DEVELOPMENT AND CONTROL OF FREEZER-BURN ON LIVER TISSUE

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Discoloration of frozen tissue

When economic considerations prevent the use of packaging materials sufficiently impermeable to water vapour, frozen goods, except when stored in jacketed rooms, are subject to noticeable weight loss during frozen storage. This weight loss arises from the sublimation of ice crystals and the evaporation of water bound to the tissue. Unfavourable changes in appearance are closely related to the desiccation of the surface. Weight losses from frozen animal tissue generally cause an increase of the concentration of the pigments and cell constituents. In addition oxidation of the pigments occurs and results in a darkening of the colour. This, however, is usually tolerated provided it is not excessive.

The same surface on which darkening occurs may also develop patches or larger areas of an unsightly greyish discoloration which is obviously different from the ordinary form of darkening. Our experience with offals has shown that very small weight losses can produce this change in appearance, commonly known as freezer-burn) (f.b.), to an extent which seriously reduces the sales value.

Another colour effect may be mentioned which occurs during the freezing process and which is occasionally confused with f.b. The surfaces of small goods such as offals which are directly exposed to cold air moving with a high velocity develop small ice crystals and a light colour while, in contrast, the sides away from the air flow where heat transfer is less rapid with consequent slower freezing rate produce larger crystals and a darker colour.

Past experience with f.b.

F.b. was observed on a large variety of frozen food products. Moran (1) described it on sheep kidneys as a spongy, corky layer with a light greyish colour resulting from the diffuse reflection of light from the holes and cavities in the surface layers which he was able to demonstrate with histological sections.

F.b. has even been found in the air pockets of wrapped poultry and in other packages where evaporation from the surface is possible by distillation on to the inside of the wall of the packaging material (2). A fatty surface offered some protection while a lean surface (as in the case of rabbits (3)) or the spots on the skin of poultry (4) left after removing the feathers favoured the onset of f.b.

To avoid f.b., low temperatures during storage, high relative humidity and still air were recommended. Cook (5) suggested storing at -12 to -15°C and 98% relative humidity to prevent f.b. in poultry stored for 6 to 10 months. Tightly applied wraps and the application of wax coatings (and also ice) were also able to control f.b. but their protection depended on the water vapour permeability of the packaging material (6). Drying of the offals before freezing was used to reduce f.b. (7) and Bergh (8) has prevented discoloration including that due to f.b. by treating the surface of meat before freezing with solutions of glycerine, hexoses, etc.

Experiments with liver

Livers from beef cattle were chosen for study because f.b. was repeatedly observed on them and frozen offals are regularly exported from Australia and New Zealand to England.

They have a reasonably uniform composition and the surface is large enough to furnish an adequate number of samples for the study of the effect of different experimental conditions. Different livers vary considerably in their fat content and there is a corresponding variation in moisture content. Even if moisture content is expressed on a fat-free wet basis, it is not completely constant but is slightly dependent on the fat content.

5 cm x 7.6 cm samples of the surface with a uniform thickness of 3 mm were obtained by the use of a mechanical cutter. Slices of the required 5 cm width and about 1.9 cm thick were moved in a guide against a razor vibrating at 1450 oscillations per minute adjusted in height to deliver slices of the required thickness of 3.0 mm. The slice was then cut into lengths and fitted with the membrane on top in stainless steel trays 5 cm x 7.6 cm and 5 mm high. The slices had an average weight of 12.1 g.

The samples were frozen either without weight loss by covering with an impermeable film or with varying weight losses by varying the rate of freezing. The time of freezing was taken as the time for the centre of the tissue to fall from 0°C to -10°C. Different times of freezing from 4 to 410 minutes were obtained by varying the resistance to heat transfer between the cooling medium and the tray. The highest rate of freezing was obtained by floating the trays in mercury at -20.5°C in a container set up in a cold room held at -13°C. The weight losses during the process of freezing increased linearly with time up to 2.0 gm/dm^2 at a freezing time of 410 minutes.

The frozen samples were stored at -10°C in drums with relative humidities of 78, 88 and 97% and with a low air flow of about 3 cm/sec on the average.

F.b. was judged subjectively by using 3 intensities as standards.- 1. the first appearance of traces, almost colourless, 2. spots or stripes which have grown to about 2-3 mm diameter or width with the development of a distinct whitish to greyish colour, 3. the increase in area ceases and the colour starts to turn dull (Fig. 1).

F.b. on samples frozen at high rates without losses

The effects of applied treatments can only be judged satisfactorily if the conditions are known under which f.b. development is at a maximum. Such conditions were obtained when fresh slices of liver from adult animals were frozen without losses at a high freezing rate (4 minutes). Under these conditions the different intensities of f.b. developed at relatively low weight losses which were practically independent of the rate of loss and of the fat content of the liver. For example intensity 2 occurred at a loss of about 0.25 g/dm^2 and intensity 3 at 1.6 g/dm^2. Drying of the surface at 0°C before freezing gave a slight but statistically significant retardation of the development of f.b. The retardation was more pronounced when the liver was stored at 0°C before freezing for a period of 4 days and especially when held for 7 days. F.b. can also occur from a liver surface from which the Glissons' capsule has been removed.

