Quality enhancement of the abundant under-valued crustacean, lobster krill (Munida spp.), during its chilled storage

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SUMMARY

Lobster krill (Munida genus) represents an under-valued crustacean frequently caught on European fishing banks. In this work, its sensory, microbiological, and biochemical qualities were evaluated during chilled storage. Additionally, the effects of a pre-storage antimelanosic treatment consisting of soaking in sodium metabisulphite (SMB) solutions at two different concentrations (0.25 and 0.75 %) were also studied. SMB pre-storage treatment provided lobster specimens that still exhibited acceptable sensory quality after 10 d of storage, while control specimens were unacceptable at that time. SMB treatment also resulted in a significant (p<0.05) inhibition of microbial growth, mainly of Enterobacteriaceae, psychrotrophes and proteolytic bacteria. Low lipid oxidation levels were observed for all batches; however, a significantly higher (p<0.05) retention of polyunsaturated fatty acids was found in SMB-treated lobster, especially in the 0.75 % SMB batch. The results presented here open the way to the potential commercialisation of currently under-utilised lobster krill as a chilled product.

Keywords: lobster krill, Munida, chilling, sodium metabisulphite, sensory acceptance, microbial activity, lipid oxidation.

Running title: Quality enhancement of chilled lobster krill.
Crustacean species provide products of great importance for the food chain in many countries worldwide (FAO, 2007). However, crustaceans exhibit a remarkably short shelf life as a consequence of several factors, such as their small size, the fact that they are not gutted and their high content of non-protein nitrogen (NPN) compounds, polyunsaturated fatty acids (PUFA) and melanin (Piclet, 1987; Finne, 1992). These facts imply that breakdown mechanisms, mainly autolytic changes, microbial spoilage and the induction of melanosis reactions, markedly affect the post-mortem quality of these products after capture (Simpson et al., 1987; Ashie et al., 1996). In order to delay crucial spoilage mechanisms, crustacean species are immediately chilled after capture by means of storage in flake ice (Mendes et al., 2002), slurry ice (Huidobro et al., 2002; Losada et al., 2006), or refrigerated seawater (Kraus, 1992). Such preservation methods have also been combined with storage under modified atmosphere (Ruiz-Capillas & Moral, 2004), immersion in brine solutions (Xiong et al., 2002) or the incorporation of chemical preservative agents (McEvily et al., 1991; Aubourg et al., 2007).

Among the factors limiting acceptability of crustaceans, melanosis plays a very important role (Slattery et al., 1991; Chinivasagam et al., 1998; Montero et al., 2001). This reaction occurs in the presence of oxygen, due to the action of polyphenoloxidase (PPO) on monophenolic compounds, which are first hydroxylated to o-diphenols and then oxidised to o-quinones. Such quinones react non-enzymatically with other compounds, giving rise to undesirable dark pigments of high molecular weight. The enzyme PPO remains active during on-board handling of fish and under chilled storage, resulting in significant economic losses for the fish industry. Sulphites and their derivatives interfere in the polymerisation of quinones, thus they have been successfully applied to delaying melanosis reactions in
crustaceans, thereby increasing shelf life as a result of the inhibition of PPO-mediated browning (Rea et al., 1998; Rotllant et al., 2002).

The marine food industry is suffering from dwindling stocks of traditional species as a result of drastic changes in their availability and legal limitations in their capture. Consequently, the fish industry is paying increasing attention to new and non-conventional sources. Accordingly, the present study is focused on lobster krill, a decapod crustacean belonging the *Munida* genus (Family Galatheidae, Order Decapoda), and which represents an under-valued crustacean frequently caught on European fishing banks and whose commercial potential is expected to increase significantly in the coming years. Lobster krill live in several areas of the Atlantic Ocean, also being abundant in the Mediterranean Sea. Other authors have previously reported the biology and ecology of *Munida* spp. (Freire et al., 1992; Company et al., 2003). Enzyme extracts obtained from these species are known to be rich in proteolytic enzymes and have been successfully used as ripening agents in cheese production (Rossano et al., 2011).

