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## Research Article

# Impact of Repeated Freezing and Thawing on the Quality of Goat Liver

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## Abstract

The present study investigated the effect of repeated freeze-thaw cycle, freezing at -20°C for whole night (12 hours) and then thawing at 4°C for whole day (12 hours) on the quality of goat liver. For this purpose, raw fresh goat liver was collected and divided into five treatment groups- Day 1 (T1, fresh group), Day 2 (T2, freeze-thaw), Day 3 (T3, freeze-thaw), Day 4 (T4, freeze-thaw) and Day 5 (T5, freeze-thaw). Liver sample was packaged in polythene bags and frozen at -20°C for overnight (12 hours) followed by thawing in refrigerator at 4°C 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 coliform count (TCC log CFU/g) and total yeast-mold count (TYMC 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 was decreased and cooking loss was increased among different treatments significantly ( $p < 0.05$ ). Biochemical properties that was FFA%, POV and TBARS were increased significantly ( $p < 0.05$ ). Microbial assessments TCC and TYMC showed significantly ( $p < 0.05$ ) higher value with the increase of freeze-thawed cycle. From these findings suggested that shelf life of raw goat liver with repeated freeze-thawed cycle up to three days can kept with significantly changes of quality of liver are safe for human health.

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## Introduction

Now a day's consumption of edible By-products from goat such as liver, kidney, spleen, and heart etc. is included in the food composition especially for their nutritional benefits. The vitamin content of edible offal is usually higher than that of lean meat issue. Riboflavin content was found to be in highest amount in kidney and liver (1.697-3.630mg/100g) and is nearly 5-10 times amount present in lean meat (Irshad and Sharma, 2015). Liver also contains good amount of niacin, cobalamin, pyridoxine, folacin, ascorbic acid and vitamin A. Livers also contain the highest amount of manganese (0.128 to 0.344 mg/100 g) as reported by Irshad and Sharma (2015). Goat 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 clotting 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 °C and domestically at -10°C is now a standard of eating quality compared to fresh meat. Both for preservation of meat and further manufacturing of meat -18 °C to -20 °C 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 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 goat liver has not been studied. Moreover in Bangladesh aspects there is lack of information regarding the shelf-life of repeated freeze-thawed goat liver. This study is aimed at undertaken the following objective: the effect of repeated freezing and thawing on the quality and shelf-life of goat liver.

## **Methodology**

### **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 (Goat liver) were obtained from the Kamal-Ranjit (KR) market of Bangladesh Agricultural University, Mymensingh. After collecting the goat 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 Goat Liver Sample**

All visible fat and connective tissue were trimmed from 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°C for the whole day (12 hours) and at evening, stored under freezing at -20°C. Analyses of other samples were performed next 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day with three replicates.

### **Measurement of parameters**

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

### **Sensory evaluation**

Different sensory attributes were examined. Each goat 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 goat 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<sup>st</sup> day and repeated at 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day of storage and stored goat liver at 4°C for whole day (12 hours) and at -20°C for whole night (12 hours).

### **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.

### **Dry Matter**

Weighed samples were taken in porcelain crucibles and dry at 100°C 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 dry matter.

### **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<sub>2</sub>SO<sub>4</sub>) in presence of K<sub>2</sub>SO<sub>4</sub>, CuSO<sub>4</sub> and Selenium powder followed by distillation of ammonia liberated by alkali

(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:**

$$\frac{\text{Titrate required (ml)} \times 0.014 \text{ (milliequivalent of N}_2\text{)} \times \text{Strengt h of HCl}}{\text{Weig ht of sample}} \times 100$$

% of CP= % of nitrogen  $\times$  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°C which took about 7-8 hours. After extraction the flask were taken out and dried in oven for 30 minutes at 100°C. The flask containing ether extract was cooled in a desiccator and weighed.

**The formula is mentioned below:**

$$\% \text{ of ether extract} = \frac{\text{Weight of the ether extract}}{\text{Weight of the sample}} \times 100$$

#### **Ash**

Weighed samples were taken in porcelain crucibles and pre-ashes at 100°C in an electric oven. The crucibles were then placed in a muffle furnace and heated at 550°C 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:**

$$\% \text{ of the ash content} = \frac{E}{C} \times 100$$

Where,

E= Weight of ash

C= Weight of the sample

### **Physicochemical properties measurement**

#### **pH measurement**

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

#### **Cooking Loss**

The fresh goat liver samples were weighted (initial weight). Firstly, weighted liver boiled at water bath to 100°C. 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**

$$\text{Cooking loss (\%)} = [(w_2 - w_3) \div w_2] \times 100$$

Where,

w<sub>2</sub> = liver weight before cooking

and w<sub>3</sub> = liver weight after cooking.

