

Effects of Frozen Storage on the Ultrastructure of Bovine Muscle

R. J. CARROLL, J. R. CAVANAUGH, and F. P. RORER

ABSTRACT

Scanning electron microscopy (SEM) studies were carried out on bovine semitendinosus samples that were subjected both to long-term frozen storage and to repeated freeze-thaw cycles. Samples frozen at -18°C and stored up to 26 wk showed essentially no change in muscle ultrastructure. Samples frozen at liquid nitrogen temperature and stored at $2-3^{\circ}\text{C}$ did show ice crystal damage within the muscle fiber. Repeated freeze-thaw produced essentially no change in muscle ultrastructure.

INTRODUCTION

IT IS GENERALLY THOUGHT that most meat and fish show a gradual deterioration in quality with frozen storage (see review articles by Urbain, 1978; Fennema, 1971; Mills, 1975). For example, after frozen storage, various meats showed increased cooking losses (Jeremiah, 1980; Miller et al., 1980; Neer and Mandigo, 1977; Sebranek et al., 1978), decreased protein extractability (Huber and Stadelman, 1970; Miller et al., 1980; Sebranek et al., 1978), increased rancidity (Jeremiah, 1980; Sebranek et al., 1978), and decreased palatability (Jeremiah, 1980; Neer and Mandigo, 1977; Winger and Fennema, 1976; Sebranek et al., 1978). The lower the holding temperature, the less the deterioration (Urbain, 1978; Huber and Stadelman, 1970). In some other studies, however, frozen storage of meat gave little deterioration (Suter et al., 1976; Baker et al., 1976; Campbell and Mandigo, 1978; Kingsley and Graham, 1978). Part of these inconsistencies may arise from the quality of the original sample, meat already of poorer quality being more subject to further deterioration upon frozen storage (Kemp et al., 1976).

The effects on fish products seem to be more uniform. There is general agreement that frozen fish are subject to lipid oxidation, to increase concentrations of free fatty acids, and to decreased protein extractability (Mills, 1975; Anderson and Ravesi, 1970a, b; King, 1966; Jarenbäck and Liljemark, 1975a, b). The quality of the original product also may play a part here (Nakayama and Yamamoto, 1977), as well as the degree of unsaturation of the fish oil (Mills, 1975). At least two studies have shown negligible effects of frozen storage (Krzynowek and Wiggin, 1979; Gibbson and Worthington, 1977).

Most of the studies to date have focused on changes in physical, chemical, or sensory properties of the frozen items. Very little work has been devoted to microscopic or ultrastructural investigations of these systems. One histological study (Bevilacqua et al., 1979) centered on the formation of ice crystals in frozen meat and was able to relate, qualitatively, ice crystal damage to other deleterious effects of frozen storage.

The first electron microscopy study to appear was an ultrastructural study on frozen cod (Jarenbäck and Liljemark, 1975a, b) by transmission electron microscopy

(TEM) of both intact fish muscle and extracted myofibrils. In the intact muscle, the myofibrillar arrays showed essentially no change with frozen storage, although there was a slight decrease in the interfibrillar spacing. TEM of the extracted myofibrils showed a decrease in the actomyosin filament lengths.

In the present study, we utilized SEM to examine the ultrastructure of frozen bovine semitendinosus muscle. Our objectives were to determine changes that take place both as a function of long-term frozen storage under conditions of fast and slow freezing, as well as a function of repeated freeze-thaw cycles. Results obtained here should shed some light on the general question of the effects of frozen storage on the properties of meat.

MATERIALS & METHODS

BOVINE SEMITENDINOSUS MUSCLES were obtained commercially from local supermarkets. For each experiment, an entire muscle minus the tapered ends, was cut perpendicularly to the longitudinal axis into 18-20 mm thick slices, which were then wrapped in freezer paper.

For long-term storage, samples from the same muscle were either immersed in liquid nitrogen for 3 hr or slow frozen in a stationary air freezer at -18°C . Slow freezing took approximately 4 hr. Both sets were kept at -18°C and removed for SEM observation after 1, 4, 12, and 26 wk. An additional sample was frozen in liquid nitrogen and then promptly thawed in order to observe the immediate effects of freezing at liquid nitrogen temperatures.

