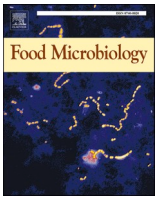
Food Microbiology 104 (2022) 103973

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm



Live holding of red king crab (*Paralithodes camtschaticus*) and snow crab (*Chionoecetes opilio*) — Effect on microbial growth in processed leg meat during refrigerated storage

Federico Lian *, Øyvind Jøstensen, Sten I. Siikavuopio, Grete Lorentzen

Nofima AS, Muninbakken 9-13, Breivika, P.O. Box 6122, NO-9291, Tromsø, Norway

ARTICLE INFO

Keywords:
Capture-based aquaculture
Cooking
Crustacean
Freezing
Frozen storage
Live holding
Microbial spoilage
Shelf-life

ABSTRACT

The red king crab (RKC, *Paralithodes camtschaticus*) and snow crab (SC, *Chionoecetes opilio*) are valuable decapods that can undergo live holding (LH) in onshore facilities before either live export or processing into two cooked-frozen sections (i.e., clusters). This study investigated the effect of the LH time (up to two months without feeding) and temperature (5|10 °C for RKC; 1|5 °C for SC) on the total viable psychrotrophic count (TVC_P) and *Pseudomonas* spp. in the leg meat of cooked RKC and SC. The effect of freezing after cooking was also evaluated. The counts were determined during storage at 4 °C after cooking on the clusters undergoing either immediate refrigeration (IR) or 24-month frozen storage before refrigeration (FBR). In the RKC cooked leg meat, the LH temperature significantly affected the TVC_P, with LH at 10 °C leading to higher counts, while the *Pseudomonas* spp. levels were mainly influenced by the freezing, with lower levels in FBR samples compared to their IR counterparts. In the SC cooked leg meat, the LH conditions did not significantly affect the counts, which were instead significantly lowered by the freezing. From an industrial and commercial standpoint, it can be concluded that LH of RKC at high temperature (10 °C) led to a shorter microbial shelf-life of cooked RKC clusters, with the clusters undergoing FBR showing longer microbial shelf-life than their IR counterparts. By contrast, with the mild cooking regime applied, the LH time and temperature of SC had a lower influence on the microbial shelf-life of cooked SC clusters than that given by the choice of the storage type (IR or FBR) after cooking.

1. Introduction

In the last decade, the red king crab (RKC, *Paralithodes camtschaticus*) and snow crab (SC, *Chionoecetes opilio*) have both become valuable resources for the Norwegian fish industry (Lorentzen et al., 2018). In 2020, the total volume and first sale value for RKC and SC were 4475 tonnes and NOK 1029 million, respectively (Norwegian Seafood Council, 2021).

The RKC and SC are typically harvested in Arctic waters and traded either as live crabs or as cooked-frozen clusters, which consist of three (for RKC) or four (for SC) walking legs and a cheliped attached to a shoulder joint (Lian et al., 2018, 2021). At the retail level, RKC and SC are often commercialized as cooked-frozen clusters or as ready-to-eat clusters, which are refrigerated either immediately after cooking or after a period of frozen storage.

The *post*-harvest management of these Arctic crab species is crucial to ensure that their quality is retained along the entire value chain, avoiding food waste and profit loss (Lorentzen et al., 2018). In this regard, there has been an increasing utilization of onshore facilities for live holding (LH) of RKC in northern Norway, as this type of capture-based aquaculture enables more flexible resource management and market adaptation (Voldnes et al., 2020). The LH has become a consolidated practice for RKC due to the high share of live export and the fact that on-board processing of RKC is prohibited (The Norwegian Ministry of Trade, 2021). Nonetheless, LH may constitute a valuable tool also for SC, not only because of the increasing interest in trading live SC but also to make the processing of SC more cost-efficient compared to the current practice of on-board processing(Lorentzen et al., 2020b). For both species, conducting LH without feeding reduces the operative costs (Woll and Berge, 2007), while the water temperature is a key factor to keep control of the metabolic rate of the crabs (Siikavuopio and James, 2015; Siikavuopio et al., 2017).

In both RKC and SC, the edible meat is located in the walking legs and chelipeds. Like other crustaceans, the muscle tissue of RKC and SC is

* Corresponding author.
E-mail address: federico.lian@nofima.no (F. Lian).

https://doi.org/10.1016/j.fm.2021.103973
Received 6 August 2021; Received in revised form 19 December 2021; Accepted 21 December 2021
Available online 23 December 2021

very susceptible to spoilage and exhibits a rapid quality deterioration *post-mortem* which starts with a systemic enzymatic breakdown (Boziaris et al., 2011; Gornik et al., 2013; Huss, 1995; Neil, 2012). Moreover, the combination of low acidity (pH > 6) and high content in non-protein nitrogen (NPN) of the crab muscle provides favorable conditions for the growth of spoilage bacteria (Boziaris and Parlapani, 2017), which can reflect the environment in which the crabs are captured (Cockey and Chai, 1991; Sofos et al., 2013). After death, the bacterial load of the crab muscle can rapidly increase from nearly-sterile levels (< 2 log CFU g^{-1}) to 5–6 log CFU g^{-1} within a few days (Condón-Abanto et al., 2018; McDermott et al., 2018; Robson et al., 2007).

The spoilage process of crustaceans has been linked to their indigenous and exogenous microbiota composition (Odeyemi et al., 2018; Zhuang et al., 2020). The indigenous contamination can be affected by a series of factors such as environmental conditions, life stage, the season of harvest, and primary processing in farming, rearing, or LH facilities (Odeyemi et al., 2021; Parlapani, 2021). The exogenous bacteria derive from sources of cross-contamination *post*-cooking, including handling, cooling media, equipment, packaging, and storage conditions, among others (Parlapani, 2021).

Crab meat can be characterized by large microbial diversity (Parlapani et al., 2019), with possible high qualitative and quantitative variation between individuals (Condón-Abanto, 2019). A large number of genera and species, including *Bacillus* spp., Enterobacteriaceae, *Pseudomonas* spp., *Aeromonas* spp., *Moraxella* spp., *Flavobacterium* spp., *Shewanella putrefaciens*, *Photobacterium phosphoreum*, *Psychrobacter* spp., *Acinetobacter* spp., *Candidatus* Bacilloplasma, and lactic acid bacteria, have been detected in Dungeness crab (*Metacarcinus magister*) (Lee and Pfeifer, 1975), blue crab (*Callinectes sapidus*) (Parlapani et al., 2019; Ward et al., 1977), blue swimming crab (*Portunus armatus*) (Olatunde et al., 2021), and edible crab (*Cancer pagurus*) (Condón-Abanto, 2019).

Applying conventional culture-dependent microbiological methods, bacteria of the genus *Pseudomonas* have been recognized to dominate the spoilage of cooked and refrigerated leg meat from both RKC and SC (Lorentzen et al., 2014, 2016). Moreover, on the basis of a proposed link between these bacterial populations and different indicators of quality deterioration (e.g., the content in total volatile nitrogen and the presence of off-odors and off-flavors), the total viable psychrotrophic count (TVC_P) and *Pseudomonas* spp. have been considered as appropriate microbial parameters to monitor the shelf-life of refrigerated cooked crab meat products (Anacleto et al., 2011; Lorentzen et al., 2014, 2016; McDermott et al., 2018; Olatunde and Benjakul, 2021).

To hinder the spoilage process, cooking should be conducted immediately after the slaughtering. The efficacy of the cooking depends on the cumulative thermal effect delivered, and therefore mainly on process parameters such as time and temperature. Recently, there has been a tendency to move towards milder cooking treatments in crab processing, aiming to improve the nutritional and organoleptic quality in light of the increasing demand for minimally processed ready-to-eat seafood products (Nanda et al., 2021). Irrespective of the cooking regime, potential cross-contamination with exogenous microflora can potentially occur after cooking (Dima et al., 2015).

Notably, it has been reported that frozen storage of cooked SC clusters can delay the microbial growth of TVC_P and *Pseudomonas* spp. during refrigerated storage, once the product is thawed (Lorentzen et al., 2019a). By contrast, there is a lack of information on whether the LH conditions can influence the TVC_P and *Pseudomonas* spp. levels in processed crab meat.

