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# Effects of conservation method and time on fatty acid composition, taste and microstructure of southern king crab (*Lithodes santolla* Molina, 1782) meat

Susana J. Risso & Amalia A. Carelli

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## Abstract

This study reports the changes in fatty acids, taste, and microstructure of cooked southern king crab meat (*Lithodes santolla*) during storage at 0°C for 10 days and at -20°C for 90 days. At the end of both storage times, the iodine value decreased by 16.5%, while 83.5% of the initial fatty acid quality remained unchanged. The polyene ratio decreased by 32% at 0°C and 35.9% at -20°C, whereas the atherogenic and thrombotic indices remained at values that do not represent any risk to human health. Free amino acids that contribute to taste (taste activity value, TAV>1) were: glycine and alanine (sweetness), arginine (bittersweetness), and histidine (bitterness). The bittersweet taste imparted by arginine (initial TAV= 16.4) was prevalent even at the end of frozen storage (TAV=7.9). The umami taste was elicited by disodium 5´-adenosine monophosphate (AMP) nucleotide. The equivalent umami concentration in g MSG/100g meat changed from 0.031 to 0.045 in refrigerated samples and to 1.6 in frozen samples. A loss of the original fibrous structure of the meat was evidenced during both treatments. Refrigerated samples presented a disintegrated and homogeneous texture at 10 days, while freezing formed a spongy tissue at 90 days.

Keywords: Southern king crab, fatty acids, taste activity value, umami flavor, scanning electron microscopy

## Disclaimer

## Introduction

The southern king crab (SKC) can be found in the cold seawaters of the San Jorge Gulf (45-47°S 65°40'W) and the Beagle Channel (55°S 68°W) off the coasts of Argentina. It is regarded as an important commercial fishery product and a delicious Patagonian seafood. There is scarce information in literature about this crab.

In previous papers, we examined the nutrient composition of raw and cooked SKC meat as well as quality indices of cooked SKC meat during refrigerated and frozen storage. We reported a proximate composition (g/100 g cooked meat) that averaged: moisture= 78.6, protein= 16.3, fat= 0.76, and ash= 2.10. Moreover, we found that the nutritional quality of the cooked meat makes it especially adequate for balanced cholesterol-restricted, low-fat meat diets (Risso and Carelli, 2012). SKC also preserves its chemical parameters of freshness and quality under the permitted limits for 10 days at 0°C and for 3 months at -20°C. In addition, spermine could be considered as a potential freshness index for refrigerated samples and spermidine for frozen storage (Risso and Carelli, in press). No information has been reported on nutrient, taste, and texture changes in cooked SKC meat during its conservation.

Although SKC meat has a very low fat level (0.76 g/100g cooked meat), its oil fraction has a high content (36%) of n-3 polyunsaturated fatty acids (PUFA) rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Risso and Carelli, 2012), which are susceptible to autoxidation. These changes in fatty acids (FA) can be studied by gas chromatography (GC) of their methyl esters or using the iodine value (IV) (Ham et al., 1998) and the polyene ratio (Lin et al., 1995), while the nutritional quality of fatty acids can be evaluated through the atherogenic index (AI) and the thrombotic index (TI) (Ulbricht and Southgate, 1991).

Free amino acids (FAAs) are considered a major part of non-protein nitrogenous compounds in marine invertebrates and play an important role in flavor. They usually contribute to a sweet, bitter, sulfurous or umami taste. The taste activity value (TAV) is widely used to evaluate the impact of FAAs on taste (Chen and Zhang, 2007; Gunlu and Gunlu, 2014; Rotzoll et al., 2006; Scharbert and Hofmann, 2005). It is calculated as the ratio of the concentration of an individual compound in the food matrix to its corresponding threshold value.

