Effects of Purslane, Stinging Nettle and Flax Seed Flours on Some Physicochemical and Sensory Properties of Naturally Fermented Turkish Style Semi-Dry Sausage (Sucuk)

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Abstract

The objective of this research was to determine the effects of seed flours of purslane, stinging nettle and flax on ripening processing and the quality characteristics of sucuk samples. Seven different formulations of sucuks were manufactured using a natural fermentation method (without starter culture) and ripened for 21 days under ambient conditions. The sucuk samples were analyzed on the related days of ripening. The pH values were decreased and in parallel lactic acid content was increased during the ripening periods. Over time, the free fatty acids and TBARS values were continuously raised. The L values were decreased, but firstly a* and b* values were increased than decreased. The highest sensory scores were found in the sucuk samples including purslane and flax seed flours. Results of this study indicated that the seed flours as natural materials could be utilized in sucuk manufacturing, in order to obtain an acceptable final product.*

Keywords: Fermentation, Flax seed, Purslane seed, Stinging nettle seed, Sucuk

1 Introduction

Meat and meat products are essential components in the diet of many countries. Among meat products, traditional Turkish semi-dry fermented sausage (sucuk) is one of the most popular fermented meat products consumed in Turkey. Sucuk dough is prepared by chopping and mincing (3 mm diameter orifice plates) meat (beef, mutton or goat) and fat with salt, sugar, spices, dry garlic and other ingredients, this mixture is then stuffed into natural or artificial casings and ripened/dried (Ensoy et al. 2010; Karslioğlu et al. 2014). The fermentation of sucuk is accomplished by microorganisms of the natural meat microflora or by added starter cultures. A quality sucuk generally contains 40–35% water, 30% fat, 30% protein, 2% NaCl, and has a pH value between 5.0 and 4.8 (Con and Gökalp 2000). Beef or lamb fat are used for sucuk production and have rich in saturated fatty acids and cholesterol.

Therefore, all health organizations have incited lowering the intake of total dietary fat, saturated fatty acids and cholesterol as a means of preventing cardiovascular heart disease. For this reason, meat producers focused to develop innovative meat products that promote better consumer health or achieve better lipid compositions (Jiménez-Colmenero et al. 2010). Vegetable oils are free of cholesterol and have a higher ratio of unsaturated to saturated fatty acids than animal fats (Liu et al. 1991). Besides, different components with antioxidant activity, most of them of plant origin (fruit, vegetables, seeds, spices, etc.), have been used as functional ingredients in meat based functional foods (Jiménez-Colmenero et al. 2001; Arihara 2006; Jiménez-Colmenero et al. 2006). Thus, the addition of vegetable seed flours in meat products may have a positive effect on consumer health.

Purslane (*Portulaca oleracea* L.) has been considered as rich sources of antioxidants, Vitamin A, B, C and E, beta-carotene and essential amino acids as well as minerals such as potassium, calcium, magnesium and iron (Rubatzky and Yamagughi 1997). The seeds and leaves of stinging nettle (*Urtica dioica* L.) contain vitamins, minerals and amino acids (Baytop1999). There have been some papers published on the useof stinging nettle plant as ingredient in sucuk product (Aksu and Kaya 2004; Karabacak and Bozkurt 2008). Flax (*Linum usitatissimum* L.) seeds contain 20% protein, 27% dietary fiber, and 41% fat (Ponnampalam et al. 2016). Flax seed contains considerable amounts of linoleic acids (Salunkhe and Kadam 1998; Ponnampalam et al. 2016).

Its oil is the richest natural source of this fatty acid, but alpha-linolenic acid is also present in large amounts in a variety of other plant oils (Charles and Myers 2000). There is more information on the use of flax seed in bakery products (Conforti and Davis 2006; Koca and Anil 2007) and flax seed oil has also been used in meat products (Pelser et al. 2007; Valencia et al. 2008). The use of seed flours of purslane, stinging nettle and flax in sucuk manufacturing as new meat ingredients which might improve as enhancer in the manufacture of healthier functional meat products. According to our knowledge, there are no reports on the use of seed flours of purslane, stinging nettle and flax as a functional ingredient in sucuk product. Therefore, the objective of this research was to determine the effects of floured seeds of purslane, stinging nettle, and flax on ripening processing and the quality characteristics of sucuk which a semi-dry fermented sausage.

2.0 Materials and Methods

2.1 Materials

Fresh boneless beef cuts and fat (sheep tail fat and beef back fat) used in the sucuk production were obtained from local meat processors (butchers) in Konya, Turkey. The beef was trimmed of visible fat and connective tissue. The beef and fat were kept at temperatures around 4C. Collagen casings (38 mm diameter; Fibran S.A., Girona, Spain) and other sucuk ingredients such as salt, garlic and powder spices were provided from a company (Hilkan Food Industry and Trade Ltd. Co., Konya, Turkey) in Konya, Turkey. Purslane (*Portulaca oleracea* L.) seeds, stinging nettle (*Urtica dioica* L.) seeds and flax (*Linum usitatissimum* L.) seeds were purchased as uncrushed from a local market (herbalist) in Konya, Turkey. Each dry seeds were separately ground to pass a 100 mesh (149 μ) screen before use. All types of the obtained seed flours were kept in covered colour bottles at room conditions until they were used in sucuk samples.

2.2 Sucuk Formulation and Preparation

The production of sucuk was performed in a commercial plant (Hilkan Food Industry and Trade Ltd. Co.) in Konya, Turkey. Each sucuk dough was added according to the formula described by Çoksever and Sariçoban (2010), with the following ingredients per kg of beef: 800 g/kg beef (about 18% fat), 200 g/kg tallow fat (sheep tail fat), 20 g/kg NaCl, 10 g/kg garlic, 4 g/kg sucrose, 5 g/kg red pepper, 5 g/kg black pepper, 5 g/kg cumin, 2 g/kg pimento and 150 ppm/kg NaNO2. Then, seven different sucuk dough were prepared according to the following formulations for each treatment: (1) Control: sucuk dough without the seed flours; (2) PSF1: sucuk dough + purslane seed flour (1%); (3) PSF2: sucuk dough + purslane seed flour (2%); (4) SSF1: sucuk dough + stinging nettle seed flour (1%); (5) SSF2: sucuk dough + stinging nettle seed flour (2%); (6) FSF1: sucuk dough + flax seed flour (1%) and (7) FSF2: sucuk dough + flax seed flour (2%). The mixture was ground through a 3 mm plate and mixed again.

