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THE INFLUENCE OF TIME FROM SLAUGHTER TO FREEZING ON THE DEVELOPMENT OF FREEZER BURN ON BEEF MUSCLE TISSUE

by

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The marked biochemical and biophysical changes which muscle tissue undergoes in the period following slaughter raises the possibility that development of freezer burn on muscle may differ from that on liver tissue. In the latter, biochemical changes are of different nature and more gradual. The main factors responsible for inhibiting the appearance of freezer burn on liver tissue were found to be (a) increases in freezing time, especially when freezing with evaporative weight loss, (b) youth of the animal (foetal tissue forms no freezer burn at all) and (c) low fat content of the liver. Weight losses in store necessary for the onset of freezer burn were only slightly increased by surface desiccation or prestorage of livers before freezing. However, these effects were statistically significant.

The present paper deals with corresponding observations on beef muscle tissue.

EXPERIMENTAL

Samples of semitendinosus muscle were taken from the carcass as soon after slaughter as possible. These were wrapped in polythene, rapidly chilled to 0°C, and then held at 0°C for periods of 1.5 to 336 hours before being frozen. Slices of the muscle 5 x 8 cm, 0.3 cm thick,

were frozen in stainless steel trays at different rates, requiring freezing times of 4 to 410 min. Freezing time was defined as the time required to lower the temperature at the centre of the slice from 0°C to -10°C. Most of the experiments were carried out with freezing times of either 4 or 220 min. Some samples were frozen without evaporative weight loss by covering with a plastic film of low water vapour permeability. Others were not covered, thus permitting evaporative weight loss during freezing.

After freezing, the samples in the trays were stored uncovered, in two drums in which air at -10°C circulated at an average speed of 3 cm/sec. The relative humidities in the two drums were 78 and 97% respectively. Weight losses of the individual samples were determined over the freezing period, and also from the end of freezing to a defined stage of freezer burn intensity. Three such intensities of "burn" were defined: (1) the first indications, which appear as areas with a thin, almost transparent, surface layer giving a pink tinge: (2) patches of increasing area which have developed a distinct whitish color; and (3) patches which have ceased to increase in area and have commenced to become dull. For each intensity, weight losses were reported as the total weight loss during freezing and storage. Further experimental details have been given elsewhere(1,3).

For histological study, additional samples were taken from fresh tissues, and also immediately after freezing, and again after an advanced stage of freezer burn had developed. Frozen samples were either freeze-fixed at -9°C at the freezing point of a 35.5% (v/v) formalin solution, or fixed at room temperature in 10% formalin. In both cases a pH close to that of the meat was maintained. Sections 10 μ

thick were prepared from the samples and stained with hematoxylin-eosin. Further experimental details have been given elsewhere (2).

RESULTS AND DISCUSSION

Weight Losses

As has been indicated in earlier publications (1), freezer burn is a discoloration which appears as a brightening of the surface caused by diffuse reflection of light from the cavities formed by dehydration of the surface. The development of freezer burn is accompanied by weight loss.

In the present work it was found that, in general, freezer burn developed most readily on samples frozen rapidly under conditions such that evaporative weight loss was prevented during freezing. As was the case with liver tissue (3), the total weight loss necessary to produce a given intensity of "burn" increased with the freezing time. Similarly, it was greater when freezing was carried out with evaporative weight loss than when evaporation during freezing was prevented. The effect of the age of the animal was small, though statistically significant: muscles from veal or from canner grade cow carcasses required a lower weight loss in store to produce a given intensity than did muscles from good quality steer carcasses.

