QUALITY AND SHELF LIFE OF THE ADDUCTOR MUSCLE OF LION’S PAW SCALLOP *Nodipecten subnodosus* TRANSPORTED AND STORED WHOLE IN REFRIGERATION


SUMMARY

The effect of refrigerated transport during 48h and four days storage on the quality and shelf life of the adductor muscle of whole lion’s paw scallops (*Nodipecten subnodosus*) was evaluated. Proximal composition, ATP and related products, K-value, total volatile bases (TVB-N) and trimethylamine (TMA-N), pH, and microbiological analyses were quantified. The muscle was found to be lean and high in protein. Levels of muscular ATP were initially low and the K-value increased linearly, but according to this parameter the product was considered edible until the end of the storage period. With respect to TVB-N and TMA-N, the allowed limits were not exceeded. The pH level showed no significant variations during storage. According to TVB-N, TMA-N values and microbiological analyses performed, adductor muscle proved to be innocuous after four days under the conditions of transportation and storage utilized.

Introduction

The lion’s paw scallop *Nodipecten subnodosus* constitutes one of the most important fishery resources on Mexico’s Baja California Peninsula. The commercial importance of this scallop rests on the size of the organisms (22cm tall) and their weight (250g at 5 years’ growth), the price (USD16/kg in the international market), flavor, and the production of their adductor muscle. Its production is concentrated in the Ojo de Liebre Lagoon, state of Baja California Sur (BCS), and in recent years it has stabilized at 100ton/year. Another important characteristic of this species is its high growth rate, as it attains a size of 7cm (commercial size) in only eight months. For the aforementioned reasons, fish farmers have become interested in producing this species and significant efforts are underway to consolidate its cultivation (*Massó-Rojas et al., 2001; Pacheco-Aguilar et al., 2001; Maldonado-Amparo et al., 2004*).

While in both Mexico and the USA the principal product consumed is the fresh refrigerated adductor muscle, other countries, mainly in Asia and Europe, have a high demand for the whole shelled bivalves. These products are usually consumed after being cooked in their shells, but are also served raw in dishes of the *sushi* or *sashimi* type (*Paust and Rice, 2001; Pacheco-Aguilar et al., 2001*).

In terms of the nutritional value of pectinids, the main edible portion is the adductor muscle, which has a high protein content and a high biological value, comparable to that of casein and meat. Moreover, it is classified as lean food due to the fact that its lipid content is ≤0.7% (*Ocaño-Higuera et al., 2001*). In addition to the nutritional aspects, another factor that must be considered in the seafood product market so as to determine the optimum means of distributing this scallop, maximize its value, and promote its consumption, is the quality and innocuous nature of the final product. Like many other aquatic organisms, upon their death the pectinids undergo a series of biochemical changes that strongly impact the quality and shelf life of finished products. These postmortem changes can be studied by monitoring certain parameters and indicators of quality and freshness (*Sikorski et al., 1990; Abbas et al., 2008; Ocaño-Higuera et al., 2009; 2011; Liu et al., 2010*).

At present there is no feasible and established strategy for exporting and distributing this particular pectinid in its

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whole, fresh refrigerated, presentation for consumption on the half shell, as is the case with oysters. In practice, handlers strive to maintain the cold chain right from the moment when the specimens are harvested in their natural habitat until they are processed and/or consumed, but this is not always achieved. It is also worth to note that when delivered fresh to intermediaries or final sellers, whole organisms can fetch a price as high as 2.00 USD/scallop. It is thus necessary to look for and design alternative storage methods during transport and distribution that will assure that the quality and adequacy of the product will be maintained until offered to the final consumer. The objective of this study was to evaluate the quality and shelf life of the adductor muscle of whole lion’s paw scallops during refrigerated transport and storage, as a possible alternative for exporting the product to international markets.

**Materials and Methods**

**Experimental organisms**

Adult organisms of lion’s paw scallops, *N. subnodosus*, with a height of ~10cm were utilized, after being harvested from a culture system located in Bahía Tortugas, BCS, in June 2009. Before transport and storage the scallops were cleaned with an oyster machine that functions on the basis of sprayers and brushes (Ets. Bertrand, Marennes, France). The cleaning process was completed with the specimens being brushed by hand and then cleared in 200 liter fiberglass tanks for 1h.