#### **Biochemical analysis**

There were two types of biochemical analysis. These are Free Fatty Acid (FFA), and Peroxide Value (POV). Two types of analysis are discussed below.

#### **Free Fatty Acid (%) analysis**

Free fatty acid value was determined according to Rukunudin et al. (1998). Five grams of sample was dissolved with 30 ml choloform using a homogenizer (IKA T25digital Ultra-Turrax, Germany) at 10,000 rpm for 1 min. The sample was filtered under vacuum through Whatman filter paper number 1 to remove particles. After five drops of 1% ethanolic phenolphthalein were added as indicator to filtrate, the solution was titrated with 0.01 N ethanolic potassium hydroxide.

**The formula is mentioned below:**

$$\text{FFA (\%)} = \text{ml titration} \times \text{Normality of KOH} \times 28.2/\text{g of sample}$$

#### **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). Chicken meatball 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°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 meatball sample.

### **Peroxide Value (POV) analysis (meq/kg)**

POV as determined according to Sallamet al. (2004). The sample (3g) was weighed in a 250-ml glass stopper Erlenmeyer flask and heated in a water bath at 60 °C for 3 min to melt the fat, then thoroughly agitated for 3 min with 30 ml acetic acidchloroform solution (3:2 v/v) to dissolve the fat. The sample was filtered under vacuum through Whatman filter paper number 1 to remove particles. Saturated potassium iodide solution (0.5 ml) was added to filtrate and continue with addition of starch solution. The titration was allowed to run against standard solution of sodium thiosulfate (25/1).

#### **The formula is mentioned below:**

POV was calculated and expressed as mill equivalent per kilogram of sample:

$$\text{POV (meq/kg)} = \frac{S \times N}{W} \times 100$$

Where,

S is the volume of titration (ml), N the normality of Sodium thiosulfate solution (n= 0.01) and W the sample weight (g).

### **Equipment Reagents**

The equipment required was meatball samples, 250-ml glass stopper Erlenmeyer flask, 30 ml acetic acid-chloroform solution (3:2 v/v), Whatman filter paper number 1, Saturated potassium iodide solution (0.5 ml), water bath, pestle and mortar, graduated cylinder, burette, 1ml Mohr pipette, 1 ml of starch solution, standard solution of sodium thiosulfate (25/1) and DDW.

### **Microbial assessment**

For microbial assessment total viable count, total coliform count and total yeast mould count were 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 TCC and Yeast-Mold count**

A quality of 10 g of goat 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 TCC and Yeast-Mold 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.

#### **Glasswares and other appliances**

Different types of glassware and appliances were used during the experiment. These were as follows: Test tubes (with or without Durham's fermentation tube and stopper), petridishes, conical flask, pipette (1ml, 5ml, 10ml and 25ml 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 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°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 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 multiplied by the dilution factor to obtain the total coliform count. The total coliform count was expressed as the number of organism of colony forming units per gram (CFU/g) of goat liver samples.

#### **Enumeration of Yeast-Mould count**

For the determination of yeast and moulds counts, 0.1 ml of each ten-fold dilution was transferred and spread on triplate 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 mould count. The yeast and mould count were calculated according to ISO (1995). The results of the yeast and mould count were expressed as the number of organism of colony forming units per gram (CFU/g) of goat 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 freeze-thawed cycles. Means were considered significantly different at  $p < 0.05$ . Data presented are shown as mean  $\pm$ SD.

## Results and discussions

### Color

The result of color score of goat liver treatments are presented in table 1. The range was 5 to 3.67 of overall observed of color score. In different treatments, color score of goat liver were significantly decreased ( $p < 0.05$ ) with increasing freeze-thaw cycle. In that treatments, most desirable color was observed in T1, and less desirable color was observed in T5 group. Gradually decreasing appearance and color score of goat liver which was thawing at 4°C for the whole day and freezing at -20°C 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). Georganteliset al., (2007) presented that the oxidative browning of the meat product still occurred during frozen storage.