Umami taste was first defined as the characteristic flavor elicited by glutamates, and it has been associated with monosodium glutamate (MSG) (Yamaguchi, 1991). Moreover, it is also elicited by disodium salts of the 5´-nucleotides: disodium 5´-inosine monophosphate (IMP), disodium 5´-guanosine monophosphate (GMP), and disodium 5´-adenosine monophosphate (AMP). These compounds are naturally present in many protein-rich foods, such as meat, fish, and fungi. There are synergistic effects between MSG, IMP, GMP, and AMP, which together in certain ratios produce a strong umami taste (Yamaguchi et al., 1971). The equivalent umami taste concentration (EUC) is the concentration of MSG equivalent to the umami intensity given by the mixture of MSG-like amino acids and flavor-enhancing 5´-nucleotides. Therefore, EUC is very useful to evaluate the umami taste in food (Chiang et al., 2006; Tseng et al., 2005; Chen and Zhang, 2007, Wang et al., 2016). Changes in the chemical composition and the degradation of muscle proteins not only affect the taste, but also

The overall objective of this study was to obtain basic information about the characteristic changes in the fatty acids, taste (evaluated by FAAs and nucleotides), and microstructure of cooked SKC meat stored for 10 days at 0°C and 90 days at -20°C.

## Materials and methods

### Sample preparation

SKC males were caught during the spring of 2008 in the San Jorge Gulf, southwestern Atlantic Ocean (45°S, 67°W), at depths between 2 and 80 m. Two samplings were performed from two captures, and a total of 15 crabs (average weight= 1055 ± 271 g, carapace length= 127 ± 11 mm) were collected in each capture. The crabs from each sampling were cooked separately in boiling water for 10 minutes. Claw, leg, and shoulder meat were hand-picked, and the meat was blended to prepare homogenate samples (H1, H2) from each capture, which were subdivided into four portions. One portion from H1 and one from H2 were immediately examined (time zero), whereas the remaining portions were placed in separate bags and kept at 0°C (one portion from each homogenate sample H1 and H2) and -20°C (two portions from each homogenate sample H1 and H2). Two independent portions (one from each homogenate sample H1 and H2) were examined after 10 days at 0°C, and after 30 and 90 days at -20°C (Figure 1).

### Lipid extraction

The oil fraction was isolated from cooked meat samples according to Bligh and Dyer's (1959) procedure.

### Fatty acids

Fatty acid composition was determined at the beginning and end of both storage conditions. For the fatty acid profile determination, the oil fraction was transmethylated using 10-15% $BF_3$/MeOH solution (Morrison and Smith, 1964). Fatty acid methyl esters (FAMEs) were analyzed by gas-liquid chromatography (GLC). FAMEs were separated on a SP 2560 capillary column [stabilized poly (90% bi cyanopropyl/10% cyanopropylphenyl siloxane)] (length= 100 m, i.d.= 0.25 mm, film thickness= 0.2 $\mu$m; Supelco, Inc., Bellefonte, PA, USA) using hydrogen as carrier gas. The oven temperature programming was: initial temperature 140°C, holding for 5 min, increasing at 4°C/min to 240°C, and holding for 15 min. The other operation conditions were: flame ionization detector (FID) temperature, 260°C; injector temperature, 175°C; splitting ratio, 1:100. FAMEs were identified by comparison with Supelco standards (Supelco 37 Component FAME mix, Supelco, Inc.). Results were expressed as mg /100g meat.

### Theoretical calculation of the iodine value

The iodine value is the number of grams of iodine absorbed per 100 g of oil or fat. The IVs were directly calculated by multiplying the percentage of each fatty acid contained in the oil fraction with calculation factors obtained from the literature (Ham et al., 1998).

expressed as:

where EPA is the eicosapentaenoic acid percentage (C20:5n-3c), DHA is the docosahexaenoic acid percentage (C22:6n-3c), and P is the palmitic acid percentage (C16:0).

## Atherogenicity and thrombogenicity indices

The propensity of crab's tissue to promote coronary heart disease can be evaluated by the atherogenic index and the thrombogenic index, which were calculated by the following equations (Ulbricht and Southgate, 1991):

where L= lauric acid (C12:0), M= myristic acid (C14:0), P= palmitic acid (C16:0), S= stearic acid (C18:0), MUFA= sum of monounsaturated fatty acids, n-6 PUFA= sum of n-6 polyunsaturated fatty acids, and n-3 PUFA= sum of n-3 polyunsaturated fatty acids. All fatty acid contents in the formulae are expressed as wt% in the lipid fraction.

