Textural and Histological Changes of Different Squid Mantle Muscle during Frozen Storage

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Mantles from three species of squids, *Loligo edulis*, *Sepia pharaonis*, and *Illex argentinus*, were frozen and stored at −20 °C for 0, 0.5, 1, 2, and 4 months, and their textural properties were investigated. The toughness of all squid mantles increased during frozen storage, and there was a significant difference between prefrozen and postfrozen storage. There was no significant difference in drip amount and SDS gel electrophoresis patterns after 4 months of frozen storage. Histology observation of mantle showed that the muscle fibers were injured and aggregated while the frozen time increased. These changes in tissue structure during frozen storage might cause the toughening of mantle.

Keywords: *Squid; frozen storage; freezing drip; electrophoresis; histological properties*

INTRODUCTION

Cephalopod, such as cuttle fish, squid is one of the most highly potential sources of fish protein. With its quantity of resources being estimated at 4 million tons (Tung, 1975) but only a total of 2.3 million tons of catch being occupied around the world currently (Li, 1990), a favorable area of exploration is still retained for the domestic squid industry. In Taiwan, squid fishing vessels mostly belong to pelagic vessels weighing 1000 tons and over, with Pacific squid (*Ommastrephes bartrami*) and Argentina squid (*Illex argentinus*) as the major squid fisheries which are being frozen and delivered back to this island for utilization. The dried products, such as dried shredded squid and dried squid slices, are the major commercial products in the area. As a result of saturation in the domestic market on dried squid products (Hwang, 1986), frozen foods and surimi-based products have gradually become a trend of development for processed foods. These frozen marine materials, therefore, need to be treated with a refrozen and a restored treatment after being thawed and processed.

The mantle muscle of squid is circumferential muscle mainly (Otwell and Hamann, 1979), with a specific toughness that is quite different from that of general fishes, and even an excellent toughness is maintained after a second freezing and thawing process. With regard to the frozen resistant nature of the squid, Stanley and Hultin (1982) found that frozen northern Atlantic squids, *Loligo pealei* and *Illex illecebrosus*, were significantly tougher than their fresh materials. Stanley and Smith (1984) also reported the freezing produced a tendency for squid muscle fibers to lose their outer membranes. Ho et al. (1991) had discovered a slight decrease on the extraction of myofibrillar protein and no nearly difference in the Ca-ATPase activity of myofibril, when the whole squid of Pacific and Argentina squids (*I. argentinus*) was stored at −20 °C for a period of 4−6 months. However the gel forming ability of the surimi-based product made from Pacific squid decreased gradually during storage, while that of Argentina squid showed no changes but had poor gel strength. There is no further literature for reference with regard to changes of the toughness and texture of the squid mantle under frozen conditions.

This research uses frozen Argentina squid (*I. argentinus*) as a raw material and compares it with unfrozen fresh neritic squid (*Loligo edulis*) and cuttle fish (*Sepia pharaonis*) to conduct an analysis of changes for muscle toughness, tissue structure, free drip, expressible drip, and protein profile of drip during frozen storage and thawing, respectively, so as to provide information for the freezing process in the future.

MATERIALS AND METHODS

Materials. Squid, *L. edulis* and *S. pharaonis*, was ice-stored for 1−2 days after harvest (purchased at the market of Tung-Kang). *I. argentinus* was frozen-stored for 40−45 days on a board before purchase. Frozen squids were thawed in running tap water and then excised (head, fin, and viscera removed). The mantles were skinned and cut into steaks about 3 × 10 cm as shown in Figure 1. All mantles thus obtained were frozen at −60 °C for 24 h using a kelvinator (series 500, Commercial Products, Inc., Manitowoc, WI) and subsequently stored at −20 °C for 4 months using a freezer (Sanyo Co., Taiwan). Samples were evaluated at each storage period (stored at −20 °C for 0, 0.5, 1, 2, and 4 months) and before frozen storage. All samples were thawed in running tap water and provided the measurements of toughness, free drip, expressible drip, and change of electrophoresis pattern of drip of the mantle muscle. Textural structures of unfrozen and frozen storage mantles were also observed with a phase contrast microscope.

