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THE EFFECTS OF SAUSAGE CASING WITH POTASSIUM SORBATE ON COLOUR, MICROBIOLOGICAL PROPERTIES AND FORMATION OF BIOGENIC AMINES OF DRY FERMENTED SAUSAGE (SUCUK)

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ABSTRACT
The sausage casings were dipped into potassium sorbate (PS) solutions (PS0; 0% (Control), PS5; 5%, PS10; 10%, PS20; 20%) before filling and the effects of this treatment were investigated on the formation of biogenic amine (BA), microbiological and physical properties of sausage during the ripening (12 days) and refrigerated storage (4 °C, 30 days). The effect of PS treatment was not found to be singificant on the pH values of the sausages. Yeast-mold and *Enterobacteriaceae* were detected only in the PS0 samples. The effect of PS treatment on the counts of total aerobic mesophilic bacteria, lactic acid bacteria and *Micrococcus/Staphylococcus* was found to be significant. Moreover, this effect on BAs except for spermine and spermidine was found to be significant. While PS treatment restricted the increase in tryptamine, 2-phenylethyl amine, putrescine, cadaverine, tyramine contents of sausages, it caused an increase in the formation of histamine. As the PS concentration increased, the redness (*a*) and yellowness (*b*) values of the sausages decreased while the lightness (*L*) values decreased in the PS20 samples.

1. Introduction

The quality of fermented sausages is closely related to the species and count of microorganisms in the flora (Tekinşen et al., 1982). During the fermentation and ripening stages, particularly development of some microorganisms is desired whereas the development of some microorganisms is not desired (Kurt, 2006). Moreover, the presence of biogenic amines (BAs), known to be toxic for humans and animals (Shalaby, 1996), in meat and meat products is considered to be indicator of undesirable microbiological activity (Lu et al., 2010). For this reason, it is important to control the formation of biogenic amines in fermented meat products (Kurt 2006).

Dry fermented sausages contain high levels of BA (Lu et al., 2010) because of that they have appropriate preconditions for BA (Komprda et al., 2009). The most important BAs in fermented sausages are putrescine, cadaverine, phenylethylamine, spermine, spermidine, histamine, tryptamine and tyramine (Lu et al., 2010) and these are main products of microbial decarboxylation of amino acids (Xie et al., 2015). Decarboxylase activity in meat products generally has been attributed chiefly to *Enterobacteriaceae*, lactic acid bacteria, *Micrococcaceae* species (Ruiz-Capillas and Jimenez-Colmenero, 2004). Furthermore, Yeasts such as *Debaryomyces* and *Candida* species isolated from fermented meats have also been reported to have histidine decarboxylase activity (Ercoşkun et al., 2005). The relationship between BAs and microbial load has been proven and it is stated that BA formation can be

restricted by controlling the count of microorganisms (Ercoşkun et al., 2000).

On the other hand, one of the most important problem in fermented sausage production is mold growth (Öztürk, 2015) due to their tolerances to low water activity and pH (Castellari et al., 2010). Molds and yeasts usually develop on the surface and near the surface of sausages (Gökalp et al., 1999). The development of undesirable mold species may adversely affect the quality properties of the product, furthermore cause the formation of toxic secondary metabolites such as mycotoxin. For this reason, mold growth on sausage surface is regarded as a toxic hazard for human health (Chaves-Lopez et al., 2012). To avoid this risk, various chemical preservatives such as potassium sorbate (PS) and sodium benzoate are added to the product formulations (Chaves-Lopez et al., 2012).

Sorbic acid and its salts, especially PS, are widely used worldwide in various foods (Davidson et al., 2005). Sorbates can be used in various forms such as direct addition to the product, spraying with sorbate solution or dipping in sorbate solution (Sofos and Busta, 1981). Sorbates are effective on many microorganisms including yeast, mold and bacteria (Davidson et al., 2005). The inhibitor effect PS on the bacteria is lower than the inhibitor effect on yeast-mold (Tekinşen et al., 1999). PS is metabolized in the human body in similar to fatty acids (Doğruer et al., 1996). It is on the GRAS list (Hoang and Vu, 2016; Robach and Sofos, 1982). There are some studies (Bozkurt and Erkmen, 2002a; Jastrzębska et al., 2016; Shalaby and El-Rahman, 1995) using PS to limit the formation of some BA in meat products. They have investigated the effect of PS by adding to meat product formulation. Any study which sausage casings are treated with PS solution before filling to limit the formation of BAs in fermented sausage production was not encountered in the literature. In addition, it is necessary to investigate the color changes that can occur in the color values due to the application of PS to the sausage casing. This study was to investigate the effects of PS treatment of sausage casing before filling on the colour values, microbiological properties and BA formation.

2. Materials and methods

One day after slaughter, beef, beef subcutaneous fat and sheep tail fat were supplied from a local meat processor (Adıyaman, Turkey). Potassium sorbate was obtained from Carlo Erba. A collagen casing (35-36 mm diameter, Naturin Darm, Germany) was used to fill the sausage batter.

