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Structural and biochemical characteristics of bovine intramuscular connective tissue and beef quality

Annabelle Dubost [a], Didier Micol [a], Brigitte Picard [a], Claire Lethias [b], Donato Andueza [a], Dominique Bauchart [a], Anne Listrat [a,*]

[a] INRA (Institut National de la Recherche Agronomique), UMR1213 Herbivores, F-63122 Saint-Genès-Champanelle, France
[b] IBCP (Institut de Biologie et Chimie des Protéines), FRE 3310 DyHTIT, Passage du Vercors, 69367 Lyon, Cedex 07, France

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ABSTRACT

The aim of the study was to evaluate the impact of structural and biochemical characteristics of muscle intramuscular connective tissue on beef quality. The experimental design was based on three muscles of three breeds sampled as fresh material and cooked at 55 °C (*Longissimus thoracis* and *Semimembranosus*) or at 70 °C (*Semimembranosus* and *Biceps femoris*) for quality assessment. The results showed that muscle characteristics influence beef quality differently from one muscle to another. In grilled LT, proteoglycan content contributed negatively to juiciness, and intramuscular lipids were linked positively to tenderness, flavour, residues and overall liking scores. In grilled SM, cross-link and lipid contents were involved in beef quality. In BF cooked to 70 °C, perimysial branch points were negatively linked to tenderness. In SM cooked to 70 °C, perimysial area was involved in beef quality. These results should allow a better understanding of the factors involved in background toughness, in juiciness and flavour of meat.

1. Introduction

Consumer perception of meat is a critical issue for the beef industry because it impacts directly on the profitability of the sector (Troy & Kerry, 2010). For the beef consumer, tenderness and juiciness remain the most sought sensory qualities which influence the decision to repurchase (Dransfield, Francombe, & Whelehan, 1984; Grunert, 1997; Troy & Kerry, 2010). Nowadays the flavour trait is growing in importance (Felderhoff et al., 2007). However, meat sensory characteristics are subject to variations suggesting that different aspects of beef management and processing need to be optimised in order to control these variations (Oddy, Harper, Greenwood, & McDonagh, 2001).

Slaughter environment and process have an impact on beef quality, but factors such as muscle fibre and connective tissue characteristics are also involved in beef quality variations (Maltin, Balcerzak, Tilley, & Delday, 2003). Muscle fibre cross-sectional area or diameter would appear to influence tenderness at very early *post-mortem* times, but not after 6 days of ageing (Crouse, Koohmaraie, & Seideman, 1991). After 14 days, ageing is optimal but background toughness in relation to connective tissue characteristics remains (structure, composition and amount) which contributes to a large part of the meat quality variations (Purslow, 2005).

Muscle connective tissue is composed of cells and extracellular matrix, consisting of collagen fibres embedded in a matrix of proteoglycans (Nishimura, 2010). The quantity of connective tissue, estimated by the quantity of collagen (its main component), influences tenderness but can lead to inconsistent conclusions. Indeed high positive correlations are found in raw meat between shear force and total collagen (Dransfield et al., 2003; Torrescano, Sanchez-Escalante, Gimenez, Roncales, & Beltran, 2003) whereas in cooked meat, only low correlations are found (Campo et al., 2000; Dransfield et al., 2003). Cross-links, which are thermo-stable covalent bounds between collagen fibrils, also show no significant nor high correlation with cooked meat tenderness (Listrat, Levieux, & Lepetit, 2007; Ngapo et al., 2002). Intramuscular lipid content affects juiciness directly and flavour and tenderness indirectly. Lipids are positively correlated with juiciness (Jeremiah, Dugan, Aalhus, & Gibson, 2003), flavour (Mottram, 1998) and tenderness (May, Dolezal, Gill, Ray, & Buchanan, 1992) of beef.

