Molecular characterization of water buffalo meat by proteomic techniques

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ABSTRACT: Buffalo breeding in Campania is aimed at milk production as the starting material for the production of Mozzarella di Bufala DOP, but it does not take into account the possibility of meat production. Buffalo meat, given its low content in fat and cholesterol, represents a good alternative to bovine meat from the dietetic standpoint. One of most interesting aspects of buffalo meat is its utilization either directly or to prepare other products. Development of these products however requires suitable technological approaches based on molecular characterization, so that product evaluation and development may be carried out on rational basis.

Key words: Water buffalo meat, Proteomics, Mass spectrometry.

INTRODUCTION - At present, water buffalo breeding in Campania (Italy) is exclusively aimed to the production of milk and derived products (essentially mozzarella cheese), while the aptitude to meat production is completely neglected. In spite of that, water buffalo meat has been recently greatly appreciated by nutritionists because of its fine sensory and nutritional characteristics (low cholesterol content, smaller lipid amount compared to bovine and pork meat, higher unsaturated/saturated fatty acids ratio, greater iron content compared to bovine, pork and rabbit meat). However, for historical reasons, scientific studies aimed to definition of the molecular components of buffalo meat are very few (Ferranti et al., 1992; Ferranti et al., 2001).

The main purpose of this work has been the structural characterization of the sarcoplasmatic protein component of the meat of buffalo and the definition of the proteolytic events taking place during the tenderization process by proteomic techniques. In fact, the peptides and the amino acids that are formed during the tenderization process, contribute to the definition of taste and aroma and to the increase of the tenderness and flavour of the buffalo meat (Etherington D.J., 1984). This study was therefore aimed to identify the molecular markers of quality of the buffalo meat compared to other species traditionally used for meat production.

MATERIAL AND METHODS - The following sample has been analysed: meat samples from three kind of muscles (Longissimus dorsi, LD, Semimembranosus, SM and Tricipitis brachii, TB) of 6 different animals taken either before and after 7 days of tenderization. Meat samples were homogenized for 10 minutes in Tris-HCl 0.2 M at pH 8.0 buffer. After centrifugation for 10 minutes at 4000g, the supernatant, containing the sarcoplasmatic protein fraction, was recovered for electrophoretic, chromatographic and mass spectrometry analysis.
Electrophoretic analysis SDS-PAGE was carried out following the procedure described by Chianese et al. (1992). For proteomic analysis, gel bands were excised and submitted to trypsin digestion. The identification of tryptic hydrolysates has been performed with mass spectrometry. For the separation of the protein components an HPLC modular system HP 1100 (Agilent) connected to a reverse phase microbore C18 column (Vydac, 218TP52) was used. The protein separation was carried out in an acetonitrile gradient from 5% to 70% in 90 minutes. The column eluent has been monitored at 220 nm. Identification of protein and peptides was performed by MALDI-TOF mass spectrometry analysis using a PerSeptive Biosystem Voyager DE-PRO apparatus. Before MALDI TOF MS analysis, the high mass protein fraction was separated by the mass peptide fraction using a diafiltration membrane 3 kDa cut-off Microcon (Millipore) by centrifugation for 20 minutes at 5° C. For peptide analysis, spectra has been acquired in linear and in reflector mode, for protein analysis in linear mode only.