Therefore, the present work aimed primarily to study the effect of LH time and temperature on microbial growth in the processed leg meat of RKC and SC clusters. Specifically, RKCs and SCs were kept without feeding in containers with seawater at two temperatures (i.e., 5 and 10 °C for RKC; 1 and 5 °C for SC) for about two months. The crabs were sampled during LH and processed into cooked clusters. Next to the effect of the LH conditions, the effect of freezing after cooking was also evaluated. The TVC_P and *Pseudomonas* spp. were determined during storage at 4 °C on the clusters undergoing either immediate refrigeration (IR) or freezing and 24-month frozen storage before refrigeration (FBR).

2. Material and methods

2.1. Harvest and live holding

The present study was conducted on samples obtained from the RKC and SC exemplars used in the LH experiments described by Lorentzen et al. (2019a,b) and Lorentzen et al. (2020a,b), respectively.

In November–December 2016, adult male RKCs and SCs were harvested in Norwegian waters of the Barents Sea and kept live without feeding at the Aquaculture Research Station in Kårvik (Tromsø, Norway). Both the RKCs and SCs were in the intermolt stage and characterized by high leg meat content (i.e., the cooked meat occupied approximately 87% of the cross-sectional area of the middle of the *merus*, the most proximal article of the walking legs). For each crab species, a LH study was performed by applying two LH temperatures, with the lowest temperature chosen as close to the crab *preferendum* while the highest temperature was simulating abusive temperature conditions (Siikavuopio and James, 2015; Siikavuopio et al., 2019a,b).

In more detail, for RKC, after an acclimatization period of seven days in tanks with running seawater at 4 °C, the LH study started (day 0) by equally distributing the crabs (n = 60) in six tanks (volume 0.7 m^3) supplied with circulating seawater at 5 °C (±0.2, three tanks, n = 10 in each tank) and 10 °C (±0.2, three tanks, n = 10 in each tank). The sampling and processing of the RKCs were performed at the start (day 0, also referred to as wild RKCs, n = 18), on day 41 (n = 9 from the group at 5 °C; n = 9 from the group at 10 °C), and on day 62 (n = 9, 5 °C; n = 9, 10 °C) of the LH study.

For SC, after an acclimatization period of three weeks in tanks with running seawater at 4 °C, the LH study was initiated (day 0) by equally allocating the crabs (n = 122) in eight tanks (volume 0.5 m^3) supplied with circulating seawater at 1 °C (±0.2, four tanks, n = 15 in three tanks and n = 16 in one tank) and 5 °C (±0.2, four tanks, n = 15 in three tanks and n = 16 in one tank). The sampling and processing of the SCs were performed at the start (day 0, also referred to as wild SCs, n = 28), on day 28 (n = 14, 1 °C; n = 14, 5 °C), and on day 68 (n = 14, 1 °C; n = 14, 5 °C) of the LH study.

The experiments had been approved by the local responsible laboratory animal science specialist under the surveillance of the Norwegian Animal Research Authority (NARA) and registered by the Authority, thereby conforming to Directive 2010/63/EU.

2.2. Processing

The sampled RKCs were processed following the procedure described by Lorentzen et al. (2019b). Briefly, the RKCs were split into two clusters which were cooked in boiling water for 16 min, reaching a core temperature of 92 °C in the *merus* of the largest walking leg of the cluster. Afterward, the clusters were cooled in ice water with 3.5% sea salt (w/v) (The Norwegian Salt Company Ltd., Bergen, Norway) for 21 min until the core temperature was below 4 °C.

The sampled SCs were processed as previously described by Lorentzen et al. (2020b).Briefly, the SCs were split into two clusters which were immersed in fresh water with 3.5% sea salt (w/v) at a temperature of 1–2 °C for 1 h, before cooking in water at 92.2–92.4 °C for 260 s. The cooking process was calibrated to achieve a core temperature of 80 °C in the *merus* of the second walking leg. The clusters were then cooled by immersion in fresh water with ice for approximately 15 min until the core temperature was below 4 °C.

After cooking and cooling, the clusters of RKC and SC originating from the same crab were allocated into two separate groups. One group of clusters (coded IR; immediately refrigerated) was packed in plastic bags (Finnvacum Oy Ab, Helsinki, Finland) closed with metallic clips and immediately stored in a climate chamber (Binder GmbH, Tuttlingen,

Germany) at 4 °C, while the other group of clusters (coded FBR; frozen before refrigeration) was frozen. Specifically, the clusters in the FBR group were placed in an air blast freezer at −40 °C with an air velocity of 2.4 m s^{-1} for 75 min before being packed in plastic bags as described above and stored at −20 °C for 24 months. Afterward, the FBR clusters were thawed and stored in the climate chamber at 4 °C. The time 0 of the refrigerated storage was defined as when the leg core temperature had reached 4 °C. This temperature was reached in 17 and 27 h for the RKC and SC clusters, respectively.

In both RKC and SC clusters, the temperature was monitored throughout cooking, cooling, freezing, thawing, and refrigerated storage using K-type thermocouples connected to data loggers (model 175H1, Testo Ltd., Hampshire, UK). The thermocouples were placed in the geometric center of the *merus*.

2.3. Microbial sampling and analysis

For both cooked RKC and SC clusters, the microbial analysis was conducted during the storage at 4 °C by sampling the leg meat extracted from the middle part of the *merus* of the walking legs. For RKC clusters, the sampling was performed on days 4, 6, 8, 10, and 13 (±4 h) of storage, except for the IR clusters originated from RKCs kept in LH for 62 days which were sampled on days 5 and 14 of storage instead of on days 4 and 13. For SC clusters, the sampling was performed on days 4, 6, 8, and 11 (±4 h) of storage, except for the IR clusters obtained from wild SCs and SCs kept in LH for 68 days which were sampled on day 7 of storage instead of on day 6. In addition, FBR clusters of SC were sampled also on day 14.

The microbiological analysis was conducted as described by Lorentzen et al. (2014), with slight modifications. Briefly, each meat sample (approximately 10 g) was transferred to a sterile Stomacher® bag (Seward Medical Ltd., Worthing, Sussex, UK) followed by a 1:5 dilution with sterile saline water 0.9% (w/v) NaCl (Oxoid) and 0.1% (w/v) peptone (Difco Laboratories Inc., Detroit, MI, USA). The sample was pummelled for 2 min in a laboratory blender (model Stomacher® 400, Seward Ltd., Worthing, UK) and plated after appropriate 10-fold dilutions. Total viable psychrotrophic count (TVC_P) was enumerated on pour-plated (with 1 mL of the 1:5 dilution) and spread-plated (with 0.1 mL of the 10-fold dilutions) plate count agar (PCA; Oxoid Ltd., Basingstoke, Hampshire, UK) supplemented with 0.5% NaCl (Oxoid) incubated at 12 °C for 7–9 days. Presumptive *Pseudomonas* spp. bacteria were enumerated on spread-plated (with 0.1 mL of the 10-fold dilutions) Pseudomonas agar base supplemented with cetrimide-fucidin-cephalosporine (CFC; Oxoid) incubated at 22 °C for 3 days.

Each independent biological sample, defined as the meat obtained from the *merus* of a crab cluster, was plated at least in duplicate in each of the microbial media.

The results were reported as the decimal logarithm of colony-forming units per gram of sample (log CFU g^{-1}) and expressed as the mean (±standard deviation) of the biological samples per storage time. For the plates accounting for the lowest dilution and without colonies detected, the level was set to half of the detection limit.

2.4. Statistical analysis

The statistical analyses were performed considering the results originated from the leg meat samples of each crab as an independent biological replicate.

The general effects of LH time and temperature and of the freezing after cooking were investigated by analysis of variance (ANOVA) using the general linear model (GLM) procedure in Statistica™ (vers. 13.5, TIBCO Software Inc., Palo Alto, CA, USA). The ANOVA model included the main effects of the explanatory variables (i.e., LH time, LH temperature, and freezing after cooking) and the interaction LH time × LH temperature. The data obtained from the RKCs sampled at the start of the LH study (i.e., day 0) were not considered for the ANOVA. The ANOVA was performed for the data obtained on days 6, 8, and 10 (RKC) or days 8 and 11 (SC) of storage at 4 °C. These time points were chosen as deemed the most important in determining the microbial shelf-life (Lorentzen et al., 2014, 2016).

Significant differences in the microbial count between LH time/temperature groups for the same storage time at 4 °C were analyzed by one-way ANOVA followed by *post-hoc* pairwise comparisons (Scheffé's test) using the software Statistica™. Independent samples (unpaired) *t*-test was used to evaluate significant differences due to the effect of freezing after cooking for data with the same LH time/temperature and storage time at 4 °C.

