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Effect of freezing/thawing process on salting kinetics and thermal properties of beef

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ABSTRACT
In this study, changes in water activity ($a_w$) values and moisture and salt contents of fresh or frozen/thawed beef samples were detected during the salting process. The salt diffusion coefficients ($D_{eff}$) in meat samples were calculated, and changes in thermal properties of meat proteins were identified. $a_w$ values in both fresh and frozen/thawed meat samples were significantly decreasing during the salting process. Salt contents of the samples significantly increased during the salting process while moisture contents decreased significantly. The $D_{eff}$ value of freshly salted samples was calculated as $1.03 \times 10^{-10} \text{m}^2/\text{s}$ and as $1.54 \times 10^{-10} \text{m}^2/\text{s}$ for frozen/thawed samples. It was found out that the freezing/thawing process significantly increased the salt $D_{eff}$ value by 50% compared with that of fresh meat. From the thermal analysis of the samples, the denaturation peak temperatures decreased or were no longer observed as a result of the decrease of the moisture content and increase of the salt concentration of the tissue.

1. Introduction
One of the most important processes used in the storage of meat or its processing into a final product is salting. This process can be done by dry salting or brine salting. In addition, the salting process can be carried out using various curing agents (Gökalkp, Kaya, & Zorba, 2002). After the salting process, the meat can become more reliable, crispier, and more delicious (Gökalkp et al., 2002; Larrea, Hernando, Quiles, Lluch, & Pérez-Munuera, 2006). One of the most important effects of salting process is that it reduces the water activity ($a_w$). Moreover, salt inhibits foodborne pathogens and spoilage bacteria growth (Liu, Qu, Sun, Pu, & Zeng, 2013). Additionally, the salting process leads to some chemical changes in meat proteins and positively contributes to the flavor of meat (Barat, Alíño, Fuentes, Grau & Romero, 2009; Gökalkp et al., 2002). However, the salting process is mainly applied during the production of various meat products. Pastirma that has an important place among these meat products is a traditional meat product specific to Turkey, which is obtained by curing, drying, and coating with a paste called çemen of the meat piece obtained from the certain areas of beef carcasses.

During the curing process of pastirma production, a curing mixture containing sodium chloride (NaCl) and potassium nitrite ($\text{KNO}_2$) is applied to the meat used for production. In the meantime, NaCl diffusion takes place into the meat, and water comes out of the meat. Barat, Rodríguez-Barona, Andrés, and Fito (2003) suggested that these processes occurred especially due to concentration and osmotic pressure differences. The speed of NaCl diffusion taking place in the meat and the amount of NaCl the meat receives at the same time, and some chemical changes that occur in the meat protein have a significant effect on the quality of the final product (Barat et al., 2009).

Salt to be used in the production of pastirma should not have too large or too small grains; salt with medium-sized grains should be used. When salt with large grains is used, the meat cannot get enough salt and if the salt grains are too small, excessive salt gain may occur (Gökalkp et al., 2002). Determination of salt diffusion coefficient is important due...
to predicting the required processing time and the salt concentration in the final product (Graiver, Pinotti, Califano, & Zarritzky, 2006). The solution of Fick’s second law of diffusion for different geometries given by Crank (1975) has been used to determine the diffusion coefficients.

In the literature, there are published studies taking into account the salt diffusion (Barat, Baigts, Aliño, Fernández, & Pérez-García, 2011; Cierach & Modzelewksa-Kapitula, 2011; Ozuna, Puig, García-Pérez, Mulet, & Cárcel, 2013; Picouet, Gou, Fulladosa, Santos-Garcés, & Arnau, 2013; Sabadini, Carvalho, Sobral, & Hubinger, 1998) and the changes in the meat proteins that may occur as a result of salting (Graiver et al., 2006; Pighin, Sancho, & Gonzalez, 2008; Tomaszewska-Gras & Konieczny, 2012). However, there is not a study analyzing the effects of freezing/thawing process on salt diffusion for beef and in the meantime the changes that may occur in the thermal properties of meat proteins. In this study, salt diffusion in the fresh or frozen/thawed beef and changes that occur in the thermal properties of meat proteins as a result of salting under the conditions of the curing stage of pastirma production were tried to be found out.

