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Observations on the solubility of the collagen in the muscles of beef animals after cooking in presence of papain.

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Proteolytic enzymes such as papain are being used increasingly for the tenderisation of meat. The most recent development in this connection is the antemortem injection process patented by Swift & Co. (1). It is important to have some data on the effect papain has under standard conditions on the solubility of the collagen (a) in the different muscles of a carcass and (b) in the muscles of animals of different ages.

Goll, Hoekstra & Bray have recently studied the effect of collagenase action on the collagen isolated from the biceps femoris muscles of groups of beef animals (3). In order of decreasing solubilisation of collagen, the age groups were veal (45 days) cows (5 years old), cows (10 years old) and finally steers (13-16 months old). The authors suggest that in the last group, enzyme action may be inhibited by a coating of lipid on the fibres.

Experimental

In the present study, observations were made on the amount of hydroxyproline (OHP) made soluble in minced muscle samples when cooked in presence of papain (4 p.p.m.) under standard conditions. Papain activity in control samples was inhibited by iodoacetate.

The samples were cooked in citrate buffer at pH 5.7 in presence of cysteine and papain (4 p.p.m.) for 18 minutes at 65°C. Filtered and dialysed 0.1 M KCl extracts of the cooked samples were hydrolysed in acid to give free amino-acids. The hydrolysates were analysed for OHP by Woessner's method (2).

The experimental animals were mostly steers bred for a study of the growth rates, yields and carcass composition of beef (Hereford) and dairy breed (Friesian) crosses. The animals were slaughtered at 12, 18 and 24 months old and the test muscles dissected after the carcasses had been held at chill temperatures for 3-4 days.

Results and discussion

The values for the control and test samples of the various animals are presented in Figure 1 and the total OHP values in Table 1. The points in Figure 1 marked with a bar (\bar{X} or \underline{X}) represent samples from animals outside the above Hereford Friesian series. The two 5 year old animals were cows; the 36 month old animal was a 2nd grade steer; the 20 month old animal was a 1st grade steer; the two 10 month old animals were barley fed steers and the calves were 3-7 days old.

Figure 1 shows that as the animals become older the collagen becomes less soluble. In the control samples, the soluble OHP decreases from values of 18.5 - 24% of the total OHP in calf muscles to 2.3 - 2.9% in the muscles of 5 year old cows. A similar decrease takes place in the soluble OHP produced in the presence of papain - a decrease from 25-34% in calf muscles to 8.6 to 9.1% in 5 year old cows.

/These changes

These changes bear no direct relation to the total OHP contents of the two muscles (psoas and longissimus dorsi), which do not show any significant change in this experiment between the age of 10 months and 5 years (Table 1). It is interesting to observe how well the muscles from the odd animals, 10, 20 and 36 months old, of mostly unknown history, fit into the general pattern of the main controlled cross breed series.

In Figure 2, the total OHP contents of 4 muscles, (biceps femoris, semimembranosus, longissimus dorsi, and psoas major), of the four 18 month old steers are plotted against the soluble OHP produced by papain under standard test conditions. With the exception of the semimembranosus, the 4 individual muscles of a group are very similar in total OHP content, soluble OHP and texture score. The differences in total OHP value within a group of the same muscles do not appear to affect the texture scores but the muscles in the different groups become tougher as the total OHP content increases. 288

The members of the psoas group with total OHP values of 346-447 $\mu\text{g/g}$ all rated tender to very tender whereas the biceps femoris muscles with total OHP values of 1089-1235 $\mu\text{g/g}$ rated very tough to tough. It would be interesting to know if this relationship between the different muscles of an animal is the same at different stages of growth.

The figures quoted in Table 2 for total OHP, soluble OHP after papain treatment and texture, together with the values in Figure 2 and other values not quoted, show that there is no simple direct correlation between the various factors. It is probable since the papain tests were done with minced tissue and the texture tests with samples of whole muscle, that this difference may be partly responsible for the lack of correlation. Most probably, however, other factors are involved which are associated not simply with the quantity of collagen present, but with the physical structure and the distribution of the connective tissue throughout the muscle.

References

- (1) U.S. Patent No. 2,903,362. Sept. 1959.
U.K. Patent No. 913,202. Dec. 1962.
- (2) Woessner, J.F. jun. Arch. Biochem. Biophys. 1961, 93, 440.
- (3) Goll, D.E., Hoekstra, W.G. & Bray, R.W. J. Animal Sci. Abstract 1962, 21, 981.