On samples from steer meat, frozen either in the pre-rigor condition (3 hr after slaughter) or in the state where rigor was almost completed (24 hr after slaughter), weight losses necessary to produce a definite intensity were up to twice as high as in the post-rigor period (e.g. at 268 hr). For restricting freezer burn, the advantage of freezing with weight loss was only apparent with slowly

frozen samples. Samples from a canner grade cow exhibited given freezer burn intensities at lower weight losses than those from steer meat, but the effects of increasing the time of storage before freezing, e.g. from 48 hr to 120 hr, were similar. The results allow the conclusion that optimum conditions obtain for the inhibition of freezer burn when muscle from good quality beef is, after a chilling period of 24 hrs, slowly frozen under conditions where evaporative weight loss occurs. Marsh (4) recommends that for commercial chilling of beef carcasses a period of 36 hr should elapse before freezing, to complete glycolytic reactions and to avoid thaw rigor. It may be considered that under the normal conditions of commercial freezing these reactions will go on (though at a reduced rate) during the period the tissue remains above and at the freezing point. From cooling curves given by Howard and Lawrie (5) this period can be estimated to be about 5 hr for thin parts of a carcase undergoing blast freezing.

Histological Examination

Pattern of ice crystals.- Physiological condition and structure of muscle tissue had a great influence on the pattern of the ice crystal formation, which in turn affected the onset of freezer burn.

Examination of samples frozen pre-rigor showed that the ice crystals were intracellular. Crystals in samples frozen in 4 min were, however, smaller in size and greater in number than in samples frozen in 220 min. Apparently crystals fuse together after the fibre membranes have been ruptured by crystal growth. In samples frozen post-rigor the ice crystals were formed between the fibres, except for a small number of intracellular crystals in rapidly

frozen samples. With a freezing time of 4 min the number was again greater and the size smaller than with a freezing time of 220 min.

Thus the rate of freezing appears to be the predominating factor determining size and number of crystals. In pre-rigor tissue, crystals always form within fibres, while in slowly frozen post-rigor tissue they are propagated between fibres. In rapidly frozen post-rigor tissue, however, propagation occurred both within and between fibres. According to Meryman (5) intracellular crystals originate when the ice front advances at a rate of 1.6 cm/min or greater.

Effects of freezing on muscle structure.- Samples frozen pre-rigor and fixed after thawing showed corrugated outlines of fibres due to thaw rigor, and the spaces occupied by ice crystals partly remained after defrosting. When the prestorage period reached 24 hr this damage was minimized.

Rapidly frozen post-rigor samples showed fibres with holes caused by piercing with extracellular crystals. In slowly frozen samples such puncture holes were only observed when the pre-storage period was extended to 14 days at 0°C, and are possibly due to weakening of the fibres through autolysis under these conditions. The rupture of fibres of fish muscle by ice crystals has been reported earlier by Reuter (7) and Love and Haraldsson (8).

Storage before freezing.- The most probable sites for the onset of freezer burn are the sites where the fibres have been penetrated during freezing and at the channels left after the sublimation of ice from the network of large crystals.

In tissue frozen pre-rigor, freezer burn cavities developed between the fibres. There was also a form of "micro-burn" in which intracellular cavities formed between the fibrils.

In rapidly frozen post-rigor samples, the mode of development of freezer burn was, in principle, very similar to that in liver, but the condensed layer which formed in front of the freezer burn layer was less pronounced and only appeared in fragments. In the late stage of freezer burn the volume of the fibres next to the surface may increase due to a looser arrangement of the fibrils.

When freezing is slow, either with or without evaporative weight loss, a condensed layer develops at the surface during storage. When evaporative loss occurred during freezing this condensed layer began to form during the freezing. As the rate of freezing was reduced, the extent to which the layer built up during storage was increased, and freezer burn developed below it. The increase in thickness of the condensed layer consequently resulted in a retardation of freezer burn development. When slow freezing was carried out pre-rigor there was again some evidence of freezer burn cavities within the cells, and they were larger than in rapidly frozen tissue. When the freezing took place after completion of rigor, intracellular cavities were rarely seen, and they were completely absent from muscle frozen post- rigor.

In slowly frozen muscle, cavities can also form in the condensed layer in the later stages of freezer burn development.

