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CHANGES IN PHYSICOCHEMICAL, MICROBIOLOGICAL AND SENSORY CHARACTERISTICS OF TRADITIONALLY PRODUCED TURKISH SUCUK DURING RIPENING AND STORAGE: NATURAL OR SYNTHETIC ADDITIVES?

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ABSTRACT
The effects of addition of nitrite, lactic acid bacteria and propolis on the physicochemical, microbiological and sensory characteristics of traditional Turkish sucuk were studied during the sun-drying and storage stages. Microbiological and physicochemical analysis were performed at the beginning of sun drying period (3rd day), at the end of the sun-drying period (14th day) and in the storage period (28th day). According to the microbiological evaluation of the samples, analyses show that total bacteria, total yeast and mould and lactic acid bacteria counts varied significantly (P <0.05) with nitrite, lactic acid bacteria and propolis addition and time. Propolis decreased the total bacteria, mould and yeast counts compared to the control sample at the end of the 28th day with acceptable sensory properties. Therefore, it can be concluded that as a natural strong antimicrobial, propolis can be added to traditional sucuk formulations to substitute nitrite to avoid negative health effects.
Keywords: Traditional sucuk, synthetic additives, natural additives, lactic acid bacteria, propolis

GELENEKSEL OLARAK ÜRETİLEN TÜRK SUCUĞUNUN OLGUNLAŞMA VE DEPOLAMA SIRASINDAKİ FİZİKOKİMYASAL, MİKROBİYOLOJİK VE DUYUSAL ÖZELLİKLERİNDEKİ DEĞİŞİKLİKLER: DOĞAL VEYA SENTETİK KATKILAR?

ÖZ
Nitrit, laktik asit bakteri ve propolis ilavesinin, geleneksel Türk sucuklarının fizikokimyasal, mikrobiyolojik ve duyusal özelliklerine etkileri güneşte kurutma ve depolama aşamalarında incelenmiştir. Mikrobiyolojik ve fizikokimyasal analizler güneşte kurutma periyodunun başında (3. gün), güneşte kurutma periyodunun sonunda (14. gün) ve depolama sürecinde (28. gün) yapılmıştır. Örneklerin mikrobiyolojik analiz sonuçlarına göre, toplam bakteri, toplam maya, küf ve laktik asit bakteri sayıları nitrit, laktik asit bakterisi ve propolis ilavesiyle ve bunun yanında zaman ile istatistiksel olarak anlamlı bir değişiklik (p <0.05) göstermektedir. Propolisin, toplam bakteri, küf ve maya sayılarını kontrol numunesine kıyasla, 28. günün sonunda, kabul edilebilir duyusal özelliklere sahip olarak azalttığı sonucuna varılmıştır. Bu nedenle, doğal ve güçlü bir antimikrobiyal olarak, olumsuz sağlık etkilerinden kaçınmak amacıyla nitrit yerine geleneksel Türk sucuğu formülasyonlarına propolis ilave edilebileceği sonucuna varılmıştır.
Anahtar kelimeler: Geleneksel sucuk, sentetik katkılar, doğal katkılar, laktik asit bakterisi, propolis

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INTRODUCTION

Sucuk, a traditional dry-fermented sausage in Turkey, has a widespread distribution in local markets and great acceptance by local consumers. Industrial and traditional methods are used in sucuk production. Traditionally produced sucuk formulation varies regionally. Sucuk generally consists of a mixture of beef, beef fat, salt, various spices (red pepper, black pepper, cumin etc.) and nitrite can be used as a preservative. The sucuk dough undergoes a bacterial fermentation followed by a ripening period (Kilic, 2009). Sucuk fermentation is carried out by native microbiota or addition of starter cultures into sucuk dough (Erkmen and Bozkurt, 2004). Then, sucuk dough is stuffed into natural bowel and air-dried in traditional method (Gokalp, Kaya and Zorba, 1999). There exists no standardized method in traditional production of sucuk (Bozkurt and Erkmen, 2002).

