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Full Length Research Paper

The biochemical, textural and sensory properties of king scallop (*Pecten maxinus*) meats frozen at different characteristic freezing times

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Post rigor scallop meats were frozen individually with characteristic freezing time of 19, 49, 89, 235, 555 and 1000 min (time to cool the thermal centre of the meats from -1 to -7°C). After freezing, the meats were thawed and their quality was evaluated with tests related to muscle integrity (β-hydroxy-acyl-coenzyme –A dehydrogenase activities in muscle extracts), freezing and thawing losses, expressible fluids and myofibrillar protein denaturation (Ca^{2+}-ATPase activities in actomyosin extracts). Instrumental texture measurements and triangular sensory evaluations were also performed. Fresh post-rigor meats were analyzed as controls. The muscle integrity test showed that the freezing process itself clearly affected the integrity of intra-cellular organelles (mitochondria). The characteristic freezing time of 89 and 49 min caused more damage to cell structure than the shorter and longer characteristic freezing time tested. Ca^{2+}- ATPase activities of actomyosin extracts suggested that the freezing process itself, but not the freezing time, caused structural damage to myofibrillar proteins and were associated with the changes in water holding capacity (sum of thawing and expressible fluids). Peak shear forces of fresh and frozen scallop meats, as measured by the Warner-Bratzler shear knife, showed that only the freezing process itself caused softening of scallop meats. Triangle sensory comparisons between fresh and frozen scallop muscles suggested that characteristic freezing times up to 555 min may not influence the sensory quality of frozen scallop meats. Freezing of meats at the short freezing time of 19 min reduced freezing and thawing weight losses compared to longer time tested.

Key words: Scallop meats, characteristic freezing times, quality, enzymes.

INTRODUCTION

In commercial seafood industries, seafoods are frozen at a range of freezing time (rates) that depends mostly on the type of seafood, the type of freezer and freezer's operating conditions (Johnston et al., 1994). Freezing of seafoods at different freezing time may furnish favourable conditions for alterations in muscle structure, muscle proteins and textural properties in general (Love, 1966; Nilsson and Ekstrand, 1994; Makri et al., 2007). These changes are related to alterations in sensory quality of frozen seafoods and may affect their market (Shenouda, 1980; Mackie, 1993). Therefore, knowledge of optimal conditions with respect to freezing rates for freezing commercially important seafoods is relevant to the fish industry.

Freezing and thawing may cause lysis of intra-cellular organelles and disintegration of membranes resulting in a loose, disorganized tissue (Karvinen et al., 1982). Cellular disintegration can be studied by the activities of enzymes in muscle tissue fluids and enzymes in fresh tissue are retained in the intra-cellular organelles. The activity of the mitochondrial enzyme β-hydroxy-acyl-coenzyme -A dehydrogenase (HADH) has been regarded as a measure of the damage caused in mitochondria by various freezing and thawing treatments of meat and fish products

Abbreviations: **HADH,** β-hydroxy-acyl-coenzyme-A dehydrogenase; **BCA,** bicinchoninic acid; **TCA,** trichloroacetic acid; **WHC,** water holding capacity; **TWL,** total weight losses.

(Gottesman and Hamm, 1983; Makri et al., 2007).

The decrease in the water holding capacity of fish muscle after thawing is one of the most obvious effects of freezing. These properties may be influenced by the rate of freezing and/or by the water holding properties of the meat product (e.g. pH) prior to freezing (Hamm, 1975). Such a decrease is associated with the fact that during freezing, water-protein associations are replaced by protein-protein associations or other interactions (Hamm, 1975). The alterations in texture and water holding capacity of fish muscle during freezing and cold storage have been associated with changes in myofibrillar proteins (Haard, 1992). Such changes can be detected in the form of reduced solubility and extractability in saline and other extracting solutions and also reductions in myosin and actomyosin ATP-ase activities, sulfhydryl groups, apparent viscosity, gel-forming ability, etc (Shenouda, 1980).

King scallop (*Pecten maximus*), a bivalve mollusc, is much prized as food and its adductor muscle is offered to the consumers mainly as fresh and frozen product (Hardy and Smith, 1986). There is hardly any information available about the effects of the freezing process on the integrity, physicochemical and sensory properties of king scallop adductor muscle. This would be useful for achieving optimal conditions for freezing the adductor muscle of scallops for commercial purposes.

Therefore, in the present study, the effects of freezing time on the quality of the adductor muscle of scallops were studied with regard to the integrity of muscle structure, myofibrillar protein denaturation, textural and sensory changes.

MATERIALS AND METHODS

Scallop meats processing and storage

A total number of one hundred and thirty five whole scallops (*Pecten maximus*), from the Orkney fishing area, were purchased from the Aberdeen fish market. The whole live scallops were delivered to the School of Life Sciences of the Robert Gordon University packed in crushed ice on the same day as their arrival at the fish market.

At the laboratory, the scallops were shucked and the striated part of the adductor muscle, that is, the scallop meat, was separated from all other tissues. In order to ensure that the scallop meats were in post rigor state, they were stored in glass jars without any washing, buried in crushed ice and stored in a chill room at 2 to 4°C for 3 days. Post-rigor scallop meats were required in order to ensure that any difference in instrumental texture measurements would indeed result from differences in freezing and not due to the development of rigor in the raw scallop meats prior to freezing. The mean weight of scallop meats was 35 ± 5.6 g (mean ±S.D).

At the end of the storage period and in order to create a range of freezing rates, the meats were individually frozen at -20, -35 and -80°C insulated in a polystyrene box (from now on named 'slow' freezing rates) and on a perforated metal sheet (from now on named 'fast' freezing rates), without any wrapping. Additionally, any washing or glazing was not applied, which avoided any variability attributed to absorbed or melted water. For the 'slow' freezing rates, five scallop muscles were weighed and placed between two cellophane sheets in a polystyrene container having external dimensions 30 x 30 x 30 cm and wall thickness of 6 cm, which was half-filled with glass wool. The container was then filled up with glass wool; the lid was placed and wrapped at the opening with packaging tape. For the three 'fast' freezing rates, five scallop muscles were weighed and placed bare on a perforated stainless steel sheet with hexagonal 7 mm perforations.

The experiments were performed in triplicate. The duration of each replicate was 3 weeks. In the first week of each replicate, the meats were frozen 'slow' and 'fast' at -20°C, the second week at -80°C and the third week at -35°C. In each day of freezing, fifteen meats were divided into 3 batches, each consisting of five meats. One batch was immediately analysed as unfrozen controls. The two other batches were weighed and frozen at the same final temperature, either 'slow' for 36 h or 'fast' for 24 h. Immediately after freezing, the meats were weighed and then thawed. The freezing facilities were a domestic freezer cabinet with working temperatures -20 and -35°C and a deep freezer with working temperature -80°C.

During freezing, the temperature of the centre of the two 'slow' frozen scallop muscles and two 'fast' frozen scallop muscles, was monitored using T type thermocouples and a recording thermometer.

Thawing of frozen scallop meats was performed by placing the scallop meats individually on a wire gauge set on top of a plastic cup. The whole apparatus was enclosed in a plastic bag to prevent evaporation and kept at +4°C in a refrigerator for 12 h. The thawed meats were re-weighed for thawing losses determinations, and subsequently sensory, expressible fluids and instrumental texture determinations were performed. The remaining tissue from those determinations and a slice of approximate weight of 2 g, which was removed from the right surface of each scallop meat were used for the preparation of the extracts for the chemical and biochemical analyses as follows: (1) The slices from five scallop meats were used for the preparation of one extract for the HADH determinations, and the remaining tissues from five meats were pooled first and then minced in a domestic mincer. The minced meats were then immediately used for the preparation of extracts.

For the chemical and biochemical analyses, three extracts were prepared for each storage period coming from five different scallop meats. The extracts were stored in an -80°C freezer until analysis. The results of the chemical and biochemical analyses and those of sensory, expressible fluids and instrumental texture determinations were the mean of three and fifteen independent determinations, respectively.

