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Vacuum-Packaged Sous-Vide Mackerel (*Scomber colias*) Fillets for School Canteens: Product Development, Acceptance, and Storage Trial

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Abstract

The Atlantic chub mackerel (*Scomber colias*) is a nutritionally valuable species with potential for inclusion in school canteens. This study aimed to develop and evaluate mackerel-based products processed through marination, vacuum packaging, and sous-vide cooking. Following collective interviews with school canteen staff to assess acceptability and logistical suitability, the preferred variants—raw, marinated, and sous-vide marinated fillets—were subjected to a 49-day refrigerated storage trial, during which physicochemical and microbiological parameters were monitored. Results showed that sous-vide processing significantly improved product stability, with enhanced water retention, reduced microbial growth (mesophile and psychrophile abundances below 7 log CFU/g up to day 21 vs. day 7 in raw and marinated fillets), and lower levels of spoilage indicators such as TVB-N, kept within acceptable limits of 25–35 mg N/100 g until day 28 of storage. Although sous-vide fillets showed slightly higher lipid oxidation (TBARS of 11.52 mg MDA/kg vs. 8.82 and 6.94 mg MDA/kg in marinated and raw fillets), they maintained superior texture and water retention. Overall, sous-vide proved highly effective in preserving the quality and extending the shelf-life of mackerel fillets, supporting its application in institutional food services as a strategy to promote healthier eating habits among children.

Keywords: vacuum-packaged; sous-vide; mackerel (*Scomber colias*); school canteens; storage trial; acceptance

Academic Editors: Monika Marcinkowska-Lesiak, Andrzej Półtorak and Iwona Wojtasik-Kalinowska

Received: 31 July 2025
Revised: 23 August 2025
Accepted: 25 August 2025
Published: 28 August 2025

Citation: Furiski, B.S.; Esteves, E.; Aníbal, J. Vacuum-Packaged Sous-Vide Mackerel (*Scomber colias*) Fillets for School Canteens: Product Development, Acceptance, and Storage Trial. *Appl. Sci.* **2025**, *15*, 9455. https://doi.org/10.3390/app15179455

1. Introduction

Global capture fisheries production reached 92.3 million metric tonnes in 2022. Notably, global trends in marine capture continue to be predominantly driven by major producing countries and on the most frequently exploited species. These dynamics reaffirm that marine fish stocks remain in decline regarding the proportion that stays within biologically sustainable limits [1,2]. However, the Atlantic chub mackerel (*Scomber colias*) represents an underexploited resource that is abundant in the Atlantic Ocean and adjacent waters [3], gradually gaining attention as a species of interest within the Portuguese market.

Of the total aquatic animal production, 89 percent was used for human consumption, equivalent to an estimated 20.7 kg per capita in 2022. The rest went to non-food uses, mostly

fishmeal and fish oil [2]. In general, aquatic foods exhibit diverse and valuable nutritional properties that may play a decisive role in addressing malnutrition and improving food security [4]. Among these, fish are recognised as a strategic component in the promotion of public health and are consistently endorsed by dietary guidelines worldwide, including the Food-Based Dietary Guidelines of the European Commission and its Member States, as well as the Dietary Guidelines for Americans (2020–2025) [5,6]. Fish are important sources of energy, high-quality protein, and polyunsaturated fatty acids (PUFAs) and account for approximately 17% of global animal protein intake and up to 50% in coastal populations [7,8].

From a nutritional standpoint, mackerel is recommended for vulnerable population groups, particularly pregnant women and young children (1–11 years), as it contributes significantly to a balanced and health-promoting diet by providing essential nutrients. Species of mackerel are high in contents of omega-3 fatty acids, such as eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), and docosahexaenoic acid (DHA, 22:6n-3), and omega-6 fatty acids. In addition, they are rich in micronutrients such as iron, iodine (especially during pregnancy), choline, and zinc—all of which are crucial for supporting immune function and proper development during early childhood [5,8,9].

Despite strong scientific and institutional support for these guidelines, current evidence indicates that many children in Western populations fail to meet recommended nutritional targets. Their diets are typically characterized by low consumption of fish, fruits, vegetables, and fiber-rich foods, alongside excessive intake of products high in sugar and saturated fats. This issue has received growing attention, as the early introduction of fish into dietary patterns is viewed as a fundamental strategy for establishing lifelong healthy eating habits [10].

In response to these challenges, recent studies suggest that interventions targeting the school food environment, such as offering healthier meals and snacks, may serve as effective tools for improving children's dietary habits in both the short term and the long term [10]. The development of new fish-based products and their inclusion in school meals has been identified as a strategy to stimulate fish consumption, especially during the early school years, when eating habits begin to form [11]. Schools thus emerge as crucial environments for implementing structured public health interventions aimed at improving childhood nutrition. By fostering the development of healthy dietary behaviors during childhood, such initiatives hold the potential to influence long-term health outcomes and reduce the burden of diet-related diseases within the general population [4,5,9,10,12–14].