## Free amino acids

Free amino acid analysis was performed without previous hydrolysis of the sample by high performance liquid chromatography (HPLC) with pre-column derivatization by AccQ·Tag agent (Waters Corp., Milford, MA, USA), followed by separation in reversed-phase column, and then fluorescent detection. The sample was subjected to derivatization using AccQ-Fluor (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate kit, Waters Corp.) at 60°C for 10 min. Amino acids were separated by HPLC with a fluorescence detector (excitation wavelength= 250 nm, emission wavelength= 395 nm) and an AccQ-Tag amino acid column Nova-Pak C18 (length= 150 mm, i.d.= 3.9 mm, particle size= 4 $\mu$m; Waters Corp.) maintained at 37ºC according to AccQ Tag methods. A gradient mobile phase consisted of eluent A (prepared from Waters AccQ Tag Eluent A concentrate by adding of 200 mL of concentrate to 2 L of Milli-Q water and mixing), eluent B (acetonitrile, HPLC grade) and eluent C (Milli-Q water). The gradient separation program was as follows with a flow rate of 1mL/min:

Table

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The identity and quality of the amino acids were assessed by comparison with the retention times and peak areas of the standard amino acids (Pierce NCI0180, Pierce, IL, USA). The results were expressed as mg amino acid/100g meat.

## Nucleotide analysis

activity value as indicated in the following section.

### Taste activity value

TAV was calculated as the ratio of the concentration of an amino acid or nucleotide in the crab meat to its corresponding recognized taste threshold. FAA threshold values were taken from Kato et al. (1989), whereas those for nucleotides were taken from Yamaguchi et al. (1971) and Fuke and Ueda (1996). The compounds whose TAV was greater than 1 were considered activity in food taste.

### Equivalent umami concentration

EUC, expressed in g of monosodium glutamate per 100g, is the concentration of MSG equivalent to the umami intensity given by the mixture of MSG-like amino acids and 5´-nucleotides. It is represented by the following equation (Yamaguchi et al., 1971):

where Y is the EUC of the mixture in terms of g MSG/100g, $a_i$ is the concentration (g/100g) of each umami amino acid (aspartic acid (Asp) or glutamic acid (Glu)), $b_i$ is the relative umami concentration (RUC) for each umami amino acid to MSG (Glu = 1 and Asp = 0.07), $a_j$ is the concentration (g/100g) of each umami 5´nucleotide (5´-IMP, 5´-GMP or 5´-AMP), $b_j$ is the RUC for each umami 5´-nucletide to 5´-IMP (5´-IMP= 1; 5´-GMP= 2.3 and 5´-AMP= 0.18), and 1218 is the synergistic constant based on the concentration of g/100g used.

### Statistical Analyses

All determinations of each independent sample (H1, H2) were carried out in triplicate. The results were expressed as mean value ± standard deviation (SD). The differences were calculated using one-way analysis of variance (ANOVA), and statistically significant differences were reported at $p < 0.05$ (Sokal and Rohlf, 1987).

### Scanning electron microscopy of cooked SKC meat

Samples were gold-sputtered (Pelco 91000 sputter coater, England). A scanning electron microscope (SEM EVO 40 (LEO), Cambridge) was used to observe their microstructure.

## Results and discussion

### Fatty acids

Lipid fraction of cooked SKC meat rendered an average 760 mg/100g meat, which represents a low fat content. Changes in the composition of FAs in cooked SKC meat during storage for 10 days at 0°C and 90 days at -20°C are shown in Table 1. As previously reported (Risso and Carelli, 2012), the most abundant FAs in cooked SKC meat were oleic acid (18:1n3c, O, 168 mg/100g meat) and eicosapentaenoic acid (20:5n-3c, EPA, 166 mg/100g meat), followed by palmitic acid (16:0, P; 117 mg/100g meat) and docosahexaenoic acid (22:6n-3c, DHA, 103 mg/100 g meat).

during refrigerated and frozen storage.

