

EFFECT OF AGEING ON PHYSICOCHEMICAL PROPERTIES AND TEXTURAL CHANGES IN HOT BONED MEAT FROM YOUNG AND OLD INDIAN WATER BUFFALOES (*BUBALUS BUBALIS*)

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**ABSTRACT**

Current study was carried out to investigate the ageing changes in hot boned meat from young (<2 years age) and old (spent animals of >10 years age) buffaloes at refrigeration temperature over a period of 6 days. The pH and water holding capacity have reduced ( $P<0.05$ ) during ageing period in both young and old buffalo meat. Protein extractability and muscle fibre diameter was higher ( $P<0.05$ ) in meat from old buffaloes relative to young buffalo meat, however no change ( $P>0.05$ ) was observed during ageing. Increase ( $P<0.05$ ) in myofibrillar fragmentation index was observed in young buffalo meat on 6<sup>th</sup> day of ageing. Reduction ( $P<0.05$ ) in Warner-Bratzler shear force values were observed on 6<sup>th</sup> day of ageing compared to 0<sup>th</sup> day in both young and old buffalo meat. The SDS-PAGE analysis revealed the appearance of 30 kDa protein bands on 4<sup>th</sup> and 6<sup>th</sup> day of ageing in young and old buffalo meat, respectively. These results suggests tenderization of hot boned buffalo meat subjected to ageing.

**Keywords:** water buffalo, *Bubalus bubalis*, hot meat, ageing, tenderization, Warner Bratzler shear force

**INTRODUCTION**

India has 112.91 million water buffaloes (*Bubalus bubalis*) accounting for 57.8% of the total world buffalo population (FAOSTAT, 2013). India also produces 1.43 million tonnes of buffalo meat annually and accounts for 36% of total buffalo meat produced in the world contributing significantly to human nutrition. Rapid increase in domestic and export markets for buffalo meat in India and South-east Asia is playing an important role in the socioeconomic development of rural Asia. Demand for buffalo meat is increasing in several parts of Asia and Europe due to relatively lean meat quality with a fat content of less than 2% and the absence of Mad Cow Disease due to feeding of grass and farm by-products. Buffaloes in India are triple purpose animals used for the production of milk, meat and draught power and are usually slaughtered at old age after completion of their milk production period making the meat coarse and tough with poor quality attributes. Domestically it is a common practice in India to process hot boned meat immediately after slaughter of animals, which further makes the meat tough. Hot boning is defined as the removal of muscles from the carcass prior to chilling (West, 1983). However,

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removal of muscles from the carcass during rigor phase allows them to contract more, than muscles conventionally chilled while attached to the skeleton (Locker, 1960). Studies have been conducted to understand the effect of hot boning in beef (White *et al.*, 2006), lamb (Kim *et al.*, 2013) and chicken (Naveena and Mendiratta, 2001).

Carcasses from young (1 to 2 years age) and old (12 years age) buffaloes were studied with respect to their physical and chemical aspects and showed that meat from young buffalo was more tender than old buffalo (Syed Ziauddin *et al.*, 1994). Kandeepan *et al.* (2009) studied the processing quality of meat produced from young and old buffaloes and concluded that young buffalo meat may be processed into whole muscle or chunked meat products whereas, meat from old buffalo is appropriate for ground/emulsion meat products. Tenderization of tough buffalo meat has been achieved using different enzymatic (Naveena *et al.*, 2004) and chemical (Naveena *et al.*, 2011) methods. Neath *et al.* (2007) have compared the tenderness and few characteristics of water buffalo meat and beef during postmortem ageing and demonstrated superior tenderness in water buffalo meat relative to Brahman beef. The ageing process typically takes 1 to 2 days in chicken, 3 to 6 days in pork, and 10 to 20 days in beef (Smulders *et al.*, 1992). During ageing, the calpain system is believed to play an important role in degradation of myofibrillar proteins and development of tenderness (Koochmaraie, 1996). Previous research indicates that postmortem ageing increases beef tenderness (Ruiz de Huidobro *et al.*, 2003), which has been shown to be a major contributing factor to consumers' perception of taste.

