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Effects of some commercial additives on the quality of sucuk (Turkish dry-fermented sausage)

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Abstract

The effects of some commercial additives on the quality of sucuk (Turkish dry-fermented sausage) were investigated during the ripening and storage periods. Microbial, chemical and sensory changes were followed for 15 days of ripening and 36 days of storage. Aerobic plate count (APC) increased ($P < 0.05$) from 5.19 to 6.09 log CFU/g during the first 10 days of ripening and afterwards decreased ($P < 0.05$) to 3.69 log CFU/g. APC and lactic acid bacteria (LAB) changed significantly ($P < 0.05$) with time and additives. LAB increased ($P < 0.05$) from 4.62 log CFU/g to about 5.47 log CFU/g during the first 10 days of the ripening period and decreased to 1.79 log CFU/g at the end of storage. Control sucuk without additives had the highest level of mould and yeast counts (5.09 log CFU/g). TBARS values increased gradually ($P < 0.05$) from 0.61 to 1.73 mg/kg and tyramine concentrations varied from 56.8 to 419 mg/kg. Control sucuk was found to have the lowest overall sensory quality ($P < 0.05$), and nitrate/nitrite concentration increased ($P < 0.05$) the overall sensory quality of the sausages. Pearson's correlation test indicated that there was a link ($P < 0.01$) between the overall sensory quality and pH, TBARS values, mould and yeast counts, and putrescine concentration.

Keywords: Sucuk; Quality; Safety; Biogenic amines

1. Introduction

Sucuk, Turkish dry-fermented sausage, is produced in a large amount in various parts of Turkey by traditional methods in small-scale enterprises by air-drying. Manufacturing of the sucuk varies regionally, and different formulations exist. The recipe preferred by many consumers is: 90 kg red meat (18% fat), 20 kg tail fat, 2 kg salt, 0.4 kg sugar (sucrose), 1 kg clean dry garlic, 0.7 kg medium bitter red pepper, 0.5 kg powdered black pepper, 0.9 kg cumin, 0.25 kg allspice, 0.033 kg $NaNO_3$, 0.005 kg $NaNO_2$ and 0.250 kg olive oil or vegetable oil (Erkmen & Bozkurt, 2004; Gökalp & Ockerman, 1985). After mixing, sausage dough is filled into natural casings, and dried under climatic conditions (Gökalp, Kaya, & Zorba, 1999).

A standard and technological method has not been developed and adapted for sucuk production throughout the country (Bozkurt & Erkmen, 2002; Gökalp, 1986). Many quality characteristics of the sucuk, i.e., colour, flavour, odour, texture may not be optimum; also some toxic materials such as biogenic amines may be formed (Erginkaya & Var, 1989; Gökalp, 1995).

Biogenic amines are basic nitrogenous compounds, formed due to decarboxylation of amino acids by the action of living organisms (Maijala, Eerola, Lievonen, Hill, & Hirvi, 1995; Shalaby, 1996). Biogenic amines are found in a wide variety of foods such as sausages (Eerola, Hinkkanen, Lindfors, & Hirvi, 1993; Hernandez-Jover, Pulido, Nogues, Font, & Carou, 1997), meat (Sayem-El-Daher, Simard, & Fillion, 1984; Vidal-Carou, Pulido, Morro, & Font, 1990; Vinci & Antonelli, 2002), milk (Ordonez, Ibanez, Torre, & Barcina, 1997), chocolate, cheese (Koehler & Eitenmiller, 1978), fish and some beverages (Shalaby, 1996). Biogenic amines

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can be found in meat products, due to microbial activity during the fermentation and ripening processes. Their production depends on the quality of raw materials and the hygienic conditions of the processing environment. These substances have been proposed as possible indicators of poor hygienic quality of raw materials and of poor processing conditions (Gonzalez-de-Liano, Cuesta, & Rodriguez, 1998; Maijala et al., 1995; Ordonez, Hierro, Bruna, & Hoz, 1999; Shalaby, 1996; Vidal-Carou et al., 1990). Changes in putrescine, cadaverine and tyramine concentrations during the storage of vacuum-packed meat are proposed as indicators of spoilage (Eerola, Sagues, Lilleberg, & Aalto, 1997). Sayem-El-Daher et al. (1984) pointed out that spermine, spermidine, putrescine and cadaverine may be used as indicators of putrefying pork; concentrations of putrescine and cadaverine correlate with microbial, physical and organoleptic attributes in pork and beef. In some studies, using good quality raw materials and some precautions, especially using amine-negative bacteria, reduces production of biogenic amines.

The stability of fat often limits the shelf life of dry sausage and cured hams (Eerola et al., 1993). Malonaldehyde is one of the by-products of lipid oxidation and it can be followed by measuring the thiobarbituric acid reactive substances (TBARS) value (Ordonez et al., 1999; Raharjo, Sofos, & Schmidt, 1993). It is formed from hydroperoxides (Sun, Faustman, Senecal, Wilkonson, & Furr, 2001). Lipid oxidation gives products that change meat quality, e.g., the colour, flavour, odour, texture and even the nutritional value (Fernandez, Perez-Alvarez, & Fernandez-Lopez, 1997).

