THE EFFECTS OF AGE ON COLLAGEN CROSSLINKS IN OVINE SKIN

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Background

Collagen molecules are crosslinked by intermolecular covalent crosslinks and the chemistry of these crosslinks is dependent on both the nature and age of the tissue (Bailey *et al.*, 1974; Eyre *et al.*, 1984). Collagen crosslink formation is initiated by lysyl oxidase, which oxidises lysyl and hydroxylysyl residues in the telopeptide region of collagen to form the aldehydes, allysine (AL) and hydroxyallysine (HAL) respectively. The AL pathway is the typical crosslink pathway present in skin, leading to the formation of dehydro-hydroxylysinonorleucine (de-HLNL) and dehydrohydroxymerodesmosine (de-HHMD) (Eyre *et al.*, 1984). The aldimine intermediate crosslinks present in immature tissue, de-HLNL and de-HHMD, decrease with increasing animal age, and are believed to be converted to stable non-reducible crosslinks which accumulate in the tissue (Bailey, 1991; Reiser *et al.*, 1992).

Controversy has surrounded HHMD since it was first described by Tanzer *et al.* in 1973, and since the concentration of this component is considerable in many tissues, it was included in this study.

Yamauchi *et al.* (1987) found that histidinohydroxylysinonorleucine (HHL) is the major non-reducible crosslink present in skin and demonstrated a precursor/product relationship between de-HLNL and HHL.

Crosslink type and content of bovine and human skin have been reported (Yamauchi *et al.,* 1987; Yamauchi *et al.,* 1988; Sims & Bailey, 1992), although these studies were based on small numbers of samples, and showed no evidence of variation in crosslink concentration at a particular age. This study differs to these reports in that a larger number of animals (72) were used.

Merino leather with an attractive appearance and feel suitable for garments has been produced. However, the product has not been consistent, and at times, has been unacceptable, even when apparently the same processing and tannage has been used. This variability may be due, in part, to the age of the animals, and hence to the nature of the collagen crosslinks present.

Objective

Alexander *et al.* (1988) found that the skins from immature sheep show different responses to the early stages of the leather manufacturing process, and since the skin is the most valuable by-product and the most valuable use for skins is the production of garment leather, these responses are of commercial importance. Furthermore, differences in the stability of skin collagen are also of importance in the conditioning of hides for gelatine manufacture. The aim of this report was to analyse and characterise the crosslink type and concentration in ovine skins of different ages.

Methods

Skin samples from 72 sheep ranging in age from 1 to 5 years were cleaned and the epidermal and underlying fat layers were removed. Samples were diced, defatted and reduced with sodium borohydride at neutral pH (Robins, 1982). Lyophilised samples were hydrolysed in 6M HCl at 115°C for 24 hours. The sample was initially separated by the method of Black *et al.* (1988). The crosslink containing fraction was eluted with distilled water and dried by rotary evaporation. The dry residue was dissolved in sodium citrate buffer, filtered and an aliquot applied to an Interaction AA911 amino acid analyser column.

Skins were fellmongered according to the procedure described in "Depilation and Preservation of Skins" (Department of Agriculture, Western Australia Bulletin 4229). The pickled pelts were tanned by Merino Tanneries, Fremantle, using a procedure developed for Merino Garment Leather. Ms Somoff, Fremantle, a designer and manufacturer of Merino garment leather, evaluated the appearance of the leather. The data was analysed by a one-way ANOVA, with p>.05 considered significant.

Results and Discussion

Ovine skin was found to contain low levels of the HAL pathway crosslink, PYR, but high concentrations of the aldimine crosslinks, HHMD, HLNL and the non-reducible crosslink, HHL (Fig. 1). There was no significant effect of age on PYR concentration at the 95% confidence level.

From Figure 1, it can be seen that the average HHL content shows a continuous increase with age from 1 to 4 years, and plateaus off from 4 to 5 years. This trend was also demonstrated for bovine skin by Yamauchi *et al.* (1988) and Sims & Bailey (1992). The skin from 1 and 2 year old animals contained significantly less HHL than skin from 3, 4 and 5 year old sheep.

The average HLNL concentration showed an approximately linear decrease with age (Fig. 1). Statistical analysis showed that 1 year old skin contained significantly more HLNL than skin from 2, 3, 4 and 5 year old sheep, and skin from 2 and 3 year old animals had significantly more HLNL than that from 5 year old sheep. Sims & Bailey (1992) demonstrated a similar decrease in HLNL concentration of bovine skin after an initial peak at approximately 15 months of age. These findings support the theory that non-reducible stable mature crosslinks replace the reducible labile crosslinks during aging. Also apparent from the graph, is the high ratio of mature crosslink HHL to its divalent aldimine precursor HLNL, supporting the precursor/product findings of Yamauchi *et al.* (1987).

