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Influence of Histological Changes of Refrigerator Preserved Buffalo Meat on Quality Characteristics

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Abstract: Buffalo meat is the only future remedy for nutritional security in India. If the quality gets deteriorated, the meat preserved in refrigerator would impact greatly on the health of consumers. Hence meat samples from five year old sixteen buffalo bulls were analyzed in the fresh state (0 day) and after 4 and 7 days in chiller (4±1°C) and 4, 7, 14, 30, 60 and 75 days in freezer (–10±1°C) in a domestic refrigerator. Chiller storage showed shrinkage and kinking of muscle fibres. Contrastingly, freezer showed separation of muscle fibres, transverse breaks and different configuration of structural damages due to ice crystal formations. The values of WHC, moisture% and protein% decreased with increasing storage period. Whereas, texture, tenderness, chilling loss and drip loss showed an increasing trend. Thus it was concluded that a storage period upto 4 days in chiller and 30 days in freezer could satisfactorily maintain the buffalo meat quality.

Key words: Buffalo meat, chiller, freezer, structural damage, whc, moisture, protein, texture, tenderness, chilling loss, drip loss, storage period

INTRODUCTION

India has 94.13million number of buffaloes, which accounts for 50.56% of the total buffalo population in the world. Presently, India credited 1.43 mMT of buffalo meat to its total meat pool (4.65 mMT) contributing 2.03% of world meat production[1]. Only the buffalo meat has the export potential from our country. Major quantity of meat is exported in frozen form from India to Malaysia and Gulf countries with an annual foreign exchange revenue to the tune of Rs. 1375.04 Crores[2].

In India, most of the meat is purchased by the consumers in fresh or frozen form. To adjust with the fast growing life style of urbanization, they hardly find time to purchase meat daily. Hence they purchase meat in bulk to meet their daily requirements. This meat is stored in refrigerator and consumed on definite intervals. Deterioration of meat quality in refrigerator storage may have great impact on the health of consumers. Considering the importance from consumer viewpoint, a program has been ascribed to study the effect of refrigerator storage on buffalo meat quality. The objective of this study was to determine the histological changes of buffalo meat preserved in domestic refrigerator and its effect on meat quality characteristics. Thereby the shelf life of buffalo meat in Chiller and Freezer was established. This study has immense importance to satisfy consumer's query relating to how long buffalo meat can be stored without any deterioration in domestic refrigerator.

MATERIALS AND METHODS

Sample collection and preservation: Meat samples each weighing 2 kg from shoulder were removed from 20 buffalo bulls of 5 years old slaughtered at Municipal slaughter house, Tangra, Kolkata. The muscles were then utilized for the study. Four meat samples from different buffalo bulls of same age group were used in a single trial. Age of the buffalo bull carcasses was estimated by observing dentition. The samples were wrapped in highly gas permeable low density polyethylene and transported to the laboratory within 1 hour postmortem.

The samples were kept in chiller (24 hrs at 4±1°C) for ageing[3]. The separable fat and connective tissue were removed. Then the samples were packaged in low-density polyethylene, each containing 250 g of meat sample and stored in chiller and freezer respectively for further study. One portion was analyzed in the fresh state (0 day) and the remaining portion after chiller storage (4±1°C) for

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4 and 7 days and freezer storage (–10±1°C) for 4, 7, 14, 30, 60 and 75 days in a domestic refrigerator (Godrej Cold Gold Model). The stored samples were then studied for histological changes to determine the influence of structural damage on meat quality.

Microscopic study: Samples of fresh, chilled and frozen meat of 0.5 mm thickness were fixed in 10% Formal Saline for 48 hrs. The Formal Saline fixed tissues were processed and embedded in Paraffin blocks. The Paraffin sections of 4-5 μ were cut by conventional methods.

The microsections were stained by Haematoxyline and Eosin, examined under microscope and photographs were taken.

