Meat Research
Vol 5, Issue 3: 1-8, Article 119, June 2025
ISSN: 2790-1971
https://doi.org/10.55002/mr.5.3.119 http://www.bmsa.info/meatresearch

1Department of Animal Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

***Corresponding Author:**

M Khan
E-mail: muckta.khan@bau.edu.bd

Keywords:
Beef liver
Freezing
Thawing
Biochemical properties
Nutritive value

Article Info:
Received: May 09, 2025
Accepted: June 15, 2025
Published online: June 30, 2025

Research Article

Effect of repeated freezing and thawing on the quality of beef liver

MA Salam[1], H Khatun[1], H Akter[1], MT Ahmed[1], MA Hashem[1], M Khan[1]*

Abstract

The present study investigated the effect of repeated freeze-thaw cycle on the quality of beef liver. For this purpose, raw fresh beef liver was collected and divided into four treatments groups- Day 1 (T_1 - fresh group, which was immediately analyzed) and other packed portions were stored under refrigeration at 4℃ for the whole day (12 hours) and at evening, stored under freezing at -20℃. Analyses of other samples were performed next 2nd (T_2, freeze-thaw), 3rd (T_3, freeze-thaw) and 4th (T_4, freeze-thaw) day with three replicates. Liver sample was packaged in polythene bags and frozen at -20℃ for overnight (12 hours) followed by thawing in refrigerator at 4℃ for whole day (12 hours). The freeze-thawed procedure was repeated for subsequent for four days. The sensory tests (color, odor, juiciness, and tenderness), the proximate components dry matter (DM), crude protein (CP), ether extract (EE) and ash, physicochemical properties (pH and cooking loss), biochemical properties free fatty acid (FFA%), Peroxide value (POV meq/kg) and Thiobarbituric acid (TBARS mg-MDA/kg), and the microbial assessments, total viable count (TVC log cfu/g) were carried out for each treatment. The result showed that color, odor, juiciness, and tenderness were significantly ($p<0.05$) decreased with the increase of freeze-thawed cycle. Dry matter and ash content were significantly ($p<0.05$) increased, whereas crude protein and ether extract were decreased with the increase of freeze-thaw cycle. pH and water holding capacity were decreased and cooking loss was increased with the increase of freeze-thaw cycle ($p<0.05$). Thiobarbituric acid values were increased ($p<0.05$) with the increase of freeze-thaw cycle. Microbial assessments like TVC values were increased ($p<0.05$) with the increase of freeze-thawed cycle. With the increased freeze-thaw cycle decreasing the quality in all parameters studied were found. Thereby, avoiding of repeated freeze-thaw cycle is more acceptable. In conclusion, these findings have been suggested that shelf life of raw beef liver freeze at -20℃ and thaw at 4℃ for whole day up to maximum three days could be acceptable.

Introduction

Animal by-products such as liver, kidney, spleen, and heart etc. has been used as an edible by-product and included in the dietary ration due to their high nutritional values. The vitamin content of edible offal is usually higher than that of lean meat issue. Liver also contains good amount of niacin, cobalamin, pyridoxine, folacin, ascorbic acid and vitamin A (NUTTAB, 2006; USDA, 2007). Livers also contain the highest amount of manganese (0.128 to 0.344 mg/100 g) as reported by Irshad and Sharma (2015). Beef liver is one of the vital organs which is actively involved in various metabolic functions of the body such as metabolism of proteins, lipids, carbohydrates, vitamin A, vitamin B, synthesis of fibrinogen, globulin, albumin clothing factors, secretion of bile, storage of glycogen, fat, excretion of urea, uric acids (Dona, 2007). Meat and meat products should be preserved by suitable technologies to maintain the quality and safety and the principles of meat preservation are to inhibit microbial spoilage and extend the shelf life of meat (Lawrie and Ledward, 2006). Different preservation methods of meat have been developed among which freezing is most useful over the world (Sultana et al., 2008). Freezing and thawing are complex processes that involve heat transfer as well as a series of physical and chemical changes which can affect the quality of meat products (Bing et al., 2002). Generally, the excess amount of meat after thawing may be put in the freezer again, especially in retail markets and restaurants, these freeze-thawed cycles may be repeated several times (Baygaret al., 2013). For assurance of food quality quick thawing at low temperature avoiding notable rise in temperature and increased dehydration of food is desirable. Longer the thawing treatment time, higher will be the microbial growth on product surface. Nutritional quality reduction due to leaching of soluble proteins, high energy consumption and large quantities of loaded wastewater are also other disadvantages of conventional thawing (Roberts et al., 1998). In recent years, food industry has relied more and more on using thawed meat in meat processing. Freezing commercially at -18 ℃ and domestically at -10℃ is now a standard of eating quality compared to fresh meat. Both for preservation of meat and further manufacturing of meat -18 ℃ to -20 ℃ freezing