Extensive studies have been conducted on the tenderisation process in beef (Huff-Lonergan *et al.*, 1996; Koochmaraie, 1996; Wheeler *et al.*, 2000)

indicating many variables that affect the tenderness of meat during postmortem ageing. However, there is no published literature comparing the quality of meat from young and old buffaloes in terms of tenderisation during postmortem ageing. Hence, this work was undertaken with an objective to evaluate physico-chemical changes in hot boned meat from young and old buffaloes during postmortem ageing. Outcomes of this research may help to develop innovative processing strategies to minimize tenderness variability as a tool of meat quality improvement from young and old buffaloes.

## MATERIALS AND METHODS

### Procurement of buffalo meat and ageing

The *Longissimus thoracis et lumborum* muscles were harvested from hot boned carcass from 18 young (<2 years) and 18 old (>10 years) water buffaloes (*Bubalus bubalis*) immediately after exsanguination from local municipal slaughterhouse of Hyderabad, India. Buffaloes were slaughtered according to traditional halal method followed in India and weighed approximately 100 kg for young and 400 kg for old animals, respectively. Each muscle was cut into 4 portions, packed in low density polyethylene (2325 to 6510 cm<sup>3</sup> O<sub>2</sub>/m<sup>3</sup>/24 h at 3°C) bags under atmospheric conditions and randomly assigned to four different ageing periods (0, 2, 4 and 6 days) at 4±1°C in domestic refrigerator. At each designated ageing period, meat samples were analysed for pH, water-holding capacity, protein extractability, muscle fibre diameter, myofibrillar fragmentation index, myoglobin content and %metmyoglobin. Cooked meat steaks were also evaluated for Warner-Bratzler shear force values and cooking yield.

## Sample analysis

### pH and water-holding capacity

Five g muscle sample was homogenized with 50 ml chilled distilled water in a tissue homogenizer (Daihan Scientifics, WiseMix, HG-15D, Korea) and pH value was measured using a cookingized electrode attached to digital pH meter (Thermo Orion, Model 420A+, USA). For determining water-holding capacity (WHC), 20 g of minced meat sample was placed in a centrifuge tube containing 30 ml, NaCl (0.6 M) and was stirred with a glass rod for 1 minute and centrifuged at 9000 g using refrigerated centrifuge (Sorvall Biofuge Stratos, Thermo electron LED GmbH, D-37520, Osterode, Germany) for 20 minutes at 4° C. The supernatant was measured and amount of water retained by samples was expressed in percentage (Wardlaw *et al.*, 1973).

### Protein extractability

Protein extractability was determined according to procedure of Joo *et al.* (1999). Sarcoplasmic and total proteins (sarcoplasmic + myofibrillar) were extracted separately by homogenizing 2 g sample with 20 ml of ice-cold 0.025 M potassium phosphate buffer (pH 7.2) or 20 ml of ice-cold 1.1 M potassium iodide in 0.1 M phosphate buffer (pH 7.2) respectively and kept overnight at 4°C with frequent shaking. All Samples were centrifuged at 5000 g for 20 minutes and concentration of protein in the supernatant was determined by the Biuret method. Myofibrillar protein extractability was calculated by obtaining difference between total and sarcoplasmic protein extractability.

### Myofibrillar fragmentation index (MFI)

Myofibrillar fragmentation index was determined as per the procedure outlined by

Davis *et al.* (1980). Ten grams of minced meat was added to 50 ml of cold sucrose (0.24 M) and Potassium chloride (0.02 M) solution in a 150 ml homogenization tube. After 5 minutes, each sample was blended for 40 seconds at high speed using tissue homogenizer (Daihan Scientifics, WiseMix, HG-15D, Korea). The resulting homogenate was filtered through a pre-weighed cheese cloth (250 µm pore size) into beaker with constant stirring using a plastic stirring rod to hasten filtration. The residue and screen were blotted twice on a Whatman No. 1 filter paper immediately and then weighed. The MFI was reported as the weight of the residue in grams times one hundred.

### Muscle fibre diameter

Muscle fibre diameter was determined as described by Tuma *et al.* (1962). A small core (1.00 cm) of muscle tissue was fixed in 10% formal saline (10% formaline, 2% sodium acetate and 2% sodium chloride) for 24 h and was blended in a homogenizer at low speed for 30 seconds. A drop of the homogenate was placed over a glass slide, covered with cover slip and observed under a microscope with 10 X eyepiece containing a calibrated micrometer.