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SUMMARY

The maximum formation of freezer burn (fb) on cuts of beef semitendinosus muscle stored at -10°C and 78% and 97% R.H. was obtained when they were frozen rapidly under conditions where evaporative weight loss was prevented. Optimum conditions for retarding fb were obtained when samples from good grade animals were frozen slowly, with permitted evaporative weight loss, at about the time that rigor was almost complete. The total weight losses corresponding to a given intensity of fb were then about twice the weight losses observed on tissues with the same intensity frozen post-rigor.

A histological study showed that ice crystals formed intracellularly in tissue frozen before the onset of rigor independently of the rate of freezing but extracellularly in tissue frozen slowly post-rigor. In tissue frozen rapidly post-rigor crystals also appeared intracellularly. After defrosting, cavities could be observed in fibres which contained intracellular crystals. Cell walls were ruptured due to the growing and piercing of ice crystals in rapidly frozen tissue and in slowly frozen tissue with an extended pre-storage period before freezing.

The basic mechanism of the development of fb corresponds with that observed on liver tissue, but on muscle tissue rapidly frozen without weight loss the condensed layer forms irregularly, often only in fragments. The physiological state of the muscle at the time of freezing influences the fb cavity formation. In tissue frozen pre-rigor, cavities frequently form intracellularly between fibrils, representing a type of micro-burn. In post-rigor

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tissue an advanced state of desiccation may increase the volume of the previously shrunken fibres by a looser arrangement of fibrils, and at this stage cavities may also form in the condensed layer of slowly frozen tissue.

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Text to figures:

(1) Effects of time after slaughter before freezing on total weight loss (during freezing and storage) necessary to produce fb of intensities I_1, I_2, I_3 on samples frozen in 4 and 220 min. S_2 steer, S_3 canner cow. Also one experiment with veal. Storage: temp. -10°C, R.H. 78%.

(2a,b) Crystal pattern in semitendinosus muscle after freezing pre-rigor (a) 4 min (b) 220 min. Sample freezefixed (transverse section).

(3a,b) As in (2) but samples frozen post-rigor.

(4a,b) Longitudinal sections from samples after freezing in 4 min, (a) fixed in defrosted state at room temperature, (b) freeze-fixed.
(a) pre-rigor sample with thaw rigor, (b) post-rigor sample with holes caused by piercing crystals.

(5a,b) Sections from samples frozen in 4 min with advanced fb, defrosted, fixed at room temperature.
(a) longitudinal section frozen pre-rigor, showing micro-burn and corrugation, (b) transverse section frozen post-rigor, showing condensed layer, fb layer.

(6) Transverse section from sample with fb of intensity I_2 frozen 220 min after precooling at 0°C for 24 hr, rigor mostly completed. Fixed at room temperature.

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ZUSAMMENFASSUNG DER ABHANDLUNG

"DER EINFLUSS VON ZEIT ZWISCHEN SCHLACHTEN UND EINFRIEREN AUF DIE ENTWICKLUNG VON GEFRIERBRAND BEI RIND-MUSKEL-GEWEBE"

von G. Kaess und J. F. Weidemann

Bei Lagerung von halbsehnigem Rindmuskel bei -10° C und 78% und 97% relat. Luftfeuchtigkeit wurde ein Höchstmass an Gefrierbrand an Schnittstellen festgestellt. Hierbei war das Fleisch schnell Eingefroren worden, wobei Gewichtsverlust durch Verdampfung verhindert wurde. Optimale Bedingungen zur Eindämmung des Gefrierbrandes wurden erreicht, als Proben von hochwertigen Tieren langsam eingefroren wurden zu einem Zeitpunkt, da das Fleisch beinah erstarrt war. Gewichtsverluste durch Verdampfen wurden dabei Zugestanden. Der gesamte Gewichtsverlust, verglichen mit einer gegebenen Intensität von Gefrierbrand, erwies sich daraufhin etwa doppelt so hoch wie die Gewichtsverluste, die an Geweben mit der gleichen Intensität beobachtet wurden, welche nach der Erstarrung eingefroren worden waren.

