



Effect of freezing-thawing on weight loss, melanosis, and microbial growth in mildly cooked snow crab (*Chionoecetes opilio*) clusters

Grete Lorentzen^{a,*}, Federico Lian^a, Amalie Agersborg Røhme^{a,b}, Eirin Johannessen^{a,b}, Karen Vanglo Grastveit^{a,b}, Adrian Eyser Grip^{a,b}, Sten I. Siikavuopio^a

^a NOFIMA – Norwegian Institute of Food, Fisheries and Aquaculture Research, P.O. Box 6122, NO-9291, Tromsø, Norway

^b NTNU, NO-7491, Trondheim, Norway

ARTICLE INFO

Keywords:

Snow crab
Mild cooking
Freezing-thawing
Refrigerated storage
Melanosis

ABSTRACT

Different mild cooking treatments of snow crab clusters in combination with immediate refrigeration (IR) or freezing-thawing before refrigeration (FBR) have been performed. The clusters were cooked in either fresh or salted (5 g NaCl/100 ml) water at two temperature/time conditions (87 °C/430 s and 96 °C/148 s) both targeting a core temperature of 81 °C in the largest cluster leg. The freezing-thawing affected quality parameters such as weight loss, melanosis and microbial growth (total viable psychrotrophic counts (TVC) and *Pseudomonas* spp.) during refrigeration. During refrigeration, FBR clusters showed a higher weight loss compared to the IR clusters, and the development of melanosis in the FBR clusters was faster and more severe compared to the IR clusters. Melanosis was also affected by the cooking treatments as the clusters cooked in water at 96 °C obtained better visual quality scores compared to those cooked in water at 87 °C ($P < 0.05$). In the FBR clusters, the levels of TVC were lower compared to the IR clusters. In summary, melanosis was the dominant shelf-life limiting factor irrespective of freezing-thawing and cooking combinations. Application of such combinations in processing of snow crabs is therefore not recommended unless measures to reduce melanosis are adopted.

1. Introduction

Snow crab (*Chionoecetes opilio*) is widely distributed in the North Pacific, the Arctic, the Northwest Atlantic, and in the Barents Sea (Siikavuopio et al., 2017). Its commercial fishery is rapidly growing under the increasing demand for snow crab products, especially in the EU, USA, Japan, and South Korea (Norwegian Seafood Council, 2019). In 2018, 2697 tons of snow crabs were captured in Norway (Norwegian Fishermen's Sales Organization, 2019).

Nowadays, the entire volume of snow crabs landed in Norway is processed into cooked and frozen clusters, i.e., sections of four walking legs and one claw attached to a shoulder (Lian et al., 2018; Norwegian Fishermen's Sales Organization, 2019). Usually, the crabs are processed on-board close to the locations of capture. Upon arrival at the destination market, the clusters are either thawed or kept frozen when displayed at points of sale (Lorentzen et al., 2018).

Cooking of snow crab clusters is usually conducted in boiling water to a target core temperature of at least 91–92 °C in the largest leg of the cluster (Lorentzen et al., 2018). However, it may be desirable to apply milder cooking conditions to reduce the risk of overcooking, which is often associated with a lower yield and a poorer eating quality

(Martínez-Alvarez, López-Caballero, Gómez-Guillén, & Montero, 2009). Also, reducing total processing time and saving of energy costs are important drivers for a milder cooking treatment.

Freezing of seafood is an effective long-term preservation method. However, freezing and frozen storage may lead to denaturation and aggregation, especially of myofibrillar proteins, resulting in changes in textural attributes, such as reduced juiciness and water holding capacity (Burgaard, 2010). The extent of this quality loss depends on the rate of freezing, duration of frozen storage, speed of thawing, storage temperature, and temperature fluctuations during storage (Boonsumrej, Chaiwanichsiri, Tantratian, Suzuki, & Takai, 2007). In shrimp and other shellfish, loss of quality during frozen storage is predominately caused by oxidation, denaturation of proteins, sublimation, and ice recrystallization (Løndahl, 1991). These quality losses often lead to off-flavors, rancidity, dehydration, weight loss, loss of juiciness, and toughening (Bhobe & Pai, 1986).

From time to time, snow crab clusters may develop dark blue spots (i.e., melanosis), that can appear shortly after slaughtering (Lian et al., 2018), leading to product rejection by consumers (García-Carreño, Cota, & Navarrete del Toro, 2008). The occurrence of melanosis is linked to the presence of enzymatic phenoloxidase (PO) activity in crab

* Corresponding author.

