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Effect of modified atmosphere packaging, storage period, and storage temperature on the residual nitrate of sliced-pastırma, dry meat product, produced from fresh meat and frozen/thawed meat ☆

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Abstract

The amount of nitrite in sliced-pastirma made, from fresh or frozen (which was stored at −18 °C for 240 days and then thawed at 10 °C for 24 h) *M. Longissimus dorsi* muscle was determined. Sliced-pastirma samples were stored in modified atmosphere packages (50% N_2 + 50% CO_2) at 4 and 10 °C for 150 days, and the amount of residual nitrite was measured after 0, 30, 60, 90, and 150 days of storage. The residual nitrite of pastirma samples made with frozen/thawed meat was higher than that of the pastirma made from fresh meat at both 0 day and at the end of the storage (150 days). The storage temperature ($p < 0.01$), storage period ($p < 0.01$) and the storage period × the storage temperature interaction ($p < 0.01$) had significant effects on the amount of the residual nitrite.

Keywords: Pastırma; Residual nitrite; Modified atmosphere; Storage; Cured meat product

1. Introduction

Pastirma, a traditional Turkish meat product, is categorized (Gökalp, Kaya, & Zorba, 1999; Leistner, 1988) as an intermediate-moisture food (IMF). It is produced from whole muscle, obtained from certain parts of beef and water buffalo carcasses and, from one carcass, 16–20 different types of pastirma can be produced. In the pastirma process, muscles are cured, dried, pressed and coated with a cement of garlic, red pepper, paprika, flour ground from seed of *Trigonella foenum graecum* and water. They are then dried again (maximum water content: 40%). Cement (paste seasoning) has a protective effect against mould growth and oxygen penetration, on and through the surface of the pastirma. High nutritional values and typical aromas from the cement have led to increase of pastirma consumption (Kaya, Aksu, & Gökalp, 1996; Tekinsen & Dogruer, 2000). Pastirma is the most popular dry-cured meat product in Turkey. A lot of dry-cured meat product is produced in the world, e.g., pastirma, bacon, Bündnerfleisch and ham, with or without a heat process. Cured-meat products differ greatly in composition and intended eating quality, but the types of bacteria growing on and in them are similar because the main factors controlling their growth are the same over a wide range of products

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(Roberts & Dainty, 1996; Topkim, 1980). Also, in cured meat products, curing may be combined with other processes, including drying, heating, smoking, and fermentation, but production of pastirma does not include heating or smoking processes.

The curing mixture used in pastirma production has a very important effect on pastirma, quality characteristics, so salt as well as nitrite and/or nitrate are used in the process of manufacturing pastirma. Also, another factor that influences quality is the penetration of curing compounds into the muscle. Numerous studies have shown that frozen/thawed meats can be penetrated more rapidly, since the salt penetrates faster than with fresh meats (Banon, Cayuela, Granados, & Garrido, 1999; Kemp, Langlois, & Johnson, 1982; Motilva, Toldra, Nadal, & Flores, 1994). In Turkey, fresh meat is usually used for pastirma production (Aksu & Kaya, 2001a, 2001b; Aksu, Aktas, & Kaya, 2002; Aksu & Kaya, 2005). However, Aksu, Kaya, and Ockerman (2005) reported that frozen/thawed meat could be used as a raw material. These researchers also determined that the quality characteristics of pastirma made from frozen/thawed meat are similar to those of pastirma made from fresh meat. Pastirma has to be sliced before being consumed without cooking. Preserving the quality characteristics of sliced pastirma can be a challenge, so sliced pastirma needs to be packaged in a modified atmosphere and stored at low temperature in order to prevent spoilage and to protect the sensory and microbiological quality.

The present study was conducted to determine the effect of modified atmosphere packaging, storage period, storage temperature and the use of fresh or frozen/thawed meat on the amount of residual nitrite remaining in sliced pastirma.

2. Materials and methods

2.1. Production of pastirma

M. Longissimus dorsi muscle from beef carcasses was used as the raw material in this study. The muscles were obtained from Eastern Anatolian Red beef carcasses (3-year-old cows) from a local slaughterhouse (Et ve Balik Kurumu Kombinasi, Erzurum, Turkey). For treatments (fresh and frozen/thawed), eight carcasses were used and one carcass was used for each replication. After removing fat and connective tissue from surfaces of the *Longissimus dorsi* muscle, the muscle was cut across the centre of the muscle into two pieces. Then half of the muscles were divided into two groups. One group was immediately used for pastirma production; the other group was frozen at −20 °C and stored at −18 °C for 240 days. The frozen muscles were then thawed at 10 °C for 24 h and the muscles were used for pastirma production. The production stages of pastirma are presented in Table 1. The curing mixture and composition of paste seasoning (cementing) used in pastirma production are shown in Table 2.

