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Effect of freezing methods, frozen storage time, and thawing methods on the quality of mildly cooked snow crab (*Chionoecetes opilio)* clusters

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ABSTRACT

Mildly cooked snow crab clusters were subjected to three freezing methods (in brine, in still air, and in circulating air), two frozen storage times (72 h and 6 months), and two thawing methods (in air and in circulating water) followed by refrigerated storage. The clusters were evaluated for weight changes during freezing, frozen storage and thawing as well as for drip loss and melanosis during the refrigerated storage. Both the freezing method and the frozen storage time significantly affected the weight changes ($P < 0.001$) and drip loss ($P < 0.015$), likely due to differences in the protein denaturation and crystallization phenomena related to the rates of freezing and thawing. Freezing in brine allowed the least weight loss during the freezing process whereas freezing in circulating air followed by short frozen storage and thawing in air minimized the weight loss during the frozen storage and thawing process. The melanosis was significantly affected by both the freezing and the thawing method ($P < 0.001$) in the case of short frozen storage, whereas for long frozen storage, only the effect of the freezing method was significant. To minimize melanosis, the clusters should be frozen in brine and thawed in water.

1. Introduction

Snow crab (*Chionoecetes opilio*) is widely distributed in the North Pacific, the Arctic, the northwest Atlantic Ocean, and in the Barents Sea (Siikavuopio et al., 2017). The commercial fishery is growing fast as a response to increasing demand, especially from the EU, USA, Japan, and South Korea (Norwegian Seafood Council, 2019). In 2018, 2697 tons of snow crabs were captured and processed in the Norwegian economic zone (Norwegian Fishermen's Organization, 2019).

In snow crabs, the edible meat is in the cluster which comprises four walking legs and one claw assembled in a shoulder joint. The shelf life of snow crab clusters cooked to a leg core temperature of 91 °C, followed by storage at 0 or 4 °C, is 14 or 10 days, respectively (Lorentzen, Rotabakk, Olsen, Skuland, & Siikavuopio, 2016). Considering the time required to distribute these products from Norway to long-distance markets, the remaining shelf life becomes a critical and limiting factor. Due to this, freezing is a suitable and preferred preservation method. Nowadays, the entire volume of snow crab captured by Norwegian vessels is preserved by cooking and freezing. Freezing does not improve the product quality; it prolongs the shelf life (Espinoza Rodezno et al., 2013). Factors influencing the frozen food quality are freezing rate, storage temperature, temperature fluctuations, freezing-thawing abuse during storage, distribution systems, thawing rate, retail display, and handling by consumers (Boonsumrej, Chaiwanichsiri, Tantratian, Suzuki, & Takai, 2007).

It is well known that irrespective of the freezing method, the formation of ice can cause substantial changes in the food structure due to the transformation of liquid water into ice. Previously, it has been demonstrated a relationship between the size of ice crystals and the freezing rate in beef (Bevilacqua, Zaritzky, & Calvelo, 1979), and apple tissue (Bomben & King, 1982). Furthermore, the influence of air blast and immersion freezing on the rate of ice crystal formation has also been investigated, leading to the conclusion that the mean ice crystal diameter varied inversely with the local freezing rate (Chevalier, Le Bail, & Ghoul, 2000).

The morphology of the ice crystals influences cell disruption as well as the denaturation of cell components. This often results in textural changes and increased drip loss during thawing and subsequent storage (Bahuaud et al., 2008). When thermal thawing methods are applied, the required thawing time depends on a series of factors such as the dimension and shape of the product, thermal conductivity, change in enthalpy, initial and final temperature, thermal properties of the

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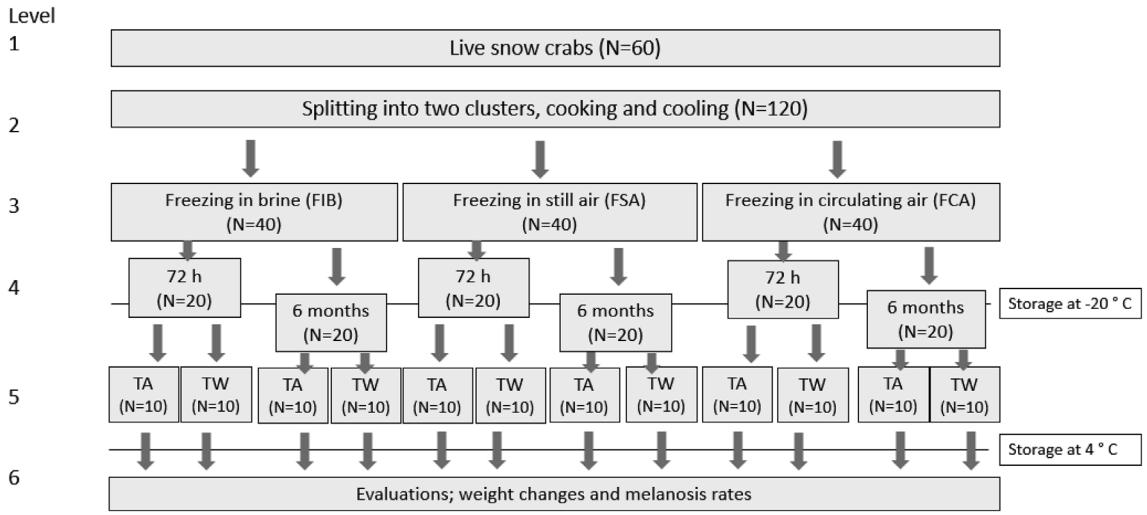

Fig. 1. Setup of the experiment with the factors freezing methods (FIB, FSA, and FCA), frozen storage intervals (72 h and 6 months), and thawing methods (TA, TW).

thawing medium and temperature, humidity (in case of thawing in air) and velocity of the thawing medium (Bøgh-Sørensen, 2006).

