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Investigation of the Quality Characteristics of Naturally Cured Sucuks with Dill, Spinach and Swiss Chard Powders during Refrigerated Storage

Ali Samet BABAOĞLU[1,*], Mustafa KARAKAYA[1]

[1]Selçuk University, Faculty of Agriculture, Department of Food Engineering, Konya, Turkey

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ABSTRACT

The purpose of this study was to analyze the R&D approaches of business The current study investigated the effects of dill, spinach and Swiss chard powders on the physicochemical (pH, TBARS, colour, residual nitrate and nitrite), microbiological (TMAB, LAB and total yeast-mould) and textural properties (TPA) of sucuks during refrigerated storage for 90 days. Five different groups of sucuk were prepared containing T1: 100 mg/kg sodium nitrite; T2: 100 mg/kg sodium nitrate; T3: dill powder 0.71%; T4: spinach powder 0.29% and T5: Swiss chard powder 0.26%. Swiss chard powder decreased the pH values of samples ($P < 0.05$). It was determined that the most effective curing agent in terms of TBARS numbers was spinach powder (T4). The residual nitrate was not detected in the groups of T4 and T5 all the refrigerated storage ($P < 0.05$). Curing with different vegetable powders did not affect the microbiological counts of sample ($P > 0.05$). Natural curing agents decreased the redness values of samples ($P < 0.05$). The highest chewiness value was determined in the group of T5 ($P < 0.05$). These results suggest that Swiss chard and spinach powders could be recommended as a natural curing agent in the sucuks.

1. Introduction

Curing in meat technology is defined as the addition of salt, nitrate and / or nitrite and various spices depending on the type of meat product (Sindelar et al., 2007). Nitrate and nitrite have been widely used in cured meat products as essential additives that inhibit pathogens (particularly against *Clostridium botulinum* and its spore germination), slow down the growth of other microorganisms, exhibit antioxidant effects, develop typical red curing color and flavor (Choi et al., 2017; Honikel, 2008; Majou and Christieans, 2018; Skibsted, 2011). Nitrate must be reduced to nitrite in order to have the stated effects (Sebranek and Bacus, 2007; Sindelar and Milkowski, 2012). However, when nitrate and nitrite are used in high concentrations in the production of cured meat products, N-nitrosamines some of which are toxic and carcinogenic compounds, can form in certain conditions (Honikel, 2014; Zarringhalami et. al., 2009). Thus, the meat processing industry searches for alternatives to solve this health risk associated with usage of nitrate and nitrite (Riel et. al., 2017). On the other hand, consumers interest in natural additives instead of synthetic additives in meat products. With the awareness of consumers, the demand for natural / organic products is increasing. In line with this demand, researches on the production and development of natural/organic products are increasing day by day (Alahakoon et. al., 2015; Jayasena and Jo, 2013). Several studies have been conducted to meet this demand of consumers. Some of the studies are on the usage of natural antioxidants, essential oils, bacteriocins and spices as a substitute to nitrite. Nonetheless, since nitrite is a multifunctional additive, it is difficult to completely substitute with simple substances (Flores and Toldrá, 2021). Due to nitrate content of some plants at considerable amount (Gassara et. al., 2016), the use of nitrite from vegetables in processed meats as a curing agent without synthetic preservatives is the most promising method. A natural nitrate source and nitrate-reducing starter culture must be used in combination to produce typical cured meat properties (Sebranek and Bacus, 2007).

The among plant-derived nitrate sources, celery, spinach, radishes and lettuce have high nitrate content with more than 2500 mg /kg (Gassara et al., 2016; Schullehner et. al., 2018). There are many studies about the usage of especially celery products as curing agent in meat products (Horsch et al., 2014; Magrinya et al., 2009; Myers et al., 2013; Riyad et. al., 2018). However, it was reported that it has allergic compounds (Ballmer-Weber et al., 2002). Therefore, the potential use of different vegetable nitrate sources as curing agent in

* Corresponding author email: *asmtbb@gmail.com*

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meat products need to be investigated. Spinach (*Spinacia oleracea*), Swiss chard (*Beta vulgaris* var. cicla) and dill (*Anethum graveolens*) contain high level nitrate together with antimicrobial compounds and antioxidant components (Jiraungkoorskul, 2016; Pyo et. al., 2004; Riel et al., 2017). In the literature, there is no study regarding the use of dill as a natural nitrate source. Considering the investigations about spinach and Swiss chard, although the studies are present on their usage as a nitrate source (Kim et al., 2017; Nasonova and Tunieva, 2017; Riyad et al., 2018; Shin et al., 2017), there is no report related with usage of them as a curing agent in sucuk. Therefore, the objective of this study was to investigate the effects of dill, spinach, and Swiss chard powders on the quality characteristics of naturally cured sucuks and evaluated their effects by comparing them with sucuks containing synthetic sodium nitrite and sodium nitrate during refrigerated storage for 90 days.

