

The effects of different processing conditions on biogenic amine formation and some qualitative properties in pastırma

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Revised: 11 May 2017 / Accepted: 31 August 2017 / Published online: 12 September 2017
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Abstract Pastırma, a Turkish dry-cured meat product, was cured at two different temperatures (4 or 10 °C) with two different curing agents (150 mg/kg NaNO₂ or 300 mg/kg KNO₃). The aim of this research was to determine the effects of these factors on biogenic amine content and other qualitative properties (pH, a_w, color, residual nitrite, TBARS, NPN-M, microbiological properties). Residual nitrite was below 10 mg/kg in all samples. Both the curing agent and temperature were found to have a very significant effect on the TBARS value, and the curing agent had a significant effect on the NPN-M content. Curing at 10 °C increased the L* value; the use of nitrate increased the a* value. The use of nitrite had a negative effect on the growth of lactic acid bacteria. *Micrococcus/Staphylococcus* showed good growth in the presence of nitrate. In all samples, Enterobacteriaceae counts were below detectable levels. Neither temperature nor curing agent had significant effects on the amounts of tryptamine, cadaverine, histamine, tyramine, or spermine. There were very significant effects of temperature on the amount of putrescine and of the curing agent on the amount of spermidine.

Keywords Nitrate · Nitrite · Curing temperature · Biogenic amine · Residual nitrite

Introduction

Pastırma, a Turkish raw cured meat product, is made from whole muscles of certain parts of beef and water buffalo. It is produced by curing, washing, drying, pressing, and covering the meat with a paste called çemen (Tekinşen and Doğruer 2000; Gökalp et al. 2012; Kaban 2013). According to the Turkish Food Codex Communiqué on Meat and Meat Products (2012), the highest pH value of pastırma must be 6.0 and the product might be contained up to 50% of moisture. Moreover, the highest amount of residual nitrite in final product must be 50 mg/kg as TS 1071 (2002). This traditional meat product is classified as an intermediate moisture food. The use of salt (without nitrate and/or nitrite) in pastırma production causes a dark color in the final product (Tekinşen and Doğruer 2000). Nitrate is generally used as a curing agent with salt (Aksu and Kaya 2002; Gökalp et al. 2012; Kaban 2013). However, both nitrite and nitrate/nitrite can be used in the production (Uğuz et al. 2011; Erdemir 2012; Kaban 2013). Ambient temperature and the kind and amount of curing agent are important factors in the final product's quality. The curing temperature is quite important for growing microorganisms and the diffusion of the curing agent into the meat (Lücke 2007; Lautenschläger 2007; Garcia-Gil et al. 2014).

Nitrate becomes an active curing agent only after it reduces to nitrite. This happens in the presence of a certain number of microorganisms that have nitrate reductase activity (Honikel 2008; Kaban 2013). Hence, the curing temperature has quite an important role in growth and nitrate reductase activity. Coagulase negative cocci, the dominant flora in pastırma, are important for nitrate reductase activity, promoting the desired red color and its stabilization. These cocci also decompose peroxides and prevent oxidative rancidity. They contribute to the flavor of

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pastırma by forming volatile and non-volatile compounds through proteolytic and lipolytic activity. Lactic acid bacteria are another important group of microorganisms for pastırma (Kaban 2009, 2013). Some species of lactic acid bacteria and coagulase-negative catalase-positive cocci have decarboxylase activity (Santos 1996; Stadnik and Dolatowski 2010). There is no study on biogenic amine formation in pastırma. On the other hand, there are a few studies on biogenic amine formation and factors affecting the formation of biogenic amine in other dry-cured meat products such as dry-cured ham and *lacón* (Alfaia et al. 2004; Landeta et al. 2007; Virgili et al. 2007; Lorenzo et al. 2007; Martuscelli et al. 2009; Stadnik and Dolatowski 2012). Pastırma differs from other cured raw meat products processed in whole pieces, such as “Lachschenken”, “Rohschinken”, “*lacon*”, “raw ham”, “prosciutto di Parma”, “country style ham”, “jambon de Savoie”, “dry cured pork shoulder”, in term of both process time and conditions and raw material. Curing takes a few days in pastırma production while it can be as high as a few months in some other meat products processed in whole pieces. There is no smoking process in pastırma production. Production time of pastırma is approximately 1 month, depending on the size of the muscle used. However, production times of such products as Serrano, Iberico, Parma, French and country-style dry-cured hams are much longer than that of pastırma. Another differing feature of pastırma is its coating with a paste called “*çemen*” at the last stage of production (Kaya and Kaban 2016). The aim of this study was to determine effects of curing agent (150 mg/kg NaNO₂ or 300 mg/kg KNO₃) and temperature (4 or 10 °C) on biogenic amine formation and some qualitative properties of pastırma.