For the cyclic freeze-thaw experiments, samples from the same muscle were frozen at -18°C for 24 hr and then thawed for 24 hr either at room temperature or at refrigerator temperature of $2-3^{\circ}\text{C}$. In another set of cyclic freeze-thaw experiments, samples were frozen for 20½ hr and then thawed at room temperature until the internal temperature of the meat reached 4°C , a time of 3½ hr (24 hr total cycle time). In all cases, samples were taken after each of 5 cycles for SEM observation.

Samples for the electron microscope were obtained from freshly cut pieces taken from the central interior portion of the thawed slice of meat and prepared as described previously (Carroll et al., 1978). A JEOL 50-A SEM operating at 15 kV was used in these investigations. Warner-Bratzler shear measurements were obtained on 1.27 cm diameter cores of raw muscle tissue. At least 3 cores were taken per sample with a minimum of 3 shears per core. The shear data were statistically analyzed by Student's t-test.

RESULTS

THE RESULTS for the freeze-thaw experiments are shown in Figures 1 and 2. The cross fracture view of the untreated raw muscle (Fig. 1a) compares favorably with similar micrographs published previously (Carroll et al., 1978). Note the open structure between muscle fibers with distinct endomysium (E). The perimysium network (P) between muscle fiber bundles is clearly delineated. Figure 1b shows a cross fracture view of a sample which was subjected to 5 cycles of 24 hr freeze, 24 hr thaw (at 4°C). Very little change from the control sample is seen except for some compaction of the muscle fibers (arrowhead). Endomysium remains unaffected.

The samples thawed for 24 hr at room temperature in the freeze-thaw experiments present a very different picture. Figure 1c displays a sample after 3 such cycles. Degrada-

Authors Carroll, Cavanaugh, and Rorer are with the USDA, Eastern Regional Research Center, SEA-AR, 600 East Mermaid Lane, Philadelphia, PA 19118.

dation was evident on exterior surfaces in these samples, both by smell and visual observation, although the sections selected for SEM study was taken from the interior portion. Figure 1c shows extensive deterioration of the muscle fiber system. The fracture plane cuts very irregularly across the fiber surface, and the fractured ends show gross pitting and distortion of structure (arrowheads).

The second set of freeze-thaw experiments was designed to minimize deterioration of the sample while thawing. A 24 hr cycle was chosen: 20½ hr freeze, 3½ hr thaw at room temperature. The latter represents the time it took for the center of the slice to reach 4°C. The results are shown in Figure 2:

The untreated control (Fig. 2a) shows structure similar to that of the control in Figure 1. Figure 2b displays in cross section a sample that was kept frozen for the entire 5 day length of the series to serve as a frozen control. This specimen shows good preservation of structure with little apparent change or deterioration from the untreated sample. Similarly, in Figure 2c, a cross section is shown of a sample subjected to 5 freeze-thaw cycles. The muscle structure is well preserved. There is some compaction of the structure in the area shown (arrowheads), but most parts have the open structure indicative of well preserved muscle. The perimysium (P) appears open and normal.

Results of the long term frozen storage are presented in Figures 3 and 4. Figure 3a, b, and c display muscle samples frozen at -18°C and stored for 4, 12, and 26 wk, respectively. In all cases, the structures are well preserved as seen in the cross sectional views of b and c and the oblique view of a. Fractures are clean, fibers are well separated, connective tissue is well maintained in all cases. There is some slight compaction of the fibers in Figure 3c (arrowhead) (sample held for 26 wk), but mostly the fibers are well separated.