Toughness. Squid mantles were skinned by hand and included inner visceral side and were cut into pieces of 1-cm square samples. Toughness of each piece was measured

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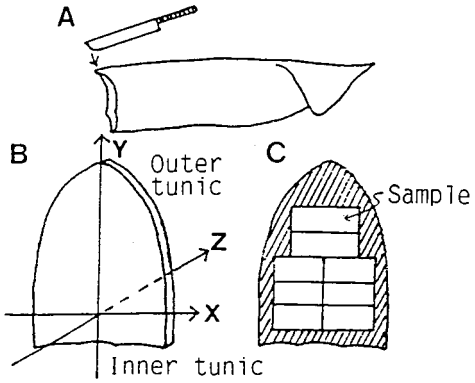

Figure 1. Sampling diagram for excising the mantle muscle from squid: (A) mantle dissected along the base of fin; (B) three dimensions of mantle used in this study; (C) illustration of a sample (about 3 × 10 cm) cut from the mantle.

according to the modified method of Kolodziejska et al. (1987), using a Rheometer (Sun Scientific Co. Ltd., CR-200D) equipped with a stainless steel plunger with a knifelike blade. The edge of the blade of the plunger which was perpendicular to the circumferential muscle fiber direction cut into muscle with its visceral side up. As the mantle surface was compressed and cut, the maximum stress was recorded and expressed as gram. More than six samples were determined in each group. Data were expressed as the mean value with standard deviation.

Histological Properties. Samples of about 0.5 × 0.5 × 0.5 cm were cut from unthawed mantle directly, and the three dimensions labeled as shown in Figure 1. Formaldehyde buffer (pH 7.0) was used for primary fixation for 24 h. The fixed samples were gradient dehydrated, dealcoholized, and embedded in paraffin. Samples were cut into 6-μm thick slices. Photomicrographs were taken using an Olympus BH-2 phase contrast microscope.

Free Drip. The free drip of squid mantles was determined by weighing the samples prior to (A1) and after (A2) frozen storage. Before weighing, the frozen mantles were thawed completely, and then immediately the surface water was removed by filter paper. Each sample had three determinations. The free drip was calculated as follows:

$$\text{free drip } (H_2O \text{ g}/100 \text{ g of meat}) = \frac{A1 - A2}{A1} \times 100\%$$

Expressible Drip. Expressible drip of squid mantles was measured according to Jauregui et al. (1981). Unthawed mantles were cut into 1-mm thick slices (about 0.5−1 g), wrapped in a Toyo 4A filter paper, and then centrifuged at 4500*g*, 4 °C, for 15 min using a Hitachi CR20B2 centrifuge. The percentage of weight loss per gram of sample was calculated. Each sample had 3−6 determinations.

SDS−Polyacrylamide Gel Electrophoresis (SDS−PAGE). To analyze the changes of proteins in expressible drip, the mantle muscle was thawed and centrifuged for 15 min at 4500*g*, and the drip was provided to SDS−polyacrylamide gel electrophoresis according to the method of Laemmli and Favre (1973). Sample was prepared by the method of Horie et al. (1975), dissolved with sample buffer (20 mM phosphate buffer containing 8 M urea, 1% SDS, and 1% mercaptoethanol, pH 6.8), and electrophoresed on 10% polyacrylamide gel to obtain the protein fraction pattern. The protein markers included phosphorylase *b* (94 kDa), bovine albumin (64 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa), and α-lactalbumin (14.4 kDa) (Pharmacia Chemical Co.).

Statistical Analysis. All data were analyzed for statistical significance by using analysis of variance (SPSS/PC program). Multiple comparisons among means were made with Duncan's new multiple range test (Puri and Mullen, 1980). Statistical significance was measured at the level of 5%.