The objective of the present work was to analyse the quality loss of lobster krill during chilled storage. This storage was combined with a pre-storage antimelanosic treatment provided by soaking in sodium metabisulphite (SMB) at two different concentrations, to assess the potential benefits on lobster krill quality. For it, sensory, microbiological, and biochemical qualities were evaluated on this marine species, to our knowledge for the first time.

**MATERIALS AND METHODS**

**Raw material, processing and sampling**

Fresh lobster krill specimens (15 kg) were caught near the Galician Atlantic coast (North-Western Spain) in February 2013 and transported on ice to the laboratory ten hours after the
catch. The length and weight of the fish specimens included were in the following ranges: 9.5-11.0 cm and 9.5-10.5 g, respectively.

Upon arrival at the laboratory, 1.5 kg of lobster krill was divided into three different groups of 0.5 kg each and analysed independently to provide baseline values (Day 0). The remaining specimens were divided into three batches of 4.5 kg each. The first batch (SMB-75) was immersed in a 0.75 % (w/v) SMB aqueous solution (4.5 kg lobster/12 l 0.75 %-SMB solution) for 10 min and the specimens were then placed in boxes and directly surrounded by ice (1:1, lobster:ice). The second batch (SMB-25) was immersed in a 0.25 % (w/v) SMB aqueous solution (4.5 kg lobster/12 l 0.25 %-SMB solution) for 10 min and the specimens were then placed in boxes and directly surrounded by ice (1:1, lobster:ice). Finally, the third batch (Control) was immersed in water for 10 min (4.5 kg lobster/12 l water) and the specimens were then placed in boxes and directly surrounded by ice (1:1, lobster:ice). In all cases, the boxes allowed draining of melt water, the ice being renewed when required.

Specimens from each batch were taken for analysis on Days 3, 6 and 10. At each sampling time, 1.5 kg of specimens from each batch were taken for analysis and divided in three groups (0.5 kg in each group) that were studied independently (n=3) in order to allow a statistical analysis.

Sensory analysis was carried out on whole specimens, while microbiological and chemical analyses were carried out on the flesh.

**Sensory analysis**

Sensory analysis was conducted by a sensory panel that consisted of five experienced judges (three males and two females) who adhered to traditional guidelines for fresh and refrigerated marine species adapted to lobster krill (Council Regulations, 1989). The panellists had participated in the sensory analysis of fish and seafood products during the previous 15 years.
Preliminary to these analyses, the judges received special training with refrigerated lobster krill of different qualities. Special attention was paid to the evaluation of sensory descriptors that were limiting factors for shelf life.

Each descriptor was ranked in four categories (Council regulation, 1989): highest quality (E), good quality (A), fair quality (B) and unacceptable quality (C), according to Table 1. Sensory assessment included the following descriptors: general odour, carapace colour, flesh odour (raw), flesh odour (cooked) and flesh taste (cooked). At each sampling time, specimens corresponding to each treatment were coded with 3-digit random numbers and presented to the panellists in individual trays, which were scored individually. Sensory evaluation began by the analysis of lobster in the raw state and was followed by the analysis in the cooked state. Cooking was accomplished by microwave heating for 2 minutes, to reach a core temperature round 65-70ºC. Each descriptor for each sample was scored once by each member of the panel. The panel members shared samples tested. Scores among panellists were averaged.

**Microbiological analysis**

Muscle samples (10 g) were dissected aseptically from chilled lobster krill specimens, mixed with 90 ml of 0.1 % peptone water (Merck, Darmstadt, Germany), and homogenised in sterilized stomacher bags (AES, Combourg, France) as previously described (Ben-Gigirey et al., 1998, 1999). In all cases, serial dilutions of the microbial extracts were prepared in 0.1 % peptone water.