Freezing time and rate determination

Temperature measurements were done with T type thermocouples and a recording thermometer. The thermocouples were placed at the centre of the thickest part of the scallop meats which was taken as the maximum distance between the right and the left surface of the scallop meats, and was measured by using a vernier instrument. The thermocouple was inserted at the centre of the thickest part from the lateral surface of the scallop meat.

The freezing time (t_e) was calculated as the time (min) required to decrease the temperature of the thermal centre from an average initial temperature of 4 ± 1°C to a final temperature of -20°C following the recommendations of the International Institute of Refrigeration (1986). The freezing rates at the thermal centre, expressed as cm h^{-1}, were obtained from the ratios of the distance from the surface to the thermal centre of the scallop muscles and the effective freezing time, t_e, (h); Chen and Pan, (1995) following the formula:

$$\text{Freezing rate (cm h}^{-1}\text{)} = \text{Half thickness of scallop muscle (cm)} \times t_e^{-1}$$

The characteristic freezing time (t_c) was calculated according to Bevilaqua et al. (1979) as the time (in minutes) for which the thermal centre of scallop muscles was in the temperature range of

maximal ice crystallization, that is, from -1 to -7°C.

Determination of the β-hydroxy-acyl-coenzyme–A dehydrogenase activity of scallop meats

The filtrates for the HADH (enzyme class [EC] 1.1.35) assays were prepared according to Fernandez et al. (1999). The HADH released in the filtrate was assayed according to Fernandez et al. (1999). Results were expressed as mill-units per gram of tissue.

Determination of weight losses due to freezing

Weight losses due to freezing were established by weighing the meats before and after freezing (Aurell et al., 1976). The weight losses were expressed as g per kg of weight before freezing.

Determination of water holding capacity parameters

Thawing losses

The method described by Chung and Merritt (1991a) was followed. The thawing loss was calculated from the weight difference between the initial weight of the frozen and the final weight of the thawed meats. The thawing losses were expressed as g per kg of weight after freezing.

Expressible fluids

Two cylindrical portions of each meat, 4 mm in thickness and 20 mm in diameter, were excised from the left surface of meats by means of a ring having 4 mm thickness and 20 mm diameter. Each cylinder was weighed accurately with an analytical balance and placed on a double thickness filter paper Whatman No 1 (diameter 42.5 mm). It was then covered with another double thickness filter paper and the pack was subjected to a 1,000 g force for 1 min using a Steven's texture analyzer fitted with a cylindrical flat probe (50mm diameter and 20 mm height). The cylinders were re-weighed and the expressible drip was expressed as g per kg of weight of frozen/thawed meats. The earlier mentioned force was chosen since it would cause the least possible damage to the cylinders (Chung and Merritt, 1991a). Two measurements per meat per treatment were taken.

Ca^{2+}- ATPase activities in actomyosin extracts

For the preparation of actomyosin, a portion (5 g) of the scallop meats' mince was washed with 25 ml of ice-cold de-ionised water for 15 min and drained through a chilled Buchner No 3 funnel under vacuum. This step was needed to deplete the mince of sarcoplasmic proteins. It was repeated twice.

A volume of 20 ml of iced-cold 5% (w/v) NaCl (pH 7) was added to the washed mince. The slurry was allowed to stand at 0 to 4°C for an extraction period of 1 h and subsequently was centrifuged for 30 min at 5,000 g at 4°C. The supernatant solution was designated 'actomyosin extract', and was used for protein content and Ca^{2+}-ATPase activity measurements.

The protein concentration in actomyosin extracts was determined by the bicinchoninic acid (BCA) procedure (Sigma Procedure TPRO-562, BCA-1, Sigma Biochemicals Co., St. Louis, Mo., U.S.A). The Ca^{2+}-ATPase activity was determined according to Carvajal et al. (1999). A portion (100 µl) of actomyosin extract was added to 50 µl of 0.5 M Tris-maleate (pH 7). To that mixture were then added 50 µl of 0.1M calcium chloride, 750µl de-ionized water and 50 µml 20 mM ATP solution (pH 7). The reaction was conducted for exactly three minutes at 25°C and terminated by adding 0.5 ml of chilled 15% (w/v) of trichloroacetic acid (TCA) solution. The mixture was then centrifuged at 10,000 g for 30 min and the inorganic phosphorus liberated in the supernatant was measured by the method of Fiske and Subbarow (1925). Specific activity was expressed as µmoles inorganic phosphate (Pi) released per mg protein/minute. A blank solution was prepared by adding chilled TCA prior to the addition of ATP.

Texture determination as measured by the texture analyzing system

Texture measurements were performed according to Chung and Merritt (1991b). Peak shear force measurements were performed with a Steven's texture analyzer at a crosshead speed of 50 mm per min. Shear strengths were measured with a V-shaped Warner-Bratzler shear probe mounted on the Steven's load cell.

From the central part of each meat, two cylinder portions (10 mm in diameter and 10 to 15 mm long) were excised longitudinal to muscle fibres using a cork borer. Individual cylinders were weighed and then inserted through the triangular opening of the blade and placed on the load cell in such a position that the meat fibres were at right angles to the blade penetration. The peak shear force, expressed in gram-force (g*) required to cut the cylinder into two pieces was read from the control panel of the analyzer. The shear peak force was adjusted to units of g*per g of meat cylinder to take into account the variations in weight of meat cylinders (Chung and Merritt, 1991b).

Sensory assessments

Meats from each freezing treatment were assessed against fresh post-rigor meats using triangular comparisons following the methods described by Jellinek (1993) and Botta (1995).

A portion (4 -5 g) of muscle was removed from the anterior part of each meat longitudinal to muscle fibres. It was steam-cooked for 10 min using a domestic steam cooker. After cooking, portions of meats were placed on wire gauze supported on a plastic cup; the whole apparatus was covered with a polyethylene bag and left at room temperature to cool for 30 min. The portions of meats were then used for triangular tests. Three coded samples, two fresh and one frozen, were simultaneously presented to five trained assessors. After tasting, the assessors were asked to indicate in a questionnaire which portion of meat was (a) Different (b) preferable and (c) that presented differences in textural properties. Fifteen assessments in three sessions per freezing rate were carried out. The number of correct judgments (those that correctly distinguished the frozen from the fresh samples) was compared to an appropriate table of minimum number of correct judgments to achieve a specified probability level. The differences between the fresh controls and frozen meats were significant when the number of correct judgments corresponded to the probability level $P < 0.05$. To determine which of the two meats (fresh or frozen) was preferred or presented textural differences, the results from the correct judgments were compared to a paired-tests table containing the minimum number of judgments to establish differences at different levels. The differences were significant when the number of judgments corresponded to the probability level of $P < 0.05$.

Statistical analyses

One way analysis of variance (ANOVA) was performed to test for the effects of characteristic freezing times (t_c) on each parameter measured. ANOVAs showing significant differences were followed

Table 1. Experimental conditions for freezing scallop meats*.

Freezing temperature (°C, ±S.D.)	Freezing environment	Freezing time (t_e , min)**	Freezing rate (r_e , cm/h)***	Characteristic freezing Time (t_c , min)****
-20±1	B.S	182±21*****	0.49±0.050	89±11
	I.S	2153±93	0.04±0.001	1000±148
-35±1	B.S	77±10	1.28±0.170	49±10
	I.S	976±98	0.09±0.070	555±120
-80±2	B.S	28±3	3.17±0.290	19±3
	I.S	417±53	0.21±0.030	235±57

B.S = Scallop meats frozen bare ('fast' frozen); I.S = scallop muscles frozen insulated in containers ('slow' frozen); *mean height of the thickest part was 29.9 mm; **from an initial temperature 4°C to reach central temperature -20°C (International Institute of Refrigeration 1986); ***freezing rate was calculated by dividing the half-thickness of the thickest part of the scallop muscles by the freezing time (Chen and Pan, 1995); ****from -1°C to reach central temperature -7°C (Bevilaqua et al., 1979); ***** values are means ± S.D., n = 6.

by a Tukey honestly significant difference (HSD) and in all statistical analyses, significance was accepted when $P < 0.05$ (Zar, 1984).

