Changes in Ice-crystal Formations in Shrimps (*Penaeus japonicus*) during Freezing and Subsequent Frozen Storage

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ABSTRACT: Fresh shrimps (*Penaeus japonicus*) were packed and frozen with three different freezing conditions; -25°C in a still air freezer and -35°C or -45°C in a semi-air blast freezer. These freezing conditions provided freezing rates of 0.70, 1.5 and 1.76 cm/hr, respectively. A Scanning Electron Microscope (SEM) was used to study the ice crystals imprinted in the frozen shrimp muscles and revealed that ice crystals in the shrimp muscles were smaller when the shrimps were frozen at the faster rate. The size distribution of the ice crystals changed during storage at -25 and -50°C for two and, especially, for 2.5 months. The relative number of large ice crystals increased faster when the shrimps were stored at the -25°C with the shrimps also showing a tendency to have a higher frozen weight loss compared to those stored at -50°C.

Key words: Ice-crystal, shrimp, frozen storage

INTRODUCTION

Shrimps are perishable food products, with the quality of shrimp meat starting to deteriorate soon after harvesting. Many food processing methods have been applied to preserve the quality of shrimp meat. Freezing is one of the best methods available in the food industry for preserving food products with high quality. Frozen storage of perishable products is normally carried out at a temperature below the freezing point (normally below -18°C). At this temperature, both the putrefying and degrading chemical reactions and the growth of bacteria are significantly slowed down.(1) The effects of freezing and frozen storage on the functional properties of protein in food materials have been reported.(2,3) During the freezing of foods, free water in the food matrix is turned into ice crystals but the formation, size and position of ice crystals in food materials depends upon the freezing rate, sample temperature and conditions of the muscle fibers.(4,5)

During the freezing and storage the changes in food quality depends on the ice crystal size.(6) Foods that have been subjected to very fast freezing have small ice crystals inside and outside of the food cells, the size and location of ice crystals are considered the most important factors that affect the textural quality of frozen foods.(7) A slow freezing process produces large extracellular ice-crystals, which cause more texture damage, accelerate enzyme activity and increase the oxidation rate during storage and after thawing,(1) compared to that seen with tissues subject to the formation of smaller ice crystals. On the other hand, rapid freezing can reduce ice-crystal size due to its high degree of super-cooling, but it may cause lethal intracellular ice crystallization in living cells and mechanical cracking.(8,9) During the storage of food materials, there are also changes in the size and number of the ice crystals. The fluctuations of temperature during frozen storage cause recrystalization and development of ice crystals size which result in deterioration of the frozen foods.(10)

The purpose of this paper was to observe the size of ice crystals which were formed during freezing at different freezing rates and upon subsequently different frozen storage temperatures. The effect of the ice crystal size on weight loss of shrimp s was also determined.

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MATERIALS AND METHODS

The effect of freezing rate and storage on the ice-crystal size in shrimp muscle.

Live shrimps (*Penaeus japonicus*) of between 10-16 g wet weight each, were cold chocked, packed in sealed plastic bags and frozen with their shell and head on at three different freezing temperatures using two types of freezers; namely at -25°C in a still air freezer and -35°C and -45°C in a semi-air blast freezer. The temperature of the shrimps was measured using a thermal-recorder with a thermocouple placed at the center of shrimp's first abdominal section. The temperature changes were monitored and data was collected every 0.5 minute until the temperature was constant. The freezing rates were calculated by using the thickness of shrimp muscle from the shell and the center divided by the time to pass the ice-crystal formation zone,[1] the latter being defined as the period of freezing, right after the temperature passed 0^{o}C, for which there was no change in the temperature of the sample.

To determine the effect of freezing rate on the ice-crystal size in shrimp muscles, the freshly frozen shrimps were cut and mounted on the frozen stage of a Cryo-SEM (Hitachi S-4000 model, Hitachi Co., Japan). Cutting and mounting were done in a -30°C environment to prevent the melting of ice crystals. The SEM images were analyzed for the size of the ice-crystals imprinted in the shrimp muscle. The size of the ice-crystals, expressed as the cross-sectional surface area (CSA), was calculated using image-analyzing program (ImageJ, NIH Washington DC, USA). The surface area about 0.296 X 0.400 mm of the sample was analyzed and calculated for the cross section area of the ice crystals imprinted in the samples. The experiment was repeated three times.