Total aerobes were determined by surface inoculation on plate count agar (PCA, Oxoid Ltd., London, UK), after incubation at 30ºC for 48 h. The anaerobe counts were also determined on PCA at 30ºC, except that an anaerobic atmosphere kit (Oxoid) was placed together with the plates inside an anaerobic jar. Psychrotrophes were also determined on PCA
but incubation was carried out at 7-8ºC for 7 d. Enterobacteriaceae were determined by pour plating using Violet Red Bile Agar (VRBA) (Merck, Darmstadt, Germany) after incubation at 37ºC for 24 h. Microorganisms exhibiting a proteolytic or lipolytic phenotype were determined on casein-agar medium or tributyrine-agar, respectively, after incubation at 30ºC for 48 h, as previously described (Ben-Gigirey et al., 2000).

In all cases, bacterial counts were transformed into log CFU g⁻¹ muscle before undergoing statistical analysis. All microbiological analyses were done in triplicate.

Assessment of moisture, lipid and sodium metabisulphite content

Moisture content was determined by the difference between the weight of fresh homogenised flesh (1-2 g) and the weight recorded after 4 h at 105ºC. Results were expressed as g water kg⁻¹ flesh.

Lipids were extracted from the crustacean homogenised flesh by the Bligh and Dyer method (Bligh & Dyer, 1959), by employing a single-phase solubilisation of the lipids using a chloroform-methanol (1:1) mixture in a solvent:tissue ratio of 4:1. Quantification was carried out on an aliquot of the resulting lipid extract and results were expressed as g lipid kg⁻¹ flesh.

SMB content in lobster krill flesh was assessed according to previous research (Aubourg et al., 2007). In it, a 5-g portion was distilled in the presence of 20 ml of water and 2 ml of concentrated HCl. The resulting sulphur dioxide was sent to a flask containing 0.5 ml of a 1 % (w/v) starch solution, 4.5 ml of water, and one drop of a saturated iodine aqueous solution. The determination of sulphur dioxide was performed with a 0.05 N iodine solution and results calculated as mg sulphur dioxide kg⁻¹ flesh.
Chemical analyses related to quality changes

The evolution of pH values in muscle with storage time was determined by means of a 6-mm diameter insertion electrode (Crison, Barcelona, Spain).

Free fatty acid (FFA) content was determined in the lipid extract of muscle by the Lowry & Tinsley (1976) method. For it, an aliquot of the lipid extract was mixed with an aqueous 5% cupric acetate solution, whose pH had been adjusted to 6.0-6.2 with pyridine. The complex formation was determined by spectrophotometric (715 nm) assessment. Results were expressed as g FFA kg\(^{-1}\) lipids, based on an oleic acid standard curve.

The peroxide value (PV) was determined spectrophotometrically (Beckman Coulter, DU 640, London, UK) in the lipid extract, according to the Chapman and McKay (1949) method. In it, hydroperoxides reacted with Fe (II) (aqueous 20 mM FeCl\(_2\) solution) in the presence of thiocyanate ions (aqueous 30% NH\(_4\)SCN solution). The purple-colour formation was determined by spectrophotometric (500 nm) assessment. Results were expressed as meq active oxygen kg\(^{-1}\) lipids, based on a standard curve prepared with a FeCl\(_3\) aqueous solution.

The thiobarbituric acid index (TBA-i) was determined in accordance with Vyncke (1970). This method is based on the reaction between an aqueous 5% trichloracetic acid extract of the marine product flesh and an aqueous 0.02 M solution of thiobarbituric acid. Content of thiobarbituric acid reactive substances (TBARS) was spectrophotometrically measured at 532 nm after heating (96-97\(^\circ\)C for 40 minutes), cooling and centrifugation and calculated from a standard curve using 1,1,3,3-tetraethoxy-propane (TEP); results were expressed as mg malondialdehyde kg\(^{-1}\) flesh.

Lipid extracts were converted into fatty acid methyl esters (FAME) by reaction with acetyl chloride. FAME were then analysed using a Perkin-Elmer 8700 gas chromatograph equipped with a fused silica capillary column SP-2330 (0.25 mm i.d. x 30 m, 0.20 \(\mu\)m film, Supelco Inc., Bellefonte, PA, USA), using nitrogen at 10 psi as carrier gas (linear flow rate of
1.0 ml/min) and a flame ionisation detector (FID) at 250°C (Aubourg et al., 1996). Peaks corresponding to fatty acids were identified by comparison to the retention times of two standard mixtures (Qualmix Fish, Larodan, Malmo, Sweden; FAME Mix, Supelco Inc., Bellefonte, PA, USA). Peak areas were automatically integrated, with C 19:0 fatty acid employed as the internal standard for quantitative purposes. The polyene index (PI) was calculated as the following fatty acid ratio: (C 20:5ω3 + C 22:6ω3)/ C 16:0.

