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Scientific Article
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Shelf life of Pacific white shrimp coated with gelatin extracted from Nile tilapia scales[1]

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ABSTRACT - Gelatin coatings obtained from fish are a renewable technology with excellent benefits for the preservation of fish products and with high nutritional value, however they are very perishable. This study evaluated the shelf life of Pacific white shrimp (*Penaeus vannamei*) coated with gelatin extracted from Nile tilapia (*Oreochromis niloticus*) scales and glycerol, stored frozen at -18 °C for 180 days. For this, the gelatin was extracted and applied at a concentration of 1.5% with 0.2% glycerol in peeled and headless shrimp (treatment G). This group was compared with uncoated shrimp (control group – C), peeled and headless, regarding psychrotrophic bacterial count (PBC), pH, total volatile bases nitrogen, trimethylamine nitrogen, and thiobarbituric acid-reactive substances (TBARS) every 30 days. As a result, the obtained gelatin extraction yield rate was 24.64% and the Total Bacterial Count (TBC) range for control group was 0.70 to 2.32 log CFU/g/estimated, while for treatment G was 0.70 to 1.48 log CFU/g/estimated. The shelf life evaluation showed that the gelatin-glycerol coating solution was effective in the preservation of *P. vannamei* in PBC, pH, TVB-N, TMA-N, and TBARS parameters. It was concluded that the use of this coating combined with frozen storage can be an excellent alternative to maintain the quality of Pacific white shrimp in the long term.

Key words: Fish waste. Gelatin coating. TVB-N. TMA-N. TBARS.

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INTRODUCTION

Gelatin is a protein derived from the denaturation of collagen, commonly extracted from by-products of poultry, beef, and fishing industries (Rigueto *et al*., 2022). However, fish-derived gelatin is increasingly preferred due to concerns over religious practices (halal and kosher) and animal diseases like foot-and-mouth disease (Senarathna; Marapana, 2021). Fish is the most produced meat globally, with 223.2 million tons produced in 2022 (FAO, 2024). Furthermore, the fishing industry generates significant waste, accounting for up to 85% of the processed weight (Liu *et al*., 2022), including fish heads, viscera, skin, bones, and scales (Boronat *et al*., 2023). This waste is often discarded, causing environmental pollution (Sousa *et al*., 2022). However, it is possible to extract from this waste valuable materials, such as gelatin (Nitsuwat *et al*., 2021), which helps reduce pollution and promotes sustainable fish consumption and production (United Nations, 2023).

Gelatin is a versatile material used in food, cosmetics, and pharmaceuticals due to its biocompatibility and biodegradability (Liao *et al*., 2021). In the food industry, gelatin enhances viscosity, texture, and stability and is used in edible films and coatings that prevent microbial growth, extending the shelf life of food products (Mirzapour-Kouhdasht; Moosavi-Nasab, 2020; Zhang *et al*., 2020). Recent studies highlight the potential of edible coatings to preserve the freshness of fishery products that, despite being rich in protein, polyunsaturated fatty acids, and essential nutrients, are prone to rapid deterioration by bacteria and enzymes (Giannakourou *et al*., 2023; Jia *et al*., 2019; Liu *et al*., 2023).

Shrimp is highly valued for its flavor and nutritional content. It contains high levels of compounds like disodium guanosine-5-monophosphate (GMP) and disodium inosine-5-monophosphate (IMP), contributing to its sweet taste (Lin *et al*., 2022). However, its short shelf life complicates distribution (Khaledian; Basiri; Shekarforoush, 2021). After death, shrimp undergoes anaerobic glycolysis, leading to rigor mortis and lactic acid production, lowering the muscle pH. As autolysis progresses, enzymes produce compounds like trimethylamine, causing the unpleasant taste (Das *et al*., 2023). Due to this fact, shrimp quality control is critical, especially for long-distance transportation to global markets (Lin *et al*., 2022).

To maintain the quality of shrimp, efficient preservation technologies are needed (Pan *et al*., 2019). Various methods, such as low-temperature storage, modified atmosphere packaging, irradiation, and ozone preservation, are used to preserve aquatic products (Nagarajan *et al*., 2021). Freezing, the most common preservation method, can negatively affect quality during transport and storage due to freeze-thaw cycles (Zuanazzi *et al*., 2020). Additionally, low temperature alone may not prevent microbial growth and lipid oxidation (Ge *et al*., 2020). With increasing demand for minimally processed foods and without chemical preservatives, there is a need for biodegradable coatings with high biocompatibility, low toxicity, and antimicrobial properties (Mehraie *et al*., 2023).

Researchers are focusing on developing sustainable packaging, such as edible and non-toxic coatings and films, to preserve shrimp by reducing the microbial load, protein degradation, melanosis, lipid oxidation, pH control and thus increase their shelf life and quality (Alcântara *et al*., 2022; Das *et al*., 2023). However, the use of coatings to extend the shelf life of frozen shrimp is a recent innovation, with limited research available. From this, the aim of this research was to extract gelatin from Nile tilapia scales, apply it as a coating on peeled, headless, and frozen Pacific white shrimp, and evaluate the shelf life of frozen shrimp.

MATERIAL AND METHODS

The material used in this study was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under the code A058F53, registered on the SISGEN platform.

