




Use of nylon net packing to increase the survival time of cultured mussels

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ABSTRACT

Mussels survive a few days after harvesting, limiting the trade of these animals when alive. The study evaluated the effect of nylon net packing on the survival and shelf life of *Perna perna* mussels farmed in Brazil. Thirty samples containing 10 or 11 market-size mussels were stored in an incubator at 4°C for four days, half packed with nylon netting and the other half maintained within opened containers. The experiment analyzed the following parameters daily: survivorship, intervalvar fluid loss, total volatile bases, pH, and mesophilic and psychrotrophic bacteria counts. By the end of the assay, chemical and microbiological parameters were all acceptable for human consumption. All packed mussels survived up to the second day of storage, while the control group recorded an average survival of 29.7% in the same period. Packed mussels survive longer, probably because the tight net prevents animals from losing liquid by maintaining their shell valves closed during storage. This packaging method extends the shelf life of live products and improves the potential trading live mussels to consumer centers far from marine farms.

Keywords: Bivalve molluscs; Shelf life; *Perna perna*.

Uso de embalagem com rede de náilon para aumentar o tempo de sobrevivência de mexilhões cultivados

RESUMO

Os mexilhões sobrevivem alguns dias após a colheita, limitando o comércio desses animais quando vivos. O estudo avaliou o efeito da embalagem com rede de náilon sobre a sobrevivência e vida útil de mexilhões *Perna perna* cultivados no Brasil. Trinta amostras contendo 10 ou 11 mexilhões de tamanho comercial foram armazenadas em incubadora a 4°C por quatro dias, metade das amostras embalada com rede de náilon e a outra metade mantida em recipientes abertos. Os seguintes parâmetros foram monitorados diariamente: sobrevivência, perda de fluido intervalvar, bases voláteis totais, pH e contagem de bactérias mesófilas e psicrotóxicas. Ao final do ensaio, os parâmetros químicos e microbiológicos estavam todos aceitáveis para consumo humano. Todos os mexilhões embalados sobreviveram até o segundo dia de armazenamento, enquanto o grupo controle registrou sobrevivência média de 29,7% no mesmo período. Os mexilhões embalados sobreviveram por mais tempo provavelmente porque a rede apertada evita que os animais percam líquido, mantendo as valvas de suas conchas fechadas durante o armazenamento. Esse método de embalagem prolonga a vida útil dos produtos vivos e melhora o potencial de comercialização de mexilhões vivos para centros consumidores distantes das fazendas marinhas.

Palavras-chave: Moluscos bivalves; Tempo de prateleira; *Perna perna*.

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INTRODUCTION

World aquaculture produced 2.1 million tonnes of mussels of different species in 2020 (FAO, 2022b), with a higher proportion traded as a live product (Hirabayasi et al., 2022). In markets like the European Union, more than 80% of the traded volume is estimated to be live mussels with shells (Monfort, 2014).

Brazil is the world's most important producer of the South American Rock mussel, *Perna perna*, with 9,800 tons produced in 2021 (Observatório Agro Catarinense, 2023). Although this species occurs in different countries in Africa, Europe, and South America, only Brazil and Venezuela are producers of these mussels based on aquaculture (FAO, 2022a). Unfortunately, there are no official statistics on the mussel trade in Brazil. However, it is notorious that the national market prefers cooked, shelled, and chilled or frozen mussels (Barni et al., 2003; Demarchi, 2003; Furlan et al., 2007), while live mussels supply local markets or specific niches, such as haute cuisine restaurants.