E-mail address: grete.lorentzen@nofima.no (G. Lorentzen).

<https://doi.org/10.1016/j.lwt.2019.03.083>

Received 4 February 2019; Received in revised form 18 March 2019; Accepted 26 March 2019

Available online 28 March 2019

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tissues including the hemolymph (Gonçalves & de Oliveira, 2016; Lindberg, Siikavuopio, Øverbø, Lorentzen, & Whitaker, 2017). Melanosis is a potential challenge in snow crabs and other crustaceans, especially in the context of mild cooking treatments (Manheem, Benjakul, Kijroongrojana, & Visessanguan, 2012), as they contain PO enzymes that are highly thermostable ($D_{90} = 3.3\text{--}12.0$ min) (Huang et al., 2014; Williams, Mamo, & Davidson, 2007).

Psychrotrophic microorganisms such as *Pseudomonas* spp., *Moraxella*, *Achromobacter* and *Acinetobacter* spp. have been reported to be major contributors to spoilage in blue crab (*Callinectes sapidus*) (Alford, Tobin, & McClesky, 1942), Dungeness crab (*Cancer magister*) (Lee & Pfeifer, 1975) and edible crab (*Cancer pagurus*) (McDermott et al., 2018) during refrigerated storage. During freezing, microorganisms suffer damage which may cause inactivation (Lund, 2000) or death. Death rates are high at the start of freezing, followed by lower inactivation rates during frozen storage. At the same time, microbial growth does not occur at temperatures below -8 °C (Farkas, 2007).

Quality parameters related to the frozen storage of raw mud crab (*Scylla serrata*) (Mishra & Dora, 2008) as well as raw (Paparella & Tatro, 1971) and cooked blue crab (Webb, Tate, Thomas, Carawan, & Monroe, 1976) have been reported. To our knowledge, corresponding studies for cooked snow crab clusters have not been published. Thus, different mild cooking treatments of snow crab clusters in combination with either immediate refrigeration (IR) or freezing-thawing before refrigeration (FBR) was performed. The clusters were evaluated for weight loss, melanosis, and microbial growth during refrigeration.

2. Material and methods

2.1. Raw material

In March 2017, male snow crabs were harvested using snow crab pots in the Barents Sea ($75^{\circ}30.372$ N- $33^{\circ}14.957$ E) at depths from 230 to 350 m and stored live onboard in holding tanks. The crabs were transported to the Aquaculture Research Station in Tromsø (Norway) and immediately placed in 6 m^3 tanks containing seawater at 3 °C. After a week, the snow crabs were transported live in a dry state, in polystyrene boxes covered with gel ice (Cold Inc., Oakland, CA, USA), from the Research Station to Nofima in Tromsø ($N = 76$). The crabs were processed at Nofima within 15 h of arrival; they were in good condition and vital before processing. The average weight and carapace length of the crabs was 604.6 ± 164.0 g and 112.8 ± 8.6 mm, respectively.

2.2. Processing, freezing and storage

Initially, experiments were performed to calibrate two temperature/time cooking conditions achieving a common core temperature of 80.8 °C in the largest cluster leg. The temperature was monitored using thermocouples connected to data loggers (model 175H1, Testo Ltd., Hampshire, UK). The core temperature of 80.8 °C represents a mild cooking treatment as compared with a core temperature of about $91\text{--}92$ °C which is commonly targeted in conventional industrial cooking protocols.

The processing and subsequent storage (IR and FBR) followed an experimental setup as illustrated (Fig. 1). The first step was slaughtering, followed by manual removal of remaining gills using a knife. The 76 cleaned left clusters were placed in one cage, and the corresponding 76 right clusters were placed in another one. To facilitate drainage of the hemolymph (i.e., de-bleeding), both cages with the clusters were soaked in fresh water at 2.5 °C for 60 min followed by draining in 10 min (Fig. 1, level 3). The de-bleeding water containing 4-hexyl-resorcinol (0.01 g/100 ml water) (4-HR; Sigma Aldrich Co., Darmstadt, Germany), was used as a melanosis inhibitor being a competitive binder of PO (Lian et al., 2018; Sae-leaw & Benjakul, 2019). Afterward, the clusters were cooked by immersion in either fresh or salted water (5 g NaCl/100 ml) (Sigma Aldrich) at 87 °C for 430 s or at

96 °C for 148 s, resulting in four different cooking treatments (Fig. 1, level 4). The two temperature/time conditions for the cooking treatments are hereafter denoted as “ 87 °C/430 s” and “ 96 °C/148 s”.

