STUDIES ON THE MORPHOLOGICAL CHANGES OF BEEF DURING FREEZING PROCESSES

Marius Mihai CIOBANU[1], Diana-Remina MANOLIU[1], Mihai Cătălin CIOBOTARU[1], Alina Narcisa POSTOLACHE[2], Bianca Georgiana ANCHIDIN[1], Elena Iuliana FLOCEA[1], Mugurel MUNTEANU[1], Paul Corneliu BOIȘTEANU[1]

e-mail: mar.ciobanu@yahoo.com

Abstract

The freezing process can cause several morphological changes in meat, with the most significant ones being the formation of ice crystals, damage to muscle fibers, and protein denaturation. During the freezing process, water molecules transform into ice crystals, which can lead to the breakdown of meat fibers. The aim of this study was to analyze the changes that occur in beef after freezing and to observe the differences between refrigerated and thawed samples using two different storage methods: vacuum-packed (wet chilling/ freezing) and unpacked (dry chilling/ freezing). Beef samples were subjected to slow freezing, both dry and wet freezing, with a temperature drop of 0.45 - 0.48ºC/hour. The most notable changes after thawing were observed in physical parameters, pH, and texture indicators. The pH of the samples decreased during thawing, with the dry freezing method showing a more pronounced decrease (from a pH value of 6.43 in the chilled sample to a pH of 5.69 after thawing). Shear force (N) and the energy required for shearing (mJ), which reflect the textural qualities of the meat, demonstrated an increase in tenderness as the values of these parameters significantly decreased after thawing. The average values shifted from 87.08 N/1351.02 mJ (wet-chilled sample) and 89.29 N/1389.80 mJ (dry-chilled sample) to the lowest values observed in the wet thawed sample, with averages of 49.14 N/778.14 mJ. The impact of the studied factors on the chemical components was minimal. Humidity decreased from initial values of 75.66% (wet refrigeration) and 75.64% (dry refrigeration) to 75.46% (wet thawing) and 74.94% (dry thawing), with a more noticeable decrease in the case of dry thawing.

Key words: beef quality, freezing / thawing, texture

The consumption of meat is largely influenced by factors such as its availability, price, and cultural traditions. Meat production is a highly intricate process, contingent not only upon consumer demand, often driven by economic factors like price and income, but also subject to a multitude of socio-economic influences, including government policies, price support mechanisms, and various interrelated factors (FAO, 2021).

In European legislation, specifically Regulation (EU) No 1169/2011 of the European Parliament and of the Council dated 25 October 2011, the term 'meat' pertains to the edible portions obtained from the carcasses of domestic animals, encompassing livestock such as cattle, pigs, sheep, goats, as well as poultry and game.

Meat ranks among the most widely consumed food items worldwide and holds significance as a vital component of a well-rounded and nutritious diet, thanks to its inherent nutritional properties. Nevertheless, despite its popularity, the meat industry is not immune to controversy. In recent years, concerns have arisen regarding the environmental impact of meat production and the welfare of animals raised for meat production (Pereira P.M. & Vicente A.F., 2013; Hartmann C., Siegrist M., 2020).

Meat and meat products constitute a significant source of essential nutrients, including protein, fats, vitamins, and minerals, which are essential for dietary nutrition (González N. *et al*, 2020). The overall quality of meat is influenced by various attributes such as taste, texture, juiciness, appearance, and odour. In the fast-paced routines of daily life, meat is often purchased in bulk for future use, necessitating a thawing process that can result in changes in nutritional quality. In many instances, the primary concern with frozen food lies in the quality of the thawing process, which often receives less attention (Akhtar S. *et al*, 2013; Balan P. *et al*, 2019).

Freezing is among the most prevalent and effective preservation methods for meat products, extending their shelf life considerably (Setyabrata

[1] Iasi University of Life Sciences, Romania
[2] Research and Development Station for Cattle Breeding Dancu, Romania

D., Kim Y.H.B., 2019; Wang Z. *et al*, 2021). The advantage of freezing meat, is, as opposed to distributing it chilled, its prolonged storage duration (Warner R.D. *et al*, 2022). However, the quality of frozen meat and subsequently thawed meat is generally regarded as inferior to that of fresh, never-frozen meat (Leygonie C. *et al*, 2012; Hou Q. *et al*, 2020).

