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EFFECTS OF VARIOUS THAWING METHODS ON PHYSICOCHEMICAL AND STRUCTURAL PROPERTIES OF BEEF

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Abstract

The effect of four meat thawing techniques including chill temperature, room temperature, tap water and microwave on quality characteristics, lipid oxidation, proteins degradation, microbial spoilage and the microstructure of beef *longissimus lumborum* muscle were compared. A fresh portion of *longissimus lumborum* muscle was obtained from eight beef carcasses after 24 h postmortem. The findings indicated that all of the thawed samples, particularly the microwave beef samples, had a markedly worse quality than those of fresh meat. Among the thawing methods, changes in the beef quality of the chill temperature samples were significantly less. The increases in malondialdehyde value and decreases in free thiol content indicated that proteins and lipids in the beef samples subjected to room temperature, tap water, and microwave thawing methods were oxidized while thawed beef at a chill temperature did not significantly affect the lipid-protein oxidation compared to fresh beef. The greater myosin degradation was observed in room temperature, tap water and microwave thawing treatments, while actin degradation was not affected. Higher growth of total bacterial counts was presented by samples subjected to tap water and at room temperature thawing compared to microwave and chill temperature thawing. The sensory traits of the chiller thawing

method were better than those of other methods. Images from scanning electron microscopy demonstrated that the myofibril configurations in the thawing samples were more loose than in the fresh beef samples, which had a compact and organized structure. This was demonstrated by the thawing samples' evidently higher myofibril gap value.

Keywords: Beef quality characteristics, Electrophoresis, Freezing-thawing, Oxidation stability, Microbial spoilage.

تأثير طرق الذوبان المختلفة على الخواص الفيزيائية والكيميائية للحم البقر

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الخلاصة

تمت مقارنة تأثير أربع تقنيات لتذويب اللحوم بما في ذلك درجة حرارة التبريد، ودرجة حرارة الغرفة، وماء الصنبور والميكروويف على الخصائص النوعية، أكسدة الدهون، تدهور البروتينات، الفساد الميكروبي والتركيب المجهرية للعضلة الظهرية الطويلة في لحم البقر. أظهرت النتائج حدوث انخفاض معنوي في الخصائص النوعية للعينات الحوم الطازجة مقارنة بالجميع عينات اللحم المذابة، وخاصة عينات لحوم البقر المذابة بطريقة الميكروويف. من بين طرق الذوبان، كانت التغيرات في جودة لحوم البقر لعينات المذابة بدرجة حرارة التبريد انخفض معنوياً. أشارت النتائج في قيمة المالون داي الدهيد (Malondialdehyde, MDA) والنقصان في محتوى الثايول إلى اكسدة البروتينات والدهون في عينات اللحم البقري المعرضة طريقة ذوبان بدرجة حرارة الغرفة، مياه الصنبور والميكروويف بينما لم تؤثر طريقة ذوبان لحم البقر في درجة حرارة الباردة بشكل كبير على أكسدة البروتين الدهني مقارنة باللحم البقر الطازج. وقد لوحظ تدهور أكبر للميوسين للعينات اللحم المذابة بدرجة حرارة الغرفة، مياه الصنبور والميكروويف في حين لم يتأثر تحلل الأكتين. النمو الإجمالي لأعداد البكتيريا كان اعلى معنوياً في عينات اللحم المعرضة لإذابة بالمياه الصنبور ودرجة حرارة الغرفة مقارنة بتلك المذابة بالميكروويف او درجة حرارة البرد. أظهر اللحوم باستخدام طريقة الذوبان المبرد كانت أفضل بالصفات الحسية. أظهرت صور المجهر الإلكتروني لترتيبات الليف العضلي لعينات الذوبان كانت أكثر مرونة من تلك الموجودة في عينات اللحم البقري الطازج ذات البنية المدمجة والمرتبة مما ادى الى الزيادة الواضحة بقيمة فجوات هايدبين الليف العضلية لعينات الذوبان.

كلمات مفتاحية: خصائص النوعية لحوم البقر، الفصل الكهربائي الهلامي، التجميد والذوبان، استقرار التاكسدي، التلف الميكروبي.

Introduction

Beef is one of the most important and widely available forms of red meat in human diets around the world. It is recognized as a lean meat with favorable dietary qualities (31). Because of its comparatively low amounts of intramuscular fat and cholesterol when compared to similar cuts of mutton, it is favored by many meat consumers (29). In spite of its low lipid content, beef contains functional fatty acids such as conjugated linoleic acid which is a good for human health and have anti-inflammatory, anti-thrombotic and anti-atherosclerotic benefits (23). This has led to the development and expansion of the beef industry worldwide in order to meet consumers' demands. However, beef is known to be a highly perishable food, therefore, it is important to use advanced technologies to ensure its nutritional value. In the past decades, freezing techniques have been widely applied in meat industry to ensure the safety and quality of meat for a long period by delaying the rate of microbial growth and biochemical reactions meat quality deterioration during long-term storage for preserving meat or distribution and sale (19). But frozen storage has its shortcomings as thawing is a crucial stage that must be completed before frozen meat can be consumed or processed further (13). During the thawing process, meat is often accompanied by protein degradation, fat oxidation, color deterioration and reduced water holding capacity due to the damage to muscle fibers (28). Myofibrillar proteins such as myosin, actin and troponin serve as the building blocks for muscle fiber and are related with the water holding capacity and the other functional properties of muscle such as gelation (1). Moreover, meat is subject to damage by the action of microorganisms during thawing (18). Consequently, the process of thawing is more challenging to achieve wholeness than freezing (20). Although this process is necessary in frozen foods before consumption and further processing, the thawing process has received less attention compared to freezing. Therefore, optimum thawing procedures must be of concern to meat technologists through adopting appropriate thawing methods.