2. Materials and Methods

2.1. Production of dill, spinach and Swiss chard powders

Fresh dill (*Anethum graveolens* L.), spinach (*Spinacia oleracea*) and Swiss chard (*Beta vulgaris* L. var. cicla) were purchased from a local market in Konya, Turkey. After the vegetables were washed, they were dried under natural laboratory conditions (at 24±1 ºC for 84 hours). The dried vegetable powders were ground using a grinder (Arzum, Mulino, AR 151, Turkey) to obtain dill (5.74±0.01 for pH), spinach (5.85±0.01 for pH) and Swiss chard (5.10±0.01 for pH) powders. The powders were sterilized for 2.5 hours at 115 °C in a dry heat sterilizer in order not to affect the microbial quality of the sucuks.

2.2. Manufacture of sucuks and experimental design

Fresh boneless beef (B*iceps femoris*, *Semitendinosus* and *Semimembranosus* muscles) and beef fat were obtained from a local meat plant (Panagro Meat Plant) in Konya, Turkey. Beef meat and fat were initially ground through a 9-mm plate. The sucuk production was conducted in Panagro Meat Plant in Konya, Turkey.

Five different groups of sucuk were produced depending on the curing agents: Treatment 1 (T1), 100 mg/kg sodium nitrite (traditionally nitrite cured); Treatment 2 (T2), 100 mg/kg sodium nitrate; Treatment 3 (T3), dill powder 0.71%; Treatment 4 (T4), spinach powder 0.29% and Treatment 5 (T5), Swiss chard powder 0.26%. The formulations of the sucuks are given in Table 1. According to initially determined nitrate level in dill, spinach and Swiss chard powders used in this study, the addition levels of vegetable powders to sucuk formulations were corresponded to an amount of 100 mg/kg nitrate.

Table 1
Formulaton of sucuks showing five different treatments

Formulation (g)	Treatments				
	T1	T2	T3	T4	T5
Beef meat	100.00	100.00	100.00	100.00	100.00
Beef fat	34.60	34.60	34.60	34.60	34.60
Garlic	3.90	3.90	3.90	3.90	3.90
Spice mixture	4.40	4.40	4.40	4.40	4.40
NaCl	2.00	2.00	2.00	2.00	2.00
Dextrose	0.15	0.15	0.15	0.15	0.15
Ascorbic acid	0.03	0.03	0.03	0.03	0.03
Starter culture*	0.05	0.05	0.05	0.05	0.05
*Curing agents***					
Sodium nitrite ($NaNO_2$)	0.01	-	-	-	-
Sodium nitrate ($NaNO_3$)	-	0.01	-	-	-
Dill powder	-	-	0.71	-	-
Spinach powder	-	-	-	0.29	-
Swiss chard powder	-	-	-	-	0.26

*Starter culture was added to sucuk batter at the level of 10^7 cfu/g.
**100 ppm sodium nitrite and sodium nitrate were added to T1 and T2 groups, respectively. Natural curing agents (dill, spinach, and Swiss chard powders) were added to sucuk batter at the level of 100 ppm nitrate equivalent.
T1: 100 ppm sodium nitrite (traditionally nitrite cured); T2: 100 ppm sodium nitrate; T3: dill powder 0.71%; T4: spinach powder 0.29%; T5: Swiss chard powder 0.26%.

For the preparation of sucuk batter, beef meat, beef fat, spice mixture, garlic, dextrose, salt and ascorbic acid were mixed in a grinder (Arı Machine, Turkey) and then selected starter cultures having nitrate reductase activity (mixture of Pediococcus pentosaceus and Staphylococcus carnosus; BFL-T03, Christian Hansen, Hoers Holm, Denmark) were added at a level of 10^7 CFU/kg of sucuk batter.