Lactic-acid bacteria (LAB) play an important role in meat preservation. They decrease the pH by lactic-acid production; produce bacteriocins, which prevent growth of some pathogenic and spoilage organisms; provide diversity of sensory properties by modification of raw material; and contribute to the development of flavor, color and texture, thereby improving the overall quality and shelf life of meat products (Holko, Hrabě, Šalaková, and Rada, 2013; Leroy and De Vuyst, 2004). However, LAB may have also negative effects. They have been noticed as main spoilage microorganisms in meat products and may be the reason of slime and sour flavor in sucuk (Wang, 2000).

On the other hand, several additives have been applied in sucuk production, which have high antioxidant and antimicrobial activity. Nitrite, nitrate and some organic acids such as benzoic acid, sorbic acid, citric acid and their salts have been used in sucuk production as commercial additives. Moreover, ascorbic acid and *a*-tocopherols are used as antioxidant additives. Several additives have been used in order to decrease the pH, improve the flavor and aroma and to reach desired color (Bozkurt, 2002).

Nitrate and nitrite are used widely to establish color stability in meat products, to control microbial growth by inhibition of pathogenic bacteria such as *Clostridium botulinum*, to improve characteristic aroma, color and taste of cured meat products. However, nitrate and nitrite have negative effects on health and they are not acceptable above certain doses. They are toxic as they cause anemia and form nitrosamines that are carcinogens by reacting with secondary amins existing in human body. Nitrate ions don't have toxic effects directly. Nitrates turn into nitrite ions by bacterial nitrate reductase activity (Bories ve Bories, 1995). Nitrites react with hemoglobin and form methemoglobin. Fe^{+2} in hemoglobin is oxidized and turns into Fe^{+3} and it avoids or reduces O_2 transport function of blood. This is called methaemoglobinaemia and causes 'blue baby syndrome' dangerous for children (Cemek ve diğ., 2007). Another negative effect of nitrite on human health is that it forms nitrosamines as a result of the reaction with secondary amines. These compounds are potentially carcinogenic, mutagenic and/or teratogenic (Connoly ve Paul, 2001). Increase of nitrate and nitrite levels cause serious health problems on human health. Their concentration should be monitored carefully. Due to the negative health effects of nitrate and nitrite addition to meat products such as Turkish dry-fermented sausage (sucuk), natural food preservative alternatives are needed to substitute these additives.

Propolis has strong antioxidant and antimicrobial activities and it is also Generally Recognised as Safe (GRAS) (Burdock, 1998). This fact makes propolis an attractive substance as natural preservative in novel food applications. This meets the demand for natural antioxidants and antimicrobials, as a result of the increasing consumer awareness for natural, minimally processed foods with natural preservatives (Tosi et al., 2007).

In view of these considerations, it is aimed in this study to compare the chemical properties such as pH, moisture content, free fatty acids and microbiological properties such as total aerobic bacteria, total yeast and mould, total coliform bacteria, total lactic acid bacteria in four different

formulations of sucuk to determine the effects of nitrite, lactic acid bacteria and propolis. Besides, these quality characteristics are monitored throughout the drying period of traditionally produced sucuk samples to evaluate the quality characteristics changing throughout this period.

MATERIALS AND METHODS

Chemicals

Violet red bile (VRB) agar, dichloran rose bengal chloramphenicol (DRBC) agar, plate count agar (PCA) and de Man, Rogosa and Sharpe (MRS) agar were obtained from Merck (Darmstadt, Germany); Brillant green bile broth was obtained from Hach (USA); peptone was purchased from Biomatik (USA). Sodium chloride, sodium hydroxide and phenolphthalein were obtained from Sigma-Aldrich (St. Louis, MO).

Starter culture for cured meat products containing *Pediococcus acidilactici* was obtained from Simbiyotek (Istanbul, Turkey).

Propolis was obtained from SBS Scientific Bio Solutions A.Ş. from Kocaeli region of year 2017.

Sucuk preparation

Four different formulations of sucuk dough was prepared as shown in Table 1. All types of sucuk contains meat, salt, sugar, garlic and spices including red pepper, black pepper, chili pepper, cumin and sumac. All formulations contain 1000 g calf meat (including about 20% fat), 6 g cumin, 6 g red pepper, 6 g black pepper, 8,8 g garlic, 8 g salt, 16 g chili pepper, 3 g sumac in their recipe.

