



Physicochemical, microbiological, and sensorial characteristics of cooked, marinated, and vacuum-packed oysters under frozen storage

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ABSTRACT

This study evaluated the quality of frozen oyster meat. Seven hundred and twenty *Crassostrea gigas* oysters (~7-cm shell length) were steam-cooked at 100°C for 30 minutes and later divided into two groups. Group 1 was submerged for 20 minutes in water and ice; and group 2 in a solution containing water, ice, and 4% vinegar (4% acidity, produced by fermentation of acetic alcohol). After cooling, the oysters were shucked, and the meat was packed with a vacuum sealer in plastic bags containing 24 oysters each. The experiment evaluated physicochemical (pH, moisture, and total volatile bases nitrogen) and microbiological (total mesophilic and psychrotrophic bacterial counts) parameters every 45 days and performed sensory analyses after 180 and 365 days of storage. Adequate physicochemical and microbial characteristics were registered for up to one year of storage for both protocols. Marination with vinegar reduced the levels of psychrotrophic bacteria during storage, and the sensory analysis judges preferred the marinated oysters.

Keywords: *Crassostrea gigas*; Frozen storage; Organic acid; Shelf life.

Características físico-químicas, microbiológicas e sensoriais de ostras cozidas, marinadas e embaladas a vácuo armazenadas congeladas

RESUMO

Este estudo avaliou um novo método de processamento, embalagem e armazenamento de ostras. O total de 720 ostras *Crassostrea gigas* (~7 cm de comprimento da concha) foi cozido a vapor a 100°C por 30 minutos e dividido em dois grupos. O grupo 1 foi submerso por 20 minutos em água e gelo; e o grupo 2, em uma solução de 4% contendo água, gelo e vinagre (4% de acidez, produzido pela fermentação de álcool acético). Após o resfriamento, as ostras foram desconchadas e a carne embalada com seladora a vácuo em sacos plásticos contendo 24 ostras cada um, em um arranjo que permite ao consumidor visualizar cada ostra individualmente. Parâmetros físico-químicos (pH, umidade e base volátil total) e microbiológicos (contagem de bactérias mesófilas totais e psicrotróficas) foram avaliados a cada 45 dias, e análises sensoriais foram realizadas após 180 e 365 dias de armazenamento. Características físico-químicas e microbianas adequadas foram registradas por até um ano de armazenamento para ambos os protocolos. A marinação com vinagre reduziu os níveis de bactérias psicrotróficas durante o armazenamento, e os juízes das análises sensoriais preferiram as ostras marinadas.

Palavras-chave: *Crassostrea gigas*; Armazenamento congelado; Acido orgânico; Shelf life.

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INTRODUCTION

Mollusks accounted for 14.5% (17.7 million tons) of the amount produced by world aquaculture in 2020, the largest share being oysters, representing 35.3% of all cultured mollusks (FAO, 2022). Oysters constitute a low-fat, low-cholesterol, and high-quality protein source containing essential vitamins. Despite their healthy characteristics, consumers' perceptions of this product harm their consumption. Loose et al. (2013) studied consumers' perceptions about oysters. The authors point out that oysters are considered hard to open, and the potential meal usage is perceived as limited, especially for shelled live oysters. The authors argued that retailers offering pre-shucked, easy-open oysters are likely to gain market share compared to unopened oysters.

In line with that perception, different processing/packing technologies have been studied or adopted worldwide, such as the high hydrostatic pressure (Cruz-Romero et al., 2008; Liu et al., 2021), mild temperature pasteurization or hot-cold pasteurization (Chung et al., 2021; Lekjing et al., 2017), modified atmosphere packaging (Rodezno et al., 2023), individual quick freezing (Dong et al., 2023; Songsaeng et al., 2010; Teng et al., 2022), and marination (Babikova et al., 2020; Essid et al., 2020; McDermott et al., 2018; Silva et al., 2021).

Botta et al. (2020) reviewed the global oyster aquaculture production and consumption and showed that the industry and markets are highly diverse. North American and European countries focus on the raw, half-shell market, which reaches a higher price per unit weight than other markets. Conversely, Asian countries focus on shucked meat products used in canning and frozen meals or for oyster sauce. Asian oyster products accounted for 63% of all oyster products imported to the United States of America in 2016, with most of the products being canned oyster meat.

