e-ISSN: 2757-8836
ORIGINAL ARTICLE
J. Raw Mater. Process. Foods vol.2(2) (2021) 63-72

Recieved: 15/10/2021 Revised: 16/12/2021 Accepted article published: 30/12/2021 Published online: 31/12/2021

Impact of Proteolytic Enzymes on Formation of Biogenic Amine In Sucuk During the Storage Period

Songül Şahin Ercan*, Hüseyin Bozkurt, Çiğdem Aykaç

Department of Food Engineering, Faculty of Engineering, University of Gaziantep, University Avenue, 27310 Gaziantep, TURKEY.
*Correspondence:
Songül Şahin Ercan
E-mail adress:
sosahin@gantep.edu.tr
ORCID: 0000-0003-1630-7552

Abstract

Reduction and/or control of biogenic amine formation in sausage is an important subject due to its undesirable effects on human health and quality of foods. There has been no research regarding the effect of proteolytic enzymes on biogenic amines in foods. Reduction of biogenic amine formation in sucuk (Turkish dry fermented sausage) by proteolytic enzymes (trypsin and chymotrypsin) is a novel study. Besides biogenic amine, some quality (pH, colour and texture) and safety (thiobarbituric acid reactive substances (TBARS)) parameters of sucuk were investigated. Trypsin and chymotrypsin enzymes reduced ($P \leq 0.05$) biogenic amine formation significantly. However, in most times use of trypsin and chymotrypsin enzymes together result in the highest reduction effect on biogenic amine formation. Approximately 70% reduction in histamine and 47% reduction in tyramine were observed at the end of the storage period. It was observed that trypsin and chymotrypsin enzymes have significant effect ($P \leq 0.05$) on pH, thiobarbituric acid reactive substances, colour and texture. These findings emphasized that application of trypsin and chymotrypsin enzymes in sucuk was found to be effective in reducing biogenic amines formation.

Keywords: Biogenic amine, chymotrypsin, storage, sucuk, trypsin.

INTRODUCTION

Sucuk is Turkish dry fermented sausage. It is consumed in large amount in Turkey. Sucuk can be produced by factory (under controlled conditions: temperature and relative humidity (%RH)) and also it can be produced by butchers under uncontrolled conditions. It is commonly produced from the mix of beef and/or sheep meat and spices (Bozkurt and Erkmen, 2007).

Biogenic amines are nitrogenous compounds and produced by decarboxylation reactions (Ercan et al., 2013). Biogenic amine formation depends on presence of free amino acids, sufficient microorganism that can produce amino acid decarboxylases and also concentration of decarboxylase enzymes (Suzzi and Gardini, 2003). Some other factors influences biogenic amine production are manufacturing processes, storage conditions, the proportion of the microbial population with decarboxylase activity, manufacturing practices, the availability of free amino acids and raw material quality (Naila et al., 2010). Cadaverine, histamine, tyramine, tryptamine, phenylethylamine, spremidine and spermine are the most detected biogenic amines in fermented sausages (Suzzi and Gardini, 2003; Lu et al., 2010). Fermentation is favourable condition for biogenic amine formation. The presence of biogenic amine has been used as an indicator of quality and/or acceptability in some foods. Intake of foods containing high concentrations of certain biogenic amine can cause health hazard through the direct toxic effect of these compounds and their interaction with some medicaments (Sahin-Ercan et al., 2016).

Histamine poisoning, is known as scombroid poisoning, is an important problem. (Naila et al.,

2010). Nout (1994) pointed out that maximum histamine content is 50-100 mg/kg for sausages. The allowable maximum level of tyramine in foods is 100-800 mg/kg and 1,080 mg/kg of tyramine is toxic for humans (Shalaby, 1996). It was reported oral toxicity levels for putrescine, cadaverine and tryptamine were 2000 mg/kg and also spermidine and spermine 600 mg/kg (Naila et al., 2010).

Over the last decades, enzymes are used by food researchers mainly for acceleration of sausage fermentation. Acceleration of fermentation reduces the cost of storage that is needed for optimum maturation. Lipases and proteases are mainly used for acceleration of fermentation (Fernandez et al., 2000). In 1980, it just studied with rats and inhibitory effect of trypsin and chymotrypsin on decarboxylase activity was reported (Yamada et al., 1980). Also, proteolytic enzymes on biogenic amine formation were studied in model system (Sahin-Ercan et al., 2016). However, there have been no research about the effect of any enzyme and also, proteolytic enzymes on biogenic amine formation in foods. The aim of this study was to determine the effect of proteolytic enzymes (trypsin and chymotrypsin) on biogenic amine and also some other quality (pH, texture and colour), and safety (thiobarbituric acid reactive substances) properties of sucuk during the storage.

