Meat Research
Vol 5, Issue 3: 1-8, Article 120, June 2025
ISSN: 2790-1971
https://doi.org/10.55002/mr.5.3.120 http://www.bmsa.info/meatresearch
[1]Department of Animal Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

***Corresponding Author:**

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

Keywords:
Liver
Proximate analysis
Microbiological analysis
Biochemical analysis
Frozen duration

Article Info:
Received: May 11, 2025
Accepted: June 17, 2025
Published online: June 30, 2025

Research Article

Effect of long-term frozen duration on the quality of cow liver

FA Sumy[1], HM Murshed[1], PR Sristi[1], NR Das[1], MT Ahmed[1], MA Hashem[1], M Khan[1]*

Abstract

The experiment was conducted to find out the effect of frozen duration (-18℃) on the quality of raw cow liver. For this purpose, raw liver samples were divided into three treatment groups. They were treated as 0 days, 180 days and 240 days as T_1, T_2 and T_3 respectively. Sensory parameters (color, odor, juiciness and tenderness), proximate analysis (DM, CP, EE, and Ash), pH value, cooking loss, water holding capacity, TBARS value and microbiological examination were determined in order to evaluate the quality of raw liver at frozen temperature (-18^{0}C). Color, odor, juiciness and tenderness were decreased among different treatments significantly ($p<0.05$). Dry matter content of all the treatments increased significantly ($p<0.05$) while the CP and EE content decreased significantly ($p<0.05$) with the increase of storage days. As well as with the advancement of days, the ash content of all the treatments was increased significantly ($p<0.05$). The cooking loss (%) at different treatments were increased significantly ($p<0.05$). The water holding capacity and pH of all the treatments decreased significantly ($p<0.05$) with the increase of storage days. Significantly higher value (0.479 mg- MDA/kg) ($p<0.05$) of thiobarbituric acid value was observed at 240days. TVC (log CFU/g), TCC (log CFU/g) and TYMC (log CFU/g) were increased significantly ($p<0.05$) among different treatments comparison to 0 days. In conclusion, from this study on sensory evaluation, nutritional content, physicochemical properties, biochemical analysis and microbial assessments, it has been found that up to 240 days is acceptable in terms of biochemical and microbial studies although nutritional values were slightly decreased with the increase of storage time.

Introduction

Edible by-products or offals, such as liver, heart, tongue, kidneys, blood, skin, and bones are significant sources of protein, vitamins, and minerals. Based on their nutritional content, offals are classified into two categories. First-grade offals include liver, tongue, brain, kidneys, meat from bones, heart, skirt meat, tail, and udder, while second-grade offals comprise rumen, pig stomach, larynx, weasand meat, pig tail, legs, ears, rennet stomach, lungs, trachea, spleen, and testes (Kawsar et al., 2006; Kakimov et al., 2016). The average offal yield by live weight is approximately 22% in cattle, 17% in pigs, and 20% in sheep and goats (Kakimov et al., 2017). The nutritional value of first-grade offals is comparable to that of standard meat, and in many cases, they provide higher levels of certain vitamins and minerals (Hossain et al., 2022). Among these, Cow liver, a nutrient-rich organ meat, is particularly valued for its high content of essential micronutrients such as vitamin A, vitamin B12, iron, and folate (Hossain et al., 2023a and 2023b; Latoch et al, 2024). Due to its dense nutritional profile, beef liver is often recommended for vulnerable populations such as pregnant women, growing children, and individuals with nutrient deficiencies. Furthermore, in low- and middle-income countries, organ meats like liver play a critical role in improving dietary diversity and reducing nutritional insecurity (Bailey et al., 2015). Liver is also considered a high-risk food because of its rich nutrient content, which makes it an ideal medium for microbial growth. Contamination often arises from unhygienic handling practices and unsanitary equipment such as cutting boards and processing tools, rather than from the tissue itself (Khatun et al., 2022; Islam et al., 2025; Masum et al., 2025). Microbial spoilage is the primary concern for liver quality, with the spoilage flora being influenced by storage conditions (Azad et al., 2022; Rahman et al., 2023; Torun et al., 2023). Inadequate handling, poor hygiene, and improper temperature control further exacerbate microbial contamination. Deterioration of quality in food manifests itself most conspicuously through changes in appearance, odor and color. pH is a good indicator to estimate the spoilage status of cow livers (Tushar et al., 2023).

