

# Effects of freezing and thawing on the quality changes of tiger shrimp (*Penaeus monodon*) frozen by air-blast and cryogenic freezing

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## Abstract

Effects of air-blast freezing and cryogenic freezing, thawing by microwave and refrigerator, and freeze–thaw cycle on changes in thio-barbituric acid (TBA), salt-soluble protein (SSP), %freezing loss (%FL), %thawing loss (%TL) and cutting force (CF) of tiger shrimp (*Penaeus monodon*) were investigated. Air-blast freezing was done at  $-28 \pm 2$  °C and air velocity of 4–8 m/s while cryogenic freezing was done at  $-70$  to  $(-100)$  °C. Shrimps frozen at the air velocity of 6 m/s had the least %FL and similar CF to the fresh samples. Thawing method did not affect SSP and CF. Samples thawed by the microwave had higher TBA than those in refrigerator. Increasing the freeze–thaw cycle increased the TBA and CF but decreased the SSP. Combined effect of thawing method and freeze–thaw cycles affected %TL only. The histological examination of cryogenic-frozen shrimps showed an increase in spacing between the muscle fiber and torn muscle fiber bundles as the number of freeze–thaw cycles increased.

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## 1. Introduction

Flesh of shrimp after death is still active and biochemically alive. The organic decomposition or the change of shrimp composition may be triggered by various factors, i.e. enzymes and microbiological activities (Pedraja, 1970). The highest shrimp quality can be obtained in the shrimp frozen immediately after harvested (Fennema, Karel, & Lund, 1975).

There are many commercial methods for freezing shrimp. Although freezing is an effective method of preserving foods, however, some deterioration in frozen food quality occurs during storage. The extent of quality loss depends on many factors, including the rate of freezing and thawing, storage temperature, temperature fluctua-

tions, freeze–thaw abuse during storage, transportation, retail display and consumption (Giddings & Hill, 1978; Sebranek, 1982; Srinivasan, Xiong, & Blanchard, 1997).

During thawing, foods are damaged by the chemical, physical, and microbiological changes. The satisfactory techniques for thawing large portions of animal tissue include thawing in a refrigerator and microwave. Microwave thawing is more rapid and much more uniform than heating by conduction which may minimize the damaging effects on the tissues (Karel & Lund, 2003). The freezing and thawing processes can have a profound effect on muscle physicochemical characteristic. Wagner and Anon (1985) studied the denaturation effect on myofibrillar proteins of bovine muscle and found that after thawing, the slow-freezing muscle tissue had the higher water loss. The denaturation observed could be a result of a partial unfolding of the myosin head being more pronounced at slow freezing.

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During frozen storage of shrimp and other shellfish products, the quality changes caused by oxidation, denaturation of proteins, sublimation and recrystallization of ice crystals are predominant (Londahl, 1997). These can result in off-flavors, rancidity, dehydration, weight loss, loss of juiciness, drip loss and toughening (Bhobe & Pai, 1986; Londahl, 1997), as well as microbial spoilage and autolysis (Bhobe & Pai, 1986). As for any other food products, rapid freezing after processing and storage at low temperature is essential for shrimp and shrimp products in order to minimize the unavoidable quality changes (Londahl, 1997).

So described above, many researches on frozen shrimp have been conducted (Kent & Stroud, 1999; Pan & Yeh, 1993; Srinivasan, Xiong, Blanchard, & Tidwell, 1998). However, as the shrimp is in the processing plant, it is frozen either in bulk by glazing with water and freezing in an air-blast freezer or individually frozen (IQF) using liquid CO<sub>2</sub> or N<sub>2</sub>. It might be thawed and refrozen during transportation and repackaging in a smaller retailer. Shrimp could be freeze–thawed many times before consumed. The objective of this research was to investigate the effects of freezing by air-blast and cryogenic, thawing by refrigerator and microwave, and freeze–thaw cycle on the physical and chemical changes of tiger shrimp.

## 2. Materials and methods

Tiger shrimps (*Penaeus monodon*), size 80–90 shrimps per kilogram, were cleaned with cooled water (~5 °C). They were then deheaded, peeled, and immediately cooled on ice before being frozen. The proximate analysis of shrimp samples were performed (AOAC, 1995).