To evaluate the effect of storage on ice-crystal sizes in shrimp muscles, freshly frozen shrimps (semi-airblast freezer at -35°C) were either evaluated immediately or stored for 2.5 months and then evaluated for ice-crystal size and weight loss. Weight loss was calculated as 100 x the difference in weight of frozen and thawed shrimps divided by the weight of frozen shrimps.[11]

All experiments were repeated three times and are reported as the mean $\pm$ one standard deviation (1 S.D.). Statistical significance of differences between means or between treatments were determined using ANOVA and Duncan's multiple means test for parametric data and Mann-Whitney test and Kruskall-Wallice test for non-parametric data, with a significant difference being established at $p<0.05$ in all cases.

RESULTS AND DISCUSSION

The effect of freezing rate on ice-crystal formation in shrimp muscle

The average water content of the shrimps determined before freezing was 70% (w/w) on a wet basis. The freezing curves obtained for the shrimps (shell and head on) during freezing at the three different conditions are summarized in Figure 1. The time required to pass the ice-crystal growing zone were found to be (mean $\pm$ 1 S.D.) 17 $\pm$ 3, 21 $\pm$ 2 and 43 $\pm$ 3 mins for -45°C,-35°C and -25°C, respectively, whilst the calculated freezing rates were 1.76 $\pm$ 0.25, 1.5 $\pm$ 0.15 and 0.70 $\pm$ 0.20 cm/hr, respectively.

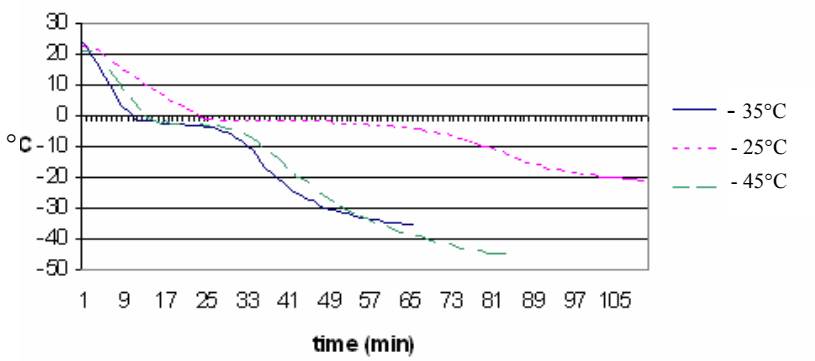

Figure 1. The temperature profile of shrimps when placed either at -25°C in a still air freezer or at -35°C or -45°C in a semi-air blast freezer.

The size of ice-crystals were measured and reported, as the CSA of the ice-crystal in micrographs. The average size of the ice-crystals formed in shrimp muscles when freezing at the rate of 1.76, 1.5 and 0.70 cm/hr were found to be 158 $\pm$ 72, 294 $\pm$ 79 and 1160 $\pm$ 190 μm^2, respectively. The relationship of the ice-crystal size and the freezing rate between 0.7 and 1.76 cm/hr is shown in Figure 2.

$$y = -1102.4 \ln(x) + 762.99 \qquad (r^2 = 0.9986) \quad \ldots\ldots\ldots\ldots \quad (1).$$

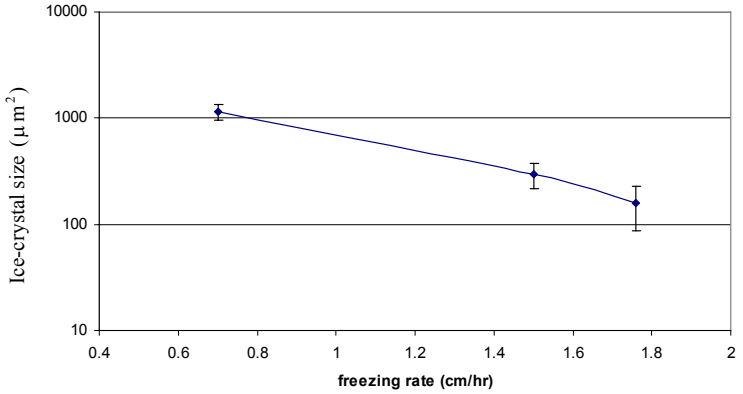

Figure 2. Relationship between the freezing rate and ice-crystal size (micrometer^2) in shrimp meat.

