PHYSICOCHEMICAL, ULTRASTRUCTURAL AND PROTEOME CHANGES IN TENDER AND TOUGH MUSCLES OF BUFFALO (*Bubalus bubalis*) MEAT

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Abstract – This study was conducted to unravel the variation in meat quality between tender (*Psoas major; PM*) and tough (*Longissimus dorsi; LD*) muscles of Indian buffaloes (*Bubalus bubalis*). Both the muscles were subjected to physicochemical analysis, ultrastructural study using electron microscopy and proteome characterization using 2-Dimensional gel electrophoresis (2-DE) and tandem mass spectrometry (MS/MS). Higher (P<0.05) muscle fibre diameter, sarcoplasmic protein extractability and Warner-Bratzler shear force was observed in LD, where as higher (P<0.05) water holding capacity, myofibrillar fragmentation index and myofibrillar protein extractability was observed in PM. Scanning electron microscopy results revealed higher inter-myofibrillar spaces in LD relative to PM. Proteome analysis using 2-DE revealed 123 differentially abundant proteins between PM and LD. Protein spots with significant differences were subjected to MALDI TOF-TOF MS and identified the proteins specific to LD or PM. The results demonstrated that both LD and PM have significant variation in meat quality attributes and requires muscle specific processing strategies for better utilization.

Key Words – 2-Dimensional gel electrophoresis, Electron microscopy, Mass spectrometry

I. INTRODUCTION

Majority of buffaloes in India are slaughtered after completion of their productive period resulting in tough meat with poor quality characteristics. However, consumer research suggests that tenderness is a very important element of eating quality and the variation in tenderness affects consumer's repurchasing decision (1). It is well established that, muscles vary substantially in tenderness internally (2, 3). The individual muscles can be better utilized by precise characterisation of the muscles by physical and chemical analysis, to develop improved understanding and know properties of individual muscles. Rhee *et al*. (3) studied 11 different muscles of beef and reported variation in texture among all muscles. In the predominant part of meat science literature, texture studies have focused mainly on *Longissimus dorsi* (LD), while other muscles in the carcass have been minimally examined (4). In many studies it is often assumed that LD is a suitable reference point for describing other muscles in the carcasses (5). *Psoas major* (PM) has been classified as most tender muscle (6) with Warner Bratzler shear value of 3.07 kg, where as LD has been classified as intermediate tender with shear value of 4.20 kg (7). Proteome is regarded as the molecular link between the genome and the functional quality characteristics of the meat (8). Proteomics tools viz, Two-dimensional gel electrophoresis (2-DE) and tandem mass spectrometry are useful techniques for separation and identification of proteins that are associated with meat quality (9). Identification of differentially expressed proteins could be implemented as molecular biomarkers for meat quality and may provide new insights into the molecular mechanisms related to tenderness (8). Therefore, present study was conducted to unravel the physicochemical, ultrastructural and proteomic variation between LD and PM muscles.

II. MATERIALS AND METHODS

Longissimus dorsi (LD) and *Psoas major* (PM) muscles from chilled buffalo (*Bubalus bubalis*) carcasses were collected from selected retail meat shops of Hyderabad, India and used for the experiments. Raw meat was evaluated for pH, water-holding capacity (10), protein extractability (11), muscle fibre diameter (12), myofibrillar fragmentation index (13) and sodium dodecyl

sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (14). Cooked meat cubes were also evaluated for Warner-Bratzler shear force values. The ultrastructural changes were observed using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (15). Protein samples were extracted, purified and separated using 2-Dimensional gel electrophoresis (2- DE) and further characterized by tandem mass spectrometry (MALDI TOF-TOF MS). The overall experiment was replicated on three separate occasions. Statistical analysis was performed with the paired samples t-test using SPSS (SPSS version 13.0 for windows; SPSS, Chicago, IL, USA).

