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THE EFFECT OF DRIED MUSHROOM (*AGARICUS BISPORUS)* ADDITION ON MICROBIOLOGICAL QUALITY AND BIOGENIC AMINE CONTENTS IN SUCUK PRODUCTION

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ABSTRACT
The aim of this study, the effect of addition to mushroom into sucuk at different concentrations (0, 0.5, 1 and 2%) on microbiological quality and biogenic amine formation were investigated at different ripening periods (0, 3, 6, 9 and 12 days). Tryptamine and phenylethylamine were found 11.42 and 42.96 mg/kg beginning of ripening time, respectively. Both mushroom concentration and ripening period had significant (P <0.01) effect on lactic acid bacteria (LAB), *Micrococcus* and *Staphylococcus* counts and pH. The *Enterobacteriaceae,* yeast and mould counts were found to be under the detectable level (<log 2.0 cfu/g) after the 3rd day of ripening period. The pH value was determined as 5.82 at the beginning of ripening time and decreased to 4.82 at the end of ripening (12 days). These results suggest that mushroom can be used as a natural functional ingredient to improve taste and flavor as well as shelf-life stability of sucuk.
Keywords: Mushroom **(***Agaricus bisporus),* biogenic amines, microbiological properties, sucuk

SUCUK ÜRETİMİNDE KURUTULMUŞ MANTAR (*AGARICUS BISPORUS*) İLAVESİNİN MİKROBİYOLOJİK KALİTE VE BİYOJENİK AMİN İÇERİĞİ ÜZERİNE ETKİLERİ

ÖZ
Bu çalışmanın amacı, sucuk üretimine farklı oranlardaki (%0, 0.5, 1 ve 2) mantar (*Agaricus bisporus*) ilavesinin, sucuğun olgunlaşma dönemlerinde (0., 3., 6., 9. ve 12. gün) mikrobiyolojik kalitesi ve biyojenik amin oluşumu üzerine etkisini belirlemektir. Triptamin ve feniletilamin olgunlaşma başlangıcında sırasıyla 11.42 ve 42.96 mg/kg bulunmuştur. Hem mantar konsantrasyonu hem de olgunlaşma süresi, laktik asit bakterileri, *Micrococcus* ve *Staphylococcus* sayıları ve pH üzerinde önemli (P <0.01) etkiye sahiptir. Olgunlaşma döneminin 3. gününden sonra *Enterobacteriaceae*, maya ve küf sayıları tespit edilebilir seviyenin altında (<log 2.0 cfu / g) bulunmuştur. Olgunlaşma başlangıcında pH değeri 5.82 olup, olgunlaşmanın sonunda 4.82'ye düşmüştür (12. gün). Bu sonuçlar, mantarın tat ve lezzeti iyileştirmek için doğal bir bileşen olarak kullanılabileceğini ve ayrıca sucuğun raf ömrü stabilitesini artırabileceğini göstermektedir.
Anahtar kelimeler: Mantar *(Agaricus bisporus),* biyojenik amin, mikrobiyolojik özellikler, sucuk

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INTRODUCTION

Sucuk is the most popular dry-fermented meat product produced in Turkey. It is produced from beef and water buffalo meat, beef fat, and sheep tail fat incorporated with some ingredients; salt, sugar, nitrite, nitrate and/or nitrite/nitrate and various spices (Kaya, 1993; Gökalp et al., 1988, 1999; Kaya et al., 1998).

Mushrooms have long been valued as delicious and nutritional foods in many countries. They are appreciated, not only for their texture and flavour but also for their chemical and nutritional characteristics (Manzi et al., 1999). Mushrooms have been reported to contain many valuable benefits such as rich in dietary fiber, protein, vitamin and mineral while having low in fat and calorific value. On a dry weight basis, they are considered to be good sources of digestible proteins (10–40%), carbohydrates (3–21%) and dietary fibre (3–35%) (Breene, 1990; Chang, 1991). Besides this facts, bioactive functional component of cell wall known as β-glucan is also found in edible mushroom. The unique functionality of β-glucan is its contribution towards healthy characteristics in edible mushroom (Manzi and Pizzoferrato 2000).

