ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2022; 27(2): 3443-3452
doi: 10.25083/rbl/27.2/3443.3452

Received for publication: December, 09, 2021
Accepted: May, 26, 2022

Original paper

Influence of using low voltage electrostatic field during freezing and thawing processes on beef quality

ATEF MOHAMED ELSBAAY[1]*, NABIHAH H. ABOUELHANA[1], ESSAM MOHAMED ELSEBAIE[2], AND MAHMOUD ABDELHAKIEM MANSOUR BASUONY[1]

[1]Agricultural Engineering Department, Faculty of Agric. Kafrelsheikh University, 33516 Egypt.
[2] Food Technology Department, Faculty of Agric. Kafrelsheikh University, Egypt.

Abstract This study examined the effects of LVEF-assisted freezing-thawing on beef loin (Longissimus dorsi) quality and texture. In this work, the quality of beef specimens at 15, 30, and 45 cm from the LVEF plate (test group) and without LVEF treatment (control group) was examined during freezing-thawing. LVEF aided freezing (LVEFF) sped up beef freezing and thawing by 32.46% and 32.60% at 30 cm layer spacing (LVEF-30). LVEF30 created the smallest, most homogeneous ice crystals and less injured muscle fibre tissue. SEM indicated that LVEF30 preserved muscle fibre and perimysium structure, and muscle fibre gaps did not expand. Z-line and M-line were generally intact, while A-band and I-band were distinct and readable, indicating that LVEF30 preserved myofibrillar structure efficiently. LVEF30's L*, a*, and C values were substantially higher than the control group (P0.05) and fresh meat (P0.05). LVEF-30 reduced thawing loss, cooking loss, and drip protein content by 52.10%, 31.313%, and 15.97%. In conclusion, LVEF can improve the quality of thawed beef by reducing quality loss during freezing-thawing, and 30 cm is the best distance from the electrostatic field generation plate.

Keywords beef, freezing-thawing, low voltage electrostatic field, texture and electric field

To cite this article: ELSBAAY AM, ABOUELHANA NH, ELSEBAIE EM, BASUONY MAM. Influence of using low voltage electrostatic field during freezing and thawing processes on beef quality. *Rom Biotechnol Lett.* 2022; 27(2): 3443-3452 DOI: 10.25083/rbl/27.2/3443.3452

*Corresponding author: atef.ahmed@agr.kfs.edu.eg

Introduction

Beef is an important animal food resource, which can supply with high-quality protein and essential nutrients such as essential amino acids, unsaturated fatty acids, minerals, and vitamins [1, 2]. Freezing is a well-acknowledged method for long-term preservation of meat by slowing microbial growth rates and loss of meat quality[3]. Currently, airblast freezing (ABF) has been mentioned to be the principal freezing method for meat industry. However, huge ice crystals could be formed in meat owing to slow freezing rate of ABF, resulting in the deterioration in meat quality including protein denaturation, texture damage and flavour loss [4]. During freezing process, the migration of the intracellular water to intermolecular water will occur, resulting in the production of massive ice crystals and subsequent mechanical damage to muscle fibers, which may cause the texture degradation [5]. On the other side, muscle cells become unable to re-absorb the water that migrates outside of the cell in the process of thawing because of the breakdown of the cell membrane and tissues construction, leading to fluid loss [6]. These unanticipated alterations may significantly impair the quality of prepared beef products and are thus undesirable to customers. Therefore, great efforts should be made on the prevention of formation and growth of huge ice crystals throughout freezing storage. The quality of frozen products consists on the quantity of ice crystals as well as their size and distribution inside the material [7]. Therefore, there have been numerous studies performed to determine the best method for increasing the number of ice crystals or reducing their size. These can be achieved either by increasing the freezing rate or applying new emerging technologies, e.g. high-pressure-assisted freezing, power ultrasound-assisted immersion freezing and magnetic fields assisted freezing [8]. Compared with the above methods, the electrostatic field assisted-freezing technology has the advantages of high efficiency, low equipment cost, and simple operation [9]. The decrease of free energy owing to the reorientation of water molecules and the development of a more ordered cluster structure might be the possible mechanism of electric field-assisted freezing [10]. Among these methods, the use of static electric fields (SEF) has been considered because to its significant influence on nucleation, ease of operation, and low energy consumption. It can also simply be incorporated with available commercially freezers [10]. Recently some investigations have been done on the impact of the electro-freezing on real food system (pork meat and lamb meat) [11, 12], but these studies emphasize on the possibility of improving quality characteristics of food materials under electro-freezing. Currently, electrostatic field applying for food preservation has been receiving considerable attention. The use of a high voltage electrostatic field is a significant non-thermal processing technique which has been used in the advancement of meat freezing and thawing [13]. However, certain limitations in the application of HVEF still exist, such as high energy consumption and security concern. In this regard, the use of low voltage electrostatic field (the output voltage does not exceed 2 500 V, and the current does not exceed 0.2 mA) is more energy-saving, safe, and widely applicable. Qian et al [14] investigated LVEF's influence on the rates of thawing and thawed beef quality, indicating that thawing with LVEF may reduce thawing time and maintain the muscular microstructure efficiently. Low-voltage electrostatic field, as a new type of non-thermal technology, has attracted widespread attention and provides new ideas for the technical innovation of meat freezing and thawing. However, the application of low-voltage electrostatic fields in food storage and preservation is still in the initial stage, and its application in meat freeze-thaw technology is rarely reported. In this study, low voltage electrostatic field at different distances from the electrostatic generating plate was utilized in beef freezing thawing process. The freezing and thawing characteristics, colour properties, textural profile, microstructure and ice crystals morphology in muscle fiber tissue of LVEF-assisted freezing (LVEFF) samples and AF samples were detected to evaluate the quality promotion of LVEF-assisted freezing-thawing process. This study provides experimental and theoretical basis for the application of low-voltage electrostatic field technology to assist meat freezing and thawing process.