The average weight of the clusters cooked at “ 87 °C/430 s” was 240.0 ± 60.5 g, while for clusters cooked at “ 96 °C/148 s”, the average weight was 183.4 ± 53.9 g. After cooking, the clusters were cooled immediately in fresh water with ice (50 kg ice mixed with 50 l water) until a corresponding core temperature of 1.5 °C was reached. In each cooling session, the total snow crab cluster weight was about 5 kg.

The clusters that underwent the same cooking treatment were then further separated into two subgroups (Fig. 1, level 5). In the IR group, 19 clusters from each of the four cooking treatments, were placed individually in plastic bags and closed with paper clips followed by storage at 4 °C in a climate chamber (Binder GmbH, Tuttlingen, Germany). The FBR group consisted of the remaining 76 clusters, which were evenly distributed on trays and placed in an air-blast freezer at -40 °C with an air velocity at 2.39 m/s for 75 min. Each cluster was then individually weighed, packed in plastic bags as described above, and stored for 3 months at -20 °C before storage at 4 °C in the climate chamber.

The core temperature of the largest legs of two selected clusters in each of the eight subgroups (Fig. 1, level 5) was monitored from cooking up to 12 days of refrigeration using thermocouples connected to data loggers (Testo 175H1). T-bar labels (Floy Tag Inc., Seattle, WA, USA) were used to keep track of each cluster from slaughtering to sample analyses.

2.3. Cooking loss

The cooking loss was calculated as the percentage weight change of the clusters after de-bleeding, cooking, cooling and draining, relative to the weight of the corresponding raw clusters (Lorentzen, Lian, & Siikavuopio, 2019). The calculations were based on the data from at least 15 clusters from each of the four cooking treatments (Fig. 1, level 4).

2.4. Drip loss, thawing/drip loss, and freezing loss

Weight loss in both IR and FBR clusters was monitored for 5 days during storage at 4 °C. In IR clusters, the weight loss (i.e., drip loss) was calculated as the percentage cluster weight change during refrigeration relative to the cluster weight after cooking and drainage. In FBR clusters, the weight loss included both thawing and drip loss (hereafter denoted as “thawing/drip loss”), and it was calculated as the percentage cluster weight change during refrigeration, relative to the cluster weight after the 3-month storage at -20 °C. To obtain uniform conditions for weight loss during refrigeration, all clusters were oriented vertically in the plastic bags with the shoulder joint pointing downwards to allow optimum conditions for drainage.

Freezing loss during the frozen storage was calculated as the percentage cluster weight after the 3-month storage at -20 °C relative to the cluster weight immediately after the air-blast freezer.

2.5. Meat content

At the end of the refrigeration period, for both IR and FBR clusters, cross sections in the middle of the largest legs in 12 selected clusters were performed to evaluate the meat content. The meat content was evaluated as the area of meat in percentage of the total inner area of the cross-section as previously described (Lorentzen et al., 2019). Average values of meat content of the clusters were calculated.

2.6. Melanosis

Melanosis was monitored during the first 48 h of refrigeration in six clusters from each of the eight subgroups (Fig. 1, level 5) by acquiring digital images in standard light condition as described by Lian et al. (2018). Four experienced judges trained at Nofima performed the evaluation independently using the digital images of the clusters.