Table 1. Concentration of nitrite/lactic acid bacteria/propolis used for production of sucuk formulations

Additives	Turkish sucuk types			
	S1	S2	S3	S4
Nitrite	0	150 ppm/ kg	0	0
Lactic acid bacteria (*Pediococcus acidilactici* KUEN 1584)	0	0	1 ml / kg	0
Propolis	0	0	0	10 g/ kg

S1: control ; S2:sample including 150 ppm/kg nitrite; S3: sample including 1 ml / kg lactic acid bacteria; S4: sample including 10 g / kg propolis

The meat was minced to about 1.5 - 2.0 cm using meat mincer (Tefal Prep'Line 1600, France). Then, spices and 150 ppm of nitrite into S2, 1 ml of starter culture *Pediococcus acidilactici* KUEN 1584 into S3 and 10 g of finely grounded propolis into S4 were added and mixed with 1000 g of minced meat. Sucuk dough was rested for 3 days at 4°C. Dry clean natural bowel was softened using perchloric acid and the dough was filled into this using sucuk dough filling machine (Kenwood mg420, USA). At the end of filling into natural bowel cases, 250 g of 4 sucuks were prepared for all formulations to produce duplicate batches. Sucuks were fermented and air-dryed for 12 days: 8th April to 19th April by relative humidity of 45% to 80% as outlined in Fig.1. After sun-drying period, sucuk samples were placed into an incubator at 30°C until 28th day by a high relative humidity of above 80%.

Sampling

Sucuks were sampled on 3rd, 14th, 28th days and total bacteria, total mould and yeast, lactic acid bacteria, total coliform bacteria counts, pH, moisture and free fatty acid contents were determined. The samples were taken at:

-3rd day: the beginning of fermentation and sun-drying period

-14th day: end of fermentation/ sun-drying period

-28th day: end of storage period

All microbiological and physicochemical analyses were performed in triplicate.

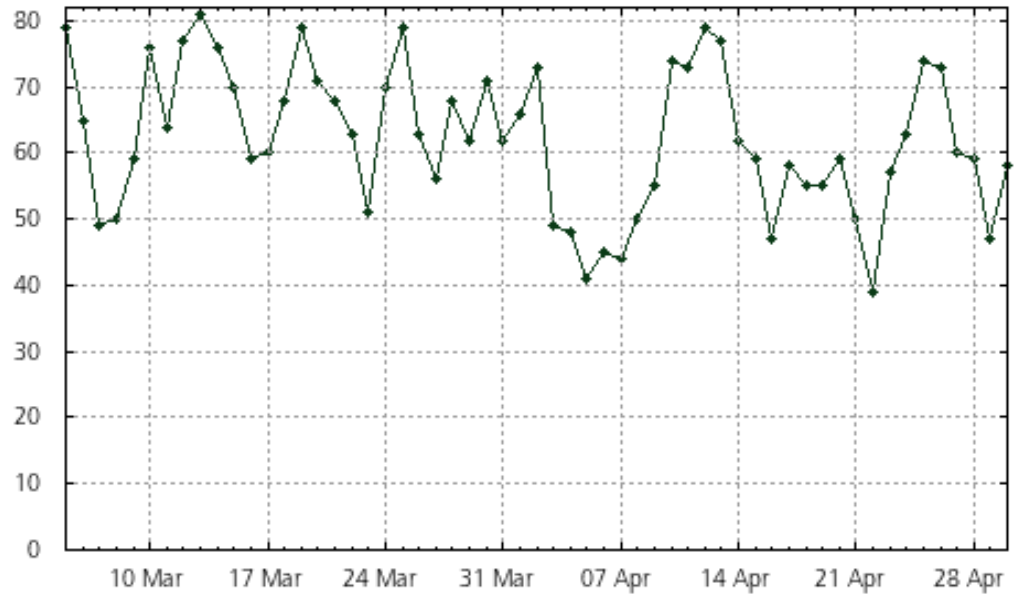

Figure 1. Relative Humidity % at Sun-Drying Period of Sucuk Samples (April 8th to April 19th, 2018)

Sample preparation and microbiological analysis

25 g of sample was collected from each sucuk types (S1, S2, S3 and S4). They are homogenized using Waring blender (Torrington, CT). Sterile peptone water (0.1%, 225 ml) was poured on the sample and serial dilutions were prepared using 0.1% peptone water for microbiological analysis (Erkmen, 2000).

