Meat Research
Vol 4, Issue 5: 1-7, Article 103, August 2024
ISSN: 2790-1971
https://doi.org/10.55002/mr.4.5.103
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
Frozen duration
Physicochemical properties
Microbial studies
Shelf life

Article Info:

Received: July 10, 2024
Accepted: August 17, 2024
Published online: October 31, 2024

Research Article

Effect of frozen duration on the quality of cow liver

MF Rabbi[1], SE Kibria[1], MM Rahman[1], MA Hashem[1], M Khan[1*]

Abstract

The experiment was conducted to find out the effect of frozen duration (-20°C) on the quality of raw cow liver. For this purpose, raw cow liver samples were divided into three treatment groups. They were treated as 0 days, 60 days, and 120 days as T1, T2, and T3 respectively. Sensory parameters (color, odor, juiciness and tenderness), proximate analysis (DM, CP, EE Ash), pH value, cooking loss, water holding capacity, TBARS value and microbiological examination were determined in order to evaluate the quality of raw cow liver at different frozen duration. Color, odor, juiciness, and tenderness were decreased among different frozen duration significantly ($p<0.05$). Dry matter content increased ($p<0.05$) with the increase of storage days. CP and EE content decreased ($p<0.05$) with the increase of storage days. As well as with the advancement of days, ash content were increased significantly ($p<0.05$). pH values were decreased with the increase of storage days. The cooking loss (%) were increased ($p<0.05$) with the increase of storage days. Water holding capacity were decreased ($p<0.05$) with the increase of storage days. Thiobarbituric acid values were increased ($p<0.05$) with the increase of storage days. TVC (log CFU/g), TCC (log CFU/g), and TYMC (log CFU/g) were increased ($p<0.05$) with the increase of storage days 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 120 days is safe for human consumption.

Introduction

Meat and meat products are a major source of complete protein, containing all essential amino acids in sufficient amounts for human consumption (Akhter et al., 2009 and 2022; Akter et al., 2009; Azad et al., 2021; Kawsar et al., 2006; Lawrie, 2006; Tushar et al., 2023). Long-term abstinence from meat and meat products and other sources of animal protein in the human diet can lead to protein deficiencies in human body, which is accompanied by dysfunction of blood formation, fat metabolism, and vulnerability to infectious and cold-related diseases (Arihara, 2006; Chakrabartty et al., 2024; Hashem et al., 2021, 2022 and 2024). Offal such as liver, heart, tongue, kidneys, blood, skin, bone etc. represent an essential source of protein, vitamin and mineral elements. In terms of nutritional value, offal is divided in two groups. The first grade of offal constitutes such organs as the liver, tongue, brain, kidneys of cattle and pigs, meat cut from bones, heart, cow skirt, tail and udder of cattle. Rumen, pig stomach, larynx, weasand meat, pig tail, pork legs and ears, rennet stomach, cattle snout and ears, lungs, trachea, spleen, testes are considered the second grade of offal (Kakimov et al., 2016). Offal yield is on an average 22% from cattle, 17% from pigs and 20% from sheep and goat by live weight (Kakimov et al., 2017). The nutritive value of first- grade offal is equal to that of normal meat; however, in some cases vitamin and mineral content is higher (Kovaleva and Shul'gina, 2014). The liver of slaughtered animals has the highest protein content of other forms of offal. It includes globulin, albumen, glycoproteins, ferritin and ferrin, vitamin B complexes and vitamin A, riboflavin, copper, foliate, iron and choline (NUTTAB, 2006; USDA, 2007). Cow liver provides us with significant amounts of protein, vitamins, and fat that keep our body healthy (Kalhy, 2011; Yasmin et al., 2022). However, liver products are considered a high-risk food as these are highly nutritious and serve as an ideal medium for bacterial growth. Contamination due to poor hygienic practices by food handlers and instruments such as cutting boards, machines, and all other related materials used for preparation of liver to sell to consumers and processing, rather than intrinsic characteristics of the tissues (Gill and Jeremiah, 1991; Sheridan and Lynch, 1988). The major cause of liver spoilage is microbial growth. The nature of the spoilage microflora is affected by storage condition (Azad et al., 2022; Hanna et al., 1982; Hernández-Herrero et al., 1999; Rahman et al., 2023; Torun et al., 2023). Their microbial quality is a function of poor product handling, unhygienic practices, and poor temperature control during collection. Freezing is most widely used methods for liver preservation and frozen food is usually stored at -18°C to -23°C. Nowadays there are some supermarkets are available in Bangladesh. They are selling different meat and meat by-products stored at freezing temperature to retard the growth of microorganisms. But in Bangladesh aspects there is lack of information regarding aright the shelf life of cow liver stored in freezing temperature. Therefore, the aim of this study to detect the effect of long-term frozen duration on the sensory, nutritional, physicochemical and microbial properties of cow liver and to predict the shelf-life of frozen 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