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Table 1 also shows the relative percentage of the major groups of FAs. Although the sum of SFA, MUFA, and PUFA contents remained constant at about 95% (~720 mg/100g meat) with 5% of unidentified FAs, each group showed variations in its relative percentage during both storage periods. The initial FAs profile was dominated by PUFA (45.7%, 348 mg/100g meat), followed by MUFA (26.8 %, 203 mg/100g meat) and SFA (21.9%, 167 mg/100g meat). However, due to an oxidation process, a decrease in PUFA (refrigerated= -18.4%, frozen= -19.5%) as well as an increase in MUFA (refrigerated= 17.1 %, frozen= 11.2%) and SFA (refrigerated= 22.4%, frozen= 32.0%) was observed under both storage conditions. These percentages confirm the higher susceptibility of PUFA to oxidation. The preservation of this group of FAs is desirable since their intake would lower the risk of cardiovascular diseases. The results indicated that 10 days at 0 °C caused a decrease similar to 90 days at -20°C.

Considering the n-3 to n-6 ratio, a decrease was observed from the initial value of 3.60 to 3.43 after 10 days of refrigeration due to a steeper decline in n-3 (-19.7%), especially EPA (from 166 to 129 and 132 mg/100g meat) and DHA (from 103 to 85.9 mg/100g meat), compared to n-6 (-15.6%, from 76 to 64 mg/100g meat). On the other hand, in the frozen samples, the ratio remained close to the initial value of 3.60, showing a reduction of approximately 20% of both n-3 and n-6 (Table 1). Although a higher ratio of n-3/n-6 PUFA is considered favorable because of the benefits to human health, the reduction of EPA, DHA, and arachidonic acid (C20:4 n-6, ARA) are not desirable.

Taking them into account individually, there are three FAs that were not initially identified in the cooked meat: SFA arachidic (C20:0), MUFA heptadecanoic (C17:1), and PUFA linoelaidic (C18:2n-6t); but they were detected after refrigerated and frozen storage. These FAs, as well as, C20:3n-6c and C20:3n-3c, which were not initially identified in the cooked SKC meat, were reported as constituents of the lipid fraction of raw crab meat (Risso and Carelli, 2012). The different FAs profile of cooked meat was attributed to the higher extractability of phospholipids richer in PUFAs together with the oil when the meat was cooked (Risso and Carelli, 2012). However, some deterioration of PUFAs could also occur with cooking. Castro-González and Carrillo-Domínguez (2015) analyzed the effect of six cooking techniques on the lipid fraction of marlin (*Makaira nigricans*). In the mentioned work, the fatty acids C18:2n-6t, C20:0, C20:3n-3, and C20:3n-6, originally present in raw marlin, were not detected when the fish was steamed cooked (Castro-González and Carrillo-Domínguez, 2015).

Initially, the cis-11,14-eicosadienoic acid (C22:2n-6c) was 0.86 mg/100g meat and increased to 2.21 mg/100g meat after 10 days at 0°C but was inexistent after 90 days at -20°C (Table 1). A significant decrease in C20:5n-3c and C20:6n-3c must also be noted, whereas the amounts of C20:0 and C20:1n-9c increased for both storage periods. These facts, as well as the increase in some C18 unsaturated FAs and most SFA for both groups, have their origin in the oxidative processes of the FAs and their relative susceptibility to oxidation. It has been reported that tissue dehydration during freezing and exposure to atmospheric oxygen are the main reasons for the oxidation of lipids in fish (Hiremath, 1973). Ke et al. (1977) indicated that lipid oxidation of mackerel during frozen storage depended on storage temperature, being much lower at -40°C than at -15°C.

different ($p < 0.05$) from that obtained for refrigerated (177 ± 1) and frozen samples (176 ± 2), showing a decrease of the same extent (-16.5%) in both storage conditions. Therefore, if we consider IV as a quality index, we can establish that after 10 days of refrigeration and/or after 90 days of freezing, the cooked SKC meat maintained 83.5% of the initial quality. Lipids of this species with a high content of unsaturated fatty acids belong to the category of drying oils, whose range has a lower limit of 130. This arbitrary classification is important from the point of view of human consumption, because the more unsaturated long-chain n-3 PUFA, namely EPA and DHA, have a positive impact on health with their cardiovascular benefits being well-known (Ruxton et al., 2005).