The quality of frozen meat is influenced by the specific processes employed in freezing, storage, and subsequent thawing. While freezing is considered a relatively mild preservation method, it does induce alterations in food, and the extent of these changes is closely linked to the speed at which freezing occurs. The rate at which freezing occurs can have a significant impact on meat quality due to the structural alterations that take place during the freezing process, primarily driven by the formation of ice crystals, known as nucleation. Nucleation is influenced by temperature, with rapid freezing rates resulting in the generation of both extra- and intra-cellular ice nuclei. Moreover, the size and shape of these ice nuclei are contingent upon the rate of freezing. For instance, conventional freezing at -20°C leads to the development of irregular and relatively large ice crystals, which in turn can increase structural damage to the meat (Leygonie C. *et al*, 2012; Muela E. *et al*, 2010; Oliveira M.R. *et al*, 2015; Wang Y. *et al*, 2020).

While freezing is a crucial method for preserving the nutritional value and sensory qualities of meat during various stages such as processing, storage, and transportation, before using the frozen meat in subsequent procedures it needs to undergo a thawing process. Thawing is the process of transforming the ice in a food product into its liquid phase, essentially representing the reversal of the freezing process. Thawing meat is often associated with protein degradation, fat oxidation, diminished color quality, and decreased water retention due to the melting of ice crystals. As a result, the adoption of suitable thawing techniques becomes imperative to enhance the quality of thawed meat (Min S.-G. *et al*, 2015; Gan S. *et al*, 2022; Köprüalan Aydın Ö. *et al*, 2023).

There are numerous thawing methods described in the literature, including thawing under refrigeration conditions, at room temperature, in cold water, in tap water, in hot water, using microwaves, heat convection, infrared, radio frequency, ohmic, pressure or even thawing while cooking (Eastridge J.S., Bowker B.C., 2011). Despite those mentioned methods, to limit the microbial growth, the increase of drip loss and other quality changes that may occur in the thawed meat, it is recommended that thawing should be performed at temperatures up to 4 °C or under controlled conditions (Eastridge J.S., Bowker B.C., 2011; Oliveira M. R. *et al*, 2015; Köprüalan Aydın Ö. *et al*, 2023).

In the case of beef consumption, the overall gustatory enjoyment derives from its harmonious blend of tenderness, juiciness, and flavour. Among these attributes, tenderness holds particular prominence for a substantial segment of consumers and is concurrently recognized as one of the most variable quality characteristics. It is well-established that the process of freezing beef has an impact on its tenderness. Notably, beef that has undergone a tenderization period of 7 days followed by freezing and subsequent thawing exhibited comparable shear force values to chilled beef that had undergone a 21-day tenderization process (Lagerstedt Å. *et al*, 2008).

Hence, this study aims to determine the morphological alterations that occur in beef during freezing and thawing processes, with the objective of discerning differences in both texture and the physico-chemical attributes of the analyzed meat. Moreover, the investigation examines the impact of the freezing process on meat quality, with particular emphasis on texture—a pivotal quality parameter known to exert a significant influence on product juiciness, tenderness, and chewiness.

MATERIAL AND METHOD

The biological material employed in this study consisted of beef (M. *longissimus dorsi*) sourced from the local market. The total quantity of beef was divided into four portions and subjected to distinct treatments as part of the experimental design:

1. Wet refrigerated
2. Dry refrigerated
3. Wet freezing for a duration of 24 hours, followed by thawing through wet refrigeration
4. Dry freezing for a duration of 24 hours, followed by thawing via dry refrigeration

The preparation procedures for the refrigerated samples involved the wet storage method, which included vacuum-sealing and placement in refrigerated cabinets maintained at temperatures between 2-4°C. For the dry refrigeration procedure, the meat was placed outside of the vacuum-sealed bag and stored in the refrigeration cabinets.

In the freezing process, a probe thermometer was inserted into the meat structure to monitor temperature changes during freezing. Subsequently, the meat was vacuum-sealed (only in the case of wet freezing) and stored in freezer crates. The thawing stage followed either the wet or dry method, with the meat left to thaw at chilling temperatures either inside or outside a vacuum-sealed bag.

The determination of the proximate chemical composition was conducted using the FoodCheck automatic analyzer, which operates on the principle of infrared spectrophotometry. This device analyzes meat samples based on their infrared absorption properties as identified in the sample spectrum.