### 2.1. Effect of freezing methods and conditions

Two freezing methods, air-blast freezing and cryogenic freezing with liquid nitrogen, were studied. The air-blast freezing was done at –28 °C and the air velocity of 4, 6 and 8 m/s in an air-blast freezer (Augusta Supply, Thailand). The cryogenic freezing was done at –70, –80, –90 and –100 °C in Cryo-test Chamber (Air Products and Chemicals model F831059E; Allentown, Penn., USA) by exposing samples with the liquid N<sub>2</sub> vapor supplied by Liquid N<sub>2</sub> tank (Taylor–Wharton model XL-55HP, USA). Twenty shrimps per batch were frozen for each condition. The changes in temperature of sample during freezing for each batch was measured by using a thermocouple type T connected to temperature recorder (Yokogawa, LR 4210) and inserted at the second abdominal segment of a shrimp. The freezing rate (Pan & Yeh, 1993) was calculated from

$$\text{Freezing rate (cm/h)} = \frac{\text{Minimum distance from the surface to the thermal center of shrimp(cm)}}{\text{Thermal arrest time(h)to reach } -18\text{ }^{\circ}\text{C}}$$

The %freezing loss of the frozen samples was determined. The cutting force of the thawed samples was measured. The freezing conditions that gave samples having the lowest %freezing loss and similar cutting force as the fresh shrimp of both methods were selected. The experiments were done in triplicate.

### 2.2. Effect of thawing methods and freeze–thaw cycles

The shrimps frozen under the selected conditions of both freezing methods (20 shrimps per condition) were vacuum packaged in nylon/HDPE plastic bags and stored at –20 °C for 3 days before being thawed in a microwave (Sharp, model R-251, using easy defrost program at 70% power level of 800 watt max. power) or in a refrigerator (~5 °C) until the temperature at the center of shrimps reached ~0 °C. The thawed shrimps were divided into two parts; the first part was immediately placed on ice for analysis (cycle 0). In order to imitate the thawing and refreezing during transportation and storage, the second part was refrozen in a still freezer at –20 °C for 3 days and thawed in a microwave or a refrigerator (cycle 1). The freeze–thaw step, i.e. 3 days stored at –20 °C and then thawed, was repeated for four cycles.

The samples were analyzed for %thawing loss, cutting force, thiobarbituric acid, total volatile base (TVB), and salt-soluble protein. The experiments were done in triplicate.

### 2.3. Freezing loss determination

The freezing loss of the frozen shrimps was determined from the known weights of shrimps before and after freezing and expressed as (AOAC, 1995)

$$\% \text{Freezing loss} = \frac{[\text{weight of raw shrimp} - \text{weight of frozen shrimp}]}{\text{weight of raw shrimp}} \times 100$$

### 2.4. Cutting force determination

The frozen shrimps were thawed at room temperature until their temperatures reached 20 °C. The cutting forces of the thawed samples were measured using a texture analyzer (Stable Micro System; TA:XT2i, England) with a knife blade attached to a 25 kg load cell. The cross-head speed of knife blade was 2 mm/s with the distance of 25 mm. The maximum force to cut transversally into the second abdominal segment of the shrimp was recorded

as the cutting force. The measurement was done in triplicate.

### 2.5. Thawing loss determination

The thawing loss of the thawed shrimps was determined from the known weights of shrimps before and after thawing and expressed as (AOAC, 1995)

$$\% \text{Thawing loss} = \frac{[\text{weight of frozen shrimp} - \text{weight of thawed shrimp}]}{\text{weight of frozen shrimp}} \times 100$$

### 2.6. Thiobarbituric acid (TBA) value determination

Oxidative rancidity, measured as thiobarbituric acid (TBA) reactive substances, was determined by the method described by Pearson (1976).

### 2.7. Total volatile base (TVB) determination

The amount of total volatile base in the sample was determined according to the method described in MFRD (1987).

### 2.8. Salt-soluble protein (SSP) determination

Myofibrillar proteins such as myosin and actin are the major proteins in marine muscle that are soluble in 3–5% salt solutions. The SSP was extracted according to the method of MFRD (1987).

### 2.9. Histological examination

Only the cryogenic samples were used for the histological investigation. The modified method of Pan and Yeh (1993) was used to prepare tissue for microscopic analysis. A light microscope (Nikon UFX-DX) and camera (Nikon FX-35DX) were used for observation.

