

Physicochemical and structural properties of beef meat thawed using various methods

Propiedades fisicoquímicas y estructurales de la carne de vacuno descongelada mediante distintos métodos

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ABSTRACT

Four meat thawing techniques that are most commonly used in daily life were used: refrigerator thawing, microwave thawing, ambient temperature thawing, and water thawing, to evaluate the physico-chemical and histological alterations in thawed beef. After thawing, the structural, chemical, and physical characteristics of beef meat were evaluated. The results showed that meat thawed in the refrigerator at 4°C was characterized by the highest pH value (5.65 ± 0.02) and a significant difference ($P < 0.05$) compared to meat thawed by other thawing methods. Also for the electrical conductivity, it reached the highest value (1.442 ± 1.012) in the microwave oven ($P < 0.05$); meanwhile, water activity decreased significantly after thawing regardless of the thawing method ($P < 0.05$). On the other hand, refrigerator thawing resulted in the least amount of water loss (1.23%) with $P < 0.05$, while high levels of microwave energy caused significant water loss, represented by thawing loss and cooking loss (4.37% and 44.47%), respectively, with $P < 0.05$. Among different thawing methods, microwave thawing had the highest level of TBARS, with a mean of $0.25 \pm 0.034 \text{ mg} \cdot \text{kg}^{-1}$ ($P < 0.05$). Regarding the color, the lightness (L^*) value in the microwave-thawed samples decreased significantly ($P < 0.05$) compared to the fresh control. Histologically, samples that were thawed in a refrigerator preserved the integrity of the fibers' structure after thawing better than other methods; samples thawed in a microwave, however, caused more structural damage. To ensure that it thaws uniformly and to retain the meat's quality as close to its fresh quality as possible, it is typically advised to thaw meat in a slower, more gradual manner, such as in the refrigerator.

Key words: Freezing; thawing methods; quality; beef; microstructure

RESUMEN

Se emplearon cuatro técnicas de descongelación de carne que son las más utilizadas en la vida diaria: descongelación en el refrigerador, descongelación en el microondas, descongelación a temperatura ambiente y descongelación en agua, para evaluar las alteraciones físico-químicas e histológicas en la carne de res descongelada. Después de descongelar, se evaluaron las características estructurales, químicas y físicas de la carne de res. Los resultados mostraron que la carne descongelada en el refrigerador a 4°C se caracterizó por el valor de pH más alto ($5,65 \pm 0,02$) y una diferencia significativa ($P < 0,05$) en comparación con la carne descongelada por otros métodos de descongelación. Además, para la conductividad eléctrica, alcanzó el valor más alto ($1,442 \pm 1,012$) en el horno de microondas ($P < 0,05$); mientras tanto, la actividad del agua disminuyó significativamente después de la descongelación, independientemente del método de descongelación ($P < 0,05$). Por otro lado, la descongelación en el refrigerador resultó en la menor cantidad de pérdida de agua (1,23%) ($P < 0,05$), mientras que los altos niveles de energía de microondas causaron una pérdida significativa de agua, representada por la pérdida de descongelación y la pérdida de cocción (4,37 y 44,47%), respectivamente, con $P < 0,05$. Entre los diferentes métodos de descongelación, la descongelación en el microondas tuvo el nivel más alto de TBARS, con una media de $0,25 \pm 0,034 \text{ mg} \cdot \text{kg}^{-1}$ ($P < 0,05$). Con respecto al color, el valor de luminosidad (L^*) en las muestras descongeladas en el microondas disminuyó significativamente ($P < 0,05$) en comparación con el control fresco. Histológicamente, las muestras que se descongelaron en el refrigerador conservaron mejor la integridad de la estructura de las fibras después de la descongelación que en los otros métodos; las muestras descongeladas en el microondas, sin embargo, causaron más daño estructural. Para asegurar que se descongelaron uniformemente y se conserva la calidad de la carne lo más cercana posible a su calidad fresca, generalmente se recomienda descongelar la carne de manera más lenta y gradual, como en el refrigerador.