### Odour

The result of odor score of goat liver treatments are presented in table 1. The observation range was 5 to 3. 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 T5. 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 flavor characteristics due to oxidative changes.

### Juiciness

The different treatments of juiciness score are shown in table 1. The result of juiciness score range was 5 to 2.67. 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 T5. 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.

### Tenderness

In table 1, the result of tenderness score range at different treatment was 5 to 2.73. Tenderness scores were significantly decreased ( $p < 0.05$ ) with the increase storage life. Among the five treatments, most acceptable tenderness was observed at T1, and less acceptable tenderness observed at T5. In frozen condition of goat 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: Values of sensory evaluation of fresh and repeated freeze-thawed goat liver**

Variables	Treatments					Level of significance
	T1	T2	T3	T4	T5	
Color	5.00 <sup>b</sup> ±0.01	4.67 <sup>b</sup> ±0.10	4.33 <sup>ab</sup> ±0.11	4.00 <sup>ab</sup> ±0.05	3.67 <sup>a</sup> ±0.02	$p < 0.05$
Odour	5.00 <sup>a</sup> ±0.45	4.67 <sup>a</sup> ±0.15	4.00 <sup>ab</sup> ±0.01	3.33 <sup>bc</sup> ±0.02	3.00 <sup>a</sup> ±0.13	$P < 0.05$
Juiciness	5.00 <sup>a</sup> ±0.01	4.67 <sup>ab</sup> ±0.42	3.83 <sup>ab</sup> ±0.06	3.33 <sup>bc</sup> ±0.13	2.67 <sup>c</sup> ±0.38	$P < 0.05$
Tenderness	5.00 <sup>bc</sup> ±0.04	4.33 <sup>bc</sup> ±0.45	3.33 <sup>ab</sup> ±0.25	2.67 <sup>ab</sup> ±0.03	2.73 <sup>a</sup> ±0.12	$P < 0.05$

Here, T1= Day1 (fresh), T2= Day2 (freeze-thawed), T3= Day3 (freeze-thawed), T4= Day4 (freeze-thawed), T5= Day5 (freeze- thawed) with significant at 5% level,  $p < 0.05$ . Values are presented as mean $\pm$ SD.

### Proximate Analysis

There were five groups of samples prepared for the determination of proximate analysis of goat liver kept at -20°C for the whole night and thawing at 4°C. In first day, DM, CP, EE, Ash was determined and then rest of samples were stored at 4°C for the whole day and -20°C for the whole night. Follow the freeze-thawed cycle, the proximate analysis had been done of goat liver sample. The determined values of proximate components are shown in Table 2.

### Dry Matter (DM)

The DM content of different treatments of goat liver are shown in table 2. The overall observation range of DM content at different treatments was 34.87% to 35.33%. Different superscript was observed for five treatments of groups indicates there were significantly ( $p < 0.05$ ) differences of DM content. 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) and they reported that DM content increased during frozen storage.



### Crude Protein (CP)

The CP content of different treatments of goat liver are shown in table 2. The overall observation range of CP content at different treatments was 17.43% to 16.96%. The same superscript was observed for five treatments of groups indicates there were significantly ( $p < 0.05$ ) differences of CP content. The highest amount of CP content indicates this product is most acceptable for consumer's health observed from first day and less acceptable CP content was observed on fifth day. The CP content was decreased with the increased storage days. The same trend was also observed by Konieczny et al., (2007) and they reported that CP content decreased during frozen storage.

### Ether Extract (EE)

The EE content of different treatments of goat liver are shown in table 2. The overall observation range of EE content at different treatments was 1.08% to 0.86%. EE content were significantly ( $p < 0.05$ ) decreased among the observation. Among these five treatments most acceptable EE content was observed from T1. The lowest amount of EE content was observed from T5 treatments, and this indicates that this product is less acceptable for consumer's health. Agnihotri (1988) reported deterioration in meat lipids took place due to intermediary activities of endogenous meat enzymes leading to hydrolysis of fat.