2.1. Sausage preparation

To prepare the sausage, beef (72%), beef fat (10%) and sheep tail fat (10%), spices (3.6%), salt (1.9%), garlic (2%), sugar (0.5%) and sodium nitrite (150ppm) were mixed and minced in a grinder with 3 mm diameter hole plate (Alveo, Turkey). Resulting batche of sausage batter was rested for 12 hour at 4°C. Collagen casing dipped into the potassium sorbate solution for 10 min before the batter was filled into the casings. Batter stuffed into collagen casings by using hydraulic filling machine (Mainca EM-30, Spain). Samples were ripened for 12 days in a climatic cabin (Qualitec Biosan, Konya). Ripening temperature was adjusted to 23 °C and then first 6 days decreased 1°C for every day. The relative humidity was started from 60±3% for 5 hours and was then increased to 90±3% and decreased every day by 1 unit. At the end of the ripening period, sausages were packed in a polyethylene bag and stored at 4°C for 30 days.

2.2. pH and microbiological analysis

Ten grams of sample was homogenized in 100 ml distilled water and the pH was determined using a pH meter (Hanna 2215, USA). For the microbiological analysis, twenty grams of sample was taken from each sample and homogenised in 180 ml of sterile salt solution (0.85% NaCl). The count of moulds-yeasts was determined on Dichloran Rose Bengal Chloramphenicol Agar (DRBCA)

incubated at 25°C for 5 days (APHA 1992), while total aerobic mesophilic bacteria on Plate Count Agar (PCA) were incubated at 37°C for 48 hours (APHA 1992) and Enterobacteriaceae was determined on Violet Red Bile Glucose Agar (VRBGA) incubated at 30 °C for 24 h (Harrigan, 1998). Lactic acid bacteria (LAB) was cultured on DeMan Rogosa Sharpe Agar (MRS) incubated at 30°C for 72 h (APHA, 1992).

2.3. Determination of biogenic amines

Determination of biogenic amines: a chromatographic method (Eerola et al., 1993) was used for the determination of cadaverine, tyramine, histamine, putrescine, tryptamine, 2-phenylethylamine, spermine and spermidine. High performance liquid chromatography (HPLC; Shimadzu, Japan) was used to separate the amines. The seperation was carried out by a gradient elution with 0.1 M ammonium acetate/acetonitrile on a reverse phase column (ODS-2; 5 µm, 125 x 4mm, Teknoroma, Spain) at the flow rate of 1 ml/min using a diode-array-detector (SPD-M20A, Shimadzu, Japan) at 254 nm.

250 µl internal standard (1.7 diaminoheptane) and 10 ml 0.4M perchloric acid solution were added to a 4 g sample and homogenised with a homogenisator (Wisd HG-15, Daihan, Korea). Then, sample was centrifuged (Universal 32R, Hettich, Germany) at 2400 g for 10 min. and the supernatant was transfered in to 25 mL bottle through filter paper. Extraction was repeated with 10 ml of 0.4 M perchloric acid solution in the remaining sample. Supernatants were adjusted to 25 ml with 0.4M perchloric acid solution.

A 500 µl extract was adjusted to alkaline using 100 µl 2N sodium hydroxide solution. Then, a 150 µl sodium bicarbonate (saturated), 1 ml dansyl chloride solution (10 mg/1 ml acetone) were added and incubated at 40 °C for 45 min. The residual dansyl chloride was then removed by addition of 50 µl of ammonia (25%). The dansylated extract was allowed to stand for 30 minutes, then adjusted to 5 ml with 0.1 M ammonium acetate/acetonitrile (1/1) and filtered through a 0.45 µm syringe filter.

The amount of biogenic amine in the sample was calculated using the following formula:

$C_u = 250 \times RF \times (H_A / H_i) \times C_i / W_S$

where C_u: mg unknown/kg sample, 250: dilution factor, RF: response factor, C_i: concentration of internal sandard, H_A: peak height of unknown, H_i: peak height of internal standard, W_S: weight of sample.

2.4. Instrumental colour analysis of sausages

Instrumental colour analysis of sausages: a portable colorimeter (Minolta CR400, Osaka, Japan) was used to measure the colour values of sausage samples. The instrument was standardized on a white standardization plate prior to each measurement. The colour was determined as *L* (lightness), *a* (redness) and *b* (yellowness) values according to CIELAB systems. Each sausage colour was measured eight times, from different places and averages of eight data were taken.

2.5. Statistical analysis

Statistical analysis: this study was carried out two replicates. Variance analysis (ANOVA) was used for the data and the results were given as mean ± standard deviation (SD). Differences between values of the samples were compared using Duncan's multiple range tests; a $p<0.05$ probability value was considered significant.

3. Results and discussions

3.1. The effects of PS on the pH

The changes in the pH values of the sausages during ripening and storage were shown in Table 1. The effect of PS on the pH values of the sausage was not found to be significant, whereas the effect of ripening and storage stages was significant. In all samples, the pH value decreased with the reason of lactic acid formation during ripening. The decrease in pH during ripening period is very important in terms of the desired flavour, colour formation and inhibition of undesired microorganisms in fermented sausage (Bozkurt, 2006). On the 30th

day of storage, the pH values showed a slightly increase in all samples. This increase in pH during the storage time was thought to be due to the formation of components such as amine and ammonia (Gök, 2006) or the increase in lactic acid degradation (Kurt, 2016).