Summary

Muscles of a group of beef animals from 3-7 days to 5 years old were cooked in presence of papain (4 p.p.m.). The hydroxyproline (OHP) present in the soluble fractions of both the control (no papain) and test samples after cooking decreased appreciably as the animals grew older.

When 4 individual muscles from each of four 18 month old steers were cooked in presence of papain, the increases in soluble OHP were 16-20% of the total OHP in the psoas samples and only 10-12% in the biceps femoris muscles. The total OHP contents of the muscles were 300-400 $\mu\text{g/g}$ and 1000-1300 $\mu\text{g/g}$ in the psoas and biceps femoris respectively.

The semimembranosus and longissimus dorsi muscles gave intermediate values.

Observations on several individual muscles from animals of different ages gave no evidence of any direct correlation between the total OHP content, the soluble OHP after papain treatment and the texture of control samples cooked without papain.

Résumé

Des muscles sélectionnés d'un nombre de boeufs âgés de 3-7 jours à 5 ans furent cuits (a) avec et (b) sans addition de papaine. L'hydroxyproline (OHP) présente dans la fraction soluble des échantillons cuits selon (a) et (b) diminuait sensiblement avec l'âge des animaux.

Quand quatre muscles individuels, provenant de chaque individu d'un groupe de quatre animaux, furent cuits à la papaine l'accroissement en OHP soluble était de 16-20% dans le psoas et seulement de 10-12% dans le biceps femoris. L'OHP totale trouvée dans les muscles était 300-400 µg/g dans le psoas et 1000-1300 µg/g dans le biceps femoris.

Le muscle semimembraneux et le muscle long. dorsal avaient des valeurs intermédiaires pour l'OHP soluble et l'OHP total.

Des observations faites sur plusieurs muscles individuels d'animaux d'âges différents et de diverses races montrent qu'il n'y a pas de corrélation directs entre le contenu total en OHP, l'OHP soluble après traitement à la papaine et la texture de la viande cuite.

Zusammenfassung

Bestimmte Muskeln von 3-7 Tagen bis 5 Jahre alten Ochsen wurden (a) mit und (b) ohne Zusatz von Papain gekocht. Das im löslichen Anteil der Proben vorhandene Hydroxyprolin (OHP), die entweder laut (a) oder (b) behandelt wurden fällt merklich mit zunehmendem Alter.

Wurden vier bestimmte Muskeln, von je einem Tier, mit Papain gekocht, so stieg der lösliche OHP-Anteil auf 16-20% für den Psoas, dagegen nur 10-12% für den Biceps femoris. Der Gesamt-OHP-Anteil war 300-400 µg/g im Psoas und 1000-1300 µg/g im Biceps femoris.

Der Semimembranosus und Longissimus dorsi ergaben dazwischenliegende Werte für den löslichen und den gesamten OHP-Anteil.

Beobachtungen an mehreren bestimmten Muskeln von Tieren verschiedener Rassen und Alter zeigten, dass keine direkte Korrelation zwischen dem Gesamt-OHP-Anteil, dem löslichen OHP-Anteil nach Behandlung mit Papain und der Struktur des gekochten Fleisches besteht.

Table 1. Total hydroxy proline (OHP) contents of psoas major (P) and longissimus dorsi (L.D.) muscles of beef animals, (for details see text). Values are μg OHP/g muscle.

Animal	Calves (3-7 days)		Steers(10)*		Steers(12)		Steers(18)		Steer (20)		Steers(24)		Steer (36)		Cows (60)	
	P.	L.D.	P.	L.D.	P.	L.D.	P.	L.D.	P.	L.D.	P.	L.D.	P.	L.D.	P.	L.D.
1	1109	1703	346	579	389	614	447	612	370	498	414	611	378	677	474	745
2	-	1299	379	609	364	525	366	528	-	-	387	715	-	-	358	627
3	-	1027	-	-	-	-	346	513	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	384	571	-	-	-	-	-	-	-	-

* Age in months

Table 2. Total hydroxy proline (OHP) content; increase in soluble OHP after cooking with papain, and the texture of various muscle samples from beef animals.