Palabras clave: Congelación; métodos de descongelación; calidad; carne de vacuno; microestructura

INTRODUCTION

Beef is one of the most important sources of protein known and widely available in the World [26]. As, it is known a highly perishable product, it is necessary apply a rigorous method to ensure its nutritional value [32, 36]. Freezing and frozen storage are one of the most important methods used in the export and import of meat, and it is widely spread to extend the shelf life and maintain the quality of meat for as long as possible [13, 18, 20]. It has been used for many years to prevent the growth and reproduction of microorganisms and to minimize metabolic activity during long-term storage or distribution and sale [7, 15]. Thawing is a necessary process prior to consumption and further processing [2]. The term "thawing" refers to the process of bringing frozen meat from freezing to a temperature between -5 and 0 degrees Celsius so that it can be cut or sliced for further use [43].

Thawing is a more difficult process to perform safely than freezing; it is generally slower than freezing [10]. However, as the thaw begins, a layer of water forms, which slows down the process. However, the thawing process has received less attention compared to refrigeration or freezing [21], although this process is a very important and integral step in frozen foods before further processing or consumption [37].

Many factors, including the thawing process, can affect meat quality [7]. Several studies have examined the effects of different thawing methods on changes in meat quality, such as water retention, tenderness, color, and flavor [15, 16, 42]; lipid oxidation [22], and microbial growth [8], but there is little information on the microstructural effects of different thawing techniques on histomorphological techniques. Official standards do not provide information on the assessment of the thawing process and its impact on food.

The official journal of the democratic and popular republic of Algeria, April 16, 2017, Art. 47, stipulates that the temperature of thawed meat should be within 4°C as a maximum to reduce the risk of multiplication of microorganisms that cause many diseases and thus become unfit for consumption [28]. Moreover, it is prohibited to re-freeze thawed foods intended for the consumer. On the other hand, home users may use various thawing methods that will affect the quality of beef, including refrigerator thawing, room temperature thawing, water thawing, and microwave thawing. The aim of this study was to determine how different thawing techniques affect the structure of meat and its physical and chemical properties by Comparing thawed meat to fresh meat allows us to determine how the freezing and thawing processes impact the meat's quality. Therefore, by understanding how different thawing techniques affect meat quality, consumers can choose the best thawing method to minimize damage and preserve the quality of the meat.

MATERIAL AND METHODS

A fresh portion of biceps *femoris* was obtained from four beef carcasses (24 hours -h- *post mortem*) from a local slaughterhouse (Batna, Algeria). Fresh beef muscle samples were cut into blocks, and each sample was snap frozen separately by polyethylene bags and frozen at -23°C for 2 months in a home freezer (CRF-NT64GF40, Condor, Algeria). Samples were thawed until the meat medium temperature reached 2°C. Four methods used for thawing are the most common in the experiment as follows:

1. Thawing in the refrigerator (R) (CRF-NT64GF40, Condor, Algeria) at +4°C;

2. Thawing at room temperature (A) (23°C);
3. Thawing in water immersion (W) (15°C);
4. Microwave (M) (MWM100, Kenwood, 800W, UK).

A minimum of five blocks were for each processing thawing methods.

Determination of physico-chemical changes pH

According to the procedure of Zhu *et al.* [42], a sample of 5 grams (g) minced beef is mixed with 45 milliliters (mL) of distilled water in order to measure the pH of the mixture. The pH was measured using an digital pH meter (INOLAB WTW 720, Germany) and the results were recorded. It is important to ensure that the pH meter is calibrated correctly and that the sample is thoroughly mixed with the distilled water before the pH measurement is taken.

Electrical conductivity (Ec)

The measurement procedure described by Jia *et al.* [12] with some modifications. Beef samples were homogenized and stirred for 10 minutes -min- after being homogenized with 30 mL distilled water. After that, a digital EC meter (FE20/EL20; Mettler Toledo, 139 Shanghai, China) was used to measure the combination.

Water activity (Aw)

To measure the Aw of a meat sample using the Hygroscope (BT-RS1 Rotronic, Germany) according to the procedure described by Lakehal *et al.* [17], the sample should be chopped into small pieces and placed in a sample cup with a volume of three quarts. The probe of the device should be inserted into the sample cup and the humidity and temperature data should be allowed to stabilize. Once the data has stabilized, the result can be read from the device.