Brazilian bivalve shellfish aquaculture concentrates in Santa Catarina state, located in the subtropical portion of the country's coast. It produced 12,000 tons of mollusks in 2021, ~95% of the national production. Observing the evolution of the production in that state, it becomes evident that the oyster (*Crassostrea gigas*) production has stagnated, ranging between 2,000 and 3,000 tons since 2003 (Observatório Agro Catarinense, 2021). There are no official reports about the proportions of oysters traded as live products or in the form of shucked meat in Brazil. However, local studies and practical experience indicate that most of these animals are sold refrigerated, shelled, and alive, therefore a product with a very limited shelf-life (four to seven days) (Tribuzi et al., 2020) and which meets a limited market niche.

The reproductive cycle of Pacific oysters in this geographical region poses challenges for their trade. In Santa Catarina,

C. gigas develops gonads during winter, reaching the highest meat yield during spring. They tend to spawn in summer, negatively impacting meat yield, flavor, and appearance during the tourist season when demand is the highest (Sühnel et al., 2017). This pattern has increased the producers' interest in alternatives such as freezing and marination that allow the harvesting and peeling of oysters at the time of best meat yield and conservation for marketing during the tourist season (Suplicy & Souza, 2022).

Despite the convenience of these methods, oyster meat undergoes a progressive decline in quality with changes in its physical, chemical, microbiological, and organoleptic characteristics during the frozen storage period. Drip loss, pH, total volatile basic nitrogen (TVB-N), and microbial counts are crucial indicators for assessing seafood quality (Min et al., 2020).

Although these methods can determine the oysters' freshness, sensory evaluation is still the most satisfactory one (Cao et al., 2009). Quality of aquatic food declines due to high moisture content, high microbial adhesion, strong enzyme activity, packaging type, and change during storage. Common ones include lipid oxidation, protein degradation, microbial proliferation, and massive loss of drips, changes in appearance or color (Tsai et al., 2022). Local restaurant chefs have reported that marination of shucked oysters with vinegar before freezing avoid them to become dark colored during frozen storage.

Combining vinegar soaking with freezing can be an alternative to increase the shelf life of cooked oyster meat. The present study aimed to analyze the combined effect of vinegar marination and freezing on the physicochemical, microbiological, and sensory properties of Pacific oysters (*C. gigas*) vacuum-packed over 365 days.

METHODS

The study used 720 *C. gigas* oysters (~7 cm shell length) harvested in May 2021 from a shellfish farm located on Santa Catarina coast (27°42'14.70"S, 48°33'34.77"W). The oysters were washed under pressurized water and transported in an insulated container with ice to an approved processing plant (1-hour shipping). There, they were steam-cooked at 100°C for 30 minutes in a convection oven (Prática, C-MAX 3 Gourmet). Afterwards, they were divided into two groups containing 360 individuals each. Group 1 was submerged for 20 minutes in water and ice (WOV), and group 2 in solution with ice and 4% vinegar obtained by fermentation of acetic alcohol (WV). After cooling, the oysters were shucked, and their meat was packed with a vacuum sealer (Orved Brock, Model-VM18, Brazil) in plastic bags containing 24 oysters each. The oysters were

positioned in bags, allowing consumers to visualize each oyster individually (Fig. 1). The oysters' packages were frozen in 5 minutes by an ultra-freezer (Pratica, UK 20 Max, Brazil) at -25°C and stored for one year in a cold chamber at -18°C . Every 45 days, each treatment package was removed from the cold chamber and brought to the laboratory for physical, chemical, and microbiological analyses. Sensory analyses were performed after six months and one year of storage.



Figure 1. Cooked, marinated, and vacuum-packed oysters in an arrangement that allows consumers to visualize each oyster individually.

The physicochemical analysis consisted of pH, moisture, and TVB-N, estimated for four samples ($n = 4$). The microbiological parameters were total mesophilic and psychrotrophic bacterial counts, quantified in two samples (duplicates). For each analysis, frozen samples were thawed for 18 hours at $4 \pm 2^{\circ}\text{C}$ before analysis. The pH was measured with a probe (Hanna-HI, 2020-20) in a thawed 10 g of ground and homogenized oyster sample. The moisture (%) was determined by weighing thawed samples (2 to 10 g) of oyster sample before and after drying in an oven at 105°C until reaching a constant weight (IAL, 2008).

