WAFP #1021023, VOL 00, ISS 00

Quality Indices of Cooked Southern King Crab (*Lithodes santolla* Molina, 1782) Meat During Storage at 0 and −20°C

Susana J. Risso and Amalia A. Carelli

QUERY SHEET

This page lists questions we have about your paper. The numbers displayed at left can be found in the text of the paper for reference. In addition, please review your paper as a whole for correctness.

Q1: Au: please provide an English translation for Planta Piloto de Ingeniería Química

PROOF ONLY

TABLE OF CONTENTS LISTING

The table of contents for the journal will list your paper exactly as it appears below:

Quality Indices of Cooked Southern King Crab (*Lithodes santolla* Molina, 1782) Meat During Storage at 0 and −20°C
Susana J. Risso and Amalia A. Carelli

JOURNAL OF AQUATIC FOOD PRODUCT TECHNOLOGY
2016, VOL. 00, NO. 00, 1–12
http://dx.doi.org/10.1080/10498850.2015.1021023

Taylor & Francis
Taylor & Francis Group

Quality Indices of Cooked Southern King Crab (*Lithodes santolla* Molina, 1782) Meat During Storage at 0 and −20°C

Susana J. Risso[a] and Amalia A. Carelli[b]

[a]Department of Biochemistry, National University of Patagonia San Juan Bosco (UNPSJB), Comodoro Rivadavia, Chubut, Argentina; [b]Planta Piloto de Ingeniería Química (PLAPIQUI), National University of South (UNS), National Scientific and Technical Research Council (CONICET), Bahía Blanca, Argentina

ABSTRACT

The aim of this work was to determine the changes in quality of cooked southern king crab (*Lithodes santolla*) meat during storage at 0°C for 5 and 10 days and −20°C for 10, 30, 60, and 90 days. Chemical indices varied from the initial to the final storage time in refrigerated and frozen samples as follows: TVB-N, 15.8–29.2 and 15.8–23.2 mg/100 g; TMA, 0.0–8.3 and 0.0–1.2 mg/100 g; formaldehyde, 0.5–8.6 and 0.5–3.7 mg/kg; indole, 3.3–20.7 and 3.3–6.6 µg/100 g; lactic acid, 16.4–39.1 and 16.4–16.6 mg/100 g; and *p*-ratio, 0–28.4 and 0–5.2%, respectively. The total nucleotide content rendered 6.77 µmol/g, with adenosine monophosphate (AMP) the main component. The nucleotide indices varied in refrigerated and frozen samples as follows: K, 7.37–27.5 and 7.37–10.3%; Ki, 58.3–86.5 and 58.3–82.7%; H, 30.2–54.9 and 30.2–38.0%; G, 19.4–57.8 and 19.4–15.8%; P, 17.2–42.3 and 17.2–14.7%; and Fr, 41.6–13.4 and 41.6–17.3%, respectively. Biogenic amines found at both storage temperatures were spermine, spermidine, tyramine, and agmatine. Spermine may be considered as a potential freshness index for refrigerated samples and spermidine for frozen storage. The tyramine level should be monitored. During the storage period tested, none of the chemical indices studied exceeded the legal limits.

KEYWORDS

southern king crab; meat crab quality; freshness indicators; nucleotide degradation; biogenic amines

Introduction

The southern king crab (SKC; *Lithodes santolla* Molina, 1782) is a common crab found in the coastal waters of the southeast Pacific Ocean (Southern Chile), Fueguian Archipelago, and the southwest Atlantic Ocean. It has a high protein content with a well-balanced essential amino acid composition and a high content of n-3 polyunsaturated fatty acid, rich in EPA and DHA in its fatty acid fraction (Risso and Carelli, 2012). Moreover, the SKC is considered an expensive and luxurious dish, and consumers demand high-quality fresh and frozen products.

A number of changes take place in the meat of seafood during storage due to undesirable reactions taking place in lipid and protein fractions. The degradation compounds of trimethylamine oxide (TMAO) as total volatile basic nitrogen (TVB-N), trimethylamine (TMA) and formaldehyde (FA), as well as other chemical compounds such as indole, lactic acid, nucleotides, and biogenic amines are usually determined to assess quality deterioration of seafood.

Total TVB-N and TMA are important indices to evaluate seafood spoilage, although they are not useful to discriminate among the early stages of degradation. These parameters represent sensitive indices in the later stages of deterioration (Ruiz-Capillas et al., 2001; Yerlikaya and Gökoğlu, 2004).

Formaldehyde, a human carcinogen, is considered an indicator of fish muscle alteration and loss of texture quality. Italian legislation sets a threshold limit value of 10 ppm for crustaceans (Renon et al., 1993).

CONTACT Susana J. Risso srisso@unpata.edu.ar Universidad Nacional de la Patagonia San Juan Bosco, Km 4, 9000 Comodoro Rivadavia, Chubut, Argentina.

© 2016 Taylor & Francis

Indole, a product of tryptophan degradation, is currently used by the United States Food and Drug Administration (FDA, 2004) to validate the sensory evaluation of shrimp decomposition. Indole maximum levels have been established at 25 μg/100 g.

Lactic acid is a freshness index, but the relationship between the degradation of glycogen and the production of lactic acid remains unclear (Matsumoto and Yamanaka, 1990).

Even though the pattern of nucleotide degradative changes varies between species, the concentrations of adenosine-5'-triphosphate (ATP) and breakdown products are considered useful indicators of freshness. Hiltz and Bishop (1975) found that the nucleotide degradative changes of Atlantic queen crabs (*Chionoecetes opilio*) resemble more closely those of vertebrate (fish) muscle than other classes of invertebrates (e.g., molluscs), in which several different pathways are found. The nucleotide degradation in this crustacean followed this pathway: ATP degraded to adenosine diphosphate (ADP), ADP to adenosine monophosphate (AMP), AMP to inosine triphosphate (IMP), IMP to inosine (Ino), and Ino to hypoxantine (Hx). The authors found that thawed muscle accumulated large amounts of IMP, whereas in cooked muscle, the main purine components were AMP and adenosine (Hiltz and Bishop, 1975). In spite of differences in the pathway of ATP breakdown between fish species, the levels of ATP decrease and most of the adenosine nucleotides are degraded to IMP. As the degradation continues, Ino and then Hx are produced. Hx has a bitter flavor, whereas IMP is desirable as a flavor component in fresh fish (Özogul et al., 2000).