2. Materials and methods

2.1. Materials

In this study, M. Longissimus lumborum muscles (pH: 5.7) removed both-sided from the 3-year old beef carcasses (Brown Swiss) obtained from Meat and Milk Board Erzurum Combine were used as the material. Three carcasses were used and one carcass was used for each replication. The salt obtained from the local market in Erzurum was passed through 10–14 mesh sieve and then that of remaining on the 14 mesh sieve was used for dry-salting process.

2.2. Preparation of samples and salting process

One side of M. Longissimus dorsi muscle from a carcass was used as fresh, and the other side was used after being frozen at −18°C for a week and then thawed at 4°C for 18 h. After crude fat and connective tissues on the surface of the muscles had been removed, 1-cm thick slices of muscle were removed for sampling. These slices were subjected to unidirectional dry-salting process with NaCl by placing on dry salt at 6 ± 1°C. For each replication three samples were taken at 0.5, 1, 2, 4, 8, 16, 32, and 48 h of salting process and the related analyses were carried out.

2.3. Determination of \( a_w \) values

\( a_w \) device (Novasina TH-500, Switzerland) was used to determine \( a_w \) values of the samples. The device was calibrated with six different salt solutions at 25°C before it was used. Samples were placed in plastic sample cups and housed in a temperature controlled measurement cabin (25°C), then \( a_w \) values were determined (Akköse & Aktaş, 2014).

2.4. Determination of moisture contents

The moisture content of the samples was determined according to the technique described by AOAC (AOAC, 2005). The values were expressed as a percentage.

2.5. Determination of salt contents

Approximately 10 g of each meat sample subjected to the salting process was removed and put into glass jars, and then 100 ml of pure water at 90–95°C were added to it and homogenized for 1–2 min with Ultra-Turrax (IKA Werk Tp 18–10 20.000 UpM). In order to adjust the ionic intensity, 2 ml of 5 M NaNO\(_3\) solution were added to the homogenized that had been cooled to room temperature. Using this homogenized, salt content in the samples was measured with the ion-selective electrode (CRISON Code: 9652, Cl− I. S. Electrode) in pH/ion meter device (CRISON GLP 22, pH and Ion Meter, Crison Instruments, S.A., Spain). Results were expressed as NaCl/100 g dry matter (Akköse & Aktaş, 2014).

2.6. Determination of salt \( D_{eff} \) values

In this study, salt uptake during dry-salting process was considered as diffusion controlled, and the analytical solution of Fick’s second law of diffusion given by Crank (1975) assuming infinite slab geometry, minimal shrinkage, uniform initial distribution, different surface concentrations, and one-dimensional diffusion was used to calculate the salt \( D_{eff} \) values (Equation (1)).

\[
\frac{M_t}{M_{\infty}} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \left( \frac{1}{(2n+1)^2} \right) \exp\left\{ -D_{eff}(2n+1)^2\pi^2t/l^2 \right\},
\]

where \( t \) is time (s), \( M_t \) is the mass of salt that has entered to the meat at time \( t \) (gNaCl/100g dry matter), \( M_{\infty} \) is the mass of salt in the meat at equilibrium (gNaCl/100g dry matter), and \( l \) is slab thickness (m).

2.7. Determination of thermal properties

Thermal properties were determined using Differential Scanning Calorimeter (DSC-60, Shimadzu Corp., Japan) device. For this purpose, the device was primarily calibrated in terms of heat flow and temperature using Indium (\( T_m = 156.6°C; \Delta H_m = 28.5 \text{ J/g} \)). Then about 10 mg of the sample were weighed in the aluminum sample container, and it was covered in a hermetic way. Then the sample was placed into the device, and heating process was carried out from 20°C to 90°C at 5°C/min heating rate using an empty container that was covered in the same way as in the reference. The thermal properties of the proteins in the sample were determined using the thermogram obtained in this way.

