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Intramuscular collagen and meat tenderness in two different beef muscles (1)

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ABSTRACT

The eating quality of meat is based predominantly on tenderness, and previous research has established a relationship between cooked beef texture and changes in the molecular structure of bovine intramuscular collagen, especially caused by maturation and stabilization of the collagen fiber network through multivalent crosslinking molecules.

We studied characteristics of intramuscular collagen and shear force values in semimembranosus and triceps brachii muscles of five Chianina bulls slaughtered at 14 months of age and at a live weight of 573 ± 10 kg (mean ± SD). Muscles were removed from each carcass, and representative subsamples were analyzed for intramuscular collagen concentration, collagen solubility, collagen type (relative proportions of types I and III), and hydroxylysyl pyridinoline concentration. Warner-Bratzler shear values were determined on cooked meat.

Triceps brachii muscle had greater (P < 0.01) collagen concentration (12.00 vs 9.49 µg/mg), soluble collagen (25.6 vs 15.1%), and type III collagen (23.2 vs 18.4%) than semimembranosus muscle. However, semimembranosus muscle had a greater (P < 0.01) hydroxylysyl pyridinoline crosslink concentration on collagen (0.250 vs 0.229 mol/mol) and higher

(P<0.01) Warner-Bratzler shear values (5.66 vs 4.85 kg).

The results suggest that real differences in both the structure and concentration of intramuscular collagen between semimembranosus and triceps brachii muscles of beef exist, probably related to a different collagen synthesis rate between muscles originating from distinctly different areas of the carcass. Since these differences are associated with variations in tenderness of cooked meat, we think that our findings help clarify the role that muscle collagen plays in

Key words: intramuscular collagen, tenderness, beef.

RIASSUNTO

COLLAGENE INTRAMUSCOLARE E TENEREZZA DELLA CARNE IN DUE DIFFERENTI MUSCOLI BOVINI

La valutazione qualitativa della carne si basa principalmente sulla sua tenerezza. Precedenti studi hanno evidenziato una relazione tra le variazioni di tenerezza della carne bovina cotta e quelle che si verificano a carico della struttura molecolare del collagene intramuscolare, legate principalmente al grado di maturazione e stabilizzazione delle fibre mediante legami crociati multivalenti.

L'obiettivo del lavoro è stato quello di studiare le caratteristiche del collagene intramuscolare ed i valori dello sforzo di taglio nei muscoli semimembranosus e triceps brachii di cinque vitelloni Chianini, macellati a 14 mesi d'età e ad un peso

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vivo di $573\pm10\,\mathrm{kg}$ (media \pm DS). I due muscoli sono stati prelevati da ciascuna carcassa e, su campioni rappresentativi, sono stati determinati la concentrazione totale di collagene, la sua frazione solubile, il rapporto tra i fenotipi I e III e la concentrazione di idrossilisil-piridinolina, uno dei principali legami crociati termostabili del collagene intramuscolare. Sono stati inoltre misurati, su carne cotta, i valori dello sforzo di taglio.

I risultati evidenziano differenze sostanziali sia nella concentrazione che nella struttura del collagene tra i muscoli bovini semimembranosus e triceps brachii. In particolare, nel triceps brachii sono stati osservati valori più elevati (P<0,01) di collagene totale (12,00 vs 9,49 µg/mg), della sua proporzione solubile (25,6 vs 15,1%) e del fenotipo III (23,2 vs 18,4%). Il semimembranosus, invece, ha evidenziato una proporzione di idrossilisil-piridinolina sul collagene più elevata (0,250 vs 0,229 mol/mol; P<0,01) e valori dello sforzo di taglio più elevati (5,66 vs 4,85 kg; P<0,01).

Dal momento che tali differenze, probabilmente legate ad una differente velocità di sintesi del collagene tra muscoli con diversa localizzazione nella carcassa, risultano associate a variazioni nello sforzo di taglio della carne cotta, riteniamo che i nostri risultati possano aiutare a chiarire il ruolo esercitato dal collagene intramuscolare sulla tenerezza della carne.

Parole chiave: collagene intramuscolare, tenerezza, bovino da carne.