Freezing is a widely adopted method for preserving the nutritional and microbiological quality of meat and offal products, including liver, typically involving storage at temperatures between -18°C and -23°C. However, despite the protective effects of freezing, prolonged storage at sub-zero temperatures may lead to deterioration in both

biochemical and sensory quality due to oxidative and enzymatic changes occurring at the molecular level (Leygonie et al., 2012).

During frozen storage, quality degradation in liver tissues may manifest through protein denaturation, lipid peroxidation, and discoloration, which collectively affect the organoleptic attributes and consumer acceptability of the product (Nooraldeen, 2025). Moreover, freeze-induced tissue damage can alter microstructure, resulting in moisture loss upon thawing and contributing to texture degradation (Du et al., 2022). Nowadays, there are some supermarkets are available in Bangladesh and they are selling different meat and meat by-products which is kept at frozen temperature to retard the growth of microorganisms. But in Bangladesh aspects there is lack of information regarding the quality of cow liver stored at frozen temperature. In this situation, this study aims to investigate the impact of extended frozen storage duration on the quality of cow liver by assessing its physicochemical, biochemical, and microbiological characteristics over time. The findings will contribute to improved post-harvest handling, storage guidelines, and shelf-life prediction for cow liver in commercial and household settings.

Materials and Methods

Study Location

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

Sample Collection

Fresh liver samples were collected at 06:00 AM from three local markets in Mymensingh: KR Market, Ganginapar Notun Bazar, and Kewatkhali. Six fresh whole livers from clinically healthy cows (aged 3 to 3.5 years) were obtained, placed in sterilized polyethylene bags within insulated containers with ice, and immediately transported to the Animal Science lab for analysis.

Instrument Sterilization

All laboratory glassware, jars, containers, and instruments were washed in hot water with detergent, rinsed, and air-dried prior to use.

Sample Preparation and Storage

Upon arrival, each liver was individually bagged, frozen to –18 °C in electronically controlled freezers, and stored for 180 and 240 days, respectively (Samples 1–6). Before analysis, samples were thawed at ambient temperature (~22 °C) for 3 hours.

Experimental Design

Analyses included sensory, proximate, physicochemical, biochemical, and microbiological evaluations, which were conducted on Day 0, Day 180, and Day 240 of frozen storage, as presented in Table 1.

Table 1: Experimental layout for quality evaluation of frozen liver

Category	Details
Treatments	T_1: Fresh liver at 0 day T_2: Stored at –18 °C for 180 days T_3: Stored at –18°C for 240 days
Number of Treatments	3
Replications	3 per treatment
Total Experimental Units	9 (3 treatments × 3 replications)
Storage Condition	–18 °C

Sensory Evaluation

Sensory attributes including color, odor, juiciness, and tenderness were assessed by a panel of six trained evaluators. The sensory evaluation was conducted using a 5-point semantic scale (1 = poor, 2 = fair, 3 = good, 4 = very good, 5 = excellent), as described by Hashem et al. (2022) and Ahmad et al. (2013). The panelists, comprising faculty members and students of the department, were trained in accordance with the American Meat Science Association (AMSA) guidelines. The evaluations were carried out in individual booths under controlled light, temperature, and humidity conditions. All liver samples were served on Petri dishes. Sensory evaluations were performed on day 0, day 180, and day 240 of storage.

Proximate Composition

The proximate composition dry matter (DM), crude protein (CP), ether extract (EE), and ash were determined in triplicate following the standard methods outlined by the Association of Official Analytical Chemists (AOAC, 2005). The results were reported as mean values.

Physicochemical Analysis

pH Measurement

The pH of liver samples was measured using a HANNA meat pH meter. A homogenate was prepared by blending 5 g of liver tissue with 10 mL of distilled water, and the pH was measured following the instrument's operating manual.

Cooking Loss

Cooking loss was determined by weighing the liver samples before and after boiling in a water bath at 100°C. After cooking, the samples were allowed to cool and excess surface moisture was removed using foil paper.

The cooking loss (%) was calculated using the following formula:

Cooking loss (%) = [(Initial wt. – Final wt.) / Initial wt.] × 100

Water Holding Capacity (WHC)

WHC was evaluated using the Grau and Hamm method (Norrung and Buncic, 2008), including thawing drip loss, natural drip loss, cooking loss, and forced drip loss. The ability to retain added water was assessed using the centrifugal method as described by Arihara (2006).

