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Physicochemical and Microbiological Properties of Sucuk produced with Different Heat Treatment Temperatures

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ABSTRACT

The aim of this study was to determine physicochemical and microbiological properties of sucuk produced with different heat treatment temperatures. The effect of heat treatment at 60 (for 15 minutes), 65 (for 10 minutes) and 70°C (as reached) on chemical (pH, moisture, fat, protein, salt, ash, free fatty acidity, thiobarbituric acid reactive substances, residual nitrite, nitrosomyoglobin, total pigments, nitrosomyoglobin conversion ratio), physical (L*, a*, b*, nitrozation, discolorization), microbiological (total mesophilic aerobic bacterial counts, Staphylococcus and *Micrococcus* counts and lactic acid bacteria counts) and sensory quality characteristics of heat treated sucuk were investigated. Results were compared with traditional sucuk samples produced under commercial conditions. Heat-treated sucuks were fermented for 3 days and traditional ones were fermented for 9 days under controlled conditions. Significant differences in physicochemical and sensory properties of produced sucuks were observed during production. The overall quality attributes of traditional sucuk were different in comparison to heat treated sucuks. Decreases in residual nitrite, nitrosomyoglobin and total pigment amounts in heat treatment process were determined. Nitrosopigment transformation ratio increased; however, in terms of physicochemical, microbiological and sensory properties, heat treatment at 60°C for 15 minutes resulted in sucuk samples with better acceptability.

Keywords: Heat treatment, Temperature, Sucuk, Physicochemical characteristics

Farklı Isıl İşlem Sıcaklıklarında Üretilen Sucukların Fizikokimyasal ve Mikrobiyolojik Özellikleri

ÖZ

Bu çalışmada farklı ısıl işlem sıcaklıklarının sucuğun özelliklerine etkisi incelenmiştir. Bu amaçla 60 (15 dakika), 65 (10 dakika) ve 70°C (anlık) ısıl işlemin kimyasal (pH, nem, yağ, protein, tuz, kül, serbest yağ asitliği, tiyobarbiturik asit, kalıntı nitrit, nitrozomyoglobin, toplam pigment, nitzomyoglobin dönüşüm oranı), fiziksel (L*, a*, b*, nitrozasyon, diskolorizasyon) mikrobiyolojik (toplam mezofilik aerobik bakteri, laktik asit bakteri, stafilokok ve mikrokok sayımları) ve duyusal kalite özellikleri incelenmiştir. Elde edilen sonuçlar endüstriyel şartlarda üretilen geleneksel sucuklarla karşılaştırılmıştır. Isıl işlem uygulaması 3 günlük fermentasyondan sonra yapılmış ve geleneksel sucuk 9 gün fermente edilmiştir. Üretim süresince sucuklarda önemli fizikokimyasal, mikrobiyolojik ve duyusal değişiklikler gözlenmiştir. Geleneksel sucuğun toplam kalite özelliklerinin ısıl işlem görmüş sucuklardan farklı olduğu görülmüştür. Isıl işlemde kalıntı nitrit, nitrozomyoglobin ve toplam pigment miktarında düşüş gözlenmiştir. Nitrozopigment dönüşüm oranı yükselmiştir. Ancak fizikokimyasal, mikrobiyolojik ve duyusal özellikler bakımından 60°C'de 15 dakikalık ısıl işlemle üretilen sucuklar daha iyi kabul edilebilirlik değerleri almıştır.