Total bacteria were measured using the spread plate method on aerobic plate count agar (Merck, Darmstadt, Germany). The Petri dishes were incubated at 30 ˚C up to 72 h (Ertas, 2010). Bacterial counts were expressed as colony-forming units per gram of sample (cfu/g) and were transformed into logarithms (TS ISO 4832, 2010).

Total mould and yeast values were counted using the spread plate method on Violet Red Bile Agar (Merck, Darmstadt, Germany) and incubated at 25 ˚C for 2–5 days (Erkmen, 2000). Bacterial counts were expressed as colony-forming units per gram of sample (cfu/g) and were transformed into logarithms (TS 6580, 1989).

Total lactic acid bacteria values were observed using the spread plate method on de Man, Rogosa and Sharpe (MRS) Agar (Merck, Darmstadt, Germany). Petri dishes were incubated at 30 ˚C for 48–72 h (Erkmen, 2000). Bacterial counts were expressed as colony-forming units per gram of sample (cfu/g) and were transformed into logarithms.

Coliform bacteria count were carried out using the pour plate method using violet red bile agar (VRBA) (Merck, Darmstadt, Germany) and incubated at 30 ˚C for 24 h. The purple-red colonies that are 0.5 mm or larger in diameter and surrounded by zone of pericipitated bile acids are counted. To confirm that the colonies are coliforms, representative colonies were transferred to tubes containing 10 ml of Brillant Green Bile Broth with Durham tubes. The tubes were examined at 24 to 48 hours for gas production (TS ISO 4832, 2010).

Sample preparation and chemical analysis

Sucuk samples that were prepared for microbiological assays were also used for physicochemical analysis. Samples were divided into around 5 x 5 x 5 mm pieces and 100 g of each sample was homogenized by Waring blender. 5 g of sucuks were used for moisture content and 10 g of samples for pH and free fatty acids determinations. All analysis of pH, moisture

content and free fatty acids were carried out in triplicate.

Moisture content of traditional sucuk types were analyzed according to the methods described by AOAC (1990). 5 g of sucuk sample was placed into hot air oven at 125 ± 2 ° C for 3 hours. Sucuk samples of 10 g were homogenized in Waring blender in 100 ml distilled water for determination of pH using a pH meter (Ohaus, ST3100).

Lipid fraction for free fatty acids (FFA) analyses was extracted from the samples according to the method of Bligh and Dyer (1959). FFA analysis was performed according to the alkaline titration method and calculated as mg KOH/g fat.

Sensory analysis

Sensory analysis of the sucuk samples was carried out in a room with 25 °C. Sensory evaluation panels were composed of 8 trained panelists from Department of Food Engineering, Istanbul Okan University. The age interval of panelists were between 20 to 30 and equal members of genders. They were selected on the basis of their sensory performances and interest and trained for 2 hours to perform sensory analysis. All of the traditionally produced sucuk samples were divided to about 20 mm diameter and 20±0.5 mm thickness pieces and grilled with a grill (Tefal Family Flavour Grill Black Edition TG8000, France) for about 1 min on each sides of sucuk pieces.

Four different types of traditionally produced sucuk samples were immediately served to panelists on a plastic white color plate and the panelists tasted the samples starting from left to right. Samples were labeled with three-digit random numbers. Assessors scored each attribute on a 0-9 category scale. Category scales were described by using the terms like 'dislike extremely' for '0' and 'like very much' for '9'. The sensory characteristics analyzed were color, flavor and aroma, texture and overall acceptability. During each session four different formulations of sucuk samples were evaluated for all the attributes and two replicates were obtained for all the assessors for every sample (4 variants x 2 replicates x 8 assessors). For panel performance, traditionally produced sucuk samples were evaluated in 2 different sessions of sensory panels.

