Korean Journal for Food Science of Animal Resources

Korean J. Food Sci. An. 2018 February 38(1):152~161
DOI https://doi.org/10.5851/kosfa.2018.38.1.152

pISSN : 1225-8563 eISSN : 2234-246X
www.kosfa.or.kr

ARTICLE

Biogenic Amine Formation in "Bez Sucuk," a Type of Turkish Traditional Fermented Sausage Produced with Different Meat: Fat Ratios

Ümran Çiçek* and Kader Tokatli

Food Engineering Department, Engineering and Natural Sciences Faculty, Gaziosmanpaşa University, Tokat 60100, Turkey

OPEN ACCESS

Received August 16, 2017
Revised January 22, 2018
Accepted January 23, 2018

***Corresponding author :** Ümran ÇİÇEK
Food Engineering Department, Engineering and Natural Sciences Faculty, Gaziosmanpaşa University, Tokat 60100, Turkey
Tel: +90 356-252 1616 (2883-ext.)
Fax: +90 356-252 1729
E-mail: umran.ensoy@gop.edu.tr; umranen@hotmail.com

Abstract This study aims to evaluate biogenic amine levels of bez sucuks (BS) produced with different meat:fat ratios. For this, three BS groups were manufactured with meat:fat ratios of 90:10 (BS10), 80:20 (BS20), and 70:30 (BS30). The pH and water activity values and biogenic amine amounts of sucuk samples were determined during processing and storage periods and the pH values of the initial mixtures of BS samples were in the range 5.51-5.74, decreasing to 4.72-4.94 by the 14th day. The water activity values of BS samples showed significant decreases as a result of the drying stage and reached to range 0.913-0.935 on the 14th day of processing ($p<0.05$). Although BS10 had the highest tyramine (434.12 mg/kg), histamine (5.69 mg/kg), cadaverine (12.48 mg/kg), putrescine (17.83 mg/kg), 2-phenylethylamine (15.43 mg/kg), and tryptamine (122.41 mg/kg) levels at the end of processing stage ($p<0.05$), spermine and spermidine levels did not differ between the BS samples due to their utilization of different meat:fat ratios ($p>0.05$). Similarly, the tryptamine (205.11 mg/kg), putrescine (43.57 mg/kg), and tyramine (766.23 mg/kg) levels of BS10 were higher than BS20 and BS30 samples at the end of storage ($p<0.05$). The results showed that BS10 with the highest meat ratio had the highest tryptamine, putrescine, and tyramine levels at the end of the processing and storage period.

Keywords bez sucuk, histamine, spontaneous fermentation, tyramine, tryptamine

Introduction

Biogenic amines (BA) are nitrogenous compounds that can be classified as aliphatic, aromatic, or heterocyclic amines according to their chemical structures (Bozkurt and Erkmen, 2007; Gençcelep et al., 2007; Papavergou et al., 2012; Suzzi and Gardini, 2003; Tosukhowong et al., 2011). In general, BAs are naturally found in various foods such as cheeses, fermented fish, meat products, and wine due to the amino acid decarboxylase activity of *Pseudomonas*, *Enterobacteriaceae,* and *Lactobacilli* (Baka et al., 2011; Suzzi and Gardini, 2003). The most important BAs in fermented foods are histamine, putrescine, cadaverine, tyramine, tryptamine, 2-phenylethylamine, spermine, and spermidine (Bover-Cid et al., 2001a; Suzzi and Gardini, 2003; Tosukhowong et al., 2011). Histamine, putrescine, tyramine, and cadaverine are the most prevalent BAs in fermented sausages; moreover, high levels of these BAs can be found in fermented sausages produced from raw materials (beef, pork, turkey meat, etc.) that have high protein content (Latorre-Moratalla et al., 2010; Papavergou, 2011; Suzzi and Gardini, 2003).

Bez sucuk (BS), which is a type of Turkish fermented beef sausage, differs from other Turkish-type fermented sausages due to the use of cloth casings sewn to size of 7 × 25 cm from uncolored cloth with 42 threads per cm^2. Although these products are mainly produced by butchers and small-scale facilities that utilize traditional technologies without adding starter cultures, a few manufacturers use curing agents such as sodium nitrite. In addition, the formulation (meat:fat ratio and seasonings) and process conditions (temperature, humidity, and ripening period) of the BS show differences between manufacturers (Çiçek and Köse, 2016). However, BS generally has three production stages: mixing the sucuk batter, filling the cloth casings, and ripening for 10-14 days (Çiçek and Köse, 2016; Çiçek and Polat, 2016). During the ripening stage for fermented sausages such as BS, lipids, proteins, and carbohydrates undergo various chemical and microbiological degradation reactions (Ensoy et al., 2010; Karslıoğlu et al., 2014; Papavergou, 2011). Although the proteolysis reactions during the ripening stage resulted in increased free amino acids, which are the precursors of BAs formation (Baka et al., 2011; Bover-Cid et al., 1999a; Karslıoğlu et al., 2014; Papavergou, 2011), the microbial counts of raw materials, process conditions, utilizing starter cultures, additives (antimicrobial agents), and the pH drop during fermentation stage affects the BA formation in fermented sausages (Bover-Cid et al., 2001a; Bozkurt and Erkmen, 2007; Suzzi and Gardini, 2003). Furthermore, the extended proteolytic reactions during ripening and storage periods with both a low pH value and high counts of bacteria having decarboxylase activity may enhance the accumulation of BAs in fermented sausages (Bover-Cid et al., 1999b; Papavergou, 2011; Papavergou et al., 2012; Suzzi and Gardini, 2003); moreover, BAs may also result from the decarboxylation activity of spoiling bacteria during the storage period (Bover-Cid et al., 2001b; Bozkurt and Erkmen, 2007; Suzzi and Gardini, 2003).