temperature is effective (Farouk et al., 2004; Soyeret al., 2010). The shelf-life of meat is normally determinate by assessing the color, microorganisms, pH value, flavor, texture, and nutritional value (Hammad, et al, 2017; McMillin, 2008). At frozen temperature, some chemical and biochemical processes in the meat may still occur. They are mainly involving lipid oxidation and discoloration. These are responsible for the deterioration of meat quality during frozen storage (Turhan et al., 2017). Liver products are considered a high-risk food as these are highly nutritious and serve as an ideal medium for bacterial growth(Karch et al, 2005). During the thawing and refreezing processes, moisture migrates from muscle cells to the space between cells (Charoenrein, 2018). Freezing, thawing, and refreezing cause damage to the cell walls, leading purge to be released more easily from the meat and moisture lost from the muscle cells not re-absorbed upon thawing (Leygonie et al., 2012; Pham and Mawson, 1997). The freeze-thawed cycles could also occur from the temperature fluctuation or abuse during storage, transportation, retail display and consumption (Srinivasan et al., 1997). Multiple freeze-thawed cycles induced repeated melting and reformation of ice crystals that caused damage of cell membrane and induced myofibrillar protein structural changes which resulted the loss of protein functionality and protein denaturation as well as protein aggregation. These will have affected the water-holding capacity (WHC) and texture of meat (Xia et al., 2010). The available meats at retail markets should be stable quality and free from pathogenic bacteria and fungi which can cause serious human disease. Fresh meats are often treated by cooling or freezing to increase their shelf-life. Freezing and refrigeration processes are the common methods used to protect foods by preventing the microorganism growth that cause food-borne illnesses (Albrecht et al., 2019; Hammad et al., 2019). Effect of repeated freezing-thawing on the muscle quality has been studied by several scientists. But till now the effect of repeated freezing and thawing on the quality of beef liver has not been studied yet. Moreover, in Bangladesh aspects there is lack of information regarding the shelf-life of repeated freeze-thawed beef liver. Therefore, the objective of the current study was to analysis the effect of repeated freezing and thawing on the quality of beef liver.

Materials and methods

Place of Experiment

The experiment was carried out in the laboratory of the Department of Animal Science at Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh.

Sample Collection

The experimental sample (beef liver) were obtained from the Kamal-Ranjit (KR) market of Bangladesh Agricultural University, Mymensingh. After collecting the beef liver sample (500gm), immediately transferred to the "Animal Science Laboratory" and carried out for sensory, proximate, physicochemical, biochemical, and microbial analysis.

Preparation of Jar and Other Instruments

For the experiment, all the necessary instrument and jar or containers were cleaned with hot water and detergent powder and then dried properly before starting the experimental activities.

Preparation of beef liver sample

All visible fat and connective tissue were trimmed from beef liver with the help of knife and the samples were sliced and individual slices were packaged in sterile plastic bags. One sample bag was immediately analyzed and other packed portions were stored under refrigeration at 4℃ for the whole day (12 hours) and at evening, stored under freezing at -20℃. Analyses of other samples were performed next 2^{nd}, 3^{rd} and 4^{th} day with three replicates.

Measurement of parameters

First day, fresh beef liver sample were analyzed (sensory properties, proximate components, physicochemical properties, Biochemical properties, and microbial analysis) and the rest of the four samples were kept in the refrigerator at 4℃ for the whole day and at evening, stored under freezing temperature at -20℃. The freeze-thawed procedure was repeated for subsequent for four days.

Sensory evaluation

Different sensory attributes were examined. Each beef liver sample was evaluated by a trained 5-member panel. The sensory questionnaires measured intensity on a 5-point balanced semantic scale (weak to strong) for the following attributes color, odor, juiciness, and tenderness. The judges evaluated the samples based on the above criterions. Panelists were selected among department member and students and trained according to the American Meat Science Association guidelines (AMSA, 2015). Sensory evaluation was carried out in individual booths under controlled conditions of light, temperature, and humidity. Prior to sample evaluation, all panelists participated in orientation sessions to familiarize with the scale attributes (color, odor, juiciness, and tenderness) of liver using an intensity scale. Sensory scores were 5 for excellent, 4 for very good, 3 for good, 2 for fair and 1 for poor (Rahman et al., 2012). All samples were served in the petri dishes. Sensory evaluations were accomplished at 1^{st} day and repeated at 2^{nd}, 3^{rd}, and 4^{th} day of storage.

Proximate Composition

Proximate composition such as dry Matter (DM), crude protein (CP), ether extract (EE) and ash were carried out according to the methods (AOAC, 2005). All determination was done in triplicate and the mean values were reported.

Dry Matter

Weighed samples were taken in porcelain crucibles and dry at 100℃ in an electric oven. The crucibles were then cooled in desiccators. The average weight in percentage of each sample of the remaining material was taken as DM.

Crude Protein

Crude protein was determined by micro kjeldahl method. Total nitrogen content of each sample was determined in triplicate by using kjeldahl apparatus. In this case total nitrogen was determined by digestion the samples with 20 ml concentrated sulphuric acid (H_2SO_4) in presence of K_2SO_4 , $CuSO_4$ and selenium powder followed by distillation of ammonia liberated by alkali 3

(NAOH) into boric acid and titrated with standard HCl. The nitrogen values thus obtained were converted to total crude protein by multiply with a factor of 6.25. This test was done at alternative days for the economic use of chemicals.

