ERAL CROSSLINKS, POLYMERIC COLLAGEN AND MEAT TEXTURE

KURTH, R. KUYPERS, D.J. HORGAN AND W.R. SHORTHOSE

0 Division of Food Processing, Meat Research Laboratory, Cannon Hill, 4170, Australia

MARY

have previously shown that the stability of crosslinks in the collagen of young animals is tissue dependent and is rmined by the relative prominence of crosslinks formed via the hydroxyallysine pathway. The principal crosslinks on pathway are pyridinoline (PYR) and a collagen-associated Ehrlich chromogen (EC). We have previously shown that concentration of PYR increases with age by about 30% from birth to old age. There is however considerable animal to al variation in PYR content at any particular age. We have been unable to show a good correlation between the PYR entration of a muscle and measures of meat toughness. Even though the concentration of EC appears to decline with asing animal age (in animals > 2 years old), the relaxation half-time determined by hydrothermal isometric tension s an increase in the number of thermally stable crosslinks in IMC. We consider these to be EC residues that no er form chromophores with the detection reagent. This could be due to the reactive structure of the EC which is e to oxidation and possibly polymerisation.

RODUCTION

agen is believed to be one of the two structural elements of meat that determine meat texture (Bailey, 1989). A ber of processing parameters are known to influence myofibrillar toughness, however when these are optimised for production of tender meat, a gradation of toughness still exists between muscles from the same animal, and between ame muscle from different animals (Shorthose & Harris, 1990). Increasing animal age, differences in collagen content crosslink stability are believed to be responsible for these variations in texture (Bailey, 1989). The primary hanism by which collagen affects meat texture is by contracting during the cooking process. This forces the ctured myofibrillar bundles closer together and results in fluid expulsion from the meat and an increase in the ber of myofibrillar bundles per unit volume (Bendall & Restall, 1983; Bailey, 1989). Additionally, the collagen asing the muscle fibre bundles will possess some residual strength following cooking. Amongst the intramuscular agen domains the strength of the perimysium, or perimysium/muscle fibre bundle interface, is likely to have a major luence on meat texture (Purslow, 1985). We have recently shown that differences in collagen thermal stability and ile strength can be related to the content of the heat stable crosslinks PYR and EC (Horgan et al., 1990; Kurth & rgan, 1991). We have proposed mechanisms for the formation of these crosslinks that show how they both may be ived from ketoamine crosslinks and hence act as "mature" collagen crosslinks (Kuypers et al., 1992a). The "mature" agen crosslinks are postulated to join adjacent molecules forming lateral arrays within a polymeric complex termed y α1 CB6 (Bailey, 1989). Thus this polymeric collagen should contain the stable crosslinks responsible for collagen ived meat toughness. In this paper we examine the change with increasing animal age in the concentration of idinohydroxymerodesmosine (HHMD), a marker for labile, immmature collagen, and the two "mature", intramuscular agen crosslinks, PYR and EC. The concentrations of the crosslinks were correlated with measures of collagen's ribution to meat texture.

THODS

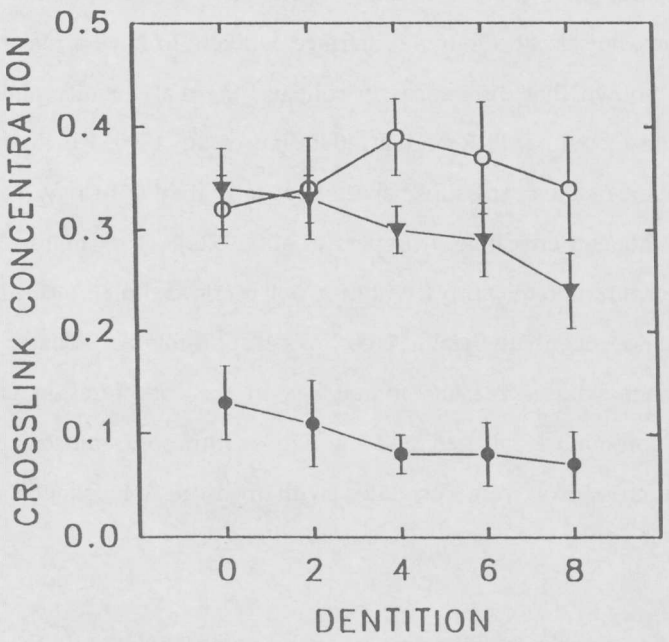
ramuscular collagen (IMC) was prepared from *Semimembranosus* muscles of 50 bovine steers by homogenisation in 0.05 phosphate, 0.15 M sodium chloride, pH 7.5 and filtration through 1 mm^2 square plastic mesh (Horgan et al., 1991a).