Numerous studies have been conducted to determine the BA levels of fermented meat products, particularly fermented sausages. The BA amounts of these products are also important from the perspective of human health. Many researchers have reported that the consumption of foods with high levels of BAs may cause various types of foodborne diseases such as hypotension, migraine, nausea, diarrhea, abdominal cramps, etc. (Bover-Cid et al., 1999a; Casquete et al., 2011; Gençcelep et al., 2007; Komprda et al., 2001; Latorre-Moratalla et al., 2008; Papavergou, 2011; Papavergou et al., 2012; Tosukhowong et al., 2011). Additionally, cadaverine and histamine levels have been proposed as chemical indicators of meat spoilage and the hygienic conditions of the manufacturing practices of fermented sausages (Bozkurt and Erkmen, 2007; Komprda et al., 2001; Tosukhowong et al., 2011); meanwhile, there is only a legal upper limit for histamine: 100 mg per kg of food (Bozkurt and Erkmen, 2002; Latorre-Moratalla et al., 2008).

The BA levels of Turkish fermented sausages that have natural or collagen casings have been investigated. Although the formulation and processing conditions of BS show differences between manufacturers and casing sewn from uncolored cloth, no study has assessed the BA levels of BS, particularly in those manufactured with different meat:fat ratios. According to the TS 1070 Turkish Sucuk (fermented sausage) Standard (Anonymous, 2002), the maximum fat contents of first, second, and third quality sucuks are limited to 35%, 40%, and 40%, respectively. In addition, according to the Turkish Food Codex-Meat Products Communique (Anonymous, 2012) the maximum fat content of fermented sausages is limited to 40%. After producing BS in accordance with both food legislation and sucuk standards, the current study aimed to determine the effects of different meat:fat ratios and the process stages/storage periods on the formation of BAs in BS.

Materials and Methods

Sausage production

In this study, experimental BS production was repeated three times, and three groups of BS named BS10, BS20, and BS30 were manufactured with respective meat:fat ratios of 90:10, 80:20, and 70:30. Cubed beef meat was subdivided into three groups and combined with salt (1.6%), garlic (2%), cumin (0.8%), hot red pepper (0.8%), red pepper (0.6%), black pepper (0.4%), sucrose (0.5%), and allspice (0.2%) using a Mainca 400 mixer (Germany). Following the mixing stage, all sucuk mixtures were stored at 4°C for 12 hours (initial mixture). Sheep tail fat was added to all sucuk batters to the meat:fat ratios mentioned above and ground twice using a grinding machine (Turkey). After grinding, cloth casings were filled with sucuk mixtures using a filling machine from Mainca EM-20 (Germany). Sucuk batons were hung on stainless steel and ripened for 14 days. The ripening steps were as follows: (step 1) at 22°C, 90% relative humidity for 48 h; (step 2) at 22°C, 85% relative humidity for 48 h; (step 3) at 20°C, 80% relative humidity for 72 h; (step 4) at 18°C, 75% relative humidity for 72 h; (step 5) at 18°C, 60% relative humidity for 96 h. During the ripening stage, all sucuk samples were pressed for three times and following the ripening stage, all sucuk samples were vacuum packed using a vacuum machine from La Minerva Pack 10B (Italy), and then stored at 4°C for six months.

Three randomly selected sucuk samples were removed during processing (initial mixture, 2nd day, 4th day, 10th day, and 14th day) and storage periods (day 0, 30th day, 60th day, 90th day, 120th day, and 180th day) for further analysis.

Methods

pH and water activity values (a_w)

The pH of the sample was determined using an Orion 420A pH-meter that was calibrated using standard buffer solutions at pH 4 and 7. The water activity values of samples were measured using AquaLab Series 3 TE model (USA) at each sampling stage (Çiçek et al., 2015).

Biogenic amine levels

The biogenic amine amounts of samples were analyzed using the method of Bozkurt and Erkmen (2002). The HPLC system, which consisted of a PerkinElmer Series 200 model UV/VIS Detector (USA), PerkinElmer Series 200 model pump (USA) and PerkinElmer Series 200 model Peltier column oven (USA) was used. The column was Waters Spherisorb® 10.0 µm, ODS2 (4.6 × 200 mm) (USA). Acetonitrile (solvent A) and 0.1 M ammonium acetate (solvent B) were the mobile phases (flow rate: 1 mL/min). The standard solution of the dansylated derivatives of tryptamine, 2-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine (SIGMA) was used and all standards were diluted to 1 mL using 0.4 perchloric acid to give concentrations in the range 0.5-10 mg/mL.

Statistical analysis

The data were statistically analyzed using the SPSS 20.0 statistical package (International Business Machines Corporation [IBM] Armonk, USA). Mean values for different meat:fat ratios and manufacturing/storage periods were compared using analysis of variance (ANOVA) with the Duncan multiple *post hoc* comparison test to evaluate statistical significance between the means (p<0.05). Comparison of means for BS groups and sampling stages was done for every tested variable. Standard deviation (SD) values are presented alongside the means in tables.