Materials and methods

Beef samples preparation

Sixteen beef loins (Longissimus dorsi) pieces (5 cm × 4 cm × 4 cm, the average weight of 85.0 ± 2.0 g per each one) were purchased from a local butchery in Kafr El-shiekh governorate, Egypt. These pieces were taken from the steer carcass (Holstein × Baladi cross breed, age of 18 months) and were kept for 24 h after slaughter at chilling temperature (5°C) and rapidly transported to the laboratory in an ice box in order to minimize the changes.

LVEF experimental apparatus

The electrostatic field device used in this experiment is composed of an electrostatic field generator (AC220V, 50/60Hz) and a plate electrode (14 cm ×12 cm). The output voltage of the electrostatic field generator is 2 500 V and the current is 0.2 mA, which is a low voltage electrostatic field (LVEF). To perform freezing treatment under LVEF, the prepared beef cubes were placed on a copper plate (14 cm × 12 cm) which is fixed on a chamber. The layer spacing was set at 15 cm, 30 cm, and 45 cm respectively. The treat-

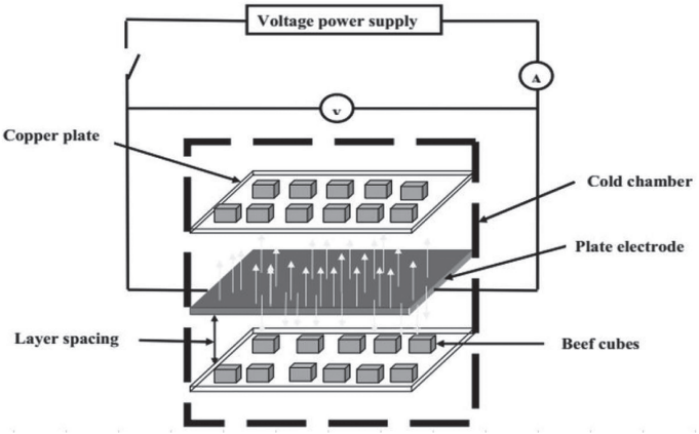

Figure 1. Schematic diagram of the LVEFF equipment.

ment chamber was placed in a cold incubator (YC-520L, MELNG, China) at -18°C or freezing process and 4°C for thawing process. The schematic diagram of the LVEFF-T system is shown in Fig. 1.

Beef samples freezing and thawing process

Sixteen meat samples were randomly divided into 4 groups. The beef that was naturally frozen and thawed (no electrostatic field was applied) was the control group, and the beef that was frozen and thawed under low voltage electrostatic field was the test group. The beef in the test group was divided into 3 groups depending on the distance away from the electrostatic field generating plate. The distances are 15, 30, and 45 cm, respectively. After packaging with transparent polyethylene film, the freezing test was carried out in a quick-freezer at –18°C. When the core temperature of the meat samples dropped to –18°C, the meat samples are considered to be completely frozen. After freezing process, the meat sample was placed in a 4°C refrigerator for thawing test. When the center temperature of the meat sample reaches to 4°C, the meat sample is considered to be completely thawed.

Temperature monitoring

Before freezing, a fiber optic thermocouple (Digi-Sense® Traceable® Kangaroo) was inserted into the center of each sample. The temperature was recorded at the interval of 1 min during freezing and thawing process and LOGGER 1.8.2 software was used to obtain the data.

Determination of color difference

Hunter Lab Colorimeter (MiniScan XE Plus, Reston, VA) has been directly used to measure the brightness value L*, redness value a*, and yellowness value b* of the sample surface. The colorimeter is calibrated with a white board before use. Each sample was tested in parallel 5 times (select the four corners of the square meat sample and the geometric center of the meat sample) and calculate the chroma value C*. The formula for calculating C* value is as follows:

$$C = \sqrt{a^{*2} + b^{*2}}$$

Drip measurement

Drip loss was determined by weighing the samples before and after thawing, and calculated as the difference between initial and final weight, and expressed in percentage, according to a modification of the method of Zhang and Ding [15]. Total protein content of the drip was determined using the biuret procedure described by Ngapo et al [16].

