



Effect of repeated freezing and thawing on the tenderness of beef[#]

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Abstract

The present study was conducted to find the effect of repeated freezing and thawing on beef tenderness. The Biceps femoris muscle from six adult crossbred female cattle were collected hygienically, portioned into a chunk size of 10×10×5 cm, and subjected to ageing for 72 hours at 4°C. Meat samples were subjected to freezing at -18°C for seven days and thawed under five different thawing methods, viz. ambient temperature (AT) at 31-34°C, chiller temperature (CT) at 4°C, microwave thawing (MT) at 2450 MHz, immersion thawing with water replacement (ITW), immersion thawing without water replacement (ITWO). Three freeze-thaw cycles were carried out. The unfrozen meat samples after ageing and meat samples after every freeze-thaw cycles were subjected to assessing myofibrillar fragmentation index (MFI), Warner-Bratzler shear force (WBSF), collagen content, collagen solubility and cooked sensory evaluation for assessing the tenderness. The results of the present study suggested that as the number of freeze-thaw cycles increased, a significant decrease ($p \leq 0.05$) was observed in the myofibrillar fragmentation index and WBSF. The collagen content and collagen solubility remained non-significant throughout the freeze-thaw cycles. The sensory scores for tenderness increased significantly ($p \leq 0.05$) with an increased freeze-thaw cycle. In conclusion, beef tenderness increased as the number of freeze-thaw cycles increased.

Keywords: Freeze-thaw cycles, shear force, tenderness, myofibrillar fragmentation index, collagen

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Frozen storage is the most common and widely used method for the preservation of meat and meat products to extend the shelf-life and maintain meat quality during storage and distribution (Xia *et al.*, 2012). To keep the quality of frozen meat from being impacted by storage circumstances, a temperature of -55°C is the optimal recommended preservation setting. Meat quality practically never degrades while stored at this temperature due to reduced microbial activity, enzymatic reactions, and oxidative deterioration. However, in the commercial scale, considering the economic feasibility, storage of frozen meat is usually done at -18°C. But some biochemical and chemical reactions may still be active at this temperature (Hansen *et al.*, 2004). These reactions mostly involve lipid oxidation and colour changes causing frozen meat quality to decline. One major challenge the frozen food sector faces is the frequent temperature variations during transit, storage and distribution. These fluctuations might result in the recrystallisation of ice in meat and micro-structural changes (Farouk *et al.*, 2004). In the actual production line, the range of temperature fluctuations will be relatively large due to imperfect cold chain technology in transportation, storage, retail, and consumption processes. Frozen meat and meat products often undergo multiple freeze-thaw cycles before they are consumed, which can degrade the quality of the meat, reduce consumer acceptability, and potentially result in financial losses for the manufacturer. However, repeated freeze-thaw cycle accelerated tenderisation of beef (Jin-ping *et al.*, 2012). The present study focused on the effects of repeated freezing and thawing on the tenderness of beef.

Materials and methods

The study was conducted at Meat Technology Unit, Mannuthy. Six adult female crossbred cattle (above six years old) were utilised in the present study. The animals were slaughtered at Meat Technology Unit, Kerala Veterinary and Animal Sciences, Mannuthy, after 12-24 h of fasting as per scientific slaughter procedures. After electrical stimulation (LVES-100-110V, upto 1.5-2 min) and deboning, *Biceps femoris* muscles were collected. Chunks of 10×10 cm (breadth × length dimensions) size with uniform thickness of approximately 4 to 6 cm were prepared and subjected to the ageing process for up to 72 h

at 2 - 4 °C. After ageing, the meat was packed in HDPE bags and frozen a temperature of -18 °C for a period of seven days. The samples were thawed by five different methods, viz., ambient temperature thawing (AT) at 31-34°C, chiller temperature thawing (CT) at 1 to 4 °C, microwave thawing (MT) , immersion thawing with water replacement (ITW) and immersion thawing without water replacement (ITWO). The meat chunks reaching the temperature of about 4°C were considered thawed. Time taken to reach 4°C for different thawing methods such as ambient temperature, chiller temperature thawing, microwave thawing, immersion thawing with water replacement and immersion thawing without water replacement were 3 hours, 24 h, 2.5 min, 2.5 h and 2 h, respectively. All the meat samples were subjected to repeated freezing (one week) and thawing cycles and after each freeze-thaw cycle, samples were drawn for analysis. There were three freeze-thaw cycles in this study. To assess the tenderness, the post-thaw meat was evaluated for myofibrillar fragmentation index, Warner-Bratzler shear force, collagen content, collagen solubility and cooked beef sensory parameters.