Statistical analysis

The data of all the analysis gathered were given as means and standard deviations of triplicate analysis. Data analysis was performed by one-way ANOVA statistical tests on physicochemical and microbial changes of time and sucuk type to determine significant changes (P <0.05). Duncan's Multiple Range Test was also performed to evaluate significant differences between means at the P <0.05 significance level by IBM SPSS Statistics 25.0 statistical software.

RESULTS AND DISCUSSION

Microbiological evaluations

Result of total bacteria counts at 3rd, 14th and 28th days are given in Table 2. Multiple range tests point out that total bacteria count increased significantly (P <0.05) over the first 14 days and after that decreased significantly until the end of 28th day.

Similar results were obtained by the studies of Bozkurt and Erkmen (2007), Samelis et al. (1998) and Bozkurt and Erkmen (2004). They have observed that aerobic plate count raised during the ripening stage and reduced during the storage period.

Multiple range tests figured out that additives changed total bacteria count. The highest (P <0.05) total bacteria count was determined at day 28 in S3, and the lowest (P <0.05) in S4 and S2 samples. Propolis significantly decrease the total bacteria count as it is a very strong antimicrobial agent. This means that propolis can be used as a natural antimicrobial in traditional sucuk production as a substitute of nitrite according to the results of total bacteria count analysis.

Total mould and yeast analysis results at 3rd, 14th and 28th days are given in Table 2. It was observed that there are statistically significant differences between traditional sucuk samples according to their additives and also according to time from 3rd to 28th days. Total mould and yeast

count decrease from 3rd to 28th days in all samples. It was observed that in S2 type sucuk containing nitrite as additive total mould and yeast count is increased from the 3rd day (4,29 log CFU/g) to 14th day (4.73 log CFU/g) and than decreased at the end of the 28th day (4.08 log CFU/g). Bruna, Ordonez, Fernandez, Herranz, and De La Hoz (2001) and Bozkurt and Erkmen (2007) also reported that during fermentation mould and yeast count of fermented sausage raised and then reduced. It was found that propolis decreased the total yeast and mould count from 3rd (5.35 log CFU/g) to 28th day (4.17 log CFU/g) significantly. Addition of starter culture also decreased total yeast and mould count from 3rd (5.24 log CFU/g) to 28th day (4.57 log CFU/g) significantly. The highest decrease was observed in S4 sucuk type containing propolis as an antimicrobial agent from the 3rd day to 28th day of analysis, but the lowest value was observed in sample S2 (4.08 log CFU/g) containing nitrite at the end of the 28th day.

Table 2. Changes in total bacteria, mould and yeast, lactic acid bacteria and total coliform bacteria counts in traditionally produced sucuk samples

Analysis	Sucuk Type	Days		
		3rd Day	14th Day	28th Day
Total bacteria analysis*	S1	$8.47\pm0.55^{B.a}$	$10.43\pm0.51^{A.a}$	$8.27\pm0.18^{B.ab}$
	S2	$7.28\pm0.18^{B.c}$	$8.77\pm0.21^{A.b}$	$7.76\pm0.63^{B.b}$
	S3	$7.66\pm0.46^{B.b}$	$8.75\pm0.21^{A.b}$	$8.81\pm0.07^{A.a}$
	S4	$7.47\pm0.21^{B.bc}$	$8.65\pm0.29^{A.b}$	$7.77\pm0.09^{B.b}$
Total mould and yeast*	S1	$5.05\pm0.03^{A.a}$	$4.96\pm0.04^{AB.a}$	$4.38\pm0.07^{B.b}$
	S2	$4.29\pm0.22^{AB.b}$	$4.73\pm0.14^{A.b}$	$4.08\pm0.07^{B.b}$
	S3	$5.24\pm0.17^{A.a}$	$4.76\pm0.04^{B.b}$	$4.57\pm0.07^{BC.a}$
	S4	$5.35\pm0.06^{A.a}$	$4.59\pm0.06^{B.bc}$	$4.17\pm0.13^{BC.b}$
Total lactic acid bacteria*	S1	$6.28\pm0.22^{B.b}$	$7.37\pm0.26^{A.a}$	$6.51\pm0.09^{B.a}$
	S2	$6.26\pm0.13^{AB.b}$	$6.36\pm0.06^{A.c}$	$5.38\pm0.07^{C.c}$
	S3	$6.30\pm0.23^{A.b}$	$6.33\pm0.19^{A.bc}$	$5.58\pm0.17^{B.bc}$
	S4	$6.90\pm0.05^{A.a}$	$6.90\pm0.05^{A.b}$	$5.90\pm0.12^{B.b}$
Total coliform bacteria*	S1	nd	nd	nd
	S2	nd	nd	nd
	S3	nd	nd	nd
	S4	nd	nd	nd