**Experiment on transport and storage**

The packing of the organisms prior to transport involved placing them in coolers made of expanded polystyrene with a capacity of 45 liters. The live organisms were arranged dorso-ventrally with elastic bands attached to prevent them from opening their valves and possibly drying out. A total of 120 organisms were collected and distributed into two coolers. During the packing process, the scallops were placed in a horizontal position and a bed of brown paper moistened with seawater was placed between the layers of mollusks. Once the organisms were packed, and just before sealing the coolers, 5kg of frozen refrigerant gel (Dolphin Blue Crabs) at -20ºC was introduced and a thermograph was placed to monitor the internal temperature of the coolers during transport, as shown in Figure 1. The refrigerant gel was separated from the upper layer of scallops by another bed of moistened brown paper.

At that moment, the adductor muscles of six scallops were frozen in liquid N₂, to be used to determine the initial biochemical conditions at the time of transport. Transportation was simulated under the most realistic conditions possible. In the first phase, the coolers with the refrigerant gel and the scallops were carried in a hardpack truck at ambient temperature for 14h. Then a second stage of transportation was simulated, in which...
the sealed coolers were placed in a refrigerated room at the installations of CIBNOR, La Paz, BCS. This procedure was meant to simulate the second stage of transportation, as when the organisms are transported in refrigerated trucks. The total simulated transportation time was 48h. This simulation of the stages of transportation was designed in accordance with the conditions assumed necessary to deliver this product to an international market, in this case that of the USA.

In the last phase, the organisms were removed from the coolers and placed in refrigerated storage (3-6°C) in polyethylene bags to simulate the actual storage facilities utilized by the final receiver, who would then undertake to sell the product. At that moment, the adductor muscles of six organisms were dissected and frozen in liquid N$_2$ for later determination of the effects of transportation and to establish the quality and shelf life at day 0. Finally, a thermograph was placed to monitor the temperature of the refrigerated room throughout product storage (Figure 1).

**Sampling for the study of refrigeration**

The organisms transported under the simulated conditions described above remained in the refrigerated storage area for a period of four days, which is a common time to market these products. During this time, the adductor muscles of six organisms were collected, dissected, and frozen in liquid N$_2$ every 24h, and then stored at -80°C until analysis. At each point of the storage period, microbiological analyses were conducted to separate the compounds. The mobile phase used was a phosphate buffer made up of 0.04M KH$_2$PO$_4$ and 0.06M K$_2$HPO$_4$. The flow rate utilized was 1ml·min$^{-1}$, and detection was carried out at 254nm in a UV-Vis Varian Prostar 325 detector. The values obtained were expressed as μmol·g$^{-1}$ of sample. The K-value (%) was calculated according to the equation described by Saito et al. (1995):

$$\text{K-value (})%\text{=}\frac{(\text{HxR}+\text{Hx})(\text{ATP}+\text{ADP}+\text{AMP}+\text{IMP}+\text{HxR}+\text{Hx})\times100}{(\text{HxR}+\text{Hx})/(\text{ATP}+\text{ADP}+\text{AMP}+\text{IMP}+\text{HxR}+\text{Hx})}$$

where ATP: adenosine 5′triphosphate, ADP: adenosine 5′diphosphate, AMP: adenosine 5′monophosphate, IMP: inosine 5′monophosphate, HxR: inosine, and Hx: hypoxanthine.

**TVB-N**

The determination of TVB-N was conducted following the method described by Woyewoda et al. (1986). Here, 2g of the sample were taken and homogenized in a distilling flask with 300ml water and 2g MgO, using a T8 Basic Ultra-turrax (IKA Works, Inc, Wilmington, NC, USA). Twenty drops of vegetable oil were added as anti-foaming agent and the solution was then distilled for 25min. The distillate was collected in 15ml of 2% boric acid and then titrated with 0.05N H$_2$SO$_4$. A control sample was distilled under the same conditions and the values obtained were expressed as mg TVB-N/100g of sample.