Both freezing and thawing methods influence the weight change of the thawed food product (Campañone, Salvadori, & Mascheroni, 2001). Weight loss can be a critical factor in terms of change in quality attributes, but also in trading, as the economic margins are minimal. A fast weight loss in thawed fish muscle can often be related to protein denaturation and reduced water content, in turn, associated with a drier and firmer muscle texture compared to fish products undergoing lower weight loss (Burgaard, 2010). To our knowledge, studies describing how freezing methods, frozen storage times, and thawing methods influence the weight change on cooked snow crab clusters, have not been published.

From time to time, snow crab clusters may exhibit a blue-hued discoloration commonly referred to as bluing or melanosis (Lorentzen et al., 2018). The melanosis is caused by the formation of dark pigments, which is linked to the presence of enzymatic phenoloxidase (PO) activity (Gonçalves & de Oliveira, 2016). Melanosis is not a food safety issue; nonetheless, it may lead to rejection of the product by consumers. Previously, it has been shown that the cooking regimen, i.e., cooking time and water temperature, in combination with freezing influences the melanosis rate (Lorentzen et al., 2019). However, the effect of different freezing methods, frozen storage times and thawing methods on the rate of melanosis, has to our knowledge not been investigated.

Therefore, the aim of this work was to study how different freezing methods, frozen storage times, and thawing methods influence the quality of mildly cooked snow crab clusters. Three freezing methods were applied, namely immersion freezing in brine (FIB), freezing in still air (FSA), and freezing in circulating air (FCA). Afterwards, the frozen clusters were stored at −20 °C for either 72 h or 6 months. For each of the six combinations of freezing methods and frozen storage periods, one group of clusters was thawed in air (TA), while the other group was thawed in circulating water (TW). The clusters were evaluated for weight changes during freezing, frozen storage and thawing as well as for drip loss and melanosis during the storage at 4 °C.

2. Material and methods

2.1. Raw material

In March 2018, male snow crabs were captured by the vessel Northeastern in the Barents Sea (75°38.62 N-33°21.81 E) at depths from 240 to 250 m using pots baited with herring and squid. The crabs (N = 60) were kept live on board in holding tanks during transport to Nofima in Tromsø, Norway. The crabs were vital and in good condition when processed at Nofima within 24 h of their arrival. The average weight and carapace length of the crabs were 754 ± 211 g and 114 ± 8 mm, respectively.

2.2. Processing

2.2.1. Cooking and cooling

The processing of the crabs into cooked clusters followed the procedure previously described by Lorentzen et al. (2019) with minor adjustments. In detail, after splitting the crabs into clusters, de-bleeding (i.e., drainage of hemolymph) was performed by soaking the clusters into the water with salt (3.5 g NaCl/100 mL) (Havsalt, GC Rieber AS, Norway) for 60 min to simulate standard on-board procedure. Afterwards, the drained clusters were cooked in water at 87 °C for 430 s, obtaining a final core temperature of 81 °C in the largest walking leg. This mild cooking regimen prevented the full inactivation of PO enzymes (Lorentzen et al., 2019), enabling to study of how different freezing methods, frozen storage time, and thawing methods affected melanosis.

Afterwards, the clusters were cooled in fresh water with ice. Within 10 min, the core temperature was below 4 °C, and the clusters were subjected to freezing. The core temperature of the largest walking leg was logged throughout all the processing steps using K-type thermocouples connected to data loggers (model 175H1, Testo Ltd., Hampshire, UK).

2.2.2. Freezing methods and frozen storage

The cooked and cooled clusters were first weighed and then randomly distributed into three groups for freezing either by immersion in brine (FIB), in still air (FSA), or in circulating air (FCA) (Fig. 1, level 3). For the FIB method, the clusters were distributed in three mesh baskets and soaked into a container (300 L) with brine (20 g NaCl/100 mL (Havsalt) at −15 °C. To ensure an effective heat exchange, the baskets were continuously moved around in the container. The FSA method was performed by putting the clusters into a freezer at −20 °C with no air circulation. The FCA method, which is comparable to air-blast freezing, was performed with the clusters evenly distributed on trays placed in a room at −40 °C with an air velocity of 2.39 ms^{-1} for 75 min.

The weight of the FSA and FCA clusters was registered immediately after freezing, while the FIB clusters were first drained from brine residues before weighing. All clusters were then single packed in plastic bags (thickness 90 μm, dimensions 220 × 600 mm, Finnvacum Sverige AB, Bandhagen, Sweden) and vacuumed at 99% (Webomatic, Webomatic Maschinenfabrik GmbH, Bochum, Germany).

