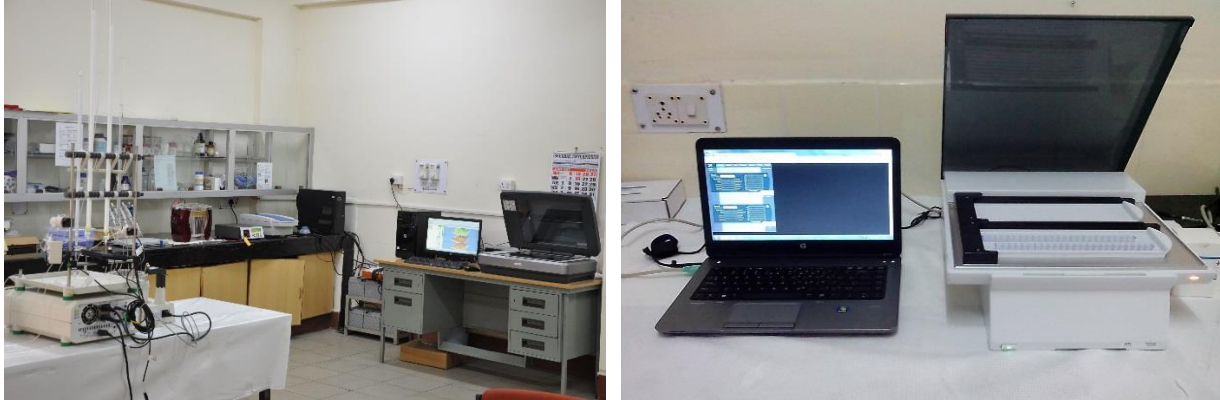


PROTEOMICS AND MEAT QUALITY LABORATORY



Proteomics facility at ICAR-NRC on Meat, Hyderabad

We are now in an exciting period where many new opportunities are presented to researchers through the application of genomics, proteomics and other ‘omic’ approaches. The great progress in biotechnology in recent years has resulted in the development of new scientific research areas such as genomics and proteomics, which are used to study the complex patterns of gene and protein expression in cells and tissues. Ability to simultaneously analyze hundreds or thousands of genetic polymorphisms (genomics), transcripts (transcriptomics), proteins (proteomics) and metabolites (metabolomics) on dedicated arrays or with specific tools have increased our knowledge of the molecular organization of living organisms. These tools have been implemented in recent years to reveal genes, proteins or metabolites whose expression level or abundance is associated with a phenotype of interest such as the quality of the meat.

After genomics, proteomics is considered the next step in the study of biological systems. Proteomics tools consist of 2-Dimensional gel electrophoresis (2-DE), mass spectrometry (MS) and bioinformatics. Proteomics permits visualisation of the protein content of the cell under varying conditions, combining powerful separation techniques with highly sensitive analytical

mass spectrometry. Modern proteomic technologies for understanding muscle biology have been successfully used for a series of investigations including mapping of muscle proteins, muscle disorders, muscle physiology, conversion of muscle to meat, understanding meat colour and texture, meat speciation, sensorial and technological meat quality traits etc.

Proteomics and Meat Quality Laboratory at ICAR-NRC on Meat has been working towards answering complex questions relating to meat colour and texture, detection of meat adulteration, identification of peptide biomarkers, understanding muscle food quality etc. using state-of-the-art proteomic approaches. Knowledge gained from these approaches are beneficial in defining and optimising management systems for quality, providing assurance of meat quality and safety and in tailoring quality to suit market needs.

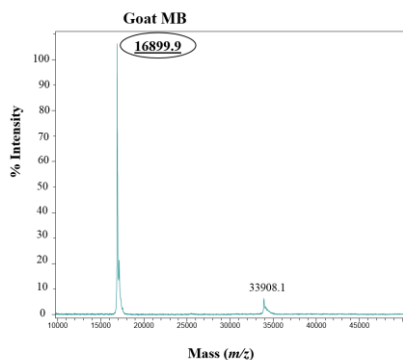
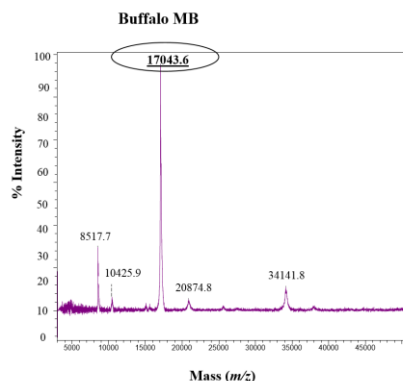
Salient achievements from Proteomics and Meat Quality Laboratory is provided below:

I. Proteomics of 4-hydroxy-2-nonenal induced oxidation of buffalo (*Bubalus bubalis*) and goat (*Capra hircus*) meat myoglobins

Myoglobin (Mb) is a sarcoplasmic heme protein primarily responsible for meat color and its chemistry is species specific. 4-hydroxy-2-nonenal (HNE) is a cytotoxic lipid derived aldehyde detected in meat and was reported to covalently adduct with nucleophilic histidine residues of Mb and predispose it to greater oxidation. In this study we characterized the Mb extracted from water buffalo and goat cardiac muscles using two-dimensional gel electrophoresis (2DE), OFFGEL electrophoresis and mass spectrometry (MS).

Purified buffalo and goat Mb samples revealed a molecular mass of 17,043.6 and 16,899.9 Daltons, respectively. The 2DE analysis exhibited 65 (sarcoplasmic protein extract) and 6 (pure Mb) differentially expressed ($P < 0.05$) protein spots between buffalo and goat samples. OFFGEL electrophoresis revealed an isoelectric point of 6.77 and 7.35 respectively, for buffalo and goat Mb's. In-vitro incubation of HNE with bright red buffalo and goat oxymyoglobin's at pH 7.4 and 37 °C resulted in pronounced ($P < 0.05$) oxidation and formation of brown metmyoglobin.

MALDI-TOF MS analysis of Mb-HNE reaction mix revealed covalent binding (via Michael addition) of 3 and 5 molecules of HNE with buffalo and goat Oxy-Mb's, respectively. ESI-QTOF MS/MS identified seven and nine histidine (HIS) residues of Mb that were readily adducted by HNE in buffalo and goat, respectively.



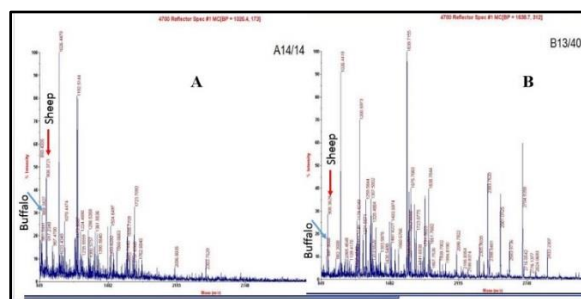
MALDI-TOF mass spectra of purified buffalo and goat myoglobins



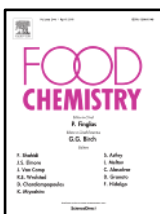
Naveena, B.M., Usha Rani, K., Praveen Kumar, Y., Kulkarni, V.V. and Rapole S. (2016). Proteomic based approach for characterizing 4-hydroxy-2-nonenal induced oxidation of buffalo (*Bubalus bubalis*) and goat (*Capra hircus*) meat myoglobins. *Proteome Science* 14(18): 1-16 (DOI: 10.1186/s12953-016-0108-7).

II. Proteomic based technique for meat species identification

Proteomic-based technology using in-gel (two-dimensional gel electrophoresis, 2DE) and OFFGEL-electrophoresis for authentication of meat species from three closely related ruminant species viz, water buffalo, sheep and goat in both raw and cooked conditions was developed. The MALDI-TOF/TOF MS analysis of proteins separated using 2DE or OFFGEL electrophoresis delineated species-specific peptide biomarkers derived from myosin light chain 1 and 2 (MLC1 and MLC2) of buffalo, sheep and goat meat mix in different proportions that were found stable to resist thermal processing. The 2DE and tandem mass spectrometry based in-gel method can detect up to 1.0 per cent substitution of sheep and goat meat in buffalo meat, whereas OFFGEL electrophoresis and tandem MS approach can detect even up to 0.1 per cent substitution of sheep and goat meat in buffalo meat. The effectiveness of OFFGEL electrophoresis over in-gel based method is its efficiency to concentrate and enrich the low abundant proteins mainly originating from myosin light chain 1 and 2. We demonstrated the accuracy and robustness of OFFGEL-based method over 2DE based in-gel approach. The aforesaid technology envisages the robustness of high throughput proteomic approach coupled with OFFGEL electrophoresis as an alternative to other existing methods for meat speciation and pave the way for future requirements of food safety and authenticity.