Both fruiting body and the mycelium contain compounds having remarkable antioxidant and antimicrobial activity (Bobek et al., 1995; Uppuluri et al., 2006). Flavonoids have been proven to display a wide range of pharmacological and biochemical actions, such as antimicrobial, antithrombotic, antimutagenic and anticarcinogenic activities (Cook and Samman, 1996; Sahu and Green, 1997). Some researchers have reported the antimicrobial activity of several mushrooms (Lee et al., 1999; Kim and Funk, 2004; Gao et al., 2005). Several compounds extracted from these mushrooms were revealed to have antifungal and antibacterial activity (Barros et al., 2007) against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* (Barros et al., 2007). The chloroform and ethyl acetate extracts of the dried mushroom were reported to have antibacterial activity against *Streptococcus mutans* and *Prevotella intermedia* (Hirasawa et al., 1999).

Biogenic amines are important for the human health because of the fact that the consumption of food with high amounts of biogenic amine content, especially histamine and tyramine, can be hazardous due to its toxic effects. Besides, their toxicological effects, biogenic amines are of concern in relation to food hygiene. Biogenic amines are also important for their role as indicators of quality and/or acceptability in some foods (Ayhan et al., 1999; Ruiz-Capillas and Jimenes-Colmenero, 2004) High amounts of amines can be found in fermented foods derived from raw material with high protein content, such as dry and semi-dry fermented sausages (Suzzi and Gardini, 2003). The amount and type of biogenic amines depend on the nature of food and microorganisms. The production of biogenic amines in meat products has been attributed to the action of several microorganisms such as *Pseudeuomonas, Enterobacteriaceae, Enterococci* and *Lactobacillus* (Santos, 1996; Shalaby, 1996; Suzzi and Gardini, 2003; Kaya and Gökalp, 2004).

No study has determined on the effect of addition of mushroom on the microbiological composition and biogenic amine formation in sucuk product. Therefore, in this study, the effect of different levels of mushroom on microbiological characteristics and biogenic amines of sucuk were investigated in different ripening storage periods.

MATERIALS AND METHODS

Preparation of mushroom

Mushroom as fruiting bodies (pileus + stipe) were dried at room temperature (until reaching approximately 10 % water content), ground and then sifted through 14 in. sieve until usage.

Starter cultures and chemical substances

Starter culture (*Lactobacillus sakei*+*Staphlococcuc carnosus* spp. carnosus, BFL-FO6 BactoFlavor, Chr. Hansen, Holdorf/Germany) was added into sucuk mix 10^7cfu/g according to the manufacturer's recommendations. Six aqueous standard solutions containing putrescine dihydrochloride, cadaverine dihydrochloride, histamine dihydrochloride, tyramine hydrochloride, tryptamine hydrochloride,

phenylethylamine hydrochloride, 1.7-diaminoheptane (as internal standard) from Sigma (St. Louis, MO, USA) were derivatized as described for sucuk samples. Acetone, ammonia (25%) and acetonitrile (HPLC grade) from Merck (Darmstadt, Germany) and perchloric acid from Reidel DeHaen (Germany) were used in the HPLC analysis. Nitrite was used in the form of sodium nitrite (Merck, Darmstadt, Germany).

Sucuk formulation and processing

Sucuk samples were produced according to the formula described by AOAC, 1997, with the following modifications. The formulation was composed of 90% beef, 10% tail fat, 2.5% salt, 1.5% garlic, 0.5% sucrose, 0.7% red pepper, 0.5% black pepper, 0.9% cumin, 0.25% pimento. After beef and tail fats were cut into small pieces, they were mixed with the respective spice mixture in a mixer. The obtained sausage mixture was ground through a grinder machine and separated into four experimental batches each of which contained different levels of dried mushrooms (DM): (1) control group added with 0% DM, (2) sausage group added with 0.5% DM, (3) sausage group added with 1% DM and sausage group added with 2% DM. Nitrite ($NaNO_2$, 150 mg/kg) was added into each batch, at this stage, which was mixed again thoroughly to achieve homogeneity. All experimental batches were kept in a refrigerator at 4±1°C for 12 h. The starter culture combination was added and then all batches were further kept in the refrigerator for 4h. Each batch was immediately stuffed into collagen casings (38 mm, Naturin Darm, Germany) using a grinder machine (6.5kg capacity, Cem Brand, Istanbul, Turkey) to obtain sucuk baton samples after the collagen casings were immersed in 5% potassium sorbate solution a 30 min. Each sucuk baton weighed approximately 200g. Each baton was rinsed with water, and sprayed with 10% potassium sorbate solution. At the first 6h. of the ripening period, the relative humidity (RH) was adjusted to 60%, and then increased to 90±3%. Following the first day, the RH was decreased 1 unit every day until the end of ripening period. The ripening temperature was 22±2°C at the first day and decreased to 18±2°C at the end of the ripening period (12 days). Sampling was performed by randomly selecting two sample of each sucuk preparation 0., 3., 6., 9. and 12. days for microbiological, chemical and biogenic amines analysis during ripening period.