All formulations were applied to a single batch of beef. For each formulation, the combined weight was 10 kg sucuk samples. Each sucuk mixture was placed in a separate plastic box and held at 4 °C for 12 h to increase the penetration of ingredients into the meat. These mixtures were separately mixed and then stuffed into collagen casings (38 mm diameter; Fibran S.A., Girona, Spain) to achieve a weight of 250 g under clean conditions using a filling machine (Tefal, Prep'Line 1600, France) at 4 °C. After filling process, the sucuks were placed in the fermentation chamber (55-58% RH at 15-16 °C) for stabilization treatment for 10 hours. Then, the sucuk samples were ripened (using a natural fermentation method without starter culture) at 90–60% relative humidity (RH) and at 25–18 °C during 21 days as follows; 26 °C for 3 days at 90% RH and; 24 °C for 3 days at 85% RH; 22 °C for 3 days at 80% RH; 20 °C for 3 days at 75% RH; 18 °C for 3 days at 70% RH; 18 °C for 3 days at 65% RH and 18 °C for 3 days at 60% RH in a controlled fermentation cabinet (Nüve, TK 252, Ankara, Turkey). After ripening, the sucuk samples were stored at room ambient conditions for 10 °C at 60% RH during analysis.

2.3 Sampling and sample preparation

Sucuk samples were taken in triplicate in two repetitions for the pH, TBARS, acidity value (as lactic acid), FFA and colour, three samples were taken from each batch at days 0, 1, 2, 3, 7, 10, 14 and 21 of ripening; for the colour measurement, five samples were taken from each batch at days 1, 2, 3, 7, 10, 14 and 21 of ripening. In addition, moisture, fat, protein and ash contents of sucuk samples were measured on the days 0 and 21. After ripening, fatty acid composition and sensory analysis of sucuk samples were determined. Sensory properties were determined in the whole sucuk and on the cut surface. A 2 cm long cross section was cut from the sausage being sampled with a sharp knife for colour analysis. Each sample was stored in a single, sealed, polyethylene box and placed in a refrigerator until its analysis. All the analyses were conducted within 30 min of sampling.

2.4 Proximate composition and chemical analysis

Moisture (hot air oven), protein (Kjeldahl, Nx6.25), fiber, ash (muffle furnace) and fat (ether-extraction) contents were determined using standard methods of the AOAC (2003). The carbohydrate content was calculated by difference. For pH determination, the sample (10 g) was homogenized in 100 mL of distilled water for 1 min using a blender (Waring Commercial Blendor®, USA). Then, pH was measured using a pH meter (pH 315i/SET WTW, Germany) (OCKERMAN 1985). TBARS values were expressed as mg malonaldehyde/kg sample and estimated colourimetrically using 2-thiobarbituric acid (Tarladgis et al. 1960) with a UV-visible spectrophotometer (Hitachi U-1800 Model, Japan). Acidity analysis was performed according to the method of Ockerman (1985) and was calculated as the percentage of total titrable acidity (TTA). For free fatty acid (FFA) analyses, fat was extracted with chloroform according to the method of Koniecko (1979) with few modifications and was calculated as the percentage of oleic acid. The fatty acid compositions were determined on the lipid extract from the sucuk samples at the end of ripening period. The lipid fractions of the sucuk samples were extracted using the method of Bligh and Dyer (1959). The fatty acid composition was determined by a gas chromatography (Model GC-2010, Shimadzu Corporation, Kyoto, Japan). Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters (Morrison and Smith 1964). The results were expressed as percentages of SFA, MUFA and PUFA. All analyses were carried out in triplicate with two repetitions.

2.5 Colour measurement

The colour measurements were performed at the surface of the sucuk samples at room temperature (20 ± 2C) using a chromameter CR-400 (Konica Minolta, Inc., Osaka, Japan) with illuminate D_{65}, 2° observer, Diffuse/O mode, 8 mm aperture of the instrument for illumination and 8 mm for measurement. The chromameter was calibrated with a white ceramic tile [L^* = 98.11, a^* = −0.53 and b^* = 2.21] before the measurements. The L^* (lightness), a^* (redness) and b^* (yellowness) colour coordinates were determined according to the CIELab colour space system. For colour measurements, American Meat Science Association guidelines were followed (Hunt et al. 1991). The colour measurements of the sucuk samples were carried out six times with two repetitions at 1st (initial), 2nd, 3th, 7th, 10th, 14th and 21st days of the ripening period.

2.6 Sensory evaluation

A sensory evaluation was performed on seven sucuk samples at the end of the ripening period (day 21) by a panel of 10 semi-trained panelists. Panel members were either graduate students or staff members of the University of Selçuk (Konya, Turkey). Four training sessions were held to familiarize the panellists with the sucuk characteristics to be evaluated and the scale to be used. A continuous scale between 1.0 and 9.0 was used for the evaluation of the each attribute. Each sucuk sample was cut into small pieces (20 mm diameter and 20 ± 0.5 mm thickness) and grilled with a grill (Tefal 1805 Serie 1, China) for 1 min for each side of the sample. After that, at each session sucuk samples were immediately served hot (approximately 50 °C) to the panellists and were subjected to sensory evaluation for colour, texture, odour and taste and general acceptability.

They were instructed to expectorate after sensory evaluation of each sample and cleanse their palates by drinking distilled water at room temperature and consuming unsalted crackers before beginning to evaluate other samples. Sensory evaluation was performed under normal white light in a separate test room (Anonymous 1988). The samples were evaluated in triplicate in two different sessions; all samples under study were evaluated in each of the two sessions. The samples were rated on a nine-point hedonic scale where 1–3 (not acceptable); 4–5 (fairly acceptable); 6–7, good (acceptable); and 8–9, very good.