Myofibril fragmentation index (MFI)

Myofibril fragmentation index of each muscle sample was determined by the procedure outlined by Davis *et al.* (1980). Ten grams of seven mm cubes of meat were added to 50 mL of 0.24 M cold sucrose and 0.02 M potassium chloride solution in a homogenisation cup. After five minutes, each sample was blended for 40 s at full speed in a homogeniser (Polytron, PT 3100, Kinematica AG, Switzerland). The resulting homogenate was then filtered through a filter assembly consisting of a pre-weighed muslin cloth (250 µ pore size) in a glass funnel and glass stirring rod. The residue and cloth were blotted twice on an absorbent towel immediately after stirring and then weighed. The fragmentation index was reported as weight of residue in grams times one hundred.

Warner-Bratzler shear force

Warner-Bratzler shear force (WBSF) of buffalo muscle was measured by the method outlined by Wheeler *et al.* (1997). Each muscle sample was cooked to an internal temperature of 80°C (monitored using a probe thermometer) and chilled overnight 2-3°C before coring. On the next day, three cores of 1.27 cm diameter

were taken from each cooked meat along the longitudinal orientation of muscle fibers. These cores were kept at 2-3°C until they were sheared. Each of the cores were sheared perpendicular to the muscle fiber on Shimadzu Texture Analyzer Model EZ-SX (Shimadzu Corporation, Kyoto, Japan) with a crosshead speed of 200 mm/min. WBSF was expressed in Newton (N).

Collagen content

Total collagen content of selected buffalo and bovine muscles was determined as per Stegman and Stalder (1967). The total collagen content was calculated from the hydroxyproline content of the sample.

About 1.2 g of dried fat-free meat sample was hydrolysed with ten milliliter of 6 N HCl in a sealed test tube at 110°C for 18-20 hours. The hydrolysate was cooled and filtered using Whatman No. 4 filter paper into a standard flask and was diluted to 100 mL using distilled water. Five milliliter of the diluted hydrolysate was neutralised to pH 6.8 using sodium hydroxide solution and the volume was made up to 100ml using distilled water. Five millilitres of the neutralised diluted tissue hydrolysate was mixed with two milliliters of oxidising agent and kept at room temperature for 20 min. Two milliliters of colouring reagent was added and incubated in a water bath at 60°C for 15 minutes, cooled under running tap water for three minutes and the absorbance of the coloured solution was measured using a single beam spectrophotometer (Systronics, India) at 560 nm. Regression equation developed from the absorbance values of known standard concentrations was used to determine the hydroxyproline content of the unknown samples. Collagen content was determined by multiplying the hydroxyproline content with 7.25 to get the total collagen content of the sample. The total collagen was expressed as per cent of fresh basis.

Collagen solubility

Collagen solubility of buffalo muscle samples was determined as per Hill (1996). Collagen solubility was calculated from the soluble hydroxyproline content of the sample.

Cooked meat sensory evaluation

The sensory evaluation of cooked

beef muscle samples was conducted by a semi-trained panel (n=6) of faculty and post-graduate students from the Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy. Meat samples used were cut into approximately equal sizes (1.5 x 1.5 x 1.9 cm) and were cooked by pressure cooking in stainless steel boxes for 20 min. All panellists received two cubes of each cooked muscle sample coded with three-digit numbers along with a scorecard (AMSA, 1983). The panellists were asked to rate the samples for tenderness on an eight-point hedonic scale (AMSA, 1983). During sensory evaluation, panellists were provided with filtered water to cleanse their palate between samples.

Statistical analysis

Data was analysed using IBM SPSS statistics software, version 24.00. Repeated measures ANOVA was used for MFI, WBSF, collagen content and collagen solubility, whereas Friedman and Kruskal-Wallis test were employed to interpret data from sensory evaluation.