Materials and methods

Pastırma manufacturing and sampling

M. Longissimus dorsi muscles from middle-aged beef carcasses (24 h post mortem), obtained from a local slaughterhouse in Erzurum (Turkey), were used to make pastırma. The production was conducted under the controlled condition in a climate chamber (Reich, Germany). Two experiments were carried out, so two carcasses were used. Each muscle was cut into two pieces and eight pastırma strips were made. Fat and connective tissue were removed from the surface of the strips, and they were rubbed and covered with salt (50 g/kg), saccharose (3 g/kg) and potassium nitrate (300 mg/kg) or sodium nitrite (150 mg/kg). Strips were cured at 4 or 10 °C for 48 h. The cured strips were hung for 6 days at 15 ± 1 °C and 80 ± 2% relative humidity. After the first drying stage,

they were pressed with 15 kg of weight per kilogram of meat for 20 h at 7 ± 1 °C. For the second drying they were hung for 5 days at 20 ± 1 °C and 70 ± 2% relative humidity. Then they were pressed for a second, again using 15 kg of weight per kilogram of meat for 7 h at 25 ± 1 °C. Strips were then hung again for 5 days at 20 ± 1 °C and 70 ± 2% relative humidity. Cured and dried strips were placed in a bowl with a seasoning mixture (*çemen*) of 500 g flour ground from fenugreek (*Trigolella foenum graecum*), 150 g red pepper, 350 g smashed fresh garlic, and 1200 ml water for 1 day at 7 °C. Subsequently, the *çemen* covering the surface of the meat was trimmed to a 2–3 mm layer. Finally, the seasoned strips were hung for 10 days at 20 ± 1 °C and 70 ± 2% relative humidity. Final products were minced and then analyzed. Two replications were carried out for all analyses.

Physical and chemical analysis

To detect pH value, 10 g sample was homogenized with 100 ml distilled water and pH value was measured with a pH meter (IKA Werk T 25, Germany). Water activity (*a_w*) was detected using an *a_w* device (TH-500 *a_w* Sprint, Switzerland) at 25 °C. Color values (*L**, *a** and *b**) were measured using a color meter (CR-400 Konika Minolta, Japan).

Residual nitrite in samples was detected according to Tauchmann (1987). Residual nitrite (mg/kg) was calculated based on sample weight, dilution factor, coefficient (calculated using standard curve) and absorbance. TBARS (Thiobarbituric acid-reactive substances) value was determined according to the method of Lemon (1975) and expressed as μmol malondialdehyde (MDA)/kg samples. For the detection of NPN-M (non-protein nitrogenous matter) content, proteins were precipitated with trichloroacetic acid. Then NPN-M was detected with the Kjeldahl method. The results were expressed as g/100 g of samples (Anonymus 1989).

Microbiological analysis

Samples of 25 g were placed in a sterile stomacher bag and then 225 ml sterile physiological saline (0.85% NaCl) was added and homogenized with a stomacher (Lab Stomacher Blander 400-BA 7021, UK) for 1.5 min. Next, 0.1 ml samples of adequate dilutions were spread on agar plates. Lactic acid bacteria, Enterobacteriaceae, and *Micrococcus/Staphylococcus* were evaluated on de Man Rogosa Sharpe Agar (MRS, Merck) in anaerobic conditions (Aneorocult A, Merck), Violet Red Bile Dextrose Agar (VRBD, Merck) in anaerobic conditions, and Mannitol Salt Red Agar (MSA, Oxoid) after 48 h at 30 °C, respectively. Yeast-mold was detected on Rose-Bengal Chloramphenicol Agar (RBC, Merck) after 5 days at 25 °C.