Introduction

The eating quality of red meat is based predominantly on tenderness. Factors influencing meat tenderness include: rate of glycolysis, post mortem pH, sarcomere length, post mortem proteolysis, and amount and solubility of collagen (Koohmaraie, 1994). Previous research has established that the tenderness of cooked meat is influenced by the effect of heat on the strength of both the myofibrillar and connective tissue components (Tornberg, 1996), even if a relationship between cooked beef texture and changes in the molecular structure of bovine intramuscular collagen was evidenced (Boccard et al., 1979; Burson and Hunt, 1986; Bosselmann et al., 1995; Nishimura et al., 1996). In addition, Davey and Gilbert (1975), Bailey and Light (1989), and Palka (1999) documented a biphasic increase in the toughening (increase in shear force) of meat as temperature increased. The first sharp increase, occurring between 40 to 50 °C, corresponds to the denaturation of the myofibrillar proteins. Beginning at 64 to 68°C, shear force again increases sharply, with a further increase in toughening corresponding to the thermal denaturation of collagen. This collagen denaturation results in shrinkage of the fibril accompanied by force or tension development, the degree of which is a function of how heavily the collagen is crosslinked with mature, heat-stable crosslinks (Bailey and Light, 1989).

Differences in meat texture are caused by maturation and stabilization of the collagen fiber network through multivalent crosslinking molecules (Bailey, 1988; Nishimura et al., 1996) that form throughout an animal's life. Reducible crosslinks that are initially formed are converted to mature non-reducible cross-

links. One of the mature crosslinks is the trivalent aminoacid pyridinoline, a fluorescent 3-hydroxypyridinium crosslink, first isolated by Fujimoto et al. (1977). This crosslink plays an important role in meat tenderness (Maiorano et al., 1993; Bosselmann et al., 1995; Field et al., 1996; McCormick, 1999).

In contrast to pyridinoline, the significance of phenotypic forms of intramuscular collagen (specifically types I and III) is not well understood. Stiffness and tensile strength, however, are usually associated with type I, whereas compliance and elasticity are usually associated with type III collagen (Weber *et al.*, 1987; Weber and Brilla, 1991). It is possible that both types could have an effect on texture of meat (Burson and Hunt, 1986; Bailey, 1988).

In addition, controversy still exists on the influence of intramuscular collagen concentration and collagen solubility on texture of meat (Hill, 1966; Cross et al., 1973; Young and Braggins, 1993).

Therefore, the objective of this study was to determine intramuscular collagen characteristics and shear force values in two bovine muscles differing in texture. Chianina cattle, where information about collagen is lacking, was studied.

Material and methods

Animals and muscle sampling

Five purebred Chianina bulls, fed a typical finishing diet for 250 days, were slaughtered at $14 \text{ months of age at a live weight of } 573 \pm 10 \text{ kg}$ (mean + SD).

Semimembranosus and triceps brachii muscle samples were removed from each carcass

7 days after slaughter, vacuum-packaged, frozen and stored at -80 °C until they were analyzed for collagen characteristics and Warner-Bratzler shear values.

Collagen analysis

Samples were trimmed of fat and epimysium, and representative subsamples were removed for intramuscular collagen concentration, solubility, typing and crosslinking.

Collagen concentration and its solubility were calculated from hydroxyproline content (Woessner, 1961) of lyophilized muscle hydrolyzed in 6 N HCl at 110 °C for 18 to 20 h. Total collagen was calculated assuming that it weighed 7.25 times the measured hydroxyproline weight. For soluble collagen, powdered muscle was heated for 70 min at 77 °C in one-fourth strength Ringer's solution, and it was separated into supernatant (heat-soluble) and residue (heat-insoluble) fractions following the procedure of Hill (1966). Collagen in the heat-soluble fraction was calculated by multiplying the hydroxyproline content by 7.25 and reported as a percentage of the total collagen.

Typing and crosslinking analyses were performed on intramuscular collagen, isolated according to Fujii and Murota (1982). The relative proportions of types I and III were determined on 100 mg of pulverized collagen after cyanogen bromide digestion (Hanson and Bentley, 1983). Identification of cyanogen bromide peptides unique to type I [α1 (I) CB8] and III collagen [α1 (III) CB5] was made by peptide mapping using 12% PAGE in the presence of SDS (Laemmli, 1970). Peptides unique to type I or III collagen were quantified by densitometry at 623 nm (LKB Ultrascan XL Laser Densitometer, Bromma, Sweden). Intramuscular collagen phenotype proportions were expressed as percent type III collagen [(III/I+ III) \times 100].