Table 1
The stages of pastirma production (Aksu, 1999; Aksu & Kaya, 2002a)

Production stage	Time (days and hours)	Temperature (°C)	Relative humidity (%)
Dry curing[a]	2 days	6 ± 0.5	80 ± 1 to 90 ± 1
First drying	4 days	15 ± 0.5	80 ± 1 to 85 ± 1
First pressing[b]	17 h	7 ± 0.5	
Second drying	3 days	20 ± 0.5	70 ± 1
Second pressing[b]	7 h	25 ± 0.5	
Paste seasoning (cementing)[c]	4 days	7 ± 0.5	
Third drying	2 days	15 ± 0.5	70 ± 1
	2 days	18 ± 0.5	65 ± 1
	6–8 days	20 ± 0.5	60 ± 1

[a] For each 1 kg of meat, 50 g of curing mixture were used and the composition can be seen in Table 2.
[b] For each 1 kg of meat, a 25 kg weight was used.
[c] 3–4 mm thickness of paste was used on the surface and the composition can be seen in Table 2.

Table 2
The composition of curing (Aksu, 1999; Aksu & Kaya, 2002a) and paste seasoning (cement) (Cankaya, 1997; Aksu, 1999) used in pastirma production

Curing mixture composition[a]		Cement mixture composition[c]	
NaCl	47.25 g	Flour (*Trigonella foenum graecum* seed)	500 g
KNO_3	0.75 g	Smashed fresh garlic	350 g
Glucose	1.0 g	Paprika	75 g
Sucrose	1.0 g	Red pepper	75 g
Starter Culture[b]	25 g/100 kg of meat	Water	1200 ml

[a] For each 1 kg of meat, 50 g of curing mixture were used.
[b] A commercial preparation of *Staphylococcus carnosus* + *Lactobacillus pentosus* (Bactoferm™ C-P-77 S) was used as the starter culture (Chr Hansen's, Pohlheim, Germany), and starter culture was introduced into muscle with the curing mixture.
[c] After the second drying step of pastirma production, the dried meat samples were pasted with the seasoning mix and the surface of the meat was covered (3–4 mm thickness) with the seasoning mixture.

2.2. Packaging of sliced pastirma

Pastirma, produced from fresh or frozen/thawed meat, was sliced 1–2 mm in thickness using a slicing machine. The sliced pastirma samples were then packaged in 90 ± 5 g portions in a moderately gas impermeable OPAEVOH/PE (water vapour transmission rate 15 g/m^2/24 h/38 °C/, 90% RH, 1 atm; O_2 transmission rate 5 cm^3/m^2/24 h/23 °C, 50% RH, 1 atm; N_2 transmission rate 1 cm^3/m^2/24 h/23 °C, 50% RH, 1 atm; CO_2 trans-

mission rate 23 cm^3/m^2/24 h/23 °C, 50% RH, 1 atm) bag. Packages were evacuated, filled with a modified atmosphere containing 50% N_2 and 50% CO_2 (the volume ratio of gas to sliced pastirma product was 1:2) and automatically heat-sealed by a Multivac packaging unit (Multivac A 300/16, Sepp Haggenmüller, D 87787 Wolfertschwenden, Germany).

2.3. Residual nitrite analysis

Residual nitrite was determined at 0, 30, 60, 90, 120, and 150 days of storage at 4 or 10 °C. Residual nitrite was spectrophotometrically determined according to the methods of Anonymous (1981) and Tauchmann (1987). Ten-grammes of minced pastirma sample were homogenized using an Ultra-Turrax moderator (Nach Prof. P. Willem, Janke and Kunkel KG, IKA Werk, Staufen I. Breisgau), in 10 ml of saturated borax solution (25 g $Na_2B_4O_7 \cdot 10H_2O$ for 500 ml deionized water) and 100 ml of boiling deionized water; then this mixture was placed in a bag to boil in a water bath for 15 min, and cooled with tap water for 3 min. The content of this mixture was transferred into a 200 ml volumetric flask 2 ml of Carrez-I (26.5 g $K_4(Fe(CN)_6 \cdot 3H_2O)$ into 250 ml deionized water) were added and also 2 ml of Carrez-II [55 g $Zn(CH_3COO)_2.2H_2O$ + 7.5 ml glacial acetic acid (99.5 %) into deionized 250 ml water] solution were also added. The total 200 ml volume was adjusted by adding deionized water. This mixture was allowed to settle at room temperature (20 °C) for 30 min and then filtered through Whatman No: 42 filter paper. Ten ml of the filtrate were combined with, 5 ml of sulfanilamide solution [1.5 g $NH_2C_6H_4SO_2NH_2$ were added to 62.5 ml HCl (37%) and made up to 250 ml with deionized water] and 5 ml of *N*(1-napthyl)-ethylenediaminedihydrochloride solution [0.25 g $C_{12}H_{14}N_2 \cdot 2HCl$ for 250 ml volume with deionized water], and this mixture was allowed to settle at room temperature (20 °C) for 30 min. At the end of 30 min, absorbances of the solutions were determined at 540 nm (utilizing a blank without the sample solution) with a spectrophotometer (Shimadzu, UV-160A UV–Visible Recording Spectrophotometer). A standard curve was prepared using pure sodium nitrite ($NaNO_2$). Results were expressed as ppm of $NaNO_2$.