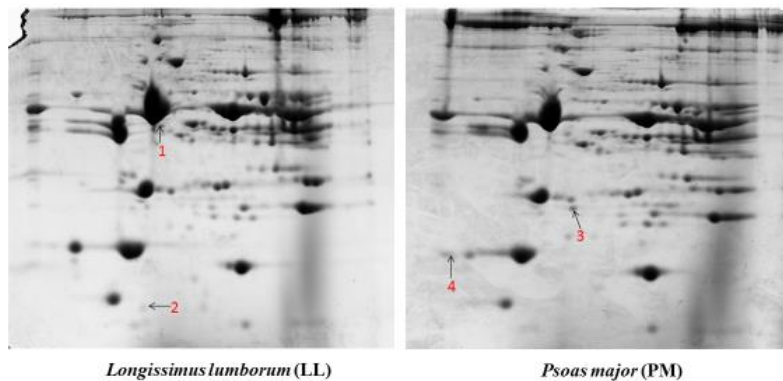
MALDI-TOF MS spectrum of sheep and buffalo meat-specific peptides derived from myosin light chain-2; A-Raw and B-Cooked.



Naveena, B.M., Deepak, S.J., Jagadeesh Babu, A., Madhava Rao, T., Veeranna, K., Vaithyanathan, S., Kulkarni, V.V. and Rapole, S. (2017). OFFGEL electrophoresis and tandem mass spectrometry approach compared with DNA-based PCR method for authentication of meat species from raw and cooked ground meat mixture containing cattle meat, water buffalo meat and sheep meat. *Food Chemistry*, 233: 311-320.

III. Muscle-specific variation in buffalo (*Bubalus bubalis*) meat texture: biochemical, ultrastructural and proteome characterization

This study was conducted to unravel the variation in meat quality between tender (*Psoas major*, PM) and less tender (*Longissimus lumborum*, LL) muscles of Indian water buffaloes (*Bubalus bubalis*). PM and LL were subjected to physicochemical analysis, ultrastructural study and proteome characterization using 2-Dimensional gel electrophoresis (2-DE) and mass spectrometry. Higher ($P < 0.05$) muscle fibre diameter and Warner-Bratzler shear force was observed in LL, whereas higher ($P < 0.05$) water holding capacity and myofibrillar protein extractability was observed in PM. Transmission electron microscopy revealed higher ($P < 0.05$) sarcomere length in PM compared to LL. Proteome analysis using 2-DE revealed 123 differentially abundant proteins in PM and LL. The MALDI TOF-TOF MS analysis of selected protein spots from LL and PM with significant ($P < 0.05$) differences identified the proteins mainly consisting of Calcium transporting ATPase.



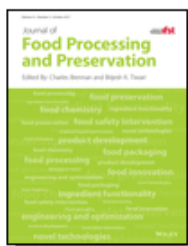
Total proteins from LL and PM muscles of water buffaloes separated through 2-dimensional gel electrophoresis



Kiran, M. Naveena, B.M., Sudhakar Reddy, K., Shashikumar, M., Ravinder Reddy, V., Kulkarni, V.V., Rapole, S., More, T. H. (2015). Muscle-specific variation in buffalo (*Bubalus bubalis*) meat texture: Biochemical, ultrastructural and proteome characterization. *Journal of Texture Studies*, 46 (4): 254-261.

IV. Effect of aging on physicochemical, textural, microbial and proteome changes in emu (*Dromaius novaehollandiae*) meat under different packaging conditions

Effect of aging on the physicochemical, textural, microbial and proteome characteristics of emu (*Dromaius novaehollandiae*) meat was studied under aerobic packaging (AP) and vacuum packaging (VP) conditions at 4±1C for 9 and 15 days respectively. Improvement ($P < 0.05$) in water holding capacity, myofibrillar fragmentation index and protein extractability with aging was observed in emu meat cubes under both AP and VP conditions. Reduction ($P < 0.05$) in Warner-Bratzler shear force values were observed on 6th and 15th day of ageing compared to 0th day in AP and VP samples, respectively. The SDS-PAGE analysis revealed the appearance of 30-kDa protein bands, indicating extensive proteolysis on 6th and 9th day of aging in AP and VP samples, respectively. Proteome analysis using 2-dimensional gel electrophoresis (2DE) revealed significant ($P < 0.01$) changes in number of differentially expressed protein spots in AP and VP samples during aging.