Results and Discussions

pH and water activity (a_w) values

The results showed that the pH values of BS decreased during the ripening stages ($p<0.05$) and reached the lowest values on day 4 due to the acidification activity of lactic acid bacteria ($p<0.05$). The occurrence of proteolytic activity during the ripening stage resulted in the formation of ammonia and amine, which slightly increased the pH values of BS10, BS20, and BS30 to 4.94, 4.78, and 4.72, respectively. Although all sucuk formulations were prepared with the same sucrose amount of 0.5%, the highest pH value was measured in BS10 on day 14 ($p<0.05$) (Table 1). This difference could be the result of proteolysis reactions in BS10 having the highest meat ratio among the three. It has been stated that sugar addition with certain concentrations to the sausage mixture can improve the rapid acidification, which limits the decarboxylase activity of bacteria (Gonzalez et al., 2003; Latorre-Moratalla et al., 2010). Similar rapid pH decreases in all BS groups were also achieved with sucrose addition to BS formulations. However, Latorre-Moratalla et al. (2010) stated that sugar addition did not result in rapid pH decreases in fermented sausages and higher amounts of sugar promoted the decarboxylase activity of aminogenic microorganisms. During the storage periods, the pH values of BS groups showed slight increases and decreases, and measured 5.21, 4.91, and 4.69 on day 180 for BS10, BS20, and BS30, respectively (Table 1). It was seen that BS10 had the highest pH value among the three ($p<0.05$).

The initial a_w values of the sucuk mixtures were 0.973-0.978 and reached 0.913-0.935 due to the increasing acidity and water release at the end of the ripening stage ($p<0.05$) (Table 1). It was observed that BS10 had the lowest a_w value on the 14th day ($p<0.05$). Although all sucuk samples were vacuum packaged, the a_w values of BS showed differences between

Table 1. pH and water activity (a_w) values of bez sucuks during processing stages and storage periods[1)]

	BS10[2)]		BS20		BS30	
	pH	a_w	pH	a_w	pH	a_w
			Process stages (day)			
Initial mixture	5.51 ± 0.21^{Aa}	0.978 ± 0.007^{Aa}	5.61 ± 0.21^{Aa}	0.976 ± 0.005^{Aa}	5.74 ± 0.26^{Aa}	0.973 ± 0.005^{Aa}
2	4.89 ± 0.20^{Bb}	0.972 ± 0.004^{BAa}	5.06 ± 0.11^{ABb}	0.974 ± 0.005^{Aa}	5.16 ± 0.19^{Ab}	0.971 ± 0.005^{Aa}
4	4.67 ± 0.14^{Ac}	0.968 ± 0.003^{Bb}	4.74 ± 0.10^{Ad}	0.971 ± 0.00^{Aa}	4.76 ± 0.13^{Ac}	0.967 ± 0.002^{Ba}
10	5.04 ± 0.16^{Ab}	0.964 ± 0.005^{Ac}	4.90 ± 0.07^{Bc}	0.949 ± 0.006^{Ab}	4.84 ± 0.08^{Bc}	0.949 ± 0.005^{Ab}
14	4.94 ± 0.13^{Ab}	0.913 ± 0.017^{Bd}	4.78 ± 0.06^{Bd}	0.922 ± 0.015^{ABc}	4.72 ± 0.07^{Bc}	0.935 ± 0.016^{Ac}
			Storage periods (day)			
0	4.94 ± 0.13^{Ab}	0.913 ± 0.017^{Ba}	4.78 ± 0.06^{Bb}	0.920 ± 0.015^{ABab}	4.72 ± 0.07^{Bbc}	0.935 ± 0.016^{Aa}
30	5.26 ± 0.24^{Aa}	0.897 ± 0.012^{Bb}	5.04 ± 0.59^{Ba}	0.908 ± 0.015^{ABcd}	4.98 ± 0.18^{Ba}	0.920 ± 0.009^{Ab}
60	5.10 ± 0.05^{Aab}	0.907 ± 0.006^{Cab}	4.83 ± 0.23^{Bb}	0.916 ± 0.008^{Babc}	4.65 ± 0.25^{Bc}	0.927 ± 0.008^{Aab}
90	5.23 ± 0.04^{Aa}	0.904 ± 0.004^{Cab}	5.04 ± 0.22^{Ba}	0.923 ± 0.005^{Ba}	4.85 ± 0.16^{Cab}	0.935 ± 0.006^{Aa}
120	5.23 ± 0.32^{Aa}	0.896 ± 0.009^{Cb}	5.01 ± 0.27^{ABa}	0.909 ± 0.010^{Bcd}	4.80 ± 0.18^{Bbc}	0.926 ± 0.011^{Aab}
150	5.12 ± 0.17^{Aa}	0.900 ± 0.008^{Bb}	4.87 ± 0.10^{Bab}	0.901 ± 0.008^{Bd}	4.67 ± 0.03^{Cc}	0.918 ± 0.010^{Ab}
180	5.21 ± 0.07^{Aa}	0.905 ± 0.004^{Bab}	4.91 ± 0.11^{Bab}	0.911 ± 0.007^{Bbcd}	4.69 ± 0.13^{Cbc}	0.923 ± 0.005^{Ab}

[1)]Data ± standard deviation (n=6).
[2)]BS10: bez sucuk having meat:fat ratio of 90:10; BS20: bez sucuk having meat:fat ratio of 80:20; BS30: bez sucuk having meat:fat ratio of 70:30.
[a-d] Means in a column of each group not having a common superscript letter are different ($p<0.05$).
[A-C] Means in a row of same processing stage not having a common superscript letter are different ($p<0.05$).

the storage periods due to sampling ($p<0.05$) and measured 0.905, 0.911, and 0.923 on day 180 for BS10, BS20, and BS30, respectively ($p<0.05$) (Table 1).