Water holding capacity (WHC): The method used for determining the WHC was a modification of the high speed centrifugation method[4].

In this method, 2 g meat samples were accurately weighed and then centrifuged at 6000 rev/min for 10 min using a centrifuge machine (REMI model). No water was added to the samples and, after centrifuging, the juice expressed was decanted off or used for other purposes, if required. The meat samples were removed from the tubes with forceps, carefully dried with tissue paper and then reweighed, to determine liquid loss. The water content of the muscles when raw and after centrifuging was determined by oven drying (105°C for 24 hrs).

Chilling loss and drip loss: Weight of the meat packaged before keeping in chiller storage and after experimental period were noted and their difference in weights were expressed as chilling loss percentage (%).

Drip loss was estimated by measuring the exuded meat juices after thawing and expressed as percentage (%) of the initial weight.

Moisture and protein content: The moisture and protein contents of buffalo meat were determined by the methods of AOAC[5].

Texture and tenderness scores: The samples were cut into cubes of uniform size, cooked in a pressure cooker at 15 pounds pressure for 5 minutes and served warm with code numbers to a trained 10 member consumer panel. They were requested to score their individual preference in a nine point sensory scale. Individual ratings of panel members for the characteristic texture and tenderness scores were subjected to statistical analysis as per the methods outlined by AOAC[5].

Statistical analysis: In the present investigation, data obtained from the experiment were statistically analyzed and interpreted for different types of estimation following the methodology as outlined by Snedecor and Cochran[6].

RESULTS AND DISCUSSION

Histological changes: Histological changes revealed significant changes in refrigerator preserved muscle structure. The fresh (Fig. 1) buffalo meat showed tremendous histological change on subsequent storage in chiller and freezer. On 4th day (Fig. 2) chiller (4±1°C) storage the muscle fibres were separated by small intracellular spaces and the nucleus retained the haematoxyline-eosin stain. Bending or kinking of the fibres was well distinct at this instant. In 7th day (Fig. 3) chilled meat the changes were comparatively greater than the 4th day chilled meat. Whereas in 4 days freezer (-10±1°C) stored meat (Fig. 4) the fibres showed mild kinking along with mild separations and initiation of breaking of muscle fibres.

7 days (Fig. 5) frozen muscle showed an increased separations and breaking of fibres but disappearance of kinking. [7]reported similar findings in cold stored meat.

In 14 days (Fig. 6) stored muscle, separations of muscle groups with longitudinal spaces and appearance of transverse breaks were noticed.

The muscle showed more separation of muscle groups and torn muscle fibres in 30 days (Fig. 7) frozen meat. The cause of transverse breaks might be due to physical stress produced by contraction and by action of autolytic enzymes. Freezing of tissue involves essentially three major possibilities of damage[7]: cellular puncture or rupture by formation of ice crystals, damage to the cell by production of increased osmotic pressure and an invisible precipitation or denaturation of the colloidal cell constituents.

60 days (Fig. 8) frozen muscle showed a great separation of muscle groups and the muscle fibres have undergone a different configuration with increased structural damage. The severe structural damage was due to ice crystals formed intracellularly exerting pressure in opposite direction and tearing the fibre in greater. The greatest increase in tenderness obtained with 60 days of storage coincided with an observed increase in frequency of fibre breaks. On 75 th day (Fig. 9) of frozen storage the degree of fibre breaks were greater than 60 th day with large extra cellular spaces. As evident from this study, ice formation causing cellular rupture was also reported by Koonz and Ramsbottom[8], Love[9] and Partman[10].