Muscle samples frozen at liquid nitrogen temperature are shown in Figure 4. Figure 4a shows the sample thawed immediately after freezing, while Figures 4b, c, and d display samples stored at -18°C for 4, 12, and 26 wk, respectively. The deterioration of the sample is evident even in the one immediately thawed and becomes progressively more apparent with storage time. In cross section (Fig. 4a), extensive pits and crevices (arrows) are seen in the muscle fiber, although the overall structure is still quite open. After 4 wk (Fig. 4b), the deterioration is more evident, with the loss of the open structure, compression of the muscle fibers, and lack of definition of the endomysium. After 12 wk (Fig. 4c), the fibers are closely compacted with deep pitting of the fibers. At 26 wk (Fig. 4d), the individual muscle fibers are no longer clearly defined with extensive

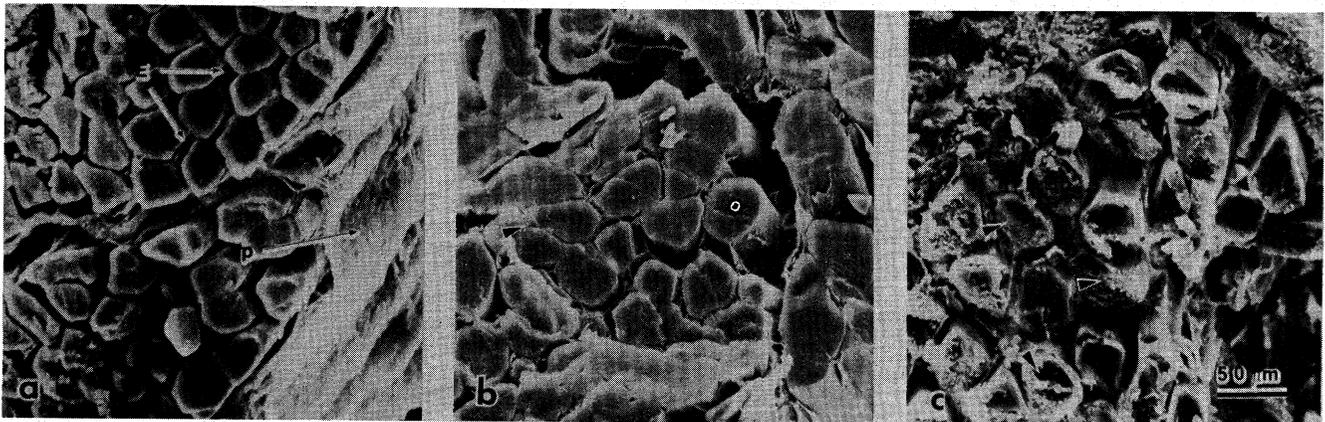


Fig. 1—Cross fracture surface of bovine semitendinosus: (a) Control, unfrozen. Structure is well preserved with well defined endomysium (E) and perimysium (P); (b) After 5 freeze-thaw cycles: 24 hr at -18°C, 24 hr at 3°C. Little change in structure observed. Some compact areas seen (arrowhead); (c) After 3 freeze-thaw cycles: 24 hr at -18°C, 24 hr at room temperature. Extensive deterioration of muscle fibers shown (arrowheads).