Biogenic amine levels

The levels of BAs measured in BS groups during the processing stages and storage periods are given in Tables 2 and 3, respectively. The spermine levels of the initial mixtures of BS groups were in the range 282.77-343.73 mg/kg ($p<0.05$) (Table 2). Many researchers noted that spermine and spermidine could be present at high levels in fresh meat used for fermented sausage manufacturing (Papavergou, 2011; Suzzi and Gardini, 2003). Papavergou et al. (2012) also stated that continued intake of foods with high amounts of spermine and spermidine may cause the facilitation of a food allergy. Spermine levels in BS groups showed slight decreases and increases during processing stages, and spermine levels were measured as 343.34, 306.95, and 321.63 mg/kg on day 14 for BS10, BS20, and BS30, respectively (Table 2). Bover-Cid et al. (1999b) also reported similar fluctuations in spermine levels during the ripening of dry fermented sausages. Contrary to our results, Kurt and Zorba (2009) stated that increasing the ripening period of sucuks resulted in higher spermine accumulation. In agreement with the results of current study, Roseiro et al. (2010) stated that spermine levels were higher than spermidine levels. The spermidine levels of BS groups increased during processing stages and reached 202.70, 141.79, and 188.42 mg/kg for BS10, BS20, and BS30, respectively ($p<0.05$) (Table 2). Kurt and Zorba (2009) also noted similar increases during processing stages. Utilizing different meat:fat ratios did not affect spermine and spermidine levels among BS groups on day 14 ($p>0.05$). Although the spermidine levels of all BS samples showed significant decreases and increases during storage periods ($p<0.05$), the changes in spermine levels for BS10 and BS20 were found to be significant ($p<0.05$) (Table 3). However, Bozkurt and Erkmen (2002) reported significant decreases in

Table 2. Biogenic amine levels of bez sucuks during processing stages (mg/kg)[1)]

Group	Process stage (day)	Tryptamine	2-Phenyl ethylamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine
BS10[2)]	Initial mixture	23.18±6.74^{Ab}	5.10±2.33^{Aab}	0.50±0.44^{Ac}	0.67±0.13^{Ab}	0.48±0.20^{Ad}	2.05±1.28^{Ad}	13.29±2.65^{Ac}	343.73±14.49^{Aa}
	2	41.67±0.73^{Ab}	ND[3)]	0.56±0.46^{Ac}	2.20±1.23^{Ab}	0.62±0.12^{Ad}	95.55±18.90^{Bcd}	62.74±6.11^{Abc}	339.40±7.48^{Aa}
	4	40.26±16.51^{Ab}	7.74±5.58^{Aab}	1.87±0.51^{Ac}	3.82±0.78^{ABb}	2.29±0.18^{Ac}	166.28±37.93^{Abc}	125.62±28.00^{Ab}	306.95±43.42^{Aab}
	10	59.75±34.57^{Ab}	14.78±7.64^{Aa}	9.05±4.66^{Ab}	13.13±2.25^{Aa}	3.60±0.01^{Ab}	247.36±100.43^{ABb}	213.23±61.57^{Aa}	245.38±93.87^{Ab}
	14	122.41±23.08^{Aa}	15.43±12.87^{Aa}	17.83±2.40^{Aa}	12.48±4.72^{Aa}	5.69±0.26^{Aa}	434.12±83.20^{Aa}	202.70±77.38^{Aa}	343.34±48.83^{Aa}
BS20	Initial mixture	16.78±3.19^{Ab}	2.28±1.11^{Bb}	0.22±0.03^{Ab}	0.78±0.10^{Ac}	0.39±0.13^{Ab}	0.95±0.46^{Ac}	8.66±3.22^{Bb}	298.10±11.11^{Ba}
	2	34.42±14.15^{Ab}	ND	0.45±0.22^{Ab}	4.00±2.55^{Ab}	0.38±0.11^{Bb}	124.13±22.39^{Ab}	146.70±96.45^{Aa}	305.51±79.76^{ABa}
	4	41.83±10.21^{Ab}	0.27±0.67^{Bc}	1.74±1.27^{Ab}	4.36±1.16^{Ab}	0.80±0.20^{Bb}	144.97±27.15^{Ab}	118.66±5.84^{ABa}	295.76±32.81^{Aa}
	10	74.98±14.24^{Aa}	18.66±3.53^{Aa}	9.06±3.43^{Aa}	8.80±1.51^{Ba}	2.16±0.81^{Ba}	311.48±33.88^{Aa}	158.44±76.99^{ABa}	321.42±8.25^{Aa}
	14	73.32±32.05^{Ba}	ND	10.34±4.20^{Ba}	8.54±1.56^{Ba}	2.70±0.04^{Ba}	303.65±82.53^{Ba}	141.79±44.75^{Aa}	306.95±41.37^{Aa}
BS30	Initial mixture	14.52±0.45^{Abc}	1.13±0.67^{Bb}	0.23±0.03^{Ac}	0.90±0.38^{Ac}	0.59±0.52^{Aa}	1.92±0.30^{Ae}	6.31±2.11^{Bc}	282.77±31.70^{Bab}
	2	ND	ND	0.23±0.08^{Ac}	1.70±1.06^{Abc}	0.31±0.02^{Ba}	66.93±7.74^{Cd}	61.82±15.28^{Abc}	232.76±107.60^{Bb}
	4	31.30±2.22^{Ab}	ND	1.14±1.43^{Ac}	2.76±1.27^{Bb}	0.38±0.27^{Ba}	103.99±12.73^{Bc}	96.06±13.14^{Bb}	294.62±22.18^{Aab}
	10	60.33±17.29^{Aa}	47.24±50.67^{Aa}	5.71±2.57^{Ab}	5.72±1.77^{Ca}	0.73±0.38^{Ca}	222.12±22.20^{Bb}	90.66±14.66^{Bb}	297.73±52.07^{Aab}
	14	73.77±14.94^{Ba}	3.68±1.93^{Bb}	9.07±2.23^{Ba}	6.44±1.94^{Ba}	0.84±0.73^{Ca}	293.99±21.47^{Ba}	188.42±89.63^{Aa}	321.63±24.77^{Aa}

[1)]Data ± standard deviation (n=6).
[2)]BS10: bez sucuk having meat:fat ratio of 90:10; BS20: bez sucuk having meat:fat ratio of 80:20; BS30: bez sucuk having meat:fat ratio of 70:30.
[3)]ND: Not detected.
$^{a-e}$ Means in a column of each group not having a common superscript letter are different ($p<0.05$).
$^{A-C}$ Means in a column of same processing stage not having a common superscript letter are different ($p<0.05$).

spermine concentrations for Turkish sucuks during storage.

