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ARTICLE

The Effects of Grape Seed Flour on the Quality of Turkish Dry Fermented Sausage (Sucuk) during Ripening and Refrigerated Storage

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Abstract

In this study, the effects of grape seed flour on the physical-chemical properties, microbiological and sensory properties of Turkish dry fermented sausage, sucuk, was investigated. After the sausages produced with beef, beef fat, sheep tail fat and spices, they were ripened for 14 d. Then they were vacuum-packaged and stored for 80 d at 4ºC. The effects of grape seed flour (GSF; 0%, 0.75%, 1.5%, 3%) on the physical-chemical properties (pH, moisture, fat, protein, free fatty acids, thiobarbituric acids, diameter reduction, ripening yield, instrumental colour), microbiological properties (total aerobic mesophilic and lactic acid bacteria, *Enterobacteriaceae*, mould and yeast) and sensory properties of the sausages were investigated. Grape seed flour decreased moisture, TBA, diameter reduction, instrumental colour (a, b) values and sensory analysis scores during the ripening period; it also decreased TBA, instrumental colour (L, a, b) values, total aerobic mesophilic and lactic acid bacteria counts during the storage period. It was concluded that grape seed flour has a potential application as an additive in dry fermented sausages.

Keywords: grape seed, Turkish dry fermented sausage, sucuk, beef, oxidation, colour

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Introduction

Sucuk is a Turkish dry fermented sausage which is mainly produced with meat, fat and spices. Microorganisms are naturally present in meat and can also contaminate the sucuk batters during processing, or can be added as starter culture. In general, it is ripened 12-15 d. It is a fatty meat product and it can undergo autoxidation which can affect the flavour, odour, and colour during ripening and refrigerated storage.

Many plant products containing polyphenolic compounds can protect fatty foods against oxidation. However, non-meat ingredients in formulations of meat products are the important factors for product quality, technological properties, and health (Bañón *et al.,* 2001; Kulkarni *et al.,* 2011; Kurt and Kilinççeker, 2012; Özvural and Vural 2011). Formulations of dry fermented sausages may include one or more non-meat ingredients (Aksu and Kaya, 2004; Shah *et al.,* 2014). Generally, they are used to improve oxidation stability, microbiological safety, and technologic properties. One of them is grape seed which can be used as a functional additive in different foods (Shrikhande, 2000). In particular, grape seed can be used in food products as a source of antioxidants (Kulkarni *et al.,* 2011; Lutterodt *et al.,* 2011; Özvural and Vural, 2011). It contains significant levels of minerals, vitamins and phenolic compounds (Konar, 2010; Shi *et al.,* 2003). Konar (2010) reported that grape seed contains 40-70% fibre, 16% oil, 11% protein and 7% phenolic compounds. The addition of ground grape seeds may affect fibre and oil contents of the meat products. The fibre content of grape seed flour can affect the physical, technological, and sensory properties of the meat products. However, phenolic compounds play an important role in the oxidative stability of meat products as an important source of antioxidants (Shah *et al.,* 2014). Moreover, the effects of antioxidants in grapes play an important role to protect against cardiovascular disease and cancer (Lutterodt *et al.,* 2011; Yilmaz and Toledo, 2004). Although the grape seed flour can be used to improve technological properties of meat products (Kyialbek, 2008), the sensory properties of meat products limit usage level (Özvural and Vural, 2011).

Grape seed flour's influence on meat products has been studied so far by only a very few researchers, so there is very little information available. Therefore, the aim of this

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study was to determine the effect of grape seed flour on the oxidative stability, microbiological and sensory properties of dry fermented sausages.

Materials and Methods

The day after slaughter, three-year-olds beef, beef subcutaneous fat and sheep's tail fat were obtained from a local meat processor (Turkey). Black grape seed flour (fiber 71%, protein 9%, oil 14%, moisture 4% and ash 2%) was obtained from Öktaş Gida Ltd. Şti. (Turkey).