Changes (%) in the amount of residual nitrite were calculated by differences at various storage days and the results are shown in Table 3 as ppm of residual nitrite and as percentage (%) change when compared to day 0.

2.4. Statistical analysis

Experimental data were statistically processed, using the SPSS version, with 10.01 software (SPSS, 1996). Data were analyzed as a completely randomized design procedure, and comparisons of treatment means were based on Duncan's multible range test. Mean values and standard deviation (±) are shown in Tables 3 and 4.

Table 3
The amount of residual nitrite (ppm) ± standard deviation of sliced and packaged (50% N_2 + 50% CO_2) pastirma samples during storage

Storage period (day)	Frozen/thawed		Fresh	
	Residual nitrite (ppm)	Changes compared to 0 days (%)	Residual nitrite (ppm)	Changes compared to 0 days (%)
0	22.42 ± 0.57 a	0.00	11.60 ± 0.10 a	0.00
30	19.82 ± 1.52 b	11.60	11.49 ± 1.23 a	0.95
60	15.04 ± 1.58 c	32.92	9.20 ± 0.37 b	20.69
90	8.15 ± 0.92 d	63.65	3.45 ± 0.14 c	70.26
120	7.59 ± 0.87 d	66.15	3.00 ± 0.15 cd	74.14
150	5.27 ± 1.62 e	76.49	2.57 ± 0.18 d	77.84
P	_[A]		_[A]	

P: Probability.
a–e: Means with different letters in the same column are significantly different at ($P < 0.05$).
[A] $P < 0.01$ (fresh vs frozen/thawed).

Table 4
The amount of residual nitrite (ppm) ± standard deviation for pastirma stored at 4 and 10 °C

Storage temperature	Frozen/thawed	Fresh
4 °C	13.55 ± 6.29 a	6.63 ± 3.98 b
10 °C	12.55 ± 7.35 b	7.14 ± 4.34 a
P	_[A]	_[A]

P: Probability.
a–b: Means with different letters in the same column are significantly different at ($P < 0.05$).
[A] $P < 0.01$ (fresh vs frozen/thawed).

3. Results and discussion

Table 3 shows the mean values and standard deviations, for residual nitrite and changes for each storage period (30, 60, 90, 120, and 150 days) compared to 0 day for the pastirma samples. Pastirma produced from frozen/thawed meat had higher ($p < 0.01$) residual nitrite than that produced from fresh meat at 0 days of storage, although the processes, of pastirma (fresh and frozen/thawed) production used the same curing mixture and the same curing time (Table 3). This difference may be explained by the properties of the raw material used for pastirma production. It has been reported, in related research, that frozen/thawed meat allows more rapid penetration of curing matter (Banon et al., 1999; Kemp et al., 1982; Motilva et al., 1994). In sliced-pastirma, produced from frozen/thawed meat, the highest amount of residual nitrite (22.4 ± 0.57 ppm) was observed in the early days of storage (0 days). This was significantly

decreased (5.27 ± 1.62 ppm) during storage. A significant decrease at 150 days of storage, by 77.84% compared to day 0 (Table 3), was also observed in sliced-pastirma produced from fresh meat.

There was a decrease ($p < 0.01$) in the amount of residual nitrite of sliced pastirma (fresh and frozen/thawed) during storage. The lowest residual nitrite values was found at 150 days in sliced pastirma produced from frozen/thawed meat, while lower residual nitrite values were observed after 90–150 days of storage for sliced pastirma produced from fresh meat (Table 3). There was also a statistically significant ($p < 0.01$) decrease in nitrite values between 90 and 150 days of storage for pastirma produced from both fresh and frozen/thawed meat (Table 3).