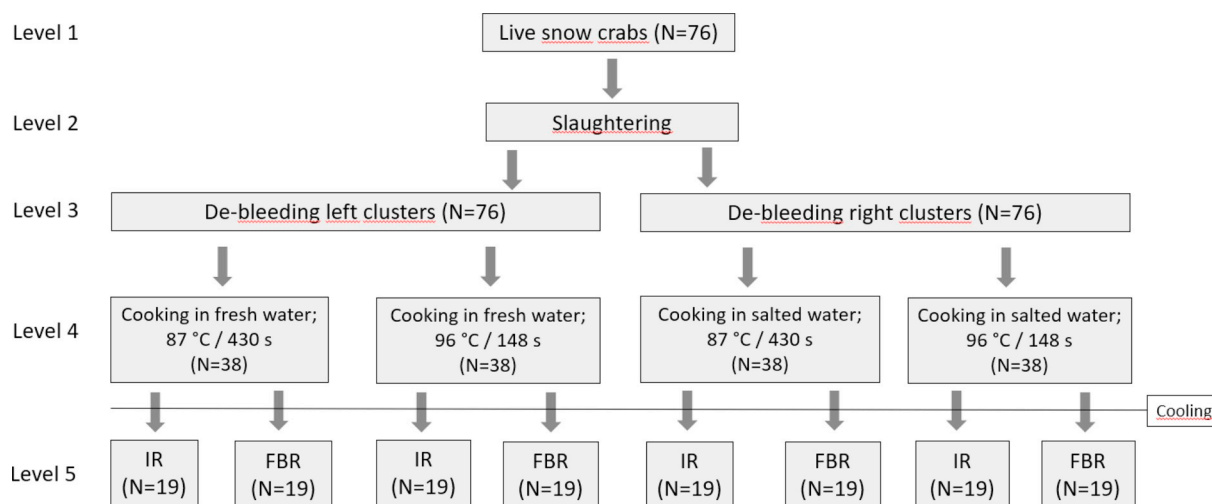


Fig. 1. Experimental setup of snow crab processing (level 2–4) and subsequent storage of clusters at 4 °C (level 5) as immediate refrigerated (IR) after processing and frozen before refrigeration (FBR).

Table 1

Weight change (%) after cooking and freezing. Weight change after cooking is expressed as the percentage weight change of the clusters after de-bleeding, cooking, cooling and drainage, relative to the weight of raw clusters. Weight change after freezing, i.e. freezing loss, is the percentage weight change after a 3-month storage at -20 °C, relative to the cluster weight immediately after the air-blast freezer. Both are expressed as mean values \pm standard deviation. $N = 15$.

Temperature of cooking water (°C)/Cooking time (s)	5 g NaCl/100 ml in cooking water	Weight change (%)	
		After cooking	After freezing
87 °C/430 s	Yes	0.02 ± 0.85^a	-2.03 ± 0.26^a
	No	-1.14 ± 1.05^b	-1.67 ± 0.36^a
96 °C/148 s	Yes	-0.53 ± 0.79^{ab}	-2.13 ± 1.03^a
	No	-0.54 ± 0.91^{ab}	-1.60 ± 0.58^a

Different letters (a or b) within the same column indicate a significant difference ($P < 0.05$).

The rate of melanosis was evaluated using a 5-point visual quality scale, in which “1” referred to *remarkable melanosis in the merus, shoulder and joints of the cluster*; “2” referred to *extensive melanosis in the shoulder and/or in the joints*; “3” referred to *a slight presence of melanosis in the space between the segments of the shoulder and/or the shoulder edge*; “4” referred to *the absence of melanosis, but slight darkening of the area around the shoulder joints*; and “5” referred to *freshly-cooked appearance and absence of melanosis* (Lian et al., 2018). Score “3” was defined as the cut-off value for sensory acceptability in accordance with a previous study on visual quality of food products undergoing enzymatic discoloration (López-Gálvez et al., 2015).

2.7. Microbiological analyses

Analyses of total viable psychrotrophic counts (TVC) and *Pseudomonas* spp. were performed as previously described (Lorentzen, Vorre Skuland, Sone, Johansen, & Rotabakk, 2014). The largest legs of three selected clusters from each of the eight subgroups (Fig. 1, level 5) were sampled at day 4, 5, 7, 9, and 12 of refrigeration. The microbial counts were expressed as \log_{10} CFU/g (mean \pm standard deviation).

2.8. Salt content

The salt content was measured in leg meat samples, obtained from the four different cooking treatments, by standard procedure (AOAC, 1990). From each cooking group, a pooled sample of meat was collected from the legs of six selected clusters.

2.9. Statistical analyses

Regression analysis, Scheffe's and Tukey's test at a 5% significance level (SPSS windows version 25, SPSS Inc., Chicago, IL, USA) were used to study differences between the eight treatment subgroups (Fig. 1, level 5). Due to an unequal number of samples in the groups, Scheffe's test was applied to results for weight change, drip loss and thawing/drip loss of IR and FBR clusters during the refrigerated storage. Tukey's test was applied to the microbial and melanosis results as the number of samples was equal in these subgroups. In the case of microbial results below the detection level ($< 1.7 \log_{10}$ CFU/g), Tukey's test was not performed.

