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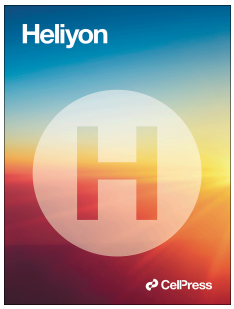

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Shelf-life of cooked meat of southern king crab (*Lithodes santolla*) and false king crab (*Paralomis granulosa)* during refrigerated storage

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Abstract

Lithodes santolla (SKC) and *Paralomis granulosa* (FSKC) are economically important resources exploited in southern South America. The effect of refrigerated storage (4 °C on flake ice) on physico-chemical (pH, thiobarbituric reactive substances (TBARs), total volatile basic nitrogen (TVB-N), water holding capacity (WHC), and water content (WC)), microbiological (total viable mesophilic bacteria (TVMC), psychrotrophic bacteria (TVPC), *Staphylococcus spp*, coliforms, enterobacteria, molds and yeasts) and sensory (odor, appearance, texture, juiciness, and taste) parameters was analyzed in the cooked SKC and FSKC *merus*. For each species, cooked *merus* from 36 animals were randomly distributed into 6 groups, corresponding to 0, 2, 5, 8, 11, and 14 days of storage. On each day, samples were taken for physico-chemical (n= 6), microbiological (n= 3), and sensory (n= 15) analyses. The pH values increased over time ($P< 0.01$ in both species), the TBARs only increased in FSKC ($P= 0.008$), whereas the TVB-N

significantly rose only in SKC (P = 0.001). The WHC and the WC did not change over time for any of the king crab species (P> 0.05) in all cases. The presence of TVCM, TVCP, and *Staphylococcus spp*. in both species was observed from day 0. Furthermore, pathogenic microorganisms (*S. aureus*, coliforms, and enterobacteria) were not detected, and only the TVCP in SFKC reached the suggested microbial limit after 11 days. All sensory scores significantly decreased (P< 0.001) over time, but the quality of both king crab species remained acceptable until the 11th day. These findings suggest that the shelf-life of cooked *merus* was 11 and 8 days for SKC and SFKC, respectively, when stored at 4 °C with the presence of flake ice. These contributions consist of elucidating the shelf-life of these economically important seafood products and providing insights into their quality maintenance during storage.

Keywords: Beagle Channel, meat quality, meat spoilage, microbial activity, decapods

1. Introduction

The southern king crab (SKC, *Lithodes santolla*) and the false king crab (FSKC, *Paralomis granulosa*) are highly prized seafood known for their delicious taste and high nutritional value [1,2]. SKC and FSKC are exploited in Chile and Argentina, specifically along the Southern Pacific coast (50 °S), the Magellan Straits (53 °S, 70 °W), the Beagle Channel (55 °S, 68 °W) and on the Atlantic coast, off the Golfo San Jorge (46 °S, 65 °W) [3] and cites there in). In Argentina, there were ~2100 t of total SKC landings [4]. Particularly, in the Beagle Channel region, both species are economically important crabs, and they are currently captured using artisanal methods with small boats [5].

The edible meat of these king crab species, is found in the walking legs and chelipeds, like other decapods such as the red king crab and snow crab [6]. The international trade of these lithodids crabs is carried out alive or frozen cooked clusters [3,6,7]. A cluster includes three walking legs and a cheliped attached to a shoulder joint [7]. At a retail level, SKC and FSKC in Tierra del Fuego, Argentina, are commercialized as peeled cooked meat under different presentations: vacuum frozen [2,8], smoked, brined and fresh crab meat [9].

After animals are sacrificed, the fast development of a series of irreversible alterations begins, so marine products are highly perishable. Seafood spoilage may take diverse forms, due to a complex process in which chemical, physical, and microbiological forms of deterioration are involved [10–13]. Spoilage is evident through changes in sensory characteristics, such as flavor, appearance, firmness, and unpleasant odor compounds [11], and it is responsible for freshness loss [10,14–16]. Volatile compounds generated by endogenous enzymes and microbial activity produce undesirable off-odors and off-flavors. Furthermore, aldehydes, ketones, and other compounds are produced as by-products of the oxidation of polyunsaturated fatty acids commonly found in marine foods, leading to changes in aroma, flavor and color [11,12]. To assess the quality of seafood during refrigeration, various analyses can be performed, such as microbiological, physico-chemical, and sensory assessments. Microbiological parameters are employed to gather information about the hygienic quality during the handling, processing, storage and shelf-life of the product as well as to detect the presence of pathogenic microorganisms [7,10,17,18]. Other factors such as animal health, slaughtering, and storage methods can also influence the quality of seafood [19–21].

Despite the various technological processes available for extending the shelf-life of food, refrigeration is the most widely used method of preservation. While cold storage is beneficial for meat preservation and microbial control it can also lead to the proliferation of psychrotrophic microorganisms. Specifically, these bacteria can thrive at temperatures near 0 ºC, resulting in meat spoilage [11,14,18]. Also, molds and yeasts can spoil different kinds of food by producing off-flavors and aromas, primarily due to their adaptable environmental requirements.

On the other hand, sensory analyses are used to assess food quality and shelf-life. Hedonic methods measure the acceptability of aquatic food products, which is a crucial factor in ensuring their success in the market [22–24]. Sensory tests have been effective with a variety of fishery products [25–28].

Numerous studies have explored the nutritional composition of SKC [1,21,29] and king crabs worldwide [6,7,17,23,24,30,31]. However, despite the economic relevance of these species, there is currently no available information on the changes in the quality of the cooked meat from these subantarctic species during refrigerated storage. In light of these considerations, the aim of this study was to evaluate the quality changes in the cooked *merus* of SKC and FSKC during 14 days at 4 ºC, in order to determine these products

shelf-life. For this reason, physico-chemical, microbiological and sensory characteristics of cooked *merus* were analyzed.

2. Materials and Methods

2.1. Ethical approval

All sampling procedures follow the guidelines of ethical use and care of animals in science approved by the Directive Board of the Southern Center of Scientific Research (CADIC-CONICET) and conform the proposals of the National Committee on Ethics in Science and Technology from Argentina (http://www.cecte.gov.ar/). The Dirección General de Biodiversidad y Conservación. Ministerio de Producción y Ambiente from Tierra del Fuego, Antártida e Islas del Atlántico Sur granted the appropriated sampling permissions.

2.2. Animal acquisition and experimental design

Thirty-six male southern king crabs (SKC, *Lithodes santolla*) and 36 male false king crabs (FSKC, *Paralomis granulosa*) were captured by commercial traps in the Beagle Channel (55 °S 68 °W). All animals were in intermoult state, with legal sizes of 110.04 ±3.79 mm and 91.04 ±4.35 mm of carapace length for the SKC and FSKC, respectively. To align with the standard procedure for processing king crabs, the animals were transported to a local processing plant (Ahumadero Ushuaia).