2. MATERIALS AND METHODS

2.1. Materials

Biogenic amine standards (histamine dihydrochloride, tyramine hydrochloride, cadaverine dihydrochloride, tryptamine hydrochloride, and putrescine dihydrochloride), 2-thiobarbituric acid, dansyl chloride, sodium hydroxide, 1,1,3,3-tetraethoxypropane (TEP), chymotrypsin, trypsin and acetone, were provided by Sigma (St. Louis, MO). sodium bicarbonate, 25% ammonium, sodium nitrite and sodium nitrate were obtained from Merck (Darmstadt, Germany) and perchloric acid were obtained from J.T. Baker (Holland).

Starter culture (BFL-F02 BactoFlavor: Chr. Hansen, Melbourne, Australia), was a mixture of *Staphylococcus carnosus* and *Pediococcus pentosaceus*, obtained from local sucuk producer.

2.2. Sucuk preparation

Sucuk dough was prepared by mixing of beef, tail fat, spices, salt, sugar, starter culture, olive oil and clean dry garlic according to the formula of Bozkurt and Bayram (2006) and held for 12 hour at 0-4°C. After mixing, trypsin and chymotrypsin enzymes were added to dough and sucuk samples were named according to their formulation (Table 1.). After that, sucuks were stuffed into 38 mm of artificial collagen casings (Naturin, Germany) and ripened under conditions represented in Table 2 and then stored at 10°C for 90 days.

Table 1. Composition of the sucuk samples

Sample	Trypsin (g/kg)	Chymotrypsin (g/kg)
TS	0.5	-
CS	-	0.5
TCS	0.5	0.5
Control	-	-

TS: Trypsin added sucuk; CS: Chymotrypsin added sucuk; TCS: mixed of enzyme added sucuk.

2.3. Sampling

Sucuks were ripened during 24 days (Table 2) and then storage period was started at 25th day. "0" day at storage period refers to 25th day of process. Samples were taken at 0, 15th, 30th, 60th and 90th days of storage for analysis. Changes of biogenic amine (histamine, cadaverine, tryptamine and tyramine, putrescine), pH, TBARS, textural attributes (Texture Profile Analysis, TPA) and colour (Hunter L, a and b) were followed during the storage period. For TPA and Hunter colour analysis, 2 cm long cut samples were used, and other analyses were carried out with homogenized samples prepared by use of Waring blender.

Table 2. Ripening conditions of the sucuk samples

Time (days)	Temperature (°C)	Relative humidity (%)
0-2	26	90
3-4	24	85
5-6	22	80
7-9	20	76
10-12	20	72
13-15	18	68
16-18	18	64
19-24	18	60

2.4. Biogenic amines

The chromatographic method was used for the determination of the biogenic amines (Eerola et al., 1993). The HPLC consisted of a Shimadzu gradient pump (Shimadzu LC 20AB, Shimadzu Solvent Delivery Module, Kyoto, Japan), a Shimadzu auto injection unit (Shimadzu SIL20AHT, Kyoto, Japan), a Shimadzu ultra violet (UV) detector (Shimadzu SPD 20A, Kyoto, Japan) and a RP-18 guard column, a SIL-20A HT auto sampler, and using a Shimadzu LC solution program (Ver. 1.25). The wavelength of UV detector was 254 nm. The HPLC column was Spherisorb ODS-3, 10 µm, 4.6x200 mm, (Inertsil, ODS-3). 0.4 M ammonium formate solution, acetonitrile were used for LC mobile phases. The flow rate was 1.0 mL/min. Acetonitrile (solvent A) and 0.4 M ammonium formate (solvent B) were used in a gradient elution program that was starting with 50% solvent A and 50% solvent B and finishing with 90% solvent A and 10% solvent B after 35 min.

2.5. pH and TBARS (2-thiobarbituric acid reactive substances) Analyses

10 g sucuk was homogenized and mixed with deionized water (90 mL) and then pH values were measured by pH meter (Jenway 3010; Essex, UK). Methods of Bozkurt (2007) was used for the thiobarbituric acid reactive substances (TBARS) .1,1,3,3-tetraethoxypropane (TEP) solutions was used for the standard curve to calculate TBARS values of sucuks and defined as mg malondialdehyde (ma) per kg product (mg ma/kg product). Duplicate measurements were performed for both pH and TBARS value.

