Comparative quality evaluation of processed clusters obtained from red king crab (*Paralithodes camtschaticus*) typical of spring and autumn harvests in the Barents Sea

Federico Lian [*], Sten I. Siikavuopio, Anette Hustad, Tina Thesslund, Stein-Kato Lindberg, Grete Lorentzen

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

ABSTRACT

The red king crab (RKC, *Paralithodes camtschaticus*) is a highly-valued decapod species of key importance for the economy of the northern Norwegian coastal region. Nearly the entire harvested volume (1727 t in 2019) is exported to high-end markets, mostly as live crabs, although RKC can also be processed into two sections (i.e., clusters) commercialized as a frozen or ready-to-eat product under refrigeration. The fact that the Norwegian RKC fishery is a year-round activity constitutes an advantage over competing RKC fisheries, which are inactive during the winter and the spring. Nonetheless, this also involves the commercialization of RKC harvested between March and May, when its quality is considered poorer due to the physiological changes related to molting. This study aimed to acquire quantitative information on the seasonal quality variation of RKC by comparatively evaluating the quality attributes of spring and autumn harvests, which are, respectively, associated predominantly with a postmolt and intermolt population. Specifically, in the processed clusters obtained from spring postmolt RKCs, it was observed significantly ($p < 0.05$) lower exoskeleton thickness, yield (by between 8 and 15 percentage points), and leg meat content. The cooked meat of spring postmolt RKCs had significantly lower textural firmness and whiteness index. Furthermore, during refrigerated storage, cooked clusters of spring postmolt RKCs were characterized by significantly higher drip loss, and their leg meat reached significantly earlier the acceptability limit of 5 log CFU g^{-1} in the total viable psychrotrophic count. The present study highlighted the risk of incurring economic losses for stakeholders along the value chain when dealing with postmolt RKC harvested in the spring. It should be questioned if the commercialization of the RKCs with poorer eating quality attributes in the spring season is an appropriate way to utilize at best this natural resource in terms of value creation, market reputation, and sustainability.

1. Introduction

The red king crab (RKC, *Paralithodes camtschaticus*) is an exclusive and highly-valued decapod species which is fished in Arctic waters and appeals to consumers worldwide for the sensory properties of its meat. The RKC fishery has become of high importance for the economy of the northern Norwegian coastal region with harvested volumes of 2312 and 1727 t, respectively, in the years 2018 and 2019, equivalent to first sale values of 391 and 302 million Norwegian kroner (NOK) (Norwegian Seafood Council, 2020). Nowadays, almost the entire captured volume is exported mainly to North America and Asia, mostly as live crabs but also as cooked-frozen clusters, which typically consist of cheliped and

walking legs jointed into a shoulder (Lorentzen, Lian, & Siikavuopio, 2019).

In the Norwegian coastal area of the Barents Sea, the commercial harvesting of RKC started in 2002 as a seasonal (September–January) activity, and it has been allowed year-round since 2008 (Norwegian Ministry of Trade, Industry, & Fisheries, 2015). On the one hand, this has provided Norwegian stakeholders with an advantage over competing countries, such as Russia, where the RKC fishery is inactive in the late winter and the spring (Lorentzen et al., 2018). On the other hand, the high export prices, attainable in the period with lower or no market competition, have led to the commercialization of RKC harvested between March and May when its quality is considered poorer

due to the physiological changes related to molting (i.e., exoskeleton shedding) (James et al., 2013; Voldnes, Kvalvik, & Nøstvold, 2020).

The periodic physiological molting cycle of RKC is responsible for seasonal quality variation, especially with regard to the content of edible meat, most of which residing in the muscle located in the appendages, such as chelipeds and walking legs, also referred to as pereiopods (Lorentzen, Lian, & Siikavuopio, 2019). In the Arctic region, the RKC typically enters a pre-molting stage during the winter (Nilssen & Sundet, 2006) when minerals and other macronutrients are progressively resorbed from the muscle tissue and the exoskeleton with the formation of a new cuticle (Stevens & Jewett, 2014), referred to as double-shell (Sainte-Marie, Raymond, & Brêthes, 1995). Shortly before molting, the RKC takes in water and undergoes a significant reduction (30–60%) of muscle mass in order to shed the old exoskeleton (Mykles & Skinner, 1990). In the postmolt stage (late spring and early summer), the muscle tissue grows rapidly in volume, and the new flexible exoskeleton is hardened by minerals (Chang & Mykles, 2011).

The biochemical changes accompanying the molting cycle can dramatically affect the compositional and structural characteristics of the muscle and, in turn, a series of quality attributes of RKC products, potentially impacting different stakeholders in the marketing chain, including the final consumer (Lorentzen, Lian, & Siikavuopio, 2019). Given the fact that RKC is such an exclusive and highly-priced delicacy, consumers consistently expect a product with adequate leg meat content and appealing eating quality attributes, such as a juicy, firm, and elastic texture, as well as high whiteness of the cooked muscle fibers. For crab processors, it is important to obtain a high yield throughout different processing operations, such as slaughtering, cooking, and freezing. At the retail level, where RKC clusters are often commercialized in a cooked ready-to-eat (RTE) form under refrigeration, it is desirable to have a low drip loss and slow microbial growth during storage. Moreover, the microbial growth in RTE crab products has been closely associated with their shelf-life (Anacleto et al., 2011; Lorentzen, Rotabakk, Olsen, Skuland, & Siikavuopio, 2016; McDermott, Whyte, Brunton, Lyng, & Bolton, 2018).

In light of these considerations, it is desirable to acquire quantitative information on the seasonal quality variation of RKC products in specific periods of the year and, indirectly, linked to the molting stage. To the best of our knowledge, there is a lack of studies that present comprehensive details about the seasonal variations in the processing- and consumer-related quality parameters of RKC.

Therefore, the present work aimed to comparatively evaluate the quality attributes of RKC representative of the spring and autumn harvests in the Norwegian coasts of the Barents Sea, which are, respectively, associated predominantly with a postmolt and intermolt population. Although the studied parameters included crab bio-morphological features, such as the thickness of the exoskeleton and the hepatosomatic and cheliped index, the main focus was placed on the quality aspects of processed clusters and their leg meat. More specifically, the yield and leg meat content of cooked clusters were determined along with the proximate composition, instrumental texture, and color attributes of cooked leg meat. Besides, the drip loss of the cooked clusters and the microbial growth in the cooked leg meat were monitored during the subsequent refrigerated storage.

2. Material and methods

2.1. Harvest and crab selection

Adult male red king crabs (RKCs) were harvested by local fishermen using square-shaped commercial pots in the North Cape area in the Barents Sea in April and October 2019.

After harvesting, the RKCs were held live indoors at a land-based facility (Honningsvåg, Norway) for one week without feeding. During the live holding, the RKCs were kept in 700 L rectangular plastic *vivier* tanks supplied with natural seawater (salinity 33‰; temperature of 4 °C

in April and 9 °C in October) pumped from the sea bottom of Honningsvåg Bay (Storbukta, Honningsvåg) at a depth of approximately 10 m. The seawater was UV-treated, filtered through a sand filter, and circulated to ensure an oxygen saturation level higher than 90% at all times.