3. Results and discussion

3.1. wt change after cooking and after freezing

Weight change after cooking varied among the four cooking treatments (Table 1). The weight change of “87 °C/430 s” with and without 5% NaCl differed significantly ($P < 0.05$). In the corresponding “96 °C/148 s” with and without 5% NaCl, the weight change did not differ significantly ($P > 0.05$), showing that the effect of salt was only present in water at 87 °C. However, the salt content in the leg meat was on average equal to $1.65 \pm 0.15\%$, and it did not differ significantly ($P > 0.05$) between cooking treatment groups.

In a previous study, the weight change of king crab clusters after cooking (i.e., cooking loss), was coherent with the meat content (Lorentzen et al., 2019). In more detail, a low cooking loss corresponded to a high meat content and *vice versa*. In the present study, a low weight change was observed (Table 1) and the meat content was calculated to about 90% or more. Consequently, a minor weight change in the cooking step can be used as an indicator of a high meat content in snow crabs as well.

For FBR clusters, the weight change after freezing (Table 1) varied among the four cooking treatments (Fig. 1, level 4), although not significantly ($P > 0.05$). The highest freezing loss was observed in clusters cooked in salted water, irrespective of water temperature and cooking time.

3.2. Drip loss and thawing/drip loss

The weight loss in terms of drip loss and thawing/drip loss during refrigeration was remarkably lower in the IR clusters compared to the corresponding FBR clusters (Table 2). After 5 days of refrigeration, the drip loss in IR clusters was about 2–3%, while the thawing/drip loss of

Table 2

Drip loss and thawing/drip loss for 5 days for cooked snow crab clusters that were immediately refrigerated (IR) at 4 °C and those that were frozen before refrigeration (FBR). N = 11.

Temperature of cooking water (°C)/Cooking time (s)	5 g NaCl/100 in cooking water	Cluster group	Drip loss				
			Day 1 of refrigeration	Day 2 of refrigeration	Day 3 of refrigeration	Day 4 of refrigeration	Day 5 of refrigeration
87 °C/430 s	Yes	IR	-2.19 ± 0.53 ^a	-2.64 ± 0.58 ^a	-2.84 ± 0.64 ^a	-2.71 ± 1.41 ^a	-3.65 ± 1.10 ^a
	No		-1.72 ± 0.78 ^a	-2.30 ± 0.78 ^a	-2.67 ± 0.91 ^a	-2.35 ± 1.58 ^a	-3.03 ± 1.08 ^a
96 °C/148 s	Yes	IR	-2.21 ± 0.71 ^a	-2.56 ± 0.81 ^a	-2.71 ± 0.77 ^a	-2.61 ± 0.97 ^a	-3.00 ± 0.99 ^a
	No		-1.57 ± 0.49 ^a	-2.12 ± 0.60 ^a	-2.38 ± 0.80 ^a	-2.51 ± 0.78 ^a	-2.72 ± 0.86 ^a
			Thawing/drip loss				
87 °C/430 s	Yes	FBR	-1.84 ± 1.80 ^a	-5.02 ± 1.59 ^{ab}	-6.33 ± 1.84 ^{ab}	-6.95 ± 1.95 ^a	-6.15 ± 2.42 ^a
	No		-2.25 ± 2.18 ^a	-6.51 ± 2.48 ^a	-8.32 ± 3.36 ^a	-8.34 ± 2.68 ^a	-7.25 ± 2.79 ^a
96 °C/148 s	Yes	FBR	-0.29 ± 0.94 ^a	-4.58 ± 1.80 ^{ab}	-6.44 ± 1.96 ^{ab}	-6.95 ± 2.07 ^a	-7.33 ± 2.25 ^a
	No		-0.22 ± 1.04 ^a	-4.22 ± 1.90 ^b	-6.13 ± 2.36 ^{ab}	-7.39 ± 2.66 ^a	-8.30 ± 2.53 ^a

Different letters (a or b) within the same column and group (IR or FBR) indicate a significant difference ($P < 0.05$).

FBR clusters was about 7%, with no significant differences ($P > 0.05$) between the four cooking groups (Fig. 1, level 4). The high standard deviation may indicate individual differences, which may, in turn, be ascribed to challenges in orientating the clusters into a vertical position during the daily handling, since the main source of weight loss is the liquid loss through the shoulder of the cluster. Similar differences in weight loss between refrigerated and frozen-thawed meat of blue crab were observed by Webb et al. (1976).

It should be noted that in the case of FBR clusters, the time to obtain a core temperature at 4 °C in the largest leg varied from 24 to 36 h, depending on the position in the climate chamber and the cluster size. Thus, a low thawing/drip loss in FBR clusters after one day of refrigeration is explained by the fact that these clusters were still in a partially-frozen state.