Thermal extraction of gelatin and yield calculation

Nile tilapia scales were purchased fresh from a fishmonger in Fortaleza, Ceará, Brazil, and taken to the Laboratory of Fishing Technology (LATEPE) of the Federal University of Ceará (UFC). Scales were immediately washed with plenty of running water to remove blood residue, pieces of meat, fins, and dirt. The scales were then dried in an oven at 35 °C for approximately 24 hours and stored at room temperature (around 25 °C). The extraction of gelatin from the scales was performed according to the method reported by Martins *et al*. (2018), with minor modifications in the thermal extraction.

For demineralization, 1,000 grams (g) of dried scales were weighed, and a 10% (w/v) sodium chloride (NaCl) solution was added in a 1:10 (m/v) ratio for leaving the mixture to stand for 24 hours at 25 °C. The scales were then washed with distilled water, immersed in 0.4 M hydrochloric acid (HCl) in a 1:10 (m/v) ratio, and left to stand for 90 min. The solution was then neutralized with sodium hydroxide (NaOH) for disposal. The flakes were then washed with distilled water until the excess solution was removed and dried in an oven at 35 °C for 24 hours, so that they were completely dry, then weighed and stored at room temperature (25 °C).

The hydrolysis process began by immersing the scales in 0.1 M acetic acid in a 1:10 (m/v) ratio for 1 hour. This was followed by alkaline treatment with 0.1 M NaOH in a 1:3 (w/v) ratio for 1 hour. Finally, treatment with 0.1 M sulphuric acid (H_2SO_4) in a 1:3 (m/v) ratio for 1 hour. The solutions used in this phase were neutralized with NaOH or HCl for disposal.

The demineralized and hydrolyzed scales were immersed in distilled water at a ratio of 1:4 (m/v) for 2 h in a water bath at 60 °C, with stirring, in a reactor equipped with a condenser (model 521-5D, Ethik Technology, Brazil) and a mechanical stirrer (model 713D, Fisatom, Brazil).

The mixture was filtered. The liquid part was left to cool and the scales were returned to the reactor for a second extraction, now at a 1:2 ratio (m/v), for another hour. A new filtration was then carried out. The scales were discarded and the second liquid mixture was added to the first, then placed in aluminum containers, frozen in an ultra-low temperature freezer (model MDF-U33V, Sanyo, Japan), and lyophilized (model LP510, Liotop, Brazil) to obtain dry gelatin, and then ground in an analytical mill (model LS-06MB-N, Skymsen, Brazil) to obtain powdered gelatin. The extraction yield was calculated from the ratio between gelatin's dry mass and scales' mass on a dry basis (Senarathna; Marapana, 2021).

Shrimp procurement and preparation

Twelve kilograms (kg) of *Penaeus vannamei*, fresh and without preservatives, with an average weight of 11 ± 0.7 g, were purchased from a shrimp farm in Russas, Ceará, Brazil, packed in isothermal boxes with ice at a ratio of 1:1 (w/w) and transported to LATEPE/UFC within 3 hours. At the laboratory they were peeled, headed, filleted, washed with cold drinking water, weighed, packed, and frozen in a freezer at -18 °C for coating the following day.

Preparation of coating solution, coating, and storage

The solution of 1.5% w/v gelatin and 0.2% glycerol 99.5% PA ACS (VETEC, Brazil) was prepared by dissolving 15 g gelatin in 1000 mL distilled water and adding 2 mL glycerol, stirring for 20 min at 30 °C, as suggested by Alcântara *et al*. (2022). The solution was then sterilized by exposure to ultraviolet light at 25 °C for 30 min.

For coating, eight kilograms of peeled, headless and frozen shrimp were separated into two treatments: the control (C) which were not coated, and the coated with gelatin (G). The shrimp from G were placed on a flat grid at room temperature and sprinkled (spray) with the aid of a sprayer with the coating solution using of 2 mL per shrimp letting it drain and dry for 2 min to form the film, turned and sprayed on the other side, letting it drain and dry for another 2 min. Then, all samples, coated (G) and uncoated (C), were packed in polypropylene and sterile styrofoam trays, sealed, identified, and frozen in a freezer at -18 °C.

Shelf life study

To study shelf life, microbiological analysis (psychrotrophic bacteria counts) and physicochemical analysis (pH, TVB-N, TMA-N and TBARS) were carried out in duplicate and with nine replicates on peeled and headless shrimp C and G treatments on the first day of the experiment and then every 30 days (T1, T30, T60, T90, T120, T150 and T180 days).

Microbiological analysis

The psychrotrophic bacteria counts (PBC) were determined according to the recommendations of the American Public Health Association (APHA, 2015) by preparing decimal dilutions of homogenized shrimp in 0.85% NaCl saline and inoculating on PCA agar plate media (Merck, Germany) using the pour plate method and incubating the plates at 7 °C for 8 days in a BOD incubator (model Q315M, Quimis, Brazil). After the incubation period, the number of colony-forming units (CFU) was determined. To calculate the standard plate count (SPC), plates with growth between 25 and 250 CFU were selected and calculated using Equation 1 (APHA, 2015). The results were transformed into logarithms of the number of colony-forming units (log CFU/g), and samples that did not grow within the specified range were estimated (log CFU/g/est.).

$$SPC = CFU \times inverse\ of\ the\ dilution\ factor \quad (1)$$

Physicochemical analysis

The pH of shrimp samples from groups C and G was measured using a bench pH meter (Kasvi, Brazil) by homogenizing 5.0 g of the macerated sample with 50 mL of distilled water at 25 °C in nine replicates (APHA, 2015).