Despite their nutritional benefits, one major challenge associated with including fish in school canteens is their high susceptibility to post-mortem spoilage due to autolytic, microbiological, and chemical processes. Fish spoilage begins immediately after death or capture and results from a series of changes induced by bacteria and enzymes [15,16]. The nutritional characteristics of fish are key contributors to this spoilage, including high water content (50–85%) and protein levels (12–24%) and a high and variable lipid content (up to 22%) [8]. Most of the protein (80–90%) is located in the muscle, with the remainder comprising non-protein nitrogenous compounds such as volatile bases (ammonia, methylamine, dimethylamine, and trimethylamine), trimethylamine oxide (TMAO), creatine, free amino acids, nucleotides, purine bases, and urea—all of which influence sensory characteristics and are relevant to the spoilage process of fishery products. In addition, lipid content can vary significantly, even within the same species. The high content of PUFAs in fatty (or blue) fish such as *Scomber colias*, while nutritionally valuable, creates conditions for hydrolysis and oxidation, leading to rancidity and the formation of characteristic off-flavors and odors (aldehydes and ketones) [8].

The literature reports several strategies to extend the shelf-life of such products, including vacuum packaging and various processing methods [15]. Additionally, species-specific and handling-related factors—such as the capture method, production, transport, and processing—directly influence quality loss and the shelf-life of fishery products [8]. In addition, in the context of collective food services such as school canteens, factors such as cost, convenience, accessibility, food safety, and variety in menu offerings play a key role in aligning with the preferences and logistical constraints of school catering services. Furthermore, parameters such as the nutritional quality of food, recipe composition, preparation methods, and the impact of cooking techniques, as well as time and temperature management during preparation and distribution (e.g., cook–chill, cook–freeze, warm-holding), must be considered to produce high-quality food on a large scale [17].

Among the techniques that influence the preparation of mackerel fillets, this study opted to apply and combine the following: (1) marination is the process of soaking in a seasoned liquid (before cooking or) to enhance sensory value (flavor, tenderness, juiciness) and extend the shelf-life. The antimicrobial properties of marinades are attributed to reduced pH, lower water activity, and the addition of herbs and antimicrobial food additives [18]; (2) vacuum packaging (VP), a technique involving the removal of air from the packaging, which, when combined with refrigeration, extends the shelf-life of seafood products by limiting the oxygen availability necessary for the growth of aerobic bacteria. Additionally, VP delays oxidative reactions such as lipid rancidity and provides an effective barrier against external contaminants, maintaining product integrity and safety throughout storage and distribution. This method not only ensures microbiological stability but also reduces economic losses associated with spoilage of fish and fisheries products. It also reduces moisture and gas permeability and provides protection from external contaminants; and (3) Sous-vide, a method involving the cooking of vacuum-sealed food at precisely controlled temperatures. This technique promotes efficient heat transfer and extends the shelf-life by reducing recontamination and oxidation. Studies have shown that sous-vide-treated fish exhibit significantly less protein denaturation, better preservation of moisture and PUFAs (such as EPA and DHA), and superior texture and structure when compared with high-temperature cooking. It also preserves the aroma and moisture, enhances cooking yields, inhibits bacterial growth, and improves flavor, texture, and nutritional quality when compared with traditional methods [19–26]. Additionally, the incorporation of natural antioxidants—such as rosemary, oregano, and basil—into sous-vide fish preparation has also proven to be effective in reducing lipid oxidation and maintaining microbiological quality, thereby further extending the shelf-life [27].

This study aimed to develop and characterize mackerel-based products processed via sous-vide, marination, and vacuum packaging—methods suitable for practical implementation in school canteens in southern Portugal. We engaged school canteen staff in groups and collected interviews pre-selection of product prototypes, assessing their feasibility, applicability, and acceptability. We then analyzed freshness and quality indicators (physicochemical and microbiological) to assess their changes and determine the storage times of the selected products under refrigerated storage.

2. Materials and Methods

2.1. Study Design

This study was conducted in two phases (Figure 1). In the first phase, four different (prototype) formulations of vacuum-packaged mackerel fillets were prepared (raw, marinated, sous-vide, and sous-vide marinated). The developed food formulations were presented during collective interviews to food service personnel from various school and university canteens within the municipality of Faro, with the aim of assessing their suit-

ability for large-scale procurement and distribution in student meal programs. In the second phase, the mackerel fillet preparations deemed most acceptable and/or of particular interest were stored under controlled refrigeration conditions (storage trial). Subsequently, a series of physicochemical and microbiological analyses were conducted to monitor quality changes.