Table 1. The effect of ripening and storage stages on the pH values of sausages[1]

Potassium Sorbate (%)	Ripening			Storage
	0th day[2]	6th day	12th day	30th day
0	5.84±0.01 ^{A}	4.63±0.04 ^{B}	4.41±0.01 ^{C}	4.60±0.01 ^{B}
5	5.84±0.01 ^{A}	4.60±0.05 ^{B}	4.40±0.01 ^{C}	4.60±0.04 ^{B}
10	5.84±0.01 ^{A}	4.62±0.05 ^{B}	4.38±0.02 ^{C}	4.58±0.05 ^{B}
20	5.84±0.01 ^{A}	4.65±0.04 ^{B}	4.39±0.06 ^{C}	4.60±0.02 ^{B}

[1]The effect of PS treatment on pH values of sausages was not significant ($p>0.05$).

[2] 0th day analyses were done on samples taken before sausage filling. They were independent for PS effect and were given for statistical analysis.

$^{A-D}$Different uppercase letters in a row show significant differences ($p<0.05$) between the groups.

3.2. The effects of PS on the microbiological properties

The effect of PS on the count of yeast-mold was found to be significant (Table 2). Yeast-mold was only detected in the PS0 sample, whereas they were not found in other samples. This is thought to be due to wide spectrum inhibitory property of PS on yeast-mold (Vasakou et al., 2003). It is suggested that sorbic acid prevents molds development by inhibiting dehydrogenase enzyme activity which involved in the oxidation of fatty acids (Sofos and Busta, 1981). PS is an effective antifungal agent used to prevent or reduce mold growth in fermented sausages (Öztürk, 2015). Ergüzel (1988) stated that the effect of dipping of sausage into 15% PS solution on the yeast-mold growth during the ripening and storage was significant. Chaves-Lopez et al., (2012) reported that dipping sausages in 20% PS solution inhibited mold growth on sample surface. Bozkurt and Erkmen (2002a) stated that the addition of some additives, including PS, inhibited yeast-mold growth and also stated that higher rate PS decreased the count of yeast-molds in fermented sausage. In another study, the addition of nitrite/nitrate or PS decreased the count of yeast-molds in fermented sausage (Bozkurt and Erkmen, 2007). Matos et al., (2007) showed that PS had an inhibitory effect on some mold species isolated from Portuguese dried-smoked sausages.

The effect of the ripening stage on the count of yeast-mold of PS0 sample was significant (Table 2). The count of yeast-mold in the PS0 sample decreased during ripening, whereas it did not change during storage. There are different findings about changes in the counts of yeast-mold in fermented sausages during ripening and storage (Bozkurt and Erkmen, 2002a; Bozkurt and Erkmen, 2007; Kaban, 2013).

PS significantly influenced the development of *Enterobacteriacea* (Table 2). These microorganisms could not be detected in PS treated sausages, it was merely detected in the PS0 sample. The absence of *Enterobacteriaceae* in PS treated sausages was thought to be due to the antimicrobial effect of PS (Sofos and Busta, 1981). It has been stated that the dipping of some meat products in PS solution was effective on the count of *Enterobacteriacea* (Gençcelep et al., 2014; Güner et al., 2004). Güner et al., (2004) reported that dipping into 2.5% and 5% PS solution of some poultry (chicken, turkey, quail, partridge) caused the decrease in the count

of *Enterobacteriaceae* during cold storage and this effect continued during storage.

The effect of ripening and storage stages on the count of *Enterobacteriacea* of PS0 sample was significant (Table 2). In the PS0 samples, *Enterobacteriaceae* showed a decrease during ripening stage, but it was not detected in the last days of storage time. Lizaso et al., (1999) stated that *Enterobacteriacea* could not be detected in the Spanish-type dry cured sausage with the development of ripening. Fernandez-Lopez et al., (2008) obtained similar results in Spanish-type dry fermented sausage and the decrease in the count of *Enterobacteriaceae* has been associated with reduction in pH occurred as a result of activity of lactic acid bacteria. Yalınkılıç et al., (2012) expressed that the *Enterobacteriaceae* in fermented sausage easily disappeared with ripening owing to the low water activity and pH susceptibility.

Table 2. The microbiological changes of sausages during the ripening and storage stages