Lactobacillus, *Micrococcus* and *Staphylococcus* microorganisms play an important role during fermentation and ripening of fermented sausage. Lactic acid bacteria enhance the physico-chemical properties of sausages and restrict the growth of some undesirable microorganisms (Gonzalez & Diez, 2002; Samelis, Metaxopoulos, Vlassi, & Pappa, 1998). However, lactic acid bacteria have also been reported as major spoilage microorganisms in meat products. These types of microorganism may cause slime and sour odour formation in sausages (Wang, 2000).

Additives used in the production of sucuk are mainly antimicrobials, antioxidants, flavouring and colouring compounds. Commercially used antimicrobials in sucuks are nitrite, nitrate, sorbic acid, benzoic acid, citric acid and their salts, while antioxidants are ascorbic acid and α-tocopherols (Bozkurt & Erkmen, 2004a). The other additives are used for decreasing the pH, enhancing the flavour and aroma, and developing the desired colour (Bozkurt, 2002).

The aim of this research was to study the quality (chemical, microbiological and sensory) changes of sucuk during the ripening and storage periods, and to determine effects of some commercially used additives on the quality of sucuk.

2. Materials and methods

2.1. Chemicals

Sodium nitrite, sodium nitrate, α-tocopherol, ascorbic acid, potassium sorbate, potassium pyrophosphate, dipotassium hydrogen phosphate, glacial acetic acid were obtained from Merck (Darmstadt, Germany); 1,1,3,3-tetraethoxypropane (TEP) and 2-thiobarbituric acid were obtained from Sigma (St. Louis, MO); β-phenylethylamine hydrochloride, histamine dihydrochloride, serotonin hydrochloride, cadaverine dihydrochloride, spermine diphosphate, spermidine diphosphate, 1,7-diaminoheptane, putrescine dihydrochloride, tryptamine hydrochloride and tyramine hydrochloride were obtained from Sigma (St. Louis, MO) and were used as biogenic amine standards; sodium hydroxide, 25% ammonium and sodium bicarbonate were from Merck (Darmstadt, Germany), acetone from Reidel De Haen (Germany), dansyl chloride from Sigma Co. (St. Louis, MO), ammonium acetate from Merck (Darmstadt, Germany), and perchloric acid from JT Baker (Holland). All chemicals except acetonitrile were analytical grade (extra pure), and acetonitrile was HPLC grade.

A culture mixture of *Pediococcus acidilactici*, *Lactobacillus plantarum* and *Staphylococcus cornosus* was obtained from Biocarna (Wiesby, Germany).

2.2. Sucuk preparation

Sucuk dough was prepared from meat (about 18% fat) mixed with tail fat, salt, sugar, clean dry garlic, spices, $NaNO_2$, $NaNO_3$, vegetable oil (generally olive oil), antioxidants and antimicrobial. Meat, fat and spices were added into sucuk dough according to the following recipe; 900 g sheep red meat (about 18% fat), 200 g tail fat, 5.5 g cumin, 1.1 g cinnamon, 11.42 g allspice, 0.48 g cloves, 5.5 g red pepper, 11 g black pepper, 20.76 g garlic, 4.4 g sugar, 18 g salt and 2.1 g olive oil were used to prepare sausage dough. A flow-chart of sucuk preparation is given in Fig. 1. The meat was minced in a meat mincer (Tefal Prep'Line 1600, France) to about 1.3–2.5 cm. After that spices and starter culture were added and mixed with minced meat. Starter culture mixture (*P. acidilactici*, *L. plantarum* and *S. carnosus*) was used as a 20 g commercial culture mixture per 100 kg meat. After that nitrate/nitrite, potassium pyrophosphate, dipotassium hydrogen phosphate, ascorbic acid and potassium sorbate which were dissolved in 25 ml of distilled water, were added into the prepared of sucuk dough (Table 1). Five formations were prepared. Sucuk type S1 was prepared as a control, without additives; S2 with 150 mg/kg nitrate, 75 mg/kg nitrite; S3 with 300 mg/kg nitrate, 150 mg/kg nitrite; S4 with 300 mg/kg nitrate, 150 mg/kg nitrite, 1.25 g/kg potassium pyrophosphate, 1.25 g/kg dipotassium hydrogen phosphate, 250 mg/kg ascorbic acid, 100 mg/kg α-tocopherol, and 100 mg/kg potassium sorbate; S5 with 300 mg/kg nitrate, 150 mg/kg nitrite, 2.50 g/kg potassium pyrophosphate,