The formula is mentioned below:

(Titrate required (ml) ×0.014 (milliequivalent of N2) × Strength of HCl /Weight of sample) ×100

% of CP= % of nitrogen × conversion factor (6.25)

Ether Extract

Ether extract content was determined by Soxhlet apparatus using diethyl ether. At first flask weight was taken. Then 5 gm sample was taken in a thimble and added 200 ml acetone in a Soxhlet. Extraction was done at 40- 45℃ which took about 7-8 hours. After extraction the flask were taken out and dried in oven for 30 minutes at 100℃. The flask containing ether extract was cooled in a desiccator and weighed.

The formula is mentioned below:

% of ether extract= (Weight of the ether extract/ Weight of the sample) ×100

Ash

Weighed samples were taken in porcelain crucibles and pre-ashes at 100℃ in an electric oven. The crucibles were then placed in a muffle furnace and heated at 550℃ for 6 hours. The crucibles were then cooled in desiccators. The average weight in percentage of each sample of the remaining material was taken as ash.

The formula is mentioned below:

% of the ash content= (E /C) ×100

Where,

E= Weight of ash

C= Weight of the sample

Physicochemical properties

pH

pH value of raw beef liver was measured using a pH meter from raw beef liver homogenate. The homogenate was prepared by blending of 5 g of beef liver with 10ml distilled water**.**

Cooking Loss

The fresh beef liver samples were weighted (initial weight). Firstly, weighted liver boiled at water bath to 100℃. After completed boiling, samples were removed from the water bath and covered with foiled paper to remove the surface water properly and final weight taken of boiled liver.

The formula of cooking loss is mentioned below:

Cooking loss (%) = [(w2-w3) ÷ w2] ×100

Where, w2 = liver weight before cooking and

w3 = liver weight after cooking.

Water Holding Capacity

Water holding capacity (WHC) was determined by the filter paper press method (Boby et al., 2021). Each piece of sample contain almost 2g of beef liver and that was covered with eight sheets of filter paper and pressed with load for two minutes.

The water holding capacity was calculated as follows:

$$\text{WHC (\%)} = \frac{(\text{Weight of sample after centrifugation})}{(\text{Weight of sample before centrifugation})} \times 100$$

Biochemical analysis

Thiobarbituric Acid Values (TBRAS) (mg-MDA/kg):

Lipid oxidation was assessed in triplates using the 2-thiobarbituric acid (TBA) method described by (Schmedes and Holmer, 1989). Beef liver samples (5g) were blended with 25 ml of trichloro acetic acid solution (200 g/L of trichloro acetic acid in 135 ml/L phosphoric acid solution) in a homogenizer for 30s. The homogenized sample was filtered with Whatman filter paper number 4, and 2 ml of 0.02 M aqueous TBA solution (3g/L) in a test tube. The test tubes were incubated at 100℃ for 30 min and cooled with tap water. The absorbance was measured at 532 nm using a UV-VIS spectrophotometer (UV-1200, Shimadzu, Japan). The TBA value was expressed as mg malonaldehyde per kg of liver sample.

Microbial assessment

For microbial assessment total viable count was undertaken. These analyses were done at alternative days for the economic use of chemicals. To determine these parameters the procedure which are described below:

Preparation of samples for TVC count

A quality of 10 g of beef liver sample was aseptically excised from stored stock sample. Each of the stored goat liver samples was thoroughly and uniformly macerated in a mechanical blender using a sterile diluent (0.1% peptone water) as per recommendation of International Organization for Standardization (ISO, 1995). A quality of ten (10) gram of the minced goat

liver sample was taken aseptically transferred into a sterile container containing 90 ml of 0.1% peptone water. A homogenized suspension was made in a sterile blender. Thus 1:10 dilution of the sample was obtained. Later, using Whirly mixture machine different serial dilutions ranging from 10-2 to from 10-6 were prepared according to the instruction of the standard method (ISO, 1995).

Media and reagent employed for TVC count Solid media and reagents

The media employed for this bacteriological analysis included plate count agar (PCA), MacConkey agar (MA) and potato dextrose agar (PDA). The commercial media were prepared according to the direction of the manufacturers. The diluent used during the study was 0.1% peptone water.

Preparation of media

A quantity of 11.50 g of PCA agar and 15.6 g of MA agar were dissolved in 500 ml and 300 ml of cold distilled water in two separate conical flasks and heated to boiling for dissolving the ingredients completely. In case of PDA, 200 g of previously peeled and sliced potato was taken in 1000 ml of distilled water and boiled for an hour. After boiling, sieving was done through clean cheese cloth. 20 g of commercial dextrose and 15 g of agar were added to the potato infusion solution and heated up to boiling to dissolve the ingredients completely. Later, the media were sterilized at 121℃ (6.795 kg pressure/ sq inch) for 15 minutes in an autoclave. The final reaction was adjusted to pH 7.0±0.1. The agar was then ready for pouring. Before pouring, the medium was kept in a boiling water bath at 45℃.

Enumeration of Total Viable Count (TCC)

For the determination of total bacterial counts, 0.1 ml of each ten-fold dilution was transferred and spread on triplicate PCA agar using a sterile pipette for each dilution. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile glass spreader. For every plate, a single sterile spreader was utilized. After that, the plates were stored for 24 to 48 hours at 35°C in an incubator. Plates showing between 30 and 300 colonies were counted after incubation. A colony counter was used to count the colonies. To get the overall viable count, the dilution factor was multiplied by the average number of colonies in each dilution. The ISO (1995) method was used to obtain the total viable count. The overall bacterial count data were reported as colony forming units (CFU/g), or the number of organisms per gram of liver samples.