Microorganism and Potassium Sorbate (%)	**Ripening**			**Storage**
	0th day[1]	**6th day**	**12th day**	**30th day**
Yeast-Mold[2] (log cfu g^{-1})				
0	3.90±0.08 ^{A}	3.52±0.04 ^{B}	3.32±0.04 ^{C}	3.31±0.08 ^{C}
Enterobacteriaceae[2] (log cfu g^{-1})				
0	2.93±0.14 ^{A}	2.35±0.26 ^{B}	2.24±0.29 ^{B}	ND
Aerobic mezophilic bacteria (log cfu g^{-1})				
0	5.35±0.03 ^{C}	8.78±0.01 ^{aA}	8.66±0.10 ^{aA}	8.17±0.07 ^{aB}
5	5.35±0.03 ^{C}	8.71±0.02 ^{aA}	8.55±0.09 ^{aA}	8.01±0.08 ^{abB}
10	5.35±0.03 ^{D}	8.57±0.14 ^{abA}	8.33±0.04 ^{bB}	7.86±0.04 ^{bcC}
20	5.35±0.03 ^{C}	8.42±0.06 ^{bA}	8.19±0.02 ^{bA}	7.66±0.18 ^{cB}
Lactic acid bacteria (log cfu g^{-1})				
0	4.35±0.04 ^{C}	8.20±0.06 ^{aA}	7.87±0.11 ^{aB}	8.11±0.03 ^{aA}
5	4.35±0.04 ^{C}	8.15±0.04 ^{aA}	7.77±0.08 ^{aB}	8.06±0.00 ^{aA}
10	4.35±0.04 ^{D}	8.10±0.01 ^{aA}	7.72±0.03 ^{aC}	8.00±0.02 ^{bB}
20	4.35±0.04 ^{C}	7.89±0.01 ^{bA}	7.49±0.01 ^{bB}	7.93±0.01 ^{cA}
Micrococcus-Staphylococcus bacteria (log cfu g^{-1})				
0	4.97±0.04 ^{A}	4.91±0.07 ^{aA}	4.43±0.06 ^{aB}	4.20±0.18 ^{aB}
5	4.97±0.04 ^{A}	4.74±0.06 ^{bA}	3.94±0.21 ^{bB}	3.67±0.04 ^{bB}
10	4.97±0.04 ^{A}	4.60±0.04 ^{cB}	3.75±0.04 ^{bcC}	3.65±0.05 ^{bC}
20	4.97±0.04 ^{A}	4.51±0.01 ^{cB}	3.54±0.04 ^{cC}	3.52±0.05 ^{bC}

[1]0th day analyses were done on samples taken before sausage filling. They were free from PS and were given for statistical analysis.
[2]Not detected in samples with PS.
ND: Not detected
$^{A-D}$Different uppercase letters in a row show significant differences between the groups ($p<0.05$).
$^{a-b}$Different lowercase letter in a column show significant differences between the groups($p<0.05$).

Another significant effect of PS was found to be on the count of TAMB (total aerobic mezophilic bacteria). As shown in Table 2, at the 6th and 12th days of ripening, TAMB counts of PS10 and PS20 samples were lower than other samples. At the storage stage, counts of TAMB

of all samples with PS were lower than PS0 sample. This change in counts of TAMB is thought to be due to the antimicrobial properties of PS (Holley, 1981) and its interaction with the enviroment conditions. There is no exact agreement among scientists about the action mechanism of sorbates against microorganisms (Sofos and Busta, 1981). However, it is suggested that the effect of sorbic acid on microorganisms is based on the inactivation of some enzymes (Sofos and Busta, 1981). Sorbates has been thought to be effective against numerous microorganisms such as gram negative and positive, catalase negative and positive, aerobic, anaerobic, mesophilic and thermophilic microorganisms (Dinçoğlu, 2002). Wareltzis et al., (1984) stated that addition of 0.20% PS instead of sodium nitrite to frankfurter sausage caused a better inhibition on TAMB. In another study (Jin et al., 2015), the count of TAMB of emulsified sausage with 0.2% PS was lower than the control sample after 4 weeks of storage.

It was observed that the effects of ripening and storage stages on the counts of TAMB of the sausages with or without PS were found to be significant (Table 2). The counts of TAMB of all samples increased during ripening. In the last days of ripening, a slight decrease was observed TAMB counts of samples. Furthermore, at the storage stage, there was a decrease in the counts of TAMB of all sausages. The results are similar to those previously reported by some researchers (Aksu and Kaya, 2004; Gök et al., 2011; Kurt, 2016). Gök et al., (2011) reported that the count of TAMB of fermented sausage increased in the first 7 days of ripening and then decreased. Kurt (2016) stated the count of these microorganisms in fermented sausage decreased during the storage.

One of the important microorganism cultures in fermented sausage is LAB. PS treatment had significant effects on the counts of LAB. On 6th and 12th days of ripening, the LAB counts of PS20 sample was lower than other samples (Table 2). At the storage time, the LAB counts of PS10 and PS20 samples were less than other samples. This change in LAB counts might be due to the antimicrobial properties of PS (Davidson et al., 2005). Another study (Öztürk, 2015), reported that the effect of dipping of 15% PS solution of sausage on the count of LAB of fermented sausage was not found significant. Howeover, Jin et al., (2015) has stated that emulsified sausages with PS contained less LAB than the control samples after cold storage.

The counts of LAB changed significantly during ripening and storage (Table 2). The count of LAB increased from 4.35 log cfu g^{-1} to 8.20 log cfu g^{-1} at the first days of ripening and decreased to 7.49 log cfu g^{-1} at the last days of ripening. This has been associated with adaptability to the meat environment of LAB (Fernandez-Lopez et al., 2008). Moreover, the counts of LAB in all samples increased during storage stage. Ercoşkun and Özkal (2011) stated that LAB count in sausage showed a rapid increase at the first days of ripening, but then, this increase was stopped.