2.8. Statistical analysis

This study was conducted according to the random complete blocks design with three replicates. General linear model analysis of variance (ANOVA) was performed to test significance (\( P < 0.05 \)) among treatments. Data were analyzed with the IBM SPSS Statistics 20 packed program (SPSS, 2010). Comparisons of mean values were made using Duncan’s multiple range tests.

3. Results and discussion

3.1. \( a_w \) values

The \( a_w \) values for both fresh and frozen/thawed samples decreased during the salting process (Figure 1). This is very
important for microbiological and chemical quality. The impact of salting time was found significant on the decline in $a_w$ values ($P < 0.05$). However, no significant effect of freezing/thawing process on $a_w$ values was observed. The salt gain and the water loss of the tissue at the same time in the samples during the salting process are thought to be effective on the decline in $a_w$ values. Similar results were determined for dry salted beef by Sabadini et al. (1998). These researchers observed that $a_w$ values decreased with the salting time and were directly influenced by the simultaneous salt penetration and water loss. Uguz, Soyer, and Dalmis (2011) found similar results for meats salted with different proportions of salts. Mujaffar and Sankat (2006) found that $a_w$ values decreased with salting time for shark meat.

3.2. Moisture and salt contents

Moisture contents declined for both fresh and frozen/thawed samples during the salting process (Figure 2). The salt content of the samples increased during the salting process (Figure 3). Bellagha, Sahli, Farhat, Kechaou, and Glenza (2007) reported that salt intake and water loss occurred together during salting process and these two events mutually affect each other. Similarly in this study, salt diffused into the meat and water outwards. It was found out that both freezing/thawing process and the duration of salting have a significant effect on the changes in the moisture and salt contents ($P < 0.05$). Similar results for moisture and salt content were also given by Sabadini et al. (1998) and Akköse and Aktaş (2014) for beef.

Salt gain and moisture loss of the meat tissue during the salting process are events taking place simultaneously. In the study, it is thought that a saturated salt solution formed on the surface of the meat contacting with the salt at the beginning of the salting process. Thus, the tissue loses water due to the concentration difference and osmotic pressure occurring. However, in the meantime the salt gain of the tissue continued. Later in the salting process, the concentration difference and osmotic pressure decrease as the tissue gains salt and thus both moisture loss and salt gain decrease gradually.

In the current study, it was found out that the moisture loss in the frozen/thawed samples was less than in the freshly salted samples (Figure 2). The protein solubility is thought to be effective in this situation. However, as can be seen in Figure 3, the salt content in the frozen/thawed samples was higher than that of the fresh samples. So, there was a higher salt gain for these samples. In this situation, the salt content of the frozen/thawed samples increased at a higher rate compared with the fresh samples and thus, protein solubility and hence water holding capacity were also higher due to the ‘salting in’ effect resulting from the gradually increasing salt concentration in the inner parts of the tissue. It is well known that high salt concentration provokes the denaturing of the protein structure and thus decreasing the water holding capacity. On the other hand, a sharp increase in the water holding capacity takes place in low salt concentrations (Barat, Rodríguez-Barona, Andres, & Fito, 2002; Thorarinssottir, Arason, Geirsdottir, Bogason, & Kristbergsson, 2002).

The salt content of the samples increased faster in the first hours of the salting process, and then this increase continued while gradually slowing down (Figure 3). Similar results were reached by Barat et al. (2011) for pork meat and by Akköse and Aktaş (2014) for beef. The initial high increase is thought to be caused by the saturated salt solution formed on the surface of the meat contacting with the salt and the high concentration difference in the inner parts of the tissue. However, the slowing down in the salt gain in the following hours is thought to be caused by the formation of a layer with high salt content that formed in the areas close to the surface salted and which acts as a barrier against receiving more salt.

