Crosslink Type and Tensile Strength of Collagen

A.B. KURTH and D.J. HORGAN
CSIRO Division of Food Processing, Meat Research Laboratory, Cannon Hill, 4170, Australia.

INTRODUCTION: Several groups (Bouton et al. 1981; Martens et al. 1982; Tornberg and Straadsson, 1988) have investigated the influence of cooking temperature on shear force values of meat. These workers observed a collagen related decline in peak shear force between 50 and 65°C after which peak force again increased. The onset temperature for this decline increases with increasing animal age. It has also been shown (Lewis and Purslow, 1989) that the tensile strength of excised perimysial collagen declines after heating for 1 h at temperatures greater than 50°C. This temperature dependent decline in tensile strength was explained in terms of the partial thermal denaturation of collagen even though thermal denaturation is generally considered to begin in the vicinity of 60 to 65°C.

The tensile properties of collagen are strongly dependent on the formation of intermolecular crosslinks between the molecules comprising collagen fibrils. Specifically, high tensile strength arises from multivalent crosslinks forming transverse linkages between molecules in register. Bi-molecular crosslinks alone may not confer tensile strength (Bailey, 1989). Several crosslinks for example, histidinohydroxymerodesmosine (HHMD), pyridinoline (PYR), and Ehrlich chromogen (EC) have been proposed to join three or four molecules, but considerable controversy surrounds their structure and authenticity (see Eyre et al. 1984, Bailey 1989, and Horgan et al. 1990, for key references). In this work we consider the properties of these crosslinks and relate them to the tensile strength of cooked collagen.

METHODS: The tendon of insertion was removed from Psoas major (PM) and Longissimus dorsi (LD) muscles derived from steers in the age range 0.9 to 2 years of age. Adhering muscle tissue and fat was removed by gently scraping with a scalpel. Beginning at the bone attachment (posterior) end, each PM tendon was divided into three segments, A,B,and C, by two cuts across the tendon so that segments A and B were each a quarter of the total length and segment C was half the total length. The tendons were cut into strips approximately 3 mm wide and equilibrated overnight in 0.1 M citrate phosphate buffer pH 7 and blotted dry the following morning. The ends of the strips were glued to polycarbonate holders with cyanoacrylate. Two sets of holders were used, a nylon unit for the gluing step and a polycarbonate unit for the heating step. The strips (3-5 replicates per treatment) were held at uniform length, but the unit used for heating allowed for a maximum 15% shrinkage. The samples were heated for 1 h in the range 20-80°C, cooled to room temperature and their tensile strength measured with an Instron testing machine. The samples were then reduced with sodium borohydride and lyophilised. The dry weight of the strips was used to calculate their tensile strength which was expressed as peak force (kg) per milligram dry weight of a constant length sample. Collagen crosslink concentration was determined by amino acid analysis and colorimetric methods (Horgan et.al. 1990).

RESULTS and DISCUSSION: Figure 1 shows the variation in mean tensile strength of PM and LD bovine tendons with temperature. The least significant difference at the 95% confidence level was 0.055. Thus, PM B tendon was stronger than PM A and LD tendon at 20 and 50°C whilst PM A was stronger than LD tendon at 50 but not 20°C. After heating at 60°C for 1h both PM A and PM B were stronger than LD tendon, but after heating for 1h at 80°C there were no significant differences in tensile strength, even though the mean values showed the order PM A > PM B > LD tendon. If the tensile strength of collagen fibers is in fact related to crosslink structure, the order of the tensile strengths reported here should be related to the concentration and types of crosslink present in the tendons.

TABLE 1

CROSSLINK CONCENTRATION IN TENDON SEGMENTS

SEGMENT	MOLES/MOLE COLLAGEN		
	PYR	EC	HHMD
PM A	0.269	0.293	0.107
PM B	0.064	0.205	0.277
LD	0.008	0.172	0.418

Table 1 shows the distribution of HHMD, PYR and EC in the three tendon types. We have previously shown that PYR and EC are thermally stable crosslinks in PM A and PM B tendons (Horgan et al. 1990). The results in Figure 1 were, therefore, predictable in that the tendons with the greatest concentrations of heat stable crosslinks, had the greatest mean tensile strengths at 60 and 80°C. However, the rapid decline in tensile strength between 50 and 60°C is difficult to explain in terms of these two crosslinks. Bouton et al. (1981) reported that the onset temperature of this decline in collagen tensile strength was age dependent. This could indicate that a slower reacting or "maturing" crosslink, such as an aldimine, is implicated.