According to Furlan et al. (2007), this preference is possibly due to the short shelf life of live mussels and the ease of storing, transporting, and marketing the shelled, chilled, or frozen products. Indeed, trading in live mussels is more challenging than cooked and shelled products. *Perna perna* mussel shells represent approximately 2/3 of their weight, and the percentage of cooked meat can reach 40% of their live weight, in a best scenario of gonad maturation (Silvestri et al., 2018). Furthermore, live mussels have a significantly greater volume than cooked and shelled mollusks, affecting product transport. Furthermore, shelf life is a major challenge related to the trade of live mollusks. In general, refrigerated mussels maintain optimal quality in the first two days of storage, and, after that, their microbiological quality, flavor, and palatability reduce gradually (Hirabayasi et al., 2022).

In Brazil, the live mussels processed in approved facilities and sold legally are transported in plastic bags packed inside styrofoam boxes with ice. In the illegal market, animals are transported without specific packaging, commonly in plastic boxes used for transporting vegetables. In countries such as Spain, Italy, Norway, New Zealand, the United States of America, and the United Kingdom, mussels and other bivalve mollusks are commonly sold alive in plastic net packages containing 1, 2, 5, or 10 kg of the product (Barrento et al., 2013), for traceability and convenience reasons. Although this practice is common for commercializing mollusks cultivated in other countries, there is no record of the use of plastic nets for packaging *P. perna* mussels in Brazil.

In order to evaluate the possibility of using this type of packaging in Brazil as a new form of presentation, this research aimed to evaluate the survival and shelf life of mussels *Perna*

perna packaged in plastic net bags. The hypothesis of the study was that mussels packaged in plastic net bags have longer survival and shelf life than non-packaged mussels.

MATERIAL AND METHODS

Perna perna mussels with an average shell length of 7.52 ± 0.55 cm (mean \pm standard deviation) were mechanically harvested, declumped, and washed by a mussel harvesting vessel from a marine farm in the municipality of Palhoça, Santa Catarina, Brazil. The mussels were transferred from the vessel's storage bins to styrofoam boxes containing ice packed in plastic bags. After that, they were transported to the laboratory (transport time of 40 minutes), where only animals with intact shells were selected for the study. Mussels transport temperature, measured at 10 minutes intervals, was $21.2 \pm 1.26^\circ\text{C}$ (mean \pm standard deviation). A sample of 24 animals was used to determine the condition index (CI). The CI of each of the 24 sampled animals was determined by the ratio between the weight of their meat and their total weight after cooking for 10 minutes in boiling water, according to Eq. 1.

$$\text{CI} = \left(\frac{\text{meat weight}}{\text{total weight}} \right) \times 100 \quad (1)$$

Fifteen samples of ~350 g each, containing 10 or 11 mussels, were packed with nylon netting with a 20 mm mesh opening (Fig. 1).

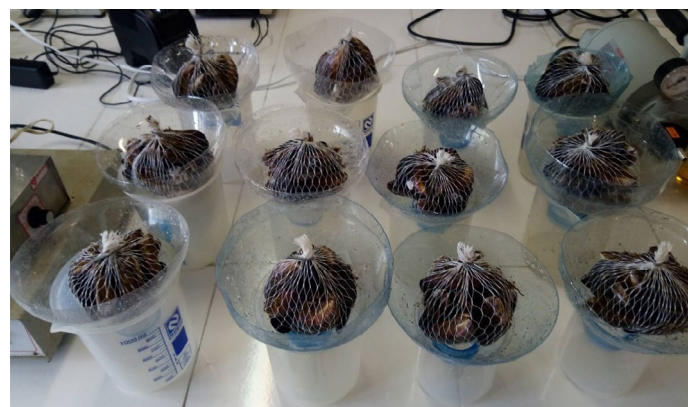


Figure 1. Mussels packed with nylon netting.

A tubular net with an 18 cm radius, 0.2 mm thick string and 25 mm distance between nodes was used to package the mussel samples. The net was cut into 45 cm long pieces, and metal clips were used to seal the packages. During the package sealing, the plastic net bags were tensioned to keep them tight, preventing mussels from opening their shells during storage. The packed samples were kept inside plastic funnels positioned over 1 L graduated beakers aimed at storing the drained water. Another 15 samples of mussels were used as control and kept under

the same conditions within the funnels, but without the nylon net packaging. All samples were stored in a DBO incubator (SolidSteel, SSBODu 342L, Brazil) at 4°C for four days.