Air thawing or water thawing are two traditional thawing methods which require longer thawing time and the surface of meat has a significant rate of microbial reproduction (13). In this regard, meat industry is implementing several thawing technique strategies to minimize the quality loss caused by meat thawing including technologies that help prevent the deterioration of physicochemical properties and microbial spoilage. Microwave thawing is primarily considered an effective modern thawing method in the meat industry. According to some research, melting frozen meat in a microwave is 100 times faster than when it thawed by air (13) and the quality of meat that thawed in a microwave was enhanced via reducing bacterial load compared with traditional thawing methods (10, 21 and 33). By comparing thawed meat to fresh meat, the current study aimed to determine how various thawing techniques impact the structure of meat and its physical and chemical properties. Therefore, the current findings would provide useful information to consumers, retailers and meat industries on the merits and demerits of thawing method of beef muscle and the factors to consider for minimizing damage and preserve the quality of the meat.

Materials and Methods

After evisceration and carcass dressing, a fresh portion of *longissimus lumborum* (LL) muscle was obtained from eight beef carcasses after 24 h postmortem from a local slaughterhouse (Erbil, Iraq). After cutting and trimming off any visible fat and connective tissue, fresh beef muscle samples were divided into blocks with 3 cm sides, individually labelled, vacuum packed in polyethylene bags and then stored at -20 ºC for one month in a home freezer. Frozen muscle samples were randomly assigned to four different thawing methods: 1- Chill temperature thawing where the frozen samples were kept in a refrigerator at about 4 ºC for 12 h; 2- Thawing at room temperature where the thawing period took roughly 90 minutes; 3- Thawing in still water in which frozen sample was placed in water at 25 ºC for 90 min; and 4- Microwave thawing, where the beef muscle samples were thawed in a microwave oven until the meat reached a central temperature of 10 ◦C. The time needed for the thawing process in a microwave oven was roughly 180 seconds, with an interval of 30 seconds every 10 seconds. The frequency of the microwave was 2450 MHz. Every frozen muscle sample was thawed until the flesh medium temperature was 2 ◦C.

Muscle pH was determined using the indirect method defined by (25). According the procedure described by (18), thawing loss of LL muscle was determined by calculating a percentage of weight loss relative to the initial weight. The cooking loss was calculated using the methodology proposed by (25). Values of shear force were calculated using the method previously outlined by (27). Using a colorimeter Flex spectrophotometer (Shenzhen 3nh Technology Co., Ltd, China), the lightness, redness, yellowness, Chroma and hue values were calculated to determine the color of the LL muscle samples. Prior use, the colorimeter was calibrated against black and white reference tile and the color values of samples that were 12 mm thick were measured (2) using illuminant D65 as the light source after a 30-minute blooming period.

A slightly modified technique described by (22) was used to isolate myofibrillar proteins using extraction buffer containing 150 mM NaCl, 25 mM KCl, 3 mM MgCl2, and 4 mM EDTA at pH 6.5. Following the method of Bradford, the concentration of total protein in the meat sample was determined. To prepare the protein standards, bovine serum albumen (BSA) was utilized. Myofibrillar protein oxidation was quantified by quantifying the free thiol concentration using 2, 2-dithiobis (5-nitropyridine) DTNP following Elman's technique as described by (22) with slight modification. Prior to being subjected to SDS-PAGE, samples were soaked in a buffer containing 30% (v/v) glycerol, 5% (v/v) mercaptoethanol, 2.3% (w/v) SDS, 62.5 mM Tris–HCl (pH 6.8), and 0.05% (w/v) bromophenol blue in a 1:1 ratio for four minutes at 90 ºC. The technique outlined by (25) was utilized to perform one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) using polyacrylamide gel measuring 8 cm (length) × 5.5 cm (width) × 0.8 cm (thickness). Actin and troponin T required 12% resolving gels, but myosin heavy chain required 5% resolve gels. Four percent stacking gel solution was applied on top of the resolving gels. Samples (25 µg protein) were separated in running buffer (0.025 M Tris base, 0.192 M glycine, 0.1 SDS, pH 8.3) using a mini PROTEAN®

Tetra system (BIO RAD, USA) set at a constant voltage of 120 V and 0.4 A for 90 min. Using a mini PROTEAN® Tetra system (BIO RAD, USA) set at a continuous voltage of 120 V and 0.4 A for 90 minutes, samples (25 µg protein) were separated in running buffer (0.025 M Tris base, 0.192 M glycine, 0.1 SDS, pH 8.3). After the electrophoretic transfer, the gels were stained with coomassie blue, and the GS-800 Calibrated Imaging Densitometer was used to view the bands of myofibrillar proteins (Figure 1).