## Polyene ratio

Polyene ratio decreased significantly from 231 ± 8 to 157 ± 7 during refrigeration and to 149 ± 6 during freezing. Thus, the polyene ratio fell by 32.0% at 0°C and 35.9% at -20°C. Its calculation takes into account the most representative PUFA, DHA (C22:6n-3c), and EPA (C20:5n-3c). Together, they showed a greater decrease than the increase in palmitic acid (Table 1). This behavior under both storage conditions has been attributed to both enzymatic hydrolysis and PUFA oxidation (Shono and Toyumizu, 1972). This ratio is considered a useful measure at advanced stages of oxidation since it reflects the loss of n-3 PUFAs (Giménez et al., 2011). Because the changes in this ratio for SKC were appreciable, we can conclude that oxidation of FAs significantly advanced after 10 days at 0°C and after 90 days at -20°C.

## Atherogenic index and thrombotic index

The initial values of atherogenic and thrombotic indices (AI= 0.21 ± 0.01, TI= 0.16 ± 0.01) for cooked SKC meat obtained in this work were higher than those for boiled cooked muscle of edible crab (*Cancer pagurus*) (AI= 0.14 ± 0.09, TI= 0.09 ± 0.07) but lower than those for steamed cooked muscle of edible crab AI= 0.26 ± 0.03, TI= 0.19 ± 0.03) (Maulvault et al., 2012). At the end of refrigeration and frozen storage for cooked SKC meat, both AI and TI showed an increase from their initial values (after 10 days of refrigeration: AI= 0.34 ± 0.01 and TI= 0.23 ± 0.0; after 90 days of frozen storage: AI= 0.35 ± 0.01 TI= 0.25 ± 0.01). However, the values of both indices remained far from those reported for raw and cooked fishes, such as raw tuna (IA=1.86, IT=0.74), raw tuna roe (IA= 0.69, IT= 0.27), boiled tuna (IA= 2.69, IT= 0.18), raw sardine (IA= 0.85, IT= 0.59), boiled sardine (IA= 0.44, IT= 0.25), raw salmon (IA= 0.54, IT= 0.21), boiled salmon (IA= 0.60, IT= 0.211), raw mackerel (IA= 3.39, IT= 0.43), and boiled mackerel (IA= 3.63, IT= 1.84) (Garaffo et al., 2011; Moussa et al., 2014). Moreover, the values of these indices for cooked SKC meat refrigerated for 10 days or after 90 days frozen can be considered low and not representing any risk to human health, which is in agreement with various reports in the literature (Ulbricht and Southgate, 1991; Garaffo et al., 2011; Moussa et al., 2014).

## Free amino acids

FAAs are good indicators for monitoring the degree of deterioration of the protein fraction. Total FAAs baseline was 1435 mg/100g meat, higher than that for mountain trout (*Salmo trutta macrostigma*) (1017-1295 mg/100g meat) (Gunlu and Gunlu, 2014) but lower than that reported for kuruma prawn (*Penaeus japonicus*) (4019 mg/100g of meat) (Matsumoto and Yamanaka, 1990), for Chinese mitten crab (*Eriocheir sinensis*) (2090 mg/100g of meat) (Chen and Zhang, 2007), and for crab *Portunus sanguinolentus* (1977 mg/100g of meat)

samples under frozen storage conditions after 90 days. It has been reported that, in general, the total value first increases with storage as a result of proteolysis, but then it may decrease due to the breakdown of FAAs by bacterial decarboxylation and deamination (Matsumoto and Yamanaka, 1990). Thus, the first stage of FAAs increase in SKC meat could have taken place in the first period (< 10 days at 0°C and < 30 days at -20°C) when the samples were not analyzed.

Table 2. Free amino acids (FAAs) of cooked southern king crab meat in mg/100g.