Results and discussion

Changes in myofibrillar fragmentation index, Warner-Bratzler shear force, collagen content, collagen solubility and sensory evaluation for tenderness are shown in Tables 1, 2 and 3.

Myofibrillar fragmentation index (MFI)

The MFI values of beef decreased significantly ($p \leq 0.05$) from raw meat to 3rd freeze-thaw cycle for all the thawing methods (Table 1). No significant difference was observed between the five thawing methods within the three freeze-thaw cycles. MFI could be used as a potential indication of tenderness when there is insufficient meat available to quantify tenderness by shear force and sensory evaluation (Veiseth *et al.*, 2001). The reduction in the MFI values with the increase in the number of freeze-thaw cycles can be attributed to mechanical damage caused by the large ice crystals that form in muscle fibres during slow freezing. Additionally, repeated freeze-thaw cycles may result in ice recrystallisation and microstructural damages in the frozen meat. This reduction can also be correlated with enhanced proteolysis by calpains resulting in myofibrillar fragmentation and increased

tenderness (González *et al.*, 2012). The results of the present study indicated that as the number of freeze-thaw cycles increased, the MFI values decreased which led to improved tenderness. Kiran *et al.* (2020) reported the MFI value of *biceps femoris* muscle as 822.8 ± 26.93 after 72 hours of ageing and in the present study the MFI value decreased as the number of freeze-thaw cycle increased indicating improved tenderness.

Warner-Bratzler shear force (WBSF)

The shear force values of beef significantly ($p \leq 0.05$) decreased from raw meat to 3rd freeze-thaw cycle for ITWO. For all other thawing methods the shear force value decreased non-significantly (Table 1). No significant difference was observed between the five thawing methods within the three freeze-thaw cycles. The Warner-bratzler shear force value can be considered an indicator of meat's comparative toughness that coincides with the collagen content and sensory evaluation. The tenderness was negatively correlated with shear force and MFI (Kavitha, 2006). The decrease in shear force values, which is associated with tenderisation, can be related to the breakdown of muscle fibers. The formation of large extracellular ice crystals in the meat during slow freezing might have damaged the structure of muscle (causing loss

of structural integrity) by breaking myofibrils which results in tenderisation. In contrast, small ice crystal formation probably releases protease enzymes which leads to meat ageing (Vieira *et al.* 2009). The results of the present study were in accordance with Qi *et al.* (2012), Wang *et al.* (2018) and Tippala *et al.* (2021) who observed similar decreases in shear force values in ovine *L. dorsi* muscle, rabbit meat and pork, respectively.

Collagen content (per cent)

The collagen content (per cent) of beef remained non-significant from raw meat to 3rd freeze-thaw cycle for all the five thawing methods (Table 2). No significant difference was observed between the five thawing methods within the three freeze-thaw cycles. The results observed in the present study might be due to the fact that collagen is a major and stable connective tissue protein of meat which helps in maintaining the texture of meat and influences meat tenderness. Meat tenderness is a major factor affecting the consumer's assessment of meat quality. However, high amounts of crosslinks of collagen can reduce tenderness and acceptability by consumers (Warriss, 2000). In the present study, thawing temperatures were within 35 °C, which was not enough to degenerate the collagen protein and the animals utilised in the present study were

Table 1: Mean ($\pm$) SE of MFI and WBSF value of beef subjected to repeated freezing and thawing