The pH determination for the meat samples was executed using a digital pH meter equipped with automated measurements for both acidity and temperature values. Before assessing the acidity of the samples under investigation, the pH meter underwent calibration using two buffer solutions with known pH values (solution 1 - pH = 4.01; solution 2 - pH = 7.01). After calibration, the electrode was inserted into the prepared meat sample. Preceding and following each measurement, the electrode was cleaned with distilled water.

Texture determination tests were conducted using a mechanical tester Mark 10 (USA), and a Mark 10 series 7 dynamometer featuring a range spanning from 0 to 1000 N and a resolution of 0.2 N. The test probe employed was of the V-blade type known as WERNER BRATZLER.

Meat samples were collected using a cylindrical stainless steel probe measuring 6 cm in length, 2 cm in width and 25 mm in diameter, ensuring uniform sample size. The maximum cutting force required to shear the cylindrical meat samples was measured, and subsequently, the mechanical work or energy needed to cut each sample was computed. The cutting process involved a knife displacement speed of 200 mm/min, and the resulting data were graphically represented as a force-versus-displacement curve. Data acquisition was performed using the MESUREGauge+ program, while further data analysis was conducted using Excel and GraphPadPrism9 software.

Each analysis entailed a minimum of five determinations for each type of refrigeration and freeze-thaw treatment applied to the meat samples. An average value was calculated based on the results obtained. The statistical analysis was carried out using the XLSTAT statistical and data analysis program (Addinsoft 2023, New York, USA), performing the Tukey test (HSD) / Analysis of the differences between the categories with a confidence interval of 95%.

RESULTS AND DISCUSSIONS

To monitor temperature variations during the freezing process for each meat type, a thermometer probe was inserted into each meat sample. These measurements were taken at a depth of 1 cm from the surface of the meat, and the rate of freezing front advancement was estimated in degrees Celsius per centimetre per hour (ºC/cm/h). After 48 hours of continuous monitoring, the average rates of freezing and thawing were calculated. This process resulted in the construction of freezing temperature curves (*figure 1*) and thawing temperature curves (*figure 2*), aiding in the characterization of the freezing process as either slow or fast.

For wet freezing, the product's temperature decreased from an initial 4.2ºC to -18.2ºC over a 24-hour period, translating to an average rate of 0.48 ºC/h, indicative of a slow freezing process. Conversely, in dry freezing, the product's temperature decreased from 4.1ºC to -18.6ºC over 24 hours, with an average rate of 0.45ºC/h, signifying a slow freezing process.

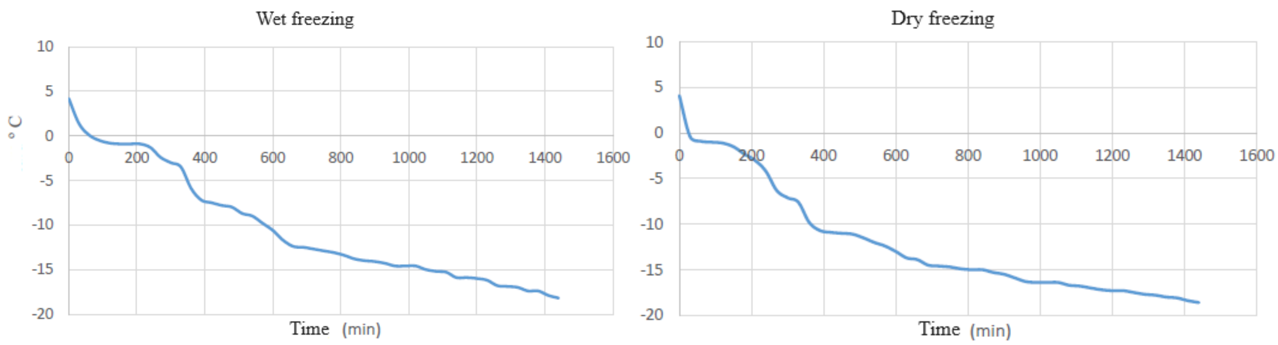

Figure 1 **Temperature graph for freezing beef**

In the wet thawing phase, the product's temperature exhibited an increase over a 24-hour period, rising from -18.1 ºC to 2.3 ºC, with an average rate of 0.42 ºC/h. Conversely, during the dry thawing process, the sample's temperature rose from -18.4 ºC to 2.4 ºC over the same 24-hour duration, at an average rate of 0.43 ºC/h (*figure 2).*