Sausage preparation

The beef, beef subcutaneous fat and sheep's tail fat pieces (~5 cm^3 in size), spices, garlic, salt, sugar, GSF, starter cultures (*Staphylococcus carnosus* and *Lactobacillus sakei*; Bactoferm™; Chr. Hansen, Denmark) and sodium nitrite were mixed and minced in a grinder (Alveo, Turkey). Sausage formulations are shown in the Table 1. Each of resulting batches of sausage batter was rested for 12 h at 4°C and stuffed into collagen casings (Naturin Darm, Germany) of 40 mm diameter. Each sample was washed under running water, and then a 10% potassium sorbate solution was sprayed on it. Samples were ripened for 14 d. Ripening temperature was started from 24°C and then first 7 d decreased 1°C for every day. For equilibration, the relative humidity was adjusted to 60±3% in the first 6 h of the ripening period and was then increased to 90±3% and decreased every day by 1 unit. At the end of the ripening period, sausages packed in a polyethylene bag by applying vacuum and stored at 4°C for 80 d.

Determination of the pH and proximate composition

Ten grams of ground sausage was homogenized in 100 mL distilled water and the pH was measured using a pH meter (Orion 3-star, USA) equipped with temperature probe as outlined by Ockerman (1985).

Moisture, fat and protein were determined according to AOAC (2000). Protein was determined as crude protein using the Kjeldahl method. Fat was determined as crude fat using the Soxhelet extraction.

Determination of the ripening yield the diameter reduction values

Ripening yield was determined as follows:

$$\text{Yield (\%)} = W_1 \times 100 / W_0$$

Table 1. Sausage formulations (SF)

Materyal	SF-1 (%)	SF-2 (%)	SF-3 (%)	SF-4 (%)
Beef	73.5	72.75	72	70.5
Beef fat	9	9	9	9
Ship tail fat	10	10	10	10
Grape seed flour	0	0.75	1.5	3
Salt	1.9	1.9	1.9	1.9
Garlic	2	2	2	2
Red pepper	1	1	1	1
Cumin	1	1	1	1
Black pepper	0.6	0.6	0.6	0.6
Allspice	0.5	0.5	0.5	0.5
Sucrose	0.5	0.5	0.5	0.5
Sodium nitrite	150 ppm	150 ppm	150 ppm	150 ppm

where W_0 is the weight before ripening, and W_1 is the weight on the 7th or 14th d of ripening period.

Diameter reduction was calculated as follows:

$$\textit{Diameter Reduction}\ (\%) = \frac{(d_0 - d_1) \times 100}{d_0}$$

where d_0 is the diameter of patties before ripening and d_1 is the diameter on the 7th or 14th d of ripening period.

Determination of Thiobarbituric acid (TBA) and free fatty acid (FFA) values

TBA (mg malonaldehyde/kg sausage) was determined according to the distillation method as outlined by Tarladgis *et al.* (1960). FFA was determined according to Egan *et al.* (1981). A 10 g ground sample was mixed with 25 mL of chloroform in the presence of anhydrous sodium sulphate for 5 min, and then filtered with filter paper. The free fatty acids in the filtrate were titrated with 0.1 N NaOH. Free fatty acid content was expressed as g oleic acid/100 g fat.

$$\text{Free fatty acid (\%)} = (S \times N \times F) \times 28.2 / W$$

where S is the volume of titration (mL), N is the normality of the sodium hydroxide solution, F is the factor of the sodium hydroxide solution and W is the fat weight (g) in the sample.

Instrumental colour analysis

The colour values of the sausages were measured by using a portable colorimeter (Minolta CR-400, Japan). The instrument was standardised against a white standardisation plate before each measurement. The colour was measured according to CIELAB systems as L (lightness), a (redness) and b (yellowness) values. Eight measurements were performed for each sausage.

Microbiological analysis

A 20 g sample was aseptically taken from each sausage and homogenised in 180 mL of sterile salt solution (0.85% NaCl; Merck). The count of total aerobic mesophilic bacteria was determined on Plate Count Agar (PCA; Merck) incubated at 37°C for 48 h (APHA, 1992), while Lactic acid bacteria (LAB) was determined on De Man Rogosa Sharpe Agar (MRS; OXOID) incubated at 30°C for 72 h. (APHA, 1992) and moulds-yeasts on Dichloran Rose Bengal Chloramphenicol Agar (DRBCA; Merck) incubated at 25°C for 5 d (APHA, 1992). Enterobacteriaceae were cultured on Violet Red Bile Glucose Agar (VRBGA; Merck) incubated at 30°C for 24 h (Harrigan, 1998).