## 3. Results and discussion

### 3.1. Effect of freezing methods

The proximate composition of the fresh shrimps were  $79.75 \pm 0.35$  %moisture,  $17.70 \pm 0.42$  %protein,  $0.86 \pm 0.05$  %fat,  $0.99 \pm 0.01$  %ash and  $0.7 \pm 0.17$  %carbohydrate.

The shrimps frozen by air-blast had the freezing rate of 6.8–7.4 cm/h while those frozen by the cryogenic freezing had the freezing rate of 11.8–21.9 cm/h (Table 1). The samples frozen with air-blast freezing at the air velocity of 4 and 6 m/s had the %freezing loss significantly lower than those at 8 m/s ( $p \leq 0.05$ ). This may be due to the higher air velocity causing an excessive dehydration of the samples (Fennema et al., 1975). The samples frozen at the air velocity of 4 m/s had the cutting force lower while those at the 8 m/s had the cutting force higher than the fresh samples. The shrimps frozen at the air velocity of 6 m/s had the cutting force similar to the fresh samples ( $p > 0.05$ ). This may be due to the higher water removal at the surface during freezing at the higher air velocity. Therefore, the air-blast freezing at the air velocity of 6 m/s was selected for further studies of thawing and freeze–thaw cycle effects.

For cryogenic freezing, it was found that all temperatures applied gave samples with similar %freezing loss ( $p > 0.05$ ). The cutting force of the shrimps frozen at  $-70$  °C was similar to the fresh samples ( $p > 0.05$ ) while those frozen at  $-100$  °C had the lowest cutting force which probably due to the cracking phenomena in the samples frozen at low temperature (Pan & Yeh, 1993). These findings were similar to that observed by Pan and Yeh (1993) that about 50% of the muscle cells surface cracking occurred in shrimps frozen at  $-120$  °C. Thus, freezing the shrimps at  $-70$  °C was selected for the best condition of cryogenic freezing.

### 3.2. Effect of thawing methods and freeze–thaw cycle

Fig. 1 showed that for both freezing methods, the combined effect of thawing methods and freeze–thaw cycles

Table 1  
Freezing time, freezing rate, freezing loss and cutting force of shrimps frozen in the air-blast freezer and cryogenic freezer

Freezing method	Freezing time (s)	Freezing rate (cm/h)	Freezing loss (%)	Cutting force (N)
Air-blast at air velocity				
4 m/s	$371.25 \pm 7.50$	$6.85^b \pm 0.10$	$2.71^b \pm 0.30$	$19.29^c \pm 0.36$
6 m/s	$363.75 \pm 7.50$	$6.90^b \pm 0.12$	$2.14^b \pm 0.29$	$21.36^b \pm 0.17$
8 m/s	$333.75 \pm 7.50$	$7.42^a \pm 0.14$	$3.43^a \pm 0.53$	$22.49^a \pm 0.30$
Fresh shrimps				
				$21.57^b \pm 0.88$
Cryogenic at				
$-70$ °C	$213.75 \pm 14.36$	$11.82^c \pm 0.79$	$1.83^a \pm 0.01$	$22.45^{bc} \pm 0.86$
$-80$ °C	$191.25 \pm 14.36$	$13.26^c \pm 0.93$	$1.81^a \pm 0.00$	$22.77^b \pm 0.61$
$-90$ °C	$153.75 \pm 14.36$	$16.25^b \pm 1.29$	$1.75^a \pm 0.01$	$23.78^a \pm 0.37$
$-100$ °C	$116.25 \pm 14.36$	$21.98^a \pm 2.74$	$1.75^a \pm 0.13$	$18.56^d \pm 0.14$
Fresh shrimps				
				$21.57^c \pm 0.88$

Means with different letters in the same column and block are significantly different ( $p < 0.05$ ).