Table 1. Effect of Frozen Storage on the Toughness of *I. argentinus*, *L. edulis*, and *S. pharaonis*[a]

time of storage at −20 °C (months)	*I. argentinus* (g)	*L. edulis* (g)	*S. pharaonis* (g)
control*	424 ± 63[a]	343 ± 51[a]	712 ± 55[a]
0**	558 ± 68[b]	756 ± 73[b]	993 ± 139[b]
0.5	587 ± 66[b]	709 ± 41[b]	1036 ± 53[b]
1	695 ± 56[c]	818 ± 86[c]	998 ± 61[b]
2	1149 ± 91[d]	1148 ± 82[d]	960 ± 143[b]
4	1778 ± 66[e]	1372 ± 61[e]	1313 ± 37[c]

[a] Data are means ± SD. Figures in the same column having the same superscript are not significantly different ($p > 0.05$). *Fresh *L. edulis* and *S. pharaonis*, stored in ice for 1 day, were used as materials, while *I. argentinus* frozen on a boat for about 40 days was used. **L. *edulis* and *S. pharaonis* were frozen quickly in a freezer at −60 °C for 24 h. *I. argentinus* was thawed in running tap water after purchasing and was subsequently frozen at −60 °C as described above.

RESULTS AND DISCUSSION

Toughness of Raw Mantle. The mantle toughness of Argentina squid (*I. argentinus*), neritic squid (*L. edulis*), and cuttle fish (*S. pharaonis*) before being frozen was 424, 343, and 712 g, respectively (Table 1). However, after the mantle was thawed immediately after having been frozen at −60 °C for 24 h, toughness increased in all cases ($p < 0.05$). When the Argentina squid and neritic squid had been frozen at −20 °C for 0.5, 1, 2, and 4 months separately, it showed that the longer the squids were frozen, the tougher their mantle was, and those values were significantly different from mantle before frozen storage ($p < 0.05$). After the cuttle fish had been frozen at −20 °C for 4 months, the mantle toughness was larger significantly than the others at different times of frozen storage and that of fresh mantle ($p < 0.05$). As shown in Table 1, the standard deviation of cuttle fish mantle toughness was slightly larger than the standard deviation of Argentina squid and neritic squid mantle toughness in the control group, which was probably because the cuttle fish mantle was thicker. The report of Stanley and Hultin (1982, 1984a,b) pointed out that squid would become slightly tougher in the frozen storage test. Our results of three species of cephalopods were similar to their findings.

Free Drip and Expressible Drip after Thawing. The increasing toughness of frozen thawed mantle may be due in part to the loss of water holding capacity of muscle protein during frozen storage. However, the total drip of three species of cephalopods was almost maintained constant during frozen storage at −20 °C for 4 months (Figure 2). Among these samples, neritic squid came out with the most drip, with 50−55 g of H_2O/100 g of meat, while Argentina squid, with about 45 g of H_2O/100 g of meat, came next, and with 25−45 g of H_2O/100 g of meat, the cuttle fish came last with the least drip. The free drip amount of all samples was in the range of 2−8 g of H_2O/100 g of meat, while drip wept out by pressure was apparently higher than the free drip. These results suggested the water holding capacity of squid mantles was poor and the thiner the mantle thickness, the higher the drip wept. Stanley and Hultin (1982) also found that weight losses of *Loligo* squid mantle over 50% were common, in either fresh or frozen samples, and were higher than that of *Illex* squid after cooking.