Six cow livers were collected from local market, Mymensingh. The samples were from physically healthy cow of almost same age (about 3 to 3.5 years old). The liver samples were then immediately transferred to the "Animal Science Laboratory" and carried out for experimental analysis.

Preparation of Jar and Other Instruments

Both hot water and detergent powder were used to clean all necessary instruments and jars or containers and then desiccated properly before starting the experimental activities.

Preparation of Cow liver Sample

The liver samples were packaged in sterilized polyethylene bags and transported to the analytical laboratory by isothermal containers with ice. Then these were stored at -18°C for further analysis. After that liver sample was taken as per requirement. Before analysis frozen samples were thawed for 3 hours at room temperature.

Sensory evaluation

Different sensory attributes were examined. Each cow liver sample was evaluated by a panel of 6-trained members. The sensory questionnaires assessed the following qualities: color, odor, juiciness, and tenderness, on a 5-point balanced semantic scale (weak to strong) (Hashem et al., 2023). Evaluation was conducted based on the above criteria. Panelists were selected among department member and students. Before the sample evaluation, all panelists were acquainted with the scale attributes (color, odor, juiciness, and tenderness) of liver using an intensity scale. They were trained according to the American Meat Science Association guidelines. The sensory evaluation took place in individual booths, with controlled light, temperature, and humidity. Sensory scores were 5 for excellent, 4 for very good, 3 for good, 2 for fair and 1 for poor (Ahmad et al., 2013). All samples were served in the Petri dishes. Finally 1st Sensory evaluation on day 0 was accomplished. The whole process was repeated on day 60 and day 120.

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 value was reported.

pH measurement

pH value of raw liver was measured using a pH meter from raw liver homogenate by using HANNA Meat pH Meter according to its operating manual. The homogenate was prepared by blending 5 g of liver with 10 ml distilled water.

Cooking loss

The fresh cow 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 for cooking loss is:

$$Cooking\ loss(\%) = \frac{w2 - w3}{w2} \times 100$$

Where, w2 = liver weight before cooking and w3 = liver weight after cooking.

Water holding capacity

The water holding capacity will be evaluated based on thawing drip loss, natural drip loss and cooking loss, forced drip loss by the Grau and Hamm method (Nørrung and Buncic, 2008), and the ability to bind added water by the centrifugal method described by(Arihara, 2006).

The formula of water holding capacity is:

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

Biochemical analysis

There was one kind of biochemical analysis which is Thiobarbituric Acid Value (TBARS). This type of analysis are discussed below.

Thiobarbituric Acid Values (TBARS) (mg-MDA/kg)

Lipid oxidation was assessed in triplicate using the 2-thiobarbituric acid (TBA) method described by (Malvestiti et al., 2007). Liver samples (5 g) were blended with 25 mL of 20% trichloro acetic acid solution (200 g/L of tricholoro acetic acid in 135 mL/L phosphoric acid solution) in a homogenizer for 30 s. The homogenized sample was filtered with Whatman filter paper number 4, and 2 mL of the filtrate was added to 2 mL of 0.02 M aqueous TBA solution (3 g/L) in a test tube. The test tubes were incubated at 100°C 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 (TVC), total coliform count (TCC) and total yeast mold count (TYMC) were undertaken. These analyses were done at alternative days for the economic use of chemicals. To determine these parameters the procedures which are described below:

Preparation of samples for TVC, TCC and TYMC

A quantity of 10 g of cow liver sample was aseptically excised from stored stock sample. Each of the stored cow 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 quantity of ten (10) gram of the minced cow 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 Yeast-Mold count

Solid media and reagents

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 organism 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 organism 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 organism 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 was calculated according to ISO (1995). The results of the yeast and mold count were expressed as the number of organism of colony forming units per gram (CFU/g) of cow liver samples.