Cooking loss

The cooking loss was calculated using the method of Hu et al [13]. Meat specimens were separately placed in polyethylene sachets and cooked at 80°C by immersion in water till its internal temperature reached 75°C. Cooked specimens were cooled with running water until they reached room temperature, then wiped dry with filter paper and weighed. The cooking loss (%) is calculated as follows:

$$\text{Cooking loss (\%)} = \frac{\text{Raw weight - Cooking weight}}{\text{Raw weight}} \times 100$$

Texture properties measurement

For meat samples textural analysis, cubes (1cm×1cm×1cm) were cut along the direction of the fiber at the geometric center of the thawed meat sample. Texture profile analyses (TPA) were done via a texture analyzer (Cometech B, Taiwan), and each processed sample is measured 3 times in parallel, and the result is the average of the 3 measurements. The selected 4 analysis indicators are elasticity, hardness, cohesiveness and

springiness. Measurement parameters: probe P35; lateral front velocity 2.0 mm/s; center measurement velocity 1.0 mm/s; post-measurement velocity 5.0 mm/s; compression ratio 40%; the interval between two probe measurements 5.00 s; trigger type is automatic.

Shear force measurement

The pre-treatment of the samples in shear force analysis was the same as that in the textural analysis. The sample was cut parallel to the fiber direction and sheared with a Warner-Bratzler shear force (WBSF) device attached to a universal testing machine (Cometech, B type, Taiwan) with a 55 Kg tension/ compression load cell and the crosshead speed was set at 200 mm/min [17]. Each processed sample was measured 5 times in parallel, and the average value of the 5 measurements was taken.

Ice crystal morphology in muscle tissue observation

The ice crystal morphology was observed by light microscopy followed the indirect method called isothermal freezing substitution technique reported by Dalvi-Isfahan et al [11]. For each freezing treatment, cut 3 small rectangular cubes (5 mm × 4 mm × 4 mm), fix them with Carnoy solution, and let them stand at 4°C for 20 h. The samples were then dehydrated at 4°C with ethanol solutions of gradient concentrations (70%–100%, v/v). Then, the dehydrated sample was immersed in n-butanol and let to stand for 30 min (repeated 3 times). The samples were soaked in wax to ensure fixation of the meat tissue. The samples were then embedded in paraffin, and let it stand for 1 h (repeated 3 times) to facilitate slicing. Slices were obtained using a microtome and were subjected to 0.4% brilliant blue water solution for 3 min, followed by microscopic analysis.

Scanning electron microscopy (SEM)

SEM analysis was performed as described by Zhang and Ding [15] with slight modification. The samples were cut into blocks (2 mm × 2 mm × 5 mm) from the central part with a scalpel. The blocks were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) for 48 h. After washing with 0.1 M phosphate buffer (pH 7.3), the blocks were fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.3) for 1 h. The blocks were then washed with distilled water before being dehydrated with a succession of ethanol solutions, dried, and coated by gold. A scanning electron microscope (LEO 440i, UK) was used to examine and photograph cross sections of myofibers from the specimens at a magnification of ×500.

Transmission electron microscopy (TEM)

The samples were cut into blocks (4 mm × 4 mm × 2 mm) from the central part with a scalpel. The blocks were fixed, rinsed, and dehydrated as described in Section 2.11. After drying, the longitudinal sections were prepared on ultra-thin microtome, stained with uranyl acetate and lead citrate, and observed with a transmission electron microscope (H-7500, Hitachi, Japan) at a magnification of × 40,000.

Statistical analysis

The measurements were determined as the results of 3 parallel determinations, expressed as the mean ± standard deviation. ANOVA were performed using the general linear regression model of SPSS (Ver.16.0, 2007) analysis software ($P \leq 0.05$).

Results and discussion

Freezing and thawing processes temperature monitoring

The temperatures used for freezing and thawing of meat samples are -18°C and 4°C respectively. In order to study the influence of the low-voltage electrostatic field on the freezing-thawing process of beef, the temperature changes in the thermal center of different processed meat samples were compared. Time spent of different freezing stages and thawing process of the control group and the test groups were presented in Table 1.

Table 1. Time consumed at various freezing phases and thawing process of beef under various freezing techniques

Treatments	Precooling stage (min)	Phase transition stage (min)	Deep cooling stage (min)	Total freezing time (min)	Total thawing time (min)
LVEF-15	162.73 ±6.32Bd	86.83±5.93Ce	289.16± 5.69Cb	538.72±7.81Ca	236.13± 3.42Cc
LVEF-30	138.41 ±8.61Dd	75.29± 4.35De	282.79± 5.28Db	496.49±7.06Da	210.32± 2.91Dc
LVEF-45	151.92 ±7.84Cd	99.37± 6.70Be	343.07± 8.09Bb	594.36±6.88Ba	267.69± 3.16Bc
AbF	186.59±9.27Ad	112.16± 9.60Ae	436.45± 8.57Ab	735.20±8.75Aa	312.08± 2.07Ac

Data are presented as mean±SD (n = 3).

AbF: air-blast freezing; LVEF: low voltage electric-field assisted freezing at different discharge gaps (15 cm, 30 cm and 45 cm).

Means with different superscripts (A-D) uppercase letters in a column are significantly different at $P \leq 0.05$.

Means with different superscripts (a-e) lowercase letters in a row are significantly different at $P \leq 0.05$.

