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Biogenic Amine Levels of *Bez Sucuks* – a Type of Traditional Fermented Beef Sausage

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Abstract: In this study, the determination of biogenic amine levels of bez sucuks was aimed. For this purpose, sucuk samples were obtained from 12 different manufacturers mainly consisting of butchers, and, the levels of tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine were measured. It was seen that putrescine, cadaverine, histamine, tyramine and spermine levels of sucuks were in the ranges of 0.97-15.77 mg/kg, 0.53-9.03 mg/kg, 0.25-19.56 mg/kg, 64.11-275.81 mg/kg and 96.39-364.14 mg/kg, respectively. Although spermidine was not detected in one of the groups obtained from butchers, spermidine levels of other groups were between 8.06 mg/kg and 103.26 mg/kg. It was determined that tryptamine levels of sucuk samples ranged between 3.54 mg/kg and 32.76 mg/kg, except the group obtained from the butcher. Although histamine and cadaverine levels of bez sucuks were low, one of the groups obtained from small scaled facility had a quite high level of spermidine known as possible precursors of carcinogenic *N*-nitrosoamines.

Keywords: fermented beef sausage, good manufacturing practice, biogenic amines, histamine, putrescine.

1. Introduction

Bez sucuk, a type of Turkish fermented sausage is manufactured by traditional methodologies without adding starter culture. The butchers and small scaled facilities are the main manufacturers of the product (Kaval *et al*. 2010). The formulation of bez sucuk mixtures generally consists of ground meat and sheep tail fat/beef carcass fat, and curing reagents (used only by small scaled facilities), with various spices including cumin, garlic, salt, allspice, red pepper and black pepper. The production of bez sucuk starts with mixing of all ingredients and stuffing the mixtures into cloth casings, and then, sucuk batones are hung for fermentation and drying stages for 10-14 days (Polat 2010). The size of cloth casings is generally 7x25 cm and prepared from noncolored cloth containing 42 threads per cm^2. The butchers start selling the product after filling stage, while the small scaled facilities sell the product after ripening period (Kaval *et al*. 2010; Polat 2010). Many researchers indicated that biochemical and microbiological properties of bez sucuks showed differences between the manufacturers due to variations in formulation and ripening conditions such as time, relative humidity and temperature (Kaval *et al*. 2010; Turhan *et al*. 2010). During the ripening period of fermented sausages such as bez sucuks, various microbiological, physical and chemical changes occur, and these degradation reactions have effects on both the final product quality and safety. Thus, the proteolysis reactions especially result in increases in the amount of free amino acids which play an important role for the flavor formation of the product; on the other hand, free amino acids are also the precursors of biogenic amines (Papavergou 2011).

Biogenic amines are nitrogenous compounds which can be found in several foods, especially in fermented sausages (Suzzi and Gardini 2003; Tosukhowong *et al*. 2011; De Mey *et al*. 2014), and these compounds are formed by decarboxylation of free amino acids during

fermentation and ripening periods of fermented sausages (De Mey *et al*. 2014). Thereby, salt content, process conditions, formulation, starter culture utilization and the microbiological quality of raw material influence the levels of biogenic amines in fermented sausages (Bover-Cid *et al*. 2001a,b,c; Suzzi and Gardini 2003; Bozkurt and Erkmen 2007; De Mey *et al*. 2014). It is known that biogenic amines have direct toxicological effects, and in addition, putrescine, cadaverine, spermine and spermidine are the precursors of the carcinogenic *N*-nitrosoamines during the heating of meat products (De Mey *et al*. 2014).

Many studies conducted on determination of biogenic amine levels of Turkish sucuks (Ayhan *et al*. 1999; Bozkurt and Erkmen 2002; Çolak and Uğur 2002; Ekici *et al*. 2004; Bozkurt and Erkmen 2004; Erkmen and Bozkurt 2004; Bozkurt and Erkmen 2007). However, biogenic amine levels of bez sucuks was not investigated before the current research. Thus, the production and the consumption ratios of this product showed increases in the last decade, from the food safety points of view, the determination of biogenic amine levels of bez sucuks was aimed in this study.