Each sucuk batter was stuffed into 38 mm collagen casings using a stuffer (Vemag, Maschinenbau, Germany). Sucuks were placed in climatic room for ripening under the following conditions: (1) at 24 ºC and 90% relative humidity (RH) for 12 hours, (2) at 20 ºC and 85% RH for 12 hours, (3) at 18 ºC and 80% RH until the pH reached 5.2-5.3, (4) at 14 ºC and 70% RH for 12 hours, (5) at 14 ºC and 50% RH for 12 hours, (6) at 11 ºC and 50% RH until the water content of sucuks reached 33-34% (end product). The air flow velocity was 0.5 m/s in all stages of the ripening period. Ready to eat sucuk samples were modified atmosphere packaged (MAP) and stored at 4 °C for 90 days. For MAP, sucuk samples were put into gas impermeable trays. Packages were evacuated, filled with a modified atmosphere containing 29.7% carbon dioxide, 0.3% oxygen and 70.0% nitrogen and automatically heat-sealed with a barrier film. The trays had a water vapor transmission rate 10 g/m^2/24 h/at 38 °C/, 90% RH, 1 atm and oxygen transmission rate 2 cm^3/m^2/24 h/at 23 °C, 50% RH, 1 atm. The film had an oxygen transmission rate of 2 cm^3/m^2/24 h/bar at 23°C and 50% RH and a water vapor permeability of 10 g/m^2/24 h at 23 °C and 90% RH.

In this study, all treatments were replicated independently twice. For each replicate, 50 sucuks were produced per treatment. Analyses of pH, TBARS, residual nitrate and nitrite were performed on days 0, 15, 30, 45, 60, 75 and 90. Color analyses were conducted on days 0, 30, 60 and 90. Microbiological analyses were performed on days 0, 45 and 90. Additionally, texture profile analyses (TPA) were conducted on day 0.

2.3. pH measurements

The pH values of samples were determined throughout the refrigerated storage. The pH measurements were conducted with a portable pH meter (WTW Series pH 720, Weilheim, Germany) according to AOAC (2000).

2.4. Determination of lipid oxidation

Thiobarbituric acid (TBARS) method described by Ockerman (1985) was used to determine the lipid oxidation of the sucuk samples. The absorbance of samples was read at 538 nm (UV-160 A, UV-Visible Recording Spectrophotometer, Shimadzu, Tokyo, Japan) against a reagent blank. The TBARS numbers were expressed as milligrams malonaldehyde per kilogram samples (mg MA/ kg sample).

2.5. Residual nitrate and nitrite analyses

The residual nitrate and nitrite contents of the samples were determined according to Cortesi et. al. (2015). For determination of nitrate contents of samples, nitrate was reduced to nitrite by means of cadmium sulphate. Afterwards, nitrite was reacted with sulphanilamide with N-1-naphthylethylenediamine dihydrochloride (NED) and the resulting pinkish dye was measured with a spectrophotometer (UV-160 A, UV-Visible Recording Spectrophotometer, Shimadzu, Tokyo, Japan) at 540 nm. The residual nitrate and nitrite contents were calculated using standard curves of sodium nitrate and sodium nitrite solutions. The residual nitrate and nitrite contents were expressed as mg nitrate per kg sample (mg/kg) and mg nitrite per kg sample (mg/kg), respectively.

2.6. Microbiological analyses

The microbiological analysis of samples was performed by following the procedure of Zhang et al. (2016) with minor modifications. 10 g of sucuk samples were hygienically transferred to the stomacher bags. Then, 90 mL of Ringer's solution (Ringer Tablet, Merck, Germany) was added and blended until a homogeneous mixture was obtained. For each sample, serial decimal dilutions were prepared with sterile Ringer's solution and 1 ml sample of the appropriate dilutions was transferred into selective agar plates. The enumeration of microorganisms was done on the plates, which contain the colonies between 30 and 300 after incubation for specific storage conditions (time, temperature, oxygen etc.). The results were expressed as log10 colony forming units per gram sucuk (log10 CFU/g).