Proximate composition

Proximate analyses of the meat and dried mushroom were based on the procedures set by the (AOAC, 1997). The muscle composition was determined as follows: crude protein, using the Kjeldahl method with a 6.25 nitrogen–to–protein conversion factor; crude fat, by petroleum ether extraction using the Soxhlet method and a SOXTEC System; moisture by drying to constant weight at 105±1°C; and total ash by incineration to constant weight at 500±2°C in a muffle furnace.

Microbiological analysis

LAB were enumerated in pour plates of De Man Rogosa Sharpe Agar (Merck, Germany), Micrococcus/Staphylococcus (Mannitol Salt Phenol Red Agar (Merck, Germany), Enterobacteriaceae (Violet Red Bile Dextrose Agar, Merck, Germany), Moulds-yeasts (Potato Dextrose Agar, Merck, Germany), Enterococci (Selenit-Barzley Agar, Merck, Germany). All microbial counts were converted to the base-10 logarithm of the number of colony forming units per g of sucuk samples (log10 cfu/g).

Physicochemical analysis

pH value was measured using a pH meter (Lab Star pH; Schott LTD 6880, Germany). Water activity (a_w) measurements were performed with an Aqualab apparatus (Decagon AquaLab LITE, Water Activity Meter, WA,USA). Residual nitrite amounts were determined by the methods of (Taucmann, 1987). In this method, *N*-1-naphthyl ethylenediamine dihydrochloride reagent, sulphanilamide reagent and nitrite standard solutions were used. The absorbance was determined at 540 nm. All measurements were duplicated.

Biogenic amine analysis

Biogenic amine contents of the samples were determined according to the method of Eerola et

al., 1993. Samples for biogenic amines determination were stored at -20 ^{0}C until analysis. Biogenic amines were extracted from 2.0 g samples with 0.4 M perchloric acid and detected as their dansyl derivatives by HPLC. The gradient-elution system was 0.1 M ammonium acetate as solvent A and acetonitrile as solvent B. The flow rate was 1.0 mL min^{-1} and column temperature 40 °C. A 20µL sample was injected into the column. Peaks were detected at 254 nm using the HPLC system equipped with a column Spherisorb ODS2 150A, 150x4.60 mm (Waters, Milford, MA, USA) and Agilent HPLC (1100 series, G1315A Diode Array Detector, MI, USA). The quantitative determinations were carried out by internal standard (1.7-diaminoheptane) from (Sigma, St. Louis, MO, USA) method, using peak heights. Biogenic amine contents were expressed as mg/kg.

Statistical analysis

All data were subjected to variance analyses and differences between means were evaluated by Duncan's multiple range test significance ($P < 0.05$) using the SPSS statistic programme (SPSS, 1996). The results of statistical analysis are shown as mean values±standard deviation in tables.

RESULTS AND DISCUSSION

The composition, microbiological properties and biogenic amine concentration of meat, dried mushroom and mixed spices are presented in Table 1.

Table 1.The results of chemical, microbiological analysis (log cfu/g) and biogenic amines analysis (mg/kg) of fresh meat and dried mushroom

	pH	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
Meat	5.74	69.32	10.20	18.78	1.02
Dried Mushroom	6.46	10.72	6.56	30.80	14.34
	LAB	M/S	Enterococci	Enterobacteriaceae	Mould/Yeast
Meat	4.50	4.34	2.25	2. 60	2.67
Dried Mushroom	4.20	4.18	2.10	2.84	3.07
Mixed Spices	3.17	6.31	2.30	3.30	4.06

	Putrescine	Cadaverine	Histamine	Tyramine	Tryptamine	Phenylethylamine
Meat	10.44	17.40	ND	ND	7.82	48.25
Dried Mushroom	ND	ND	ND	ND	21.05	86.08
Mixed Spices	ND	ND	ND	ND	46.41	78.33