Statistical Analysis

Statistical analysis was performed by using SPSS 17.0. One-way analysis of variance (ANOVA) was used to analyze the differences between fresh and freeze-thawed cycles. Means were considered significantly different at $p<0.05$. Data presented are shown as mean ±SD.

Results and Discussions

Effect of repeated freeze-thaw cycle on the sensory evaluation of beef liver

The parameters for sensory evaluation have been shown in Table 1. The scores of color, odor, juices and tenderness were high ($P<0.05$) in fresh liver and decreased with the increase of freeze-thaw cycles.

The range was 5 to 3.35 for color score of fresh and repeated freeze-thawed beef liver. Similar color (5 to 3.67) was found by Akter et al., (2023) in repeated freeze-thawed goat liver. Among the treatments, most desirable color was observed in T1, and less desirable color was observed in T4 group. Gradually decreasing appearance and color score of beef liver which was thawing at 4℃ for the whole day and freezing at -20℃ for whole night. It might be due to pigment and lipid oxidation, resulting in non-enzymatic browning between lipids and amino acids. Some authors reported that oxidation of myoglobin is responsible for browning of meat during storage (Mancini and Hunt, 2005). Georgantelis et al., (2007) presented that the oxidative browning of the meat product still occurred during frozen storage.

The odor score range was 5 to 3.31 in the present study. Similar odor score (5 to 3) was found by Akter et al., (2023) in repeated freeze-thawed goat liver. Odor score was significantly decreased ($p<0.05$) with increase of freeze-thaw cycle. The most preferable odor was observed from T1 and the less preferable odor from T4. The lower odor scores may be related to the increased of malonaldehyde formation due to oxidation of fat, which has detrimental effect on the flavor and firmness of the product (Miller et al., 1980). J. Kanner (1994) said that one of the common problems encountered during meat storage is the development of undesirable odor characteristics due to oxidative changes.

The juiciness score range was 5 to 3.29 in fresh and repeated freeze-thawed beef liver. Similar decreasing juiciness score was found by Akter et al., (2023) in repeated freeze-thawed goat liver. Juiciness scores were significantly decreased ($P<0.05$) with the increase of freeze-thaw cycle. Among the treatments most acceptable juiciness score was observed from T1 and less acceptable score was observed from T4. Thomas et al., (2006) reported that a decline in the juiciness scores of different meat products during frozen storage. Aaslyng et al., (2003) Showed that the lower juiciness of frozen stored meat most likely resulted from the loss of water during thawing and cooking loss which was only slightly lower relative to fresh meat.

The ranges of tenderness score at different treatment was 5 to 3.00 in fresh and repeated freeze-thawed beef liver. Similar decreasing tenderness score was found by Akter et al., (2023) in repeated freeze-thawed goat liver. Among the treatments, most acceptable tenderness was observed at T1, and less acceptable tenderness observed at T4. In frozen condition of liver, ice crystals form inside the cells of muscle tissue and puncture the cell walls. It is the causes of liver leak moisture when they were cooked. Tenderness is interrelated with dry matter content of liver. With the increasing of storage period dry matter was increased consequently, tenderness was decreased with day's intervals. The result of this experiment is also related to (Lui et al., 2010) findings. Several researchers have associated with tenderness of meat with the breakdown of myofibrillar proteins affected by the presence of calcium-dependent proteases or calpains (Muchenje et al., 2009). Further, similar findings were supported by Syuhairah et al., (2016).

Table 1. Effect of repeated freeze-thaw cycle on the sensory evaluation of beef liver

Parameters	Treatments				Level of significance
	T_1	T_2	T_3	T_4	
Color	5.00^{a}±0.01	4.67^{ab}±0.10	4.00^{b}±0.11	3.35^{c}±0.05	P<0.05
Odor	5.00^{a}±0.41	4.75^{a}±0.17	4.00^{b}±0.01	3.31^{c}±0.02	P<0.05
Juiciness	5.00^{a}±0.01	4.50^{ab}±0.42	4.00^{b}±0.06	3.29^{c}±0.13	P<0.05
Tenderness	5.00^{a}±0.04	4.00^{b}±0.45	3.35^{c}±0.25	3.00^{c}±0.03	P<0.05

Here, T_1= Day1 (fresh), T_2= Day2 (freeze-thawed), T_3= Day3 (freeze-thawed), T_4= Day4 (freeze-thawed) with significant at 5% level, P<0.05. Values are presented as mean ± SD.

Effect of repeated freeze-thaw cycle on proximate composition of beef liver

The values of proximate components have been shown in Table 2.