Sensory analysis

Sensory analyzes were performed at the end of the ripening period. The sausages were sliced to ~2 mm thickness and coded with geometric shapes. They were served in a random order. Water and sliced bread pieces were served after each sample to remove traces of the previous sample from mouth. Eleven trained panel (academic staff of Adiyaman University, Food Process, Food and Beverage, and Cookery Departments) who were selected and trained according to Yetim and Kesmen (2009) assessed the sensory properties using a hedonic scale for the general appearance, cross-sectional surface appearance, outer surface color, cross-sectional surface colour, brittleness, odour, flavour, and overall acceptability. The values in the scale indicated the following range of reactions: 1: dislike extremely to 9: like extremely.

Statistical analysis

Two replicates were performed for this study. The data were subjected to analysis of variance (ANOVA), and the results were expressed as mean±standard deviation (SD). When there were differences among the samples, the differences were compared using Duncan's multiple-range tests; a probability value of $p<0.05$ was considered significant.

Results and Discussion

The composition and pH values of sausages

The effects of GSF on the pH values of the sausages were not found to be significant ($p>0.05$, Table 2). Moreover, the effects of ripening and storage periods on the pH values of the sausages were found to be significant ($p<0.01$). As shown in Table 2, pH values decreased during the ripening period. However, the storage period increased pH values. LAB of fresh meat and starter cultures produces lactic acid which reduces the pH of dry fermented sausages (Bover-Cid *et al.,* 2001). The increased pH in the storage period might result from basic components such as amines. Generally, such basic components are produced from the degradation of amino acids by proteolytic enzymes. Moreover, such increases in pH could result from decreasing lactic acid formation and increasing degradation of lactic acid. The pH values of the samples were found to be lower than the pH of typical sucuk. This result might be due to the use of an acidifying starter culture.

The effects of GSF on the moisture values of the sausages were found to be significant ($p<0.01$, Table 3). The addition of GSF decreased the moisture values of the sausages on the 7th d of the ripening period. Although the addition of GSF decreased the moisture values of the sausage on 0 and 14th d, these decreases were not found to be significant. Moreover, the effects of the ripening period on the moisture values were found to be significant ($p<0.01$). The moisture values of sausages were decreased during ripening period (Table 3). Gökalp *et al.* (1999) reported that moisture loss in sucuk increased with decreasing pH values to the isoelectric points of meat proteins. The decrease in the pH and relative humidity of the medium increased the moisture loss of the sucuk (Kurt, 2006). The addition of GSF did not cause a significant difference

Table 2. The effects of ripening and storage periods on the pH values of sausages*

GSF (%)	Ripening			Storage		
	0 d	7 d	14 d	20 d	40 d	80 d
0.00	5.72±0.018 ^{A}	5.01±0.138 ^{B}	4.65±0.018 ^{D}	4.88±0.092 ^{BC}	4.92±0.141 ^{B}	4.82±0.127 ^{BC}
0.75	5.77±0.117 ^{A}	5.04±0.050 ^{B}	4.64±0.078 ^{C}	4.94±0.000 ^{B}	4.99±0.103 ^{B}	4.86±0.004 ^{B}
1.50	5.88±0.046 ^{A}	5.04±0.007 ^{B}	4.63±0.046 ^{D}	4.97±0.057 ^{B}	5.02±0.042 ^{B}	4.87±0.018 ^{C}
3.00	5.84±0.057 ^{A}	5.01±0.074 ^{B}	4.60±0.078 ^{D}	4.95±0.014 ^{B}	4.97±0.007 ^{B}	4.80±0.053 ^{C}

*The effects of GSF on the pH values of the sausages were not found to be significant.

$^{A-D}$Different uppercase letters in a row show significant differences between the groups ($p<0.05$).

GSF, Grape seed flour.

