



Omics integrating physical techniques: Aged Piedmontese meat analysis



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ABSTRACT

Piedmontese meat tenderness becomes higher by extending the ageing period after slaughter up to 44 days. Classical physical analysis only partially explain this evidence, so in order to discover the reason of the potential beneficial effects of prolonged ageing, we performed omic analysis in the *Longissimus thoracis* muscle by examining main biochemical changes through mass spectrometry-based metabolomics and proteomics. We observed a progressive decline in myofibrillar structural integrity (underpinning meat tenderness) and impaired energy metabolism. Markers of autophagic responses (e.g. serine and glutathione metabolism) and nitrogen metabolism (urea cycle intermediates) accumulated until the end of the assayed period. Key metabolites such as glutamate, a mediator of the appreciated umami taste of the meat, were found to constantly accumulate until day 44. Finally, statistical analyses revealed that glutamate, serine and arginine could serve as good predictors of ultimate meat quality parameters, even though further studies are mandatory.

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1. Introduction

The Piedmontese is a typical breed of cattle from the region of Piedmont, in north-west Italy. The breed originated when migrating Zebu (*Bos indicus*) cattle crossed with the autochthonous Aurochs, approximately 25 thousand years ago (Piedmontese – Origins of Breed. Breeds of Livestock, 2014). However, processes of natural selection and domestication, especially from the late nineteenth century, resulted in the selection of characteristic postpartum hypertrophic muscle growth (“double muscling”), a peculiarity that stems from the inherited inactivation of the myostatin gene and favours muscle growth in this breed (Wheeler, Shackelford, Casas, Cundiff, & Koohmaraie, 2001).

Piedmontese has been historically considered a triple feature/attribute/characteristic (meat, milk and work). In 1976 the Piedmontese breed became a specialised variety for meat. The individuals reach the average slaughter weight (males 550–650 kg, females 400–450 kg) in about 14–18 months. The Piedmontese breed is mainly known for its superior yields of lean and tender meat. In comparison to other breeds, the double-muscling Piedmontese meat is characterised by higher water and protein

contents. Normally the intramuscular fat content is about 1% or lower. Consequently, the triacylglycerol content is greatly reduced, resulting in lower fat deposition, with a positive increase of the polyunsaturated/saturated fatty acid ratio (Brugiapaglia, Lussiana, & Destefanis, 2014). The meat of the hypertrophied Piedmontese animals is also very tender, because of a large reduction in muscle collagen and a lower proportion of stable non-reducible cross-links (Destefanis, Barge, and Brugiapaglia, 1993; Destefanis, Brugiapaglia, and Barge, 1993). As a result, the Piedmontese is amongst the most important Italian autochthonous beef breed, and it contributes for 37% to the beef production and for about 50% to the gross sealable product in Piedmont, with approximately 300,000 heads of cattle (Destefanis, Barge, et al., 1993; Destefanis, Brugiapaglia, et al., 1993).

Tenderness is a key factor influencing consumers' repurchase intention, and biochemical models have been proposed over the years to describe the main events driving muscle to meat conversion and meat tenderization processes (Ouali et al., 2006; D'Alessandro & Zolla, 2013a). Overall, the complexity of the process of muscle to meat conversion process can be summarized in three main steps, with (i) a short pre-rigour phase during which muscle still remains excitable; (ii) the rigour phase, during which high energy phosphate compounds (ATP, phosphocreatine) and glycogen are exhausted, while tissue reaches its maximum toughness; and (iii) the post-rigour tenderizing phase, largely depending on ageing duration and temperature, muscle types, individual

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animals and animal species (Becila et al., 2010). During phase three, tenderization is driven by the activity of proteases (calpains, cathepsins, proteasomes, caspases, serineproteases and metalloproteases) on skeletal muscle (Ouali et al., 2006; D'Alessandro & Zolla, 2013a). Other than proteolysis, non-enzymatic aspects such as temperature, pH, calcium concentration, sarcomere length, and connective tissue/collagen content of the muscles can all affect meat quality, as these variables have an impact on proteolytic activity in the muscle (Ouali et al., 2006; D'Alessandro & Zolla, 2013a).

During the last eight years, several Authors (Becila et al., 2010; Kemp & Parr, 2012) have produced compelling evidence about the likely involvement of apoptotic mechanisms in the processes driving meat tenderization. More recently, such theory has been further confirmed and expanded by pondering the resemblance of the biochemical environment in *post mortem* muscles that are induced through the process of animal's slaughter and exsanguination to the hypoxic/ischaemic conditions that have been investigated in other biological models, such as in the case of neuronal or cardiac ischaemia and reperfusion injury (Solaini, Baracca, Lenaz, & Sgarbi, 2010). In such cases, hypoxia is accompanied by altered mitochondrial metabolism (mitochondrial uncoupling) and production of reactive oxygen species (ROS), utterly promoting either apoptotic or autophagic events (Sierra & Oliván, 2013), and affecting meat physiological parameters, such as colour (D'Alessandro & Zolla, 2013a).