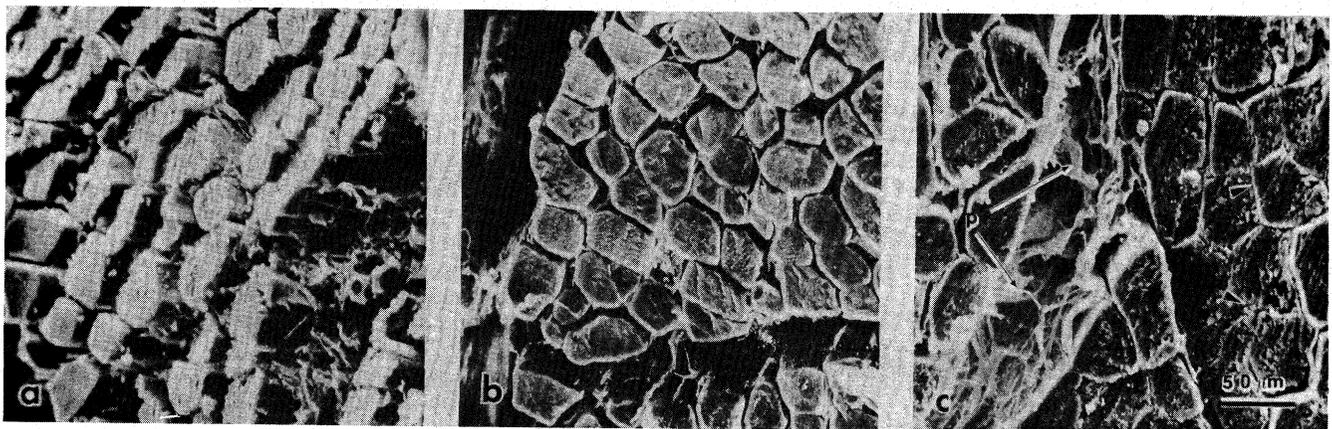


Fig. 2—Cross fracture surface of bovine semitendinosus: (a) Control, unfrozen; (b) Control, frozen for 5 days at -18°C. No changes observed. (c) After 5 freeze-thaw cycles: 20½ hr at -18°C, 3½ hr at room temperature. Structure is well preserved with intact perimysium (P); some compact areas shown (arrowhead).

deterioration and pitting (arrows) of the surface of the cross fracture.

DISCUSSION

FROM OUR EXPERIMENTS on the cyclic freeze-thaw of muscle tissue, we can conclude that little change takes place in the meat ultrastructure as a result of the repeated re-freezing. This is in agreement with an earlier study on refrozen chicken broilers which showed essentially no change in tenderness, juiciness, flavor, acceptability, or color after 5 cycles (Baker et al., 1976). In our work, we saw some slight compaction of the muscle fibers after 5

cycles (Fig. 1b and 2c); this is in accord with the reported increases in total drip volume in the above study.

The one set of our experiments that revealed drastic alterations in the meat ultrastructure was that in which the samples were allowed to thaw for 24 hr at room temperature. After 1 cycle, the meat appeared fresh and no changes in the meat ultrastructure were detected. After the second cycle, even though the exterior portions of the meat slice showed evident deterioration (both by smell and visual observation), the internal portions supplied for SEM observation showed well preserved ultrastructure. However, after the third cycle, the interior portions showed

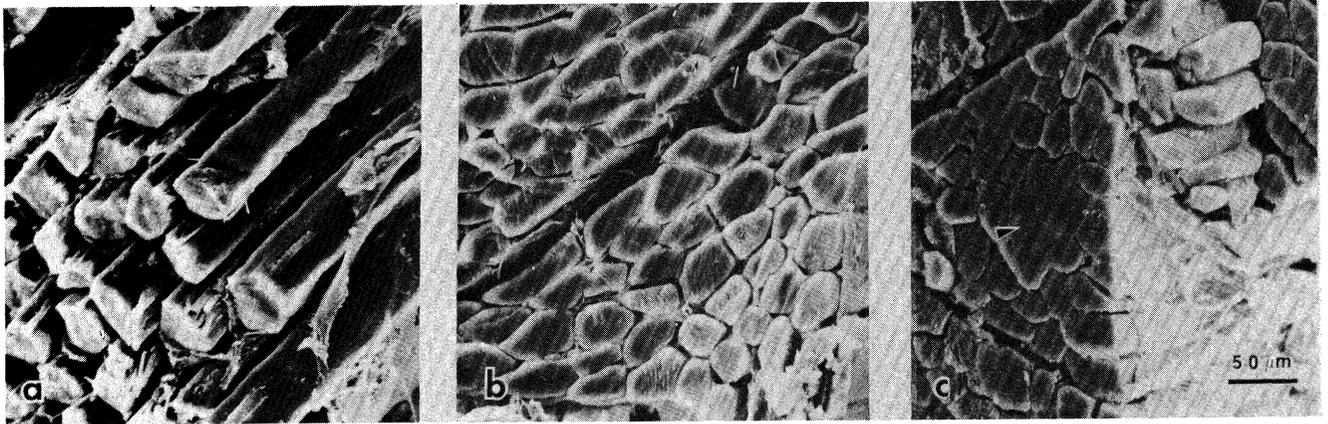


Fig. 3—Cross fracture surface of bovine semitendinosus frozen and stored at -18°C : (a) After 4 wk; (b) After 12 wk; (c) After 26 wk. Very little change in structure is observed with some compactness (arrowhead) after 26 wk.

