

Nutritional Quality Changes of Cooked Beef Due to Refrigeration Storage

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Abstract: This study was undertaken to assess nutritional quality of cooked beef stored at refrigeration (4 ± 1 °C) for 1 day, 3 days, and 5 days. A traditional method was used to cook the collected beef and subjected to analyze proximate composition, peroxide value (PV), total volatile base nitrogen (TVBN), bacteriological load and sensory quality. The moisture percentage of fresh cooked, 1 day, 3 days, and 5 days storage samples were 78.03, 65.78, 63.58, and 63.02 respectively. There was no significant difference ($P > 0.5$) in ash content with increasing time in refrigeration. The percentage of fat content in all the samples was almost same (6.94 ± 0.05) and showed a declining trend. The content of protein was found to be increased significantly ($p > 0.05$) from fresh cooked beef to 5 days storage samples. The PV in 5 days storage sample was 7.5 meq/kg fat indicates slight rancidity whereas other samples showed the value around 2 meq/kg. The TVBN content in fresh, 1 day, and 3 days storage samples was found to be (17 ± 2.6) mg/100gm while in 5 days storage samples it was increased to 36.4 mg/100gm protein. The fresh cooked beef was free from bacterial contamination whereas 1 day, 3 days, and 5 days storage samples recovered 5.8×10^5 , 7.8×10^5 and 8.8×10^5 (CFU/g). The sensory attributes of cooked beef showed a declining trend with increasing the storage period. Up to 3 days store at refrigeration, there are no major changes in nutritional and sensory quality of cooked beef.

Keywords: Bacterial load, Cooked beef, Nutritional quality and Refrigeration storage

Date of acceptance: 21-11-2017

I. Introduction

Meat and meat products constitute an important source of protein in man's diet. Intake of meat and meat products depends on several factors such as socio-economic conditions, ethical issues, religious beliefs and tradition (Font-I-Furnols and Guerrero, 2014). Among all meats, beef plays an important role in human nutrition providing a good source of high quality protein, vitamins and minerals especially iron and zinc (Jensen et al., 2014). The ICMR recommendation for protein consumption of 1 g/kg body wt. per day with Net Protein Utilization (NPU) of 65 could be achieved only by introducing the animal proteins in regular diet (Bhat et al., 2013). Fresh beef is not usually consumed readily which, unless cooked properly due to its unhygienic condition and microbial load (Donald and Sun, 1999). The cooked beef is also considered as a delicious item especially in Asia.

Being a working woman, it is difficult to manage time for cooking. Thus people always seek ready to eat or processed foods for consumption (Juul and Hemmingsson, 2015; Moubarac et al., 2014). Usually, the people of Bangladesh and many other Asian countries consume beef after cooking. The beef is cooked by using different recipe in different cultures. In Bangladesh, traditionally the beef is cooked by boiling in water with mixing several spices to increase its palatability. The cooking of beef usually takes enough time. As beef is a prestigious and palatable food, most people are intended to consume cooked beef by storing it for several days avoiding cooking hassles every day. Thus preservation of cooked beef is necessary.

Since refrigeration is employed to control the growth of microorganisms (Vithanage et al., 2017), storing cooked foods at refrigeration temperature ($4-7^{\circ}$ C) has been widely used to keep pleasant nutritional quality. Moreover, it is beneficial to store cooked beef at refrigeration temperature instead of raw beef because cooked beef contains different types of food additives such as oils, salts, spices etc. which enhance the keeping quality by protecting microbial growth and limiting undesirable physical and chemical changes such as lipid oxidation resulting in rancid flavor (Baljeet et al., 2015; Babuskin et al., 2014; Neilsen and Rios, 2000). However, people are flurried whether the cooked food stored at refrigeration is nutritionally safe. Although there is not enough study to confirm the changes occur in cooked food stored at refrigeration. Hong et al. (2015) indicated that cooked beef stored at refrigeration undergoes several changes which are undesirable to

consumers. Even the eating quality of refrigeration storage cooked beef could be reduced (Marchetti et al., 2017). With increasing the storage period in the refrigeration the sensory attributes of the cooked beef demonstrate declining trends (Bhat et al., 2013).