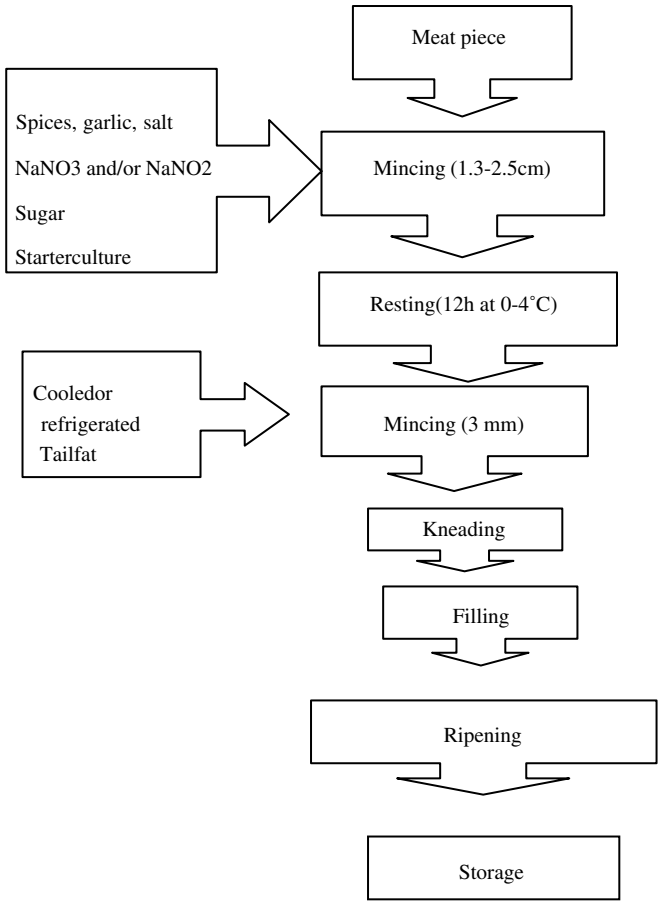

Fig. 1. General production flow-chart of sucuk.

Table 1
Concentrations of various additives used for preparation of sucuk

Additives	Sucuk type				
	S1	S2	S3	S4	S5
Nitrate (mg/kg)	0	150	300	300	300
Nitrite (mg/kg)	0	75	150	150	150
Potassium pyrophosphate (g/kg)	0	0	0	1.25	2.5
Dipotassium hydrogen phosphate (g/kg)	0	0	0	1.25	2.5
Ascorbic acid (mg/kg)	0	0	0	250	500
α-Tocopherol (mg/kg)	0	0	0	100	200
Potassium sorbate (mg/kg)	0	0	0	100	200

2.50 g/kg dipotassium hydrogen phosphate, 500 mg/kg ascorbic acid, 200 mg/kg α-tocopherol, and 200 g/kg potassium sorbate. Each dough was rested for 12 h at 0–4 °C. The minced refrigerated tail fat was added and mixed into the sucuk dough. After that, the dough was filled into artificial collagen casings (Naturin RL2, Germany), of 38 mm diameter, under aseptic conditions, using a filling machine (Tefal, Prep'Line 1600, France) at 2 °C. Duplicate batches were prepared; therefore, 24 sucuks of each type in duplicate, each about 100 g in weight, were prepared from each dough recipe.

Sucuks were fermented and matured from 95% to 60% RH and from 22 to 18 °C during 15 days, as outlined in Table 2. Sucuk samples were then stored at 50% RH and 30 °C for 36 days.

2.3. Sampling

Two sucuks of each type were removed on the 0th, 5th, 10th, 15th day of ripening and 0th, 7th, 11th, 18th, 27th and 36th day of storage and the aerobic plate counts, mould and yeast counts lactic acid bacteria count, pH values, TBARS values, biogenic amine contents, flavour, colour and cutting scores were determined. The chemical analyses were carried out in duplicate.

2.4. Sample preparation and microbiological counts

A sample (25 g) was removed from each sucuk type under aseptic condition and homogenized in a sterile Waring blender (Torrington, CT) containing 225 ml of 0.1% peptone water. Samples were serially diluted with 0.1% peptone water (Erkmen, 2000). Aerobic plate counts (APC) were measured using the spread plate method on aerobic plate count agar (Merck, Darmstadt, Germany). The Petri dishes were incubated at 37 °C for 24–72 h (Erkmen, 2000). Mould and yeast counts were measured using the spread plate method on potato dextrose agar (Merck, Darmstadt, Germany). Petri dishes were incubated at 25 °C for 2–5 days (Erkmen, 2000). Total lactic acid bacteria counts (LAB) were carried out using the spread plate method on MRS sharp agar (Merck, Darmstadt, Germany). Petri dishes were incubated at 30 °C for 48–72 h (Erkmen, 2000).

2.5. Sample preparation for chemical analysis

Sucuk samples that remained after microbiological analysis were used for chemical analysis. Samples cut into small pieces (about $5 \times 5 \times 5$ mm) and 50 g were homogenized using the Waring blender. Ten grams of the homogenized samples were used for the determination of pH, TBARS values and biogenic amines contents.

2.5.1. Determination of pH

The measurement of pH was carried out on 10 g of sample homogenised in 100 ml distilled water. The pH value of sample was determined using a Jenway pH meter (Jenway 3010; Jenway Ltd., Essex, UK) equipped with an electrode (J95, 924001; Jenway Ltd., Essex, UK).

2.5.2. Determination of 2-thiobarbituric acid reactive substances (TBARS) value

TBARS values of sausage were determined by the spectrophotometric method (Byun, Lee, Jo, & Yook, 2001).