The relationship between the ice crystal size and freezing rate (between 0.70 to 1.76 cm/hr) showed a log-linear relationship which fitted equation 1. A reduction in the mean ice crystal size (CSA) of about 90% was attained when the rate of freezing was 1 cm/hr faster.

Damage to shrimp muscle tissues during frozen storage

The ice-crystal size in frozen shrimp tissues were evaluated as CSA under Cryo-SEM. Freshly frozen shrimps had an ice-crystal CSA with a mean ($\pm$ 1 S.D.) of 294 $\pm$ 79 μm^2 but with a fairly broad size distribution within the range of 11 - 1100 μm^2, with 43% of ice-crystals being smaller than 100 μm^2 and with no crystal being recorded with a CSA above 1200 μm^2 (Figures 3, 5).

In contrast, after storage at -25^{o}C for 2 or 2.5 months, ice-crystals within the shrimp muscle tissues showed a larger average size and broader size range of ice-crystals in both storage periods (Figure 4), compared to those found in freshly frozen shrimps (Figures 3, 5), with an average CSA of 617 $\pm$ 127and 1362 $\pm$ 11 μm^2 at 2 and 2.5 months storage respectively. The analysis of the data using Kruskull-Wallis test showed that these distributions were significantly different ($p<0.05$). The largest CSA of the ice-crystals found was in the range of 2800-3200 μm^2 and in the samples stored for 2.5 months, the shift in the ice-crystal CSA size distribution showed a marked decrease and increase in the proportion of crystals with a CSA of 0-100 and over 1200 μm^2, respectively.

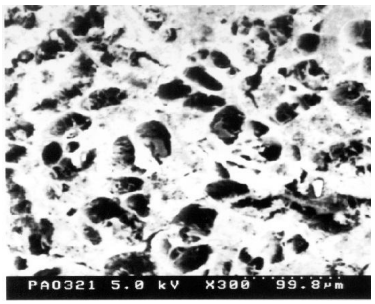

Figure 3. Representative cryo-SEM image (x300) of ice-crystals formed in shrimp muscles immediately after being frozen in a semi-air blast freezer at -35^{o}C. The micrograph is representative of three independent repeats.

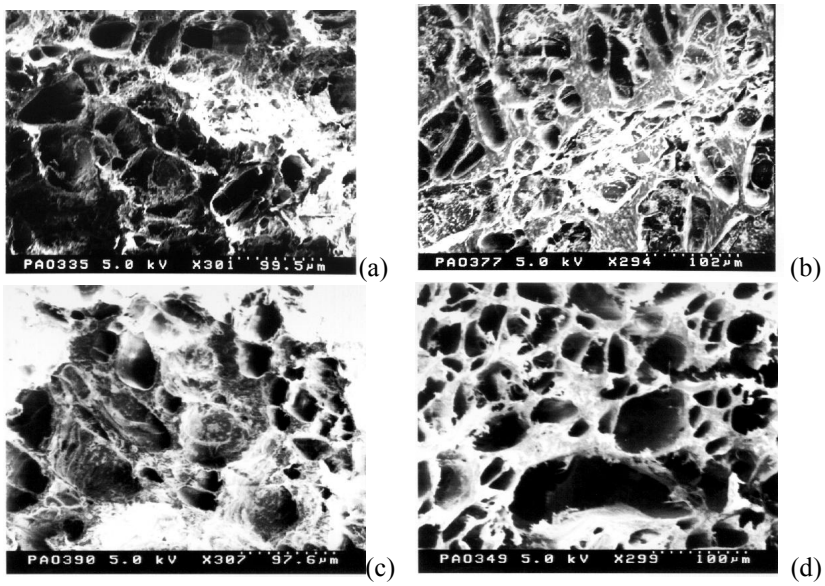

Figure 4. Representative cryo-SEM images (x300) of shrimp muscle tissues frozen in a semi-air blast freezer at -35°C and then stored at (a) -25°C for two months, (b) -25°C for 2.5 months, (c) -50°C for two months and (d) -50°C for 2.5 months. The micrographs are representative of three independent repeats.