Statistical analysis

Data were analyzed statistically by using MSTATC package in one way analysis of variance test as per Completely Randomized Design (CRD). Means were considered significantly different for ($p<0.05$). Data presented are shown as means ± SD.

Results and Discussion

Sensory Evaluation

The observation of color score of different frozen duration on liver are shown in Table 1. The range of overall observed color score at different treatment was 5 to 3.1. Color scores were decreased ($p<0.05$) with the increase of storage duration. Most preferable color was observed from 0 days among three treatments group and less preferable color was observed from 120 days. Gradual decline in appearance and color scores of cow liver stored at frozen conditions might be due to pigment and lipid oxidation resulting in non-enzymatic browning between lipids and amino acids. A similar result was reported by (Hernández-Herrero et al., 1999) conducted an experiment on shelf life of Ostrich (Struthio camelus) liver stored under different packaging conditions. Changes in color of the muscle and blood pigments determine the attractiveness of fresh red meat, which in turn influences the consumer's acceptance of meat products (Pearson, 1994).

The flavor score of different frozen duration on liver are shown in Table 1. The range of flavor score among three storage duration was 5 to 3.2. Flavor scores were decreased ($p<0.05$) with the increase of storage life. The most preferable flavor was observed from 0 days and the less preferable flavor from 120 days. The lower flavor 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 flavor during storage might be due to microbial growth, formation of FFA and oxidative rancidity.

The juiciness score of different frozen duration on liver are shown in Table 1. The range of overall observed juiciness score at different storage duration was 5 to 2.83. Juiciness scores were decreased ($p<0.05$) with the increase of storage life. Among these three storage duration most preferable juiciness score was observed from 0 days and less preferable juiciness score was observed from 120 days. That's why cow liver leak juices when they were stored. The result of this experiment is also related to (Leygonie, et al., 2011) findings. Similar sensory values were also found by Sharker et al., (2024) in doe liver.

Table 1: Sensory parameters of liver at different frozen duration

Parameters	Frozen duration			Level of Significance
	0 d	60 d	120 d	
Color	$5.00^{a}\pm0.01$	$4.56^{ab}\pm0.11$	$3.10^{b}\pm0.02$	$P < 0.05$
Flavor	$5.00^{a}\pm0.45$	$4.13^{ab}\pm0.01$	$3.20^{b}\pm 0.13$	$P < 0.05$
Juiciness	$5.00^{a}\pm0.01$	$3.16^{ab}\pm0.06$	$2.83^{b}\pm0.38$	$P < 0.05$
Tenderness	$5.00^{a}\pm0.04$	$3.85^{ab}\pm0.25$	$2.97^{b}\pm0.12$	$P < 0.05$

Means in each row having different superscript vary significantly at values $p < 0.05$. Values are presented as mean±SD.

The range of overall observed tenderness score at different frozen duration was 5 to 2.97 (table no 1). Tenderness score was decreased ($p<0.05$) with the increase of storage life. Among these three storage duration most preferable tenderness score was observed on 0 day and less preferable tenderness score was observed at 120 day. Tenderness is interrelated with DM content of the liver. With the increasing of storage period DM was increased consequently, tenderness was decreased with day's intervals. The result of this experiment is also agreed with Leygonie et al., (2011) findings. Several researches have associated tenderness of meat with the breakdown of myofibrillar proteins affected by the presence of calcium-dependent proteases or calpains.

Proximate analysis

The values of proximate components are shown in Table 2.

Table 2: Proximate components of liver at different frozen duration

Parameters	Frozen duration			Level of Significance
	0 d	60 d	120 d	
DM (%)	$28.89^{b}\pm0.68$	$29.37^{ab}\pm0.89$	$30.87^{a}\pm0.62$	$P < 0.05$
CP (%)	$24.09^{a}\pm0.31$	$22.69^{a}\pm0.37$	$20.80^{b}\pm0.26$	$P < 0.05$
EE (%)	$4.39^{a}\pm0.01$	$3.96^{a}\pm0.09$	$3.38^{b}\pm0.09$	$P < 0.05$
Ash (%)	$1.31^{c}\pm0.27$	$1.54^{b}\pm0.36$	$1.74^{a}\pm0.28$	$P < 0.05$

Means in each row having different superscript significantly at values $p < 0.05$.Values are presented as mean±SD.