Total mesophilic aerobic bacteria (TMAB) were calculated by using Plate Count Agar (PCA, Merck, Germany) after incubation at 37°C for 48 h and then enumerated (Babuskin et al., 2014). The lactic acid bacteria (LAB) were cultured on Man-Rogosa-Sharpe (MRS) agar anaerobically incubated at 37°C for 72 h and then enumerated (Zhang et al., 2016). Yeast-mold were counted on Potato Dextrose Agar acidified by sterile tartaric acid (10 %) (Merck, Germany) incubated at 25℃ for 5 days (Gökalp et. al., 1999). The total coliform bacteria medium Violet Red Bile agar (VRBA; Merck, Germany) on the plates was incubated at 37 °C for 24 h and then enumerated (Sagdic et. al., 2011).

2.7. Texture profile analysis

Texture profile analyses of sucuks were conducted using the method of Crehan et. al. (2000) and Herrero et al. (2007). TPA was conducted in accordance with the two-compression method using a texture analyzer (TA-HD Plus Texture Analyser, UK). A cylindrical plate which has diameter of 20 cm and 50 kg load cell were used. The sample was compressed twice, with a 0.1-sec delay between the descents, pre-test speed of 1 mm/sec, test speed of 5 mm/sec, post-test speed of 5 mm/sec and compression of 50%. The following texture profile parameters were determined: hardness (N), adhesiveness (N.s), cohesiveness, springiness and chewiness (N). Sucuk samples were sliced at 1.5 cm height for texture analysis and analyses were performed as 3 parallel slices for each group.

2.8. Colour measurements

Colour properties of sucuks were measured according to Hunt et al. (1991). Chroma meter CR-400 (Konica Minolta, Inc., Osaka, Japan) with illuminant D65, 2° observers, Diffuse/O mode was used for color determination. $L*$ (lightness), $a*$ (redness) and $b*$ (yellowness) parameters of the samples were determined. The measurements were carried out on the outer surfaces of the sucuk samples. Three readings were taken on different parts of outer surfaces for each sample.

2.9. Statistical analysis

This study was conducted in two independent replicates with triplicate sampling and a completely randomized design was employed. A one-way analysis of variance (ANOVA) was performed for all variables (pH, residual nitrate, residual nitrite, TBARS, microbial counts, TPA and colour) by using MINITAB release 18.0 programme. The interaction between curing agent treatment and storage was also analyzed with two-way Anova using the GLM procedure.

The curing agent treatments (T1, T2, T3, T4 and T5) and storage days were analyzed as a fixed factor while the replicate was considered as a random factor. Tukey Multiple Comparison Tests were used to determine the statistical significance among the means at a 5% significance level.

3. Results and Discussion

3.1. pH values

Table 2 indicates the pH values of the sucuks during the refrigerated storage for 90 days. As the refrigerated storage progressed, the pH values of all samples decreased (P < 0.05). The lowest pH values of samples were determined on days 75 and 90 (P < 0.05). It is thought that this decrease in pH values during the refrigerated storage may be due to the activities of lactic acid bacteria in the sucuks (Rubio et al., 2007). While the pH value of the sucuks cured with spinach powder (T4) was higher than the other groups during the storage period, the pH values of the T5 group were the lowest (P < 0.05). The lower pH value (5.10±0.01) of Swiss chard powder compared to other vegetable powders is thought to may be the reason for this situation. These results are in accordance with Shin et al. (2017) who describe that the use of Swiss chard powder decreases the pH values of pork patties. Similarly, red beet in meat emulsion (Choi et al., 2017) and fermented red beet extracts in frankfurters (Hwang et al., 2017) decreased the pH values of samples.

Table 2.
pH values, residual nitrate and nitrite contents of sucuks during refrigerated storage (Mean ± standard error)