Biogenic amine analysis

Two-gram samples were weighed and 0.1 ml internal standard (1,7-diaminoheptane, Aldrich, D17408) was added. Samples were homogenized using an ultra-turrax (IKA Werk T 25, Germany) with 10 ml of 0.4M perchloric acid and then centrifuged (3000 rpm/10 min.) and filtered in a volumetric flask (25 ml). Next, 0.4M perchloric acid (10 ml) was added and the process was repeated. The volumetric flask was filled to 25 ml with 0.4M perchloric acid. The 1 ml extract was made the basic characteristic with 2N NaOH and 300 μ l saturated NaHCO₃ was added for derivatization. After adding 2 ml dansyl chloride (D2625, Sigma), samples were kept at 40 °C for 45 min. 100 μ l ammonia (25%) was put in the samples. Volume was completed to 5 ml with acetonitrile. After this stage, samples were centrifuged (2500 rpm/5 min) and filtered with a 0.45 μ m filter. Standards of biogenic amines were used for the preparation of a standard curve. The gradient-elution system was 0.1 M ammonium acetate as solvent A and acetonitrile as solvent B. The gradient-elution program was started at 50% solvent B and reached at 90% solvent B at the end of 25 min. Flow rate was 1 ml/min, column temperature was 40 °C, and measurements were done at 254 nm using the HPLC system (Agilent 1100 series, Germany), with a Spherisorb S5 ODS2-150A column (Waters-15 cm \times 4.60 mm). Putrescine (P5780, Sigma), tyramine (T2879, Sigma), histamine (H7250, Sigma), cadaverine (C8561, Aldrich), tryptamine (246557, Aldrich), spermidine (Sigma, S2501), and spermine (Sigma, S2876) standards were obtained from Sigma. Results were calculated according to Eerola et al. (1993). Biogenic amine contents were expressed as mg/kg.

Statistical analysis

Two different curing agents (300 mg/kg KNO₃ and 150 mg/kg NaNO₂) and curing temperatures (4 and 10 °C) were selected as factors and experiments were designed according to a randomized complete block design with two replicates. The data were tested by variance analysis and significant means were compared with Duncan's multiple range tests (SPSS 2011).

Results and discussion

Table 1 shows the effects of curing agent and temperature on pH, a_w, residual nitrite, *Micrococcus/Staphylococcus*, and yeast-mould count. Low pH value was detected in the presence of nitrate at 10 °C. However, pH values in all samples were not under 5.5, as in other studies on pastirma (Aksu and Kaya 2001; Kaban 2009). In dry cured meat

products such as ham and pastirma, water activity is an important hurdle effect for microbiological stability (Leistner 1988; Kaban 2009). In all pastirma samples, the a_w value was under 0.90. Neither curing temperature nor curing agent affected a_w value (Table 1). In these type products, water activity is usually affected by the ripening process. The water activity progressively decreases with processing time because of salt diffusion and water loss that occurred during the processing (Lautenschläger 2007; Martuscelli et al. 2015).

Residual nitrite was under 10 mg/kg in all samples. The interaction of temperature and curing agent had a very significant effect ($P < 0.01$) on residual nitrite (Fig. 1). Curing temperature showed a slight effect on residual nitrite in the presence of nitrite (Fig. 1). In the presence of nitrate, residual nitrite was significantly lower at 10 °C than 4 °C. This result may be explained by a good nitrate reductase activity of microorganisms at 10 °C.