The animals were sacrificed by separating the clusters (*merus* and shoulders) from the bodies (cephalothorax and abdomen), followed by boiling in tap water (100 °C) for 10 minutes. After that, the clusters were cooled in tap water at 15 °C for 10 minutes and finally peeled with specific scissors to extract the cooked *merus* from exoskeleton, following the decortication process. Our research only considered cooked meat from the *merus* whereas the meat from the shoulders of the animals was discarded.

After being peeled, the cooked *merus* from each animal were placed in individual polystyrene trays and covered with plastic wrap, resulting in a total of 36 trays per species. Then, the trays were randomly distributed into 6 groups (n = 6 for each group). The trays

were placed in a container with flake ice at the bottom and stored at 4 ±0.5 °C. The flake ice was replaced twice a day.

At each sampling time (0, 2, 5, 8, 11 and 14 days), the samples from one group of trays were analyzed for their water holding capacity, water content, pH, microbiological and sensory characteristics. The rest of the *merus* were frozen at -80 °C for the total volatile basic nitrogen (TVB-N) and thiobarbituric acid reactive substances (TBARs) determinations. The *merus* of the day 0 were analyzed immediately after peeled. The same protocol was applied for both species (Fig. 1). The processing and storage conditions, including temperature and the use of flake ice, followed the local practice typical of Tierra del Fuego, Argentina.

2.3. Physico-chemical parameters of cooked merus

pH determination

pH was measured by a modified protocol from Tribuzi et al. [32], on 20 g of *merus* homogenized in distilled water in a ratio 1:10 (w/v) using a digital pH meter (Arcano, PHS-3E). Determinations were carried out in triplicate.

Lipid oxidation

Thiobarbituric acid reactive substances (TBARs) values were determined according to the method of Ohkawa et al. [33], based on the reaction of thiobarbituric acid (TBA) with the secondary products of lipid peroxidation, measured at 535 nm (see details in Schvezov et al. [34]). Data were expressed as μmol TBARs·g wet tissue^{-1}. Determinations were done in triplicate.

Total volatile basic nitrogen

Total volatile basic nitrogen (TVB-N) values were determined by Kjeldahl method [35]. Briefly, 10 g of cooked *merus* were digested with trichloroacetic acid 5% w/v and then filtered. Supernatants were distilled using a Buchi equipment with NaOH (10 N), and the distillate was collected in 50 mL of boric acid (20 g·L^{-1}) containing 5 mL of indicator (100 mL ethanol, 0.05 g methyl red, 0.075 g bromocresol green), to a final volume of 230

159 mL. Finally, it was titrated with sulphuric acid 0.01 N. Results were expressed as mg
160 TVB-N·100 g of wet tissue^{-1} (mg%). Determinations were done in duplicate.
161
162 *Water Holding Capacity*

163 Water holding capacity (WHC) was analyzed following Lorentzen et al. procedure [7].
164 Five g of cooked *merus* were wrapped in filter paper and centrifuged at 465 g for 10 min
165 at 4 °C, using 15mL falcon tubes with cotton at the bottom. WHC was calculated using
166 (Wo - ΔC)/Wo)·100, where Wo is the initial water content and ΔC is the *merus* weight
167 difference before and after centrifugation and expressed as a percentage of initial meat
168 weight.
169
170 *Water content*
171 Water content (WC) was determined by drying 5 g of *merus* in an oven at 60 °C (Tecno
172 Dalvo, Argentina), until constant weight was achieved and was calculated as the
173 difference in weight before and after drying. Results were expressed in % water content
174 (g·100g sample $^{-1}$).
175
176 *2.4. Microbiological analyses*

177 On each sampling day, a total of three biological samples (n= 3) from each group were
178 analyzed. Each sample consisting of 10 g of *merus* from 2 different crabs (5 g of each),
179 were transferred to a sterile beaker with 90 mL of peptone water 0.1 % (Britanialab, ISO
180 6579, CABA, Argentina) and homogenized for 90 seconds. From this homogenate, serial
181 decimal dilutions were prepared with 0.1 % peptone water, according to the standard
182 methodology proposed by APHA [36]. One mL of the dilutions was inoculated in
183 duplicate on the different 3M Petrifilm^{TM} plates corresponding to each microbiological
184 analysis for later quantification [37].

185 The total viable mesophilic bacteria (TVMC) and psychrotrophic bacteria (TVPC) counts
186 were performed according to ICMSF [37]. Samples were inoculated on 3M Petrifilm™
187 count plates (AC 6400), incubated at 37 °C for 48 h and 7 °C for 10 days, for mesophilic
188 and psychrotrophic bacteria, respectively.

To determine *Staphylococcus spp.*, sample dilutions were inoculated on 3M Petrifilm™ Staph Express count plates (STX 6446), then incubated at 35 °C for 24 h. and red-violet colonies were counted. If colonies with a different color than red-violet were observed (e.g., black or blue-green), a Staph express disc (STX 6492) was used to confirm *S. aeurus*.

To detect coliforms and enterobacteria, 3M Petrifilm™ count plates (CC 6410 and EB 6420, respectively), were used and incubated at 35 ºC for 24 h [37].

The presence of molds and yeasts was evaluated by using 3M Petrifilm™ count plates (YM 6400), which were incubated at 25 °C for 5 days [37,38].

Microbiological results were expressed as the decimal logarithm of colony-forming units per gram of tissue ($\log_{10}$ CFU·g^{-1}) and calculated as the mean (± standard error) of three independent samples in each experimental time.

2.5. Sensory analysis

The degree of freshness of cooked king crab meat from both species during storage was assessed using a descriptive sensory test. Fifteen trained panelists, aged between 30 and 60 years old, assessed the attributes odor, appearance, texture, juiciness, and taste using a demerit scoring system on a structured linear scale ranging from 9 (highest quality/maximum freshness) to 1 (lowest quality/ inedible product) (Table 1). These attributes were selected based on considerations by Lorentzen et al.[23] , and customized to the product characteristics by the panel in preliminary evaluation sessions in which specific attributes and descriptors were defined. Also, some of these attributes are considered to be the most representative and important for consumers and the food industry [39]. Samples were prepared in a separate room from the testing area. Cooked *merus* (refer to section 2.2) were cut transversally into pieces of 2-2.5 cm in length. Samples were served at room temperature (20°C) to the panelists on plastic plates coded with a random three-digit number. Each panelist received once pieces of each sample and was provided with a fork, knife, napkin, and water to rinse their mouth. The sensory tests were conducted on days 0, 2, 5, 8, 11, and 14 at 11 a.m. The score sheet consisted of linear structured scales of 8 cm for each attribute, with three anchor points at 1, 5, and 9, representing the corresponding descriptors listed in Table 1. Data obtained from the

position on the scales were assigned scores between 1 and 9. The average score for each attribute was calculated, with scores in the range of 5 to 9 considered acceptable.

2.6. Statistical Analyses

Statistical analyses were performed by species. For the physic-chemical parameters the *merus* of each crab was considered as a biological replica (n= 6 in each group). For microbiological analyses one sample was represented by *merus* of two crabs (n= 3 in each group). For the sensory analyses 15 panelist (n= 15 in each group) were considered. In both species each group was analyzed in each sampling day. Data were presented as mean ± standard error.