*log cfu/g; nd: not determined.
S1: control ; S2:sample including 150 ppm/kg nitrite; S3: sample including 1 ml / kg lactic acid bacteria; S4: sample including 10 g / kg propolis
Values in columns represents the mean of 3 replications and standard deviation.
Values followed by the same small letters in columns are not significantly different ($P < 0.05$).
Values followed by the same capital letters in rows are not significantly different ($P < 0.05$).

Total lactic acid bacteria analysis results at 3rd, 14th and 28th days are given in Table 2. According to the statistical analysis performed, it was observed that the time and addition of nitrite, lactic acid bacteria and propolis changed ($P < 0.05$) the lactic acid bacteria count in traditionally produced sucuk samples.

LAB increased in S1 type sucuk ($P < 0.05$) from 6.28 to 7.37 log CFU/g during 12 days of the sun-drying stage and reduced to 6.51 log CFU/g in storage stage. The results of this study are in agreement with previous studies that reported LAB raised while ripening stage and reduced during the storage stage (Bozkurt and Erkmen (2007); Samelis et al. (1998); Mora-Ventura (1999); Roig-Sagues, Hernandez-Herrero, Lopez-Sabater, Rodriguez, and, Bruna et al. (2001); Gonzalez and Diez (2002).

Addition of nitrite, lactic acid bacteria and propolis influenced (P <0.05) the LAB in traditionally produced sucuk types. The lactic acid bacteria counts for all samples were decreased from 14th to 28th day. This finding is also agreed with Bozkurt and Erkmen (2007) as they have observed that adding nitrite/nitrate to sucuk formulations reduce (P <0.05) the lactic acid bacteria.

Coliform bacteria in particular, *Staphylococcus aureus, Clostridium perfringens, Campylobacter jejuni, Escherichia coli* and *Salmonella,* in meat products, are considered as potential sources of risk (*Balpetek and Gürbüz, 2010*). Coliform bacteria are not found in our analysis as it is indicated in Table 2.

Physicochemical changes

pH values of sucuks decreased from 14th to 28th days significantly (P <0.05). The decrease of pH can be explained by the production of lactic acid and other organic acids by lactic acid bacteria and other acid-producing bacteria (Gökalp, 1986; Gökalp and Ockerman, 1985; Lücke, 1994). According to the Turkish Food Codex (Anon., 2012) fermented sucuks should have pH values of maximum 5.4 for high quality characteristics and only the S4 sample is accepted for this value.

It was found that addition of nitrite, lactic acid bacteria and propolis changed (P <0.05) pH values significantly. According to the results all sucuk types have statistically different pH values. At the end of the 28th day, the pH values were ordered as S2>S3>S1>S4. As a result it can be concluded that addition of nitrite increase the pH value of sucuk at the 3rd and 14th days. This could be explained by the phosphate buffering activity (Bozkurt and Erkmen, 2002).

Table 3 shows moisture content of sucuk types prepared with no additive, nitrite, lactic acid bacteria and propolis during sun-drying and storage. Moisture contents of sucuk samples were in average 37-43% at the end of storage and there were significant difference in the moisture contents of the samples ($p < 0.05$) at 14th and 28th day of analysis.