Hydroxylysyl pyridinoline crosslinks were determined in 100 mg (wet weight) of hydrolyzed intramuscular collagen. They were concentrated and purified by selective elution from a CF 1 cellulose column (Skinner, 1982), and then quantified by reverse phase HPLC, using pyridoxamine as the internal standard (Eyre et al., 1984). The HPLC was equipped with a data system Kontron 450 MT2 (Kontron Instruments, Milan, Italy) and with an Altex (Beckman)

Ultrasphere-ODS (C-18; small pore; 4.6×250 mm) column. Hydroxylysyl pyridinoline was identified by comparison with a purified hydroxylysyl pyridinoline standard extracted from bovine articular cartilage and the known relationship between the elution time of hydroxylysyl pyridinoline and pyridoxamine (Eyre et al., 1984). Its concentration, expressed as mol/mol of collagen, was calculated assuming that the molecular weight of collagen was 300000 and the molar fluorescence yield of pyridoxamine was 3.1 times that of hydroxylysyl pyridinoline (Eyre et al., 1984).

Shear tests

For Warner-Bratzler shear force determination, samples from semimembranosus and triceps brachii were thawed overnight at 4°C and dry roasted at 163 °C in a forced air oven until an internal temperature of 71°C was reached. They were then cooled for 2 h at room temperature, and stored overnight at 4°C before Warner-Bratzler shear values were determined. Three 1.2 cm cores were removed from each of three sections (central, medial and lateral) of the cooked meat, parallel to the muscle fibers orientation. Each core was sheared three times using a shear-force attachment of the Instron Universal Testing Machine Model 1140 (Instron, Milan, Italy) at a crosshead speed of 5 cm/ min, and the nine values for each muscle were averaged.

Statistical analysis

Differences between means were detected employing the GLM procedure of SAS (1990), with a model including the animal effect.

Results and discussion

Collagen characteristics (table 1) varied between muscles. *Triceps brachii* muscle had greater (P < 0.01) collagen concentration and soluble collagen percentage, than the *semimembranosus* muscle. McKeith *et al.* (1985) also found a higher collagen concentration in *triceps brachii* than in *semimembranosus* muscle of steers. Also Boccard *et al.* (1979) and Maiorano and Nicastro (1993) observed the

TABLE 1. – Intramuscular collagen characteristics and shear force values from semimenbranosus and triceps brachii muscles.

TABELLA 1. - Caratteristiche del collagene intramuscolare nei muscoli semimembranosus e triceps brachii.

		Muscle - Muscolo		SEM
		semimembranosus	triceps brachii	ESM
Animals Animali	no. <i>n</i> .	5	5	
Total collagen Collagene totale	μg/mg	9.49 ^A	12.00 ^B	0.21
Soluble collagen Collagene solubile	%	15.1 ^A	25.6 в	1.16
Type III collagen (¹) Collagene tipo III (¹)	»	18.4 ^A	23.2 ^B	0.54
Hydroxylysyl pyridinoline Idrossilisil piridinolina	mol/mol	0.250 ^B	0.229 ^A	0.01
Warner-Bratzler shear force Sforzo di taglio	kg	5.66 ^B	4.85 ^A	0.15

(1) $[III/(I + III)] \times 100$.

Values with different letters in the same row are significantly different (P < 0.01). Lettere diverse sulla stessa riga indicano differenze significative (P < 0.01).

same trend on total and soluble collagen, in triceps brachii and semimembranosus muscles of 16- and 14-month-old bulls, respectively. Various researchers (Wu et al., 1981; Miller et al., 1987; Abouheif et al., 1995) suggested that an increase in collagen solubility is probably due to an increase in the rate of collagen biosynthesis. In addition, in our previous works on lambs (Maiorano et al., 1998, 1999) we found total collagen strongly correlated to soluble collagen.

Maiorano et al. (1993) found that increased solubility corresponded with an increase in type III collagen, that is generally considered an embryonic form of collagen (Kovanen and Suominen, 1989). In the present study, percentage of type III collagen was greater (P<0.01) for triceps brachii than for semimembranosus muscle. The elevated proportions of type III collagen and larger soluble collagen pools, as well as subsequent accretion of newly synthesized muscle collagen (McCormick, 1994), probably reflect the different metabolic activity of collagen among different muscles (McCormick, 1999).