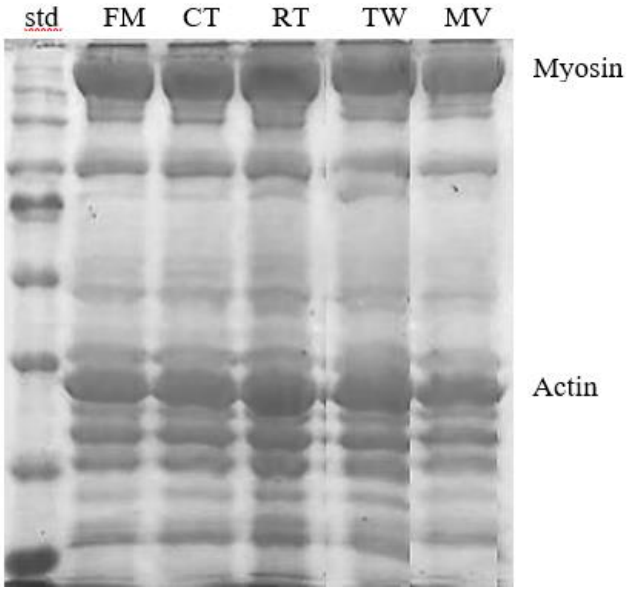

Fig. 1: Representative SDS-PAGE showing the myofibrillar protein bands of *longissimus lumborum* muscle in beef subjected to chill temperature (CT), room temperature (RT), tap water (TW) and microwave (MW) thawing in comparison to fresh meat (FM).

After SDS-PAGE, myosin heavy chain proteins were transferred for 123 minutes at 250 mA per gel with a voltage limit of 25 while actin proteins were transferred for 40 minutes at the same voltage and amperage to polyvinylidene difluoride (PVDF) membranes (Bio TraceTM polyvinylidene fluoride Transfer Membrane 0.45 µm, Pall Corporation, Mexico) via Trans-Blot® SD semi-dry transfer system (BIORAD, USA). In blocking solution (5% BSA in TBST buffer, which contains 100 mM Tris-HCl, 150 mM NaCl, and 0.05% Tween 20), the membranes were blocked at room temperature for three hours. In order to detect myosin, the membranes were incubated with a primary antibody (produced in rat monoclonal; Cat no. MAC147 from ABCAM, USA) dilution of 1: 500 for 12 hours. The anti-Actin antibody produced in mouse; Cat no. AB11003 from ABCAM, USA, was the primary antibody used for actin. After that, the membranes were incubated for a further 90 minutes at room temperature in a 1:10000 dilution of secondary antibody (IgG-peroxidase; developed in rabbit; Cat no. 610-4302 from Rockland, USA) in 3% BSA in TBS-T buffer. The membranes were then detected using a DAB substrate kit (Thermo Scientific™ 34002, USA). Myosin and actin band intensities were measured by Quantity one 1-D software on Calibrated Imaging Densitometer (Bio-Rad, USA).

Approximately 30 g of LL muscle samples were added to 270 ml of 0.1% sterile peptone water to prepare the serial dilutions. After homogenizing the sample for 2.5

minutes in an aseptic food bag, the homogenized solution was mentioned the stock solution (dilution 10^{-1}). Following the instructions provided by (24), the first dilution of 10^{-1} (1 ml was transferred to 9 ml peptone water) and serial decimal dilutions up to 10-6 were organized. For purpose of determining the total aerobic count, ten-fold dilutions were spread out in duplicate on petri dishes and then the plates were incubated at 32 ºC for 72 h. The overall population was estimated as log10 colony forming units (cfu) per gram of beef following the incubation phase

A histological investigation was conducted to look into potential changes in the microstructure of beef after various thawing techniques in comparison to fresh samples. The preparation of muscle sample was done based on the technique described by (18). Images of the microstructure of muscle were obtained using a scanning electron microscope (XSZ-107bn, Zenith Lab Inc, China) coupled with camera (AmScope MU300, California, USA).

Using the method outlined by (26), sensory analysis was carried out with ten panel members. The thawed LL muscle samples were cut into steaks of 5 cm in length, 5 cm in width and 2 cm in height. To achieve an internal temperature of 70 ºC, the steaks were grilled for 20 minutes at 180 ºC. A five-point rating system ranging from 5 (denoted strong appreciation) to 1 (implied extreme dislike) was used to record the tenderness, juiciness, flavor and overall acceptability of cooked steaks beef.

A completely randomized design was used for the experiment. The generalized linear model (GLM) technique of the Statistical Analysis System package (SAS) Version 9.1.3 software (SAS Institute Inc., Cary, NC, USA) was applied to the data obtained for all parameters. Where significant results were found, comparison among means was made by Duncan's multiple range test with a p value of 0.05.