The K value proposed by Saito et al. (1959) is a biochemical index for fish quality assessment based on nucleotide degradation (Özogul et al., 2006b). Due to the rapid disappearance of ATP, the Ki value, which excludes ATP, ADP, and AMP, was proposed (Karube et al., 1984). In addition, the H-value (Luong et al., 1992) and G-value (Burns et al., 1985) have been described as quality indices of freshness due to the wide diversity in patterns of nucleotide catabolism (Özogul et al., 2006a). Considerable data exist on nucleotides in fish (Aubourg et al., 2005; Murata and Sakaguchi, 1986; Özogul et al., 2008; Özogul et al., 2006b; Özogul et al., 2006c; Rodríguez et al., 2006), shrimp and prawn (Matsumoto and Yamanaka, 1990), and in several other marine invertebrates such as crabs: king crab (*Paralithodes camtschatica*; Porter, 1968), tanner crab (*Chionoecetes bairdi*; Stone, 1970), Atlantic queen crab (*Chionoecetes opilio*; Hiltz and Bishop, 1975), and mud crab (*Scylla serrata*; Chiou and Huang, 2003).

Biogenic amines are nonvolatile amines that are produced in fish and shellfish products after death. They include agmatine, β-phenylethylamine, cadaverine, putrescine, spermidine, spermine, tyramine, tryptamine, and histamine. Biogenic amines are usually generated by microbial decarboxylation of specific free amino acids in fish or shellfish tissue (Anderson, 2008; Rawles et al., 1996). When held for excessive periods of time, the microflora of fish converts free amino acids into biogenic amines, which produce characteristic symptoms in certain humans after fish consumption. Low levels of biogenic amines in food are not considered a serious risk. However, if the amount consumed is high enough, or the normal pathways of amine catabolism are inhibited, various physiological effects may occur—such as hypotension or hypertension, nausea, headache, rash, dizziness, cardiac palpitation, emesis, and even death (Anderson 2008; Rawles et al., 1996). Biogenic amines are also considered precursors of carcinogens, such as N-nitrosamines, and they are also an indicator of food quality (Mietz and Karmas, 1977). Histamine is the only biogenic amine for which maximum legal levels (10 mg/100 g) have been established by the European legislation (EC Commission Regulation No. 2073/2005, 2005) for tuna and other types of fish of the Scombridae and Scomberesocidae families. However, no data are available on the formation of biogenic amines in SKC meat during storage.

The aim of the present work was to investigate the chemical changes and shelf life of cooked SKC meat during storage at 0°C for 5 and 10 days and at −20°C for 10, 30, 60, and 90 days. The study focuses on TVB-N, TMA, FA, indole, lactic acid, nucleotides, and biogenic amines content to analyze their potential use as freshness quality indices or spoilage indices of SKC meat during refrigerated and frozen storage.

PROOF ONLY

Materials and methods

Sample preparation

Male SKC were caught during the spring of 2008 in the San Jorge Gulf, southwestern Atlantic (45° S, 67° W), at depths from 2 to 80 m. Two samplings were performed from two captures, and a total of 15 crabs were collected in each capture. The average weight and carapace length of the crabs were 1,055 ± 271 g and 127 ± 11 mm, respectively. The crabs from each sampling were cooked separately in boiling water for 10 min. 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 seven portions. One portion from H1 and one from H2 were immediately examined (time zero), while the remaining portions were placed in separate bags and kept at 0°C (two portions from each homogenate sample H1 and H2) and −20°C (four portions from each homogenate sample H1 and H2). Two independent portions (one from each homogenate sample H1 and H2) were examined after 5 and 10 days at 0°C and after 10, 30, 60, and 90 days at −20°C.

Total volatile basic nitrogen

TVB-N content was measured according to the method described by Antonacopoulos and Vyncke (1989). Briefly, 10 g of sample, 1.5 g magnesium oxide, and 300 mL distilled water were added into a flask. The samples were boiled and distilled into 10 mL of boric acid 3% solution with Tashiro indicator in a 500 mL conical flask. After distillation, the content of the conical flask was titrated with 0.1 N sulfuric acid. The results were expressed as mg TVB-N per 100 g crab muscle.

Trimethylamine

TMA values were determined by the picrate method, as previously described by Wekell and Barnett (1991). A 10 g sample was blended with 50 mL of 6% trichloroacetic acid (TCA) solution and filtered to remove gross tissue debris. Then, 1 mL aliquot, 3 mL anhydrous toluene, and 1.0 mL KOH (45%) solution were added in three test tubes. The tubes were shaken in a vortex for 15 s, allowed to stand for 5 min, and shaken again for 15 s. After 5 min, 1.0 mL of the toluene phase was transferred to another tube containing 80–100 mg anhydrous sodium sulfate as drying agent. After a few minutes, 3 mL picric acid (2% in toluene) was added and shaken. The supernatant was then transferred into a cuvette. Absorbance at 410 nm was measured (Metrolab 1700 UV/Vis spectrophotometer, Buenos Aires, Argentina) using a blank as reference. At the same time, a calibration curve was obtained using 0.5, 1, 2, 3, and 4 mM TMA solutions prepared from a 98% TMA.HCl standard (trimethylamine hydrochloride, T72761; Sigma Chemical Co., St. Louis, MO, USA). The results were expressed as mg TMA/100 g meat.

The P Ratio = TMA/TVBN%

This index gives the possibility of using TMA and TVB-N simultaneously to evaluate the degree of freshness by studying the ratio between TMA and TVB-N and the level of decomposition (Malle and Poumeyrol, 1989).

Formaldehyde

FA content was determined by the standard chromotropic acid method (Nordic Committee on Food Analysis, 1964). Free and reversibly bound FA was steam-distilled from a 5 g homogenate sample, 0.5 mL phosphoric acid (85%), and 50 mL water. Distillate aliquots (2 mL) were mixed with 7 mL chromotropic acid reagent (0.1% w/v concentrated sulphuric acid solution of 1,8-dihydroxynaphthalene-3,6-disulphonic acid sodium salt, 98% purity, from Sigma Chemical Co.), heated 30 min at 100°C, cooled, and its absorbance was measured at 570 nm. Simultaneously, a distilled water blank and standard solutions

PROOF ONLY

containing 4, 3, 2, and 1 μg FA/mL were also analyzed. In order to prepare standard solutions, a stock solution (2.0 mg/mL) was obtained by dissolving 778 mg of hexamethylene-tetramine (Sigma Chemical Co.) in 50 mL of 10 N sulphuric acid, left to stand at room temperature for 2 days, and then diluting it to 500 mL with distilled water (Boeri et al., 1993). The analyses were performed in triplicate, and the results expressed as mg/kg.