Aldimine crosslinks are known to contribute to the tensile strength of collagen (Davison, 1989) but are thermally labile. The only aldimine crosslink that is capable of conferring tensile strength by forming transverse linkages between molecules in register is HHMD. Figure 2 shows the effect of heat on the HHMD concentration of bovine tendon at post-mortem pH. It can be seen that HHMD was destroyed at temperatures between 50 and 60°C. Thus, the decline in tensile strength of the tendons coincides with the breakdown of HHMD. Davison (1989) showed a similar correlation, however, in this case the tensile strength of rat tail tendons declined when the pH was lowered in a manner known to lower (Robins and Bailey, 1973) HHMD concentration. When the tendons were reduced with sodium borohydride, thus stabilising HHMD, there was no longer a pH dependent decline in tensile strength. Viidik (1980) also showed age and pH dependent variation in the tensile strength of tendons that was consistent with cleavage of HHMD in the aldimine crosslinked tissues. These results cannot be taken as proof of the existence of HHMD, but clearly, the occurrence of the compound correlates with changes in the tensile strength of tendons.

Lewis and Purslow (1989) observed an increase in the strength of perimysial collagen between 20 and 50°C and attributed this to straightening of the collagen crimp. We did not observe consistent results for the different tendon types in this temperature range. Since the temperatures are below the range when even pH affected HHMD is destroyed, it is unlikely that cleavage, due to thermal denaturation, of any crosslink is involved. Whilst it may be considered unlikely that chemical differences in crosslink type affect physical properties of native tissues, this contention is by no means certain (Bailey, 1989). The presence in a tissue of a higher proportion of crosslinks capable of forming transverse linkages between molecules in lateral register, could be expected to increase the strength of that tissue compared to another containing fewer such crosslinks and more bi-molecular bonds. Such a situation would approximate the normally held view of maturation, where bi-molecular crosslinks react or "mature" to form lateral arrays (Bailey 1989).

Further work is now underway to investigate the role of PYR and EC with particular reference to the number of collagen molecules that these compounds link. Elucidation of the fate of HHMD as animals get older is also being investigated.

CONCLUSIONS: The tensile strength of tendon collagen showed a marked decline between 50 and 60°C. The only multi-molecular crosslink that is destroyed by heating in this temperature range is the putative crosslink HHMD. Tendons containing higher proportions of PYR and EC had higher mean tensile strengths after heating at 60 and 80°C. The question of the existence of HHMD as a lateral crosslink, and the elucidation of the number of collagen molecules linked by PYR and EC are crucial to an understanding of the strength of collagen fibers.

REFERENCES:

BAILEY, A.J. (1989): The chemistry of collagen crosslinks and their role in meat texture. Proc. Recip. Meat Conf. 42: 127-135.

BOUTON, P.E., HARRIS, P.V. and RATCLIFF, D. (1981): Effect of cooking temperature and time on the shear properties of meat. J. Food Sci. 46: 1082-1087.

DAVISON, P.F. (1989): The contribution of labile crosslinks to the tensile behaviour of tendons. Conn. Tiss. Res. 18: 293-305.

MORGAN, D.J., KING, N.L., KURTH, L.B. and KUYPERS, R. (1990): Collagen crosslinks and their relationship to the thermal properties of calf tendons. Arch. Biochem. Biophys. 281: 21-26.

LEWIS, G.J. and PURSLOW, P.P.(1989): The strength and stiffness of perimysial connective tissue isolated from cooked meat. Meat Sci. 26: 255-269.

MARTENS, H., STABURSVIK, E. and MARTENS, M. (1982): Texture and colour changes in meat during cooking related to thermal denaturation of muscle proteins. J. Text. Stud. 13: 291-309.

ROBINS, S.P. and BAILEY, A.J. (1973): The chemistry of the collagen crosslinks. The characterization of fraction C, a possible artefact produced during the reduction of collagen fibers with sodium borohydride. Biochem J. 135: 657-665.

TORNBERG, E. and PERSSON, K. (1988): The viscoelastic properties of whole beef (M. Biceps femoris) during cooking as related to its sensory and structural characteristics. Proc. International Congress Meat Science and Technology A: 190-193.

VIIDIK, A. (1980): Interdependence between structure and function in collagenous tissues. In: "Biology of Collagen" (A. Viidik and J. Vuust eds.). Academic Press. pp 257-280.