Thawing loss

The method for determining the thawing loss was based on Xia *et al.*'s approach [38]. Initially, the samples were weighed with Scale (Kern EW 620-3NM, Germany) before freezing, and subsequently, the frozen samples were thawed with different thawing methods. After thawing, the samples were dried with paper towels and weighed again immediately. To calculate the thawing loss, the following equation was used:

$$\text{Thawing loss (\%)} = \left[\frac{(\text{initial weight of raw material} - \text{weight after thawing})}{\text{initial weight of raw material}} \right] \times 100$$

Cook loss

The methods given by Choi *et al.* [8] was used to calculate cooking loss. The thawed sample (50 g) was placed in a polyethylene bag and cooked for 25 min at 80°C in a water bath (GFL 1052, Germany) until it reached 75°C. Cooking loss was determined using the following equation:

$$\text{Cooking loss (\%)} = \left[\frac{(\text{thawed meat weight} - \text{meat sample weight after cooking})}{\text{thawed meat weight}} \right] \times 100$$

Color

In the method developed by Minz and Saini [24], color was quantified using a computer vision system (CVS) that measures three color parameters: lightness (L*), redness (a*), and yellowness (b*). The experimental setup involved using a Canon DS126621 digital camera placed vertically at a distance of 30 centimeters (cm) from the sample.

To illuminate the CVS, two lamps with standard illumination (6500 K) were employed. These lamps, measuring 60 cm in length, were positioned at a 45° angle above the samples. A cubical wooden box was used to house both the lamps and the camera, with the internal walls of the box coated in black opaque photographic cloth to reduce background light. Adobe Photoshop CS3 software was used for measuring and analyzing color values in images.

Lipid oxidation

Thiobarbituric acid-reactive compounds (TBARS) were used to assess lipid oxidation, according to a protocol described by Buege and Aust [6]. For 1 min, 5 g of minced beef was combined with 50 mL of distilled water. Two mL of a 20 millimolar (mM) 2-thiobarbituric acid/15 percent trichloroacetic acid/chlorhydric acid (TBA/TCA/HCl) solution were mixed with one mL of the sample solution. Afterwards, sample solutions were transferred to a water bath at 90°C for 20 min. The resultant solutions were chilled for 10 min under running water. The absorbance of the resultant top layer was measured at 532 nanometers (nm) by a UV-vis spectrophotometer (Shimadzu, Japan). The concentrations of TBARS were represented as nanomolars (nmol) of malondialdehyde per g of beef using a molar extinction value of $1.56 \times 10^5 \cdot \text{M}^{-1} \cdot \text{cm}^{-1}$.

Microstructure observation under light microscope

Histological analysis was performed to investigate possible changes in beef microstructure during different thawing methods compared to fresh samples. For histological examination, specimens were prepared according to Lakehal *et al.* [17], with some modifications. The samples were fixed in 10% formalin for 48 h and then dehydrated with graduated ethanol for 10 h. After dehydration, the samples were clarified by soaking them in xylene for 45 min, twice. Then, after embedding the samples in a paraffin bath at 58°C for 8 h, each sample was embedded in a block of paraffin and sectioned transversely to the muscle fiber over a thickness of 6 micrometers (μm) on a microtome (Leica Jung-histocut 820, Germany) into thin slices. Glass slides were used to mount the selected sections and for staining, it was used hematoxylin and eosin by soaking for 2 min in each staining solution. After marking out the intracellular ice crystals (white voids), it was calculated the location of each ice crystal within the cell and the number and average area of each intracellular ice crystal.

Statistical analysis

The current study's results were statistically evaluated using the SPSS software version 20 (IBM SPSS Statistics v22). Analysis of variance (one-way ANOVA) techniques, as well as Tukey multiple comparison tests were employed to examine differences for the data acquired in the experimental study. The information was presented as a mean value with a standard deviation.