RESULTS AND DISCUSSION

Freezing times and rates

It is a commercial practice to freeze scallop meats individually in air blast freezing systems (Mason, 1983; Hardy and Smith, 1986). Plate freezers are normally designated for the production of frozen foodstuffs into blocks, but they can also be used for freezing of individual particles (Aurell et al., 1976). Typical freezing rates that can be met by freezing of seafood by such freezing systems range from 0.3 to 3 cm/h. Freezing rates of 0.2 to 0.1 cm/h can be met by bulk freezing of seafood in batch air blast–rooms (International Institute of Refrigeration, 1986). Although quick freezing of scallop meats is a common commercial practice, slow freezing of scallop meats can occur in badly designed and operated freezers. Therefore, the experimental conditions of freezing scallop meats in the present study produced freezing time and rates (Table 1) that can be met in commercial practice of freezing scallop meats.

Effect of freezing times on β-hydroxy-acyl-coenzyme –A dehydrogenase activity in scallop meats

The activities of HADH in frozen and thawed meats were significantly higher than those in fresh meats ($P < 0.05$; Figure 1), in agreement with the findings for plaice, whiting, mackerel (Dulfos et al., 2002) and gilthead seabream (Makri et al., 2007). Therefore, the freeze-thaw process itself affects the integrity of mitochondria of scallop meats. The freeze damage of mitochondria has been attributed to dehydration of the mitochondrial inner membrane and/or mechanical disruption of mitochondrial membranes by ice crystals (Hamm and Gottesmann, 1982).

The results of the present study show that the activities of HADH in frozen meats at times (t_c values) from 1000 to 235 min were significantly less than those in meats frozen at times from 89 to 19 min ($P < 0.05$; Figure 1). It has been reported by Love (1966) that freezing times of about 100 min marked the changeover from intra- to inter-cellular ice formation in post-rigor cod. In addition, this process of ice formation was accompanied by a migration of water from the muscle fibers into the inter-cellular spaces causing dehydration of cod muscle cells. Thus, it is suggested that freezing of meats at times from 1000 to 235 min should result in inter-cellular ice formation and hence the freeze damage of mitochondria could be due to the dehydration of mitochondrial membranes. Freezing of meats at times (t_c values) from 89 to 19 min should mainly, result in intra-cellular ice formation (Love, 1955). Thus, the additional damage of mitochondrial membranes observed at times from 89 to 19 min compared to that of the times from 1000 to 235 min could be due to mechanical damage and dehydration of mitochondrial membranes caused by ice crystals formation in the vicinity or even inside of the mitochondria. Similar observations and results have been reported by previous workers for bovine muscle (Hamm and Gottesmann, 1982), trout (Garcia de Fernando et al., 1992), squid and hake (Pavlov et al., 1994). However, the characteristic freezing time of 19 min seems to cause less release of the HADH enzyme from mitochondria into meats than the characteristic freezing time of 35 and 89 min (Figure 1). This observation implies that freezing of scallop meats at the characteristic freezing time of 19 min might have caused less damage to intra-cellular organelles than the longer times of 35 and 89 min. This suggestion may be attributed to the smaller and less destructive intra-cellular ice crystals, which would have formed in the scallop muscles frozen at the short characteristic freezing time of 19 min compared to the larger and more destructive intra-cellular ice crystals formed in the scallop meats frozen at longer time (t_c values) of 35 and 89 min (Love, 1955).

Overall, the results show that the freezing process itself

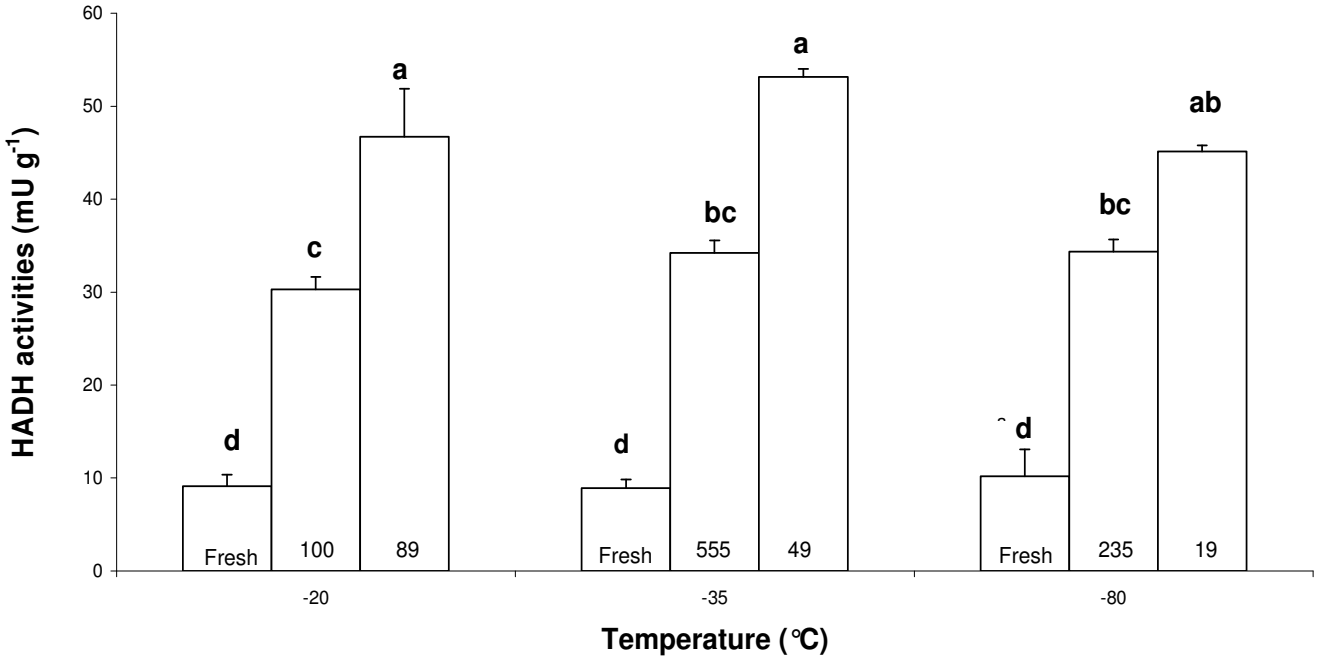

Figure 1. β-hydroxy-acyl-coenzyme-A dehydrogenase activities (mU g^{-}). Values are means ± SEM, n = 3; numbers inside the bars are the characteristic freezing time (tc values) in minutes. Groups with different letters (a, b, c and d) are significantly different (P < 0.05).

Table 2. Weight losses due to freezing.

Scallop meats frozen in the container		Scallop meats frozen bare	
Characteristic freezing time (t_c, min)	Weight losses (g kg^{-1})	Characteristic freezing Time (t_c, min)	Weight losses (g kg^{-1})
1000	13.8±0.9	89	39.4±1.1a
555	13.5±0.5	49	18.2±0.7b
235	14.0±0.5	19	11.1±0.3c

t_c = Characteristic freezing time in minutes. Values (means ± S.E.M) in columns, n = 9; values in the same row without letters are not significantly different (P >0.05).

clearly affected the integrity of scallop meats' mitochondria. Moreover, freezing of scallop muscles at times (t_c values) from 1000 to 235 min caused less damage to mitochondria than the shorter time tested.

Effect of freezing time on freezing weight losses

The results of the present study show that an increase in freezing time (t_c values) caused an increase in weight loss during freezing of the unpacked meat (Table 2).