LAB: Lactic Acid Bacteria, , M/S: *Micrococcus/Staphylococcus,* ND: Not Detected

Microbiological and physicochemical analysis

The statistical analysis results indicating the effect of mushroom addition on the biogenic amine formation and microbiological counts in the sucuk samples are shown in Tables 2, 3, 4 and 5. The concentration of mushroom had no significant effect on LAB, yeast-mould, *Enterococci* counts, moisture, aw, tryptamine and phenylethylamine contents ($P > 0.05$). But, it had a significant ($P < 0.01$) effect on *Micrococcus /Staphylococcus*, *Enterobacteriaceae* counts and pH. Putrescine, cadaverine, histamine and tyramine were not detected in the sucuk samples, while phenyletylamine and tryptamine were detected in all the sucuk samples. Ripening period had significant ($P < 0.01$) effect on LAB, *Micrococcus/Staphylococcus* and *Enterococci* counts, pH, moisture and aw.

The results of Duncan test showed that the number of LAB was higher in control group (8.06 log10 cfu/g) than in the groups added with mushroom (Table 2). But, the addition of mushrooms did not cause a change in the counts of LAB. The highest LAB count was determined

in the 3rd day of the ripening period (Table 3). The interaction of the mushroom concentration x ripening period resulted in a significant (P <0.01) effect on LAB count. LAB count increased the 3rd day of the ripening period. This could result from the addition of starter cultures and their adaptation to the meat fermentation environment (Bover-Cıd et al., 2001; Kaban and Kaya, 2006). This is an important result, because sucuk quality is closely related to the LAB counts (Gençccelp et al., 2007). In the beginning of the ripening time, the inoculated meat mixtures contained levels of LAB (>10^7 cfu/g), confirming the effectiveness of the added starter culture (Table 3). In this respect, fermentation period was rapid and effective, bringing the pH to the levels lower than 4.6 after 3 days of ripening period. After the 3rd day, the LAB count did not change in control samples but it decreased in the samples added with mushroom (Fig. 1). However, the sucuks added with mushroom had approximately 1 log lower count of LAB than the control sucuk at the end of ripening period. LAB were also present after 3 days of ripening period, indicating the values >10^8 cfu/g. LAB counts increased during the initial days and decreased slightly during the last days of the ripening period. Similar results were found by Aksu and Kaya (2004) and Gençcelep et al. (2007) who confirmed again that short acidification times were mainly due to the use of starter cultures, and therefore it was a necessary step to produce safer and higher quality products (Coloretti et al., 2008). As presented in the Table 2, the LAB count of the control group sample was higher than that of the samples added with different concentrations of mushroom, indicating the effectiveness of the mushroom addition on the LAB conts of the sausage samples.

Micrococcus/Staphylococcus counts decreased until the 12th day of the ripening period (Table. 3). It was reported that these microorganisms were sensitive to pH changes and anaerobic media were tolerant to aw (Gökalp et al., 1999; Kaban and Kaya, 2006). The concentration of mushroom had no effect on the *Micrococcus/Staphylococcus* count except for the concentration of 2.0% where the highest counts were found (Table 2). The interaction of mushroom concentration x ripening period affected (P <0.01) the *Micrococcus/Staphylococcus* counts (Fig. 2). The interaction decreased the *Micrococcus/Staphylococcus* count in sucuk, which might be explained by the fact that the microorganisms uses up all of the oxygen source mixed in the sausage matrix by chopping process at the first day of fermentation. This could have allowed the redox potential to reduce and made the nitrite more effective, thus restricting the growth of aerobic spoilage bacteria (Erkkila, 2001). Accordingly, Aksu and Kaya (2004) and Gençcelep et al. (2007) was found similar results.

Table 2. The influence of addition of mushroom on microbiological status of sucuk (log cfu/g mean counts±SD) (n=10)

The levels of mushroom	Lactic acid bacteria	Micrococcus/ Staphylococcus	Yeast-mould	Enterobacteriaceae	Enterococci
Control	8.06±1.01a	4.87±0.28b	3.19±0.27a	2.78±0.21d	2.78±0.37a
0.5%	7.77±0.82b	4.86±0.29b	3.73±1.45b	3.44±0.19c	2.61±0.43a
1.0%	7.76±0.67b	4.92±0.49b	3.97±0.27b	4.35±0.20b	2.74±0.63a
2.0%	7.82±0.50b	5.44±0.51a	3.73±0.63b	4.83±0.70a	2.81±0.62a

±Standard deviation. (a–d) Any two means in the same column having the same letters are not significantly different at (P <0.05).