After one week of live holding, a selection was carried out on April 30 and October 22, 2019, in order to pick the RKC individuals that were to be used for the investigation. The selection was conducted by randomly picking RKCs from five different live holding tanks followed by verification that the RKCs met pre-defined criteria for representing the most frequent type of RKCs harvested in the spring or in the autumn. Specifically, the spring sampling focused on postmolt individuals ($n = 17$), selected after inspection and verification of low meat content of the walking legs (i.e., raw muscle filling less than 65% of total volume in the most proximal article of the walking leg, also regarded to as *merus*), high sharpness of the dactyl of walking legs, and orange-pink hued mother-of-pearl-like exoskeleton color. The autumn sampling ($n = 17$) focused on intermolt individuals, characterized by high meat content (i.e., raw muscle filling more than 85% of total *merus* volume) in the walking legs.

The meat content of the *merus* of the second leg on each side of the crabs was screened using a non-destructive methodology, previously developed and successfully applied for estimating meat content in live edible crab (*Cancer pagurus*) (Wold, 2018; Wold, Kermit, & Woll, 2010). This technology, based on near-infrared (NIR) spectroscopy, enables estimation of the volume of the *merus* occupied by the muscle ($\pm 5\%$). Before sampling, the crabs were also checked for the integrity of their limbs and a good vitality response upon being lifted out of the seawater (Siikavuopio et al., 2014).

The average weights ($\pm$standard deviations) of the crabs sampled in the spring and autumn were 1783 g (± 162) and 1629 g (± 255), respectively. The corresponding average carapace widths were 144 mm (± 7) and 140 mm (± 6).

Immediately after the selection, the crabs were transported live in a dry state covered with gel ice (Cold Ice Inc., Oakland, CA, USA) in polystyrene boxes by air freight in approximately 3 h to Nofima (Tromsø, Norway). The crabs were kept live in the boxes, stored overnight at 2 °C, and processed the following day within 15 h of their arrival.

2.2. Processing and sample preparation

The procedure for processing the red king crabs into clusters reflected the industrial processing and followed the steps described by Lorentzen, Lian, and Siikavuopio (2019) with some modifications (Fig. 1).

Briefly, the processing started with registering the weight of the live red king crabs, which were then split into two clusters using a butchering iron. Each cluster was labeled using a T-bar tag (FD-94, Floy® tag & mfg) for traceability purposes. Immediately after splitting, the hepatopancreas was collected from the carapace and weighed. Afterward, the chelipeds, which are the limbs bearing the claw, were removed from the cluster and weighed.

The clusters were cleaned of residues of entrails while being kept in a vertical position with the shoulder joint pointing downwards to facilitate drainage of the free body fluid, which is the liquid containing hemolymph and water that drips freely from the limbs when the exoskeleton is cut (Mizuta, Kobayashi, & Yoshinaka, 2001).

Afterward, the clusters underwent a "de-bleeding" step, which consists of the immersion of the clusters into a container with fresh water (100 L) at 4 °C for 2 h. Next, the clusters were drained for at least 30 min, and the weight of each de-bled and drained cluster was registered.

Subsequently, the clusters were distributed into wire mesh baskets and cooked by immersion into a cooking bath containing fresh water at 95 °C (± 0.5 °C), which was continuously recirculated to increase the homogeneity of heat exchange and minimize cold spots. In each season sampling, cooking was conducted in two separate cooking batches, each
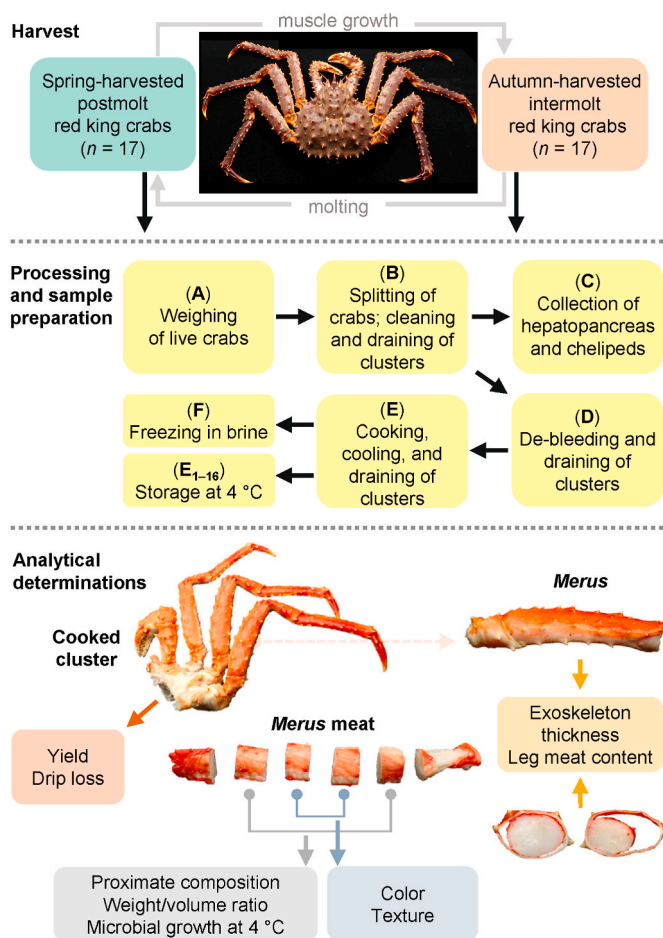
Fig. 1. Overview of the procedure followed for the spring and autumn sampling.

including 17 clusters with a balanced number of right- and left-side clusters generated from different crabs. The cooking treatment targeted a core temperature of 92 °C in the *merus* of the second walking leg (Fig. 1). This temperature was achieved in 11.5 and 12 min in the clusters sampled in the spring and autumn, respectively. The core temperature was logged using K-type thermocouples connected to data loggers (model 175H1, Testo Ltd., Hampshire, UK). Immediately after cooking, the clusters were cooled by immersion into a container with salted water added with ice (100 L, 3.5% w/v NaCl, Havsalt, GC Rieber AS, Norway) for approximately 20 min until the core temperature was below 4 °C. The clusters were then drained for at least 30 min before their weights were registered.

After cooling and draining, one of the two clusters from each crab was air-packed into plastic bags (thickness 80 μm, dimensions 220 × 600 mm, Finnvacum, Helsinki, Finland) closed with metallic clips and stored in a climate chamber (BINDER GmbH, Tuttlingen, Germany) at 4 °C for up to 16 days. The complementary cooled and drained clusters were distributed into two wire mesh baskets and frozen by immersion into a container with saturated brine (275 L, 27% w/v NaCl, Havsalt) at −18 °C for 15 min, reaching a core temperature of −15 °C in the muscle of the *merus* of the second walking leg. The frozen clusters were weighed and air-packed into plastic bags (Finnvacum) closed with metallic clips and stored in a freezer room at −20 °C for subsequent studies.

2.3. Hepatosomatic index, cheliped index, and thickness of leg exoskeleton

The hepatosomatic index (HSI) and the cheliped index (CI) were calculated as described by Lorentzen et al., 2020:

$$HSI = (C_H/A) \times 100 \qquad (1)$$

$$CI = (C_C/A) \times 100 \qquad (2)$$

where C_H and C_C are the weights of the hepatopancreas and of the two chelipeds, respectively, and A is the live red king crab weight.