RESULTS AND DISCUSSION

pH

The pH is a determining factor for the organoleptic characteristics of the meat [25]. Normal pH levels in living muscle are around 7.4. After slaughter, the pH of the meat decreased from 5.6 to 5.7 within 6 to 8 h [30]. In this study (FIG. 1A), the pH of beef meat ranged from 5.53 to 6.65, indicating that freezing and thawing had an effect on pH. By comparison between different thawing methods, meat thawed

in the refrigerator at 4°C was characterized by the highest pH value (5.65 ± 0.02) with a significant difference ($P < 0.05$), while meat thawed by other thawing methods showed no significant difference. Meat protein denaturation can be attributed to higher pH values of frozen or thawed meat than fresh control meat [23]. According to Ho *et al.* [11], accumulation of free amino acids, ammonia and organic sulphides derived from the hydrolysis of proteolytic amines might be considered to be the primary cause of the elevated pH. However, other studies have not reported any change in pH after thawing [19, 41, 43].

Water activity (Aw)

The Aw of a food is an important factor in determining its shelf life and the risk of microbial contamination [40]. Foods with low Aw are less likely to support the growth of microorganisms, while those with high water activity are more susceptible to spoilage and foodborne illness [4]. The Aw results of different meat samples depending on the thawing method are shown in FIG. 1B. In fresh meat samples, the average Aw was 0.944. After freezing/thawing, Aw decreased significantly in all frozen meat samples. However, Oliveira *et al.* [29] found no differences in Aw in chicken (*Gallus gallus domesticus*) breast meat thawed using different thawing methods. Medić *et al.* [22] found that variations in Aw are related to fluid migration and ice crystallization.

Electrical conductivity (Ec)

FIGURE 1C depicts the Ec of frozen beef thawed using various methods. The value of Ec at the beginning of the experiment was $1,341 \text{ second} \cdot \text{cm}^{-1} (\text{s} \cdot \text{cm}^{-1})$. There was an upward trend after thawing. The Ec of refrigerator thawing (R), immersion thawing (W), room temperature thawing (A), and microwave thawing (M) increased significantly ($P < 0.05$) to 1,436, 1,394, 1,370, and 1,442, respectively. The reason for this may be that when the membrane permeability of muscle fibers increases, there is a greater influx of ions, such as sodium and chloride, into the extracellular space. This leads to an increase in the concentration of ions in the extracellular space, which increases the Ec of the tissue. At the same time, the increase in the fluid losses after thawing of muscle fibers can lead to an increase in extracellular volume, further contributing to the increase in Ec [10].

Water losses

It is widely acknowledged that freezing and thawing have a negative impact on the water retention capacity, often assessed as loss from thawing and loss from cooking [40]. FIGURE 1D shows the water loss results. High levels of microwave energy can cause significant water loss as represented by thawing loss and cooking loss 4.37 and 44.47%, respectively ($P < 0.05$), which can negatively affect humidity levels [14]. Excessive heat can cause this, altering muscle protein structure and causing protein denaturation, resulting in a high amount of thawing loss, while refrigerator thawing results in the least amount of water loss (1.23%) with ($P < 0.05$).

According to FIG. 1D, the cooking losses of the samples showed similar trends which comparable to those in the samples' thawing losses. Meats frozen/thawed by different methods are characterized by higher cooking losses than fresh meat, in agreement with Xia *et al.* [39]. Thawing methods had a significant influence on cooking losses, which tended to be higher in microwave-thawed meat (44.47%). In general, the highest cooking loss values of thawed meat could be related to the aforementioned tissue damage due to the formation of ice crystals during the freezing process.