Anahtar Kelimeler: Isıl işlem, Sıcaklık, Sucuk, Fizikokimyasal özellikler

INTRODUCTION

Sucuk, like other fermented sausages, is one of the most appreciated meat products that people enjoy to consume. Sucuk is a traditional fermented, semi-dry or dry sausage produced from a mixture of meat (beef, sheep and/or water buffalo meat), fat (beef fat and sheep tail fat), salt, sugar, garlic and other ingredients like spices and seasonings, and this mixture is stuffed into a casing where fermentation is carried out until a semi-dry or dry product is obtained [1, 2]. Production technology of sucuk has changed with modern plants that use controlled ripening chambers in recent decades. Moreover, these plants have changed their production method from traditional fermentation-drying to fermentation-heat treatment to shorten the production time and to improve the hygiene quality of the final product [3]. Nowadays, heat process has become a part of sucuk production [3, 4]. Heat process of sucuk production is used to eliminate pathogens, to extend shelf life, to shorten production time and to decrease production costs [3, 4]. Most of the industrial sucuk manufacturers apply similar heat process as prescribed in the regulation of the United States of America, Food Safety and Inspection Service (9 CFR 318.17) in which a 5-log^{10} relative reduction of *E. coli* O157:H7 can be achieved [4]. Legislation of Meat and Meat Products in Turkish Food Codex (5) has recently described heat-treated red meat sucuk with a minimum heat treatment temperature of 68°C.

Studies on heat-treated sucuks are limited and there is no study about the effect of heat treatment temperatures of sucuks. Therefore, the targets of this work were to determine the effect of different heat treatment temperatures on the quality parameters of sucuk before and after heat treatment as well as to compare traditional sucuk.

MATERIALS and METHODS

Three batches of sucuk were produced in a commercial meat processing plant, which is one of the most known sucuk establishment located in İzmir, Turkey. The beef from round and brisket of carcass, beef fat, spices, salt, sugar and nitrite were obtained from the same plant facility. A lyophilized starter culture mixture of *Stapylococcus carnosus* and *Lactobacillus plantarum* was obtained from Chr. Hansen Laboratory (Chr. Hansens Lab., Hørsholm, Denmark).

Sucuk Preparation

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, 50% brisket and 50%round), 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 $NaNO_2$. Beef, nitrite, garlic and spices were added before mincing of the meat using a 1.3 cm plate mincer. Starter culture was added during the mixing stage which were a mixture of *Lactobacillus plantarum* and *Staphylococcus carnosus*, and each was added at a dose of 10^8 cfu/kg of sucuk dough. The mixture was kept at 4°C for 12 h. Frozen fat was added slowly to the dough during mincing using a 3 mm plate to form a mosaic appearance and was stuffed into fibrous casing (Ø: 38 mm) with a vacuum stuffer. Fermentation was carried out at 20°C and 1 m/s air velocity while initial relative humidity was 90% and decreased 3% per day to obtain 81% RH at the 3rd day. After 3 days of fermentation sucuks were heat treatment until the core temperature reached to 60 (for 15 minutes), 65 (for 10 minutes) and 70°C (as reached). The fermentation conditions of traditional sucuk were the same with heat-treated sucuks until the 3rd day, and then conditions were 20°C, 1 m/s air velocity and 75% RH until 9th day.

Sampling and Sample Preparation

Thee replications of sucuk were prepared and samples were collected before and after heat treatments. Moisture, salt, ash, fat, protein, pH, residual nitrite, free fatty acidity, 2-thiobarbituric acid reactive substances, nitrosomyoglobin conversion ratio, nitrosomyoglobin, total haem pigments contents and instrumental colour and texture analyses were determined in heat treated and traditional sucuks. Heat-treated and traditional products were also subjected to sensory analyses. Analyses were duplicated.

Chemical and Microbiological Analyses

Analyses of salt, fat, protein, moisture, ash and residue nitrite contents, pH, free fatty acidity (FFA), thiobarbituric acid reactive substances (TBARS) values were described in previous study [6]. Nitrosomyoglobin conversion ratio and nitrosomyoglobin, total haem pigments contents were measured according to Zaika et al. [7].

Instrumental colour measurements were taken after slicing the samples to prevent effect of oxygen and light. American Meat Science Association's advices were followed on colour determination [8]. Minolta 508d colorimeter (Chuo-Ku, Osaka, Japan) using the D65 illuminant was used in measuring CIE L*, a*, b* values and reflectance values. The samples were covered with a layer of stretch film and a uniform, air gap free surface was obtained. The readings were carried out on the surface of the wrapped samples by the colorimeter. The average of six readings were taken for per replication. Pigment nitrozation (NI) and pigment discoloration (RSI) values were calculated from percent reflectance values according to Hunt [9].