The thickness of the leg exoskeleton was measured in the center of the *merus* of the second walking leg by using a caliper (precision ± 0.05 mm).

2.4. Cluster yield and changes in cluster weight during processing

To obtain an overview of the effect of the processing steps on the cluster yield and the changes in cluster weight during processing, six separate calculations were performed as described by Lorentzen et al., 2020.

In detail, the cluster yield (CY) of the raw clusters was calculated as:

$$CY_{raw}(\%) = (2D/A) \times 100 \qquad (3)$$

where 2D is the sum of the weight of the right and left cluster from the same crab after splitting, cleaning, de-bleeding, and draining (Fig. 1, step D), and A is the live weight of the corresponding crab (Fig. 1, step A).

Furthermore, the yield of the cooked clusters was calculated as:

$$CY_{cooked}(\%) = (2E/A) \times 100 \qquad (4)$$

where 2E is the sum of the weight of the two cooked, cooled, and drained clusters obtained from the same crab (Fig. 1, step E), and A is the live weight of the corresponding crab.

In addition, the yield of the frozen clusters was calculated as:

$$CY_{frozen}(\%) = (2 \times F/A) \times 100 \qquad (5)$$

where F is the weight of the single cluster, generated from each crab, which was frozen in brine (Fig. 1, step F), and A is the live weight of the corresponding crab.

The change in cluster weight relative to the effect of the de-bleeding step was calculated as:

$$\Delta Cluster_{de-bled}(\%) = [(D - B)/B] \times 100 \qquad (6)$$

where D is the weight of a single cluster after de-bleeding and draining (Fig. 1, step D), and B is the weight of the corresponding single raw cluster after splitting and draining before the de-bleeding (Fig. 1, step B).

The change in cluster weight relative to the combined effect of the de-bleeding and cooking was calculated as:

$$\Delta Cluster_{cooked}(\%) = [(E - B)/B] \times 100 \qquad (7)$$

where E is the weight of a single cluster after cooking, cooling, and draining, and B is the weight of the corresponding single raw cluster as described above.

The cluster weight change relative to the combined effect of the de-bleeding, cooking, and freezing was calculated as:

$$\Delta Cluster_{frozen}(\%) = [(F - B)/B] \times 100 \qquad (8)$$

where F is the weight of the single cluster, generated from each crab, which was frozen in brine (Fig. 1, step F), and B is the weight of the corresponding raw cluster as described above.

2.5. Cluster drip loss during refrigerated storage

The cooked clusters were monitored for drip loss during the first five days of storage at 4 °C by measuring the cluster weight. The liquid accumulated at the bottom of the plastic bags was discharged after each

weight measurement. The drip loss was expressed as:

$$\text{Drip loss}(\%) = [(E - E_{1-5})/E] \times 100 \tag{9}$$

where E_{1-5} is the weight of the cooked, cooled, and drained cluster measured daily in the first five days of storage at 4 °C (Fig. 1, step E_{1-16}), and E is the weight of the cooked, cooled, and drained cluster before storage at 4 °C.

2.6. Meat content of cooked legs

The meat content of the walking legs of cooked clusters was measured by digital analysis of images of a cross-section of the middle of the *merus* as described by Lorentzen, Lian, and Siikavuopio (2019). The meat content was calculated as the proportion of the surface area of the leg cross-section occupied by meat:

$$\text{Meat content}(\%) = (\text{Area occupied by meat/Total inner area}) \times 100 \tag{10}$$

The meat content was determined in the *merus* of each of the three walking legs of the cooked clusters.

2.7. Analytical determinations on cooked leg meat

Analytical determinations were performed on different leg meat chunks extracted from the *merus* of the walking legs of cooked clusters, as depicted in Fig. 1. Specifically, the second walking leg of the cluster was used for the measurements of proximate composition, weight/volume ratio, color, and instrumental texture, whereas the first and third walking legs were used for the determination of the microbial growth during storage at 4 °C.

2.7.1. Proximate composition and weight/volume ratio

For proximate composition analysis, the moisture was determined according to the AOAC (2000) method 950.46, the ash content was determined according to the method ISO 936:1998, the protein content was measured by the Dumas method using a LECO TruMac nitrogen analyzer (LECO Corp., St. Joseph, MI, USA) with a conversion factor of 6.25, and the fat content was quantified on dried samples by pulse nuclear magnetic resonance after equilibration at 50 °C in a heating block and calibration against certified olive oil standard. Results were expressed as percentages of wet sample weight and were obtained from three analytical replicates in which the leg meat of at least five crabs per season group was pooled together.

The weight/volume ratio was calculated by approximating the volume of the leg meat chunks to that of an elliptic cylinder according to the following formula:

$$\text{Weight/volume ratio} = W_{meat}/[(\pi \times r_1 \times r_2) \times h] \tag{11}$$

where W_{meat} is the weight (g) of the cooked meat chunks, r_1 and r_2 are the length (cm) of the semi-axes of the elliptical cross-section area of the meat chunks, and h is the height (cm) of the meat chunks.

2.7.2. Instrumental texture

The instrumental textural properties were determined using a texture profile analyzer (TA-HD plus, Stable Micro System Ltd., Godalming, Surrey, UK) following the method described by Martínez-Maldonado, Ramírez-De León, Méndez-Montealvo, Morales-Sánchez, and Velazquez (2018) with some modifications.

Briefly, for the texture profile analysis, a chunk of cooked meat (thickness of approximately 15 mm) was compressed to 60% of the initial height at a crosshead speed of 60 mm min^{-1} using a flat compression plate of 100 mm diameter. The sample was equilibrated at room temperature in plastic bags for 20 min before the analysis, and the compression was directed parallelly to the muscle fibers of the meat. For each crab, two cooked leg meat chunks were analyzed separately

(Fig. 1).

The texture parameters hardness, gumminess, and chewiness were calculated with the software Exponent (version 6.1.14.0, Stable Micro System Ltd.) and expressed as values normalized over the surface area of the meat chunk in contact with the compression plate (Bland et al., 2018; Bourne, 2002, pp. 107–188).

2.7.3. Color

The color was evaluated using a portable color-spectrophotometer (CM-600d, Minolta Ltd., Osaka, Japan) with a D65 illuminant and calibrated against a white tile before measurements. The measurements were conducted by placing the head of the instrument in contact with five different spots on the cut surface of the cross-section of the meat chunks (Fig. 1).

The color was expressed in the CIELAB scale as lightness ($L*$), green-red ($a*$), and blue-yellow ($b*$) coordinates.

Also, the whiteness index (WI) was calculated as (Pathare, Opara, & Al-Said, 2012):

$$WI = \left[(L* - 100)^2 + (a*)^2 + (b*)^2 \right]^{\frac{1}{2}} \tag{12}$$

where $L*$, $a*$, and $b*$ are the CIELAB color coordinates.