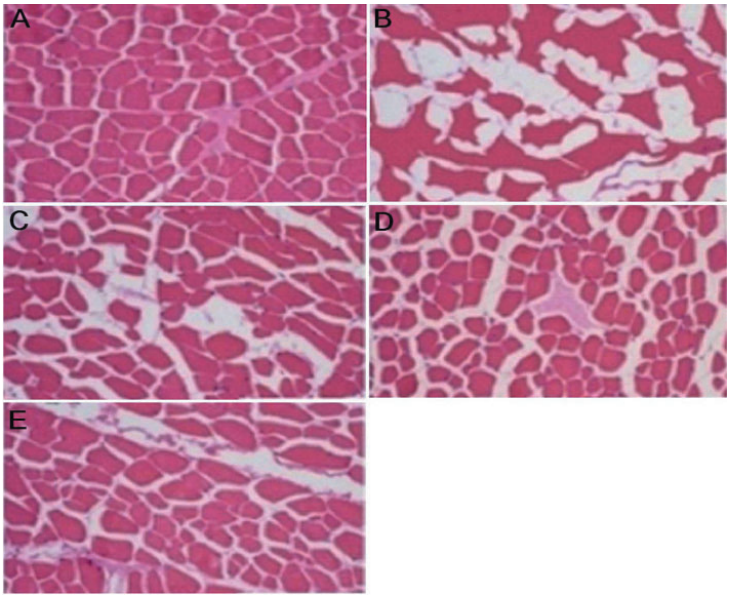

Figure 2. Micrograph of beef muscle fibers and ice crystals formed after different freezing treatments (200×)

A = Fresh sample, B = samples freeze and thawed in air blast freezer (AbF), C = LVEF-15, D = LVEF-30, and E = LVEF-45.

The freezing process was divided into three distinct stages, namely, the pre-cooling phase (in which the meat is cooled from its initial temperature to the freezing point), the phase transition (- 1°C~ - 5°C) (which represents the crystallization process of the water in muscle), and the deep freezing stage (in which the temperature decreased to the final temperature) [18].

Treatments under layer spacing of 15 cm (LVEF-15), 30 cm (LVEF-30) and 45 cm (LVEF-45) passed the maximum ice crystal formation stage lasted 86.83±5.93, 75.29±4.35 and 99.37±6.70 min respectively, while the control group (AbF) passed the maximum ice crystal formation stage for 112.16±9.60 min. It can be seen that the three groups of processed samples shorten the time taken to pass the maximum ice crystal formation stage when the meat is frozen to various degrees, indicating that the electrostatic field affects the water phase change process during meat freezing. Among them, LVEF-30 lasted the shortest time, which was 59.3% shorter than the control (AbF). The entire freezing process begins to cool down from 5°C, and ends when the sample center temperature reaches –18°C. The freezing time required for the treatment group LVEF15, LVEF30, and LVEF45 were 538.72±7.81, 496.49±7.06 and 594.36±6.88 min, while the freezing time required for the control group was 735.20±8.75 min. The freezing time required for the samples under the electrostatic field was significantly lower than that of the control group where LVEFF-30 reduced freezing time the most (47.1%). In this experiment, the meat sample is considered to be completely thawed when the center temperature of the meat sample reaches 1°C. As can be seen from Table 1, the thawing time required for the test group LVEF15, LVEF30, and LVEF45 are 236.13±3.42, 210.32±2.91 and 267.69±3.16 min, while the thawing time required for the control group was 312.08±2.07 min. Moreover, thawing process under the electrostatic field significantly ($P \leq 0.05$) reduced the thawing time of the frozen meat, and LVEFF-30 was the most efficient one, where the thawing process was accelerated by 32.69%. These results suggested that, under the low-voltage electrostatic field, the meat freezes fast, and the ice crystals grown are small in size. When thawed, transitioning to the water molecules state is easy.

In addition, some studies stated that meat thawing in the same way, in an electrostatic field environment, can accelerate the breaking of hydrogen bonds in the ice structure, and the thawing speed will increase [14]. The electric field strength at a distance of 30 cm from the discharge plate can effectively improve the freezing-thawing efficiency of meat.

Morphology of ice crystals in frozen beef tissues

Fig. 2 shows the ice crystal morphology after freezing under different LVEFF and AbF conditions. It can be seen from Fig. 2 that the muscle fiber tissue structure of fresh beef is uniform and dense, with very small gaps between muscle