Table 3. Changes in physicochemical properties of traditionally produced sucuk samples

Analysis	Sucuk Type	Days		
		3rd Day	14th Day	28th Day
pH	S1	$5.54\pm0.04^{AB,b}$	$5.66\pm0.12^{A,a}$	$5.46\pm0.02^{B,a}$
	S2	$5.72\pm0.01^{A,a}$	$5.76\pm0.02^{A,ab}$	$5.48\pm0.02^{B,a}$
	S3	$5.65\pm0.02^{A,ab}$	$5.65\pm0.03^{A,a}$	$5.47\pm0.01^{B,a}$
	S4	$5.47\pm0.02^{A,ab}$	$5.45\pm0.03^{A,b}$	$5.36\pm0.02^{B,b}$
Moisture (g/100 g)	S1	$31.0\pm0.005^{C,ab}$	$35.0\pm0.02^{B,c}$	$37.0\pm0.02^{A,c}$
	S2	$32.0\pm0^{B,a}$	$37.0\pm0.06^{A,a}$	$43.0\pm0.07^{C,a}$
	S3	$27.0\pm0.03^{C,bc}$	$34.0\pm0.01^{B,b}$	$38.0\pm0.02^{A,bc}$
	S4	$22.0\pm0.01^{C,c}$	$34.0\pm0.01^{B,b}$	$41.0\pm0.01^{A,b}$
Free fatty acid (mg KOH/g)	S1	$0.33\pm0.03^{C,a}$	$0.59\pm0.01^{B,bc}$	$0.94\pm0.04^{A,c}$
	S2	$0.29\pm0.01^{C,a}$	$0.77\pm0.04^{B,b}$	$1.04\pm0.01^{A,b}$
	S3	$0.36\pm0.03^{C,a}$	$0.89\pm0.08^{B,a}$	$2.18\pm0.08^{A,a}$
	S4	$0.34\pm0.04^{C,a}$	$0.55\pm0.01^{B,c}$	$1.16\pm0.05^{A,b}$

*log cfu/g;

S1: control ; S2:sample including 150 ppm/kg nitrite; S3: sample including 1 ml / kg lactic acid bacteria; S4: sample including 10 g / kg propolis.

Values in columns represents the mean of 3 replications and standard deviation.

Values followed by the same small letters in columns are not significantly different ($P < 0.05$).

Values followed by the same capital letters in rows are not significantly different ($P < 0.05$).

Fermentation and drying periods are two main procedures applied in sucuk production. Weight loss during the drying stage results in the reduction of moisture content (Bozkurt and Bayram, 2006). Water content of sucuk samples also significantly change in the ripening stage. Soyer, Ertaş and Üzümcüoğlu (2005) applied two different ripening stages and temperature intervals as 20-22 ˚C and 24-26 ˚C. They have observed that the moisture content of sucuk ripened at 22-24 ˚C and 24-26 ˚C as 40.28 and 38.25 g/100 g, respectively. In another study, Saricoban, Karakaya, and Caner (2006) observed after 5 days fermentation period of sucuk that moisture content of sucuk ripened at 24-26˚C in 85-95% RH as 38.15 g/100 g. As a result of the high ripening temperature, they have observed that after 5 days, moisture content was reduced from 57.36 g/100 g to 38.15 g/100 g. In comparison, the results of this study is similar to previously mentioned studies.

Kayaardi and Gök (2004) have fermented sucuk under ambient conditions around 15-20 ˚C and 70-85% RH for 21 days with a longer ripening stage and lower temperature and RH. They have reported that the moisture content of sucuk after 21 days fermentation period was 35.17 g/100 g which is quite lower compared to values in the this study according to long fermentation time of sucuk. Yildiz-Turp and Serdaroğlu (2008) also observed that the moisture content of the sucuk was 34.5 g/100 g at the end of 12 days fermentation of sucuk. In this study, it is assumed that moisture content at the end of 28th day is increased compared to 14th day because of the high relative humidity and temperature values.

Table 3 shows free fatty acid content of sucuk types prepared with no additive, nitrite, lactic acid bacteria and propolis during sun-drying and storage. Table 3 gives free fatty acid contents of traditionally produced sucuk samples in mg KOH/g fat.