Daily, all the samples were removed from the incubator for analysis. In order to record the intervalvar liquid loss, the liquid content of each beaker was transferred to a graduated cylinder, and their volumes were recorded (in mL). Mussels survival, physical-chemical and microbiological analyses were carried out daily in three of the samples of each treatment. The analyses are described below.

Total volatile bases

The total volatile bases (TVB) analysis was performed following Brazilian official methods for animal-source foods (Brasil, 2019), with some modifications. Five grams of mussel meat were homogenized with 45 mL of 6% perchloric acid solution using a homogenizer Turrax (IKA, T18 digital, China) for 2 minutes and then filtered on filter paper. A 25 mL aliquot of the filtrate was placed in the steam distillation apparatus along with five drops of phenolphthalein and, when necessary, a few drops of antifoam and 3.25 mL of 20% sodium hydroxide. The distillate was collected in an Erlenmeyer flask with 50 mL of 3% boric acid and five drops of Tashiro indicator. Distillation was considered completed when a final volume of 100 mL was obtained (50 mL of distillate + 50 mL of solution), and then titration with 0.1M hydrochloric acid was performed. Finally, a blank test was performed, replacing the 25 mL filtrate with 25 mL of 6% perchloric acid solution. The results were expressed in mg TVB-N per 100 g of mussel meat sample.

pH

The mussel meat pH was determined using a pH meter (Hanna-HI 2020-02), placing the sensor in approximately 10 g of homogenized mussel meat sample.

Microbiological analyses

Sterile bags were filled with 25 g of mussel meat sample and 225 mL of 0.1% peptone water with 0.05% sodium chloride (PEP), and these samples were homogenized in a stomacher. The mesophilic bacteria count followed the ISO 4833:2015 (ABNT, 2015) method. Serial decimal dilutions were inoculated using the pour plate technique in sterile Petri dishes with the subsequent addition of plate count agar (PCA), previously melted and cooled. The plates were incubated at $30 \pm 1^\circ\text{C}$ for 48 ± 2 hours, and the results were expressed in $\text{CFU}\cdot\text{g}^{-1}$. Psychrotrophic bacteria counts followed the method of the American Public Health Association (APHA, 2015). Serial decimal dilutions were inoculated by surface seeding on PCA agar. Plates were incubated at $7 \pm 1^\circ\text{C}$ for 10 days. The results were expressed in $\text{CFU}\cdot\text{g}^{-1}$.

Survival of mussels

The animals were left at room temperature for half an hour before the assessments to prevent the low temperature from affecting the vitality assessment. We considered alive the animals that had their shell valves closed or those with slightly opened valves that closed them after being pressed with the fingers. Mussels were considered dead when their shell valves remained open after 10 quick squeezes to stimulate their shell movement. This method is commonly used for survival assessment and is known as the British standard squeeze method (Dunphy et al., 2015).

Statistical analysis

The datasets did not meet the assumptions of normality and homoscedasticity. Therefore, the analyses were carried out through classic statistical tests using ranked data. Two-way analysis of variance (ANOVA) was used to study the effect of packaging and storage time on the different parameters analyzed. Tukey's test was used as a post-hoc analysis to compare the results obtained at different storage times. Unlike the other analyzed parameters, which had results generated in triplicates, fluid loss results were generated for a decreasing number of samples throughout the study since part of the samples was destined daily for microbiological and physicochemical analysis until only six remained. Therefore, six results were randomly selected from each treatment's time steps to avoid biases due to the greater number of results available at the beginning than at the end of the assay.