Similar results for other food products have been reported by other workers. Aurell et al. (1976) evaluated the effects of freezing by liquid freon freezant (freezing times from 50 s to 3 min) and air blast (freezing times from 6 to 22 min) on weight losses of lemon sole, shrimps and scallop muscles. According to the results of that study, weight losses of all products were less by freezing in liquid freon freezant than by air blast. Nusbaum et al. (1983) investigated the effects of four freezing time, that is, 6, 30 80 and 100 min, on weight losses during freezing of unpacked beef patties. The results showed that short freezing time caused less weight losses of the patties compared to longer time. Petrovic et al. (1993) showed that an increase in freezing rate caused a decrease in weight loss during freezing of unpacked beef steaks. Altogether, these findings suggest that freezing time affected weight losses during freezing of the unpacked meats with the freezing time of 19 min showing the lowest losses. This can be due to the fact that freezing of meats over a time of 19 min decreased the surface temperature of the meats quickly to a value where the rate of moisture evaporation or sublimation was lower than the evaporation values at the other freezing times

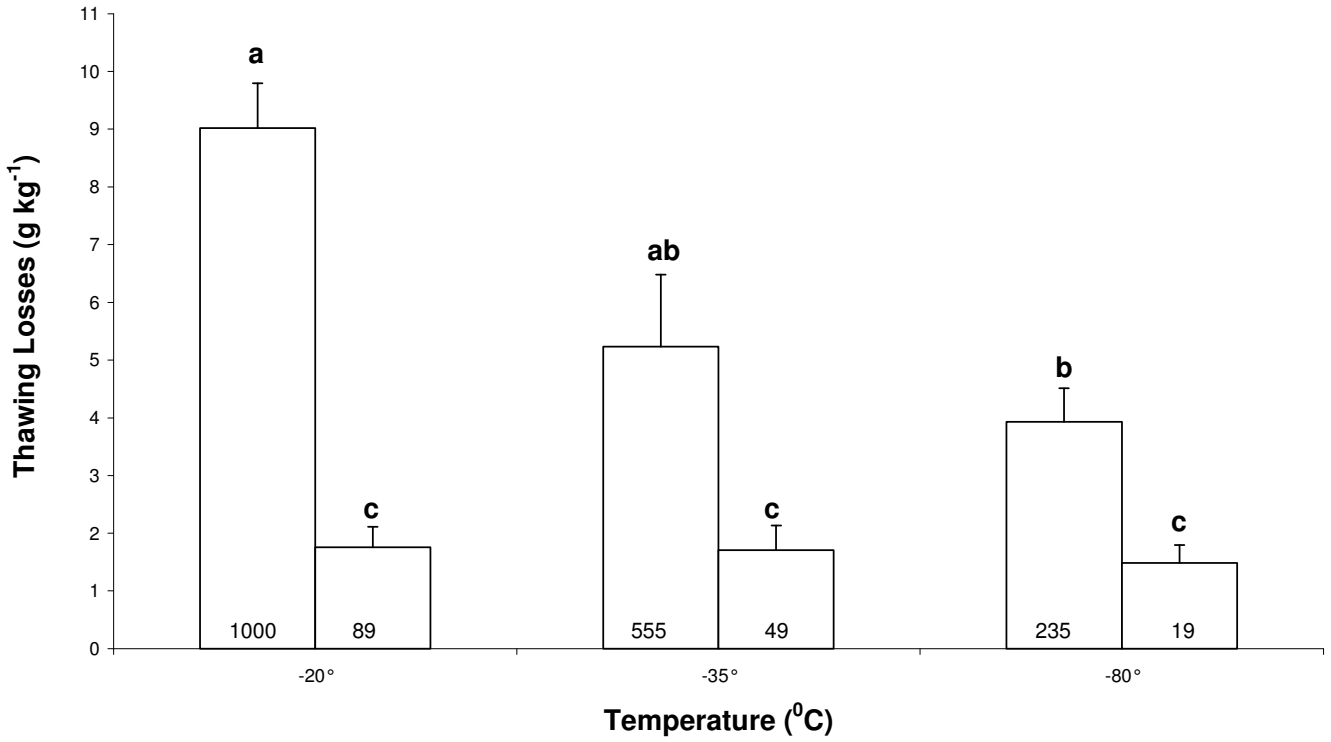

Figure 2. Thawing weight losses (g kg^{-1}). Values are means ± SEM, n = 7 - 9. Numbers inside the bars are the characteristic freezing times (t_c values) in minutes. Groups with different letters (a, b and c) are significantly different ($P < 0.05$).

(International Institute of Refrigeration, 1986).

Effects of freezing time on water holding parameters

Thawing weight losses of meats frozen from 1000 to 235 min were higher than those of scallop muscles frozen at t_c values of 19 to 89 min (Figure 2).

Several studies have reported increases in thawing weight losses of seafood products with increase in freezing times. It has been reported that the drip losses after thawing of scallop meats were higher when using an air blast freezer (freezing time 15 min) compared to a liquid freon freezant (freezing time 3 min) (Aurell et al., 1976). Whiting fillets frozen at a freezing rate of 0.77°C/min showed less thawing losses than those frozen at a rate of 0.14°C/min (Chevalier et al., 1999). Thawing losses of gilthead seabream fillets frozen at a characteristic freezing time of 640 min were higher than those of fillets frozen at characteristic freezing time of 2, 18 and 17 min (Makri et al., 2007).

The differences in thawing weight losses of meat products frozen at different freezing time have been attributed mainly to changes in myofibrillar proteins and/or to distortion and destruction of muscle fibres by ice-crystals. However, as will be shown in the following section, the freezing times tested in the present study did not seem to cause an irreversible denaturation of myofibrillar proteins of 'fast' and 'slow' frozen meats (in terms of Ca^{2+}-ATPase activities of actomyosin), possibly because no storage time was included in the present study. Thus, the differences in the amount of exudates released from meats during thawing might be due to differences in the size and location of ice crystals and to differences in the mechanical damage of muscle fibres. Scallop meats frozen at the 'slow' characteristic freezing times (t_c values of 1000, 555 and 235 min) would have inter-cellular ice that led to internal water diffusing out of the cells, leaving little ice in the cells. It is, therefore, suggested that when the slow frozen meats were thawed, much thawing drip exuded, presumably because a quantity of the inter-cellular water formed by melting of the inter-cellular ice in the inter-cellular spaces was free to escape from the inter-cellular spaces before it could be reabsorbed by the fibres. Scallop meats frozen at a 'fast' characteristic freezing time (t_c) of 19 min would have intra-cellular ice crystals. These ice crystals would be small in size since it took only 28 min to freeze the meats at -20°C (freezing time (t_e value) of 28 min) (Table 1). Thus, it is likely that when these meats were thawed, the water being *in situ* already did not to any appreciable extent, escape as drip. The scallop meats frozen at the characteristic freezing time (t_c values) of 49 and 89 min might have frozen fast enough to form intra-cellular ice, but these scallop meats continued to freeze at a much slower rate such that the intra-cellular ice might have time (freezing times (t_e values)