2.6. Colour

Hunter Lab ColorFlex (A60-1010-615 Model, Reston, VA) was used for measurement of Hunter L, a and b values. Black and white ceramic plates ($L_\circ$= 93.01, $a_\circ$ = -1.11, and $b_\circ$= 1.30) were used for standardization of instrument for each time. Illuminant D 65 10° observer was used. Samples were equilibrated at *room temperature and triplicate measurements were done.*

2.7. Texture Profile Analysis (TPA)

Sucuks were equilibrated at ~20°C (room temperature) and cut into cylinders (20±0.5 mm height and 20 mm diameter and peeled prior to analysis). Samples were analyzed by use of TA.XT2i Texture Analyzer (Stable Micro System Ltd., Surrey, UK) as explained by Bozkurt and Bayram (2006). An aluminum rectangular probe was used (5cm x 4cm). Test speed was 1mm/s; compression (strain) 25%; and 25 kg load cell. Duplicate measurements were performed.

2.8. Statistical analysis

SPSS 16.0 (SPSS Inc, Chicago, IL, USA) was used to perform one way ANOVA test ($P<0.05$) was performed for all parameters followed during the storage period as a function of time and sucuk samples used to determine significant differences at $P\leq0.05$. Also, Duncan's multiple range test was used to evaluate any significant differences due to the changes among time and sucuk samples for all parameters at storage period.

3. RESULTS AND DISCUSSION

3.1. pH

pH of enzyme added sucuks mostly increased ($P\leq0.05$) during the storage period (Table 3). However, this change was not significant ($P\geq0.05$) for control. Enzyme added sucuks had higher ($P\leq0.05$) pH values than control sucuk. It could be due to the proteolytic activity of trypsin and chymotrypsin. Diaz et al., (1993) were concluded

Table 3. Changes of pH and TBARS (mg ma/kg product) values of sucuks during storage period.

		Samples			
Storage Time (day)		TS	CS	TCS	Control
0	pH	7.23±0.01cdC	7.31±0.01dD	7.10±0.03cdB	6.80±0.04bA
15		7.26±0.01dC	7.32±0.03dD	7.18±0.01dB	6.80±0.01bA
30		7.18±0.04cC	7.09±0.01bB	7.0±0.02bB	6.60±0.07aA
60		6.95±0.01aB	6.99±0.01aB	6.92±0.03aB	6.60±0.07aA
90		7.07±0.02bB	7.25±0.13cC	7.08±0.5bcB	6.80±0.49bA
0	TBARS	2.64±1.42aA	2.97±0.19cA	3.34±0.7eA	1.56±0.06bA
15		2.54±0.7aAB	2.67±0.13bBC	3.22±0.2dB	1.54±0.03abA
30		2.51±0.7aB	2.53±0.36aAB	3.02±0.1cB	1.52±0.03abA
60		2.32±0.5abAB	2.44±0.47aBC	2.87±0.4bC	1.46±0.28aA
90		2.26±0.1aB	2.40±0.32bC	2.80±0.5aD	1.45±0.02aA

TS: Trypsin added sucuk; CS: Chymotrypsin added sucuk; TCS: mixed of enzyme added sucuk.
Different small letters indicate statistical difference at α = 0.05 level in each sample at different time in each parameter.
Different capital letters indicate statistical difference at α = 0.05 level among products at each time in each parameter.

concentration as a result of accelerated the proteolysis.that use of proteolytic enzymes caused high pH Also, it was explained that when proteinases are added to sausage an intense proteolysis can be achieved with an increase in different nitrogen fractions (water soluble non protein nitrogen total volatile basic nitrogen) (Fernandez et al., 2000). Fernando and Fox (1991) reported that pH of sausage stayed at high values due to the production of nitrogenous compounds which is a result of proteolysis.

3.2.2. Thiobarbituric acid reactive substances (TBARS) value

TBARS value is used for degree of lipid oxidation in meat and meat products. TBARS values of all sucuks decreased ($P \leq 0.05$) during the storage period (Table 3). These results were in agreement with the literature that TBARS values of sausage increased at the beginning of the fermentation and then decreased (Bozkurt, 2006, 2007; El Adab and Hassouna, 2016; Ferial et al., 2010; Sojic et al., 2015). Wojciak et al., (2015) were also explained that reaction of malondialdehyde with sugars, amino acids and other compounds could cause lower TBARS value during the storage period. It was explained that 3 mg/kg is limit for TBARS that indicates the oxidative rancidity of meat (Chouliara et al., 2008). In this study, none of samples did not exceed this value until the end of storage.