RESULTS AND CONCLUSIONS - The proteomic analysis of the sarcoplasmatic protein extracts from different muscle tissues of water buffalo meat were performed by SDS-PAGE coupled to MALDI TOF MS of the bands after excision and tryptic hydrolysis. In figure 1 the electrophoretic analysis of proteins from samples before (BT) and after (AT) meat tenderization are shown. In all muscle tissues, the intensity of the bands of kinase creatine, aldolase, glyceraldehyde-3-phosphate dehydrogenase and α– and β-globin decreased sharply after meat tenderization. From the pattern of proteolysis observed on SDS-PAGE, it can be concluded that the above mentioned proteins were more susceptible to proteolysis by action of indigenous enzymes in the early stages of the tenderisation process compared to the other sarcoplasmic proteins. To confirm this data and to evidence the differences occurring in the different muscles during the tenderization process, proteomic characterization was carried out on the same samples by MALDI-TOF MS. In figure 2 the MALDI-TOF-MS spectra of sarcoplasmic proteins from Longissimus dorsi (LD), Semimembranosus (SM) and Tricipitis brachi (TB) muscles before tenderization are compared. Here, a difference in initial composition of the proteins in the three muscles may be immediately observed from the mass signal intensity, for instance the higher abundance of creatine kinase (CK, MW 43097 Da) in LD muscle tissue, as well as that of glyceraldehyde-3-phosphate dehydrogenase (G3PD, MW 35700 Da) in TB muscle tissue. In the same way it was possible to identify the structural changes occurring in the various muscles during tenderization, in order to monitoring the proteolytic process of sarcoplasmic proteins. For example, in figure 3, the MALDI TOF mass spectra of Semimembranosus (SM) muscle before and after tenderization are compared. Figure 1. SDS-PAGE electrophoreto-grams of sarcoplasmic protein before and after meat tenderization.
Figure 2.  The comparison among different muscle tissue of water buffalo meat before tenderization.

Figure 3.  Maldi-TOF-MS spectra of SM muscle before and after tenderization.
shown, where it is possible to observe the disappearance of the peaks relating to glyceral-
dehyde-3P-DH, to aldolase and to creatine kinase, after tenderization, in agreement with
the previously shown data.

Furthermore, in order to characterise the peptides produced during tenderization, the pep-
tide fraction was analyzed by RP-HPLC coupled to MALDI-TOF MS. Data related to protein
identification are summarized in Table 1. The peptides identified all derive from proteolysis
of the CK and G3PD proteins, confirming the tendency of this proteins to degradation du-
ring tenderization.

From the data provided by proteomic analysis we can conclude that in water buffalo meat
samples analysed, being in an early stage of the tenderisation process, only two proteins,
namely CK and G3PD, appear to be more susceptible to proteolysis during tenderisation
in all kind of muscles. On the contrary, for the other sarcoplasmic proteins, including eno-
lase and triosophosphate isomerase, protein degradation is tissue-dependent. This means
that, in all examined individuals, protein hydrolysis occurs at different extent in the three

| Fraction | T_R (mm: ss) | Peptide mass (Da) | Position | Identification |
|----------|--------------|-------------------|----------|----------------|
| 4        | 32' 32”      | 598.90            | 293-298  | Creatine kinase|
|          |              | 853.63            | 26-32    | Creatine kinase|
| 5        | 41' 10”      | 1192.33           | 237-247  | Creatine kinase|
| 7        | 44' 03”      | 1948.67           | 269-284  | Enolase         |
|          |              | 2283.08           | 28-49    | Enolase         |
| 8        | 47' 21”      | 1870.83           | 15-31    | Enolase         |
|          |              | 2427.75           | Aug-31   | α globin       |
|          |              | 2952.58           | 62-90    | α globin       |
| 9        | 51' 05”      | 2089.63           | 40-58    | β globin       |
| 12       | 55' 05”      | 820.21            | 1-Jul    | β globin       |
|          |              | 1491.93           | 43-55    | Aldolase        |
| 13       | 57' 55”      | 1310.64           | 1-Nov    | Creatine kinase|
| 14       | 59' 50”      | 1217.70           | 184-194  | G3PD            |
| 15       | 61’          | 1360.59           | 321-333  | G3PD            |
|          |              | 3361.48           | 160-191  | G3PD            |
| 17       | 64’          | 1071.89           | 32-40    | α globin       |
|          |              | 2165.99           | 232-251  | G3PD            |
|          |              | 3344.80           | 30-58    | β globin       |
| 18       | 66’          | 1121.91           | 57-68    | α globin       |
|          |              | 1513.05           | 17-31    | α globin       |
muscles, being more pronounced in LD compared to the other muscles. Furthermore, the characterization of the peptides formed during tenderization could be used to evaluate the degree of proteolysis in given a meat sample as well as to correlate the rheological and sensory characteristic with formation of particular compounds.

ACKNOWLEDGMENTS - The authors gratefully acknowledge the Centro di Competenza per la Produzioni Agroalimentari della Regione Campania for permitting the use of the MS facilities. Lina Chianese was partly funded by project “Molecular characterization of water buffalo meat”, in the frame of PRIN 2005.

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