### Myoglobin content and %metmyoglobin

Myoglobin was extracted from raw patties using a modified procedure of Warris (1979). Samples were blended with 5 volumes of cold 0.04 M phosphate buffer at pH 6.8 for 10 seconds in a homogenizer. After keeping at 1°C for 1 h, the mixtures were centrifuged at 3500 g (Sorvall, Biofuge Stratos, Thermo Electron LED GmbH, D-37520 Osterode, Germany) and 4°C for 30 minutes. The supernatant was further clarified by filtration through Whatman No. 1 filter paper. The absorbance of filtrate was

measured at 525, 572, and 700 nm using a UV-VIS spectrophotometer (SHIMADZU, Model: UV-1700 PharmaSpec, Japan). The myoglobin concentration, %metmyoglobin (Met Mb) were calculated according to Trout (1989).

$$\text{Mb (mg/g)} = (A_{525} - A_{700}) \times 2.303 \times \text{dilution factor}$$

Where Mb = Oxy Mb + Deoxy Mb + Met Mb

$$\% \text{ Met Mb} = 1.395 - [(A_{572} - A_{700}) / (A_{525} - A_{700})] \times 100$$

### Cooking yield

Meat samples were sealed in low-density polyethylene bags and placed in water bath maintained at 100°C for 30 minutes. The weight of samples were recorded before and after cooking and cooking yield was expressed in percentage.

### Warner-Bratzler shear force

Muscle samples were cooked in water bath maintained at 85°C for 30 minutes, followed by overnight chilling at 4°C (Kandeepan *et al.*, 2009). Chilled samples were equilibrated to room temperature and 1.25 cm cores were taken using tissue borer with muscle fibres parallel to the direction of the borer. The Warner-Bratzler shear force (WBSF) of the cores were measured using Texturometer (Tinius Olsen, Model H1KF, 6 Perrywood Business park, Redhill, RH1 5DZ, England) with V-shaped stainless steel blade (60° angle). The cores were sheared perpendicular to the muscle fibre orientation with 75 Newton load range and a crosshead speed set at 200 mm/minutes. The force required to shear the samples were recorded in Newton (N).

### Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried

out using the method of Laemmli (1970) with midi-electrophoresis apparatus (GE Healthcare, Uppsala, Sweden, Model: SE-600 Ruby). About 50 µl of total protein sample extracted under protein extractability procedure (containing 50 µg of protein) was used for loading the 12% gel. Electrophoresis was performed at a constant voltage mode of 100 V/slab at 60 mA for 7 to 8 h or until the sample reached the lower end of the gel. The gel was removed and stained with Coomassie blue for 4 to 5 h followed by overnight destaining and then scanned using Image Scanner-III, LabScan 6.0 (GE Healthcare, Uppsala, Sweden) and IQTL calibration converter was used to obtain image.

### Statistical analysis

Statistical analysis was performed with the analysis of variance (ANOVA) using SPSS (SPSS version 13.0 for windows; SPSS, Chicago, IL, USA). Least square means for F-tests were calculated by using Duncan's multiple range tests and were considered significant at  $P < 0.05$ . The experimental design was a completely randomized block design with three replicates ( $n=3$ ). The effects of animal age (young and old) and ageing period (0, 2, 4 and 6 days) were analyzed using two-way analysis of variance (ANOVA) option in SPSS and the differences among means were detected using the least significance difference (LSD) at a 5% level.

## RESULTS AND DISCUSSION

Fresh meat undergoes significant biochemical changes after slaughter of an animal till the consumption, which is technically known as "conversion of muscle to meat". During this process three important stages *viz.*, 1. Reduction

in physiological live animal pH (7.0) to ultimate post-mortem pH (5.6 to 5.8); 2. Development of rigor-mortis (stiffening of muscles); and 3. Resolution of rigor and tenderization of meat (conditioning or ageing) takes place. Under normal Industrial processing of buffalo meat, either the whole carcasses or carcass halves are chilled for 24 to 48 h to facilitate the completion of rigor mortis, followed by separation of muscles from bones (deboning), packaging and chilling/freezing. However for domestic condition, the carcasses are deboned immediately after slaughter (hot boning) without any chilling and the hence present work discusses the variation in meat quality (tenderness) and ageing changes in young and old buffaloes that are hot boned.