**Microbiological analyses**

Microbiological analyses were carried out according to the recommended methods set out in NOM-031-SSA1-1993 (NOM, 1995) every 24h for 4 days. A total of six organisms
were collected for each 24h period. The moist tissues were extracted from the scallops and homogenized to obtain a representative sample. These procedures were conducted at the CIBNOR Microbiological Diagnostics Laboratory in La Paz, BCS, where the number of aerobic bacteria and fecal coliforms were determined in tryptone yeast extract agar media (48h at 35 ±2°C) and EC media (44 ±2°C), respectively. Also, samples were taken to be analyzed for Vibrio cholerae and Salmonella spp. (six whole organisms) for days 1 and 4 of refrigerated storage. The V. cholerae and Salmonella spp. tests were carried out in TCBS media (24h at 35-37°C) and brilliant green (24h at 35 ±0.5°C), respectively.

Statistical analysis

A one-way variance analysis (ANOVA) was conducted. When significant differences were found, the multiple comparison Tukey test was applied using the NCSS Ver. 2000 program (Hintze, 2001). Also, in some cases regression analyses were run. A significance level of 5% was set for all of the analyses conducted.

Results and Discussion

Proximal analysis

The percentage of moisture in the adductor muscle was 75.18 ±0.94 and was characterized by the presence of a high protein content of 17.88 ±0.46% and a low lipid content of 0.11 ±0.01%. For this reason, and following the classification reported by Domínguez and Gutiérrez (1993), the muscle was considered lean (<5% lipids). As is the case with most pectinids, N. subnodosus also contains considerable amounts of carbohydrates, mostly glycogen, that serve as an energy reserve in the adductor muscle; the value was 7.08 ±1.58%. Finally, the percentage of ash was 1.40 ±0.10 and, as was the case for the values of the rest of the components, similar to that reported by Beltrán-Lugo et al. (2006) and Pacheco-Aguilar et al. (2008) for N. subnodosus and by Ocaño-Higuera et al. (2006) for the catarina scallop Argopecten ventricosus.

pH

Table I presents the data on the pH of the adductor muscle of the lion’s paw scallop transported in immersion and stored whole under refrigeration. The initial value of this parameter in the organisms immediately after collection and before conditioning and storage (pH= 7.02) is similar to that reported by Kimura et al. (1999) for P. yessoensis, but higher than the value of 6.80 reported by Beltrán-Lugo et al. (2006), also for N. subnodosus. The difference between the latter figure and the initial value found in the present study may be due to the conditions employed by Beltrán-Lugo et al. (2006) during transport to the site where analyses were conducted, as the specimens spent 30min in a simple cooler conditioned with seawater. Under those conditions, a first stage of anaerobic glycolysis may have taken place, with the concomitant lactic acid production and pH reduction (Durán et al., 2008; Mørkøre et al., 2008).

The initial neutral pH values recorded in the recently collected organisms diminished significantly (P<0.05) during the stage of simulated transport and on the first day of refrigerated storage. However, after that day and up to the end of the study, no further changes were observed. According to Riaz and Qadri (1985), an increase <0.1 units during storage indicates deterioration and loss of freshness, as occurs when alkaline-type metabolites are produced as a result of bacterial proliferation. In the present study, the pH of the adductor muscle did not increase during storage, so it can be said that in relation to this parameter the adductor muscle retained its edible quality throughout the period and under the conditions used in this study. This behavior and similar values have been reported for N. subnodosus and for A. ventricosus by Pacheco-Aguilar et al. (2008) and by Ocaño-Higuera et al. (2006), respectively.