2.7 Statistical analysis

Two replications of the study were performed and measurements of all parameters were made in triplicate. Mean values for various parameters of seven different sucuk formulations were calculated and compared by analysis of variance using MINITAB for Windows Release 16® (Minitab 2010). Means of fatty acids and sensory properties of products was analyzed using one-way analysis of variance in order to test for significant differences between treatments. Also, data collected for all other parameters were analyzed using two-way ANOVA with treatment and the ripening period as main effects. When a significant ($P < 0.05$) main effect was found, the mean values were further analyzed using Duncan's Multiple Range Test (MstatC 1986) (Snedecor and Cochran 1994). The results of the treatments and the ripening periods are shown in the tables as the mean values and standard errors. Correlation coefficients between the variables were analyzed by Pearson's test using Minitab 16.0 software. Each parameter was tested in triplicate samples with two replications.

3.0 Results and Discussion

3.1 Proximate analysis of the raw materials

Proximate and fatty acid compositions of the raw materials used in the manufacture of the sucuks are shown in Table 1. The beef had a pH value of 5.87 in average and contained 19.30 % protein, 62.20 % moisture, 17.71 % fat and 0.74 % ash.

Table 1: Proximate and fatty acid compositions of meat, purslane seed, stinging nettle seed and flax seed

Parameters		Meat	Purslane seed	Stinging nettle seed	Flax seed
Moisture (%)		62.20	9.02	7.36	4.85
Protein (%)		19.30	20.37	24.00	21.21
Fat (%)		17.71	9.21	31.00	35.29
Ash (%)		0.74	13.55	7.56	3.21
Carbohydrates (%)		-	32.54	22.07	24.89
Fiber (%)		-	15.31	8.01	9.15
pH		5.87	5.72	5.80	5.93
*L**		40.35	46.17	56.06	58.46
*a**		17.47	3.25	0.93	5.60
*b**		9.79	5.01	8.89	18.08
Fatty acid composition (%)					
Myristic	C14:0	4.14	ND	ND	ND
Palmitic	C16:0	30.49	18.08	7.35	5.86
Stearic	C18:0	6.82	23.86	3.81	ND
Arachidic	C20:0	ND	ND	0.56	ND
ΣSFA		41.44	41.95	11.73	5.86
Myristoleic	C14:1	2.18	ND	ND	ND
Palmitoleic	C16:1	ND	ND	0.09	ND
Oleic	C18:1	54.79	30.71	20.49	28.34
Gadoleic	C20:1	ND	ND	0.77	ND
ΣMUFA		56.96	30.71	21.35	28.34
Linoleic	C18:2	1.60	21.01	66.18	15.28
Linolenic	C18:3	ND	6.34	0.74	46.86
Arachidonic	C20:4	ND	ND	ND	3.65
ΣPUFA		1.60	27.35	66.92	65.79
ΣUFA		58.56	58.06	88.27	94.13

SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; UFA; Unsaturated fatty acid. ND: Not detected.

3.2 Chemical composition of the sucuks

Mean percentages of moisture, protein, fat and ash contents of the sucuk samples added with seed flours at different levels (0%, 1% and %2) at the beginning and the end of ripening period are given in Table 2.

As seen in Table 2, moisture contents of sucuk samples declined as the fermentation time passed, and this decline was found to be statistically significant (P <0.05). Moisture content decreased from initial values of 51.10 - 52.01% to 19.54 - 23.23% after 21 days of ripening and no differences were recorded in moisture content among sucuk samples except on the 21st day of ripening. On day 21, the moisture contents of control (22.69%), PSF1 (22.95%) and FSF2 (22.40%) samples were significantly higher than those of the other groups (P< 0.05) (Table 2). According to Declaration of Meat Products by Turkish Food Codex, moisture content in fermented sucuks should be maximum 40% (Anonymous 2002). Moisture contents of sucuk samples show similarities in previously performed studies (Soyer et al. 2005; Çoksever and Saricoban 2010). As a result of moisture loss during ripening period, percentage protein and fat contents of the samples are inclined to increase in the final products. Control sample had higher moisture content than all the samples added with the floured seeds. This moisture reduction explains the proportional increment observed in fat content compared to those sucuk samples with added seed flours (Table 1). Differences in composition of the sucuk samples might be attributed to the type of meat or fat or the raw materials used in sucuk production, being the only variables.

Table 2: Changes in chemical composition (%) of the sucuk samples at 0 day and 21 days of the ripening period

Treatments	Moisture	Protein	Fat	Ash
Day 0				
Control	52.01±0.29^{aA}	12.52±0.09^{dB}	17.33±0.18^{cB}	2.60±0.09^{bcdB}
PSF1	51.81±0.51^{aA}	14.07±0.07^{aB}	19.00±0.29^{bB}	2.51±0.04^{cB}
PSF2	51.11±0.34^{aA}	14.13±0.06^{aB}	20.90±0.26^{aB}	2.67±0.02^{abcB}
SSF1	51.18±0.18^{aA}	13.67±0.02^{bB}	21.18±0.09^{aB}	2.70±0.01^{abB}
SSF2	51.35±0.40^{aA}	14.06±0.02^{aB}	16.22±0.35^{dB}	2.79±0.03^{aB}
FSF1	51.99±0.36^{aA}	13.46±0.20^{bcB}	17.82±0.26^{cB}	2.79±0.03^{aB}
FSF2	51.10±0.52^{aA}	13.14±0.09^{cB}	21.01±0.31^{aB}	2.75±0.01^{abB}
Day 21				
Control	22.69±0.12^{aB}	19.96±0.25^{aA}	43.24±0.37^{aA}	4.38±0.15^{cA}
PSF1	22.95±0.17^{aB}	20.84±0.27^{abA}	41.95±0.21^{bA}	4.62±0.09^{abcA}
PSF2	19.54±0.59^{bB}	21.00±0.22^{abA}	37.96±0.38^{cA}	4.57±0.07^{bcA}
SSF1	23.23±0.44^{aB}	20.45±0.44^{abA}	40.90±0.26^{bA}	4.71±0.12^{abA}
SSF2	20.61±0.64^{abB}	19.98±0.43^{abA}	43.36±0.39^{aA}	4.62±0.03^{abcA}
FSF1	20.88±1.27^{abB}	20.56±0.19^{bA}	35.57±0.23^{dA}	4.80±0.05^{abA}
FSF2	22.40±0.82^{aB}	21.62±0.09^{bA}	36.75±0.38c^{dA}	4.89±0.06^{aA}

$^{a-d}$Within treatments, means presented in the same column with different superscript lower letters are significantly different (P<0.05). $^{A-B}$Within ripening periods, means presented in the same column with different superscript upper letters are significantly different (P<0.05). Means based on six values (Σn=12 for treatment; Σn=42 for ripening period). Data are the mean value ± standard error. PSF: Purslane seed flour; SSF: Stinging nettle seed flour; FSF: Flax seed flour Control, no seed flours added sucuk formulation; PSF1–PSF2 and SSF1–SSF2 and FSF1-FSF2: Purslane seed flour and stinging nettle seed flour and flax seed flour at 1% and 2%, respectively.