The TVB-N analysis was performed according to the Brazilian official methods for animal-source foods (Brasil, 2019), with some modifications. Five grams of oyster meat were homogenized with 45 mL of 6% perchloric acid solution using a Turrax homogenizer (IKA, T18 digital, China) at 12,000 rpm for 2 minutes and 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 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 oyster meat sample.

For microbiological analysis, sterile bags were filled with 25 g of oyster meat and 225 mL of 0.1% peptone water with 0.05% sodium chloride, 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. 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 $\text{CFU} \cdot \text{g}^{-1}$.

Sensory analyses were carried out under the authorization of the Research Ethics Committee of Universidade Federal de Santa Catarina (No. 5,405,325, CAAE No. 58147722.5.0000.0121). The sensory properties of the frozen oysters were evaluated by eight panelists at 180 days of storage, and by seven panelists at 365 days of storage. The panelists were asked to score the intensity of each characteristic appearance, color, odor, taste, and texture using the modified guidelines from Cao et al. (2009), Lee et al. (2017), Songsaeng et al. (2010), and Tsai et al. (2022), with a scale from 1 to 9, as follows:

- 1: unacceptable;
- 2: extremely poor;
- 3: very poor;
- 4: poor;
- 5: acceptable;
- 6: good;
- 7: very good;
- 8: extremely good;
- 9: excellent.

The panelists were also asked to indicate their order of preference for the samples. Frozen oysters were thawed at 4°C for 18 hours and then kept at room temperature for approximately 2 hours until they reached 25°C . Three samples containing one oyster each were served in disposable dishes at room temperature (25°C) and duly identified by random three-digit codes, along with a wheat cracker and water, to clean taste buds between samples. The samples were a

recently harvested oyster cooked and vacuum-packed the day before (control); a thawed oyster from the freezing chamber (WOV); and an unfrozen marinated oyster from the freezing chamber (WV).