3.3. Salt $D_{eff}$ values

The $D_{eff}$ value of freshly salted samples was calculated as $1.03 \times 10^{-10} m^2/s$ and as $1.54 \times 10^{-10} m^2/s$ for frozen/thawed samples. Table 1 provides information on the salt
$D_{\text{eff}}$ values defined for red meat in some studies mentioned in the literature, and the relevant application conditions together. As can be seen in the table, salt $D_{\text{eff}}$ values obtained in this study are similar as those determined by Picouet et al. (2013) for pork meat. However, Sabadini et al. (1998) and Akköse and Aktaš (2014) determined much higher values for dry salting, compared to the values in this study. This was thought to be caused by the high salting temperatures used by Sabadini et al. (1998) and the incision procedure (šak) applied to the beef for pastirma by Akköse and Aktaš (2014). Again, the values obtained in some other studies carried out using brine salting (Cierach & Modzelewksa-Kapitula, 2011; Ozuna et al., 2013) are similar to the salt $D_{\text{eff}}$ values obtained in this study. However, it is possible to say for all studies that the determined salt $D_{\text{eff}}$ values are affected by the properties of the meat used, temperature, salting methods, and the mathematical approaches used in the calculations.

In the study, it was found out that the freezing/thawing process applied to the meat samples increased the salt $D_{\text{eff}}$ value by 50% compared with that of fresh meat. This is very important for salting kinetics. Probably the freezing/thawing pretreatment modified the structure of proteins and facilitated the intake of salt and thus $D_{\text{eff}}$ values increased. A similar result was found for pork meat by Picouet et al. (2013), and it was reported that the freezing/thawing process increased salt $D_{\text{eff}}$ values by 30%. Thus, it is thought that in the production of various meat products such as pastirma to which curing process is applied, freezing/thawing process can be applied to fresh meat at the beginning stage of the production in order to increase curing effectiveness and decrease the curing duration.

### 3.4. Thermal analysis results

In the thermograms obtained from the thermal analysis applied to both fresh and frozen/thawed samples, three peaks were observed for non-salted samples (Figure 4). These peaks were associated with myosin ($T_1$, $\Delta H_1$), the sarcoplasmic proteins and collagen ($T_2$, $\Delta H_2$), and actin ($T_3$, $\Delta H_3$) denaturation. It was observed that, depending on the salting time, the denaturation peaks obtained varied due to the decrease in the moisture content of the tissue and the increase of the salt concentration and the peak temperatures and enthalpies gradually declined or were not observed anymore. In addition, it was also found out that the freezing/thawing process applied to fresh meat was effective on the $T_1$ and $T_3$ peak temperatures and $\Delta H_1$ enthalpy obtained ($P < 0.05$). This process led to a more effective denaturation than that of fresh meat during the salting. Average denaturation peak temperatures and enthalpies obtained from DSC thermograms are shown in Table 2.

The $T_1$ temperatures and $\Delta H_1$ enthalpies for both fresh and frozen/thawed samples significantly decreased during the first 2-h period of the salting process, and could not be determined for the following periods. The $T_2$ temperatures and $\Delta H_2$ enthalpies could not be determined for both fresh and frozen/thawed samples after half an hour of salting. The $T_3$ temperatures and $\Delta H_3$ enthalpies were determined for all periods and gradually decreased in general. However, only $\Delta H_3$ values were significantly increased after 0.5 h of salting. After this time, the second peak of thermograms was not observed and probably these enthalpies were added to $\Delta H_3$ values. Thus, it is possible to say that increasing salt concentration in the meat samples during the salting process.