The predominant bacteria associated with spoilage of refrigerated beef and pork, are *Rochothrithermosphacta*, *Amobacterium* spp., *Enterobacteriaceae*, *Leucostoc* spp., *Lactobacillus* spp., *Pseudomonas* spp. The defects occurred in refrigerated meat due to spoilage by these bacteria are sour off-Ravours, discolouration, gas production, slime production and decrease in pH (Borch et al., 1996). The initial mesophiic bacterial count on meat and cooked meat products is about 100 to 1000 cfu/g, consisting of a large variety of species (Blickstad et al., 1981; Jackson et al., 1992). Only 10% of the bacteria initially present are able to grow at refrigeration temperatures, which is basically responsible to initiate spoilage (Gill and Molin, 1991). Therefore this study was undertaken to know how long the cooked meat could be kept nutritionally safe for consumption in refrigeration.

II. Materials and Methods

2.1 Place of Experiment

This study was conducted at the food Technology laboratory of Food Technology and Nutritional Science (FTNS) Department in MawlanaBhashani Science and Technology University (MBSTU), Santosh, Tangail and the laboratory of Department of Livestock Service (DLS), Tangail.

2.2 Sample Collection

About 1 kg of bonelessrawbeef was collected from the butcher shop of local market in Tangail. During collecting beef, it was noticed that the cow was immediately slaughteredand free from diseases. The fresh beef was identified visually by its reddish and bright color. It was firm, elastic and soft also. Natural odour of beef and intramuscular marbling was present.

2.3 Sample preparation

Collected beef was first cut into appropriate sizes for traditional cooking and washed with pure water to remove inedible substances. Afterwards these meats were mixed with salts, chilies, powders of dried chilies & turmeric, cumin, garlic, onion, cinnamon, ginger and other spices as necessary. Then it has been cooked with water and soybean oiluntil it gets palatable and delicious. Generally it takes 45 to 50 minutes to be cooked in a suitable pan with lid. Then the cooked beef was cooled and immediately transferred to a refrigerator at $4 \pm 1^{\circ}\text{C}$. The fresh cooked beef, 1- day, 3-days and 5-days stored beef were taken as samples respectively.

2.4 Moisture Content

Moisture content was determined in triplicate by placing an accurately weighed (5 g) amount of ground sample in a pre-weighed porcelain crucible in a thermostat oven at 105°C for 24 hours until a constant weight was obtained (AOAC, 2005). Moisture content was calculated by the following equation.

Moisture content (%) = $(\text{Initial weight} - \text{final weight}) / \text{weight of sample} \times 100$

2.5 Ash Content

The defatted samples are used for ash content analysis. The samples were subjected to a temperature of 600°C in a muffle furnace for 4 hours (AOAC, 2005). After completing the cycle the samples are removed from the furnace and kept into a desiccator to cool. Then ash content was weighing in an electronic balance.

2.6 Crude Protein Content

Kjeldahl method of analysis (AOAC, 2005) was used to determine crude nitrogen present in the beef samples. Then the resulting nitrogen was multiplied by 6.25 to obtain crude protein.

2.7 Fat Content

Fat content was determined according to the method outlined by Gheisari et al. (2010) and Lee (1996). A 5 g of sample was homogenized with 50 ml chloroform/methanol mixture (2/1, v/v) for 1.5 minutes in a blending jar on a warning blender. The homogenate was filtered through fast-speed filter paper (Whatman No. 1) into a separating funnel and 20 ml of 0.5% NaCl was added to separate the filtrate into two phases. The mixture was gently shaken and allowed to stand until a clear separation was visible. The methanol–water phase was discharged and the chloroform phase was taken into a test tube then the chloroform was evaporated in a vacuum oven at 60°C for 30 minutes. The following equation was used to calculate the fat content.

$$\text{Fat content (\%)} = \frac{\text{amount of fat extracted from samples (g)}}{\text{weight of samples (g)}} \times 100$$

2.8 Peroxide Value (POV)

The peroxide value (POV) was determined according to the method of Sallam et al. (2004). The samples (3 g) were weighed in a 250-mL glass stopper Erlenmeyer flask. Then it was heated for 3 min at 60°C in a water bath to melt the fat. After that the flask thoroughly agitated for 3 min with 30 mL acetic acid-chloroform solution (3:2 v/v) to dissolve the fat. Whatman filter paper number 1 was used in filtration process to remove beef particles from the filtrate. After adding saturated potassium iodide solution (0.5 mL) and starch

solution as indicator to filtrate, titration was continued against standard solution of sodium thiosulfate until the color disappeared. POV was calculated by following equation and expressed as milli equivalent peroxide per kilogram of sample.