The effects of storage time on the physic-chemical and microbiological parameters were assessed for each species by analysis of variance (one-way ANOVA). The assumptions of normality of distribution and homogeneity of variance were checked by Shapiro-Wilk and Levene tests, respectively [40]. Tukey HSD post-hoc tests were done when the ANOVA was significant ($P< 0.05$).

The effects of storage time on the sensorial attributes were assessed for each species by analysis of non-parametric Friedman ANOVA by ranks [41]. A Kendall concordance coefficient was used to test the hypothesis that each parameter ranked was in agreement among panelists more than that expected by chance. The range of this coefficient is from 0 to 1, where values close to 1 represent perfect agreement in the ranking of the particular attribute among panelists. Dunn´s tests were performed when Friedman test was significant ($P< 0.05$). Statistical analyses were performed using GraphPad Prism software version 9.0.0, employing a minimum significance level of $\alpha= 0.05$ with $P< 0.05$ being considered significantly different.

3. Results

3.1. Physico-chemical parameters of cooked merus

Figure 2A shows the evolution of pH in cooked *merus* where it was observed a significant increase with time in both species (ANOVAs: $F_{SKC}= 4$, $P_{SKC}= 0.007$ and $F_{FSKC}= 18$, $P_{FSKC} <0.001$). Particularly, the pH in SKC significantly increased from the 11th day and

maintained until the end of the experiment, whereas in FSKC significantly increased from the 5th day.

TVB-N significantly increased only in SKC on the 14th day (ANOVA: F_{SKC}= 7.2, P_{SKC}< 0.001) from 13.89 until 21.57 mg% (Fig. 2B). In contrast, in FSKC, the TVB-N values remained constant throughout the experimental time (ANOVA: F_{FSKC}= 1.1, P_{FSKC}= 0.392) with a mean value of 11.69 ±0.19 mg% (Fig. 2B).

TBARs significantly increased with time only in FSKC (ANOVA, F_{FSKC}= 4.2, P_{FSKC}= 0.008), from 10.63 until 16.01 μmol·g^{-1}, whereas TBARs in SKC did not vary during storage (ANOVA: F_{SKC}= 1.1, P_{SKC}= 0.389) with mean values of 15.47 ±0.74 umol·g^{-1} (Fig. 2C).

In both species, water holding capacity (WHC) values remained unchanged throughout the experiment (ANOVAs: F_{SKC}= 1.7, P_{SKC}= 0.178 and F_{FSKC}= 0.8, P_{FSKC}= 0.560). Both species presented similar WHC mean values of 72.13 ±0.69 and 70.60 ±0.79 % for SKC and FSKC, respectively (Fig. 2D).

Water content (WC) values did not present significant differences (ANOVAs: F_{SKC}= 0.81, P_{SKC} = 0.556 and F_{FSKC}= 1.2, P_{FSKC} = 0.318) during the experiment. Also, the WC was similar throughout the storage in both species (80.29 ±0.41 and 78.67 ±0.62 % for SKC and FSKC, respectively; Fig. 2E).

3.2. Microbiological analyses

In both species, initial TVMC (3.17 log CFU·g^{-1} in SKC and 3.61 log CFU·g^{-1} in FSKC) significantly decreased with storage (ANOVAs: F_{SKC}= 9.9, P_{SKC}= 0.001 and $F_{FSKC=}$ 10.86, $P_{FSKC<}$ 0.001) (Fig. 3A). Starting from the 5th day, the TVMC values were significantly different from the initial values, and they continued to decrease until the end of the experiment, reaching values of 2.38 and 3.09 log CFU·g^{-1} in SKC and FSKC, respectively.

The initial *Staphylococcus spp.* values were 2.66 and 3.10 log CFU·g^{-1} in SKC and FSKC, respectively, and varied significantly over time (ANOVAs: F_{SKC}= 8.2, P_{SKC}= 0.001 and F_{FSKC}= 5.1, P_{FSKC}= 0.01). Furthermore, coagulase-positive *Staphylococcus* was not detected in any of the analyzed species. In SKC, *Staphylococcus spp.* counts decreased

281 from the 1st to the 5th day, and then increased by the end of the experiment (Fig. 3B). In
282 FSKC, a significant decrease in *Staphylococcus spp.* counts was observed on the 2nd day
283 and persisted until the last day of analysis (Fig. 3B).
284
285 In both species, initial TVPC values were 2.46 and 2.51 log CFU·g^{-1} in SKC and FSKC,
286 respectively. These values remained nearly unchanged, staying below 4 log CFU·g^{-1} until
287 the 8th day, after which they significantly increased (ANOVAs: F_{SKC}= 55.9, P_{SKC}< 0.001
288 and F_{FSKC}= 124.4, P_{FSKC}< 0.001) (Fig. 3C). At the end of the experiment, TVPC reached
289 values of 4.7 and 5.93 log CFU·g^{-1} for SKC and FSKC, respectively.
290 Enterobacteriae and coliforms were not detected neither in SKC nor in FSKC. Molds and
291 yeasts were only detected in SKC and their values remained constant throughout the
292 storage (ANOVA: F_{SKC}= 1.3, P= 0.65), with a mean value of 1.32 log CFU·g^{-1}.
293
294 *3.3. Sensory analysis*
295 For both species, the initial sensory attribute scores were close to 9, indicating a high level
296 of freshness and quality. Over the 14-day study period, significant differences were
297 observed in all attributes for both species, with a consistent decreasing trend. The patterns
298 of scores for odor, appearance, and texture were highly similar between the two species
299 (Fig. 4).
300
301 In general, the quality of both species significantly deteriorated during storage, with a
302 loss of its fresh smell, aspect, firmness, and increasing its dryness and tastelessness
303 (Friedman test for SKC and FSKC, P< 0.001 in all cases, see Table 2). Also, the ranking
304 of the attributes done by panelist was confident (Kendall coefficient of concordance> 0,6 in all
305 cases, see Table 2). Hence, a change in each attribute of both species was clearly and
306 statistically detected after storage.
307
308 However, all attributes remained acceptable until 11th day (Fig. 4A, B, D and E), except
309 for texture, which was acceptable until the last day of the experiment (Fig. 4C).
310 In both species, by the last sampling day, the *merus* exhibited a pronounced unpleasant
311 odor characterized by a distinct ammonia scent that exceeded the acceptability limit (Fig.
312 4A). In addition, the *merus* appearance was less shiny (Fig. 4B) and dry (Fig 4D),
313 compared to its initial state at the beginning of the storage. Even more, the panelists stated

an incipient rancid taste in the *merus* of both king crabs species after 14 days of storage (Fig 4E).

4. Discussion

4.1. Physico-chemical parameters of cooked merus

After 14 days of storage there was a pH increase of 4 and 8 %, for SKC and FSKC, respectively. This pH rise during storage could be related to the accumulation of alkaline molecules (ammonia and dimethyl and trimethyl amine) due to the decomposition of tissue protein, which are produced by endogenous enzymes and microbes during seafood spoilage [11,14,42–44].