Analyses	Storage periods (Day)	Treatments				
		T1	T2	T3	T4	T5
pH	0	5.25±0.01^{Aa}	5.22±0.02^{Ba}	5.25±0.00^{Aa}	5.25±0.01^{Aa}	5.21±0.02^{Ba}
	15	5.23±0.00^{Ba}	5.19±0.01^{Ba}	5.23±0.01^{Ba}	5.24±0.00^{Aa}	5.20±0.01^{Ba}
	30	5.15±0.01^{Bb}	5.13±0.01^{Bb}	5.16±0.01^{Bb}	5.23±0.00^{Aa}	5.18±0.00^{Bab}
	45	5.14±0.00^{Cb}	5.14±0.01^{Cb}	5.13±0.01^{Cb}	5.23±0.01^{Aa}	5.15±0.01^{Bab}
	60	5.13±0.02^{Bc}	5.13±0.01^{Bb}	5.13±0.00^{Bb}	5.22±0.01^{Aa}	5.12±0.03^{Bbc}
	75	5.07±0.01^{Bc}	5.11±0.01^{ABb}	5.06±0.00^{Bc}	5.17±0.00^{Ab}	5.05±0.02^{Bc}
	90	5.06±0.00^{Bc}	5.10±0.01^{ABb}	5.06±0.01^{Bc}	5.14±0.01^{Ab}	5.03±0.00^{Cc}
Residual nitrate (ppm)	0	23.34±0.55^{Aa}	1.51±1.12^{C}	14.50±0.97^{B}	nd	nd
	15	22.22±0.48^{Aa}	nd	nd	nd	nd
	30	8.27±0.45^{Ab}	nd	nd	nd	nd
	45	8.19±0.51^{Abc}	nd	nd	nd	nd
	60	6.87±0.82^{Abc}	nd	nd	nd	nd
	75	4.44±1.15^{Acd}	nd	nd	nd	nd
	90	1.30±0.51^{Ad}	nd	nd	nd	nd
Residual nitrite (ppm)	0	2.30±0.00^{C}	2.85±0.03^{Ba}	3.64±0.16^{Aa}	2.95±0.06^{Ba}	3.08±0.13^{Ba}
	15	2.23±0.06^{B}	2.59±0.04^{Bab}	3.59±0.24^{Aa}	2.49±0.07^{Bb}	2.26±0.03^{Bb}
	30	2.49±0.07^{BC}	2.39±0.03^{BCbc}	3.25±0.03^{Aab}	2.49±0.06^{Bb}	2.23±0.00^{Cb}
	45	2.43±0.13^{B}	2.36±0.00^{Bbc}	3.02±0.14^{Aab}	2.33±0.03^{Bbc}	2.23±0.00^{Bb}
	60	2.65±0.23	2.36±0.00^{bc}	2.88±0.13^{ab}	2.33±0.04^{bc}	2.22±0.00^{b}
	75	2.23±0.00^{AB}	2.20±0.10^{ABc}	2.62±0.13^{Ab}	2.06±0.03^{Bc}	2.10±0.07^{Bb}
	90	2.49±0.07^{A}	2.23±0.06^{ABc}	2.59±0.10^{Ab}	2.06±0.03^{Bc}	2.10±0.07^{Bb}

Within the same row, values with different uppercase superscript letters ($^{A-C}$) indicate significant differences (P < 0.05).
Within the same column, values with different lowercase superscript letters ($^{a-c}$) indicate significant differences (P < 0.05).
T1: 100 ppm sodium nitrite (traditionally nitrite cured); T2: 100 ppm sodium nitrate; T3: dill powder 0.71%; T4: spinach powder 0.29%; T5: Swiss chard powder 0.26 %.

3.2. Lipid oxidation

Figure 1 shows the effects of different curing agents and refrigerated storage on TBARS numbers of sucuks. The curing with vegetable powders and refrigerated storage significantly affected the TBARS numbers of samples (P < 0.05). As expected, TBARS numbers increased as the refrigerated storage process progressed (P < 0.05) and the highest TBARS number were determined on day 90. The samples cured with spinach powder had the lowest the TBARS numbers, while the group of T3 had the highest lipid oxidation level compared to the other groups (P < 0.05). This situation is probably due to incomplete the reduction of nitrate to nitrite in the group of T3 group. Similar findings have been reported that Swiss chard powder inhibited lipid oxidation in the pork patties (Shin et al., 2017).3.3. Residual nitrate and nitrite contents of fermented sucuks

The residual nitrate and nitrite contents of sucuks are given in Table 2. In the production of sucuks (sucuk batter), 100 ppm nitrate was added to T2 T3, T4 and T5 groups and 100 ppm nitrite was added to T1. In ready-to-eat samples, in other words, at the beginning of the refrigerated storage, the nitrate contents of T1, T2 and T3 were determined as 23.34, 1.51 and 14.50 ppm, respectively. Interestingly, although T1 cured with sodium nitrite (no addition of nitrate), the highest nitrate content was determined in this group on day 0 (P < 0.05). A possible explanation for this might be that nitrogen dioxide, which is formed as a result of the reduction of nitrate or nitrite, react with the water in the medium and it cause nitrate formation again (Pegg and Shahidi, 2008; Sebranek, 2009). Due to the completely reduction of nitrate in T4 and T5 groups, nitrate was not detected in these groups during refrigerated storage. In T2 and T3, nitrate was not detected on day 15 and after. In the T1 group, the nitrate amount decreased over time (P < 0.05) and the nitrate content was determined as 1.30 ppm on day 90. The results of the current study are consistent with those of Riel et al. (2017) who determined that Mortadella type sausages cured with sodium nitrite had the higher nitrate contents than samples cured with vegetable extract.