Results of changes in various quality parameters with ageing in young and old buffalo meat was presented in Table 1.

### pH

The pH has reduced ( $P < 0.05$ ) from 6.22 to 5.55 and 5.79 to 5.42 in young and old buffalo meat, respectively after 6 days of ageing. Young buffalo meat had a mean pH value of 5.76 compared to 5.57 in old buffalo meat. The ultimate pH 5.69 (Naveena *et al.*, 2004) was also reported in buffalo meat. Postmortem pH decline of buffalo meat was reported to be significantly slower than beef (Neath *et al.*, 2007) and these authors have reported a reduction in pH of buffalo meat from 6.7 to 5.4 over a period of 48 h. White *et al.* (2006) have reported an ultimate pH of 5.47 to 5.50 in hot-boned beef after 48 h of chilling at 4°C.

### Water holding capacity

Water holding capacity (WHC) of young and old buffalo meat decreased ( $P < 0.05$ ) from 35.33 and 31.33 on day' 0 to 17.67 and 17.00 on

6<sup>th</sup> day of ageing, respectively. The overall mean WHC of 23.92% and 22.41% was observed for young and old buffalo meat respectively. Similar to our findings, Kandeepan *et al.* (2009) have also not reported any difference in WHC between young and old buffalo meat. In contrast to our findings, increase in WHC with ageing was reported in buffalo meat (Irurueta *et al.*, 2008) and beef (Ruiz de Huidobro *et al.*, 2003). The reduction in WHC with ageing in our study may be due to processing of hot boned meat resulting in rapid contraction and denaturation of proteins which may reduce binding of water (Locker, 1960). Pre-rigor excision of muscles leads to severe muscle shortening (Jeremiah *et al.*, 1999) and shorter sarcomeres which may results in lower WHC. The changes in WHC observed in the present study are in agreement with the findings of Pinto *et al.* (2013) who reported reduction in WHC in hot boned *Bos indicus* Nelore steers from the 2<sup>nd</sup> to the 14<sup>th</sup> day post mortem.

### Protein extractability

The results of changes in protein extractability during ageing was shown in Figure 1. Sarcoplasmic, myofibrillar and total protein extractability reduced ( $P > 0.05$ ) during ageing period in both young and old buffalo meat. No difference ( $P > 0.05$ ) in extractability was observed between young and old buffaloes except lower ( $P < 0.05$ ) total and myofibrillar protein extractability for old buffalo meat on 6<sup>th</sup> day of ageing. Due to higher pH and high ATP level in hot boned meat, dissociation of actomyosin and better solubility of myofibrillar proteins and superior water holding capacity was observed during initial stages of processing (Pisula and Tyburcy, 1996). However, these functional properties will diminish during development of rigor which might be the

Table 1. Effect of aging on physico-chemical and histological properties of hot boned raw meat from young and old buffaloes.