As seen in Table 2, protein content in sucuk samples increased from initial values of 12.52 – 14.13 to 19.96 – 21.62% after 21 days of ripening. Soyer et al. (2005) found out the protein content to be 22.20% in their 20% fat additive sucuk sample which was fermented at 20-22 °C. The case can be justified in a way that as the moisture ratio of sucuk declines, its dry mass ratio increases. Fat ratios of sucuk samples on the first day were in the range 16.22 - 21.18%, and these ratios increased to the range of 35.57 to 43.36% at 21st day. During their production fermented meat products lose moisture due to desiccation. Depending on moisture loss in product, an increase in fat content occurs that is 30-35% for 7-9 day long productions (Soyer et al. 2005), and 40- 50% in 4 week long productions (Ordonez et al. 1999). When the ash content of sucuk samples are analyzed, the initial ash values ranged from 2.51 to 2.79 and the final pH on the 21st day ranged from 4.38 to 4.89 (Table 2). An increase in ash content determined in sucuk was in parallel with the increase in dry mass of sucuk samples.

3.3 pH value

The effects of the treatment and ripening period on pH values of sucuk samples are shown in Table 3. As can be seen in Table 3, both treatment and ripening period had a statistically significant (P < 0.05) effect on pH values.

The initial pH values ranged from 5.97 to 4.83 and the final pH on the 21st day ranged from 5.02 to 4.83 (Table 3). In general during the ripening period, the pH values decreased continuously due to the increase in lactic acid content as a result of carbohydrate (dextrose and lactose) breakdown by microbial metabolism (Bozkurt and Erkmen 2002; Aleson-Carbonell et al. 2005; Fernández-López et al. 2008). At the end of the ripening period, the pH values of sucuks added with seed flours were lower than those of control samples and sample with SSF2 had the lowest pH (4.83) ($P < 0.05$) (Table 3). These pH values may be due to the acid pH of the added seed flours.

The pH of fermented sausages depends on several factors such as ripening temperature, ripening time, and fat level (Soyer et al. 2005). Similar to our results, pH values of Spanish dry-fermented sausage enriched with orange fibre were reported by Fernández-López et al. (2008). Turkish Standard Institute (Anonymous 2002) states that high quality ripened sucuks should have pH values between 4.7 and 5.2. In our study, all of sucuks were found in this range at the end of the ripening period.

Pearson correlation coefficients were calculated in order to assess the strength and direction of a linear relationship among pH, TBARS, acidity value, FFA, L^*, a^* and b^* values (data not shown). It was found from correlation test that pH value was negatively related to TBARS value ($r = -0.40$; $P < 0.01$), acidity value ($r = -0.81$; $P < 0.01$) and FFA value ($r = -0.74$; $P < 0.01$), and positively correlated to L^* ($r = 0.80$; $P < 0.01$), a^* ($r = 0.39$; $P < 0.01$) and b^* ($r = 0.74$; $P < 0.01$).

3.4 TBARS value

The effects of the treatment and ripening period on the TBARS values of sucuk samples are shown in Table 3. Both treatment and ripening period statistically had a strong significant ($P < 0.05$) effect on the TBARS values. The initial TBARS values of the fresh product ranged from 0.36 to 0.58 mg malonaldehyde/kg sample and the TBARS value at the end of the ripening period ranged from 0.45 to 2.05 mg malonaldehyde/kg sample. As can be seen in Table 3, seeds added to SSF2 increased its TBARS values higher compared to PSF and FSF. This may be caused by fatty acid constituents of seeds. As seen in Table 3, when the concentration of floured seed addition increased, usually the TBARS values also increased. TBARS values of sucuks, which are produced by addition of different seed flours used in this study, were observed to increase continuously. Together with increase in the ripening period, TBARS values were also observed to increase. Since the unsaturated fatty acids are exposed to oxidation, fatty acids are degraded into smaller chain secondary components such as malonaldehyde, so an increase in TBARS values is observed. Bozkurt and Erkmen (2004) reported that sucuks gathered from factories had TBARS values ranging between 0.51 and 2.11 mg malonaldehyde/kg and samples from butchers had TBARS values ranging between 0.65-3.34 mg malonaldehyde/kg; moreover, they specified that this difference in values was due to avoiding the use of antioxidant substances, differences in production techniques, pH values, differences in starter cultures used and raw material used.

Highest TBARS value in SSF2 added samples is found to be greater compared to those in PSF and FSF (Table 3). The reason may be explained as existence of constituents having antioxidant property in PSF and FSF which show. While the TBARS values, in PSF and FSF added samples are similar in magnitude, and stay constant; in SSF added samples TBARS values, as opposed to other two, showed an increasing trend during the following periods of ripening (Table 3). This may be explained by the existence of catalytic enzymes in SSF constituents which catalyse the oxidation reactions and result in an increase in TBARS values (Gümüş 2007). When Table 3 is analysed; as the additional seeds concentration and ripening period increase, an increase in TBARS values is observed firstly, then it is constant, following which a decline is noted. However in SSF added samples TBARS values are observed to increase continuously. The TBARS value was positively related to acidity value ($r = 0.64$; $P < 0.01$) and FFA value ($r = 0.76$; $P < 0.01$), and negatively correlated to L^* ($r = -0.29$; $P < 0.01$), a^* ($r = -0.38$; $P < 0.01$) and b^* ($r = -0.40$; $P < 0.01$).

3.5 Acidity value

The effects of the treatment and ripening period on acidity value of sucuks are shown in Table 3. When Table 3 is analysed, lactic acid amount, which is ranged from 0.53 to 0.60% at the beginning, shows an increasing trend as the ripening proceeds and is found to be ranging 1.08 to 1.99% on the 21st day. The reason is the action of lactic acid bacteria which continuously produce lactic acid throughout the ripening and are examined to reach a maximum value during the ripening (Çoksever and Saricoban 2010). Lactic acid, produced during ripening period, is the most important compound produced as a result of carbohydrate oxidation. Gök (2015) reported the pH values of all sucuk decreased due to the production of lactic acid by lactic acid bacteria.