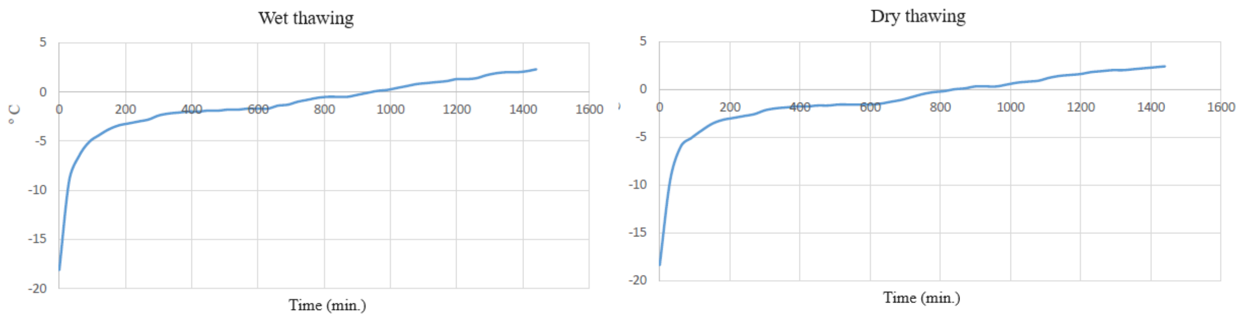

Figure 2 **Temperature graph for thawing beef**

The physicochemical properties examined for the studied meat included pH values, shear force, and cutting energy (*table 1*), as well as water content, dry matter, protein, collagen, and fat content (*table 2*). The obtained values were compared among themselves based on the applied treatments.

The results obtained for the physical parameters of the four samples are presented in *table 1*. The pH values of the samples ranged from 5.69 ± 0.05 (thawed dry sample) to 6.43 ± 0.13 (refrigerated dry sample). Higher pH values were observed for the fresh meat samples compared to the thawed ones, with similar findings reported by Leygonie C. *et al* (2012b). Min S.-G. *et al* (2015) also reported decreases in pH values for beef following conventional thawing. This decrease in pH after thawing can be attributed to the production of exudate, which leads to denaturation of buffering proteins and the release of hydrogen ions, subsequently resulting in a decrease in pH. Statistical analysis revealed significant differences in pH among the four samples, as determined by the Tukey test. Based on the Type III sum of squares, the variable that does not bring significant information to explain the variability of the dependent variable pH is the storage method, whereas variable storage type is the most influential.

The highest shear force was observed in the dry chilled sample, measuring 89.20 ± 1.79 N, whereas the lowest shear force was noted in the wet thawed sample, amounting to 49.14 ± 1.71 N. Statistically, a significant decrease in shear force was observed following the thawing process, with the storage type variable exerting the most substantial influence. The lower shear forces observed in the thawed samples can be attributed to the thawing process, which induces the breakdown of muscle fibers as a result of protein denaturation, thereby diminishing the cutting force.

The storage method (wet/dry) exhibited statistically significant differences solely in the thawed samples, where the shear force was notably higher in the dry thawed sample by a margin of 10.84 N. Elevated shear forces in the dry refrigerated / thawed samples can be explained by the enhancement of beef tenderloin cutting resistance due to the reinforcing effect of connective tissue on the meat's structural integrity, along with moisture loss resulting from exposure to air currents.

These findings align with previous studies by other researchers who have reported an increase in meat tenderness as a consequence of freezing and thawing (Lagerstedt Å. *et al*, 2008; Leygonie C. *et al*, 2012a).

Table 1

Analysis of the differences between the categories for physical parameters.

Parameter	Refrigerated		Thawed		Significance levels of p-value		
	W.	D.	W.	D.	A	B	A x B
pH	6.19 ^{c} ± 0.06	6.43 ^{d} ± 0.13	5.88 ^{b} ± 0.05	5.69 ^{a} ± 0.05	***	ns	***
Shear force (N)	87.08 ^{c} ± 2.16	89.20 ^{c} ± 1.79	49.14 ^{a} ± 1.71	59.98 ^{b} ± 1.42	***	***	***
Total energy (mJ)	1351.02 ^{c} ± 4.11	1389.80 ^{d} ± 8.15	778.14 ^{a} ± 2.40	909.48 ^{b} ± 3.06	***	***	***

a, b, c, d - The same superscript letter within the same row means there is no significant difference between the same parameter analysed (p>0.05). W. - wet refrigeration / thawing; D. - dry refrigeration / thawing; A - storage type (R/T); B - storage method (W/D)