The addition of different levels of mushroom in sucuk batters had increased (P <0.01) the *Enterobacteriaceae* count, and the lowest *Enterobacteriaceae* was found in the control sucuks (Table 2). The initial counts of Enterobacteriaceae were 3.85 log cfu/g but fell below detectable levels (<2.00 log cfu/g) in all samples of sucuk on the 3rd day of ripening. This different behaviour

could be ascribed to the presence of the LAB presence in the starter culture; in fact, several species of lactobacilli have been shown to have capability of inhibiting the growth of many organisms involved in food spoilage by decreasing the pH values of the fermented products (Suzzi and Gardini, 2003). Accordingly, a rapid and sharp reduction in pH in the sausage samples is known to reduce the growth of the amine-positive microorganisms, particularly *Enterobacteriaceae* (Maijala et al., 1993; Bover-Cid et al., 2001). The Figure 1, 2, 3

Enterobacteriaceae presence is an important factor in control of the histamine, cadaverine and putrescine formation in the fermented sausages (Maijala et al., 1993; Suzzi and Gardini, 2003; Kaban and Kaya, 2006). Aksu and Kaya (2004) and Gençcelep et al. (2007) reported that *Enterobacteriaceae* counts were found to be lower than <2.00 log cfu/g in sucuk on the 3rd day. In this study, we found similar results with these authors.

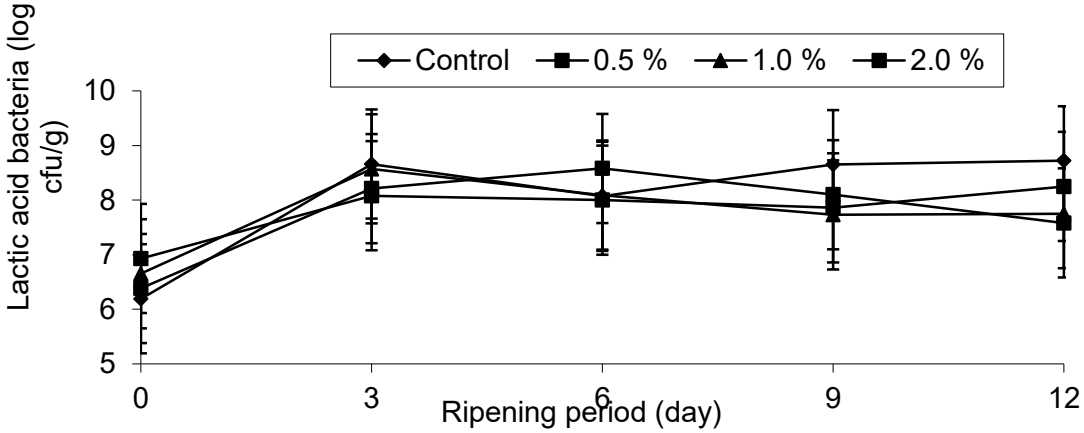

Figure 1. The effect of the interaction between mushroom x ripening period on lactic acid bacteria count.

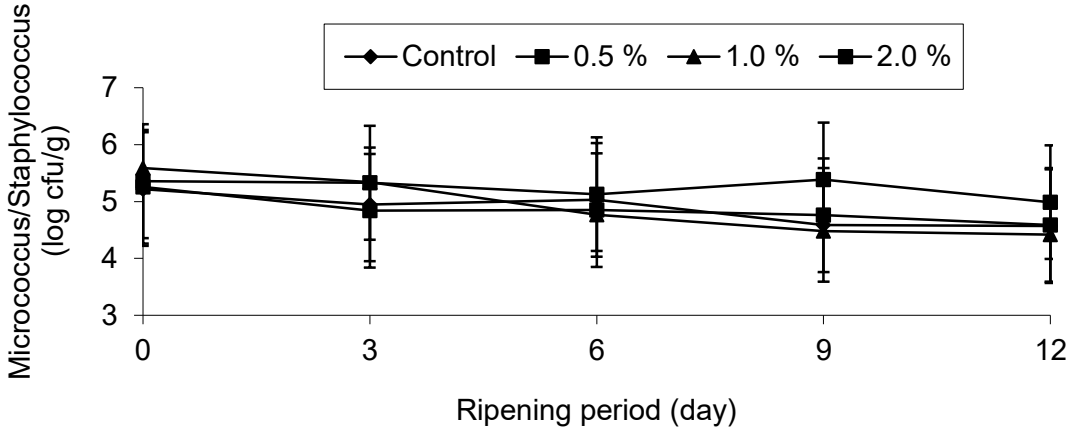

Figure 2. The effect of the interaction between mushroom x ripening period on Micrococcus /Staphylococcus count.

