GERMAN

start writing here ▶

Eine differentielle abtast calorimetrische Studie von intramuskulärem Verbindungseisebe Kollagen.

R. Chizzolini, D.A. Ledward and R.A. Lawrie.
Food Science Laboratories, Department of Applied Biochemistry and Nutrition, School of Agriculture, Sutton Bonington, Loughborough, England.

Zusammenfassung

Mit Hilfe von differentiellen abtast calorimetrischen Methode wurden Veränderungen des Wärmestabilität des im KCl unlöslichen Kollagen des intramuskulären Verbindungsgewebes (IMVG) verfolgt. Während der Lagerung von l.dorsi Muskeln bei 1°C und 2 Wochen ging die Stabilität des im KCl unlöslichen Kollagen von Hammelfleisch, Reh und 2 Schweine Muskeln zurück weil 3 Kaninchen und 1 Schwein Muskel unverändert blieben. Das unbehandelte Epimysium von 2 Schwein Muskeln verlohr auch Stabilität im Lager.

Etwas Kollagen löste sich nach Entfernung von Mukopolysaccharid des IMVG mit -Gluk-ronidase und das Rest Kollgen des gelagerten Muskel war immer weniger stabil als das Kollagen von Pre-rigor Muskeln.

Es wird der Schluss gezogen, dass die beobachteten Veränderungen im IMVG von Fleisch während des Lagern nicht ausschliesslich auf Mukopolysaccharid Hydrolyse zurückzuführen sind.

FRENCH

Une étude du collagène du tissue conjonctif intramusculaire par calorimétrie différentielle spectrale: R.Chizzolini, D.A.Ledward and R. A.Lawrie, Food Science Laboratories,Department of Applied Biochemistry and Nutrition,School of Agriculture,Sutton Bonington,Loughborough,England.

Sommaire

La calorimétrie différentielle spectrale a été employée pour étudier les variations de la stabilité thermique du collagène,insoluble en KCl,du tissu conjonctif intramusculaire(TCIM).Pendant la conservation de muscles l.dorsi,à 1°C pour 2 semaines,la stabilité thermique du collagène du TCIM des muscles de 1 agneau,1 daim et 2 porcs diminuait, mais aucune différence ne fut observée avec les muscles de 3 lapins et 1 porc.Le collagèn de l'epimysium non traité des muscles de 2 porcs diminuait aussi en stabilité pendant la conservation.

La libération des mucopolysaccharides du TCIM par la β-glucuronidase entrainait la solubilization partielle du collagène.Toutefois le collagène restant des muscles conservés était,dans tous les cas,moins stable que le collagène des muscles pre-rigor.

On conclut que les variations du TCIM pendant la conservation ne sont pas dues seulement à l'hydrolyse des mucopolysaccharides.

ENGLISH

A differential scanning calorimetric study of intramuscular connective tissue collagen :-

R. Chizzolini, D.A. Ledward and R.A. Lawrie,
Food Science Laboratories, Department of Applied Biochemistry and Nutrition, School of Agriculture, Sutton Bonington, Loughborough, England.

Summary

Differential scanning calorimetry was used to follow changes in the thermal stability of the KCl insoluble collagen of intramuscular connective tissue (IMCT). During storage of l.dorsi muscles, at 1°C for 2 weeks, the collagen of IMCT of lamb, deer and 2 pig muscles decreased in stability but no differences were observed in 3 rabbit and 1 pig muscles. The untreated epimysium collagen of 2 pig muscles also decreased in stability on storage.

Removal of the mucopolysaccharide of IMCT with β-glucuronidase led to some solubilisation of collagen. However the remaining collagen from the stored muscles was, in all cases, less stable than the collagen from the pre-rigor muscles.

It is concluded that the changes occurring in the IMCT of meat during storage are not due solely to mucopolysaccharide hydrolysis.

Изучение коллагена в внутримышечной соединительной ткане при помощи дифференциального сканирующего калориметра.

R. Chizzolini, D.A. Ledward and R.A. Lawrie,
Food Science Laboratories, Department of Applied Biochemistry and Nutrition, School of Agriculture, Sutton Bonington
Loughborough, England.