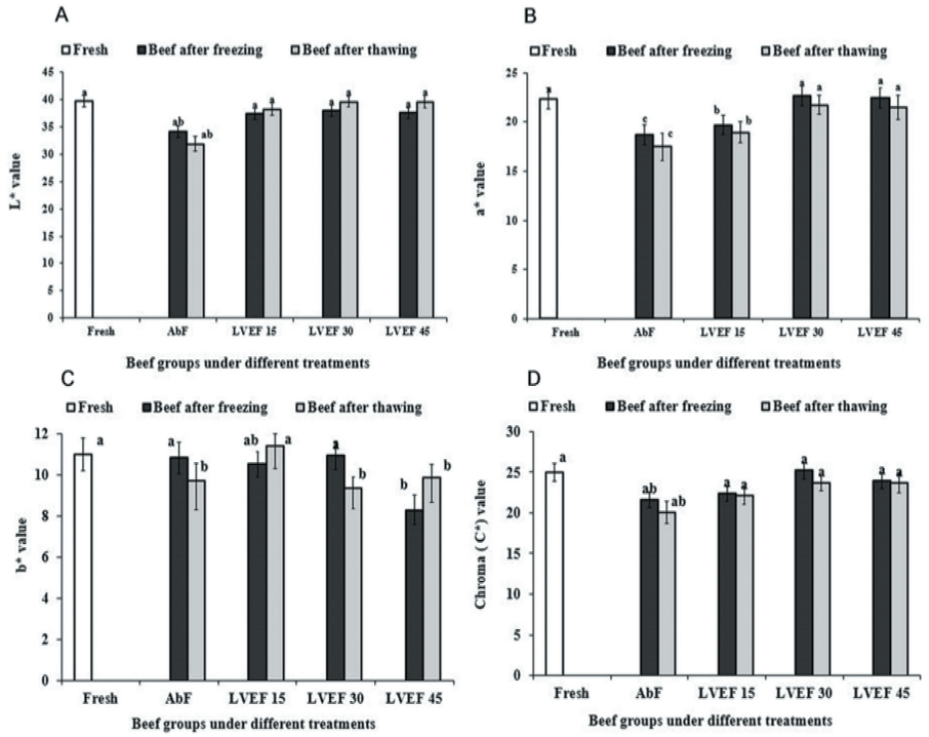

Figure 3. Changes in beef color after different freezing and thawing treatments.

Data are presented as mean±SD.

Means with different lowercase letters are significantly different at $P \leq 0.05$.

fibers. During the freezing process of muscles, water crystallizes in the tissues and increases in volume. The growth of ice crystals leads to the destruction of muscle tissues. The ice crystals formed in the control group (AbF) were large in size and small in number, and distributed chaotically in the muscle tissue.

However, the ice crystals produced in the test group were small in shape, large in number and evenly distributed. Control group and test group muscle fiber tissue was damaged by ice crystals to different degrees: the beef muscle fibers of the control group were obviously damaged and suffered severe mechanical damage while, the test group beef muscle fiber tissue structure was maintained well and was less affected by ice crystals. Among all groups, the LVEF30 treatment maintained muscle fiber tissue structure well and caused a relatively small degree of damage to the muscle fiber by ice crystals. This is due to the fact that the meat sample is frozen at a distance of 30 cm from the discharge plate and had ice crystals small in volume and uniformly distributed. Our results are consistent with those in the study of Kiani et al [19] and Dalvi-Isfahan et al [11] where they reported that in the perspective of the thermodynamic law, electric-field can modify and lower the free energy barrier for ice nucleation, leading to the enhancement of the nucleation rate and the number of ice nuclei, and consequently, the size of the resulted ice crystals is relatively smaller.

Beef color parameters

Changes in beef color within the normal range will not have much impact on its nutritional value and flavor. However, as an important indicator of meat sensory quality, the color and luster largely affect consumers' preferences. The L* value and a* value of the flesh color represents the brightness value and the redness value of the flesh sample. The higher L* value means the better meat gloss, and the higher a* value refers to better meat color and fresher meat. The higher C (chroma) value indicates better meat brightness [20]. It can be seen from Fig. 3 that, the L* value, a* value and C value of the frozen meat sample have decreased to various degrees compared with fresh meat samples. The L* value, a* value and C value of the control group (AbF) after freezing were 34.12, 18.72 and 21.63, which were sig-

nificantly lower than 39.71, 22.40 and 24.96 registered for the fresh meat sample (P≤ 0.05). However, the test group frozen under low-voltage electrostatic field conditions maintained the better color of the meat sample, and the LVEF30 test group had the most obvious effect. The L*, a* and C values of the LVEF30 group after freezing were 37.95, 22.68 and 25.19, which were significantly higher than those of the control group (P≤ 0.05), and there was no significant difference compared with fresh meat samples (P≤ 0.05). The meat sample loses too much water after thawing and the L* value is significantly lower than that of the fresh meat sample. In addition, if the meat sample has been in contact with air for too long, the increase in the oxidation rate of myoglobin reduces the a* and C values [19]. Hence, the beef in the test group after thawing has a higher color and freshness compared with the control. The L*, a* and C values of the LVEF30 in the test group after thawing are of 39.56, 21.75 and 23.68 which were significantly higher than 31.87, 17.52 and 20.03 in the control group (P≤ 0.05).

Thawing loss, drip protein content and cooking loss

After the muscle is frozen, the water crystal volume increases, causing the muscle cell membrane break-cracking, drip loss occurs when thawing, and a large amount of soluble protein is lost with the drip, resulting in a serious decline in the meat nutritional value [21]. As shown in Fig. 4A, the thawing loss of frozen beef cubes specimens exposed to LVEF15, LVEF30 and LVEF45 were 5.48±0.52%, 4.10±0.31%, and 4.20±0.44%, respectively, which were significantly lower (P ≤ 0.05) than that of AbF specimens (8.56±0.47%). In the four treatment groups, the LVEF30 specimen showed the lowest rate of thawing loss, while the thawing loss rate of the LVEF45 specimen was somewhat less than that of LVEF15. According to Leygonie et al [3], ice melting in cell exo-spaces could lead to water flow into endo-spaces and their eventual re-absorption via dehydrated fibers and denatured proteins.