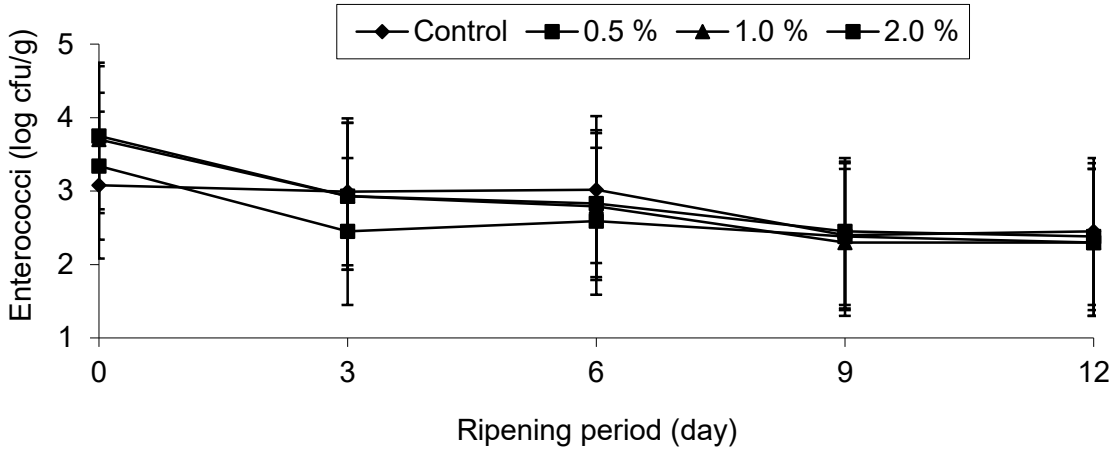

Figure 3. The effect of the interaction between mushroom x ripening period on Enterococci count.

It was determined that the *Enterococci* count was not changed by the addition of mushroom into the sucuk samples, (Table 2). It was also observed that the *Enterococci* count was decreased during ripening period (Table 3). Fig. 3 shows the effect of interaction of mushroom concentration x ripening period, indicating that the *Enterococci* count in the all samples started to decrease in the initial day of ripening time. However, no significant change water activity observed betwwen the treatment groups in respect of the *Enterococci* count in the end of ripening period. It can be concluded that mushroom did not show between effect on *Enterococci* species. In the initial time of storage period, these microorganisms were present at amounts of $>10^3$ cfu/g; however, they decreased to values of $<10^2$ cfu/g in batches in the prolonged ripening period (Table 3). The prevalence of lactobacilli in the sausage flora appeared to allow a partial control of the proliferation of enterococci (Coloretti et al., 2008).

Table 3. The influence of ripening period on microbiological status of sucuk (log cfu/g mean count±SD) (n=8)

Ripening period(day)	Lactic acid bacteria	Micrococcus/ Staphylococcus	Yeast-mould	Enterobacteriaceae	Enterococci
0	6.54±0.31c	5.60±0.49a	3.16±1.32a	3.85±0.85a	3.46±0.31a
3	8.38±0.26a	5.11±0.26b	<2.00	<2.00	2.82±0.25b
6	8.18±0.25b	4.94±0.18c	<2.00	<2.00	2.81±0.18b
9	8.08±0.42b	4.80±0.42cd	<2.00	<2.00	2.23±0.29c
12	8.07±0.50b	4.64±0.23d	<2.00	<2.00	2.35±0.32c

±Standard deviation. (a–d) Any two means in the same column having the same letters are not significantly different at (P <0.05)

The yeast and mould counts were found to be under the detectable level (<log 2.0 cfu/g) after the 3rd day of ripening period (Table 3). This could have resulted from the aforementioned immersion of the sausage samples in the 5% potassium sorbate solution for 30 min, which might indicate an antimicrobial effect on these microorganisms.

