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

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# Effect of storage periods on the quality and shelf life of beef liver at refrigerated temperature

MS Akhter<sup>1</sup>, H Khatun<sup>1</sup>, MA Hashem<sup>1</sup>, MM Rahman<sup>1</sup>, M Khan<sup>\*1</sup>

# Abstract

The current study investigated the quality and shelf life of beef liver storage under refrigerated temperature (4°C). For this purpose, raw beef liver sample was divided into five treatments groups as  $T_1$  (day 1 or control),  $T_2$  (day 2),  $T_3$  (day 3),  $T_4$  (day 4) and  $T_5$  (day 5). Sensory attributes, proximate composition, pH value, cooking loss, free fatty acids (FFA), peroxide value (POV) and microbial load such as total viable count (TVC), total coliform count (TCC) and total yeast mould count (TYMC) analysis were carried out for each treatment. The results show that color, odor, juiciness, and tenderness were significantly (P<0.05) decreased with increase the storage periods. Dry matter and ash contents were significantly (P<0.05) increased, whereas, crude protein and either extract were decreased with increase storage periods. Consequently, the cooking loss (%), FFA and POV values were significantly (P<0.05) increased, while pH value was significantly (P<0.05) decreased with the increase of storage periods. Moreover, TVC (log CFU/g), TCC (log CFU/g), and TYMC (log CFU/g) were also showed significantly (P<0.05) higher value for  $T_3$  and  $T_5$  compared to  $T_1$ . These findings suggest that shelf life of raw beef liver at refrigeration temperature (4°C) may be maximum three days with minor changes of quality.

## Introduction

Liver is an important slaughterhouse by product, generally known as edible by-products are produced and they can be used by humans as food or processed as secondary products (Liu, 2002; Sadakuzzaman et al., 2021). Depending on the live weight of animal, by-products yield is on an average 22% from cattle, 17% from pigs and 20% from sheep and goat (Kakimov et al., 2017). The nutritive value of these by-products is equal to that of normal meat; however, vitamin and mineral content is higher (Kovaleva and Shulgina, 2014, Akter et al., 2009). The liver is 1-2% of the live weight of cattle and is an important edible organ that is richer in minerals and vitamins than other tissue and muscle (Ercan and El, 2011). Numerous studies reported the livers are a good source of protein including globulin, albumen, glycoproteins, ferritin and ferrin (Lai et al., 2012; Nunes et al., 2013). However, liver products are considered a high-risk food as these are serving as an ideal medium for bacterial growth. Contamination occurs 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. Indeed, livers (and other offals) are necessarily of poor hygienic quality, are prone to rapid spoilage, and have a high incidence of pathogenic organisms (Gill et al., 1988). Their microbial quality is a function of poor product handling, unhygienic practices, and poor temperature control during collection and processing, rather than intrinsic characteristics of the tissues (Gill and Jeremiah, 1991; Sarker et al., 2021; Sheridan and Lynch, 1988).

The major cause of liver spoilage is microbial growth. The nature of the spoilage microflora is affected by storage conditions (Hanna et al., 1982; Hernandez-Herrero et al., 1999; Rashid et al., 2013). 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 beef livers (Hanna et. al, 1982; Hossain et al., 2021). Refrigeration is one kind of process of storage liver for short term period. Nowadays there are some supermarkets are available in Bangladesh are selling different meat and meat by-products stored at refrigeration temperature to retard the growth of microorganisms. But in Bangladesh aspects there is lack of information regarding the shelf life of beef liver stored in refrigeration temperature. Therefore, the present study aim was to investigate whether storage periods of raw beef liver at refrigerated temperature (4 $^{\circ}$ C) influences the shelf life and quality of beef livers, from the viewpoint of sensory attributes, nutritional value, physico-chemical and biochemical properties as well as microbial load.

## **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.

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## \*Corresponding author:

Muckta Khan Department of Animal Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. E-mail: muckta.khan@bau.edu.bd

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## **Sample Collection and Preparation**

Beef livers of 2.5 kg were collected from local market at 9.00 am and immediately transferred to the Animal Science Laboratory, BAU. All visible fat and connective tissue were trimmed off as far as possible with the help of sharp knife and the samples were sliced and individual slices were packaged in sterile plastic bags. One sample bags were analyzed immediately after preparation and remaining bags were stored at refrigeration temperature ( $4^{\circ}$ C) followed by analyzed on 2nd, 3rd, 4th, and 5th day of storage. All samples were used for sensory, proximate, physicochemical, biochemical and microbial analysis.