### Ash

The ash content of different treatments of goat liver are shown in table 2. The overall observation range of ash content at different treatments was 2.18% to 2.61%. Among these five treatments most acceptable ash content was found from T1 and less acceptable found from T5. The ash content was significantly increased with the increased of freeze-thaw cycle and the high amount of ash content indicates that this product is less acceptable for consumer's health. 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.

**Table 2.** Value of proximate components in fresh and repeated freeze-thawed goat liver

Variables	Treatments					Level of Significance
	T1	T2	T3	T4	T5	
DM%	34.87 <sup>b</sup> ±0.56	34.96 <sup>b</sup> ±0.85	35.07 <sup>ab</sup> ±0.54	35.19 <sup>ab</sup> ±0.26	35.33 <sup>a</sup> ±0.10	P<0.05
CP%	17.43 <sup>a</sup> ±0.27	17.28 <sup>a</sup> ±0.36	17.17 <sup>ab</sup> ±0.21	17.03 <sup>bc</sup> ±0.91	16.96 <sup>a</sup> ±0.86	P<0.05
EE%	1.08 <sup>a</sup> ±0.08	1.02 <sup>ab</sup> ±0.05	0.98 <sup>ab</sup> ±0.05	0.89 <sup>bc</sup> ±.11	0.86 <sup>c</sup> ±0.05	P<0.05
Ash%	2.18 <sup>bc</sup> ±0.03	2.28 <sup>bc</sup> ±0.13	2.39 <sup>ab</sup> ±0.01	2.48 <sup>ab</sup> ±0.11	2.61 <sup>a</sup> ±0.05	P<0.05

Here, T1= Day1 (fresh), T2= Day2 (freeze-thawed), T3= Day3 (freeze-thawed), T4= Day4 (freeze-thawed), T5= Day5 (freeze-thawed) with significant at 5% level,  $p < 0.05$ . Values are presented as mean±SD.

### Physicochemical properties

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

#### pH

The pH of different treatments of goat liver with days interval are given in table 3. The overall observation of pH range at different treatments was 6.85 to 6.48. With the increase of freeze-thaw cycle pH value were significantly ( $p < 0.05$ ) decreased. Among the five treatments observation, most acceptable pH value was from T1, and less acceptable pH value was from T5. In this treatment, the highest amount of pH indicates the product is most beneficial for consumer's health than other group of treatments. The data of the treatments showed a slight decrease in the pH values and an increase in the acidity values for all samples along with freeze-thaw cycle. During 5<sup>th</sup> days of freeze-thaw cycle of liver, increase of free fatty acids due to rancidity. The decreasing pH was probably due to the secretion of microorganisms in the goat liver. A similar result was reported by Ali et al. (2015) who examined chicken meat.

#### Cooking loss

The cooking loss of different treatments of goat liver with days interval are given in table 3. The overall observation of cooking loss range at different treatments was 13.38% to 27.85%. With the increase of storage days, cooking loss were significantly ( $p < 0.05$ ) increased. Among the five treatments results, most acceptable cooking loss was observed at T1 and less acceptable cooking loss observed at T5. In this treatment, the lowest amount of cooking loss indicates this product is most beneficial for consumer's health than other treatment groups. Cooking loss refers to the reduction in weight of liver during the cooking process (Jama et al., 2008; Muchenje et al., 2009). Such losses are lower following a rapid freezing compared with slow freezing. This is because of small crystallization formed by the rapid freezing (Hui, 2004). Cooking loss in liver is very important for maintaining an attractive retail display of goat 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 (Jama et al., 2008). 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).

**Table 3.** Values of physicochemical properties in fresh and repeated freeze-thawed goat liver

Variables	Treatments					Level of significance
	T1	T2	T3	T4	T5	
pH	6.85 <sup>a</sup> ±0.07	6.73 <sup>ab</sup> ±0.03	6.64 <sup>bc</sup> ±0.09	6.60 <sup>bc</sup> ±0.04	6.48 <sup>c</sup> ±0.11	P<0.05
Cooking Loss %	13.38 <sup>b</sup> ±0.24	18.96 <sup>c</sup> ±0.39	22.24 <sup>bc</sup> ±0.86	25.62 <sup>ab</sup> ±1.18	27.85 <sup>ab</sup> ±0.73	P<0.05

Here, T1= Day1 (fresh), T2= Day2 (freeze-thawed), T3= Day3 (freeze-thawed), T4= Day4 (freeze-thawed), T5= Day5 (freeze-thawed) with significant at 5% level,  $p < 0.05$ . Values are presented as mean±SD.

