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Meat quality

COLLAGEN CROSSLINK PROFILE AND MEAT TEXTURE IN TWO BEEF MUSCLES

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Abstract

Heifers of three different breed types (Angus x Hereford, Piedmontese x Hereford, pure Brahman) and of similar age (approx. months), were used to study in two muscles (Longissimus dorsi, Semitendinosus) the influence of the crosslinking characteristics of intramuscular connective tissue (IMCT) on its heat stability and on the texture of cooked meat. Differences were found between the muscles in the concentration of the major crosslinks in the IMCT (pyridinoline, Ehrlich chromogen, DHLNL, HLNL, HHMD). No differ was found between breed types. Within each muscle, collagen heat solubility and shear value were not significantly correlated with cross concentrations in IMCT. On the other hand, when expressed per unit of muscle mass, the crosslink concentrations were generally significa correlated with the muscle collagen content. Significant correlation coefficients were found between crosslink concentrations in the muscle shear value when pooling the data over the two muscles, but not within each muscle. It was concluded that within a small range of age crosslink profile of IMCT was independent of breed, and that meat texture was mainly determined by the IMCT concentration in the muscle.

Introduction

The intramuscular connective tissue (IMCT), which includes mainly collagen and, to a lesser extent, elastin, is a major contributo meat toughness, though it is quantitatively a minor constituent of the muscle (Lawrie, 1974). When post-mortem conditions allow normal tenderization, those muscles with highest IMCT contents yield usually the toughest meats. The resistance of IMCT to heat solubiliza during the cooking of meat is also a factor of meat toughness. It is determined by the extent of crosslinking, viz. the amount and the type covalent bonds stabilizing the collagen and the elastin. It was demonstrated that collagen heat stability increases from birth to maturity, mai through an increase in the concentration of heat stable crosslinks (Horgan et al., 1991; Smith and Judge, 1991; McCormick, 1994), resulting in the toughening of meat. However, the influence of the variations in the crosslink composition not related to age differences has not been investigated as thoroughly. The aim of this work was to study the effect of crosslink composition on IMCT heat stability and cook meat texture in animals of similar age.

Materials and methods

Twenty-eight heifers of similar age (20.4 ± 0.6 months) and of three different breed types (Angus × Hereford, A×H, n = Piedmontese × Hereford, P×H, n = 9; pure Brahman, B×B, n = 9) were slaughtered. The carcasses were chilled for 24 h at 5°C, and Longissimus dorsi, pars thoracis (LD) and Semitendinosus (ST) muscles were excised, vacuum packed, then stored for 14 days at 0°C finally frozen at -20°C until analysis. Total collagen concentration and collagen heat solubility (90°C, 2 h) were determined on freeze dr muscle homogenates (ISO, 1978). Total collagen was also determined on isolated IMCT (Neuman and Logan, 1950). The IMCT was isola by homogenizing a muscle sample in a physiological saline solution, passing it through a sieve, and repeating this process 3 more times on residue obtained at each step (Light and Champion, 1984). The Ehrlich chromogen (EC) concentration was determined on an IMCT samp according to Horgan et al. (1990). For the other crosslinks, another IMCT sample was reduced with $NaBH_4$ (Robins, 1982), and hydrolysed 6M HCl at 105°C for 16 h. The hydrolysate was run on a CF1 cellulose column in a butanolic eluent (Black et al., 1988), and the crosslink fraction was then eluted with deionized water, and evaporated. Pyridinoline (PYR) was determined by fluorescence detection using the method of Eyre et al. (1984) ; the quantification of this crosslink was made by comparing the peaks with those obtained with a known molar amount PYR standard prepared as described by Fujimoto et al. (1977). The reducible crosslinks were eluted on an ion exchange column using sodium citrate buffers, first with 0.2M, pH 4.61 for 150 min, then with 0.35M, pH 5.28 for 50 min ; they were then quantified by measuring absorbance at 546 nm after post-column derivatization with ninhydrin, and using leucine equivalent factors of 1.75, 1.84 and 3.5 hydroxylysinonorleucine (HLNL), dihydroxylysinonorleucine (DHLNL), and histidinohydroxymerodesmosine (HHMD) respectively (Horg et al., 1990). Crosslink concentrations were expressed both in mole/mole collagen and µmole/100 g muscle. Meat texture was evaluated the peak force developped by a muscle sample during a shear test after cooking at 80°C for 60 min in a water bath (Bouton et al., 1971).