The observed differences in weight loss between IR and FBR clusters are probably due to the physical damage caused to the muscle cell wall by the pressure exerted by the ice crystals formed in the freezing process and during frozen storage (Löndahl, 1991). Notably, almost all the claws of the clusters presented visible cracks after air-blast freezing, probably due to the high meat content and freezing rate. Indeed, the cracks could have contributed to the thawing/drip loss.

3.3. Melanosis

The first spots of melanosis were observed as early as only a few hours after cooking, and this was observed in proximity to the cluster joints. Subsequently, it also occurred in other parts of the cluster.

The development of melanosis was faster and more severe in FBR clusters than in the IR clusters. It should be noted that, considering that FBR clusters were still in a partially-frozen state after 24 h of refrigeration, the effect of freezing-thawing on melanosis should be evaluated by comparing the visual quality scores obtained for IR clusters after 24 h of refrigeration with the scores for FBR clusters after 48 h of refrigeration. At this time, the visual quality scores in FBR clusters were below the acceptability threshold except for the clusters cooked in salted water at 96 °C (Table 3).

Similarly, in a study on pre-cooked Pacific white shrimps (*Litopenaeus vannamei*), freezing and thawing induced higher melanosis levels, likely mediated by increased PO activity (Manheem, Benjakul, Kijroongrojana, & Visessanguan, 2013). In crustaceans, melanosis is catalyzed by enzymes with PO activity which can also be displayed by hemocyanin, an oxygen carrier protein present in the hemolymph. Hemocyanin-derived POs are stable at freezing temperatures and retain their ability to cause melanosis even after several months of frozen storage (Le Bris et al., 2016). Furthermore, the freezing-thawing process disrupts the cells, allowing previously compartmentalized contents to

Table 3

Visual quality score (\pm standard deviation) of cooked snow crab clusters immediately refrigerated (IR) after processing or after freezing before refrigeration (FBR). Score of «5» refers to absence of melanosis, while a score of «1» refers to a remarkable melanosis. Scores 4 and 5 are defined as acceptable, while scores of 3 and below are considered as not acceptable. N = 6.

Temperature of cooking water (°C)/Cooking time (s)	5 g NaCl/100 ml cooking water	IR (refrigeration time)		FBR (refrigeration time ^a)	
		6 h	24 h	24 h	48 h ^b
87 °C/430 s	Yes	4.7 ± 0.5 ^a	3.1 ± 0.9 ^a	3.2 ± 0.7 ^a	2.1 ± 0.7 ^a
	No	4.5 ± 0.6 ^a	3.1 ± 0.9 ^a	3.8 ± 0.5 ^{ab}	2.4 ± 0.9 ^a
96 °C/148 s	Yes	4.8 ± 0.4 ^a	4.5 ± 0.7 ^b	4.4 ± 0.5 ^b	3.6 ± 0.5 ^b
	No	4.9 ± 0.3 ^a	4.4 ± 0.7 ^b	3.9 ± 0.6 ^{ab}	2.6 ± 0.8 ^a

Different letters (a or b) within the same column indicate a significant difference ($P < 0.05$).

^a Clusters were air-blast frozen, and then stored at -20 °C for 3 months before refrigeration.

^b All clusters thawed.

react together (García-Carreño et al., 2008), thus exacerbating the development of melanosis.

Regarding the effect of different mild cooking treatments on melanosis, after 24 h of refrigeration, the visual quality score for IR clusters was significantly higher ($P < 0.05$) in the clusters cooked in water at 96 °C compared with their counterparts cooked in water at 87 °C, irrespective of the presence of salt in the cooking water. The same trend was also observed for FBR clusters, although significant differences ($P < 0.05$) between cooking treatments were present only for the clusters cooked at 96 °C in salted water.

The difference in visual quality scores observed between cooking treatment groups indicates that the melanosis rate is more a function of the heat exposure of the cluster surface rather than the core temperature of the leg muscle. This might be ascribed to the presence of POs in the joints and in the subcuticular membrane in proximity to the calcareous layer of the shell (Gonçalves & de Oliveira, 2016). These parts of the cluster reach the temperature of the cooking water very quickly, allowing a higher PO inactivation. However, irrespective of the temperature/time conditions applied, the thermal load was not sufficient to provide adequate inactivation of POs to inhibit or satisfactorily delay the melanosis (Williams et al., 2007).