| Parameters                       | 0 day                   | 2 day                    | 4 day                    | 6 day                   | Overall mean |
|----------------------------------|-------------------------|--------------------------|--------------------------|-------------------------|--------------|
|                                  | Young buffalo meat      |                          |                          |                         |              |
| pH                               | 6.22±0.02 <sup>c</sup>  | 5.65±0.01 <sup>b</sup>   | 5.63±0.04 <sup>b</sup>   | 5.55±0.01 <sup>a</sup>  | 5.76±0.08    |
| Water holding capacity (%)       | 35.33±2.40 <sup>c</sup> | 23.67±1.20 <sup>b</sup>  | 19.00±1.15 <sup>ab</sup> | 17.67±0.88 <sup>a</sup> | 23.92±2.19   |
| Myofibrillar fragmentation index | 72.53±0.57 <sup>a</sup> | 73.33±0.88 <sup>a</sup>  | 73.67±1.20 <sup>a</sup>  | 82.33±2.03 <sup>b</sup> | 75.47±1.32   |
| Muscle fibre diameter (µ)        | 47.26±1.94              | 44.87±0.60               | 43.20±0.49               | 46.26±2.15              | 45.40±0.79   |
| Myoglobin (mg/g tissue)          | 2.36±0.06 <sup>c</sup>  | 2.04±0.13 <sup>b</sup>   | 1.77±0.02 <sup>a</sup>   | 2.23±0.01 <sup>bc</sup> | 2.10±0.07    |
| % Metmyoglobin                   | 31.60±0.59 <sup>a</sup> | 42.91±0.22 <sup>b</sup>  | 49.86±2.71 <sup>c</sup>  | 57.63±2.94 <sup>d</sup> | 45.40±3.01   |
| Old buffalo meat                 |                         |                          |                          |                         |              |
| pH                               | 5.79±0.02 <sup>d</sup>  | 5.57±0.01 <sup>c</sup>   | 5.52±0.03 <sup>b</sup>   | 5.42±0.01 <sup>a</sup>  | 5.57±0.41    |
| Water holding capacity (%)       | 31.33±1.76 <sup>b</sup> | 20.67±0.88 <sup>a</sup>  | 20.69±0.92 <sup>a</sup>  | 17.00±1.00 <sup>a</sup> | 22.41±1.69   |
| Myofibrillar fragmentation index | 73.05±2.25              | 74.33±2.60               | 75.33±1.45               | 77.67±3.75              | 75.09±1.24   |
| Muscle fibre diameter (µ)        | 50.88±1.76              | 48.93±1.54               | 49.66±0.69               | 50.44±1.75              | 49.97±1.09   |
| Myoglobin (mg/g tissue)          | 3.59±0.25 <sup>b</sup>  | 2.27±0.09 <sup>a</sup>   | 2.45±0.07 <sup>a</sup>   | 2.46±0.05 <sup>a</sup>  | 2.69±0.17    |
| % Metmyoglobin                   | 23.94±1.08 <sup>a</sup> | 30.95±2.58 <sup>ab</sup> | 31.90±1.50 <sup>ab</sup> | 34.16±4.72 <sup>b</sup> | 30.24±1.67   |

Means bearing same superscripts row-wise do not differ significantly ( $P>0.05$ ).