POV (meq / kg) = $\{(S \times N) / W\} \times 100$, Where “S” is the volume of titration (ml), “N” is the normality of sodium thiosulfate solution (N=0.01) and “W” is the sample weight (g).

2.9 Total Volatile Base Nitrogen (TVBN)

Total volatile base nitrogen (TVBN) was determined according to the method describe in the official methods of analysis of AOAC international (AOAC, 2005). Exactly 10 gm of each ground sample was weighed and mixed with 90 ml of 6% perchloric acid. The samples were homogenized for 2 minutes with a blender under cooled condition. Afterwards the extract with 4–6 drops phenolphthalein was taken in Kjeldahl flask. 20 ml of 20% NaOH solution were added into the flask. The distillation outflow tube was submerged in a conical flask with 50 ml 3% boric acid solution containing 1 -2 drops of mixed indicator. After 15-20 minutes boiling in the flask, the distillate accumulated in conical flask was titrated with 0.01N HCl and appearing violet color of mixed indicator confirmed the end point. The following equation was used to calculate TVBN.

$$\text{Amount of TVB-N (mg/100gm sample)} = \frac{\text{ml of titrant} \times 0.14}{\text{sample weight}} \times 100$$

2.10 Total Bacterial Count

For bacterial count, after preparing nutrient agar media it was subjected to sterilize. Then the media was poured into petri-dishes in the laminar airflow. After then the samples were ground and diluted serially. Thereafter the diluted samples were transferred to the media containing dishes and placed in an incubator at 36°C temperature for 24 hours to grow out the bacterial colony. After 24 hours bacterial colonies were counted.

2.11 Sensory Evaluation

The sensory evaluation of the products was carried out for quality attributes such as appearance, flavor, softness, texture and overall palatability. Samples from each group were randomly assigned to panel members for sensory evaluation according to Sahoo and Anjaneyulu (1997). A seven educated and aristocrat panel member were chosen to evaluate the sensory attributes of cooked storage meat. The scores for sensory attributes were obtained by using 5 point hedonic scale where 1 = very unpleasant, 2 = moderately unpleasant, 3 = moderately pleasant, 4 = pleasant and 5 = very pleasant

2.12 Statistical analysis

Experiment was replicated 3 - 4 times and means and standard deviations were calculated for each parameter. The data obtained against different parameters were subjected to statistical analysis using SPSS 20. A 95% confidence level was used to analyze data.

III. Results and Discussion

The cooked meat samples stored in refrigeration were analyzed in order to elucidate whether the meat is fit for consumption after certain days of storage. To characterize the samples, several analyses were performed including proximate analysis, spoilage rate quantification and evaluation of sensory attributes.

3.1 Proximate Composition

The moisture content of cooked beef was gradually decreased from fresh cooked to 5 days storage in refrigeration. The fresh cooked beef sample contained 78.03% whereas the moisture content of 5 days storage sample was found to be 63.02% and indicated the lowest among all the samples (Figure 1). It could be due to drying of cooked beef samples during refrigeration. Though Kenny et al. (2008) said that moisture content of fresh meat should be around 75%. A significant difference ($p < 0.5$) was observed in the fresh cooked sample and 1 day storage samples with other samples though the content was almost same in the 3 days and 5 days storage sample ($p > 0.5$). Leygonie (2012) also reviewed that the moisture content of meat was decreased during freezing and refrigeration.

The ash content of all the samples was almost same. The highest percentage of ash content (1.98%) was found in the 5 days storage sample and the lowest content (1.90%) was observed in the fresh cooked samples (Figure 2) though the ash content between fresh cooked samples and other samples was significantly differed ($p < 0.5$). But the difference between refrigeration storage samples was not significant ($p > 0.5$) Due to decreasing the moisture content the overall mineral concentration has increased in the samples. Actually cooking and refrigeration have no impact on ash content. However addition of salt and spices during cooking may subsequently increase ash content during refrigeration storage.

About 6.98% of fat was extracted from fresh cooked beef sample which was highest among all other samples. And the lowest fat content (6.53) was observed in 5 days storage sample (Figure 3). The fat content of all the samples were gradually decreasing. The 5 days storage sample was significant different ($p < 0.5$) from other samples in terms of fat content however the difference between fresh cooked sample and 1 day storage samples was not significant ($P > 0.5$). The loss of lipid content in the refrigerated storage samples is mainly considered to be related with auto-oxidation of lipids (Sampaio et al., 2012). Sabow et al. (2016) and Soyer et al.

