AGEING OF BOVINE COLLAGEN
SKIN COLLAGEN AS MODEL FOR THE AGEING OF INTRAMUSCULAR COLLAGEN

SOPHIE VANDERHAEGHE and CLAUDE DEROANNE
Centre Agronomique de Recherche sur la Viande (I.R.S.I.A.-I.W.O.N.L.)
U.E.R. de Technologie des I.A.A.
Faculté des Sciences Agron. de l'Etat
B-5800 GEMBLOUX BELGIUM

INTRODUCTION

For the consumer, tenderness seems to be the most important meat quality characteristic.
It is now well established that the amount and mostly the properties of collagen are important factors in determining toughness of meat.
Many workers have shown that the collagen properties change with increasing age (BAILEY et al., 1974 ; BAILEY, 1988 ; FLANDIN et al., 1984 ; HILL, 1966 ; HEINZE et al., 1986 ; JUDGE and MILLS, 1986 ; JUDGE and ABERLE, 1982 ; LE LOUS et al., 1982 ; SHIMOKOMAKI et al. 1972 ; ASGHAR and HENRICKSON, 1982 ; LIGHT and BAILEY, 1980).
HILL (1966) and HEINZE et al. (1986) have shown that the intramuscular collagen content decreases with increasing age.
This decrease would be due to the fact that the muscle development is more important than the synthesis of connective tissue in young animals (HILL, 1966).
Thermal solubility of collagen decreases as age increases. This decrease in solubility is related to the formation of heat-labile reducible crosslinks first, and then to the appearance of heat-stable non-reducible crosslinks (BAILEY et al., 1974 ; BAILEY, 1988 ; HILL, 1966 ; HEINZE et al., 1986 ; LIGHT et al., 1985 ; SHIMOKOMAKI et al. 1972 ; ASGHAR and HENRICKSON, 1982).
Those modifications have been shown to have an influence on meat texture (BAILEY, 1988 ; HILL, 1966 ; SHIMOKOMAKI et al., 1972).
Intramuscular collagen with a large crosslinking extent confers a higher toughness to meat.
The crosslinking extent can be measured by determination of the thermal solubility of collagen (HILL, 1966 ; CROSS, 1973 ; BOCCARD, 1979 ; HEINZE et al., 1986 ; LIGHT et al. 1985 ; SHIMOKOMAKI et al., 1972) or by differential scanning calorimetry (DSC) (FLANDIN et al., 1984 ; JUDGE and MILLS, 1986 ; JUDGE and ABERLE, 1982 ; LEDWARD et al., 1975 ; LE LOUS et al., 1982).
Ageing of collagen is naturally related to the chronological age of the animal, but some external factors such as sex, caloric intake, hormonal status of the animal, dietary components (ASGHAR and HENRICKSON, 1982) could accelerate this ageing.
It is thus better to define a physiological age which will be related to the ageing stage of collagen.
It should obviously be interesting to have a method able to give informations about meat quality on alive animals.
Our purpose, in this experiment, was thus to look for a relationship between the ageing of skin collagen and of intramuscular collagen, in order to estimate the physiological age in vivo.

MATERIAL AND METHODS

Animal material

Samples of skin and of Longissimus dorsi were obtained from animals slaughtered at the University of GHENT (BELGIUM). Five groups were studied in this experiment :

- I 17 four-years-old cows
- II 16 one-year-old steers
- III 13 BB cows of 4 to 10 years old (average : 5 1/2 ± 1 1/2 years)
- IV 1 eight-months-old foetus
- V 8 one-year-old steers

After slaughtering, meat samples were stored at 4°C during 7 days. Then they were frozen at -20°C. Hair and epidermis were removed from skin samples and the dermis was directly frozen at -20°C.

Methods

Collagen content

Collagen content was calculated by multiplying hydroxyprolin content by 8.
Hydroxyprolin content was determined by the ISO-3496-1978 method.
Collagen content is expressed in function of the total protein content.