Total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) were measured using the steam distillation method, as described by Malle and Tao (1987). To measure TVB-N, 50 g of shrimp muscle was homogenized with 100 mL of 7.5% (v/v) trichloroacetic acid (TCA) and left to stand for 30 minutes, in three replicates. The homogenate was then filtered through the Whatman No. 1 filter paper. 5 mL of 10% (w/v) aqueous NaOH solution was added to 25 mL of the filtrate, which was added to a Kjeldahl distillation tube and steam distilled in a distiller (TE-036/1, Tecnal, Brazil), in three replicates of each, totaling nine replicates. 40 mL of the distillate was collected in a beaker containing 10 mL of 4% (v/v) aqueous boric acid solution and 0.04 mL of the indicator methyl red and bromocresol green. The collected distillate was titrated with a sulfuric acid solution (0.1 N) until the turning point. TVB-N values were calculated

by multiplying the volume (mL) of 0.1 N sulphuric acid in the titration by 16.8, with results expressed in mg of nitrogen per 100 g of sample.

The procedure for measuring TMA-N followed the same steps as for TVB-N, with the exception that 20 mL of 16% (v/v) formaldehyde was added to the distillation tube inhibit primary and secondary amines, thus allowing the reaction to proceed only with tertiary amines.

Lipid oxidation was analyzed using thiobarbituric acid reactive substances (TBARS) according to Vyncke (1970). To measure TBARS, a 10 g shrimp sample was macerated and homogenized with 50 mL of a 7.5% TCA solution. This process was performed in triplicate. The mixture was then filtered through Whatman No. 1 filter paper. From the filtrate, 5 mL was taken and added to a culture tube containing 5 mL of 0.02 M thiobarbituric acid (TBA) solution. This mixture was shaken using a tube shaker in three replicates, totaling nine replicates. The tubes were then heated in a water bath at 90 °C for 10 minutes and cooled in an ice bath. The absorbance of the samples and the blank were measured at 532 nm using a digital spectrophotometer (UV-2000A, Instrutherm, Brazil). TBARS value in mg malondialdehyde equivalent per kilogram of shrimp (mg MDA eq/kg muscle) was determined using the standard curve of 1,1,3,3-tetraethoxypropane (TEP) calculated using Equation 2:

$$TBARS = (Absorbance - 0.032) / 0.0789 \quad (2)$$

Excel was used to create graphs of the results of the shelf life study.

Statistical analysis

Using Excel software, the gelatin yield was subjected to descriptive statistics and the result was expressed as mean ± standard deviation. The physicochemical shelf life parameters for the two treatments were analyzed using Jamovi(R) software, version 2.4.6. First, the Shapiro-Wilk test was applied to check the normality of the values obtained, while the Levene test was used to check the homogeneity of the data. The values were then subjected to the Student's t-test to check for significant differences ($p < 0.05$) between treatments.

RESULTS AND DISCUSSION

Gelatin extraction yield

The gelatin yield from Nile tilapia scales was 24.64%. This value was higher than other yields obtained from the same source and species such as 14.32% extracted in a water bath at 60 °C and ultrasound for 3 h by Senarathna and Marapana (2021); and 12.10% by acid-alkaline-acid extraction and water bath at 60 °C for 1 h by Martins *et al*. (2018).

It was also higher than the gelatin yield obtained from other species, such as 24% from *Labeo rohita* scales (Das *et al*., 2017); 11.88% in the extraction of gelatin from black tilapia (*Oreochromis mossambicu*s) scales, in a water bath at 65 °C for 8 h (Sockalingam; Abdullah, 2015); 8.63% and 9.27% of gelatin from spotted golden goatfish scales with 6 and 12 h extraction, respectively (Chuaychan; Benjakul; Kishimura, 2017); and 9.55% from sea bream scales in a water bath at 60 °C for 12 h (Akagündüz *et al*., 2014).

However, the yield obtained in this study was lower than some fish skin extraction values reported in the literature. Liao *et al*. (2021) achieved yields of 35.24%, 41.91%, 31.66%, and 36.27% of tilapia skin gelatin extracted at 60 °C and 75 °C at pH 3 and 60 °C and 75 °C at pH 5, respectively. Sinthusamran *et al*. (2018) achieved 44.83% to 71.5% yields of giant barramundi (*Lates calcarifer*) skin gelatin, extracted at 45 °C to 75 °C. This is because fish scales and bones generally contain less collagen than skin and collagen can be lost during processing steps such as washing, pre-treatment, and extraction (Sockalingam; Abdullah, 2015).

Extraction yields depend on many factors including species, age, tissue type, and process conditions such as pH, ionic strength, pretreatment and extraction time, temperature, and acid type (Martins *et al*., 2018; Tkaczewska *et al*., 2018). The high yield of gelatin from fish scales correlates with the high proline and hydroxyproline content of these structures (Das *et al*., 2017). In tilapia, it may be correlated with the elevated collagen content, as well as the effective pretreatment and extraction methods used (Sockalingam; Abdullah, 2015), especially the extraction time and elevated extraction temperature, which significantly increase the gelatin yield (Tan *et al*., 2019).