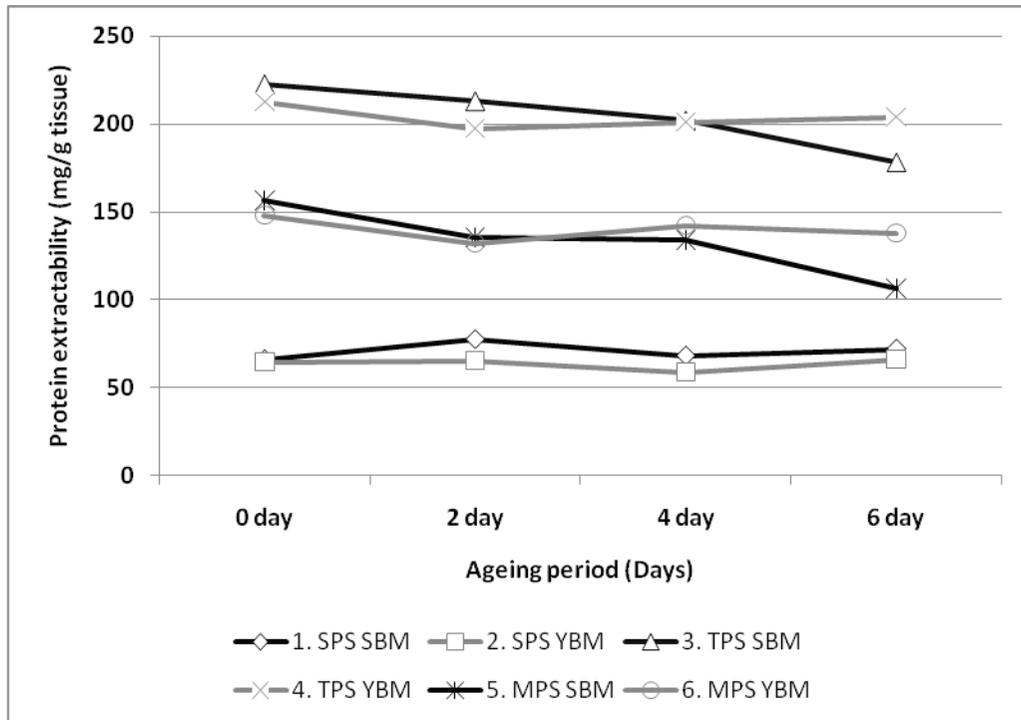


Figure 1. Effect of ageing on protein solubility of old and young buffalo meat. SPS, sarcoplasmic protein solubility; MPS, myofibrillar protein solubility; TPS, total protein solubility. Values are Mean $\pm$ SE of three independent determinations. Standard error bars are indicated.

reason for reduction in water holding capacity and protein extractability in both young and old buffalo meat during ageing.

The tenderization process is estimated to begin soon after slaughter (Veiseth *et al.*, 2001). Current evidence suggests that proteolysis of key myofibrillar proteins is the cause of meat tenderization. Proteolytic degradation of myofibrillar proteins was suggested to weaken myofibrils leading to tenderization (Koochmaraie *et al.*, 2002).

#### Myofibrillar fragmentation index and muscle fibre diameter

Myofibrillar fragmentation index (MFI) is an indicator of extent of tenderization. Higher MFI is positively correlated with improvement in

tenderness. No difference ( $P>0.05$ ) was observed in overall mean MFI of young (75.47) and old (75.09) buffalo meat. The MFI increased ( $P>0.05$ ) from 73.05 to 77.67 during ageing in old buffalo meat, where as higher ( $P<0.05$ ) MFI was seen on 6<sup>th</sup> day (83.33) compared to 0<sup>th</sup> day (72.53) in case of young buffalo meat. The increased MFI in young buffalo meat clearly indicate higher rate and extent of tenderization compared to old buffalo meat. Proteolytic degradation of myofibrillar proteins was suggested to weaken myofibrils leading to tenderization (Huff Lonergan *et al.*, 2010). The MFI observed in the present study are in agreement with the findings of Kandeepan *et al.* (2009) who reported 84.77% and 72.23% MFI for young and old buffalo meat respectively.

The overall mean muscle fibre diameter

of old buffalo meat (51.47  $\mu$ ) was higher ( $P < 0.05$ ) than young buffalo meat (45.40  $\mu$ ). No variation in muscle fibre diameter was observed during ageing in both young and old buffalo meat. Higher muscle fibre diameter was associated with an increase in age of river buffaloes (Ragab *et al.*, 1966).

### **Myoglobin and %metmyoglobin**

The mean myoglobin content of young buffalo meat (2.10 mg/g) was lower ( $P < 0.05$ ) than old buffalo meat (2.69 mg/g). Myoglobin oxidation as indicated by %metmyoglobin has increased from 31.6 to 57.63 and 23.94 to 34.16 in young and old buffalo meat respectively. This will indicate better colour stability in old buffalo meat relative to young buffalo meat. This is in agreement with findings of Robertson *et al.* (1986). A higher meat pigments in old buffaloes was attributed to greater amount of heame and myoglobin (Mamino and Horn, 1996) compared to young buffaloes. Similarly increase in dark colour with increasing age was reported in buffaloes (Valin *et al.*, 1984).

### **Cooking yield and Warner-Bratzler shear force**

Cooking loss/yield is dependent on the type of meat, ageing, temperature and cooking method. Significantly higher cooking yield was observed for old buffalo meat relative to young buffalo meat. During ageing cooking yield reduced ( $P > 0.05$ ) in both young and old buffalo meat. Reduction in cooking yield with increased ageing may be due to decreased WHC with ageing observed in the present study for hot boned meat. Increase in cooking loss with ageing was also reported in buffalo meat (Irueta *et al.*, 2008).