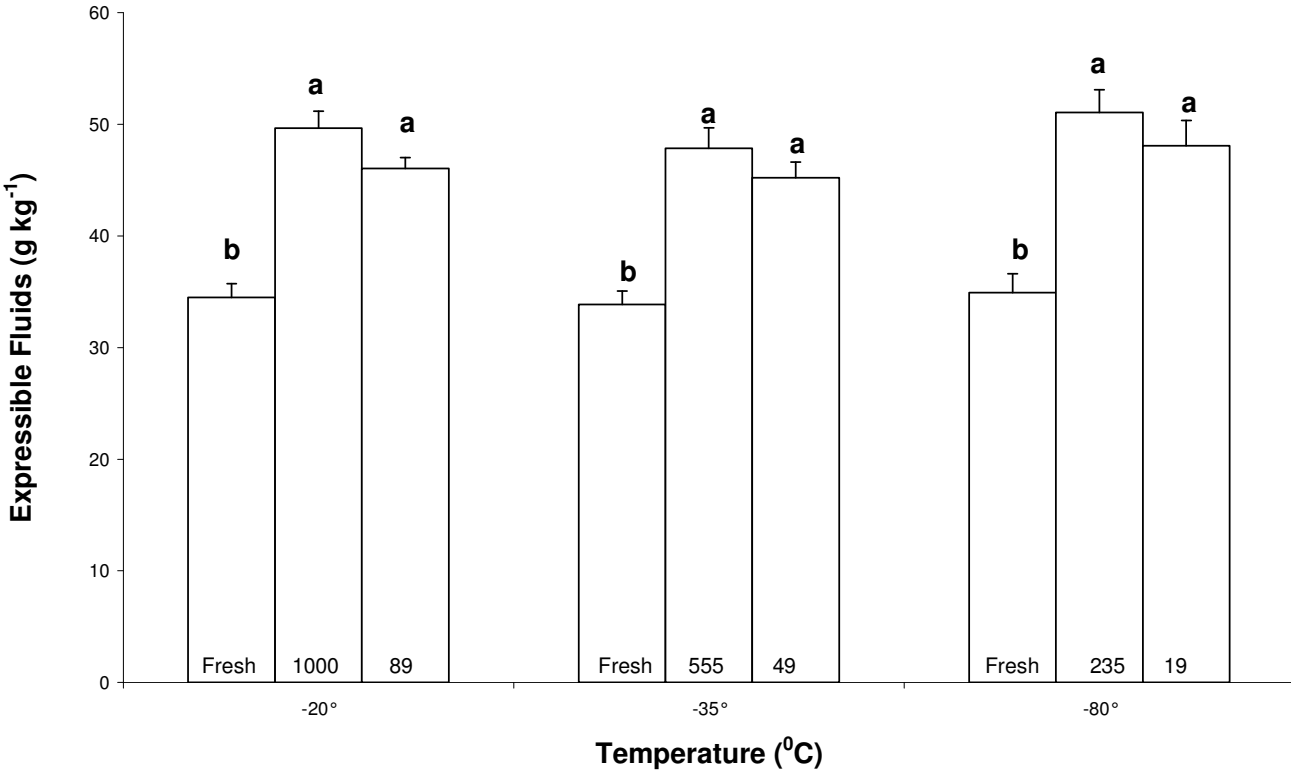

Figure 3. Expressible fluids, g kg-1. Values are means ± SEM., n = 15. Numbers inside the bars are the characteristic freezing times (t_c values) in minutes. Groups with different letters (a, b) are significantly different ($P < 0.05$).

of 77 and 189 min, respectively) (Table 1), to grow to disruptive size and rupture the cells before the meats reached the final respective temperatures, that is, -35 and -20°C. As a consequence of such cell damage, more drip loss during thawing of the meats frozen at the characteristic time of 49 and 89 min compared to those frozen at 19 min would be expected. However, this was not the case; thawing weight losses from meats frozen at a characteristic freezing time of 19 min were equal to 14.9 g kg^{-1} of tissue and slightly less than those of meats frozen at characteristic freezing time of 49 (17 g kg^{-1} of scallop tissue) and 89 min (17.6 g kg^{-1} of scallop tissue) (Figure 2). This might have been due to the higher dehydration losses the meats suffered during freezing at the characteristic freezing time of 49 and 89 min compared to those of 19 min (Table 2). Thus, a part of the water that could have been released during thawing of scallop muscles frozen at characteristic freezing time of 49 and 89 min might have been evaporated during freezing. These suggestions are in agreement with the statement made by Hamm (1986) that there is a direct relation between the freezing time, the movement of the water within muscle and the weight loss during thawing of a meat product. It must be mentioned, also, that the total weight losses due to freezing and thawing of scallop meats frozen at a characteristic freezing time of 19 min (26 g kg^{-1} of scallop meat) were less compared to those of scallop muscles frozen at characteristic freezing time of 49 min (35.2 g kg^{-1} of scallop muscle) and 89 min (60 g kg^{-1} of scallop muscle), respectively.

In contrast to thawing weight losses, the values of the expressible fluids were similar for all frozen groups of scallop meats and significantly higher compared to those of fresh meats (Figure 3). In the present study, expressible fluid measurements were taken by pressing the meats after thawing, that is, after the meats had released part of the unbound or loosely bound water. These measurements can be considered as indicators of the state of the remaining quantity of loosely bound water in thawed meats, as reported by Petrovic et al. (1993) for frozen beef. Since water holding capacity of a meat product is indicative of the water loosely bound to protein (Trout, 1988), the total losses during thawing and pressing were taken as a measure of the water holding capacity (WHC) of the fresh and frozen scallop meats. Total weight losses (TWL) were significantly higher in frozen than in fresh scallop muscles (Figure 4), but differences between the frozen groups themselves were not observed. Since changes in WHC are very sensitive indicators of the changes in the charges and structure of myorfibrillar proteins (Mackie, 1993), the results suggest that the freezing process itself, but not the freezing time, affected the properties of myofibrillar proteins of scallop muscles. This suggestion was further investigated by

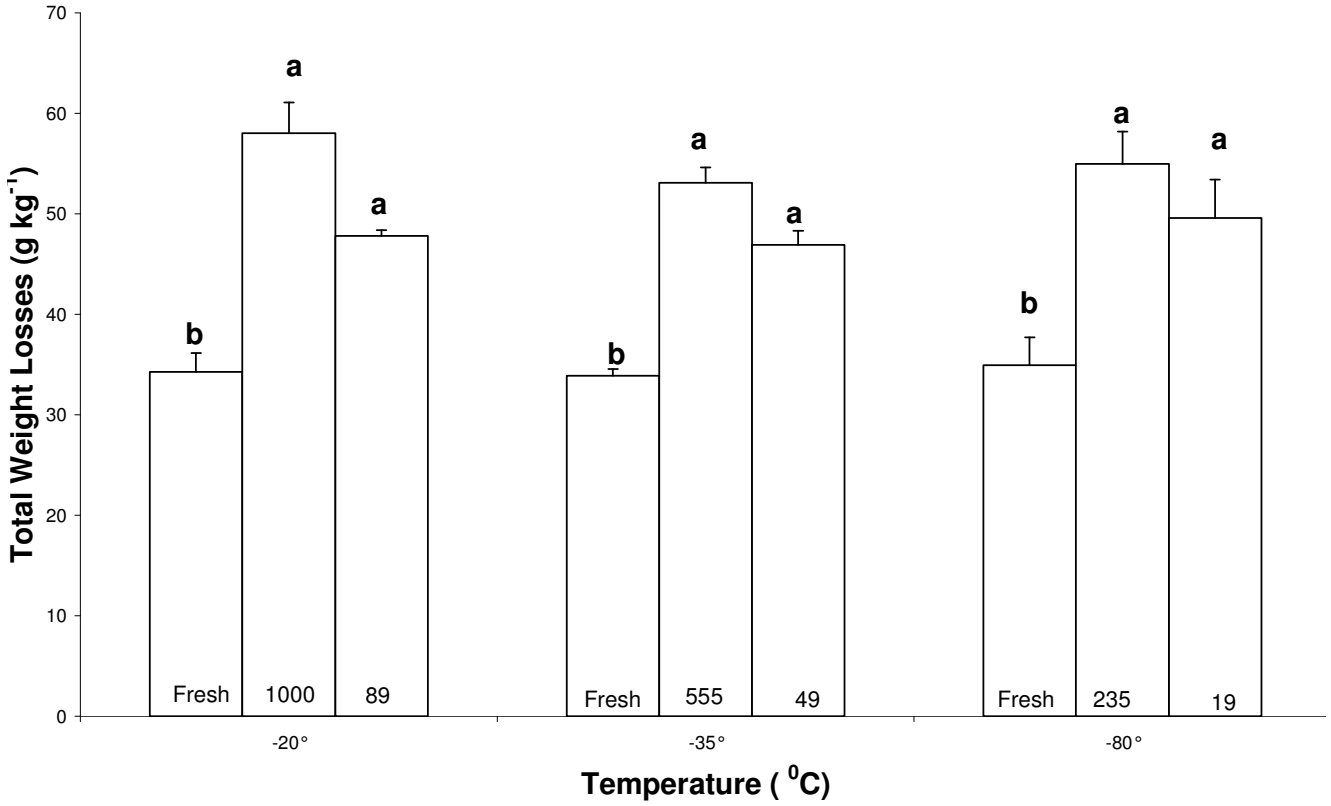

Figure 4. Total weight losses, (g kg^{-1}). Values are means ± SEM, n = 3. Numbers inside the bars are the characteristic freezing time (t_c values) in min. Groups with different letters (a, b) are significantly different (P < 0.05).

analyses related to myofibrillar protein denaturation.