Results and Discussion

In the current study, the pH of the beef meat varied from 5.52 to 6.68, suggesting that the process of freezing and thawing had an impact on the pH value. However, different thawing methods had no significant impact on the pH value of meat (Table 1). Higher thawed meat pH values than fresh control meat are linked to meat protein denaturation. (14) suggest that the main reason for the higher pH of thawed meat could be due to the accumulation of free amino acids, ammonia and organic sulphides generated from the hydrolysis of proteolytic amines. Meat can retain its natural water content. The industry and consumers both depend on this capability, which is known as water-holding capacity. Generally, the two popular methods used to determine the water holding capacity of meat and meat products are thawing loss and cooking loss. The degree of damage of the beef's cell and tissue structure upon freezing and thawing is reflected in the thawing loss and cooking loss. More tissue and cell structural damage in the beef sample causes more juice to be drained from the cells, which increases nutritional loss (3). Table 1 illustrates the cooking and thawing losses of beef using various thawing techniques. In the current study, the thawing loss values ranged from 3.041% for microwave followed by room temperature 2.968%, tap water 2.711% and chill temperature 2.436% thawing methods respectively. This finding made it clear that whereas beef samples thawed by chill temperature had significantly the lowest value of thawing loss while beef samples thawed in the

microwave had the highest thawing loss value. The energy and heat produced by microwave thawing can change muscle protein structure and cause protein denaturation, which could be the cause of the significant thawing loss values. Even with a brief thawing period (three minutes), denaturation leads to the protein's decreased ability to hold water which results in greater exudate losses. These findings are in line with those of (30), who discovered that evaporating water at an instantaneous high temperature during microwave thawing was the primary cause of the high thawing loss of red meat. The quality of the meat samples is somewhat reflected in the cooking loss. There are more cooking losses in meat that has been frozen and thawed through various techniques compared to fresh meat. The creation of ice crystals during the freezing process which is known to cause tissue damage, may be the reason behind the greatest cooking loss values of thawed beef. Table 1 indicates an increase in the cooking loss of beef during thawing, with the microwave-thawed beef samples exhibiting the largest cooking loss 33.917% among all the thawed samples. According to (18), this is because microwave thawing produces an environment with a greater temperature which contributes to protein aggregation and deformation. Tenderness is evaluated by measuring the shear force of the muscles. which is an important indicator of meat quality. Meanwhile, there is a strong correlation between the level of meat tenderness and its moisture content. As shown in Table 1, the shear force value of beef samples after thawing at chill temperature, room temperature, tap water and microwave was obviously increased compared with fresh meat ($p < 0.05$), which showed that the degree of tenderness reduced with each thawing sample. The substantial thawing and cooking loss, as well as the shrinking with the breaking of muscle fibres, could be the reason for the decrease in tenderness in frozen and thawed samples. (17) found that the reason for the lower tenderness value was fluid loss during thawing, which resulted in less water being available to hydrate the muscle fibers. Moreover, sarcomere shortening during the thawing process results in toughening. In line with the present findings, (17) reported that shear force values of meat samples after thawing were higher than that of fresh meat. (30) also reported that shear force value in meat subjected to freezing and thawing processes was higher than that of fresh meat.

Table 1: Impact of thawing methods on pH, water holding capacity and shear force values of beef *longissimus lumborum* muscle.

Traits	Fresh meat	Thawing methods			
		Chill temperature	Room temperature	Tap water	Microwave
pH	5.515±0.02 a	5.603±0.03 ab	5.665±0.01 b	5.668±0.01 b	5.672±0.02 b
Thawing loss (%)	-	2.436±0.07 b	2.768±0.03 a	2.611±0.12 a	3.041±0.14 a
Cooking loss (%)	29.911±0.18 c	31.603±0.79 b	32.408±0.21 a	32.256±0.17a	33.917±0.14 a
Shear force (Kg)	1.607±0.01 b	1.731±0.07 ab	1.869±0.11 a	1.860±0.05 a	1.901±0.09 a

a,b Least square means with different superscripts in the same row indicate significant difference at $p< 0.05$.

Meat color is an important aspect for the meat industry since it is among the main quality attributes that influence consumer buying decisions. Table 2 displays the variations in color coordinates between various samples of thawed beef. When compared to samples of fresh beef, the thawed samples showed significant increases in lightness and hue value, but significant decreases in redness and chroma value. Destruction of the muscle structure and decreased muscle water holding capacity could result in a greater amount of light reflection from the sample meat. The lightness value is a useful indicator to determine the degree of muscle loss. It is well known that the higher quality of muscle is strongly correlated with the lightness values. Myoglobin's chemical state changed and its quantity decreased during the thawing process, which was the primary cause of the redness value decline. In fresh muscle, the metmyoglobin reducing enzyme is very active, causing the metmyoglobin to be rapidly reduced to deoxymyoglobin and then oxygenated back to oxymyoglobin, thereby preserving its color. On the other hand, after thawing, the sarcoplasmic environment may lose the enzyme that reduces metmyoglobin, which could result in an accumulation of metmyoglobin on the muscle surface and accelerate the loss of redness. Research has indicated that frozen and thawed red meat has a higher percentage of metmyoglobin and a lower redness compared to its fresh form (13, 18 and 33). Among the different thawing methods, a higher lightness value from microwave thawing samples was obtained. The lightness values for the beef samples subjected to the chill temperature thawing method were the same as those of fresh meat. Greater light reflection and a lighter color of microwave-thawed samples could be resulted from the high thawing loss. (33) established that samples with lower water content possessed higher lightness values. Likewise, the significantly lower redness value from microwave thawing samples may be attributed to the loss of methemoglobin reducing enzyme with the exudation. Furthermore, (34) showed that the high level of microwave energy increases the amount of denaturation of other protein in the meat and decreases the solubility of myoglobin which is the main pigment in the meat. This observation contrasts those of (30) who observed that meat samples obtained from microwave thawing showed significantly lower redness value. The change in value of yellowness from freezing-thawing meat samples was comparable to that in lightness value (4). The yellowness value from microwave thawing beef samples was higher than that from other thawing methods samples. Meanwhile, the increase in hue angle from the thawed samples can be used to illustrate the change in yellow value. The hue angle was employed to represent the change from redness to yellowness and a greater value of hue angle suggested that the muscle samples that had been thawed had a stronger yellowish hue (22). The higher yellow value and hue angle of microwave thawing beef samples could be attributed to the high degree of lipid-protein oxidation. (6) discovered that lipid-protein oxidation might increase yellowness values and hue angle of meat samples following the process of freezing and thawing. In this study, Chroma value represents color intensity and has been considered a good indicator of meat stability (22). The results of this investigation indicate that the thawing process was not conducive to maintaining the stability of muscle color as the Chroma value from the thawed samples significantly decreased. It was demonstrated that room temperature, tap