3.3. Colour

L- values of sucuks decreased significantly ($P \leq 0.05$) during the storage period (Table 4). Enzyme added sucuks had lower *L*- values than control sucuk. Decrease of *L*- values showed dark colour formation could be due to the browning reaction (Bozkurt, 2006). The *a*-values of all sucuk samples decreased significantly ($P \leq 0.05$) during storage period. Decrease of *a*-value could be due to the denaturation of nitrosomyoglobin. The order of *a*-value at the end of the storage period was control>TS>CS>TCS. The *b*-values decreased significantly ($P < 0.05$) during the storage period (Table 4). The decrease in *b* values indicated the color of sucuks turned to blue rather than yellow (Bozkurt and Bayram, 2006).

Table 4. Changes of Hunter values (L, a, b) during storage period.

Hunter parameters	Storage Time (days)	Control	TS	CS	TCS
L	0	36.45±2.62cB	34.21±0.78cAB	32.60±0.99dA	30.37±1.46cA
	15	34.95±2.47bcB	30.25±0.84bA	29.10±0.34cA	26.85±0.21bA
	30	29.70±1.69abB	29.05±1.96abB	27.70±0.14bB	24.15±0.37abA
	60	29.0±1.69aC	27.70±0.79abBC	25.30±0.07aAB	23.15±1.37aA
	90	28.85±1.62aB	27.20±0.62aAB	25.30±0.26aA	24.45±1.54bA
a	0	11.50±0.85cB	11.50±0.71dB	10.30±0.56dB	9.60±0.42dA
	15	10.95±0.77bcC	9.30±0.42cB	8.30±0.28cAB	7.35±0.07cA
	30	10.55±0.75abcB	7.50±0.45bA	7.10±0.63bA	6.15±1.20bcA
	60	9.60±0.56aC	6.60±0.28bB	5.55±0.35aA	5.30±0.14abA
	90	9.25±0.50aC	5.28±0.30aB	5.25±0.63aB	4.60±0.35aA
b	0	11.05±0.07eA	12.50±0.28bB	13.30±0.14dC	16.05±4.45dD
	15	8.35±1.06dAB	8.00±0.70aA	9.90±0.70cBC	11.30±0.42cC
	30	7.65±0.49cB	6.80±0.35aA	8.45±0.45bC	9.40±0.28bC
	60	6.95±0.08bA	6.40±1.06aA	6.65±0.63aA	7.45±0.49aB
	90	6.25±0.63aA	6.55±0.63aAB	6.90±0.84aAB	7.55±0.07aB

TS: Trypsin added sucuk; CS: Chymotrypsin added sucuk; TCS: mixed of enzyme added sucuk.
Different small letters indicate statistical difference at α = 0.05 level in each sample at different time in each color values.
Different capital letters indicate statistical difference at α = 0.05 level among products at each time in each color values.

Control sucuk had the lowest *b*-value than that of all other sucuks. The order of *b*-value at the end of the storage period was TCS>CS>TS>control.

3.4. Texture profile analysis

Hardness values of sucuks increased ($P \leq 0.05$) significantly during the storage period (Table 5). Also, similar increasing trend was reported by Kargozari, et al. (2014) and Bozkurt and Bayram (2006). During the storage period, enzyme added sucuks had lower hardness values than control. Order of the hardness values was control>TS>CS>TCS at the end of the storage period.

Chewiness and gumminess values of sucuks increased ($P \leq 0.05$) significantly during the storage period. Control sucuk had highest chewiness and gumminess values than those of enzyme added sucuks during the storage period. Order of chewiness and gumminess values of sucuks were control>TS>CS>TCS at the end of the storage period.

At storage period, adhesiveness values of sucuks were significantly affected by time and addition of enzyme. Adhesiveness values significantly ($P \leq 0.05$) increased during the storage period (Table 5). Cohesiveness values of sucuks increased significantly ($P \leq 0.05$) during the storage period. Enzyme added sucuks had lower cohesiveness values than control sucuk.