Table 3. The effects of GSF on the moisture, fat and protein values of sausages during ripening period*

GSF (%)	0 d	7 d	14 d
Moisture			
0.00	59.48±2.25 ^{A}	53.51±0.48 ^{a,B}	42.75±1.53 ^{C}
0.75	58.29±1.80 ^{A}	51.00±0.73 ^{b,B}	41.64±2.27 ^{C}
1.50	59.23±0.30 ^{A}	49.73±0.21 ^{b,B}	41.47±0.46 ^{C}
3.00	58.22±0.04 ^{A}	49.77±0.68 ^{b,B}	41.72±2.92 ^{C}
Fat			
0.00	17.89±2.84 ^{B}	20.70±0.84 ^{AB}	25.74±1.09 ^{A}
0.75	18.50±2.40 ^{B}	22.15±0.20 ^{AB}	25.84±0.90 ^{A}
1.50	17.07±0.11 ^{C}	22.24±0.69 ^{B}	25.47±1.10 ^{A}
3.00	17.19±0.04 ^{B}	21.74±0.81 ^{AB}	26.27±2.63 ^{A}
Protein			
0.00	17.76±0.13 ^{C}	20.11±0.42 ^{B}	22.75±0.06 ^{A}
0.75	17.95±0.27 ^{C}	19.99±0.16 ^{B}	22.76±0.02 ^{A}
1.50	17.66±0.10 ^{C}	20.12±0.44 ^{B}	23.26±1.59 ^{A}
3.00	17.68±0.02 ^{C}	20.06±0.69 ^{B}	21.86±0.33 ^{A}

*Columns without lowercase letters show no significant differences between groups.
^{a,b}Different lowercase letters in a column show significant differences between the groups (p<0.05).
$^{A\text{-}D}$Different uppercase letters in a row show significant differences between the groups (p<0.05).
GSF, Grape seed flour.

in fat and protein values of sausage (Table 3). However, the fat and protein values increased significantly (p<0.01) during the ripening period. Moisture loss in the sausages increased protein and fat values.

The oxidation stability of the sausages

Although the addition of GSF decreased FFA values of the sausages, such differences were not found to be significant (p>0.05, Table 4). However, FFA values of the sausages increased significantly (p<0.01) during ripening and storage periods. The level of free fatty acids in dry fermented sausages depends on the hydrolytic activity of the lipases, the microbial metabolic processes, and the oxidative reactions (Soriano *et al.,* 2006). Such lipases are mainly of endogenous origin. Lipase activities increase with decreasing pH value in dry cured meat products (Chizzolini *et al.,* 1998; Toldrá, 1998; Vestergaard *et al.,* 2000). Toldrá (1998) reported that phospholipid hydrolysis generated significant free fatty acids in dry cured meat products. Moreover, Toldrá (2006) reported that long processes with mild ripening conditions allowed for relatively higher enzyme activity.

The effects of GSF on the TBA values were found to be significant during ripening (p<0.05) and storage (p<0.01) periods (Table 4). 1.5% and 3% of the GSF decreased TBA values at the 14th day of the ripening period. Moreover, the addition of GSF decreased TBA values during the storage period. Such effects of GSF might result from its phenolic content which affects the oxidative stability of meat products (Lutterodt *et al.,* 2011). Lutterodt *et al.* (2011) reported that the most abundant phenolic compound in concord and ruby red grape seed flours was catechin. Özvural and Vural (2011) reported that TBARS values in frankfurters decreased with increasing grape seed flour content.

The effects of the ripening and storage periods increased TBA values significantly (p<0.01). Sausage can be easily oxidized during ripening and refrigerated storage periods because of its fat content and environment (Gökalp *et al.,* 1999). Increasing oxidation can increase secondary products, such as malonaldehyde. The effects of the ripening period on TBA values were in accordance with the results of Kurt (2006), who reported that TBA values increased with increasing ripening period of sucuk.

The size and ripening yield of sausages

The effects of GSF on the diameter of sausages were found to be significant (p<0.01, Table 5). The addition of GSF decreased the diameter reduction of sausages during the ripening period. In particular, the rate of decrease on the 7th d of the ripening period was greater than that on the 14th d. The addition of GSF did not cause significant difference in ripening yield values (p>0.05, Table 5). The changes in the diameter reductions might be related to the fibre content of the GSF.