3.4. Microbial growth

The microorganisms analyzed were below the level of detection ($< 1.7 \log_{10}$ CFU/g) up to 4 days of refrigeration in both IR and FBR

Table 4

Total viable psychrotrophic counts (TVC) and *Pseudomonas* spp. of cooked snow crab clusters that were immediately refrigerated (IR) at 4 °C and those that were frozen before refrigeration (FBR). N = 3.

Temperature of cooking water (°C)/ Cooking time (s)	5 g NaCl/100 ml cooking water	Cluster group	TVC/ <i>Pseudomonas</i> spp. (log ₁₀ CFU/g)			
			Day 5 of refrigeration	Day 7 of refrigeration	Day 9 of refrigeration	Day 12 of refrigeration
87 °C/430 s	Yes	IR	3.7/ND	5.4 ^a /4.7 ^a	6.5 ^a /5.1 ^a	8.4 ^a /7.4 ^a
	No		ND/ND	4.6 ^a /3.9 ^a	5.8 ^a /4.7 ^a	7.8 ^a /6.9 ^a
96 °C/148 s	Yes	IR	3.7/ND	5.6 ^a /4.0 ^a	6.5 ^a /4.4 ^b	8.0 ^a /6.5 ^b
	No		4.0/ND	5.5 ^a /4.2 ^a	7.6 ^a /5.8 ^a	8.1 ^a /8.6 ^a
87 °C/430 s	Yes	FBR	ND/ND	3.5/ND	4.8 ^a /ND	5.5 ^a /ND
	No		ND/ND	3.0/3.0	4.3 ^a /3.6	6.3 ^a /5.8
96 °C/148 s	Yes	FBR	ND/ND	ND/ND	2.4 ^b /1.7	3.2 ^b /ND
	No		2.3/ND	1.7/ND	2.8 ^b /1.7	3.0 ^b /ND

Different letters (a or b) within the same column and group (IR and FBR) indicate a significant difference ($P < 0.05$).

ND = Not detected, below 1.7 log₁₀ CFU/g.

clusters (data not shown). In IR clusters, no significant differences were observed in TVC ($P > 0.05$) between cooking treatments at day 7, whereas at day 9 and 12 of refrigeration, the treatment “96 °C/148 s” with 5% NaCl resulted in significantly lower levels of *Pseudomonas* spp. ($P < 0.05$) compared to the other cooking treatments.

In FBR clusters, at day 9 and 12 of refrigeration, the cooking treatment “96 °C/148 s” resulted in significantly lower levels of TVC ($P < 0.05$) compared to “87 °C/430 s” irrespective of the presence of salt in the cooking water (Table 4). Notably, in FBR clusters, *Pseudomonas* spp. was detected at low levels and only sporadically, except for the clusters cooked at 87 °C for 430 s in fresh water.

In the FBR clusters, lower levels of TVC and *Pseudomonas* spp. were observed as compared with the IR clusters. Considering the time required for the frozen clusters to thaw during refrigeration (24–36 h), the results of microbial growth at day 5, 7, and 9 in IR clusters can reasonably be compared with the counts at day 7, 9, and 12 in FBR clusters. When performing such a comparison, the TVC levels were within the same log unit for “87 °C/430 s” samples, while the corresponding differences for “96 °C/148 s” samples were in the range of 2-log unit. Thus, the freezing-thawing process had a higher limiting effect on TVC during refrigeration in clusters cooked at 96 °C compared to their counterparts cooked at 87 °C. The reduction in TVC level observed in FBR clusters is in accordance with previous studies. For example, a 2-log reduction in TVC was observed in meat of cooked marine crab (*Portunus pelagicus*) after freezing to –41 °C (Subramanian, 2007). Furthermore, a reduction by 2–3 log units in TVC was observed in Jonah crab (*Cancer borealis*) meat after 40 weeks of storage at –23 °C (Rebach, Stribling, & Wilber, 1990).

A similar pattern was observed for *Pseudomonas* spp. levels. In the clusters cooked at “87 °C/430 s”, *Pseudomonas* spp. counts differed by 1-log unit between the values observed for IR clusters at day 7 and 9 of refrigeration and for FBR clusters at day 9 and 12 of refrigeration. In contrast, in the clusters cooked at “96 °C/148 s” the *Pseudomonas* spp. level differed by up to 4-log units when comparing the counts at day 7 and 9 of refrigeration for the IR clusters with corresponding counts at day 9 and 12 for the FBR clusters. Thus, also the growth of *Pseudomonas* spp. was limited by the freezing-thawing process to a higher degree in the clusters cooked at 96 °C, compared to their counterparts cooked at 87 °C. The reduction in *Pseudomonas* spp. levels observed in FBR clusters is in accordance with the study of Baixas-Nogueras, Bover-Cid, Veciana-Nogués, and Vidal-Carou (2007) reporting a 1–2 log reduction of *Pseudomonas* spp. in raw hake stored at –20 °C for 6 months.