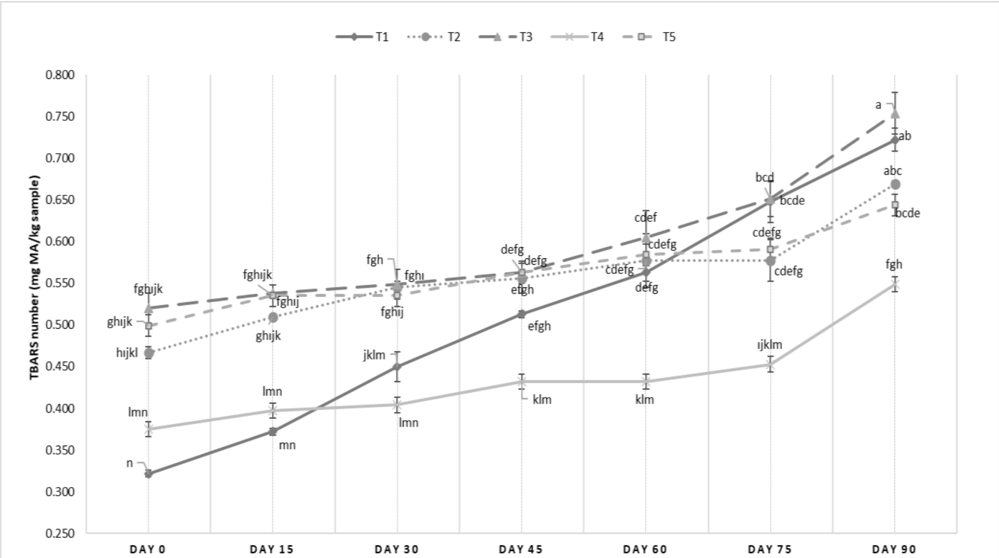

Figure 1
TBARS numbers of sucuks during refrigerated storage. T1: 100 ppm sodium nitrite (traditionally nitrite cured); T2: 100 ppm sodium nitrate; T3: dill powder 0.71%; T4: spinach powder 0.29%; T5: Swiss chard powder 0.26%.

Curing with different vegetable powders and refrigerated storage significantly affected the residual nitrite contents of sucuks ($P < 0.05$). As the refrigerated storage progressed, nitrite contents of samples (except for T1) were generally decreased ($P < 0.05$). T3 had the highest nitrite content at the beginning of the storage while the groups of T1 and T3 had higher nitrite contents than other group on day 90 ($P < 0.05$). It is thought that as a result of the reduction of nitrate in the group of T3 at the beginning of storage, the nitrite content is higher than the other groups ($P < 0.05$). The reason for the fluctuations in the nitrite contents of the T1 group during the refrigerated storage is the reduction of nitrate, which is present in high amounts at the beginning of storage, to nitrite over time. Curing with spinach and Swiss chard powders (T4 and T5) had the lowest residual nitrite contents on days 75 and 90 ($P < 0.05$). These observations are in accordance with Sindelar (2014) and Riel et al. (2017) who describe that residual nitrite contents are lower in cured meat products with natural agents than in synthetic nitrite cured samples.

3.4. Microbiological enumeration

Microbiological counts (log CFU/g) of sucuks during refrigerated storage are given in Table 3. Curing with different vegetable powders did not affect the TMAB, LAB, yeast and mould and total coliform counts of samples compared to control groups (T1 and T2) ($P > 0.05$). The refrigerated storage affected the TMAB and LAB counts of the sucuks ($P < 0.05$). The differences between the TMAB counts of samples were insignificant on days 0 and 45 ($P > 0.05$) while an increase was determined after 45 days in all groups ($P < 0.05$). It is thought that the progress of the storage period and the change of gas concentrations in the modified atmosphere package over time may be the reasons for the increase in the TMAB counts of sucuks