Micrococcus/Staphylococcus are important microorganisms for pastirma because of their nitrate reductase and catalase activities. They have also proteolytic and lipolytic activities. Hence these microorganisms play an important role in color formation and stabilization, aroma development and delaying autoxidation (Kaban 2013). According to the variance analysis of the *Micrococcus/Staphylococcus* count, curing temperature had no significant effect on these microorganisms (Table 1). However, catalase-positive cocci showed better growth in the presence of nitrate than nitrite (Table 1). In contrast, yeast-mould count was affected by curing temperature ($P < 0.05$). However, the effect of curing agent on yeast-mould count was found to be insignificant ($P > 0.05$), while the interaction of both factors had a very significant effect on yeast-mould count ($P < 0.01$) (Table 1). As it can be seen from Fig. 2, there were no significant changes in yeast-mould count due to curing temperature factor in the presence of nitrite. On the other hand, a lower average count was detected at 10 °C in comparison with at 4 °C in the presence of nitrate. This result is caused by better growth of *Micrococcus/Staphylococcus* in the presence of nitrate (Table 1) and probably due to faster degradation of nitrate to nitrite and nitrite to NO at 10 °C.

The lactic acid bacteria count was below detectable levels (<2 cfu/g) in all samples cured with nitrite. However, these microorganisms were generally above 1×10^4 cfu/g in samples cured with nitrate. According to this, nitrite repressed the growth of lactic acid bacteria at both curing temperatures. The Enterobacteriaceae count was below detectable level in all samples. In other studies, it has been found that Enterobacteriaceae do not survive during ripening of pastirma (Aksu and Kaya 2001; Kaban 2009).

Table 1 Overall effect of curing agent and curing temperature on the pH, a_w and microbiological properties of pastirma (mean \pm SD)

	a_w	pH	Residual Nitrite (mg/kg)	<i>Micrococcus/Staphylococcus</i> (log cfu g ⁻¹)	Yeast-mould (log cfu g ⁻¹)
Curing agent (CA)					
Nitrite	0.853 \pm 0.01 ^a	5.77 \pm 0.03 ^a	6.19 \pm 1.28 ^a	5.92 \pm 0.30 ^b	4.61 \pm 0.22 ^a
Nitrate	0.848 \pm 0.01 ^a	5.74 \pm 0.04 ^b	4.90 \pm 1.51 ^b	6.46 \pm 0.40 ^a	4.71 \pm 0.63 ^a
Significance	NS	*	**	**	NS
Curing temperature (CT)					
4 °C	0.849 \pm 0.01 ^a	5.77 \pm 0.03 ^a	6.00 \pm 1.51 ^a	6.32 \pm 0.32 ^a	4.87 \pm 0.51 ^a
10 °C	0.852 \pm 0.01 ^a	5.74 \pm 0.04 ^b	5.09 \pm 1.45 ^b	6.07 \pm 0.53 ^a	4.45 \pm 0.29 ^b
Significance	NS	*	*	NS	*
CA \times CT	NS	NS	**	NS	**

NS Not Significant ($P > 0.05$); SD Standard Deviation

* $P < 0.05$; ** $P < 0.01$

a–b Any two means in the same column having the same letters in the same section are not significantly different at $P > 0.05$

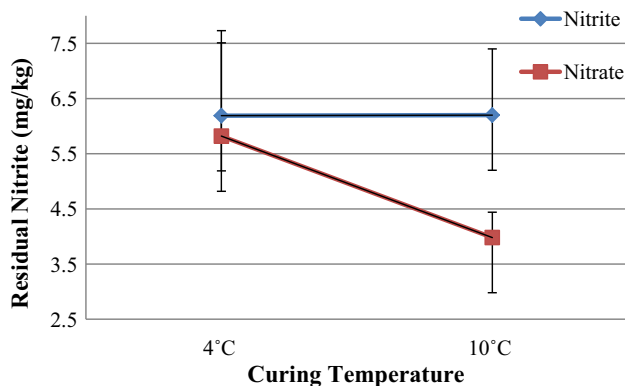


Fig. 1 The effect of the interaction of curing agent \times curing temperature on residual nitrite

