THE ULTRASTRUCTURE OF WHOLE PORCINE MUSCLES FROZEN WITH AN INTERNAL THERMAL GRADIENT

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SUMMARY

Strips of whole porcine muscle were frozen with unidirectional heat flow and an internal thermal gradient. Cartons of porcine muscle were also frozen. Cryo-scanning electron microscopy was used to study the meat ultrastructure. Cavities created after sublimation were used to estimate the cross-sectional areas of ice crystals. Upon freezing, as the distance from the refrigerated border increased the average ice crystal area also increased. After 4 weeks storage at -16°C, these differences in crystal areas were no longer apparent. An internal thermal gradient was observed along the longitudinal diagonal axis of the cartons of meat during freezing. After 3 weeks storage, large crystals were observed in all samples along this axis. Compression of the meat occurred during sampling and the microscopy technique could not confidently be used on cartons of meat. Ultrastructural similarities between the refrigerated border of strips and small samples used in an earlier study support suggestions that the small sample is representative of the periphery of a sample with an internal thermal gradient.

INTRODUCTION

In earlier studies in our laboratories the effect of freezing rate on muscle ultrastructure was investigated (Ngapo *et al.*, 1997). Small samples of porcine muscle were used so that the sample centre had a similar thermal history to the sample surface permitting the effects of freezing rate only to be investigated. No significant differences were observed in the medians of the cross sectional areas of cavities, which were used to estimate ice crystal areas, at six freezing rates. The small sample did not simulate typical industrial freezing conditions, but was suggested to mimic the periphery of a large piece of meat. At this periphery ice crystal nucleation and growth occur simultaneously, while at the centre only growth of the crystals formed at the periphery occurs (Anon and Calvelo, 1980).

The present study aims to provide ultrastructural information about the ice crystals formed along an internal thermal gradient within a muscle and across a carton of meat using the cryo-scanning electron microscopy (cryo-SEM) technique described by Payne *et al.* (1994). Variation in the crystal sizes along the thermal gradient will validate the conclusions reached in the study using small samples of muscle (Ngapo *et al.*, 1997).

MATERIALS AND METHODS

Meat. Meat was obtained from a local abattoir where a 1:1 ratio of male to female pigs are slaughtered (22-24 weeks old) derived from crossbreeds of Landrace, Large White and Duroc. Porcine muscles *biceps femoris*, *semimembranosus* and *rectus femoris* were obtained from hindquarters of carcasses stored for 24 hours at 4°C and stored for a further 24 hours before use.

Freezing strips from individual muscles. Porcine muscle *biceps femoris* was cut into strips (15 x 2 x 2 cm), wrapped in polyethylene film and encased in polystyrene (6 cm thick) leaving one surface (2 x 2 cm) exposed, this being the refrigerated border. The heat flow was unidirectional along the longitudinal axis. Meat fibres were perpendicular to the direction of heat flow. The encased samples were placed in a domestic freezer at about -16°C. Three strips were frozen, each from different muscles. Cores were removed for electron microscopy after at least 48 h. Two of the strips were stored at -16°C for a further 4 weeks after which cores were removed for electron microscopy.

Freezing cartons of meat. Porcine muscles *biceps femoris*, *semimembranosus* and *rectus femoris* were packed into plastic liners (approx. 20 kg of meat in each) and placed in 4 corrugated cardboard cartons (58 x 37 x 15 cm, head space between plastic liner and box of approx. 4 cm, cardboard thickness of 1.5 mm). The cartons of meat were frozen and stored for 3 weeks in still air at about -28°C at a commercial cold storage facility. Two cartons were frozen each week for two successive weeks. Plugs (17 mm diam.) of meat were removed from the frozen cartons using a hydraulic press and stored at -18°C for up to 8 h prior to sampling for cryo-SEM.