Table 3**.**
Microbiological counts (Log CFU/g) of sucuks during refrigerated storage (Mean ± standard error)

Microbiological analyses (Log CFU/g)	Storage periods (Day)	Treatments				
		T1	T2	T3	T4	T5
Total mesophilic aerobic bacteria	0	5.57±0.04^b	5.52±0.04^b	5.38±0.05^b	5.51±0.11^b	5.26±0.05^c
	45	5.41±0.01^b	5.50±0.01^b	5.44±0.04^b	5.44±0.03^b	5.50±0.01^b
	90	8.03±0.01^a	8.07±0.09^a	8.07±0.02^a	7.99±0.01^a	8.03±0.03^a
Lactic acid bacteria	0	7.97±0.02^b	7.73±0.09^b	7.84±0.02^b	7.96±0.05^b	7.99±0.04^b
	45	8.15±0.03^a	7.97±0.03^a	7.87±0.03^b	7.90±0.15^b	8.09±0.02^a
	90	8.17±0.00^a	7.97±0.02^a	8.05±0.00^a	8.06±0.02^a	8.09±0.01^a
Yeast-mold	0	ndg	ndg	ndg	ndg	ndg
	45	ndg	ndg	ndg	ndg	ndg
	90	ndg	ndg	ndg	ndg	ndg
Total coliform	0	ndg	ndg	ndg	ndg	ndg
	45	ndg	ndg	ndg	ndg	ndg
	90	ndg	ndg	ndg	ndg	ndg

Within the same column, values with different lowercase superscript letters ($^{a-c}$) indicate significant differences ($P< 0.05$) for each different microbial criteria. ndg: No detectable growth
T1: 100 ppm sodium nitrite (traditionally nitrite cured); T2: 100 ppm sodium nitrate; T3: dill powder 0.71%; T4: spinach powder 0.29%; T5: Swiss chard powder 0.26%.

It was determined that the LAB counts of sucuks increased with the progress of the refrigerated storage and the highest results were determined on the 90th day ($P < 0.05$). The yeast-mould and total coliform group bacteria growth were not detected in the samples during the refrigerated storage. It has been reported that the metabolites formed as a result of the activities of lactic acid bacteria and the decrease in pH play an important role in the inhibition of coliform bacteria (de Oliveira Mendonca, et. al., 2004).

On the other hand, it has been stated that nitrate/nitrite inhibits some microorganisms and pathogens that cause deterioration in meat and meat products (Weiss et. al., 2010). Similarly, Bağdatli and Kundakci (2016) stated that there was no growth of coliform group bacteria in sucuks.

3.5. Textural characteristics

Table 4 shows the textural characteristics of the sucuks. Curing treatment affected the hardness, cohesiveness and chewiness values of samples ($P < 0.05$) whereas the springiness and the adhesiveness were not ($P > 0.05$).

Table 4.
Textural properties of the sucuks during refrigerated storage (Mean ± standard error)

Texture parameters	Treatments				
	T1	T2	T3	T4	T5
Hardness (N)	148.70±0.49^{D}	172.90±0.46^{C}	184.30±0.31^{A}	181.50±0.23^{B}	183.80±0.14^{A}
Adhesiveness (N.s)	4.23±0.52	2.90±0.04	2.94±0.42	2.65±0.03	3.14±0.22
Cohesiveness	0.398±0.01^{AB}	0.401±0.00^{AB}	0.385±0.00^{B}	0.397±0.00^{AB}	0.406±0.00^{A}
Springiness	0.471±0.01	0.446±0.01	0.424±0.01	0.431±0.02	0.463±0.02
Chewiness (N)	27.93±0.75^{B}	30.97±0.37^{AB}	30.04±0.56^{AB}	31.07±2.03^{AB}	34.64±1.29^{A}

Within the same row, values with different uppercase superscript letters ($^{A-D}$) indicate significant differences ($P < 0.05$).
T1: 100 ppm sodium nitrite (traditionally nitrite cured); T2: 100 ppm sodium nitrate; T3: dill powder 0.71%; T4: spinach powder 0.29%; T5: Swiss chard powder 0.26%.