Eine histologische Untersuchung zeigte, dass sich Eiskristalle innerhalb der Gewebezellen formten, die vor Erstarrungsbeginn eingefroren waren unabhängig von der Einfriergeschwindigkeit. Eiskristalle formten sich ausserhalb der Zellen in Geweben, die nach der Erstarrung langsam eingefroren wurden, In Geweben, die nach der Erstarrung schnell eingefroren wurden, erschienen die Kristalle ebenfalls innerhalb der Zellen. Nach dem Auftauen wurden Hohlräume zwischen den Fasern beobachtet, die Kristalle in den Zellen enthalten hatten. Die Zellwände waren zerstört infolge von Vergrösserung und Durchdringung von Eiskristallen in schnell gefrorenem Gewebe. Das gleiche geschah bei langsam gefrorenen Geweben, die vor dem Einfrieren längere Zeit gelagert hatten.

Der Hauptvorgang beim Entstehen von Gefrierbrand ist dem bei Leber beobachteten ähnlich, nur bildet sich bei Muskelgewebe die kondensierte Schicht unregelmässig, oft nur teilweise. Der physiologische Zustand von Muskelfleisch beim Zeitpunkt des Einfrierens beeinflusst die Bildung von Gefrierbrand-Hohlräumen. In Geweben, eingefroren vor der Erstarrung, bilden sich häufig Hohlräume zwischen den Fäserchen innerhalb der Zellen und bilden damit eine Art Mikro-Brand. In Geweben, eingefroren nach der Erstarrung, kann fortgeschrittene Aus-

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trocknung das Volumen der vorher geschrumpften Fasern vergrössern durch lockere Anordnung von Fäserchen. Zu diesem Zeitpunkt können sich Hohlräume ebenfalls in der kondensierten Schicht in langsam gefrorenen Geweben bilden.

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PRECIS D'EPREUVE "INFLUENCE DU TEMPS, DEPUIS L'ABATTAGE JUSQU'A LA CONGELATION, DANS LE DEVELOPPEMENT DE BRULURE PAR CONGELATION SUR LES TISSUS MUSCULEUX DU BOEUF"

Par G. KAESS et J. F. WEIDEMANN

La formation maximum de la brûlure par congélation (freezer burn :fb) sur des morceaux de boeuf à muscles semi tendineux, entreposés à -10°C et 78 % et 97 % R.H., était obtenue lors d'une congélation rapide dans des conditions telles que la perte de poids par évaporation était évitée. Les conditions les plus favorables au retardement de fb étaient obtenues quand des échantillons de viande de bonne qualité étaient congelés lentement, avec une perte de poids allouée à l'évaporation, aux environs du moment où la rigidité était presque complète. Les pertes de poids totales correspondant à une intensité donnée de fb, étaient alors de deux fois les pertes de poids observées sur des tissus de même intensité, congelés après rigidité.

Une étude histologique a montré que les cristaux de glace se formaient intracellulairement dans les tissus congelés avant le début de rigidité, indépendamment du taux de congélation, mais extracellulairement dans les tissus congelés lentement après rigidité. Dans les tissus congelés rapidement après rigidité des cristaux apparaissent également intracellulairement. Après le dégivrage, des cavités pouvaient être observées dans les fibres ayant contenu des cristaux intracellulaires. Les parois des cellules étaient rompues en raison de la formation et de la pénétration des cristaux de glace dans des tissus congelés rapidement et dans des tissus congelés lentement avec un entrepôt prolongé avant la congélation.

Le mécanisme de base du développement de fb correspond à celui observé sur les tissus d'un foie; mais, sur des tissus musculeux congelés rapidement, sans perte de poids, la couche de condensation se forme irrégulièrement, et souvent uniquement en fragments. L'état physiologique du muscle au moment de la congélation influence la formation de la cavité fb. Dans les tissus congelés avant rigidité des cavités se forment fréquemment intracellulairement entre les fibrilles ce qui représente un type de micro-brûlure. Dans des tissus congelés après rigidité un état avancé de dessiccation peut augmenter le volume des fibres préalablement rétrécies par un relâchement des fibrilles et, à cette phase, des cavités peuvent aussi se former dans les couches de condensation des tissus congelés lentement.