Monitoring temperature changes. Temperature change was monitored using copper constantan thermocouples connected as differential inputs to an DL600 Datataker data logger (DT5 Series, Data Electronics Australia Pty Ltd). Thermocouples were inserted (0.5, 3.5, 7.0, 10.5 and 14.5 cm from the refrigerated border) into the centre of the strips of meat perpendicular to both the longitudinal axis and the fibre direction. For cartons of meat, thermocouples were inserted from the top of the carton at 4 points along its diagonal axis (3, 13, 24 and 35 cm from the surface to the centre of the carton) and to the meat centre.

Cryo-SEM. Cryo-SEM was conducted using a Cambridge Instruments Stereoscan 90 scanning electron microscope fitted with an Oxford Instruments CT1000A cryostage. Images were captured using an Image Slave Software Package. Sample preparation was conducted at -18°C. Cores (3 mm diam.) were removed from the meat samples parallel to the fibre direction and inserted into a hole (3 mm diam.) drilled into the centre of a cryo-microscope stub. The stubs were held in a polystyrene cup over liquid N_2 for up to 2 h before plunging into liquid N_2. The meat sample was freeze fractured perpendicular to the fibre direction, mounted on the cryostage and heated to -60°C until the ice had sublimed. The tissue surface was examined with an accelerating voltage of 2.5 kV and at a constant working distance of 7 mm. At least two samples were examined at each thermocouple site in the strips of meat and from each of the plugs from the cartons of meat. Cores were taken from the longitudinal centre of the plugs.

RESULTS AND DISCUSSION

Cavities created after sublimation were used to estimate the cross-sectional areas of the ice crystals. Samples taken from the refrigerated border of strips of muscle with an internal thermal gradient showed ice crystal cross-sectional area and uniformity similar to that observed in the small samples used in earlier experiments (Ngapo *et al.*, 1997). As the distance from the refrigerated border increased, the average crystal area also increased. Small crystals were present in all samples, but decreased in number as a

consequence of the increased area occupied by the greater number of large crystals. The apparently random location of these large crystals resulted in decreasing uniformity of tissue structure as the distance from the refrigerated border increased.

Image analysis was able to be undertaken on the samples closest to the refrigerated border. However, it became increasingly difficult to fracture the samples without appearing to distort the fibres as the distance of the sample from the border increased resulting in little surface area after fracturing that was perpendicular to the fibre direction and causing inaccuracies in image analysis. It is suggested that this difficulty in fracturing is a result of contrasting adjacent textures within a sample created by the large crystals, causing compaction of muscle tissue. Localised areas of tissue are softer and more malleable than the large adjacent ice crystals. The comparatively uniform samples at the refrigerated border would fracture more evenly and "cleanly".

During freezing of the strips of muscle the entire sample cooled to about -1°C within 2 h. The temperature at the refrigerated border continued to decrease, but at a reduced rate. As the distance from the refrigerated border increased the time the sample locale remained at -1°C increased so that the regions furthest from the border did not appear to begin decreasing below -1°C for approximately 12 h. The rate of decline of temperature below this plateau increased with increasing distance from the refrigerated border. The freezing times from -1°C to -7°C ranged from 17 to 22 h (0.0059 to 0.0045°C/min) from the refrigerated border to the opposite end of the strip, respectively. The temperatures continued to decline to about -16°C.

The existence of a thermal gradient in the strips of meat results as nucleation and crystal growth occur at the periphery of the sample. Nucleation occurs only at the refrigerated border and the crystals formed grow into the sample creating a freezing front. The movement of the freezing front from the border to the centre of the sample results in a thermal gradient. The formed ice crystals grow towards the centre of the system as protuberances accompanied by lateral diffusion of solutes (Bevilacqua *et al.*, 1979). The protuberances do not tend to expand on their sides, producing a columnar structure. These structures grow in the direction of the heat flow and away from the refrigerated border. Only those crystals that have their fastest growth direction nearly parallel to the direction of heat flow will subsist. As a consequence, as they grow in length they increase their cross-sectional area.