The residual nitrite values of sliced and packaged pastirma in modified atmosphere samples stored at 4 and 10 °C are shown in Table 4. The storage temperature (4 and 10 °C) had an effect ($p < 0.01$) on the amount of residual nitrite. Higher residual nitrite value was found in the pastirma produced from frozen/thawed meat and the samples stored at 4 °C had higher residual nitrite values than those stored at 10 °C (Table 4).

An interaction ($p < 0.01$) of the storage period × the storage temperature was found for residual nitrite of both pastirma groups (fresh and frozen/thawed), as shown in Fig. 1. This figure illustrates that the fastest decrease in residual nitrite, among both pastirma groups, occurred between 30 and 90 days of storage.

Nitrite, if used improperly, can be dangerous from a human health standpoint; hence, the level of usage is limited, and in some countries, the use of nitrite has been banned. It is also advisable to use a mixture of salt with nitrite to reduce accidental formulation mistakes. Nitrite and/or nitrate need to be added to the curing mixture for cured meat products to enhance colour, and flavour, reduce bacterial growth and reduce oxidation properties of the final product (Gökalp et al., 1999; Kaya, 1995; Kaya et al., 1996; Wirth, 1986; Prändl, Fischer, Schmidhofer, & Sinell, 1988). According to the Turkish Food Additive Codex (Anonymous, 1997), the maximum amount of residual nitrite for cured meat products is 50 mg/kg. In the present study, the determined residual nitrite values in all the pastirma samples, produced by the current procedure from fresh and frozen/thawed meat were below this level (<50 mg/kg, $NaNO_2$). There has been some research on the amount of residual nitrite of pastirma produced in Turkey. For instance, El-Khateib, Schmidt, and Leistner (1987) reported that the amount of residual nitrite in Turkish pastirma ranged from 2 to 58 mg/kg (average: 12 mg/kg). Soyutemiz and Özenir (1996) found that the amount of residual nitrite in pastirma was 16.0 mg/kg of product found in the market place in Bursa. Cankaya (1997) reported that the amount of residual nitrite in pastirma was 0.43–6.37 mg/kg. Yagli (Gür) and Ertas (1998) made similar observations (13.4–25.8 mg/kg) during investigations of products also containing sodium ascorbate in the production of pastirma. Tyrpenou, Gouta, Tsigouri, and Vlasiotis (2000) reported that the amount of residual nitrite in Greek pastirma ranged from 0.85 to 190 mg/kg as sodium nitrite. Aksu and Kaya (2001a) reported that the amount of residual nitrite of pastirma marketed in Erzurum was 0.93–11.6 mg/kg. Aksu and Kaya (2002a, 2002b) also reported that the amounts of residual nitrite in pastirma produced by different curing methods (dry- and brine-curing) and pastirma produced using potassium nitrate and a starter culture were 44.1–

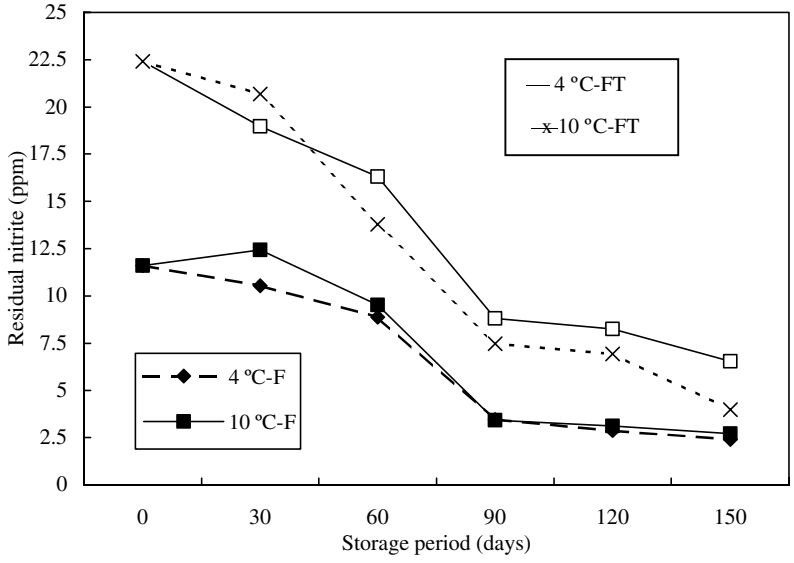

Fig. 1. Effect of fresh and frozen/thawed raw material, storage period and storage temperature on the residual nitrite content of pastirma samples (Raw material for pastirma: F, Fresh meat and FT, Frozen/Thawed meat).