Homogenized sucuk sample (2 g) were taken and TBARS was extracted twice with 10 ml of 0.4 M perchloric acid. Extracts were collected and made up to 25 ml with 0.4 M perchloric acid and then centrifuged for 5 min at 1790*g*. After centrifugation, 1 ml of extract was poured

Table 2
Ripening and storage conditions of the sucuk

	Time (days)	Temperature (C)	Relative humidity (%)
Ripening	1–2	25 ± 1	90 ± 3
	3–4	20 ± 1	80 ± 2
	5–7	18 ± 1	75 ± 2
	8–10	18 ± 1	67 ± 2
	11–15	18 ± 1	60 ± 2
Storage	0–36	30 ± 1	50 ± 2

into a glass stoppered test-tube. TBARS reagent (5 ml) was added and the extract was heated in a boiling water bath for 35 min. After cooling in tap water, the absorbance of the sample was read against the appropriate blank at 538 nm. A standard curve was prepared using 1,1,3,3-tetraethoxypropane (TEP).

2.5.3. Determination of biogenic amines

The chromatographic method of Eerola et al. (1993) was used for the determination of the biogenic amines. The HPLC system consisted of a quadratic gradient pump (Shimadzu Solvent Delivery Module, LC-10ADvp, Kyoto, Japan), a Hewlett–Packard UV detector, a RP-18 guard column, and a computer containing a Borwin package program (Borwin, Ver. 1.21, JMBS Developments, Le Fontanil, France). The HPLC column was Spherisorb ODS2, 10 μm, 200 × 4.6 mm (Phenomenex, Torrance, CA).

Ammonium acetate solution (0.1 M) was prepared by dissolving 7.7 g ammonium acetate in 100 ml of triple distilled water and filtering through a 0.45 μm Millipore filter (Billerica, MA). Acetonitrile was filtered through a 0.45 μm Millipore filter. Ammonium acetate and acetonitrile were used as the LC mobile phases. A gradient elution program was used with mobile phases of acetonitrile (solvent A) and 0.1 M ammonium acetate (solvent B), starting with 50% solvent A and 50% solvent B and finishing with 90% solvent A and 10% solvent B after 20 min. The flow rate was 1.0 ml/min.

Sample (2 g) was homogenized in 10 ml of 0.4 M perchloric acid, using a Waring blender. The sample was centrifuged for 10 min at 1790*g* and filtered. Extraction was repeated with a further 10 ml of 0.4 M perchloric acid solution and the supernatants were combined and made up to 25 ml with 0.4 M perchloric acid.

One millilitre of the extracted sample was made alkaline by adding 200 μl of 2 N NaOH solution; 300 μl of saturated sodium bicarbonate were also added as buffer. Two millilitres of dansyl chloride solution was added to each sample and incubated for 45 min at 40 °C. Residual dansyl chloride was removed by adding 100 μl of 25% ammonia. After 30 min, the solution was adjusted to 5 ml with acetonitrile, centrifuged for 5 min at 1790*g*. The supernatant was filtered (0.45 μm), and 20 μl was then injected onto the HPLC. The standard solution of the dansylated derivatives was diluted to 1 ml with 0.4 M perchloric acid to give concentrations from 0.5 to 10 μg/ml.

Starter cultures used in the production of sucuks were activated in 10 ml of MRS broth (Merck, Darmstadt, Germany) and incubated for 24 h at 30 °C anaerobically. After incubation, 0.1 ml was transferred to a tube containing MRS broth, supplemented with 0.1% L-histidine and 0.1% L-tyrosine and incubated for 24 h at 30 °C anaerobically. Activated culture was also inoculated into a tube containing 10 ml MRS broth supplemented with 0.1% L-histidine and 0.1% L-tyrosine (Roig-Sagues & Eerola, 1997). Tubes were incubated at 20 °C for 72 h. This test was performed using duplicate samples.

2.6. Sensory analysis

Sensory attributes (flavour, colour of cut surface and ease of cutting) of 25 g sucuk samples were determined at intervals during the ripening and storage periods, twice for each sample, by a panel of 10 trained panellists. Panellists were trained with high quality sucuks before the sensory analysis. Panellists gave scores for each sample, with respect to their perceptions of each sensory attribute, for flavour and colour as 1 (worst) to 10 (best). Cutting scores were evaluated as 1 (worst), that is not sliced, to 10 (best), which is sliced perfectly. The overall sensory quality of sucuks was evaluated from the same expression described by Bozkurt and Erkmen (2004a) as:

$$\text{Overall sensory quality} = (\text{flavour} \times 0.50) + (\text{colour} \times 0.25) + (\text{cutting} \times 0.25)$$

2.7. Statistical analysis

An ANOVA was performed for both the chemical and microbial changes as a function of time and sucuk type (recipe) to determine significant differences ($P < 0.05$), by using the Duncan's multiple range test. Multiple range tests were also carried out on the different recipes during ripening and storage. Pearson correlation coefficients were calculated between the overall sensory quality and APC, mould and yeast count, LAB, pH, TBARS values and biogenic amines (histamine, tyramine and putrescine).