Texture Analyzer (TA Plus Texture Analyzer Lloyd Instruments, Hants, England) equipped with a cone probe (FGC045) was used to evaluate textural properties of sucuks Approximately 40 mm thick sucuks were peeled and allowed to achieve room temperature (20°C). The samples were pressed with 1 mm/s probe speed and 0.01 Newton force to compress 35% of their original thickness. The average of ten readings was taken per replication.

Sucuks were sensory evaluated after 30 days by a panel of ten individuals who were previously trained and experienced on the sensory evaluation of sucuks. Quantitative Descriptive Analysis (QDA) was used. Two sessions per day were conducted in which panel members were served four randomized samples per session with a break of at least 1 h between sessions to reduce fatigue. Evaluations were performed in individual booths, under white fluorescent lighting. Bread and room temperature water were provided to clean the palate between samples. The tests were carried out between meals. Sucuk samples sliced into 5-mm thickness were randomly served for evaluation as raw and fried. Unsalted bread and water were given between the samples. Sensory outer colour, inner colour, consistency, appearance, taste, odour, flavour, chewiness and overall acceptability properties of the samples were evaluated with a continuous scale between 0 and 10, where 0 was worst and 10 was best.

Total mesophilic aerobic bacterial (TMAB) counts were determined by spreading on plate count agar (PCA, Merck). Plates were incubated at 30°C for up to 96 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.

Statistical Analyses

Data were analysed by the analysis of variance (ANOVA). Tukey'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.

RESULTS

pH and Proximate Composition

Table 1 shows some of the physicochemical properties of sucuk samples. The pH value was 4.99 before heat treatment and increased after the treatment ($P<0.05$). Sucuk samples heat treated at 70°C showed significant greater pH value than the others ($P<0.05$). The pH values of sucuk before heat treatment and traditional sucuk were in same statistical group ($P<0.05$). The pH increment during heat treatment may be a result of thermal protein denaturation since the pH value of heat treated sucuk at 70°C showed the highest value. Ercoşkun et al. [6] and Ercoşkun [4] reported pH increment during heat treatment of sucuk at 60 and 68°C, respectively. According to Turkish food legislation, the pH value of the final product should be less than 5.6 in heat treated sucuks and 5.4 in fermented sucuk.

Table 1. The results of Proximate Composition and pH. FFA and TBARS Values

	Before Heating	Heat Treatment Conditions			Traditional Sucuk
		60°C	65°C	70°C	
pH	4.99±0.03^{C}	5.12±0.01^{B}	5.15±0.01^{B}	5.24±0.01^{A}	4.99±0.01^{C}
Moisture (%)	49.92±0.20^{A}	48.80±0.20^{B}	48.65±0.24^{B}	48.41±0.18^{B}	35.39±0.26^{C}
Fat (%)	28.46±0.12^{C}	29.09±0.15^{B}	29.18±0.16^{B}	29.47±0.06^{B}	36.41±0.20^{A}
Protein (%)	14.63±0.08^{C}	14.96±0.03^{B}	15.00±0.08^{B}	15.00±0.21^{B}	18.72±0.23^{A}
Salt (%)	2.72±0.02^{C}	2.78±0.04^{BC}	2.79±0.01^{B}	2.80±0.01^{B}	3.48±0.01^{A}
Ash (%)	3.43±0.02^{C}	3.50±0.01^{B}	3.51±0.02^{B}	3.54±0.03^{B}	4.38±0.04^{A}
FFA	4.34±0.05^{B}	3.63±0.05^{C}	3.44±0.06^{CD}	3.26±0.03^{D}	6.66±0.17^{A}
TBARS	0.34±0.01^{D}	0.62±0.01^{B}	0.63±0.01^{AB}	0.65±0.01^{A}	0.40±0.01^{C}

Values are given as mean ±S.D from duplicate determinations; A-D Means in the same line with different superscripts are significantly different ($P<0.05$); FFA: free fatty acidity (mg KOH/g fat); TBARS: thiobarbituric acid reactive substances (mg malonaldehyde /kg product).

