore, the microbial reduction rate for the sample from each fermentation stage upon heat treatment seems to be more related to destructive effect of the heat rather than pH. On the other hand, based on the log reduction for each fermentation interval, except for the SM and TE counts, log reduction remained almost unchanged, between 4 and 5 log cfu/g, suggesting that, after heat treatment the number

### Table 4

<table>
<thead>
<tr>
<th>Microbial group</th>
<th>Fermentation time (day)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Traditional†</th>
</tr>
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<tr>
<td>TMAB§</td>
<td>BH†</td>
<td>7.91 ± 0.03&lt;sup&gt;a&lt;/sup&gt;A</td>
<td>8.03 ± 0.05&lt;sup&gt;a&lt;/sup&gt;A</td>
<td>8.17 ± 0.59&lt;sup&gt;a&lt;/sup&gt;A</td>
<td>7.72 ± 0.05&lt;sup&gt;a&lt;/sup&gt;B</td>
<td>7.39 ± 0.32&lt;sup&gt;a&lt;/sup&gt;B</td>
<td>6.51 ± 0.12&lt;sup&gt;a&lt;/sup&gt;C</td>
<td>5.23 ± 0.28&lt;sup&gt;a&lt;/sup&gt;B</td>
</tr>
<tr>
<td></td>
<td>AH†</td>
<td>2.58 ± 0.03&lt;sup&gt;a&lt;/sup&gt;B</td>
<td>3.19 ± 0.05&lt;sup&gt;a&lt;/sup&gt;B</td>
<td>2.99 ± 0.06&lt;sup&gt;a&lt;/sup&gt;B</td>
<td>2.87 ± 0.12&lt;sup&gt;a&lt;/sup&gt;B</td>
<td>2.37 ± 0.15&lt;sup&gt;a&lt;/sup&gt;B</td>
<td>2.15 ± 0.11&lt;sup&gt;a&lt;/sup&gt;B</td>
<td></td>
</tr>
<tr>
<td>LAB§</td>
<td>BH†</td>
<td>7.60 ± 0.07&lt;sup&gt;a&lt;/sup&gt;A</td>
<td>7.83 ± 0.04&lt;sup&gt;a&lt;/sup&gt;A</td>
<td>7.93 ± 0.02&lt;sup&gt;a&lt;/sup&gt;B</td>
<td>7.63 ± 0.09&lt;sup&gt;a&lt;/sup&gt;A</td>
<td>6.85 ± 0.01&lt;sup&gt;a&lt;/sup&gt;C</td>
<td>6.59 ± 0.12&lt;sup&gt;a&lt;/sup&gt;C</td>
<td>5.45 ± 0.10&lt;sup&gt;a&lt;/sup&gt;B</td>
</tr>
<tr>
<td></td>
<td>AH†</td>
<td>2.37 ± 0.13&lt;sup&gt;a&lt;/sup&gt;B</td>
<td>2.79 ± 0.02&lt;sup&gt;a&lt;/sup&gt;B</td>
<td>3.93 ± 0.04&lt;sup&gt;a&lt;/sup&gt;B</td>
<td>2.78 ± 0.32&lt;sup&gt;a&lt;/sup&gt;B</td>
<td>2.30 ± 0.34&lt;sup&gt;a&lt;/sup&gt;B</td>
<td>2.25 ± 0.10&lt;sup&gt;a&lt;/sup&gt;B</td>
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</tr>
<tr>
<td>SM§</td>
<td>BH†</td>
<td>7.90 ± 0.05&lt;sup&gt;a&lt;/sup&gt;A</td>
<td>7.56 ± 0.16&lt;sup&gt;a&lt;/sup&gt;B</td>
<td>6.85 ± 0.09&lt;sup&gt;a&lt;/sup&gt;C</td>
<td>5.96 ± 0.10&lt;sup&gt;a&lt;/sup&gt;D</td>
<td>5.69 ± 0.01&lt;sup&gt;a&lt;/sup&gt;D</td>
<td>5.67 ± 0.05&lt;sup&gt;a&lt;/sup&gt;D</td>
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<td>AH†</td>
<td>2.70 ± 0.04&lt;sup&gt;a&lt;/sup&gt;B</td>
<td>2.66 ± 0.05&lt;sup&gt;a&lt;/sup&gt;B</td>
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<tr>
<td>TE§</td>
<td>BH†</td>
<td>5.60 ± 0.06&lt;sup&gt;a&lt;/sup&gt;A</td>
<td>5.30 ± 0.31&lt;sup&gt;a&lt;/sup&gt;B</td>
<td>4.90 ± 0.02&lt;sup&gt;a&lt;/sup&gt;B</td>
<td>4.36 ± 0.15&lt;sup&gt;a&lt;/sup&gt;C</td>
<td>3.86 ± 0.06&lt;sup&gt;a&lt;/sup&gt;D</td>
<td>3.46 ± 0.25&lt;sup&gt;a&lt;/sup&gt;D</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>AH†</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