The packed frozen clusters were stored in a freezer room at −20 °C

with circulating air. For each of the three freezing methods applied, one group of clusters was thawed after 72 h, whereas the other group was thawed after 6 months (Fig. 1, level 4).

2.2.3. Thawing methods and refrigerated storage

The thawing in air (TA) was performed by placing the bags with the frozen clusters directly into a climate chamber (Binder GmbH, Tuttlingen, Germany) at 4 °C, while the thawing in water (TW) was performed by soaking the bags with the clusters into a container with circulating water at 4 °C (Fig. 1, level 5). Upon reaching a core temperature of 4 °C in the largest walking leg, the thawing process was considered completed, and each bag was opened allowing measurement of the cluster weight. Afterwards, the clusters were closed with paper clips and oriented vertically in their plastic bags in order to obtain equal conditions of drainage during storage at 4 °C for 5 days.

2.3. Weight changes

The weight changes of the clusters were measured during freezing, thawing, and subsequent storage at 4 °C.

The weight change due to the freezing process (F1) was calculated as (AOAC, 1995):

$$F1\ (\%) = [(\text{weight of cluster immediately after FIB, FSA, or FCA} - \text{weight of cooked and cooled cluster}) / (\text{weight of cooked and cooled cluster})] \times 100 \quad (1)$$

The weight change during the frozen storage period (72 h or 6 months) and the thawing process (F2) was determined as:

$$F2\ (\%) = [(\text{weight of the thawed cluster} - \text{weight of the frozen cluster before frozen storage}) / (\text{weight of the frozen cluster before frozen storage})] \times 100 \quad (2)$$

The weight change of the thawed clusters after the first three days of storage at 4 °C, also referred to as drip loss when considered in absolute value, was expressed as:

$$D3\ (\%) = [(\text{weight of thawed cluster}_{day(3)} - \text{weight of thawed cluster}_{day(0)}) / (\text{weight of thawed cluster}_{day0})] \times 100 \quad (3)$$

2.4. Melanosis

The clusters were monitored for melanosis daily during the 5-day refrigerated storage (Fig. 1, level 6). The melanosis was evaluated by acquiring digital images as previously described (Lian et al., 2018), with minor modifications. The images were acquired in a room with downlights (Master TL-D Super 80 58 W/840, Philips, Norway). Four experienced judges evaluated the images independently, using a 5-point visual quality score scale (Lian et al., 2018). Score “5” referred to a *freshly cooked appearance and absence of melanosis*, while score “1” referred to *remarkable melanosis in the merus, shoulder and joints of the cluster*.

2.5. Statistical analyses

The melanosis scores were modelled as a linear kinetic equation (Lian et al., 2018):

$$S(t) = k \times t + S_0 \quad (4)$$

where S(t) is the score at storage time t (day), S_0 is the score at time zero which was imposed equal to “5”, and k is the kinetic constant or melanosis rate (day^{-1}). Equation (4) was fitted individually to the melanosis scores obtained from each judge for each cluster until the fourth day of storage at 4 °C using the software Statistica™ (vers. 13, TIBCO Software Inc., Palo Alto, CA, USA).

The values of the response variables F1, F2, and D3 were expressed as mean ± standard deviation. For the melanosis rate, the values of k calculated by Equation (4) were expressed as absolute values ($|k|$) (± standard error), hence with higher values indicating a faster rate of melanosis. Each treatment group, resulting from the combination of the freezing method, frozen storage time and thawing method, included 10 snow crab clusters (Fig. 1, level 5). The statistical analyses were performed considering each cluster as a replicate.

The significance of the effects of the studied factors (i.e., freezing method, frozen storage time and thawing method) was investigated by carrying out a full-factorial analysis of variance (ANOVA) using the general linear model (GLM) procedure in the software Statistica™. For the response variable F1, it was conducted a one-way ANOVA considering only the freezing method as a factor as, by definition (Eq. (1)), the values of F1 could not be dependent on the frozen storage time or thawing method. In addition, for the response variables F2, D3 and melanosis rate, a one-way ANOVA was carried out on the data grouped by the combination of freezing method, frozen storage time and thawing method in order to highlight significant differences between each group which were assessed by *post-hoc* multiple comparisons (Tukey's HSD test). For ANOVA and *post-hoc* comparisons, the significance level was set at 5% probability ($P = 0.05$).

Principal component analysis (PCA) was also carried out to identify correlations between all the response variables (i.e., F1, F2, D3, and melanosis rate) and to assess the overall contribution of the factors of the study to data variance using the package *FactoMineR* within the software R (vers. 3.6.1) (R Core Team, 2019).

3. Results and discussion

3.1. Temperature profiles

The freezing methods FIB, FSA, and FCA allowed to reach leg core temperatures of −15.6, −20.3, and −37.6 °C, in 14, 430, and 43 min, respectively (Fig. 2A). However, it should be noted that the freezing rate was particularly slow for the clusters frozen in still air where after 320 min of freezing, the leg core temperature was still as high as −3.8 °C. Therefore, at this time point, the FSA clusters were moved to the freezer room at −20 °C with circulating air in order to reduce the time necessary to complete the freezing process.