The high yield values were probably due to the extraction time. Thus, the process used to extract gelatin from Nile tilapia scales was found to have yields compatible with literature values. The extraction method proved to be simple and low-cost. In this research, 1 kg of Nile tilapia scales was obtained for free from the fishmonger (it would be discarded) and generated around 246 g of gelatin. This volume can produce around 16.4 liters of coating solution. Each liter of coating of 1.5% gelatin and 0.2% glycerol costs approximately US$ 1.79 and could coat around 5 kg of peeled, head-on shrimp. This equates to an approximate cost of US$ 0.36 per kilo of peeled and headless shrimp coated. In other words, it is a cost-effective solution to maintain the quality of the fish for longer and protect customers from consuming unhealthy shrimp.

Microbiological analysis

Microbial spoilage of fish is mainly due to proliferation of bacteria and the products of their metabolism, which cause

sensory changes such as discoloration, physical changes, changes in texture, slime or gas formation, or unpleasant odors and flavors (Jia *et al*., 2019).

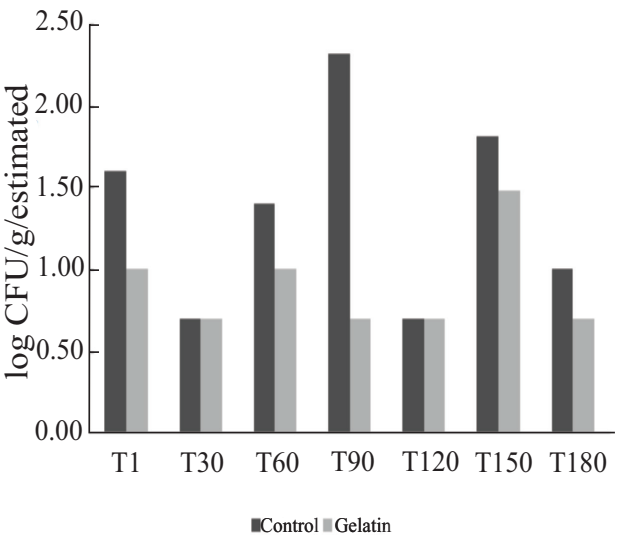
Psychrotrophic bacteria can grow and multiply at low refrigeration temperatures and perform proteolytic and lipolytic activities and include species of *Acinetobacter, Aeromonas, Alcaligenes, Arthrobacter, Bacillus, Brochothrix, Carnobacterium, Chromobacterium, Citrobacter, Clostridium, Corynebacterium, Enterobacter, Escherichia, Flavobacterium, Klebsiella, Lactobacillus, Leuconostoc, Listeria, Microbacterium, Micrococcus, Moraxella, Pseudomonas, Psychrobacter, Serratia, Shewanella, Streptococcus*, *Weissella*, *Alteromonas, Photobacterium,* and *Vibrio* (APHA, 2015).

Figure 1 shows the average decimal logarithm of the colony-forming units (CFU) of psychrotrophic bacteria detected in shrimp (*P. vannamei*) muscle.

From Figure 1, the psychrotrophic bacterial count (PBC) of control group (C) was always higher than that of treatment G, except in two moments when both had the same count: at T30 and T120 where the values were equal to 0.70 log CFU/g/estimated. In addition, the PBC range for control group was 0.70 to 2.32 log CFU/g/est., whereas for treatment G it was only 0.70 to 1.48 log CFU/g/est. This may suggest that the combined gelatin and glycerol coating used in this study appears to have slowed microbial growth in frozen shrimp, possibly due to the antimicrobial action of gelatin reported by other researchers (Das *et al*., 2023; Nagarajan *et al*., 2021).

The International Commission on Microbiological Specifications for Food (Stewart, 1987) sets a maximum acceptable limit of 10^7 CFU/g (equivalent to 7.0 log CFU/g) for the population of these Bacteria in fish intended for human consumption. Considering this limit, all samples from treatments C and G were within the standards for PBC in shrimp muscle throughout the storage period.

Figure 1 - Quantification of psychrotrophic bacteria (log CFU/g/estimated) in samples of *Penaeus vannamei* frozen for 180 days from the control and gelatin treatments



Farajzadeh *et al*. (2016) found that the PBC of the control group increased significantly ($p < 0.05$) faster than the shrimp group (*P. vannamei*) coated with chitosan and commercial bovine gelatin. Both groups were stored at 4 ºC, from an initial 2.5 log CFU/g to the maximum allowable limit of 7.0 log CFU/g according to the International Commission on Microbiological Specifications for Food (ICMSF) in only 8 days (control), while the coated group reached this limit after 14 days of storage.

Mirzapour-Kouhdasht and Moosavi-Nasab (2020) also observed that the PBC of the control group increased much more and faster ($p < 0.05$) than the samples of *Penaeus merguiensis* shrimp coated with gelatin extracted from *Scomberomorus commerson* skin, after 12 days of storage at 4 °C. Jiang, Liu and Wang (2011) found that coatings of gelatin solution (5% w/w) from catfish (*Ictalurus punctatus*) skin, glycerol plus potassium sorbate and/or sodium tripolyphosphate were effective against bacterial growth and extended the shelf life of fresh white shrimp (*Penaeus vannamei*) by up to 10 days when stored on the ice at 0 °C for 31 days.