3. Results and discussion

3.1. Microbial changes

3.1.1. Aerobic plate count

APC was determined during the ripening and storage periods and results are given in Fig. 2. It increased from 5.19 to 6.09 log CFU/g during the first 10 days of ripening and afterwards decreased to 3.69 log CFU/g. Statistical analysis, ANOVA and multiple range tests (Duncan) were carried out, to determine the effect of time and additives on APC. These analyses show that APC changed significantly ($P < 0.05$) with time and additives. Multiple range tests indicate that APC increased significantly ($P < 0.05$) over

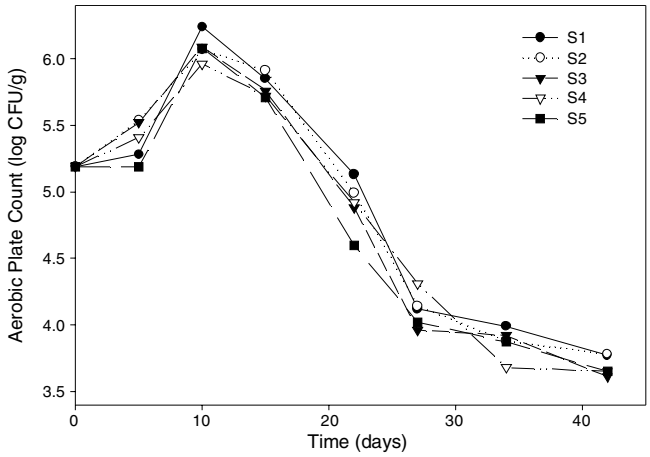

Fig. 2. Aerobic plate count (log CFU/g) of sucuk during ripening and storage.

the first 10 days of the ripening period, due to higher RH (85–95%) and temperature (22–25 °C). After that, it decreased significantly ($P < 0.05$), during storage, due to adjustment of RH to 50% RH. Samelis et al. (1998) and Bozkurt and Erkmen (2004a) observed similar results. They found that APC increased during the ripening period and decreased during the storage period. Multiple range tests indicated that additives changed APC. The highest ($P < 0.05$) APC was observed in sucuk type S1, and the lowest ($P < 0.05$) in sucuk type S5. It was observed that the combined effect of nitrite/nitrate and potassium sorbate ($P < 0.05$) on APC was significant. However, addition of low levels of potassium sorbate did not affect ($P > 0.05$) APC, but increasing the level of potassium sorbate decreased ($P < 0.05$) APC in sucuks. This means that commercial additives especially nitrate, nitrite and potassium sorbate, could be used to control APC in sucuks.

3.1.2. Mould and yeast counts

Mould and yeast counts of sucuks during the ripening and storage periods are given in Table 3. It was observed that time and additives had a significant affect on the mould and yeast count. Their numbers increased ($P < 0.05$) from 4.54 to 5.09 log CFU/g up to 5 days of ripening and after that their numbers decreased ($P < 0.05$) to 2.70 log CFU/g at the end of the storage. Bruna, Ordonez, Fernandez, Herranz, and De La Hoz (2001) observed that the mould and yeast count of fermented sausage increased during fermentation and afterwards decreased. The Turkish Standard Institute (Anon., 1983; TS, 1070) states that sucuks should not have a mould and yeast count greater than 2 log CFU/g of sucuk.

It was found that additives reduced the mould and yeast count in sucuk. The control sucuk (S1) had the highest mould and yeast counts. Additions of either nitrite/nitrate or potassium sorbate into sucuks decreased ($P < 0.05$) the mould and yeast counts. Higher mould and yeast count reductions ($P < 0.05$) were observed in sucuk type S5, containing high levels of nitrite/nitrate and potassium sorbate, than in S4, S3, S2 and S1 after 10 days of storage. This means that reduction of mould and yeast in sucuks depends on the concentration of additives.

3.1.3. Lactic acid bacteria count (LAB)

LAB counts in sucuks during the ripening and storage periods are given in Table 3. Statistical analyses were performed and it was found that time and additives affected the LAB in sucuks significantly. LAB increased ($P < 0.05$) from 4.62 to 5.47 log CFU/g during the first 10 days of the ripening period and decreased to 1.79 log CFU/g during storage. Our results agree with Samelis et al. (1998), Roig-Sagues, Hernandez-Herrero, Lopez-Sabater, Rodriguez, and Mora-Ventura (1999), Bruna et al. (2001), and Gonzalez and Diez (2002), who observed that LAB increased during the ripening period and decreased during the storage period.

Additives affected ($P < 0.05$) the LAB in sucuks. The highest ($P < 0.05$) LAB was observed in the control sucuk (S1), and the lowest ($P < 0.05$) in sucuk type S4. Addition of nitrite/nitrate decreased ($P < 0.05$) the LAB. However, addition of other additives with high level of nitrite/nitrate did not decrease ($P > 0.05$) the LAB in sucuks. This means that, only nitrite/nitrate were able to reduce LAB in sucuks.

