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Contribution of cross-links and proteoglycans in intramuscular connective tissue to shear force in bovine muscle with different marbling levels and maturities

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ABSTRACT

To explain the factors contributing to the changes in shear force, pyridinoline cross-links and proteoglycans (decorin and glycosaminoglycans) were investigated in *longissimus thoracis* (LT) taken from Qinchuan steers. In LT muscles with different marbling levels (C, B, A, S) and the same teeth maturity (group I), the contents of cross-links, decorin and glycosaminoglycans (GAGs), as well as the shear force declined gradually with increasing marbling while the collagen heat solubility increased. In group II, the heat solubility gradually declined by 58.22% along with the teeth maturity from 0 to 8 permanent incisors at the same marbling level, whereas the shear force increased by 37.49%. The contents of cross-links, decorin and GAGs increased to 0.72 μmol/g collagen, 34.86 μg/g collagen and 25.46 mg/g collagen respectively. Multivariate statistical results indicated that the shear force was positively correlated with the inherent intramuscular connective tissue traits (content of mature cross-links and proteoglycans) and relied more on the collagen heat solubility than on the content of total collagen. The distribution of the score plots definitely indicated that "youthful and marbled" beef had similar beef characteristics, such as high collagen heat solubility and low shear force.

1. Introduction

Tenderness is a paramount factor in the determination of meat quality. Shear force is an objective method to evaluate meat texture. The intramuscular connective tissue (IMCT) plays a significant role in determining meat tenderness (Nishimura, 2010).

Early researches showed that shear force generally increased (Nishimura, Hattori, & Takahashi, 1996; Nishimura, Ojima, Liu, Hattori, & Takahashi, 1996) and tenderness declined with animal age due to many factors, such as collagen content and perimysium thickness (Nishimura, Hattori, & Takahashi, 1999; Purslow, 2005; Schönfeldt & Strydom, 2011). In Torrescano, Sánchez-Escalante, Giménez, Roncalés, and Beltrán (2003), a high positive correlation between the total collagen and shear force of raw beef was observed in Swiss Brown young bull (485 days). Nevertheless, in Christensen et al. (2011), there was no correlation between collagen characteristics and the raw and cooked meat texture in *longissimus thoracis* from 15 different European breeds (15 months). Chriki et al. (2013) also concluded that total collagen was not a good predictor of overall tenderness in more than 5000 animals from 1 to 120 months of age belonging to 20 different breeds but this conclusion depended on muscle type.

Un-reducible (mature) cross-links between the collagen molecules in macromolecular fibrils provide connective tissue with the required physical-chemical properties and biomechanical stability (Lepetit, 2008; McCormick, 1999; Purslow, 2005). The known mature cross-links are hydroxylysyl pyridinoline (HP) and lysyl pyridinoline (LP). However, the exact changes that occur in IMCT and the contribution of cross-links to the tenderness during the process of maturity still require further research.

Meanwhile, fatness may play a more important role than maturity in the improvement of meat tenderness (Pflanzer & De Felicio, 2009). The decreased shear force in beef from marbled Japanese Black cattle was attributed to mechanical "disorganisation and weakening" of the IMCT structure caused by the development of adipose tissue (Nishimura et al., 1999). The honeycomb structure of the endomysium was partially broken, and the perimysium was

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separated into thinner collagen fibres. Although collagen heat solubility was taken into account, it could not explain the primary reasons for tenderisation in highly marbled beef. Cross-links were only used to explain the slight increase in heat solubility of collagen in theory.

In addition, other principal components in IMCT are proteoglycans (PGs) (Eggen, Pedersen, Lea, & Kolset, 2001; Hannesson, Pedersen, Ofstad, & Kolset, 2003). Decorin is a major type of PG in striated muscle (Eggen, Malmstrøm, & Kolset, 1994). A number of glycosaminoglycans (GAGs) attach to the core protein in PGs. Some studies have proposed that PGs likely interact with both collagen and non-collagen materials and play an important role in tissue function, architecture and morphogenesis (Nishimura, Futami, Taneichi, Mori, & Hattori, 2002; Nishimura, Hattori, et al., 1996; Velleman, Patterson, & Nestor, 1997). Nevertheless, there have been few reports on the contributions of PGs (decorin and GAGs) to meat texture at different marbling levels and maturities.

Thus, the objectives of the present study were (1) confirm the role of heat solubility on shear force; (2) to quantify the contributions of cross-links and PGs in IMCT to the tenderisation of highly marbled beef and the toughening of old cattle beef; and (3) investigate the content of pyridinoline cross-links, decorin, GAGs, collagen and the changes in shear force in bovine *longissimus thoracis* (LT) with different marbling levels and maturities.