Changes in microbial populations provide useful information for understanding population changes associated with spoilage related to food storage conditions (Jia *et al*., 2019).

Physicochemical analysis

pH

Shortly after the death of the aquatic products, some substances in the muscle begin to be converted into alkaline substances, raising the pH of the muscle. Proteins are then broken down into these compounds, leading to a further increase in pH to rise, indicating the deterioration of aquatic products (Zuanazzi *et al*., 2020). pH values below 4.0 indicate that bacterial growth is suppressed and yeasts and molds grow abundantly; above pH 5, proteolytic bacteria can become active (GMIA, 2019). The pH values of the muscles of frozen *L. vannamei* shrimp samples from treatments C and G are shown in Figure 2.

On day one (T1), the initial pH of control group was 6.55 ± 0.09, while the average pH of treatment G significantly higher ($p < 0.05$), reaching 6.66 ± 0.02. At 30 days (T30), there was a decrease in pH values: 6.45 ± 0.05 for C and 6.40 ± 0.10 for G, with no significant differences ($p > 0.05$). Then, at T60, there was an increase in the pH of the two treatments, with a significant difference ($p < 0.05$) between them (6.57 ± 0.10 in C and 6.69 ± 0.07 in G).

This behavior was to be expected, as anaerobic glycolysis and lactic acid formation begin soon after the shrimp die, causing the muscle's pH to drop. Days later, autolysis begins through the action of the shrimp's natural enzymes, producing low-molecular-weight compounds such as trimethylamine, which causes the pH of the muscle tissue is to rise (Das *et al*., 2023; Ge *et al*., 2020). Thus, the times between T1-T30 and T30-T60 respectively correspond to these two moments after shrimp death.

The fact that treatment G had higher values than C in T1 and T60, and the pH dropped at T120, which may be due to the sampling factor since, although they are individuals from the same batch, each individual may have different degradation states. However, as the days passed, in analysis T150 and T180, the expected pH was re-established, and the coating effect significantly ($p < 0.05$) reduced the pH of the shrimp muscle in treatment G compared to the control with 150 days of storage.

Analyzing only the initial (T1) and final (T180) values, treatment G hardly changed its pH (6.66 ± 0.02 and 6.67 ± 0.08, respectively), while control group increased by 0.14 (6.55 ± 0.09 and 6.69 ± 0.09, respectively) on pH scale, indicating that the shrimp had entered the autolysis and protein degradation phase. Nevertheless, on the last day of analysis, there were no significant differences ($p > 0.05$) between treatments C and G. Throughout the experiment, the pH of the shrimp flesh remained well below the limit of 7.85 recommended for consumption of crustaceans by current Brazilian legislation (Brazil, 2020).

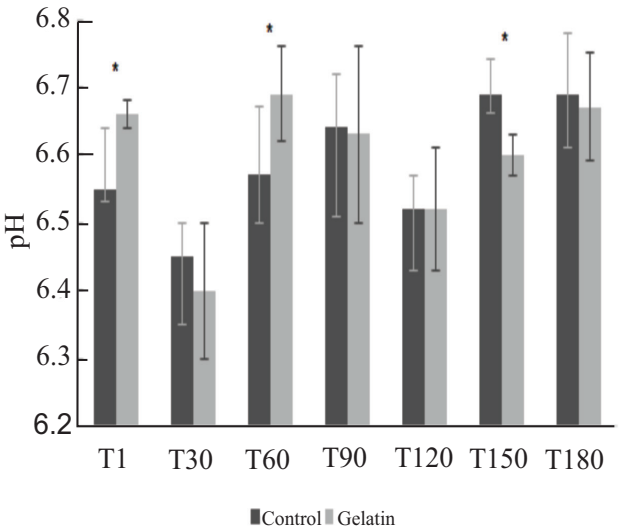
Looking at control group in Figures 1 and 2, there is a similarity in the behavior of the microbiological analysis and pH curves behave similarly, indicating that the higher the PBC, the more alkaline the pH of the samples and, consequently, the greater the action of enzymes and bacteria. Therefore, it can be seen that using coatings can be an excellent practice to inhibit the degradation of shrimp.

The values obtained in this research are consistent with those reported in the other literature. Ge *et al*. (2020), studying swordfish (*Parapenaeopsis hardwickii*), observed that the pH of shrimp in the control group was significantly higher ($p < 0.05$) from the 4th day of storage at -5 ºC until the final day of analysis (23 rd day) when compared to shrimp treated with acid-chlorogenic gelatin. Farajzadeh *et al*. (2016) found that the pH of the control samples (ranging from 6.32 and 7.91) was significantly higher values ($p < 0.05$) than that of P. vannamei shrimp coated with gelatin and chitosan (ranging from 6.22 and 6.44), with both groups stored under refrigeration at 4 ºC. Remembering that p values indicate statistical significance: $p < 0.05$ demonstrate statistical difference and $p > 0.05$ there is no statistical difference between the samples.