Another important microorganism cultures in fermented sausages are *Micrococcus/Staphylococcus* species and they can improve color by reducing nitrate to nitrite and contribute to the development of specific aroma with lipolytic and proteolytic activities (Gökalp et al., 1998). *Micrococcus/Staphylococcus* counts of sausages were significantly affected by PS application (Table 2). There was a decrease in *Micrococcus/Staphylococcus* counts depending on PS concentration. During ripening and storage, the count of *Micrococcus/Staphylococcus* of PS0 sample was higher than other samples. Öztürk (2015) stated that dipping of the fermented sausages into 15% PS solution was a significant effect on the count of *Micrococcaceae*. Moreover, our findings were similar with the results of other meat products using PS (Doğruer et al., 1996; Güner et al., 2004). Tekinşen et al., (1999) reported that count of *Micrococcus/Staphylococcus* increased depends on garlic reduction rate in cemen of pastrami, but this increase was limited by PS addition.

The effect of ripening stage on *Micrococcus/Staphylococcus* count was significant, whereas the effect of storage stage on *Micrococcus/Staphylococcus* count was not found to be significant (Table 2). There was no significant difference between the *Micrococcus/Staphylococcus* counts of PS0 and PS5 samples in the 0th days and 6th of ripening. On the other hand, *Micrococcus/Staphylococcus* counts of PS10 and PS20 samples decreased at the first 6 days of ripening. After 6th day of ripening, the count of *Micrococcus/Staphylococcus* decreased in all samples. Kaban and Kaya (2009), Yalınkılıç et al., (2012) reported that the effect of ripening stage on the count of *Micrococcus/Staphylococcus* of fermented sausage was important. Another study (Kaban and Kaya, 2009), has been reported that the count of *Micrococcus/Staphylococcus* increased at the first days of fermentation and then their counts remained relatively stable throughout the ripening. Moreover, no increase was observed in these microorganisms at the first days of fermentation in our study (Table 2). Kaban (2013) reported that the growth and viability of these microorganisms was depending on acidity during fermentation. However, in some cases, it has been reported that the rapid growth of lactic acid bacteria may cause inhibition against these slowly growing microorganisms (Kaban and Kaya, 2009).

3.3. The effects of PS on the biogenic amine (BA) formation

The BA contents of the samples during the ripening and storage stages were given in Table 3. The use of PS treated casings in the sausage was found to be effective considering BAs with the exception of spermine and spermidine. However, PS treatment was not caused significant changes in spermine and spermidine contents of sausages.

The effect of PS treatment on tryptamine contents was found to be significant. The contents of tryptamine of all samples has been shown an increase at the ripening and storage. The highest tryptamine content was determined in the PS0 sample during ripening and storage stage. At the 6th day of ripening, tryptamine contents of PS10 and PS20 samples were considerably lower than PS0 and PS5 samples. After the 6th day of ripening, all PS concentrations provided a reduction in tryptamine content compared to the PS0 sample. Shalaby and El-Rahman (1995) reported that the content of tryptamine in fermented sausages with PS and starter culture increased during fermentation and then decreased to undetectable level. In another study (Bozkurt and Erkmen, 2002a), it was stated that the use of additives, including PS, did not affect the content of tryptamine in fermented sausages.

PS treatment caused significant differences in 2-phenylethylamine contents of samples. As shown in Table 3, the contents of 2-phenylethylamine of sausages with or without PS were increased at the ripening and storage. The effect of PS treatment on contents of 2-phenylethylamine were not found significant at 6th day of ripening. However, the contents of 2-phenylethylamine of PS10 and PS20 samples were lower than other samples at the 12th day of ripening. During the storage stage, the contents of 2-phenylethylamine of all samples were found the less than PS0 sample. Bozkurt and Erkmen (2002b) reported that 2-phenylethylamine could not be detected in fermented sausage produced with additives including PS. In another study (Gençcelep et al., 2014), it was reported that 2-phenylethylamine content of *pearl mullet* which dipping into 1-5% PS solution increased during cold storage. It has been reported that *Micrococcus/Staphylococcus* in the microflora of dry fermented sausages can produce 2-phenylethylamine (Kurt, 2006). In addition, some yeast species can generate 2-phenylethylamine (Ercoşkun et al., 2005). It is not desirable that the content of 2-phenylethylamine is above 30 ppm in terms of "good manufacturing practices" (Shalaby, 1996). In our study, the contents of 2-phenylethylamine were below this level. In a research (Gençcelep et al., 2008), 2-

phenylethylamine was detected maximum 25 mg kg^{-1} in 17 of 30 fermented sausage collected from local markets in Turkey.

Putrescine was the most abundant biogenic amine in PS treated samples (Table 3). The contents of putrescine of all samples increased at the ripening and storage. PS treatment limited the increase of putrescine contents from the first days of ripening and the content of the highest putrescine was determined in the PS0 sample. Shalaby and El-Rahman (1995) investigated the effect of PS on the formation of BA in fermented sausage, and stated that putrescine cannot be determined. The putrescine content of fresh meat is related to the count of total aerobic organism (Ruiz-Capillas and Jimenez-Colmenero, 2004). It is also reported that *Enterobacteriaceae* species produce considerable amounts of putrescine and there is a relation between the counts of these bacteria and the content of putrescine (Kurt, 2006). Ba et al., (2016) reported that one of the two highest biogenic amines in fermented pork sausage produced was putresin. Komprda et al., (2004) reported that the two highest concentration of BAs were putrescine and tyramine.