2. Materials and methods

2.1. Sample preparation

According to Table 1, eighty Qinchuan steers were harvested and selected at Shaanxi Kingbull Animal Husbandry Development Co., Ltd. After 24 h of rest, the cattle were slaughtered according to halal method. The slaughter was executed by a throat cut using a sharp knife in order to bring the animal to a quick death without suffering. Carcasses were carefully selected according to GB/T29392-2012 “Beef cuts grading for high rib, ribeye, striploin, tenderloin of national beef” (AQSIQ & SAC, 2012) and NY/T676-2010 “Beef Quality Grading” (Ministry of Agriculture, 2010) in China. The number of permanent incisors was recorded by looking directly at the teeth after removal of the animal's head. The marbling was evaluated by observing a section of the ribeye (12–13th rib cross-section). In China, 36-h post-mortem is usually adopted as the content of intramuscular fat in LT muscle is less than 15%. LT muscle samples (7th to 12–13th thoracic vertebrae) were selected from the carcasses at 4 °C, and then were split into two groups. The selection scheme for LT muscle can be found in Table 1. All of the LT muscles were individually vacuum packaged and stored at −20 °C until sampling. In group I, all of the test samples were at different marbling levels with the same number of permanent incisors. The experiments were executed to explore the changes in the inherent traits of the IMCT (mature cross-links, PGs), heat solubility and shear force at different levels of marbling in beef. In group II, all of the test muscles were at the same marbling level to observe the changes affected by maturity. The content of fat in each sample was measured according to the method described by Folch, Lees, and Sloane-Stanley (1957). The fat content of each muscle was in accordance with the corresponding marbling level described in GB29392-2012 (AQSIQ &SAC, 2012).

The whole LT muscle was cut into two segments (approximately 7–10th and 10–12th from cranial to caudal). The cranial sections were used for the quantification of total collagen, collagen heat solubility, cross-links, decorin, and GAGs. The caudal sections were arranged to measure the shear force. Before the test, muscle samples were thawed at 4 °C overnight. The cranial sections were cut into pieces of 1-cm cross-sections and then fully minced and mixed in a pulveriser. Visible fat and noticeable connective tissue were removed. The mixed samples were freeze-dried and stored at −20 °C until the next experiment. In the caudal sections, the medial central portions of the muscles were used for the analysis of shear force to avoid interferences of haemorrhagic spots, tendons, thick perimysium or epimysium, and extra fat.

2.2. Methods

2.2.1. Measurement of shear force

Shear force was determined according to a previously published method (Xu, Zhou, Peng, Zhao, & Yao, 2009). Samples from each group were sealed in polyethylene bags and cooked in a water bath (80 °C) until the internal temperature of each sample reached 75 °C. Next, the samples were cooled to room temperature (25 °C). Muscle strips of approximately 1 cm × 1 cm were taken parallel to the fibre direction of the muscle, avoiding visible fat and IMCT. The shear force was measured with a digital meat tenderness meter (Model C-LM3B, Northeast Agricultural University, Harbin, China). Strips were sheared perpendicular to the fibre's longitudinal axis. At least three strips from each muscle specimen were sheared into 6–8 cuts.

2.2.2. Total collagen and heat solubility of collagen

To estimate the total collagen content, approximately 300 mg of freeze-dried samples were randomly selected and hydrolysed in 10 mL of 6 mol/L HCl for 16 h at 110 °C. The hydroxyproline content was measured in the method described by Xu et al. (2009). The amount of hydroxyproline was determined and converted to the collagen content with a factor 7.25.

The heat solubility of collagen was determined by the procedure

Table 1
Selection scheme of *longissimus thoracis* (LT) muscle according to teeth maturity and marbling level.

	Permanent incisors	8	8	8	8	/
Group I	Equivalent to animal age[a]	54–60 months	54–60 months	54–60 months	54–60 months	/
	Marbling level	C	B	A	S	/
	Equivalent to intramuscular fat content[b]	<5%	5–10%	10–15%	>15%	/
	n	10	10	10	10	/
	Permanent incisors	0	2	4	6	8
Group II	Equivalent to animal age[a]	<18 months	18–24 months	30–36 months	42–48 months	54–60 months
	Marbling level	B	B	B	B	B
	Equivalent to intramuscular fat content[b]	5–10%	5–10%	5–10%	5–10%	5–10%
	n	10	10	10	10	10

n: The number of steers.
[a] Equivalent to animal age described in NY/T676-2010 “Beef Quality Grading” in China.
[b] Equivalent to intramuscular fat content described in GB/T29392-2012 “Beef cuts grading for high rib, ribeye, striploin, tenderloin of national beef” in China.