WHC was calculated using the following formula:

Water holding capacity (%) = (Weight after centrifugation / Weight before centrifugation) ×100

Biochemical Analysis

Thiobarbituric Acid Reactive Substances (TBARS)

Lipid oxidation was determined using the 2-thiobarbituric acid (TBA) assay according to Malvestiti et al. (2007). Briefly, 5 g of liver sample was homogenized with 25 mL of 20% trichloroacetic acid solution in a blender for 30 seconds. The homogenate was filtered through Whatman No. 4 filter paper. Two milliliters of the filtrate was mixed with 2 mL of 0.02 M aqueous TBA solution and incubated in a water bath at 100°C for 30 minutes, then cooled under tap water. Absorbance was measured at 532 nm using a UV-VIS spectrophotometer (UV-1200, Shimadzu, Japan). TBARS values were expressed as mg malondialdehyde (MDA) per kg of liver sample.

Microbial Assessment

Total viable count (TVC), total coliform count (TCC), and total yeast mold count (TYMC) were performed for microbiological assessment. For the most cost-effective use of chemicals, these studies were carried out on different days.

Sample Preparation

A quantity of 10 g of liver sample was aseptically excised from stored stock sample. Each of the stored 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). 10g of the minced 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 samples was obtained. Later on using whirly mixture machine different serial dilutions ranging from 10-2 to 10-6 were prepared according to the instruction of the standard method (ISO, 1995).

Media and reagent employed for TVC, TCC and TYMC

The media employed for these bacteriological analyses 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.

Glassware and other appliances

Different types of glassware and appliances were used during the course of the experiment. These were as follows: Test tubes (with or without Durham‟s fermentation tube and stopper), petri dishes, conical flask, pipette (1 ml, 5 ml, 10 ml and 25 ml capacities), glass rod spreader, test tube stand, mortar and pestle, whirly mixture machine, blender machine, water bath, incubator, refrigerator, sterilizing instruments, hot air oven, ice boxes, electronic balance, electronic pH meter etc.

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 respectively 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 15g 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°C (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°C.

Enumeration of total viable count

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. One sterile spreader was used for each plate. The plates were then kept in an incubator at 35°C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted. Colonies were counted with the aid of a colony counter. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the total viable count. The total viable count was calculated according to ISO (1995). The results of the total bacterial count were expressed as the number of organisms of colony forming units per gram (CFU/g) of cow liver samples. Enumeration of total coliform count (TCC) for the determination of total coliform counts, 0.1 ml of each ten-fold dilution was transferred and spread on triplicate MA 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. One sterile spreader was used for each plate. The plates were then kept in an incubator at 35°C for 24-48 hours. Following incubation, plates exhibiting 30- 300 colonies were counted. Colonies were counted with the aid of a colony counter. The average number of colonies in a particular dilution was multiple by the dilution factor to obtain the total coliform count. The total coliform count was calculated according to ISO (1995). The results of the total coliform count were expressed as the number of organisms of colony forming units per gram (CFU/g) of cow liver samples.

Enumeration of total coliform count

For the determination of total coliform counts, 0.1 ml of each ten-fold dilution was transferred and spread on triplicate MA 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. One sterile spreader was used for each plate. The plates were then kept in an incubator at 35°C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted. Colonies were counted with the aid of a colony

counter. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the total coliform count. The total coliform count was calculated according to ISO (1995). The results of the total coliform count were expressed as the number of organisms of colony forming units per gram (CFU/g) of liver samples.

Enumeration of Yeast-Mold count

For the determination of yeast and mold counts, 0.1 ml of each ten-fold dilution was transferred and spread on triplicate PDA 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. One sterile spreader was used for each plate. The plates were then kept in an incubator at 25°C for 48-72 hours. Following incubation, plates exhibiting 30-300 colonies were counted. Colonies were counted with the aid of a colony counter. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the yeast and mold count. The yeast and mold count were calculated according to ISO (1995). The results of the yeast and mold count were expressed as the number of organisms of colony forming units per gram (CFU/g) of cow liver samples.

Statistical Analysis

All data were analyzed using the MSTATC software package following one-way analysis of variance (ANOVA) under a Completely Randomized Design (CRD). Significant differences among means were considered at $p < 0.05$. Results are presented as mean ± standard deviation (SD).