The DM content of different storage duration are shown in Table 2. The range of overall observed DM content at different storage duration was 28.89% to 30.87%. DM content was increased ($p<0.05$) with increase of storage days among these observation. The same trend was also observed by (Garrett & Hinman, 1971) and they reported that DM content increased during frozen storage.

The CP content of different storage duration is shown in Table 2. The range of overall observed CP content at different storage duration was 24.09% to 20.80%. The CP content was decreased with the increased storage days. The most preferable CP content was observed from day 1. The same trend was also observed by (Garrett & Hinman, 1971) and they reported that CP content decreased during frozen storage.

The EE content of different storage duration is shown in Table 2. The observation of EE at different storage duration was 4.39% to 3.38%. Among these three storage duration most preferable EE content was observed from 0 days. The lowest amount of EE

content indicates this product is less preferable for consumer health. Less preferable EE content was observed at120 day. EE content was significantly ($p<0.05$) decreased among these observations.

The ash content of different storage duration is shown in Table 2. The range of overall observed ash content at different storage duration was 1.31% to 1.74%. Ash content was increased ($p<0.05$) among these observations. A non-significant decrease in ash percentage was reported by (Kalhy, 2011) which coincided with this study. Kakimov et al., 2017 found that cow liver contains about 1.30% ash. Similar results for proximate components were also found by Sharker et al., (2024) in doe liver.

Physicochemical properties

The physicochemical properties such as pH, and cooking loss were determined and the results obtained are shown in Table 3.

Table 3: Physicochemical properties of liver at different frozen duration

Parameters	Frozen duration			Level of Significance
	0 d	60 d	120 d	
Water holding capacity (%)	$89.09^{a}\pm0.50$	$87.01^{b}\pm0.49$	$84.01^{c}\pm0.35$	$P < 0.05$
pH	$6.30^{a}\pm0.09$	$5.97^{ab}\pm0.12$	$5.73^{b}\pm0.14$	$P < 0.05$
Cooking loss (%)	$15.14^{c}\pm0.79$	$22.84^{b}\pm0.73$	$26.33^{a}\pm0.97$	$P < 0.05$

Means in each row having different superscript vary significantly at values $p< 0.05$. Values are presented asmean±SD.

The range of overall observed raw pH at different storage duration was 6.30 to 5.73. pH values were decreased ($p<0.05$) with increase of storage days among these observations. Among these three storage duration most preferable pH was observed from 0 day. The data showed a slight decrease in the pH values and an increase in the acidity values for all samples along with storage time during 120 days of storage as a result of the increase of free fatty acids due to rancidity. The pH of fresh liver is 6.72 to 6.94. pH of frozen liver ranges from 5.5-6.2 is acceptable limit for human consumption (ES., 2007).The range of overall observed cooking loss at different storage duration was 15.14% to 26.33%. Cooking loss were increased ($p<0.05$) with increase of storage days. Among these storage duration most preferable cooking loss was observed at 0 day. The lowest amount of cooking loss indicates this product is most preferable for consumers' choices than others. The less preferable cooking loss was observed at 120. Cooking loss refers to the reduction in weight of liver during the cooking process (Kovaleva and Shul'gina, 2014). Major components of cooking losses are thawing, dripping and evaporation. Thawing loss refers to the loss of fluid resulting from the formation of exudates following freezing and thawing (Kovaleva and Shul'gina, 2014). Such losses are lower following a rapid freezing compared with slow freezing. This is because of small crystallization formed by the rapid freezing. Dripping is the loss of fluid from liver and water evaporation from the shrinkage of proteins (actin and myosin). Cooking loss in liver is important for maintaining an attractive retail display of liver. For example, meat and their products are a rich source of proteins, essential minerals and vitamins. The increased loss of such nutrients deteriorates the meat nutritional quality and lowers its purchase (Kovaleva and Shul'gina, 2014). The meat also tends to shrink during the cooking process due to the denaturation of meat protein; the loss of water and fat also contribute to the shrinking process.

The range of overall observed WHC at different treatments was 89.09% to 84.01%. Water holding capacity were decreased ($p<0.05$) with increase of storage days. Among these storage duration most preferable WHC was observed at 0 day. The lowest value of WHC was observed at 120 day.

Biochemical properties

Biochemical properties indicate the good or bad quality of liver. The value of biochemical components such as TBARS is shown in Table 4.