Table 5. Changes of textural properties of sucuks during storage period

Textural properties	Storage Time (days)	Control	TS	CS	TCS
Hardness (g)	0	4271.20±30	4700.46±547.2a	2522.04±178.34a	1061.63±75.07aA
	15	4260.0±549	4964.0±208.31a	2769.43±84.43aB	2315.90±137.2aA
	30	4715.20±44	5308.50±14.33a	3116.52±220.36aA	3231.90±228.53b
	60	5328.80±118.	5188.80±351.0ab	4915.07±347.55bB	3269.10±372.58bA
	90	5650.40±30	5548.5±199.40b	5022.80±355.16bB	3797.0±268.48bA
Adhesiveness(g	0	-	-195.44±6.38aB	-165.93±18.39aB	-69.84±4.94aC
	15	-	-185.70±1.15aB	-153.90±1.36bC	-48.15±1.85bD
	30	-	-175.85±0.33bB	-158,99±2.05bC	-51.33±1.37bD
	60	-	-183.14±9.41aB	-160.05±0.01aC	-60.96±0.14aD
	90	-	-184.50±2.80aB	-167.44±0.78aC	-59.03±1.36aD
Chewiness	0	1406.29±178.	469.0±35.9.9aA	310.43±21.96aA	352.30±24.91aA
	15	1522.25±40	403.70±45.53aA	354.69±25.08aA	431.81±30.53abA
	30	1958.21±212.	643.20±60.80bA	567.14±40.10aA	668.94±47.29bA
	60	2429.44±38	1860.0±131.51dB	1220.25±86.28bA	1142.91±10.15cA
	90	2135.41±327	1434.2±101.42cB	1296.26±91.66bA	1109.76±7.61cA
Gumminess	0	2904.41±25	1081.6±382.7aC	680.95±68.61aB	244.17±27.95aA
	15	3109.80±292	1489.2±70.51bC	941.60±42.98aB	717.92±49.21aA
	30	3630.70±6.6	1539.46±101.89b	872.62±62.90aA	969.57±70.25bA
	60	4263.04±121	1556.64±191.53b	1474.52±105.22bA	1144.18±13.26cA
	90	4576.82±33	1775.52±126.62b	1757.98±124.72bA	1366.92±9.75cA
Cohesiveness	0	0.68±0.01aB	0.23±0.02aA	0.27±0.04aA	0.23±0.01aA
	15	0.73±0.03bB	0.30±0.02bA	0.34±0.02bA	0.31±0.02bA
	30	0.77±0.04bc	0.29±0.01bA	0.28±0.02aA	0.30±0.01bcA
	60	0.80±0.02cB	0.30±0.02bA	0.30±0.02abA	0.35±0.02cdA
	90	0.81±0.04cB	0.32±0.02bA	0.35±0.03bA	0.36±0.03dA

TS: Trypsin added sucuk; CS: Chymotrypsin added sucuk; TCS: mixed of enzyme added sucuk.
Different small letters indicate statistical difference at α = 0.05 level in each sample at different time in each textural attributes.
Different capital letters indicate statistical difference at α = 0.05 level among products at each time in each textural attributes.

3.5. Biogenic amines

Cadaverine concentration increased (P≥0.05) at storage period (Table 6). Enzyme added sucuks had lower cadaverine concentration than control at the end of the storage period. Formation of cadaverine was reduced 46.09%, 28.14% and 13.08% by TCS, CS and TS, respectively (Tables 7). Result showed that use of trypsin and chymotrypsin enzymes together result in lowest cadaverine concentration. These founding was in agreement with the result of Sahin-Ercan, et al. (2016). On the other hand, some authors such as Lu, et al. (2010), Papavergou (2011), Papavergou et al., (2012), Ercan et al., (2013) and Latorre-Moratalla et al., (2008) reported the cadaverine concentrations in sucuk as 1435.24 mg/kg, 1014.08 mg/kg, 689.83 mg/kg, 129.0 mg/kg, and 610.96 mg/kg, (maximum cadaverine concentrations) for sausages. At the end of the storage period, obtained maximum cadaverine concentration (425.70 mg/kg,) was lower than most of these reported values.

Table 6. Changes of biogenic amine concentrations in sucuks during storage period