2.7.4. Microbiological analyses during refrigerated storage

Microbiological analyses were conducted as described by Lorentzen, Skuland, Sone, Johansen, and Rotabakk (2014), with slight modifications. Briefly, each meat sample (approximately 10 g) was transferred to sterile bags containing sterile saline water with 0.9% (w/v) NaCl (Oxoid Ltd., Basingstoke, UK) and 0.1% (w/v) peptone (Difco Laboratories Inc., Detroit, MI, USA) yielding a 1:10 dilution. The sample was blended in a laboratory blender (model Stomacher® 400, Seward Ltd., Worthing, UK) for 2 min and inoculated after appropriate 10-fold dilutions. Total viable psychrotrophic count (TVC$_P$) and total viable mesophilic count (TVC$_M$) were enumerated on spread-plated plate count agar (PCA; Oxoid) supplemented with 0.5% NaCl (Oxoid) incubated at 12 °C for 7–9 days and 30 °C for 3 days, respectively. Total aerobic plate count (APC) was enumerated on Lyngby iron agar (IA; Oxoid) containing 0.06% (w/v) L-cysteine after incubation at 22 °C for 3–5 days. Black colonies growing on IA were taken as indicators of H$_2$S-producing bacteria (e.g., *Shewanella* spp.). Spread-plated Pseudomonas agar base supplemented with cetrimide-fucidin-cephalosporine (CFC; Oxoid) was used for the enumeration of presumptive *Pseudomonas* spp. bacteria after incubation at 22 °C for 3 days.

The analyses were performed after 5, 7, 9, 11.5, 13.5, and 15.5 (± 4 h) days of storage at 4 °C. At each sampling point, a total of seven biological samples ($n = 7$), each consisting of two meat chunks extracted from the *merus* of clusters (Fig. 1) generated from different crabs, were plated at least in duplicate in each of the microbial enumeration media considered. Results were reported as decimal logarithm of colony-forming units per gram of sample (log$_{10}$ CFU g^{-1}) and expressed as the mean ($\pm$standard deviation) of the seven biological samples per time point. For plates with no colonies detected, the level was set to half of the detection limit.

Besides, to quantitatively determine the time necessary to reach a pre-defined microbial acceptability limit, the reparametrized Gompertz-Zwietering model was fitted to microbial growth data as follows (Corbo, Del Nobile, & Sinigaglia, 2006; Zwietering, Jongenburger, Rombouts, & van't Riet, 1990):

$$N(t) = MAL - a \times \exp(-\exp(e \times \mu_{max} \times (\lambda - MSL) \times a^{-1})) + a$$
$$\times \exp(-\exp(e \times \mu_{max} \times (\lambda - t) \times a^{-1})) \tag{13}$$

where N(t) is the microbial count enumerated at time t of storage (ln CFU g^{-1}), MAL is the pre-defined microbial acceptability limit (ln CFU g^{-1}), e is the Euler's number, t is the storage time (day), and the model parameters a, μ_{max}, λ, and *MSL* are, respectively, the asymptotic

microbial count increase (ln CFU g^{-1}), the maximum growth rate (ln CFU g^{-1} day^{-1}), the lag phase (day), and the estimated time (day) to reach the MAL, also referred to as microbial shelf-life.

2.8. Statistical analyses

The statistical analyses were performed by considering each crab as a biological replicate. The values of the response variables, which were measured on both the clusters generated from a single crab (i.e., CY$_{raw}$, CY$_{cooked}$, ΔCluster$_{de\text{-}bled}$, and ΔCluster$_{cooked}$), were averaged by crab. Unless otherwise specified, the values reported for each measured variable are the mean and standard deviation resulting from analyses conducted on each of the crabs ($n = 17$) of the two seasons.

Differences between spring and autumn harvests were investigated by a two-sided independent samples *t*-test, whereas a paired *t*-test was used to compare the meat content of different walking legs within the same season.

A correlation analysis was carried out by computing the Pearson correlation matrix of selected response variables based on pairwise-complete observations ($n = 14$ for the microbial counts and $n = 34$ for all the other response variables considered) followed by a pairwise two-sided Pearson's correlation significance test.

The *t*-tests and the correlation analysis were performed in the software R (version 3.6.2) (R Core Team, 2020).

Nonlinear regression was carried out to fit Equation (13) to microbial growth data using the software GraphPad Prism (version 5.00 for Windows, GraphPad Software, Inc., La Jolla, CA, USA). The significance of the effect of the harvest season on the model parameter estimates was tested by means of the extra-sum-of-squares *F*-test in GraphPad Prism.

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

3. Results and discussion

3.1. Quality aspects linked to crab bio-morphological features

3.1.1. Hepatosomatic and cheliped index
The RKCs harvested during the autumn presented a significantly higher hepatosomatic index (HSI) compared to spring RKCs (Table 1).

The HSI can be a valuable indicator of the biological and nutritional status of the RKC (Albalat et al., 2019) as the hepatopancreas, which is located in the carapace, is the organ chiefly deputed to the digestion and absorption of nutrients and storage of reserves (Cervellione, McGurk, & Van den Broeck, 2017).

The RKCs sampled in the spring were postmolt individuals. Molting demands energy, and RKCs do not feed for about one month during the period immediately preceding and following the actual process of shifting the exoskeleton (i.e., ecdysis) (Morado, Shavey, Ryazanova, & White, 2014). During this period, reserves, such as proteins, lipids, sugars, and other carbohydrates, are mobilized from the hepatopancreas towards the newly-forming cuticle through the hemolymph. At the same time, ionic constituents of the hemolymph increase during the phases prior to and immediately after the ecdysis, leading to a rise in internal osmotic pressure and, in turn, increased water absorption (Morado et al., 2014). This explains the difference in HSI between the spring and autumn harvests. Moreover, the HSI values obtained in the present study are in line with the ones previously reported for RKC immediately after molting (3.2 ± 0.3) (James et al., 2013) as well as for other king crab species, such as false southern king crab (*Paralomis granulosa*) (5.8 ± 1.2) (Comoglio, Smolko, & Amin, 2005) and southern king crab (*Lithodes santolla*) (5.4 ± 0.6) (Sacristán, Di Salvatore, Fernández-Gimenez, & Lovrich, 2019), harvested in the intermolt stage.

As for the cheliped index (CI), the mean value obtained for the RKCs harvested in the spring was significantly higher than the value for the autumn harvest (Table 1). This may indicate the tendency for RKC to preserve throughout the molting process the functionality of the muscle tissue in the chelipeds for defense and foraging purposes, similar to what was recently observed in snow crab (*Chionoecetes opilio*) during live holding in the absence of feeding (Lorentzen et al., 2020).

3.1.2. Thickness of leg exoskeleton
The thickness of the exoskeleton of the walking legs was significantly lower (65% thinner) in RKCs harvested in the spring as compared to the autumn harvest (Table 1).

Although it is well known that postmolt crabs have a thin and soft exoskeleton (James et al., 2013), there is a paucity of quantitative information on the exoskeleton thickness of the walking legs of RKC, especially as a function of molting stage and harvest season. This parameter is directly related to the hardness and resilience of the exoskeleton (Chen, Lin, McKittrick, & Meyers, 2008), which, in turn, is an important factor in reducing the risk of damage during handling and processing.