The cadaverine and putrescine levels of initial mixtures were in the ranges of 0.67-0.90 mg/kg and 0.22-0.50 mg/kg, respectively (Table 2). The accumulation of cadaverine and putrescine significantly increased during processing stages and reached 6.44-12.48 mg/kg and 9.06-17.83 mg/kg, respectively ($p<0.05$). Similar increases in putrescine and cadaverine levels in Turkish sucuks were also noted by Gençcelep et al. (2007). The presence of especially high levels of cadaverine and putrescine in BAs has been attributed to meat spoilage (Baka et al., 2011; Suzzi and Gardini, 2003). Thus, the formation of cadaverine is mainly related to the decarboxylase activity of Enterobacteriaceae and *Pseudomonas* (Baka et al., 2011; Latorre-Moratalla et al., 2010). The low cadaverine levels of BS groups could be explained by the contribution of good hygienic conditions when handling raw materials and the manufacturing conditions. Latorre-Moratalla et al. (2010) noted that high cadaverine levels of spontaneously fermented Portuguese *chouriços* were due to their high counts of Enterobacteriaceae. The cadaverine levels of BS samples showed increases during storage periods, and respectively measured 27.66, 17.89, and 11.48 mg/kg for BS10, BS20, and BS30 on day 180 (Table 3). It was seen that utilizing different meat:fat ratios did not affect the cadaverine levels of BS samples at the end of storage ($p>0.05$). The significant increment in putrescine levels of all BS groups was observed, especially after day 10 of the ripening stage ($p<0.05$). Bover-Cid et al. (1999a) also stated similar putrescine production that increased after the first week due to a pH drop. In our study, it was seen that no significant putrescine formation was observed during the first two days of the ripening stage ($p>005$) and, although the putrescine accumulation was significant on day 10, the pH value of the BS groups dropped dramatically after day 4 of ripening. Additionally, it was determined that BS10, with the highest meat ratio and pH value, had the highest amounts of cadaverine and putrescine on day 14 ($p<0.05$). The results indicated that the putrescine levels of the BS groups increased during storage and BS10, with the highest meat ratio, had the highest putrescine level (43.57 mg/kg) on day 180 ($p<0.05$) (Table 3). Baka et al. (2011) and Papavergou (2011) stated that putrescine was the major amine in fermented sausages. The results obtained from Turkish fermented sausages showed that utilizing only nitrite/nitrate or starter cultures limited putrescine formation (Bozkurt and Erkmen, 2007; Gençcelep et al., 2007).

The histamine levels of the BS10 and BS20 groups showed significant increases during the processing stages ($p<0.05$) and were respectively measured as 5.59, 2.70, and 0.84 mg/kg for BS10, BS20, and BS30 (Table 2), on day 14. This could be the result of pH decreases in the first days of ripening as the pH values of all BS groups significantly decreased after the 2nd day of the ripening stages and reached the range of 4.89-5.16 ($p<0.05$). Many researchers also noted that an inadequate decrease of pH at the beginning of the ripening stage with extended ripening times could increase the accumulation of histamine in fermented sausages (Papavergou, 2011; Suzzi and Gardini, 2003). Bozkurt and Erkmen (2007) also stated that the ripening period had an increasing effect on the histamine levels of Turkish sucuks, but, in contrast, the researchers also noted decreases in the histamine levels after the 5th day of ripening. Furthermore, an increasing meat ratio resulted in higher histamine levels. Thus, BS10, having the highest meat ratio, had the highest histamine level ($p<0.05$) (Table 2); on the other hand, the histamine levels of the BS samples were under the values causing health concerns (100 mg/kg). Although the histamine levels of BS20 and BS30 showed significant decreases and increases during storage periods ($p<0.05$), the differences between the BS groups were not significant on day 180 ($p>0.05$) (Table 3).

The tyramine levels of all initial mixtures were between 0.95 and 2.05 mg/kg ($p>0.05$). The accumulation of tyramine showed increases during the processing stages, and the tyramine levels of BS10, BS20, and BS30 were respectively measured as 434.12, 303.65, and 293.99 mg/kg (p<0.05), on day 14 (Table 2). In all the BS groups, the major tyramine formation rate occurred during the first two days due to the pH dropping. Bover-Cid et al. (2001a) also stated that gradual biogenic amine accumulation occurred from the first week of the ripening stage, coinciding with the pH decrease, especially in noninoculated fermented sausages. Similarly, Bozkurt and Erkmen (2007) determined significant increases in the tyramine levels of Turkish sucuks during the first five days of ripening and then decreases during the ripening stage. Contrary to these findings, Komprda et al. (2001) reported a substantial increment in tyramine content after the second week of ripening. Furthermore, tyramine was the main amine in all the BS groups with the exception of the naturally occurring polyamines spermine and spermidine at the end of the processing stages. Similarly, many researchers also stated that tyramine was the major amine in fermented sausages and/or sucuks (De Mey et al., 2014; Gençcelep et al., 2007; Papavergou et al., 2012). It was also determined that utilizing different meat ratios affected the tyramine levels of the BS samples. Thus, BS10, with the highest meat ratio, had the highest tyramine level on the 14th day (p<0.05) (Table 2). The tyramine levels of BS showed significant increases during the storage periods (except BS30), and the tyramine levels of BS10, BS20, and BS30 were respectively measured as 766.23, 539.60, and 453.48 mg/kg on day 180 (Table 3). The maximum allowable limit for tyramine in foods is 100-800 mg/kg (Bozkurt and Erkmen, 2002), and it was seen that the tyramine levels of all BS groups were in this range both at the end of the processing stages and storage periods.