Effects of freezing times on Ca^{2+}-ATPase activities

In order to examine the possibility of denaturation of myofibrillar proteins, Ca^{2+}-ATPase activities in actomyosin extracts from fresh and frozen groups of scallop meats were measured (Figure 5). The Ca^{2+}-ATPase activities from all fresh groups of scallop meats were significantly higher (P < 0.05) compared to those of frozen and thawed scallop meats at all freezing times. Since Ca^{2+}-ATPase activity can be used as an indicator for the integrity of myosin molecules, the decrease of Ca^{2+}-ATPase activity due to freezing could indicate a change on the myosin head, which contains the active site of the enzyme. This change could be a result of partial tertiary structural change (unfolding) of myosin due to a weakening of intra-molecular hydrophobic bonds. This could be a result of local increase of ionic strength as a consequence of freezing and water migration from the myofibrillar space or dehydration of myofibrillar proteins (Benjakul and Bauer, 2000). Similar suggestions were reported by Wagner and Anon (1985) after studying the effects of freezing and freezing times on the denaturation of myofibrillar proteins of beef muscle.

There was no clear effect of the freezing times on myofibrillar proteins (in terms of Ca^{2+}-ATPase activity) of scallop meats (Figure 5). Although more research is needed to confirm this, it may be related to the very short time that the meats remained in the frozen state, that is, 24 h for the 'fast' frozen scallop muscles and 36 h for the 'slow' frozen scallop muscles. During freezing of fish muscle, part of the intra-cellular water freezes out but a considerable portion of it remains unfrozen. Consequently, the concentration of solutes in the unfrozen phase increases considerably. An increase in solute concentration may affect protein properties. At high ionic strength solutions, as might be the case of 'slow' frozen scallop muscles, myosin molecules undergo a rapid, but reversible dissociation into heavy cores and light polypeptide chains. Extended storage of myosin in those solutions could cause irreversible aggregation between myosin's subunits (Shenouda, 1980). Several previous studies have shown no changes in myofibrillar proteins of meat products frozen at different freezing times. Reid et al. (1986) found no difference in Ca^{2+}-ATPase activities between fast (2 h) and slowly (24 h) frozen Pacific rockfish immediately after freezing. Srinivasan et al. (1997) showed no significant changes in myosin of freshwater prawns after blast (fast) and still (slow) freezing. Ngapo et al. (1999) showed that freezing times from 12 min to

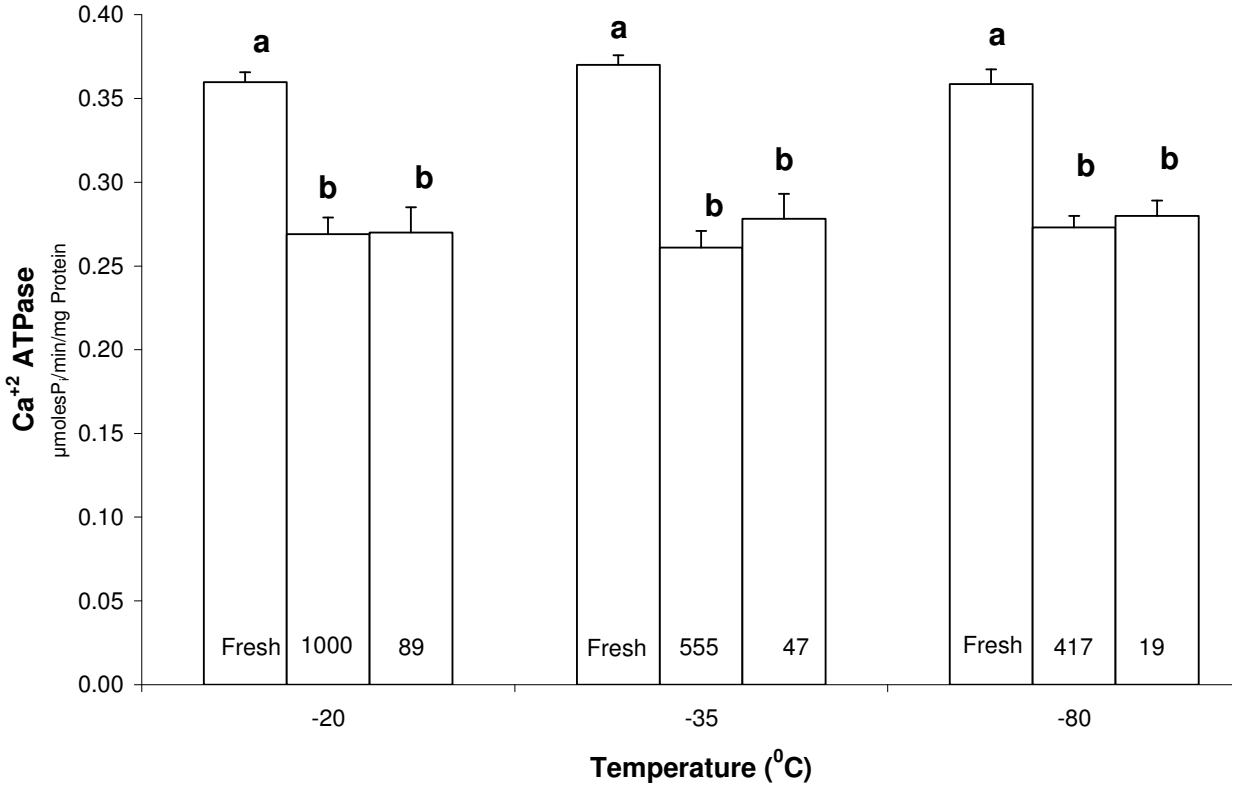

Figure 5. Ca^{2+}-ATPase activities, µmoles Pi/mg protein/min. Means ± SEM, n = 3. Numbers inside the bars are the characteristic freezing times (t_c values) in minutes. Groups with different letters (a, b) are significantly different ($P < 0.05$).

more than 900 min did not cause protein denaturation of small pork samples immediately after freezing, but the thawing losses from samples frozen at

freezing time of 240 and 900 min were significantly more than those of samples frozen from 12 to 120 min. Adenosine triphosphatase (ATPase) activities of acto-myosin extracts from fresh and frozen at characteristic freezing time of 2, 18, 74 and 640 min gilthead sea-bream, suggested that the freezing process itself, but not the freezing time caused structural changes to myofibrillar proteins (Makri et al., 2007).

Therefore, the results of this section suggest that there was no clear effect of freezing time on Ca^{2+}-ATPase activities of myofibrillar proteins of scallop meats, but that the freezing process itself might have caused changes in myofibrillar proteins of scallop meats (in terms of Ca^{2+}-ATPase activities).