3.2. Quality parameters of processed clusters

3.2.1. Cluster yield and changes in cluster weight during processing
The RKCs harvested in the spring had significantly lower cluster yields throughout all processing steps compared to the ones obtained from RKCs harvested in the autumn (Table 1). Specifically, in spring RKCs, the yield of raw clusters after slaughtering (CY$_{raw}$) was, on average, 8.5 percentage points lower than in autumn RKCs. This difference was exacerbated after cooking, as the yield of cooked (CY$_{cooked}$) and cooked-frozen clusters (CY$_{frozen}$) differed, respectively, by 15.0 and 14.4 percentage points between spring and autumn RKCs.

The changes in cluster weight calculated after de-bleeding (ΔCluster$_{de\text{-}bled}$), de-bleeding and cooking (ΔCluster$_{cooked}$), or de-bleeding, cooking, and freezing in brine (ΔCluster$_{frozen}$) indicate that, for spring RKCs, the highest proportion of cluster yield loss occurs as an effect of cooking. This can be explained by high liquid loss, including water and water-soluble protein constituents, caused by the heat treatment and linked to physiological phenomena related to molting. In particular, the leg muscle of postmolt RKCs may have weaker muscle fibers or connective tissue and be, therefore, more susceptible to thermal denaturation (Lorentzen, Lian, & Siikavuopio, 2019). Furthermore, as a result of the water absorption before and immediately after molting, it can be assumed that postmolt RKCs may contain a high level of free body fluid, which is loosely bound to the muscle structure or poorly physically entrapped in the larger volume delimited by the new exoskeleton, and

Table 1
Seasonal difference in the quality parameters (mean ± standard deviation) of red king crabs and processed clusters thereof.

Parameter	Harvest season (molting stage)		*p*-value
	Spring (postmolt)	Autumn (intermolt)	
HSI (%)	4.6 ± 1.0	5.8 ± 0.9	0.001
CI (%)	11.0 ± 0.7	9.4 ± 0.6	<0.001
Shell thickness (mm)	0.47 ± 0.05	0.72 ± 0.05	<0.001
CY$_{raw}$ (%)	48.2 ± 3.1	56.7 ± 1.5	<0.001
CY$_{cooked}$ (%)	41.7 ± 4.2	56.7 ± 1.6	<0.001
CY$_{frozen}$ (%)	42.1 ± 4.1	56.5 ± 1.8	<0.001
ΔCluster$_{de\text{-}bled}$ (%)	-4.2 ± 5.0	-0.6 ± 0.6	0.007
ΔCluster$_{cooked}$ (%)	-13.2 ± 9.0	-0.1 ± 1.5	<0.001
ΔCluster$_{frozen}$ (%)	-12.5 ± 7.9	0.1 ± 1.7	<0.001
Drip loss (%) during refrigerated storage			
Day 1	8.8 ± 1.9	2.6 ± 1.2	<0.001
Day 3	14.2 ± 4.1	6.0 ± 3.0	<0.001
Day 5	16.4 ± 4.5	7.5 ± 3.4	<0.001

Abbreviations. HSI, hepatosomatic index; CI, cheliped index; CY$_{raw}$, raw cluster yield; CY$_{cooked}$, cooked cluster yield, CY$_{frozen}$, cluster yield after freezing in brine; ΔCluster$_{de\text{-}bled}$, cluster weight change relative to the effect of the de-bleeding; ΔCluster$_{cooked}$, cluster weight change relative to the combined effect of the de-bleeding and cooking; ΔCluster$_{frozen}$, cluster weight change relative to the combined effect of the de-bleeding, cooking and freezing in brine.

hence highly prone to be lost upon cooking (Mizuta et al., 2001).

3.2.2. Cluster drip loss during refrigerated storage

The drip loss, monitored in the first five days of storage at 4 °C, was significantly higher for the clusters obtained from the RKCs harvested in the spring as compared to their autumn counterparts (Table 1). Specifically, the clusters obtained from spring RKCs were characterized by a rapid drip loss, which, as early as in the first day of storage, was higher than for the autumn samples after five days of storage.

Similar to the case of snow crab clusters, the primary source of drip loss in RKC clusters is the liquid physically entrapped in the legs in the interstitial space between the cooked meat and the exoskeleton, which drains out through the shoulder joint of the cluster (Lorentzen, Lian, Røhme, et al., 2019). Furthermore, it has been suggested that in crab meat, the drip loss may, at least in part, be associated with proteolytic activity in the muscle during storage (Sun et al., 2017). However, it has been shown that a significant proteolytic activity of the meat in RKC clusters only occurs at a later stage during refrigerated storage (Lorentzen et al., 2014). Moreover, as described by the parameters CY_{cooked} and $\Delta Cluster_{cooked}$, a significantly higher share of liquid was lost as an effect of cooking in spring RKC clusters compared to autumn RKC clusters. Most likely, the observed differences in drip loss between spring and autumn RKC clusters might be ascribed to different characteristics of the muscle of postmolt and intermolt crabs at a macro- and micro-structural level (Benjakul & Sutthipan, 2009) that is reflected in different capacities to withhold the liquid present in the inter- and intra-myofibrillar space during cooking and subsequent refrigerated storage.

3.3. Meat content of cooked legs

The meat content in cooked clusters was, on average and within-season, highest in the first walking leg (56.5% ± 9.1 in the spring, 91.1% ± 2.3 in the autumn) followed by the second (55.7% ± 7.2 in the spring, 91.0% ± 2.8 in the autumn) and the third (53.0% ± 5.5 in the spring, 87.8% ± 4.5 in the autumn) walking leg (Fig. 2).

It has to be highlighted that differences in meat content due to the harvest season were expected as the study deliberately focused on sampling specifically RKCs representative of the type predominantly harvested in the spring or in the autumn: Typical postmolt individuals with low meat content (i.e., raw muscle filling less than 65% of total *merus* volume) in the spring and typical intermolt individuals with high meat content (i.e., raw muscle filling more than 85% of total *merus* volume). At the same time, it is interesting to note the differences in meat content between legs within-season, although these were statistically significant within-crab only for the RKCs sampled in the autumn, specifically between the first and third leg ($p = 0.019$) and between the second and third leg ($p = 0.004$). The third walking leg was the pereiopod, which contained, on average, the lowest meat content irrespective of the harvest season. In particular, the third walking leg presented lower meat content than in the first and second leg in 7 (spring) and 11 (autumn) clusters out of the 17 clusters sampled for each season. Similar results were reported by Mizuta et al. (2001) for hard-shell (i.e., intermolt) snow crabs where the relative proportion of muscle in the third walking leg was lower than in the first and second walking legs.