All chemical analyses were carried out in duplicate. All solvents and chemical reagents used were of reagent grade (Merck, Darmstadt, Germany).

**Statistical analysis**

Data obtained from the different microbial and chemical analyses were subjected to the ANOVA method to explore differences by two different ways: pre-storage soaking condition effect and chilling time effect. For this, the PASW Statistics 18 software for Windows (SPSS Inc., Chicago, IL, USA) was employed. The comparison of means was performed using the least-squares difference (LSD) method. Differences among batches and among icing times were considered significant for a confidence interval at the 95 % level (p<0.05) in all cases.

Correlation analyses between quality (microbiological and chemical) indices and chilled storage time were also carried out; in each case, the best fit obtained is reported.

**RESULTS AND DISCUSSION**

**Sensory analysis**

The results of sensory analysis are compiled in Table 2. Sensory quality decreased in all batches as storage time progressed. However, this decline was less pronounced in the case of SMB-25 and SMB-75 batches as regards general odour and carapace colour, as compared with the control batch. Thus, the control batch exhibited unacceptable quality on Day 10, with
general odour being the factor limiting acceptability. In contrast, the two batches subjected to a pre-storage SMB soaking exhibited acceptable quality even after 10 d of storage. Moreover, pre-storage treatment with SMB also resulted in a better maintenance of carapace colour, as compared with the control batch. Flesh odour, both in raw and cooked products, and flesh taste did not seem to be affected by SMB treatment (Table 2).

A previous report on Norway lobster (*Nephrops norvegicus*) outlined the beneficial effect of a pre-storage treatment with 0.5 % SMB combined with storage in slurry ice on sensory quality of the lobster (Aubourg *et al.*, 2007). A shelf life increase during the refrigerated storage of the same species was also attained by applying a pre-storage potassium metabisulphite (1.25, 2.50 and 6.25 %) soaking (Rea *et al.*, 1998) or by dusting with a commercial sulphite-based antimelanosis formulation (6 %) (Martínez-Alvarez *et al.*, 2008). Pre-storage SMB treatment also led to quality enhancement in crustacean shrimp species such as *Penaeus aztecus* and *Penaeus duorarum* (McEvily *et al.*, 1991) and *Pandalus borealis* (Martinsdóttir *et al.*, 2001).

Carapace browning is caused by melanin oxidation. Melanin has been reported to be a substrate of enzymatic oxidation during the processing of crustacean species, this leading to undesirable losses in their sensory quality (McEvily *et al.*, 1991; Chen *et al.*, 1997). In keeping with this, the results presented in this study indicate that a rapid and controlled pre-storage soaking in SMB resulted in a better maintenance of carapace colour and general odour of lobster krill at intermediate storage times, permitting a shelf life extension from 6 d to 10 d, as compared with the untreated control batch (Table 2).

**Microbial activity assessment**

The results of microbial analyses are compiled in Table 3, Fig. 1 and Fig. 2. Pre-storage soaking in SMB did not significantly (p>0.05) affect the growth of the bacterial groups most
relevant as indicators of microbial quality. Thus, the numbers of aerobes, anaerobes (3-6-day period) and lipolytic bacteria for each batch increased as storage time progressed ($r = 0.94-0.96$ and $r = 0.88-0.92$ for aerobe and lipolytic counts, respectively; both linear fittings), with no significant ($p>0.05$) difference among batches being observed at any sampling time (Table 3).