The instrumental colour of the sausages

The effects of GSF on the L values of sausages were

Table 4. The effects of GSF on the FFA and TBA values of sausages during ripening and storage periods*

GSF (%)	Ripening			Storage		
	0 d	7 d	14 d	20 d	40 d	80 d
FFA						
0.00	0.27±0.04 E	1.26±0.23 D	1.46±0.11 CD	1.82±0.23 BC	1.96±0.01 AB	2.28±0.11 A
0.75	0.23±0.06 D	1.16±0.09 C	1.38±0.13 C	1.85±0.17 B	1.84±0.03 B	2.17±0.06 A
1.50	0.20±0.04 E	1.06±0.06 D	1.33±0.03 C	1.72±0.04 B	1.81±0.12 B	2.15±0.05 A
3.00	0.20±0.01 F	1.05±0.01 E	1.31±0.02 D	1.63±0.03 C	1.84±0.07 B	2.08±0.07 A
TBA						
0.00	0.222±0.011 F	0.387±0.005 E	0.636±0.003 a,D	0.718±0.008 a,C	0.799±0.006 a,B	0.892±0.006 a,A
0.75	0.206±0.011 F	0.376±0.023 E	0.631±0.006 a,D	0.710±0.002 ab,C	0.800±0.013 a,B	0.864±0.011 b,A
1.50	0.228±0.005 F	0.363±0.024 E	0.626±0.005 ab,D	0.705±0.006 ab,C	0.782±0.005 ab,B	0.867±0.008 b,A
3.00	0.212±0.009 F	0.362±0.008 E	0.617±0.003 b,D	0.696±0.002 b,C	0.766±0.005 b,B	0.824±0.006 c,A

*Columns without lowercase letters show no significant differences between groups.

a-c Different lowercase letters in a column show significant differences between the groups ($p<0.05$).

A-D Different uppercase letters in a row show significant differences between the groups ($p<0.05$).

GSF, Grape seed flour.

Table 5. The effects of GSF on the diameter reduction and ripening yield values of sausages during ripening period*

GSF (%)	Dimeter reduction (%)		Ripening Yield (%)	
	7 d	14 d	7 d	14 d
0.00	9.50±0.001 a	12.87±0.587	85.11±0.933	73.19±1.655
0.75	9.00±0.354 a	12.23±0.955	85.08±0.948	73.12±1.181
1.50	7.63±0.177 b	12.04±0.438	84.94±0.771	72.58±1.082
3.00	7.38±0.177 b	12.05±0.071	84.40±2.608	72.71±1.230

*Columns without lowercase letters show no significant differences between groups.

a,b Different lowercase letters in a column show significant differences between the groups ($p<0.05$).

GSF, Grape seed flour.

found to be significant ($p<0.01$) during the storage period (Table 6). Although the addition of GSF decreased L values during the ripening period (Table 6), its effect was found to be significant ($p<0.05$) on the 80th day of the storage period. Moreover, L values decreased during the ripening period and then increased significantly ($p<0.01$) with the storage period. Such decreases might be associated with moisture loss, in particular drying of the surface of the sausages. Bozkurt and Bayram (2006) reported that the L values of sucuk decreased from 38 to 28 during ripening period. Vacuum packaging and equilibration of the moisture content of the sausages might have affected L values during the storage period.

The effects of GSF on the a (redness) values of the sausages were found to be significant ($p<0.01$, Table 6). The addition of GSF decreased the a values of the sausages during the ripening and storage periods. The differences in the a values might be due to the colour pigment contents of GSF. The colour black grape seed flour was darker than the beef. This difference in the colour might be affected the colour values of beef patties. Özvural and Vural (2011) reported that grape seed, the by-product of the production of red wine, decreased the colour values of sausages significantly.

Although the ripening and storage periods decreased a values of the sausages significantly ($p<0.01$), the effects in control group were not found to be significant during storage period (Table 6). Moreover, GSF decreased the a values of the sausages more than the control group during the ripening period. Decreasing a values might be related to the moisture loss and natural colour pigments of the grape seed flour. Similarly, Kargozari *et al.* (2014) reported that the ripening period decreased a and b values of Turkish dry fermented sausage. Decreasing a values during the ripening period might have resulted from denaturation of colour pigments due to lactic acid formation. Pérez-Alvarez *et al.* (1999) reported that a decline in red values is related to partial or complete denaturation of myoglobin, nitrosomyoglobin and oxymyoglobin.