The flattening of all the three freezing curves below 0 °C, termed as “thermal arrest region”, was at least about 20 and 40 times longer for the clusters frozen in still air compared to the clusters frozen in circulating air and in brine, respectively. It has been shown that a long freezing time results in a small number of large ice crystals, most of which are extracellular while a short freezing time results in many small ice crystals located both intra- and extra-cellularly (Kolbe & Kramer, 2007). Therefore, it can be assumed that different freezing rates resulted in the formation of larger ice crystals in the FSA clusters compared to the crystals formed in the clusters belonging to the FCA and FBI groups. This has also been demonstrated in a study on frozen claw meat of Southern Ocean swimming crab (*Ovalipes trimaculatus*) and Patagonian stone crab (*Danielethus patagonicus*) (Dima, Santos, Baron, Califano, & Zaritzky, 2014). The authors reported that crab meat tissue frozen in circulating air at −40 °C and velocity of 4.16 ms^{-1} presented significantly smaller intracellular ice crystals (average diameter 15 ± 2 μm) compared to the counterpart undergoing slower freezing rate (average ice crystal diameter 62 ± 4 μm) in a freezing process with air at −27 °C and velocity of 3.64 ms^{-1}.

Thawing in air (TA) and in water (TW) resulted in a cluster core temperature of 4 °C (± 0.3) after 950 and 135 min, respectively (Fig. 2B). The short thawing time obtained when applying the TW method can be explained by the characteristic higher thermal conductivity of water compared to air.

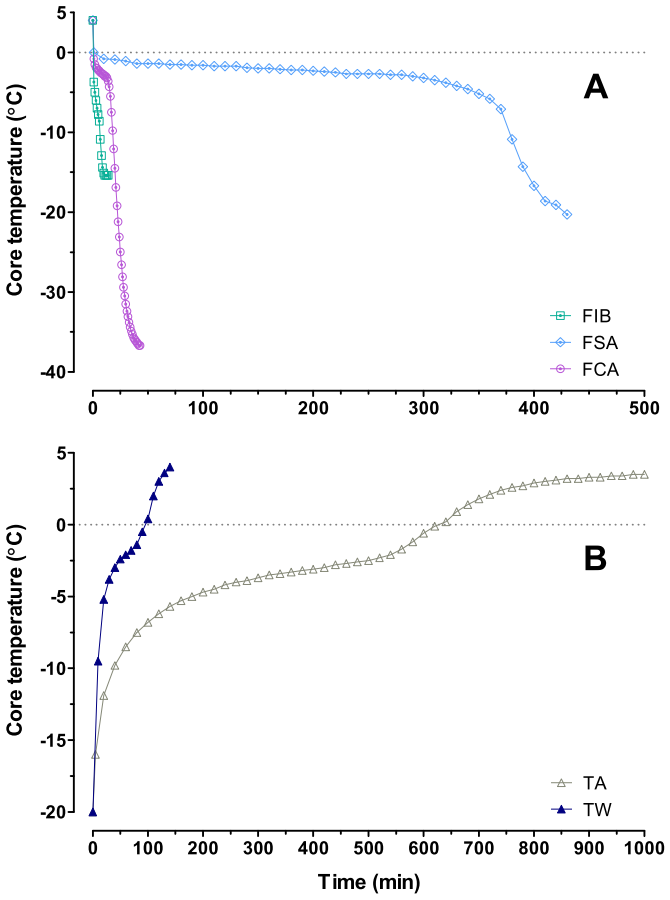

Fig. 2. Leg core temperature during freezing (A) and thawing (B). Immersion freezing in brine (FIB), freezing in still air (FSA), and freezing in circulating air (FCA). Thawing at 4 °C in circulating air (TA) and in circulating water (TW).

3.2. Weight changes

3.2.1. Weight changes during the freezing process

The weight changes due to freezing (F1) differed significantly ($P < 0.001$) with the freezing methods applied (Table 1). More specifically, the values of F1 were 1.8% (± 1.8), −4.5 (± 1.1), and −2.0 (± 0.6), for the FIB, FSA, and FCA method, respectively. These differences may be ascribed to the different freezing rates associated with the studied freezing methods (Fig. 2A). In fact, it has been reported that fast freezing rates, as in the case of the FIB and FCA methods, can result in a low weight loss due to a low sublimation (Campañone et al., 2001). On the contrary, a slow freezing rate, as in the case of the FSA method, can be associated with high sublimation and thereby a high weight loss. Moreover, in the FCA method, the fast freezing rate may result in the formation of an ice crust on the surface, acting as a glaze that reduces the moisture transfer from the product to the atmosphere (Espinoza Rodezno et al., 2013). Compared to both FCA and FSA, the FIB method resulted, on average, in an increase in the weight of the cluster. The weight increase can be explained by the presence of residues of brine which adhered to the cluster surface during the freezing process.