2. Materials and methods

2.1. Materials

Bez sucuk samples were obtained from 12 different manufacturers consisting of 10 butchers and two small scalled facilities at each sampling period (sampling was planned according to the different production periods of each manufacturer) and the samples collected from butchers were coded as 2^{nd}, 3^{rd}, 5^{th}, 6^{th}, 7^{th}, 8^{th}, 9^{th}, 10^{th}, 11^{th} and 12^{th} group while the bez sucuk samples obtained from small scaled facilities were named as 1^{st} and 4^{th} group.

2.2. Methods

2.2.1. pH and titratable acidity values

The pH of the samples was determined using an Orion 420A pH-meter and sample slurries were then titrated with 0.1 N NaOH to an endpoint of pH 8.30 to measure the titratable acidity (TA) values. The meq of NaOH were converted to and expressed as percent lactic acid (Candoğan 2000).

2.2.2. Water activity values

The water activity (a_w) values of the samples were measured by using AquaLab Series 3 TE model (USA) at each sampling stages (Çiçek *et al*. 2014).

2.2.3. Biogenic amine levels

The chromatographic method of Bozkurt and Erkmen (2002) was used for the determination of the biogenic amines. The HPLC system consisted of PerkinElmer Series 200 model UV/VIS Dedector (USA), PerkinElmer Series 200 model pump (USA) and PerkinElmer Series 200 model Peltier column oven (USA). The HPLC column was Waters Spherisorb® 10.0 µm, ODS2 (4.6mmx200mm) (USA). A gradient elution programme was used with mobile phases of acetonitrile (solvent A) 0.1 M ammonium acetate (solvent B). The flow rate was 1 ml/min. The standard solution of the dansylated derivatives of tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine (SIGMA) was diluted to 1 ml with 0.4 perchloric acid to give concentratios from 0.5 to 10 mg/ml.

2.2.4. Statistical analysis

The data were statistically analyzed by using the statistical package SPSS 20.0 (International 154 Business Machines Corporation [IBM] Armonk, NY, USA). Mean values for bez sucuk manufacturers 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).

3. Results and Discussions

The pH, titratable acidity (TA) and a_w values of sucuk groups were in the ranges of 5.08 - 5.66, 1.02 - 2.25% lactic acid and 0.843 - 0.958, respectively (Table 1). Similar pH, TA and a_w values were noted by Kaval *et al*. (2010) and Turhan *et al*. (2010). In this study, the highest and the lowest a_w values were measured in the 3^{rd} and

8th groups, respectively, which were obtained from butchers. It was seen that 6th group had the highest pH value while the lowest pH was measured in the 9th group (P<0.05). On the other hand, the lowest TA was determined in the 2nd group (P<0.05). It is known that fermented sausages containing high amounts of protein with low pH values are one of the sources of biogenic amines (Papavergou *et al*. 2012). It was stated by many researchers that the acidic pH of the fermented sausages improves the amino acid decarboxylase activity of bacteria (Bover-Cid *et al*. 2001c; Suzzi and Gardini 2003; Latorre-Moratalla *et al*. 2010; Papavergou *et al*. 2012). [Table 1]

Table 1. The pH, titratable acidity (TA) and water activity (a_w) values of bez sucuks*
Çizelge 1. *Bez sucukların pH, titrasyon asitliği (TA) ve su aktivitesi (a_w) değerleri**

Manufacturer	pH	TA (lactic acid%)	a_w
1	5.18±0.35^{abc}	2.04±0.23^{a}	0.912±0.007^{c}
2	5.56±0.37^{abc}	1.02±0.25^{d}	0.936±0.010^{abc}
3	5.47±0.59^{abc}	1.04±0.38^{cd}	0.958±0.012^{a}
4	5.29±0.49^{abc}	1.89±0.26^{ab}	0.925±0.017^{abc}
5	5.25±0.21^{abc}	1.11±0.17^{cd}	0.953±0.005^{ab}
6	5.66±0.33^{a}	1.14±0.19^{cd}	0.926±0.029^{abc}
7	5.36±0.13^{abc}	2.25±0.95^{a}	0.939±0.024^{abc}
8	5.64±0.19^{ab}	2.11±0.15^{a}	0.843±0.054^{d}
9	5.08±0.09^{c}	1.70±0.34^{abc}	0.929±0.019^{abc}
10	5.33±0.42^{abc}	1.29±0.35^{bcd}	0.957±0.007^{a}
11	5.16±0.19^{abc}	1.63±0.73^{abcd}	0.952±0.003^{ab}
12	5.14±0.17^{bc}	1.69±0.28^{abcd}	0.918±0.024^{bc}

***** *Data are the mean ± standard deviation (n=6)*

$^{a, b, c, d}$ Means in a column not having a common superscript letter are different (P<0.05).