Indole

The levels of indole in the homogenate samples were determined according to the official colorimetric AOAC Method 948.17 (AOAC, 2000) from a 30 g homogenate sample. The results were expressed as μg per 100 g of meat.

Lactic acid

Lactic acid was extracted by AOAC Method 944.05 (AOAC, 2000) from a 40 g homogenate sample and then determined by the enzymatic method with lactate dehydrogenase (LDH; Wiener Lab SAIC, Rosario, Argentina). The lactic acid content was spectrophotometrically quantified and the results expressed as mg per 100 g of meat (Matsumoto and Yamanaka, 1990).

Nucleotides

The determination of nucleotides and related compounds was performed by high performance liquid chromatography (HPLC) after sample preparation according to the procedure described by Ryder (1985). Briefly, a 5 g sample was homogenized with 25 mL of 0.6 perchloric acid at 0°C for 1 min. The suspension was centrifuged at 3,000 *g* for 10 min, and 10 mL of the supernatant was immediately neutralized to pH 6.5–6.8 with 1 M potassium hydroxide. The extract was placed on ice for 30 min and filtrated through Whatman No. 1 filter paper to remove potassium perchlorate. Then, the filtrate was diluted to 20 mL with distilled water and stored at −20°C until HPLC analysis.

A Perkin Elmer Series 200 UV/Vis HPLC system (Perkin Elmer; Waltham, MA, USA) with UV detector set at 254 nm, a Supelcosil LC-18-T column (length = 15 cm, I.D. = 4.6 mm, particle size = 3 μm; Cod. 58970-U; Sigma-Aldrich Corp.) maintained at 30°C, and a TotalChrom workstation (Perkin Elmer) were employed. Two solutions were used as mobile phase: Solution A, made with 0.1 M potassium, phosphate buffer/4 mM tetrabutylammonium hydrogen sulfate (pH 6.0); and Solution B, consisting of methanol:water 70:30 (pH 7.2). The gradient elution program was set at 1.5 mL/min, starting with 100% A for 3 min. The program proceeded linearly, first to 70% A/30% B over 2.5 min, then to 40% A/60% B over 5 min, and to 0% A/100% B over 3 min; then, isocratic elution at 0% A/100% B over 4 min and finally linearly to 100% A over 1 min. For quantitative analyses of ATP breakdown products, the external standard method was applied. Calibration curves were determined using solutions with varying amounts (0.002–0.1 mg/mL) of ATP, ADP, AMP, IMP, Ino, and Hx standards (Sigma Chemical Co.). All the solutions and samples were passed through a 0.22 μm aqueous filter prior to injection onto the column.

Nucleotide indices: K, Ki, H, G, P, and Fr

Freshness indices that relate degradation products of nucleotides such as K, Ki, H, G, P, and Fr values were calculated by the procedures described by Saito et al. (1959), Karube et al. (1984), Luong et al. (1992), Burns et al. (1985), and Özogul et al. (2006c). In the present study, the nucleotide ratios were expressed as percentages, and the formulas used are the following:

PROOF ONLY

$$\%K \text{ value} = [(Ino + Hx)/(ATP + ADP + AMP + IMP + Ino + Hx)] \times 100,$$
$$\%Ki \text{ value} = [(Ino + Hx)/(IMP + Ino + Hx)] \times 100,$$
$$\%H \text{ value} = [(Hx)/(IMP + Ino + Hx)] \times 100,$$
$$\%G \text{ value} = [(Ino + Hx)/(AMP + IMP + Ino)] \times 100,$$
$$\%P \text{ value} = [(Ino + Hx)/(AMP + IMP + Ino + Hx)] \times 100.$$
$$\%Fr \text{ value} = [(IMP)/(IMP + Ino + Hx)] \times 100.$$

Biogenic Amines

Biogenic amines were analyzed using an HPLC method (Hwang et al., 1995). The sample was prepared as follows: a 5 g sample was transferred into a 50 mL centrifuge tube and homogenized with 20 mL of 6% trichloroacetic acid for 3 min. The homogenate was centrifuged at 14,000 rpm and 4°C for 10 min, and filtered using Whatman No. 2 filter paper. The filtrate was placed in a volumetric flask and diluted to 50 mL. Each extract (2 mL) was derivatized with benzoyl chloride (Riedel-de Häen, Laborchemikalien GmbH & Co. KG, Munich, Germany). Standard amine solutions in water (10 mg/mL, free base) of β-phenylethylamine hydrochloride (phen), spermidine trihydrochloride (spd), spermine trihydrochloride (spn), histamine dihydrochloride (hi), tryptamine hydrochloride (tr), tyramine hydrochloride (ty), and agmatine sulphate (agm; Sigma Chemical Co.) were prepared, and aliquots of 50 µL were derivatized. The benzoyl derivates were obtained according to the method described by Yen and Hsieh (1991) with minor modifications. Sodium hydroxide solution (2 M, 1 mL) was added to the amine solution, followed by 10 µL of benzoyl chloride, and then mixed using a vortex mixer and allowed to stand for 20 min. The benzoylation was stopped by adding 2 mL saturated sodium chloride solution, and the amide was extracted with 3 mL diethyl ether. After centrifugation, the upper organic layer was transferred into a tube and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 500 µL methanol, and 5 µL aliquots were injected for HPLC analysis.

Amines were determined using a Perkin Elmer Series 200 UV/Vis HPLC system (Perkin Elmer) with UV detector set at 254 nm. A Lichrospher 100 RP-18 reverse-phase column (length = 125 mm, I. D. = 2.5 mm, particle size = 5 µm; Merck, KGaA, Darmstadt, Germany) was used for separation. The gradient elution program was set at 1.1 mL/min, starting with a methanol-water mixture (55:45, v/v) for 4 min. The program proceeded linearly to methanol-water (80:20, v/v) over 2 min. This was followed by the same composition and flow rate for 6 min, and then methanol concentration decreased to methanol-water (55:45, v/v) over 2 min.

Statistical analyses

All the determinations of each independent sample (H1 and 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).