3.2. Chemical changes

3.2.1. pH

Changes of pH during the ripening and storage periods of sucuks are given in Fig. 3. pH values of sucuks decreased significantly ($P < 0.05$) during the first 10 days of ripening from 5.78 to 4.62. During this period, lactic acid bacteria and other acid-producing bacteria produce lactic acid and other organic acids (Gökalp, 1986; Gökalp & Ockerman, 1985; Lücke, 1994). After that time, an increase in pH value ($P < 0.05$) was observed and this may be due to decomposition of acids and production of basic nitrogenous compounds. Turkish Food Codex (Anon., 2000) states that high quality ripened sucuks should have pH values between 5.2 and 5.4. All of the sucuks were in this range.

It was found that additives affected ($P < 0.05$) pH values of sucuks stored at 50% RH, since some additives, especially potassium pyrophosphate and di-potassium hydrogen phosphate have buffering capacity. At the end of 36 days storage, the pH values were in the order of S5 > S4 > S3 for those samples that contained the same concentration of nitrite/nitrate. Increasing nitrite/nitrate concentration also decreased ($P < 0.05$) pH values and the order of pH values were found to be S3 > S2 > S1. Addition of other additives with high concentration of nitrite/nitrate increased ($P < 0.05$) the pH values in sucuks. This could be due to the buffering capacity of phosphates (Bozkurt & Erkmen, 2002).

Table 3
Changes in mould and yeast counts, lactic acid bacteria count, 2-thiobarbituric acid values, and overall sensory quality of the sucuk during ripening and storage periods

Sucuk types	Time (days)		Mould and yeast (log CFU/g)	Lactic acid bacteria (log CFU/g)	TBARS (mg/kg)	Overall sensory quality
S1	Ripening	0	4.54	4.62	0.61	NM
		5	5.09	4.86	1.57	3.75
		10	4.66	5.47	1.34	5.25
	Storage	0[a]	4.48	5.22	1.64	7.25
		7	4.42	4.16	1.82	6.75
		11	4.28	3.89	1.69	6.00
		18	4.10	2.70	1.65	4.75
		27	3.65	2.00	1.78	4.50
		36	NM	NM	2.20	4.25
S2	Ripening	0	4.54	4.62	0.61	NM
		5	4.99	4.72	1.61	4.25
		10	4.61	5.25	1.73	6.75
	Storage	0[a]	4.49	5.12	1.65	7.75
		7	4.42	4.81	1.72	7.50
		11	4.18	2.98	1.88	7.25
		18	4.00	2.57	1.73	6.00
		27	3.60	2.00	1.75	6.00
		36	NM	NM	1.93	4.50
S3	Ripening	0	4.54	4.62	0.61	NM
		5	4.91	4.77	1.47	4.25
		10	4.49	5.29	1.31	7.50
	Storage	0[a]	4.40	5.14	1.67	8.00
		7	4.34	4.38	1.65	7.75
		11	4.14	2.83	1.72	7.00
		18	3.78	2.49	1.54	6.50
		27	3.54	2.13	1.59	6.75
		36	NM	NM	1.82	6.00
		5	4.51	4.75	1.46	5.50
		10	4.45	5.28	1.54	8.00
		15	4.34	5.02	1.33	8.75
	Storage	0[a]	4.34	5.02	1.33	8.75
		7	4.17	4.25	1.59	8.25
		11	4.01	3.67	1.55	8.75
		18	3.51	2.00	1.46	7.50
		27	3.24	1.79	1.53	6.50
		36	NM	NM	1.69	6.00
		5	4.48	4.93	1.18	5.50
		10	4.41	5.26	1.09	7.25
		15	3.99	5.12	1.03	8.75
	Storage	0[a]	3.99	5.12	1.03	8.75
		7	3.88	4.70	1.50	8.50
		11	3.66	3.08	1.47	8.50
		18	3.34	2.92	1.19	7.75
		27	2.70	1.79	1.43	7.50
		36	NM	NM	1.31	6.50

Nm: not measured.
[a] Immediately after ripening period (15 days).

3.2.2. 2-Thiobarbituric acid reactive substances (TBARS) value

Changes of TBARS values of sucuks were followed during the ripening and storage and are given in Table 3. It was found that TBARS values were affected significantly ($P < 0.05$) by time and additives. TBARS values increased gradually ($P < 0.05$) from 0.61 to 1.73 mg/kg during the ripening period. After that, TBARS values became nearly constant ($P > 0.05$) during the storage period. The rate of TBARS formation was faster during the first 5 days ($P < 0.05$). It can be concluded that the lipid oxidation started from the first 5 days of ripening and continued during the further ripening and storage periods.