Similar increase of meat pH during storage was shown in other species of crustaceans [19,45]. pH of cooked meat from *Paralithodes platycus* increased 7 % after 14 days at 4 °C [23] and 12% in brown crab *Cancer pagurus,* under similar storage conditions [10]. Furthermore, the initial pH values of 7.5 and 7.4 obtained in this study for SKC and FSKC, respectively, were similar to those observed for SKC (7.6, [21]), for *Paralithodes camtschaticus* (7.3 or 7.2, [7,23]), for *C. pagurus* (7.5, [10]), and for *Parapenaeus longirostris* (7.5, [45]), among others.

The muscle pH of *L. santolla* is slightly basic, around 7.7 [29], due probably to their high amount of non-protein nitrogenous compounds [23]. In cooked shrimp, pH values between 7.5 and 8.6 were observed, with an acceptability limit of 8.3 corresponding to the 8th day of storage at 2 °C [45]. In shrimp, there was a correlation between sensory analysis and pH values, indicating quality loss at pH values over 7.5 [46]. A pH of 7.8 was reported as the critical threshold for determining the acceptability of shrimps and prawns. Therefore, this parameter could serve as an indicator of crustacean freshness [45,47,48].

The *merus* pH variation would affect the tissue in physiological terms. However, in the context of meat quality, such as in our study, this variation would be unnoticed by a regular consumer, since the *merus* pH remains within a neutral to slight basic range [21].

While pH is a good indicator of freshness [45], it should not be relied upon as the sole method for assessing freshness [49].

As meat pH is linked to the formation of TVB-N, which quantifies volatile nitrogen amines, so both parameters tend to increase during storage [50]. Specifically, the TVB-N increase in SKC, could coincide with spoilage and microbial growth. This was observed in other crab species in different time of storage as in *Chioneocetes opilio* (TVB-N of 140 mg% and TVCP of log 5.5 UFC·g^{-1} after 14 and 10 days, respectively; [24]); in *P. camtschaticus* (TVCP of log ~7 UFC g^{-1} after 15 days [51], and log 7.53 UFC·g^{-1} to 11days [6]) and in the seafish *Engraulis anchoita* (TVB-N of 30 mg% after 10 days [49]). In general, TVB-N values obtained in our analysis were lower than those normally found in cooked crab meat as *Calinectes sapidus* [52], in *Cancer Pagurus* [10] and in *Scylla serrata* [53], in similar conditions of storage.

In SKC, the highest TVB-N value detected (22 mg%) at the end of the experiment (14 days) could be associated with the presence of psychrotrophic bacteria and *Staphylococcus spp.* (see Fig. 3B and 3C). During meat storage, the increase in TVB-N concentration generally coincides with other biomarkers of spoilage such as microbial count and changes in sensory acceptability [54]. TVB-N is one of the most widely used indicators to assess meat quality [55]. It includes the measurement of trimethylamine, which is produced by bacterial degradation. The rise observed in TVB-N values by the end of storage can be related to the low scores observed for all the sensory attributes (Fig. 4). However, TVB-N values in FSKC remained constant (12 mg%), which were consistent with the observed behavior of *Staphylococcus spp.* bacteria. There are precedents that establish a positive association between the presence of *Staphylococcus* spp. and TVB-N [56].

The final TVB-N values obtained for both king crab species did not exceed the limit value of 30 mg% established by our national legislation for raw or frozen fish [9]. Also, these values are lower than those proposed by international standards of good seafood quality and the European Commission Regulation for fishery products (~ 25.8 mg%; [57]) . It should also be noted that the natural content of TMAO (Trimethylamine *N*-Oxide), the substrate for microbial trimethylamine (TMA) formation, is in crustaceans usually lower than it is in fish [58].

Lipid peroxidation (TBARs) can negatively impact the quality and shelf life of cooked fish products. Upon reaching TBARs values of 15-18 μmol g^{-1}, consumer acceptance is limited because it is closely related to the development of spoilage and off-flavors [59]. Additionally, it is influenced by changes in the taste and smell [15,60]. Also, increases of temperature, either during cooking or storage, can lead to lipid oxidation [61,62].

However, lipid peroxidation final values observed in this study were lower (18.50 and 16.01 μmol·g^{-1} for SKC and FSKC, respectively), than the maximum recommended level of 350 μmol·g^{-1} (5 mg of MDA·kg^{-1}) for fish muscle [63]. Since there are no established maximum values of lipid peroxidation for crustaceans, we considered the reference value for fish [64]. SKC has a low percentage of total lipids, <1 % [1,21]. Although this information was not found for FSKC, we dare to assume a similar fat content for this species, considering data of other edible crab species such as <1 % in *P. camtschaticus*; 0.6 % in *P. camtschaticus* and *P. platypus*, ~1 % in *C. opilio*, *C. angulatus* and *C. japonicus*; <6 % in *Homalaspis plana* and <1 % in *Chaceon chilensis*; and <1 % in *Carcinus maenas*, *C. pagurus*, *Callinectes sapidus*, among others [65–67]. Therefore, the effect of lipid peroxidation on the meat quality of both king crab species could be considered almost negligible [21].

WHC affects meat aspects, both qualitatively and quantitatively, such as the retention of vitamins, minerals or salts, and the volume of water retained [68]. In our study, WHC did not change in any of both species after 14 days of storage (Fig. 2D). Also, values observed at day 0 were similar to those found for *P. camtschaticus* (67.8 %; [7]). Since an increase of pH and protein denaturation might produce a decrease in the WHC [11], we expected to observe similar results. However, it seems that longer storage periods are necessary for changes in WHC to be evident, as reported in *Portunus trituberculatus* after 60 days of storage [69].

L. santolla cooked *merus* contain about 18.7 ±0.2 g·100g^{-1} of proteins [1,21]. From this, more than 60 % would correspond to myofibrillar and structural proteins, of which actin and myosin represent the most important ones [70]. Given that the WHC is mainly due to these proteins, most of the water in the living muscle is held within the myofibrils (>80 %), in the spaces between thick and thin filaments [71]. Thus, the fresher the state of the

myofibrillar proteins, the more water retaining capacity they will have [72]. Therefore, the amount of myofibrillar proteins is considered as a key factor for meat quality [70]. WHC represents the muscle tissue's natural ability to retain moisture, which in turn affects the sensory and textural properties of the product, such as tenderness, juiciness or color [73].

In our study, the water content (WC) in both species also remained constant throughout the 14 days of storage. Similar WC values were found in *P. camtschaticus* at the beginning of the experiment, stored ~4 °C (78.5 %; [7]), and in *P. trituberculatus* which were steamed by ten minutes (78.3 %; [74]). Although our WC values were not significantly different, there was a tendency to decrease in the last sampling day. This pattern might be due to the exoskeleton presence during boiling, that prevents the evaporation of moisture, and to protein denaturation, caused by heating induced myofibrillar proteins contraction which would reduce the muscle's ability to retain water [75].