TVB-N

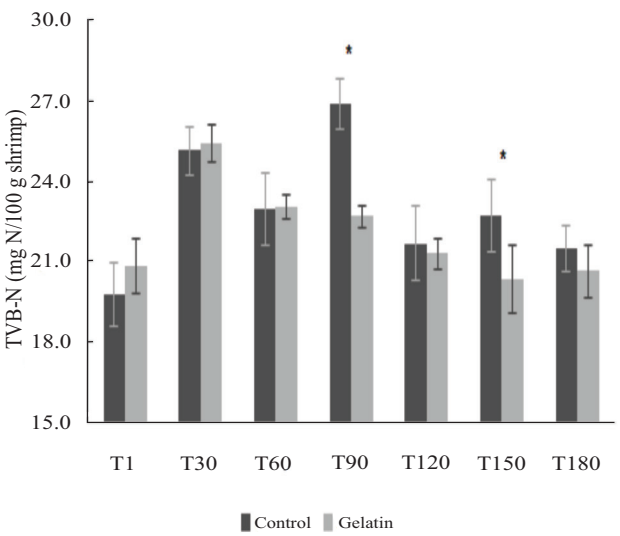
Total volatile basic nitrogen (TVB-N) is a widely used index to evaluate the deterioration of aquatic products due to the action of endogenous animal enzymes and bacterial action that degrade nitrogen compounds in muscle proteins such as peptides, amino acids, and nucleotides (Pan *et al*., 2019). The TVB-N value is derived from trimethylamine oxide (TMAO), ammonia, dimethylamine (DMA), trimethylamine (TMA), and other volatile basic nitrogen compounds in aquatic product muscle during storage that causes loss of freshness (Ge *et al*., 2020). Figure 3 shows the TVB-N values of treatments C and G during 180 days of frozen storage.

Figure 2 - Graph of pH of *Penaeus vannamei* muscle frozen for 180 days in the control and gelatin treatments



Legend: *indicates a statistically significant difference ($p < 0.05$) between treatments using Student's t-test

Figure 3 - Graph of TVB-N (mg N/100 g shrimp) in the muscle *of Penaeus vannamei* frozen for 180 days in the control and gelatin treatments



Legend: *indicates a statistically significant difference ($p < 0.05$) between treatments using Student's t-test

Figure 3 shows that treatments C and G do not have a statistically significant difference ($p > 0.05$) in the analysis at 1, 30 and 60 days. However, at 90 days, control group showed the highest value of TVB-N recorded during the 180 days of storage (26.88 ± 0.94 mg N/100 g shrimp), with a significant difference ($p < 0.05$) between it and treatment G (22.68 ± 0.42 mg N/100 g shrimp), and where the highest value of PBC was also recorded (Fig. 1), showing a correlation between the microbiological results and those of shrimp deterioration.

The G samples showed the highest TVB-N values at 30 days of the experiment (25.39 ± 0.70 mg N/100 g shrimp) and the lowest at 150 days of the experiment (20.35 ± 1.24 mg N/100 g shrimp), with a significant difference ($p < 0.05$) between C and G treatments at T150. On the last day of the experiment (T180), TVB-N values were 21.47 ± 0.85 mg N/100 g for C and 20.63 ± 0.95 mg N/100 g for G, with no significant differences ($p > 0.05$) between C and G treatments. These results indicate that storage time contributed to preserving the chemical quality of the samples and inhibiting TVB-N formation.

Lannelongue *et al*. (2006) presented the following TVB-N acceptance scales for raw shrimp <12 mg N/100 g for fresh; 12-20 for edible but slightly decomposed; 20-25 for borderline; and >25 mg N/100 g for inedible and decomposed. According to current Brazilian legislation (Brazil, 2020), the total volatile base value must be less than 30 mg nitrogen/100 g muscle tissue. Thus, shrimp from both treatments kept their TVB-N levels below the limits recommended in the literature. The low TVB-N values found in this study may be due to the good initial freshness of the shrimp samples analyzed and the low storage temperature (-18 °C) throughout the storage period.

The results of this study align with findings from previous research. Mirzapour-Kouhdasht and Moosavi-Nasab (2020) found that *Penaeus merguiensis* shrimp coated with gelatin extracted from the skin of *Scomberomorus commerson* had significantly lower TVB-N levels ($p < 0.05$) than the control group over 12 days of storage at 4 °C. In their study, the control samples exceeded the limit of 30 mg N/100 g of muscle by the 9 th day. Similarly, Huang, Zelaya and Shiau (2016) reported that TVB-N formation in uncoated *P. vannamei* increased significantly from 4.2 mg/100 g (fresh) to a final value of 49.0 mg/100 mg after 24 h of storage at 25 °C. However, when the storage temperature was reduced to 4 °C, TVB-N levels increased more gradually, reaching 52.8 mg N/100 g after 14 days. These findings reinforce the importance of temperature control and protective coatings in delaying the formation of TVB-N and preserving shrimp quality.