III. RESULTS AND DISCUSSION

The results of various physico-chemical analysis of *Longissimus dorsi* (LD) and *Psoas major* (PM) muscles are shown in Table 1. Water holding capacity and myofibrillar fragmentation index was higher ($P<0.01$) in PM compared to LD. Myofibrillar fragmentation index (MFI) was reported to be negatively correlated with the shear force values in buffalo meat (16). Muscle fibre diameter of PM was lower ($P<0.05$) than LD. This is in accordance with findings of Tuma *et al.* (12) who indicated that fibre diameter and tenderness are negatively related

Table 1 Physicochemical qualities of *Psoas major* (PM) and *Longissimus dorsi* (LD) muscles from buffaloes

Parameters	LD	PM	SEM
pH	5.87	5.70	0.08
Water-holding capacity (%)	15.75	21.00	1.11*
Myofibrillar fragmentation index (%)	73.00	77.75	1.11*
Muscle fibre diameter (μ)	44.22	42.12	0.51*
Sarcoplasmic protein extractability (mg/g)	64.00	51.00	1.29*
Myofibrillar protein extractability (mg/g)	111.0	125.0	2.94*
Total protein extractability (mg/g)	175.0	176.0	3.41
WBSF (N)	36.73	26.89	3.76*

* Significant at ($P<0.05$)
SEM- Standard error of mean

Higher ($P<0.01$) myofibrillar protein extractability was observed in PM compared to LD. Higher myofibrillar protein extractability is positively correlated with higher level of tenderness in PM compared to LD (17). PM was significantly tender when compared to LD as evidenced by lower ($P<0.01$) Warner-Bratzler shear force values. This is in accordance with reports of Sullivan and Calkins (7), who established PM as a most tender muscle relative to LD with intermediate tenderness.

The morphology of muscle structures can be related to meat tenderness. Hence, in order to understand the ultrastructural variation between PM and LD, scanning and transmission electron microscopy was performed. Scanning electron microscopy photographs (Fig. 1) did not reveal any major structural changes between PM and LD except for increased interfibrillar gaps in LD.

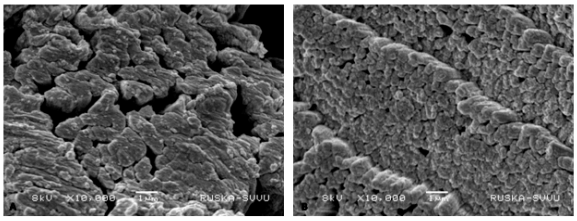

Figure 1. Scanning electron micrographs (transverse section, 10000 X) of A. *Longissimus dorsi* B. *Psoas major*

SDS-PAGE (figures not shown) did not reveal any change in band intensity/pattern among LD and PM. Hence, further proteome characterization was carried out using 2-DE and tandem MS. Using homogenization, extraction, passive rehydration, 2-DE and Coumassie blue staining, we were able to separate around 710 proteins on a single gel. 2-DE profile of LD and PM demonstrated differential expression of about 123 spots between these two muscles in the present study. Selected differentially expressed spots were picked and subjected to MALDI TOF-TOF MS and proteins were identified. The differentially expressed proteins were identified to be Myosin light chain 1 and troponin T, slow skeletal muscle isoforms from LD and Vitamin K dependent protein Z precursor in case of PM.

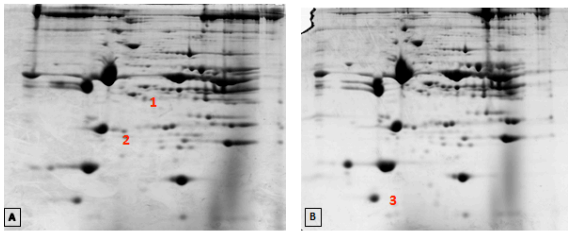

Figure 2. Coumassie stained 2- DE gels of A. *Longissimus dorsi* B. *Psoas major* (Spots picked for MS are numbered in red)

IV. CONCLUSION

The study demonstrates significant difference in textural characteristics and variation in ultrastructural qualities between LD and PM muscles of Indian buffaloes. Proteomic characterization using 2-DE and tandem MS revealed several differentially expressed proteins between LD and PM and identified the peptides/proteins that correlates with tenderness. The results of the present study suggest the necessary to develop muscle-specific tenderization strategies in order to minimize the variation between tender and tough muscles.

ACKNOWLEDGEMENTS

Authors would like to thank Indian Council of Agricultural Research (ICAR) and University Grants Commission (UGC), India for financial support.

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