4.2. Microbiological analyses

TVCM bacterial and *Staphylococcus spp*. values in cooked *merus* of both species were generally low. The *Staphylococcus spp.* observed were not pathogenic and belonged to the mesophilic group. The reduction of TVMC and *Staphylococcus spp*. counts observed in SKC and FSKC *merus* can be attributed to bacterial cell injury resulting from exposure to temperatures lower than the optimal range for the growth of these bacterial groups [37]. Some cells have demonstrated the ability to recover from such chilling stresses, [76] which could explain the increase in *Staphylococcus spp*. counts registered in SKC samples on the 14th day.

TVPC values remained relatively stable until the 2nd and 5th day for SKC and FSKC, respectively, after which they increased rapidly. A lag phase can be observed during chilled storage and depends on the time that bacteria require to adjust to the new environment. Lorentzen et al. [23] did not detect total viable counts in meat of red king crab until day 5 of storage at 4°C. Moreover, Boziaris et al. [77] observed lag phases of 24 and 48 h for the total bacterial population of norway lobster flesh stored at 5 and 0°C, respectively. Drip loss and the physicochemical changes that occur during storage release dissolved nutrients that can be used by bacteria for rapid growth [78].

446

447 It is important to highlight that, until 8th day, in both species, TVMC and TVPC did not
448 exceed the microbial acceptability limit of 5 log CFU·g^{-1}, which is recommended for total
449 viable counts in cooked crab meat [37]. Furthermore, after 14 days of storage at 4 °C,
450 pathogenic microorganisms were not found in any of the species. Regarding
451 *Staphylococcus spp*. counts, SKC reached the microbial limit of 3 log CFU·g^{-1} [37] on
452 the 14th day. Also, similar values to those of our study for psychrophiles were found in *P.*
453 *camtschaticus* leg meat stored in similar conditions, where initial values were ~2.5 log
454 CFU·g^{-1} and the microbiological acceptability limit was reached on the 8th day [23]. Also,
455 in *C. Pagurus* cooked meat, a similar pattern of TVPC was observed with initial values
456 close to 3 until 5 log CFU·g^{-1} on the 6th day of storage [79].

457

458 Very low mold and yeast counts were found in SKC and completely absent in FSKC,
459 during our experiment. These microorganisms can serve as indicators of contamination
460 resulting from procedures of inadequate hygienic and sanitary conditions. The absence or
461 low concentration of these microorganisms can be attributed to proper handling and
462 appropriate storage conditions.

463

464 *4.3. Sensory analysis*

465 Considering the threshold of acceptance in a score of 5, the shelf-life of cooked *merus* of
466 SKC and FSKC stored at 4 °C was 11 days. Similar results were observed for cooked
467 clusters of the snow crab (*C. opilio*) stored at 4 and 0 °C, where the shelf-life was 10 and
468 14 days, respectively [10]. In cooked edible crab of *C. pagurus merus* was approximately
469 13 days [10] while clusters of king crabs (*P. camtschaticus*) shelf-life stored at 4 °C was
470 of 8 days [23]. These differences among species may arise from variations in handling,
471 hygiene practices during processing, and storage conditions, in addition to specific
472 characteristics of each species.

473

474 During the storage of the cooked *merus* of SCK and FSKC, the texture scores did not
475 exceed the quality rejection threshold, but there was a tendency to decrease over time. So,
476 we could assume that there is a deterioration of the myofibrillar structure during chilling,
477 which coincides with the increase in proteolytic microorganisms, mainly psychrotrophic

bacteria. A similar tendency was found in shrimp (*Macrobrachuim rosenbergii*) stored at 5 °C, where texture became very soft after 6 days [26].

In the present study, the juiciness decreased in both species of king crab below the acceptance threshold after the 11th day. This decrease may be attributed to increased enzymatic activity, leading to greater water loss through dripping [24]. So, this attribute is determined by the amount of water retained within its structure [80]. Regarding juiciness, a similar situation was observed in the snow crab (*C. opilio*) clusters, where this attribute reached 2.7 after 13 days at 4 °C [24]. Also, these authors found that the level of moisture tended to be higher for cooked clusters stored at 4 °C compared to 0 °C. Therefore, we could assume that by storing the *merus* at less than 4 °C the initial juiciness could be maintained for 11 days.

Our results showed that the odor fell below the acceptance limit only towards the end of the storage, making it sensory unacceptable at that point. Prolonged storage of *merus* at low temperature (4 °C) can promote putrefaction through enzymatic decarboxylation of free amino acids by psychrotrophic pseudomonas [81]. We only detected a faint odor after 11 days of storage at 4 °C, which was coincidental with a significant increase in psychotropic bacteria in both species.

The decrease in odor and taste scores in both species over time are related to an increase in both pH and TBVN levels. As deterioration progresses during storage, a variety of unpleasant-smelling volatile compounds are produced, including TMA. TMA is formed through the bacterial reduction of TMAO and is known for its distinctive 'fishy' odor [11,15]. These results are consistent and according to Lorentzen [24] they are probably the main reason for the sensory rejection of the *merus*. Microbial spoilage is the most common cause of food detriment. It can manifest in various forms, including visible bacteria or yeast growth (such as slime or colonies), textural changes (degradation of polymers), and the development of unpleasant odors and flavors, ultimately resulting in sensory rejection [82].

In general, maintaining the initial quality, optimizing the processing method, and implementing an effective preservation system are crucial factors in ensuring the shelf-life of edible crab and guaranteeing high-quality products to customers. Our findings

512 demonstrate that storing the cooked crab meat at 4 °C on flake ice significantly delayed
513 the deterioration based on sensory quality.
514
515 Overall, sensory analyses conducted by trained judges exhibited a high degree of
516 similarity between both species of king crabs. Thus all attributes were well accepted until
517 day 11, where in both species, the sensory scores for all the analyzed attributes gradually
518 decreased with storage time, resulting in a progressive decline in the sensory quality of
519 cooked crab *merus*. Though incipient deterioration changes occurred from the 5^{th} day, the
520 quality of all the sensory attributes was acceptable until the 11^{th} day, except texture, which
521 showed acceptability until the end of the experiment. Degradation became more evident
522 at the end of the storage, characterized by a slight ammonia odor, loss of the *merus*
523 original white color, significant dryness and a rancid taste. So, from a sensory perspective,
524 the *merus* was considered unacceptable after the 11^{th} day.
525
526 It is widely known that the general public does not read scientific bibliography about food
527 safety and seafood consumption. This facts, added to the misinformation on the internet
528 about these topics, make us reconsider the needed of use social media platforms to show
529 scientific advances as has been developed for University of Bologna [83]. These
530 platforms could be an alternative to reach a broader audience and ensure accurate
531 information accessible to different community actors as consumers, policymakers, and
532 industry stakeholders. Thus, sharing scientifically validated findings on platforms we can
533 contribute to informed decision-making and promote public trust in the safety and quality
534 of seafood products.

535
536
537 **5. Conclusions**
538
539 Based on physico-chemical, microbiological and sensory evaluation we could suggest
540 that shelf-life of cooked *merus* was 11 and 8 days, for SKC and FSKC, respectively, when
541 stored at 4 °C with flake ice. Since our study exclusively focused on *merus* meat,
542 deviations from these conditions would directly affect the meat quality of these king
543 crabs. Future studies could include the effects of storage on the entire cluster of these
544 species.