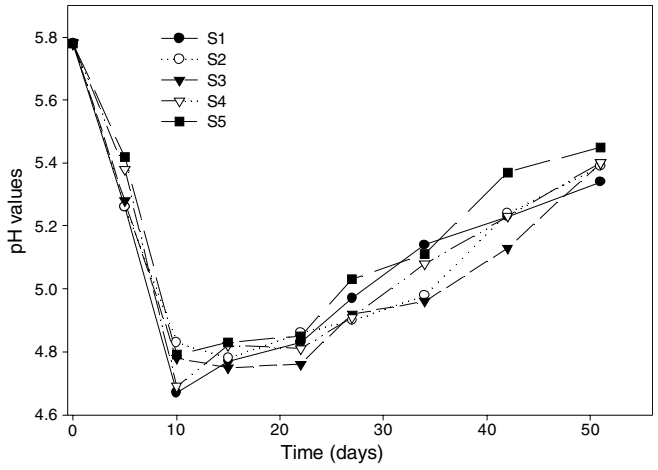

Fig. 3. Changes of pH values in sucuks during ripening and storage.

Additives decreased ($P < 0.05$) the TBARS values. TBARS values were higher ($P < 0.05$) in sucuks made without additives (S1) than made with additives (S2, S3, S4 and S5). A multiple range test indicated that addition of a high level of nitrite/nitrate into sucuks decreased ($P < 0.05$) the TBARS values. However, low levels of nitrite/nitrate did not reduce ($P > 0.05$) the TBARS values. Either low or high levels of other additives combined with nitrite/nitrate decreased ($P < 0.05$) the TBARS values in sucuks. It can be concluded that the effects of nitrite and nitrate on the TBARS values were found to be less than α-tocopherol, ascorbic acid and phosphates, since these compounds have antioxidant properties. The highest TBARS value was found in the control sucuk. The sucuk type S5, made with the highest level of additives (α-tocopherols, ascorbic acid, nitrite and nitrate) had the lowest TBARS value.

Table 4
Changes of biogenic amine concentrations in sucuk during ripening and storage periods

Sucuk types	Time (days)		Biogenic amine concentration (mg/kg)						
			TA	PUT	HA	Dia	SER	TYR	Sm
S1	Ripening	0	ND	11.2	ND	ND	ND	67.9	ND
		5	ND	117	191	2.17	8.62	419	5.58
	Storage	0[a]	10.7	211	147	3.92	12.5	382	2.62
		11	23.2	140	142	ND	10.8	326	2.37
		18	28.0	133	138	ND	12.0	296	2.29
		27	32.9	129	71.7	ND	13.2	232	2.20
		36	35.8	126	31.0	2.06	11.6	145	2.55
S2	Ripening	0	ND	11.2	ND	ND	ND	67.9	ND
		5	ND	119	144	ND	10.10	412.	4.49
	Storage	0[a]	14.2	212	126.30	4.35	13.1	359	3.60
		11	46.5	128	126	ND	13.8	275	4.71
		18	48.9	120	113	ND	13.6	245	3.39
		27	51.4	119	87.1	ND	13.5	201	2.06
		36	57.6	118	37.4	ND	13.7	137	2.89
S3	Ripening	0	ND	11.2	ND	ND	ND	67.9	ND
		5	0.72	115	134	3.05	8.56	287	5.73
	Storage	0[a]	6.47	193	140.	1.45	10.4	257	2.85
		11	54.8	139	138.	1.07	14.9	280	3.35
		18	60.4	134	125.	0.54	13.2	250	2.76
		27	66.0	129	83.1	ND	11.5	176	2.17
		36	53.3	114	30.9	ND	11.9	142	2.36
S4	Ripening	0	ND	11.2	ND	ND	ND	67.9	ND
		5	ND	116	127	3.76	9.00	305	5.56
	Storage	0[a]	13.2	215	114	ND	13.0	275	4.36
		11	43.1	138	112	2.45	15.1	296	3.70
		18	45.6	125	122	1.22	12.6	266	3.21
		27	48.0	112	103	ND	9.97	147	2.71
		36	50.1	106	30.1	ND	7.95	124	2.81
S5	Ripening	0	ND	11.2	ND	ND	ND	67.9	ND
		5	ND	123	135	4.25	11.2	301	5.87
	Storage	0[a]	10.4	209	119	4.87	10.7	271	6.18
		11	21.9	134	115	ND	14.9	227	ND
		18	29.9	126	106	ND	13.1	197	1.99
		27	37.9	118	47.9	ND	11.2	93.3	3.97
		36	25.3	102	24.6	ND	12.6	56.8	2.38

Biogenic amine concentrations were an average of duplicate determinations. Cadaverine, β-phenylethylamine and spermidine were not detected. TA, tryptamine; PUT, putrescine; HA, histamine; Dia, 1,7-diaminoheptane; SER, serotonin; TYR, tyramine; Sm, spermine.
ND: Not detected.
[a] Immediately after ripening period (15 days).

3.2.3. Biogenic amine formation

Biogenic amines were not detected in sucuk dough. Also, starter culture did not produce any biogenic amine under in vitro conditions, using the procedure reported by Roig-Sagues and Eerola (1997). Biogenic amines were produced by natural flora and contaminants during the ripening and storage periods. Table 4 shows the changes of biogenic amine concentrations (tryptamine (TA), putrescine (PUT), histamine (HA), 1,7-diaminoheptane (Dia), serotonin (SER), tyramine (TYR), and spermine (Sm)) in the sucuk during the ripening and storage periods. Cadaverine, β-phenylethylamine, and spermidine were not detected in any sucuk types.