The prominent nucleus retaining haematoxyline stain in all the figures showed that the tissues were not

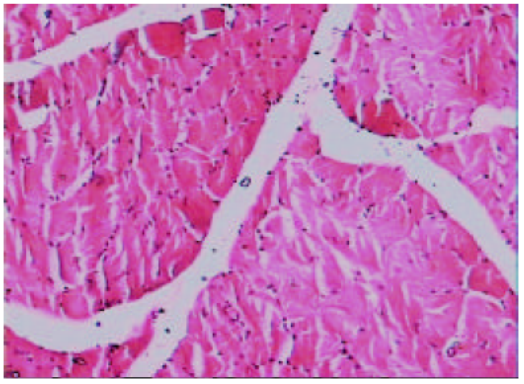

Fig. 1: The section of the muscle showing normal histological structure. The nucleus retained haematoxyline stain (H and E, × 100)

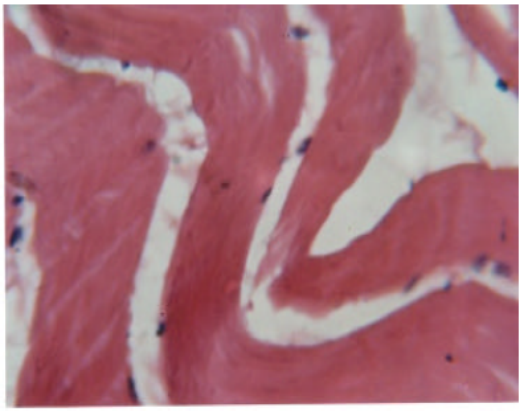

Fig. 2: The section of the muscle showing separation of muscle fibres by small intracellular spaces and the extent of kinking and waves of fibres are prominent. The nucleus retained haematoxyline stain (H and E, × 450)

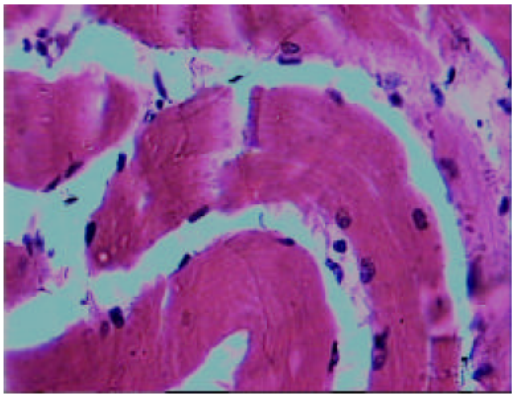

Fig. 3: The section of the muscle showing separation of muscle fibres by prominent large intercellular spaces, kinking and breaking of fibres. The nucleus retained haematoxyline stain (H and E, × 450)

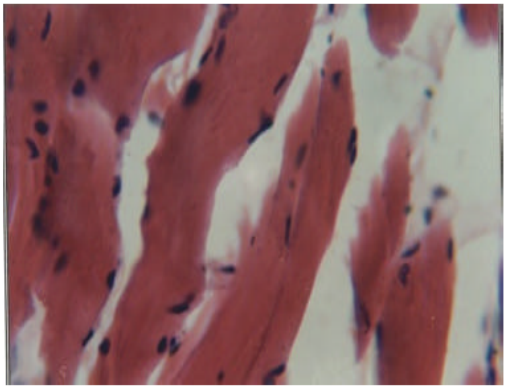

Fig. 4: The section of the muscle showing separation and breaking of the muscle fibres along with bending and kinking of the fibre. The nucleus retained haematoxyline stain (H and E,× 450)

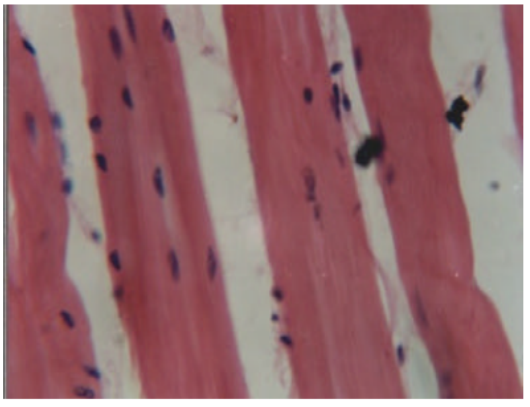

Fig. 5: The section of the muscle showing increased separation and breaking of the fibre but disappearance of kinking. The nucleus retained haematoxyline stain (H and E, × 450)