Çoksever and Saricoban (2010) in their study reported the lactic acid amounts to be between 0.79% and 1.78%. As seen in Table 3, SSF1 and SSF2 addition in sucuk sample increase lactic acid amounts at a greater rate compared to other two seed types. SSF has a bountiful supply of enzymes which explains high metabolic activity of choline acetyl transferase and transferases involved in nitrogen metabolism (Gümüş 2007). This increase in lactic acid amount with the increase in concentration is thought to be related to the constituents of seeds. The acidity value was positively related to the FFA value ($r = 0.76$; $P < 0.01$), and negatively correlated to L^* ($r = -0.59$; $P < 0.01$), a^* ($r = -0.30$; $P < 0.01$) and b^* ($r = -0.55$; $P < 0.01$).

3.6 Free fatty acid (FFA) composition

The effects of the treatment on FFA content of sucuk samples are shown in Table 3.

Table 3: Changes of pH, TBARS, Acidity and Free Fatty Acid values in the sucuk samples during the ripening period

Ripening period (days)	Treatments						
pH values	Control	PSF1	PSF2	SSF1	SSF2	FSF1	FSF2
0	5.97±0.00^{aA}	5.96±0.00^{bA}	5.96±0.00^{bA}	5.94±0.00^{cA}	5.94±0.00^{cA}	5.95±0.00^{bA}	5.97±0.00^{bA}
1	5.80±0.00^{cdB}	5.79±0.01^{dB}	5.78±0.01^{dB}	5.79±0.00^{dB}	5.83±0.01^{aB}	5.81±0.01^{bB}	5.81±0.00^{bcB}
2	5.17±0.01^{bcC}	5.23±0.01^{aC}	5.18±0.00^{bC}	5.16±0.00^{bcdC}	5.17±0.00^{bcC}	5.12±0.01^{dC}	5.14±0.01^{cdC}
3	5.14±0.01^{aD}	5.10±0.00^{bcD}	5.05±0.01^{dD}	5.11±0.01^{bD}	5.11±0.00^{bD}	5.08±0.01^{cD}	5.09±0.01^{bcD}
7	5.10±0.01^{aE}	5.09±0.01^{aD}	5.02±0.06^{abcDE}	4.96±0.02^{bcE}	4.91±0.02^{cE}	5.04±0.02^{abE}	4.95±0.01^{bcG}
10	5.08±0.02^{aE}	5.02±0.00^{abE}	5.01±0.03^{bE}	4.91±0.02^{cdF}	4.89±0.01^{dF}	4.93±0.01^{cdF}	4.97±0.01^{bcF}
14	5.03±0.01^{aF}	4.95±0.00^{bF}	5.00±0.02^{abDE}	4.86±0.02^{cH}	4.87±0.02^{cG}	4.83±0.01^{cH}	4.99±0.02^{abE}
21	5.02±0.00^{aF}	4.93±0.00^{bF}	4.90±0.00^{cF}	4.88±0.00^{dG}	4.83±0.01^{eH}	4.88±0.00^{dG}	4.85±0.01^{deH}
TBARS values							
0	0.41±0.00^{dE}	0.36±0.00^{fF}	0.58±0.00^{aB}	0.52±0.01^{bF}	0.47±0.00^{cH}	0.51±0.00^{bA}	0.40±0.00^{eG}
1	0.39±0.01^{cF}	0.31±0.00^{fG}	0.42±0.00^{bG}	0.37±0.01^{dG}	0.61±0.01^{aG}	0.42±0.01^{bF}	0.32±0.00^{eH}
2	0.43±0.01^{eE}	0.45±0.00^{dE}	0.49±0.01^{cE}	0.60±0.00^{bE}	0.87±0.01^{aE}	0.48±0.00^{cCD}	0.49±0.00^{cD}
3	0.46±0.00^{deD}	0.50±0.01^{cC}	0.51±0.01^{cD}	0.72±0.02^{bD}	1.38±0.01^{aE}	0.45±0.01^{eE}	0.47±0.00^{dE}
7	0.58±0.00^{cA}	0.53±0.01^{dA}	0.44±0.00^{gF}	0.75±0.00^{bD}	1.53±0.00^{aD}	0.47±0.00^{fD}	0.53±0.00^{eC}
10	0.57±0.00^{dAB}	0.52±0.00^{fAB}	0.57±0.00^{cB}	0.78±0.00^{bC}	1.61±0.00^{aC}	0.49±0.00^{gBC}	0.54±0.00^{eB}
14	0.56±0.00^{dBC}	0.52±0.00^{eB}	0.70±0.00^{cA}	0.81±0.00^{bB}	1.69±0.00^{aB}	0.51±0.01^{cAB}	0.55±0.00^{dA}
21	0.54±0.00^{dC}	0.48±0.01^{fD}	0.55±0.00^{cC}	1.27±0.00^{bA}	2.05±0.00^{aA}	0.49±0.00^{eBC}	0.45±0.00^{gF}
Acidity values							
0	0.57±0.00^{bD}	0.53±0.00^{dD}	0.51±0.00^{eD}	0.57±0.00^{bD}	0.60±0.00^{aF}	0.60±0.00^{aE}	0.55±0.00^{cF}
1	0.64±0.01^{cD}	0.59±0.00^{dD}	0.64±0.00^{cE}	0.67±0.00^{bD}	0.79±0.00^{aE}	0.63±0.01^{cE}	0.63±0.00^{cE}
2	1.03±0.01^{dB}	1.02±0.01^{deB}	1.07±0.01^{bcC}	1.09±0.01^{bC}	1.13±0.01^{aD}	0.99±0.00^{eC}	1.04±0.01^{cdC}
3	1.17±0.02^{aA}	1.13±0.02^{aA}	1.18±0.02^{aB}	1.23±0.03^{aB}	1.15±0.01^{aD}	1.17±0.02^{aA}	1.20±0.02^{aB}
7	0.79±0.02^{dC}	1.11±0.03^{aA}	1.02±0.02^{bC}	1.09±0.00^{abC}	1.16±0.02^{aD}	0.88±0.03^{cD}	1.03±0.00^{bC}
10	1.04±0.05^{dB}	1.10±0.01c^{dAB}	1.01±0.02^{dC}	1.24±0.07^{bB}	1.55±0.01^{aB}	1.08±0.01^{cdB}	1.21±0.02^{bcB}
14	0.86±0.03^{cC}	0.91±0.03^{cC}	0.89±0.01^{cD}	1.00±0.02^{bC}	1.26±0.00^{aC}	1.00±0.01^{bC}	0.91±0.00^{cD}
21	1.17±0.04^{deA}	1.08±0.04^{eAB}	1.23±0.01^{cdA}	1.62±0.02^{bA}	1.99±0.03^{aA}	1.20±0.03c^{deA}	1.32±0.04^{eA}
FFA values							
0	1.17±0.01^{aH}	1.17±0.02^{aH}	1.15±0.05^{aH}	1.03±0.00^{bH}	1.16±0.01^{aH}	1.13±0.00^{abH}	1.05±0.02^{bH}
1	1.28±0.00^{eG}	1.46±0.02^{dG}	1.48±0.02^{dG}	1.77±0.01^{bG}	2.18±0.02^{aG}	1.57±0.05^{cG}	1.45±0.02^{dG}
2	1.65±0.01^{eF}	1.84±0.06^{cF}	1.80±0.01^{cdF}	2.18±0.02^{bF}	2.61±0.03^{aF}	1.83±0.03^{cF}	1.71±0.04^{deF}
3	2.02±0.01^{cdE}	2.22±0.11^{cE}	2.12±0.01^{cdE}	2.60±0.03^{bE}	3.04±0.04^{aE}	2.10±0.01^{cdE}	1.97±0.06^{dE}
7	2.91±0.04^{dD}	2.92±0.03^{dD}	2.86±0.02^{dD}	4.30±0.05^{bD}	5.57±0.02^{aD}	2.95±0.02^{dD}	3.14±0.06^{cD}
10	3.32±0.00^{cC}	3.38±0.02^{cC}	3.14±0.01^{eC}	4.57±0.03^{bC}	5.96±0.02^{aC}	3.24±0.00^{dC}	3.33±0.03^{cC}
14	3.73±0.03^{dB}	3.83±0.01^{cB}	3.40±0.01^{eB}	4.84±0.02^{bB}	6.35±0.03^{aB}	3.53±0.02^{eB}	3.51±0.01^{eB}
21	4.15±0.04^{dA}	4.13±0.06^{dA}	4.27±0.05^{dA}	6.41±0.05^{bA}	7.92±0.06^{aA}	5.03±0.14^{cA}	4.36±0.01^{dA}