3.2.2. Weight changes during the frozen storage and thawing

The weight change during the storage at −20 °C and the thawing process (F2) was significantly affected ($P < 0.001$) by the freezing method and frozen storage time but not by the thawing method ($P = 0.104$) (Table 1). Irrespective of the freezing method, length of the frozen storage period and thawing method, the storage at −20 °C and the thawing process resulted in weight loss in all the clusters, i.e., negative F2 values were obtained (Eq. (2)) (Fig. 3A and B). In detail, for clusters thawed in air (TA) after 72 h of frozen storage (Fig. 3A), the weight loss was 14.4% (± 1.7), 12.9% (± 4.3), and 10.7% (± 3.4) applying the FIB, FSA, and FCA methods, respectively. The corresponding weight loss after 6 months of frozen storage was 21.3% (± 2.6), 21.8% (± 5.1), and 16.5% (± 3.9). For clusters thawed in water (TW) after 72 h of frozen storage (Fig. 3B), the weight loss for the FIB, FSA, and FCA clusters was 15.3% (± 3.0), 16.3% (± 3.0), and 13.4% (± 3.4), respectively. The corresponding weight loss after 6 months of frozen storage, was 19.5% (± 5.4), 22.1% (± 2.6), and 17.8% (± 4.9).

It can be noted that, in general, FSA clusters underwent the highest weight loss especially after long frozen storage (6 months) followed by thawing in water. Moreover, irrespective of the freezing and thawing methods, the weight loss during the frozen storage and subsequent thawing was faster in the clusters stored at −20 °C for 6 months compared to their counterparts stored for only 72 h.

This can be explained by protein denaturation phenomena that may occur during the freezing process and the frozen storage period linked to alterations in which intermolecular cross-linking takes place. Wagner and Añon (1985) studied the denaturation effect of freezing on myofibrillar proteins of bovine muscle and found a faster liquid loss in the thawed muscle which had been exposed to slower freezing rates. This was reported to be a consequence of partial unfolding of the myosin head being more pronounced at slow freezing rates (Boonsumrej et al., 2007). Next to protein denaturation, the average crystal size will increase during frozen storage (Skåra, Stormo, & Nilsen, 2019). This involves an increase in the average size and a reduction in the average number of crystals caused by the growth of larger crystals at the expense of smaller crystals, also referred to as migratory recrystallization. Migratory recrystallization is the most important in most foods and is largely caused by fluctuations in storage temperature (Delgado & Sun, 2010). Although the freezer temperature was set to −20 °C, temperature fluctuations due to defrosting during the frozen storage period was observed. Thus, small ice crystals were assumed to melt/rearrange at a faster rate compared to larger crystals. When the temperature in the freezer room decreased after the defrosting, the water deriving from the melted crystals were expected to re-freeze onto the existing crystals, leading to the formation of larger ice crystals as previously described by Shenouda (1980).

Table 1
Overview of p-values as determined by full-factorial ANOVA for the response variables F1, F2, D3, and melanosis rate.

Factor effect	Response variable			
	F1	F2	D3	Melanosis rate
Freezing method	< **0.001**	< **0.001**	**0.015**	< **0.001**
Frozen storage time		< **0.001**	< **0.001**	0.539
Thawing method		0.104	0.317	< **0.001**
Frozen storage time × Freezing method		0.369	**0.007**	0.664
Frozen storage time × Thawing method		0.089	0.756	0.338
Freezing method × Thawing method		0.272	**0.012**	0.385
Frozen storage time × Freezing method × Thawing method		0.857	0.730	0.332