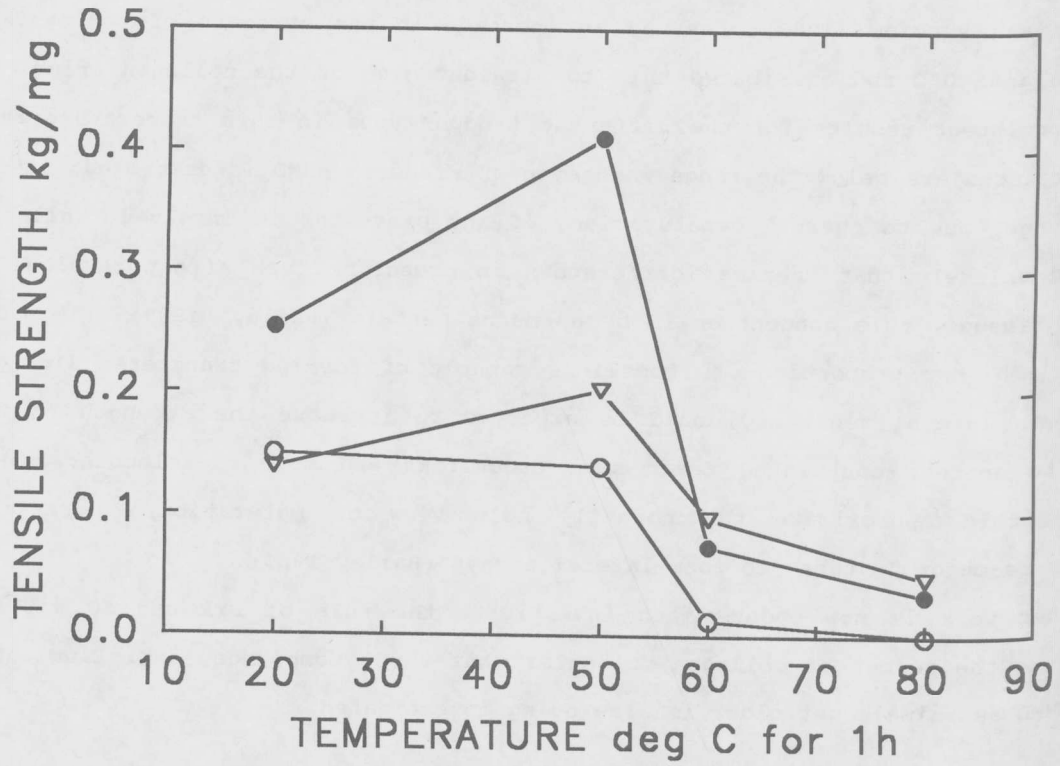

FIGURE 1: Variation in tensile strength of LD tendon ○, PM A tendon ▽, and PM B tendon ●, with cooking temperature.

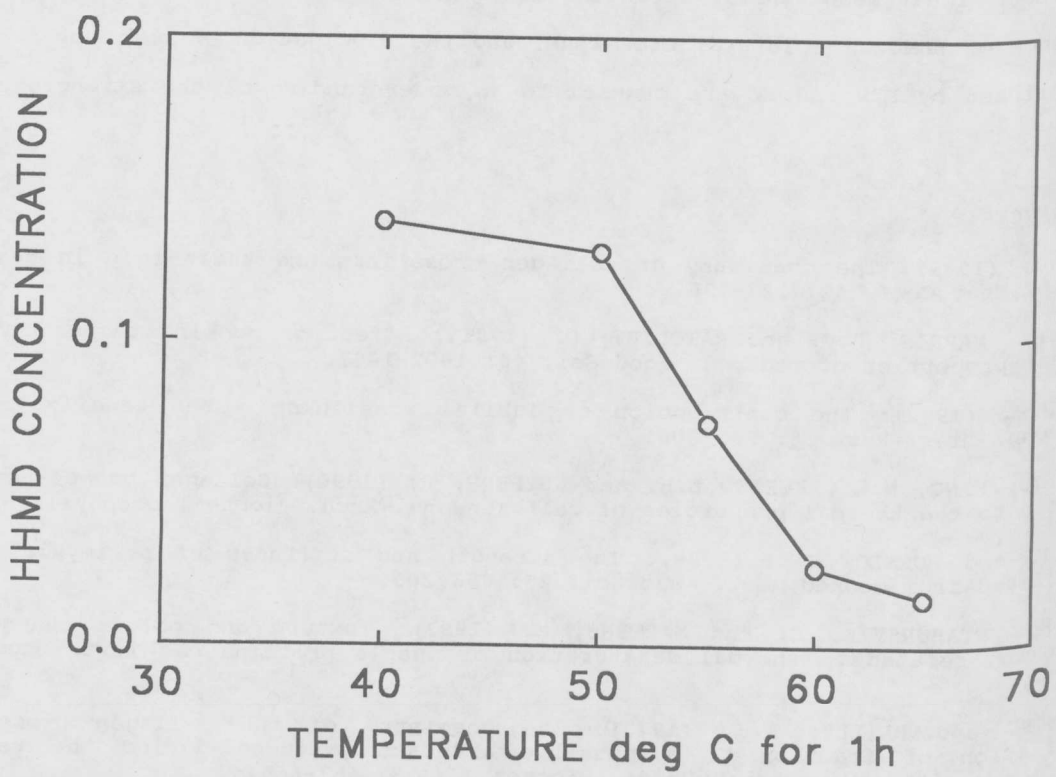

FIGURE 2: Variation in HHMD concentration (expressed as moles HHMD/ mole of collagen), with cooking temperature.