While the molecular mechanisms underlying muscle to meat conversion and meat tenderization have yet to be fully disclosed, precious insights have been gained during the last few years upon the introduction of omics technologies in the field of farm animal proteomics (D'Alessandro & Zolla, 2012; Ibáñez et al., 2013) and, in particular, in meat science (D'Alessandro & Zolla, 2013b), and their integration with standard physical assays to investigate meat quality parameters (for example, on pig and bovine meat quality (D'Alessandro, Gevi, & Zolla, 2011; D'Alessandro, Marrocco, Zolla, D'Andrea, & Zolla, 2011; D'Alessandro et al., 2012)).

Biochemical evidence accumulated so far has provided a scientific rationale supporting the beneficial effects on meat tenderness of prolonged ageing for a limited time span (from 7 to 15 days, on average), while no significant improvements are gained by further extending such period. The rationale behind such conclusion is that a series of factors end up negatively affecting protease activities in the long term, including pH lowering, altered cation homeostasis, oxidative stress and proteolytic cleavage mediated by cross-interactions (Ouali et al., 2006; D'Alessandro & Zolla, 2013a).

Nevertheless, recent empirical evidence in Piedmont farms has suggested the possibility to obtain highly marketable tender meat from cull cows simply by extending the ageing period up to more than forty days. While the producers insisted on the effectiveness of their approach, firmly supported by consumers' appraisal of their products, no scientific experimental evidence has been produced to underpin their statements.

Therefore, we hereby investigated whether the prolonged ageing (up to 44 days at 1 °C) of Piedmontese cull cow meat was actually correlated to improved palatability (mostly affected tenderness, juiciness) and desirability (colour), as gleaned by standard biomechanical assays (Warner Bratzler Shear force measurement – Wbs; water holding capacity – WHC; Minolta values). Physiological/mechanical assays were then supported by 'Omic' analyses, as to delve into the biochemical events driving muscle to meat conversion in Piedmontese *Longissimus thoracis* in a time course-wise fashion. Particularly, the effects of long ageing were studied in the *Longissimus thoracis* muscle (by monitoring 5 different time points, including day 0, 1, 10, 17 and 44) examining the main biochemical changes by means of mass spectrometry-based metabolomics and proteomics. In order to correlate and integrate

'omics' reading of biochemical changes regarding prolonged-stored meat with standard meat quality assays, we performed principal component analyses (PCA) and Pearson's correlations between omics and physiological/mechanical results.

2. Materials and methods

2.1. Animals

Ten Piedmontese cull cows between 4 and 13 years old, were raised in farms belonging to Consorzio La Granda (CN, Italy), located in Piedmont, a north-west region in Italy.

All the animals were slaughtered in an industrial slaughterhouse, the carcasses were stored in a chilling room at 2 °C. Average slaughter weight of the carcasses was 389.99 ± 6.18 kg. Carcasses were transported to a meat processing plant on post-slaughter day 1.

Longissimus thoracis (LT) muscle (that is, the thoracic region of *Longissimus dorsi*) was removed and stored in a cooler at 1 °C (steady or dynamic) and a relative humidity of 78%.

At 0, 1, 10, 17 and 44 days of ageing, a 10 cm section was removed from the LT muscle and used for all subsequent analyses.

2.2. Classical standard analyses

Classical standard analyses on meat samples include the measurement of microbial safety, together with physical and chemical meat parameters, in order to assess meat safety and quality.

2.2.1. Microbiological analysis

The hygienic status of meat samples were assessed through the Total Bacteria Counts (TBC), the Enterobacteriaceae counts analysis and the standardised methods ISO 4833 (2004) and AFNOR NF V08-054 (1999). For the assessment of faecal contamination, *Escherichia coli* and *Listeria monocytogenes* levels were also measured according to ISO 16649-2:2001 and ISO 11290-1:1996/Amd 1:2004 (2004) procedures, respectively. Colonies with a typical *L. monocytogenes* appearance were identified using a species-specific PCR, according to D'Agostino et al. (2004).

2.2.2. Chemical and physical analysis

The evaluation of meat quality at d0 and d1 was assessed carrying out the following analysis:

- pH measurements at 1–3 h after slaughter and 24 h after slaughter, made by a Crison pH metre with an Ingold Spear electrode and automatic temperature compensator.
- Sarcomere length according to the diffraction method by Cross, West, and Dutson (1981). The diffraction patterns from muscle samples compressed between glass microscope slides were obtained using a helium–neon laser (632.8 nm) as the light source.
- Haem iron content ($\mu\text{g/g}$ muscle) according to Hudzik (1990).
- Water, protein and ether extraction contents (AOAC, 1970). We applied the Kjeldhal method to achieve the determination of nitrogen using a Buchi System apparatus (Buchi Labortechnik, Flawil, Switzerland); crude protein was calculated by multiplying $N \times 6.25$. We used a Buchi extraction system for the determination of lipids content, according to the Soxhlet method.
- Lightness (L), redness (a) and yellowness (b) on meat samples at d1, d10, d17 and d44. We used a Minolta CR-331C Chroma Meter (Minolta Camera Co., Japan) (Petracci, Betti, Bianchi, & Cavani, 2004) in the CIELAB space (CIE, 1978) calibrated on the D65 illuminant. The measures were carried out after 1 h of blooming on a 3 cm thick steak. Chroma and hue were calculated from a and b values according to expressions: $C = (a^2 + b^2)^{1/2}$ and hue ($h = \tan^{-1}(b/a)$).

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