The effects of GSF on the b values of the sausages were found to be significant ($p<0.01$, Table 6). The addition of GSF decreased b values of the sausages during the ripening and storage periods. Such effect was the highest on the 14th d of the ripening period. The colour components of grape seed, such as polyphenols might have affected the b values of the sausages.

Table 6. The effects of GSF on the L, a and b values of sausages during ripening and storage periods*

GSF (%)	Ripening			Storage		
	0 d	7 d	14 d	20 d	40 d	80 d
L						
0.00	36.95±0.55 ^{A}	30.53±1.35 ^{B}	26.31±0.80 ^{C}	28.29±0.47 ^{BC}	28.64±2.73 ^{BC}	30.27±0.71 ^{a,B}
0.75	34.21±0.50 ^{A}	30.50±0.40 ^{B}	25.83±0.18 ^{D}	28.87±0.83 ^{C}	27.64±0.63 ^{C}	28.84±0.28 ^{b,C}
1.50	34.71±1.15 ^{A}	29.39±0.14 ^{B}	25.91±0.22 ^{C}	29.10±0.32 ^{B}	28.85±0.81 ^{B}	28.48±0.17 ^{b,B}
3.00	34.82±2.30 ^{A}	28.76±0.85 ^{B}	24.98±0.93 ^{C}	28.49±0.34 ^{B}	27.65±0.16 ^{BC}	27.67±0.52 ^{b,BC}
a						
0.00	10.67±0.37 ^{a,A}	8.62±0.92 ^{a,B}	6.38±0.34 ^{a,C}	7.12±0,21 ^{a,C}	7.03±0.71 ^{a,C}	6.91±0.44 ^{a,C}
0.75	9.27±0.05 ^{b,A}	7.62±0.12 ^{ab,B}	5.62±0.31 ^{ab,D}	6.50±0.04 ^{b,C}	6.15±0.21 $^{ab, DC}$	5.81±0.54 ^{ab,CD}
1.50	8.21±0.12 ^{c,A}	6.74±0.32 ^{bc,B}	4.96±0.30 ^{b,D}	6.02±0.19 ^{c,C}	5.90±0.37 ^{ab,C}	5.32±0.40 ^{bc,CD}
3.00	7.94±0,04 ^{c,A}	5.85±0.21 ^{c,B}	4.07±0.17 ^{c,D}	5.04±0.08 ^{d,C}	4.98±0.16 ^{b,C}	4.20±0.55 ^{c,D}
b						
0.00	9.76±0.39 ^{a,A}	5.62±0.02 ^{a,B}	3.68±0.10 ^{a,D}	4.39±0.01 ^{a,C}	4.45±0.51 ^{a,C}	4.73±0.01 ^{a,C}
0.75	7.74±0.22 ^{b,A}	4.71±0.41 ^{b,B}	3.24±0.09 ^{b,D}	4.12±0.01 ^{a,BC}	3.95±0.15 ^{ab,C}	3.99±0.45 ^{ab,C}
1.50	6.32±0.36 ^{c,A}	4.07±0.35 ^{bc,B}	2.92±0.02 ^{c,D}	3.68±0.19 ^{b,BC}	3.35±0.18 ^{bc,CD}	3.51±0.30 ^{bc,BCD}
3.00	5.55±0.38 ^{c,A}	3.47±0.36 ^{c,B}	2.61±0.08 ^{d,C}	3.23±0.07 ^{c,BC}	3.07±0.04 ^{c,BC}	2.86±0.30 ^{c,BC}

*Columns without lowercase letters show no significant differences between groups.

$^{a-c}$Different lowercase letters in a column show significant differences between the groups (p<0.05).

$^{A-D}$Different uppercase letters in a row show significant differences between the groups (p<0.05).

GSF, Grape seed flour.

The b values of the sausages decreased significantly (p<0.01) during the ripening period. A GSF level of 3% decreased the b values of the sausages less than other levels of GSF during ripening period. Drying of the sausages during the ripening period could have contributed to the decreased in b values. Pérez-Alvarez *et al.* (1999) reported that a decline in yellowness values of Spanish-type dry-cured sausage may have been related to a decrease in oxymyoglobin due to oxygen consumption by microorganisms. Casaburi *et al.* (2007) and Lorenzo and Franco (2012) have agreed about the decreasing in L, a and b values during ripening period of sausages.