ATP, Products of its Breakdown, and the K-value

Table I shows the values for ATP and related products obtained in the adductor muscle of the lion’s paw scallop at the moment of collection, during transport in emersion, and while stored whole in refrigeration. The first organisms obtained immediately after the cleaning process and those collected after the 48h period of simulated transport showed low levels of muscular ATP, values that may be the result of the stress generated during the conditioning and cleaning of the organisms, which fostered the breakdown of this energy metabolite. The concentration of this metabolite increased on the first day (P<0.05), but then remained with no significant variation during the rest of the storage period (P>0.05). This increase on day 1 may be due to the regeneration of ATP through the action of the adenylate kinase enzyme, or to the utilization of the phosphoarginine phosphagen in a reaction catalyzed by the arginine kinase enzyme, as has been reported for other species, such as the abalone Haliotis discus (Watanabe et al., 1992) and the scallop Zygoclamys patagonica (Massa et al., 2001).

The concentration of muscular ADP showed a significant variation during transport (P<0.05), but no important variations during the storage (P>0.05), whereas AMP diminished significantly (P<0.05) after day 1 of storage, but then became stable at ~1μmol·g⁻¹, and IMP was found in concentrations <0.15μmol·g⁻¹ through-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Initial organisms</th>
<th>Post-transport (day 0)</th>
<th>Storage day</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.08 ±0.02 a</td>
<td>6.43 ±0.05 b</td>
<td>6.29 ±0.10 c</td>
</tr>
<tr>
<td>ATP</td>
<td>0.03 ±0.00 bd</td>
<td>0.02 ±0.00 cde</td>
<td>0.05 ±0.01 a</td>
</tr>
<tr>
<td>ADP</td>
<td>0.33 ±0.01 a</td>
<td>0.25 ±0.03 b</td>
<td>0.26 ±0.08 b</td>
</tr>
<tr>
<td>AMP</td>
<td>3.95 ±0.37 a</td>
<td>3.61 ±0.68 a</td>
<td>1.44 ±0.46 b</td>
</tr>
<tr>
<td>IMP</td>
<td>0.05 ±0.00 b</td>
<td>0.08 ±0.01 a</td>
<td>0.12 ±0.05 a</td>
</tr>
<tr>
<td>HxR</td>
<td>0.10 ±0.02 c</td>
<td>0.76 ±0.01 d</td>
<td>1.43 ±0.30 c</td>
</tr>
<tr>
<td>Hx</td>
<td>0.12 ±0.03 c</td>
<td>0.10 ±0.09 c</td>
<td>0.37 ±0.07 b</td>
</tr>
<tr>
<td>TBBV-N</td>
<td>6.19 ±1.62 b</td>
<td>6.97 ±2.35 b</td>
<td>11.66 ±1.76 a</td>
</tr>
<tr>
<td>TMA-N</td>
<td>0.16 ±0.00 c</td>
<td>0.19 ±0.01 a</td>
<td>0.17 ±0.01 b</td>
</tr>
</tbody>
</table>

Values are mean and standard deviation of n= 6. Means in the same row with different superscript letters are statistically different (P<0.05).
out the experiment. For the latter two compounds, which are related to the sweet, pleasant flavor of seafood products, a behavior similar to that reported in *P. yessoensis* (Kawashima and Yamanaka, 1992), *Z. patagonica* (Massa et al., 2001), *A. ventricosus* (Ocaño-Higuera et al., 2006), and *N. subnodosus* (Pacheco-Aguilar et al., 2008) was observed. On the other hand, HxR and Hx showed significant variations during the experiment, but their concentrations during the storage period were always below those reported by Ocaño-Higuera et al. (2006) and Pacheco-Aguilar et al. (2008) for *A. ventricosus* and *N. subnodosus*, respectively. This finding is relevant, since these compounds have been related to a bitter taste in seafood products. The concentrations found in this experiment could be the result of low autolytic and bacterial activity in the adductor muscle.

In the case of the K-value for the adductor muscle (Figure 2), as was to be expected in the organisms analyzed directly from the culture site, the value obtained was low, 4.58 ±0.41% (data not shown on the graph), although it increased significantly (P<0.05) during simulated transport, reaching a value of 19.77 ±7.11% on day 0 of the refrigerated storage experiment. This is due to the increased HxR concentration at this time.