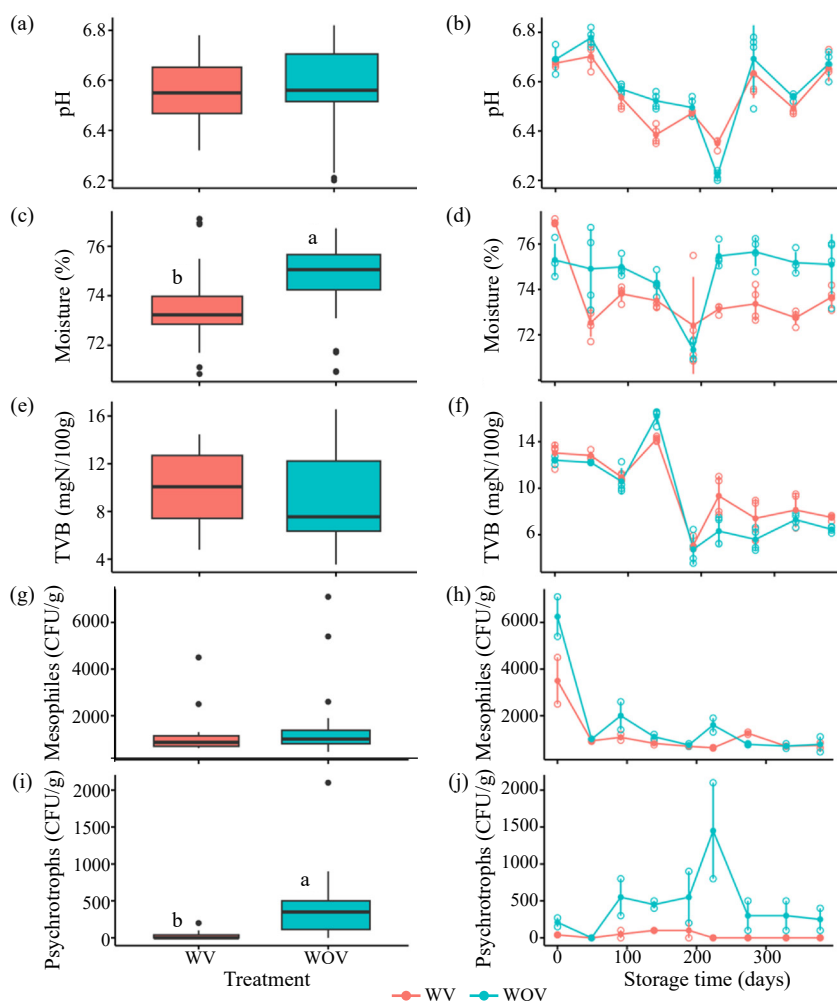
STATISTICAL ANALYSIS

The data obtained for most analyzed parameters differed from normality according to Shapiro-Wilk's test. For this reason, the physicochemical and microbiological results obtained for WOV and WV oysters were compared using Mann-Whitney's U test. Sensory analysis results also differed from normality in most cases, and the comparison among treatments was performed using the Kruskal-Wallis' H test, followed by pairwise comparisons using the Wilcoxon rank sum test with continuity correction. Acceptability

indexes were also inferred based on the sensory analysis results, dividing the mean grade obtained for each treatment by the overall maximum grade attributed for that parameter. Indexes above 70% were considered good acceptability (Dutcosky, 2011).

RESULTS

According to Fig. 2, the pH, TVB-N, and mesophilic microorganisms displayed no significant differences ($p > 0.05$) (Figs. 2a, 2e, 2g, respectively). The analysis of data obtained for all measurement efforts combined pointed out that marinated oysters (WV) presented lower proportions of moisture ($p < 0.001$) (Fig. 2c) and lower levels of psychrotrophic microorganisms ($p < 0.0001$) (Fig. 2i) than non-marinated ones (WOV).



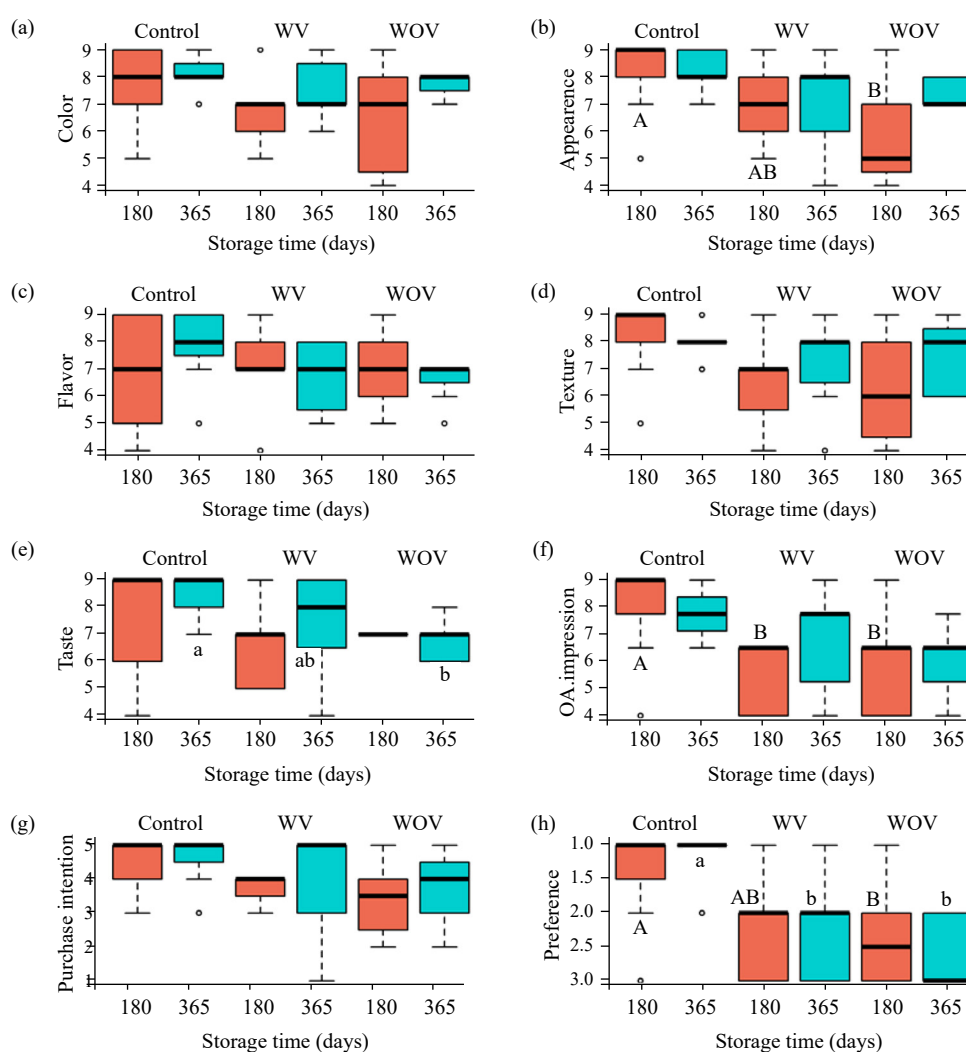
TVB: total volatile basic.