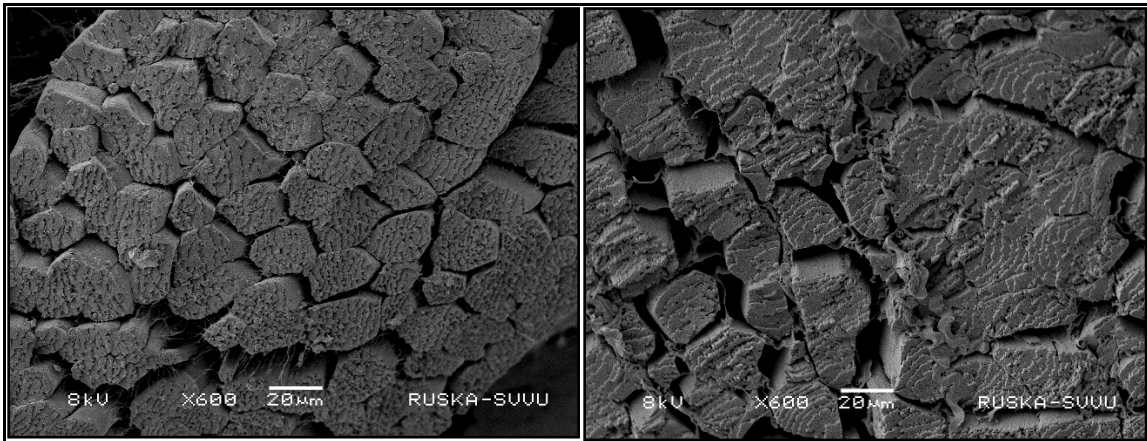


Naveena, B.M., Muthukumar, M., Kulkarni, V.V., Praveen Kumar, Y., Usha Rani, K. and Kiran, M. (2015). Effect of aging on the physicochemical, textural, microbial and proteome changes in emu (*Dromaius novaehollandiae*) meat under different packaging conditions. *Journal of Food Processing and Preservation* 39(6): 2497-2506.

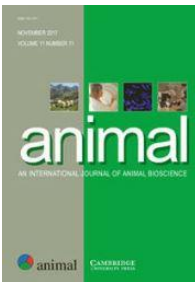
V. Understanding tenderness variability and ageing changes in buffalo meat: Biochemical, ultrastructural and proteome characterization

This study was conducted to unravel the differences in biochemical, ultrastructural and proteome profile of *Longissimus dorsi* muscle between buffaloes (*Bubalus bubalis*) of different age groups (young vs. old). Higher ($p < 0.05$) myofibrillar and total protein extractability, muscle fibre diameter, and Warner-Bratzler shear force (WBSF) values was observed in old buffalo meat relative to meat from young buffaloes. Scanning electron microscopy photographs revealed reduced fibre size with increased inter-myofibrillar space in young compared to old buffalo meat. Transmission electron microscopy results revealed longer sarcomeres in young buffalo meat relative to meat from old buffaloes. Proteomic characterization using two-dimensional gel electrophoresis (2DE) found 93 differentially expressed proteins between old and young buffalo

meat. Proteome analysis using 2DE revealed 191 and 95 differentially expressed protein spots after 6 days of ageing in young and old buffalo meat, respectively. The MALDI-TOF/TOF analysis of selected gel spots helped in identifying molecular markers of tenderness mainly consisting of structural proteins. Protein biomarkers identified in the present study have the potential to differentiate meat from young and old buffaloes and pave the way for optimizing strategies for improved buffalo meat quality.



Scanning electron microscopy (SEM) images showing the ultrastructure of young and old buffalo meat



Kiran, M. Naveena, B.M., Sudhakar Reddy, K., Shashikumar, M., Ravinder Reddy, V., Kulkarni, V.V., Rapole, S., More, T. H. (2016). Understanding Tenderness Variability and Ageing Changes in Buffalo Meat: Biochemical, Ultrastructural and Proteome Characterization. *Animal: An International Journal of Animal Bioscience* 10(6): 1007-1016.