The drip wept out from the mantle muscle being frozen and thawed contained water soluble components, which mainly came from the sarcoplasmic protein

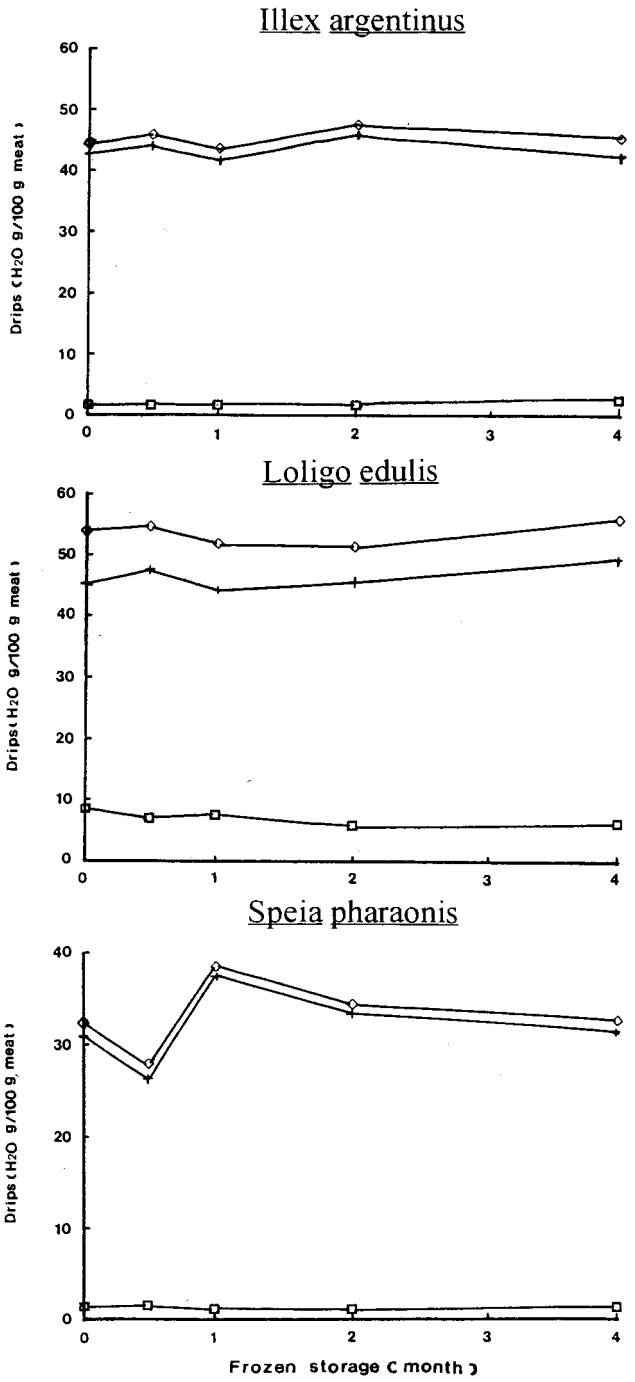

Figure 2. Changes in the free drip (□), expressible drip (+), and total drip (◇) contents of the mantle muscle from *I. argentinus, L. edulis, and S. pharaonis* during frozen storage at −20 °C; 0, sample was frozen at −60 °C for 24 h without storage.

existing in the sarcoplasmic core of the muscle fiber. As shown in Figure 3, the electrophoresis pattern showed the bands of proteins whose molecular weights were mostly from 20 to 100 kDa as well as the band of the sarcoplasmic protein. The report of Hultin et al. (1995) pointed out that myofibrillar proteins were soluble in low ionic strength and could even be soluble in water, and actin, paramyosin, and myosin may be soluble in part. Some of these bands could be these proteins. However, there were some proteins above 100 kDa existing in the drip. These high molecular weight proteins might come from myofibrillar protein. After