(2010) also reported that refrigeration storage has significant influence on lipid and protein oxidation resulting in loss of fat. This is the agreement with the findings reported by Maqsood et al. (2015) and Igene et al. (1979). They said that decrease in total fats in meat during refrigeration storage is could be due to changing in the triglycerides levels.

Although the fat content and moisture content was gradually decreasing, the protein content likewise the ash content, showed a linear increasing trend with increasing the storage time in refrigeration. A significant difference ($p < 0.5$) in protein content was observed in all the samples (Figure 4). Although the 3 days storage sample did not vary that much with 1 day and 5 days storage samples in terms of protein content. The increase of protein might be due to synthesis of protein from non-protein nitrogenous substances by resident microorganisms as the number of microorganisms was gradually increased (figure 7) with increasing storage time (Agunbiade et al., 2010). This result also agreed with the study of Ellis and Goodcre (2011).

3.2 Evaluation of Spoilage Rate

Peroxide value indicates the overall picture of rancidity of fat present in the beef. Lipid peroxidation is one of the prime mechanisms of quality deterioration in stored meat and meat products (Pearson et al., 1983). It is widely used method to measure oxidative rancidity (Salih et al., 1989). Increasing peroxide value triggers lots of undesirable reactions which enhance the unacceptable taste in meat. Up to 3 days storage in the refrigeration the peroxide value of cooked meat was increased to 2.6 meq kg^{-1} from 1.8 meq kg^{-1} of fresh coked sample (Figure 5). And the difference between the samples from fresh cook to 3 days storage was not significant ($P > 0.5$). However after 5 days of storage the peroxide value was increased to 7.5 meq kg^{-1} and made a significant difference ($P < 0.5$) with all other samples. It is also agreed with the study of Gheisari (2011). It has also been reported that peroxide value and thiobarbituric acid reactive substances increased significantly with increasing storage time in refrigeration in both minced chicken meat and beef (Gheisari, 2010). They reported that it could be due to increasing peroxide formation during storage rather than degrading the peroxides into other oxidative products.

The total volatile base nitrogen (TVBN) could be used as quality indicator for meat and meat products and is associated with decarboxylation of amino acids activity of microorganisms (Jay, 1992). It could be used as index of meat quality under refrigeration storage according to (Pearson, 1975) which stated that TVBN of fresh cooked meat should be 13 and the acceptable value is ≤ 17 . The TVBN was increased with increasing time of storage in the refrigeration. Up to three days storage the value was increasing slowly but the samples stored for 5 days showed an abrupt increase of TVBN value reached to $36.4 \text{ mg}/100\text{g}$ whilst the TVBN value of fresh cooked sample was $14.4 \text{ mg}/100\text{g}$ (figure 6). This result agreed with the study conducted by Abd El-Aal (2005).

Standard plate count for bacteria in fresh cooked beef sample was 0 CFU/g and the value was increased gradually with increasing storage period. The bacterial colony recovered from 1 day, 3 days and 5 days cooked beef samples was $5.8 \times 10^5 \text{ CFU/g}$, $7.8 \times 10^5 \text{ CFU/g}$ and $8.8 \times 10^5 \text{ CFU/g}$ respectively (figure 7). According to Dempster (1986) the total microbial count of meat and meat products should be between $10^3 - 10^7/\text{cm}^2$. The growth of microbes in meat is one of the main factors for discoloration and spoilage. This is the agreement with the study conducted by Bhat et al. (2013). They reported that microbes in meat could be extended up to 21 days of storage in refrigeration. Under refrigeration, a linear increase in total plate counts of chicken was also reported by Nath et al. (1995). Kalaikannan et al. (2007) also reported that a gradual increase of psychrophilic counts was observed during refrigeration storage of chicken products.