Result and Discussion

Sensory Evaluation

The parameters for sensory quality (color, odor, juiciness, and tenderness) have been shown in Table 2. The range of overall observed color score at different treatments was 5 to 3.1. Color scores were significantly decreased ($p<0.05$) with the increase of storage duration. A similar result was reported by Fernandez-Lopez et al. (2006). Changes in color of the muscle and blood pigments determine the attractiveness of fresh red meat, which in turn influences the consumers' acceptance of meat products (Tushar et al., 2023; Hossan et al., 2024). Georgantelis et al. (2007) presented that the oxidative browning of the meat product still occurred during frozen storage. Most preferable color was observed from 0 day among three treatments group and less preferable color was observed from 240 days.

The range of odor score among three treatments was 5 to 3.21. Odor scores were significantly decreased ($p<0.05$) with the increase of storage life. The most preferable odor was observed from 0 days. The lower odor scores may be related to the increased malonaldehyde formation due to oxidation of fat, which has detrimental effect on the flavor and firmness of the product (Malvestiti et al., 2007). Deterioration of odor during storage might be due to microbial growth, formation of FFA and oxidative rancidity.

Table 2: Values of sensory parameters in fresh and frozen liver

Parameters	Treatments			Level of significance
	T_1	T_2	T_3	
Color	5.00^{a}±0.01	4.16^{b}±0.10	3.10^{c}±0.11	p<0.05
Flavor	5.00^{a}±0.02	4.11^{b}±0.15	3.21^{c}±0.01	P<0.05
Juiciness	5.00^{a}±0.01	4.55^{b}±0.41	3.01^{c}±0.06	P<0.05
Tenderness	5.00^{a}±0.02	4.67^{b}±0.52	3.11^{c}±0.25	P<0.05

Here, T_1= Day 0, T_2= Day 180, T_3= Day 240. Means in each row having different letter vary significant at 5% level, $p<0.05$. Values are presented as mean±SD.

The range of overall observed juiciness score at different treatments was 5 to 3.01. Juiciness scores were significantly decreased ($p<0.05$) with the increase of storage life. Among these three treatments most preferable juiciness score was observed from 0 days and less preferable juiciness score was observed from 240 days. That's why cow liver leak juices when they were stored. The result of this experiment is also similar to Leygonie et al. (2011) findings. Thomas et al. (2006) and Sharker et al. (2024) 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.

Tenderness scores were significantly decreased ($p<0.05$) with the increase of storage life. Among these three treatments most preferable tenderness score was observed from 0 days and less preferable tenderness score was observed from 240 days. Tenderness is interrelated DM content of the liver (Yasmin et al., 2022). With the increasing of storage period DM was increased consequently tenderness was decreased with day's intervals (Yasmin et al., 2022). The result of this experiment is also related to Lui et al. (2010) findings. Several researchers have associated tenderness of meat with the breakdown of myofibrillar proteins affected by the presence of calcium-dependent proteases or calpains (Syuhairah et al., 2016).

Proximate analysis

There were three groups of treatments for the determination of proximate components. In 0 day, DM, CP, EE, and ash were determined and then rest samples were stored at -18º C and analyzed. The values of proximate components are shown in Table 3. The range of overall observed DM content at different treatments was 28.00% to 31.15%. DM content was significantly ($p<0.05$) increased with increase of storage days among these observations. The same trend was also observed by Garrett and Hinman, (1971) and Konieczny et al. (2007). They reported that DM content increased during frozen storage. The highest amount of DM content indicates the product is less acceptable.

The range of overall observed CP content at different treatments was 23.81% to 18.87%. The observation from different treatment indicates there was significant ($p<0.05$) differences of CP content. The CP content was decreased with the increased storage days. The most preferable CP content was observed from 0 days. The same trend was also observed by (Garrett and Hinman,1971) and they reported that CP content decreased during frozen storage. The highest amount of CP content indicates this product is most acceptable for consumer's health observed from 0 day and less acceptable CP content was observed on third treatment. The same trend was also observed by Konieczny et al. (2007) and Kakimov et al. (2017) they reported that CP content

decreased during frozen storage.

Table 3: Value of proximate components in fresh and frozen liver

Parameters	Treatments			Level of significance
	T_1	T_2	T_3	
DM%	28.00^{c}±0.73	30.71^{b}±0.78	31.15^{a}±0.85	P<0.05
CP%	23.81^{a}±0.37	20.39^{b}±0.52	18.87^{c}±0.42	P<0.05
EE%	4.04^{a}±0.11	3.65^{b}±0.32	3.16^{c}±0.18	P<0.05
Ash%	1.15^{a}±0.04	1.72^{b}±0.12	1.76^{b}±0.08	P<0.05

Here, T_1= Day 0, T_2= Day 180, T_3= Day 240. Means in each row having different letter vary significant at 5% level, p<0.05. Values are presented as mean±SD.