## **Sensory evaluation**

Sensory attributes were analyzed 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 beef liver using a 5-point balanced semantic scale (weak to strong). Sensory scores were 5 for excellent, 4 for very good, 3 for good, 2 for fair and 1 for poor (Rahman et al., 2012). Panelists were selected among department member and students and trained according to the American Meat Science Association guidelines (AMSA, 1995).

#### **Proximate Composition**

Proximate composition such as dry matter (DM), crude protein (CP), ether extract (EE), and ash were carried out as per the standard procedures of AOAC (1995).

# Measurement of Physicochemical properties of beef liver

Physicochemical properties in terms of pH value and cooking loss (%) were determined in fresh and preserved samples. A pH meter was used to measure the pH value of beef liver homogenate. The homogenate was prepared by blending 5 g of beef liver with 10 ml distilled water. For measuring the cooking loss, the fresh beef liver samples were weighted (initial weight) followed by boiled at 100°C in at water bath. 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

Cooking loss (CL %) is expressed as the principle expressed by Saba et al. (2018):

$$CL (\%) = \frac{(Weight before cooking of sample - weight after cooking)}{Weight before cooking of sample} \times 100$$

where,  $w_2 = liver$  weight before cooking and  $w_3 = liver$  weight after cooking.

# Analysis of Free Fatty Acid (FFA)

FFA value was determined according to Rukunudin et al. (1998). Five grams of sample was dissolved with 30 ml chloroform using a homogenizer (IKA T25 digital 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:

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

## Analysis of Peroxide Value (POV) (meq/kg)

POV as determined according to Sallam et al. (2004). The sample (3 g) was weighed in a 250-ml glass stopper Erlenmeyer flask and heated in a water bath at 60C for 3 min to melt the fat, then thoroughly agitated for 3 min with 30 ml acetic acid-chloroform 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 milli equivalent peroxide per kilogram of sample:

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).

## **Microbial assessment**

For microbial assessment, total viable count (TVC), total coliform count (TCC) and total yeast mould count were undertaken according to the procedure described by Parvin et al. (2017).

# **Experimental designs**

In this study, total five treatments were undertaken to find out the effect of storage at refrigerated temperature (4 °C) on the quality and shelf life of beef liver. These five treatments were considered based on the storage day such as -  $T_1$  = fresh beef liver (control) collected at day 1;  $T_2$ = stored beef liver until day 2;  $T_3$ =stored beef liver until day 3;  $T_4$  = stored beef liver until day 4;  $T_5$  = stored beef liver until day 5.

#### **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 were presented as means  $\pm$  SD.

## **Results and discussions**

#### Effect of storage periods on sensory attributes of beef liver

The parameters for sensory attributes have been shown in Table 1. The range of overall observed color score at different treatment was 5 to 3. All parameters color, odor, juiciness and tenderness scores were significantly decreased (P<0.05) with the increase of storage life. Most preferable color and odor were observed in  $T_1$  whereas less preferable were found in  $T_5$  group. Gradual decline in appearance and color scores of beef liver stored at refrigeration conditions might be due to pigment and lipid oxidation resulting in non-enzymatic browning between lipids and amino acids. Tenderness is interrelated with DM content of the beef liver. In addition, decreasing tenderness also supported by the increasing of DM content of beef liver in this study. A similar result was reported by Juana et al. (2006) 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 decrease the attractiveness of fresh red meat, which in turn influences the consumers' acceptance of meat products (Pearson, 1994). The lower odor scores may be related to the increased malondealdehyde formation due to oxidation of fat, which has detrimental effect on the flavor and firmness of the product (Miller et al., 1981). Deterioration of odor during storage might be due to microbial growth, formation of FFA and oxidative rancidity (Devatkal and Mendiratta, 2007). Several researchers have associated tenderness of meat with the breakdown of myofibrillar proteins affected by the presence of calcium-dependent proteases or calpains (Muchenje et al., 2008).