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Of the eighteen amino acids tested, five represent 88% of total FFAs initially present in cooked SKC meat in the following descending order: arginine (57%), glycine (14.8%), proline (7.4%), histidine (5.2%), and alanine (4.4%) (Table 2). Although SKC protein contains high amounts of glutamic and aspartic acid (Risso and Carelli, 2012), they make a minor contribution to FAAs.

In the refrigerated SKC samples, a significant increase in glycine and a significant decrease in phenylalanine were statistically appreciated (Table 2). When frozen samples are considered, aspartic acid, glutamic acid, threonine, histidine, tyrosine, arginine, methionine, valine, tryptophan, proline, and cysteine did not evidence significant changes after 30 days of storage. Only phenylalanine decreased in value to 13.08 mg/100g meat, whereas the remaining five FAAs increased their initial value.

After 90 days at -20°C, FAAs values for aspartic acid and phenylalanine remained constant at their initial value, whereas isoleucine returned to its original level. Only glutamic acid, threonine, and cysteine increased their value in the 30-90 day period (Table 2). Moreover, twelve amino acids decreased their value with respect to the 30 day storage, with the major reductions corresponding to arginine (475 mg/100g meat), glycine (82 mg/100g meat), histidine (66.9 mg/100g meat), proline (30.6 mg/100g meat), alanine (29.4 mg/100g meat), serine (19.56 mg/100g meat), and tyrosine (19.53 mg/100g meat).

Discrepancies in the literature about FAAs distribution and changes during storage originate in the different storage conditions and species studied. Matsumoto and Yamanaka (1990) studied the FAAs changes in the muscle of kuruma prawn for a storage of 9 days at 5°C and 12 days at 0°C. The authors evaluated thirty-six amino acids, six of them represented 97% of total FAAs, mainly glycine (43.2%), arginine (23.5%), proline (21.3%), glutamic acid (3.8%), alanine (3.4%), and taurine (2.1%). Total FAAs reached maximum value at day 9 (4138 mg/100g flesh) and then dropped after 14 days (2378 mg/100g) as decomposition progressed; these changes were mainly influenced by the contents of glutamic acid, proline, glycine, alanine, and arginine. In contrast, the snow crab (*Chionoecetes opilio*) presented taurine, proline, glycine, and arginine as its major FAAs components, with the concentration of free proline, glycine, and arginine increasing until day 3 and then decreasing at 0°C (Miyagawa et al., 1990). A more similar distribution to that exhibited by SKC meat was found for the Chinese mitten crab, with arginine (32%), glycine (24%), proline (6%), histidine (5.2%), and alanine (4.4%) being the main FAAs (Chen and Zhang, 2007); and for crab *Portunus sanguinolentu*, arginine (28%), glycine (26%), glutamine (11%), alanine (10%) and proline (9%) were the main FAAs (Rethna Priva et al., 2015).

In Table 3 the amino acids with a TAV greater than 1 contribute to the taste of the meat. Initially, these were arginine, histidine, glycine, and alanine, whose values remained constant even after 10 days of refrigerated and 30 days of frozen storage. At 90 days of frozen storage, a significant increase in glutamic acid and threonine TAVs was observed. This was accompanied by a decrease in arginine, alanine, and histidine TAVs; the latter two reaching TAVs of less than 1.

Table 3. Taste activity values (TAVs), taste threshold value (TTV, mg/mL) and taste attributes (+ = pleasant, - = unpleasant) of free amino acids (FAAs) at initial time and during refrigerated and frozen storage.

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The glycine, alanine, and arginine amino acids contribute to the sweet taste; although the contribution of alanine was not significant at 90 days of frozen storage. Arginine and histidine imparted a neutral bitter taste; but in the case of arginine, it is mixed with a sweet taste, resulting in a pleasant sweet-bitter taste (Kato et al., 1989).