The observation of EE at different treatments was 4.04% to 3.16%. EE content was significantly (p<0.05) decreased among these observations. Among these three treatments most preferable EE content was observed from 0 days. The lowest amount of EE content indicates this product is less preferable for consumers health. Less preferable EE content was observed from 240 days. 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 Konieczny et al. (2007) and they reported that ether extract content decreased with the increase of storage days.

The range of overall observed ash content at different treatments was 1.15% to 1.76%. Ash content was significantly (p<0.05) increased among these observations. A non-significant decrease in ash percentage was reported by Ziauddin et al. (1993) which coincided with this study. The same trend was also observed by Konieczny et al. (2007) and they reported that ash content decreased with the increase of storage days.

Physicochemical properties

The physicochemical properties such as pH, Water holding capacity and cooking loss were determined and the results obtained are shown in Table 4. The pH values of raw cow liver samples varied across different treatments and storage periods, ranging from 6.33 to 4.83 (Table 4). A significant (p<0.05) decrease in pH was observed with the progression of storage time up to 240 days. Among the three treatment groups, the samples analyzed at 0 days exhibited the highest pH values, indicating the least amount of acidity and the most stable condition. As storage time increased, the pH values gradually decreased across all treatments, reflecting chemical and microbial changes occurring during frozen storage. The pH declined slightly during storage due to increased free fatty acids from rancidity and microbial activity. Acidity rose over time, consistent with earlier findings by Ali et al. (2016) in meat. The higher initial pH is considered more preferable for consumer health compared to stored samples.

Table 4: Values of physicochemical properties in fresh and frozen temperature of liver

Parameters	Treatments			Level of significance
	T_1	T_2	T_3	
pH	6.33^{a}±0.09	5.37^{b}±0.38	4.83^{c}±0.09	P<0.05
Cooking Loss (%)	15.63^{c}±0.34	26.91^{b}±0.53	27.73^{a}±0.45	P<0.05
Water holding Capacity (%)	89.77^{a}±.50	83.86^{b}±0.93	80.97^{c}±.76	p<0.05

Here, T_1= Day 0, T_2= Day 180, T_3= Day 240. Means in each row having different letter vary significant at 5% level, p<0.05. Values are presented as mean±SD.

Cooking loss ranged from 15.63% to 27.73% across treatments and significantly (p<0.05) increased with storage time (Table 4). The lowest cooking loss was observed at 0 days, while the highest was at 240 days. Cooking loss increased due to protein denaturation and moisture/fat loss during cooking. Rapid freezing resulted in lower loss due to smaller ice crystals. Greater nutrient loss with longer storage reduces meat quality (Kovaleva et al., 2014). Lower cooking loss at 0 days indicates better product quality and higher consumer preference compared to extended storage periods.

Table 5: Biochemical properties of liver at fresh and frozen temperature

Parameters	Treatments			Level of significance
	T_1	T_2	T_3	
TBARS (mg- MDA/kg)	0.272^{c}±0.05	0.363^{b}±0.04	0.479^{a}±0.03	P<0.05

Here, T_1= Day 0, T_2= Day 180, T_3= Day 240. Means in each row having different letter vary significant at 5% level, p<0.05. Values are presented as mean±SD.

The range of overall observed water holding capacity at different treatments was 89.77% to 80.97% (Table 4). Water holding capacity was significantly (p<0.05) decreased with increase of storage days. Among these treatments most preferable water holding capacity was observed at 0 days. The lowest amount of cooking loss indicates this product is most preferable for consumers' choices than other treatment groups. The less preferable water holding capacity was observed 240 days and most preferable water holding capacity was observed 0 days. The same trend was observed by Rabbi et al., (2024).

Biochemical properties

Biochemical properties indicate the quality of liver. The value of biochemical components TBARS that was examined was shown in Table 5. The range of overall observed thiobarbituric acid value at different treatments was 0.272 to 0.479. Among these three treatments, the lower amount of TBARS value was observed at 0 days group and highest amount observed at 240 days. The TBARS values increased significantly (p<0.05) with increase the storage time, similar results were reported for meat and meat products during frozen storage by (Rukunudin et al, 1998). The lowest TBARS value results of the products indicate that the product is beneficial for human health. Similar results were reported for meat and meat products during frozen storage (Ganhao et al., 2011). Also, similar phenomenon was observed in chicken meat by Hasan et al. (2016), Sagar et al. (2024) and in goat liver by Sharker et al. (2024).