Table 3. Biogenic amine levels of bez sucuks during storage periods (mg/kg)[1)]

Group	Storage periods (day)	Tryptamine	2-Phenyl ethylamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine
BS10[2)]	0	122.41±23.08^{Abc}	15.43±12.87^{Abc}	17.83±2.40^{Ad}	12.48±4.72^{Aa}	5.69±0.26^{Aa}	434.12±83.20^{Ab}	202.70±77.38^{Aab}	343.34±48.83^{Aa}
	30	104.19±26.31^{Ac}	0.80±0.95^{Ac}	13.38±3.80^{Ad}	10.95±0.72^{Aa}	2.44±2.10^{Aa}	417.02±88.72^{ABb}	131.42±47.25^{ABb}	317.42±49.43^{Aab}
	60	150.18±40.74^{Aabc}	18.99±12.70^{Abc}	26.89±4.79^{Abc}	18.79±11.13^{Aa}	6.30±5.68^{Aa}	554.34±113.07^{Aab}	235.86±71.61^{Aa}	319.35±28.74^{Aab}
	90	111.59±82.96^{Ac}	12.27±7.72^{ABbc}	20.07±12.25^{Acd}	11.71±8.87^{Aa}	2.80±3.47^{Aa}	425.27±266.83^{Ab}	128.25±113.63^{Ab}	224.35±113.17^{Ab}
	120	157.27±47.94^{Aabc}	21.92±18.95^{Abc}	31.68±8.00^{Ab}	18.29±13.20^{Aa}	3.58±4.02^{Aa}	631.57±178.73^{Aab}	223.29±65.68^{Aa}	327.81±38.84^{Aa}
	150	184.97±54.01^{Aab}	64.45±19.81^{Aa}	35.63±6.06^{Aab}	23.42±18.29^{Aa}	3.48±3.74^{Aa}	681.73±163.02^{Aa}	256.16±45.76^{Aa}	276.03±49.99^{Aab}
	180	205.11±25.64^{Aa}	29.85±23.27^{Ab}	43.57±4.96^{Aa}	27.66±24.15^{Aa}	5.53±6.42^{Aa}	766.23±171.00^{Aa}	297.31±37.02^{Aa}	358.58±120.47^{Aa}
BS20	0	73.32±32.05^{Bb}	ND[3)]	10.34±4.20^{Bc}	8.54±1.56^{Ba}	2.70±0.04^{Ba}	303.65±82.53^{Bb}	141.79±44.75^{Ab}	306.95±41.37^{Aab}
	30	120.91±39.02^{Aab}	11.62±10.89^{Aabc}	19.00±7.10^{Abc}	14.76±8.94^{Aa}	3.77±3.73^{Aa}	481.84±123.88^{Aab}	214.63±76.77^{Aab}	299.83±32.77^{Aab}
	60	153.29±40.08^{Aa}	4.48±2.00^{Bc}	23.55±3.42^{ABab}	12.02±2.44^{ABa}	1.82±1.88^{Ba}	498.21±83.05^{Aab}	189.05±34.96^{Aab}	331.18±12.57^{Aa}
	90	112.71±72.65^{Aab}	24.34±19.93^{Aa}	23.89±13.32^{Aab}	17.39±15.42^{Aa}	2.65±3.46^{Aa}	441.25±268.81^{Aab}	138.13±80.66^{Ab}	242.47±97.03^{Ab}
	120	123.79±34.35^{ABab}	9.52±9.31^{ABbc}	25.84±6.84^{Aab}	13.70±5.44^{Aa}	3.48±4.30^{Aa}	457.19±122.22^{ABab}	209.28±45.81^{Aab}	299.85±63.16^{Aab}
	150	150.68±18.57^{ABa}	16.48±12.93^{Babc}	32.30±4.43^{ABa}	16.13±7.47^{Aa}	3.54±4.38^{Aa}	551.88±93.70^{ABa}	239.53±44.02^{Aa}	312.29±43.04^{Aab}
	180	146.10±54.38^{Ba}	18.20±7.19^{Aab}	30.10±8.17^{Ba}	17.89±8.27^{Aa}	3.04±3.14^{Aa}	539.60±167.06^{Ba}	249.52±84.71^{Aa}	246.31±23.19^{Bb}
BS30	0	73.77±14.94^{Bb}	3.68±1.93^{ABb}	9.07±2.23^{Bc}	6.44±1.94^{Bb}	0.84±0.73^{Cb}	293.99±21.47^{Ba}	188.42±89.63^{Aab}	321.63±24.77^{Aa}
	30	83.51±30.49^{Aab}	ND	11.50±4.48^{Abc}	6.80±2.15^{Aab}	0.75±0.58^{Ab}	200.77±64.68^{Ba}	104.88±5.59^{Bb}	255.65±49.90^{Aa}
	60	104.65±27.79^{Aab}	0.97±2.38^{Bb}	18.47±5.92^{Babc}	8.14±0.72^{Bab}	0.77±0.48^{Bb}	374.14±70.54^{Ba}	192.17±70.41^{Aab}	298.49±55.83^{Aa}
	90	90.72±57.35^{Aab}	ND	18.68±10.92^{Aabc}	6.75±3.54^{Aab}	1.39±1.62^{Aab}	339.60±180.84^{Aa}	121.52±76.13^{Ab}	258.44±110.53^{Aa}
	120	99.77±45.19^{Bab}	0.14±0.35^{Bb}	21.12±9.55^{Aab}	8.39±4.60^{Aab}	1.89±2.30^{Aab}	356.66±150.06^{Ba}	136.16±46.49^{Bb}	249.66±85.30^{Aa}
	150	109.46±42.32^{Bab}	34.87±34.01^{ABa}	24.93±10.64^{Ba}	9.87±5.38^{Aab}	1.57±0.98^{Aab}	397.09±155.19^{Ba}	234.41±104.52^{Aa}	273.73±85.85^{Aa}
	180	132.04±14.08^{Ba}	10.91±4.18^{Ab}	28.81±5.25^{Ba}	11.48±4.16^{Aa}	9.32±15.32^{Aa}	453.48±62.92^{Ba}	278.66±61.90^{Aa}	284.58±46.43^{ABa}