All statistical analyses were carried out at a 95% confidence level ($\alpha = 0.95$).

3. Results and discussion

3.1. Preliminary considerations

It should be highlighted that the length of the acclimatization period and the sampling time points during the LH at two temperatures were different for RKC and SC and were chosen according to current practice in the crab industry, research and industry interest, and practical constraints. Therefore, each species was the object of an independent LH study. It should also be noted that the cooking process applied to RKC and SC clusters differed in terms of cumulative thermal effect; a conventional industrial cooking regime was chosen for RKC, whereas in the case of SC, a milder-than-conventional cooking protocol was applied in an attempt to explore the potential for obtaining a minimally processed product. Adding to the differences in the length of the acclimatization period and LH sampling intervals, the difference in the cumulative thermal effect delivered to the RKC and SC clusters suggests that the microbial data discussed in the present manuscript should not necessarily be interpreted for cross-species comparison.

3.2. Red king crab (RKC)

The levels of total viable psychrotrophic count (TVC_P) and *Pseudomonas* spp. in the leg meat of cooked RKC clusters (IR and FBR) during storage at 4 °C are reported in Table 1.

For cooked RKC meat, the levels of TVC_P and *Pseudomonas* spp. have been shown to correlate negatively with positive sensory attributes during refrigerated storage, and therefore can be considered important spoilage indicators to monitor the shelf-life (Lorentzen et al., 2014). In this regard, the microbial acceptability limit (MAL) of 5 log CFU g^{-1} for TVC_P and 4 log CFU g^{-1} for *Pseudomonas* spp. have been proposed as the microbial shelf-life endpoint of the leg meat of cooked RKC clusters (Lian et al., 2021; Lorentzen et al., 2014).

3.2.1. Wild RKC

The TVC_P in IR leg meat samples obtained from wild RKCs (i.e., LH day 0) was characterized by a lag phase between day 4 and 6, reaching 5 log CFU g^{-1} between day 8 and 10 and exceeding 7 log CFU g^{-1} between day 10 and 13. This microbial growth was similar to that reported in a previous shelf-life study for cooked-refrigerated clusters obtained from wild intermolt RKCs (Lorentzen et al., 2014). By contrast, a similar lag phase but lower TVC_P levels at the later stages of storage were reported for the leg meat of cooked-refrigerated clusters obtained from wild autumn intermolt RKC (Lian et al., 2021). With regard to *Pseudomonas* spp., the observed levels in the present study for wild RKCs followed an evolution similar to that reported in the abovementioned studies (Lian et al., 2021; Lorentzen et al., 2014), exceeding 4 log CFU g^{-1} between day 8 and 13.

The differences in the TVC_P may be explained by the variability in the physiological status of the crabs together with the effect of factors such as the location and season or period of harvest and *post*-harvest

Table 1
Total viable psychrotrophic count (TVC_P) and *Pseudomonas* spp. in leg meat of cooked red king crab clusters that were immediately refrigerated (IR) or frozen and stored at −20 °C for 24 months before refrigeration (FBR) at 4 °C. The clusters were obtained from wild crabs (i.e., live holding day 0) or crabs undergoing live holding at 5 or 10 °C.

Microbial population	Freezing after cooking	Live holding		Storage time at 4 °C (day)									
		Time (day)	Temp. (°C)	4		6		8		10		11	
				Log CFU g^{-1}	*n*	Log CFU g^{-1}	*n*	Log CFU g^{-1}	*n*	Log CFU g^{-1}	*n*	Log CFU g^{-1}	*n*
TVC_P	No (IR)	0 (wild)		*0.40*	3	2.26 ± 0.51[b]	3	4.74 ± 1.54	3	5.12 ± 1.27	3	7.19 ± 1.65	3
		41	5	*0.40*	3	3.34 ± 0.45[ab]	3	4.41 ± 0.43	3	5.79 ± 0.78	3	7.53 ± 0.33	3
			10	*0.40*	3	3.37 ± 0.47[ab]	3	4.17 ± 0.24	3	6.08 ± 0.42	3	7.88 ± 0.38	3
		62	5	3.34 ± 0.77[a]	3	1.99 ± 0.17[b]	3	4.26 ± 0.66*	3	5.78 ± 1.04	3	7.74 ± 1.41[b]	3
			10	2.59 ± 1.06[a]	3	4.74 ± 0.64[a]*	3	5.05 ± 0.31	3	6.74 ± 0.46*	3	7.42 ± 1.30[b]	3
	Yes (FBR)	0 (wild)		2.13 ± 0.36	3	1.93 ± 0.52	3	2.82 ± 0.49[b]	3	4.44 ± 0.12	3	5.51 ± 0.03	3
		41	5	1.21 ± 0.54	3	2.69 ± 1.24	3	3.28 ± 0.83[ab]	3	5.18 ± 0.53	2	6.27 ± 0.71	3
			10	2.16 ± 0.57	3	4.55 ± 0.42	3	5.78 ± 0.72[a]	3	5.83 ± 0.12	2	7.30 ± 0.07	3
		62	5	2.15 ± 0.59	3	1.87 ± 0.80	3	1.87 ± 0.12[b]*	3	4.00 ± 1.92	3	4.43 ± 0.89	3
			10	1.38 ± 0.07	3	3.26 ± 1.58*	2	4.91 ± 1.01[ab]	2	4.41 ± 0.07*	2	6.69 ± 0.67	3
Pseudomonas spp.	No (IR)	0 (wild)		*1.40*	3	1.65 ± 0.12	3	3.52 ± 1.06	3	na	3	5.43 ± 1.28	3
		41	5	*1.40*	3	2.20 ± 0.29	3	4.35 ± 0.55*	3	5.35 ± 0.62	3	7.07 ± 0.22	3
			10	*1.40*	3	3.23 ± 0.45	3	4.71 ± 0.71	3	5.69 ± 0.18*	3	7.72 ± 0.30	3
		62	5	2.08 ± 0.47[a]	3	*1.40*	3	4.15 ± 0.60*	2	4.70 ± 1.10*	3	5.48 ± 0.79[b]	3
			10	*1.40*[a]	3	3.44 ± 1.11	3	4.90 ± 1.51*	3	6.63 ± 1.06*	3	6.31 ± 1.21[b]	3
	Yes (FBR)	0 (wild)		*1.40*	3	*1.40*	3	*1.40*	3	*1.40*[b]	3	1.70 ± 0.19	3
		41	5	*1.40*	3	*1.40*	3	2.11 ± 0.48*	3	5.09 ± 0.47[a]	2	5.61 ± 0.13	3
			10	*1.40*	3	2.32 ± 0.56	3	4.14 ± 1.36	3	4.18 ± 0.79[a]*	2	6.77 ± 0.35	3
		62	5	*1.40*	3	*1.40*	3	*1.40**	3	*1.40*[b]*	3	*1.40*	3
			10	*1.40*	3	*1.40*	2	*1.40**	2	*1.40*[b]*	2	4.51 ± 0.27	3

Note. Results are expressed as mean values (±standard deviation) of the decimal logarithm of colony-forming units per gram of sample (log CFU g^{-1}) obtained from independent biological replicates (*n*) for each combination of live holding time and temperature, storage time, and microbial population. na = not available. The values in italics are the result of independent biological samples all below the detection limit. For the plates accounting for the lowest dilution and without colonies detected, the level was set to half of the detection limit. Different superscript letters within the same subcolumn indicate significantly different mean values ($p \leq 0.05$, one-way ANOVA followed by Scheffé's test). For a same live holding time and temperature and storage time at 4 °C, the symbol (*) indicates the mean values which are significantly different due to the effect of freezing after cooking ($p \leq 0.05$, *t*-test).

[a] Sampled on day 5 of storage at 4 °C.

[b] Sampled on day 14 of storage at 4 °C.

handling practices (Calliauw et al., 2016). In addition, the microbial growth during refrigerated storage can be affected by the thermal exposure received by the clusters during the cooking process. Based on the cooking conditions (i.e., the cumulative thermal effect), it is possible to achieve different degrees of inactivation of the indigenous bacteria (Condón-Abanto et al., 2019). The present study targeted the same leg core temperature as the cooking endpoint (92 °C) adopted by Lorentzen et al. (2014) and Lian et al. (2021). However, in the latter study, the homogeneity of the heat exchange was improved by continuous recirculation of the cooking water, which might explain the lower TVC_P detected.