### Table 1. Determined salt $D_{\text{eff}}$ values in red meat.

| Sample   | Salting method | Concentration (%) | Temperature (°C) | $D_{\text{eff}} (10^{-10} \text{m}^2/\text{s})$ | Reference                  |
|----------|----------------|-------------------|------------------|---------------------------------|---------------------------|
| Beef     | Brine          | Saturated         | 10               | 0.25                            | Sabadini et al. (1998)    |
|          | Dry            | –                 | 20               | 0.26                            |                           |
|          | Dry            | –                 | 10               | 17.21                           |                           |
|          | Dry            | –                 | 20               | 19.37                           |                           |
| Pork     | Brine          | 0–20              | 21               | 5–7                             | Vestergaard, Andersen, and Adler-Nissen (2007) |
| Pork     | Brine          | 20                | 15               | 3–7                             | Hansen, Berg, Ringgaard, Stadkilde-Jørgensen, and Karlsson (2008) |
| Pork (different pH or fat) | Brine (tumbling/ultrasound) | 4               | 5                 | 2.4–42                          | Siró et al. (2009)        |
| Pork (different pH or fat) | Brine | 16               | 4                 | 0.12–3.46                       | Cierach and Modzelewksa-Kapitula (2011) |
| Pork     | Brine (ultrasound) | 5–28             | 5                 | 1.24–2.93                       | Ozuna et al. (2013)       |
| Pork     | Dry (fresh)    | –                 | 3                 | 0.72–1.06                       | Picouet et al. (2013)     |
| Pork     | Dry (freezed/thawed) | –             | 3                 | 0.99–1.41                       |                           |
| Beef     | Dry (incision made) | –                 | 4                 | 14.9–40.8                       | Akköse and Aktaš (2014)   |
| Pork     | Brine (massaging) | 5–13            | 4                 | 4.3–17                          | Sharedeh, Mirade, Venien, and Daudin (2015) |
| Beef     | Dry (fresh)    | –                 | 6                 | 1.03                            | This study                |
| Beef     | Dry (freezed/thawed) | –              | 6                 | 1.54                            |                           |
Table 2. Denaturation peak temperatures and enthalpies obtained from DSC thermograms.

| Time (h) | 0 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 48 |
|---------|---|-----|---|---|---|---|----|----|----|
| Peak temperatures (°C) and enthalpies (J/g) | | | | | | | | | |
| Fresh | T<sub>1</sub> | 54.40 ± 0.27<sup>a</sup> | 50.59 ± 0.41<sup>b</sup> | 50.88 ± 0.66<sup>b</sup> | 50.82 ± 0.11<sup>b</sup> | – | – | – | – |
| | ΔH<sub>1</sub> | 0.23 ± 0.05<sup>c</sup> | 0.22 ± 0.05<sup>d</sup> | 0.18 ± 0.01<sup>e, b</sup> | 0.15 ± 0.02<sup>b</sup> | – | – | – | – |
| | T<sub>2</sub> | 63.19 ± 0.34 | – | – | – | – | – | – | – |
| | ΔH<sub>2</sub> | 0.32 ± 0.09 | – | – | – | – | – | – | – |
| | T<sub>3</sub> | 77.25 ± 0.16<sup>c</sup> | 69.83 ± 0.79<sup>cd</sup> | 69.49 ± 0.95<sup>de</sup> | 68.51 ± 0.42<sup>d</sup> | 68.14 ± 0.52<sup>e</sup> | 68.02 ± 0.14<sup>f</sup> | 68.79 ± 0.28<sup>def</sup> | 70.66 ± 1.31<sup>b</sup> | 71.27 ± 0.03<sup>b</sup> |
| | ΔH<sub>3</sub> | 0.56 ± 0.08<sup>c</sup> | 0.92 ± 0.02<sup>a</sup> | 0.87 ± 0.02<sup>a</sup> | 0.86 ± 0.02<sup*e</sup> | 0.68 ± 0.00<sup>d</sup> | 0.54 ± 0.04<sup>c</sup> | 0.59 ± 0.04<sup>e</sup> | 0.58 ± 0.01<sup>c</sup> | 0.53 ± 0.01<sup>c</sup> |
| Freezed/thawed | T<sub>1</sub> | 55.03 ± 0.66<sup>a</sup> | 51.04 ± 0.72<sup>b</sup> | 49.45 ± 0.12<sup>cd</sup> | 48.99 ± 0.87<sup>c</sup> | – | – | – | – |
| | ΔH<sub>1</sub> | 0.21 ± 0.02<sup>c</sup> | 0.20 ± 0.04<sup>d</sup> | 0.08 ± 0.01<sup>b</sup> | 0.06 ± 0.02<sup>b</sup> | – | – | – | – |
| | T<sub>2</sub> | 62.67 ± 0.60 | – | – | – | – | – | – | – |
| | ΔH<sub>2</sub> | 0.36 ± 0.02 | – | – | – | – | – | – | – |
| | T<sub>3</sub> | 77.26 ± 0.15<sup>c</sup> | 68.79 ± 1.16<sup>d</sup> | 68.22 ± 0.34<sup>cd</sup> | 67.68 ± 0.29<sup>cd</sup> | 67.40 ± 0.07<sup>cd</sup> | 66.95 ± 1.06<sup>cd</sup> | 68.26 ± 0.52<sup>cd</sup> | 68.24 ± 1.23<sup>cd</sup> | 70.42 ± 0.55<sup>b</sup> |
| | ΔH<sub>3</sub> | 0.61 ± 0.03<sup>cd</sup> | 0.94 ± 0.03<sup>c</sup> | 0.85 ± 0.04<sup>cd</sup> | 0.81 ± 0.03<sup>b</sup> | 0.66 ± 0.05<sup>b</sup> | 0.56 ± 0.02<sup>de</sup> | 0.56 ± 0.03<sup>de</sup> | 0.56 ± 0.01<sup>de</sup> | 0.52 ± 0.03<sup>c</sup> |