Results and discussion

TVB-N and TMA

The chemical parameters TVB-N and TMA are widely used as freshness indices in fish and shellfish. According to Argentine regulations (Código Alimentario Argentino [CAA], 1971), the TVB-N legal limit for seafood is 30 mg/100 g, and for the European legislation (EC Commission Regulation No. 2074/2005, 2005) the limiting value is 35 mg/100 g for *Salmo salar* and fish of the families Merlucciidae and Gadidae. In the case of TMA, it is considered that a level of 10 mg TMA/100 g indicates acceptable quality in

PROOF ONLY

international trading (Connell, 1995; Yerlikaya and Gökoğlu, 2004). During the refrigerated and frozen storage of cooked SKC meat, the limit values for TVB-N and TMA were not reached for the time considered (Table 1). As TMA results from the reduction of TMAO by bacterial activity and possibly partly by intrinsic enzymes, some bacterial activity was present at 0°C, but it was very low at −20°C. In raw blue crab stored for 3 months at −18°C, TVB-N and TMA were reported as 23 and 0.95 mg/100 g, respectively (Yerlikaya and Gökoğlu, 2004); whereas in sea bream fish (*Sparus auratus*), TMA reached a value of 1.13 mg/100 g under the same storage conditions (Abdalla et al., 1989). These values are comparable to those obtained for SKC (Table 1). When SKC samples were stored at 0°C, TVB-N and TMA values presented sa linear increase with time (TVB-N = 1.34 days + 15.08, R^2 = 0.9668; TMA = 0.88 days + 0.4667, R^2 = 0.9674); whereas at −20°C, TVB-N increased curvilinearly (TVB-N = 0.0012 days − 0.0372 days + 16.17, R^2 = 0.9618) and TMA linearly (TMA = 0.0131 days − 0.0179, R^2 = 0.9923).

The P ratio

The ratio of TMA to TVB-N renders the P ratio. The P ratio values are only slightly dependent on species, amount of fat, and various treatments such as dehydration and freezing (Malle and Poumeyrol, 1989). In the present study, this index value was 28.4% after 5 and 10 days of refrigerated storage, a value near the maximum limit (30%) recommended for fish (Pons Sánchez-Cascado, 2005). As expected, frozen samples gave lower P ratios, showing a linear regression (P ratio = 0.0588 days + 0.2044, R^2 = 0.978) and reaching 5.17% after 3 months. According to these results, the P-ratio would be a more rigorous indicator to estimate the freshness of SKC than TMA and TVB-N individually, especially in refrigerated samples.

Formaldehyde

FA contents for both storage conditions are shown in Table 1. After 10 days of refrigerated storage, the FA value (8.6 mg/kg) in cooked SKC samples was lower than the 160 mg/kg reported for headed/eviscerated lizardfish (*Saurida tumbil*; Benjakul et al., 2003) and the 23.3 mg/kg value for saithe (*Pollachius virens*) after 9 days (Mackie and Thomson, 1974). In the frozen samples of cooked SKC meat, a drastic FA increase during the first month followed by a slight increase to the end of storage time was observed (Table 1). A similar behavior was observed in mud crabs (*Scylla serrata*), with values of 3.2, 3.5, and 3.8 mg/kg after 1, 2, and 3 months of frozen storage, respectively (Benjakul and Sutthipan, 2009). However, higher FA values were reported for marine fish. For example, values of 7.7 mg/kg were found in saithe (*Pollachius virens*) after 11 weeks at −15°C (Mackie and Thomson, 1974). In fish species, formaldehyde formation and its reaction with muscle proteins during frozen storage are considered to be a major factor affecting

Table 1. Changes in total volatile basic nitrogen, trimethylamine, formaldehyde, indole, and lactic acid contents in cooked southern king crab meat during refrigerated and frozen storage.

Time (days)	*TVB-N (mg/100 g)*	*TMA (mg/100 g)*	*FA (mg/kg)*	*Indole (μg/100 g)*	*Lactic acid (mg/100 g)*
0	15.8 ± 0.12^{a}	n.d.^{a}	0.50 ± 0.03^{a}	3.30 ± 0.01^{a}	16.4 ± 0.77^{a}
Storage at 0°C					
5	20.4 ± 0.35^{b}	5.83 ± 0.10^{b}	6.27 ± 0.48^{b}	8.67 ± 0.00^{b}	40.2 ± 0.51^{b}
10	29.2 ± 0.06^{c}	8.29 ± 0.63^{c}	8.56 ± 0.18^{c}	20.7 ± 0.02^{c}	39.1 ± 0.92^{b}
Storage at −20°C					
10	16.1 ± 0.71^{a,b}	0.34 ± 0.02^{b}	2.02 ± 0.18^{b}	3.30 ± 0.01^{b}	37.2 ± 2.34^{b}
30	16.9 ± 0.06^{b}	0.41 ± 0.05^{b}	3.15 ± 0.08^{c}	4.89 ± 0.02^{c}	28.4 ± 1.42^{c}
60	17.3 ± 0.03^{c}	0.70 ± 0.03^{c}	3.29 ± 0.06^{d}	6.03 ± 0.01^{d}	28.2 ± 1.35^{c}
90	23.2 ± 0.14^{d}	1.25 ± 0.00^{d}	3.70 ± 0.06^{e}	6.81 ± 0.02^{e}	16.6 ± 1.42^{a}

TVB-N, total volatile basic nitrogen; TMA, trimethylamine; FA, formaldehyde; n.d., not detected. Mean values from triplicate analyses of two independent samples ± standard deviation. For each storage condition, values with different letters in the same column are significantly different ($p < 0.05$).

PROOF ONLY

texture and functionality. However, in our work, cooked SKC meat did not exceed the maximum limit permitted in Italy of 10 mg/kg (Renon et al., 1993) after 10 and 90 days of refrigerated and frozen storage, respectively.

Indole

Indole has a strong and characteristic odor, and it is not detected in fish and crustaceans (seafood) after fishing or harvest (Chang et al., 1983). Just after cooking, indole content in SKC meat was 3.3 μg/100 g, and then increased rapidly up to 20.7 μg/100 g at 10 days of refrigerated storage; whereas a slow increase was observed in the samples stored at −20°C, reaching 6.81 μg/100 g after 3 months (Table 1). The values obtained under both storage conditions were lower than the acceptance limit of 25 μg/100 g recommended by the Food and Drug Administration (FDA, 2004) in shrimps and crabmeat. As indole is produced by bacteria, its formation rate is affected both by the initial degree of contamination and storage temperature, and therefore careful handling of the meat is very important. Experiments with golden crab (*Chaceon chilensis*) cooked in boiling water and stored at 4–6°C gave different results after 3 days of storage depending on whether the meat was removed by hand after boiling (179 μg/100 g) or not (whole specimen, 20 μg/100 g; Cifuentes and Quiñinao, 2000).