The differences in the microbial growth between IR and FBR clusters could be due to stress imposed by cooking in combination with freezing-thawing, leading to sublethal cell damage, and therefore, limiting the survival and growth of microorganisms in the subsequent refrigeration. Moreover, the freezing-thawing process was more effective in delaying the microbial growth, especially of *Pseudomonas* spp., in the clusters cooked with the treatment “96 °C/148 s” as compared with

“87 °C/430 s”. This suggests that, when applying a mild cooking treatment, a short cooking time at a higher temperature is more effective in delaying microbial growth than a long cooking time at a lower temperature.

3.5. Shelf-life considerations

In literature, there is a lack of studies describing the shelf-life of cooked snow crab clusters. A shelf-life of 10 days was reported for snow crab clusters processed according to the conventional industrial protocol in boiling water to a core temperature of minimum 91 °C in the claw and stored at 4 °C (Lorentzen, Rotabakk, Olsen, Vorre Skuland, & Siikavuopio, 2016). Melanosis was not observed, and the shelf-life was defined as the time to reach the acceptability threshold for off-odors, in turn, corresponding to TVC and *Pseudomonas* spp. levels of approximately 5–6 and 3–4 log₁₀ CFU/g, respectively.

In the present study, melanosis appeared very early and severely during storage at 4 °C in both IR and FBR clusters. The occurrence of melanosis was therefore the shelf-life limiting factor, as it resulted in visual quality scores below the acceptability threshold as early as after 48 h of refrigeration. Consequently, when applying the mild cooking treatments of the present study, further measures should be implemented to inhibit melanosis and thereby prolong shelf-life. In this regard, considering that oxygen is a key factor in the melanosis cascade (Sae-leaw & Benjakul, 2019), potential solutions may include mild cooking in combination with oxygen-free packaging (i.e., *sous-vide* cooking, vacuum or modified atmosphere during storage) (Mohan, Ravishankar, & Srinivasa Gopal, 2017).

Furthermore, it should be noted that while the exoskeleton of the cluster was severely affected by melanosis in the early refrigeration period, the leg meat did not present any discoloration in the first 4–6 days of storage, irrespective of the cooking treatment and storage conditions. At points of sale, consumers make their purchasing choice on the basis of the visual appearance of the outer part of the cluster. Therefore, another possibility for prolonging the shelf-life of mildly cooked snow crab clusters is to perform the extraction of the meat in the legs and claws immediately after the cooking treatment (Jun, Jung, Jeong, Kim, & Kim, 2019).

4. Conclusion

The freezing-thawing clearly affected the quality parameters of mildly cooked clusters of snow crab. The weight loss during refrigeration was significantly higher in frozen-thawed clusters compared to the clusters that were refrigerated immediately after cooking. However, neither drip loss or thawing/drip loss was influenced by the different temperature/time conditions of mild cooking.

The freezing-thawing delayed the microbial growth during storage at 4 °C, as suggested by the lower levels of both total viable count and

Pseudomonas spp. observed in frozen-thawed clusters compared to the clusters that were refrigerated immediately after cooking. In addition, in frozen-thawed clusters, the microbial growth was influenced also by the temperature/time conditions applied for mild cooking, with a short treatment time at a higher temperature (96 °C/148 s) resulting more effective in delaying the microbial growth than a long treatment time at a lower temperature (87 °C/430 s).

Melanosis was the dominant shelf-life limiting factor irrespective of freezing-thawing and cooking combinations. The development of melanosis was faster and more severe in the clusters that were subjected to freezing-thawing before refrigeration. In clusters cooked in water at “96 °C/148 s”, a better quality score of melanosis was obtained compared to those cooked in water at “87 °C/430 s”.

Declaration of interest

None.

Acknowledgment

This work was partly funded by Nofima and the Bionær program of The The Research Council of Norway (SnowMap, grant number 267763). The authors would like to thank Svein Kristian Stormo and Heidi Nilsen, Nofima, for useful input to the experiment and proof-reading.

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