While the molting stage and hence the season of harvest are known to influence the meat content of RKC (Hjelset & Sundet, 2004), there is a lack of information on the meat content distribution among the three walking legs. A possible explanation for the lower meat content observed in the present work in the third walking leg might be related to the fact that this leg plays a less active role in the motility than the first and second walking legs. Close observations of the movements of RKCs indicated that the first two legs produce most of the thrust (T. Thesslund, personal communication, August 2020). It might be assumed that the third walking leg is exposed to lower mechanical stress and hence lower stimulation of muscle growth.
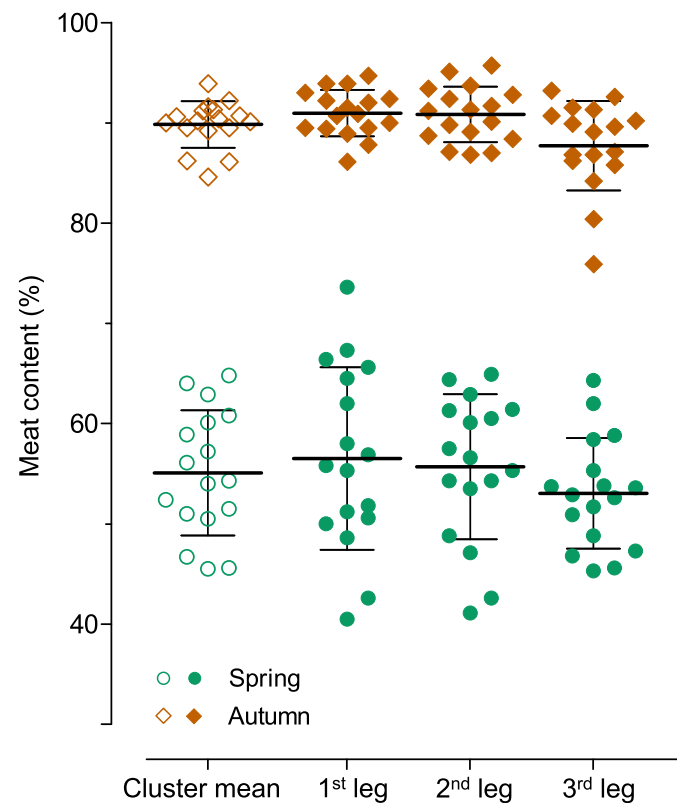

Fig. 2. Box plots depicting the meat content (%) in the *merus* of each walking leg of cooked clusters of red king crabs sampled in the spring ($n = 17$) and in the autumn ($n = 17$). Cluster mean refers to the mean value of the three legs of each cluster. The thick horizontal line indicates the mean value within season, whereas the error bars indicate the standard deviation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Furthermore, it is noteworthy that the average meat content for the postmolt RKCs harvested in April (55.1% ± 6.3) was similar to the meat content reported in a previous study for intermolt RKCs harvested in October and held live without feeding at 5 °C for 92 days (53.7% ± 9.0) (Lorentzen, Lian, & Siikavuopio, 2019).

3.4. Quality parameters of cooked leg meat

3.4.1. Proximate composition and weight/volume ratio

Regarding the proximate composition of the cooked leg meat, no significant differences between harvest seasons were observed except for moisture, which was slightly higher in the autumn samples (Table 2). In general, the difference observed in the proximate composition can be considered negligible from a commercial standpoint. This also indicates that the differences in the cluster yield between harvest seasons cannot be ascribed to changes in the macromolecular composition of the cooked leg meat.

The ratio between the weight and the volume of the cooked leg meat did not differ significantly with the harvest season. From a methodological standpoint, it should be highlighted that the measurement of the weight/volume ratio carries some limitations related to conducting manual measurements and approximating the volume of meat chunks to that of an elliptic cylinder. In this regard, a more accurate volume determination of meat chunks may be obtained through three-dimensional digital acquisitions.

3.4.2. Instrumental texture

Texture profile analysis (TPA) showed that the cooked leg meat obtained from the RKCs harvested in the autumn was characterized by

Table 2
Seasonal difference in the quality parameters (mean ± standard deviation) of the meat obtained from the *merus* of cooked red king crab clusters.

Parameter	Harvest season (molting stage)		*p*-value
	Spring (postmolt)	Autumn (intermolt)	
Proximate composition			
Moisture (%)	79.1 ± 0.1	79.5 ± 0.2	0.031
Protein (%)	18.4 ± 0.1	18.5 ± 0.9	0.952
Fat (%)	0.53 ± 0.12	0.40 ± 0.00	0.116
Ash (%)	1.83 ± 0.12	1.77 ± 0.12	0.518
Ratio weight/volume (g cm^{-3})	1.16 ± 0.09	1.15 ± 0.04	0.663
Instrumental texture			
Hardness (N cm^{-2})	4.73 ± 0.85	6.42 ± 1.02	<0.001
Gumminess (N cm^{-2})	1.96 ± 0.34	2.10 ± 0.43	0.320
Chewiness (N cm^{-2})	1.40 ± 0.30	1.56 ± 0.35	0.169
Color			
L*	76.9 ± 1.7	79.9 ± 1.1	<0.001
a*	−3.16 ± 0.73	−3.04 ± 0.51	0.580
b*	2.04 ± 0.94	3.16 ± 0.73	0.001
WI	76.5 ± 1.6	79.4 ± 1.0	<0.001
Time to reach microbial acceptability limit (MAL)			
TVC$_P$ (MAL = 5 log CFU g^{-1})	10.6 ± 0.6	12.7 ± 0.6	0.020
TVC$_M$ (MAL = 5 log CFU g^{-1})	13.1 ± 0.6	13.6 ± 0.6	0.557
APC (MAL = 5 log CFU g^{-1})	11.1 ± 0.7	12.1 ± 0.6	0.252
Pseudomonas spp. (MAL = 4 log CFU g^{-1})	11.6 ± 0.7	10.4 ± 0.7	0.249

Abbreviations. WI, whiteness index; TVC$_P$, total viable psychrotrophic count; TVC$_M$, total viable mesophilic count; APC, aerobic plate count.

significantly higher hardness but not significantly different gumminess and chewiness compared to the counterpart extracted from spring RKCs (Table 2).

In previous sensory studies regarding cooked RKC meat, trained panelists associated high sensory scores with a series of texture attributes, including firmness (James et al., 2013; Lorentzen et al., 2014). Furthermore, good correlations between instrumental and sensory assessment of hardness have been reported for several solid food matrices, including fish muscle tissue (Coppes, Pavlisko, & Vecchi, 2002; Meullenet & Gross, 1999). Instrumental determination of texture is an objective method, usually preferred over sensory evaluation in the context of commercial activities (Lorenzo, Tomac, Tapella, Yeannes, & Romero, 2021).

Based on the TPA results and the above-mentioned considerations, it can be speculated that the higher hardness found for autumn RKCs may be linked to higher sensory textural quality, although a specific study to establish the relationship between sensory and instrumental texture for cooked leg meat of RKC is warranted.

Previous studies have suggested the presence of an association between the hardness (i.e., firmness) of crab meat and the level of myofibrillar proteins and collagen in the crab muscle (Martínez-Maldonado et al., 2018; Mizuta, Yoshinaka, Sato, & Sakaguchi, 1994; Zhang, Wang, Zhou, Zheng, & Wang, 2020). The proportion of myofibrillar proteins in mud crab (*Scylla serrata*) was lower in soft-shell (i.e., postmolt) individuals as compared to their hard-shell counterparts (Benjakul & Sutthipan, 2009). Furthermore, the histological characteristics of the muscle of soft-shell snow crab indicated a structural weakening of muscle fibers or connective tissue compared to hard-shell snow crab, possibly linked to changes occurring during molting (Mizuta et al., 2001). It can be of interest to explore whether also the differences found in the present study in the texture parameters of the cooked leg meat of postmolt and intermolt RKC are ascribable to differences in the histochemical characteristics of the leg muscle.