При помощи дифференциального сканирующего калориметра, мы исследовали изменениями термической устойчивости коллагена, нерастворимого в KCl, в внутримышечной соединительной ткане (ВСТ). В мускулах 'Latissimus dorsi', храненных на две недели при 1°С., устойчивость коллагена уменьшилась в двух образцах мускула свиньи, и в мускулах агнёнка и оленя. В одной другой мускуле свиньи, и в трех мускулах кролика, устойчивость не изменяла. Эпимизиальный коллаген, необработанный с KCl, двух мускулов свиньи тоже оказал при хранении уменьшение устойчивости.

Когда мы убирали мукополисахарид бета-глюкуронидазой, коллаген делался частично растворим. Остаточный коллаген храненных мускулах был однако всегда меньше устойчивей чем коллаген из мускулов перед жесткость.

Мы заключаем что гидролиз мукополисахарида не только причины изменений в ВСТ при храненной мясе.

A differential scanning calorimetric study of intramuscular connective tissue collagen.

R. Chizzolini, D.A. Ledward and R.A. Lawrie

INTRODUCTION

A recent differential scanning calorimetric (DSC) study of sow tendon has shown a marked decrease in thermal stability of the collagen can occur during storage at 1°C (Ledward, Chizzolini and Lawrie, 1975). Recent studies also suggest that changes in the nature of intramuscular connective tissue (IMCT) collagen also occur during storage (Stanley & Brown, 1973; Dutson & Lawrie, 1974).

One possible cause of the loss in stability of collagen during storage is that the mucopolysaccharides of the ground substance may breakdown (McIntosh, 1967) and thus lower the stability of the collagen (Gelman & Blackwell, 1974). In support of this hypothysis Dutson and Lawrie (1974) found that β-glucuronidase, an enzyme capable of hydrolysing glucose-galactose moities present in collagen (Blumenfield et al., 1963) and also of destroying the β-glucuronidic disaccharide linkages between the protein and polysaccharide of the connective tissue matrix (Roden, 1965), was liberated during storage of beef l.dorsi muscles.

The present study was undertaken to see if DSC could be used to demonstrate changes in the collagen of IMCT during storage and, if changes did occur, to see if these were similar to changes induced by β-glucuronidase treatment of the IMCT of prerigor samples.

MATERIALS AND METHODS

Meat Samples.

l.dorsi muscles from lamb, rabbit and pig were all obtained within 1 hr of death from animals slaughtered at the School of Agriculture. l.dorsi muscle from deer was obtained about 40 hr after slaughter.

The IMCT from about 50g portions of each muscle was extracted(a) as soon as possible after death and (b) after storage of the muscle, in polythene, at

1 ± 1°C for 2 weeks.

Extraction of IMCT

The samples, freed from epimysium and fat, were ground through a 4.8 mm plate and blended in a Waring Blender with cold, isotonic NaCl (Field, 1970). The extracted IMCT was washed, at 1 ± 1°C, in excess M KCl for 3 days (Mohr & Bendall, 1969). Previous work has shown that this treatment has little effect on the thermogram of tendon collagen (Ledward et al., 1975). Finally the IMCT was washed in a large excess of cold, isotonic NaCl over at least 4 days.

Treatment with β-glucuronidase

Portions of the extracted IMCT were incubated with 25 ml of β-glucuronidase solution for 24 hr at 37°C. Following the digestion the samples were centrifuged and washed in excess, cold isotonic NaCl for at least 4 days.

The β-glucuronidase was of bacterial origin (Sigma) and was made up to a concentration of 1 mg/ml in 0.1M phosphate buffer (pH 6.8).

Epimysium

The epimysium used in some analyses was from the l.dorsi muscles of 2 pigs. It was scraped clean of fat and myofibrillar proteins and then used without any further treatment.

Calorimetry

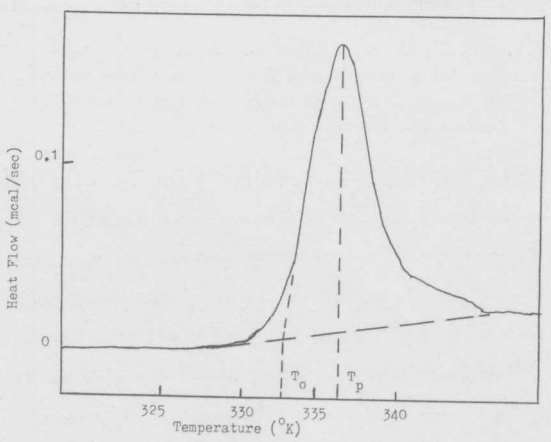
Thermograms of the IMCT samples soaked in 0.9% NaCl or the untreated epimysium samples were determined at a scan rate of 5°K/min in a Perkin-Elmer DSC-1I differential scanning calorimeter. The chart speed was 2 cm/min. The actual calibration and measurement procedure has been described previously (Finch and Ledward, 1972).