<sup>a</sup>Means in the same column with different lowercase superscripts are significantly different \((P < 0.05)\).
<sup>a</sup>Means in the same row with different capital superscripts are significantly different \((P < 0.05)\).
<sup>c</sup>Microbial counts reported in comparable studies can be attributed to the use of different starter cultures, composition of the product, different level of initial contamination of the meat, and different processing conditions.
<sup>d</sup>Values are given as mean ± S.D. from duplicate determinations.
<sup>e</sup>BH: before heat treatment; AH: after heat treatment.
<sup>f</sup>Traditional: 9 days fermented.
of surviving microbial cells might have been spore forming or heat resistant ones.

3.3. Sensory panel

Sensory evaluation indicated that the taste of heat-treated sucuks from all fermentation intervals was not significantly different (P > 0.05) to those for traditional sucuk samples while scores for odor and flavor of heat-treated sucuks from the 3rd and 4th days of fermentation were higher (P < 0.05), respectively (Table 5).

Scores for spice ratio were lower for day 0 and 1st days of fermentation and there were insignificant differences in spice ratio values between the 2nd and 5th days of fermentation. Redness, color, and appearance scores increased (P < 0.05) gradually to the 3rd day of fermentation, and then stabilized (P > 0.05) while redness and color were lower (P < 0.05) and appearance were higher (P < 0.05) than those for the traditional sucuk samples. This could be due to the formation of a desirable red color. The rate of nirosomyoglobin formation may be slower due to the decreasing amount of nitrite and myoglobin or denaturation of formed pigment resulting from heat treatment at lower pH. Traditional sucuks received the highest scores for both redness and color parameters (P < 0.05) while having lower values (P > 0.05) for appearance and consistency. During fermentation, heat-treated sucuks between day 3 and 5 had the highest (P > 0.05) scores for appearance. Chewiness scores for the heat-treated sucuk samples from the 1st and 5th days of fermentation were similar (P > 0.05) but higher than the scores for the traditional sucuk samples (P < 0.05). Overall acceptability scores were similar to those for traditional sucuk (P < 0.05) except for the 5th day.

4. Conclusion

Results of pH, proximate composition, FFA, TBARS, microbial counts and sensory analyses indicated that heat-treated sucuks from the fermentation at 0–5 day intervals were significantly different to the non heat-treated and traditional sucuk. Heat-treated sucuks from each fermentation interval were different from each other in terms of physicochemical, sensory and microbiological properties. On the other hand, Turkish official standard for heat-treated sucuk (TSE, 2007) neither refers to any fermentation period nor pH range. Instead, terms like “partial ripening” in the description of “sucuk-like meat product” and a maximum pH level of 5.8 in the final product are mentioned. In this study, it was shown that pH level attained after heat treatment for each fermentation interval differs considerably. Therefore, heat-treated sucuks with different fermentation times should be legally classified as un-

fermented heat-treated sucuk, semi-fermented heat-treated sucuk, and fermented heat-treated sucuk.

Application of heat treatment after fermentation gave satisfactory bacterial load reduction, and at least 3 days of fermentation of sucuk followed by a heat treatment can lead to reasonable reduction of microbial load and an acceptable product with desirable physical, chemical and sensory attributes. However, further study should be considered to determine changes of quality attributes of heat-treated sucuks during storage. Moreover, reduction of pathogenic microorganisms should be studied to assure safer products.

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References