Furthermore, microbiological and sensory analyses were very important parameters in the decomposition of the *merus* meat of these edible king crabs. In both species, the growth of psychrotrophic bacteria, as well as changes in odor and taste were the most reliable spoilage indicators.

Finally, from a commercial perspective, this work provides fundamental insights that can enhance the control and management of the quality of cooked and refrigerated king crab meat. The application of this knowledge will prove invaluable information for ensuring and enriching bromatological control at sales points in Tierra del Fuego.

Additionally, the information provided in this and future studies may be used to develop social media platforms to counter the vast amount of misinformation available on the web and reach a broader audience with accurate information for consumers, policymakers and industry. Future studies should explore alternative storage methods to extend the shelf-life of king crab meat and thus provide valuable insights for industry stakeholders. Thus, the use of high hydrostatic pressure and vaccum packaging is considered, as well as the use of quitosane coating or any other natural antioxidant that have provided promising results in the preservation of seafood products.

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Declarations of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics statement

Panelists signed a consent form.

Data availability statement

The data will be available on request of the corresponding author.

CrediT authorship contribution statement

Laura L. Cocito (LLC): conceptualization, data curation, formal analysis, writing original draft, writing – review & editing

Sabrina Permigiani (SIP): data curation, formal analysis

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878 **Figure captions**

879 Fig. 1. Diagram of southern king crab (SKC, *L. santolla*) and false southern king crab (FSKC, *P.*
880 *granulosa)* processing and sample preparation for later analysis.

881 Fig 2. Evolution of physico-chemical parameters of cooked *merus* of *L. santolla* (SKC, ●) and *P.*
882 *granulosa* (FSKC, Δ), stored at 4 °C. A) pH, B) Total basic volatile Nitrogen (TVB-N), C)
883 Thiobarbituric acid reactive substances (TBARs), D) Water holding capacity (WHC), E)
884 Water content (WC). Data are expressed as mean values ± standard error. Printing and italics
885 letters indicate significant differences (Tukey, $P< 0.05$) among days, for SKC and FSKC,
886 respectively.

887 Fig. 3. Microbial growth in cooked *merus* of *L. santolla* (SKC, ●) and *P. granulosa* (SFKC,Δ)
888 stored at 4 °C. A) Total viable mesophilic counts (TVMC); B) *Staphylococcus spp* counts. C)
889 Total viable psychrotrophic counts (TVPC). Data are expressed as mean log $CFU \cdot g^{-1}$ ± standard
890 error. Printing and italics letters indicate significant differences (Tukey, $P< 0.05$) among days, for
891 SKC and FSKC respectively.

892 Fig. 4. Sensory attributes scores of cooked *merus* of *L. santolla* (SKC, ●) and *P. granulosa*
893 (SFKC,Δ) stored at 4 °C. A) Odor, B) Appearance, C) Texture, D) Juiciness, and E) Taste. Values
894 are expressed as mean score ± standard error. Acceptance threshold is indicated by dot line (score
895 = 5). Printing and italics letters indicate significant differences (Tukey, $P< 0.05$) among days, for
896 SKC and FSKC respectively.

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898 Table 1: Sensory attributes with their respective scores according to the state of the
899 cooked *merus* of *L. santolla* (SKC) and *P. granulosa* (FSKC) taking as reference extremes
900 and medium values: 9 (optimal quality), 5 (medium quality) and 1 (poor quality). Scores
901 of 5 or higher are considered acceptable for consumption (Adapted from Lorentzen et al.,
902 2014).

Cooked *Merus*	**Score 9**	**Score 5**	**Score 1**
Odor	Fresh Seaweed	Neutral Slight ammonia	Rotten Stronge ammoniacal Hydrogen sulfide or sulfide
Appearance	On the surface Shiny Red/Orange color Inside the *merus* Shiny white color	On the surface Loss of gloss Incipient discolored to pale orange color Inside the *merus* White color	On the surface Absence of shine Strongly discolored to pale orange color Inside the *merus* Loss of white color with yellowish hues tones
Texture	Firm and elastic Integral	Less firmness Less elasticity	Loss of elasticity Disintegrable
Juiciness	Juicy	Less juicy	Dry Very dry
Taste	Sweet Fresh	Less fresh Slight bitter flavors	Intense off flavors Rotten crustacean Rancid

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905 Table 2: Results of Friedman ANOVAs test (χ^2: stadigraph; *P*: probability and K: Kendall
906 coefficient of concordance) to compare the effect of storage in each attribute of the
907 cooked *merus* of *L. santolla* (SKC) and *P. granulosa* (FSKC).
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	Lithodes santolla (SCK)			*Paralomis granulosa* (FSCK)		
Attributes	χ^2	*P*	K	χ^2	*P*	K
Odor	61.04	<0.001	0.87	44.17	<0.001	0.68
Appearance	60.75	<0.001	0.88	44.93	<0.001	0.69
Texture	60.44	<0.001	0.86	46.57	<0.001	0.72
Juiciness	49.65	<0.001	0.83	51.89	<0.001	0.80
Taste	56.34	<0.001	0.94	46.37	<0.001	0.77