In contrast, pre-storage soaking in SMB allowed a slight but significantly ($p<0.05$) better control of Enterobacteriaceae (Table 3), of psychrotrophes (Fig. 1) and of proteolytic bacteria (Fig. 2) after 6 d of chilled storage, as compared with the control batch. In the case of the Enterobacteriaceae counts, these were also significantly ($p<0.05$) lower in the SMB-75 batch after 10 d of storage, as compared with the control batch (Table 3). In all cases, the counts for Enterobacteriaceae (Table 3), psychrotrophes (Fig. 1; $r = 0.93-0.94$, quadratic fitting) and proteolytic bacteria (Fig. 2; $r = 0.92-0.95$, linear fitting) progressed with storage time.

A previous study on Norway lobster subjected to a pre-storage antimelanosic treatment with 0.5 % SMB also reported slight beneficial effects at the microbial level (Aubourg et al., 2007). Another study on Norway lobster reported that pre-storage soaking in potassium metabisulphite led to a reduction in the numbers of aerobic mesophiles, while no effect on psychrotrophes was observed (Rea et al., 1998).

While alive, the immune system of marine species prevents bacterial growth (Cyprian et al., 2008). However, the immune system collapses post-mortem and, consequently, bacteria are able to invade the flesh during chilled storage (Sveinsdóttir et al., 2002). From the results presented in the current study, pre-storage soaking in SMB was not detrimental at the microbial level. In contrast, it exerted a slight antimicrobial effect on Enterobacteriaceae, psychrotrophes and proteolytic bacteria. This better microbial control correlated well with the results of the sensory analysis that indicated an extended shelf life in batches subjected to
SMB treatment, as compared with the control batch. Previous reports had also described the potential antimicrobial effect of sulphites on fish specimens (Chinivasagam et al., 1998; Maldhavi et al., 1995).

Moisture, lipid and sodium metabisulphite content

Moisture scores ranged between 790 and 830 g kg\(^{-1}\) flesh in all samples, while lipid contents ranged between 6.7 and 7.7 g kg\(^{-1}\) flesh on a wet basis. Differences found in both constituents among specimens may be explained in terms of individual variations, so that no significant differences (p>0.05) were obtained as a result of pre-storage soaking in SMB or storage time. Both constituents (water and lipids) exhibited in lobster krill values similar to those reported for Norway lobster (*Nephrops norvegicus*) (Losada et al., 2006), but were different from those found in both pink shrimp (*Parapenaeus longirostris*) (Cadun et al., 2005) and Dungeness crab (*Metacarcinus magister*) (King et al., 1990). Thus, lobster krill exhibited higher moisture and lower lipid levels than the latter two species.

Residual sulphur dioxide content in lobster krill flesh ranged between 25 and 45 mg kg\(^{-1}\) in the SMB-25 batch, while values in the 30-70 mg kg\(^{-1}\) range were determined in the SMB-75 batch. Remarkably, the preliminary treatment applied in the current study provided chilled lobster krill flesh with a residual content of the antimelanosic agent (sulphur dioxide content) below the legal limit of 150 mg kg\(^{-1}\) flesh established by the European legislation (Rotllant et al., 2002).

Chemical parameters related to quality changes

A progressive pH increase with storage time was observed in all batches of lobster krill (r = 0.93-0.94, linear fitting; Table 4). However, no significant (p>0.05) effect of the pre-storage SMB soaking on the flesh pH of lobster krill was found. A marked pH increase (from 6.61 to...
7.18 on Days 0 and 9, respectively) had been observed during the chilled storage of Norway lobster (Aubourg et al., 2007). However, in that study the pre-storage antimelanotic treatment provided a better control of alkalinising mechanisms during flake ice storage. Lower pH values were reported for other crustacean species, Penaeus brasilensis (Moura et al., 2003) and Macrobrachium rosenbergii (Kirschnik & Viegas, 2004), kept on flake ice for similar storage times (8-10 d).

The initial FFA content (11.4±7.2 g kg⁻¹ flesh, Table 4) in lobster krill was similar to that previously reported for Norway lobster (Nephrops norvegicus) (Losada et al., 2006). FFA formation progressed with storage time (r = 0.89-0.92, logarithmic fitting, Table 4). However, a clear effect of the pre-storage soaking in SMB on FFA formation could not be demonstrated in lobster krill (p>0.05).