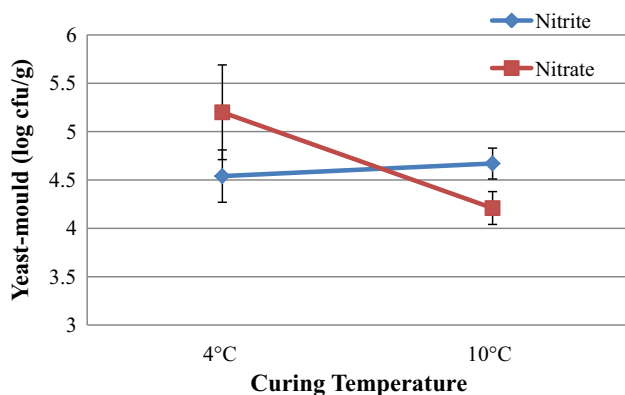


Fig. 2 The effect of the interaction of curing agent \times curing temperature on yeast-mould

The curing agent had a very significant effect ($P < 0.01$) on the TBARS value, which is an indicator for lipid autoxidation; nitrite decreased the TBARS value. TBARS value was lower at 4 °C than 10 °C (Table 2). Nitrate and nitrite are used as curing agent in the production of

pastirma. However, nitrate reductase positive microorganisms are very important especially in pastirma cured with nitrate. Nitrate could be transformed to nitrite by these microorganism and its effect occurs (Kaban 2013). In other studies conducted on pastirma, it has been reported that TBARS value increases during the ripening period and reaches about 30 μ mol MDA/kg in final product (Aksu and Kaya 2001; Kaban 2009). The lowest NPN-M mean value was detected in a sample cured with nitrite. However, curing temperature had no significant effect on NPN-M. The increase of NPN-M or NPN value may be explained by means of formation of peptides and amino acids that caused by endogenous enzymes (Martuscelli et al. 2015). Statistical analyses displayed that instrumental color parameters (L^* , a^* and b^* —values) are showed in Table 2. L^* value was very significantly ($P < 0.01$) influenced by curing temperature, and samples cured at 10 °C showed higher L^* than at 4 °C, indicating a brighter red color. However, curing agent had a significant effect ($P < 0.05$) only on a^* value (Table 2). According to these results, color, a significant criterion for this product, was affected positively by using nitrate as a curing agent at 10 °C.

Amine content and profile may be influenced by many factors such as pH, redox potential, temperature, additives, curing, the size of product (Jairath et al. 2015). Table 3 shows the effects of curing agent and curing temperature on biogenic amine formation in pastirma. Putrescine had higher values than other biogenic amines. Tyramine and cadaverine generally had lower values. Histamine was also low. In a study on Italian dry-cured ham, histamine was not detected, in addition to low cadaverine levels and high tyramine and putrescine levels (Virgili et al. 2007). Putrescine, cadaverine, and tyramine levels increased

Table 2 Overall effects of curing agent and curing temperature on the NPN-M, TBARS and color values of pastirma (mean \pm SD)

	NPN-M (g/100 g)	TBARS (μ mol)	L*	a*	b*
Curing agent (CA)					
Nitrite	5.13 \pm 0.26 ^b	33.96 \pm 8.40 ^b	39.52 \pm 4.68 ^a	37.43 \pm 1.91 ^b	23.97 \pm 2.47 ^a
Nitrate	5.49 \pm 0.28 ^a	40.76 \pm 5.64 ^a	38.19 \pm 2.74 ^a	38.86 \pm 1.55 ^a	22.96 \pm 2.57 ^a
Significance	*	**	NS	*	NS
Curing temperature(CT)					
4 °C	5.22 \pm 0.31 ^a	31.68 \pm 6.24 ^b	37.74 \pm 3.45 ^b	38.67 \pm 1.69 ^a	23.25 \pm 2.04 ^a
10 °C	5.39 \pm 0.33 ^a	43.04 \pm 4.01 ^a	39.97 \pm 3.97 ^a	37.61 \pm 1.92 ^a	23.68 \pm 3.00 ^a
Significance	NS	**	**	NS	NS
CA \times CT	NS	NS	NS	NS	NS