Table 1: Effects of storag	e periods at refrigerate	d temperature (4 C)	) on sensory <b>p</b>	parameters of beef liver
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Daramatara			Treatments		
Farameters	$T_1$	T <sub>2</sub>	$T_3$	$T_4$	T <sub>5</sub>
Color	$5.00^{a} \pm 0.01$	$4.67^{ab} \pm 0.10$	$4.00^{bc} \pm 0.11$	$3.33^{cd} \pm 0.05$	$3.00^{d} \pm 0.02$
Odor	$5.00^{a} \pm 0.45$	4.33 <sup>ab</sup> ±0.15	$4.00^{bc} \pm 0.01$	3.33 <sup>cd</sup> ±0.02	$3.00^{d} \pm 0.13$
Juiciness	$5.00^{a} \pm 0.01$	4.33 <sup>ab</sup> ±0.42	$3.67^{bc} \pm 0.06$	$3.00^{cd} \pm 0.13$	$2.33^{d} \pm 0.38$
Tenderness	$5.00^{a}\pm0.04$	$4.00^{b} \pm 0.45$	$3.67^{bc} \pm 0.25$	$3.00^{cd} \pm 0.03$	$2.67^{de} \pm 0.12$

 $T_1$ =Day 1,  $T_2$ =day 2,  $T_3$ =Day 3,  $T_4$ =Day 4,  $T_5$ =Day 5. Means in each row having different superscripts vary significantly at values P<0.05. Values are presented as mean  $\pm$  SD.

### Effect of storage periods on proximate composition of beef liver

The values of proximate components have been shown in Table 2. Among the treatments, the DM content was significantly (P<0.05) increased whereas CP and EE content were decreased with the increased of storage days. Accordingly, table 2 shows that ash content at different treatments varies from 1.23% to 1.71%, indicating that ash content were significantly (P<0.05) increased with increase storage days. The same trend was also observed by Konieczny et al. (2007), reported that DM and CP content increased and decreased during frozen storage respectively. Agnihotri (1988) reported deterioration in meat lipids took place due to intermediary activities of endogenous meat enzymes leading to hydrolysis of fat. A non-significant decrease in ash percentage was reported by Ziauddin et al. (1993) which coincided with this study.

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Deremators (0/)			Treatments		
	$T_1$	T <sub>2</sub>	T <sub>3</sub>	$T_4$	T <sub>5</sub>
DM	$26.86^{b} \pm 0.56$	$26.87^{b} \pm 0.85$	$28.07^{ab} \pm 0.54$	28.31 <sup>ab</sup> ±0.26	$28.71^{a}\pm0.10$
CP	$18.43^{a} \pm 0.91$	-	$18.14^{a}\pm0.86$	-	$16.25^{a} \pm 2.19$
EE	$4.60^{a} \pm 0.18$	$4.49^{ab} \pm 0.15$	4.43 <sup>ab</sup> ±0.21	$3.92^{bc} \pm 0.02$	$3.6^{2c} \pm 0.10$
Ash	$1.23^{bc} \pm 0.03$	1.33 <sup>bc</sup> ±0.13	$1.44^{ab} \pm 0.01$	$1.51^{ab} \pm 0.11$	$1.71^{a} \pm 0.07$

 $T_1$ =Day 1,  $T_2$ =day 2,  $T_3$ =Day 3,  $T_4$ =Day 4,  $T_5$ =Day 5. Means in each row having different superscripts vary significantly at values P<0.05. Values are presented as mean ± SD. DM=Dry Matter, CP=Crude Protein, EE= Ether Extract.

#### Effect of storage periods on pH value and percentage of cooking loss of beef liver

The value of pH, and cooking loss (%) have been shown in table 3. The pH value and cooking loss were significantly (P<0.05) decreased and increased with increase of storage days respectively. The range of pH value at different treatments was 6.72 to 6.15 whereas the percentages of cooking loss were 11.38% to 23.68 %. The highest amount of pH indicates this product is most preferable for consumers' health. The decreasing pH was probably due to the secretions of microorganisms in the beef liver. Generally, the pH of fresh liver is 6.72 to 6.94. Previous study reported that pH values lower than 6.15 may be considered as indicator of beef liver spoilage (Hernandez-Herrero et al., 1999). Elsaaid (1993) found that pH of fresh beef liver 6.26 to 6.91. The cooking loss refers to the reduction in weight of beef liver during the cooking process (Jama et al., 2008). Major components of cooking losses are thawing, dripping and evaporation. Cooking loss in liver is important for maintaining an attractive retail display of beef liver. For example, meat and their products are a rich source of proteins, essential minerals and vitamins. The increased loss of such nutrients of meat decreases the nutritional quality and consumer demands (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 (Serdaroglu et al., 2005).