In global terms, lipid oxidation mechanisms in chilled lobster krill were not relevant (Table 4). Thus, PV and TBA-i scores below 4.50 and 0.15, respectively, were determined. No significant (p>0.05) differences were observed for any of these parameters as a result of the pre-storage SMB soaking or storage time. The only exception was the peroxide content on Day 10, when a significantly (p<0.05) lower content in both SMB batches was observed.

In spite of the lack of differences in lipid oxidation parameters, a higher retention of PUFA was observed in SMB-treated batches (Fig. 3). Thus, PI values were found to be lower in the control batch as compared with their counterparts in the SMB-25 (Day 3) and SMB-75 (Days 6 and 10) batches. PI scores decreased with storage time in control and SMB-25 batches. However, the correlation values were poor. PI values were in all cases in the 2.44-2.59 range, which can be considered as relatively high scores with marked positive effects from a nutritional point of view (Simopoulos, 1997).
CONCLUSIONS

Chilled lobster krill (control batch) exhibited acceptable sensory quality until Day 6, and was no longer acceptable after that time, with the general odour being the factor limiting acceptability. An enhancement of sensory acceptance was attained when lobster krill individuals were pre-storage soaked in aqueous 0.25 % or 0.75 % SMB solutions prior to storage chilled, so that SMB-treated samples were still acceptable at the end of the study (Day 10). A partial inhibition (p<0.05) of the growth of Enterobacteriaceae, psychrotrophes and proteolytic bacteria could be observed as a result of the pre-storage SMB soaking. Although there were no differences in relevant lipid oxidation mechanisms among the batches, a higher (p<0.05) retention of PUFA content was found in SMB-treated lobster krill, especially in the SMB-75 batch.

The results presented in this study open the way to the potential commercialisation of currently under-utilised lobster krill as a chilled marine product. Moreover, a simple preservation step, SMB soaking prior to chilling, was successfully applied to retain quality. Further research is needed to reinforce the commercial and technological possibilities to provide the consumer with a new, attractive and nutritious crustacean species. Thus, the effect of the catching season on quality changes during processing ought to be checked.

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REFERENCES


FIGURE LEGENDS

**Fig. 1.** Psychrotrophes (log CFU g⁻¹ flesh) in chilled lobster krill during chilled storage. Mean values of three replicates (n=3); standard deviations are indicated by bars. For each chilled storage time, values accompanied by different letters (A, B) denote significant differences (p<0.05) as a result of the pre-storage soaking condition. For each pre-storage soaking condition, values accompanied by different letters (a, b, c) denote significant differences (p<0.05) as a result of the chilled storage time. No letters are included when no significant differences (p>0.05) are found. Initial lobster krill values: 4.05±0.06. Treatments given to batches Control, SMB-25 and SMB-75 are as explained in Table 2.

**Fig. 2.** Proteolytic bacteria (log CFU g⁻¹ flesh) in chilled lobster krill during chilled storage. Mean values of three replicates (n=3); standard deviations are indicated by bars. For each chilled storage time, values accompanied by different letters (A, B, C) denote significant differences (p<0.05) as a result of the pre-storage soaking condition. For each pre-storage soaking condition, values accompanied by different letters (a, b, c) denote significant differences (p<0.05) as a result of the chilled storage time. No letters are included when no significant differences (p>0.05) are found. Initial lobster krill values: 2.58±0.28. Treatments given to batches Control, SMB-25 and SMB-75 are as explained in Table 2.

**Fig. 3.** Polyene index measurement in chilled lobster krill during chilled storage. Mean values of three replicates (n=3); standard deviations are indicated by bars. For each chilled storage time, values accompanied by different letters (A, B) denote significant differences (p<0.05) as a result of the pre-storage soaking condition. For each pre-storage soaking condition, values accompanied by different letters (a, b) denote significant differences (p<0.05) as a result of...
the chilled storage time. No letters are included when no significant differences (p>0.05) are found. Initial lobster krill values: 2.56±0.06. Treatments given to batches Control, SMB-25 and SMB-75 are as explained in Table 2.