Lactic acid

Table 1 also shows the changes in lactic acid content in SKC meat during storage at 0 and −20°C. Maximum values can be found between 5 and 10 days of storage at 0°C and after 10 days in frozen samples. In a similar way, the formation of lactic acid in kuruma prawn (*Penaeus japonicus*) muscle was dependent on storage temperatures (5, 0, −1°C), showing maximum levels (50 mg/100 g) after 1 day at 5°C, 7 days at 0°C, and 10 days at −1°C (Matsumoto and Yamanaka, 1990). The fact that the concentration of lactic acid decreases during frozen storage to the initial value of fresh SKC meat makes it an unsuitable freshness index for SKC.

Nucleotides

Total nucleotide content at the beginning of storage was 6.77 μmol/g SKC meat. Similar values were reported for the muscle of cultured mud crab (*Scylla serrata*; 6–12 μmol/g; Chiou and Huang, 2003) and queen crab (*Chionoecetes opilio*; 5–6 μmol/g; Hiltz and Bishop, 1975), while the total nucleotide content in the leg muscle of Alaska king crab was 3.47 μmol/g (Porter, 1968).

Changes in nucleotide contents in cooked SKC meat during refrigerated and frozen storage are shown in Table 2. During the ATP postmortem metabolism, ATP concentration decreased from 2.07 to 0.72 and 0.58 μmol/g at the final time of refrigerated and frozen storage, respectively. AMP rather than IMP was accumulated during both storage conditions. Stone (1970) reported a similar behavior for the Alaska king crab (*Paralithodes camtschatica*), suggesting that the enzymatic deamination of AMP will contribute to the IMP content of salmon fillets, but will not contribute significantly to the IMP content of crab or scallop muscles (Stone, 1970). However, Hiltz and Bishop (1975) found that thawed leg muscle of queen crab accumulated a large amount of IMP, whereas cooked muscle did not. Therefore, the pretreatment of the meat affects the pathway of nucleotide degradation.

Regarding hypoxanthine changes during SKC storage (Table 2), Hx content increased from 0.26 to 1.16 and 0.32 μmol/g at 0 and −20°C, respectively. These values are low compared with those found for iced herring, which showed an Hx level of about 4 μmol/g after 10 days (Özogul et al., 2000). Hx has a bitter taste and is regarded as a contributor to off-flavors. In addition, Hx content has been reported as a freshness index of fish, since it reflects the initial phase of autolytic deterioration and subsequent bacterial spoilage (Özogul et al., 2008). However, its accumulation

PROOF ONLY

Table 2. Changes in nucleotide contents (μmol/g) in cooked southern king crab meat during refrigerated and frozen storage.

Time (days)	*Hx*	*Ino*	*IMP*	*AMP*	*ADP*	*ATP*
0	0.26 ± 0.01[a]	0.24 ± 0.01[a]	0.36 ± 0.01[a]	2.04 ± 0.10[a]	1.80 ± 0.05[a]	2.07 ± 0.06[a]
Storage at 0°C						
5	0.53 ± 0.01[b]	0.40 ± 0.04[b]	0.29 ± 0.01[b]	2.23 ± 0.09[b]	1.88 ± 0.06[a]	1.32 ± 0.08[b]
10	1.16 ± 0.03[c]	0.67 ± 0.05[c]	0.28 ± 0.01[b]	2.21 ± 0.06[b]	1.61 ± 0.09[b]	0.72 ± 0.06[c]
Storage at −20°C						
0	0.26 ± 0.01[a]	0.24 ± 0.01[a]	0.36 ± 0.01[a]	2.04 ± 0.10[a]	1.80 ± 0.05[a]	2.07 ± 0.06[a]
10	0.26 ± 0.01[a]	0.25 ± 0.01[a]	0.26 ± 0.01[b]	2.90 ± 0.15[b]	1.85 ± 0.05[a]	1.12 ± 0.50[b]
30	0.27 ±0.01[a,b]	0.32 ± 0.02[b]	0.20 ± 0.01[c]	3.26 ± 0.20[c]	1.87 ± 0.06[a]	0.73 ± 0.05[c]
60	0.29 ± 0.02[b,c]	0.37 ± 0.02[c]	0.18 ± 0.01[c]	3.49 ± 0.16[c]	1.61 ± 0.05[b]	0.71 ± 0.07[c]
90	0.32 ± 0.03[c]	0.37 ± 0.02[c]	0.14 ± 0.01[d]	3.80 ± 0.14[d]	1.43 ± 0.04[c]	0.58 ± 0.05[d]

Hx, hypoxanthine; Ino, inosine; IMP, inosine monophosphate; AMP, adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate. Mean values from triplicate analyses of two independent samples ± standard deviation. For each storage condition, values with different letters in the same column are significantly different ($p < 0.05$).

varies within and between species (Huss, 1988) and with body location (Murata and Sakaguchi, 1986).

The K, Ki, H, G, P, and Fr values of cooked SKC meat under refrigerated and frozen storage are shown in Table 3. Initial values of these freshness indices were as follows: K, 7.37%; Ki, 58.3%; H, 30.2%; G, 19.4%; P, 17.2%; and Fr, 41.6%. In the refrigerated samples, linear increases ($R^2 > 0.9$) were observed for K, Ki, H, G, and P (Table 3), whereas Fr decreased linearly to 13.4%. A similar behavior was found by Özogul et al. (2006a) for wild sea bass stored at 4°C, in ice, wrapped in aluminum foil at 4°C, and wrapped in cling film at 4°C, observing linear increases for K, Ki, H, and G values. In contrast, for cooked SKC meat (Table 3), variations were smaller at −20°C than 0°C, especially for K, H, and G. While K, Ki, H, and Fr showed linear regressions, G and P did not present significant regressions, and their values oscillated in the 14.8–16.3% range for G and 13.8–15.3% for P.

The K value of 20% is regarded as the optimum freshness limit (Pacheco-Aguilar et al., 2000), and values in the 20–40% range are considered acceptable (Pons Sánchez-Cascado, 2005). The K index for cooked SKC meat reached 27.5% after 10 days at 0°C and 10.3% after 90 days at −20°C; this implies that cooked SKC meat stored under these conditions is fit for consumption. The other freshness indices are usually considered to estimate the formation of intermediates in the ATP pathway degradation, and no stipulated limits are fixed, as it is highly variable according to species.