It was found that time and additives had significant effects ($P < 0.05$) on the formation of putrescine. Putrescine concentration increased gradually ($P < 0.05$) up to 211 and 209 mg/kg in sucuk types S1 and S5, respectively (Table 4). After that, its concentration decreased during the storage period. The highest putrescine concentration was observed ($P < 0.05$) in the control sample (S1). Addition of a low level of nitrite/nitrate reduced ($P < 0.05$) putrescine formation. However, putrescine concentration did not change on further addition of nitrite/nitrate. Also, other additives did not reduce putrescine formation.

Histamine concentrations were affected ($P < 0.05$) by both time and additives. Histamine concentration increased ($P < 0.05$) during the first 5 days of ripening to about 191 and 135 mg/kg in samples S1 and S5, respectively (Table 4). After that time, histamine level decreased ($P < 0.05$) to about 40 mg/kg. Similar results were observed by Dierick, Vandekerckhove, and Demeyer (1974), who found that the histamine concentration increased during the first days of ripening. It was observed in a multiple range test that histamine level was the highest biogenic amine in the sucuk made without additives (S1) and the levels decreased ($P < 0.05$) in sucuks made with additives. Increasing the concentration level of nitrite/nitrate did not reduce the histamine formation in sucuks. However, addition of other additives with nitrite/nitrate decreased ($P < 0.05$) the histamine level in sucuks. Bozkurt and Erkmen (2004b) also found that increasing the amount of nitrite and nitrate decreased the histamine formation. Nout (1994) pointed out that histamine contents should be in the range of 50–100 mg/kg in sausages processed according to Good Manufacturing Practice. In this study, histamine content was found to be higher than the acceptable range during the ripening period and around the maximum level during the first 10 days of storage.

Tyramine concentrations varied from 145 to 56.8 mg/kg at the end of the storage in sucuk types S1 and S5, respectively (Table 4). The allowable maximum level of tyramine in foods is 100–800 and 1080 mg/kg of tyramine is toxic (Shalaby, 1996). Time had a significant effect ($P < 0.05$) on the tyramine formation. Tyramine formation increased gradually during first 5 days of the ripening periods and then decreased. Eerola et al. (1997) observed that tyramine concentrations in sausages increased during 7 days of storage at 4 °C, and then decreased during further storage. Vinci and Antonelli (2002) found that the tyramine concentration of red meat increased during 36 days of storage at 4 °C. This low temperature may reduce the growth of tyramine-producing microorganisms but in our study storage temperature was 30 °C. This high temperature may have reduced the activity of tyramine-producing microorganisms. Eitenmiller, Koehler, and Reagan (1978) found that tyramine concentrations increased during the first 3 days of storage then decreased. In this study, additives decreased ($P < 0.05$) tyramine formation. The highest tyramine concentration was observed in S1, and the lowest tyramine formation was in S4.

Spermine concentrations varied between 2.36 and 2.89 mg/kg at the end of the storage. Spermine concentration decreased ($P < 0.05$) during storage and the highest spermine formation was observed in the control sample.

3.3. Overall sensory quality

Overall sensory scores were evaluated from an equation proposed by Bozkurt and Erkmen (2004a) and results are given in Table 3. Statistical analysis was performed, to determine the effect of time and additives on the overall sensory quality. The overall sensory quality of sucuks was affected by both time and additives ($P < 0.05$).

The overall sensory scores increased ($P < 0.05$) during the ripening periods, in parallel to the flavour scores of samples, and then decreased ($P < 0.05$) during the storage periods. It was observed that control sample (S1) was found to be the worst ($P < 0.05$) sample, and increasing the nitrate/nitrite concentration increased ($P < 0.05$) the overall sensory quality of sucuks. It was found that the most acceptable ($P < 0.05$) sucuk was S5, and the order of acceptability was found to be S5 > S4 > S3 > S2 > S1.

Pearson's correlation test indicates that there was a link ($P < 0.01$) between the overall sensory quality and pH, TBARS values, mould and yeast counts, and putrescine concentration. The simple correlation coefficients were −0.346, −0.293, −0.254 and 0.579 between overall sensory score and pH, TBARS values, mould and yeast counts, and putrescine concentration, respectively. A negative correlation coefficient means that at low pH, TBARS values, and mould and yeast counts high sensory scores were recorded and a positive correlation coefficient means that the higher the concentration of putrescine, the higher the sensory scores. These results indicate that pH, TBARS values, mould and yeast counts and putrescine formation could be used to predict overall sensory quality, and shelf life of sucuk could be determined using these parameters.

4. Conclusion

This study showed that the use of additives improved the qualities of sucuks. Additives decreased ($P < 0.05$) the APC, mould and yeast count, biogenic amine and TBARS formation. From the point of human health, biogenic

amine and TBARS formation should be limited. In order to further limit the formation of biogenic amines and TBARS, APC, and mould and yeast count, hygienic conditions should be applied and ripening periods should be strictly controlled (temperature and relative humidity).

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