$^{a-e}$ Within treatments, means presented in the same row with different superscript lower letters are significantly different ($P<0.05$). $^{A-H}$ Within ripening periods, means presented in the same column with different superscript upper letters are significantly different ($P<0.05$). Means based on six values ($\Sigma n=12$ for treatment; $\Sigma n=42$ for ripening period). Data are the mean value ± standard error. TBARS = TBA reactive substances; FFA = Free fatty acid (%) as oleic acid. PSF: Purslane seed flour; SSF: Stinging nettle seed flour; FSF: Flax seed flour Control, no seed flours added sucuk formulation; PSF1–PSF2 and SSF1–SSF2 and FSF1-FSF2: Purslane seed flour and stinging nettle seed flour and flax seed flour at 1% and 2%, respectively.

As seen in Table 3, as the additional seed concentration increases, the amount of free fatty acids also increases. Moreover, SSF out of the other seed types increases its free fatty acid amount at a greater rate compared to other two seed types. This is due to the end products produced by catabolic enzymes which are found especially in SSF (Gümüş 2007). Free fatty acid contents increase depending on the ripening period (day) in sucuk samples.

On the first day of production, free fatty acid contents are measured to be the least, 1.05% (FSF2), and then on 21st day the value is observed to be 7.92% (SSF2). When Table 3 is analysed, FFA values in PSF and FSF added samples are observed to be similar whereas FFA values of SSF added samples increased at a greater amount, which were observed to enhance on increasing seed concentration. Free fatty acidity, is a chemical reaction occurring as a result of hydrolysis rather than oxidation. Free fatty acidity is an acidity obtained by free fatty acids which are produced as a result of hydrolysis of ester bonds between fatty acids that constitute all together a fat molecule, influenced by factors such as enzyme activity (lipolysis), heat and moisture (Nawar 1996). O'brien (1998) stated that one of the main reasons of increase in free fatty acid amounts is the hydrolytic breakdown occurring as a result of high amount of moisture inside the product. Zanardi et al. (2004) notified that FFA values of fermented sausages increased 2-3 times during ripening period. Enlightened by this information, it may be determined that FFA values of SSF containing samples are greater compared to that of other see containing samples due to enzyme activity in SSF constituents. It was found from correlation test that the FFA value was negatively correlated to L^* ($r = -0.75$; $P < 0.01$), a^* ($r = -0.75$; $P < 0.01$) and b^* ($r = -0.82$; $P < 0.01$).

3.7 Colour properties

The effects of the treatment and ripening period on exterior surface colour of sucuks are shown in Table 5. According to Table 4, among the seed types added into samples, L^* values of SSF added samples were found to be greater compared to those of control group, PSF and FSF added samples. As seen in Table 4, when the ripening period was analysed in terms for days, L^* values was seen to be decreasing depending on time. L^* value of samples was found to be ranging from 40.36 to 43.15 on the first day and on the 21st day this value was measured to be ranging from 33.39 to 35.79. In meat and meat products L^* value is accepted to be the most apparent indicator for measuring the colour change (Gimeno et al. 2000). A decline in L^* value depending on time can be explained with oxidation dependent browning reactions (Bozkurt and Bayram 2006). Üren and Babayiğit (1996), analysed colour properties of Turkish sucuk, and determined L^* values for 11 different types of sucuks ranging between 35.87 and 45.92. The L^* value was positively correlated to a^* ($r = 0.65$; $P < 0.01$) and b^* ($r = 0.89$; $P < 0.01$).