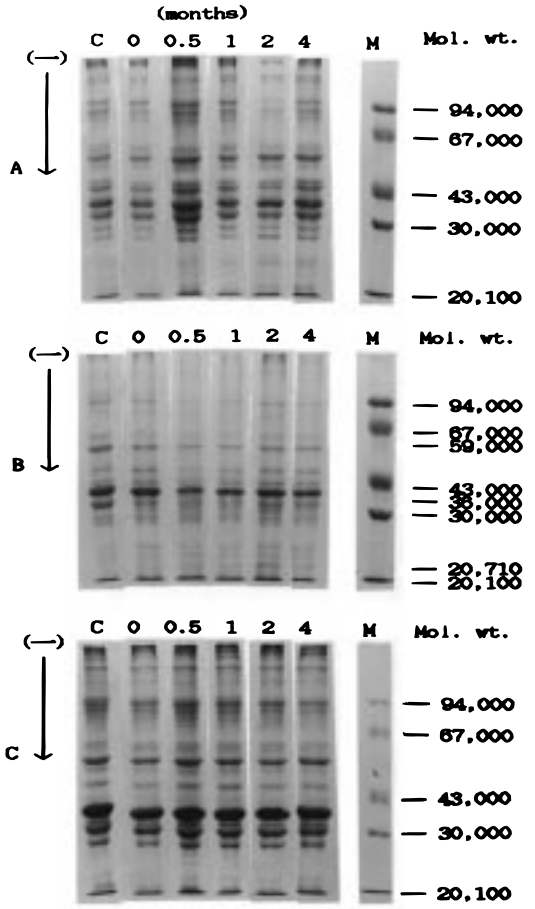

Figure 3. SDS−PAGE patterns of expressible drip in 10% gel from *I. argentinus* (A), *L. edulis* (B), and *S. pharaonis* (C) mantle muscle during frozen storage at −20 °C for 4 months. M represents the protein markers of phosphorylase *b* (94 kDa), bovine albumin (64 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa), and α-lactalbumin (14.4 kDa). C represents the control (see Table 1).

being frozen at −60 °C for 1 day and thawed, sarcoplasmic and myofibrillar proteins flowed out along with expressible drip from the mantle muscle. These phenomena indicated that the tissue of muscle fiber had probably been damaged by freezing and thawing.

Though the Argentina squid (*I. argentinus*) mantle was refrozen and thawed in this experiment, the protein patterns in the drip were similar to those from cuttle fish. After Argentina squid and cuttle fish had been frozen at −60 °C for 4 months, the SDS−PAGE patterns of their drip of mantle muscle showed no obvious changes. The muscle protein component of neritic squid at molecular weights of 36 kDa decreased with increasing frozen time and produced a few new proteins consisting of 20.7 kDa. The phenomenon of slight change could be possible due to aggregation of protein or reaction of enzyme. A report once pointed out that neritic squid possessed alkaline proteinase with stronger activity. Although the optimal temperature was 60 °C and the optimal pH was 7.6 (Cheng et al., 1979; Lanier et al., 1981; Rodger et al., 1984), there was still enzyme activity, though very little, under the frozen temperature (−20 °C) which might cause the protein to decompose gradually. Nevertheless, the changes were extremely slow.

Tissue of Frozen Thawed Mantle Muscle. Histology observation of mantle muscle is shown in Figures