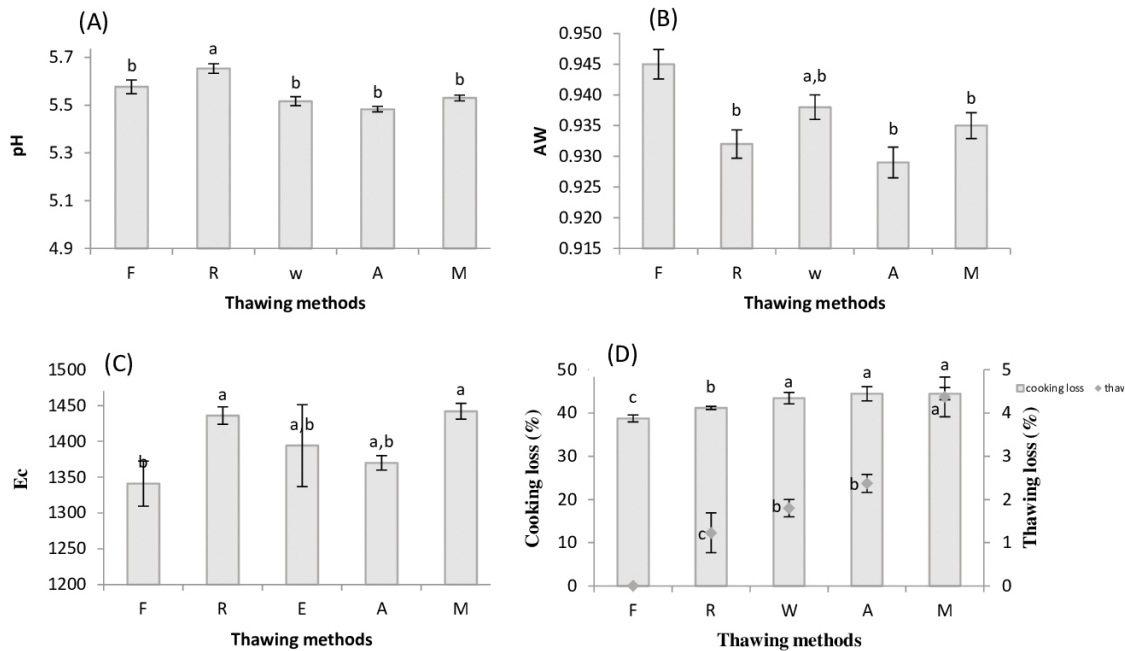


FIGURE 1. Impact of thawing methods on pH (A), water activity (B), electrical conductivity (C), cooking loss and thawing loss (D). F: Fresh meat, R: refrigerator thawing (4°C), A: ambient temperature thawing (23°C), W: water immersion thawing (15°C), M: microwave thawing. (a, b, c) differ significantly ($P < 0.05$)

It should be noted that the volume lost by cooking is generally made up of a mixture of liquid and soluble matter coming from the muscle during cooking as reported by Zhang *et al.* [40]. For that reason, differences in meat fat and protein content may be partly responsible for the amount of cooking loss [1].

Because the consumer prefers to use color as an indicator of freshness and health, color is a major evaluation factor affecting the appearance, presentation, and acceptance of many foods, particularly meat [3, 41]. Several studies have recorded a greater proportion of metmyoglobin and less redness in thawed red meat than fresh state [19, 27, 31, 40]. FIGURE 2 shows the influence of various thawing procedures on the color characteristics of beef samples. When compared to fresh control, the lightness (L^*) value in the microwave-thawed samples reduced significantly ($P < 0.05$), which was consistent with the results of Zhang *et al.* [41]. The (L^*) values for the thawing in a refrigerator, in Water immersion and thawed room temperature did not differ from that of fresh meat. The thawing at the room temperature sample had the lowest redness (a^* value) ($P < 0.05$). Furthermore, there is significant variation in the (b^*) value was detected in the microwave thawing and water immersion thawing methods ($P < 0.05$) compared to fresh control, which was different with the results of Kim *et al.* [13] and Leygonie *et al.* [19]. Protein oxidation and pigment degradation are major elements that contribute to the color stability of meat during the freezing and thawing processes, according to several researchers [19].

TBARS, or thiobarbituric acid reactive substances, is a measure of lipid oxidation in food. It is typically measured in milligrams per kilogram of sample ($\text{mg}\cdot\text{kg}^{-1}$). The higher the TBARS value, the more oxidized the lipids in the sample are [25].