3.4.3. Color

The cooked leg meat of RKCs harvested in the autumn presented significantly higher values for lightness (*L**), yellowness (*b**), and whiteness index (*WI*) than the counterpart obtained from spring RKCs

(Table 2).

The whiteness of cooked muscle tissue can be ascribed to surface properties and the ratio between absorbed and reflected light (Kruk et al., 2011), which is linked to the changes occurring during the cooking process, such as shrinkage of meat fibers and aggregation/denaturation of myofibrillar proteins (Condón-Abanto, Raso, Arroyo, Lyng, & Álvarez, 2019). Higher light scattering, hence whiter meat, may be linked to the presence of a structurally denser myofibrillar network. It has been hypothesized that in Chinese mitten crab (*Eriocheir sinensis*) the density of the muscle filament lattice could be closely related to the maturity stage (Zhang et al., 2020). Whether this association also applies to the meat of RKCs at different molting stages should be the object of further investigation.

Overall the visual appearance and color play a key role in the consumer purchasing choice of crab products (Requena, Hale, Green, McClure, & Farkas, 1999). Previous works have considered white color as a positive sensory attribute for cooked RKC meat (James et al., 2013; Lorentzen et al., 2014). Furthermore, the degree of lightness was correlated to higher sensory scores for the meat of blue crab (Martínez et al., 2017), and color attributes such as white and bright were associated with higher consumer liking in cooked claw meat of edible crab (Lian, Lyng, & Brunton, 2017). In light of these considerations, the higher lightness and whiteness of the meat obtained for autumn RKCs in the present study might, therefore, be linked to higher perceived quality, although this contention requires verification.

3.4.4. Microbial growth during refrigerated storage of the cooked clusters

The microbial growth in the leg meat, monitored during refrigerated storage of the cooked clusters, was, in general, higher for spring samples (Fig. 3).

The total viable psychrotrophic count (TVC$_P$) was the microbial population showing the largest difference between spring and autumn samples for all the time points between 7 and 15.5 days of storage (Fig. 3A). More specifically, the TVC$_P$ was, on average, higher in leg meat of spring RKCs as compared to their autumn counterparts throughout the storage time. It may be hypothesized that the mobilization of nutrients (i.e., peptides, free amino acids, and nonprotein-nitrogen), occurring during the molting process, may generate a more favorable substrate for the growth of psychrotrophic bacteria in the leg meat of spring postmolt RKCs (Dooley, Crouch, & West, 2002).

The presence of bacteria producing hydrogen-sulfide (H$_2$S) (e.g., *Shewanella* spp.) was detected only in 14 out of the 42 leg samples analyzed in each season. Moreover, no specific pattern in the prevalence of H$_2$S-producing bacteria was observed during storage and in relation to the season.

Previous studies have suggested a microbial acceptability limit (MAL) linked to shelf-life of 5 log CFU g^{-1} for total viable counts or aerobic plate count in crab meat (Gilbert et al., 2000; McDermott et al., 2018; Wentz, Duran, Swartzentruber, Schwab, & Read, 1983). Furthermore, a level of 4 log CFU g^{-1} of *Pseudomonas* spp. was associated with the limit for sensory acceptability of leg meat of cooked RKC clusters (Lorentzen et al., 2014). By fitting the reparametrized Gompertz-Zwietering model (Eq. (13)) to microbial growth data, it was possible to estimate the time necessary to reach the MAL (i.e., microbial shelf-life, MSL) for each of the microbial populations investigated (Table 2).

The MSL was significantly different between harvest seasons only when the TVC$_P$ was considered. Nonetheless, it should be noted that, in all instances, the estimated time-to-MAL was longer than 10 days, which is the shelf-life limit recommended for packaged refrigerated RTE seafood products to which the most severe heat treatment applied had a cumulative lethality (*F* value) in the coldest spot which did not meet the criteria for a 6-log reduction of non-proteolytic *Clostridium botulinum* ($F_{90}^{7-10} = 10$ min) (ACMSF, 2007; ECFF, 2006).
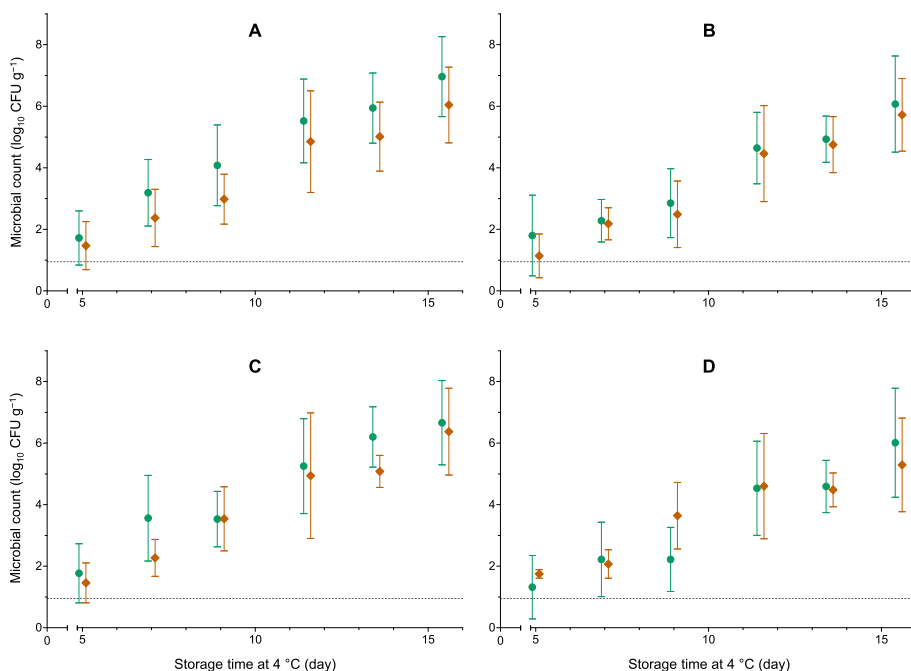
Fig. 3. Total viable psychrotrophic count (TVC_P) (A), total viable mesophilic count (TVC_M) (B), total aerobic plate count (APC) (C), and presumptive *Pseudomonas* spp. (D) enumerated after 5, 7, 9, 11.5, 13.5, and 15.5 ($\pm$4 h) days of storage at 4 °C in the leg meat of cooked clusters of spring postmolt (●) and autumn intermolt red king crabs (◆). The counts are reported as decimal logarithm of colony-forming units per gram of sample ($\log_{10}$ CFU g^{-1}) and expressed as the mean $\pm$ standard deviation indicated by error bars ($n = 7$ per time point and season group). The dotted horizontal line indicates the detection limit. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.5. Correlation between quality parameters