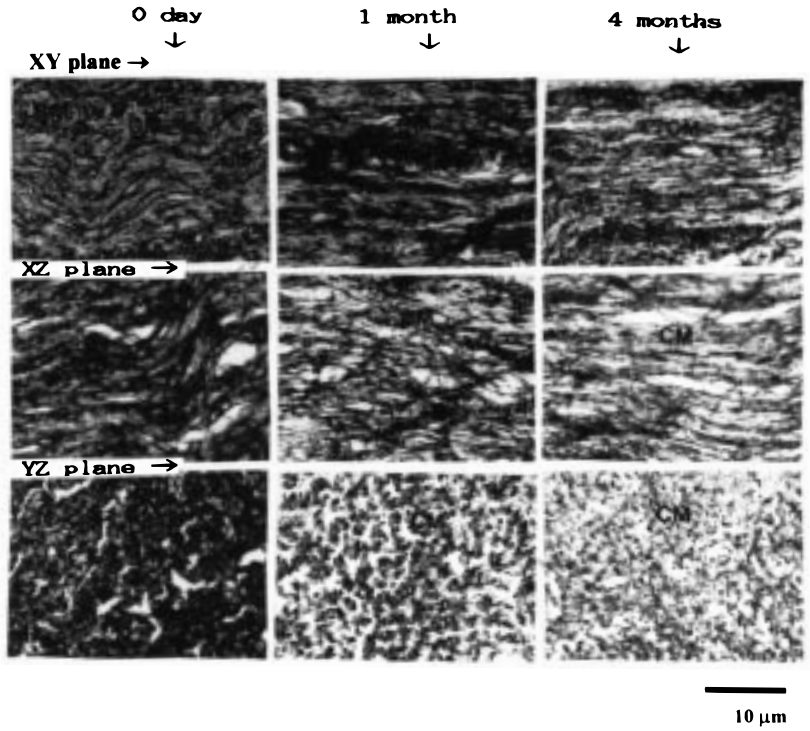

Figure 4. Phase contrast micrographs observed of mantle muscle fibers from *I. argentinus* during frozen storage at −20 °C. The bar represents 10 μm; CM, circumferential muscle; RM, radial muscle.

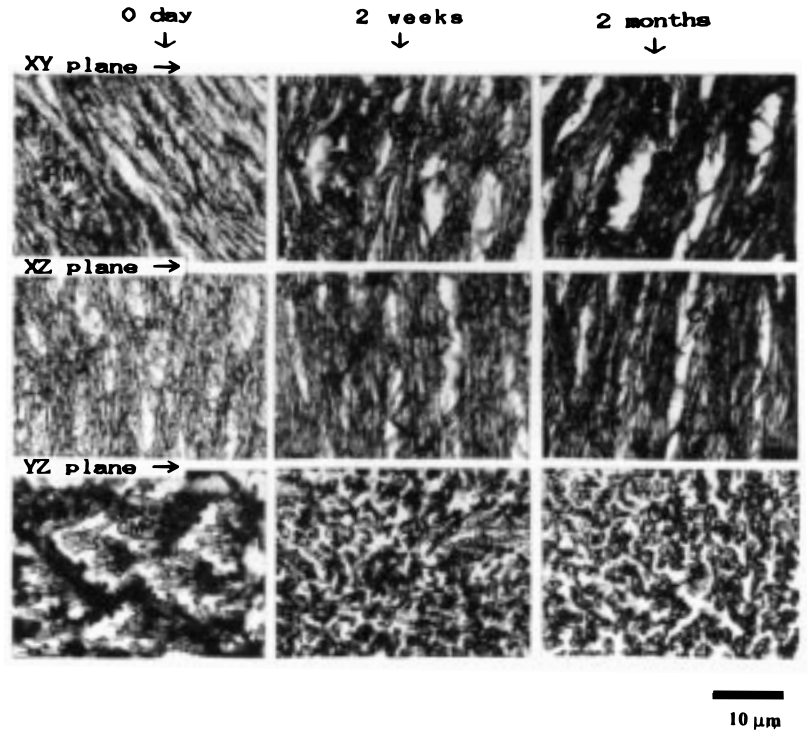

Figure 5. Phase contrast micrographs observed of mantle muscle fibers from *L. edulis* during frozen storage at −20 °C. The bar represents 10 μm; CM, circumferential muscle; RM, radial muscle.

4−6. As Argentina squid had been frozen and stored on the fishing vessel for approximately 6 weeks before sampling without fresh material available for comparison, this frozen material was, therefore, refrozen at −60 °C after being thawed and frozen at −20 °C for 4 months. Muscle fibers became crowed gradually after being refrozen for 2 months (Figure 4) and parts of muscle fibers were broken. The YZ phase also showed that the shape of muscle fiber fasciculus became smaller. It indicated the mantle muscle was injured by freezing and storing. The XY, XZ, YZ phases of the fresh mantle muscle fiber of neritic squid were greatly different from those of Argentina squid; especially, the YZ phase of muscle fiber fasciculus was cross-linking closely (Figure 5). However, after going through different storage times, the muscle fiber fasciculus became smaller, while XY and XZ phases became rougher and condensed as the frozen time became longer. Therefore, the tough-