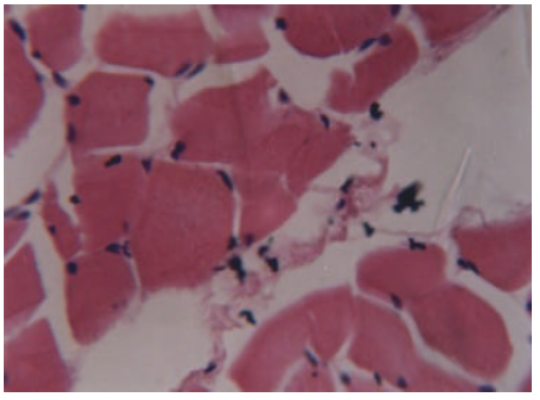

Fig. 6: The section of the muscle showing separation of muscle groups with longitudinal spaces and the appearance of transverse breaks. The nucleus retained haematoxyline stain (H and E, × 450)

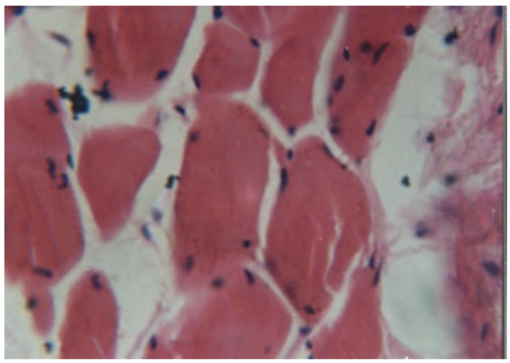

Fig. 7: The section of the muscle showing more separation of muscle groups and torn muscle fibres. The nucleus retained haematoxyline stain (H and E, × 450)

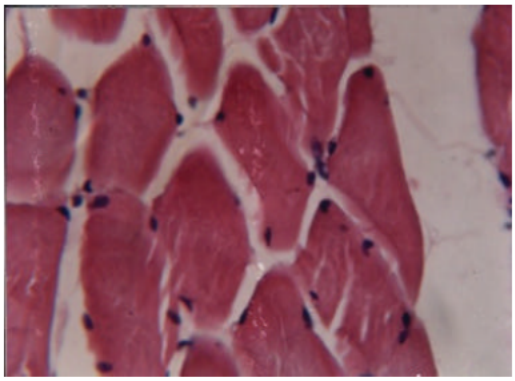

Fig. 8: The section of the muscle showing great separation of muscle groups and muscle fibres have undergone a different configuration with increased structural damage. The nucleus retained haematoxyline stain (H and E, × 450)

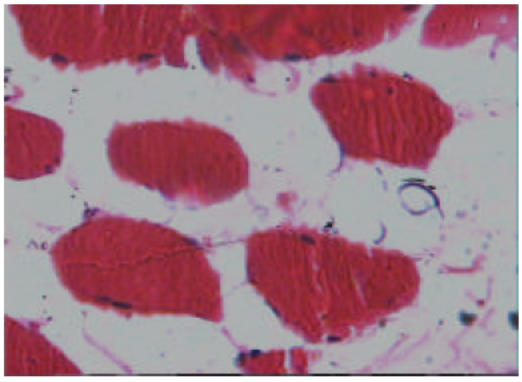

Fig. 9: The section of the muscle showing great separation of muscle groups and muscle fibres have undergone increased serrated structural damage . The nucleus retained haematoxyline stain (H and E, × 450)

necrosed at any extent. Indirectly, it showed the palatability characteristics of the meat.