Table 3. Changes in K, Ki, H, G, P and Fr values (%) in cooked southern king crab meat during refrigerated and frozen storage.

Time (days)	*K*	*Ki*	*H*	*G*	*P*	*Fr*
0	7.37 ± 0.11[a]	58.3 ± 0.37[a]	30.2 ± 0.68[a]	19.4 ± 0.42[a]	17.2 ± 0.37[a]	41.6 ± 0.40[a]
At 0°C						
5	13.4 ± 0.71[b]	75.8 ± 0.82[b]	43.2 ± 1.15[b]	31.7 ± 1.65[b]	26.9 ± 1.31[b]	24.1 ± 0.90[b]
10	27.5 ± 1.17[c]	86.5 ± 0.53[c]	54.9 ± 1.33[c]	57.8 ± 2.04[c]	42.3 ± 1.30[c]	13.4 ± 0.58[c]
a*	2.010	14.05	12.45	19.45	12.50	−14.05
b*	6.216	45.53	17.9	−2.666	3.833	54.46
R^2	0.960	0.979	0.998	0.962	0.982	0.979
At −20°C						
10	7.62 ± 0.22[a]	66.1 ± 1.26[b]	34.0 ± 0.98[b]	14.8 ± 0.46[b]	13.8 ± 0.40[b]	33.8 ± 1.26[b]
30	8.79 ± 0.39[b]	74.3 ± 0.91[c]	34.2 ± 1.37[b,c]	15.5 ± 0.83[b,c]	14.4 ± 0.72[b,c]	25.6 ± 0.91[c]
60	9.95 ± 0.29[c]	78.2 ± 1.19[d]	34.3 ± 1.43[c]	16.3 ± 0.44[d]	15.3 ± 0.38[d]	21.7 ± 1.19[d]
90	10.3 ± 0.31[c]	82.7 ± 1.56[e]	38.0 ± 2.96[d]	15.8 ± 0.42[c,d]	14.7 ± 0.34[c,d]	17.3 ± 1.56[e]
a*	0.035	6.060	1.601	—	—	−6.061
b*	7.543	53.90	29.50	—	—	46.10
R^2	0.949	0.971	0.814	—	—	0.991

*Coefficients of the linear regression equation y = a days + b; where y represents K, Ki, H, G, P, or Fr values. Mean values from triplicate analyses of two independent samples ± standard deviation. For each storage condition, values with different letters in the same column are significantly different ($p < 0.05$).

PROOF ONLY

However, limit values can be established taking into account chemical parameters such as TMA, TVB-N, and sensory analysis. For SKC, TMA and TVB-N values are close to the limits at the end of refrigerated storage; therefore, the values of Ki, 86.5%; H, 54.9%; G, 57.8%; P, 42.3%; and Fr, 13.4% could be taken as limits. At the limit of acceptability, a similar Ki value (84%), a lower H value (39%), and a higher G value (137%) were found for iced white grouper after 16 days of storage (Özogul et al., 2008).

Based on the results for SKC, K, G, and P values were useful freshness indices for refrigerated storage due to their slow linear increase at the initial stage of decomposition. On the other hand, K, Ki, and H values, which showed a linear increase during frozen storage, can be used as freshness indices for frozen SKC meat.

Biogenic amines

Seven biogenic amines were examined—namely, spermidine (spd), spermine (spn), tyramine (ty), agmatine (agm), histamine (hi), 2-phenylethylamine (phen), and tryptamine (tr). Hi and tr were not detected in any sample under both storage conditions, whereas phen was detected only during frozen storage. Hi and tr were reported in iced wild European eel (Özogul et al., 2006b), but not in iced white grouper (Özogul et al., 2008).

Agm was pointed out as a potential indicator for freshness of common squid (*Todarodes pacificus*). In this specimen, changes in agm seemed to be similar to the bacterial growth curve (Yamanaka et al., 1987): it was detected in small amounts in the fresh muscle, and it increased with storage time (0, 3.5, 15°C), exceeding 30 mg/100 g at the initial stage of decomposition and reaching 40 mg/100 g at the advanced stage of decomposition. In contrast, in SKC (Table 4), agm presented low values during refrigeration. But during frozen storage, agm was detected after 10 days, and only a slight increase was observed in the 10–60 days period; after that, agm increased up to 123 mg/100 g at the end of frozen storage (Table 4). Based on these results, agm is not an adequate freshness index for SKC.

Spn and spd are usually the major amines present in fresh muscle in concentrations that depend on the species considered (Mackie et al., 1997). They were detected in refrigerated wild European eel (Özogul et al., 2006b), but not in iced white grouper (Özogul et al., 2008). In the case of SKC, spn and spd were the only biogenic amines initially detected, and they increased significantly under both storage conditions, with spn showing a level fluctuation at −20°C. These amines may serve as quality indicators of SKC meat: spn as a potential index to assess freshness during refrigeration, and spd during frozen storage, since their values steadily increase with time under both storage conditions.

Phen was only observed during frozen storage. The maximum value presented by this amine (2.95 mg/100 g at 30 days) is lower than the legal limit of 3 mg/100 g from a "Good Manufacturing Practice" point of view.

Table 4. Changes in biogenic amines contents (mg/100 g meat) in cooked southern king crab meat during refrigerated and frozen storage.

Time (days)	phen	spd	spn	hi	tr	ty	agm
0	n.d.	5.66 ± 0.08^{a}	17.9 ± 1.3^{a}	n.d.	n.d.	n.d.	n.d.
Storage at 0°C							
5	n.d.	4.72 ± 0.10^{b}	45.7 ± 0.2^{b}	n.d.	n.d.	23.6 ± 2.2^{b}	0.08 ± 0.01^{b}
10	n.d.	516 ± 45^{c}	161 ± 15^{c}	n.d.	n.d.	3.96 ± 0.01^{c}	0.01 ± 0.00^{c}
Storage at −20°C							
10	2.12 ± 0.21^{b}	6.31 ± 0.32^{b}	87.1 ± 8.3^{b}	n.d.	n.d.	0.61 ± 0.01^{b}	n.d.
30	2.95 ± 0.01^{b}	19.8 ± 1.3^{c}	176 ± 15^{c}	n.d.	n.d.	3.75 ± 0.12^{c}	3.11 ± 0.21^{b}
60	1.11 ± 0.04^{c}	39.2 ± 3.7^{d}	61.3 ± 5.4^{d}	n.d.	n.d.	12.8 ± 0.04^{d}	4.74 ± 0.29^{b}
90	1.05 ± 0.01^{c}	106 ± 7^{d}	147 ± 12^{e}	n.d.	n.d.	24.6 ± 1.73^{e}	123 ± 11^{c}

phen, β-phenylethylamine; spd, spermidine; spn, spermine; hi, histamine; tr, tryptamine; ty, tyramine; agm, agmatine; n.d., not detected. Mean values from triplicate analyses of two independent samples ± standard deviation. For each storage condition, values with different letters in the same column are significantly different ($p < 0.05$).