Effects of freezing times on instrumental texture measurements

The peak shear forces obtained from the fresh meats were significantly higher than those of frozen scallop muscles (Figure 6). This means that the freezing process itself caused softening of raw scallop meats.

Freezing and thawing cause lysis of lysosomes and release into sarcoplasm of proteases, which cause break-down of muscle proteins and consequent tissue-softening (Pan and Yeh, 1993; Civera et al., 1996). Therefore, it is likely that the freezing process itself causes release of proteolytic enzymes from lysosomes of scallop meats with concomitant softening of their tissue. Although significant differences between the frozen experimental groups of meats were not observed, meats frozen at freezing times (t_c values) from 19 to 89 min showed slightly higher peak shear forces compared to those frozen from 235 to 1000 min. Nilsson and Ekstrand (1993) showed that short freezing times released less lysosomal enzymes in trout muscle compared to longer ones. Hence, the freezing time from 19 to 89 min might have caused less damage to lysosomes, and therefore, less softening, than the times from 235 to 1000 min. Therefore, the freezing process itself appeared to cause softening of scallop meats.

Effects of freezing times on sensory attributes

The results from the sensory assessments of the present study may suggest that meats frozen at a freezing time (t_c value) of 1000 min were less tender than the fresh ones. However, differences in the textural attributes between the fresh meats and the other frozen groups of scallop

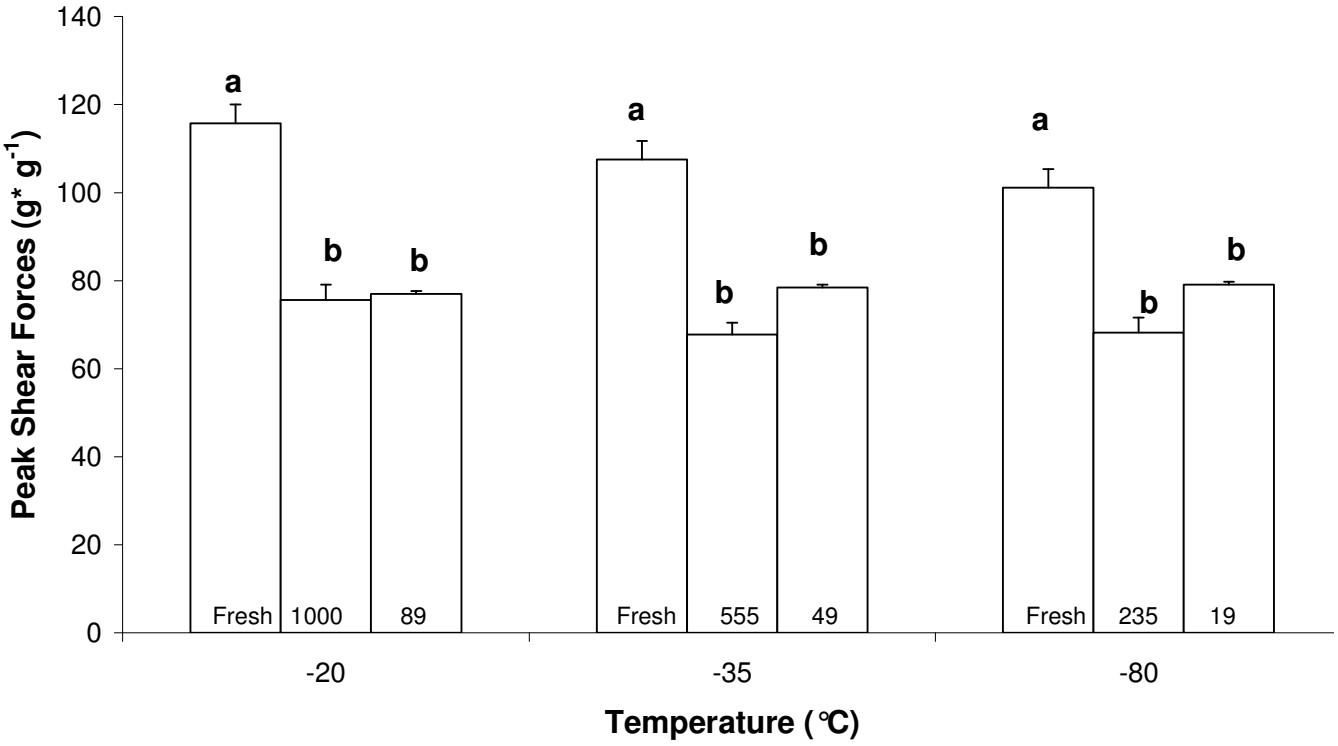

Figure 6. Peak shear forces, g* g^{-1} Means ± S.E.M., n = 15. Numbers inside the bars are the freezing times (tc values) in minutes. Groups with different letters (a, b) are significantly different ($P < 0.05$).

meats were not observed (Table 3). Other reported studies on the effects of short and moderate freezing times on sensory attributes of frozen seafoods have failed to show any freezing time effect. This was the case with scallop meats frozen from 3 to 45 min, lemon sole frozen from 4 to 41 min and shrimps frozen from 50 s to 30 min (Aurell et al., 1976). In addition, Desrosier and Tressler (1977) reported that a freezing time of several hours to as long as 26 h did not significantly influence the quality of the frozen fish as judged by organoleptic examination. Moreover, Love (1966) stated that the differences, which may exist in the sensory quality of foodstuffs frozen at different rates and thawed after freezing, that is, without cold-storage, are not great enough to be obvious to many of the investigators.

Therefore, the results of the present study suggest that freezing time (t_c values) up to 555 min may not influence the sensory quality of frozen scallop meats. Differences may exist in the sensory quality of frozen groups of scallop meats, but these were not large enough to be obvious to the panel of this study.

Conclusions

In summarizing the main findings and discussion of the effects of freezing time on quality parameters of scallop meats, it can be said that: 1. There was a clear effect of freezing times only on the integrity of intra-cellular organelles (mitochondria) and weight losses due to freezing and thawing of scallop meats; 2. the longer freezing time tested (from 235 to 1000 min) caused less injury to mitochondria and more thawing losses than the shorter time tested; 3. the short freezing time of 19 min reduced the total weight losses due to freezing and thawing more than the time of 49 and 89 min, to cause less damage to intra-cellular organelles (mitochondria) than the freezing time of 49 and 89 min appeared also; 4. Triangle sensory comparisons between fresh and frozen scallop muscles suggested that characteristic freezing times up to 555 min may not influence the sensory quality of frozen scallop meats.

Thus, post rigor scallop muscles can be frozen at a variety of freezing time and rates that can be met in most common commercial freezing methods (e.g. air blast freezing systems and plate freezers) without considerable change in their sensory quality. However, freezing of scallop meats at the short freezing time of 19 min reduced freezing and thawing weight losses compared to longer time tested. From the work reported in the literature, increased weight losses on freezing and thawing of frozen seafoods may influence adversely their quality and consequently their market. It is suggested, therefore, that short characteristic freezing times such as that of 19 min (freezing time of 28 min and freezing rate of 3.17 cm/h), may be beneficial for freezing post-rigor scallop meats

Table 3. Results of the triangle comparisons between fresh and frozen scallop meats.

t_c (min)	Number of evaluations	Correct number of evaluations	Incorrect number of evaluations	More chewy		More juicy		More firm		Preferred	
				Frozen	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen	Fresh
19	15	7	8	-	-	-	-	-	-	-	-
49	15	9 x	6	3	6	2	7	6	3	5	4
89	15	7	8	-	-	-	-	-	-	-	-
235	15	8	7	-	-	-	-	-	-	-	-
555	15	7	8	-	-	-	-	-	-	-	-
1000	15	10 xx	5	9 x	1	3	7	5	5	3	7

tc = Characteristic freezing time; numbers followed by x were significantly different at 5% level. Numbers followed by xx were significantly different at 1% level.

compared to longer characteristic freezing time tested.