Table 3. Biogenic amine concentrations of sausages during the ripening and storage stages

Biogenic amines (mg kg^{-1})	Samples	Ripening			Storage
		0th day[1]	6th day	12th day	30th day
Tryptamine	PS0	0.54±0.01 ^{C}	47.51±4.94 ^{aB}	70.28±4.82 ^{aA}	72.56±6.25 ^{aA}
	PS5	0.54±0.01 ^{C}	39.07±4.40 ^{aB}	53.42±1.51 ^{bA}	55.89±1.86 ^{bA}
	PS10	0.54±0.01 ^{D}	25.58±0.94 ^{bC}	40.64±2.08 ^{cB}	47.04±3.23 ^{bA}
	PS20	0.54±0.01 ^{C}	18.85±1.58 ^{bB}	21.16±2.67 ^{dAB}	23.71±0.95 ^{cA}
2-phenylethyl amine	PS0	2.79±0.05 ^{C}	4.80±0.88 ^{aB}	7.81±0.43 ^{aA}	9.22±0.32 ^{aA}
	PS5	2.79±0.05 ^{C}	4.28±1.52 ^{aBC}	6.33±0.91 ^{aAB}	7.75±0.89 ^{abA}
	PS10	2.79±0.05 ^{C}	3.86±1.27 ^{aAB}	4.71±0.35 ^{bAB}	5.90±0.86 ^{bcA}
	PS20	2.79±0.05 ^{B}	3.38±0.68 ^{aB}	3.76±0.35 ^{bB}	5.09±0.48 ^{cA}
Putrescine	PS0	136.78±0.35 ^{D}	841.52±48.91 ^{aC}	1168.20±45.69 ^{aB}	1365.90±63.79 ^{aA}
	PS5	136.78±0.35 ^{D}	790.82±5.64 ^{abC}	1097.90±33.33 ^{abB}	1337.40±64.96 ^{aA}
	PS10	136.78±0.35 ^{D}	736.65±3.68 ^{bC}	1004.40±24.34 ^{bcB}	1220.00±58.68 ^{abA}
	PS20	136.78±0.35 ^{D}	611.39±34.27 ^{cC}	912.94±34.82 ^{cB}	1151.70±16.22 ^{bA}
Cadaverine	PS0	0.00±0.00 ^{D}	16.10±0.23 ^{aC}	61.61±0.98 ^{aB}	78.28±2.42 ^{aA}
	PS5	0.00±0.00 ^{D}	16.01±0.26 ^{aC}	58.85±2.25 ^{aB}	70.96±2.35 ^{bA}
	PS10	0.00±0.00 ^{D}	14.60±0.71 ^{bC}	56.19±2.12 ^{aB}	67.58±0.90 ^{bA}
	PS20	0.00±0.00 ^{D}	9.43±0.01 ^{cC}	40.85±5.35 ^{bB}	51.89±2.60 ^{cA}
Histamine	PS0	2.46±0.01 ^{B}	2.74±0.08 ^{bB}	3.54±0.00 ^{cA}	3.73±0.22 ^{bA}
	PS5	2.46±0.01 ^{D}	2.72±0.09 ^{bC}	3.63±0.04 ^{bcB}	3.88±0.04 ^{bA}
	PS10	2.46±0.01 ^{C}	2.89±0.02 ^{abB}	3.86±0.13 ^{abA}	4.06±0.09 ^{abA}
	PS20	2.46±0.01 ^{C}	3.00±0.05 ^{aB}	4.04±0.18 ^{aA}	4.38±0.19 ^{aA}
Tyramine	PS0	2.52±0.06 ^{C}	158.54±2.39 ^{aB}	252.10±16.41 ^{aA}	274.96±5.72 ^{aA}
	PS5	2.52±0.06 ^{D}	139.86±5.93 ^{bC}	236.05±12.50 ^{aB}	260.90±1.30 ^{aA}
	PS10	2.52±0.06 ^{C}	128.62±10.56 ^{bcB}	220.24±6.46 ^{aA}	246.05±16.52 ^{aA}
	PS20	2.52±0.06 ^{C}	115.89±0.57 ^{cB}	172.58±15.94 ^{cA}	189.91±17.40 ^{bA}
Spermidine	PS0	7.41±0.11 ^{B}	9.90±0.93 ^{aA}	11.17±0.26 ^{aA}	11.30±0.46 ^{aA}
	PS5	7.41±0.11 ^{C}	9.72±0.69 ^{aB}	11.16±0.26 ^{aA}	11.29±0.53 ^{aA}

	PS10	7.41±0.11 ^{C}	8.98±0.48 ^{aB}	11.01±0.22 ^{aA}	11.03±0.16 ^{aA}
	PS20	7.41±0.11 ^{B}	8.62±0.85 ^{aB}	10.95±0.27 ^{aA}	11.01±0.16 ^{aA}
Spermine	PS0	55.21±0.33 ^{B}	57.70±8.88 ^{aAB}	70.85±3.31 ^{aA}	58.93±0.03 ^{aAB}
	PS5	55.21±0.33 ^{B}	52.30±8.25 ^{aAB}	65.66±1.70 ^{aA}	58.74±0.19 ^{aAB}
	PS10	55.21±0.33 ^{AB}	41.86±9.90 ^{aB}	62.42±1.14 ^{aA}	58.68±0.29 ^{aA}
	PS20	55.21±0.33 ^{AB}	39.74±10.52 ^{aB}	57.97±5.01 ^{aA}	58.82±0.05 ^{aA}

[1]0th day analyzes were done on samples taken before sausage filling. They were free from PS and were given for statistical analysis.