water, and microwave thawing were not effective in preserving the stability of muscle color because the Chroma values from these samples were lower than those from samples that were chilled.

Table 2: Impact of thawing methods on color coordinates of beef *longissimus lumborum* muscle.

Traits	Fresh meat	Thawing methods			
		Chill temperature	Room temperature	Tap water	Microwave
Lightness	40.455±0.69b	41.181±0.51 ab	44.320±0.23 a	44.635±0.22a	45.953±0.41a
Redness	26.044±0.28a	23.945±0.18 b	22.711±0.31 cb	22.739±0.16bc	19.678±0.68c
Yellowness	10.440±0.03b	9.981±0.13 b	12.115±0.11 a	12.362±0.15a	13.735±0.12a
Chroma	28.447±0.48a	23.470±0.21ab	21.005±0.51 b	21.911±0.21b	21.055±0.37b
Hue	21.225±0.44a	22.300±0.40 b	23.682±0.75 b	22.955±0.22b	22.920±0.17b

a,b,c Least square means with different superscripts in the same row indicate significant difference at $p< 0.05$.

Under pro-oxidative conditions, lipid oxidation is the primary non-microbial cause of meat and meat product deterioration. Malondialdehyde (MDA) is an essential aldehyde which can be formed during the oxidation of lipids containing a carbon-carbon double bond, especially polyunsaturated fatty acids (8). In the present study, malondialdehyde concentration was monitored by thiobarbituric acid reactive substances (TBARS) test as a marker to measure lipid oxidative degradation (Table 3). The TBARS values of beef increased in varying degrees after thawing compared to fresh beef samples. According to (16), lipid oxidation may be accelerated during the post-mortem processing of muscles, which includes freezing and thawing. When compared to fresh beef, thawing meat at room temperature, using tap water, or in a microwave greatly raises the TBARS value. However, thawing beef at a cool temperature had no discernible effect on the TBARS value. When using the traditional thawing procedure (room temperature or tap water), the meat samples are exposed to air for a longer period of time, which causes a larger degree of oxidation. This could be the cause of the higher TBARS levels. Generally, higher levels of lipid oxidation are observed in meat samples that have been thawed for longer time (12). Similarly, one explanation for this could be that more oxidative enzymes and pro-oxidants are released from ruptured cellular organelles due to the high temperatures produced during microwave thawing, which would accelerate lipid oxidation. (13) provided a comparable justification which attributed the higher TBARS values of porcine *longissimus lumborum* during microwave thawing compared to the refrigerator thawing to the fact that the instantaneous high electromagnetic heating made by microwave thawing encouraged the protein and lipid oxidation of muscle through the release of pro-oxidative substances linked with free radicals and oxidative enzymes.

The results for the effect of different thawing methods on free thiol content of beef *longissimus lumborum* muscle are presented in Table 3. After freezing and thawing treatments, the meat samples thawed by microwave had the lower quantification of protein thiol groups than beef samples subjected to chill thawing which indicated that chill thawing has lower effect on oxidative properties of protein. The majority of oxidative modifications in proteins take place on the side chains of amino acids, leading to the reduction in thiol groups (9). This observation is in line with the findings of the lipid oxidation analysis, which revealed that the lipid oxidation value of the beef samples that were thawed in a microwave was higher than that of the samples. (11) explained that protein oxidation is caused by the interaction of proteins, particularly the nitrogen or sulfur centers of reactive amino acid residues in proteins, with lipid hydroperoxide or secondary lipid oxidation products such malondialdehyde. Furthermore, it appears that the oxidation of lipids in meat and meat products starts earlier than the oxidative degeneration of protein. Thus, lipid-derived radicals and hydroperoxides are more likely to promote protein oxidation (8). The observations of the current study are consistent with those of (13 and 30) who showed that microwave thawing had higher high protein oxidation of muscle induced by instantaneous high temperature during thawing.