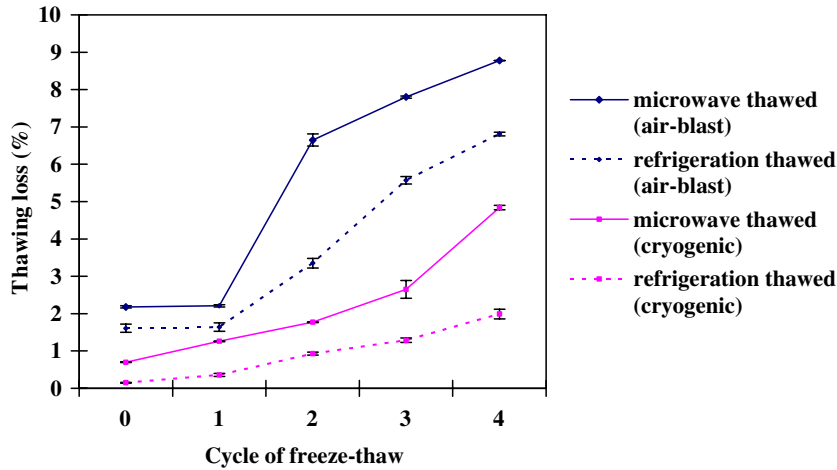


Fig. 1. Thawing loss of shrimp frozen by air-blast freezing at 6 m/s air velocity and  $-28^{\circ}\text{C}$  (—◆—, ---◆---) and cryogenic freezing at  $-70^{\circ}\text{C}$  (—■—, ---■---).

affected %thawing loss. Microwave thawing gave the samples with the higher %thawing loss than those thawed in refrigerator temperature in every freeze–thaw cycle. This may be because the microwave thawing gave a rapid heating to the samples which enhanced the vaporization of water from the samples. The frozen foods were not homogeneous since they always contained frozen and unfrozen phases and a non-uniform distribution of lipids. These components differed greatly in their abilities to absorb radiofrequency energy and this tended to cause localized areas to overheat before other areas had thawed (Fennema et al., 1975). Therefore, this resulted in the high drip loss from the microwave thawing samples. Although microwave thawing produced fast thawing, it might cause pronounced protein denaturation and destabilization. It was also an undesirable method to thaw shrimps due to the asymmetric shape of the samples (Srinivasan et al., 1997). Moreover, for fresh foods, where texture was important,

it seemed that a slow thawing process in cool environment was preferable because it allowed time for diffusion to take place in the thawed tissue and the water might return to its original positions in the tissue (Jul, 1984). An increase in number of freeze–thaw cycles resulted in the higher %thawing loss (Fig. 1) and cutting force (Fig. 2). Repeated melting during thawing and reformation of ice crystals during freezing in multiple freeze–thaw situations was clearly detrimental to muscle tissues by causing mechanical damage to cell membranes and the loss of water holding capacity (Srinivasan, Xiong, Blanchard, & Tidwell, 1997). However, it was found that thawing method did not significantly affect the cutting force ( $p > 0.05$ ).

The TBA value in shrimps frozen under both air-blast and cryogenic freezing during multiple freeze–thaw cycles were shown in Fig. 3. The shrimps thawed under the microwave had a slightly higher TBA value than those thawed under refrigerator temperature in every freeze–thaw cycle.

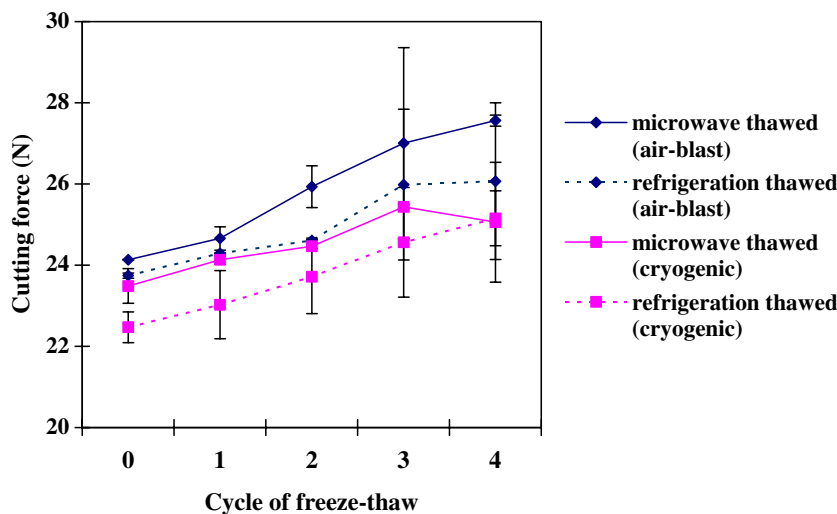


Fig. 2. Cutting force of shrimp frozen by air-blast freezing at 6 m/s air velocity and  $-28^{\circ}\text{C}$  (—◆—, ---◆---) and cryogenic freezing at  $-70^{\circ}\text{C}$  (—■—, ---■---) during multiple freeze–thaw cycles.