Dry Matter (DM)

The DM content of different treatments of beef liver are shown in table 2. The overall observation range of DM content at different treatments was 27.04% to 29.65%. It was observed that DM contents were increased (P<0.05) with the increase of repeated freeze-thawed cycles. Among these five treatments most acceptable DM content was observed at T1 group, and the lowest amount DM content indicates this product is most acceptable. The highest amount of DM content indicates the product is less acceptable. The same trend was also observed by Konieczny et al., (2007) in beef jerky and Akter et al., (2023) in repeated freeze-thawed goat liver, they reported that DM content increased during frozen storage. Among these four treatments most acceptable DM content was observed at T_1 group, and the lowest amount DM content indicates this product is most acceptable. The highest amount of DM content indicates the product is less acceptable. The same trend was also observed by (Konieczny *et al.,* 2007) and they reported that DM content increased with frozen duration.

Crude Protein (CP)

The CP content of different treatments of beef liver are shown in table 2. The range of CP content at different treatments was 21.70% to 21.45%. It was observed that CP contents were decreased (P<0.05) with the increase of repeated freeze-thawed cycles. The same trend was also observed by Akter et al., (2023) in repeated freeze-thawed goat liver.

Ether Extract (EE)

The EE content of different treatments of beef liver are shown in table 2. The range of EE content at different treatments was 4.28% to 3.02%. EE content were significantly (p<0.05) decreased with the increase of repeated freeze-thawed cycles. The lowest amount of EE content was observed from T4 treatments. Agnihotri (1988) reported deterioration in meat lipids took place due to intermediary activities of endogenous meat enzymes leading to hydrolysis of fat. The same trend was also observed by Akter et al., (2023) in repeated freeze-thawed goat liver.

Ash

The ash content of different treatments of beef liver are shown in table 2. The range of ash content at different treatments was 1.30% to 1.74%. The ash content was increased (p<0.05) with the increase of freeze-thaw cycle. A non-significant decrease in ash percentage was reported by Ziauddin et al. (1994) which coincided with this study. The same trend was also observed by Konieczny et al., (2007) and they reported that ash content increased during frozen time. Similar trend was also observed by Akter et al., (2023) in repeated freeze-thawed goat liver.

Table 2. Effect of repeated freeze-thaw cycle on proximate composition of beef liver

Parameters	Treatments				Level of significance
	T_1	T_2	T_3	T_4	
DM%	27.04^{c}±1.06	27.18^{c}±1.00	27.96^{b}±0.76	29.65^{a}±0.53	P<0.05
CP%	21.70^{a}±0.70	21.61^{a}±0.72	21.50^{ab}±0.71	21.45^{b}±0.74	P<0.05
EE%	4.28^{a}±0.18	3.80^{b}±0.15	3.30^{c}±0.21	3.02^{c}±0.10	P<0.05
Ash%	1.30^{c}±0.02	1.47^{b}±0.01	1.60^{a}±0.03	1.74^{a}±0.11	P<0.05

Here, T_1= Day1 (fresh), T_2= Day2 (freeze-thawed), T_3= Day3 (freeze-thawed), T_4= Day4 (freeze-thawed) with significant at 5% level, p<0.05. Values are presented as mean ± SD.

Effect of repeated freeze-thaw cycle on physicochemical properties of beef liver

The results of physicochemical properties such as pH, cooking loss and water holding capacity are presented in Table 3. The values of pH at different treatments was 6.62 to 5.47. Similar pH values was also observed by Akter et al., (2023) and Ali et al. (2015) in repeated freeze-thawed goat liver and chicken meat respectively. With the increase of freeze-thaw cycle pH values were (p<0.05) decreased. According to Leygonie *et al.,* (2011) the pH of frozen or thawed meat is usually lower than the pH of fresh meat. This may be due to the denaturation of protein buffer systems, release of hydrogen ions, and increased concentrations of water-soluble compounds in meat in consequence of drip loss during thawing as well as the release of hydrogen atoms following protein deamination by microorganisms and enzymes.

In the present study, the values of cooking loss ranges from 18.57% to 26.55%. Cooking loss were increased by repeated freeze and thawed (p<0.01) cycles. Similar increased cooking losse trend was also observed by Akter et al., (2023) in goat liver. Cooking loss in liver is very important for maintaining an attractive retail display of beef liver. As an example, meat and their products are a good source of proteins, essential minerals, and vitamins. The increased loss such nutrients deteriorates the meat nutritional quality and lowers its purchase. The meat also tended to shrink during the cooking process due to the denaturation of meat protein; the loss of water and fat also contributed to the shrinking process (Serdaoglu *et al.,* 2005). Cooking yield is an important data that are used by the meat industry to predict the behavior of their products during processing (Ulu, 2006).

A significant difference was noted for WHC among beef samples subjected to repeated freeze-thawed cycles. The initial WHC of fresh sample was 9.53%. It is noted that thawing and then refreezing, mechanically disrupts the muscle cell integrity initiating a

series of changes. Protein and lipid oxidation are commonly linked to decreases in muscle protein functionalities, such as reduced water-holding capacity and weakened gels strength (Xia et al., 2012).

Table 3. Effect of repeated freeze-thaw cycle on physicochemical properties of beef liver

Parameters	Treatments				Level of significance
	T_1	T_2	T_3	T_4	
pH	6.62^{a}±0.07	5.88^{b}±0.04	5.74^{b}±0.09	5.47^{c}±0.14	P<0.05
Cooking loss%	18.57^{d}±0.21	23.89^{c}±0.68	24.22^{b}±0.89	26.55^{a}±1.68	P<0.05
Water holding capacity %	89.52^{a}±0.95	88.96^{ab}±0.87	88.20^{b}±0.32	87.67^{c}±0.15	P<0.05

Here, T_1= Day1 (fresh), T_2= Day2 (freeze-thawed), T_3= Day3 (freeze-thawed), T_4= Day4 (freeze-thawed with significant at 5% level, p<0.05. Values are presented as mean ± SD.