T<sub>1</sub>: peak temperature of myosin denaturation, ΔH<sub>1</sub>: enthalpy of myosin denaturation, T<sub>2</sub>: peak temperature of sarcoplasmic proteins and collagen denaturation, ΔH<sub>2</sub>: enthalpy of sarcoplasmic proteins and collagen denaturation, T<sub>3</sub>: peak temperature of actin denaturation, ΔH<sub>3</sub>: enthalpy of actin denaturation.

Means with different superscript letters in the same row represent values significantly different (P < 0.05).

± Standard deviation of samples.

T<sub>1</sub>: temperatura pico de la desnaturalización de miosina, ΔH<sub>1</sub>: entalpía de desnaturalización de miosina, T<sub>2</sub>: temperatura pico de la desnaturalización de proteínas sarcoplásicas y colágeno, ΔH<sub>2</sub>: entalpía de desnaturalización de proteínas sarcoplásicas y colágeno, T<sub>3</sub>: temperatura pico de la desnaturalización de actina, ΔH<sub>3</sub>: entalpía de desnaturalización de actina.

Los promedios con diferentes superíndices en la misma fila representan valores significativamente distintos (P < 0.05).

± Desviación estándar de las muestras.
has, in general, a destabilizing effect on proteins. Similar results have been found out by Graiver et al. (2006) for pork meat, Pighin et al. (2008) for beef, and Tomaszewskas-Gras and Konieczny (2012) for chicken meat.

In general, peak denaturation temperatures and enthalpies determined for frozen/thawed samples decreased at a higher rate compared with the freshly salted samples, depending on the salting duration, hence a decrease of moisture content and increase of salt concentration in the tissue. This shows that the freezing/thawing process affects the thermal stability of proteins and has a destabilizing effect. However, it should also be taken into account that there is a higher salt diffusion in frozen/thawed samples, and thus the frozen/thawed samples gain more salt compared with the freshly salted samples at the same salting period. Consequently, the salt gain of the tissue at a higher rate will decrease the thermal stability of meat proteins at a higher rate as well. Thus, denaturation temperatures determined in the frozen/thawed samples at the same salting period occurred at a lower level.

4. Conclusions

The $a_w$ values determined both in fresh and frozen/thawed meat samples greatly decreased with the salting process. Salt content in the samples during the salting process increased steadily, and moisture content decreased. The changes in the salt and moisture content of the samples took place simultaneously at a gradually decreasing speed. The freezing/thawing process applied to the samples was effective on salt gain, moisture loss, and salt $D_{eff}$ values. It was thought that the freezing/thawing process applied before salting to the meats to be salted has positive effects in terms of salting kinetics. The freezing/thawing process applied to fresh meat were effective on the denaturation peaks and enthalpies. The freezing/thawing process led to a more effective denaturation than that of fresh meat during the salting.

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