NS Not Significant ($P > 0.05$); SD Standard Deviation

* $P < 0.05$; ** $P < 0.01$

^{a-b} Any two means in the same column having the same letters in the same section are not significantly different at $P > 0.05$

Table 3 Overall effect of curing agent and curing temperature on biogenic amines of pastirma (mean \pm SD)

	Tryptamine (mg/kg)	Putrescine (mg/kg)	Cadaverine (mg/kg)	Histamine (mg/kg)	Tyramine (mg/kg)	Spermidine (mg/kg)	Spermine (mg/kg)
Curing agent (CA)							
Nitrite	17.30 \pm 6.63 ^a	38.14 \pm 27.08 ^a	1.37 \pm 1.14 ^a	4.32 \pm 1.37 ^a	4.09 \pm 3.67 ^a	8.36 \pm 2.84 ^a	13.37 \pm 2.91 ^a
Nitrate	17.62 \pm 3.85 ^a	44.55 \pm 28.37 ^a	1.68 \pm 1.68 ^a	4.56 \pm 1.72 ^a	1.45 \pm 0.86 ^a	5.53 \pm 2.42 ^b	11.54 \pm 6.61 ^a
Significance	NS	NS	NS	NS	NS	**	NS
Curing temperature(CT)							
4 °C	16.25 \pm 2.66 ^a	33.73 \pm 19.15 ^b	1.82 \pm 1.36 ^a	4.54 \pm 1.50 ^a	2.77 \pm 2.04 ^a	7.44 \pm 2.28 ^a	14.49 \pm 4.98 ^a
10 °C	18.67 \pm 6.96 ^a	48.96 \pm 32.60 ^a	1.23 \pm 1.46 ^a	4.34 \pm 1.61 ^a	2.77 \pm 3.74 ^a	6.45 \pm 3.57 ^a	10.43 \pm 4.46 ^a
Significance	NS	**	NS	NS	NS	NS	NS
CA \times CT	NS	NS	NS	NS	NS	**	NS

NS Not Significant ($P > 0.05$); SD Standard Deviation

** $P < 0.01$

^{a-b} Any two means in the same column having the same letters in the same section are not significantly different at $P > 0.05$

rapidly during the ripening of Portuguese dry-cured meat, which had low histamine and spermidine levels and a decrease in spermine during the ripening period. It was suggested that this was related to microbial utilization for biological reactions (Alfaia et al. 2004). Córdoba et al. (1994) stated that biogenic amine levels were too low to be toxic in Iberian ham. Toxicologic levels of biogenic amines changes according to individual characteristics and other amines. Legal upper limit for histamin is reported as 100 mg/kg food. There are limited information available on the toxic doses other amines. Nevertheless, maximum levels for tyramine and phenylethylamine are reported as 100–800 mg/kg food and 30 mg/kg food, respectively (Stadnik and Daltovski 2010). Lorenzo et al. (2007) reported that the use of additives (glucose + sodium nitrate + sodium nitrite + sodium ascorbate + sodium citrate) in dry-cured lacón significantly increased the total amine content as well as tryptamine, histamine and

tyramine. In addition, the mean levels of tryptamine and cadaverine were determined as 36.92 and 39.15 mg/kg in dry-cured lacón without additives, as 57.50 and 35.55 mg/kg in the product with additives, respectively. The researchers have also expressed that this may be resulting from glucose's stimulation of the growth of lactic acid bacteria. On the other hand, similar results regarding cadaverine and tryptamine were determined in dry-cured pork loins (300 ppm KNO₃) with probiotics and their average values were found as 39.6 and 49.2 mg/kg, respectively (Stadnik and Dolatowski 2012). These levels are much higher than biogenic amine levels in pastirma, in which the highest mean tryptamine and cadaverine levels were 18.67 and 1.82 mg/kg, respectively.