Microbiological assessment

This study observed the presence of micro-flora (TVC), TCC and TYMC on fresh and preserved samples were presented on Table 6.

According to the results the initial value of TVC for fresh liver was significantly lower compared to stored samples and the value was 3.98 log CFU/g liver (Table 6), indicating good quality liver which is most preferable for consumers' health. 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. Some bacteria may be present in the sample, but their growth is controlled under storage conditions (Koutsoumanis et al., 2008). 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. Some bacteria may be present in the sample, but their growth is controlled under thawing conditions (Kondraratowiez et al., 2006). The observation from different treatments indicates there were significant differences ($p<0.05$) of TVC values. The plate count in the control sample. (3.98 logCFU/g) was significantly lower than the other treatments. The lower amount of TVC value indicates this product is most preferable for consumer's health 0 days. The range of overall observed of different treatments of TVC value was 3.98 logCFU/g to 5.01 logCFU/g. The amount of TVC was increased with the storage periods.

Table 6: Microbiological assessment of liver among different treatments at fresh and frozen temperature

Parameters	Treatments			Level of significance
	T_1	T_2	T_3	
TVC (log CFU/g)	3.98^{b}±0.09	4.77^{a}±0.31	5.01^{a}±0.49	$p<0.05$
TCC (log CFU/g)	1.97^{c}±0.09	2.46^{a}±0.03	2.97^{a}±0.02	$p<0.05$
TYMC (log CFU/g)	1.07^{c}±0.06	1.97^{b}±0.03	2.34^{a}±0.01	$p<0.05$

Here, T_1= Day 0, T_2= Day 180, T_3= Day 240. Means in each row having different letter vary significant at 5% level, $p<0.05$. Values are presented as mean±SD.

The range of overall observed total coliform count from the liver was 1.97 to 2.97 (logCFU/g) at different treatment levels (Table 6). The observation from different treatments indicates there were significant differences ($p<0.05$) of TCC values. Among these treatments, the total coliform count in the fresh sample (1.97 logCFU/g) was significantly lower than other treatments. Most preferable TCC content was observed at 0 days group. TCC value was increased due to increasing storage period deteriorating of fat and helped prevent the metabolism of fat by bacteria. As a result, bacterial growth was lower in cow liver at 0 day. The antioxidant compounds blocked the deteriorating of fat and helped prevent the metabolism of fat by bacteria. The TCC was increased with the increase of storage period. As a result, bacterial growth was lower in cow liver at 0 day. Aziz et al. (2020) also, similar result was observed in broiler chicken meat. TCC value was increased due to temperature fluctuation as well as deteriorating of fat and helped prevent the metabolism of fat by bacteria (Clarence et al., 2009).

The range of overall observed TYMC from the liver was 1.07 to 2.34 (logCFU/g), at different treatment levels (Table 6). The observation indicates that there were significant differences ($p<0.05$) of TYMC values among these treatment groups. Among these treatments, TYMC in the fresh sample (1.07 log CFU/g) was significantly lower than others and highest TYMC was 240 days. The less amount of TYMC value indicates this product is most preferable for consumers" health. During increasing storage period TYMC value was increased. Aziz et al. (2020) Also, Similar result was observed in broiler chicken meat. Low amount of TYMC value indicates that this method is most preferable for thawing. When increased thawing period, fluctuate temperature and humidity, TYM value would be increased (Stopforth et al., 2006).

Similarly to our findings, a study in beef also stated that the mean value of TVC, TCC and TYMC for fresh sample is lower than preserved samples (Haider, 2018).

Conclusion

Based on the results of sensory evaluation, nutritional analysis, physicochemical measurements, biochemical assessments, and microbial evaluations including total viable count (TVC), total coliform count (TCC), and total yeast and mold count (TYMC), it was concluded that cow liver stored at −18°C remains microbiologically and biochemically acceptable for up to 240 days. Although a slight decline in nutritional content was observed over time, the product remained within safe consumption limits throughout the storage period. These findings suggest that frozen cow liver can be safely stored at −18°C for up to 240 days without compromising overall quality and safety.

Reference

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.

Adesogan AT, Havelaar AH, McKune SL, Eilittä M, Dahl GE. 2020. Animal source foods: Sustainability problem or malnutrition and sustainability solution? Perspective matters. Global Food Security, 25:100325.

Agnihotri MK. 1988. A comparative study on shelf life and microbial spoilage of refrigerated buffalo meat. PhD Thesis, IVRI, Izatnagar, India.