It was found that additives affected (P <0.01) pH values of sucuks. Mushroom levels had very significant effects on the pH (P <0.01) of sucuk

(Table 4). The lowest average pH value was determined in control groups and this was statistically different from that of mushroom groups (P <0.05). It was determined that an increase in the mushroom concentration increased (P <0.05) the pH values (Table 4). Because, pH values of mushrooms had determined much higher than meat (Table 1). Ripening period also had a significant effect (P <0.01) on the pH (Table 5). The initial pH of all the sucuk samples was approximately 5.82 (Table 5). The pH values were found below 5.0 beginning from the 3rd day of ripening period. The pH levels started to decrease in all groups after 0 days of ripening (Fig. 4). The interaction of mushroom concentration x ripening period decreased (P <0.01) pH values (Fig. 4). As also can be seen in the Table 5, pH values decreased (P <0.01) during the ripening period by ranging from 5.82 to 4.48. During this period, a rapid and sharp reduction in pH in sausages was reported to be due to the increase in growth rate of the LAB and other acid-producing bacteria (Gökalp and Ockerman, 1985; Lücke, 1994). pH is one of the important technological quality traits for the fermented meat products, in order to be considered "shelf-stable" the ultimate pH of finished products must be around 5.3 or lower (Ba et al., 2017). Therefore, these obtained pH results could consider enough to ensure the stability of the products.

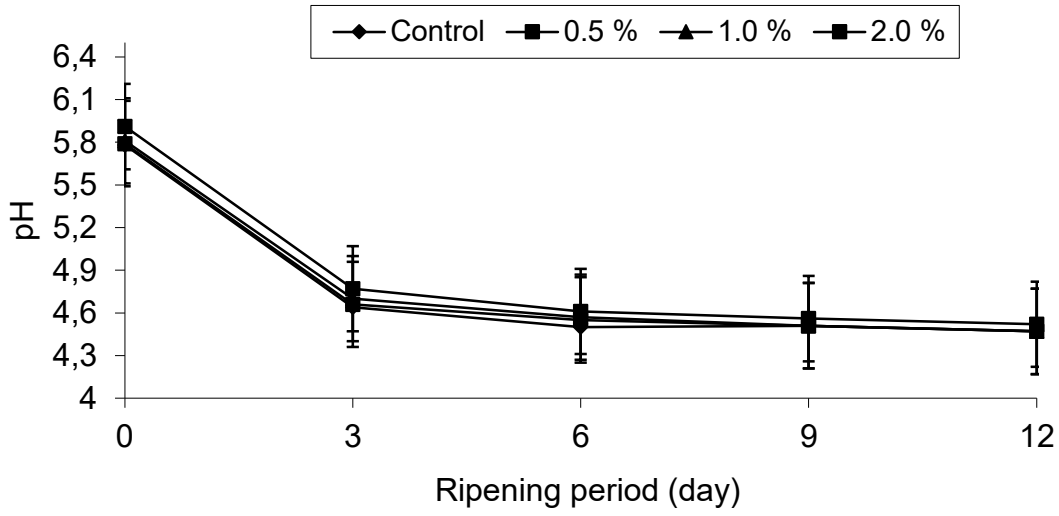

Figure 4. The effect of the interaction between mushroom x ripening period on pH level.

Water activity is considered as an important technological quality trait; food products with lower aw-values usually have higher self-life stability and vice versa (Ba et al., 2017). mushroom addition did not affect the aw and moisture values (Table 4); however they were decreased (P <0.01) throughout the ripening period (Table 5). The aw values decreased in all samples during the ripening period; at the beginning of the ripening, with a mean value of 0.974±0.003, and 0.888 ± 0.009 at the end of ripening (Table 5). The moisture values decreased in all samples during the ripening period; at the beginning of the ripening, with a mean value of 60.51±0.71, and 39.63±1.38 at the end of ripening (Table 5).

Nitrite are added into sucuk dough to attain the colour and aroma, to prevent the lipid oxidation, and to inactivate the unwanted microorganisms. Residual nitrite was not affected by neither the mushroom concentration, nor the ripening period (Tables 4, 5). The interactions of dried mushroom level and ripening period had also no significant effect on the residual nitrite levels.