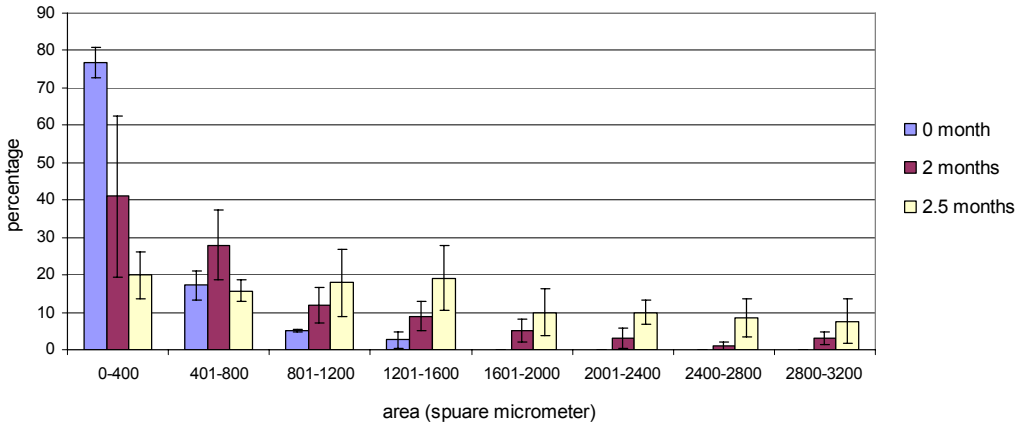

Figure 5. The size (as the cross-sectional surface area) distribution of ice-crystals in shrimp muscle tissues frozen at -35°C and then stored at -25°C for 0, 2 or 2.5 months.

Storage of shrimps at -50°C resulted in an increasing ice crystal size with time, with an average CSA of 531 ± 132 and 603 ± 89 μm^2 after 2 and 2.5 months, respectively. The analysis of the data using Kruskull-Wallis test showed that these distributions were significantly different ($p<0.05$). The distribution of the ice crystals, however, were less heterogeneous (Figure 6) and varied less over time than that seen with storage at -25°C (Figure 5). However, the number of very small ice-crystals (CSA of <400 μm^2) was reduced as shrimps were stored for longer than two months at -50°C (Figure 6). Thus, although the ice-crystals were larger in shrimps after storage at -50°C, compared to freshly frozen shrimps, this increase in crystal CSA was significantly ($p<0.05$) smaller than that seen after storage at -25°C for the same time periods, as summarized in Figure 7. The analysis of data using ANOVA was found that the crystal CSA in shrimp samples of -25°C differed from -50°C. Indeed, ice crystals in shrimp muscles stored at -25°C grew about two times faster than those stored at -50°C. During extended storage (2.5 months), the growth rate of the ice crystals increased in both storage conditions. Such changes in the size distribution of the ice crystals during frozen storage have been reported in other frozen foods.[12]

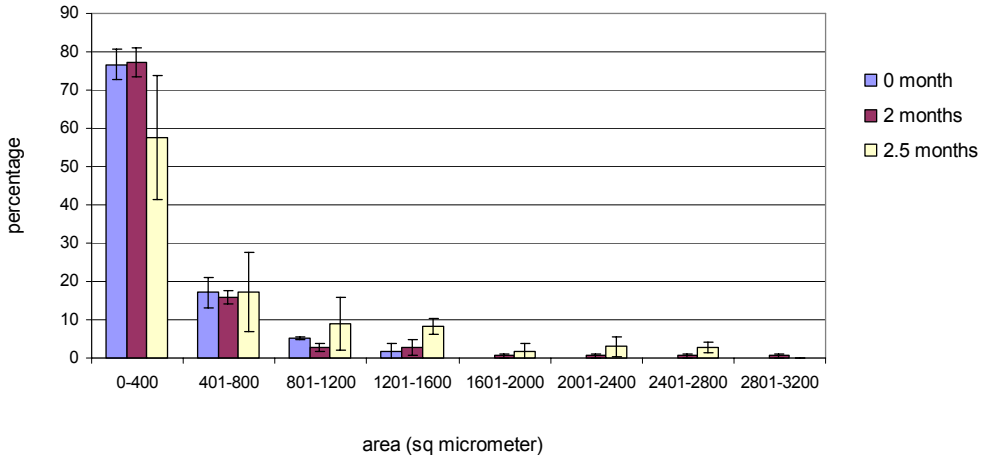

Figure 6. The size (as the cross-sectional surface area) distribution of ice-crystals in shrimp muscle tissues frozen at -35°C and then stored at -50°C for 0, 2 or 2.5 months.