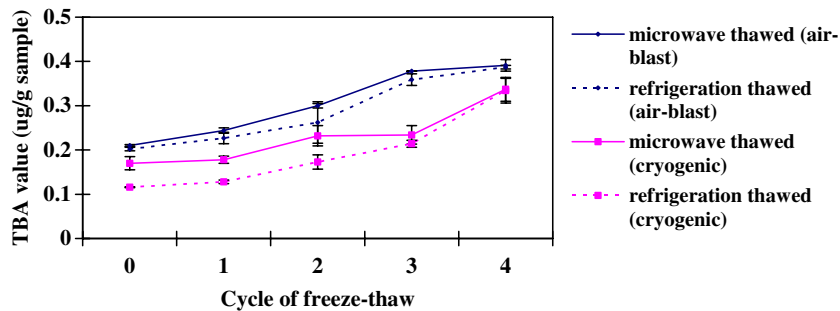


Fig. 3. TBA value in shrimps frozen under the air-blast freezing at 6 m/s air velocity and  $-28^{\circ}\text{C}$  (—◆—, ---◆---) and cryogenic freezing at  $-70^{\circ}\text{C}$  (—■—, ---■---) during multiple freeze–thaw cycles.

It was probably due to the fact that high energy generating under the microwave thawing might activate the lipid oxidation in the shrimps and thus gave a higher TBA value than those thawed in refrigerator. Siu and Draper (1978) reported that lipolysis also occurred at higher temperature. Although cooking was the most common method used to avoid enzymatic deterioration during frozen storage, the extent of lipid oxidation in cooked meat appeared to be related to the intensity of heat treatment. In a survey of malonaldehyde (MA) content of retail meats and fish (Siu & Draper, 1978), it was reported that 38% of all fresh meats samples tested had MA contents less than  $1\ \mu\text{g/g}$  where as 60% of the cooked products were in the range  $1\text{--}6\ \mu\text{g/g}$ .

For both freezing methods, the TBA value of samples increased during freeze–thaw cycles indicating an increase in lipid oxidation. This could be due to the release of oxidative enzymes and prooxidants from various ruptured cellular organelles. Moreover, the removal of shrimp shells that contained phenolic antioxidants also meant an elimination of the oxidation protection of the samples (Srinivasan et al., 1998). The quality of the frozen samples was also determined from the TVB values. The TVB values of all freeze–thawed samples were found to be  $10.2\text{--}14.6\ \text{mg N}/100\ \text{g sample}$ . There were references (Botta, 1994; TIS, 1986) related the TVB values with the freshness of the shrimps. The TVB value of  $\leq 20\ \text{mg N}/100\ \text{g sample}$  was

considered fresh,  $\leq 30\ \text{mg N}/100\ \text{g sample}$  was acceptable and  $>40\ \text{mg N}/100\ \text{g sample}$  was not suitable for consumption. According to the Thai standard for frozen shrimps and prawns of  $30\ \text{mg N}/100\ \text{g sample}$  (TIS, 1986).

Fig. 4 showed the changes in the salt-soluble protein (SSP) in frozen shrimps during multiple freeze–thaw cycles. Figs. 2 and 3 indicated that only the number of freeze–thaw cycle affected the cutting force and the SSP for each freezing method. Fig. 5 showed the changes of the SSP in shrimps frozen under the selected conditions of both freezing methods during multiple freeze–thaw cycles when we observed only the effect of freeze–thaw cycles. The denaturation of muscle proteins may occur during multiple freeze–thaw cycles and a decrease in SSP could be due to the denaturation of proteins caused by the interaction of free fatty acid with SSP and the consequent lower solubility of proteins (Verma & Srikar, 1994). In addition, the toughness of frozen shrimp was attributed to myosin denaturation, as well as cross-linking and aggregation of myofibrillar proteins (Sikorski, 1977). As the SSP in shrimps decreased when the freeze–thaw cycles increased, the cutting force of the shrimp muscle also increased. The cutting force increased up to two freeze–thaw cycles and then leveled off after that for both freezing methods (Fig. 5). An increase in the cutting force indicated that the samples became toughen which might be due to the fiber shrinkage and drip loss (Hale & Waters, 1981).