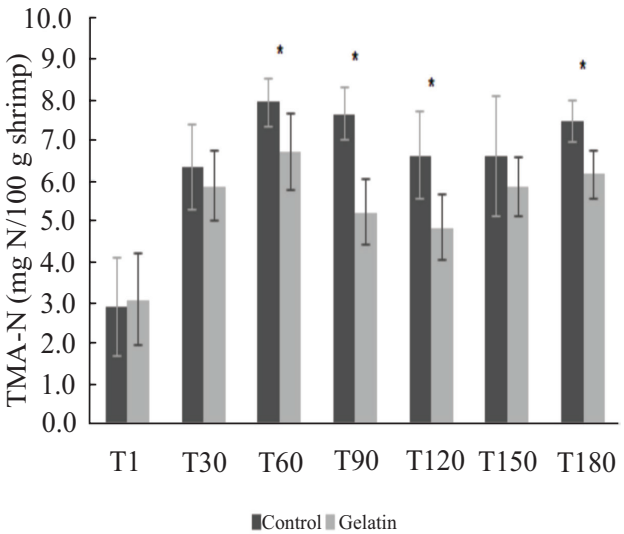
Farajzadeh *et al*. (2016) observed that the initial TVB-N content of the control group increased exponentially ($p < 0.05$) until the 8th day of storage under refrigeration at 4 ºC (10.48 to 33.58 mg N/100 g), while shrimp coated with gelatin and chitosan took 14 days to reach the same level (10.43 to 33.27 mg N/100 g). According to these authors, low-temperature storage combined with edible coating technologies significantly reduced the formation of TVB-N.

TMA-N

The accumulation of nitrogenous compounds such as TVB-N, trimethylamine (TMA), and biogenic amines in aquatic products is caused by the metabolism of spoilage bacteria and enzymes, affecting the sensory quality, flavor, nutritional value, and safety of the products (Li *et al*., 2018). TMA is one of the main substances responsible for fishy odor, and some biogenic amines (such as histamine, cadaverine, and putrescine) are potentially toxic to humans (Yu *et al*., 2018). This study used a formaldehyde method to quantify TMA by blocking primary and secondary amines (Huang; Zelaya; Shiau, 2016). Figure 4 shows the evolution of TMA-N in the muscles of *P. vannamei* shrimp samples from treatments C and G over 180 days of frozen storage.

Figure 4 shows that the treatments started with no significant difference ($p < 0.05$) in the TMA-N content of the muscle of frozen shrimps. However, after 60, 90, 120 and 180 days of storage, the G samples (6,72 ± 0,94 mg N/100 g; 5,23 ± 0,82 mg N/100 g; 4,85 ± 0,82 mg N/100 g;

Figure 4 - Graph of TMA-N (mg N/100 g shrimp) in the muscle of *Penaeus vannamei* shrimps frozen for 180 days in the control and gelatin treatments



Legend: *indicates a statistically significant difference ($p < 0.05$) between treatments using Student's t-test

and 6,16 ± 0,59 mg N/100 g, respectively) showed lower values ($p < 0.05$) compared to control group (6,35 ± 1,04 mg N/100 g; 7,65 ± 0,66 mg N/100 g; 6,63 ± 1,07 mg N/100 g; and 7,47 ± 0,50 mg N/100 g, respectively). Nevertheless, the highest value for control group was 7.93 ± 0.61 mg N/100 g of shrimp and, according to Connell (1995), the maximum level of TMA recommended for human consumption is 10 to 15 mg N/100 g. Thus, none of the samples exceeded this limit.

The changes in TVB-N and TMA-N appear to be somewhat consistent with changes in the pH of the shrimp muscle resulting from the accumulation, however small, of basic compounds induced by bacterial or enzymatic activity. The increase in pH, especially in group C, reflected the production of alkaline bacterial metabolites in shrimp muscle.

The results obtained in this research were lower than those of Tsironi *et al*. (2009), who found values of up to 25 and 14 mg N/100 g TVB-N and TMA-N, respectively, after 8 months in samples of uncoated whole shrimp frozen and stored between -5 and -15 °C. Huang, Zelaya and Shiau (2016) reported a 50% lower increase in TMA content after 14 days of storage of *P. vannamei* at 4 °C compared to 25 °C, both without coating. According to the authors, TMA content is widely used as a indicator of the bacterial contamination level of seafood.

According to Jia *et al*. (2019), the microbiota present in shrimp produces various compounds associated with deterioration, such as TMA, hydrogen sulfide, ammonia, and acetic acid, which cause shrimp tissue to lose elasticity and produce unpleasant odors and flavors. In this research, gelatin combined with low temperature slowed the production of volatile compounds.

TBARS

Lipid oxidation in seafood leads to discoloration, rancid flavors, potentially toxic compounds, reduced protein functionality, and nutritional loss of some amino acids (Yu *et al*., 2018). The thiobarbituric acid reactive substances (TBARS) test determines the state of lipid oxidation in aquatic products. Thiobarbituric acid (TBA), the main reagent used in this method, reacts with the tissues to produce a pink color as a result of the formation of a complex between TBA and oxidized lipid compounds, mainly malondialdehyde (MDA) (Vyncke, 1970). The quantification of the levels of malonaldehyde, in mg equivalent/kg shrimp muscle, present in the muscles of *P. vannamei* samples from treatments C and G is shown in Figure 5.