Thermal solubility of collagen

A 10 g meat sample (1 g skin sample) was solubilized at 75°C in Ringer's solution (NaCl 0.9 %) during 70 minutes.
The solution was then filtered and the soluble fraction was concentrated by evaporation.
Collagen content of both fractions was determined by the method described above.
Solubility of collagen is given by the following formula :

$$Cs\ (\%) = \frac{Fs}{Fs + Fi} \times 100$$

Cs = soluble collagen in %
Fs = soluble fraction in mg
Fi = non-soluble fraction in mg

Tenderness of meat

Samples of Longissimus dorsi were cooked in a waterbath at 75°C and cutted with a meat borrer in small cores.
The shear forces were measured with the Warner-Bratzler cell.

Differential Scanning Calorimetry (DSC)

Skin samples are rich in collagen and an extraction is not necessary.
In the case of meat, intramuscular collagen was extracted with physiological water.
Samples of about 100 mg collagen were sealed in DSC cells. DSC curves were obtained with a Setaram Calorimeter between 2 and 110°C at the heating rate of 2°C/min.
Interpretation of the thermograms of skin collagen was made as described by FLANDIN et al. (1984). The DSC curves present a main peak with two shoulders before and after this peak.
The interpretation of those curves is made by determination of six characteristic temperatures (fig. 1).

- T1 is the temperature at which the curve leaves the baseline
- T2 is the maximum of the first peak
- T3 is the extrapolated onset of the main peak
- T4 is the maximum of the main peak
- T5 is the transition between the main peak and the third peak
- T6 is the recovery temperature

In the case of meat, the extraction was not sufficient, and it was impossible to determine those temperatures.
Only the maximum of the main peak was determined.

RESULTS

Collagen content

As shown in table I, chronological age by itself cannot explain collagen content variations.
Collagen content of foetus muscle is clearly higher than in adult animals, what can be explained by the fact that, during growth, muscles have a more rapid development than connective tissue. The relative amount of intramuscular collagen thus decreases.
There are small differences between the first group of cows and both groups of steers (groups I, II and V).
On the other hand, the second group of cows (group III) has a lower intramuscular collagen content.
Actually, the animals of that group had a better conformation. The muscles are much more developped than in animals of normal conformation.

Collagen solubility

We see clearly that collagen solubility decreases with increasing age (Table 1).
But, again, the groups of cows are different and chronological age only is thus not sufficient to explain solubility differences.
In young animals, skin collagen is much more soluble than intramuscular collagen.
On the other hand, in older animals, skin collagen solubility and intramuscular collagen solubility are almost the same.
Both groups of steers are very similar.

Meat tenderness

When we look at the results of Table II, it seems that there is no logical evolution of the tenderness with increasing age.
The meat of the first group cows is clearly tougher than the meat of the other groups, which give very close results.
It was impossible to measure shear forces on the foetus because the meat was too flabby.

Differential Scanning Calorimetry (DSC)

The DSC curves of skin collagen at different ages are shown in figure 2.
The DSC curve of foetal skin shows one peak only.
With the steer skin, a shoulder appears just before the main peak, which is moved to the higher temperatures in comparison with the foetus.
The DSC curves of old cows present two shoulders before and after the main peak.
The maximum temperature of the main peak is quite higher than that of the steers.
The onset temperature (T1), the maximum of the main peak (T4) and the recovery temperature (T6) increase with increasing age (Table III), whereas the maximum of the first peak (T2) and the extrapolated onset of the main peak (T3) are similar for steers and cows.
T3 of the foetus is clearly weaker.
With the steers, the limit between the main peak and the second shoulder (T5) is not clear. It is the main peak which gets wider.
In the case of the cows, the transition is evident and the third peak is well marked.

The DSC curves of intramuscular collagen are not so nice because the extraction of collagen was not sufficient (Fig. 3). Results of Table III show that there is a small difference between young steers and old cows. The intramuscular collagen denaturation temperature of cows is quite higher than that of steers.