It is noteworthy also to mention that in a preliminary study on microbial growth in cooked RKC clusters stored at 4 °C, high variability in the microbial counts was observed not only between individuals belonging to the same harvest and cooking batch but also within individuals between samples obtained from different walking legs of the same cluster (unpublished data).

3.2.2. Effect of LH time and temperature in RKC

The general effects of LH time and temperature (5.0 ± 0.2 °C or 10.0

Table 2
Probability values (*p*) determined by analysis of variance (ANOVA) relative to total viable psychrotrophic count (TVC_P) and *Pseudomonas* spp. in leg meat of cooked red king crab clusters on days 6, 8, and 10 of storage at 4 °C. The ANOVA model included the main effects of the factors (i.e., LH time, LH temperature, and freezing after cooking) and the interaction LH time × LH temperature.

Microbial population	Storage time at 4 °C (day)	Factor				Total *df*
		Live holding			Freezing after cooking	
		Time (t)	Temp. (T)	t × T		
TVC_P	6	0.252	**0.005**	0.852	0.121	22
	8	0.308	**0.002**	0.567	0.104	22
	10	0.087	**0.018**	0.183	**0.016**	20
Pseudomonas spp.	6	0.085	**0.027**	0.592	**0.014**	22
	8	0.111	0.334	0.459	**< 0.001**	21
	10	**0.009**	0.328	0.283	**< 0.001**	20

Note. df, degrees of freedom. Significant effects ($p \leq 0.05$) are highlighted in bold.

± 0.2 °C), determined specifically after 6, 8, and 10 days of storage at 4 °C, are reported in Table 2.

The temperature was the LH factor influencing the TVC_P significantly, with LH at 10 °C leading to higher TVC_P. This effect was more evident for IR clusters on day 6 and, in general, for FBR clusters, especially on day 8. By contrast, *Pseudomonas* spp. levels were significantly affected by the LH temperature only at the early stage of storage (day 6), also in this case increasing with the LH temperature. In IR clusters, the MAL for the TVC_P was reached between 8 and 10 days of storage but earlier, in the interval of 6–8 storage days, for the samples obtained from RKC kept for 62 days at 10 °C. Considering *Pseudomonas* spp., the MAL was reached earlier (6–8 days) for all LH time and temperature combinations compared to their wild counterparts (8–13 days).

The significant effect of the LH temperature on microbial growth may be related to a series of physio-chemical modifications occurring in the leg muscle during LH without feeding, which are exacerbated by higher LH temperature. Water temperature is, in fact, an important factor for crab physiology. At higher water temperatures, lower oxygen levels are present, which has a repercussion on the metabolic functions and the stress-immunity axis of decapod species (Coates and Söderhäll, 2020). The RKC is a cold-water-adapted species typically found at 2–7 °C although tolerating up to 12 °C (Loher and Armstrong, 2005). Conducting LH at 10 °C, beyond the temperature *preferendum* of RKC (2.5–3.5 °C) (Christiansen et al., 2015), can induce stress conditions and thereby increase the metabolic energy requirements (Nilssen and Sundet, 2006). Previously, it has been shown that in crustaceans, such as Norway lobster (*Nephrops norvegicus*), *post-mortem* biochemical processes can be strongly influenced by *ante-mortem* stressors (Gornik et al., 2010).

In a study on RKCs from the same LH experiment as these microbial data originate from, it was reported a decrease in the leg meat content with LH time which was significantly faster in the RKCs held at 10 °C than at 5 °C (Lorentzen et al., 2019b). By combining the meat content with corresponding proximate and fatty acid composition data, it could be hypothesized a higher rate of catabolization of the leg muscle tissue for the RKCs held at 10 °C as a strategy to cope with starvation when

Table 3
Total viable psychrotrophic count (TVC_P) and *Pseudomonas* spp. in leg meat of cooked snow crab clusters that were immediately refrigerated (IR) or frozen and stored at −20 °C for 24 months before refrigeration (FBR) at 4 °C. The clusters were obtained from wild crabs (i.e., live holding day 0) or crabs undergoing live holding at 1 or 5 °C.

Microbial population	Freezing after cooking	Live holding		Storage time at 4 °C (day)									
		Time (day)	Temp. (°C)	4		6		8		11		14	
				Log CFU g^{-1}	*n*	Log CFU g^{-1}	*n*	Log CFU g^{-1}	*n*	Log CFU g^{-1}	*n*	Log CFU g^{-1}	*n*
TVC_P	No (IR)	0 (wild)		2.13 ± 0.46	6	4.35 ± 0.77[a]	6	4.53 ± 0.54[a]*	6	5.81 ± 0.72[a]*	6	na	6
		28	1	0.54 ± 0.08	3	0.60 ± 0.08	3	3.14 ± 0.33[ab]*	3	2.48 ± 0.00[b]	3	na	3
			5	0.60 ± 0.08	3	0.60 ± 0.08	3	2.79 ± 1.17[b]	3	3.99 ± 0.79[ab]	3	na	3
		68	1	*0.40*	3	2.36 ± 0.49[a]	3	2.56 ± 0.72[b]*	3	3.51 ± 1.04[b]	2	na	3
			5	*0.40*	3	2.89 ± 0.89[a]	3	2.64 ± 0.22[b]	3	3.54 ± 1.04[b]	2	na	3
	Yes (FBR)	0 (wild)		1.05 ± 0.48	6	1.92 ± 0.85	6	2.47 ± 1.06*	6	3.07 ± 1.49*	6	4.62 ± 2.09	6
		28	1	na	5	*0.40*	5	1.87 ± 0.82*	5	3.08 ± 1.26	5	4.66 ± 1.26	5
			5	na	5	*0.40*	5	1.53 ± 0.67	5	3.28 ± 1.39	5	4.83 ± 1.66	5
		68	1	na	5	*0.40*	5	0.74 ± 0.29*	5	2.76 ± 0.77	5	4.44 ± 1.25	5
			5	na	5	*0.40*	5	*0.40*	5	3.87 ± 1.58	5	4.68 ± 1.97	5
Pseudomonas spp.	No (IR)	0 (wild)		2.04 ± 0.45	6	3.67 ± 0.85[a]	6	4.50 ± 0.30[a]*	6	5.84 ± 0.77[a]*	6	na	6
		28	1	*1.40*	3	*1.40*	3	2.29 ± 0.50[b]	3	2.45 ± 0.67[b]	3	na	3
			5	*1.40*	3	*1.40*	3	2.91 ± 0.75[b]*	3	5.73 ± 1.68[ab]*	3	na	3
		68	1	*1.40*	3	*1.40* [a]	3	2.26 ± 0.57[b]	3	3.72 ± 0.00[ab]	3	na	3
			5	*1.40*	3	2.96 ± 0.92[a]	3	3.04 ± 0.96[b]	3	3.09 ± 0.92[ab]	3	na	3
	Yes (FBR)	0 (wild)		*1.40*	6	*1.40*	6	*1.40**	6	2.48 ± 0.68*	6	4.50 ± 1.45	6
		28	1	na	5	*1.40*	5	*1.40*	5	*1.40*	5	2.34 ± 0.61	5
			5	na	5	*1.40*	5	*1.40**	5	*1.40**	5	3.96 ± 1.30	5
		68	1	na	5	*1.40*	5	*1.40*	5	*1.40*	5	*1.40*	5
			5	na	5	*1.40*	5	*1.40*	5	*1.40*	5	*1.40*	5

Note. Results are expressed as mean values (±standard deviation) of the decimal logarithm of colony-forming units per gram of sample (log CFU g^{-1}) obtained from independent biological replicates (*n*) for each combination of live holding time and temperature, storage time, and microbial population. na = not available. The values in italics are the result of independent biological samples all below the detection limit. For the plates accounting for the lowest dilution and without colonies detected, the level was set to half of the detection limit. Different superscript letters within the same subcolumn indicate significantly different mean values ($p \leq 0.05$, one-way ANOVA followed by Scheffé's test). For a same live holding time and temperature and storage time at 4 °C, the symbol (*) indicates the mean values which are significantly different due to the effect of freezing after cooking ($p \leq 0.05$, *t*-test).

[a] Sampled on day 7 of storage at 4 °C.

kept at higher water temperature (Lian et al., 2022). The mobilization and breakdown of proteic compounds may lead to higher availability of nutrients, especially in the form of NPN, which can act as a readily available substrate for bacteria (Madigan et al., 2018). In this regard, it should be noted that, in RKC, the level of specific free amino acids, which are a form of NPN, can increase in association with stress conditions as they play an essential physiological role in the adaptive response of crustaceans (Mota et al., 2021).