Results and discussion

The concentrations of collagen and of the different crosslinks in the IMCT of LD and ST muscles are presented in Table 1. The IMC of ST contained significantly less collagen than that of LD ($P < 0.001$), but the sums of collagen plus elastin in the IMCT were similar for th two muscles (results not presented). The crosslink concentrations were significantly different between the 2 muscles ($P < 0.05$) except for DHLNL. However, the differences were noticeable only for PYR and HHMD, and the concentrations were higher in the collagen of LD than in that of ST. Actually, the PYR concentrations found in the LD of three animals were much higher (around 0.70 mole/mole collagen) than those found in the other animals (all less than 0.50 mole/mole collagen). When the data obtained on those animals were removed, the difference between LD and ST was reduced by half (0.30 and 0.26 mole/mole collagen, respectively). There was no significant effect of breed on any of the crosslink concentrations in the IMCT, except for HHMD, which concentration was greater in B×B than in A×H heifers (0.42 and 0.36 mole/mole collagen, respectively; $P < 0,05$), the P×H heifers being intermediate. These results show that the crosslink profile of the IMCT little differs between beef muscles such as LD and ST, and also between contrasted breed types.

Table 1. Collagen concentration and crosslink profile of the intramuscular connective tissue (IMCT) of two beef muscles.

IMCT constituent	Muscle LD	Muscle ST	*rsd*
Total collagen (% dry weight)	66a	47b	*5*
EC (*)	0.27a	0.25b	*0.03*
PYR (*)	0.34a	0.26b	*0.10*
DHLNL (*)	0.16	0.16	*0.07*
HLNL (*)	0.05a	0.04b	*0.01*
HHMD (*)	0.43a	0.35b	*0.06*

(*) mole/mole collagen
Within row, means with different superscripts differ significantly ($P < 0.05$).

Muscle ST contained on average 58 % more total collagen than muscle LD (Table 2), and the heat solubility of its collagen was slightly, but significantly lower than that of LD (24 and 27 %, respectively; $P < 0.001$). The muscle collagen concentration was similar in the A×H and B×B heifers, and it was significantly higher ($P < 0.001$) than in the P×H heifers, probably as a result of the presence of the double muscling gene in the latter breed type. On the basis of dry muscle mass, the crosslinks concentrations were higher in ST than in LD by 30 to 50 %, and differences were again all significant ($P < 0.01$) except for HLNL (Table 2). The effect of breed type was significant only for EC

and HHMD ($P < 0.05$). However, differences between breeds were not consistent, EC concentration being lower in P×H heifers and HHMD concentration being higher in B×B heifers as compared to the corresponding two other breed types. The shear value of ST was significantly greater than that of LD ($P < 0.001$; Table 2), but it was not significantly affected by breed type.

Table 2. Collagen concentration, crosslink profile and shear value of two beef muscles.

Muscle constituent	Muscle		*rsd*
	LD	ST	
Collagen (% dry weight):			
- Total	1.8a	2.9b	*0.28*
- Heat insoluble	1.3a	2.2b	*0.26*
Collagen heat solubility (%)	27a	24b	*4*
EC (*)	1.62a	2.36b	*0.37*
PYR (*)	2.02a	2.47b	*0.68*
DHLNL (*)	0.97a	1.45b	*0.64*
HLNL (*)	0.31	0.37	*0.11*
HHMD (*)	2.59a	3.33b	*0.67*
Shear value (peak force, kg)	3.3a	4.5b	*0.6*

(*) µmole/100 g dry muscle.
Within row, means with different superscripts differ significantly $P < 0.05$).

Correlation coefficients between crosslink concentrations and meat tenderness-related variates were calculated within each muscle, and also after pooling the data over the two muscles (Table 3). In the LD muscle, collagen solubility was negatively correlated to the concentrations of the heat stable crosslinks (EC, PYR, DHLNL) in IMCT, and positively correlated to those of the heat labile crosslinks (HLNL, HHMD). No such trend was not found in the ST muscle, except for PYR. However, in both muscles, and also on pooled data, collagen solubility was always poorly, and generally not significantly, correlated with crosslink concentrations in the IMCT ($r \leq 0.30$). The correlation coefficient between PYR concentration and collagen solubility was comparable to that reported by Smith and Judge (1991) in beef semimembranosus ($r = -0.26$), but it was lower than those found by Damergi (1996) in three beef muscles (-0.25 to 0.41) and by Young et al. (1994) in the sheep semimembranosus muscle ($r = -0.71$).