## Biochemical Properties

Three types of biochemical properties indicate the good or bad quality of meat and meat by-products. They are Free Fatty Acid (FFA %), Peroxide Value (POV meq/kg), Thiobarbituric Acid Value (TBARS mg-MA/kg). Goat liver biochemical properties were determined, and the results are given in Table 4.

### Free Fatty Acid Value (FFA %)

FFAs are the products which is result of enzymatic or microbial degradation of lipids. It's also giving information about stability of fat during storage. FFA% of different treatments of goat liver with day's interval are given in table 4. The overall observation of FFA% range at different treatments was 1.14% to 1.81%. With the increase of freeze-thaw cycle, FFA% were significantly ( $p<0.05$ ) increased. Among the five treatments observation, most acceptable FFA% was observed at T1 and less acceptable FFA% observed at T5. The chemical component in liver is reflected in liver quality, including organoleptic characteristics. The combination of two sensory factors: aroma and taste are determining the palatability of meat and liver. Polyunsaturated fatty acids increase sensitivity to peroxidation, leading to unpleasant odors (Coulon and Priolo, 2002). Changes in proportions between saturated and unsaturated acids are also an adverse phenomenon from the dietary point of view.

### Peroxide value (POV)

Lipid oxidation is the product which is the causes of undesirable rancid off-flavors and potentially toxic products, leading to the qualitative deterioration. Peroxide value (POV) is the primary products of lipid oxidation generated by oxygen attacking on the double bond in fatty acids. Peroxide value of different treatments of goat liver with days interval are given in table 4. The overall observation of peroxide value range at different treatments was 1.58 to 1.99. With the increase of freeze-thaw cycle, peroxide value was significantly ( $p<0.05$ ) increased. Among the five treatments result, most acceptable peroxide value was observed at T1 and less acceptable peroxide value observed at T5. The lowest Peroxide value results of the products indicate that the product is beneficial for human health. Therefore, it seemed reasonable to determine the concentration of peroxide in liver samples to clarify the extent of oxidation (Donald, 1998).

### Thiobarbituric Acid Value (TBARS)

TBARS of different treatments of goat liver with day's interval are given in table 4. The overall observation of TBARS range at different treatments was 0.13 to 0.16. With the increase of freeze-thaw cycle, TBARS were significantly ( $p<0.05$ ) increased. Among the five treatments observation, most acceptable TBARS value was observed at T1 and less acceptable TBARS value observed at T5. 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). Similar phenomenon was observed in chicken meat by Ali et al. (2016).

**Table 4: Value of biochemical properties in fresh and repeated freeze-thawed goat liver**

Variables	Treatments					Level of significance
	T1	T2	T3	T4	T5	
FFA(%) analysis	1.14 <sup>c</sup> ±0.01	1.20 <sup>bc</sup> ±.20	1.36 <sup>bc</sup> ±0.26	1.55 <sup>ab</sup> ±0.27	1.81 <sup>a</sup> ±0.11	P<0.05
POV (meq/kg)	1.58 <sup>c</sup> ±0.25	1.70 <sup>bc</sup> ±0.17	1.76 <sup>bc</sup> ±0.02	1.89 <sup>bc</sup> ±0.01	1.99 <sup>ab</sup> ±0.12	P<0.05
TBARS (mg MA/kg)	0.13 <sup>c</sup> ±0.01	0.13 <sup>bc</sup> ±0.05	0.14 <sup>bc</sup> ±0.01	0.15 <sup>a</sup> ±0.01	0.16 <sup>a</sup> ±0.02	P<0.05

Here, T1= Day1 (fresh), T2= Day2 (freeze-thawed), T3= Day3 (freeze-thawed), T4= Day4 (freeze-thawed), T5= Day5 (freeze-thawed) with significant at 5% level,  $p<0.05$ . Values are presented as mean±SD

### Microbiological assessment

This study observed the presence of micro-flora (TCC) and food borne pathogens (Coliform and Yeast-Mould) on goat liver at refrigerator temperature. Goat liver microbial assessment were determined, and the results are given in Table 5.