Figure 2. Results of different physicochemical and microbiological parameters obtained for cooked and vacuum-packed oysters, marinated with vinegar (WV) or not (WOV) (boxplots), and the evolution along storage time (line plots) at -18°C . The boxplots on the left display the median (horizontal line), interquartile range (box), extremes (vertical lines), and outliers (points). The line plots on the right display the results in terms of mean (filled circles) and standard deviation (vertical lines), and the outlined circles represent the raw values.

The differences observed for moisture and psychrotrophic bacteria can be also noted by analyzing these parameters over time. Concerning moisture, higher proportions were registered for non-marinated oysters virtually during the storage period, with a median value of 75.2% (range = 70.9 to 76.6), with only a punctual variation by 200 days of storage (Fig. 2d). The median moisture of marinated oysters was 73.3% (range = 70.8 to 75.8%). The psychrotrophic microorganism counts in non-marinated oysters increased from the 100th day of storage on, remaining higher than the marinated ones until the end of the assay (Fig. 2j). The maximum values reached for marinated

and non-marinated oysters were 200 (median = 0) and 2,100 (median = 350) CFU·g⁻¹, respectively. For parameters that did not differ significantly between WV and WOV, overall median values and range (between parentheses) were: pH = 6.54 (6.2 to 6.76); TVB-N = 7.96 mgN·100g⁻¹ (3.56 to 16.56 mgN·100g⁻¹); and mesophiles = 890 CFU·g⁻¹ (450 to 2,600 CFU·g⁻¹).

The sensorial analysis results showed that WV and WOV did not differ significantly ($p > 0.05$) in any of the studied parameters, including those of acceptance (Figs. 3a–3g) and preference (Fig. 3h), for products stored both for 180 and 365 days. The grades attributed to thawed oysters, as referred to the



OA.impression: overall impression.

Figure 3. Results of sensorial analysis comparing cooked and vacuum-packed oysters submitted to different processing: recently harvested animals (control), and thawed oysters marinated with vinegar (WV) or not (WOV). The boxplots describe the median (horizontal line), interquartile range (box), extremes (vertical lines), and outliers (points). Letters within the plots indicate homogeneous groups according to pairwise comparisons using the Wilcoxon's rank sum test with continuity correction. Capital letters refer to the comparisons among results obtained for oysters stored for 180 days, and lowercase ones refer to those stored for 365 days.

acceptance parameters, did not differ significantly from recently harvested ones for most of the studied parameters. The exceptions were higher grades for the appearance of recently harvested oysters than for that of marinated oysters stored for 180 days (KW $\chi^2 = 6.6$, $p = 0.03$) (Fig. 3b); higher grades for the taste of recently harvested oysters than for the one of marinated oysters for products stored for 365 days (KW $\chi^2 = 6.68$, $p = 0.03$) (Fig. 3e); and higher grades for the overall impression of recently harvested oysters than for the

one of thawed oysters (WV and WOV), for products stored for 180 days (KW $\chi^2 = 7.8$, $p = 0.02$) (Fig. 3f). On the other hand, the acceptability indexes (Table 1), inferred based on the same database, highlighted that recently harvested oysters tended to reach higher indexes than thawed oysters. This result was consistent with the preference analysis since judges generally pointed out recently harvested oysters as their preference (Figs. 3g and 3h). WV and WOV displayed no apparent differences for preference results.

Table 1. Acceptability indexes for different attributes of cooked and vacuum-packed oysters submitted to different processing: recently harvested animals (control), and thawed oysters marinated with vinegar (WV) or not (WOV). The analyses were performed after 180 and 365 days of storage.