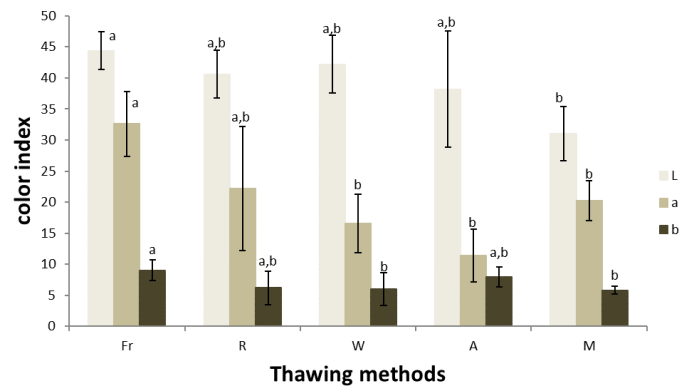


FIGURE 2. Impact of thawing methods on color of beef meat. F: Fresh meat, R: refrigerator thawing (4°C), A: ambient temperature thawing (23°C), W: water immersion thawing (15°C), M: microwave thawing. (a, b) differ significantly ($P < 0.05$)

Lipid oxidation

The results presented in the FIG. 3 suggest that thawing beef in a refrigerator or at room temperature does not significantly affect the TBARS value compared to fresh beef ($P > 0.05$), while thawing using water immersion or microwave methods significantly increases the TBARS value ($P < 0.05$). This suggests that these methods of thawing may contribute to lipid oxidation in the beef. One possible explanation for this is that the high temperatures generated in microwave thawing may release more oxidative enzymes and pro-oxidants from ruptured cellular organelles, leading to increased lipid oxidation. Similarly, the electromagnetic heating during microwave thawing may also contribute to lipid oxidation [9, 39].

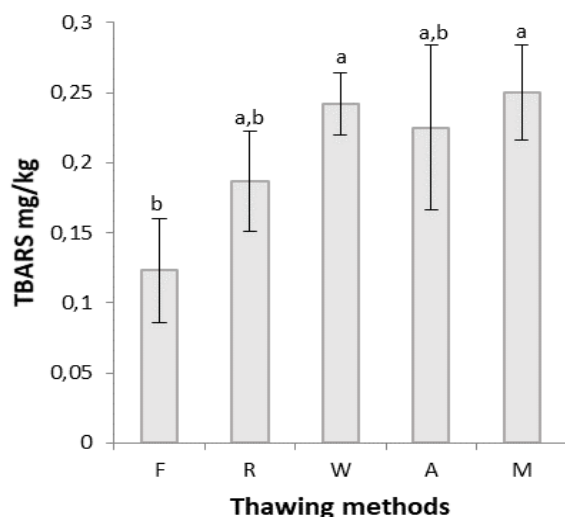


FIGURE 3. Impact of thawing methods on TBARS of beef meat. F: Fresh meat, R: refrigerator thawing (4°C), A: ambient temperature thawing (23°C), W, water immersion thawing (15°C), M: microwave thawing. (a, b) differ significantly ($P < 0.05$)

Morphological differences under microscopic observation

The results of histological examinations on fresh and thawed beef muscle tissues following different thawing treatments are depicted in FIG. 4. The analysis revealed varying degrees of gaps between muscle fibers in all thawing methods, possibly due to mechanical damage sustained during the process. Unfrozen beef exhibited uniformly distributed and regularly-formed fibers. Notably, the refrigerator (R) thawing method had the least detrimental effect on meat microstructure, with tight muscle fibers and minimal gaps between them closely resembling that of fresh meat, which may be due to the small changes in ambient temperature. However, using a microwave to thaw meat resulted in important widening of muscle fiber gaps and breaking of muscle fiber bundles. These results are consistent with previous studies about extracellular space expansion due to cellular and myofibrillar compression between ice crystals during freezing, frozen storage, and thawing of beef muscle [33, 34, 35].

Moreover, the presence of intracellular spaces in the form of vacuoles differs in shape and size in most muscle cells. According to Bozzetta *et al.* [5], the presence of intracellular vacuoles of varying shape and size in most muscle cells is a key indicator associated with freezing. It was also observed in several areas of damaged and partially deformed muscle fibers in which the fiber boundaries could not be determined (FIG. 4E). This tissue damage during the processes of freezing and thawing is an inevitable consequence of the formation of ice crystals inside and outside the cell, resulting in structural changes.