Biogenic amine (mg/kg)	Storage Time (days)	Control	TS	CS	TCS
Histamine	0	195.05±11.95aC	87.05±0.78bB	71.05±0.64bA	67.35±0.78bA
	15	218.80±8.06bB	76.80±3.81aA	66.0±0.56aA	60.30±1.76aA
	30	210.0±9.54bC	76.10±2.19aB	73.21±1.13bB	62.33±0.70aA
	60	205.0±1.83aC	79.20±4.80abB	74.80±1.34bB	65.61±0.56abA
	90	200.0±3.18aB	78.10±0.71aAB	73.60±0.28bA	64.86±1.55abA
Cadaverine	0	441.45±18.46aC	355.05±41.75aAB	305.90±11.52aA	229.45±18.51aA
	15	475.0±33.16aD	364.30±31.18aC	275.60±15.76aB	175.20±38.85aA
	30	480.0±64.27aC	361.50±29.76aBC	277.0±33.30aAB	175.70±40.72aA
	60	452.90±14.73aC	361.30±38.39aB	289.40±27.71aAB	188.10±18.38aA
	90	425.70±13.81aC	370.0±13.37aB	305.90±56.42aAB	229.50±75.87aA
Tyramine	0	620.25±7.57aC	332.70±27.27aC	258.55±0.7aA	222.80±23.62aA
	15	620.70±21.56aB	320.18±84.85aA	268.51±32.59aA	243.94±13.69aA
	30	615.50±20.50aC	382.91±60.1aB	290.86±80.53aA	283.56±19.30aA
	60	608.0±71.41aC	382.91±42.32aB	300.20±77.0aA	291.57±27.08aA
	90	600.60±40.51aC	398.91±72.61aB	315.56±33.23aA	322.68±34.01aA
Tryptamine	0	1108.15±80.82aD	392.15±48.86aA	508.40±56.99aB	784.0±26.92aC
	15	1160.20±23.90bD	420.0±48.86aA	539.40±39.31aB	849.20±6.01abC
	30	1180.0±99.63bC	464.30±36.55abA	566.40±68.09abA	924.30±10.26abcB
	60	1104.0±89.09aB	493.70±27.71bA	589.0±85.41bA	989.0±30.32bcB
	90	1108.15±95.95aB	557.70±79.54bA	632.90±84.14bAB	998.90±70.35cB
Putrescine	0	922.75±25.81bB	683.30±94.89aAB	620.55±195.09aAB	503.25±60.60abA
	15	958.70±24.53bC	735.50±12.86aB	665.50±19.94aAB	558.70±10.11abA
	30	862.60±18.95bD	752.0±18.38abAB	682.60±134.77abAB	573.30±10.33bA
	60	880.80±50.69bD	773.50±50.84bC	692.0±103.02bB	590.20±39.31bA
	90	798.50±1.06aC	789.40±126.14bC	725.0±117.94bB	612.20±8.83bA

TS: Trypsin added sucuk; CS: Chymotrypsin added sucuk; TCS: mixed of enzyme added sucuk.

Different small letters indicate statistical difference at α= 0.05 level in each sample at different time in each biogenic amine.

Different capital letters indicate statistical difference at α = 0.05 level among products at each time in each biogenic amine

.

Tyramine concentration of enzyme added sucuks increased (P≥0.05) during the storage period (Table 6). Vinci and Antonelli (2002) reported the increase of tyramine concentration of red meat during 36 days of storage. Tyramine concentration varied from 600.60 to 315.56 (mg/kg) at the end of the storage period. Concentration of tyramine of all sucuks were within the acceptable level at the end of the storage period (<800 mg/kg). Use of enzymes result in lower tyramine concentration. At the end of the storage period, CS had lowest TS had highest tyramine concentration amoung the enzyme added sucuks (Table 6). Tyramine formation was reduced 33.58%, 47.45% and 46.27% by TS, CS and TCS compared to control sucuk (Table 7). Reduction of tyramine formation is an important subject due to the toxic effects of tyramine.

Tryptamine is a biogenic amine found in sausages, formed from decarboxylation of tryptophan. Tryptamine concentration of TS, CS and TCS increased (P≤0.05) during the storage period. However, tryptamine concentration of control increased (P≤0.05) during the 30th days of storage and then decreased (P≤0.05). Control sucuk had the highest and TS had the lowest tryptamin concentration at the end of the storage period (Table 6) TS showed highest inhibitory effect on tryptamin formation (49.67%) and it followed up with CS (42.89%) at the end of the storage period (Table 7). Tryptamine concentration in the control sample increased up to the 30th days of storage and then decreased thereafter. Tryptamine decarboxylase enzyme activity could decrease after 30th days of storage. Also, it is probably that produced amines were consumed by microorganisms. Histamine concentration increased initially and then decreased (P≤0.05) for control during the storage period (Table 6). At the end of the storage period, it was observed that TS had 60.95% inhibitory effect on histamine formation and it is followed by 63.20% and 67.57% by CS and TCS, respectively (Table 7). Histamine concentrations of enzyme added sucuks were not exceed the values of health concern (above 100 mg/kg). In this study, histamine range of sucuks was 64.86-200.0 mg/kg. Hernandez-Jover et al., (1997) and Montel et al., (1999) explained that histamine concentration range change as 0-314 mg/kg in Spanish sausage and 16 to 151 mg/kg in French sausage, respectively..