Curing with vegetable powder increased the hardness values of the samples and the highest values were determined in T3 and T5 groups ($P < 0.05$). On the contrary, some studies indicated that the use of red beet powder in emulsified pork sausage (Jin et. al., 2014), the parsley extract powder in mortadella-type sausages (Riel et al., 2017) and the beetroot powder in Turkish fermented beef sausage (Sucu and Turp, 2018) did not affect the textural properties of samples.
A possible explanation for this might be the differences in the treatments (production conditions, addition level of the additives, the form of additive etc.) and sausage compositions (fat and water content) in different studies (Barbieri et. al., 2013). In addition, since the results are directly related to the texture analyser used and the device settings are not given in detail in the studies, it may not be very accurate to compare the differences between the studies (Riel et al., 2017).

3.6. Colour properties

The effects of different curing agents on L^*, a^* and b^* values of sucuks are presented in Table 5. Curing treatment did not affect the L^* values of samples ($P > 0.05$), except the day 60. The groups of T1 and T2 had the highest L^* values on day 60 ($P < 0.05$). In terms of refrigerated storage, the lowest lightness values were determined on day 0, and the L^* value of the samples increased with the progress of storage ($P < 0.05$).

Table 5.
Color characteristics of sucuks during refrigerated storage (Mean ± standard error)

Analyses	Storage periods (Day)	Treatments				
		T1	T2	T3	T4	T5
*L**	0	27.90±0.72^{b}	25.50±0.61^{b}	25.88±0.29^{c}	27.56±0.10^{b}	29.33±0.74^{b}
	30	39.23±0.67^{a}	40.14±0.31^{a}	38.09±0.19^{ab}	38.41±0.18^{a}	38.64±0.43^{a}
	60	40.25±0.72^{Aa}	39.91±0.70^{Aa}	35.53±0.72^{Bb}	38.25±0.08^{ABa}	39.18±0.76^{ABa}
	90	40.09±0.44^{a}	39.03±0.18^{a}	38.93±0.70^{a}	38.72±0.55^{a}	39.03±0.04^{a}
*a**	0	16.51±0.17^{A}	15.30±0.28^{AB}	12.53±0.23^{B}	12.86±0.01^{B}	12.13±1.19^{B}
	30	14.22±0.02^{A}	12.15±0.03^{AB}	10.90±0.28^{B}	11.16±0.24^{B}	12.06±0.91^{AB}
	60	14.25±0.31^{A}	12.75±0.06^{B}	11.83±0.20^{B}	11.96±0.09^{B}	11.91±0.19^{B}
	90	14.01±0.87^{A}	13.30±0.49^{AB}	10.79±0.45^{B}	11.62±0.39^{AB}	12.47±0.25^{AB}
*b**	0	9.99±0.18	7.85±0.04^{b}	8.01±0.04^{b}	7.50±0.35^{b}	5.99±0.74
	30	10.27±0.24	8.16±0.06^{ab}	8.57±0.31^{ab}	8.66±0.22^{ab}	8.26±0.73
	60	11.26±0.91	9.04±0.17^{ab}	9.92±0.41^{a}	9.82±0.00^{a}	8.93±0.28
	90	10.63±1.50	9.81±0.36^{a}	9.26±0.21^{ab}	9.94±0.52^{a}	8.33±0.70

Within the same row, values with different uppercase superscript letters ($^{A-B}$) indicate significant differences ($P< 0.05$). Within the same column, values with different lowercase superscript letters ($^{a-c}$) indicate significant differences ($P< 0.05$). T1: 100 ppm sodium nitrite (traditionally nitrite cured); T2: 100 ppm sodium nitrate; T3: dill powder 0.71%; T4: spinach powder 0.29%; T5: Swiss chard powder 0.26%.

Similarly, Sucu and Turp (2018) reported that L^* values of the fermented beef sausages increased during refrigerated storage ($P < 0.05$). Natural curing treatment affected the redness values of samples ($P < 0.05$), but the effect of storage was not significant ($P > 0.05$). The highest a^* values were determined in the group of T1 whereas T3 had the lowest. The curing with vegetable powders decreased the a^* values of the samples compared to the control groups. It is thought that this is probably due to the lower a^* values of the vegetablepowders.

In agreement with our results, Ko et. al. (2017) indicated that the use of young radish and vegetable powder caused a decrease in sausages. Different curing agents did not change the *b** values of the sucuks, while progress of refrigerated storage increased the yellowness of T2, T3 and T4. This result was in accordance with that Sucu and Turp (2018) of who put forth that beetroot powder did not change the *b** values of fermented beef sausages after day 0.

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