RESULTS

The mean CI of the mussels used in the experiment was $24.77\% \pm 4.03$ (mean \pm standard deviation). Two-way ANOVA showed that the main parameters affected by packaging, either directly or through interaction with storage time, were survival, fluid loss, and pH. Both packaging ($p = 9.587 \times 10^{-8}$) and storage time ($p = 5.366 \times 10^{-9}$) had significant effects on mussel survival (Figs. 2a and 2b). Mussels packed in plastic netting showed the highest survival rates, and decreasing survival values were observed between days 1 and 3 of storage.

Furthermore, mussel survival was affected by a significant interaction [$F(4,20) = 6.7066$, $p = 0.001$] between packaging and storage time. Figure 2b shows that this is a significant interaction and that the effect of storage time on survival is more remarkable for the control (CT) than for the net treatment (NT). NT maintained a survival of 100% in all experimental units until the second day of storage, presenting an average survival of 53.3% at the end of the assay. In the CT, an average survival of

less than 30% was recorded on the second day of storage, and virtually no live mussels were found from the third day on.

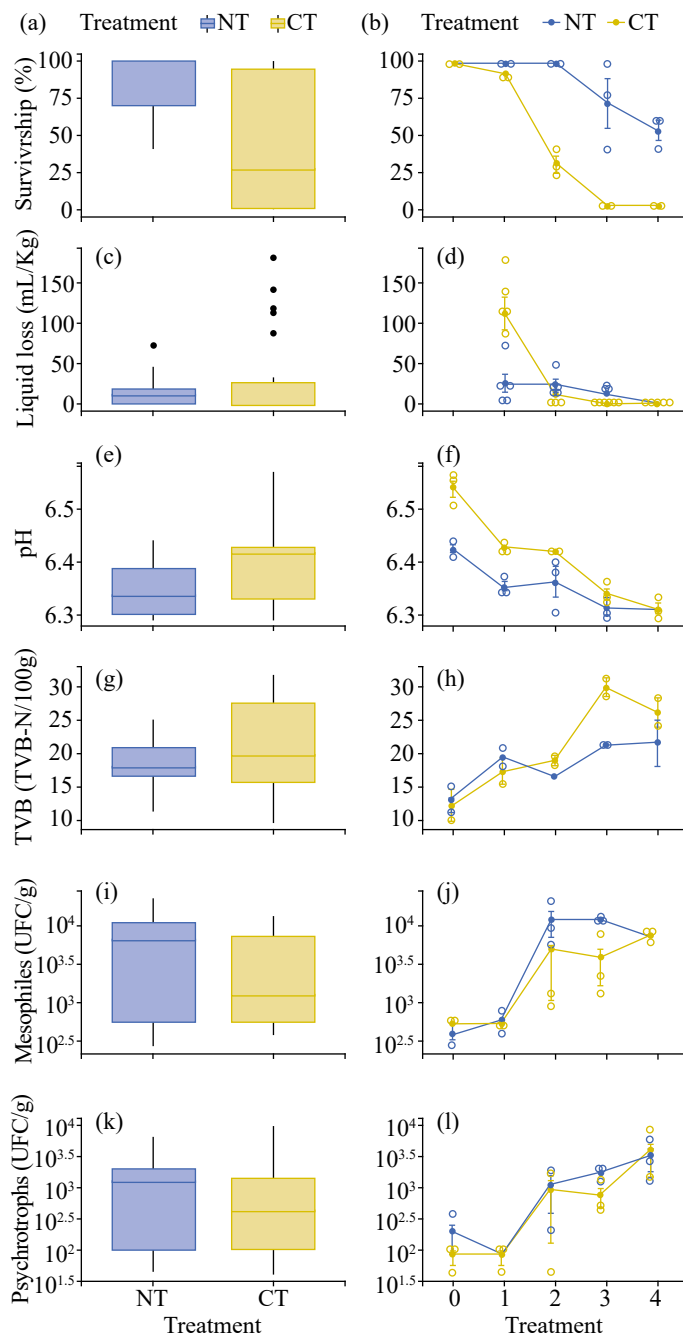
There was no significant effect of packaging ($p = 0.30$) on liquid loss (Fig. 2c). However, storage time ($p = 1.39 \times 10^{-11}$) and the interaction between this factor and packaging [$F(4,20) = 7.4327$, $p = 0.0004$] showed significant effects. A decreasing fluid loss was observed, with significant differences ($p < 0.05$) between the analyzed days. Regarding the interaction between the factors, it is possible to note (Fig. 2d) that the effect of time on fluid loss was more relevant in the CT, which showed an initial peak and a clear drop between the first and the second day of storage. NT tended to maintain more stable values over time.