Water holding capacity (WHC): Prolonged storage of buffalo meat showed a significantly lower WHC than its fresh state (Table 1). Freezing produces some changes in the tissue, which reduces the WHC after thawing[11]. Chilling resulted in poor WHC than freezing[12]. WHC in the 4th day freezer stored meat was higher than the 4th day chiller stored meat. A high level of WHC in freezer was due to better water retention of meat caused by an immobilization of tissue water within the myofibrillar system[13]. In 7 day chilled meat the WHC was observed to be lower than on 7th and 14th days of freezer stored meat. The loss of WHC observed was partly due to increased denaturation of protein and partly due to enhanced movement of water into extracellular spaces. The values decreased much lower on 30th, 60th and 75th days of freezer storage. The loss of WHC in prolonged storage of meats may be due to the rate in post mortem pH falls, ice crystal formation, high ionic strength, protein denaturation, drip loss and above all, the bulk of meat stored and the capacity of the refrigeration facility[14].

Chilling loss and drip loss: On the 4 th day in the present study, the observations showed a significant chilling loss in the stored buffalo meat (Table 1). On the same day, the frozen (-10±1°C) buffalo meat showed a significant drip loss. Van der wal *et al.*[15] observed a drip loss in buffalo longissimus lumborum as 3.9% in conventional chilling when meat was nearer to the ultimate pH. As the storage period in the freezer increased, the amount of drip got increased[16-19], as recorded in the present study (Table 1). This marked increase in drip on later days of storage were due to shortening of the sarcomere[20], increased enzyme activity[18], the degree of fibre distribution and translocation of water. Curie and Wolfe[21] showed that the amount of juice squeezed from beef muscles increased from 1-30% as the extracellular space increased with storage time.

Moisture and protein content: The moisture content of the freshly slaughtered buffalo meat was higher than the chiller and freezer stored meat (Table 1). A slight difference in moisture content was observed between the 4th day chiller and freezer stored meat. This loss in moisture was due to evaporation of moisture from meat in chiller[22]. Whereas, it was due to sublimation of surface water of the meat to colder surfaces in the vicinity of the freezer[23]. Gradually the moisture content of the meat decreased on 7th and 14th days of freezer storage. Kondaiah *et al.*[12] studied the moisture content of frozen buffalo meat at-10°C for 7 days as 76.4%. Subsequently, on 30th, 60th and 75th days of freezer storage a significant ($p<0.05$) decrease in moisture

Table 1: A comparative appraisal between meat quality parameters at different storage periods

	0	4C°	4F°	7C°	7F°	14F°	30F°	60F°	75F°
WHC (%)	75.98±0.57^{a}	71.83±0.47^{ab}	73.69±0.67^{ab}	67.68±0.02^{c}	71.91±1.10^{b}	67.92±1.10^{c}	62.98±1.52^{d}	57.84±1.08^{e}	53.88±0.07^{f}
CLDL (%)	0	0.17±0.01^{fh}	3.24±0.23^{g}	0.67±0.01^{f}	5.03±0.41^{e}	10.91±0.29^{d}	12.08±0.54^{c}	14.99±0.60^{b}	
Moisture %	76.87±0.01^{a}	76.31±0.06^{a}	76.18±0.09^{a}	72.94±0.04^{d}	75.66±0.03^{ab}	74.21±0.04^{c}	70.73±0.03^{e}	68.25±0.04^{f}	65.17±0.03^{g}
Protein %	20.25±0.06^{a}	18.95±0.04^{b}	19.80±0.06^{ab}	15.70±0.02^{d}	19.69±0.09^{ab}	19.23±0.09^{ab}	18.10±0.06^{cb}	16.57±0.07^{de}	16.19±0.01^{d}
Texture	5.00±0.16^{dc}	5.03±0.20^{c}	5.34±0.13^{c}	NR	5.44±0.18^{c}	5.56±0.18^{c}	6.38±0.29^{b}	7.13±0.18^{a}	7.09±0.10^{a}
Tenderness	5.13±0.47^{c}	5.25±0.19^{c}	5.44±0.24^{bc}	NR	5.56±0.16^{bc}	5.94±0.27^{b}	6.38±0.24^{b}	7.44±0.16^{a}	7.52±0.03^{a}

Means bearing different superscripts differ significantly ($p<0.05$), C°represents Chiller (4±1° C) storage on respective days, F° represents Freezer (-10±1° C) storage on respective days, NR: Value was not recorded since the meat was spoiled

content was recorded. The marked moisture losses encountered in later storage periods may be accounted for the myofibrillar distortion undergone by the meat in the freezer that led to the poor water retention ability of the meat.