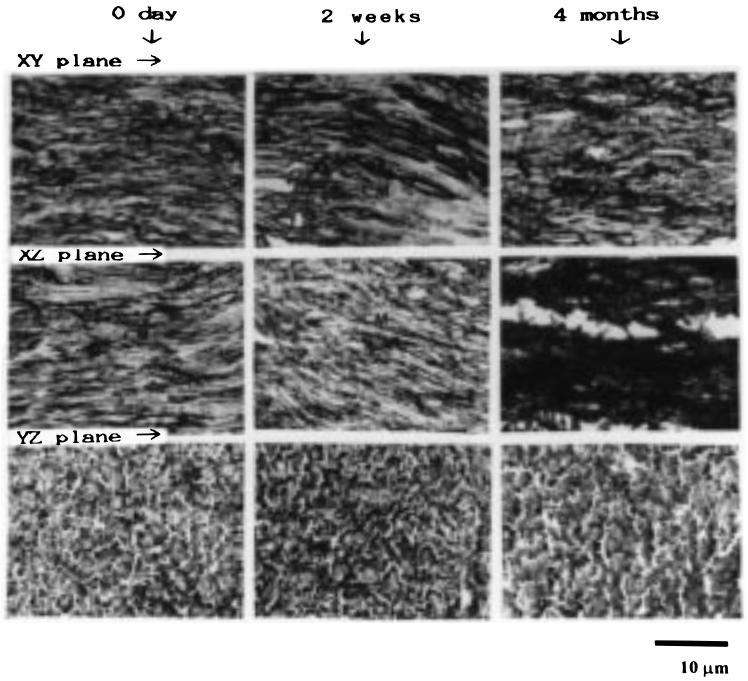

Figure 6. Phase contrast micrographs observed of mantle muscle fibers from *S. pharaonis* during frozen storage at −20 °C. The bar represents 10 μm; CM, circumferential muscle.

ness of mantle muscle of Argentina and neritic squid in Table 1 increased as the frozen time became longer, which was probably due to the changes of the shape of muscle fiber fasciculus. The tissue of cuttle fish muscle fiber stored for 0.5 month showed no obvious changes when compared with the sample frozen at −60 °C for 24 h and then thawed (Figure 6). However, after the tissue had been frozen for 4 months, the muscle fiber aggregation of XZ phase became more compact. The results in Table 1 show that the value of cuttle fish frozen at −20 °C for 4 months was larger than the values at the other frozen time periods, which was probably due to the above-mentioned facts.

The moisture content of cephalopods was 76.2−80%, crude protein content was 18.5−21.6% (Chu et al., 1992), and the myofibril protein ratio was only 52−60%. Once those squids were frozen, the formation of ice crystals would injure the muscle fiber and affect the water holding capacity of muscle protein. This might be the reason why the sample frozen at −60 °C for 24 h and then immediately thawed wept drip as well as those frozen and then stored. Stanley and Hultin (1982) reported that the toughening of frozen squid mantle might be caused by protein cross-linking because of the existence of high levels of DMA and formaldehyde. Stanley and Smith (1984) observed that outer membranes of squid mantle muscle fiber were lost by freezing. Ho et al. (1991) also found that the extraction ratio of mantle myofibrillar protein and the gel forming ability of surimi-based products decreased gradually with time of frozen storage. In this study, muscle fiber damage and aggregation during frozen storage were also observed. Therefore, the frozen mantles were tougher than the unfrozen ones, and the longer-period frozen-stored mantles were tougher than the shorter-period frozen-stored ones. The formation and growth of ice crystals might have injured the muscle fiber and enhanced the protein aggregation that caused toughening of the mantle. Further research is needed to elucidate these details.