F.b. on samples frozen slowly without losses

When the freezing time exceeded 40 minutes but losses were prevented by covering the samples, there was a retarding effect on the development of f.b. apparently due to more pronounced denaturation of proteins at low rates of freezing.

F.b. on samples frozen with weight losses

The onset of f.b. was markedly retarded when the surface was dried during the process of freezing. The weight losses necessary to provide the different intensities were then increased. At low fat content ($\lesssim$ 10%) a freezing time of 40 minutes was sufficient to prevent intensity 3 occurring within the time necessary to produce weight loss of $\sim$10 g/dm^2 at which stage darkening is excessive. With a freezing time of 220 minutes the development was restricted to intensity 1. However even with a freezing time of 220 minutes only intensity 3 could be avoided when the fat content of the liver was high. Generally the increased freezing time restricted the development of f.b. on the surface, and at freezing times of 73 minutes and over, especially with livers of high fat content, f.b. develops under the surface and can be seen through a thin layer of tissue.

In contrast to freezing without losses, where the rate of evaporation had no influence on the extent of freezer burn development, when losses occur during freezing the onset of f.b. was noticeably accelerated when the rate of weight losses was decreased.

Additional treatments with dipping solutions

The experiments in which a period of storage of 4 days or more was used prior to freezing and the experiments in which losses occurred before freezing suggested that treatments which produce a viscid condition in the extracellular fluid remaining in the tissue after the formation of ice crystals would retard the onset of f.b. When solutions of glycerol and hexoses etc., are frozen rapidly there is a reduction in the size and rate of growth of the ice crystals, and the volume of the unfrozen liquid increases (9). It was assumed that the presence of such liquids in the tissue could enhance these effects and further retard the onset of f.b. Livers were dipped for 10 minutes in solutions of glycerol, sorbitol, glucose, fructose and also NaCl and urea. The solutions readily penetrated into the tissue; with urea and NaCl the depth of penetration was about 1½ mm. The development of f.b. on slices from these livers frozen in 4 minutes without losses was drastically reduced. The concentrations which entirely controlled f.b. in tissue with a fat content of $\lesssim$ 10% and reduced that of fatty livers to a moderate intensity were glycerol 20%, sorbitol 25%, glucose 30%, fructose 25%, NaCl 15% and urea 20%. Moreover fatty livers showed f.b. only when the weight losses had increased to a level which is undesirable because of the development of excessive dark discoloration. Increasing the dipping time to 100 minutes did not reduce the development of f.b. noticeably.

Higher concentrations of the alcohols and hexoses produce a surface gloss and cannot be recommended. With the alcohols and urea the usual dark discoloration was not more pronounced than in untreated livers, but with hexoses, and especially with NaCl, the dark discoloration is noticeably accelerated.

When the dipping treatments are used the effect overrides that of losses during freezing. Treated liver slices with a high fat content frozen with losses in 220 minutes show the first development of f.b. at weight losses of about the same order as those for treated slices frozen without losses (in 4 minutes).

Histological examination of sections of slices with f.b.

Sections from tissue with f.b. which have been fixed in neutral formalin solution (25°C) and stained with H aE showed surface layers with the typical f.b. cavities which gain in depth as weight losses increase (Fig.2). This is in good agreement with the picture of a section from kidney with f.b. already shown by Moran (1).

Samples treated this way defrost before fixing is completed and the shape and locations of the ice crystals cannot be shown. We therefore fixed pieces of tissue at the freezing point of a formalin solution leaving ice crystals intact till the end of the fixing process. Sections obtained from the freeze-fixed tissue (Fig.3) showed that the typical f.b. cavities are not just free spaces left by the sublimation of the ice crystals. It appears rather that after sublimation of the ice a condensed layer forms from which the f.b. cavities originate by subsequent evaporation of moisture from the tissue. With further weight losses the layer containing the cavities increases in depth and a condensed layer develops in front of it also increasing in thickness. There is a sharply defined boundary between the condensed layer and the zone with ice crystals. The size of the ice crystals is scarcely changed in the immediate vicinity of this boundary.

Summary

Slices of beef liver 5 cm x 7.6 cm., 3 mm thick taken from the surface layer developed a maximum of freezer-burn (f.b.) when fresh samples taken from adult slaughtered animals, were rapidly frozen without loss of weight. F.b. appeared at comparatively low subsequent weight losses, and these were independent of fat content and the rate of losses at -10°C (+14°F), the relative humidities 78, 88 and 97% and a low air speed (average about 3 cm/s). Removal of moisture from the surface before freezing resulted only in a minor reduction of f.b. of samples frozen without losses. A more pronounced, significant reduction on samples frozen likewise was obtained when the livers were stored for 4 or 7 days at about 0°C before freezing.