Table 3: Impact of thawing methods on lipid and protein oxidation of beef longissimus lumborum muscle.

Traits	Fresh meat	Thawing methods			
		Chill temperature	Room temperature	Tap water	Microwave
MDA (mg /kg)	0.306±0.01 c	0.314±0.01 c	0.355±0.01 b	0.381±0.01 ab	0.417±0.02 a
Free thiol content (nmol/mgprotein)	26.14±0.19 a	24.57±0.27 a	23.612±0.14 ab	23.89±0.21 ab	21.24±0.16 b

a,b,c Least square means with different superscripts in the same row indicate significant difference at $p< 0.05$. MDA - Malondialdehyde.

Electrophoresis was performed in order to observe modifications induced by different thawing methods in beef muscle myofibrillar proteins. SDS-PAGE patterns showed a decrease of bands corresponding to myosin band after thawing while the actin band was relatively more stable (Figure 1). In their study on beef muscle myofibrillar proteins degradation, (32) showed that increased protein oxidation enhanced the degradation of myosin but had little influence on the degradation of actin. The intensities of myosin and actin were determined by measuring the densitometry analysis of concentrations of each detected band. Table 4 shows that the concentrations of myosin were significant differences among the thawing methods while that of actin was stable. Beef muscle samples subjected to chill temperature thawing had the highest value that was significantly different from other thawing methods and the myosin concentration of beef samples after chill temperature thawing was closest to that of fresh meat samples. However, there were no significant differences between room temperature and tap water thawing methods. The SDS-PAGE profiles coincide with protein oxidation indicator (free thiol content). The observation in the present study is consistent with that of (1) who found that the

highest optical density of myosin protein was presented by meat samples subjected to refrigerator thawing while microwave thawing had the lowest value.

Table 4: Impact of thawing methods on the concentrations of myofibrillar proteins of beef longissimus lumborum muscle.

Traits	Fresh meat	Thawing methods			
		Chill temperature	Room temperature	Tap water	Microwave
Myosin (mg)	13.122±0.12[a]	12.992±0.11 [a]	11.702±0.15 [b]	11.891±0.17 [b]	10.481±0.19 [c]
Actin (mg)	4.336±0.02	4.282±0.08	4.244±0.09	4.237±0.03	4.149±0.07

a,b,c Least square means with different superscripts in the same row indicate significant difference at $p< 0.05$.

The results achieved in Figure 2 shows the microbial levels of different thawing beef samples. The value of total aerobic bacterial counts in beef muscle samples that were thawed in microwave was 2.967 ± 0.03 while more significant growth of total bacterial counts was indicated by beef muscle samples thawed under tap water and at room temperature which were 3.877 ± 0.18 and 3.875 ± 0.12, respectively. Moreover, data revealed that there were no significant differences in the average bacterial counts between the beef samples that thawed in microwave and at chill temperature. A similar trend was also reported by (10 and 15) who indicated less growth of microorganisms in frozen beef samples subjected to microwave or chiller thawing method. In comparison to samples thawed in a microwave and at chilled temperature, samples thawed at room temperature and under tap water showed higher levels of bacterial growth. This could be because the traditional thawing method involves a longer thawing time, which exposes the meat samples to air for a longer period of time resulting in a higher degree of microbial load. However, the mean value of total aerobic bacterial counts in beef samples was 3.228 log cfu/g meat, which is in line with Dempster's (7) findings regarding standardization and quality control, which state that the total counts should fall between 103 and 107 cfu/g of meat.

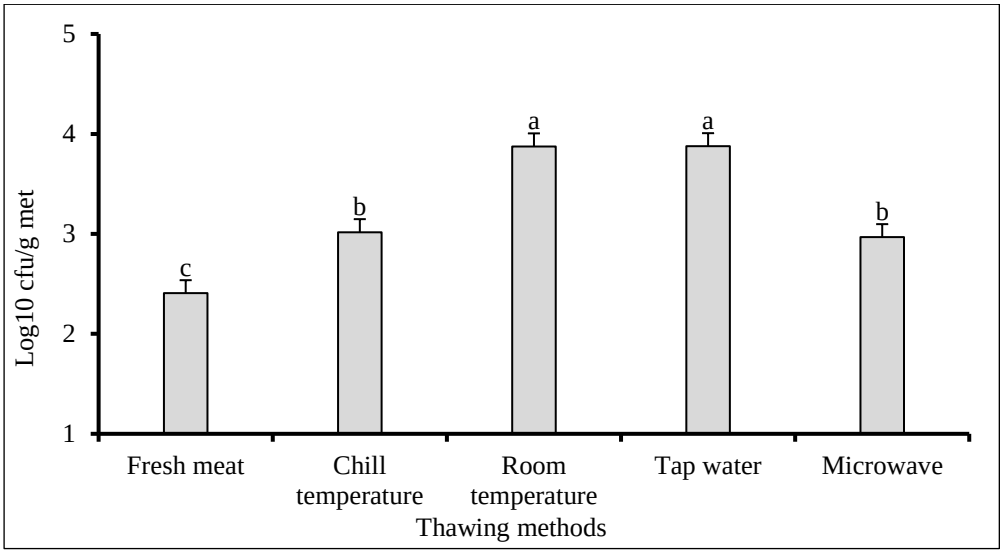

Fig. 2: Total bacterial count of beef *longissimus lumborum* muscle subjected to different thawing methods.

a,b,c Means with different letters differ significantly at $p<0.05$.