Initial moisture content of sucuks before heat treatment was 49.92 and this value significantly decreased with the heat application ($P<0.05$). Traditional sucuk showed lowest moisture content ($P<0.05$). The decrease in moisture content on heat treated samples could be due to the rapid drying during heat process. Being a part of dry matter content in sucuk; salt, ash, fat and protein contents of heat treated samples showed similar increase due to the decrease in moisture level. Decrement of moisture content and increment of salt, ash, fat and protein contents due to drying in heat treatment of sucuk and were reported by other researchers [3, 4, 6, 10-12].

Lipid Parameters

The lipid reactions were followed by FFA and TBARS values, which are shown in Table 1. The FFA value of sucuk was 4.37 mg KOH/g fat before heat treatment and heat treatment significantly decreased FFA values ($P<0.05$). As the heat treatment temperature increased the FFA value decreased ($P<0.05$) may be due to breaking of unsaturated FFA with the effect of heat. Traditional sucuk showed highest FFA value ($P<0.05$). Heat treatment significantly increased TBARS value in accordance with heat treatment temperature ($P<0.05$) indicating the acceleration of oxidative reactions. Peroxides formed by free fatty acids degradation may be decomposed to thiobaribituric acid reactive substances. Lipids are one of the main components of

fermented sausages which are source of several flavour substances, due to lipolytic and oxidative reactions [13, 14]. Since FFA and TBARS values of traditional sucuk had significant differences comparing to heat treated sucuks, changes in lipid reactions would have a direct effect on the sensory attributes as well as lipid stability of the sucuk [4]. Coşkuner, Ertaş and Soyer [15] reported decrement in FFA and increment in TBARS values in sucuks heat treated at 73°C.

Pigment and Colour Parameters

Residual nitrite content pigment amounts and colour parameters are shown in Table 2. Residual nitrite content of sucuk before heat treatment was significantly decreased during heat treatment (P<0.05). As the heat treatment temperature increased the residual nitrite content decreased (P<0.05). Sodium nitrite used in cured meat products interacts with various constituents in the complex biological systems of meat and heat treatment may accelerate these reactions. Ercoşkun [4] observed that heat treatment at 60°C (for ten minutes) decreased residual nitrite contents from 12.47 ppm to 7.28 ppm after a three days of fermentation. Yürür [12] heat treated sucuks which originally 25, 50, 75 and 100 ppm nitrite added at 68°C after 3 days of fermentation and reported that the residual nitrite contents were 1.90, 4.35, 7.55 and 9.28, respectively. Similarly, Ercoşkun *et al.* [6] reported that heat treatment at 68°C decreased residual nitrite contents from 13.69 ppm to 5.75 ppm after three days of fermentation.

Heat treatment decreased the total pigment and nitrosomyoglobin amounts (P<0.05) and as the heat treatment temperature increased the total pigment and nitrosomyoglobin amounts decreased (P<0.05). Contrary, heat treatment increased percentage of heme pigments transformed to nitrosyl-heme pigments (P<0.05). These results clearly indicate that nitrosomyoglobin formation was occurred more than nitrosomyoglobin denaturation which resulted with increment in pigment conversion ratio during the heat treatment. Decrement of residual nitrite levels is also confirming this hypothesis. Üren and Babayiğit [16] reported the nitrosomyoglobin, total haem pigments contents and nitrosomyoglobin conversion ratios of 47.85–203.58 ppm 61.20-319.6 ppm and 40.19-81.71 ppm, in retail sucuks, respectively. This was reported that heat treatment of sucuk at 68°C after three days of fermentation decreased nitrosomyoglobin and total haem pigments contents and increased nitrosomyoglobin conversion ratios [4, 6, 12].