Table 4: Biochemical properties of liver at different frozen duration

Parameters	Frozen duration			Level of Significance
	0 d	60 d	120 d	
TBARS (mg-MDA/kg)	$0.282^{b}\pm0.05$	$0.363^{b}\pm0.05$	$0.497^{a}\pm0.03$	$P < 0.05$

Means in each row having different superscript vary significantly at values $p<0.05$. Values are presented as mean±SD.

The range of overall observed TBARS value at different storage duration was 0.282 to 0.497 (mg-MDA/kg). Among these three storage duration, the lower amount of TBARS value was observed at 0 day group and highest amount observed at 120 day. The TBARS values increased ($p<0.05$) with increase the storage duration. With the TBARS values >0.5 mg/kg, unpleasant flavor would be detected (Hansen *et al*., 2004). So, in the present research upto 120 days frozen storage of cow liver the TBARS value did not exceed the secure level. Similarly, TBARS values increased ($p<0.05$) with increases the frozen duration but not exceed the secure level at 120 day in doe liver (0.41±0.01) founded by Sharker et al., (2024).

Microbiological assessment

The present study observed the presence of micro-flora (TVC) and food borne pathogens (Coliform and Yeast-Mold) on fresh and frozen stored cow liver. The effect of frozen duration on microbial population of cow liver is presented in Table 5. Though the TVC, TCC and TYMC values were increased with the increase of frozen duration, the TVC, TCC and TYMC values were in secure range up to 120 days. All microbial values did not exceed reference limit (maximum secure values for TVC <6 log cfu/g, TCC <3.5 log cfu/g, TYMC <2.50 log cfu/g Cheng et al., 2017) up to 120 days.

Table 5: Microbiological assessment of liver at different frozen duration

Parameters	Frozen duration 0 d	60 d	120 d	Level of Significance
TVC (*log*CFU/g)	4.62^{c}±0.0 19	4.92^{b}±0.017	5.01^{a}±0.049	P < 0.05
TCC (*log*CFU/g)	2.35^{c}±0.009	2.56^{b}±0.030	2.97^{a}±0.029	P < 0.05
TYMC (*log*CFU/g)	1.18^{c}±0.070	1.62^{b}±0.090	1.98^{a}±0.00	P < 0.05

Means in each row having different superscript vary significantly at values p < 0.05. Values are presented as mean±SD.

The TVC value of different storage duration are shown in Table 5. The initial value of TVC for fresh cow liver was 4.62 log CFU/g liver, indicating good quality liver. 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). The observation from different storage duration indicates there were differences ($p<0.05$) of TVC values. The plate count in the fresh sample. (4.62 logCFU/g) was significantly lower than the frozen stored samples. The less amount of TVC value indicates this product is most preferable for consumers' health. The range of overall observed treatment values of TVC was 4.62 log CFU/g to 5.01 log CFU/g. The amount of TVC was increased with the storage periods.

The TCC value of different frozen storage are shown in Table 5. The range of overall observed TCC from the cow liver was 2.35 to 2.97 (log CFU/g) at different storage duration. The observation from different storage duration indicate there were significant differences of TCC values. Among this storage duration, the TCC in the control sample (2.35 log CFU/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 to prevent the metabolism of fat by bacteria.

The TYMC value of different storage duration are shown in Table 5. The range of overall observed TYMC from the cow liver was 1.18 to 1.98 (log CFU/g), at different storage duration. The observation indicate that there were significant differences ($p<0.05$) of TYMC values among these storage duration. Among these storage duration, the TYMC in the fresh sample (1.18 log CFU/g) was significantly lower than others and highest TYMC was at 120 day. The less amount of TYMC value indicates this product is most preferable for consumers' health. With the increased of storage period TYMC values were increased. Similar microbial results were also found by Sharker et al., (2024) in doe liver.

Conclusion

In conclusion, from this study on sensory evaluation, nutritional content, physicochemical properties, biochemical analysis and microbial assessments it has been found that upto 120 days frozen stored cow liver is safe for human consumption. Further studies are recommended with the increased frozen duration.

References

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.

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

Akhter S, Rahman MM, Hossain MM, Hashem MA. 2009. Effects of drying as a preservation technique on nutrient contents of beef. Journal of Bangladesh Agricultural University, 7(1): 63-68.