Animals were of different ages as indicated by dentition which ranged from 0 - 8 tooth. Samples of the same muscle were cooked at 80°C for 1 h prior to taste panel tenderness and Instron texture evaluation (Shorthose & Harris, 1990). Warner-Bratzler peak force - initial yield (PF - IY) and Instron compression were used to assess the contribution of collagen to meat toughness (Bouton et al., 1975). Total collagen content of the muscles was determined by the International Standards Organisation method (3496). The concentrations of collagen crosslinks in the isolated IMC samples were determined by ion exchange amino acid analysis of the sodium borohydride reduced material (Horgan et al., 1991a). The concentration of the EC crosslink was determined by colorimetric assay (Horgan et al., 1991). Interrelationships between textural parameters and collagen crosslink concentration were examined by regression and analysis of variance. Relaxation half times of IMC samples from 8 bovines of accurately known age were determined by the method of Le Lous et al., (1982) following hydrothermal isometric tension measurement. This method was modified for IMC by rolling the long strands of IMC into a rope-like structure suitable for clamping in the Instron.

RESULTS

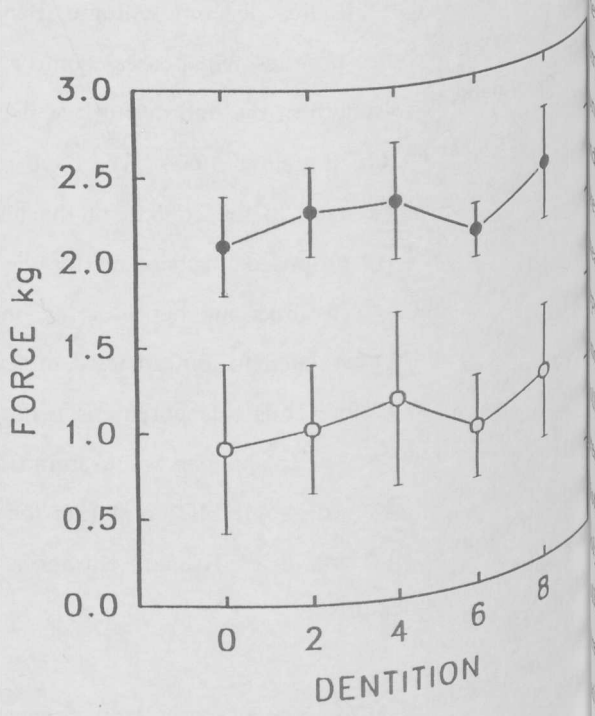
When the data was examined within each dentition group there were considerable variations in the HHMD, PYR and EC concentrations within the age groups (Graph 1). This was in agreement with our previous results using goat muscle (Horgan et al., 1991). Additionally, the total collagen content ranged from 0.93-3.47 g/100 g meat with a mean of 1.9 g/100 g meat. The collagen content could be combined with the concentration of crosslink per mole of collagen to obtain a figure for the amount of heat stable collagen in 100 g of meat. A major difficulty arises from biological diversity in data of this type and is evidenced by the range of values obtained. The objective textural data showed similar variability (Graph 2) to the crosslink concentrations. No significant correlations could be detected between textural parameters (tas