Furthermore, the decreasing leg meat content with longer LH time and higher LH temperature may have indirectly influenced the thermal exposure of the meat during the cooking process at different LH sampling points, potentially with contrasting effects. The reduced amount of meat in the legs, on the one hand, can contribute towards a higher cumulative thermal exposure with higher inactivation of bacteria; on the other hand, it can cause more severe denaturation of muscle proteins and degradation of the muscle structure, leading to higher levels of NPN and accessibility of nutrients for bacteria.

3.3. Snow crab (SC)

The levels of total viable psychrotrophic count (TVC_P) and *Pseudomonas* spp. in the leg meat of cooked SC clusters (IR and FBR) during storage at 4 °C are reported in Table 3. Similarly to the case of RKC, also for cooked SC meat, MALs of 5 log CFU g^{-1} for TVC_P and 4 log CFU g^{-1} for *Pseudomonas* spp. have been proposed as the microbial shelf-life endpoint (Lorentzen et al., 2016).

3.3.1. Wild SC

The TVC_P in IR leg meat samples obtained from wild SCs (i.e., LH day 0) showed a lag phase of about 4 days, approaching 6 log CFU g^{-1} at the end of the monitoring period (day 11) and reaching the MAL between day 8 and 11 of storage. The corresponding levels of *Pseudomonas* spp. were very similar to the TVC_P, indicating a relatively high abundance of this microbial population throughout storage. The MAL for *Pseudomonas* spp. was reached between days 6 and 8 of storage.

Previously, a shelf-life study on cooked-refrigerated SC leg meat reported a similar microbial growth for TVC_P, but lower levels of *Pseudomonas* spp., which reached the MAL at a later stage between day 10 and 12 of storage (Lorentzen et al., 2016). However, it should be noted that the cooking process applied by Lorentzen et al. (2016) was targeted to achieve a cheliped core temperature of 91 °C and, therefore, was characterized by a remarkably higher cumulative thermal effect compared to this study. In this regard, a better comparison can be made with the work of Lorentzen et al. (2019a) in which SC clusters were cooked at 87 °C for 430 s or 96 °C for 148 s, aiming a leg meat core temperature of 80–81 °C as the cooking endpoint. Compared to the present study, Lorentzen et al. (2019a) reported similar levels of *Pseudomonas* spp. but higher TVC_P during storage.

Furthermore, next to differences in the cumulative thermal effect of the cooking process, as for the case of RKC, it can be hypothesized that different season and harvest locations influence the type of microflora associated with the microbial growth during the refrigerated storage period after cooking (Calliauw et al., 2016).

3.3.2. Effect of LH time and temperature in SC

No significant general effect of the time and temperature (1.0 ± 0.2 °C or 5.0 ± 0.2 °C) of LH was observed on TVC_P and *Pseudomonas* spp. levels for the time points 8 and 11 days of storage at 4 °C (Table 4).

Although caution should be applied when comparing the LH effects in RKC and SC given the different cooking treatments applied, the results for SC appear in contrast with the ones obtained for RKC, where especially the TVC_P was significantly influenced by the LH temperature. As the case of RKC, also SC may be sensitive at LH temperatures above its *preferendum* (1.0–1.6 °C) (Siikavuopio et al., 2019a). Nonetheless, our results are in line with a previous study conducted on the SCs in the same LH experiment, showing that the LH time and temperature did not have a significant effect on the leg meat content during the first 68 days of LH without feeding (Lorentzen et al., 2020b). It was suggested that SC might compensate for the absence of feed resources with a different degradation pattern than RKC, predilecting the mobilization of energy reserves from the hepatopancreas while limiting the muscle degradation in the walking legs (Lorentzen et al., 2020b).

Table 4
Probability values (p) determined by analysis of variance (ANOVA) relative to total viable psychrotrophic count (TVC_P) and *Pseudomonas* spp. in leg meat of cooked snow crab clusters on days 8 and 11 of storage at 4 °C. The ANOVA model included the main effects of the factors (i.e., LH time, LH temperature, and freezing after cooking) and the interaction LH time × LH temperature.

Microbial population	Storage time at 4 °C (day)	Factor				Total *df*
		Live holding			Freezing after cooking	
		Time (t)	Temp. (T)	t × T		
TVC_P	8	0.070	0.603	0.422	**< 0.001**	31
	11	0.586	0.805	0.354	**0.017**	31
Pseudomonas spp.	8	0.657	0.454	0.873	**< 0.001**	31
	11	0.737	0.479	0.069	**< 0.001**	29

Note. *df*, degrees of freedom. Significant effects ($p \leq 0.05$) are highlighted in bold.

Although the time of LH did not have a significant influence, it is noteworthy that on days 8 and 11 of storage, the microbial levels for wild SCs in IR samples were significantly different from those observed for several of the other LH groups. For example, on day 8, in the samples obtained from wild SCs, the values of TVC_P and *Pseudomonas* spp. were significantly higher than in the leg meat of the SCs kept in LH irrespective of time and temperature, except for TVC_P in the group LH day 28/5 °C. Similarly, on day 11, compared to wild SCs samples, significantly lower microbial levels were found in the leg meat obtained from the SCs in the group LH day 28/1 °C (for both microbial populations) and on day 68 from both temperature groups (for TVC_P). Consequently, the MAL in IR samples obtained from SCs kept in LH was not reached in the monitored period (until day 11) except for the case of *Pseudomonas* spp. in the group LH day 28/5 °C.

The lower microbial growth generally found in IR samples after LH may be theoretically ascribed to aspects that influence indigenous and exogenous microflora activity. Given that the cooking treatment applied to SC clusters was relatively mild, it may be postulated that these changes are linked to the LH itself rather than cross-contamination after cooking.

A hypothesis might be that the LH in controlled conditions induces an immunomodulatory response in SCs, activating or increasing the production of compounds with antimicrobial properties. Previous research has demonstrated the presence of functional molecules, such as antimicrobial peptides (AMPs), which exhibit antimicrobial activity in marine crustacean decapods, including SC (Beaulieu et al., 2010; El Menif et al., 2018; Haug et al., 2002; Sperstad et al., 2011). AMPs are small cationic peptides that display a broad range of antimicrobial activity against Gram-positive and Gram-negative bacteria (Yue et al., 2010); they can typically be found in the hemocytes of the hemolymph of the marine crabs as an integral component of their immune system (Anbuchezian et al., 2018), and their levels and activity can be related to the stress status of the crab (Coates and Söderhäll, 2020). For some crustacean species, including the spider crab (*Hyas araneus*) and shore crab (*Carcinus maenas*), AMPs have displayed stability to heat, specifically when exposed to 85 °C for 15 min (Haug et al., 2002) and 100 °C for 10 min (Relf et al., 1999), respectively. The mild cooking treatment applied to SC clusters may have avoided the denaturation of any AMPs present. In this regard, it is noteworthy that the cooking treatment applied to SC clusters in the present study did not deactivate polyphenoloxidases (POs) as evidenced by the development of melanosis discoloration after cooking (Lorentzen et al., 2019a; Lorentzen et al.,

2020a). Furthermore, it is known that the melanization reaction and associated activities promoted by the POs activating system can play a significant immunological contribution (Cerenius and Söderhäll, 2021). A specific investigation is warranted to assess whether certain LH conditions can elicit enhanced AMPs levels and whether such compounds can play a role in modulating the microbial growth in the leg meat after cooking.

3.4. Effect of freezing after cooking and frozen storage in RKC and SC

Regarding RKC, the application of freezing after cooking significantly influenced the TVC_P on day 10 and *Pseudomonas* spp. on days 6, 8, and 10 of storage at 4 °C (Table 2). Specifically, lower microbial levels were found in the FBR leg meat compared to their IR counterparts (Table 1). Consequently, the freezing-thawing process led to a general extension of the time to reach the MAL during storage, which was more evident for the wild RKCs and the ones kept in LH at 5 °C, irrespective of the LH time.