REFERENCES

Aurell T, Dagbjartsson B. Salomonsdottir E (1976). A comparative study of freezing qualities of seafoods obtained by using different freezing methods. J. Food Sci. 41: 1165-1167.

Benjakul S, Bauer F (2000). Physicochemical and enzymatic changes of cod muscle proteins subjected to different freeze - thaw cycles. J. Sci. Food Agric. 80: 1143-1150.

Bevilaqua A, Zaritzky NE, Calvelo A (1979). Histological measurements of ice in frozen beef. J. Food Technol. 14: 237-251.

Botta JR (1995). Evaluation of seafood freshness quality. New York, USA: VCH Publishers, Inc.

Carvajal PA, MackDonald GA, Lanier TC (1999). Cryostabilization mechanism of fish muscle proteins by maltodextrins. Cryobiology, 38: 16-26.

Chen YL, Pan BS (1995). Freezing tilapia by air blast and liquid nitrogen-freezing point and freezing rate. Inter. J. Food Sci. Technol. 30: 167-173.

Chevalier D, Bail AL, Chourot JM, Chantreau P (1999). High pressure thawing of fish (Whiting): Influence of the process parameters on drip losses. *Lebensm.-Wiss. u.*-Technol. 32: 25-31.

Chung SL, Merritt JH (1991a). On - board handling and freezing of the sea scallop, *Placopecten magellanicus*. Int. J. Food Sci. Technol. 26: 695-705.

Chung SL, Merritt JH (1991b). Physical measures of sensory texture in thawed sea scallop meat. Inter. J. Food Sci. Technol. 26: 207-210.

Civera T, Rebufati P, Parisi E (1996). Pesce decongelato: Validita e limiti di alcuni metodi analitici e proposte per il loro utilizzo. Industrie Alimentari, 35: 813-818.

Desrosier NW, Tressler DK (1977). Fundamentals of food freezing. Westport, Connecticut, USA: AVI Publishing Company, Inc.

Dulfos G, Fur BL, Mulak V, Becel P, Malle P (2002). Comparison of methods of differentiating between fresh and frozen-thawed fish or fillets. J. Sci. Food Agric. 82: 1341-1345.

Fernandez M, Mano S, Garcia de Fernando GD, Ordóñez JA, Hoz L (1999). Use of β-hydroxy-acyl-CoA-dehydrogenase (HADH) activity to differentiate frozen from unfrozen fish and shellfish. Eur. Food Res. Technol. 209: 205-208.

Fiske C, Subbarow Y (1925). The colorimetric determination of phosphorus. J. Biol. Chem. 66: 375-400.

Garcia de Fernando, GD, Fernández M, Diaz O, Ordóñez JA, Hoz L (1992). An objective method to differentiate between fresh and thawed trout meat. Arch. Lebensmittelhygiene, 43: 13-14.

Gottesman P, Hamm R (1983). New biochemical methods of differentiating between fresh meat and thawed, frozen meat. *Fleischwirtsch.* 63: 219-221.

Haard NF (1992). Biochemical reactions in fish muscle during frozen storage. In Seafood Science and Technology, pp. 176-209 (Ed Bligh E). Oxford, UK: Fishing New Books.

Hamm R (1975). Water holding capacity of meat. In Meat, Vol. 16, p. 321 (Eds Cole BJA and Lawrie RA). London, UK: Butterworth Publications.

Hamm R (1986). Functional properties of the myofibrillar system and their measurements. In Muscle as Food, pp. 135-199 (Ed Bechtel PJ). Florida, USA: Academic Press, Inc.

Hamm R, Gottesmann P (1982).Release of mitochondrial enzymes by freezing and thawing of meat: structural and analytical aspects. In 28th European meeting. Meat Research Workshop Congress, Vol. 1, 152-155 Madrid.

Hardy R, Smith JGM (1986). Catching and processing scallops and queens. Torry Advisory Note 46. Aberdeen, UK.

International Institute of Refrigeration (1986). Recommendations for the processing and handling of frozen foods. Paris, France: International Institute of Refrigeration.

Jellinek G (1993). Sensory evaluation of food: Theory and Practice. Chichester, U.K: Ellis Horwood Ltd.

Johnston WA, Nicholson FJ, Roger A, Stroud GD (1994). Freezing and refrigerated storage in fisheries. FAO Fisheries Technical Papers-T340. Rome, Italy: Food and Agricultural Organization.

Karvinen VP, Bamford DH, Granroth B (1982). Changes in muscle sub cellular fractions of Baltic herring (*Clupea harengus membras*) during cold and frozen storage. J. Sci. Food Agric. 33: 763-770.

Love RM (1955). The expressible fluid of fish fillets. I. - Nucleic acid as an index of cell damage in fillets frozen from both sides. J. Sci. Food Agric. 6: 30-37.

Love RM (1966). The freezing of animal tissue. In Cryobiology, pp. 317-405 (Ed HT Meryman). London, UK: Academic Press.

Mackie IM (1993). The effects of freezing on flesh proteins. Food Rev. Int. 9: 575-610.

Makri M, Melvin M, Xotos G, Doubi X (2007). The bio-chemical and sensory properties of gilthead seabrem (*Sparus aurata*) frozen at different characteristic freezing times. J. Food Qual. 30: 970-992.

Mason J (1983). Scallop and queen fisheries in the British Isles. Farnha, Surrey, UK: Fishing News Books.
Ngapo TM, Babare IH, Reynolds J, Mawson RF (1999). Freezing and thawing rate effects on drip loss from samples of pork. Meat Sci. 53: 149-158.
Nilsson K, Ekstrand B (1993). The effect of storage on ice and various freezing treatments on enzyme leakage in muscle tissue of rainbow trout (*Oncorhynchus mykiss*). Z Lebensm Unters Forsch 197: 3-7.
Nilsson K, Ekstrand B (1994). Enzyme leakage in muscle tissue of rainbow trout (*Oncornychus mykiss*) related to various thawing treatments. Z Lebensm Unters Forsch, 198: 253-257.
Nusbaum RP, Sebranek JG, Topel DG, Rust RE (1983). Structural and palatability relationships in frozen ground beef patties as a function of freezing treatments and product formulation. Meat Sci. 8: 135-146.
Pan BS, Yeh WT (1993). Biochemical and morphological changes in grass shrimp (*Penaeus monodon*) muscle following freezing by air blast and liquid nitrogen methods. J. Food Biochem. 17: 147-160.
Pavlov A, Garcia de Fernando GD, Diaz O, Fernández O, López D, Ordóñez, JA, Hoz L (1994). Effect of freezing on the β-hydroxy-acyl-CoA- dehydrogenase (HADH) activity of fish meat. Z Lebensm Unters Forsch, 198: 456-468.
Petrovic L, Grujic R, Petrovic M (1993). Definition of the optimal freezing rate-2. Investigation of the physico-chemical properties of *M. longissimus dorsi* frozen at different freezing rates. Meat Sci. 3: 319-331.
Reid DS, Doong NF, Snider M, Foin A (1986). Changes in the quality and microstructure of frozen rockfish. In Seafood quality determination, 1-15 (Ed DE. Kramer, JListon). Amsterdam, Netherlands: Elsevier Science Publishers.
Shenouda SYK (1980). Theories of protein denaturation during frozen storage of fish flesh. Adv. Food Res. 26: 275-311.
Srinivasan S, Xiong YL, Blanchard SP (1997). Effects of freezing and thawing methods and storage time on thermal properties of freshwater prawns (*Macrobrachium rosenbergii*). J. Sci. Food Agric. 75: 37-44.
Trout GR (1988). Techniques for measuring water-binding capacity in muscle foods-A review of methodology. Meat Sci. 23: 235-252.
Wagner JR, Anon MC (1985). Effect of freezing rate on the denaturation of myofibrillar proteins. J. Food Technol. 20: 735-744.
Zar JH (1984). Biostatistical analysis. U.S.A: Prentice Hall International.