According to Qian et al [14], the electrostatic field can enhance the renaturation of freezing-induced denaturated myofibrillar proteins and preserve the bonding capability amongst protein and water throughout the thawing step. This might account for the lower thawing loss of frozen beef cubes when treated with LVEF. Data presented in Fig. 4B indicated that the drip protein content of LVEF15, LVEF30 and LVEF45 in the test group were 10.84±0.19%, 9.31±0.25% and 9.72±0.13%, which were significantly lower (P≤ 0.05) than the control group (11.08± 0.16%). Fig. 4C revealed that the LVEF30 specimen had the lowest cooking loss (19.98± 1.16%), which was significantly lower (P ≤0.05) than that of

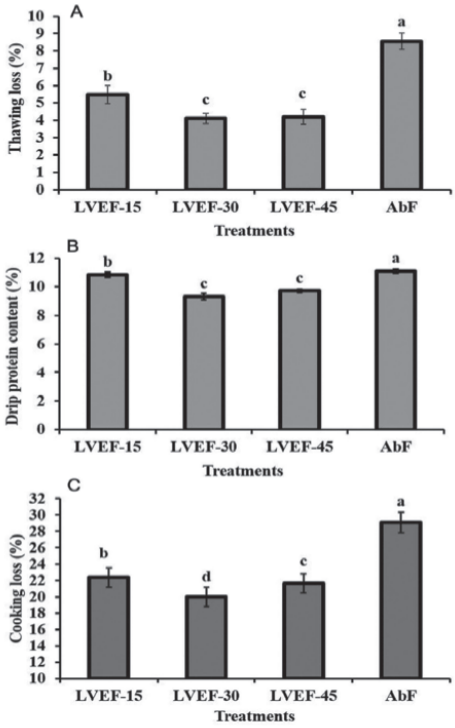

Figure 4. Effects of different freezing and thawing treatments on thawing loss (A), Drip protein content (B) and cooking loss(C).

Data are presented as mean±SD.

Means with different lowercase letters are significantly different at P ≤ 0.05.

AbF (29.09±1.25%), LVEF15 (22.35±1.20%) and LVEF45 (21.62±1.13%). The obtained results showed that the electrostatic field-assisted freeze-thaw process can effectively improve the water retention of beef and significantly reduce the nutrient loss of beef after thawing. Among all investigated groups, the LVEF30 treatment group resented a reduced thawing loss rate, cooking loss rate, and drip protein content by 4.46%, 9.11%, and 1.77% compared with the control group (AbF). Generally, water retention of meat samples was the best in all meat samples treated with LVEF. Also, results in Figure 4 (A, B and C) show that meat muscle fibers treated with LVEF30 are minimally damaged by ice crystals during freezing, and that drip loss is better suppressed during thawing. Same results were reported by Qian et al [14], where applying LVEF in the thawing process of raw beef could significantly decrease the thawing loss of the thawed beef compared with air thawing.

Table 2. Effects of different freezing and thawing treatments on texture of beef

Treatments	Elasticity/ N	Hardness/ N	Cohesiveness	Springiness /N	Shear force/N
Fresh	783.42±56.37a	423.89±20.12d	0.56±0.02a	0.98±0.01a	2.33±0.07c
LVEF-15	641.65±38.09b	457.92±17.27b	0.52.±0.04a	0.95±0.03a	2.26±0.04c
LVEF-30	613.57±33.18b	439.76±21.50cd	0.56±0.03a	0.97±0.02a	2.01±0.09d
LVEF-45	594.10±56.53c	448.30±22.29c	0..53±0.02a	0.96±0.01a	2.58±0.07b
AbF	479.81±41.29d	512.73±18.32a	0.46±0.05b	0.81±0.03b	3.96±0.05a

Data are presented as mean±SD (n = 5).

AbF: air-blast freezing; LVEF: low voltage electric-field assisted freezing at different discharge gaps (15 cm, 30 cm and 45 cm).

Means with different superscripts (a-d) lowercase letters in a column are significantly different at $P \leq 0.05$.

Beef texture characteristics and shear force

Textural parameters are also an essential quality indicator for meat products, which may be assessed by hardness, chewiness and cohesiveness of the meat [22]. In this experiment, the texture characteristics and shear force of fresh meat samples and freeze-thaw beef under different conditions were analyzed (Table 2). The results indicated that the elasticity of LVEF15, LVEF30 and LVEF45 beef in the experimental group were 641.65±38.09N, 613.57±33.18N and 594.10±56.53N, which were significantly greater ($P \leq 0.05$) than the control group (479.81±41.29N). Notably, the hardness of the experimental group LVEF30 and LVEF45 was 439.76±21.50N and 448.30±22.29N, which was significantly lower ($P \leq 0.05$) than the control group (512.73±18.32N). This could be due to the dry and matte surface of meat caused by the thawing loss. However, the cohesiveness of the LVEF groups was not significantly different from that of the fresh specimen ($P \leq 0.05$).