Values are mean ± standard error.

Figure 3 illustrates the change in the microstructure of beef muscle samples subjected to various thawing techniques in comparison to fresh samples. Fresh beef muscle samples presented uniformly distributed and regularly formed fibers. After thawing, the microstructure of beef muscle samples was disrupted and the intermuscular gap grew significantly. This could be explained by the fact that the extreme dehydration of muscle caused by protein denaturation may be the cause of the increase in intermuscular gap during thawing and the mechanical damage to the epimysium, perimysium and endomysium. Furthermore, according to (3), the development of ice crystals inside and outside of cells during the freezing and thawing processes leads to cell muscle breakdown and tissue damage. The method of thawing meat in the refrigerator was shown to have the least negative impact on the microstructure of the meat. The tight muscle fibers and small gaps between them closely resembled the texture of fresh meat. This could be attributed to the slight variations in the surrounding temperature. Nevertheless, there was a significant expansion of muscle fiber gaps and breakdown of muscle fiber bundles when beef was thawed in a microwave. These findings are in line with other research on the growth of extracellular space in beef muscle frozen and thawed beef muscle as a result of cellular and myofibrillar compression between ice crystals (14 and 28).

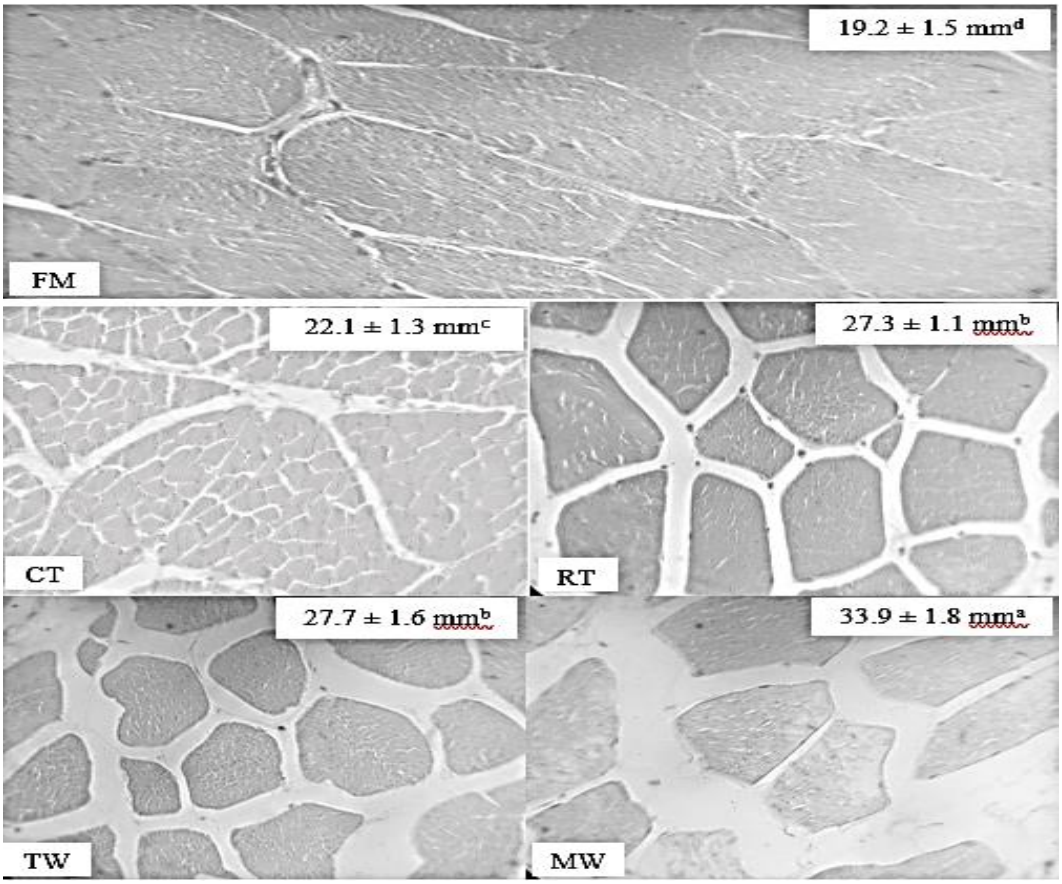

Fig. 3: Change in microstructure of longissimus lumborum muscle subjected to chill temperature (CT), room temperature (RT), tap water (TW) and microwave (MW) thawing in comparison to fresh meat (FM).

a-d Means with different letters differ significantly at $p<0.05$.
Values are mean ± standard error.