### Total Coliform Count (TCC)

TCC value of different treatments of goat liver with days interval are given in table 5. The overall observation of TBARS range at different treatments was 4.35 to 4.43 log CFU/g. With the increase of storage days, TCC values were significantly ( $p<0.05$ ) increased. Among the five treatments observation, most acceptable TCC value was observed at T1 and less acceptable TCC value observed at T5. The lowest TCC value results of the products, the initial value of TCC value for fresh goat liver was 4.35 log CFU/g indicate that the product is good quality and beneficial for human health. During storage TCC value was increased. The antioxidant compounds blocked the deteriorating of fat and helped for prevent the metabolism of fat by bacteria. The TCC was increased with the increased of storage period. As a result, bacterial growth was lower in goat liver at 1<sup>st</sup> day. Aziz et al. (2020) Also, Similar phenomenon was observed in broiler chicken meat.

### Total Yeast-Mould Count (TYMC)

TYMC value of different treatments of goat liver with days interval are given in table 5. The overall observation of TYMC range at different treatments was 5.10 to 5.20 logCFU/g. With the increase of storage days, TYMC values were significantly ( $p<0.05$ ) increased. Among the five treatments observation, most acceptable TYMC value was observed at T1 and less acceptable TYMC value observed at T5. The lowest TYMC value results of the products, the initial value of TYMC value for fresh goat liver was 5.10 log CFU/g indicate that the product is good quality and beneficial for human health. During storage TYMC value was increased. Aziz et al. (2020) Also, Similar phenomenon was observed in broiler chicken meat.

**Table 5.** Value of microbial assessments in fresh and repeated freeze-thawed goat liver

Variables	Treatments					Level of significance
	T1	T2	T3	T4	T5	
TCC ( <i>log</i> CFU/g)	4.35 <sup>c</sup> ±0.01	4.37 <sup>bc</sup> ±0.01	4.38 <sup>bc</sup> ±0.05	4.40 <sup>b</sup> ±0.01	4.43 <sup>a</sup> ±0.01	P<0.05
TYMC ( <i>log</i> CFU/g)	5.10 <sup>c</sup> ±0.01	5.13 <sup>bc</sup> ±0.05	5.14 <sup>bc</sup> ±0.01	5.18 <sup>b</sup> ±0.01	5.20 <sup>a</sup> ±0.02	P<0.05

Here, T1= Day1 (fresh), T2= Day2 (freeze-thawed), T3= Day3 (freeze-thawed), T4= Day4 (freeze-thawed), T5= Day5 (freeze-thawed) with significant at 5% level, p<0.05. Values are presented as mean±SD.

## Conclusions

The study was conducted to find out the impact of repeated freeze-thawed goat liver quality and establish the shelf life of goat liver. For this purpose, raw goat liver was collected and divided into five treatment groups. They were treated as Day 1 (T1, fresh group), Day 2 (T2, freeze-thawed), Day 3 (T3, freeze-thawed), Day 4 (T4, freeze-thawed) and Day 5 (T5, freeze-thawed) respectively and there were three replications for each sample. The sensory tests (color, odor, juiciness, and tenderness), the proximate composition test (DM, CP, EE and ash), physicochemical properties (pH and cooking loss), biochemical properties (FFA%, POV and TBARS), and the microbial assessments (TCC and TYMC) of goat liver were determined. The results of sensory test like color, odor, juiciness, and tenderness were decreased with the increase of freeze-thawed cycle. DM and ash content was increased significantly among different treatments of liver with the increase of freeze-thawed cycle, whereas CP and EE content of liver was decreased with the increase of freeze-thawed cycle. Physicochemical quality like pH was decreased and cooking loss was increased among different treatments with the increase of freeze-thawed cycle. Biochemical properties FFA, POV and TBARS were increased with the increase of freeze-thawed cycle. Microbial assessments TCC, and TYMC were increased with the increase of storage days.

From the results of present study, it may be concluded that the number of repeated freeze-thawed cycle increased, it affected sensory, proximate composition, physicochemical properties, biochemical and microbial quality of goat liver and up to three days is acceptable in terms of biochemical and microbial studies. In conclusion, these findings have been suggested that shelf life of raw goat liver freeze at -20 °C and thawed at 4°C for whole day up to maximum three days.

## Conflict of interest

The authors declare that they have no conflict of interest.

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