Regarding SC, it was freezing after cooking that affected the levels of both TVC_P and *Pseudomonas* spp. significantly on days 8 and 11 of storage (Table 4). Also, in the case of SC, the freezing-thawing process led to lower microbial levels than their IR counterparts, thus extending the time to reach the MAL (Table 2). The reduction in the TVC_P was significant for the wild SCs (on days 8 and 11) and for the SCs kept at 1 °C (on day 8), whereas the decrease in *Pseudomonas* spp. levels was significant for wild SCs and SCs held for 28 days at 5 °C. For FBR samples obtained from SC kept in LH for 28 and 68 days, the TVC_P and *Pseudomonas* spp. levels did not differ significantly from their wild counterparts.

The differences in the microbial growth between IR and FBR clusters of both RKC and SC can be ascribed to sublethal damage caused by the combination of cooking and subsequent freezing, leading to a limited survival and growth of the bacteria in the subsequent refrigeration (Lee and Jiang, 2004). As an effect of freezing, bacteria become dormant; they may regain their activity after thawing, but the associated microbial spoilage is slowed down (Leygonie et al., 2012). Although the literature on microbial quality and shelf-life after freezing and thawing is scarce, the reduction in the TVC_P and *Pseudomonas* spp. observed in FBR clusters is in accordance with previous findings for other seafood species. As an example, freezing of cooked marine crab (*Portunus pelagicus*) and a 40-week storage period at −23 °C in Jonah crab (*Cancer borealis*) meat led, respectively, to a reduction by 2 and 2–3 log CFU g^{-1} of the TVC_P (Rebach et al., 1990; Subramanian, 2007). Similarly, a reduction by 1–2 log CFU g^{-1} in *Pseudomonas* spp. levels was reported by Baixas-Nogueras et al. (2007) for raw hake stored at −20 °C for 6 months. Furthermore, Lorentzen et al. (2019) reported that the freezing-thawing process effectively delayed microbial growth, especially of *Pseudomonas* spp., in the leg meat of mildly-cooked SC clusters stored at −20 °C for 3 months.

From a commercial standpoint, our results show the potential in prolonging the shelf-life at 4 °C by applying freezing and an extended period of frozen storage before thawing and refrigeration. This effect appears to be more evident on *Pseudomonas* spp. and in the leg meat of SC clusters as compared to that of RKC.

The freezing process and the subsequent frozen storage time may influence the quality of the product, with a possible negative impact on the drip loss and sensory attributes (e.g., juiciness), making the choice of applying freezing more complex (Yang et al., 2020). In this regard, it is important to carefully evaluate both the freezing method, since it determines the freezing rate, and the length and conditions of the frozen storage (Lorentzen et al., 2020a; Tan et al., 2021).

4. Conclusion

This study showed that the pattern of microbial spoilage and, therefore, the microbial shelf-life of cooked crab meat could be affected by the live holding (LH) conditions.

Considering the LH conditions investigated (i.e., time and temperature) and the cooking and freezing procedures applied, the LH temperature significantly affected the total viable psychrotrophic count of the leg meat of cooked red king crab (RKC) clusters, with LH at 10 °C leading to higher counts compared to LH at 5 °C; the *Pseudomonas* spp. levels were mainly influenced by the application of freezing after cooking, with lower levels detected in the samples that underwent 24-month frozen storage before refrigeration (FBR) compared to their immediately refrigerated (IR) counterparts. In the case of the leg meat of cooked snow crab (SC) clusters, the LH conditions did not significantly influence the microbial counts, which were instead significantly lowered by the freezing after cooking.

From an industrial and commercial perspective, it can be concluded that high LH temperature (10 °C) of RKC caused a shorter microbial shelf-life of the cooked clusters, with the clusters undergoing FBR showing longer microbial shelf-life than their IR counterparts. By contrast, with the mild cooking regime applied, the LH time and temperature of SC had a lower influence on the microbial shelf-life of the cooked clusters than that given by the choice of the storage type (IR or FBR) after cooking.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgments

The results presented are deliverables from the project “Harvest and live holding of snow and king crab, the relevance of time in pots and storage time onshore and its effects on welfare and quality” funded by The Norwegian Seafood Research Fund (project no. 901254), and the project “SnowMap” funded within the Bionær program by The Research Council of Norway (project no. 267763). Besides, this work is partly funded by the Irish Department of Agriculture, Food and the Marine within the project QualCrust (project no. 13/F/529), the Erasmus+ traineeship program (2016/2017), and Nofima AS. We would like to thank Prof. Margrethe Esaiassen (UiT, The Arctic University of Norway, Tromsø) as well as Adrian Eyser Grip, Elin Sandaker, Tor Evensen, Anette Hustad, and Tina Thesslund at Nofima for valuable help.

References

Anacleto, P., Teixeira, B., Marques, P., Pedro, S., Nunes, M.L., Marques, A., 2011. Shelf-life of cooked edible crab (*Cancer pagurus*) stored under refrigerated conditions. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 44 (6), 1376–1382. https://doi.org/10.1016/j.lwt.2011.01.010.

Anbuchezian, R., Ravichandran, S., Karthick Rajan, D., Tilivi, S., Prabha Devi, S., 2018. Identification and functional characterization of antimicrobial peptide from the marine crab *Dromia dehaani*. Microb. Pathog. 125, 60–65. https://doi.org/10.1016/j.micpath.2018.08.056.

Baixas-Nogueras, S., Bover-Cid, S., Veciana-Nogués, M.T., Vidal-Carou, M.C., 2007. Effects of previous frozen storage on chemical, microbiological and sensory changes during chilled storage of Mediterranean hake (*Merluccius merluccius*) after thawing. Eur. Food Res. Technol. 226 (1–2), 287–293. https://doi.org/10.1007/s00217-006-0538-z.

Beaulieu, L., Thibodeau, J., Desbiens, M., Saint-Louis, R., Zatylny-Gaudin, C., Thibault, S., 2010. Evidence of antibacterial activities in peptide fractions originating from snow crab (*Chionoecetes opilio*) by-products. Probiot. Antimicrob. Protein. 2 (3), 197–209. https://doi.org/10.1007/s12602-010-9043-6.

Boziaris, I.S., Kordila, A., Neofitou, C., 2011. Microbial spoilage analysis and its effect on chemical changes and shelf-life of Norway lobster (*Nephrops norvegicus*) stored in air at various temperatures. Int. J. Food Sci. Technol. 46 (4), 887–895. https://doi.org/10.1111/j.1365-2621.2011.02568.x.

Boziaris, I.S., Parlapani, F.F., 2017. Specific spoilage organisms (SSOs) in fish. In: Bevilacqua, A., Corbo, M.R., Sinigaglia, M. (Eds.), The Microbiological Quality of Food. Woodhead Publishing, pp. 61–98. https://doi.org/10.1016/b978-0-08-100502-6.00006-6.

Calliauw, F., De Mulder, T., Broekaert, K., Vlaemynck, G., Michiels, C., Heyndrickx, M., 2016. Assessment throughout a whole fishing year of the dominant microbiota of peeled brown shrimp (*Crangon crangon*) stored for 7 days under modified atmosphere packaging at 4 °C without preservatives. Food Microbiol. 54, 60–71. https://doi.org/10.1016/j.fm.2015.10.016.