GRAPH 1 : Variation in collagen crosslink concentration with animal age



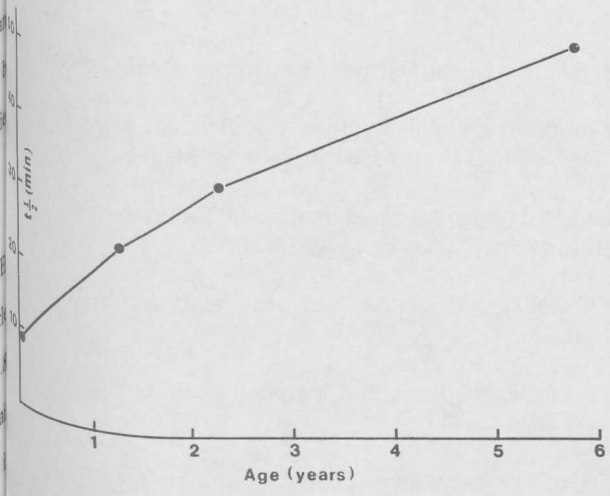
● = moles HHMD/mole collagen
○ = moles PYR/mole collagen
▼ = moles EC/mole collagen
error bar = ±1 standard deviation

GRAPH 2 : Variation in IMC strength with animal age



○ = Warner-Bratzler peak force - initial yield
● = Instron compression
error bar = ±1 standard deviation

APH 3 : Relaxation half-time of IMC from animals ifferent ages.



panel tenderness, peak force-initial yield and Instron compression) and collagen crosslink concentration (either on a mole/mole collagen or, moles/100 g meat basis). However, when IMC was prepared from the 8 animals with known birth dates, the relaxation half-times were seen to increase with increasing animal age (Graph 3). This clearly indicates an increase in the number of heat stable crosslinks in these samples.

DISCUSSION

The EC and PYR have been clearly established as heat-stable mature collagen crosslinks on the hydroxyallysine pathway (Horgan et al., 1990; Horgan et al., 1991a; Kuypers et al., 1992a). Furthermore, PYR has been identified as a crosslink in poly α1 CB6 (Kurth, 1991). We have shown that PYR and EC crosslinks are involved in the maintenance of tension when collagen is heated, and contribute to the residual tensile strength of heat denatured collagenous networks (Horgan et al., 1990; Kurth & Horgan, 1991). However, we have been unable how any correlation between the PYR content of the SM muscles examined and their tenderness. The results ented here clearly demonstrate the difficulties encountered when moving from a model system, where tendons with ict crosslink profiles were used to examine the effects of specific crosslinks, to the examination of those crosslinks in C and the correlation of their concentrations to textural parameters.

re are several possible reasons for this. Perhaps the major complication arises from the fact that meat is a 2 ponent system and the effects of myofibrillar toughness might compromise the measurement of collagen's tribution to meat toughness. Thus PF - IY or IC might not act as accurate measures of collagen toughness if the PF, IY d IC values are influenced abnormally by myofibrillar toughness brought about by mild muscle shortening. It has viously been shown that collagen's contribution to meat toughness is more readily discernible by objective asurement in muscles with relatively long sarcomere lengths (Bouton et al., 1975). The divergence of the results orted here from that of Shorthose and Harris (1990) could possibly be due to this factor.

ther major difference between the correlation of collagen strength to crosslink concentration in IMC and tendon llagens, is the fact that IMC contains two predominant collagen types (I and III) whereas tendons are composed of dominantly one (type I). Although the significance of the type III content of IMC is unclear, recent results show the tribution of PYR and EC crosslinks in type I and III collagen of IMC is not random and that PYR linked type I-III co-mers exist (Kuypers et al., 1992b). Therefore the role of PYR and EC in stabilising the lateral arrays of polymeric llagen in IMC and tendon could be quite different. Collagen crosslink loci could well have an effect on the tensile ength of IMC. We believe the measurement of EC concentration in animals >2 years old is inaccurate because dation or polymerisation reactions would lead to a lack of reactivity with the detection reagent used in the assay. Such

reactions would be consistent with the proposed pyrrolic structure of the EC (Scott et al., 1981; Kuypers et al., 1992a). Furthermore, a decline in the concentration of the thermally stable EC crosslink would be inconsistent with the observed increase in relaxation half-time data. The increase in the relaxation half-times of IMC with increasing animal age indicated that in this group of animals the number of thermally stable crosslinks increased with increasing animal age. These results indicate that further work is required on the structure of the EC and the complex interactions between IMC and meat texture.

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