Treatment	Control		WV		WOV	
Storage time (days)	180 (%)	365 (%)	180 (%)	365 (%)	180 (%)	365 (%)
Colour	87.5	90.5	77.5	84.1	72.5	85.7
Appearance	92.5	92.1	80.0	77.8	65.0	82.5
Flavour	77.5	87.3	80.0	74.6	80.0	73.0
Texture	92.5	88.9	72.5	79.4	70.0	82.5
Taste	85.0	93.7	75.0	82.5	80.0	74.6
O.A. impression	92.5	88.9	72.5	79.4	75.0	73.0
Purchase intention	90.0	91.4	75.0	77.1	67.5	74.3

O.A. impression: overall impression.

DISCUSSION

According to Loose et al. (2013), consumers believe they have lack of knowledge about seafood preparation, which they consider too time-consuming, and preparation format is regarded as the second strongest barrier to oyster consumption, besides the price. The same authors argued that smart retailing and packaging solutions can potentially overcome the consumers' impression, making transport, preparation, and storage more manageable for customers.

The shucked frozen products are a widely adopted alternative (Botta et al., 2020). Despite the convenience, frozen oyster meat might undergo a progressive decline in quality with changes in its physical, chemical, microbiological, and organoleptic characteristics (Dong et al., 2023). This study evaluated physicochemical, microbiological, and sensory characteristics of pre-cooked frozen shucked oysters, marinated with vinegar, and vacuum-packed. We expected this technique to maintain the product overall quality and provide a better consumer experience, providing an almost ready-to-eat product with visual appeal.

The results showed that the thawed vacuum-packed pre-cooked oysters maintained satisfactory quality since all the

physicochemical and microbiological parameters studied were in accordance with adequate levels during the storage time. The *Escherichia coli* concentrations of frozen oyster meat were lower than 230 MPN, as established by the Brazilian regulation (Brasil, 2022). The pH values registered range was above 6.0, but below the 6.85 limit established by Brazilian regulations (Brasil, 2017). Similar levels of pH were previously reported for frozen oysters: pH ranging between 5.72 and 6.45 for *C. gigas* stored at -20°C for 12 months (Lee et al., 2017), between 6.98 and 6.39 for *C. gigas* stored at -20°C for 28 days (Dong et al., 2023), and between 6.32 and 6.4 for *Crassostrea belcheri* stored at -20°C for 12 months (Songsaeng et al., 2010). The obtained moisture results (range = 70.8 to 75.8%) were not as high as the 76.6% reported by Cruz-Romero et al. (2004) for fresh *C. gigas*, and not as low as the 72.5% for steamed *Crassostrea hongkongensis*, reported by Liu et al. (2021), either. The TVB-N values (range = 3.56 to 16.56 mgN·100 g⁻¹) were all below the 30 mg of TVB-N·100 g⁻¹ maximum limit established for fish products by Brazilian regulations (Brasil, 2017). Concerning the microbiological parameters, the maximum values of mesophiles recorded by the end of our assay, of 10^{3.4}, were lower than

the maximum level of 10^7 CFU·g⁻¹ in fish samples intended for human consumption suggested by the International Commission on Microbiological Specifications for Foods (1986).

These results evidenced the excellent preservation of the studied oysters. Changes in pH can be used as a postmortem indicator as glycogen transforms into lactic acid (decreasing pH), and after manipulation of the muscle into components such as proteins and nucleotides (increasing pH) during long-term storage (Min et al., 2020; Songsaeng et al., 2010). Moisture levels were intermediate between those expected for fresh and cooked oysters, the low levels of TVB-N indicated the presence of low levels of metabolites that might be produced if the product were degrading, and the low bacterial count demonstrated no critical contamination.

It was also possible to detect a subtle effect of the vinegar on the microbiological quality of the products. Vinegar was the chosen preservative for this study, considering the growing demand for products with fewer chemical preservatives and using molecules of natural origin, which act as potent antimicrobials when dissociated. Vinegar is recognized as a natural antimicrobial and antioxidant that can improve safety, increase shelf life, and provide acceptable sensory quality to the products (Di Toro et al., 2019). Vinegar used for marination, as it happened in this study, is an ancient technique used to improve the texture of hard meat, provide pleasant flavors, and increase the shelf life of perishable foods (Björkroth, 2005). Despite the overall low bacterial counts registered during the present study, the analyses detected lower levels of psychrotrophic bacteria in marinated oysters. On the other hand, these oysters also underwent higher dehydration, evidenced by significantly lower moisture, which could cause changes in the product's texture, softness, color, and juiciness.

