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Proteolytic pattern of myofibrillar protein and meat tenderness as affected by breed and aging time

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ABSTRACT

The effects of breed and aging time (1, 7, 14, 21 days) were evaluated on meat tenderness and on proteolysis in 24 young bulls from Romagnola × Podolian crossbreed, Podolian and Friesian breed. Shear force decreased with aging in all breeds and showed the highest values at 1 and 7 days in Podolian meat. Myofibrillar fragmentation index significantly increased in Podolian meat throughout aging whereas in Friesian and in Crossbreed meat it increased only in the first week. Proteolysis was investigated by SDS-PAGE and 2-dimensional electrophoresis showing a different quantity and expression profile of myofibrillar proteins among breeds. In all breeds a decrease of troponin-T and an increase of troponin-T derived polypeptides during aging were observed. The highest decrease of troponin-T together with the presence of fragments of MHC in Podolian meat during aging was an outcome of a more extensive proteolysis in this breed. Data suggest that tenderness and proteolytic changes during aging are related to animal's breed.

1. Introduction

Breed is an important factor that can influence the characteristics of the raw muscle tissue (Cuvelier et al., 2006) and hence of the finished product. A large number of genetically distinct cattle breed are reared in Italy and this genetic diversity produces meat with many different qualities.

Chianina, Marchigiana, Romagnola, Maremmana, and Podolian cattle, which descended from the same original stock, are indigenous Italian breeds widespread throughout Italy. The farms which follow the cow-calf line are small or middle sized and most of them are located in hilly or mountainous areas. These autochthonous breeds currently number a total of 150,000 head of cattle involved in selection activities (A.N.A.B.I.C., 2011).

Podolian breed is a rustic and frugal breed exploiting grazing areas covered with shrubs, stubbles and thickets. It is a typical breed well adapted to the difficult climatic conditions of the rural areas of Southern Italy (d'Angelo et al., 2006). Physical activity along with the leanness of Podolian breed can account for the low tenderness which often characterizes its meat and may give rise to reduced consumer acceptability (Napolitano, Caporale, Carlucci, & Monteleone, 2007). Podolian breed has been crossbred with other beef breeds to improve sensorial properties (Braghieri, Cifuni, Girolami, Riviezzi, & Napolitano, 2005). Romagnola breed derives from the Podolian breed, maintaining the morphological features of a grazing animal. However, in the last decades, this breed has been subjected to a selection work gaining the characteristics of a modern beef-cattle type (Sbarra, Mantovani, & Bittante, 2009). Indeed meat production characteristics of Romagnola breed are comparable to Limousine breed as investigated by Keane and Allen (2002). Friesian is a worldwide spread dairy breed with a high potential for milk production, male progeny of this breed is sometimes raised in intensive system for beef production.

Tenderness is considered one of the most important components of meat quality, inadequate meat tenderness has been identified as the major cause of consumer dissatisfaction (Grunert, 1997; Miller, Carr, Ramsey, Crockett, & Hoover, 2001) so any improvement in tenderness would increase the commercial value of the final product (Brooks et al., 2000). Meat tenderness is the result of different factors such as the amount and solubility of connective tissue, sarcomere shortening during rigor development and post mortem proteolysis of myofibrillar proteins (Koohmaraie & Geesink, 2006). Aging is one of the most influential factors affecting meat tenderness involving complex changes in muscle metabolism in the post slaughter period and is dependent on animal breed, metabolic status and environmental factors such as rearing system and stress (Cifuni, Napolitano, Riviezzi, Braghieri, & Girolami, 2004.

Many studies focused the effect of breed type and aging on sensorial and texturial meat quality parameters of bovine meat (Monson, Sanudo, & Sierra, 2005; Vieira, Cerdeño, Seffano, Lavín, & Mantecón, 2007). Analysis of muscle proteins during aging time is necessary to understand the biological basis of changes in meat tenderness and their differences among breeds. In the last years, several studies on

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proteolysis of bovine meat have been performed (Bjarnadottìr et al., 2012; Jia, Hollung, Therkildsen, Hildrum, & Bendixen, 2006), however, limited data are available on the differences in meat proteolysis among cattle breed with different purposes during aging.

Three breeds that have been chosen for their different purposes: dairy (Friesian), beef (Romagnola × Podolian crossbreed) and rustic (Podolian) have been involved in this study. The effects of breed and aging time were evaluated on meat tenderness and on proteolytic pattern of myofibrillar proteins in *longissimus dorsi* muscles.

2. Materials and methods

2.1. Animals and meat sampling

A total of 24 Friesian, Podolian, and Romagnola × Podolian crossbred young bulls were involved in the study. Eight animals from each breed were reared under comparable management conditions for about 120 days during the finishing period and were slaughtered at 19 months of age, according to the EU rule n. 119/1993. The mean weights at slaughter were 510 ± 15.8 (SE) kg, 600 ± 14.5 (SE) kg and 625 ± 13.2 (SE) kg for Podolian, Friesian and Crossbred, respectively.

Longissimus dorsi (LD) muscle was removed from each half carcass 24 h post mortem; each muscle was divided into two sections resulting in four samples for each animal. LD was stored at 2 °C under vacuum packaging and analyzed at 1, 7, 14 and 21 days of aging; cranial and caudal sections were randomized across aging time.

2.2. Meat chemical composition

Each sample was ground to homogeneous consistency using a food processor. Analysis of moisture, protein, lipid and ash contents was performed, in duplicate, according to AOAC methods (1995).

The concentration of hydroxyproline was determined using the method described by Hutson, Crawford, and Sorkness (2003). The amount of total collagen was calculated from the hydroxyproline concentration using a conversion factor of 7.7. The collagen content was expressed in mg per gram of wet meat.