An overview of the correlation pattern between quality parameters is given in Fig. 4. The highest positive correlations (r between 0.87 and 0.99) were found between the different cluster yields (CY_{raw}, CY_{cooked}, and CY_{frozen}), the shell thickness, and the leg meat content. Interestingly, the HSI, the WI, and the textural hardness were significantly positively correlated between them (r between 0.54 and 0.67) and with the cluster yields, the shell thickness, and the leg meat content (r between 0.52 and 0.77). By contrast, the CI and the drip loss (after 5 days of refrigerated storage) were significan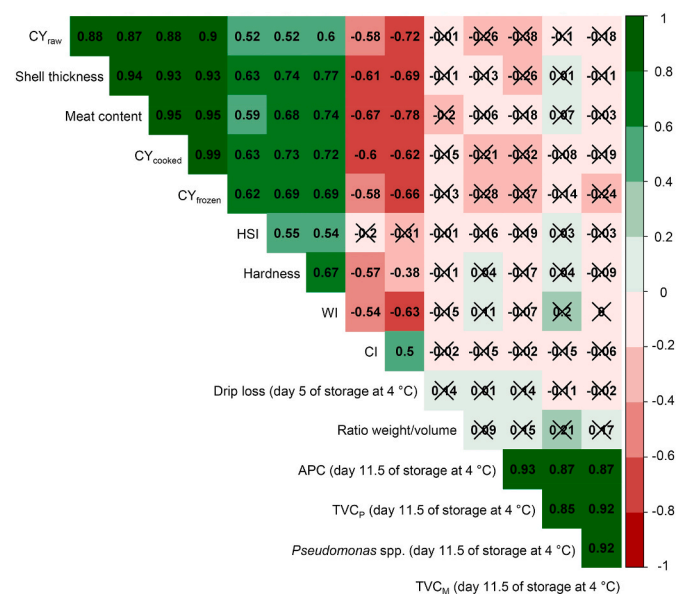tly negatively correlated with all the above-mentioned parameters (r between -0.38 and -0.90). The weight/volume ratio of the meat in the *merus* and all the microbial counts after 11.5 days of refrigerated storage were not significantly correlated with the other parameters considered. The correlation pattern may be used by the RKC processors and traders to infer valuable information on the product quality based on measurable parameters.

3.6. Implications of commercial relevance and for fishery management

The results showed that the harvest season and, indirectly, the molting stage have a substantial impact on the quality attributes of RKC clusters and their leg meat with potential implications of commercial relevance.

The thin exoskeleton observed in the postmolt RKCs caught in the spring increases the risk of damage during handling and transport. The presence of bruises on the exoskeleton not only impairs the commercial value of the product but also constitutes an animal welfare issue, especially in all instances in which it is allowed to return the crabs to the sea after catch and handling on-board.

The cluster yield and the drip loss are quality parameters of key importance to the profitability of the utilization and commercialization of RKC. The cluster yields of postmolt RKCs harvested in the spring were from 8 to 15 percentage points lower than the ones obtained from intermolt RKCs harvested in the autumn. Business operators purchasing live RKCs or raw RKC clusters to be processed and further marketed as cooked products may incur substantial economic losses when trading postmolt RKCs harvested in the spring. The same applies to retailers selling RTE clusters of RKC in light of the higher drip loss and shorter time for the TVC_P to reach the MAL observed during refrigerated storage in the cooked clusters obtained from postmolt RKCs harvested in the spring.

The meat content, defined as the percentage of the volume of claws and walking legs occupied by the muscle, is one of the decisive attributes of cooked RKC clusters for final consumer satisfaction (Siikavuopio & James, 2015). Given the low leg meat content observed, there is a high risk that the clusters obtained from spring postmolt RKCs do not meet consumer expectations (Voldnes et al., 2020). The same might apply to the cooked leg meat in terms of eating quality attributes such as texture and color, given the lower firmness and whiteness of the meat observed

Fig. 4. Correlogram illustrating the Pearson's correlation coefficient (r) between selected response variables. The computation of the correlation matrix was based on pairwise-complete observations ($n = 14$ for the microbial counts and $n = 34$ for all the other response variables considered). Crosses mark insignificant correlations ($p > 0.05$) resulting from a pairwise two-sided Pearson's correlation significance test.

in the present study.

The commercialization of postmolt RKCs harvested in the spring is the direct consequence of the extension of RKC fishing to year-round in Norway since 2008 (Norwegian Ministry of Trade, Industry, & Fisheries, 2015). The RKC fishing in the spring is mostly conducted in the open access area (OAA, east of 26°E), which is not subjected to a quota limitation, but where it is mandatory to keep and land the whole catch, including females and undersized individuals. Therefore, it is not possible for fishermen to keep selectively the RKCs that are in the intermolt phase, which are, however, the minority in the spring (Sundet & Hoel, 2016). Nonetheless, it remains valuable for fishing vessels to undertake RKC harvesting in the spring in the OAA, especially in April and May right after the cod fishing period (S. I. Siikavuopio, personal communication, August 2020), despite the substantially lower first sale value attainable (120–139 NOK kg^{-1} in 2018 and 2019) compared to that of RKCs harvested in the autumn (174–176 NOK kg^{-1} in 2018 and 2019) (The Norwegian Fishermen's Sales Organization, 2020).

In comparison to the price paid to fishermen, the export price of live RKC is considerably less affected by the actual crab quality, as it is high throughout the year (304 NOK kg^{-1} annual average in 2018 and 2019) with only marginal fluctuation (around 15%) between seasons (Norwegian Seafood Council, 2020). This is due to the fact that the availability of RKCs in the spring, when competing fisheries in other countries are inactive, places Norwegian traders in the privileged position of being the sole actors capable of supplying high-end markets (e.g., South Korea) with RKCs (Voldnes et al., 2020). Nonetheless, while the establishment of the OAA aimed to limit the expansion of the RKC westwards (Sundet & Hoel, 2016), it has been pointed out that there is a risk of long-term dissatisfaction and loss of reputation when supplying the market with low-quality RKCs (Voldnes et al., 2020).

The present study has highlighted the quality difference between RKCs representative of the spring and autumn harvest in the northern Norwegian coastal region. These findings may be used to strengthen the marketing orientation by improving transparency in the information stream along the value chain. The results also indicate that the commercialization of the RKCs with poorer eating quality attributes in the spring season might not be the most appropriate way to utilize this resource in terms of value creation, market reputation, and sustainability. Furthermore, in this scenario, it should be explored whether or not feeding postmolt RKCs during post-capture live holding in land-based facilities can improve the quality conditions before commercialization.

4. Conclusions

The harvest season and, indirectly, the molting stage of red king crab (RKC) harvested in the Norwegian coastal region of the Barents Sea had a substantial impact on the quality attributes with potential implications of commercial relevance. Specifically, it was observed that the clusters obtained from spring postmolt RKCs had lower exoskeleton thickness and yield compared to autumn intermolt RKCs. The cooked leg meat of spring postmolt RKCs was also characterized by significantly lower textural firmness and whiteness index, which may be associated with lower overall eating quality. Furthermore, during refrigerated storage, cooked clusters of spring postmolt RKCs showed significantly higher drip loss, and their leg meat reached significantly earlier the acceptability limit of 5 log CFU g^{-1} in the total viable psychrotrophic count.

The commercialization of postmolt RKC harvested in the spring may not only lead to economic losses for stakeholders along the value chain but also cause consumer dissatisfaction, thereby undermining the market reputation and profitability in the long run.

Furthermore, research should be carried out to evaluate if feeding spring postmolt RKCs during post-capture live holding can improve their quality conditions before commercialization.

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