The protein content of fresh buffalo meat decreased significantly as the storage period increased (Table 1). In fact, Krishnan and Sharma[24] reported buffalo skeletal meat containing 20.32% total protein. The main reason behind decline in protein on prolonged storage was due to protein denaturation exhibited in drip loss and proteolysis induced by enzymatic activities of psychrotrophic microbial growth[25]. Buffalo meat stored for 4 days in chiller (4±1 °C) showed a significantly lower protein content than the freezer (-10±1 °C) stored meat. The lower protein content of chilled meat might be due to increased microbial growth resulted from higher water activity (a_W) and enzymatic autolysis[26] In 7 day chilled meat the protein content was observed to be lower than 7th and 14th days of freezer stored meat. In fact, on 30th, 60th and 75th days of frozen storage the meat has lost a significant ($p<0.05$) amount of protein. This significant loss in later days of freezer storage might be a result of ice formation raising the solute concentration in the tissue.

Texture and tenderness scores: The texture scores of buffalo meat improved quite significantly ($p<0.05$) with prolonged storage period in chiller and freezer (Table 1). 4 day stored buffalo meat in chiller (4±1°C) showed a lower texture score than the freezer (-10±1°C). The scores increased gradually with increase in freezer storage. This increased score in freezer storage was also studied by Arief *et al.*[22] Freezing tenderized meat by splitting fibre and breaking or stretching connective tissue surrounding muscle fibres and fibre bundles[27]. Texture scores of 30th and 60th days of frozen meat showed a significant ($p<0.05$) variation.

The present study showed an increase in tenderness scores of buffalo meat with increase in storage period (Table 1). 4 day freezer stored buffalo meat showed an increase in tenderness score than the chilled meat. An increased tenderness score in chilled meat was reported by Dushyanthan *et al.*[28] Spoilage was observed in 7th day chilled meat. Since that meat couldn't be used for consumption, the scores were not recorded on that day. The results showed a gradual increase in tenderness scores in 7,14,30,60 and 75 days of frozen buffalo meat. The results showed a significant ($p<0.05$) improvement in tenderness scores at 30 and 60 days of storage. Guenther *et al.*[29] indicated that the formation of intercellular and intracellular ice crystals during frozen condition indirectly contributed to tenderisation of meat.

CONCLUSION

Histological changes revealed shrinkage and kinking of muscle fibres in chiller storage. As the storage period increased in freezer, separation of muscle fibres, transverse breaks, different configuration of structural damages due to ice crystal formations were evident.

The results distinctly revealed a significant ($p<0.05$) variation in WHC, moisture, protein, texture and tenderness scores at different storage periods of chiller and freezer. It was evident that at a given instant freezer maintained the meat better than chiller with respect to physicochemical parameters. The values of WHC, moisture and protein content decreased with increasing storage period. Where as, texture, tenderness, chilling loss and drip loss exhibited an increasing trend.

Sixty days of frozen storage improved some of the sensory qualities of buffalo meat Owing to the fact that meat is praised for its richness in protein, domestic frozen meat would be best if consumed before 30 days of storage. The loss of protein as drip with increased frozen storage was mainly attributed to the large extracellular ice crystal formation in the muscle fibres. Taking into account of all the above considerations, it may be concluded that a storage period upto 4 days in chiller (4±1°C) and 30 days in freezer (-10±1°C) would satisfactorily maintain the buffalo meat quality.

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