Animal : Muscle	Total OHP $\mu\text{g/g}$.	Δ sol OHP as % of T.OHP	Texture
Steer (36 months) Epimysium from Tri. brachii	1733	16	-
" Tri. brachii	1100	9.8	-
" Sternomandibularis	2200-3300	10-13	very tough
Calves Long. dorsi (3-7 days)	1027-1703	26-34	tough to very, very tough
Cow Psoas	358	8.6	very tender
(60 months) Long. dorsi	627	9.1	very tough

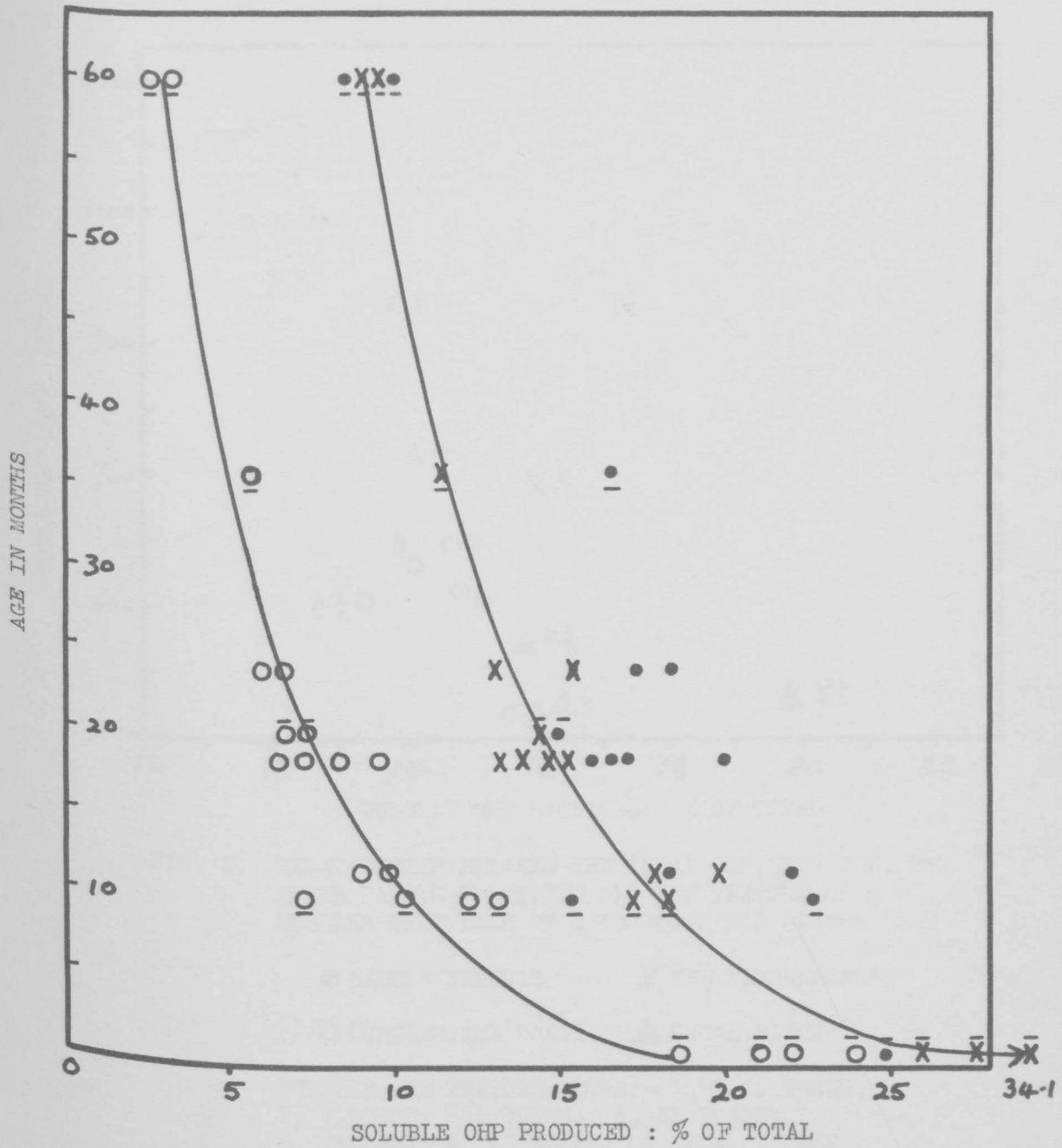


FIG. 1. RELATIONSHIP BETWEEN AGE OF BEEF ANIMALS AND THE SOLUBILITY OF COLLAGEN AFTER COOKING WITH AND WITHOUT 4 ppm. PAPAIN.