The presence of biogenic amines is undesirable, because of their toxicological effects to consumers such as hypertension, headache and diarrhea (Ayhan *et al*. 1999; Gençcelep 2006). The microbial activity, pH value, proteolysis, the quality of raw material, contamination, and, the conditions of processing and storage also have effects on the biogenic amine formation in fermented meat products (Bozkurt and Erkmen 2007). Thus, the unstandardized manufacturing conditions especially spontaneous fermentation stage may affect the biogenic amine formation and the levels in bez sucuks. In this research, to evaluate the biogenic amine amounts of bez sucuks tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermine and spermidine levels were measured (Table 2). Tryptamine levels of bez sucuks were in a range of 5.45-32.76 mg per kg sample except the 3rd group in which tryptamine was not detected. While the highest tryptamine level was measured in the 12th group produced by butcher, the tryptamine levels of 1st and 4th groups manufactured by small scalled facilities were 11.41 and 11.20 mg per kg sample, respectively. Şenöz *et al*. (2000) reported that the tryptamine levels of Turkish sucuks produced with or without starter culture were between 0.00 and 46.80 mg/kg and between 25.00 and 619.00 mg/kg, respectively. On the other hand, Gençcelep (2006) stated that the tryptamine was not detected in Turkish sucuks produced with starter culture, while the tryptamine levels of sucuks produced without starter culture were in a range of 123.45-127.70 mg/kg which were higher than the bez sucuk groups that fermented by natural microflora. Similarly, Bover-Cid *et al*. (2001c) also indicated that the tryptamine was detected

only in fermented sausage produced by spontaneous fermentation. In another study, Erkmen and Bozkurt (2004) observed the tryptamine levels of 50 different Turkish sucuks, and, the researchers determined that the tryptamine levels of sucuks were between 0.00 and 126.90 mg/kg.

Phenylethylamine was measured in the groups of 6, 7, 8 and 11 as 5.16, 16.10, 7.88 and 4.23 mg/kg, respectively (Table 2). The 7th group had the highest phenylethylamine levels while the 11th group had the lowest level (P<0.05). Ayhan *et al*. (1999) reported that the toxicity level of phenylethylamine was 30 mg/kg in foods, thus, the phenylethylamine levels of bez sucuks in our study were lower than the limit stated by the researchers. Bover-Cid *et al*. (2001a) detected phenylethylamine only in sulphite added fermented sausage group during the ripening period, in another study of the researchers, they reported that only spontaneously fermented sausages contain phenylethylamine (Bover-Cid *et al*. 2001c). Ansorena *et al*. (2002) also indicated that phenylethylamine was not detected in the fermented sausage group manufactured with *Staphylococcus carnosus*. Bozkurt and Erkmen (2002) and Gençcelep (2006) also demonstrated similar effects of starter culture utilization on phenylethylamine formation in Turkish sucuks. In contrary to the results of these researchers, phenylethylamine was detected in some of the bez sucuk samples produced without starter culture.

Although the putrescine levels of bez sucuks produced by butchers were between 0.97 and 15.77 mg/kg (P<0.05), putrescine levels of 1st and 4th groups manufactured by small scalled facilities were 1.19 and 8.94 mg/kg, respectively (Table 2). The putrescine levels of sucuk samples obtained from small scaled facilities showed that utilization sodium nitrite did not have a decreasing effect on putrescine level. However, Suzzi and Gardini (2003) stated that the using sodium nitrite reduced the putrescine level of Italian fermented sausages. On the other hand, Ayhan *et al*. (1999), noted that utilization of starter culture mix which consist of *Lactobacillus sakei*, *Pediococcus pentasaceus*, *Staphyloccus carnosus* and *Staphylococcus xylosus* limited the putrescine formation in Turkish sucuks, thus, many researchers found a noticeable decreasing effect of starter culture on putrescine levels of fermented sausages (Bover-Cid *et al*. 2001a; Gençcelep 2006).