Determination of collagen content

Hydroxyproline contents were determined by the method of Woessner (1961) and converted to collagen concentration as described by Mohr and Bendall (1969).

RESULTS

Fig. 1. Thermogram of 1.89 mg of KCl treated prerigor pig l.dorsi IMCT collagen swollen in isotonic NaCl. The scan rate was 5°K/min.



A typical thermogram of IMCT is shown in Figure 1 and the extrapolated onset temperature, T_o and peak temperature, T_p, were determined as shown. The percentage collagen melting before 335 or 337°K was determined from the ratio of the area before 335 or 337°K to the total peak area.

The effect of storage on these parameters is shown in Table 1. It is seen that, except for 3 rabbit and 1 pig muscle the collagen in both the KCl insoluble IMCT and untreated epimysium becomes less stable during storage. This is shown by the decreased T_o values and higher proportion of collagen melting before 335 or 337°K. T_p decreased in an analogous way to T_o. Thus the decreased collagen stability observed on storage of some sow tendons (Ledward et al. 1975) is confirmed for IMCT and epimysium collagen.

Table 1. The effect of storage at 1°C on the stability of the KCl insoluble collagen of l.dorsi IMCT and untreated collagen of the epimysium.

Storage Time / Species	1 hr after death		2 weeks		Stability
	% less stable*	T_o °K	% less stable*	T_o °K	
Pig A	17.3 ± 7.0	332.9 ± 0.2	28.5 ± 6.4	331.9 ± 0.2	Decreased
Pig A Scald	21.0 ± 8.2	333.0 ± 0.3	28.7 ± 1.4	331.9 ± 0.2	Decreased
Pig A^E	34.3 ± 8.8	330.6 ± 1.2	43.9 ± 3.8	329.2 ± 0.2	Decreased
Pig A^{E}Scald	30.7 ± 10.3	331.1 ± 0.6	44.5 ± 8.7	328.8 ± 1.0	Decreased
Pig B Scald	11.0 ± 3.9	333.8 ± 0.2	21.5 ± 5.6	331.9 ± 0.5	Decreased
Pig B^{E}Scald	30.1 ± 5.6	331.8 ± 0.3	43.0 ± 6.5	330.4 ± 0.3	Decreased
Pig C Scald	37.1 ± 6.4	331.2 ± 0.2	37.3 ± 5.2	331.4 ± 0.3	No change
Rabbit A	34.5 ± 6.0	331.6 ± 0.4	37.8 ± 4.8	331.3 ± 0.2	No change
Rabbit B	39.5 ± 2.4	331.3 ± 0.3	36.7 ± 7.1	331.1 ± 0.2	No change
Rabbit C	30.7 ± 8.4	331.7 ± 0.5	34.2 ± 5.0	331.3 ± 0.2	No change
Lamb	38.5 ± 3.1	334.0 ± 0.1	51.2 ± 2.9	332.8 ± 0.4	Decreased
Deer#	46.5 ± 8.6	332.1 ± 0.3	59.7 ± 4.2	332.0 ± 0.2	Decreased

*% melting before 335°K (pig & rabbit) or 337°K (lamb and deer)
Scald = carcase had been through the scalding process before the samples were removed.
E = Epimysium; # the samples were received 40 hr after death.
Each value is the mean ± standard deviation on 6 samples

To see if the loss of stability during storage was due to β-glucuronidase digestion of susceptible linkages, the extracted IMCT was treated with this enzyme. The thermal characteristics of the resulting insoluble IMCT collagen are described in Table 2.

Table 2. The effect of storage at 1°C on the stability of the KCl/β-glucuronidase insoluble collagen of l.dorsi IMCT.