Cerenius, L., Söderhäll, K., 2021. Immune properties of invertebrate phenoloxidases. Dev. Comp. Immunol. 122 https://doi.org/10.1016/j.dci.2021.104098. Article 104098.
Christiansen, J.S., Sparboe, M., Saether, B.-S., Siikavuopio, S.I., 2015. Thermal behaviour and the prospect spread of an invasive benthic top predator onto the Euro-Arctic shelves. Divers. Distrib. 21 (9), 1004–1013. https://doi.org/10.1111/ddi.12321.
Coates, C.J., Söderhäll, K., 2020. The stress–immunity axis in shellfish. J. Invertebr. Pathol. https://doi.org/10.1016/j.jip.2020.107492. Article 107492.
Cockey, R.R., Chai, T., 1991. Microbiology of crustacea processing: Crabs. In: Ward, D.R., Hackney, C. (Eds.), Microbiology of Marine Food Products. Springer, pp. 41–63. https://doi.org/10.1007/978-1-4615-3926-1_3.
Condón-Abanto, S., 2019. Adding Value to Ready-To-Crustacean Products: Process Optimization for "entire" Crustaceans Using Novel Technologies (Accessed on June 30, 2021). PhD thesis. Available at: https://zaguan.unizar.es/record/99210/files/TESIS-2021-040.pdf?version=1.
Condón-Abanto, S., Arroyo, C., Álvarez, I., Brunton, N., Whyte, P., Lyng, J.G., 2018. An assessment of the application of ultrasound in the processing of ready-to-eat whole brown crab (*Cancer pagurus*). Ultrason. Sonochem. 40, 497–504. https://doi.org/10.1016/j.ultsonch.2017.07.044.
Condón-Abanto, S., Raso, J., Arroyo, C., Lyng, J., Álvarez, I., 2019. Quality-based thermokinetic optimization of ready-to-eat whole edible crab (*Cancer pagurus*) pasteurisation treatments. Food Bioprocess Technol. 12 (3), 436–446. https://doi.org/10.1007/s11947-018-2222-2.
Dima, J.B., Barón, P.J., Zaritzky, N., 2015. Pasteurization conditions and evaluation of quality parameters of frozen packaged crab meat. J. Aquat. Food Prod. Technol. 25 (5), 745–759. https://doi.org/10.1080/10498850.2014.923083.
El Menif, E., Offret, C., Labrie, S., Beaulieu, L., 2018. Identification of peptides implicated in antibacterial activity of snow crab hepatopancreas hydrolysates by a bioassay-guided fractionation approach combined with mass spectrometry. Probiot. Antimicrob. Protein. 11 (3), 1023–1033. https://doi.org/10.1007/s12602-018-9484-x.
Gornik, S.G., Albalat, A., Atkinson, R.J.A., Coombs, G.H., Neil, D.M., 2010. The influence of defined ante-mortem stressors on the early post-mortem biochemical processes in the abdominal muscle of the Norway lobster, *Nephrops norvegicus* (Linnaeus, 1758). Mar. Biol. Res. 6 (3), 223–238. https://doi.org/10.1080/17451000903147468.
Gornik, S.G., Albalat, A., Theethakaew, C., Neil, D.M., 2013. Shelf life extension of whole Norway lobster *Nephrops norvegicus* using modified atmosphere packaging. Int. J. Food Microbiol. 167 (3), 369–377. https://doi.org/10.1016/j.ijfoodmicro.2013.10.002.
Haug, T., Kjuul, A.K., Stensvåg, K., Sandsdalen, E., Styrvold, O.B., 2002. Antibacterial activity in four marine crustacean decapods. Fish Shellfish Immunol. 12 (5), 371–385. https://doi.org/10.1006/fsim.2001.0378.
Huss, H., 1995. Quality and Quality Changes in Fresh Fish (Accessed on June 30, 2021). FAO Fisheries Technical Paper 348. Available at: http://www.fao.org/3/v7180e/v7180e00.htm.
Lee, J.S., Pfeifer, D.K., 1975. Microbiological characteristics of Dungeness crab (*Cancer magister*). Appl. Microbiol. 30 (1), 72–78. https://doi.org/10.1128/aem.30.1.72-78.1975.
Lee, T.-C., Jiang, S.-T., 2004. Freezing seafood and seafood products; principles and applications. In: Hui, Y.H., Cornillon, P., Legaretta, I.G., Lim, M.H., Murrell, K.D., Nip, W.-K. (Eds.), Handbook of Frozen Foods. Marcel Dekker, pp. 245–294.
Leygonie, C., Britz, T.J., Hoffman, L.C., 2012. Impact of freezing and thawing on the quality of meat: Review. Meat Sci. 91 (2), 93–98. https://doi.org/10.1016/j.meatsci.2012.01.013.
Lian, F., Måge, I., Lorentzen, G., Siikavuopio, S.I., Øverbø, K., Vang, B., Lindberg, D., 2018. Exploring the effect of inhibitors, cooking and freezing on melanosis in snow crab (*Chionoecetes opilio*) clusters. Food Control 92, 255–266. https://doi.org/10.1016/j.foodcont.2018.04.055.
Lian, F., Siikavuopio, S.I., Harrison, S.M., Vang, B., Brunton, N.P., Esaiassen, M., Lorentzen, G., 2022. Fatty acid profile of cooked leg meat and raw hepatopancreas of red king crab (*Paralithodes camtschaticus*) during three-month live holding without feeding at 5 and 10 °C. Article 104206 J. Food Compos. Anal. 105. https://doi.org/10.1016/j.jfca.2021.104206.
Lian, F., Siikavuopio, S.I., Hustad, A., Thesslund, T., Lindberg, S.-K., Lorentzen, G., 2021. Comparative quality evaluation of processed clusters obtained from red king crab (*Paralithodes camtschaticus*) typical of spring and autumn harvests in the Barents Sea. Article 107826 Food Control 123. https://doi.org/10.1016/j.foodcont.2020.107826.
Loher, T., Armstrong, D.A., 2005. Historical changes in the abundance and distribution of ovigerous red king crabs (*Paralithodes camtschaticus*) in Bristol Bay (Alaska), and potential relationship with bottom temperature. Fish. Oceanogr. 14 (4), 292–306. https://doi.org/10.1111/j.1365-2419.2005.00337.x.
Lorentzen, G., Hustad, A., Lian, F., Grip, A.E., Schrødter, E., Medeiros, T., Siikavuopio, S. I., 2020a. Effect of freezing methods, frozen storage time, and thawing methods on the quality of mildly cooked snow crab (*Chionoecetes opilio*) clusters. Article 109103 LWT 123. https://doi.org/10.1016/j.lwt.2020.109103.
Lorentzen, G., Lian, F., Røhme, A.A., Johannessen, E., Grastveit, K.V., Grip, A.E., Siikavuopio, S.I., 2019a. Effect of freezing-thawing on weight loss, melanosis, and microbial growth in mildly cooked snow crab (*Chionoecetes opilio*) clusters. LWT 108, 283–288. https://doi.org/10.1016/j.lwt.2019.03.083.
Lorentzen, G., Lian, F., Siikavuopio, S.I., 2019b. Quality parameters of processed clusters of red king crab (*Paralithodes camtschaticus*) - effects of live holding at 5 and 10 °C up to 92 days without feeding. Food Control 95, 142–149. https://doi.org/10.1016/j.foodcont.2018.07.041.
Lorentzen, G., Lian, F., Siikavuopio, S.I., 2020b. Live holding of snow crab (*Chionoecetes opilio*) at 1 and 5 °C without feeding — quality of processed clusters. Article 107221 Food Control 114. https://doi.org/10.1016/j.foodcont.2020.107221.
Lorentzen, G., Rotabakk, B.T., Olsen, S.H., Skuland, A.V., Siikavuopio, S.I., 2016. Shelf life of snow crab clusters (*Chionoecetes opilio*) stored at 0 and 4 °C. Food Control 59, 454–460. https://doi.org/10.1016/j.foodcont.2015.06.019.
Lorentzen, G., Skuland, A.V., Sone, I., Johansen, J.-O., Rotabakk, B.T., 2014. Determination of the shelf life of cluster of the red king crab (*Paralithodes camtschaticus*) during chilled storage. Food Control 42, 207–213. https://doi.org/10.1016/j.foodcont.2014.02.019.
Lorentzen, G., Voldnes, G., Whitaker, R.D., Kvalvik, I., Vang, B., Gjerp Solstad, R., Thomassen, M.R., Siikavuopio, S.I., 2018. Current status of the red king crab (*Paralithodes camtschaticus*) and snow crab (*Chionoecetes opilio*) industries in Norway. Rev. Fish. Sci. Aqua. 26 (1), 42–54. https://doi.org/10.1080/23308249.2017.1335284.
Madigan, M.T., Bender, K.S., Buckley, D.H., Sattley, W.M., Stahl, D.A., 2018. Microbial growth and its control. In: Brock Biology of Microorganisms, fifteenth ed. Pearson Education, pp. 173–208.
McDermott, A., Whyte, P., Brunton, N., Lyng, J., Fagan, J., Bolton, D.J., 2018. The effect of organic acid and sodium chloride dips on the shelf-life of refrigerated Irish brown crab (*Cancer pagurus*) meat. LWT 98, 141–147. https://doi.org/10.1016/j.lwt.2018.08.039.
Mota, V.C., Siikavuopio, S.I., James, P., 2021. Physiological responses to live air transport of red king crab (*Paralithodes camtschaticus*). Article 105882 Fish. Res. 237. https://doi.org/10.1016/j.fishres.2021.105882.
Nanda, P.K., Das, A.K., Dandapat, P., Dhar, P., Bandyopadhyay, S., Dib, A.L., Lorenzo, J. M., Gagaoua, M., 2021. Nutritional aspects, flavour profile and health benefits of crab meat based novel food products and valorisation of processing waste to wealth: a review. Trends Food Sci. Technol. 112, 252–267. https://doi.org/10.1016/j.tifs.2021.03.059.
Neil, D.M., 2012. Ensuring crustacean product quality in the post-harvest phase. J. Invertebr. Pathol. 110 (2), 267–275. https://doi.org/10.1016/j.jip.2012.03.009.
Nilssen, E.M., Sundet, J.H., 2006. The introduced species red king crab (*Paralithodes camtschaticus*) in the Barents Sea: II. Growth increments and moulting probability. Fish. Res. 82 (1–3), 319–326. https://doi.org/10.1016/j.fishres.2006.05.008.
Norwegian Seafood Council, 2021. Red king crab. Snow crab. Monthly export volume and prices (Accessed June 30, 2021) Available at: https://en.seafood.no/.
Odeyemi, O.A., Burke, C.M., Bolch, C.C.J., Stanley, R., 2018. Seafood spoilage microbiota and associated volatile organic compounds at different storage temperatures and packaging conditions. Int. J. Food Microbiol. 280, 87–99. https://doi.org/10.1016/j.ijfoodmicro.2017.12.029.
Odeyemi, O.A., Dabadé, D.S., Amin, M., Dewi, F., Kasan, N.A., Waiho, K., 2021. Microbial diversity and ecology of crustaceans: influencing factors and future perspectives. Curr. Opin. Food Sci. 39, 140–143. https://doi.org/10.1016/j.cofs.2021.01.001.
Olatunde, O.O., Benjakul, S., 2021. Sous-vide cooking as a systematic approach for quality maintenance and shelf-life extension of crab lump meat. Article 111004 LWT 142. https://doi.org/10.1016/j.lwt.2021.111004.
Olatunde, O.O., Chantakun, K., Benjakul, S., 2021. Microbial, chemical qualities and shelf-life of blue swimming crab (*Portunus armatus*) lump meat as influenced by in-package high voltage cold plasma treatment. Article 101274 Food Biosci. 43. https://doi.org/10.1016/j.fbio.2021.101274.
Parlapani, F.F., 2021. Microbial diversity of seafood. Curr. Opin. Food Sci. 37, 45–51. https://doi.org/10.1016/j.cofs.2020.09.005.
Parlapani, F.F., Michailidou, S., Anagnostopoulos, D.A., Koromilas, S., Kios, K., Pasentsis, K., Psomopoulos, F., Argiriou, A., Haroutounian, S.A., Boziaris, I.S., 2019. Bacterial communities and potential spoilage markers of whole blue crab (*Callinectes sapidus*) stored under commercial simulated conditions. Food Microbiol. 82, 325–333. https://doi.org/10.1016/j.fm.2019.03.011.
Rebach, S., Stribling, J., Wilber, M., 1990. Frozen storage quality changes in whole Jonah crabs. J. Food Qual. 13 (3), 203–208. https://doi.org/10.1111/j.1745-4557.1990.tb00017.x.
Relf, J.M., Chisholm, J.R.S., Kemp, G.D., Smith, V.J., 1999. Purification and characterization of a cysteine-rich 11.5-kDa antibacterial protein from the granular haemocytes of the shore crab, *Carcinus maenas*. Eur. J. Biochem. 264 (2), 350–357. https://doi.org/10.1046/j.1432-1327.1999.00607.x.
Robson, A.A., Kelly, M.S., Latchford, J.W., 2007. Effect of temperature on the spoilage rate of whole, unprocessed crabs: *Carcinus maenas, Necora puber* and *Cancer pagurus*. Food Microbiol. 24 (4), 419–424. https://doi.org/10.1016/j.fm.2006.07.016.
Siikavuopio, S.I., Bakke, S., Sæther, B.S., Thesslund, T., Christiansen, J.S., 2019a. Temperature selection and the final thermal *preferendum* of snow crab (*Chionoecetes opilio*, Decapoda) from the Barents Sea. Polar Biol. 42 (10), 1911–1914. https://doi.org/10.1007/s00300-019-02530-3.
Siikavuopio, S.I., James, P., 2015. Effects of temperature on feed intake, growth and oxygen consumption in adult male king crab *Paralithodes camtschaticus* held in captivity and fed manufactured diets. Aquacult. Res. 46 (3), 602–608. https://doi.org/10.1111/are.12207.
Siikavuopio, S.I., James, P., Olsen, B.R., Evensen, T., Mortensen, A., Olsen, S.H., 2017. Holding wild Snow crab, *Chionoecetes opilio*: effects of stocking density and feeding on survival and injury. Aquacult. Res. 48 (4), 1590–1595. https://doi.org/10.1111/are.12993.
Siikavuopio, S.I., Johansson, G.S., James, P., Lorentzen, G., 2019b. Effect of starvation on the survival, injury, and weight of adult snow crab, *Chionoecetes opilio*. Aquacult. Res. 50 (2), 550–556. https://doi.org/10.1111/are.13926.
Sofos, J.N., Flick, G., Nychas, G.-J., O'Bryan, C.A., Ricke, S.C., Crandall, P.G., 2013. Meat, poultry, and seafood. In: Doyle, M.P., Buchanan, R.L. (Eds.), Food Microbiology: Fundamentals and Frontiers, fourth ed. ASM Press, pp. 111–118. https://doi.org/10.1128/9781555818463.ch6.