Effect of repeated freeze-thaw cycle on biochemical properties of beef liver

In the present study, the observation of TBARS range at different treatments was 0.22 to 0.43 (Table 4). Unpleasant flavor would be detected with the TBARS values >0.5 mg MA/kg (Hansen *et al*., 2004), though in the present research with the increase of freeze-thawed cycle, TBARS were increased (p<0.05) but it was remained below this level (>0.5 mg MA/kg) after 4 days. Similar results were reported Daszkiewicz *et al*., (2018), TBARS values were high in both fresh and frozen-thawed meat samples. Farouk and Freke, (2008) also reported that malondialdehyde concentration was lower in samples of red deer meat which were vacuum-packaged and stored in the freezer than in samples that were not vacuum-packaged.

Table 4. Effect of repeated freeze-thaw cycle on biochemical properties of beef liver

Parameters	Treatments				Level of significance
	T_1	T_2	T_3	T_4	
TBARS (mg MA/kg)	0.22^{c}±0.01	0.23^{c}±0.07	0.27^{b}±0.01	0.43^{a}±0.19	P<0.05

Here, T_1= Day1 (fresh), T_2= Day2 (freeze-thawed), T_3= Day3 (freeze-thawed), T_4= Day4 (freeze-thawed with significant at 5% level, p<0.05. Values are presented as mean ± SD.

Effect of repeated freeze-thaw cycle on microbial load of beef liver

The present study (Table 4) showed that the values of TVC, were significantly increased with the increase of freeze-thaw cycle but not exceeded the maximum secure values (6 log cfu/g, Cheng et al., 2018) up to fourth freeze-thaw cycle. Cross-contamination from the environment (i.e., the air or food handlers) or from the survival of spores or resistant cells was possible in this study as well as in commercial operations. In frozen state the formation of the ice crystal leads to death or injury for the microbes by damaging their cell walls and membranes, thus increase meat shelf-life (Pizato et al., 2015). Generally, in frozen state the nutritional and sensory quality of meat are stored, because of the inactivity of the microbes at a lower temperature (Löndahl et al.,1993) but the microbe's activity resumes during thawing, and exposing the meat to more favorable conditions for microbial growth because thawing is a slower and less uniform process compared to freezing (Leygonie et al., 2012).

Table 5. Effect of repeated freeze-thaw cycle on microbial load of beef liver

Parameters	Treatments				Level of significance
	T_1	T_2	T_3	T_4	
TVC (*log* cfu/g)	5.21^{d}±0.15	5.34^{c}±0.03	5.80^{b}±0.04	5.97^{a}±0.06	P<0.05

T_1= Day1 (fresh), T_2= Day2 (freeze-thawed), T_3= Day3 (freeze-thawed), T_4= Day4 (freeze-thawed) with significant at 5% level, p<0.05. Values are presented as mean ± SD.

Conclusions

The results of this study reported that with the increasing of freezing and thawing cycle decreasing the quality in all parameters such as sensory evaluation, nutritional composition, physicochemical properties, biochemical and microbial analysis. Thereby, avoiding of repeated freeze thaw cycle is more essential. Overall, these findings suggested that shelf life of raw beef liver freeze at -20℃ and thaw at 4℃ for whole day up to maximum three days could be acceptable. Further, the findings of the current study will contribute for advance research in the processing of meat and meat products.

Conflicts of Interest

The authors declare that there is no potential conflict of interests.

References

Aaslyng MD, Bejerholm C, Ertbjerg P, Bertram HC, Andersen HJ 2003: Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. Food Quality and Preference 14(4): 277-288.

Akhter MS, Khatun H, Hashem MA, Rahman MM, Khan M. 2022. Effect of storage periods on the quality and shelf life of beef liver at refrigerated temperature. Meat Research, 2: 2, Article 17. https://doi.org/10.55002/mr.2.2.17

Albrecht A, Hebel M, Mittler M, Hurck C, Kustwan K, Heitkönig B, Bitschinski D, Kreyenschmidt J 2019: Influence of different production systems on the quality and shelf life of poultry meat: a case study in the German sector. Journal of Food Quality37 18-57.

AMSA 2015 Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of fresh meat. Chicago III. American Meat Science Association at Nutritional Livestock and Meat Board.

AMSA. 1995. Research guidelines for cookery sensory evaluation, and Instrumental tenderness measurements of fresh meat. Animal Feed Science and Technology, 63: 211-228.

Baygar T, Alparslan Y, Cakli S 2013: Effect of multiple freezing and refrigerator thawing cycles on the quality changes of sea bass (DicentrarchusIabrax). Iran Journal fish Science12 289-300.

Boby F, Hossain MA, Hossain MM, Rahman MM, Azad MAK, Hashem MA. 2021. Effect of long coriander leaf (*Eryngium foetidum*) extract as a natural antioxidant on chicken meatballs during at freezing temperature. SAARC Journal of Agriculture, 19(2): 271-283.