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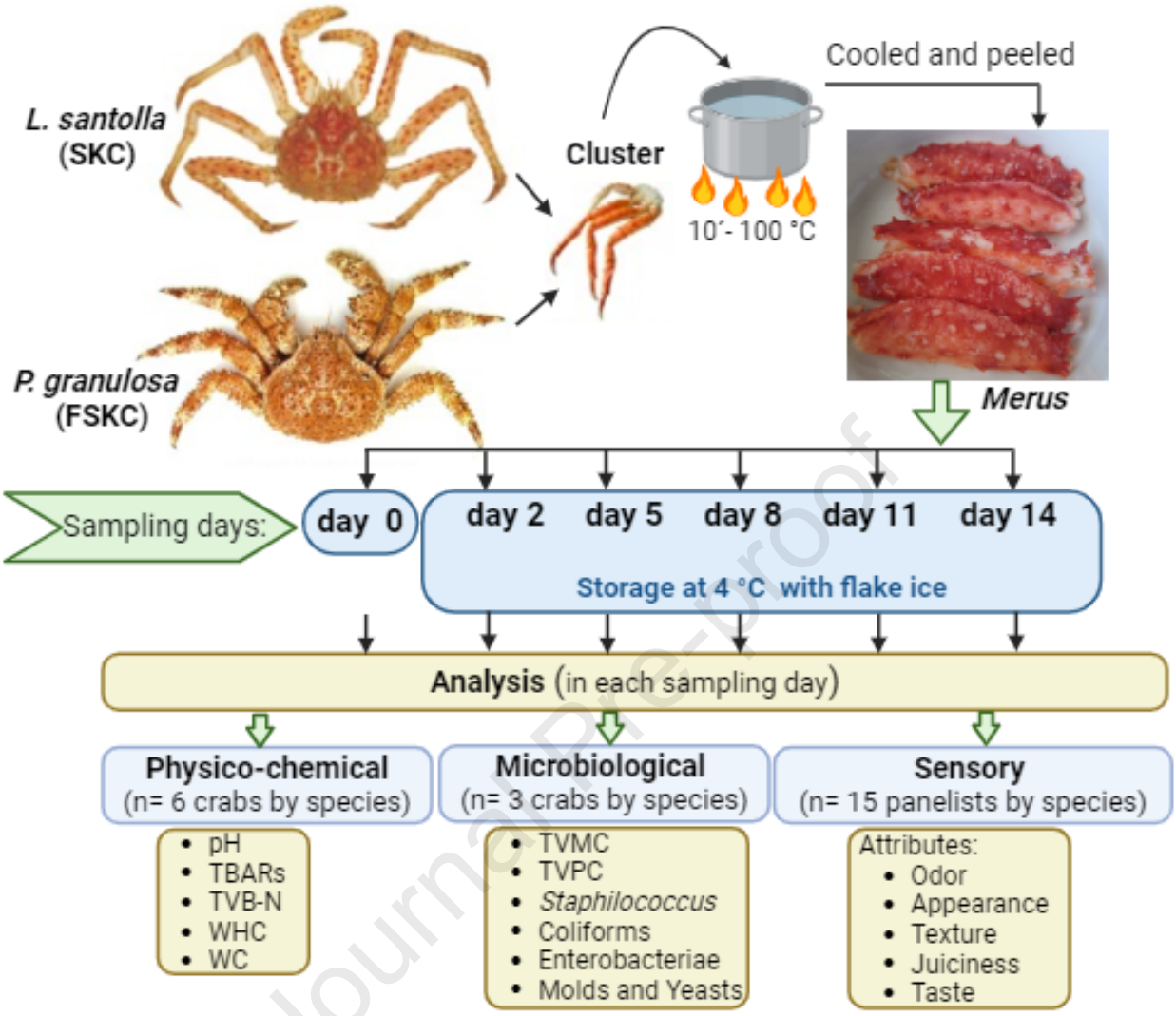