Shear force values have (Figure 2) reduced ( $P < 0.05$ ) from 41.79 to 26.09 Newton and 54.28 to 32.06 Newton in young and old buffalo meat respectively after 6 days of ageing. Young buffalo

meat had significantly lower shear force values relative to old buffalo meat. The reduction in shear force during ageing indicate improvement in tenderness in both young and old buffalo meat. Our findings are in agreement with Irueta *et al.* (2008) who reported the reduction in shear force values from 33.45 to 22.95 Newton in buffalo meat subjected to ageing under vacuum for 25 days at 2°C. Reduction in shear force from 7.39 kg on 2<sup>nd</sup> day to 5.14 kg on 14<sup>th</sup> day was observed in hot boned *Bos indicus* Nelore steers chilled at 0°C (Pinto *et al.*, 2013). Tenderness was reported to be higher in young bulls followed by steers and then cows (Reid and Swan, 1995). Syed Ziauddin *et al.* (1994) showed that meat from young buffaloes (1 to 2 years) was more tender than from old buffaloes (12 years).

### **SDS-PAGE**

The SDS-PAGE photograph (Figure 3) of total soluble proteins revealed the reduction in number and intensity of certain protein bands with progress in ageing in both young and old buffalo meat indicating fragmentation and breakdown of key proteins. The appearance of low molecular weight protein band (~30 kDa) became evident in old buffalo meat on 6<sup>th</sup> day of ageing whereas, same appeared on 4<sup>th</sup> day of ageing in case of young buffalo meat. This clearly suggests difference in rate and extent of ageing in young and old buffalo meat. The appearance of the 30 kDa component, a result of troponin-T degradation, has been considered a good indicator of postmortem tenderisation in muscle (MacBride and Parrish, 1977) and therefore, it is used as an indicator for proteolysis. Appearance of 30 kDa protein band after 7 and 14 days of ageing at either 2°C or 10°C chilling temperature was also observed in hot boned beef muscles (White *et al.*, 2006). These authors opined that chilling rate of

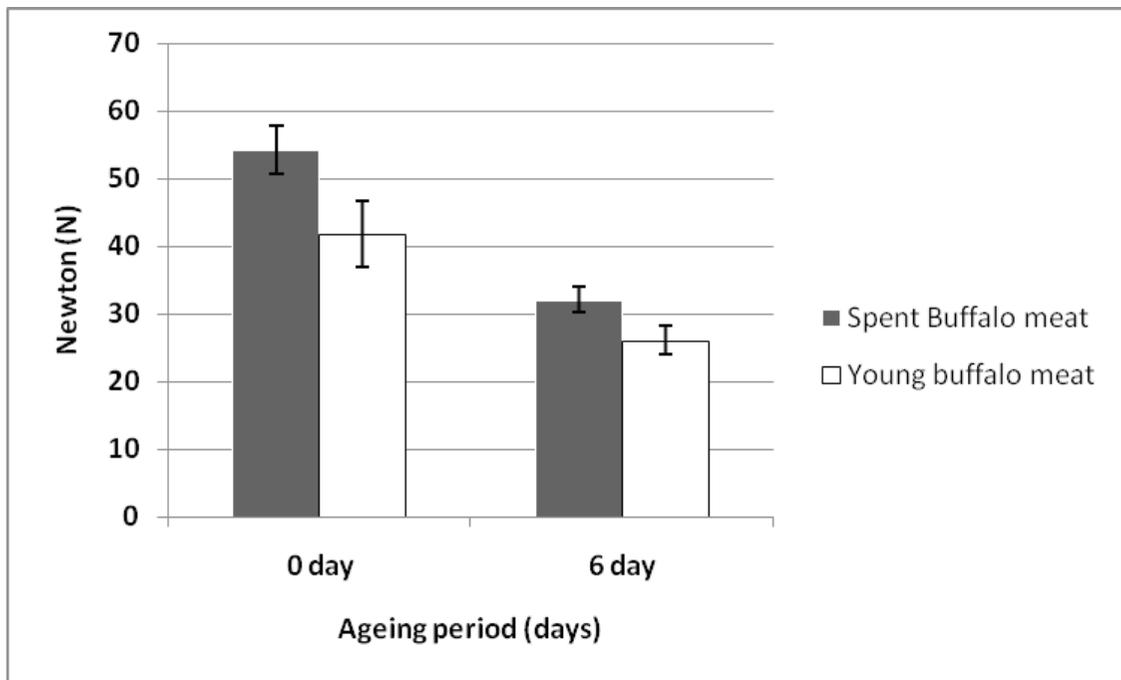


Figure 2. Effect ageing on Warner-Bratzler shear force (WBSF) values of old and young buffalo meat. Values are Mean  $\pm$  SE of three independent determinations. Standard error bars are indicated.

hot-boned muscles is faster than that of the whole carcasses, which disrupts the high temperature/low pH balance that may enhance proteolytic activity.