Many researchers have shown that Enterobacteriaceae, produce biogenic amine, which plays an important role in and directly relates to high cadaverine levels (Suzzi and Gardini 2003; Genççelep et al. 2008; Lorenzo et al. 2007).

Low cadaverine levels in pastirma could be related to a low Enterobacteriaceae count ($<2 \log \text{ cfu/g}$). Lorenzo et al. (2007) reported an average of $35.55 \pm 5.88 \text{ mg/kg}$ cadaverine in Spanish dry-cured ham after ripening. In another study, histamine, putrescine, tyramine, and phenylethylamine values varied between 38.2–271, 41.3–588, 123–618 and 0.1–215 mg/kg, respectively (Pechanek et al. 1983). These findings are higher than the biogenic amine content of pastirma. A low biogenic amine content of pastirma could be related to the short time of curing and ripening/production. On the other hand, pH value was above 5.5 in all of pastirma groups. In the formation of biogenic amine, the pH value is accepted as a key factor because of the influencing amino acid decarboxylase activity, showed an optimum activity in acid pH. Thereby, when the pH value decreases, as a result of decarboxylase activity increases the production of biogenic amines (Jairath et al. 2015).

The curing temperature is one of the main sources of variation, and it significantly affected the putrescine. The putrescine content was higher at 10°C than 4°C ($P < 0.05$) (Table 3). This is probably related to the better growth of *Micrococcus/Staphylococcus* and lactic acid bacteria at 10°C . It has been reported that some strains of *Lactobacillus* and *Staphylococcus* can produce putrescine (Beutling 1996). In another study, high putrescine content was detected at the end of the ripening period (Virgili et al. 2007). As can be seen in Table 3, the curing agent affected ($P < 0.01$) spermidine levels. Samples cured with nitrite showed higher average spermidine levels than samples cured with nitrate (Table 3). Hence, putrescine is a good precursor for spermidine. Similar results were reported in other studies (Smělá et al. 2003; Karahan 2003; Vidal-Carou et al. 2007). The interaction of curing temperature and agent had a very significant effect ($P < 0.01$) on spermidine (Fig. 3). In samples cured at 4°C , spermidine was not affected by the curing agent. In contrast,

spermidine levels increased at 10°C when nitrite was used. As shown in Fig. 3, the presence of nitrate at the same curing temperature caused a decrease in spermidine. According to these results, putrescine can be converted to spermidine in samples cured at 4°C and spermidine can be converted to spermine or putrescine in samples cured at 10°C (Vidal-Carou et al. 2007).

Conclusion

In conclusion, using nitrate in pastirma production as curing agent showed a range of positive effects on some properties of the product. Similarly, some characteristics were affected positively by curing at 10°C . The residual nitrite in the final product decreased to below 10 mg/kg in the presence of 150 mg/kg NaNO_2 or 300 mg/kg KNO_3 . The formation of biogenic amines in pastirma is limited if production is carried out according to hygienic and technological rules.

Acknowledgements This study has been supported by the Research Council of Atatürk University (BAP 2013/136). The financial support of Atatürk University is gratefully acknowledged.

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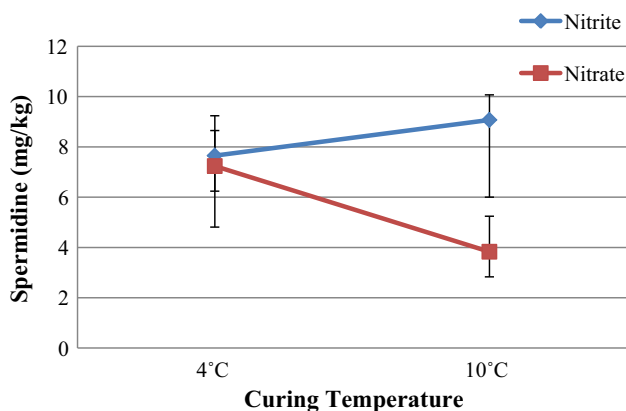


Fig. 3 The effect of the interaction of curing agent \times curing temperature on spermidine amount

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