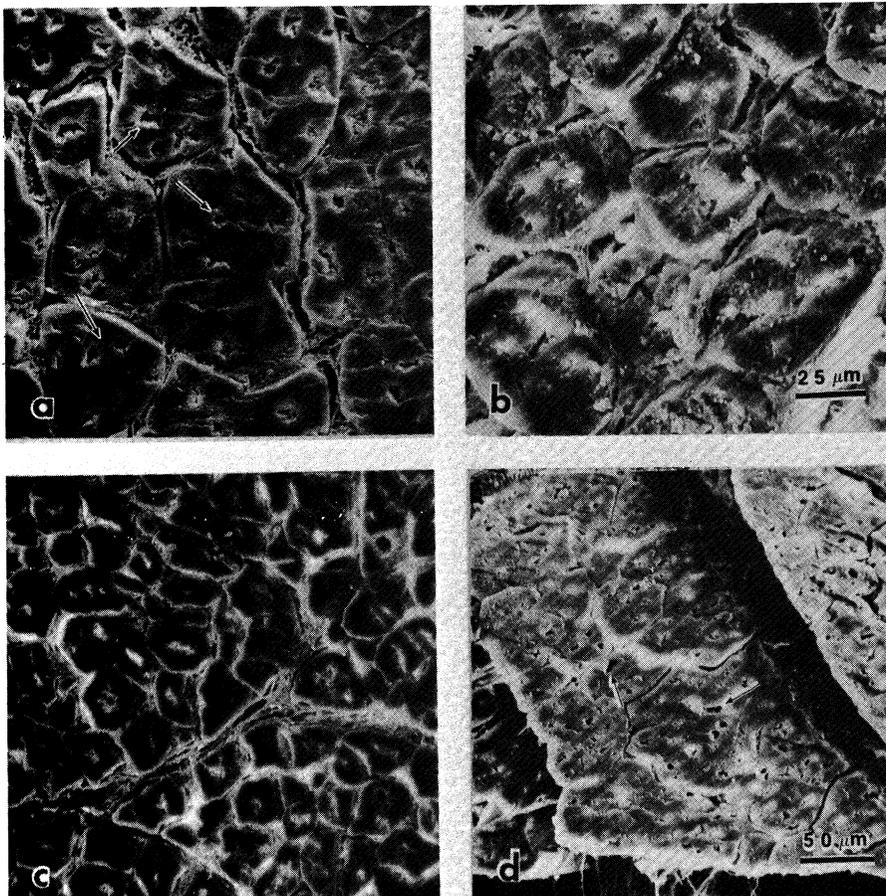


Fig. 4—Cross fracture surface of bovine semitendinosus frozen in liquid nitrogen and stored at -18°C : (a) Immediately thawed. Fibers appear distorted with many pits and crevices (arrows); (b) After 4 wk. More deterioration is evident with loss of open structure; (c) After 12 wk. Extensive deterioration with compression of muscle fibers; (d) After 26 wk. Extreme compactness with deep pitting of fibers is seen (arrows).

age of fish: A review. *J. Food Technol.* 10: 483.
Nakayama, T. and Yamamoto, M. 1977. Physical chemical and sensory evaluations of frozen-stored deboned (minced) fish flesh. *J. Food Sci.* 42: 900.
Neer, K.L. and Mandigo, R.W. 1977. Effects of salt, sodium triphosphate and frozen storage time on properties of a flaked, cured pork product. *J. Food Sci.* 42: 738.
Sebranek, J.G., Sang, P.N., Ruct, R.E., Topel, D.G., and Kraft, A.A. 1978. Influence of liquid nitrogen, liquid carbon dioxide and mechanical freezing on sensory properties of ground beef patties. *J. Food Sci.* 43: 842.
Suter, D.A., Marshall, W.H., Dutson, T.R., and Carpenter, Z.L. 1976. Effect of freezing on the mechanical properties of lamb loin chops. *J. Food Sci.* 41: 1455.

Urban, W.M. 1978. Meat preservation. In "The Science of Meat and Meat Products," Ed. J.F. Price and B.S. Schweigert, 2nd ed. Food & Nutrition Press, Inc., Westport, Conn.
Winger, R.J. and Fennema, O. 1976. Tenderness and water holding properties of beef muscle as influenced by freezing and subsequent storage at -3 or 15 °C. *J. Food Sci.* 41: 1433.
Ms received 8/30/80; revised 2/5/81; accepted 2/18/81.