Koohmaraie *et al.* (1984) reported the disappearance of Troponin-T and simultaneous appearance of low-molecular weight (30 kDa) components in their study on myofibrillar proteins of bovine muscles during 14 days of post mortem storage at 2°C. Previous researchers have also reported that, degradation of high molecular weight proteins (65 to 200 kDa) and increase in the number of low-molecular weight proteins (<25 kDa) is a result of proteolysis and degradation of myofibrillar proteins (Huff-Lonergan *et al.*, 2010; Naveena and Mendiratta, 2001).

## CONCLUSION

From this study, it can be concluded that hot boning and ageing of young and old buffalo meat significantly improved ( $P < 0.05$ ) the meat quality especially tenderness and colour. However, the rate and extent of ageing varies considerably between meat from young and old buffaloes. Significant reduction in WBSF values and increase in MFI was observed in both young and old buffalo meat during ageing. Appearance of 30 kDa protein bands and increased number of differentially expressed protein spots as evident from SDS-PAGE indicate the proteolysis and tenderisation of hot boned buffalo meat in both young and old animals. Further characterization of proteins using mass spectrometry is required to identify the specific proteins responsible for variation in

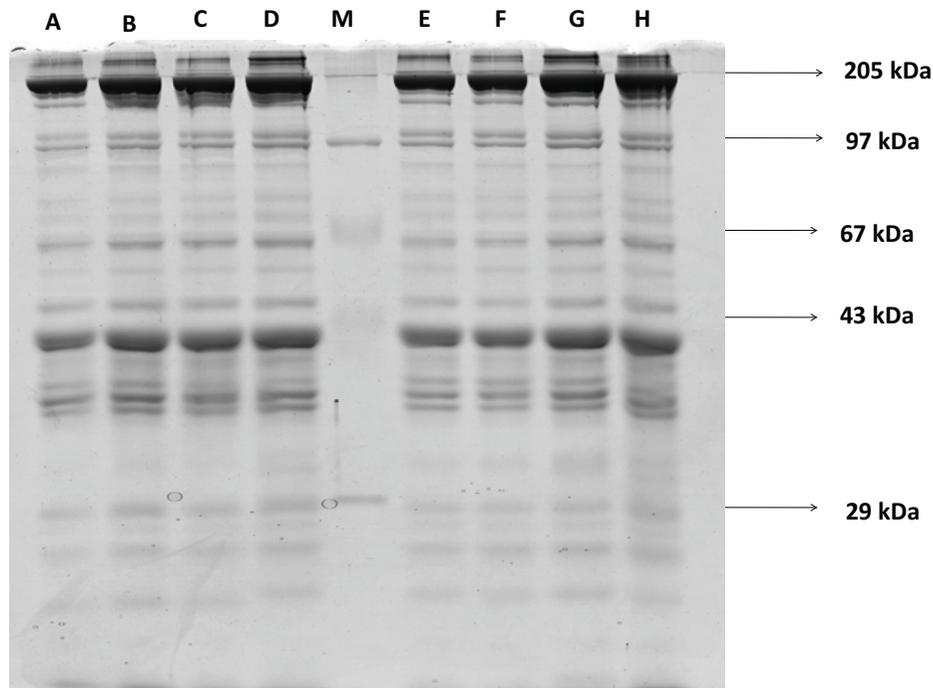


Figure 3. Effect of ageing on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) pattern of old and young buffalo meat.

Lane A-D, old buffalo meat; E-H, young buffalo meat; A and E-0 day; B and F-2<sup>nd</sup> day; C and G-4<sup>th</sup> day; D and H-6<sup>th</sup> day; M-Molecular weight marker.

tenderness of buffalo meat.

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