Bing L, Sun DW 2002: Novel methods for rapid freezing and thawing of foods-a review. Journal of Food Engineering 54 175-182.
Chakrabartty S, Akter S, Murshed HM, Rahman MM, Azad MAK. 2024. Effects of ageing on longissimus thoracis et lumborum (LTL) muscle of local beef. Meat Research, 4: 1, Article No. 83.
Charoenren S. 2018: Microstructural changes and their relationship with quality and stability of frozen foods. In Food microstructure and its Relationship with Quality and Stability, pp. 123-138.
Clarence SY, Nwinyi O, Chinedu SN 2009. Assessment of bacteriological quality of ready to eat food (Meat pie) in Benin City metropolis, Nigeria. African Journal of Microbiology Research, 3(6): 390-395.
Daszkiewicz, Dorota K, Agata P 2018: The Effect of Long-Term Frozen Storage on the Quality of Meat (Longissimus thoracis et Lumborum) from Female Roe Deer (Capreolus capreolus L.). Journal of Food Quality 7 10-719.
Dona AS 2007: Textbook of Veterinary Histology. Saunders Elsvier, Westline industrial drive St, Louis Missouri. 358-366.
Hammad H, Ma M, Damaka AW, Elkhedir A, Jin G 2019: Effect of freeze and re-freeze on chemical composition of beef and poultry meat at storage period 4.5 months SP4. 5. Journal of Food Processing and Technology 10(5) 07-10.
Hammad HH, Ma M, Jin G, Jin Y, Khalifa I, Zeng Q, Liu Y 2017: Nitroso-hemoglobin increased the color stability and inhibited the pathogenic bacteria in a minced beef model: A combined low-field NMR study. Food Science of Animal Resources 39 (5) 704.
Irshad A, Sharma BD, 2015: Abattoir by-product Utilization for Sustainable Meat Industry: A Review Journal of Animal Production Advances 5(6) 681-696.
Islam MR, Hasan MM, Masum MMR, Khan M, Rahman MM. 2025. Effect of organic acid and natural antioxidant on the quality and shelf life of raw chicken meat at refrigerated storage. Meat Research 5 (1), Article No. 111. https://doi.org/10.55002/mr.5.1.111
ISO 1995: Recommendation of the meeting of the subcommittee, International Organization for Standardization, on meat and meat products. ISO/ TC-36/SC-6 10-18.
Karch H, Tarr P, Bielazewska M 2005: Enterohaemorrhagic Escherichia coli in human medicine. International Journal of Medical Microbiology, 295 (6-7): 405-418.
Kondraratowiez J, Chwastowska I, Matusevicius P. 2006. Sensory quality of pork and total microbial count depending on deep-freeze storage time and thawing method. Veterinarija ir zootechnika, 33(55): 43-46.
Konieczny P, Stangierski J, Kijowski J 2007: Physical and chemical characteristics and acceptability of home style beef jerky. Journal of Meat Science 76 253–257.
Leygonie C, Britz TJ, Hoffman LC. 2011. Oxidative stability of previously frozen ostrich Muscularis iliofibularis packaged under different modified atmospheric conditions. International Journal ofFood Science & Technology, 46(6): 1171-1178.
Leygonie C, Britz TJ, & Hoffman LC 2012: Impact of freezing and thawing on the quality of meat. Meat Science, 91(2): 93-98.
Liu Z, Xiong YL, Chen J 2010. Protein oxidation enhances hydration but suppresses water-holding capacity in porcine longissimus muscle. Journal of Agricultural and Food Chemistry, 58(19): 10697-10704.
Liza FA, Hosssan MI, Hashem MA, Rahman MM. 2024. Effect of cattle age on the physio-chemical properties of beef. Meat Research, 4: 4. Article No. 98. https://doi.org/10.55002/mr.4.4.98
Masum MMR, Mahmud S, Liza FA, Islam MR, Azad MAK, Rahman MM. 2025. Effect of natural and synthetic antioxidant on the quality of broiler meat during refrigeration. Meat Research 5 (1), Article No. 110. https://doi.org/10.55002/mr.5.1.110
Muchenje V, Dzama K, Chimonyo M, Strydom PE, Hugo A, Raats JG 2009: Some biochemical aspects pertaining to beef eating quality and consumer health: A review. Journal of Food Chemistry 112 279-289.
NUTTAB 2006. Food Standards Australia New Zealand. Online database of the nutritional composition of Australian foods. Canberra: FSANZ, 2007. Cited 25 April 2007.
Rahman MM, Hashem MA, Azad MAK, Choudhury MSH, Bhuiyan MKJ. 2023. Techniques of meat preservation-A review. Meat Research. 3 (3): Article No. 55.
Rahman MM 2000. Fundamentals of Meat Hygiene. Bismillah Farming and Frozen Meat Ltd. Dhaka, 76-101.
Rahman MH, Hossain MM, Rahman SM, Hashem MA, Oh DH 2014: Effect of repeated freeze-thaw cycles on beef quality and safety. Korean Journal for Food Science of Animal Resources 34(4) 482.
Roberts JS, Balaban MO, Zimmerman R, and Luzuriaga D 1998: Design and testing of prototype ohmic thawing unit. Computers and Electronics in Agriculture 19 211-222.
Sadakuzzaman M, Rahman MM, Hashem MA, Ali MS, Alam AMMN. 2024. Synergistic effects of gamma irradiation and butylated hydroxyanisole on the sensory, physicochemical, and microbiological quality of beef. Applied Food Research, 4 (2): 100547.
Schmedes A, Holmer S. 1989. A newthiobarbituric acid (TBA) method for determining free malondialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation. Journal of the American Chemist's Society, 66(6): 813-817.
Shohiduzjaman M, Biplob MAKA, Hashem MA, Rahman MM. 2024. Effects of natural and synthetic antioxidant on the quality of beef in short-term preservation. Meat Research, 4: 6. Article No. 107. https://doi.org/10.55002/mr.4.6.107
Serdaroğlu M, Yıldız-Turp G, Abrodímov K 2005. Quality of low-fat meatballs containing legume flours as extenders.Meat Science70 (1): 99-105.
Soyer A, Ozalp B, Dalm SU, Bilgin V 2010: Effects of Freezing temperature and duration of frozen storage on lipid and protein oxidation in chicken meat. Food Chemistry 120 1025-1030.
Srinivasan S, Xiong YL, Blanchard SP, Tidwell JH 1997: Physicochemical changes in pwarns (Machrobrachiumrosenbergii) subjected to multiple freeze-thaw cycles. Journal of Food Science62 123-127.
Stopforth JD, Lopes M, Shultz JE, Miksch RR, Sarnadpour M. 2006. Microbiological status of fresh goat cuts. Journal of Food Production, 69(6): 1456-1459.
Sultana, A, Nakanishi A, Roy B C, Mizunoya W, Tatsumi R, Ito T, Tabata S, Rashid H, Katayama S, Ikeuchi Y 2008: Quality Improvement of Frozen and Chilled Beef biceps femoris with the Application of Salt- bicarbonate Solution. Asian-Austral Journal of Animal Science 21 903-911.
Sultana, A, Nakanishi A, Roy B C, Mizunoya W, Tatsumi R, Ito T, Tabata S, Rashid H, Katayama S, Ikeuchi Y 2008: Quality Improvement of Frozen and Chilled Beef biceps femoris with the Application of Salt- bicarbonate Solution. Asian-Austral Journal of Animal Science 21 903-911.
Torun MMR, Khan MMH, Rahman MM, Sadakuzzaman M, Hashem MA. 2023. Influence of degree of doneness on the sensory, physiochemical, nutri-tional, and microbial properties of beef. Meat Research, Vol 3, Issue 5: 1-9, Article 69. https://doi.org/10.55002/mr.3.5.69
Turhan S, Ustun NS, Bank I 2006: Effect of Freeze-thaw cycles on total and haeme iron contents of bonito (Sardasarda) and bluefish (Pomatomussaltator) fillets. Journal Food Composition and Analysis19: 384-387.
Tushar ZH, Rahman MM, Hashem MA. 2023. Metmyoglobin reducing activity and meat color: A review. Meat Research, 3:5. Article No. 67.