Among the bez sucuk groups, the lowest cadaverine level (0.53 mg/kg) was obtained from 1st group manufactured by one of the small scalled facilities. The cadaverine levels of bez sucuks produced by the butchers were between 0.76 and 9.03 mg/kg (P<0.05) (Table 2). In agreement with the results of the current study, Çolak and Uğur (2002) indicated that cadaverine level of Turkish sucuk was 4.30 mg/kg at the beginning and reached to a level of 10.10 mg/kg at the end of the ripening stage. Gençcelep (2006) studied different starter cultures and different levels of sodium nitrite to produce Turkish sucuks, the researcher noted that both presence of starter culture and the increasing amount of sodium nitrite had a decreasing effect on cadaverine level. Bozkurt and Erkmen (2004) studied the effects of different ripening temperature, relative humidity, and additives on the biogenic amine amounts of Turkish sucuks produced with the starter culture mixture consisting of *Pediococcus acidilactici*, *Lactobacillus plantarum* and *Staphylococcus carnosus*. The researchers reported that none of the sucuk groups contained cadaverine.

In the present study, the histamine levels of bez sucuks were in the range of 0.25-19.56 mg/kg, while the 1st and 4th groups manufactured by small facilities had the histamine levels of 0.25 and 1.75 mg/kg (P>0.05), respectively (Table 2). While the 1st, 7th and 8th sucuk groups had the highest TA values, the highest histamine levels were obtained from the 7th and the 8th groups.

Table 2. Biogenic amine levels of bez sucuks (mg/kg)*
Çizelge 2. *Bez sucukların biyojenik amin seviyeleri (mg/kg)**

Manufacturer	Tryptamine	Phenylethylamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine
1	11.41± 7.89^{abc}	ND	1.19± 0.34^{c}	0.53± 0.19^{c}	0.25± 0.18^{b}	88.73± 1.44^{bc}	103.26± 26.73^{a}	364.08± 81.49^{a}
2	11.20± 17.36^{abc}	ND	6.16± 8.66^{bc}	2.37± 2.47^{bc}	0.32± 0.08^{b}	120.03± 82.24^{abc}	ND	316.12± 20.34^{ab}
3	ND	ND	0.97± 1.15^{c}	1.78± 1.17^{c}	0.46± 0.21^{b}	64.11± 44.42^{c}	8.06± 12.55^{cd}	248.35± 23.37^{bc}
4	15.37± 12.13^{abc}	ND	8.94± 8.77^{abc}	0.95± 0.28^{c}	1.75± 2.15^{b}	112.67± 74.97^{abc}	93.93± 72.77^{ab}	197.07± 126.54^{cd}
5	10.07± 8.09^{bc}	ND	1.21± 0.44^{c}	1.73± 0.59^{c}	1.18± 1.20^{b}	127.86± 15.63^{abc}	99.47± 4.43^{ab}	274.33± 10.85^{abc}
6	5.45±6.06^{bc}	5.16±8.06^{b}	8.85±6.59^{abc}	3.29±2.47^{bc}	1.67±2.06^{b}	138.64±77.02^{abc}	42.24±32.99^{cd}	364.14±19.54^{a}
7	20.42± 23.99^{abc}	16.10± 24.64^{a}	5.86± 2.52^{bc}	2.97± 1.21^{bc}	19.56± 16.01^{a}	275.39± 137.17^{a}	42.86± 23.02^{cd}	202.95± 71.58^{cd}
8	20.98± 32.50^{abc}	7.88± 10.45^{ab}	15.77± 12.17^{a}	9.03± 7.69^{a}	18.06± 13.68^{a}	257.10± 193.78^{ab}	54.83± 50.29^{bc}	198.17± 131.98^{cd}
9	3.54± 5.10^{bc}	ND	1.38± 0.60^{c}	1.07± 0.40^{c}	0.50± 0.24^{b}	275.81± 293.44^{a}	45.28± 19.95^{cd}	240.91± 37.88^{bc}
10	25.39± 20.04^{ab}	ND	13.71± 10.26^{ab}	5.83± 4.85^{ab}	1.35± 0.68^{b}	166.17± 76.89^{abc}	55.12± 41.65^{bc}	124.42± 103.49^{de}
11	19.61± 16.04^{abc}	4.23± 6.55^{b}	7.63± 9.49^{abc}	2.75± 1.57^{bc}	7.45± 5.78^{b}	187.37± 127.25^{abc}	44.31± 39.40^{cd}	102.50± 62.91^{e}
12	32.76± 5.44^{a}	ND	3.86± 1.96^{c}	0.76± 0.68^{c}	3.02± 3.76^{b}	147.29± 100.44^{abc}	26.11± 42.22^{cd}	96.39± 25.60^{e}