The microbiological quality of the sausage

The effects of GSF on the counts of Enterobacteriaceae, moulds and yeasts were not found to be significant (p> 0.05) on the 0th d of ripening period. Moreover, Enterobacteriaceae, moulds and yeasts were not detected on the 7th and 14th d. The decreasing pH values of the sausages might have prevented the growth of Enterobacteriaceae. However, the application of potassium sorbate on the sausages casings' might have prevented the growth of moulds and yeasts. Özdemir *et al.* (1996) reported that the counts of moulds and yeasts in sucuk may decrease below the detection limit until 7th d of ripening period.

The effects of GSF on the count of total aerobic mesophilic bacteria were found to be significant (p<0.05) during the storage period (Table 7). As shown in Table 7, 1.5% and 3% levels of GSF decreased the count of total aerobic mesophilic bacteria during the storage period. Such effect might be due to the flavonoid contents of GSF. Anastasiadi *et al.* (2009) reported that flavonoids and flavonoid derivatives in the grape seed play an important role in its antimicrobial activity.

The effects of the ripening and storage periods on the count of total aerobic mesophilic bacteria were found to be significant (p<0.01, Table 7). The count of total aerobic mesophilic bacteria increased on the first day of the ripening period. The results of some studies (Ayhan *et al.,* 1999; Gönülalan *et al.,* 2004; Özdemir *et al.,* 1996) have agreed with this result. The addition of starter cultures and ripening conditions might be responsible for the increased count of total bacteria on the first day of the ripening period. However, the storage period decreased the count of such bacteria. Decline in the count of total aerobic mesophilic bacteria might be associated with the vacuum packaging and refrigerated storage condition.

The effects of GSF on the count of Lactic acid bacteria (LAB) were found to be significant (p<0.05) during the storage period (Table 7). The addition of 1.5% and 3% GSF decreased the count of LAB on the 40th and 80th d of the storage period. Such an effect might be associated with the flavonoid content of GSF. Perumalla and Hettiarachchy (2011) reported that grape seed extract has an important antimicrobial activity. Metabolic activities of starter culture such as LAB are required for the desirable

Table 7. The effects of GSF on the counts of viable aerobic mesophilic bacteria (AMB; Log CFU/g) and lactic acid bacteria (LAB; Log CFU/g) during ripening and storage periods*

GSF (%)	Ripening			Storage		
	0 d	7 d	14 d	20 d	40 d	80 d
AMB						
0.00	5.94±0.039 ^{E}	8.95±0.040 ^{A}	8.98±0.008 ^{AB}	8.90±0.007 ^{a,B}	8.78±0.011 ^{a,C}	8.55±0.025 ^{a,D}
0.75	5.89±0.011 ^{E}	8.92±0.011 ^{A}	8.96±0.023 ^{AB}	8.89±0.010 ^{a,B}	8.75±0.001 ^{a,C}	8.49±0.050 ^{a,D}
1.50	5.93±0.051 ^{D}	8.94±0.066 ^{A}	8.93±0.017 ^{B}	8.85±0.013 ^{b,A}	8.75±0.009 ^{ab,B}	8.47±0.002 ^{a,C}
3.00	5.90±0.014 ^{D}	8.93±0.007 ^{A}	8.92±0.029 ^{B}	8.85±0.006 ^{b,AB}	8.72±0.022 ^{b,B}	8.23±0.132 ^{b,C}
LAB						
0.00	5.53±0.134 ^{D}	9.05±0.127 ^{A}	8.85±0.028 ^{B}	8.81±0.007 ^{BC}	8.76±0.021 ^{a,BC}	8.61±0.028 ^{a,C}
0.75	5.30±0.078 ^{D}	8.91±0.071 ^{A}	8.81±0.021 ^{AB}	8.79±0.035 ^{B}	8.75±0.028 ^{a,B}	8.55±0.021 ^{a,C}
1.50	5.21±0.120 ^{D}	8.90±0.021 ^{A}	8.80±0.028 ^{AB}	8.72±0.064 ^{AB}	8.67±0.050 ^{b,B}	8.47±0.092 ^{ab,C}
3.00	5.30±0.226 ^{C}	8.88±0.007 ^{A}	8.78±0.071 ^{A}	8.72±0.000 ^{A}	8.62±0.001 ^{b,A}	8.32±0.057 ^{b,B}

*Columns without lowercase letters show no significant differences between groups.
^{a,b}Different lowercase letters in a column show significant differences between the groups (p<0.05).
$^{A-D}$Different uppercase letters in a row show significant differences between the groups (p<0.05).
GSF, Grape seed flour.