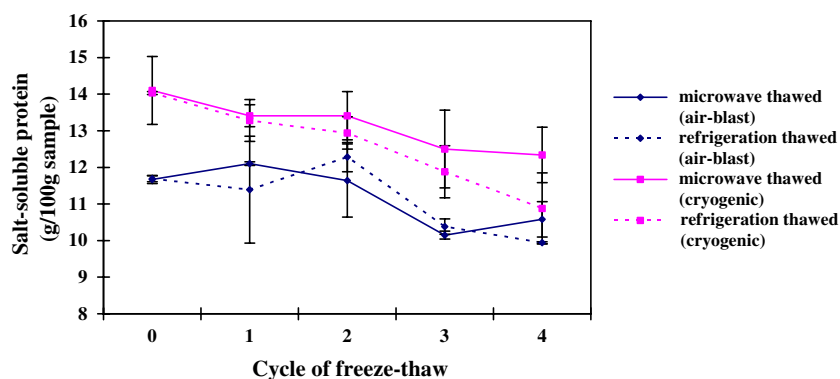


Fig. 4. The salt-soluble protein content (g/100g sample) in shrimps frozen under air-blast freezing at 6 m/s air velocity and  $-28^{\circ}\text{C}$  (—◆—, ---◆---) and cryogenic freezing at  $-70^{\circ}\text{C}$  (—■—, ---■---) during multiple freeze–thaw cycles.

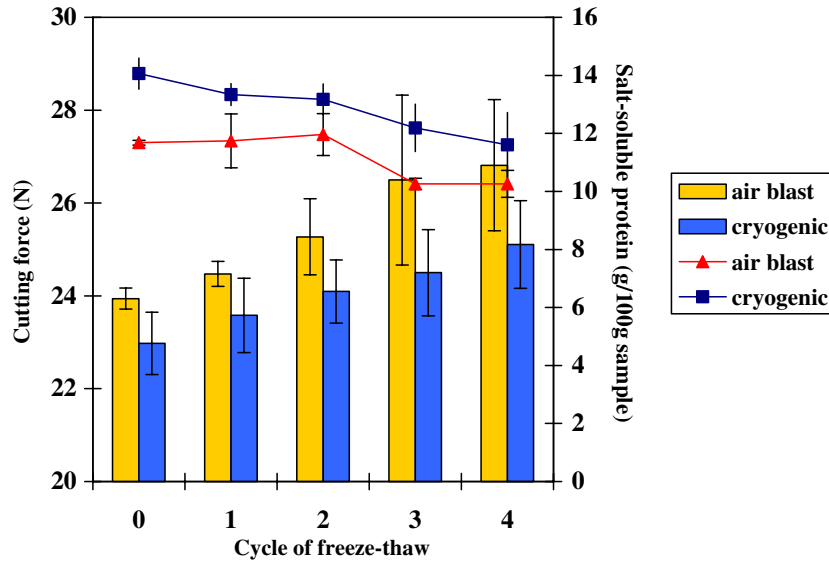


Fig. 5. The cutting force (bar graph) and salt-soluble protein (connected line graph) in shrimps frozen under air-blast freezing at 6 m/s air velocity and  $-28^{\circ}\text{C}$  and cryogenic freezing at  $-70^{\circ}\text{C}$  during multiple freeze–thaw cycles.

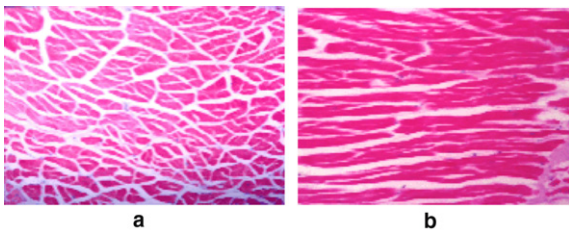


Fig. 6. Light micrographs of the fresh shrimp mainly focusing on (a) the inner part of meat, (b) the part around the subcuticular membrane (magnification 140 $\times$ ).

