PROTEOME CHARACTERIZATION OF BUFFALO (*Bubalus bubalis*) AND GOAT (*Capra hircus*) MYOGLOBINS

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Abstract- This study was conducted to characterize myoglobin (Mb) from water buffalo (*Bubalus bubalis*) and goat (*Capra hircus*) cardiac muscles using two-dimensional gel electrophoresis (2DE) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Purified buffalo and goat Mb samples revealed a molecular mass of 17,043.6 Daltons and 16,899.9 Daltons, respectively. The 2DE analysis of Mb's from buffalo and goat samples revealed 65 (crude Mb) and 6 (pure Mb) differentially expressed spots (P<0.05) between them. Significant variation in 2DE protein spot numbers was observed for crude Mb extract between buffalo and goat samples. Peptide mass fingerprinting (PMF) of Mb protein from 2DE gels confirmed the buffalo and goat Mb's.

I. INTRODUCTION

Water buffalo meat is darker compared to beef and the darkness is attributed to higher Mb content. Darkness of buffalo meat depends on factors other than the oxidation rate of its Mb (1). Goat meat was reported to be darker, more red and had higher sarcoplasmic protein content than sheep meat (2). These observed differences in color attributes between different species indicated possible variation in Mb chemistry.

Recently, using different proteomic tools researchers have demonstrated the effect of muscle source (3) and role of lipid oxidation products (4, 5, 6) on meat color stability. All these studies have reported species-specific effect of Mb on meat color and majority of these studies have used purified myoglobin protein which could differ slightly from the true structure of the native protein in complex biological media. Almost all the studies related to meat color have used liquid chromatography-mass spectrometry (LC-MS) based tools and to our knowledge no studies have been reported on use of two-dimensional gel electrophoresis (2DE) coupled with mass spectrometry to characterize crude and or/purified Mb's especially from buffalo and goat meats. Therefore, present study was undertaken to characterize the Mb using 2DE and mass spectrometry from two important emerging meat animals, buffalo and goat. Study also included the identification of Mb using peptide mass fingerprinting (PMF).

II. MATERIALS AND METHODS

Cardiac muscles from water buffalo and goat were minced, homogenized and subjected to different centrifugation and ammonium sulfate precipitation steps as described by Faustman and Phillips (7). Myoglobin (Mb) pellet was dialysed, filtered using 0.45 µm and 0.2 µm syringe filters and purified through gel filtration column chromatography. SDS-PAGE was performed to check the purity of Mb at each step during the extraction. The purified Mb fraction was subjected to MALDI-TOF MS to determine its intact mass. For 2DE, roughly 200 µl of crude (sarcoplasmic extract) and purified Mb (gel filtered fraction) were loaded on 11 cm immobilized pH gradient strips (pH 3-10), followed by passive rehydration, iso-electric focusing in a Ettan IPGPhor-3 (GE health care, Uppsala, Sweeden) gel apparatus, equilibration and SDS-PAGE (second dimension) with the SE 600 Ruby apparatus as per the procedure standardized at our

laboratory. Gels were stained using Colloidal Coomassie, destained followed by scanning on an Image Scanner III using labscan 6.0 software. Spot detection and quantification were performed with Image Master Platinum7.0 software (GE Healthcare, Uppsala, Sweeden). The myoglobin spot from 2DE gels were also subjected to Trypsin digestion and analyzed on the MALDI TOF/TOF ULTRAFLEX III instrument and further analysis was done with FLEX ANALYSIS SOFTWARE for obtaining the Peptide mass fingerprinting (PMF).

III. RESULTS AND DISCUSSION

Extraction and purification of Mb from buffalo and goat is minimally investigated and to our knowledge only two papers from Dosi et al. (1) and Suman et al. (8) are available in the literature for buffalo and goat Mb, respectively. In the present study, we could able to successfully extract and purify both buffalo and goat Mb's as per the procedure suggested by Faustman and Phillips (7) with 70% ammonium sulfate precipitation and gel filtration chromatography using Sephacryl S-200 HR. The SDS-PAGE of pooled fractions from second peak which is supposed to be Mb consistently revealed the presence of single band at approximately 17 kDa level in both buffalo and goat samples. The MALDI-TOF MS analysis of intact buffalo Mb revealed the mass of 17,043.6 Daltons (Fig. 1A) which is 9.6 Daltons higher than the report of Dosi et al. (2002). The MS analysis of goat Mb revealed a mass of 16,899 Daltons (Fig. 1B).