0.00±0.00: Not detected

$^{A-D}$Different uppercase letters in a row show significant differences between the groups ($p<0.05$).

$^{a-b}$Different lowercase letter in a column show significant differences between the groups($p<0.05$).

PS also significantly affected the formation of cadaverine (Table 3). Cadaverine was not detected before the filling, however an increase in cadaverine contents was detected together with ripening. Whereas, in some studies (Ba et al., 2016; Bozkurt, 2002; Shalaby and El-Rahman, 1995), it has been reported that cadaverine cannot be detected in fermented sausage. In our study, the highest cadaverine content was determined in the PS0 sample. It was found that PS10 and PS20 samples contained less cadaverine than the PS0 during ripening. Also, samples with PS contained less cadaverine than the PS0 sample during storage. Particularly during the storage, the cadaverine content of the PS20 was found significantly lower than the other samples. Previous studies (Shalaby and El-Rahman, 1995; Bozkurt and Erkmen, 2002a) have reported that cadaverine was not detected in fermented sausages produced using PS.

Although PS treatment limited the formation of tryptamine, 2-phenylethylamine, putrescine and cadaverine, it increased histamine formation during ripening and storage. As shown in Table 3 shows that histamine contents slightly increased with PS treatment. The highest content of histamine during ripening and storage stages was determined in the PS20 sample. Shalaby and El-Rahman (1995) reported that histamine content initially increased in fermented sausages with PS addition, but fell below undetectable level with ripening. Taylor and Speckhard (1984) reported that 0.5% PS concentration inhibited the development of certain histamine-producing bacteria. It was reported that histamine in fermented sausages occurs more between 2th and 4th weeks and it depends on the count of LAB rather than ripening conditions (Stratton et al., 1991). It was also stated that *Enterobacteriaceae* species can produce histamine in significant amounts in addition to cadaverine and putrescine production.[8] Debaryomyces and Candida species isolated from fermented meats have been reported to exhibit high decarboxylase activity (Ercoşkun et al., 2005). Histamine and tyramine are known to be the most toxic amines that cause intoxication (Anastasio et al., 2010). However, it is known that histamine is found in fermented sausages at a lower level than other Bas (Latorre-Moratalla et al., 2012). 50-100 ppm of histamine is considered acceptable for "good production practices" (Ekici et al., 2004). In some studies (Ekici et al., 2004; Gençcelep et al., 2008) reported that the histamine contents of dry fermented sausages were less than this level.

Tyramine contents of sausages had been shown to increase with ripening. The highest tyramine is detected in PS0 sample during the ripening and storage. It can be said that all PS concentrations were effective on the formation of tyramine at the first days of ripening. However 20% PS concentration was effective on tyramine formation at the 12th day of ripening and storage stage. The most abundant BA following putrescine was tyramine. It is stated that the most commonly BA in fermented sausages is tyramine and is produced mostly by LAB (Latorre-Moratalla et al., 2012). Moreover,

this BA is generated more rarely by coagulase negative *Staphyloccoccus* (Latorre-Moratalla et al., 2012).

Although significant differences were not found in spermidine and spermine contents of the samples, the effect of ripening and storage stages on contents of spermidine and spermine was significant. Spermidine slightly increased during ripening and it also remained nearly stable throughout the storage. In addition, spermine contents of sausages apart from PS0 decreased during the first days of ripening and then spermine contents of all samples increased. Also during the storage, the spermine contents of sausages except for PS20 sample slightly decreased. It has been known that fresh meat contains considerably spermidine and spermine (Hernandez-Jover et al., 1996) and that the formation of these amines does not originate from factors such as food deterioration, fermentation process (Hernandez-Jover et al., 1997). Shalaby and El-Rahman (1995) stated that spermidine and spermine were not detected in fermented sausag es with PS.

3.4 The effects of PS on the colour values of sausages

The colour values of sausages were given in Table 4. The effects of PS treatment on *L*, *a* and *b* values were found to be significant. The effect of only 20% PS treatment on the *L* (lightness) value was significant during ripening and storage stages. From the 6th day of ripening, it was determined that the *L* value of the PS20 sample was lower than the other samples. Öztürk (2015) stated that the *L* value of sausage which was dipped into PS solution, decreased during ripening. In another study (Jin et al., 2015), the *L* value of sausage with PS was found to be similar to the *L* value of control sample. The effect of ripening and storage stages on the *L* values of the sausages was found to be significant. *L* values of all samples decreased during ripening stage and it was too little increased during the storage stage. In previous studies (Bozkurt, 2006; Ercoşkun and Özkal, 2011), it has been stated that the *L* value of the sausage is decreased during the ripening stage. Üren and Babayiğit (1997) reported that the decrease in the *L* value represents the dark coloration of sausage after drying.