Both packaging ($p = 0.0005$) and storage time ($p = 9.775 \times 10^{-08}$) had significant effects on mussel meat pH (Figs. 2e and 2f). A higher pH was registered in CT than in NT, and a decreasing pH trend over time was observed, with significant reductions recorded between days zero and 1, and between days 2 and 3. However, there was no significant interaction between storage time and packaging ($p = 0.21$).

No significant effects of packaging ($p = 0.55$) or of storage time ($p = 0.36$), nor the interaction between these factors ($p = 0.97$), were detected on TVB (Figs. 2g and 2h). The analyses did not show significant effects of packaging on the investigated microbiological parameters, mesophiles ($p = 0.19357$) and psychrotrophs ($p = 0.11$) either, nor of their interaction with storage time [mesophiles $F(4,20) = 2.53$, $p = 0.072$, psychrotrophs $F(4,20) = 0.42$, $p = 0.79$] (Figs. 2i and 2k). Only the effect of storage time was detected for both factors ($p < 0.0001$), with a bacterial count increase over time. Mesophiles displayed a significant increase between the storage days 1 and 2 of storage (Fig. 2j), and, in the case of psychrotrophs, between days 2 and 3 of storage (Fig. 2l).

DISCUSSION

Mussels of different species cultivated around the world, in general, survive a few days out of water. However, Barrento et al. (2013) report the transport of live mussels packed in nets and refrigerated (3-5°C) for up to 72 hours in countries such as Norway and Ireland. The results of the present study showed that, without packaging, most of the *P. perna* mussels (~70%) die up to the second day of storage in the refrigerator. In addition, they showed that packaging in plastic nets significantly increases the survival of these mussels, which were all still alive after two days of storage. This result can be considered an essential gain with significant implications for the trade of live *P. perna* mussels.



TVB: total volatile bases.

Figure 2. Results of different parameters obtained for mussels packed with nylon net (NT) and non-packed mussels (CT). The graphs on the left (boxplots) and on the right (line plots) show the same data in different presentations. The boxplots display the results obtained during the whole assay combined and presented as median (horizontal line), interquartile range (box), extremes (vertical lines), and outliers (points). The line plots display the evolution of the results over storage time, in terms of mean (filled circles) and standard deviation (vertical lines), and the outlined circles represent the recorded raw values.

The results also showed the effect of packaging on liquid loss and pH, directly or through interaction with the effect of storage time. Different dynamics were observed between the treatments, with more significant initial fluid losses recorded in the CT than in NT. Desiccation is mentioned in the literature as a factor related to mussel mortality, especially after a loss of 20% or more of body weight (or intervalvar fluid) (Barrento et al., 2013). *P. perna* is a mussel species with gaping behavior (periodically opening and closing their shell valves when exposed to the air) (Nicastro et al., 2012), and we hypothesize that the pressure exerted by the nylon net kept their shell valves closed, preventing mussels from losing internal liquid during storage.