### 3.3. Histological examination

The results of the histological examination under light microscope of fresh shrimp and the shrimp frozen under the cryogenic and thawed under the microwave and refrigerator were shown in Figs. 6–8. It could be seen that the spacing between the muscle fiber increased and the muscle fibers were torn as the number of freeze–thaw cycles increased. In the cycle 0 (thawing immediately after freezing) and the first cycle (Fig. 7), the shrimp structure was still similar to the fresh sample (Fig. 6). The muscle fiber around the subcuticular membrane arranged in parallel and the spacing between the muscle fiber in the inner part of the shrimp trunk was similar to the fresh sample. The changes of muscle fiber structure were obvious when the shrimps were freeze–thawed more than two cycles. After the third and the fourth cycle, the muscle fiber was torn apart and showed disordered arrangement and the spacing between muscle fiber increased. Moreover, the boundary of muscle fiber was not clear. These changes were the results of the mechanical damage of the tissues. These results agreed with the changes in TBA value (Fig. 3) and %thawing loss of shrimp frozen in cryogenic freezer and thawed

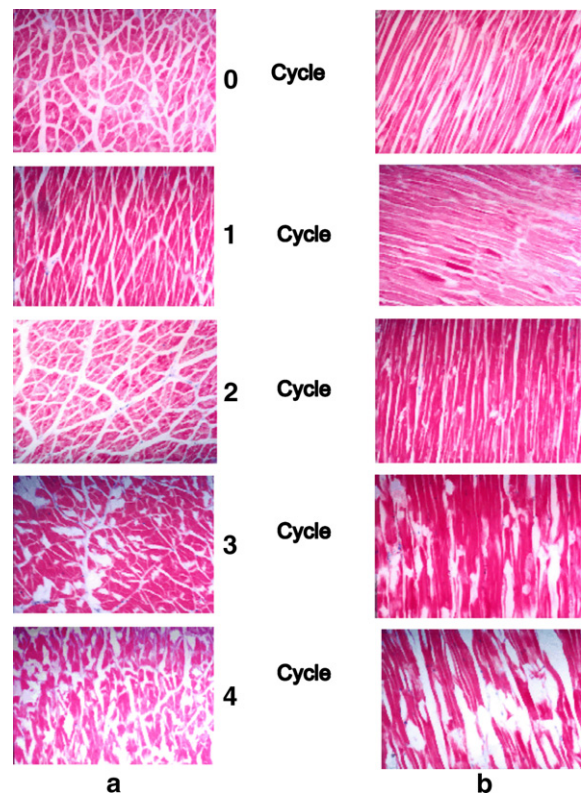


Fig. 7. Light micrographs of the shrimp frozen in cryogenic freezer and thawed under the microwave during the multiple freeze–thaw cycles, mainly focusing on (a) the inner part of meat, (b) around the subcuticular membrane (magnification 140 $\times$ ).

under microwave (Fig. 1). The TBA value in shrimp thawed under the microwave after freeze–thawed 1 cycle increased 4.71% from the cycle 0. From the first cycle to the second cycle the TBA increased 30.34%, then slightly increased 0.86% after the third cycle. After the fourth cycle,