Treatment	Freeze-Thaw Cycle					p-value
	Raw meat	C1	C2	C3	Overall mean	
Myofibrillar fragmentation index						
AT	880.23±20.24 ^c	836.10±29.05b ^c	798.35±22.5 ^b	695.33±20.14 ^a	802.50±17.91	0.000*
CT	880.23±20.24 ^b	860.28±22.48 ^b	778.83±23.65 ^a	712.67±26.92 ^a	808.00±17.72	0.000*
MT	880.23±20.24 ^c	826.22±32.3b ^c	792.42±23.87 ^b	683.00±14.81 ^a	795.46±18.65	0.000*
ITW	880.23±20.24 ^c	886.35±18.80 ^c	797.72±9.75 ^b	678.33±16.43 ^a	810.66±19.19	0.000*
ITWO	880.23±20.24 ^c	879.82±35.64 ^c	778.25±12.68 ^b	681.83±24.16 ^a	805.03±20.66	0.000*
Level of sig.		NS	NS	NS		
Warner-Bratzler shear force						
AT	53.89±0.68	50.69±1.79	49.12±0.81	45.75±5.71	49.86±1.54	0.322 ^{NS}
CT	53.89±0.68	49.79±2.51	43.65±0.78	43.26±5.42	47.65±1.69	0.063 ^{NS}
MT	53.89±0.68	50.58±3.18	50.00±2.50	48.96±2.27	50.86±1.16	0.495 ^{NS}
ITW	53.89±0.68	48.24±2.65	48.47±2.40	47.84±1.30	49.61±1.04	0.120 ^{NS}
ITWO	53.89±0.68 ^b	48.38±2.46 ^a	47.24±1.49 ^a	46.58±1.52 ^a	49.02±0.98	0.022*
Level of sig.		NS	NS	NS		

Means bearing different superscripts between columns (a,b,c) and between rows (A,B,C) differ significantly ($P \leq 0.05$). *- Significant, NS- Non significant (AT- Ambient temperature thawing, CT- Chiller temperature thawing, MT- Microwave thawing, ITW- Immersion thawing with water replacement, ITWO- Immersion thawing without water replacement)

Table 2: Mean ($\pm$) SE of collagen content and collagen solubility (per cent) of beef subjected to repeated freezing and thawing

Treatment	Freeze-Thaw Cycle					
	Raw meat	C1	C2	C3	Overall mean	p-value
Collagen content (per cent)						
AT	0.71±0.05	0.69±0.02	0.67±0.06	0.66±0.09	0.68±0.03	0.925 ^{NS}
CT	0.71±0.05	0.65±0.05	0.68±0.05	0.67±0.03	0.68±0.02	0.744 ^{NS}
MT	0.71±0.05	0.73±0.05	0.74±0.07	0.69±0.03	0.72±0.02	0.920 ^{NS}
ITW	0.71±0.05	0.68±0.05	0.70±0.06	0.65±0.04	0.69±0.02	0.829 ^{NS}
ITWO	0.71±0.05	0.78±0.07	0.71±0.05	0.75±0.06	0.74±0.03	0.845 ^{NS}
Level of sig.		NS	NS	NS		
Collagen solubility (per cent)						
AT	1.20±0.13	1.80±0.30	2.13±0.72	2.29±0.13	1.85±0.21	0.284 ^{NS}
CT	1.20±0.13	2.08±1.08	1.78±0.42	2.33±0.34	1.85±0.30	0.601 ^{NS}
MT	1.20±0.13	1.56±0.38	1.71±0.29	2.22±0.43	1.67±0.17	0.201 ^{NS}
ITW	1.20±0.13	2.18±0.30	2.09±0.55	2.41±0.89	1.97±0.27	0.446 ^{NS}
ITWO	1.20±0.13	2.17±0.49	1.98±0.28	2.47±0.32	1.96±0.18	0.074 ^{NS}
Level of sig.		NS	NS	NS		

Means bearing different superscripts between columns (a,b,c) and between rows (A,B,C) differ significantly ($P \leq 0.05$). *-Significant, NS- Non significant (AT- Ambient temperature thawing, CT- Chiller temperature thawing, MT- Microwave thawing, ITW- Immersion thawing with water replacement, ITWO- Immersion thawing without water replacement)

of same age group, which could be the reason for non-significance observed in the collagen content.

Collagen solubility (per cent)

The collagen solubility (per cent) remained non-significant for beef, but a gradual increase in the solubility was observed from the raw to 3rd freeze-thaw cycle (Table 2). No significant difference was observed between the five thawing methods within the three freeze-thaw cycles. The soluble fraction of intramuscular collagen is a major determinant of the background tenderness of the meat. As the age of animals increases, collagen cross-links will get stabilised making it less soluble (Warriss, 2000). The thawing temperatures undertaken in current study were within 35 °C, which was not enough to degenerate the collagen protein nor solubilise the collagen. The improved tenderness of beef during repeated freeze-thaw cycles could be due to mechanical damage caused by large ice crystals and enhanced proteolysis by calpains (González *et al.*, 2012), rather than the effect of multiple freezing and thawing on collagen. This conclusion is based on the lack of significant changes in collagen solubility after three freeze-thaw cycles.