** Data are the mean ± standard deviation (n=6)*
ND: Not detected
$^{a, b, c, d, e}$ *Means in a column not having a common superscript letter are different (P<0.05)*

This could be explained by the utilizing curing agents in the production of 1st group that has a decreasing effect on biogenic amine formation. Bover-Cid *et al*. (2001c) also noted that acidic conditions of fermented sausages increased the biogenic amine formation. Ayhan *et al*. (1999) stated that histamine levels of 8-40 mg/kg may cause slight poisoning, thus, the histamine levels of 7th and 8th groups were in this range. Ekici *et al*. (2004) reported that the histamine levels of Turkish sucuks obtained from 5 different manufacturers were between 19.64 and 87.47 mg/kg. Erkmen and Bozkurt (2004) indicated that the histamine levels of 50 different Turkish sucuk samples were ranged from 1.50 to 478.20 mg/kg. Bozkurt and Erkmen (2004) noted that the histamine levels of Turkish sucuks that they produced were between 0.00 and 242.20 mg/kg.

Many researchers noted that the most important biogenic amine detected from fermented sausages is tyramine (Ayhan *et al*. 1999; Bozkurt and Erkmen 2002; Çolak and Uğur 2002; Gençcelep 2006; Şenöz *et al*. 2000). While the tyramine levels of bez sucuks were in the range of 64.11-275.81 mg/kg ($P<0.05$), it was determined that highest tyramine levels were obtained from the bez sucuk groups such as 7th, 8th and 9th groups with high TA values (Table 2). Ayhan et al. (1999) noted that tyramine contents over 100 mg/kg may cause migraine. It was seen that only the groups of 1 and 3 had tyramine levels lower than 100 mg/kg. Şenöz et al. (2000) indicated that the tyramine levels of sucuks were ranged from 125 to 1173.28 mg/kg, while Gençcelep (2006) reported that tyramine levels of the sucuks without starter culture were in a range of 76.80-157.00 mg/kg.

Altough spermidine was not detected in the 2nd group, spermidine levels of other groups were between 8.06 and 103.26 mg/kg ($P<0.05$) (Table 2). Bozkurt and Erkmen (2002) reported lower spermidine levels of sucuks that ranged from 10.18 to 18.64 mg/kg, while Gençcelep et al. (2007) indicated that the spermidine levels of sucuks were between 13.34 and 14.55 mg/kg.

Bez sucuks contained higher levels of spermine in comparison with spermidine levels. Many researchers also noted similar results (Bover-Cid *et al*. 2001b, Çolak and Uğur 2002; Bozkurt and Erkmen 2004; Gençcelep 2006; Gençcelep *et al.* 2007). Thus, spermine and spermidine can be present at high levels in the fresh meat (Suzzi and Gardini 2003; Papavergou 2011). The spermine levels of bez sucuks were in a range of 96.39-363.14 mg/kg (Table 2). The highest spermine levels were measured in 1st and 6th groups obtained from small scaled facility and butcher, respectively, while the 12th group had the lowest spermine level ($P<0.05$). Furthermore, the high levels of spermine could be a risk especially in the 1st group containg curing agents due to formation of *N*-nitrosocompounds.

4. Conclusions

Bez sucuks are traditionally produced by spontaneous flora which might produce biogenic amines. Although, some of these nitrogenous compounds especially cadaverine and histamine have been proposed as chemical indicators of the hygienic conditions of both raw material and manufacturing process, it was seen that histamine and cadaverine levels of bez sucuks were low. On the other hand, 1st group which contained curing agents and produced by small scaled facility had quite high level of spermidine that can be precursor of *N*-nitrosoamines. Furthermore, among the manufacturers the levels of biogenic amines showed a wide variety. This could be the result of selling strategies of butchers, the different production practices of manufacturers and the quality of raw materials. The results of the study indicated that adoption of good manufacturing practices and utilizing standard bez sucuk formulation (starter culture, curing agents)/process conditions are necessary in order to ensure the safe consumption of bez sucuks with respect to their biogenic amine levels.

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