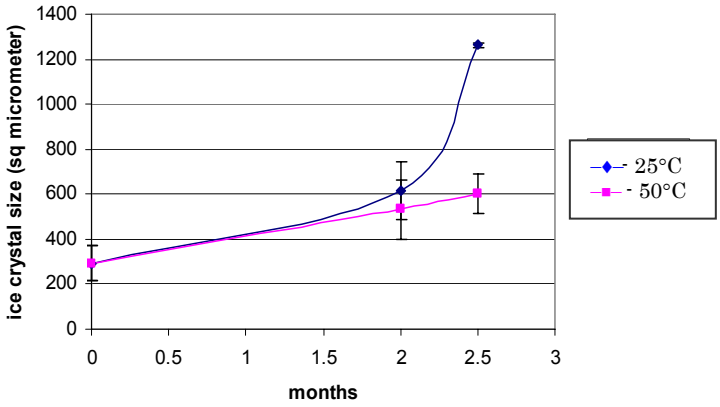

Figure 7. Changes in the average ice-crystal size during storage at -25°C and -50°C.

After thawing, shrimps were weighed and the calculated loss of wet weight during storage is summarized in Figure 8. The average loss in the samples stored at -25°C showed a marked trend to be greater than those stored at -50°C, but these results were not statistically significant ($p>0.05$) when the data was analyzed with Mann-Whitney test. Nevertheless, the observed increase in the weight loss during storage correlated well with the increase in the ice crystal CSA during storage. The loss in weight of thawed shrimps may well reflect protein denaturation in shrimps during frozen storage. The reduction of free water in the shrimp muscle cell, during ice crystal formation, can cause proteins to denature with a resultant lose in water solubility, enzyme activity and protein thermostability.[13,14] Certainly, the reduction in actomyosin solubility and Ca^{2+}ATPase activity in frozen marine products during storage at -20°C has been reported.[15,16]

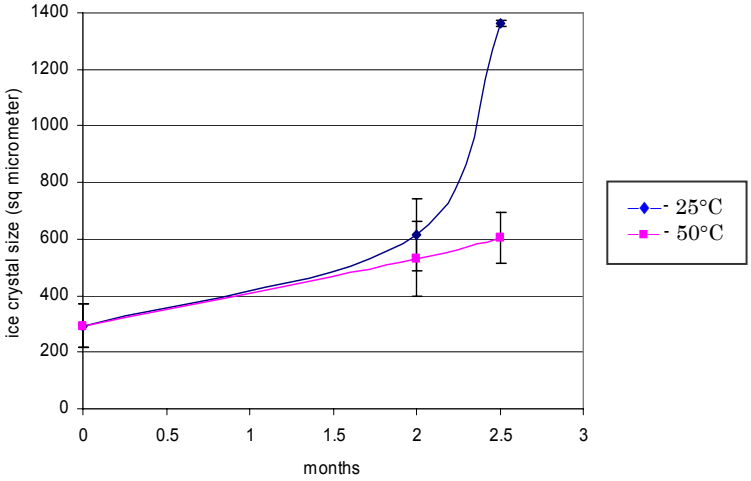

Figure 8. The weight loss in percentage of shrimps frozen at -35°C and then stored at either -25°C or -50°C for two or 2.5 months.

CONCLUSION

Freezing at a faster rate resulted in a smaller average size (CSA) of ice-crystals in the shrimp muscle tissues. The duration and temperature of frozen storage also affected the average size of the ice-crystals, with an increase in both average size (CSA) and number of large ice-crystals being formed with increasing storage time up to 2.5 months. Ice-crystals in shrimps stored at -25°C grew about two times faster than those stored at -50°C and also tendency displayed a greater wet weight loss.

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