DISCUSSION

We have seen that collagen content by itself cannot explain meat tenderness variability.
DRANSFIELD (1977) has found a correlation between total collagen content and muscle toughness.
However, many workers have shown that it was rather the properties of collagen which play a role in determining meat tenderness (BAILEY, 1972 ; SHIMOKOMAKI et al., 1972 ; BAILEY and SIMS, 1977 ; BAILEY et al., 1979).
If we consider the combination between collagen content and collagen solubility, a relationship with meat tenderness appears.
The second group of reform cows (group III) contains 30 % less intramuscular collagen than the first (group I). Thus, despite the lower solubility of this collagen, intramuscular connective tissue of the second group cows has little influence on meat tenderness since the concentration is weak.
On the other hand, intramuscular collagen content of groups I, II and V is very similar. In that case, the meat with the less soluble collagen (group I) is clearly tougher.
All those observations are valid for cooked meat. In the case of raw meat, LEPETIT (1988) has found a correlation between tenderness and total collagen content.
Evolution of the skin collagen solubility and the intramuscular collagen solubility with increasing age is very different.
With steers and foetus, skin collagen is much more soluble than intramuscular collagen (IMC). That means that the IMC is more stabilized by the intermolecular crosslinks.
This is in agreement with the findings of BAILEY et al. (1974) who have found that skin collagen contains mainly dehydro-hydroxylysinonorleucine. In fact, this component confers a high tensile strengh to collagen, but does not have any influence on its solubility.
In intramuscular collagen, 50 % of the lysine residues in the N- and C-terminal non-helical regions are hydroxylated, leading to the formation of hydroxylysino-5-keto-norleucine (BAILEY et al., 1974). This crosslink is responsible of the lower solubility of IMC.
In the case of older animals, heat-labile crosslinks are replaced by heat-stable non-reducible crosslinks (JUDGE and MILLS, 1986). With old cows, the solubility of skin collagen and intramuscular collagen is very similar.
Thus the transformation of the heat-labile reducible crosslinks in heat-stable non-reducible crosslinks would lead to the thermal stabilization of both crosslinks (dehydro-hydroxylysinonorleucine and hydroxylysino-5-keto-norleucine), resulting in similar collagen solubility.
We have seen that collagen solubility gives an indication on the crosslinking extent of collagen. But Differential Scanning Calorimetry is more suited to explain changings which occur when collagen ages.
The DSC technique measures the denaturation of hydrogen bonds in the transition collagen --> gelatin.
According to LE LOUS et al. (1982), the collagen crosslinks would have a protective effect on the hydrogen bonds which stabilize the helical structure of collagen.
The first shoulder of the curve represents the denaturation of native collagen, stabilized by hydrogen bonds with a few thermolabile crosslinks. In the case of the foetus, this first peak is predominant, showing that the crosslinking is still weak.
The main peak (second peak) represents a collagen fraction with a larger crosslinking extent.
The third peak would be due to the thermostable crosslinks. This peak becomes more and more important when animal gets older, showing that the relative proportions of thermolabile and thermostable crosslinks evoluate with increasing age.
SHIMOKOMAKI et al. (1972) have seen that in intramuscular collagen, the amount of heat-labile crosslinks increases up to 12-18 months of age. After that age, these crosslinks are converted in heat-stable crosslinks (JUDGE and MILLS, 1986).
This appears clearly in our DSC curves of skin collagen (fig. 2).

CONCLUSION

Our purpose was to find a relationship between the ageing of intramuscular collagen and skin collagen in order to determine physiological age in vivo.
We have seen that the ageing of both collagens is characterized by a stabilization of their structure.
This stabilization can be measured by determination of the collagen solubility and by differential scanning calorimetry.
But it seems that those two collagen types behave very differently with increasing age.
Actually, we have worked on two groups of age only and our results should be completed by data obtained from animals of a larger range of ages.