LVEFF had a significant enhancement on parameter ($P \leq 0.05$). After the freezing procedure the springiness of the beef cubes diminished. None significant variation ($P \leq 0.05$) was found in the springiness values among LVEF-15, LVEF-30, LVEF-45 and the fresh beef specimen. Frozen beef tends to have more strength and lower softness than fresh meat owing to muscle fibers deteriorating and muscle drip loss after thawing stage [3]. The water is released from the myofibrils, and muscle fibers are less water-hydrated throughout the thawing process. The decreased hardness and increased springiness may potentially be due to muscular cell physical disruption (which will be shown later in Fig. 5A). Further, the shear force values of LVEFF samples were significantly lower than that of control (AbF) sample. As shear force reflects the muscle tenderness, and better tenderness is associated with lower shear force value [23]. Thus, electrostatic field assisted freeze-thaw can significantly improve the texture characteristics and maintain better tenderness of thawed beef. Additionally, the textural characteristics of the sample frozen with LVEFF-30 were the best among all samples. Our results were consistent with Dalvi-Isfahan et al [11].

Microstructure of beef Myofibril

Scanning electron microscope

Fresh and frozen-thawed beef under different conditions were observed by scanning electron microscopy to analyze the integrity of muscle fiber bundles and perimuscular membranes, as well as the gaps between muscle fiber bundles. The results are shown in Figure 5A (photographed at ×500). It can be seen from Figure 5A, that the muscle fiber bundles and perimuscular membrane structure of fresh beef are complete, the muscle fiber bundles are tightly arranged, and the gaps in the muscle bundles are small. After thawing, the integrity of beef muscle fibers is lost, the arrangement of muscle fiber bundles is loose, the space between muscle bundles is large, and the structure of the myofibril cells membrane is broken. The beef muscle fibers of the control group were severely deformed, and some areas were even hollow. Compared with the control group, the muscle microstructure of the test group is relatively complete, the muscle fiber bundles are arranged tightly and the gaps are smaller, and the degree of damage to the fascia is less. Among all investigated groups, the LVEF15 and LVEF30 meat samples in the test group effectively maintained the structure of muscle fiber bundles and perineurium after thawing, and the gaps between muscle fiber bundles did not expand significantly. The perimysium in muscle is elastic and can maintain the integrity of muscle tissue and the dense arrangement of muscle fiber bundles. The destruction of the perimysium will cause the gaps between the muscle fiber bundles to increase, and the water will leak out more easily, the water retention of the muscles will decrease, and serious drip loss will occur.

Transmission electron microscope

Muscle fiber bundle longitudinal sections of fresh and frozen-thawed beef under different conditions were observed by transmission electron microscope and the results are shown in Fig. 5 (magnification 40000×). Fig. 5B shows that the fresh meat myofibril structure is completed, the muscle fiber bundles are tightly arranged, the A-band and the I-band are clearly distinguishable, and the Z-line and M-line

are obvious and complete. In the control group after thawing, the Z-line of the meat sample was broken, the M-line was blurred, and the A and I zones were severely damaged. Thawing meat samples under LVEFF15 or LVEF30 kept Z-line and M-line relatively compelled, and the A and I belts are still clearly identifiable. This shows that the low electrostatic field assisted freezing and thawing can effectively maintain the structural integrity of beef muscle fiber tissue. Feng et al [24] found a significant correlation between the integrity of myofibrils and the water retention characteristics of muscles by studying the relationship between muscle tissue changes and water retention.

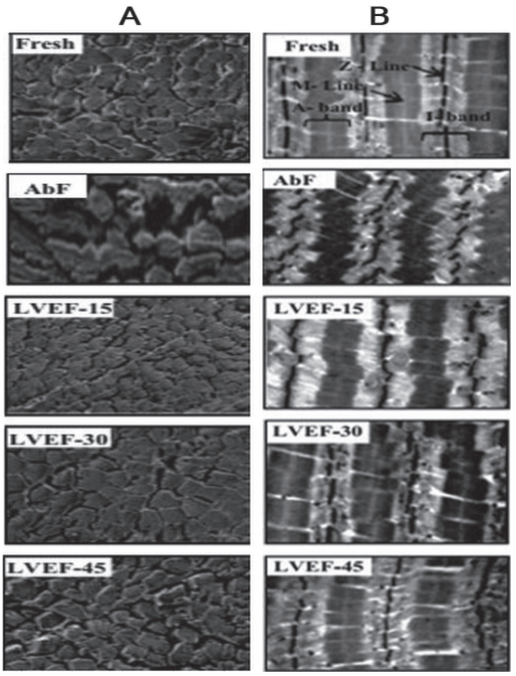

Figure 5. Scanning electron microscopy (SEM) images (×500) (A) and transmission electron microscopy (TEM) images (×40000) (B) of beef after different freezing and thawing treatments.

At the same time, the destruction of the complete and dense tissue structure of the muscle fiber bundles will lead to the reduction of the elasticity and chewiness of the meat. Low electrostatic field assisted freeze-thaw process maintained the structure of fleshy muscle fiber bundles being more complete and dense, which also verifies its low drip loss rate and better maintenance of texture properties.

Conclusion

The quality of beef has deteriorated after freezing and thawing, and different freezing and thawing methods will affect the quality of beef to various degrees. In this study, the use of an electrostatic field to assist the freeze-thaw process of beef, compared with natural freeze-thaw, can effectively slow down the quality deterioration of beef during the freeze-thaw process and improve the quality of thawed beef as follows:

1) The freezing-thawing efficiency of beef under low-voltage electrostatic field has been significantly improved ($P \leq 0.05$). The ice crystals grown during the freezing process are small in size and evenly distributed in the muscle tissue. The damage to the muscle tissue is light, and the muscle fiber bundle structure is more complete and dense.