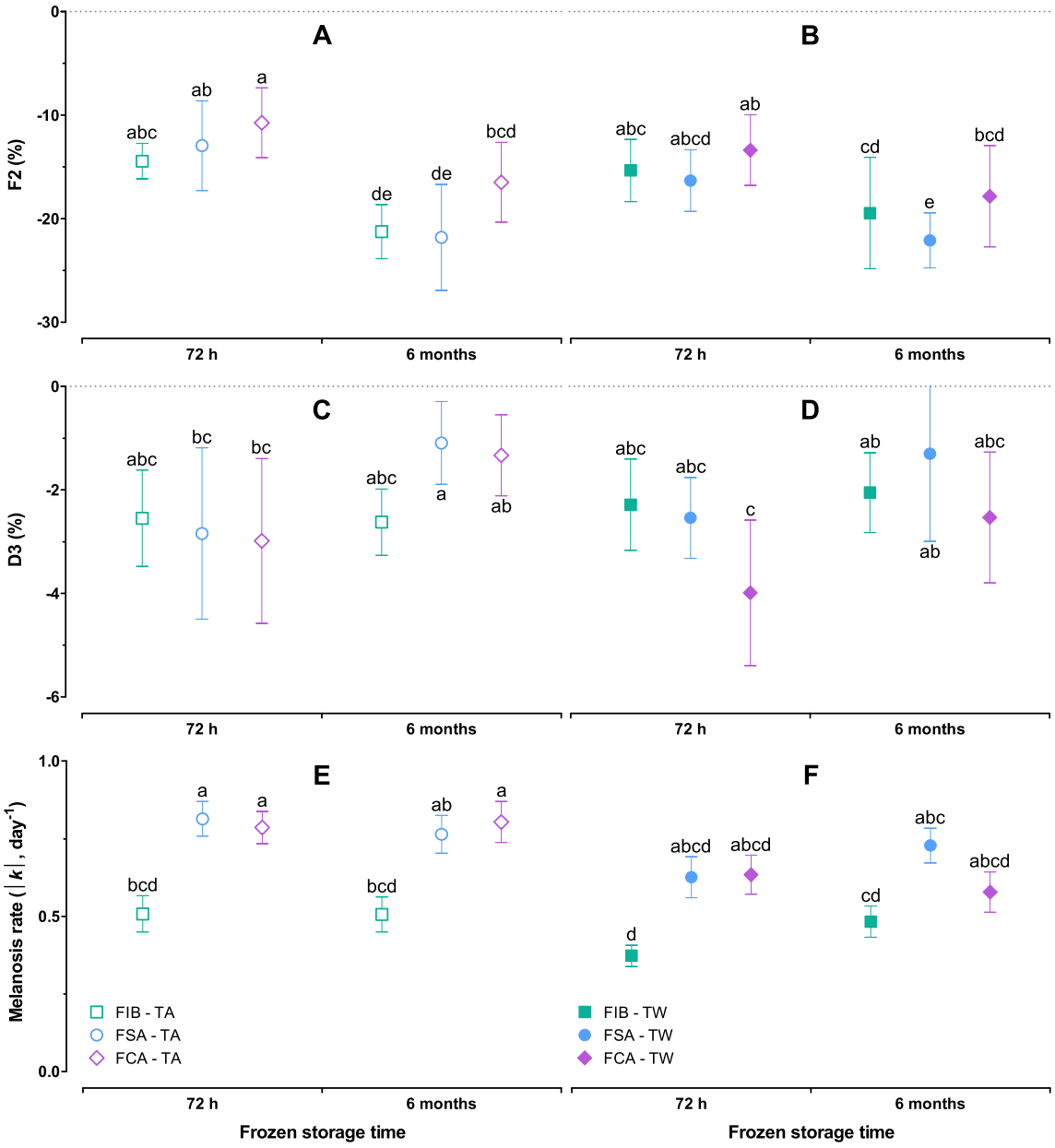

Fig. 3. Average values for weight changes during storage at −20 °C and the thawing process (F2), weight change during the first three days of storage at 4 °C (D3), and estimated melanosis rates ($|k|$) for mildly cooked snow crab clusters stored as frozen for 72 h and 6 months. The error bars indicate standard deviation for F2 and D3, and standard error for $|k|$. Freezing methods were immersion freezing in brine (FIB), freezing in still air (FSA), and freezing in circulating air (FCA), while thawing methods were thawing in air (TA) (A, C, E) and thawing in circulating water (TW) (B, D, E). Values with different letters are significantly different ($P < 0.05$).

The differences observed in F2 as an effect of freezing method and frozen storage time can be ascribed to both ice crystal growth and protein denaturation. These conclusions have been reached also by previous comparable studies on blue crabs and shrimps (Giddings & Hill, 1978), pre-cooked pacific white shrimps (Manheem, Benjakul, Kijroongrojana, & Visessanguan, 2013), and freshwater prawns (Srinivasan, Xiong, Blanchard, & Tidwell, 1998).

3.2.3. Weight changes during the refrigerated storage

The weight change of the thawed clusters stabilized from the second day of storage at 4 °C (data not shown). Thus, the data of weight change at day 3 (D3) also referred to as drip loss when considered in absolute values were selected for statistical analysis. Drip loss was significantly affected by the freezing methods ($P = 0.015$) and frozen storage time ($P < 0.001$), but not by the thawing methods ($P = 0.317$). In addition, the effect of the freezing method on the drip loss was influenced by the frozen storage time and by the thawing method as the interactions "frozen storage time × freezing method" and "freezing method × thawing method" were found statistically significant with *P*-values of 0.007 and 0.012, respectively (Table 1).

In detail, the drip loss (Eq. (3)) of clusters stored at −20 °C for 72 h and thawed in air (TA) was 2.5% (± 0.9), 2.8% (± 1.7), and 3.0% (± 1.6) for the FIB, FSA, and FCA clusters, respectively (Fig. 3C). After 6 months of frozen storage, the corresponding drip loss values were 2.62% (± 0.64), 1.09% (± 0.80), and 1.33% (± 0.78). The drip loss of clusters thawed in water (TW) after 72 h of frozen storage was 2.3% (± 0.9), 2.5% (± 0.8), and 4.0% (± 1.4) applying the FIB, FSA, and FCA methods, respectively (Fig. 3D). After 6 months of frozen storage, the corresponding values were 2.0% (± 0.8), 1.3% (± 1.7), and 2.5% (± 1.3). In general, the choice of freezing method did not influence D3