The authors thank Mrs. Ruth Zabarsky for excellent technical assistance during the course of this research.

The mention of commercial items is for convenience and does not constitute an endorsement by the USDA over other items of a similar nature not mentioned.

large-scale degradation of the ultrastructure (Fig. 1c), even though the color of these interior samples appeared normal.

The critical aspect of the preservation of structure in the repeated refreezing of meat seems to be in the time/temperature of the thaw cycle. When thawed at 2°C for 24 hr or room temperature for 3½ hr, the meat ultrastructure showed no signs of deterioration. Only when the meat samples were subjected to the 24 hr thaw at room temperature did the meat ultrastructure show extensive degradation after repeated freeze-thaw.

The long-term storage of meat frozen in a conventionally slow manner also does not appear to affect the meat ultrastructure, even after 26 wk of storage. Some slight compaction of structure occurred with the longer times, and this would be consistent with the known increase in drip loss (or decrease in cooking yield) with long term storage (Jeremiah, 1980; Miller et al., 1980; Neer and Mandigo, 1977; Sebranek et al., 1978). The preservation of structure is in agreement with a study of restructured pork patties in which no differences in shear-force values, percent cooking loss, protein, moisture, fat or ash content were found as a result of frozen storage (Campbell and Mandigo, 1978). Similarly, most of the mechanical properties of lamb loin chops changed either minimally (10–15%) or not significantly with frozen storage (Suter et al., 1976).

On the other hand, these results conflict with much of the literature on the effects of frozen storage. However, the original stage of preservation of the meat material appears to influence greatly the result obtained. Ground meats, for example, have a greater chance for bacterial contamination and often yield the poorest results on long-term frozen storage (Miller et al., 1980; Sebranek et al., 1978). A flaked cured pork product gave lower cooking yields, increased shear values, color fade, and increased rancidity with frozen storage (Neer and Mandigo, 1977), but all samples initially rated acceptable were still acceptable after long term storage. Even in this case, the quality degradation was not sufficient to make the products unacceptable.

Our results with the liquid nitrogen frozen samples present an interesting contrast to our results on samples frozen at -18°C. The cryogenically cooled samples showed wide-scale imperfections in the ultrastructure, the deterioration increasing with storage time. The imperfections seen here are apparently due to the formation of very small ice crystals which disrupt the central portions of the muscle fibers. Slow freezing, on the other hand, seems to leave the fiber ultrastructure intact. This result is particularly curious since slow freezing promotes the formation of large ice crystals (Bevilacqua et al., 1979) which would be thought to create more structural damage than small ones.

At the same time, shear measurements taken on these systems reveal no significant differences between the two freezing treatments except after 1 wk of storage. These results are shown in Table 1. With either treatment, there is

Table 1—Mean values of Warner-Bratzler test measurements on bovine semitendinosus samples held in frozen storage^a

Temp of freezing	Shear values ^b			
	Weeks in storage			
	1	4	12	26
-18°C	17.2 ^c (6.7)	13.3 (3.8)	14.0 (4.3)	15.6 (6.4)
Liquid nitrogen	12.5 ^c (1.9)	15.8 (3.3)	14.8 (2.5)	16.2 (2.2)

^a At -18°C

^b In pounds. Mean value for control sample was 13.6 lb. Figures in parentheses are standard deviations.

^c Significantly different ($p < 0.05$).

no significant change in the shear measurements due to the length of storage.

Therefore, the ultrastructural changes taking place do not affect at least this one macroscopic property of the meat system, namely, the shear measurements. Moreover, several studies suggest that other properties may not be affected as well. In particular, a study of ground beef patties shows less deterioration of quality with the liquid nitrogen frozen samples than with conventionally frozen samples (Sebranek et al., 1978). Similarly, poultry broilers froze at liquid nitrogen temperatures yielded a better quality product and a longer shelf life than immersion chilled carcasses (Arafa and Chen, 1978).