REFERENCES

ALLAIN J.C. et al., 1978
Biochim. et Biophys. Acta, 533, 147-155

ASGHAR A. and HENRICKSON R.L., 1982
Advances in Food Research, 28, 231-372

BAILEY A.J., 1972
J. Sci. Fd Agric., 23, 995

BAILEY A.J. et al., 1974
Nature, 251(13), 105-109

BAILEY A.J. and SIMS T.J., 1977
J. Sci. Fd Agric., 28, 565-570

BAILEY A.J. et al., 1979
J. Sci. Fd Agric., 30, 203-210

BAILEY A.J., 1988
Proc. 34th ICOMST, BRISBANE, 152-160

BOCCARD R. et al., 1979
Meat Science, 3, 261-280

CROSS H.R. et al., 1973
J. Food Sci., 38, 998-1003

DRANSFIELD E., 1977
J. Sci. Fd Agric., 28, 833

FLANDIN F. et al., 1984
Biochim. et Biophys. Acta, 791, 205-211

HARRIS P.V., 1988
Proc. 34th ICOMST, BRISBANE, 169-172

HEINZE P.H. et al., 1986
Proc. EMMRW, GHENT, 169-173

HILL F., 1966
J. Food Sci., 31, 161-166

JEREMIAH L.E. and MARTIN A.H., 1981
Can. J. Anim. Sci., 61, 53-61

JUDGE M.D. and ABERLE E.D., 1982
J. Anim. Sci., 66(9), 1380-1382

JUDGE M.D. and MILLS E.W., 1986
Fleischwirtsch., 54(1), 68-71

LEDWARD D.A. et al., 1975
J. Fd Technol., 10, 349-357

LE LOUS M. et al., 1982
Biochim. et Biophys. Acta, 717, 295-300

LEPETIT J., 1988
Personnal communication

LIGHT N. et al., 1985
Meat Science, 13, 137-149

SHIMOKOMAKI M; et al., 1972
J. Fd Sci., 37, 892-896

SHORTHOSE W.R. et al., 1988
Proc. 34th ICOMST, BRISBANE, 194-196

VIIDIK A. and VUUST J., 1980
Biology of collagen
Academic Press

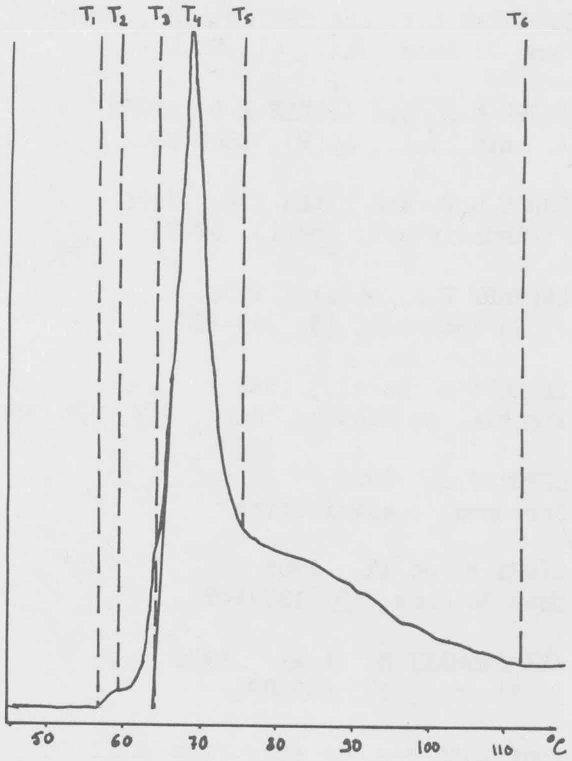

Figure 1 DSC curve of a 4-years-old cow skin with the characteristic temperatures

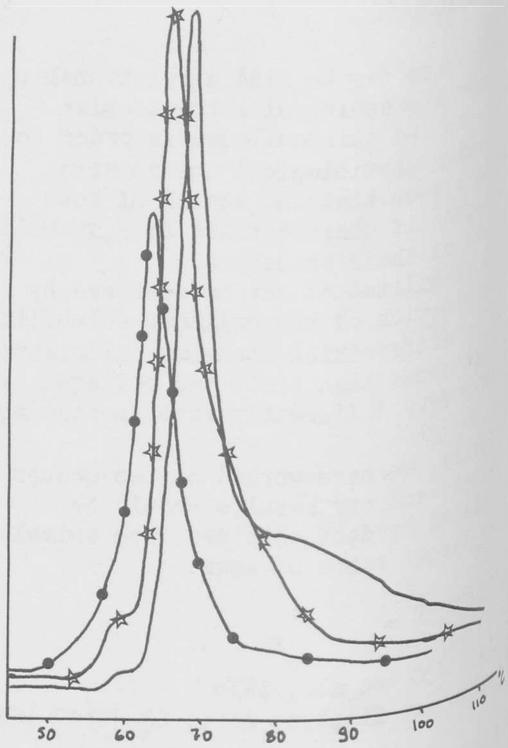

Figure 2 DSC curve obtained on skin collagen of a 8-months-old foetus (-●-), a 1-year-old steer (-☆-) and a 4-years-old cow (—)