Ahmad MUD, Sarwar A, Najeeb MI, Nawaz M, Anjum AA, Ali MA, Ansur NM. 2013. Assessment of microbial load of raw meat at abattoir and retail. The Journal of Animal and Plant Sciences, 23(3): 745–748.

AOAC. 2005. Official Methods of Analysis (20th Ed). Association of Official Analytical Chemists, Washington, D.C., USA.

Arihara K. 2006. Strategies for designing novel functional meat products. Meat Science, 74(1): 219–229.

Azad MAK, Rahman MM, Hashem MA. 2022. Meat microbiota: A conceptual review. Meat Research, 2(2), Article No. 20.

Aziz MF, Hayat MN, Kaka U, Kamarulzaman NH, Sazili AQ. 2020. Physico-chemical characteristics and microbiological quality of broiler chicken pectoralis major muscle subjected to different storage temperature and duration. Foods, 9(6): 741.

Bailey RL, West KP Jr, Black RE. 2015. The epidemiology of global micronutrient deficiencies. Animal Nutrient Metabolism, 66(Suppl 2):22–33.

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.

Du X, Wang B, Li H, Liu H, Shi S, Feng J, Pan N, Xia X. (2022). Research progress on quality deterioration mechanism and control technology of frozen muscle foods. Comprehensive Reviews in Food Science and Food Safety, 21(1), 604–632.

Fernandez-Lopez J, Yelo A, Sayas-Barbera E, Sendra E, Navarro C, Perez-Alvarez JA. 2006. Shelf life of ostrich (Struthio camelus) liver stored under different packaging conditions. Journal of Food Protection, 69(8): 1920–1927.