2.3. Warner–Bratzler shear force (WBSF) and myofibril fragmentation index (MFI)

WBSF was tested on cooked meat using a Warner–Bratzler blade attachment on an Instron 3343 universal testing machine (Instron Ltd., High Wycombe, United Kingdom) which measured the peak force (kg) required to cut steak sample in half perpendicular to its length. Steak samples were placed on aluminum-folded strips and grill-cooked to a core temperature of 80 °C, previously the grill was heated to 250 °C. For each meat sample, 6 blocks with sides at right angles and a 1 cm^2 in cross-section area cut parallel to the muscle fiber direction were obtained to evaluate WBSF. Each meat block was sheared perpendicular to the fiber at 100 mm/min cross-head speed using 100 kg load cell.

MFI was measured according to Culler, Parrish, Smith, and Cross (1978); the protein concentration was determined by spectrophotometric assay at 540 nm.

2.4. Extraction of myofibrillar protein

Samples were freed of connective and adipose tissue, and 2.5 g was homogenized with an Ultra Turrax homogenizer (IKA T18 basic, Germany) with 10 ml of 0.003 M phosphate buffer at pH 7, for 3 min. The homogenate was centrifuged under refrigeration at 4 °C and 8000 ×g (Eppendorf 5810 R, Eppendorf AG, Hamburg, Germany) for 20 min. After centrifugation the supernatant was discarded and 0.100 g of pellet was resuspended with 1 ml of denaturing solution (8.3 M Urea, 2M Thiourea, 64mM Dithiothreitol (DTT), cholamidopropyl dimethyl hydroxypropane sulfonate (CHAPS) 2%, NP-40 2%, Glycerol 10% and 20 mM Tris–HCl, pH 8) and kept in contact overnight. The next day they were centrifuged at 15,000 ×g for 20 min at 10 °C. After centrifugation the supernatant was aliquotated and frozen at −80 °C.

2.5. SDS-PAGE analysis of myofibrillar proteins

Myofibrillar proteins were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a gradient gel 8–18%. The run was performed in a continuous buffer system using a Protean II xi vertical slab gel unit (Bio-Rad Laboratories, Hercules, CA). Gels were stained with Coomassie blue G250. Destained gel images were acquired by the Chemi Doc EQ system (Bio-Rad Laboratories, Hercules, CA) using a white light conversion screen and analyzed with the Quantity One software (Bio-Rad Laboratories, Hercules, CA) to determine the signal intensity (optical intensity) of the defined bands. Identification of the protein molecular weight was done by comparison with a known molecular weight standard (precision plus protein standard-broad range, Bio-Rad, Laboratories, Hercules, CA). With the sum of the intensity of the defined bands in a lane set a 100%, the relative quantity of each band was determined as the percentage of the signal intensity of the defined band in a lane.

2.6. Two-dimensional gel electrophoresis (2-DE) of myofibrillar protein

Protein separation in the first dimension was made in immobilized pH gradient (IPG) dry strips (17 cm IPG strips, Bio-Rad Laboratories, Hercules, CA), spanning the pH regions 4–7 for the myofibrillar extract using an IPG Protean IEF Cell (Bio-Rad Laboratories, Hercules, CA). The myofibrillar extract was suspended in isoelectrofocusing IPG sample buffer (ready-Prep Rehydratation/Sample Buffer, Bio-Rad Laboratories, Hercules, CA). The IPG strips were equilibrated at room temperature for 15 min in equilibration buffers I and II (Bio-Rad Laboratories, Hercules, CA). Two dimensional separation was performed on a Protean II xi vertical slab gel unit (Bio-Rad Laboratories, Hercules, CA) using SDS-PAGE gradient 8–18%. A broad range molecular weight electrophoresis calibration kit (Bio-Rad Laboratories, Hercules, CA) was used as standard. Gels were fixed and stained with Coomassie silver blue G250 (Bio-Rad Laboratories, Hercules, CA). The destained gels were acquired by the Chemi Doc EQ system (Bio-Rad Laboratories, Hercules, CA) using a white light conversion screen and analyzed with the ImageMaster 2DE Platinum software 5.0 (GE Healthcare, Piscataway, NJ) to determine the number of spot in the different zones, the number of total spots, the isoelectrical point (pI), the molecular weight (MW), the volume, area and the intensity of each spot. On this software, the gel images were processed first by automatic spot detection and spot matching, and then each spot was checked manually for matching and quantification. All the spots were identified on the basis of their isoelectric point (pI) and molecular weight (MW) and by comparison with maps reported in literature (Bouley, Chambon, & Picard, 2004).

2.7. In-gel digestion and protein identification by MALDI-TOF mass spectrometry

Protein of interest from SDS-PAGE and 2DE preparative gels was in-gel digested according to Di Luccia et al. (2005). Prior to mass spectrometric analysis peptide digests were desalted on C_{18} Zip-Tip micro-columns (Millipore, Bedford, CA, USA), washing with 0.1% TFA and eluting with 7 μl of 70% acetonitrile/0.1% TFA. MALDI-TOF mass spectra were acquired on a Voyager DE-Pro spectrometer (PerSeptive BioSystems, Framingham, MA) equipped with a N2 laser (λ = 337 nm), using α-cyano-4-hydroxy-cinnamic acid as the matrix (10 mg/ml in 50% acetonitrile, v/v, containing 0.1% TFA). The instrument operated with an accelerating voltage of 20 kV. External mass calibration was performed using a standard low-mass peptide mixture. The mass spectra were acquired in the reflector mode