When comparing the initial content of those FAAs that contributed to the flavor of SKC meat in mg/100g meat (Table 2) (glycine= 212, TAV= 1.6; arginine= 818, TAV= 16.4; alanine= 63.7, TAV=1; histidine= 75.2, TAV=3.7) with those that contributed to the flavor of snow crab (glycine= 623, TAV=4.8; arginine= 579, TAV= 11.6; alanine= 187, TAV= 3.11; proline= 327, TAV= 1.1) (Hayashi et al., 1981) and Chinese mitten crab (glutamic acid= 62, TAV= 2.1; glycine= 497, TAV= 3.8; arginine= 664, TAV= 10.3; alanine= 369, TAV= 6.2) (Chen and Zhang, 2007), it can be observed that SKC had a lower contribution to the sweet taste, especially that elicited by glycine and alanine; but it presented a higher amount of arginine, with a sweet-bitter taste, and histidine, which also provides a bitter taste.

Aspartic acid and glutamic acid have a sour taste, but in the presence of sodium salts they give sodium glutamate, contributing to the umami taste, which is comparable to that imparted by MSG and the nucleotides AMP, GMP, and IMP. The umami taste, represented by the FAA, aspartic acid, and/or glutamic acid, was present in frozen SKC samples stored for 90 days (glutamic acid content= 49 mg/100g meat; TAV= 1.6). Similarly, the Chinese mitten crab (*Eriocheir sinensis*) initially had an umami taste due to the glutamic acid (62 mg/100g, TAV= 2.1) (Chen and Zhang, 2007).

The calculated TAVs for the nucleotides are shown in Table 4. Three nucleotides (AMP, GMP, and IMP) contributed to the umami taste, but only AMP presented a TAV higher than 1 for the SKC meat (refrigeration: 71-77 mg/100g, TAV= 1.5; freezing: 71-132 mg/100g, TAV=1.5-2.7), thus being responsible for the umami taste of the SKC meat and showing an increase during frozen storage (Table 4). Fuke and Ueda (1996) analyzed taste changes in synthetic prawn extracts and found that the contribution of AMP alone depends on concentration. For example, at low concentrations (0.5-1 mg/mL), AMP contributed to the sweet taste and not the umami taste, and in the 1-2 mg/mL range, it was sweet and slightly salty. But, when 0.04 mg/mL extract of IMP was added along with AMP, the umami taste was perceived and sweetness increased although the concentration of IMP was very small (Fuke and Ueda, 1996). For that reason, although IMP in SKC meat presented a TAV <1, it

Table 4. Taste activity values (TAVs), taste threshold value (TTV, mg/mL), attribute, and equivalent umami concentration (EUC, g MSG/100g meat) of nucleotides during refrigerated and frozen storage.

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Compared with those for other crabs, results are very diverse. No nucleotide presented a TAV > 1 for the snow crab (*Chionoecetes opilio*) (Hayashi et al., 1981), whereas the nucleotides AMP (75.3 mg/100g, TAV= 1.5) and IMP (34.4 mg/100g, TAV= 1.4) contributed to the umami taste in Chinese mitten crab (Chen and Zhang, 2007). These authors also mention a synergistic action of AMP and IMP exalting the umami taste.

In a previous paper, we reported a mineral content for SKC of sodium as 594 and potassium as 203 mg/100g cooked meat (wet weight) (Risso and Carelli, 2012). Rotzoll et al. (2006) reported that the thresholds of sodium chloride and potassium chloride are 180 and 130 mg/100mL, respectively. Therefore, these minerals with TAVs greater than 1 could contribute to the salty taste of the crab meat. As is usual in crab cooking, salt is added and so the contribution of these minerals would be minor (Chen and Zhang, 2007).