Furthermore, refrigeration equipment tends to dehydrate products (Wucher et al., 2020), what could potentialize this effect. It is important to note that the effect promoted by the refrigerator influenced the two tested treatments. Therefore, it is reasonable to expect that *P. perna* mussels, independently of net packing, stored under conditions that do not promote dehydration, for example, in styrofoam boxes with ice, might have longer shelf lives. As a reference, preliminary studies on the survival of mussels *Perna canaliculus* packed in nets and stored in styrofoam boxes with ice extended their shelf life to up to 84 hours (Yap and Orano, 1980).

Our pH results are similar to that of Furlan et al. (2007), who reported pH values between 6.3 and 6.5 in *P. perna* mussel meat after four days of storage in a domestic refrigerator at $10^{\circ}\text{C} \pm 1^{\circ}\text{C}$. Despite varying within a small range, our results showed that the NT presented a more stable pH along the storage period, while a steeper decline was seen for the CT. Ashie et al. (1996) explain that bivalves store energy in their tissues as glycogen and that the lactic acid produced, resulting from the glycogenolysis that occurs after mortality, reduces the pH. Therefore, the lower survival in the CT and the expected pH reductions caused by the glycogenolysis might justify the different pH dynamics between treatments. It is worth mentioning that different authors disregard pH as a trustable index of seafood freshness since this parameter varies from sample to sample and fluctuates during the storage period (Ogawa and Maia, 1999).

Brazilian microbiological standards for foods (Normative Instruction No. 161, of July 1, 2022) do not include limits to the parameters evaluated in the present study for bivalve mollusks (Brasil, 2022). As a reference, the International Commission Microbiological Specifications for Foods (ICMFS, 1986) suggests a mesophile maximum level of 10^7 CFU·g⁻¹ in fish samples intended for human consumption. This limit is higher than the maximum values recorded by the end of our assay, of $10^{4.3}$, suggesting

that the product from both treatments was suitable for human consumption after four days of storage. However, it is essential to note the lack of evidence regarding gains in the microbiological quality of the packed product. The levels of mesophilic and psychrotrophic bacteria increased from the second day on for both animals packed or not with plastic nets, and statistical tests could not detect differences between treatments. Therefore, it is impossible to state that plastic net packaging increases the shelf life of live mussels based on this aspect.

Finally, the results of the present study should not be directly extrapolated to mussels from natural banks. As they inhabit the intertidal zone, these mussels are periodically challenged by atmospheric conditions during low tides and are selected to withstand them. Mussels farmed in suspended systems (as in most countries that are important producers) remain submerged during all the cultivation time (Barrento et al. 2013) and do not face the same challenges, which results in more fragile animals with shorter times of post-harvest survival (Weldon, 1999).

CONCLUSION

Farmed *P. perna* mussels can be stored at the temperature of 4°C in a refrigerator for four days maintaining chemical and microbiological parameters acceptable for human consumption. Packaging them with nylon netting can significantly increase the post-harvest survival time of these mussels, extending the shelf life of live products and improving the potential of trading live mussels to consumer centers far from marine farms. Packed mussels survive longer, probably because the tight net prevents animals from losing liquid, maintaining their shell valves closed during storage. These findings indicate that packaging live *P. perna* mussels with nylon netting can benefit the industry, retailers and the shellfish consumers, and this method can be a candidate to replace other practices currently adopted in Brazil and other *P. perna* producing countries. To confirm that, future studies can investigate the effect of nylon net packing under different storage conditions, for example, within styrofoam boxes with ice, a method currently used for mussels processed in Brazilian authorized facilities.

CONFLICT OF INTERESTS

Nothing to declare.

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AUTHORS' CONTRIBUTIONS

Conceptualization: Suplicy FM; **Investigation:** Bernardi F; **Formal Analysis:** Bernardi F, Miotto M; **Data curation:** Suplicy FM, Souza RV; **Resources:** Tribuzi G; **Supervision:** Tribuzi G; **Writing – original draft:** Suplicy FM; **Writing – review & edition:** Suplicy FM, Bernardi F, Souza RV, Miotto M.

DATA AVAILABILITY STATEMENT

Data will be available on request.

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