The results obtained for the energy required for cutting are inherently linked to the shear force, as a greater force needed to cut the meat sample corresponds to higher energy consumption. In this context, the energy required for cutting in the case of thawed samples was notably lower (wet thawed sample: 778.14 mJ; dry thawed sample: 909.48 mJ) compared to that observed for the chilled samples. Consequently, thawing demanded considerably less energy for meat cutting, with reductions of up to 44%, indicative of increased tenderness in the samples.

Moisture loss in meat is an inherent post-mortem occurrence owing to pH fluctuations. However, the extent of these losses varies depending on various influencing factors. In the present study, the moisture content of the samples fell within the range of 74.94 ± 0.18 (dry thawed samples) to 75.66 ± 0.09 (wet refrigerated samples). Significant differences were only noted in the case of the dry thawed sample, which exhibited the lowest moisture content, potentially attributed to its higher lipid content (2.96%). Given that the samples underwent a slow freezing process, water losses were minimal.

In terms of dry matter content, a higher concentration was observed in the dry thawed sample (25.12%), which also exhibited the highest fat level. The dry matter content, inversely related to moisture content, displayed higher values in the thawed samples compared to the chilled ones, regardless of the storage method.

Regarding the protein content in the analyzed samples, the average values ranged from a minimum of 21.54% (wet thawed sample) to a maximum of 21.86% (dry refrigerated sample). Lower values were noted in the frozen and thawed samples, indicating some degree of protein denaturation likely induced by slow freezing, resulting in the formation of ice crystals.

Collagen content ranged from 19.88% (wet thawed sample) to 20.24% (dry thawed sample). Collagen is a structural protein found in connective tissues of meat, and it plays a crucial role in determining the meat's texture and tenderness. Collagen does not undergo major alterations when meat is frozen and subsequently thawed.

Table 2

Analysis of the differences between the categories for chemical components

Parameter	Refrigerated		Thawed	
	W.	D.	W.	D.
Moisture	$75.66^{b} \pm 0.09$	$75.64^{b} \pm 0.09$	$75.46^{b} \pm 0.15$	$74.94^{a} \pm 0.18$
D.M.	$24.32^{ab} \pm 0.17$	$24.09^{a} \pm 0.13$	$24.54^{b} \pm 0.15$	$25.12^{c} \pm 0.11$
Protein	$21.84^{b} \pm 0.05$	$21.86^{b} \pm 0.05$	$21.54^{a} \pm 0.05$	$21.78^{b} \pm 0.04$
Collagen	$20.22^{b} \pm 0.08$	$20.18^{b} \pm 0.08$	$19.88^{a} \pm 0.04$	$20.24^{b} \pm 0.11$
Fat	$2.26^{a} \pm 0.11$	$2.22^{a} \pm 0.13$	$2.46^{ab} \pm 0.15$	$2.96^{b} \pm 0.11$

a, b, c, d - The same superscript letter within the same row means there is no significant difference between the same parameter analysed (p>0.05). D.M. – dry matter; W. - wet refrigeration / thawing; D. - dry refrigeration / thawing; A - storage type (R/T); B - storage method (W/D)

CONCLUSIONS

Freezing is an extensive preservation method widely employed in the meat industry, known for its capability to extend the shelf life of meat by several months. However, it is crucial to acknowledge that the freezing process can induce significant morphological alterations in the meat's structure, which can significantly influence its physico-chemical and textural attributes. In this study, following the process of slow freezing applied to beef tenderloin samples, noteworthy changes were observed, primarily in terms of texture. Post-thawing, a notable reduction in shear force was evident, particularly in the case of the wet thawed sample when compared to the chilled sample.

Concerning the chemical characteristics of the samples, despite the slow freezing process, there were minimal differences in moisture content between the refrigerated and thawed samples, attributed to the controlled thawing conditions maintained at 2-4°C. The freezing process indeed impacts the morphological attributes of the meat, notably its texture, rendering it softer and more tender upon thawing. However, depending on the thawing method employed, it is possible to preserve elasticity and juiciness while minimizing moisture loss.

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