PROOF ONLY

Ty showed maximum values after 5 days of refrigeration (23.6 mg/100 g) and 90 days of freezing (24.6 mg/100 g; Table 4). Ty values between 10 and 80 mg/100 g are considered acceptable, and more than 10 mg/100 g may cause migraine (Saaid et al., 2009; Shalaby, 1996). Therefore, the level of the biogenic amine ty should be monitored in both storage conditions for SKC meat.

Conclusions

This study shows that cooked meat of SKC (obtained in the San Jorge Gulf, Argentina) 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. Potential freshness indices are suggested for this crustacean for both storage conditions. The data obtained in this study are important for the fishing industry in the South Atlantic, contributing to the knowledge of the quality and shelf life of the southern king crab.

Acknowledgments

The authors would like to thank Mr. Pedro Forbito for his experimental assistance.

Funding

This work was financially supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), the Universidad Nacional del Sur (UNS), and the Universidad Nacional de la Patagonia San Juan Bosco (UNPSJB), Argentina.

References

Abdalla, M. A., Hassan, I. M., Shalaby, A. R., and Nabuib, K. 1989. Correlation between biogenic amines, chemical changes and stability of sea bream fish during storage at -18 °C. Grasas Aceites 40: 406–412.

Anderson, A. K. 2008. Biogenic and volatile amine-related qualities of three popular fish species sold at Kuwait fish markets. Food Chem. 107: 761–767.

Antonacopoulos, N., and Vyncke, W. 1989. Determination of volatile basic nitrogen in fish: A third collaborative study by Western European Fish Technologists' Association (WEFTA). Z. Lebensm. Unters. Forsch. 189: 309–316.

AOAC. 2000. Official Methods of Analysis of Association of Official Analytical Chemists (17th ed.). Horwitz, W. (Ed.). Washington, DC: Author.

Aubourg, S. P., Piñeiro, C., Gallardo, J. M., and Barros-Velazquez, J. 2005. Biochemical changes and quality loss during chilled storage of farmed turbot (*Psetta maxima*). Food Chem. 90: 445–452.

Benjakul, S., and Sutthipan, N. 2009. Muscle changes in hard and soft shell crabs during frozen storage. LWT-Food Sci. Technol. 42: 723–729.

Benjakul, S., Visessanguan, W., and Tueksuban, J. 2003. Changes in physico-chemical properties and gel-forming ability of lizardfish (*Saurida tumbil*). Food Chem. 80: 535–544.

Boeri, R. L., Almandós, M. E., Ciarlo, A. S., and Giannini, D. H. 1993. Formaldehyde instead of dimethylamine determination as a measure of total formaldehyde formed in frozen argentine hake (*Merluccius hubbsi*). Int. J. Food Sci. Technol. 28: 289–292.

Burns, G. B., Ke, P. J., and Irvine, B. B. 1985. Objective procedure for fish freshness evaluation based on nucleotide changes using a HPLC system. Can. Tech. Rep. Fish. Aquat. Sci. 1373: 1–35.

CAA. 1971. Ley 18284: Poder Ejecutivo Nacional, República Argentina. Retrieved from http://www.anmat.gov.ar

Chang, O., Cheuk, W. L., Nickelson, R., Martin, R., and Finne, G. 1983. Indole in shrimp: Effect of fresh storage temperature, freezing and boiling. J. Food Sci. 48: 813–816.

Chiou, T. K., and Huang, J. P. 2003. Chemical constituents in the abdominal muscle of cultured mud crab *Scylla serrata* in relation to seasonal variation and maturation. Fish. Sci. 69: 597–604.

Cifuentes, A., and Quiñinao, J. 2000. Composición y vida útil de la carne cocida de cangrejo dorado (*Chaeceon chilensis*) proveniente del archipiélago de Juan Fernández, Chile [Composition and shelf-life of golden crab (*Chaeceon chilensis*) cooked meat from Juan Fernández archipelago, Chile]. Invest. Marit. 28: 195–202.

Connell, J. J. 1995. Control of Fish Quality (4th ed.). London, UK: Fishing News Books.

Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Off. J. Eur. Commun. L338 (22 December 2005), 1–26.

PROOF ONLY

Commission Regulation (EC) No. 2074/2005 of 5 December 2005 on laying down implementing measures for certain products under Regulation (EC) No. 853/2004 of the European Parliament and of the Council and for the organization of official controls under Regulation (EC) No. 854/2004 of the European Parliament and of the Council and Regulation (EC) No. 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No. 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No. 853/2004 and (EC) No. 854/2004. Off. J. Eur. Commun. L 338 (22 December 2005), 27–59.

FDA. 2004. Seafood Chemistry: Indole. Retrieved from http://www.fda.gov/ScienceResearch/FieldScience/ucm172196.htm#9_2_2

Hiltz, D. F., and Bishop, L. J. 1975. Posmortem glycolytic and nucleotide degradative changes in muscle of the Atlantic queen crab (*Chionoecetes opilio*) upon ice storage of unfrozen and of thawed meat, and upon cooking. Comp. Biochem. Physiol. B 50: 453–458.

Huss, H. H. 1988. Fresh Fish: Quality and Quality Changes. Rome, Italy: Food and Agriculture Organization of the United Nations.

Hwang, D. F., Chang, S. H., Shiua, C. Y., and Cheng, C. C. 1995. Biogenic amines in the flesh of sailfish (*Isiophorus platypterus)* responsible for scombroid poisoning. J. Food Sci. 60: 926–928.

Karube, I., Atsuoka, H., Sufzuki, S., Watanabe, E., and Toyama, K. 1984. Determination of fish freshness with an enzyme sensor system. J. Agric. Food Chem. 32: 314–319.

Luong, J. H. T., Male, K. B., Masson, C., and Nguyen, A. L. 1992. Hypoxanthine ratio determination in extract fish using capillary electrophoresis and immobilized enzymes. J. Food Sci. 57: 77–81.