Saito et al. (1959) and Sikorski et al. (1990) elaborated a rather general classification of the quality of seafood products with respect to their K-value. In it, a K-value <20% indicates a ‘very fresh’ product, while products with values between 20 and 50% are considered ‘moderately fresh’, and those >70% are ‘not fresh’. In terms of this classification, the object of this study can be considered ‘very fresh’ after two days of simulated transport and, indeed, maintained that standard until the first day of refrigerated storage, when it reached 44.87 ±3.24%. After that, and despite the significant increase in the K-value (P<0.05), according to this parameter the product remained edible until the end of the storage period. In a regression analysis, the K-value of the adductor muscle during the refrigerated storage phase showed a linear increase (y= 12.07x +27.04, r² = 0.90, P<0.05). This behavior was reported previously by Ocaño-Higuera et al. (2006) for *A. ventricosus*.

**TVB-N**

Determining TVB-N allowed to quantify the low molecular weight volatile bases and amino compounds produced by the microbial decarboxylation of amino acids and nitrogenated compounds, which are widely used as indicators of shelf life and, more specifically, to measure stages of deterioration or loss of freshness. Table I presents the TVB-N values for the adductor muscle of lion’s paw scallop *N. subnodosus* transported in immersion and stored whole in refrigerated conditions. It shows the initial low values of TVB-N (6.97 ±2.35mg/100g), which are below those found in this same species by Pacheco-Aguilar et al. (2008) and those obtained by Ocaño-Higuera et al. (2006) for *A. ventricosus*. In the present study, an increase (P<0.05) of TVB-N was seen on day 1 of storage in refrigeration, but no additional significant change was observed (P<0.05). In this regard, it is important to point out that the NOM-029-SSAI-1993 norm (NOM, 1994) establishes a maximum limit of 30mg/100g. According to this parameter and our analysis, the adductor muscle was considered apt for human consumption at the end of the study in refrigeration.

**TMA-N**

One indicator that is commonly used to evaluate bacterial deterioration in seafood products is the determination of TMA-N, a compound with a characteristic ammonia odor that may reflect bacterial deterioration (Ryder et al., 1984). As the results obtained from the muscle shown in Table I reveal, the initial values of TMA-N of organisms obtained directly from the lagoon increased during the transport stage, and variations were also observed during storage (P<0.05). However, the TMA-N concentrations found for the muscle under the conditions of transport and storage used were lower than those reported in other studies conducted with this same species and with *A. ventricosus*, even though the latter included only adductor muscles stored at a temperature of 0ºC (Ocaño-Higuera et al., 2006; Pacheco-Aguilar et al., 2008). This indicator has been associated with unpleasant odors in fish and other seafood species when it exceeds certain limits. In this regard, limits of 5-10mg/100g of muscle have been established (Sikorski et al., 1990), which are higher than those found in the present study.

**Microbiological Analyses**

The results of the microbiological analyses carried out on lion’s paw scallop during refrigerated storage, shown in Table II, reveal differences in the amount of mesophilic aerobic bacteria on different days of storage (P<0.05). These indexes did not reach at any moment of the experiment the maximal limit of 5×10⁵ CFU/g (5.69 log₁₀ CFU/g) allowed for human consumption according to the NOM-031-SSAI-1993.

### TABLE II

**MICROBIOLOGICAL ANALYSIS OF THE TOTAL WET BIOMASS IN THE LION’S PAW SCALLOP N. subnodosus TRANSPORTED AND STORED WHOLE IN REFRIGERATION CONDITIONS (3-6°C)**

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>Aerobic mesophiles (log₁₀ CFU/g)*</th>
<th>Faecal coliforms (MPN/100 g)**</th>
<th><em>Vibrio cholerae</em></th>
<th><em>Salmonella spp</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.42 ±0.08*</td>
<td>&lt; 30</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1</td>
<td>3.16 ±0.02*</td>
<td>&lt; 30</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>2</td>
<td>4.32 ±0.09*</td>
<td>40</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>2.24 ±0.09*</td>
<td>40</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>3.49 ±0.06*</td>
<td>40</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>

*Values are mean and standard deviation of n=2. Means in the same column with different superscript letters are statistically different (P<0.05).

**Statistical values calculated from Table 4 of the NMX-AA-042-1987 (NMX, 1987).
ND= Not Determined.
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REFERENCES