3.3 Acceptability and Preference of Sensory attributes

The mean values and standard deviations of various sensory attributes of fresh cooked, 1 day, 3 days and 5 days refrigeration storage samples are represented by table 1. The sensory qualities such as appearance, flavour, softness, texture and overall palatability of cooked and storage samples were showed a declining trend with increasing storage time in refrigeration. A significant difference in quality attributes was observed between all samples. Even one day refrigeration storage sample was significantly different from fresh cooked beef sample. However, the overall palatability and appearance score was not significantly different till 1 day storage at refrigeration. Till 3 days of refrigeration storage, the acceptance of cooked beef was moderately pleasant. But until it reaches to 5 days of storage, it has lost its acceptability though none has remarked that it was very unpleasant. The decrease in appearance scores might be due to lipid and protein oxidation. A decrease in appearance or colour score of meat products with increase in storage period was also reported by Kalaikannan et al. (2007) and Nag et al. (1998). Decreasing flavour score with increasing storage time was also reported by Nag et al. (1998) and Sharma et al. (1988). Softness or Juiciness scores also followed a decreasing trend in the cooked beef samples. It could be due to loss of some moisture from the samples during storage (figure 1). This results also supported by Kaliakannan et al. (2007). Texture scores were also changed with significant differences ($P < 0.05$) between the samples. It might be due to dehydration of cooked samples during refrigeration storage resulting hardening texture. Breakdown of fat as well as protein also might be contributed

to changing the texture. The findings were strongly supported by the study conducted by Kumar and Sharma (2004).

IV. Tables And Figures

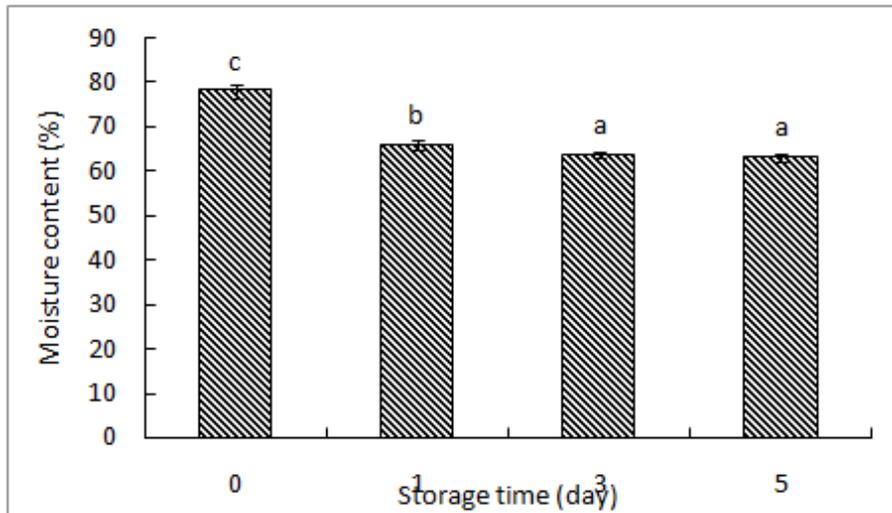


Figure 1: Moisture content of cooked beef in refrigeration storage (Bars with different superscript are significantly different ($p < 0.05$))