Mackie, I. M., Pirie, L., Ritchie, A. H., and Yamanaka, H. 1997. The formation of non-volatile amines in relation to concentrations of free basic amino acids during postmortem storage of the muscle of scallop (*Pecten maximus)*, herring (*Cuplea harengus*) and mackerel (*Scomber scombrus*). Food Chem. 60: 291–295.

Mackie, I. M., and Thomson, B. W. 1974. Decomposition of trimethylamine oxide during iced and frozen-storage of whole and comminuted tissue of fish. Proc. IV Int. Cong. Food Sci. Technol. 1: 243–250.

Malle, P., and Poumeyrol, M. 1989. A new chemical criterion for the quality control of fish: Trymethylamine/total volatile basic nitrogen (%). J. Food Prot. 52: 419–423.

Matsumoto, M., and Yamanaka, H. 1990. Post-mortem biochemical changes in the muscle of kuruma prawn during storage and evaluation of the freshness. Nippon Suisan Gakk. 56: 1145–1149.

Mietz, J. L., and Karmas, E. 1977. Chemical quality index of canned tuna as determined by high-pressure liquid chromatography. J. Food Sci. 42: 155–158.

Murata, M., and Sakaguchi, M. 1986. Storage of yellowtail (*Seriola quinqueradiata)* white and dark muscles in ice: changes in content of adenine nucleotides and related compounds. J. Food Sci. 51: 321–326.

Nordic Committee on Food Analysis. 1964. Determination of formaldehyde in foods. Nordisk Metodik Komite for Levnesdsmidler, D C 547.281.1, Nº 54.

Özogul, F., Gökbulut, C., Özogul, Y., and Özyurt, G. 2006a. Biogenic amine production and nucleotide ratios in gutted wild sea bass (*Dicentrarchus labrax*) stored in ice, wrapped in aluminium foil and wrapped in cling film at 4°C. Food Chem. 98: 76–84.

Özogul, F., Özogul, Y., and Kuley, E. 2008. Nucleotide degradation and biogenic amine formation of wild white grouper (*Epinephelus aeneus*) stored in ice and chill temperature (4°C). Food Chem. 108: 933–941.

Özogul, F., Taylor, K. D. A., Quantick, P., and Özogul, Y. 2000. Chemical, microbiological and sensory evaluation of Atlantic herring (*Clupea harengus*) stored in ice, modified atmosphere and vacuum pack. Food Chem. 71: 267–273.

Özogul, Y., Özogul, F., and Gökbulut, C. 2006b. Quality assessment of wild European eel (*Anguilla anguilla)* stored in ice. Food Chem. 95: 458–465.

Özogul, Y., Özogul, F., Kuley, E., Özkukut, A. S., Gökbulut, C., and Köse, S. 2006c. Biochemical, sensory and microbiological attributes of wild turbot (*Scophthalmus maximus)*, from the Black Sea, during chilled storage. Food Chem. 99: 752–758.

Pacheco-Aguilar, R., Lugo-Sánchez, M. E., and Robles-Burgueño, M. R. 2000. Postmortem biochemical and funtional characteristic of Monterrey sardine muscle stored at 0 ºC. J. Food Sci. 65: 40–47.

Pons Sánchez-Cascado, S. 2005. Estudio de alternativas para la evaluación de la frescura y la calidad del boquerón (*Engraulis encrasicholus*) y sus derivados (Doctoral thesis). Retrieved from http://hdl.handle.net/10803/2427

Porter, R. W. 1968. The acid-soluble nucleotides in king crab muscle. J. Food Sci. 33: 311–314.

Rawles, D. D., Flick, G. J., and Martin, R. E. 1996. Biogenic amines in fish and shellfish. Adv. Food Nutr. Res. 39: 329–365.

Renon, P., Malandra, R., and Biondi, P. A. 1993. Produzione di formaldeide in crostacei congelati, preventivamente tratati con SO_2. Ind. Aliment. XXXII: 254–258.

Risso, S. J., and Carelli, A. A. 2012. Nutrient composition of raw and cooked meat of male southern king crab (*Lithodes santolla* Molina, 1782). J. Aquat. Food Prod. T. 21: 433–444.

Rodríguez, O., Barros-Velázquez, J., Piñeiro, C., Gallardo, J. M., and Aubourg, S. 2006. Effects of storage in slurry ice on the microbial, chemical and sensory quality and on the shelf life of farmed turbot (*Psetta maxima*). Food Chem. 95: 270–278.

Ruiz-Capillas, C., Gillyon, C. M., and Horner, W. F. A. 2001. Determination of different volatile base components as quality control indices in fish by official methods and flow injection analysis. J. Food Biochem. 25: 541–553.

PROOF ONLY

Ryder, J. M. 1985. Determination of adenosine triphosphate and its breakdown products in fish muscle by high-performance liquid chromatography. J. Agric. Food Chem. 33: 678–680.
Saaid, M., Saad, B., Hashim, N. H., and Salhin, A. 2009. Determination of biogenic amines in selected Malaysian food. Food Chem. 113: 1356–1362.
Saito, T., Arai, K., and Matsuyoshi, M. 1959. A new method for estimating the freshness of fish. Bull. Jap. Soc. Sci. Fish. 24: 749–750.
Shalaby, A. R. 1996. Significance of biogenic amines to food safety and human health. Food Res. Int. 29: 675–690.
Sokal R. R., and Rohlf, F. J. 1987. Introduction to Biostatistics. New York, NY: W.H. Freeman.
Stone, F. E. 1970. Enzymatic deamination of adenosine monophosphate (AMP), adenosine and adenine by salmon, crab and scallop muscle extracts. J. Food Sci. 35: 565–567.
Wekell, J. C., and Barnett, H. 1991. New method for analysis of trimethylamine oxide using ferrous sulfate and EDTA. J. Food Sci. 56: 132–135.
Yamanaka, H., Shiomi, K., and Kikuchi, T. 1987. Agmatine as a potencial index for freshness of common squid (*Todarodes pacificus*). J. Food Sci. 52: 1133–1135.
Yen, G. C., and Hsieh, C. L. 1991. Simultaneous analysis of biogenic amines in canned fish by HPLC. J. Food Sci. 56: 158–160.
Yerlikaya, P., and Gökoğlu, N. 2004. Quality changes of blue crab (*Callinectes sapidus*) meat during frozen storage. J. Food Qual. 27: 83–89.

PROOF ONLY