#### Table 3: Effects of storage periods and refrigerated temperature (4 °C) on physico-chemical properties of beef liver

Donomotors			Treatments		
Parameters	T <sub>1</sub>	$T_2$	T <sub>3</sub>	$T_4$	T <sub>5</sub>
pH	$6.72^{a}\pm0.07$	6.57 <sup>ab</sup> ±0.03	$6.46^{abc} \pm 0.07$	6.39 <sup>b</sup> c±0.04	6.15 <sup>c</sup> ±0.11
Cooking Loss %	$11.38^{d} \pm 0.24$	$16.96^{\circ} \pm 2.02$	$19.24^{bc} \pm 1.18$	21.63 <sup>ab</sup> ±2.06	23.68 <sup>ab</sup> ±0.73

 $T_1$ =Day 1,  $T_2$ =day 2,  $T_3$ =Day 3,  $T_4$ =Day 4,  $T_5$ =Day 5. Means in each row having different superscripts vary significantly at values P<0.05. Values are presented as mean  $\pm$  SD.

#### Effect of storage periods on biochemical properties of beef liver

The value of biochemical components such as FFA (%) and POV (meq/kg) have shown in Table 4. Both the FFA and POV values were increased with increase of storage days. The most preferable FFA was observed from 1st day and less preferable FFA was observed from 5th days of observation. Biochemical properties indicate the good or bad quality of beef liver. The lowest amount peroxide value indicates this product is most preferable for consumes health. 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.

Daramatara			Х		
Farameters	$T_1$	$T_2$	T <sub>3</sub>	$T_4$	T <sub>5</sub>
FFA (%)	1.14 <sup>c</sup> ±0.01	$1.27^{bc} \pm 0.20$	1.31 <sup>bc</sup> ±0.26	1.88 <sup>ab</sup> ±0.26	$2.07^{a} \pm 0.26$
POV (meq/kg)	$1.53^{\circ} \pm 0.25$	$1.73^{bc} \pm 0.02$	$1.73^{bc} \pm 0.02$	$1.74^{bc} \pm 0.01$	$1.94^{ab} \pm 0.01$
			4 4 41.00		

 $T_1$ =Day 1,  $T_2$ =day 2,  $T_3$ =Day 3,  $T_4$ =Day 4,  $T_5$ =Day 5. Means in each row having different superscripts vary significantly at values P<0.05. Values are presented as mean ± SD. FFA=Free Fatty Acid, POV=Peroxide Value.

#### Effect of storage periods on microbial load of beef liver

In the present study, we also observed the presence of micro-flora (TVC) and TYMC on fresh and preserved samples. According to the Table 5, the initial value of TVC, TCC and TYMC for fresh beef liver were significantly lower compared to storage samples, indicating that all these value were increased with increasing the storage days. The lower value indicates the freshness of product which is most preferable for consumers' health. Similarly, Haider (2018) stated that the mean value of TVC, TCC and TYMC for fresh beef sample is lower than preserved beef samples.

Table 5: Effects of s	storage periods a	at refrigerated t	temperature (4 C	) on microbial	population (	log cfu/g
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Daramatara		Treatments	
Farameters	$T_1$	$T_3$	T <sub>5</sub>
TVC	4.84 <sup>c</sup> ±0.05	$5.98^{b} \pm 0.02$	$7.05^{a}\pm0.01$
TCC	2.32 <sup>c</sup> ±0.01	$3.10^{b} \pm 0.01$	$3.74^{a}\pm0.02$
ТҮМС	$1.99^{c} \pm 0.01$	2.16 <sup>b</sup> ±0.03	$4.24^{a}\pm0.01$

 $T_1$ = Day 1,  $T_3$ = Day 3,  $T_5$ = Day 5. Means in each row having different superscripts vary significantly at values P<0.05. Values are presented as mean  $\pm$  SD.

### Conclusion

In conclusion, the results obtained from sensory attributes, proximate composition, physico-chemical properties, biochemical and microbial analysis suggest that shelf life of raw beef liver at refrigerated temperature  $(4^{\circ}C)$  is maximum three days with minor changes of quality. Therefore, the findings of the current study will contribute for further research in preservation of meat and meat products.

#### **Declaration of interest**

The authors have declared that no competing interests exist.

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