Table 2. The results of pigment and colour parameters*

Parameter	Heat Treatment Conditions				
	Before Heating	60°C	65°C	70°C	Traditional Sucuk
Residual nitrite (ppm)	11.84±0.46^{A}	6.50±0.08^{B}	4.48±0.25^{C}	3.49±0.08^{D}	6.59±0.25^{B}
Total pigment (ppm)	241.77±0.52^{A}	187.25±1.82^{C}	181.37±1.45^{C}	175.12±1.53^{D}	200.96±4.17^{B}
Nitrosomyoglobin (ppm)	168.83±2.68^{A}	153.70±3.14^{B}	150.80±1.05^{B}	148.84±3.23^{B}	167.33±2.36^{A}
Pigment convesion ratio (%)	78.61±1.39^{B}	82.09±2.08^{AB}	82.81±0.79^{AB}	83.86±2.40^{A}	83.29±1.09^{A}
L*	43.20±0.55^{AB}	44.11±0.16^{A}	43.86±0.35^{A}	43.89±1.07^{A}	42.28±0.10^{B}
a*	13.23±0.12^{A}	12.66±0.09^{AB}	12.63±0.22^{AB}	12.46±0.43^{B}	12.27±0.12^{B}
b*	10.75±0.13^{A}	10.53±0.14^{A}	10.59±0.10^{A}	10.64±0.11^{A}	9.59±0.12^{B}
NI	1.18±0.01^{AB}	1.17±0.01^{AB}	1.18±0.01^{AB}	1.19±0.01^{A}	1.15±0.01^{B}
RSI	0.39±0.01^{B}	0.41±0.01^{AB}	0.42±0.01^{A}	0.42±0.01^{A}	0.40±0.01^{AB}

*: Values are given as mean ± SD from duplicate determinations; A-D Means in the same line with different superscripts are significantly different (P<0.05); NI: nitrozation; RSI: discolorization; nd: not determined.

The instrumentally colour parameters of the samples (L*, a*, b*) significantly changed by heat treatment (P<0.05). Colour was brighter in heat treated samples (P<0.05) that may be result of denaturation of the main meat pigment [17]. This increase in L* value may also be accounted for by increased scattering resulted by gel formation due to protein denaturation during heat treatment. Heat process decreased a* values of sucuks (P<0.05) that may be result of denaturation of the main meat pigment, too. The b* values of the samples during heat treatment did not changed (P>0.05). The L*, a* and b* values of marketed sucuks have been observed in the range of 42.57-54.49, 11.67-20.15 and 11.50-26.20, respectively [16].

Sucuk heat treated at 70°C showed highest nitrosation value (NI) (P<0.05). The nitrosation value is a ratio of nitrosomyoglobin and myoglobin that is calculated by R560/R500. Therefore, NI values can be used as nitrosomyoglobin formation ratio [16, 18]. Üren and Babayiğit [19] reported that the NI value of fermented sucuks ranked between 0.97-2.15. The NI values of the sucuks used in this study were lower than the findings of Üren and Babayiğit [19]. Ercoşkun et al. [6] and Yürür [12] reported that heat treatment had no effect on NI values of heat treated sucuks.

Pigment discolouration values (RSI) of sucuks are increased with heat treatment (P<0.05). The RSI value is a ratio of Fe II pigments (myoglobin, oxymyoglobin and nitrosomyoglobin) and Fe III pigment, (metmyoglobin) and is defined as R570/R650. Small RSI values reflecting low levels of metmyoglobin [16, 18]. Üren and Babayiğit [19] reported that the RSI values of fermented sucuks ranged between 0.406-0.689. Ercoşkun et al. [6] reported that the RSI value of heat treated sucuks between 0.43-0.47. Heat treated and fermented sucuks showed RSI values between 0.39-0.42 in this study.

Textural Attributes

Heat treatment significantly affected textural attributes of heat treated sucuk (P<0.05) as shown in Table 3. Hardness increased from 6.29 N to 16.03 N, 16.22 N and 19.17 N in heat treated sucuks at 60, 65 and 70°C (P<0.05). The hardness value of traditional sucuk was 8.61 N that was significantly different comparing to the heat treated sucuks (P<0.05). Adhesiveness value of heat treated sucuks increased with the heat treatment temperature. Springiness and cohesiveness values of traditional sucuk showed significant difference with all other elaborated samples (P<0.05). Gumminess value increased from 2.15 N to 5.53–6.64 N in heat treated sucuks during the heat treatment. Heat treatment significantly increased the chewiness value (P<0.05) but there were no differences in the chewiness values of heat treated samples (P>0.05). There were no differences in the chewiness values of sucuk before heat treatment and traditional sucuk (P>0.05).