Ganhao R, Estevez M, Morcuende D. 2011. Suitability of the TBA method for assessing lipid oxidation in a meat system with added phenolic-rich materials. Food Chemistry, 126: 64–72.
Garrett W, Hinman N. 1971. Fat content of trimmed beef muscles as influenced by quality grade, yield grade, marbling score and sex. Journal of Animal Science, 33(5): 948–957.
Georgantelis D, Ambrosiadis I, Katikou P, Blekas G, Georgakis SA. 2007. Effect of rosemary extract, chitosan and α-tocopherol on microbiological parameters and lipid oxidation of fresh pork sausages stored at 4°C. Meat Science, 76(1): 172–181.
Haider MR. 2018. Assessment of the quality of raw and cooked beef through laboratory analysis. MS Thesis, Department of Animal Science, Bangladesh Agricultural University, Mymensingh, Bangladesh.
Hasan MM, Shohiduzjaman M, Hashem MA, Khan M, Rahman MM. 2024. Effects of edible oil on the quality of chicken meat in short-term preservation. Meat Research. 4 (4): Article No. 99. https://doi.org/10.55002/mr.4.4.99
Hashem M, Islam M, Hossain M, Alam A, Khan M. 2022. Prediction of chevon quality through near infrared spectroscopy and multivariate analyses. Meat Research, 2(6): 1–7. https://doi.org/10.55002/mr.2.6.37
Hossain MA, Rahman MM, Hashem MA. 2023a. Carcass and meat quality attributes of native sheep in Bangladesh: A review. Meat Research, 3: 2, Article 49.
Hossain MA, Rahman MM, Rahman MW, Hossain MM, Hashem MA. 2023b. Effect of supplementary feeding on the production traits, carcass and meat quality of Jamuna basin lambs. Journal of Animal Science and Technology. 65 (1): 209-224.
Hossain MA, Numan AA, Haque ME, Haque MR, Rahman MM, Hashem MA. 2022. Effect of castration on carcass and meat quality attributes in native lambs of Bangladesh. Meat Research, 2: 6, Article 38.
Hossan MI, Biplob MAKA, Khan MRI, Rahman MM. 2024. Effect of oats on sensory and physico-chemical properties of chicken meatball. Meat Research, 4: 5. Article No. 104. https://doi.org/10.55002/mr.4.6.104
Islam MR, Hasan, 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. International Organization for Standardization Protocol. Geneva, Switzerland.
Kakimov A, Suychinov A, Mayorov A, Yessimbekov Z, Okuskhanova E, Kuderinova N, Bakiyeva A 2017: Meat- bone paste as an ingredient for meat batter, effect on physicochemical properties and amino acid composition. Pakistan Journal of Nutrition 16: 797-804.
Kakimov AK, Yessimbekov Z, Kabulov B, Bepeyeva A, Kuderinova N, Ibragimov N 2016: Studying chemical composition and yield stress of micronized grinded cattle bone paste. Research Journal of Pharmaceutical, Biological and Chemical Sciences **7(2):** 805-812.
Kawsar SM, Rahman MM, Rahman SME, Hossain MM, Huq MA. 2006. Growth, carcass and non-carcass traits of Black Bengal goats due to urea molasses block supplementation. International Journal of Bio-Resources. 2 (1): 1-5.
Khatun MM, Hossain MA, Ali MS, Rahman MM, Azad MAK, Hashem MA. 2022. Formulation of value-added chicken nuggets using carrot and ginger as a source of dietary fiber and natural antioxidant. SAARC J. Agric., 20 (1): 185-196.
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. Meat Science, 76: 253–257.
Koutsoumanis KP, Stamatiou AP, Drosinos EH, Nychas G-JE. 2008. Control of spoilage microorganisms in minced pork by a self-developed modified atmosphere induced by the respiratory activity of meat microflora. Food Microbiology, 25: 915–921.
Latoch A, Stasiak DM, Siczek P. 2024. Edible offal as a valuable source of nutrients in the diet—A review. Nutrients, 16(11):1609.
Leygonie C, Britz TJ, Hoffman LC. 2011. Protein and lipid oxidative stability of fresh ostrich M. iliofibularis packaged under different modified atmospheric packaging conditions. Food Chemistry, 127(4): 1659–1667.
Leygonie C, Britz TJ, Hoffman LC. 2012. Impact of freezing and thawing on the quality of meat: Review. Meat Science, 91(2):93–98.
Lui ZY, 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: 10697–10704.
Malvestiti L, Giannuzzi L, Ferrero C. 2007. Mixtures of beef tripe, beef liver and soybeans applied to food development. Journal of Food Processing and Preservation, 31(3): 270–285.
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
Nooraldeen FA. 2025. Effect of cold storage on lipid and protein oxidation, microbial spoilage, and physicochemical properties of chicken meat. Kirkuk University Journal for Agricultural Sciences, 16(2):1–10.
Norrung B, Buncic S. 2008. Microbial safety of meat in the European Union. Meat Science, 78: 14–24.
Rabbi MF, Kibria SE, Rahman MM, Hashem MA, Khan M. 2024. Effect of frozen duration on the quality of cow liver. Meat Research, 4(5): Article 103. https://doi.org/10.55002/mr.4.5.103
Rahman MM, Hashem MA, Azad MAK, Choudhury MSH, Bhuiyan MKJ. 2023. Techniques of meat preservation-A review. Meat Research, 3 (3): Article No. 55
Rukunudin IH, White PJ, Bern CJ, Bailey TB. 1998. A modified method for determining free fatty acids from small soybean oil sample sizes. Journal of the American Oil Chemists' Society, 75(5): 523–525.
Sharker BC, Mia N, Ali MH, Rahman MM, Hashem MA, Khan M. 2024. Effect of freezing period on the quality of doe liver. Meat Research. Vol 4, Issue 2: 1-8, Article 90, ISSN: 2790-1971. https://doi.org/10.55002/mr.4.2.90.

Stopforth JD, Lopes M, Shultz JE, Miksch RR, Sarnadpour M. 2006. Microbiological status of fresh beef cuts. Journal of Food Protection, 69(6): 1456–1459.
Syuhairah A, Huda N, Syahariza ZA, Fazilah A. 2016. Effects of vegetable incorporation on physical and sensory characteristics of sausages. Asian Journal of Poultry Science, 10(1): 117–125.
Thomas R, Anjaneyulu A, Kondaiah N. 2006. Quality and shelf-life evaluation of emulsion and restructured buffalo meat nuggets at cold storage (4 ± 1°C). Meat Science, 72: 373–379.
Torun MMR, Khan MMH, Rahman MM, Sadakuzzaman M, Hashem MA. 2023. Influence of degree of doneness on the sensory, physiochemical, nutritional, and microbial properties of beef. Meat Research, 3(5): 1-9.
Tushar ZH, Rahman MM, Hashem MA. 2023. Metmyoglobin reducing activity and meat color: A review. Meat Research, 3:5. Article No. 67.
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
Ziauddin KS, Mahendrakar NS, Rao DN, Amla BL. 1993. Effect of freezing, thawing and frozen storage on microbial profiles of buffalo meat. Journal of Food Science and Technology, 35(3): 331–340.