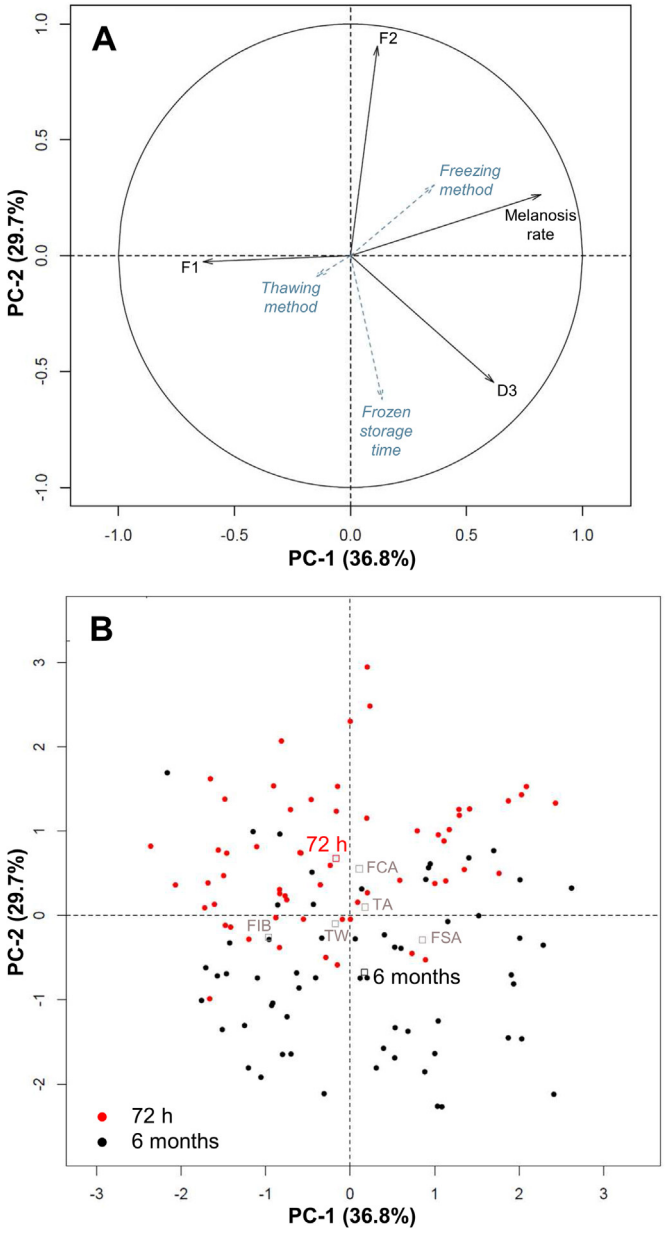

Fig. 4. Loading plot (A) and score plot (B). In the loading plot, the response variables (Melanosis rate, F1, F2, D3) are active variables whereas the factors (Freezing method, Frozen storage time, and Thawing method) are supplementary variables. The score plot shows the grouping of the observations according to frozen storage time of 72 h (●) and 6 months (●). In addition, the centroid of each factor level is indicated (□).

significantly after 72 h of frozen storage at −20 °C ($P > 0.050$). After 6 months of frozen storage time, however, the FSA method resulted in a significantly lower drip loss when thawed in air compared to the FIB method (Fig. 3C). Such differences were not observed for the clusters thawed in water. It is worthwhile noticing that faster weight loss (F2) after 6 months of frozen storage (Fig. 3A and B), resulted in lower drip loss (D3) for the corresponding clusters after storage at 4 °C for 3 days (D3) (Fig. 3C and D). Thus, clusters having lost a major share of fluid during the frozen storage and the subsequent thawing process had less fluid to lose during the following storage period at 4 °C. This was irrespective of freezing and thawing method.

Regardless of the freezing method applied, it was observed that widespread cracks were present in the exoskeleton of the claws and of some walking legs. Consequently, the weight loss observed during thawing and the storage at 4 °C (F2 and D3) was contributed by a fluid loss from these cracks.

3.3. Melanosis rate

The melanosis rates obtained were characterized by a high variability also within each treatment group (Fig. 3E and F). This could be explained by a different PO activity as a response to uneven thermal exposure in the cooking step. Likely, this could be due to the heterogenicity in cluster size, also considering that the temperatures in the range of 60–80 °C can actually exhibit a heat-induced activation of PO (Williams, Davidson, & Mamo, 2003).

During the frozen storage, some of the vacuum bags were punctured by the exoskeleton of the clusters, leading to oxygen exposure. Before thawing, these bags were transferred into new plastic bags and re-vacuumed. Despite this, the rate of melanosis in these clusters did not differ significantly ($P > 0.050$) compared to clusters with an intact vacuum. Thus, all 120 clusters were included in the dataset for calculation of melanosis rates.

The rate of melanosis ($|k|$) was significantly affected by the freezing methods and thawing methods ($P < 0.001$), whereas the frozen storage time did not influence the melanosis rate significantly ($P = 0.539$) (Table 1). In detail, the melanosis rates for clusters thawed in air (TA) after 72 h of frozen storage were 0.51 (± 0.06), 0.81 (± 0.06), and 0.79 (± 0.05) applying FIB, FSA, and FCA methods, respectively (Fig. 3E). The corresponding melanosis rates for clusters stored for 6 months were 0.51 (± 0.06), 0.76 (± 0.06), and 0.80 (± 0.07). For clusters thawed in water (TW) after 72 h of frozen storage time, the melanosis rates were 0.37 (± 0.03), 0.63 (± 0.07), and 0.63 (± 0.06) applying the FIB, FSA, and FCA methods, respectively (Fig. 3F). After 6 months of frozen storage, the corresponding melanosis rates were 0.48 (± 0.05), 0.73 (± 0.06), and 0.58 (± 0.07).

The average values for melanosis rates for cooked clusters thawed in the air (Fig. 3E) were faster compared to those thawed in water (Fig. 3F). Furthermore, for the clusters thawed in air after 72 h of frozen storage, the freezing method FIB resulted in a significantly slower melanosis rate compared to both the FSA and FCA methods (Fig. 3E). Although not being significant, the same pattern was observed for clusters thawed in water after 72 h frozen storage time (Fig. 3F).