CONCLUSION

OUR STUDIES of bovine semitendinosus muscle show that under the proper conditions, essentially no change takes place in the meat ultrastructure either under long-term storage or repeated freeze-thaw conditions. There is some question on the optimum temperature for the initial freezing; cryogenic freezing gives better preservation of quality according to literature studies but yields ice crystal damage within the muscle fiber according to our work. The latter may not affect the desirable properties of the meat system and consequently may be quite acceptable.

It is likely that more and more of the meat consumed by the public will be coming from frozen sources. These studies add continued weight to the evidence that frozen methods can provide safe and wholesome means of meat delivery.

REFERENCES

- Anderson, M.L. and Ravesi, E.M. 1970a. On the nature of altered protein in cod muscle stored at -29°C after aging in ice. *J. Food Sci.* 35: 199.
- Anderson, M.L. and Ravesi, E.M. 1970b. On the nature of the association of protein in frozen-stored cod muscle. *J. Food Sci.* 35: 551.
- Arafa, A.S. and Chen, T.C. 1978. Liquid N₂ as an alternative means of chilling poultry. *J. Food Sci.* 43: 1036.
- Baker, R.C., Darfler, J.M., Mulnix, E.J., and Nath, K.R. 1976. Palatability and other characteristics of repeatedly refrozen chicken broilers. *J. Food Sci.* 41: 443.
- Bevilacqua, A., Zaritzky, N.E., and Calvelo, A. 1979. Histological measurements of ice in frozen beef. *J. Food Technol.* 14: 237.
- Campbell, J.F. and Mandigo, R.W. 1978. Properties of restructured pork patties as affected by cooking method, frozen storage, and reheating method. *J. Food Sci.* 43: 1648.
- Carroll, R.J., Rorer, F.P., Jones, S.B., and Cavanaugh, J.R. 1978. Effect of tensile stress on the ultrastructure of bovine muscle. *J. Food Sci.* 43: 1181.
- Fennema, O.R. 1971. Rates of chemical deterioration in frozen foods. *Proc. of the Meat Ind. Res. Conference*, p. 35.
- Gibson, T.A. and Worthington, R.E. 1977. Lipid changes in frozen stored channel catfish grown by tank culture: effects of dietary fat, freezing method, and storage temperature. *J. Food Sci.* 42: 355.
- Huber, C.S. and Stadelman, W.J. 1970. Effect of freezing rate and freeze drying on the soluble proteins of muscles. 1. Chicken muscle. *J. Food Sci.* 35: 229.
- Jarenbäck, L. and Liljemark, A. 1975a. Ultrastructural changes during frozen storage of cod (*Gadus morhua* L.). 1. Structure of myofibrils as revealed by freeze etching preparations. *J. Food Technol.* 10: 229.
- Jarenbäck, L. and Liljemark, A. 1975b. Ultrastructural changes during frozen storage of cod (*Gadus morhua* L.). 2. Structure of extracted myofibrillar proteins and myofibril residues. *J. Food Technol.* 10: 309.
- Jeremiah, L.E. 1980. Effect of frozen storage and protective wrap upon the cooking losses, palatability, and rancidity of fresh and cured pork cuts. *J. Food Sci.* 45: 187.
- Kemp, J.D., Montgomery, R.E., and Fox, J.D. 1976. Chemical, palatability, and cooking characteristics of normal and low quality pork loins as affected by freezer storage. *J. Food Sci.* 41: 1.
- King, F.J. 1966. Ultracentrifugal analysis of changes in the composition of myofibrillar protein extracts obtained from fresh and frozen cod muscle. *J. Food Sci.* 31: 649.
- Kingsley, G.R. and Graham, P.P. 1978. Effects of frozen storage and dry-curing on ham triglyceride fatty acids. *J. Food Sci.* 43: 479.
- Krzynowek, J. and Wiggin, K. 1979. Seasonal variation and frozen storage stability of blue mussels. *J. Food Sci.* 44: 1644.
- Miller, A.J., Ackerman, S.A., and Palumbo, S.A. 1980. Effects of frozen storage on functionality of meat for processing. *J. Food Sci.* 45(6): 1466.
- Mills, A. 1975. Measuring changes that occur during frozen stor-

—Continued on page 1102