According to Table 4, a^* (redness) values in terms of seed type; highest a^* value was observed in FSF1 added samples. In PSF2 and SSF2 added samples a^* values are found to be less which could be justified as, a^* value of FSF being at a greater ratio compared to other two seed flours. In terms of concentration, as the addition of seed flours amount increases, a^* values showed a decreasing trend. When ripening time periods (days) in Table 4 are analysed, a^* values of samples show an increasing trend in first 3 days of ripening and then decrease during the following days. In the course of ripening, a^* values change between 6.68 and 18.85. Bozkurt and Bayram (2006) determined that redness values in sucuk samples increase until 5th days and then declines. Perez-Alvarez et al. (1999) found similar results and stated that decline in red colour values is due to partial or complete denaturation of myoglobin, nitrosomyoglobin and oxymyoglobin. Üren and Babayiğit (1996) in their research, detected a* values of 11 different sucuk types to be 6.86-14.14. It was found from correlation test that the a^* value was positively correlated to b^* value ($r = 0.85$; $P < 0.01$).

In Table 4, in terms of seed type, b^* values of FSF added samples are observed to be greater compared to other two seed type containing samples. The reason can be explained with flax seed having a greater b^* values are in a greater ratio compared to that of other two seed types (Table 1). In terms of concentration, as seen in a^* values, the b^* values also decrease as the seed concentrations increase (Table 4). When b^* values are analysed in terms of ripening periods; an increase for the first 3 days is observed, followed by a decrease during the rest of the days, and in last week a steady state is maintained (Table 4). Chasco et al. (1996), investigated colour changes, throughout production period, of 13 sucuk samples gathered from local marketing and declared to find the b^* values as 7.82 for dough and 6.11 at the end of 4th week.

They signified that b^* values decreased with a ratio of 44 % during fermentation period. For samples in which 3 seeds are added separately, b^* values are observed to decrease with increasing seed concentration together with ripening period (Table 4).

Table 4: Changes of surface colour values (CIE *L, *a**, *b**) in the sucuk samples during the ripening period**

Ripening period (days)	Treatments						
L*values	Control	PSF1	PSF2	SSF1	SSF2	FSF1	FSF2
1	41.45 ± 0.04^{bA}	43.14 ± 0.28^{aA}	40.36 ± 0.16^{cA}	42.85 ± 0.31^{aA}	42.53 ± 0.03^{aA}	41.48 ± 0.16^{bA}	42.95 ± 0.11^{aA}
2	41.78 ± 0.24^{abA}	40.11 ± 0.25^{cdB}	38.15 ± 0.43^{dB}	41.70 ± 0.09^{abB}	42.78 ± 0.23^{aA}	40.57 ± 0.44^{bcB}	39.94 ± 0.28^{cdB}
3	39.77 ± 0.18^{bB}	38.44 ± 0.19^{cC}	39.24 ± 0.09^{cC}	39.87 ± 0.09^{bC}	40.96 ± 0.39^{aB}	37.59 ± 0.06^{cC}	36.65 ± 0.32^{dC}
7	34.32 ± 0.11^{dC}	37.39 ± 0.11^{abD}	36.11 ± 0.07^{cD}	37.47 ± 0.06^{aD}	37.09 ± 0.24^{abC}	36.83 ± 0.23^{abcCD}	36.55 ± 0.44^{bcC}
10	34.11 ± 0.26^{dC}	36.07 ± 0.20^{bcE}	35.77 ± 0.02^{cDE}	37.25 ± 0.07^{aD}	36.77 ± 0.09^{abCD}	36.48 ± 0.06^{bcD}	36.18 ± 0.24^{bcC}
14	34.48 ± 0.04^{dC}	34.59 ± 0.15^{dF}	$35.10\pm0.19c^{dE}$	35.26 ± 0.33^{cE}	36.05 ± 0.06^{abD}	35.59 ± 0.14^{bcE}	36.30 ± 0.06^{aC}
21	34.66 ± 0.31^{bC}	33.85 ± 0.06^{cG}	33.39 ± 0.13^{cF}	34.68 ± 0.08^{bE}	33.75 ± 0.24^{cE}	34.59 ± 0.23^{bF}	35.74 ± 0.14^{aC}
a* values							
1	14.88 ± 0.34^{aB}	14.28 ± 0.13^{aC}	12.15 ± 0.04^{bB}	14.13 ± 0.36^{aB}	11.64 ± 0.39^{bB}	14.38 ± 0.12^{aB}	14.91 ± 0.28^{aB}
2	18.85 ± 0.43^{aA}	18.54 ± 0.41^{abA}	16.31 ± 0.18^{cA}	16.97 ± 0.62^{bcA}	13.85 ± 0.31^{dA}	18.09 ± 0.45^{abA}	17.24 ± 0.33^{abcA}
3	19.60 ± 0.16^{aA}	17.38 ± 0.11^{bcB}	16.09 ± 0.49^{cA}	17.33 ± 0.44^{bcA}	13.45 ± 0.44^{dA}	18.69 ± 0.21^{abA}	17.73 ± 0.63^{bcA}
7	12.96 ± 0.14^{aC}	9.96 ± 0.39^{cD}	8.40 ± 0.22^{dC}	9.91 ± 0.24^{cC}	12.89 ± 0.18^{aA}	13.15 ± 0.10^{aC}	11.13 ± 0.18^{bC}
10	10.94 ± 0.32^{aD}	8.98 ± 0.33^{cdDE}	7.43 ± 0.31^{dCD}	9.20 ± 0.26^{bcC}	7.83 ± 0.26^{cdC}	11.58 ± 0.56^{aD}	10.57 ± 0.59^{bcC}
14	10.41 ± 0.03^{aDE}	8.46 ± 0.25^{cE}	7.02 ± 0.26^{dD}	7.70 ± 0.31^{dD}	7.41 ± 0.05^{dC}	9.56 ± 0.05^{bE}	10.06 ± 0.07^{abC}
21	9.39 ± 0.21^{aE}	7.95 ± 0.28^{cE}	7.09 ± 0.05^{deD}	7.60 ± 0.05^{cdD}	6.68 ± 0.20^{eC}	8.73 ± 0.09^{bE}	7.75 ± 0.08^{cD}
b* values							
1	17.76 ± 0.47^{abA}	17.75 ± 0.53^{abA}	15.00 ± 0.25^{cAB}	18.41 ± 0.15^{aA}	13.36 ± 0.14^{dA}	18.06 ± 0.19^{abA}	16.79 ± 0.43^{bA}
2	18.39 ± 0.64^{aA}	17.50 ± 0.67^{abA}	$15.51\pm0.13b^{cA}$	15.57 ± 0.70^{bcB}	14.28 ± 0.49^{cA}	16.82 ± 0.18^{abB}	16.40 ± 0.52^{abcA}
3	17.10 ± 0.81^{aA}	14.34 ± 0.51^{bcdB}	14.74 ± 0.17^{abcB}	16.33 ± 0.29^{abB}	11.88 ± 0.48^{dB}	13.85 ± 0.66^{bcdC}	12.03 ± 1.19^{cdB}
7	5.42 ± 0.04^{abB}	3.70 ± 0.33^{cdC}	2.72 ± 0.04^{dC}	5.87 ± 0.03^{aC}	3.09 ± 0.04^{dC}	5.50 ± 0.12^{aD}	4.49 ± 0.54^{bcC}
10	4.59 ± 0.17^{aBC}	2.28 ± 0.13^{cCD}	2.24 ± 0.25^{cCD}	3.24 ± 0.23^{bcD}	1.99 ± 0.33^{cCD}	3.07 ± 0.42^{bcE}	3.63 ± 0.45^{abC}
14	3.81 ± 0.40^{aBC}	3.43 ± 0.32^{abC}	1.98 ± 0.11^{cDE}	3.03 ± 0.40^{abD}	1.76 ± 0.06^{cD}	2.52 ± 0.08^{bcE}	3.45 ± 0.14^{abC}
21	2.81 ± 0.05^{aC}	1.68 ± 0.21^{cD}	1.44 ± 0.05^{cE}	1.92 ± 0.09^{bcD}	1.76 ± 0.19^{cD}	2.33 ± 0.06^{abE}	2.47 ± 0.11^{aC}