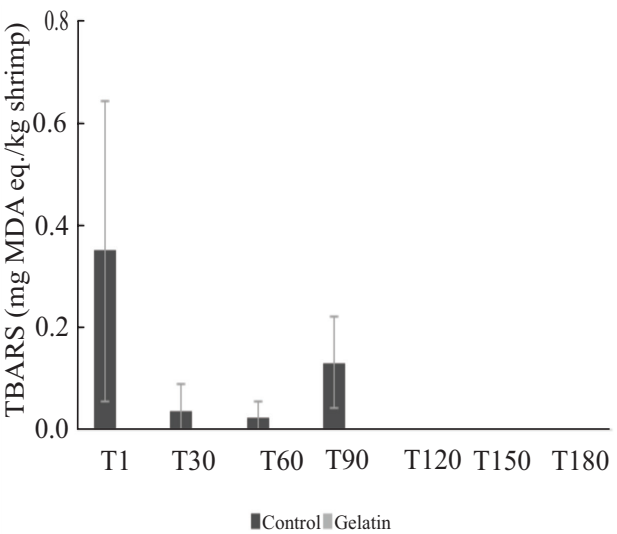
Figure 5 shows that the TBARS value of control group was 0.349 ± 0.29 mg MDA eq./kg shrimp at T1 and then showed a decreasing trend until its values reached zero after 120 days of storage, except at T90 when there was an increase in TBARS. At this time, the samples may have been exposed to the environment for longer after thawing (until the time of analysis) or have been contaminated during handling in the analysis processing stages, which would also justify the peak in psychrotrophic bacterial count (PBC) and TVB-N of the control treatment at 90 days.

On the other hand, the gelatin treatment exhibited undetectable TBARS value throughout the 180 days of frozen storage, demonstrating that the coating prevented lipid oxidation of the shrimp flesh. According to Connell (1995), TBA levels between 1.0 and 2.0 mg MDA/kg in fish muscle are associated with unpleasant taste and odor. Therefore, based on these parameters, the samples from both treatments were below this limit.

The results of this analysis those reported in the literature. Bono *et al*. (2012) evaluated the TVB-N levels of frozen marine shrimps during storage at -18 °C for one year and found that the lipid oxidation, also measured by MDA content, of all their samples (control, modified atmosphere, sulfite treatment and vacuum packaging) remained below the limit proposed by Connell (1995) throughout the 12 months of the experiment, with values between 0.1 and 0,6 mg MDA eq./kg; however, the control samples deteriorated more rapidly in the second half of the year than the other samples.

Das *et al*. (2023) observed that TBARS levels in uncoated shrimp (control) were significantly higher than in shrimp coated with commercial gelatin and peppermint oil at the beginning and end of the storage period. However, all samples remained below the upper limit of 1-2 mg MDA/kg shrimp. Yu *et al*. (2018) found that edible coatings effectively slowed the lipid oxidation of seafood products during storage, mainly by acting as a barrier to oxygen and through their antioxidant properties.

Figure 5 - Graph of TBARS (mg MDA eq./kg shrimp) in *Penaeus vannamei* muscle frozen for 180 days in the control and gelatin treatments



Coatings help to improve food safety and shelf life by slowing down lipid oxidation, preventing moisture loss and loss of protein functionality, and reducing unpleasant odors and discoloration (Farajzadeh *et al*., 2016). Gelatin is a promising coating material due to its film-forming or gelling ability and its resistance to drying, light, and oxygen (Feng *et al*., 2017), which can efficiently extend the shelf life and ensure the acceptability of frozen fish products (Zhang *et al*., 2020).

Using gelatin from fish waste is an excellent alternative to reduce waste and environmental impact and add value to the product (Martins *et al*., 2018). According to FAO (2024), *Oreochromis niloticus* is the third most produced species in the world and 5.3 million tons (live weight) of this fish will be produced in 2022. Considering this production, the residual scale of about 5% (Boronat *et al*., 2023) and the yield obtained in this study (24.64%), a total of 65,296 tons of Nile tilapia scale gelatin could be produced annually, an edible, non-toxic, biodegradable gelatin that is biocompatible with aquatic products (Abdelhedi *et al*., 2019). It could be used at low temperatures to inhibit the degradation of seafood significantly, effectively extending the shelf life and ensuring the acceptability of frozen fish products (Liu *et al*., 2023b), especially *Penaeus vannamei*, a very noble but highly perishable product (Alcântara *et al*., 2022).

CONCLUSIONS

1. This research confirmed the suitability of Nile tilapia scales as a good source of gelatin and demonstrated the efficiency of adapting the methodology used to extract gelatin. It guaranteed a high yield of material (24.64%) and low production costs (US$1.79/l of coating);

2. The study showed that many of the deterioration analysis (psychrotrophic bacteria growth, pH, TVB-N, TMA-N, and TBARS) of the peeled, headless and frozen shrimp samples coated with a solution of Nile tilapia scale gelatin and glycerol showed lower values than the control samples, suggesting that coating is a good technique to inhibit deterioration and preserve the quality of *Penaeus vannamei* for longer;

3. Hence, the importance of this research and the need to develop others to help increase the shelf life and food safety of this noble product, such as: combining gelatin with substances with antibacterial and/or antioxidant properties, coating shrimp, and evaluating the synergistic effects of gelatin and bactericidal and/or antioxidant agents on microorganisms in frozen shrimp; testing oxidation control and enzyme inhibition mechanisms to optimize their application in shrimp preservation; and comparing gelatin-coated and uncoated shrimp under refrigeration and freezing by sensory analysis.

CONFLICTS OF INTEREST

The authors report there are no competing interests to declare.

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