The effect of different thawing methods on sensory attributes of beef samples was assessed and compared to fresh meat sample to suggest the thawing treatment with the minimum sensory losses (Figure 3). The fresh meat samples received the highest scores by the tasters for tenderness and juiciness. The tasters found that the beef samples that were thawed at room temperature, in the microwave, and under tap water were not as tender and juicy as the samples that were thawed at chill temperature. This sensory outcome was explained by the fact that less water was available to hydrate the muscle fires as a result of the fluid loss during thawing. Consequently, more fibers per surface area appeared to increase toughness as judged by the sensory panel (22). The reason for the low tenderness and juiciness grades was the sudden high temperature during thawing which caused significant thawing and cooking loss as well as protein oxidation in both microwave and traditional thawing samples. Meanwhile, the findings showed that chill temperature beef samples were judged by the tasters to be more tender and juicier than those at room temperature, tap water and microwave thawing samples influenced the overall acceptability.

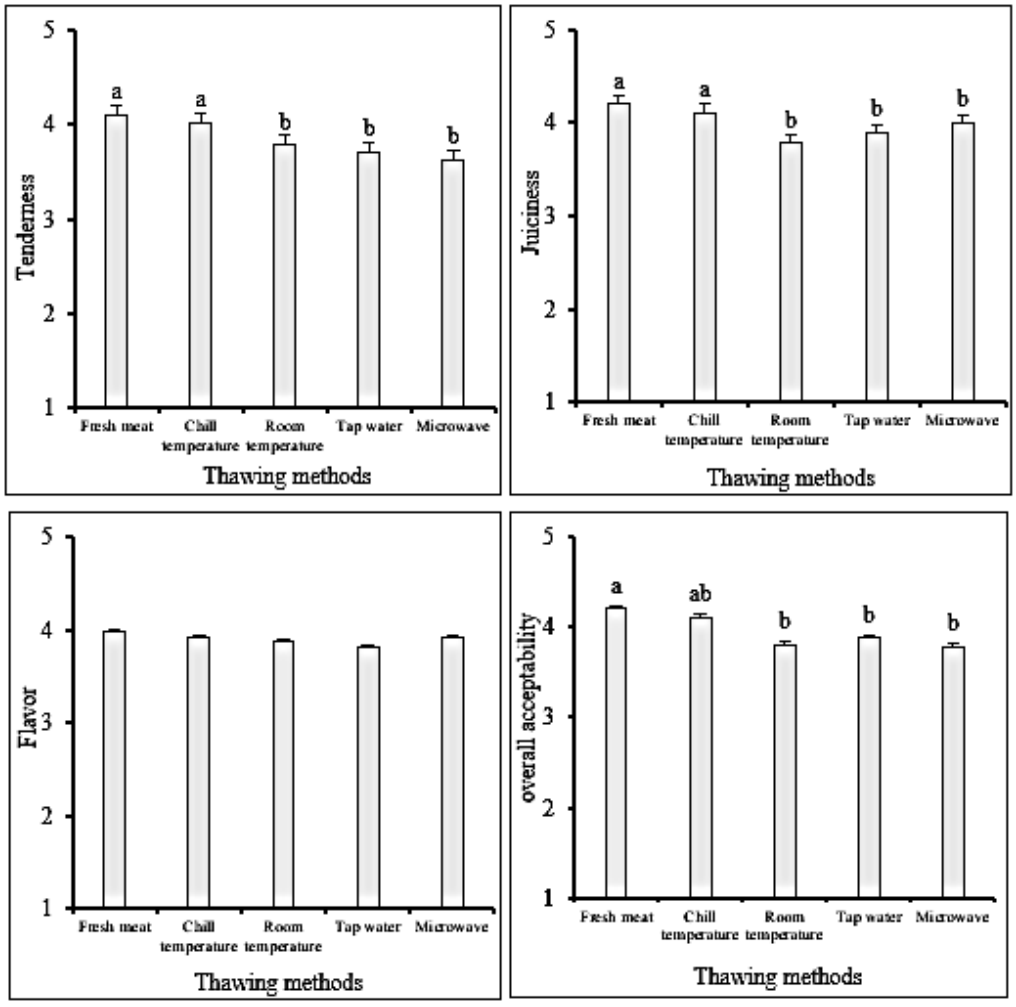

Fig. 4: Sensory meat quality parameters of beef longissimus lumborum muscle subjected to different thawing methods.

a-d Means with different letters differ significantly at $p<0.05$.
Values are mean ± standard error.

Conclusions

In the current investigation, the quality of frozen beef was significantly impacted by thawing techniques. The pH of the thawed beef increased noticeably. Chiller thawing resulted in the least water loss while microwave thawing resulted in a significant loss of water as evidenced by both thawing and cooking loss. Based on the observation color characteristics values, room temperature tap water and microwave thawing were not conducive to maintaining the stability of muscle color. Additionally, the highest degree of lipid-protein oxidation and less growth of total aerobic bacteria counts were seen after microwave thawing. The profile of protein myofibrillar bands showed that beef muscle samples subjected to chill temperature thawing was closest to that of fresh meat samples. Histologically, it was found that beef that had been thawed in the chiller appeared to have suffered relatively less damage to its structural composition. Sensory analysis revealed that the chiller thawing beef samples were more accepted than other thawing methods.

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No Supplementary Materials.

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Author A. B. Sabow; methodology, writing—original draft preparation, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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No Data Availability Statement.

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The authors declare no conflict of interest.

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