Correlation coefficients between peak force and crosslink concentrations were low and non significant within each muscle ($r \leq 0.35$). This could be attributed to the relatively small number of observations, namely 28 per muscle. But on almost twice this number of animals, Field et al. (1996) reported correlation coefficients ($r = 0.33$) between shear value of beef loin and PYR concentration (mole/mole collagen) of the same order as that found in the present experiment in the LD muscle ($r = 0.29$). This is also consistent with the correlation found by Damergi et al. (1995) in the ST and BF muscles of growing cattle, and by Young et al. (1994) in sheep semimembranosus ($r = 0.23$ and 0.36 respectively). However, on a muscle mass basis (µmole/100 g muscle), the latter workers found an even lower correlation ($r = 0.16$), and, in the present experiment, it was only 0.25 and 0.35 for the LD and ST muscles respectively. However, the combination of the two muscles produced coefficients that were much higher and all positive, particularly with the heat stable crosslinks EC and PYR ($r = 0.50$ and 0.41 respectively; significant, $P < 0.01$). Actually, peak force was also highly correlated with total collagen concentration over the two muscles ($r = 0.67$; significant, $P < 0.001$), but not within muscle. Thus, it may be suspected that the apparent dependence of peak force upon crosslink concentration was actually the result of its dependence upon total collagen concentration. This is evidenced by the high correlation coefficients found between crosslink concentrations and total collagen concentration, both within muscle and in combined muscles (in µmole/100 g muscle; Table 3).

Then it can be concluded that (1) within a narrow range of animal age, the variations in the heat stability of beef intramuscular collagen were not explained by differences in the collagen crosslink profile, (2) total collagen concentration was the major determinant of the variations in crosslink concentrations in the muscle, and finally of meat toughness. The shear values of the two muscles studied were notably low, in particular that of the ST muscle, thus indicating that the meat was tender. Further investigation on muscles of more contrasted levels of toughness is needed to support the present conclusions.

Table 3. Pearson correlation coefficients between crosslink concentrations in IMCT or in muscle, and meat tenderness-related variates (a).

	Collagen solubility			Muscle collagen conc.			Peak force		
	LD	ST	LD+ST	LD	ST	LD+ST	LD	ST	LD+ST
Crosslink concentration in IMCT (mole/mole collagen)									
EC (1)	-0.27	0.04	0.05	0.02	-0.03	-0.27*	0.11	-0.27	-0.30*
PYR (2)	-0.19	-0.32	-0.03	-0.07	-0.15	-0.34**	0.29	0.13	-0.11
DHLNL (3)	-0.15	0.26	0.15	-0.02	-0.28	-0.14	0.02	-0.25	-0.13
HLNL (4)	0.11	-0.09	0.22	-0.08	0.12	-0.42**	0.29	0.17	-0.23
HHMD (5)	0.15	0.04	0.29*	0.02	0.06	-0.43**	0.13	-0.34	-0.45***
Heat stable (1+2+3)	-0.25	0.10	0.07	-0.06	-0.30	-0.38**	0.26	-0.24	-0.21
Heat labile (4+5)	0.16	0.02	0.30*	0.01	0.08	-0.46***	0.17	-0.31	-0.45***
Crosslinks concentration in muscle (µmole/100 g dry muscle)									
EC (1)	-0.61***	-0.17	-0.47***	0.83***	0.64***	0.85***	0.01	0.03	0.50***
PYR (2)	-0.36	-0.47*	-0.46***	0.22	0.53**	0.44***	0.25	0.35	0.41**
DHLNL (3)	-0.39*	0.19	-0.11	0.45*	0.06	0.31*	-0.01	-0.18	0.17
HLNL (4)	-0.15	-0.18	-0.25	0.47*	0.51**	0.47***	0.21	0.30	0.35**
HHMD (5)	-0.20	-0.10	-0.31*	0.69***	0.58**	0.68***	0.03	-0.10	0.30*
Heat stable (1+2+3)	-0.52**	-0.15	-0.48***	0.48*	0.46*	0.70***	0.19	0.04	0.49***
Heat labile (4+5)	-0.20	-0.12	-0.32*	0.69***	0.62***	0.70***	0.06	-0.06	0.32*
Collagen solubility				0.54***	-0.28	-0.51***	0.13	-0.20	-0.31*
Muscle. collagen conc.							-0.06	0.34	0.67***

(a) LD, ST: 28 observations; LD+ST: 56 observations.
Correlation coefficients differ significantly from zero at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***).

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