## Equivalent umami concentration

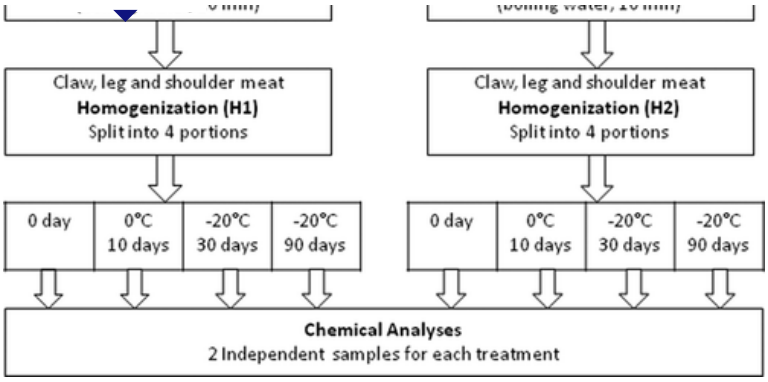
The initial EUC value for SKC meat (0.03) was low and far from those reported for other crabs, such as snow crab (EUC= 0.5) (Hayashi et al., 1981) and the Chinese mitten crab (EUC= 1.99-4.2) (Chen and Zhang, 2007; Wang et al., 2016). It remained low (0.03-0.05) throughout the refrigerated and frozen storage (Table 4), only exceeding the unit after 90 days of frozen storage (EUC= 1.6). This EUC value is equivalent to 1.6 g MSG /100 g meat and is considered not intense.

## Scanning electron microscopy of cooked SKC meat

Texture of the SKC meat is an important sensorial property from the point of view of the consumer. Electron microscopy of the cooked SKC meat at the beginning of storage showed a compact structure and defined forms with fibers and crystals (Figure 2a). When samples were stored at 0°C for 10 days, loss of the original structure was evidenced, and a disjointed and homogeneous texture was observed (Figure 2b). This change from a fibrous structure to a pasty one influences not only the texture of the food but also its quality.

During frozen storage, changes in the texture were also observed. Electronic photographs taken after 30 days at -20°C are shown in Figure 2c, where a fibrous structure and a spongy tissue are present; but after 90 days, deeper and more cavernous spaces appear, which could be formed by meat dehydration (Figure 2d).

Figure. 1.

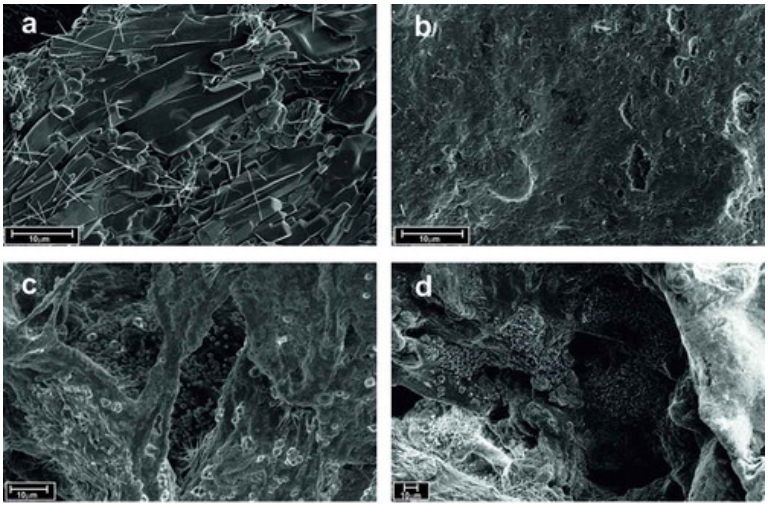



PowerPoint slide Original jpg (125.00KB) Display full size

## Conclusions

This study showed that cooked southern king crab (*Lithodes santolla*) meat was susceptible to quality changes during refrigerated and frozen storage resulting in a decrease of nutritional values. It was found that the lipid fraction of SKC preserves 83.5% of the initial quality of the FAs after storage at 0°C for 10 days and at -20°C for 90 days. The FAs oxidation produced a decrease in the polyene ratio of 32% at 0°C and 35.9% at -20°C, whereas the AI and TI indices maintained values considered low. Total FAAs decreased after 90 days of freezing, indicating their transformation into lower molecular-weight compounds. Glycine and alanine contributed to sweetness, histidine to bitterness, and arginine to bitter-sweetness, the latter being prevalent. The EUC value was low over both storage conditions. The meat of *L. santolla* preserved a bittersweet taste provided by the FAA during both storages, with mild umami taste imparted by the AMP. In contrast, a loss of the original structure was observed, with the increase of both storage conditions affecting its quality.

Figure. 2.

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In this article

Nacional del Sur (UNS), and the Universidad Nacional de la Patagonia San Juan Bosco (UNPSJB), Argentina.

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