Storage time / Species	1 hr		2 weeks		Stability
	% less stable*	T_o °K	% less stable*	T_o °K	
Pig C	28.4 ± 5.8	332.0 ± 0.4	42.9 ± 5.9	331.3 ± 0.3	Decreased
Rabbit A	22.8 ± 3.0	332.4 ± 0.3	36.9 ± 5.0	331.4 ± 0.2	Decreased
Rabbit B	24.8 ± 5.1	332.0 ± 0.2	33.3 ± 6.1	331.5 ± 0.4	Decreased
Rabbit C	21.0 ± 3.2	332.5 ± 0.4	46.6 ± 7.8	330.8 ± 0.6	Decreased
Lamb	27.7 ± 5.2	334.7 ± 0.3	33.7 ± 5.4	333.9 ± 0.4	Decreased
Deer ‡	41.0 ± 3.1	333.0 ± 0.4	67.0 ± 4.0	331.1 ± 0.3	Decreased

* % melting before 335°K (pig & rabbit) or 337°K (lamb and deer)

‡ Initial samples about 40 hr after death

Each value in the mean ± standard deviation of 6 samples

In all cases β-glucuronidase solubilised some collagen, the amount varying from 3.4 to 5.2% in the prerigor samples and 3.7 to 5.8% in the stored samples. In spite of this loss of collagen it is seen from Table 2 that the collagen of the IMCT from the stored muscles was, in all cases, less stable than that from the prerigor samples. This effect was especially marked in deer and was even true for the pig and rabbit muscles which showed no apparent change on just KCl purification (Table 1).

DISCUSSION

As the storage effects are seen both before and after β-glucuronidase hydrolysis it is apparent that the changes found on storage are not due solely to the action of β-glucuronidase released during storage (Dutson and Lawrie, 1974).

The solubilisation of collagen by β-glucuronidase will explain the apparent increase in stability of prerigor IMCT collagen following this treatment (Tables 1 and 2). However, unlike the effect of storage, there is no apparent destabilising of the remaining collagen verifying that the changes observed on storage are not due solely to β-glucuronidase digestion of the susceptible polysaccharide linkages. However β-glucuronidase hydrolysis may well contribute to the increase in soluble collagen observed in some stored meats (Dutson and Lawrie, 1974).

It has been shown that the decreased stability of stored sow tendon collagen can not be adequately explained by rupture of intra and inter-molecular crosslinks (Ledward et al., 1975). The present results show the β-glucuronidase hydrolysis of the glucose-galactose moities in collagen and/or the protein-polysaccharide linkages in the connective tissue will not fully explain the observed changes in collagen stability. Therefore, to explain the decrease in stability of IMCT collagen on storage a further factor must be important and this may well be the action of cathepsins and other enzymes that are released in stored meat along with β-glucuronidase (Ono, 1970, 1971; Valin, 1970; Canonico & Bird, 1970).

Although both the present and previous studies have demonstrated that changes in IMCT do occur during storage it is still not known wrether this is a major factor in the conditioning of meat.

REFERENCES

Blumenfield, O.O., Mercedes, A.P., Gallop, P.M. and Seifter, S.S. (1963). J. Biol. Chem. 238, 3835.

Canonico, P.G. & Bird, J.W.C. (1970). J. Cell Biol. 45, 321.

Dutson, T.R. & Lawrie, R.A. (1974). J. Fd. Technol. 9, 43.

Field, R.A. (1970). Proceedings MSIBAS, 21, 242.

Finch, A. & Ledward, D.A. (1972). Biochim. Biophys. Acta, 278, 433.

Fujiman, M.A. & Blackwell, J. (1974). Biochim. Biophys. Acta, 342, 254.

Ledward, D.A., Chizzolini, R. & Lawrie, R.A. (1975). J. Fd. Technol. 10,

Lightbody, [illegible] (1967). J. Fd. Sci. 32, 210.

Mohr, W. & Kendall, J.K. (1969). Nature 224, 404.

Ono, K. (1970). J. Fd. Sci. 35, 256.

Ono, K. (1971). J. Fd. Sci. 36, 858.

Perdon, L. (1965) In "Structure and Function of Connective and Skeletal Muscle" (Ed. by S. Fitton Jackson, S.K. Partridge, R.D. Harkness & G.R. Tristram), pp. 141-195, Butterworth, London.

Stanley, D.W. & Brown, R.G. (1973) XIXth Réunion Europeenne des Chercheurs en viande, Paris, I (B4), 36.

Valin, C. (1970) Annls. Biol. anim Biochim. Biophys. 10, 313

Weissmann, J.B. Jr.(1961) Arch. Biochem. Biophys. 93, 440.

ACKNOWLEDGEMENTS

We thank the Agricultural Research Council for the funds to purchase the Perkin-Elmer DSC-II and Mrs. B. Ellington for technical assistance.