$^{a-e}$Within treatments, means presented in the same row with different superscript lower letters are significantly different ($P<0.05$). $^{A-F}$Within ripening periods, means presented in the same column with different superscript upper letters are significantly different ($P<0.05$). Means based on six values ($\Sigma n=12$ for treatment; $\Sigma n=42$ for ripening period). Data are the mean value ± standard error. PSF: Purslane seed flour; SSF: Stinging nettle seed flour; FSF: Flax seed flour Control, no seed flours added sucuk formulation; PSF1–PSF2 and SSF1–SSF2 and FSF1-FSF2: Purslane seed flour and stinging nettle seed flour and flax seed flour at 1% and 2%, respectively.

3.8 Sensory evaluation

The effects of the treatment on sensory properties of sucuks are shown in Table 5. During sensory evaluation of sucuks: cross-sectional colour, cross-sectional appearance, homogeneity, taste flavours and general acceptances are evaluated by panel lists by using 9- point hedonic scale. When the variance analysis results are investigated; effects of seed types added into samples are considered to be statistically important ($P < 0.05$). According to Table 5, among the parameters used in sensory evaluation in terms of appearance the most appreciated sucuks were FSF2 samples whereas SSF2 and PSF2 including samples scored the lowest points from the panellists. In terms of homogeneity, the most appreciated samples were FSF2 including samples whereas SSF2 including ones scored the lowest points. In terms of colour, highest points were given to control group, FSF1 and FSF2 including samples whereas SSF1, SSF2 and PSF2 including samples scored the lowest points. In terms of taste and flavour, FSF1, FSF2 and control group samples got the highest points whereas the lowest points were given to SSF1 and SSF2 added samples. In terms of general acceptance points, considered to be the average of appearance, homogeneity, taste and flavour aspects; the highest score for general acceptance belonged to the FSF2 added samples. On sensory evaluation of sucuk samples on a general basis, FSF1 and FSF2 including samples were determined to receive a greater acceptance (Table 5).

Table 5: Sensory scores of the sucuk samples at 21 days of the ripening period (1 = dislike extremely, 9 = like very much)

Treatment	Sensory properties				
	Colour	Texture	Odour	Taste	General acceptability
Control	7.05±0.32^{ab}	6.80±0.33^{ab}	6.95±0.32^{ab}	6.05±0.43^{a}	6.85±0.35^{ab}
PSF1	6.00±0.36^{bc}	5.85±0.42^{bc}	5.85±0.31^{bc}	5.55±0.38^{ab}	6.05±0.35^{bc}
PSF2	5.60±0.36^{c}	5.75±0.47^{bc}	5.50±0.39^{c}	6.05±0.42^{a}	5.90±0.38^{bc}
SSF1	5.70±0.33^{c}	5.50±0.37^{bc}	6.00±0.36^{abc}	5.50±0.43^{ab}	5.60±0.34^{bc}
SSF2	5.15±0.44^{c}	5.10±0.43^{c}	5.35±0.38^{c}	4.20±0.37^{b}	4.80±0.35^{c}
FSF1	7.30±0.30^{ab}	6.50±0.33^{abc}	6.95±0.35^{ab}	6.60±0.37^{a}	6.75±0.32^{ab}
FSF2	7.55±0.30^{a}	7.40±0.33^{a}	7.30±0.33^{a}	6.85±0.37^{a}	7.50±0.33^{a}

$^{a-c}$Within treatments, means presented in the same column with different superscript letters are significantly different (P <0.05). Means based on twenty values (Σn=140 for treatment). Data are the mean value ± standard error. Control, no seed flours added sucuk formulation; PSF: Purslane seed flour; SSF: Stinging nettle seed flour; FSF: Flax seed flour PSF1–PSF2 and SSF1–SSF2 and FSF1-FSF2: Purslane seed flour and stinging nettle seed flour and Flax seed flour at 1% and 2%, respectively.

4.0 Conclusion

Sucuks added with the seed flours had higher TBARS values than control sucuk. Based on the results of sensory analysis, it can be concluded that seed flours can be used in the production of sucuks. Hence the seed flours as a material source can be successfully formulated into semi-dry fermented sausages, like sucuk. The results suggest that the seed flours in the production of sucuk could give a healthier option to consumers. Correlation results indicate that the pH, TBARS, acidity value, free fatty acid, and colour properties could be used to predict a quality sucuk, and shelf life of sucuk could be determined using these parameters.

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