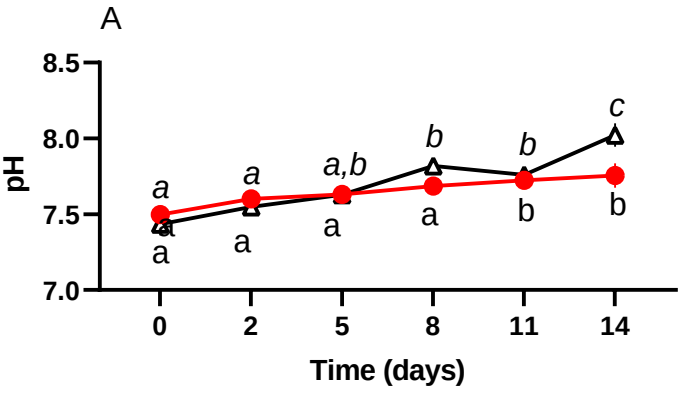

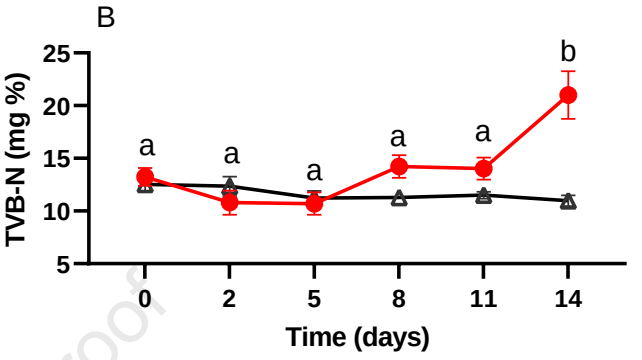

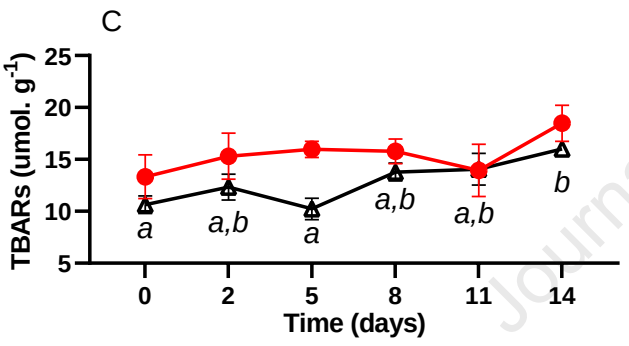

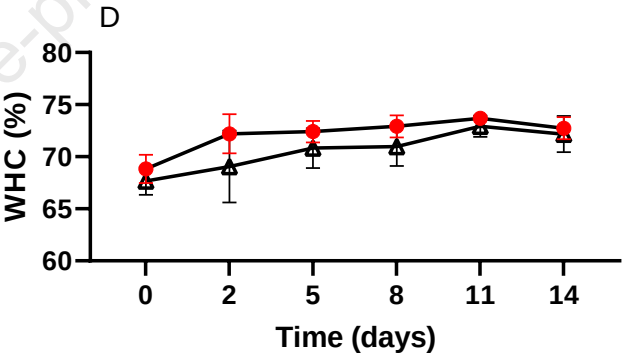

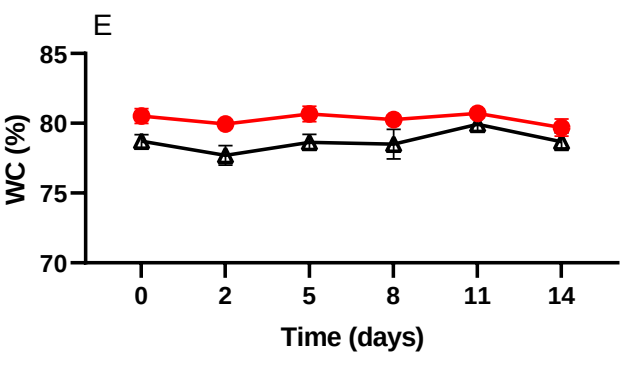

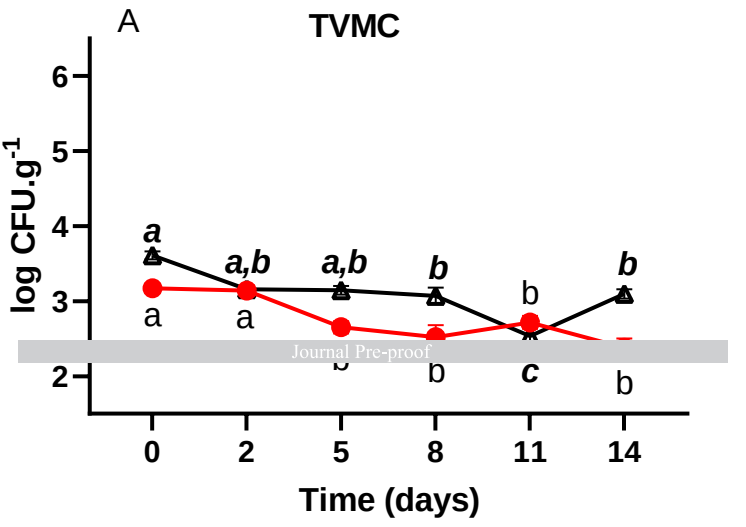

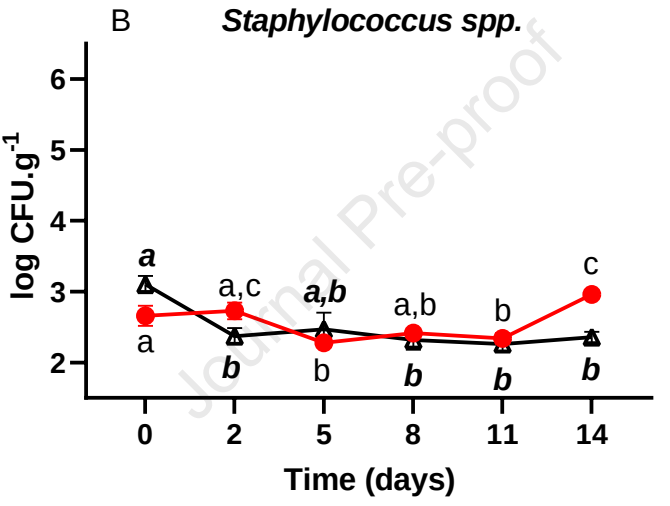

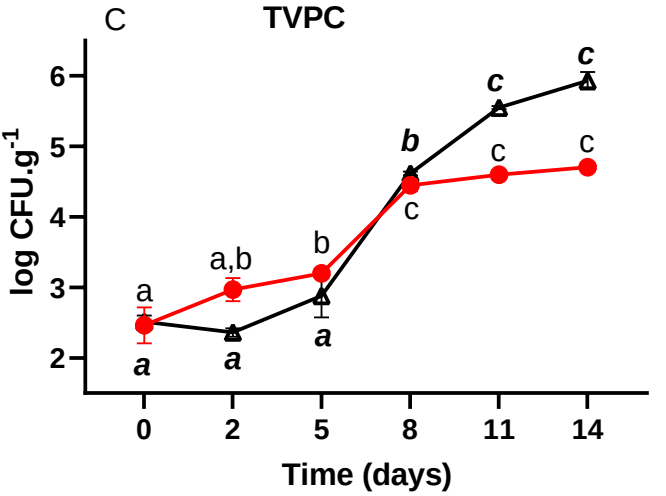

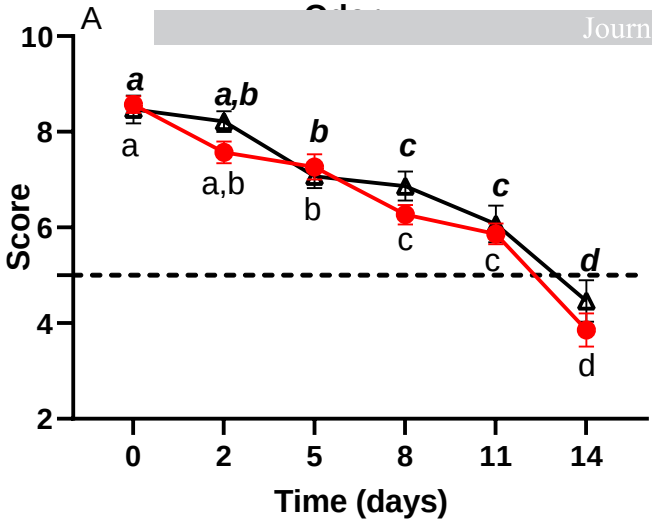

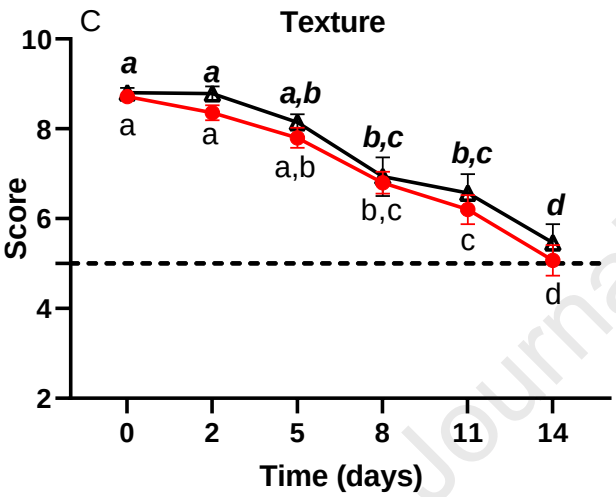

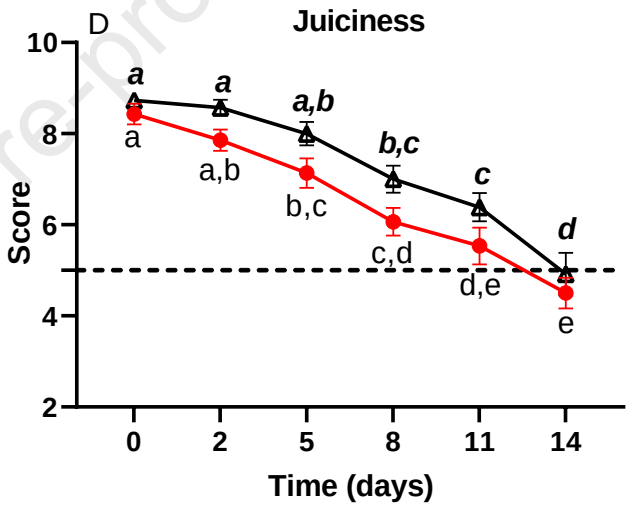

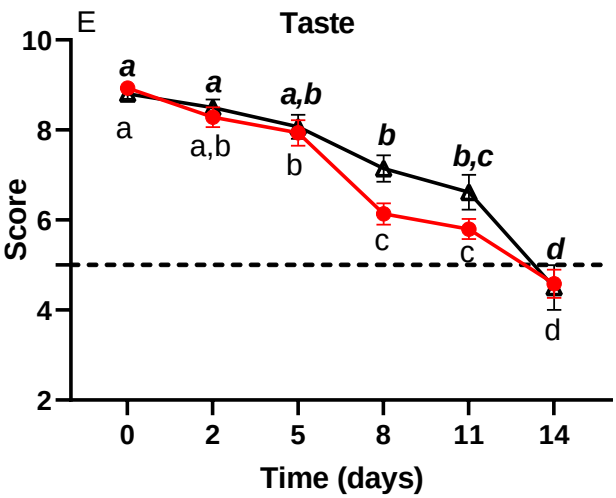

Highlights

- We studied the quality of two king crab species cooked *merus* meat over 14 days at 4°C
- Sensory attributes of *L. santolla* and *P. granulosa* remained acceptable for 11 days
- The chemical indexes (pH, TVB-N, TBARs) increased but remained within tolerable values
- *S. aureus*, coliforms, or enterobacteria were not detected during storage
- Shelf-life at 4°C was 8 days for *P. granulosa* and 11 days for *L. santolla* cooked meat

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.