After 4 weeks storage of the strips, the cross-sectional areas of the crystals had increased at the refrigerated border and resembled the samples taken furthest from this border prior to storage. At these latter sites, differences in crystal area or uniformity of location were not apparent. Water movement in frozen meat takes place due to recrystallisation during which large crystals grow at the expense of small ones (Devine *et al.*, 1996). This process is driven by the difference in surface energy between small and large crystals; the lower surface energy of large crystals favours growth.

Unlike the freezing of insulated strips, when freezing cartons of meat the heat flow is not unidirectional. It was expected that meat freezing would occur along the shortest axis. However a thermal gradient was observed along the horizontal diagonal axis suggesting that the thermal gradient front moved from the surface to the carton centre uniformly from all directions, as it might into a sphere. This effect would relate to the carton head space and the impingement of air blast on the sides of the carton. In the cartons of meat it was observed that in less than 4 h the entire sample had attained a temperature of approximately -2°C. The area near the carton's periphery decreased in temperature most rapidly; the temperature at sites across the rest of the sample appeared to decrease almost as if one site. The temperature near the surface of the carton continued to decline at the initial rate. As the distance along the longitudinal diagonal axis increased, the time the meat temperature remained at approximately -2°C also increased. However, the longer the time at this temperature, the more rapid the rate of decline to reach the freezer temperature. Times to freeze from -1 to -7°C ranged from 13 to 43 h (0.008 to 0.0023°C/min) from the refrigerated border to the carton centre, respectively.

Layers of fat and collagen in whole muscles might act as barriers to crystal growth and nucleation could occur in the inner regions of a carton where the surface of one muscle acts as a refrigerated border to another. Furthermore, each muscle has fibres in different directions from the next, possibly affecting the rate of penetration of the ice front. Therefore, in cartons large crystals might exist at the muscle surface when frozen packed with other muscles. Commercially, it is common to find 2-3 muscles in a bag, and 2-3 bags in a carton. The bags would act as barriers to crystal growth, as suggested fat and collagen might. Ice crystals might therefore terminate longitudinal growth at the bag and nucleation could occur in an adjacent meat sample. However, nucleation might not occur around the entirety of the bags and is dependent on the location of the bag in the carton.

No differences were observed in the crystal location or areas for samples taken along the internal thermal gradient of the cartons of meat. These cartons were stored frozen for 3 weeks prior to examination by cryo-SEM and recrystallisation during storage would have occurred. Furthermore, difficulties were encountered in the method of preparation for cryo-SEM resulting in inaccuracies in the images. In particular, a corer with a tapered end was used to minimise compression within the plug during sampling. Therefore, the cryo-SEM method and image analysis could not confidently be used on the meat frozen in cartons.

CONCLUSIONS

Using the cryo-SEM technique it was observed that ice crystal cross-sectional area increased along the thermal gradient from refrigerated border to the opposite end of a strip of a single muscle frozen with unidirectional heat flow. After 4 weeks of storage at -16°C, the differences between samples had decreased so that the samples at the refrigerated border resembled those furthest from this border. The ultrastructural similarities between the refrigerated border of strips and small samples used in earlier support suggestions that the small sample is representative of the periphery of a sample with an internal thermal gradient.

REFERENCES

1. Añón, M.C. and Calvelo, A. (1980). *Meat Sci.*, **4**, 1.
2. Bevilacqua, A., Zaritzky, N.E. and Calvelo, A. (1979). *J. Fd. Technol.*, **14**, 237.
3. Devine, C.E., Bell, R.G., Lovatt, S. and Chrystall, B. (1996). In *Freezing Effects on Food Quality.* L.E. Jeremiah (Ed.), Marcel Dekker Inc., New York, USA, p51.
4. Ngapo, T.M., Babare, I.H., Murphy, K.L., Reynolds, J. and Mawson, R.F. (1997). *Proc. Int. Congress Meat Sci.Technol.*, **43**, 674.
5. Payne, S.R., Sandford, D., Harris, A. and Young, O.A. (1994). *Meat Sci.*, **37**, 429.