Table 8. The effects of GSF on the sensory properties of sausages*

GSF (%)	General appearance	Cross-sectional surface appearance	Outer surface colour	Cross-sectional surface colour	Brittleness	Odour	Flavour	Overall acceptability
0.00	7.39±0.11 ^{a}	7.37±0.08 ^{a}	7.39±0.22 ^{a}	7.23±0.21 ^{a}	7.24±0.54	7.27±0.06 ^{a}	7.08±0.00 ^{a}	7.50±0.06 ^{a}
0.75	7.15±0.00 ^{a}	7.24±0.13 ^{a}	7.04±0.06 ^{a}	6.81±0.06 ^{a}	6.27±0.27	7.08±0.11 ^{ab}	6.93±0.11 ^{a}	7.27±0.06 ^{a}
1.50	7.46±0.00 ^{a}	7.13±0.18 ^{a}	7.39±0.22 ^{a}	7.27±0.06 ^{a}	7.15±0.33	7.00±0.11 ^{b}	7.04±0.06 ^{a}	7.39±0.11 ^{a}
3.00	6.58±0.28 ^{b}	6.73±0.15 ^{b}	6.35±0.38 ^{b}	6.08±0.43 ^{b}	6.66±0.16	6.89±0.05 ^{b}	6.54±0.22 ^{b}	6.85±0.22 ^{b}

*Columns without lowercase letters show no significant differences between groups.
^{a,b}Different lowercase letters in a column show significant differences between the groups (p<0.05).
GSF, Grape seed flour.

changes that determine the particular characteristics of dry fermented sausages (Erkkilä, 2001). Therefore, such antimicrobial activity of GSF is important during storage instead of ripening period.

LAB play the most important role in producing the required quality of sausages. Their count increased in the first days and then significantly (p<0.01) decreased during the last days of the ripening period (Table 7). Moreover, the count of LAB decreased during the storage period significantly (p<0.01). The addition of starter cultures and their adaptation to the meat fermentation environment might have increased the count of LAB in the first days of the ripening period. Increasing moisture loss and decreasing pH values in the last days of the ripening period could account for the decrease in LAB during that period. Some researchers (Aksu and Kaya, 2004; Gönülalan *et al.,* 2004; Özdemir *et al.,* 1996) reported that the count of LAB in the sausages increased during the first days of the ripening period and decreased slightly during the last days of the ripening period. Moisture loss and decreasing in pH values might have caused decrease in LAB count during the last days of the ripening period. However, low moisture, pH and refrigerated conditions could also have affected the count of LAB.

The sensory quality of the sausage

The effects of GSF on the sensory scores of the sausages were found to be significant (p<0.05) with the exception of brittleness scores (Table 8). The addition of grape seed flour at 1.5% did not cause a statistically significant difference in the sensory properties, with the exception of odour. Odour scores decreased with the addition of GSF significantly. The addition of GSF at the 3% level caused decreasing scores of the sensory parameters, as shown in Table 8. The decreasing sensory scores might be related to the components of GSF such as tannins. Lesschaeve and Noble (2005) reported that many phenolic compounds are characterized by bitterness and astringency.

Conclusion

Grape seed flour added into dry fermented sausages inhibited lipid oxidation rate during ripening and storage periods due to its antioxidant activity. Moreover, It inhibited microbial growth rate during storage period due to its antimicrobial activity. The addition of GSF did not affect pH, fat and protein values of sausage. However, higher levels of GSF decreased the sensory quality of the dry fermented sausages. The results suggested that grape seed may have a potential application as a natural additive in dry fermented sausages. However, grape seed flour addition into the sausages up to the level of 3% can be recommended.

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