The 2DE gel analysis (Fig. 2) revealed separation of 508 and 563 spots respectively in buffalo and goat crude sarcoplasmic extracts (crude Mb). The class analysis table by analysis of variance (ANOVA) of buffalo and goat sample gels indicated 65 differential spots which had protein spot expression of 1.5 fold or more between them. For gel-filtered Mb (pure Mb), 19 spots were separated in buffalo relative to 20 spots in goat samples with 6 spots being differentially expressed between them. These findings suggest significant variation in sarcoplasmic proteome between buffalo and goat samples.

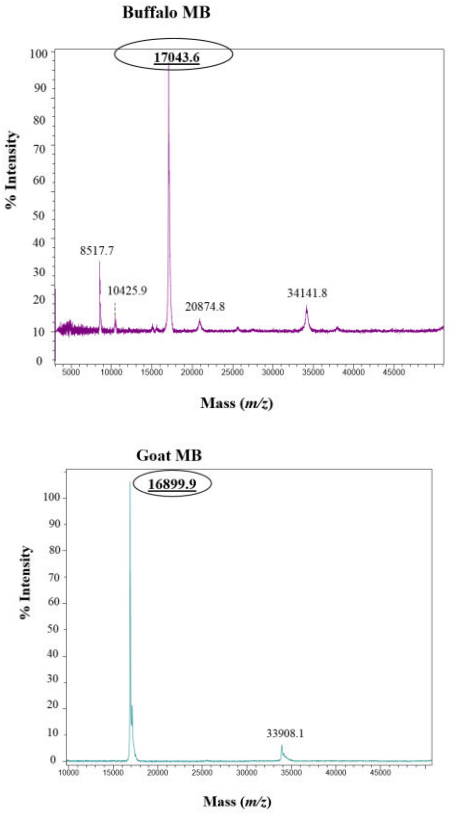

Fig. 1. MALDI-TOF mass spectra of (A) buffalo and (B) goat Mb's

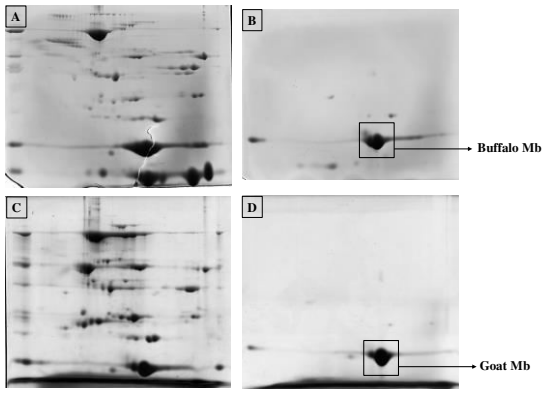

Fig. 2. Two-DE photographs of (A) crude & (B) pure buffalo Mb; (C) crude & (D) pure goat Mb

Purified Mb protein from buffalo and goat samples separated by 2DE gel was identified by peptide mass fingerprinting using MALDI-TOF/TOF mass spectrometry (data not shown). The peptide with a *m/z* value of 748 is most

abundant in buffalo compared to goat samples wherein peptide with 1592 *m/z* is abundant. Peptide masses detection has been matched the buffalo and goat Mb protein in a database which showed the sequence coverage of 39.61% for buffalo and 41.55% for goat. These results confirmed the identity of purified protein spot on 2DE gel as buffalo and goat Mb.

IV. CONCLUSION

Buffalo and goat Mb's were similar in behavior during isolation and purification and exhibited a molecular mass of 17,043.6 Daltons and 16,899 Daltons respectively. The 2DE of crude Mb and purified Mb from buffalo and goat samples revealed significant variation in abundance of proteins between them. The PMF using MALDI-TOF/TOF mass spectrometry of buffalo and goat Mb digested from 2DE gels confirmed the identification of Mb to their respective species. Present study has demonstrated the species-specific variation in 2DE properties of buffalo and goat Mb's

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