Table 3. The results of textural attributes*

Parameters	Heat Treatment Conditions				
	Before Heating	60°C	65°C	70°C	Traditional Sucuk
Hardness (N)	6.29±0.08^{B}	16.03±045^{A}	16.22±0.99^{A}	19.17±4.41^{A}	8.61±0.82^{B}
Adhesiveness (N mm)	0.13±0.03^{C}	0.15±0.10^{C}	0.31±0.04^{B}	0.47±0.03^{A}	0.28±0.03^{BC}
Springiness (mm)	8.18±0.24^{A}	8.12±0.18^{A}	8.30±0.20^{A}	8.57±0.39^{A}	6.89±0.45^{B}
Cohesiveness	0.35±0.02^{A}	0.35±0.01^{A}	0.35±0.01^{A}	0.35±0.01^{A}	0.32±0.01^{B}
Gumminess (N)	2.15±0.04^{A}	5.53±0.17^{B}	5.60±0.43^{B}	6.64±1.72^{B}	2.74±0.26^{A}
Chewiness (N mm)	17.62±0.35^{B}	44.99±1.09^{A}	46.44±3.41^{A}	57.89±8.10^{A}	18.79±0.73^{B}

*: Values are given as mean ± SD from duplicate determinations; A-D Means in the same line with different superscripts are significantly different (P<0.05).

The differences of textural attributes of traditional sucuk may be attributed to the decreased moisture content. The differences of heat treated sucuks could be attributed due to thermal denaturation in which as the heat treatment temperature increased textural attributes changed. Heat treatment hardened sucuk and formed gel elastic gummy product.

Microbial Counts

The total mesophilic aerobic bacteria (TMAB), lactic acid bacteria (LAB) and *Staphlococcus – Micrococcus* spp (SM) counts in traditional sucuk respectively 7.37-7.36-4.84 log cfu/g. Ercoşkun et al. [6] reported that the TMAB, LAB and SM counts of traditional sucuk were counted as 6.52-6.60-5.68 log cfu/g respectively.

TMAB, LAB and SM counts of sucuks were significantly decreased with heat treatment (P<0.05). TMAB, LAB and SM level of sucuks heat treated at 60°C were 4.61-4.98 and 4.93 log cfu/g. Heat process at 65°C TMAB, LAB and SM level were 3.89-2.48 and 3.60 log cfu/g and 70°C this parameters were 3.44-2.36 and 2.17 log cfu/g respectively. Tayar [6] reported similar counts.

Sensory Quality

Sensory outer and inner colour scores of traditional sucuk were significantly higher than heat treated ones (P<0.05) that is confirming the instrumental colour measurements. The sensory outer colour score of heat treated sucuk at 70°C was the lowest value representing the degradation of nitrosomyoglobin. The sensory inner colour scores of heat treated sucuks decreased with increasing heat treatment temperature (P<0.05). Consistency scores of sucuks heat treated at 65 and 70°C were significantly higher than traditional sucuk (P<0.05) which is confirming the thermal protein denaturation. Taste and odour scores of traditional sucuk took highest values (P<0.05). Sucuks heat treated at 60°C took the best acceptability scores and acceptability scores of heat treated sucuks decreased with increasing heat treatment temperature.

CONCLUSIONS

Results of the study are clearly showing the difference of heat treated and traditional sucuks. Heat-treatment had significant effect in terms of physicochemical, microbiological and sensory and showed a very wide variation. The results of analyses showed that a highly acceptable heat treated sucuk could be manufactured with lower heat treatment temperatures as 60°C (for 15 minutes). Contrary Legislation of Meat and Meat Products in Turkish Food Codex (5) described a minimum heat treatment temperature of 68°C for red meat sucuk.

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