2) After thawing, the water holding capacity of beef is improved and the nutrient loss is reduced. Among them, the freeze-thaw at a distance of 30 cm from the electrostatic plate, the drip loss rate, cooking loss rate and the drip protein content are reduced by 4.46, 9.11, and 1.77 percentage points respectively compared with the natural freeze-thaw, the difference being significant ($P \leq 0.05$).

3) The color and texture characteristics of thawed beef are effectively maintained. The L* value, a* value and C value of the frozen-thawed meat sample at a distance of 30 cm from the electrostatic plate were significantly higher ($P \leq 0.05$) than the natural freeze-thaw (AbF); Elasticity, hardness, cohesiveness and springiness were significantly higher than those of natural frozen-thawed meat ($P \leq 0.05$).

4) Compared with the other test groups, the frozen-thawed meat sample at a distance of 30 cm from the electrostatic plate has a brighter color, better texture and water retention performance.

Conflict of interest

The authors have declared no conflicts of interest for this article.

References

1. Cabrera, M. and A.J.M.s. Saadoun, *An overview of the nutritional value of beef and lamb meat from South America.* 2014. **98**(3): p. 435-444.
2. Oh, M., et al., *Chemical compositions, free amino acid contents and antioxidant activities of Hanwoo (Bos taurus coreanae) beef by cut.* 2016. **119**: p. 16-21.
3. Leygonie, C., T.J. Britz, and L.C.J.M.s. Hoffman, *Impact of freezing and thawing on the quality of meat.* 2012. **91**(2): p. 93-98.
4. Hou, Q., et al., *Quality changes of pork during frozen storage: Comparison of immersion solution freezing and air blast freezing.* 2020. **55**(1): p. 109-118.

5. Jiang, Q., et al., *Changes in quality properties and tissue histology of lightly salted tuna meat subjected to multiple freeze-thaw cycles.* 2019. **293**: p. 178-186.
6. Pietrasik, Z. and J.J.F.R.I. Janz, *Utilization of pea flour, starch-rich and fiber-rich fractions in low fat bologna.* 2010. **43**(2): p. 602-608.
7. Zhu, Z., et al., *Measuring and controlling ice crystallization in frozen foods: A review of recent developments.* 2019. **90**: p. 13-25.
8. Cheng, X., et al., *The principles of ultrasound and its application in freezing related processes of food materials: A review.* 2015. **27**: p. 576-585.
9. Le-Bail, A., et al., *Electrostatic field assisted food freezing.* 2011. **2**: p. 685-692.
10. Dalvi-Isfahan, M., et al., *Review on the control of ice nucleation by ultrasound waves, electric and magnetic fields.* 2017. **195**: p. 222-234.
11. Dalvi-Isfahan, M., et al., *Effect of freezing under electrostatic field on the quality of lamb meat.* 2016. **37**: p. 68-73.
12. Jia, N., et al., *Changes in the structural and gel properties of pork myofibrillar protein induced by catechin modification.* 2017. **127**: p. 45-50.
13. Hu, F., et al., *Combined impacts of low voltage electrostatic field and high humidity assisted-thawing on quality of pork steaks.* 2021: p. 111987.
14. Qian, S., et al., *Effects of low voltage electrostatic field thawing on the changes in physicochemical properties of myofibrillar proteins of bovine Longissimus dorsi muscle.* 2019. **261**: p. 140-149.
15. Zhang, Y. and C.J.I.A. Ding, *The Study of Thawing Characteristics and Mechanism of Frozen Beef in High Voltage Electric Field.* 2020. **8**: p. 134630-134639.
16. Ngapo, T., et al., *Freezing and thawing rate effects on drip loss from samples of pork.* 1999. **53**(3): p. 149-158.
17. Shackelford, S., T. Wheeler, and M.J.J.o.a.s. Koohmaraie, *Evaluation of sampling, cookery, and shear force protocols for objective evaluation of lamb longissimus tenderness.* 2004. **82**(3): p. 802-807.
18. Castro-Giráldez, M., et al., *Thermodynamic approach of meat freezing process.* 2014. **23**: p. 138-145.
19. Kim, Y., et al., *Evaluation of antioxidant capacity and colour stability of calcium lactate enhancement on fresh beef under highly oxidising conditions.* 2009. **115**(1): p. 272-278.
20. Sales, L.A., et al., *Effect of freezing/irradiation/thawing processes and subsequent aging on tenderness, color, and oxidative properties of beef.* 2020. **163**: p. 108078.
21. Xia, X., et al., *Physicochemical change and protein oxidation in porcine longissimus dorsi as influenced by different freeze–thaw cycles.* 2009. **83**(2): p. 239-245.
22. Martinez, O., et al., *Texture profile analysis of meat products treated with commercial liquid smoke flavourings.* 2004. **15**(6): p. 457-461.
23. Zhang, M., et al., *Changes in microstructure, quality and water distribution of porcine longissimus muscles subjected to ultrasound-assisted immersion freezing during frozen storage.* 2019. **151**: p. 24-32.
24. Feng, Q., et al., *Effect of different cooking methods on sensory quality assessment and in vitro digestibility of sturgeon steak.* 2020. **8**(4): p. 1957-1967.