Table 7. Percent reduction of biogenic amines concentrations of sucuks with respect to control at the end of the storage period

	Reduction (%)				
Samples	Histamine	Cadaverine	Tyramine	Tryptamine	Putrescine
TS	60.95	13.08	33.58	49.67	1.14
CS	63.20	28.14	47.46	42.89	9.20
TCS	67.57	46.09	46.27	9.86	23.33

TS: Trypsin added sucuk; CS: Chymotrypsin added sucuk; TCS: mixed of enzyme added sucuk

Putrescine is a polyamine that is formed from ornithine which is produced from arginine. Putrescine concentration of enzyme added sucuks increased significantly (P≤0.05) during the storage period (Table 6). Enzyme added sucuks had lower putrescine concentration than control sucuk. It was found that TCS result in the highest (23.33%) reduction effect and TS had the lowest (1.14%) reduction effect on putrescine (Table 7).

4. CONCLUSION

The results showed that trypsin and chymotrypsin enzymes effective in reduction (*P*≤0.05) of biogenic amine formation in sucuk during storage

period. However, mixed form of these enzymes as more effective in reduction than the separately used form. The highest reduction was observed in histamine (67.57%) by TCS. Higher pH, TBARS, Hunter *b*-values and also lower Hunter *L* and *a*-values were observed in enzyme added sucuks compared to control ($P \leq 0.05$). Therefore, our findings suggested that use of trypsin and chymotrypsin can be used to reduce biogenic amine formation. However further research is also needed on the impact of these enzymes on some safety and quality parameters of sucuk.

ACKNOWLEDGEMENT

We wish to thank to Scientific Research Projects Executive Council of University of Gaziantep (Project No: MF.10.03) for financial support.

REFERENCES

Bozkurt H. (2006). Utilization of natural antioxidants: Green tea extract and Thymbra spicata oil in Turkish dry-fermented sausage. *Meat Science,* 73(3), pp: 442-450.

Bozkurt H. (2007). Comparison of the effects of sesame and Thymbra spicata oil during the manufacturing of Turkish dry-fermented sausage. *Food Control,* 18(2), pp: 149-156.

Bozkurt H., & Bayram M. (2006). Colour and textural attributes of sucuk during ripening. *Meat Science,* 73(2), pp:344-350.

Bozkurt H., & Erkmen O. (2007). Effects of some commercial additives on the quality of sucuk (Turkish dry-fermented sausage). *Food Chemistry,* 101(4), pp: 1465-1473.

Chouliara E., Badeka A., Savvaidis I., & Kontominas M. G. (2008). Combined eVect of irradiation and modiWed atmospherepackaging on shelf-life extension of chicken breast meat:microbiological, chemical and sensory changes. *European Food Research and Technology,* 226, pp: 877-888.

Diaz O., Fernandez M., García de Fernando G. D., de la Hoz L., & Ordonez J. A. (1993). Effect of the addition of pronase E on the proteolysis in dry fermented sausages. *Meat Science,* 34(2), pp: 205-216.

Eerola S., Hinkkanen R., Lindfors E., & Hirvi T. (1993). Liquid chromatographic determination of biogenic amines in dry sausages. Journal of AOAC International, 76(3), pp: 575-577.

El Adab S., & Hassouna M. (2016). Proteolysis, Lipolysis and Sensory Characteristics of a Tunisian Dry Fermented Poultry Meat Sausage with Oregano and Thyme Essential Oils. Journal of Food Safety, 36(1), pp: 19-32.

Ercan S. Ş., Bozkurt H., & Soysal Ç. (2013). Safety and quality attributes of sucuk-like products made with mecanically deboned broiler/beef. *Journal of Food and Science and Engineering,* 3, pp: 246-254.

Ferial M., Salem A., & Ibrahim H. M. (2010). Dry fermented buffalo sausage with sage oil extract: Safety and quality. *Grasas Y Aceites,* 11, pp: 201-209.

Fernandez M., Ordonez J. A., Bruna J. M., Herranz B., & de la Hoz L. (2000). Accelerated ripening of dry fermented sausages. Trends in Food Science & Technology, 11(6), pp: 201-209.

García de Fernando G. D., & Fox P. F. (1991). Study of proteolysis during the processing of a dry fermented pork sausage. *Meat Science,* 30(4), pp: 367-383.