Sperstad, S.V., Haug, T., Blencke, H.-M., Styrvold, O.B., Li, C., Stensvåg, K., 2011. Antimicrobial peptides from marine invertebrates: challenges and perspectives in marine antimicrobial peptide discovery. Biotechnol. Adv. 29 (5), 519–530. https://doi.org/10.1016/j.biotechadv.2011.05.021.

Subramanian, T.A., 2007. Effect of processing on bacterial population of cuttle fish and crab and determination of bacterial spoilage and rancidity developing on frozen storage. J. Food Process. Preserv. 31 (1), 13–31. https://doi.org/10.1111/j.1745-4549.2007.00101.x.

Tan, M., Mei, J., Xie, J., 2021. The formation and control of ice crystal and its impact on the quality of frozen aquatic products: a review. Article 68 Crystals 11 (1). https://doi.org/10.3390/cryst11010068.

The Norwegian Ministry of Trade, Industry and Fisheries, 2021. Forskrift Om Regulering Av Fangst Av Kongekrabbe I Kvoteregulert Område Øst for 26 Grader Øst Mv. I 2021 [Regulation of the Catch of King Crab in Quota-Regulated Area East of 26 Degrees East Etc. In 2021] (In Norwegian) (Accessed on June 30, 2021). Available at: https://lovdata.no/dokument/SF/forskrift/2020-12-21-3157/%C2%A74#%C2%A74.

Voldnes, G., Kvalvik, I., Nøstvold, B., 2020. Taking care of a highly valuable resource throughout the value chain - lack of market orientation in red king crab export? Article 103965 Mar. Pol. 117. https://doi.org/10.1016/j.marpol.2020.103965.

Ward, D.R., Pierson, M.D., Tassell, K.R., 1977. The microflora of unpasteurized and pasteurized crabmeat. J. Food Sci. 42 (3), 597–600. https://doi.org/10.1111/j.1365-2621.1977.tb12557.x.

Woll, A.K., Berge, G.M., 2007. Feeding and management practices affect quality improvement in wild-caught edible crab (*Cancer pagurus*). Aquaculture 269 (1–4), 328–338. https://doi.org/10.1016/j.aquaculture.2007.04.022.

Yang, S., Hu, Y., Takaki, K., Yuan, C., Yu, H., 2020. The impact of thawing on the quality attributes of swimming crab (*Portunus trituberculatus*) frozen by liquid nitrogen freezing. CyTA - J. Food 19 (1), 33–39. https://doi.org/10.1080/19476337.2020.1850531.

Yue, F., Pan, L., Xie, P., Zheng, D., Li, J., 2010. Immune responses and expression of immune-related genes in swimming crab *Portunus trituberculatus* exposed to elevated ambient ammonia-N stress. Comp. Biochem. Physiol. Mol. Integr. Physiol. 157 (3), 246–251. https://doi.org/10.1016/j.cbpa.2010.07.013.

Zhuang, S., Hong, H., Zhang, L., Luo, Y., 2020. Spoilage-related microbiota in fish and crustaceans during storage: research progress and future trends. Compr. Rev. Food Sci. Food Saf. 20 (1), 252–288. https://doi.org/10.1111/1541-4337.12659.