The onset of f.b. was markedly delayed when the slices were frozen with weight losses.

Increasing fat content and decreasing rate of weight losses during storage favoured the appearance of f.b.

Dipping samples (10 minutes) in solutions of glycerol, sorbitol, hexoses, NaCl, urea before freezing without losses prevented f.b. entirely on livers with a fat content $< 10\%$ and to a considerable extent on livers $> 10\%$ fat. The effect depends on the concentration applied. With the alcohols and hexoses the concentration has to be limited to avoid gloss. Hexoses and especially NaCl also accelerate dark discoloration. Decreasing rates of losses in the storeroom caused an earlier appearance of f.b.

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Zusammenfassung

Scheiben 5 cm x 7.6 cm, 3 mm dick, der Oberflaechenschicht von Rinderlebern entnommen, entwickelten ein Maximum an "freezer burn" (f.b.) wenn die Proben von ausgewachsenen, frischgeschlachteten Tieren stammten und ohne Gewichtsverluste schnellgefroren wurden. F.b. erschien nach verhaeltnismässig geringen Verlusten waehrend der Lagerung (-10°C, relative Feuchtigkeiten 78, 88 and 97%, mittlere Luftgeschwindigkeit etwa 3 cm/S), unabhaengig vom Fettgehalt und den Gewichtsverlusten je Zeiteinheit. Verdunstungsverluste an der Oberflaeche, vor dem Gefrieren ohne Gewichtsverluste, verminderten f.b. nur wenig. Eine ausgepraegtere, signifikante Einschraenkung wurde erzielt, wenn ebenso gefrorene Proben vor dem Gefrierungsvorgang 4 order 7 Tage bei etwa 0°C vorgelagert wurden.

Der Befall mit f.b. war betraechtlich verzoegert, wenn die Proben mit Gewichtsverlusten gefroren wurden. Die Verzoegerung nahm mit der Gefrierzeit zu. Die Gefriergeschwindigkeit verminderte auch den Befall mit f.b. bei der anschliessenden Lagerung, wenn ohne Gewichtsverluste gefroren wurde und die Dauer zum Gefrieren der 3 mm Proben mehr als 40 Min. betrug. Zunehmender Fettgehalt und eine Abnahme der Zeitlichen Gewichtsverluste bei der Lagerung beguenstigten das Auftreten von f.b.

Durch Tauchen von Proben (10 Minutes) in Loesungen von Glyzerin, Sorbitol, Hexozen, NaCl und Harnstoff vor dem Gefrieren ohne Gewichtsverluste konnte f.b. voellig vermieden werden wenn der Fettgehalt kleiner als 10% war, aber auch eine bedeutende Einschraenkung war moeglich wenn der Fettgehalt groesser als 10% war. Die Wirkung hing von der Konzentration der Loesungen ab. Die Konzentration der Alkohole und Hexosen war begrenzt durch die Ausbildung von Oberflaechenglanz. Hexosen und besonders NaCl beschleunigten die Dunkelfaerbung. Sehr kleine zeitliche Gewichtsverluste bei der Lagerung bedingten eine Verkuerzung der Zeitspanne bis zum sichtbar werden von f.b.

Resume

Des tranches de foie de bovins 5 cm x 7.6 cm et de 3 mm d'épaisseur prises de la couche de la surface developèrent un maximum de "freezer burn" (f.b.) quand les échantillons, pris des animaux grandis et abattus, furent congelés rapidement sans perte de poids.

A 10°C(14°F), humidité relative de 78, 88 et 97% et bas courant d'air (moyenne de 3 cm/sec), f.b. apparut avec des pertes de poids comparativement basses indépendemment de la teneur en gras et de la vitesse des pertes.

Desiccation de la surface avant la congélation ne produisit q'une faible diminution de f.b. sur des échantillons congelés sans perte de poids. Une diminution significante et plus prononcée de f.b. sur des échantillons congelés de même façon fut obtenue quand les foies furent gardés pendant 4 à 7 jours à 0°C avant la congélation.

Le commencement de f.b. fut notamment retardé quand les tranches furent congelées avec des pertes de poids. Ce retard fut augmenté en augmentant la période de congélation. La vitesse de congélation produisit aussi un délai quand la période de congélation dépasse 40 minutes et les échantillons sont congelés sans perte de poids. Une augmentation de la teneur en gras et une diminution de la vitesse de perte de poids pendant l'entreposage sont tous deux favorables à l'apparition de f.b.

Trempage des échantillons (10 minutes) dans des solutions de glycerol, sorbitol, hexoses, NaCl ou Urée avant la congélation sans perte de poids empêche le f.b. complètement sur les foies ayant une teneur en gras plus basse que 10% et considérablement sur les foies d'une teneur en gras plus grande que 10%, l'effet dépendant de la concentration appliquée. Avec les alcohols et les hexoses la concentration doit être limitée pour empêcher le lustre. Les hexoses et NaCl accélerèrent le noircissement. Diminution des vitesses des pertes de poids causèrent une apparition prématurée de f.b.