The role of proteoglycans in tenderness and juiciness is not known. One hypothesis is that they could impact on the juiciness by interacting with water molecules, contributing to trap them and to create a water compartment (Velleman, 2012). In addition, degradation of proteoglycans associated with collagen fibres during the *post-mortem* period would result in a weakening of connective tissue, thus increasing meat

* Corresponding author. Tel.: +33 473624105.
E-mail address: anne.listrat@clermont.inra.fr (A. Listrat).

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tenderness (Eggen, Ekholdt, Host, & Kolset, 1998; Nishimura, Ojima, Liu, Hattori, & Takahashi, 1996; Velleman, 2012).

Intramuscular connective tissue is highly structured, both by the perimysium that bundles muscle fibres into fascicles and by the endomysium that individualizes skeletal muscle fibres. Structural characteristics of peri- and endomysium vary from one animal to another and from one muscle to another (Purslow, 2005). Few references are available on the influence of connective tissue structure on tenderness, except a potential role of the perimysial thickness (Brooks & Savell, 2004; Fang, Nishimura, & Takahashi, 1999; Nishimura, Fang, Wakamatsu, & Takahashi, 2009).

In the literature, authors were interested either in structural or biochemical aspects of intramuscular connective tissue, and mainly in connection with mechanical tenderness. To our knowledge there are no studies taking into account both the structural and the biochemical composition of intramuscular connective tissue with respect to beef quality. This is an integrative study since it assesses not only the role of structural characteristics of perimysium and endomysium on tenderness, juiciness and flavour of meat but also those of the main biochemical components of intramuscular connective tissue, that is, collagen and its cross-links, proteoglycans and lipids.

2. Materials and methods

The study was carried out in compliance with French recommendations and those of the Animal Care and Use Committee of the National Institute for Agricultural Research (INRA, Institut National de la Recherche Agronomique) of Clermont-Ferrand/Theix, France, for the use of experimental animals including animal welfare.

2.1. Beef production and muscle sampling

The experiment was performed on 40 pure breed young bulls: Aberdeen Angus (n = 12), Limousin (n = 14) and Blond d'Aquitaine (n = 14) (Micol et al., 2010). Animals were assigned to a 100-day finishing period. The animals were housed in straw bedded pens, individually fed and weighed every 2 weeks. Diets consisted of concentrate (75%) and straw (25%). The percentages of straw and concentrate referred to daily diet weight on dry matter basis.

Animals were slaughtered at 17 months on average and at a final live weight of 670 kg. They were slaughtered at the experimental slaughter-house of the INRA Research Centre. Three muscles were taken from each animal: *Longissimus thoracis* (LT), *Semimembranosus* (SM) and *Biceps femoris* (BF). For all muscles and analyses, samples were taken from the 9th rib for LT muscle and from the centre of the tissue for SM and BF muscles.

For structural (histology) and proteoglycan measurements, muscle samples were taken 15 min after exsanguination. For histological analysis, muscle samples of 1.5 × 1.5 × 1 cm were taken, fixed on a piece of cork and frozen in isopentane chilled in liquid nitrogen. For proteoglycans, muscle samples (60–80 g) were cut up into small pieces, frozen and powdered in liquid nitrogen. The two sets of samples were stored at −80 °C until analyses.

Carcasses were chilled in a cold room (+2 °C) and muscle samples were taken at 24 h *post-mortem*. For collagen and cross-link measurements, muscle samples (about 150 g) were cut into pieces of 1 cm cross-section, sealed under vacuum in plastic bags and frozen. Frozen muscle was homogenized in a household cutter, freeze-dried for 48 h, pulverized in a horizontal blade mill and finally stored at +4 °C in stopper plastic flasks until analyses. For lipid content, 60–80 g of muscle was cut into small pieces and frozen in liquid nitrogen. They were ground in liquid nitrogen in a mill to produce a fine homogeneous powder and stored at −80 °C. For sensorial and mechanical quality evaluations, meat samples were aged in vacuum-packs at +4 °C for 14 days. After these 14 days of ageing at +4 °C, sample muscles were packed under vacuum and stored at −20 °C until analyses.