Sensory evaluation of cooked meat for tenderness

The tenderness scores of beef increased significantly ($p \leq 0.05$) between the freeze-thaw cycles for all the thawing methods from raw meat to 3rd freeze-thaw cycle (Table 3). viz; Ambient temperature (6.90 $\pm$ 0.08 to 7.19 $\pm$ 0.08), chiller temperature (6.90 $\pm$ 0.08 to 7.16 $\pm$ 0.08), microwave thawing (6.90 $\pm$ 0.08 to 7.26 $\pm$ 0.07), immersion thawing with water replacement (6.90 $\pm$ 0.08 to 7.30 $\pm$ 0.05), immersion thawing without water replacement (6.90 $\pm$ 0.08 to 7.28 $\pm$ 0.06), respectively. No significant difference was observed between the five thawing methods within the three freeze-thaw cycles. The improved tenderness might be due to the mechanical damage caused by large ice crystal formation in the meat during repeated freezing (slow freezing) and thawing. Rahman *et al.* (2014) reported that during multiple freeze-thaw cycles, moisture loss is accelerated due to refreezing of meat, contributing to dry and dense texture in cooked meat. Kavitha (2006) reported that tenderness could be correlated with and MFI and sheer force of meat. The present study was not in agreement with observations of Rahman *et al.* (2014) in beef samples. Prajwal *et al.* (2017) recorded low tenderness score (4.37 $\pm$ 0.20) for *biceps femoris* muscle of beef after 72 hour of

Table 3: Mean ($\pm$) SE of sensory scores for tenderness of cooked beef subjected to repeated freezing and thawing

Treatments	Raw meat	Freeze-Thaw Cycle			χ^2 - value	Level of significance (P value)
		C1	C2	C3		
Tenderness Score						
AT	6.90±0.08 ^a	6.90±0.10 ^{ab}	6.94±0.07 ^b	7.19±0.08 ^c	85.559	0.000*
CT	6.90±0.08 ^a	7.08±0.08 ^{ab}	7.08±0.06 ^{ab}	7.16±0.08 ^b	87.643	0.000*
MT	6.90±0.08 ^a	6.96±0.08 ^a	7.16±0.05 ^b	7.26±0.07 ^b	94.865	0.000*
ITW	6.90±0.08 ^a	6.92±0.07 ^a	7.01±0.07 ^a	7.30±0.05 ^b	91.012	0.000*
ITWO	6.90±0.08 ^a	6.99±0.07 ^a	7.08±0.06 ^{ab}	7.28±0.06 ^b	91.059	0.000*
Level of significance		NS	NS	NS		

Means bearing different superscripts between columns (a,b,c) and between rows (A,B,C) differ significantly ($P \leq 0.05$).

*- Significant, NS- Non significant

(AT- Ambient temperature thawing, CT- Chiller temperature thawing, MT- Microwave thawing, ITW- Immersion thawing with water replacement, ITWO- Immersion thawing without water replacement)

ageing. The tenderness score in the present study ranged between extremely tender to very tender for all the thawing methods even after third freeze-thaw cycle.

Conclusion

The present study concluded that as the number of freeze-thaw cycles increased, irrespective of thawing methods, the tenderness of beef improved. The parameters like myofibrillar fragmentation index and Warner-Bratzler shear force values declined significantly ($p \leq 0.05$) with the increased number of freeze-thaw cycle, which indicated improved tenderness. On sensory evaluation, the tenderness score of beef increased significantly ($p \leq 0.05$) upon repeated freeze-thaw cycles. But the difference in collagen content and collagen solubility remained non-significant throughout the freeze-thaw cycle. The different thawing methods had no significant effect on the tenderness of beef since the results obtained did not show any significant differences between the thawing methods.

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Conflict of interest

The authors declare that they have no conflict of interest.

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