The decrease in the tetravalent reducible aldimine crosslink, HHMD, is also approximately linear with age (Fig. 1). There was a significant effect of age, where skin from 1 year old sheep contained significantly more HHMD than skin from 2, 3, 4 and 5 year old animals. Skin from 2 and 3 year old sheep had significantly higher concentrations of HHMD than skin from 5 year old animals. Shimokomaki *et al.*

(1972) and Robins *et al.* (1973) reported HHMD in bovine skin and showed that it reached a maximal value at approximately 18 months (compared to approximately 12 months for HLNL), whereafter both decreased at 4-5 years of age before plateauing off.

Considerable variation in the crosslink content of sheep skin is evident between animals of the same age group (Fig. 2). This result can be interpreted as evidence that animals of the same age undergo variable rates of maturation of collagen crosslinks.

Grain damage caused during processing results in garment quality leather (valued at $8-$10 per pelt) being downgraded to chaois grade (valued at around $1.20 per pelt). There are linear relationships between % of skins having good grain and the concentrations of both the reducible crosslinks (HHMD r^2=0.91; HLNL r^2=0.90), but not with the non-reducible crosslink HHL (Fig. 3). Note that it is an inverse relationship, which argues that it is the conversion of the reducible crosslinks that protects the skin from damage during processing. The question arises as to why some are damaged and others are not. The observed behaviour is quantitative, consistent with the proposal that if the HHMD concentration is below the highest value measured in the 5 year sample (0.43 mole/mole collagen), then no grain damage will occur when processed in this manner. Using this data, the relationship in Figure 4 is obtained (r^2=0.90).

Conclusions

HHL was the major non-reducible collagen crosslink detected in ovine skin. The concentrations of the reducible crosslinks, HHMD and HLNL, decreased with increasing animal age. There was a strong correlation between the decrease in reducible crosslink concentration and the decrease in grain damage evident in the sheepskin leathers. Further investigation of the relationship between collagen crosslink concentration and leather quality is warranted.

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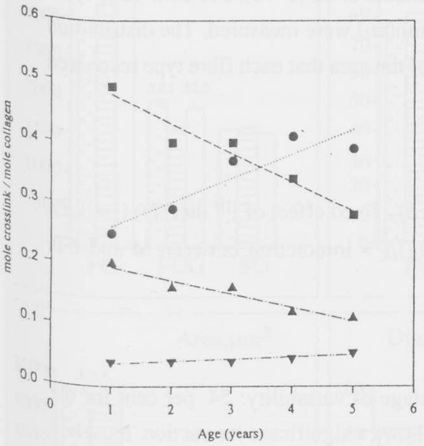

Figure 1 : Crosslink concentration in ovine skin of various ages.

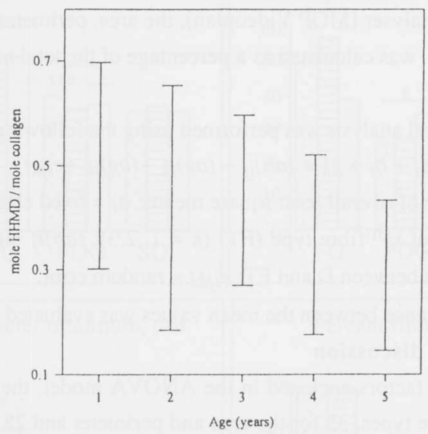

Figure 2 : Variation of HHMD concentration in ovine skin within age groups.

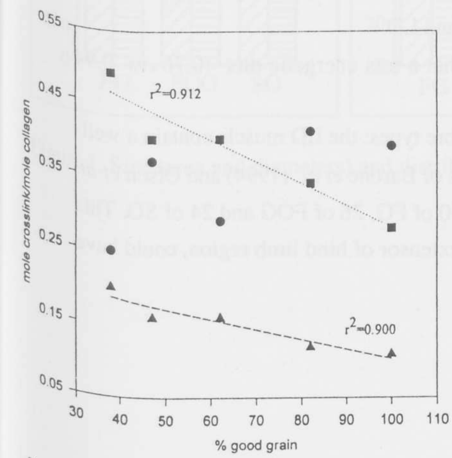

Figure 3 : Relationship between skins with good grain and crosslink concentration.

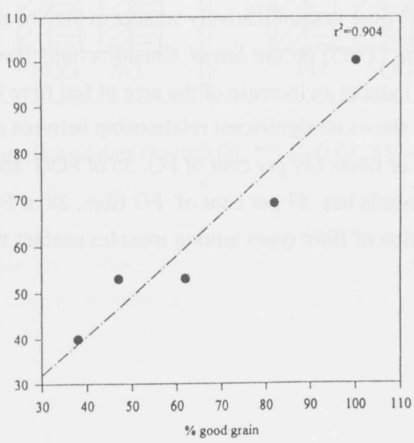

Figure 4 : Relationship between skins with specific HHMD concentration and skins with a good grain.