Table 4. Colour values of sausages during the ripening and storage stages

Instrumental Colour	Sample	Ripening			Storage
		0th day	6th day	12th day	30th day
L	PS0	36.06±0.03 ^{aA}	27.45±0.00 ^{aB}	26.03±0.57 ^{aC}	27.08±0.11 ^{aB}
	PS5	35.17±0.57 ^{aA}	26.96±1.24 ^{aB}	25.70±0.47 ^{aB}	26.84±0.25 ^{aB}
	PS10	35.34±1.21 ^{aA}	27.12±0.04 ^{aB}	25.63±0.58 ^{aB}	26.65±0.33 ^{aB}
	PS20	35.24±0.47 ^{aA}	24.61±0.12 ^{bBC}	24.41±0.23 ^{bC}	25.38±0.25 ^{bB}
a	PS0	6.76±0.36 ^{aA}	6.77±0.55 ^{aA}	4.99±0.11 ^{aB}	5.31±0.21 ^{aB}
	PS5	5.40±0.28 ^{bAB}	5.76±0.15 ^{abA}	4.33±0.08 ^{bC}	5.04±0.23 ^{aB}
	PS10	5.62±0.58 ^{bA}	5.22±0.38 ^{bAB}	4.12±0.08 ^{bB}	4.71±0.40 ^{aAB}
	PS20	5.38±0.00 ^{bA}	3.74±0.41 ^{cB}	3.02±0.18 ^{cC}	3.37±0.17 ^{bBC}
b	PS0	5.94±0.53 ^{aA}	4.61±0.24 ^{aB}	3.55±0.01 ^{aC}	3.66±0.01 ^{aC}
	PS5	4.90±0.21 ^{aA}	4.39±0.54 ^{aA}	3.26±0.06 ^{abB}	3.53±0.03 ^{aB}
	PS10	5.67±0.61 ^{aA}	4.06±0.11 ^{aA}	3.10±0.04 ^{bC}	3.32±0.19 ^{aBC}
	PS20	6.14±0.58 ^{aA}	2.86±0.18 ^{bB}	2.59±0.22 ^{cB}	2.81±0.26 ^{bB}

[A-D]Different uppercase letters in a row show significant differences between the groups ($p<0.05$).

[a-b]Different lowercase letter in a column show significant differences between the groups($p<0.05$)

The *a* (redness) values of samples decreased with the PS treatment. Moreover, the effect of only 20% PS concentration on the *a* values of sausages during the storage was found to be significant. PS0 sample had highest *a* value and the *a* values of sausages decreased with the increasing PS concentration during the ripening. In the storage stage, *a* value of PS20 sample was found to be lower than the other samples. Öztürk (2015) reported that sausage which was dipping into PS had the lowest *a* value. It was determined that the effect of ripening and storage stages on the *a* values of sausages was significant (Table 4). The *a* values of all samples showed a decrease during the ripening stage. During the storage stage, the *a* values of all samples slightly increased. Ercoşkun and Özkal (2011) stated that the *a* value of sausage decreased from the 4th day of ripening. Bozkurt and Bayram (2006) reported that the *a* value of sausage increased during the first 5 days of ripening but then declined. It has been reported that this decrease in *a* value is due to the myoglobin denaturation caused by lactic acid (Ercoşkun and Özkal, 2011; Perez-Alvarez et al., 1999).

PS treatment also significantly affected the *b* (yellowness) values of sausages (Table 4). At 6th day of ripening, *b* value of PS20 sample was significantly lower than the other samples. However, all PS concentrations caused decrease in *b* value during 12th day of ripening. Only 20% PS concentration was found to be effective on *b* value during the storage stage. Moreover, *b* values in all samples decreased during ripening. Karabacak and Bozkurt (2008) stated that the *b* value of sausage decreased during the ripening stage. In another study (Perez-Alvarez et al., 1999), it was reported that *b* value of Spanish type dry-cured sausage decreased during ripening. This decrease in *b* value was associated with a decrease in the oxymyoglobin, which is contributed by the yellow color to the oxygen consumption by the microorganisms (Perez-Alvarez et al., 1999).

4. Conclusion

PS treatment to sausage casings decreased the counts of LAB, TAMB and *Micrococcus/Staphylococcus*. It was effective when applied at higher concentrations on LAB. No yeast-mold and *Enterobacteriaceae* were detected in sausages using PS-treated casings. Moreover, PS application to the casing significantly prevented the formation of biogenic amines with the exception of histamine. While the effect of PS on spermin and spermidine was not significant, it significantly increased histamine concentration. However, PS adversely affected red color, and with the application of more than 15% PS, lightness and yellowness were adversely affected. This process which casings were dipped into PS solution before filling, can be used effectively to inhibit microbial activity and to limit the formation of BA in sausage production. When the color values are taken into consideration, this treatment can be carried out at low PS concentrations such as 5-15%.

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