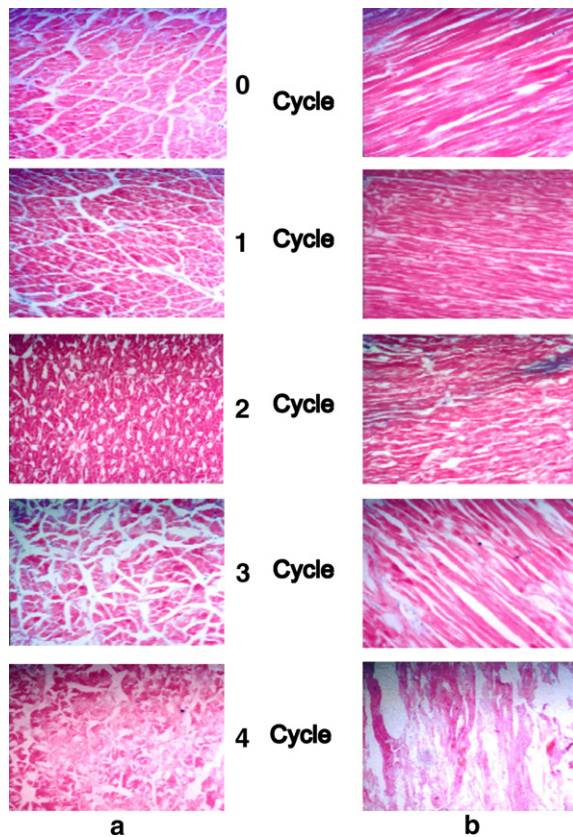


Fig. 8. Light micrographs of the shrimp frozen in cryogenic freezer and thawed under the refrigeration temperature during the multiple freeze–thaw cycles, mainly focusing on (a) the inner part of meat, (b) around the subcuticular membrane (magnification 140 $\times$ ).

the TBA value increased 44.02% from the third cycle. The %thawing loss rapidly increased from 40.48% to 82.64% from the second cycle to fourth cycle (Fig. 6).

Fig. 8 showed the changes in structure of muscle fiber in shrimps frozen in cryogenic freezer and thawed under the refrigeration temperature, it could be seen that the spacing between the muscle fiber increased and the muscle fiber were torn as the number of freeze–thaw cycles increased similar to that found in microwave-thawing shrimps. In the cycle 0 (thawing immediately after freezing) and the first cycle, the shrimp structure was still similar to the fresh sample (Fig. 6). The muscle fiber lined continuously (mainly focusing the part around the subcuticular membrane) and the spacing between the muscle fiber was similar to the fresh sample (mainly focusing on the inner part of meat). The change of muscle fiber structure was obvious when the shrimps were freeze–thawed more than two cycles. In the shrimp freeze–thawed for two cycles, large holes were found (Fig. 8(a)). These holes were the result of the large ice crystals formed during multiple freeze–thaw situation. The storage of thawed shrimp under  $-20\text{ }^{\circ}\text{C}$  may result in crystallization and these phenomena were the same as the crystallization in slow freezing. The ice crystals grew between the cells making the extracellular liquid more concentrated. The cells would lose water by osmosis which led to an extensive dehydration of the cells and the cells

contraction. When thawing, the water frozen out was not reabsorbed during thawing, leading to a high drip loss (Boegh-Soerensen & Jul, 1985). After the third and the fourth cycle, the muscle fiber was torn apart without continuity and the spacing between muscle fiber increased. Moreover, the boundary of muscle fiber was not clear. These changes were the results of the mechanical damage of the tissues. These findings were similar to the changes in TBA value (Fig. 3) and %thawing loss (Fig. 1). The TBA values in shrimp thawed under the refrigerator temperature and freeze–thawed 1 cycle increased 10.34% from cycle 0. From the first cycle to the second cycle it increased 35.16%, then dropped to 23.70% after the third cycle. After the fourth cycle, the TBA value increased 56.07%. The %thawing loss increased 58.33%, 38.71%, and 54.26% after the freeze–thaw cycle second, third, and fourth, respectively.

#### 4. Conclusions

Freezing the shrimps under the air-blast freezer at the air velocity of 6 m/s gave the least %freezing loss and same cutting force as the fresh shrimps. For cryogenic freezing, it was found that all temperatures used did not affect the %freezing loss with the shrimps frozen at  $-70\text{ }^{\circ}\text{C}$  had the same cutting force as the fresh samples. Samples thawed under the microwave had slightly higher TBA value than those thawed at the refrigerator temperature. An increase in the freeze–thaw cycles resulted in an increase in TBA value and cutting force and a decrease in SSP value. The combined effect of thawing method and freeze–thaw cycle of the shrimps frozen under both methods had the influence on %thawing loss. The microwave thawing gave the samples with the higher %thawing loss than those thawed at refrigerator temperature in every cycle and the increase in the freeze–thaw cycles gave the samples having the higher %thawing loss. The samples could be freeze–thawed up to 2 cycles. Therefore, it is important to prevent temperature fluctuations during transportation and storage to avoid the freezing and thawing effect and to maintain the quality of the frozen shrimps.

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