CONCLUSIONS

With an increasing concern among people regarding their health while consuming red meat, particularly frozen imported beef, research into the physical, biochemical, and histological changes that occur during beef thawing is gaining more significance. This study aimed to investigate how commonly used thawing procedures affect the histological structure of beef and its physico-chemical properties. In this study, the quality of frozen meat was significantly affected

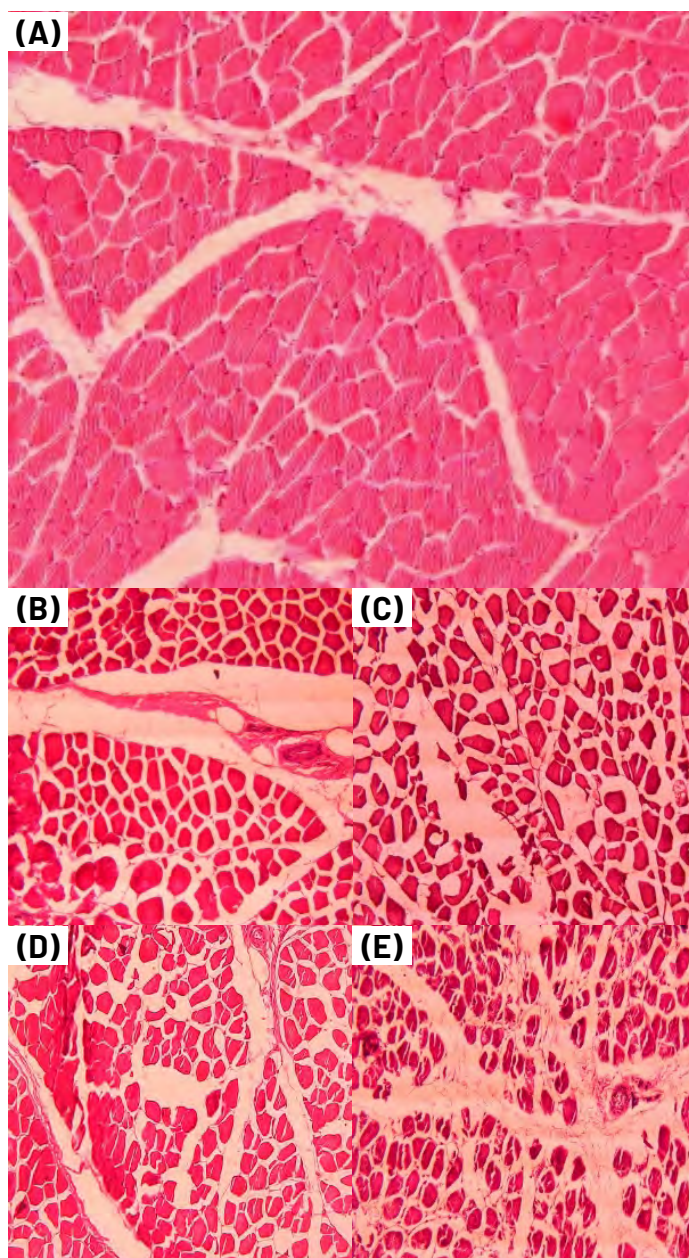


FIGURE 4. Impact of thawing methods on microstructure of beef meat: (A) fresh meat, (B) thawing in refrigerator, (C) thawing in water, (D) thawing in air temperature and (E) thawing in microwave

by thawing techniques. The pH level of thawed meat was increased ($P < 0.05$). In contrast, Aw decreased significantly after being thawed regardless of the thawing method used ($P < 0.05$). While refrigerator thawing resulted in the least water loss at 1.23% ($P < 0.05$), so the brightness was closer to fresh meat, microwave thawing caused a loss of significant water demonstrated by thawing loss and cooking loss at 4.37 and 44.47%, respectively ($P < 0.05$). Microwave thawing also resulted in the highest level of TBARS at $0.25 \pm 0.034 \text{ mg} \cdot \text{kg}^{-1}$ ($P < 0.05$). The light value (L^*) of the microwave-thawed samples decreased significantly ($P < 0.05$) compared to the fresh control. Histologically, upon analyzing meat samples, it was discovered that beef subjected to refrigerator thawing seemed comparatively lesser harm to its

structural composition. Additionally, the organization of the muscle fibers was successfully preserved.

Conflict of interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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