2.2. Structural characterization of connective tissue

Muscle blocks (1.5 × 1.5 × 1 cm) were fixed with Tissue-Tek® OCT™ (Sakura) in a cryostat (HM 500 M, Microm, France) maintained at −25 °C, and cross-sections of 10 μm thick were prepared. The cross-sections were stained according to the Picro-Sirius red method (Fig. 1) previously described by Flint and Pickering (1984) with slight modifications, as previously described (Dubost, Micol, Meunier, Lethias, & Listrat, 2013).

Stained cross-sections were then analysed by image analysis with two programmes developed using the Visilog 6.7 Professional Software (Noesis, Gif-sur-Yvette, France) and previously described (Dubost et al., 2013). For the perimysium study, each stained muscle section (1.5 × 1.5 cm) was scanned entirely in transmission mode using an EPSON Expression 10000XL PRO A3 scanner (resolution 2400 dpi, corresponding to a spatial resolution of 10.58 μm/pixel). For endomysium and muscle fibre, stained sections were visualized in bright field mode with an Olympus BX51 microscope (Olympus, Tokyo, Japan) using a 10× objective (NA = 0.3). Five images were acquired randomly from the stained sections of 1.5 × 1.5 cm (the same as used for the perimysium study) with a DP-72 colour camera and Cell-D image acquisition software (Olympus Soft Imaging Solutions, Münster, Germany). The resolution was 1360 × 1024 pixels, representing an 879 × 662 μm field of view. The resulting spatial resolution was 0.65 μm/pixel.

For perimysium and endomysium, we determined: area (% of the total muscle section area), total length (mm/mm^2), width (μm), number of branch points per cm^2 for perimysium and number of connection points per mm^2 for endomysium (Fig. 1), unit length (expressed in mm for perimysium and in μm for endomysium). For muscle fibre, we determined: fibre number per mm^2 and mean fibre area (μm^2).

2.3. Biochemical characterization of connective tissue

2.3.1. Collagen and cross-link measurements

For total collagen and cross-links, about 250 mg of muscle powder were weighed, acid hydrolysed with 10 mL of 6 N HCl, over-night at 110 °C in a screw-capped glass tube. For total collagen, the acid hydrolysate was diluted 5 times in water and the subsequent procedure used was that previously described and updated by Listrat et al. (Listrat &

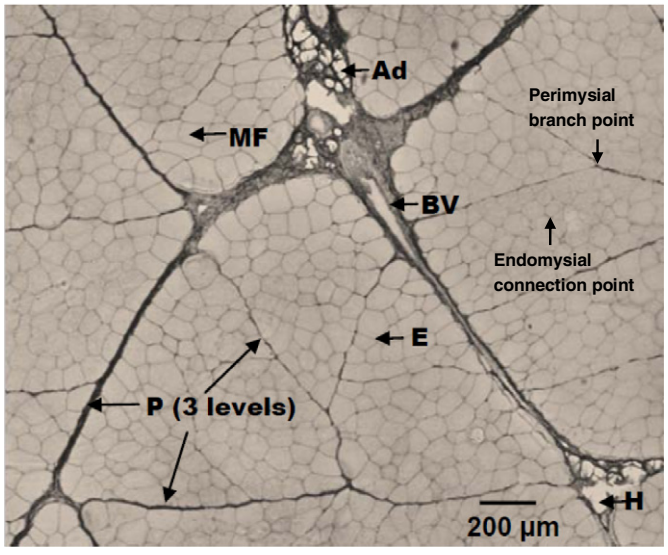

Fig. 1. Histological section of BF muscle stained according to the Picro-Sirius red method. Intramuscular connective tissue (P: perimysium and E: endomysium) appeared in dark grey and muscle fibres (MF) in light grey. Adipocytes (Ad) or holes (H) were not stained and appeared in white. The wall of blood vessels (BV) appeared in an intermediate level of grey. Perimysial branch point were ramifications of perimysium network; endomysial connection points were intersection between two muscle cells.