According to the one way ANOVA test results, there are no statistical differences between sucuk types at the 3rd day (P >0.05), but there are statistically significant differences between samples (P <0.05) at the 14th and 28th days. Free fatty acid content increase up to 2.18 mg KOH / g fat in S3 sample at the end of 28th day. During maturation of sausage, endogenous enzymes (lipase and phospholipase) and exogenous enzyme activity result in lipolysis cause a rapid increase in the amount of free fatty acids (Ensoy et al., 2010). As a result of addition of lactic acid bacteria to sample S3, it has reached the highest value compared to other samples. Besides, propolis added sample has a higher value compared to the nitrite added sample. Free fatty acid content of sucuk samples increased about 3 fold at the end of the 28th day. Coşkuner (2002) and Ersoy (2004) in their study also observed that free fatty acid content of sucuk samples constantly increase during the ripening of sucuk samples. In the study of Ercoşkun (2006) he has also observed free fatty acid value of 3.44 mg KOH / g fat, 6.74 mg KOH / g fat. Zanardi et al. (2004) fermented sausages, free fatty acidity values, have increased 2-3 fold during ripening which is similar to our results. However Salgado et al. (1999) have observed higher increase in the free fatty acid content which is about 9-10 fold of the initial value of the ripening process.

Sensory evaluation

Table 4 shows results of the sensory analysis of traditionally produced sucuk types. The overall acceptability of traditional sucuk samples were higher than 7.0 which means all of the samples have a good overall acceptability. Addition of propolis, nitrite and lactic acid bacteria significantly resulted in changes (P < 0.05) of sensorial properties. As a result, all the sucuk samples have overall acceptability, but there is a slight decrease in the sensory properties of propolis added sucuk samples. In order to evaluate overall acceptability, the sample S2 has the maximum score as nitrite establish color stability in sucuk, and improve characteristic flavor and aroma resulting in the overall acceptability of sucuk. Bozkurt and Erkmen (2007) also performed sensory analysis to sucuk samples added with several commercial additives. Their results also confirm that nitrate/nitrite addition to sucuk production differentiate the overall sensory quality of the sucuk samples.

Table 4. Evaluation of sensory analysis results of traditionally produced sucuk samples (1=dislike extremely, 9= like very much)

Samples	Color	Flavor and aroma	Texture	Overall acceptability
S1	7.33±0.78[a]	7.08±0.90[a]	7.33±1.30[a]	6.92±1.24[a]
S2	7.58±0.90[a]	7.25±1.06[a]	7.33±1.30[a]	7.08±1.24[a]
S3	7.17±1.03[a]	7.17±0.83[a]	7.17±0.83[a]	6.92±1.24[a]
S4	5.92±0.90[b]	6.00±0.85[b]	6.58±0.90[b]	6.25±0.97[b]

*log cfu/g; nd: not determined
S1: control ; S2:sample including 150 ppm/kg nitrite; S3: sample including 1 ml / kg lactic acid bacteria; S4: sample including 10 g / kg propolis
Values in columns represents the mean of 3 replications and standard deviation.
Values followed by the same small letters in columns are not significantly different ($P < 0.05$).

CONCLUSION

The results of this study showed that the addition of nitrite, lactic acid bacteria and propolis into traditional sucuk samples have provided significant changes ($P < 0.05$) in the microbiological and physical properties. Propolis decreased the total bacteria count and mould and yeast count compared to the control sample at the end of the 28th day with acceptable sensory properties. Besides, propolis added sucuk sample has reached the most acceptable pH value at the end of the 28th day compared to other samples. According to free fatty acid results, lactic acid bacteria added sucuk sample has reached the maximum value at the 28th day, but propolis added sample's value is higher than the control and nitrite added samples. Therefore, it can be concluded that as a natural strong antimicrobial, propolis can be added to traditional sucuk formulations to substitute nitrite in order to avoid the negative health effects of nitrite. Further studies should be performed in the future about propolis addition to cured meat products instead of nitrite/nitrate to improve quality characteristics of sucuk and to avoid harmful effects of nitrite without causing any sensorial defect. Effects on physicochemical, microbiological and sensory characteristics of traditional sucuk by addition of different propolis concentrations together with starter cultures (lactic acid bacteria) should be examined in further studies.

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