[1)]Data ± standard deviation (n=6).
[2)]BS10: bez sucuk having meat:fat ratio of 90:10; BS20: bez sucuk having meat:fat ratio of 80:20; BS30: bez sucuk having meat:fat ratio of 70:30.
[3)]ND: Not detected.
[a-e] Means in a column of each group not having a common superscript letter are different (p<0.05).
[A-C] Means in a column of same processing stage not having a common superscript letter are different (p<0.05).

Although tryptamine was not detected in BS30 on the 2nd day of processing, the tryptamine levels of all initial mixtures increased during manufacturing and reached the range of 73.32-122.41 mg/kg at the end of the processing stages (Table 2). It was seen that BS10, having the highest meat ratio and pH on day 14, had the highest tryptamine level (p<0.05) (Table 2) and a significant increment in the tryptamine value of BS10 occurred after the 14th day of ripening, while the tryptamine levels of BS20 and BS30 increased after the 10th day of ripening (p<0.05). Tasic et al. (2012) stated that the tryptamine levels of *Petrovska klobasa* - a traditional fermented sausage - ranged between 14.7 and 75.1 mg/kg at the end of the drying stage. Papavergou (2011) indicated that the tryptamine levels of Greek fermented sausages were between 0 and 60.53 mg/kg. In another study, Ikonic et al. (2013) reported higher tryptamine levels (24.3-150 mg/kg) for *Petrovska klobasa*. The tryptamine levels of all the BS groups showed decreases and increases during storage and reached the range of 132.04-205.11 on day 180 (p<0.05) (Table 3). The highest tryptamine level was measured in BS10, having the highest meat ratio and pH value, on day 180 (p<0.05).

In the initial mixtures of all groups, the 2-phenylethylamine levels were in the range of 1.13-5.10 mg/kg (p<0.05). On the 2nd day of processing, no 2-phenylethylamine was determined in any groups. Additionally, 2-phenylethylamine was not detected in BS30 on the 4th day (Table 2). Roseiro et al. (2010) and Bozkurt and Erkmen (2007) noted that 2-phenylethylamine was not detected in any ripening stages of traditional fermented sausages. In the present study, on the 14th day, the 2-phenylethylamine levels of BS10 and BS30 increased to the values of 15.43 and 3.68 mg/kg, respectively (Table 2). Similar findings were also reported by Bover-Cid et al. (2001b). Furthermore, Bover-Cid et al. (2001a) detected 2-phenylethylamine only in spontaneously fermented sausages; thus, BS is a type of spontaneously fermented sausage., the 2-phenylethylamine amounts of all BS groups varied during the storage periods, and the 2-phenylethylamine levels of BS10, BS20, and BS30 were respectively measured as 29.85, 18.20, and 10.91 mg/kg (p>0.05), on day 180 (Table 3).

Conclusions

Although the histamine levels of all the BS groups were lower than 100 mg/kg, the findings of the current study showed that increasing meat ratio resulted in higher biogenic amine levels; thus, BS10, with the highest meat ratio, had the highest amounts of tryptamine, putrescine, cadaverine, and tyramine on the 14th day (ripening) and of tryptamine, putrescine, and tyramine at the end of the storage period. The effect of the ripening period was also found to be significant in tryptamine, putrescine, cadaverine, tyramine, and spermine formation in all the BS groups. The study results indicated that the meat:fat ratio of 70:30 could be suitable for BS production to decrease the BA levels of BS.

Acknowledgements

This study was supported by Science, Industry and Technology Ministry of Republic of Turkey (00148.STZ.2007-2).

References

Anonymous. 2002. Turkish Sucuk Standard, TS 1070, Türk Standardları Enstitüsü, Ankara, Available from: http://www.tse.org.tr. Accessed at Jan 01 2018.

Anonymous. 2012. Republic of Turkey Ministry of Food, Agriculture and Livestock. Turkish Food Codex - Meat and Meat Products Communique. Communique number, 2012/74, Available from: http://tarim.gov.tr/Mevzuat/Turk-Gida-

Kodeksi. Accessed at December 05, 2012.

Ayhan K, Kolsarıcı N, Alsancak Özkan G. 1999. The effects of a starter culture on the formation of biogenic amines in Turkish soudjouks. Meat Sci 53:183-188.

Baka AM, Papavergou EJ, Pragalaki T, Bloukas JG, Kotzekidou P. 2011. Effect of selected autochthonous starter cultures on processing and quality characteristics of Greek fermented sausages. LWT- Food Sci Technol 44:54-61.

Bover-Cid S, Izquierdo-Pulido M, Vidal-Carou MC. 2001a. Changes in biogenic amine and polyamine contents in slightly fermented sausages manufactured with and without sugar. Meat Sci 57:215-221.

Bover-Cid S, Izquierdo-Pulido M, Vidal-Carou MC. 2001b. Effect of the interaction between a low tyramine-producing Lactobacillus and proteolytic staphylocci on biogenic amine production during ripening and storage of dry sausages. Int J Food Microbiol 65:113-123.