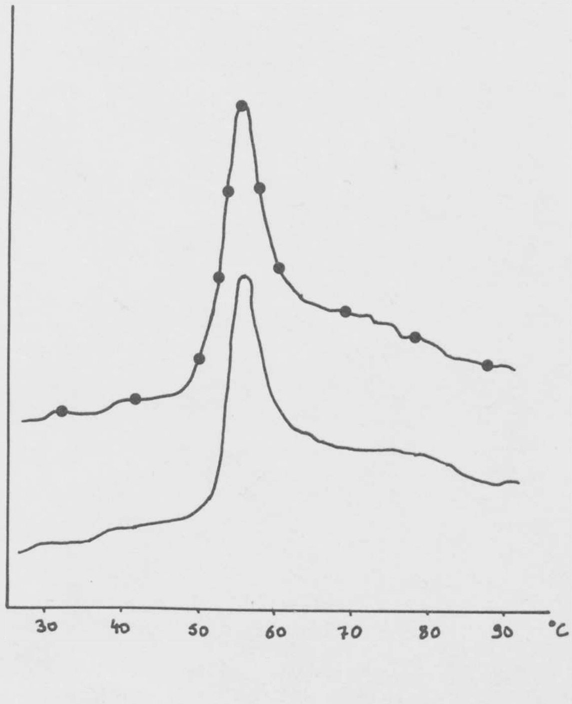

Figure 3 DSC curve obtained on intramuscular collagen of a 4-years-old cow (—) and a 1-year-old steer (-●-).

TABLE I Collagen content and collagen solubility of muscle Longissimus dorsi and skin

Group	Age	Conformation	Sample	N	Coll/Prot %	Solubil %
I cows	4 Y	normal	L.D.	17	2.6±0.5	10.8±1.6
			skin	17	89.1±4.5	7.1±3.6
II steers	1 Y	normal	L.D.	16	2.9±0.3	14.5±3.2
			skin	8	91.4±4.0	37.4±3.9
III cows	4-10 Y	BB	L.D.	13	2.0±0.2	4.6±1.3
			skin	13	96.5±2.7	4.3±2.3
IV foetus	8 M	-	L.D.	1	12.2	35.9
			skin	1	57.3	78.3
V steers	1 Y	normal	L.D.	8	2.6±0.2	14.9±2.6
			skin	8	88.8±4.3	31.6±6.2

TABLE II Shear forces measured with the Warner-Bratler cell on muscle Longissimus dorsi

Group	Age	Conformation	N	Shear force (N)
I cows	4 Y	normal	17	63.01 ± 16.64
II steers	1 Y	normal	16	37.65 ± 2.37
III cows	4-10 Y	BB	13	32.17 ± 6.58
IV foetus	8 M	-	1	-
V steers	1 Y	normal	8	30.20 ± 5.30

TABLE III Characteristic temperatures measured on the DSC curves of intramuscular collagen(IMC) and skin collagen

Group	IMC	SKIN					
	Tmax	T1	T2	T3	T4	T5	T6
I	-	55.7±1.0	58.8±0.8	63.1±1.3	68.0±0.9	76.9±1.3	96.1±1.2
II	65.3±0.6	54.5±1.6	58.8±0.5	62.1±0.8	66.8±0.5	-	89.8±1.4
III	66.2±0.7	55.4±0.9	59.2±0.4	62.9±1.1	68.1±1.0	77.0±1.1	103.6±2.6
IV	-	49	-	59	64	77	91
V	-	54.3±1.3	-	62.0±0.5	67.5±0.5	-	93.5±0.8