LITERATURE CITED

Cheng, C. S.; Hamann, D. D.; Webb, N. D. Effect of the thermal processing on minced fish gel texture. *J. Food Sci.* **1979**, *44*, 1080.

Chu, Y. J.; Ueng, Y. E.; Chow, C. J. Comparative study on the characteristics of Cephalopod mantle muscle for surimi-based products processing. *J. Fish Soc. Taiwan* **1992**, *19*, 75−83.

Ho, T. P.; Chow, C. J.; Chu, Y. J. Comparison between the mantle muscle toughness of *Ommastrephes bartrami* and *Illex argentinus* after frozen storage. A thesis collection of the 6th R.O.C. Technology & Vocation Education Seminar, 1991; pp 40087−40093.

Horie, K.; Tsuchiya, T.; Matsumoto, J. J. Studies on ATPase activity of actomyosin of squid mantle muscle. *Bull. Jpn. Soc. Sci. Fish.* **1975**, *41*, 1039−1045.

Hultin, H. O.; Feng, Y.; Stanley, D. W. A reexamination of muscle protein solubility. *J. Muscle Foods* **1995**, *6*, 91−107.

Hwang, Y. S. The status quo and future prospects of squids industry in R. O. C. Special issue on the seminar of squids & squids industry development; Taipei, Fishery Bio. Laboratory of National Taiwan University, 1986; pp 39−41.

Jauregui, C. A.; Regenstein, J. M.; Baker, R. C. A simple centrifugal method for measuring expressible moisture, a water-binding property of muscle foods. *J. Food Sci.* **1981**, *46*, 1271−1273.

Kolodziejska, I.; Sikorski, Z. E.; Sadowska, M. Texture of cooked mantle of squid *Illex argentinus* as influenced by specimen characteristics and treatments. *J. Food Sci.* **1987**, *52*, 932−935.

Laemmli, U. K.; Favre, M. Maturation of the head of bacteriophage T4, I. DNA packaging events. *J. Mol. Biol.* **1973**, *80*, 575−599.

Lanier, T. C.; Lin, T. S.; Hamann, D. D.; Thomas, F. B. Effects of alkaline protease in minced fish on texture of heat-processed gels. *J. Food Sci.* **1981**, *46*, 1643.

Li, Y. M. Important records of the 1st international meeting of invertebrates (abstract tranlated). *China Marine Prod.* **1990**, *450*, 63−65.

Otwell, W. S.; Hamann, D. D. Textural Characterization of squid (*Loligo pealei* Lesuer): scanning electron microscopy of cooked mantle. *J. Food Sci.* **1979**, *44*, 1629−1635.

Puri, S. C.; Mullen, K. Multiple comparisons. *Applied Statistics for Food and Agricultural Scientists*; G. K. Hall Medical Publishers: Boston, MA, 1980; pp 146−162.

Rodger, G.; Weddle, R. B.; Craig, P.; Hastings, R. Effect of alkaline protease activity on some properties of comminuted squid. *J. Food Sci.* **1984**, *49*, 117−119, 123.

Stanley, D. W.; Hultin, H. O. Quality facters in cooked north atlantic squid. *Can. Inst. Food Sci. Technol. J.* **1982**, *15*, 277−282.

Stanley, D. W.; Hultin, H. O. Amine and formaldehyde production in North American squid and their relation to quality. *Can. Inst. Food Sci. Technol. J.* **1984a**, *17*, 157−162.

Stanley, D. W.; Hultin, H. O. Proteolytic activity in North American squid and its relation to quality. *Can. Inst. Food Sci. Technol. J.* **1984b**, *17*, 163−167.

Stanley, D. W.; Smith, A. K. Microstructure of squid muscle and its influence on texture. *Can. Inst. Food Sci. Technol. J.* **1984**, *17*, 209−213.

Tung, Y. S. Squids and squids resourse development. *Special J. Agric. Renaissance Assoc.* **1975**, 21.

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