The results of sensory analyses showed that judges attributed similar grades to oysters marinated or not with vinegar. This favorable evidence indicated that this preservative substance in a dilution with beneficial effects reducing psychrotrophic bacterial counts does not deteriorate the product's organoleptic characteristics. Furthermore, the acceptability indexes suggested that marinated oysters tended to have higher acceptability than those not. Marinated samples obtained slightly higher acceptability indexes; non-marinated were the only ones that reached indexes lower than the 70% limit, considered a threshold for good acceptability (Dutcosky, 2011). The purchase intention results also corroborated the judges' preference for marinated oysters rather than non-marinated ones: 57% of judges pointed out that they would buy the product after one year of storage.

In comparison, only 29% would buy the non-marinated oysters. It is worth mentioning that the same marinated oysters

did not reach as good grades during the sensory analysis carried out after 180 days of storage. Such variations might be expected since sensory evaluations have a subjective element that is affected not only by the physical property of the sample but also by the psychological perspectives of the panel involved in the tests (Stone & Sidel, 2004). Anyway, sensory methods can be considered the most comprehensive and analogous to consumers' expectations for quality, linked to product acceptance in the market (Cao et al., 2009). The panelists could not detect differences in the color of the product among marinated and non-marinated oysters; therefore, the allegation of some restaurant chiefs that acetic acid can promote color maintenance on frozen oyster could not be confirmed in this study.

Finally, the sensorial analysis evidenced that the judges preferred the recently harvested oysters. The acceptability indexes, in general, were much higher for recently harvested oysters, and higher grades were attributed to most of the evaluated parameters, even when the statistical tests could not detect significant differences. Previous studies with frozen oysters also noted changes in the sensory attributes of the frozen product. Songsaeng et al. (2010) processed shucked oysters (*C. belcheri*) using a freezing tunnel at -40 and -60°C and stored them at -20°C for 12 months and observed changes in color and texture, while odor and appearance were less affected.

In agreement with findings for sensory attributes, the purchase intention also showed preference for recently harvested oysters: more than 72% of judges would buy them. Interestingly, this preference seemed more noticeable in the comparison with frozen oysters after 180 days of storage than after one year of storage, suggesting that the sensory characteristics of the frozen oysters were better after 365 days than after 180, which seems illogical. Therefore, we hypothesized that the result is linked to the quality of control-group oysters and not to the frozen oysters' characteristics, as the control oysters probably were fatter in the 180-days essay than in the 365-days essay. This fact is explained by the influence of the fresh oysters' seasonal variation (Kim et al., 2014; Orban et al., 2004), since they were collected at different times (for the analysis after 180 days, the control oysters were harvested during the winter and for 360 days they were gathered in the autumn). The oysters' organoleptic characteristics are expected to vary over time, according to their reproductive cycle (Sühnel et al., 2017); therefore, consumers' sensory perceptions about recently harvested oysters are also expected to change over time.

From a commercial perspective, this could be an indication that, in Brazil's subtropical coast, for example, the consumers'

interest for frozen oysters might be higher in the summer, when farmed oysters tend to spawn, negatively impacting meat yield, flavor, and appearance. Future studies can confirm this hypothesis.

CONCLUSION

Both methods for processing, packing, and storing oysters maintained adequate physicochemical and microbiological characteristics for up to one year of storage. Evidence proved that consumers preferred the thawed oysters marinated with vinegar before freezing. This observation is relevant to the industry since vinegar marination is a simple and inexpensive technique that could be easily incorporated, and good news for shellfish farmers since oysters harvested and shucked at the time of best meat yield could be conserved and traded during the highest demand season using the proposed method. Future studies can investigate whether the proposed packing arrangement has a visual appeal that could positively impact the trade of the products.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

The data will be available upon request.

AUTHORS' CONTRIBUTION

Investigation: Bernardi, F., Suplicy, F.M., Miotto, M.; **Writing – Original Draft:** Bernardi, F., Suplicy, F.M.; **Writing – Review & Editing:** Bernardi, F., Suplicy, F.M., Souza, R.V., Miotto, M., Tribuzi, G.; **Conceptualization:** Suplicy, F.M.; **Methodology:** Suplicy, F.M.; **Project Administration:** Suplicy, F.M.; **Formal Analysis:** Souza, R.V.; **Visualization:** Souza, R.V.; **Supervision:** Tribuzi, G.; **Final approval:** Suplicy, F.M.

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