A muscle effect on crosslink concentration is also apparent. In fact, the pyridinoline concentration, used to estimate degree of intramuscular collagen maturation (Bosselmann et al., 1995), was greater (P < 0.01) for semimembranosus than for triceps brachii muscle. No data about a direct comparison of pyridinoline in intramuscular connective tissue of semimembranosus and triceps brachii muscles exist. Smith and Judge (1991) found pyridinoline in semimembranosus bovine muscle was lower than that in the present study. This might be due to the differences in animal genotype, carcass maturity grade, or analytical procedure. However, Maiorano et al. (1996) have observed a hydroxylysyl pyridinoline concentration of 0.244 mol/mol in semimembranosus muscle of 12-month-old bulls of Chianina breed. Moreover, researches in sheep (Young et al., 1994) and in goat (Horgan et al., 1991) reported that the semimembranosus is a muscle abundant in hydroxylysyl pyridinoline content. The increase in mature collagen crosslinking in semimembranosus muscle, associated with the low levels of collagen concentration and of soluble collagen, may be due to a slower rate of collagen synthesis than in triceps brachii muscle, with a steady maturation of existing fibrillar collagen (McCormick, 1994). On the other hand, stabilization of mature collagen is manifested by decreased solubility (Cross et al., 1984; Augustini and Temisan, 1985; Smith and Judge, 1991). In addition, Young et al. (1994) believed that pyridinoline concentration in sheep is a reasonable indicator of heat-dependent solubility between muscles as well as within muscles. Generally, greater fractions of both heat-soluble collagen and type III collagen are indicative of more youthful, labile, less crosslinked collagen, even if McCormick (1994, 1999) sustains that the exact nature of the interactions between crosslink concentration and collagen type remain uncertain. This is probably due to a lack of knowledge of the mechanisms that regulate crosslinking in muscle, even if recent studies have suggested that the proteoglycan decorin could have a role in collagen fibrillogenesis (McCormick, 1999; Velleman, 1999).

Warner-Bratzler shear force values showed that the semimembranosus muscle was tougher than triceps brachii (P < 0.01) (table 1). Acciaioli et al. (1995), also, found semimembranosus tougher than triceps brachii muscle, in Chianina males slaughtered at different ages. These findings are in agreement with Dransfield (1977) and Stolowski et al. (1995), and with sensory evaluations by Carmack et al. (1995). Some studies, in contrast to our data, have reported that muscles richer in collagen tend to be tougher (Light et al., 1985; Young and Braggins, 1993), while others suggested that variations in collagen concentration are not closely associated with meat texture (Field et al., 1970; McKeith et al., 1985; Light, 1987; Bailey, 1988). Boccard et al. (1979) observed greater collagen concentration associated to lower shear force value in triceps brachii, than in semimembranosus muscle. McCormick (1999) concluded that mature crosslink and collagen concentration have an additive effect on the toughening of meat, even if Nishimura et al. (1996) suggested that mechanical strength of the intramuscular connective tissue does not depend on the amount of collagen.

No studies, involving the relationship between collagen qualitative characteristics (solubility, type and crosslinking) and tenderness of meat among different muscles of the same animal, are available. However, some researches showed, within a muscle type, that shear values increase with decreases in soluble collagen percentage (Miller et al., 1987; Abouheif et al., 1995; Boleman et al., 1996), with increases in crosslinking (Maiorano et al., 1993; Bosselmann et al., 1995; Field et al., 1996) and with decreased type III percentage (Burson and Hunt, 1986). This trend agrees with our findings on differences between semimembranosus and triceps brachii muscles.

Conclusions

Results from the present study confirm that differences in both the structure, and concentration of intramuscular collagen in *semimembranosus* and *triceps brachii* muscles, exist. This probably is due to a different collagen synthesis rate between muscles originating from distinctly different areas of the carcass.

In a muscle with a greater collagen amount, as *triceps brachii* when compared to *semimembranosus* muscle, collagen is more soluble, less mature (more type III), and less crosslinked. Since these characteristics are associated with an improvement in tenderness of *triceps brachii* cooked meat, we think that our findings help clarify the role that muscle collagen plays in meat texture.

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