Akter H, S Akhter, SME Rahman, MM Rahman, MM Hossain, CS Ra, JM Kim, DH Oh. 2009. Effect of different preservation methods on physicochemical qualities of beef. Journal of Food Hygiene and Safety, 24 (3): 217-225.

AOAC 2005. Official Methods of Analysis. Association of Official Analytical Chemists (20 Ed), Washington, D.C., U.S.A

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. https://doi.org/10.55002/mr.2.3.20

Azad MAK, Kikusato M, Zulkifli I, Rahman MM, Ali MS, Hashem MA, Toyomizu M. 2021. Comparative study of certain antioxidants - electrolyzed reduced water, tocotrienol and vitamin E on heat-induced oxidative damage and performance in broilers. Meat Research, 1(1), Article 7. https://doi.org/10.55002/mr.1.1.7

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.

Cheng S, Wang N, Zhang J, Brennan C, Guan W, Wang Z. 2017. The effects of electron beam application on the microbiological stability and physical–chemical quality of mince beef (M. longissimus Dorsi) during cold storage. Journal of Food Processing and Preservation, 42(2): 13448.

Garrett W, Hinman N. 1971. Fat content of trimmed beef muscles as influencedby quality grade, yield grade, marbling score and sex. Journal of Animal Science, 33(5): 948-957.

Gill CO, Jeremiah LE. 1991. The storage life of non-muscle offals packaged under vacuum or carbon dioxide. Food Microbiologhy, 8: 339-353.

Hanna MO, Smith GC, Savell JW, McKeith FK, Vanderzant C. 1982: Microbial flora of livers, kidneys and hearts from beef, pork and lamb: effects of refrigeration, freezing and thawing. Journal of Food production, 45: 63-73.

Hashem MA, Tule SA, Khan M, Rahman MM, Azad MAK, Ali MS. 2021. Prediction of beef quality traits through mini NIR spectrophotometer and multivariate analyses. Meat Research, 1(1): Article 6.

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

Hashem MA, Ambia J, Mia N, Ali M, Rahman M, Ali M. 2024. Detection of adulteration of goat and sheep meat through NIRS and chemometric analysis. Meat Research, 4(2). https://doi.org/10.55002/mr.4.2.86

Hernandez-Herrero MM, Roig-Sague"s AX, Lo" pez-Sabater EI, Rodri"guez-Jerez JJ, Mora-Ventura MTY. 1999. Influence of storage temperature on the quality of beef liver; pH as a reliable indicator of beefliver spoilage. Journal of Science and Food Agriculture, 79: 2035-2039.

ISO. 1995. International Organization for Standardization Protocol.
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.
Kalhy LM. 2011. Benefits of eating liver our most nutrient-dense food article. Available at: http://blog.radiantlifecatalog.com/bid/26773/Benefits-of- Eating-Liver-Our-Most-Nutrient-Dense-Food.
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.
Koutsoumanis KP, Stamatiou AP, Drosinos EH, Nychas GJE. 2008. Control of spoilage microorganisms in minced pork by a self-developed modified atmosphere induced by the respiratory activity of meat microflora. FoodMicrobiology, 25: 915-921.
Kovaleva OA, Shul"giva LV. 2014. Offal meat as the ingredient in the formulationof canned meat products with functional properties. Proceeding of XXXVIII International Conference, Novosibirsk SibAK, 9 (34).
Lawrie RA, Ledward DA. 2006. Chemical and biochemical constitution of muscle. CRC Press, New York. Kawrie"s Meat Science, 87-93.
Leygonie C, Britz TJ, Hoffman LC. 2011. Oxidative stability of previously frozen ostrich M. Iliofibularis packaged under different modified atmospheric conditions. International Journal of Food Science andTechnology, 46: 1171-1178.
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.
Norrung B, Buncic S. 2008. Microbial safety of meat in the European Union. Meat Science**,** 78: 14-24.
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.
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, 4 (2): 1-8.
Sheridan JJ, Lynch B. 1988. The influence of processing and refrigeration on the bacterial numbers on beef and sheep offals. Meat Science, 24(2)**:** 143-150
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, 3(5): 1-9, https://doi.org/10.55002/mr.3.5.69
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
USDA, (2007). US Department of Agriculture, USDA National Nutrient Database for Standard Reference. Release 19. Washington DC: Cited 25 April 2007.