47.9 and 64.3–75.6 mg/kg, respectively. Dogruer, Guner, Gurbuz, and Ucar (2003) reported that the residual nitrite of pastirma produced by using sodium and potassium nitrate was 22.1–143 mg/kg. Aksu and Kaya (2001b, 2002c) in another studies, determined that the use of commercial starter cultures in pastirma production, decreased the amount of residual nitrite [8.50–21.3 mg/kg (2001b); 22.7 mg/kg (2002c)]. Furthermore, various researchers have reported that use of starter cultures, in dry-cured raw meat products like pastirma, increased the quality properties of the final (end) product (Aksu & Kaya, 2001b, 2002a, 2002b; Aksu et al., 2002; Katsaras, Lautenschlager, & Bosklova, 1996). Some types (*Micrococcaceae*, such as *Staphylococcus carnosus*, *Staphylococcus xylosus*, etc) generally contain nitrate reductase, and proteolytic and lipolytic enzymes (Johansson, Berdague, Larsson, & Borch, 1994; Krockel, 1995), and these microorganisms can be used as starter cultures in cured meat products (Hammes & Hertel, 1998). Nitrate has to be converted to nitrite to improve the quality properties of meat products (Geisen, Lücke, & Kröckel, 1992; Lücke, 1985). This conversion is enzymatic and due to the activity of nitrate reductase which is an intracellular enzyme (Jessen, 1995). Also, Geisen et al. (1992) reported that nitrite is also reduced by *Micrococcus* and *Staphylococcus*; therefore, the amount of nitrite in the final products is lower than in products produced without *Micrococcus* and *Staphylococcus*. In addition, these microorganisms (starter cultures) grow easily in muscle tissue, because they are facultative anaerobes or have anaerobic properties. Katsaras et al. (1996) have reported that the *Micrococcus*/*Staphylococcus* count in the centre of muscle, at the end of curing, is increased (5.0–7.53 log CFU/g) due to the starter cultures (*S. carnosus*, *L. curvatus* and *M. Varians)* utilized in pastirma production. Also, Aksu (1999) and Aksu and Kaya (2002a) reported that the *Micrococcus*/*Staphylococcus* and lactic acid bacteria counts in samples with starter culture, at the end of curing, of the pastirma process, were higher than without starter culture. Also, Hammes and Knauf (1993) reported that lactic acid bacteria promoted activity of nitrate reductase. *L. plantarum* and *L. pentosus* have higher nitrate reductase activity than other lactic acid bacteria (Hammes & Knauf, 1993; Jessen, 1995; Krockel, 1995; Wolf & Hammes, 1988). This study showed that nitrate, both near the surface and in the centre of the muscle, was reduced by the addition of the starter cultures. In frozen/thawed meats, the penetration of the curing mixture has been shown to be more rapid than fresh meats, because pastirma produced from frozen/thawed meat was determined to have higher residual nitrite than that produced from fresh meat (Table 3). The structure of muscle tissue is damaged by freezing and thawing. Muscle proteins are also denatured by freezing and thawing (James & James, 2002), and so the curing mixture (both salt and nitrate and starter cultures) penetrates faster in frozen/thawed muscle tissues than in intact muscles.

Gas dissolution, which causes changes during pastirma aging, depends on the storage temperature and properties of the gas. Gases such as carbon dioxide (CO_2) and nitrogen (N_2) helps to maintain the quality of some foods during storage. Many gases and gas combinations are used, depending on the kind and properties of the meat products. Carbon dioxide (CO_2) and/or nitrogen (N_2) combinations are usually used for processed meat products. Carbon dioxide has the most significant effect on the food shelf life because of the increased dissolution of CO_2 at low temperatures. Jo, Ann, Son, Lee, and Byun (2003) reported that the amount of residual nitrite of sausage samples packaged with CO_2 (100%) was lower than that with vacuum and aerobic packaging, and the redox potential changes to a reduced state in vacuum or CO_2 packaging and can increase the possibility of transforming the nitrite ion to nitric oxide, resulting in a reduction of residual nitrite in sausages.

4. Conclusion

The results from the present study indicate that there is no risk due to residual nitrite associated with using frozen/thawed meat for pastirma manufacturing. This research suggests that the curing time (in process) for frozen-thawed meat may be shortened, and/or less nitrate can be used in the curing and storage of this product. However, from the results, it was observed that additional experiments are needed to determine the most appropriate curing time and initial nitrate concentration, when frozen–thawed meat is used for pastirma manufacturing.

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