Hernández-Jover T., Izquierdo-Pulido M., Veciana-Nogues M. T., Marine-Font A., & Vidal-Carou M. C. (1997). Biogenic Amine and Polyamine Contents in Meat and Meat Products. *Journal of Agriculture and Food Chemistry,* (45), pp: 2098–2102.

Kargozari M., Moini S., Akhondzadeh Basti A., Emam-Djomeh Z., Ghasemlou M., Revilla Martin I., Gandomi H., Carbonell-Barrachina Á. A., & Szumny A. (2014). Development of Turkish dry-fermented sausage (sucuk) reformulated with camel meat and hump fat and evaluation of physicochemical, textural,

fatty acid and volatile compound profiles during ripening. *LWT - Food Science and Technology,* 59(2), pp: 849-858.

Latorre-Moratalla M. L., Veciana-Nogués T., Bover-Cid S., Garriga M., Aymerich T., Zanardi E., Ianieri A., Fraqueza M. J., Patarata L., Drosinos E. H., Lauková A., Talon R., & Vidal-Carou M. C. (2008). Biogenic amines in traditional fermented sausages produced in selected European countries. *Food Chemistry,* 107(2): 912-921.

Lu S., Xu X., Shu R., Zhou G., Meng Y., Sun Y., Chen Y., & Wang P. (2010). Characterization of Biogenic Amines and Factors Influencing Their Formation in Traditional Chinese Sausages. *Journal of Food Science,* 75, pp: 366-372.

Lu S. L., Xu X. L., Shu R. H., Zhou G. H., Meng Y., Sun Y. M., Chen Y. P., & Wang P. (2010). Characterization of Biogenic Amines and Factors Influencing Their Formation in Traditional Chinese Sausages. *Journal of Food Science,* 75(6), pp: M366-M372.

Montel M. C., Masson F., & Talon R. (1999). Comparison of biogenic amine content in traditional and industrial French dry sausages *International Journal of Food Microbiology,* 32, pp: 199-207.

Naila A., Flint S., Fletcher G., Bremer P., & Meerdink G. (2010). Control of biogenic amines in food: existing and emerging approaches. *Journal of Food Science and Engineering,* 75, pp: 139-150.

Nout M. J. R. (1994). Fermented foods and food safety. Food Research International, 27(3), pp: 291-298.

Papavergou E. J. (2011). Biogenic amine levels in dry fermented sausages produced and sold in Greece. *Procedia Food Science,* 1, pp: 1126-1131.

Papavergou E. J., Savvaidis I. N., & Ambrosiadis I. A. (2012). Levels of biogenic amines in retail market fermented meat products. *Food Chemistry,* 135(4), pp: 2750-2755.

Sahin-Ercan S., Bozkurt H., & Soysal C. (2016). Reduction of Cadaverine and Tyramine Formation by Proteolytic Enzymes in Model System. *International Journal of Food Properties,* 19(7), pp: 1465-1474.

Shalaby A. R. (1996). Significance of biogenic amines to food safety and human health. *Food Research International,* 29(7), pp: 675-690.

Sojic B., Tomovic V., Kocic-Tanackov S., Skaljac S., Ikonic P., Dzinic N., Zivkovic N., Jokanovic M., Tasic T., & Kravic S. (2015). Effect of nutmeg (Myristica fragrans) essential oil on the oxidative and microbial stability of cooked sausage during refrigerated storage. *Food Control,* 54, pp: 282-286.

Suzzi G., & Gardini F. (2003). Biogenic amines in dry fermented sausages: a review. *International Journal of Food Microbiology,* 88(1), pp: 41-54.

Şahin Ercan S., Bozkurt H., & Soysal Ç. (2013). Significance of Biogenic Amines in Foods and Their Reduction Methods. *Journal of Food Science and Engineering,* 3, pp:395-410.

Vinci G., & Antonelli M. L. (2002). Biogenic amines:quality index of freshness in red and white meat. *Food Control,* 13, pp: 519-524.

Wojciak K. M., Karwowska M., & Dolatowski Z. J. (2015). Fatty acid profile, color and lipid oxidation of organic fermented sausage during chilling storage as influenced by acid whey and probiotic strains addition. *Scientia Agricola,* 72, pp: 124-351.

Yamada M., Watanabe T., Harino S., Fukui H., & Wada H. (1980). The Effects of Protease Inhibitors on Histidine Decarboxylase Activities and Assay if Enzyme in Various Rat Tissues. *Biochimica et Biophysica Acta (BBA)—Enzyme,* 615, pp: 458–463.