Bover-Cid S, Izquierdo-Pulido M, Vidal-Carou MC. 1999a. Effect of proteolytic starter cultures of *Staphylococcus* spp. on biogenic amine formation during the ripening of dry fermented sausages. Int J Food Microbiol 46:95-104.

Bover-Cid S, Schoppen S, Izquierdo-Pulido M, Vidal-Carou MC. 1999b. Relationship between biogenic amine contents and size of dry fermented sausages. Meat Sci 51:305-311.

Bozkurt H, Erkmen O. 2002. Effects of starter cultures and additives on the quality of Turkish style sausage (sucuk). Meat Sci 61:149-156.

Bozkurt H, Erkmen O. 2007. Effects of some commercial additives on the quality of sucuk (Turkish dry-fermented sausage). Food Chem 101:1465-1473.

Casquete R, Benito MJ, Martin A, Ruiz-Moyano S, Hernandez A, Cordoba MG. 2011. Effect of autochthonous starter cultures in the production of "*salhichon*", a traditional Iberian dry-fermented sausage, with different ripening process. LWT- Food Sci Technol 44:1562-1571.

Çiçek, Ü.; Kolsarıcı, N.; Candoğan, K. 2015. The sensory properties of fermented turkey sausages: effect of processing methodologies and starter culture. J Food Process Preserv 39:6, 663-670.

Çiçek Ü, Köse T. 2016. Physical and biochemical quality parameters of fermented beef sausages: Bez Sucuk. Acta Aliment Hung 45:3, 363-370.

Çiçek Ü, Polat N. 2016. Investigation of physicochemical and sensorial quality of a type of traditional meat product: Bez sucuk. LWT- Food Sci Technol 65:145-151.

De Mey E, De Klerck K, De Maere H, Dewulf L, Derdelinckx G, Peeters MC, Fraeye I, Heyden YV, Paelinck H. 2014. The occurrence of N-nitrosoamines, residual nitrite and biogenic amines in commercial dry fermented sausages and evaluation of their occasional relation. Meat Sci 96:821-828.

Ensoy Ü, Kolsarıcı N, Candoğan K, Karslıoğlu B. 2010. Changes in biochemical and microbiological characteristics of turkey sucuks as affected by processing and starter culture utilization. J Muscle Foods 21:142-165.

Gençcelep H, Kaban G, Kaya M. 2007. Effects of starter cultures and nitrite levels on formation of biogenic amines in sucuk. Meat Sci 77:424-430.

Gonzalez-Fernandez C, Santos EM, Jaime I, Rovira J. 2003. Influence of starter cultures and sugar concentrations on biogenic amine contents in chorizo dry sausage. Food Microbiol 20:275–284.

Ikonic P, Tasic T, Petrovic L, Skaljac S, Jokanovic M, Mandic A, Ikonic B. 2013. Proteolysis and biogenic amines formation during the ripening of *Petrovska klobasa*, traditional dry-fermented sausage from Northern Serbia. Food Control 30:69-75.

Karslıoğlu B, Ensoy-Çiçek Ü, Kolsarıcı N, Candoğan K. 2014. Lipolytic changes in fermented sausages produced with turkey meat: Effects of starter culture and heat treatment. Korean J Food Sci An 34:1, 40-48.

Komprda T, Nezlova J, Standara S, Bover-Cid S. 2001. Effect of starter culture and storage temperature on the content of biogenic amines in dry fermented sausage polican. Meat Sci 59:267-276.

Kurt Ş, Zorba Ö. 2009. The effects of ripening period, nitrite level and heat treatment on biogenic amine formation of "sucuk" – A traditional Turkish dry fermented sausage. Meat Sci 82:179-184.

Latorre-Moratalla ML, Veciana-Nogues T, Bover-Cid S, Garriga M, Aymerich T, Zanardi E, Ianeri A, Fraqueza MJ, Parata L, Drosinos EH, Laukova A, Talon R, Vidal-Carou MC. 2008. Biogenic amines in traditional fermented sausages produced in selected European countries. Food Chem 107:2, 912-921.

Latorre-Moratalla ML, Bover-Cid S, Talon R, Garriga M, Zanardi E, Iaieri A, Fraqueza MJ, Elias M, Drosinos EH, Vidal-Carou MC. 2010. Strategies to reduce biogenic amine accumulation in traditional sausage manufacturing. LWT-Food Sci Technol 43:20-25.

Papavergou EJ. 2011. Biogenic amine levels in dry fermented sausage produced and sold in Greece. Procedia Food Sci 1: 1126-1131.

Papavergou EJ, Savvaidis IN, Ambrosiadis IA. 2012. Levels of biogenic amines in retail market fermented meat products. Food Chem 135:2750-2755.

Roseiro LC,Gomes A, Gonçalves H, Sol M, Cercas R, Santos C. 2010. Effect of processing on proteolysis and biogenic amines formation in a Portuguese traditional dry-fermented ripened sauage “*Chouriço Grosso* de Estremoz e Borba PGI”. Meat Sci 84:172-179.

Suzzi G, Gardini F. 2003. Biogenic amines in dry fermented sausages: a review. Int J Food Microbiol 88:41-54.

Tasic T, Ikonic P, Mandic A, Jokanovic M, Tomovic V, Savatic S, Petrovic L. 2012. Biogenic amines content in traditional dry fermented sausage *Petrovska klobasa* as possible indicator of good manufacturing practice. Food Control 23:107-112.

Tosukhowong A, Visessanguan W, Pumpuang L, Tepkasikul P, Panya A, Valyasevi R. 2011. Biogenic amine formation in Nham, a Thai fermented sausage, and the reduction by commercial starter culture, *Lactobacillus plantarum* BCC 9546. Food Chem 129:846-853.