USDA, (2007). US Department of Agriculture, USDA National Nutrient Database for Standard Reference. Release 19. Washington DC: Cited 25 April 2007.
Ulu, H. 2006. Effects of carrageenam and guar gum on the cooking and textural properties of low-fat meatballs. Food Chemistry 95 (4): 600-605.
Xia X, Kong B, Xiong Y, Ren Y 2010: Decreased Gelling And emulsifying properties of myofibrillar protein from repeatedly frozen-thawed porcine longissimus muscle are due to protein denaturation and susceptibility to aggregation. Meat Science85 481-486.
Yasmin T, Khatun H, Hashem MA, Rahman MM, Khan M. 2022. Assessment of quality and shelf life of goat liver stored at refrigerated temperature. Meat Research, 2: 3, Article 25. https://doi.org/10.55002/mr.2.3.25
Akter H, Hashem MA, Hassan MM and Khan M. 2023. Impact of Repeated Freezing and Thawing on the Quality of Goat Liver. Meat research. Vol 3, Issue 2 Article 51 ISSN: 2790-1971. https://doi.org/10.55002/mr.3.2.51.
Pizato, S.; Cortez-Vega, W.R.; Prentice, C. Quality assessment of cooked chicken breast meat at different storage temperatures. Int. Food Res. J. 2015, 22, 143–154.
Löndahl, G.; Nilaaon, T. Storage of Frozen Foods. In Encyclopaedia of Food Science and Nutrition, 2nd ed.; Academic Press: Oxford, UK, 1993; pp. 2732–2735.
Leygonie, C.; Britz, T.J.; Hoffman, L.C. Impact of freezing and thawing on the quality of meat: Review. Meat Sci. 2012, 91, 93–98.
Cheng S, Wang N, Zhang J, Brennan C, Guan W, Wang Z. The effects of electron beam application on the microbiological stability and physical–chemical quality of mince beef (M. longissimus Dorsi) during cold storage. J Food Process Preserv. 2018; 42:e13448. https://doi.org/10.1111/ jfpp.13448