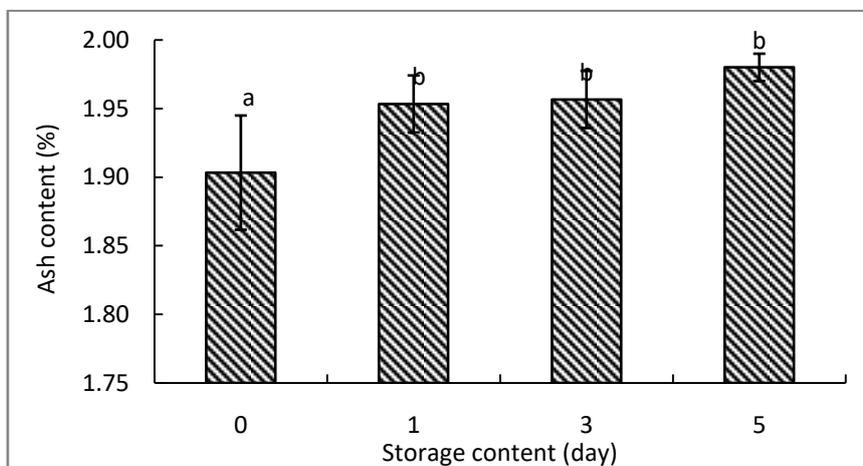


Figure 2: Ash content of cooked beef in refrigeration storage

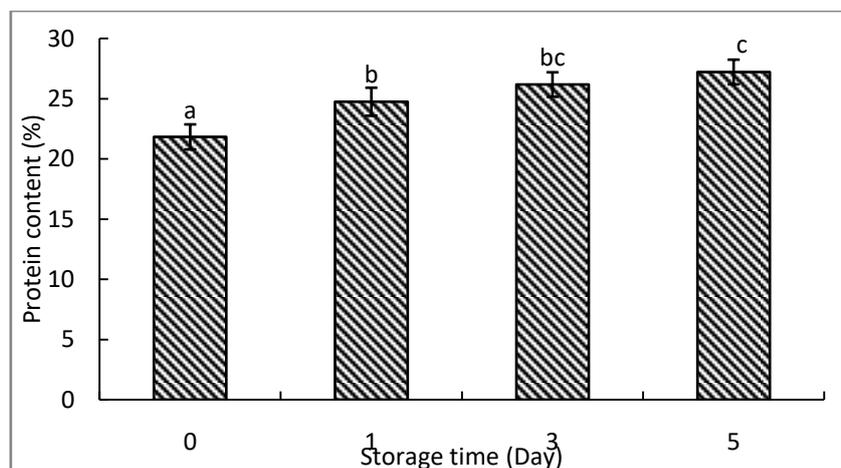


Figure 3: Protein content of cooked beef in refrigeration storage

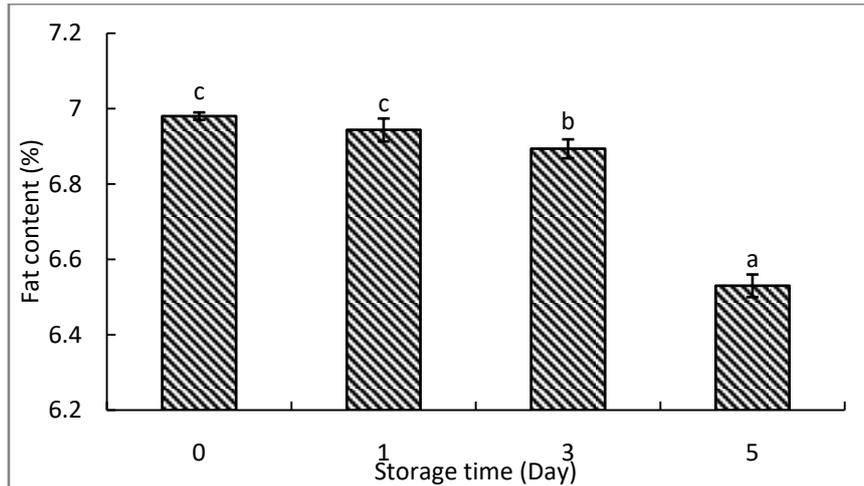


Figure 4: Fat content of cooked beef in refrigeration storage

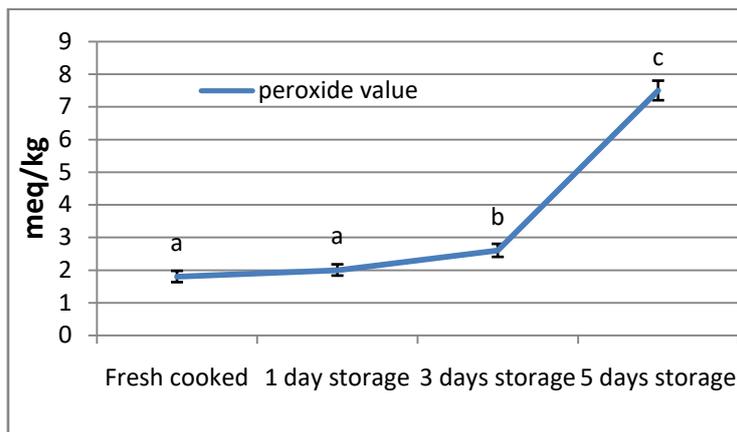


Figure 5: Peroxide content (meq/kg) of cooked beef in refrigeration storage

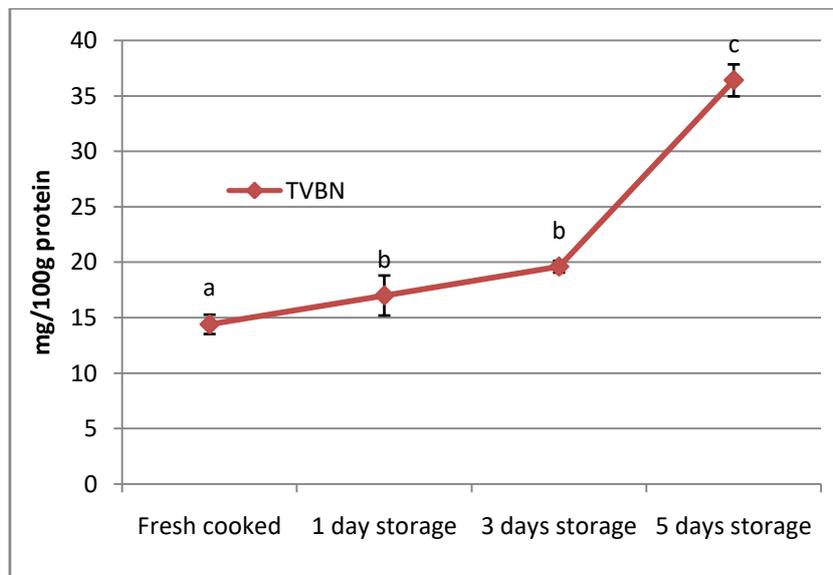


Figure 6: TVBN (mg/100g protein) content of cooked beef in refrigeration storage

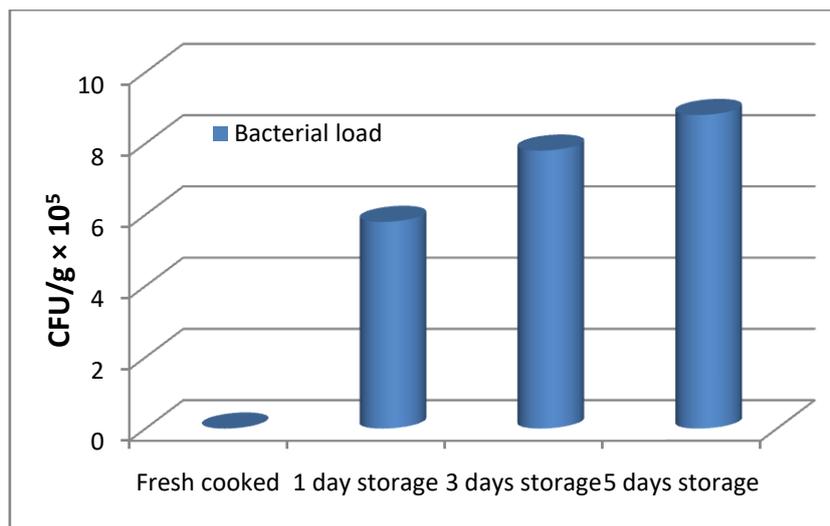


Figure 7: Bacterial load in the cooked beef during refrigeration

Table 1: Evaluation of sensory attributes

Sensory parameter	fresh cooked	1day storage	3 days storage	5 days storage
appearance	4.71±0.49 ^c	4.29±0.49 ^c	3.14±0.9 ^b	2±0.58 ^a
flavor	5.00±0 ^d	4.29±0.76 ^c	3.14±0.69 ^b	1.86±0.69 ^a
softness	4.57±0.53 ^d	3.71±0.49 ^c	3±0.82 ^b	2.29±0.49 ^a
texture	5±0 ^d	4.14±0.69 ^c	3.14±1.07 ^b	2.14±0.69 ^a
overall palatability	4.43±0.53 ^c	4±0.58 ^c	3.14±0.38 ^b	1.43±0.53 ^a

Mean±SD. Different superscripts indicate significantly different from others (p< 0.05).

V. Conclusion

The quality parameters of beef samples indicate positive significance in the aspect of human nutrition and considered as dietary source of first class protein. It also plays an important role to meet protein requirements. Preservation of cooked beef by refrigeration temperature has been suggested as an efficient way to keep well of its overall quality. After completing this study, it can be thus concluded that a significant change occurs in the cooked beef stored at refrigeration though storage till 3-days retains acceptable quality. However, concerning bacterial load the refrigerated cooked beef should be reheated before consumption. Whenever it exceeds 3 days, the cooked beef stored at refrigeration, started to be unfit for consumption though the protein content increased significantly.

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Md. NannurRahman Nutritional Quality Changes of Cooked Beef Due to Refrigeration Storage.” *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, vol. 11, no. 10, 2017, pp. 49-56.