It should be noted that for clusters that underwent frozen storage for 72 h, both the freezing and the thawing method were influencing significantly the melanosis rates. By contrast, after 6 months of frozen storage time, the melanosis rate was significantly affected only by the freezing method.

Next to the freezing rate, the availability of oxygen is crucial for the melanosis rate (Sea-leaw & Benjakul, 2019). The FIB method implied a lower oxygen exposure and a faster freezing rate of the cooked clusters compared to both the FSA and FCA methods.

Previously, it has been demonstrated that freezing and thawing of white leg shrimp (*Penaeus vannamei*) (García-Carreño, Cota, & Navarrete del Toro, 2008) results in cell disruption allowing previously compartmentalized contents to mix, resulting in a faster melanosis rate. The growth of ice crystals during the 6-month frozen storage was thereby expected to contribute with cell disruption leading to a faster melanosis rate than the clusters stored for only 72 h at −20 °C before thawing. In the present study, a slight increase in melanosis rate was, on average, observed for clusters thawed in water (Fig. 3F), while this was not observed for clusters thawed in air (Fig. 3E). Thus, a slow thawing rate appears to be beneficial in terms of minimizing melanosis after frozen storage.

3.4. Considerations of main effects

To obtain an overview of correlations between weight changes and melanosis rates due to the freezing methods, frozen storage time, and thawing methods, a principal component analysis (PCA) was performed

(Fig. 4.).

The loading plot obtained by plotting the first and second principal component (PC-1 and PC-2) is illustrated in Fig. 4A. The first two principal components, PC-1 and PC-2, explained 66.5% of the total variance. It is a low correlation between the response variables, except for the F1 and melanosis rate which are negatively correlated along PC-1, and F2 and D3 which are negatively correlated along PC-2. Overall, among the factors studied, the frozen storage time accounted for the highest portion of variance, followed by the freezing method and thawing method.

In the score plot (Fig. 4B), the frozen storage time of 72 h and 6 months were grouped. The grouping followed mainly PC-2 and was also associated with the weight change during the frozen storage and the thawing process (F2) as well as with the drip loss at day 3 (D3) at 4 °C.

The combination of the loading and score plot shows that freezing in brine allows the least weight loss during the freezing process. Furthermore, to minimize the weight loss during frozen storage time and the thawing process, freezing in circulating air, followed by a short frozen storage time and thawing in air should be performed. From a retailer's point of view, if the focus is to minimize the drip loss during storage at 4 °C, the best performing clusters would be the ones frozen in still air and stored at −20 °C for a long period of time. However, if the goal is to minimize the melanosis rate, the best performing clusters would be the ones frozen in brine and thawed in water, irrespective of the frozen storage time.

4. Conclusion

Mildly cooked clusters of snow crabs were exposed to three separate freezing methods, namely freezing in brine, freezing in still air, and freezing in circulating air. The different freezing methods significantly affected weight changes ($P < 0.001$). The frozen clusters were stored for 72 h or 6 months at −20 °C before thawing either in the air or circulating water. The frozen storage time significantly affected weight change. The observed variations in weight changes are assumed to be due to level of protein denaturation, size of ice crystals, and extent of cracks in the clusters as a response to different freezing rates, temperature fluctuations during the frozen storage, and thawing rates. Thus, these factors are crucial in controlling the weight change of the product.

The melanosis rates were significantly affected by both freezing and thawing methods. After 72 h frozen storage time, both the freezing and thawing method were influencing significantly the melanosis rates. By contrast, after 6 months of frozen storage time, the melanosis rate was significantly affected only by the freezing method. Presence of melanosis is assumed to be due to active PO enzymes as the core temperature during cooking was only about 80 °C. The standard commercial cooking procedure involves reaching a core temperature of minimum 92 °C, which allows inactivation of PO enzymes, hence the prevalence of melanosis is minimized once the clusters are thawed.

From an industrial point of view, freezing in brine allows for the least weight loss during the freezing process. To minimize the weight loss during the frozen storage time and the following thawing process, freezing in circulating air, a short frozen storage time, and thawing in air is recommended. From a retailer's point of view, if the focus is to minimize the drip loss during storage at 4 °C, the best performing clusters would be the ones frozen in still air and stored at −20 °C for a long period of time. However, if the goal is to minimize the melanosis rate, the best performing clusters would be the ones frozen in brine and thawed in water, irrespective of the frozen storage time.

This study has revealed the choice of freezing methods, frozen storage time, and thawing methods highly influences the weight change and melanosis of mildly cooked clusters of snow crab. Thus, better and more optimal methods should be further developed to obtain high quality products with less variation.

CRediT authorship contribution statement

Grete Lorentzen: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Anette Hustad:** Investigation, Data curation. **Federico Lian:** Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision. **Adrian Eyser Grip:** Validation, Formal analysis, Investigation, Data curation. **Espen Schrødter:** Validation, Investigation, Data curation. **Tatiana Medeiros:** Validation, Investigation, Data curation. **Sten I. Siikavuopio:** Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition.

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