- X LONG. DORSI SAMPLES WITH PAPAIN.
- PSOAS " " " .
- O CONTROL " WITHOUT " .

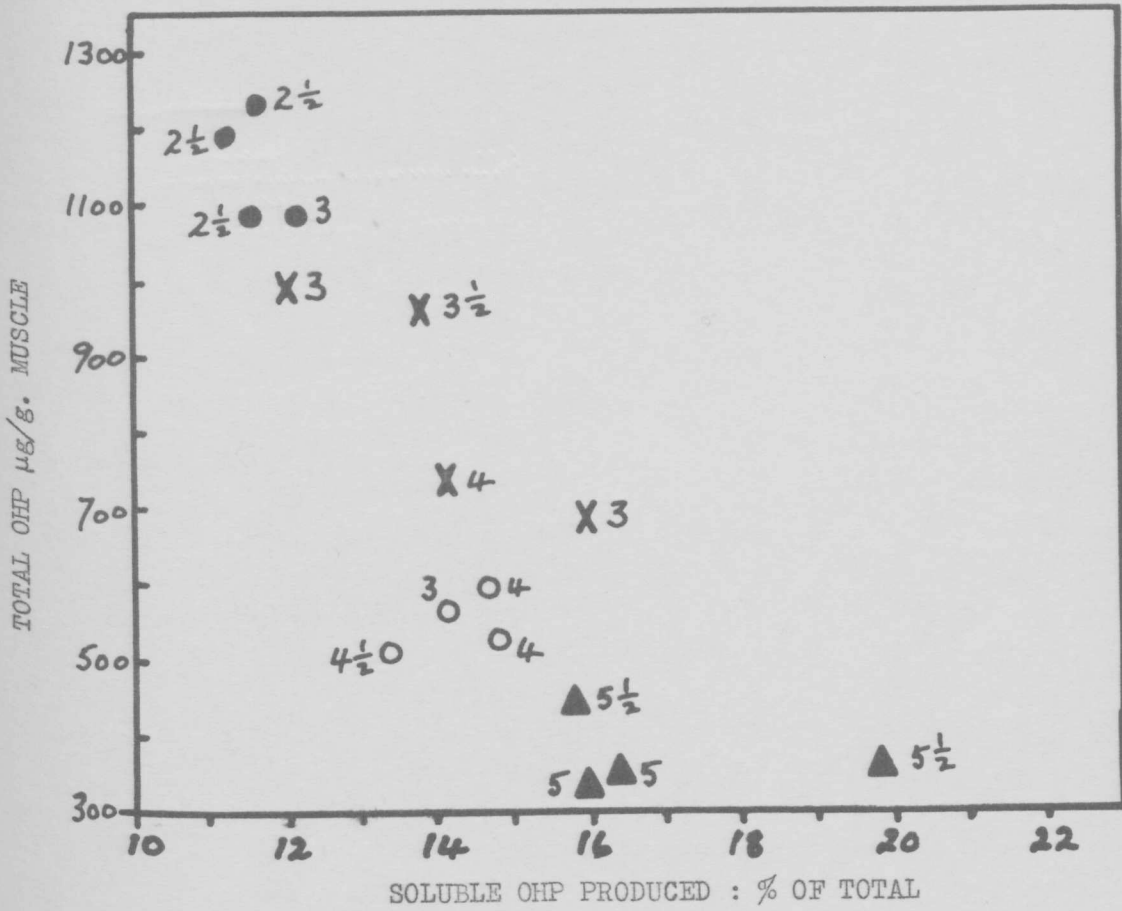


FIG. 2. RELATIONSHIP BETWEEN THE TOTAL OHP, THE SOL. OHP AFTER PAPAIN TREATMENT AND THE TEXTURE OF 4 MUSCLES FROM EACH OF 4-18 MONTH OLD STEERS.

● BICEPS FEMORIS X SEMIMEMBRANOSUS
 ○ LONGISSIMUS DORSI ▲ PSOAS MAJOR

NUMBERS ARE TEXTURE SCORE: - 1, V.V. TOUGH;
 2, V.TOUGH; 3, TOUGH; 4, SL.TOUGH;
 5, TENDER; 6, V.TENDER; 7, V.V. TENDER.