Table 4. The influence of addition of mushroom on chemical properties and biogenic amines of sucuk (mean values±SD) (n=10)

The levels of mushroom	pH	Moisture (%)	aw	Nitrite (mg/kg)	TRY (mg/kg)	PHE (mg/kg)
Control	4.78±0.12c	49.83±8.3a	0.940±0.03a	4.01±0.50a	11.51±3.71a	54.07±19.04a
0.5%	4.80±0.13b	49.51±8.0a	0.940±0.03a	3.90±0.83a	12.95±4.10a	46.45±21.39a
1.0%	4.81±0.12b	49.95±7.4a	0.933±0.03a	4.10±0.54a	15.99±2.89a	50.80±15.42a
2.0%	4.87±0.15a	49.78±6.6a	0.941±0.03a	4.22±0.61a	14.33±3.89a	51.78±20.00a

±Standard deviation. TRY; Tryptamine. PHE; Phenylethylamine. (a–d) Any two means in the same column having the same letters are not significantly different at (P <0.05)

Table 5. The influence of addition of mushroom on chemical properties and biogenic amines of sucuk (mean values±SD) (n=8)

Ripening period (day)	pH	Moisture (%)	aw	Nitrite (mg/kg)	TRY (mg/kg)	PHE (mg/kg)
0	5.82±0.05a	60.51±0.71a	0.974±0.003a	--	11.42±5.74a	42.96±22.77a
3	4.69±0.05b	54.10±1.05b	0.970±0.004a	4.52±0.37a	12.19±2.34a	45.35±17.69a
6	4.56±0.05c	49.81±0.46c	0.942±0.007b	4.45±0.19a	16.39±3.03a	51.93±19.93a
9	4.52±0.04d	44.39±1.06d	0.925±0.006c	4.27±0.35a	14.07±3.86a	53.20±19.27a
12	4.48±0.03e	39.63±1.38e	0.888±0.009d	4.19±0.69a	14.42±2.19a	58.62±13.47a

±Standard deviation. TRY; Tryptamine. PHE; Phenylethylamine. (a–e) Any two means in the same column having the same letters are not significantly different at (P <0.05)

Biogenic amines

Tryptamine and phenylethylamine were found to be small amounts in this study samples. However, putrescine, cadaverine, histamine and tyramine were not detected in the sausage samples (data not present). Tryptamine and phenylethylamine levels in the sucuk samples were not affected by the mushroom concentration and ripening period (Tables 4, 5). The hygienic quality of raw materials is a crucial factor that could affect the biogenic amine content of final products. High levels of biogenic amines in final products are usually related with the high occurrence of microflora possessing amino acid decarboxylase activity (Brink et al., 1990; Halasz et al., 1994; Larotte-Moratalla, 2008). In this study, the using raw material were good hygiene quality (Table 1).

Especially, adding nitrite is important for the prevention of formation of putrescine. Gençcelep et al. (2007) reported that the addition of sodium nitrite (75 mg/kg) with starter cultures was enough to reduce tyramine and cadaverine formation in sucuk. In this study, no tyramine and cadaverine content was detected in the sucuk samples, which was thought to be due to the nitrite (150 mg/kg) and starter culture addition. In addition, *Enterobacteriaceae* was reported to influence histamine concentration in sucuks (Halasz et al., 1994; Durlu-Özkaya et al., 2001; Suzzi and Gardini, 2003), and these microorganisms were not found at detectable levels (<2.00 log cfu/g) in any samples of sucuk by the 3rd day of ripening period. For this reason, histamine might not have formed during the ripening period.

Several authors have shown that tyramine and putrescine are usually decreased by using a starter culture and nitrite in the production of sausages (Hernandez-Jover et al., 1997; Martuscelli et al., 2000; Bover-Cid et al., 2001). Gençcelep et al. (2007) and Kurt and Zorba (2009) reported that the effect of nitrite was found to be significant on tyramine and putrescine, as tyramine values decreased with increasing nitrite levels. These could be attributed to the antimicrobial effect of nitrite on proteolytic organisms, which causes tyramine formation.

CONCLUSION

LAB counts increased and *Micrococcus-Staphylococcus* and *Enterococci* counts decreased during the ripening period. Increasing mushroom levels increased counts of *Enterobacteriaceae*. Both the mushroom and ripening period had significant effect on pH. The results from this study showed that muhroom had not effects on the formation of biogenic amines in sucuk. In addition, there could not be determined very important antimicrobial effects of the use of mushroom in sucuk production. The effects of the other features of mushroom sausage-related studies are recommended.

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