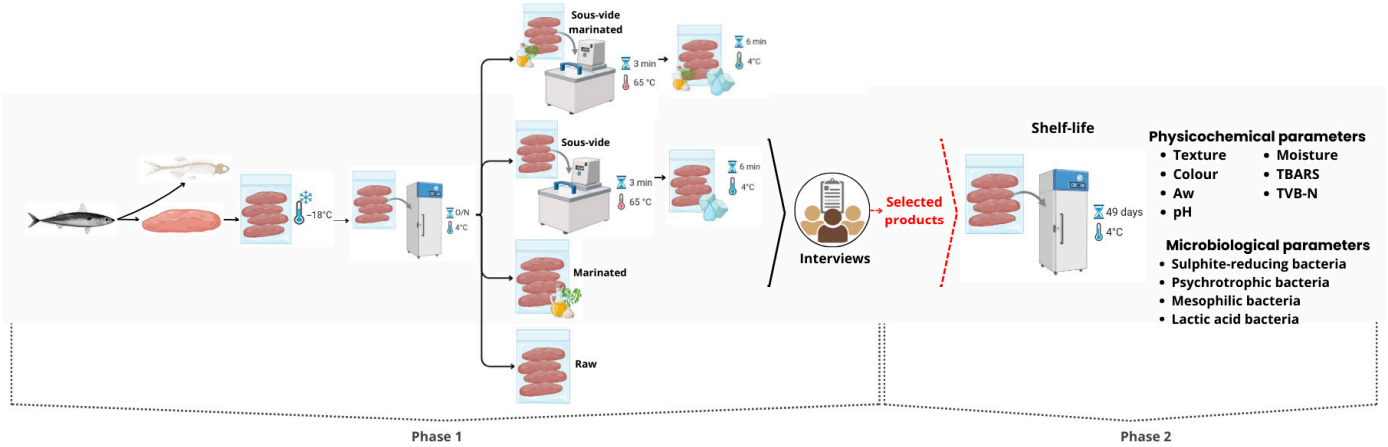

Figure 1. Overview of the two-phase experimental design for formulation development and shelf-life assessment of vacuum-packaged mackerel fillets.

2.2. Preparation of Fillets

Fresh mackerel (*Scomber colias*) were purchased (ca. 30 kg, two commercial boxes) at the local fish market in Faro (southern Portugal). The specimens were transported to the laboratory at the University of Algarve in Faro using insulated boxes containing flake ice (Figure 2a). Upon arrival, the fish were washed with tap water and filleted under hygienic conditions, discarding the head and taking care to remove all spines from the dorsal, ventral, anal and caudal areas. Special attention was given to the removal of the internal spines adjacent to the lateral line (Figure 2b) since the products are intended for school canteens and may be consumed by very young children who lack the fine motor skills required to remove these bones. From a total of fresh mackerel, a random subsample of twenty specimens (n = 20) was selected to assess body weight (BW) (138.8 ± 22.9 g, mean ± SD), fillet weight (FW) (60.2 ± 10.7 g), and fillet yield percentage (FY) (43.4% ± 3.7). Also, fillet yield was calculated as FY = (FW / BW) × 100, in accordance with the method described in Sang et al. [28].

(**a**) (**b**)

Figure 2. The different stages of fillet preparation: (**a**) fresh mackerel stored in boxes containing flake ice; (**b**) final product obtained after filleting under hygienic conditions, with removal of all the internal bones.

Immediately after filleting, groups of eight fillets were packaged per bag (300 × 400 mm and a thickness of 90 µm; Tecknopack, Venda do Pinheiro, Portugal) and vacuum-sealed at 99% vacuum (650 mbar) for 15 s in accordance with Henkelman [29] using a vacuum-packing machine (Boxer 42, Henkelman, 's-Hertogenbosch, The Netherlands) and then frozen at −18 ± 2.5 °C (Figure 3). The packaging film consisted of an outer cast polyamide (PA) layer and an inner polyethylene (PE) barrier layer, providing an oxygen transmission rate (OTR) of 40.5 cm^3 m^{-2} d^{-1} atm^{-1} at 23 °C and 0% relative humidity. This procedure was carried out to minimize lipidic oxidation during the storage period.

Figure 3. Mackerel fillets vacuum-packed prepared for subsequent frozen storage.

2.3. Product Processing

Prior to the collective interviews or storage trial, the frozen fillets were thawed under refrigeration at 4 ± 1 °C for 8–10 h. After thawing, the fillets were divided into four groups (Figure 4): raw (A), marinated (B), sous-vide (C), and sous-vide marinated (D).

Fillets in Group A were not further processed, but repackaged using the same material and procedure as pre-packaging and refrigerated at 4 ± 1 °C. This experimental group functioned as the control. The remaining groups were subjected to additional processing steps, including marination and sous-vide cooking, applied either separately or in combination, before returning to refrigeration at 4 ± 1 °C.

Fillets assigned to groups B and D were marinated in a mixture consisting of olive oil (1:4 oil-to-fillet ratio), garlic (10% of oil's weight), and oregano (1 g) in accordance with Martins [30]. The fillets remained in the marinade for 10 min, followed by a 1 min draining period to remove excess marinade. After draining, the fillets in Group B were vacuum packaged following the procedure described above.

Fish fillets from groups C and D were vacuum-packaged and subsequently immersed in a thermostatic water bath (model SUB36, Grant, Royston, UK) heated by a circulating heating unit (model DC10, Haake, Karlsruhe, Germany) at 65 ± 1 °C for 3 min—i.e., the sous-vide. Following the sous-vide cooking process, the products were subjected to an immediate cooling procedure in ice-chilled water at a temperature of 3 ± 1.5 °C for 6 min. This cooling step was intended to halt the ongoing cooking process. Thereafter, the products were stored under refrigeration conditions at a temperature of 4.0 ± 1.0 °C.

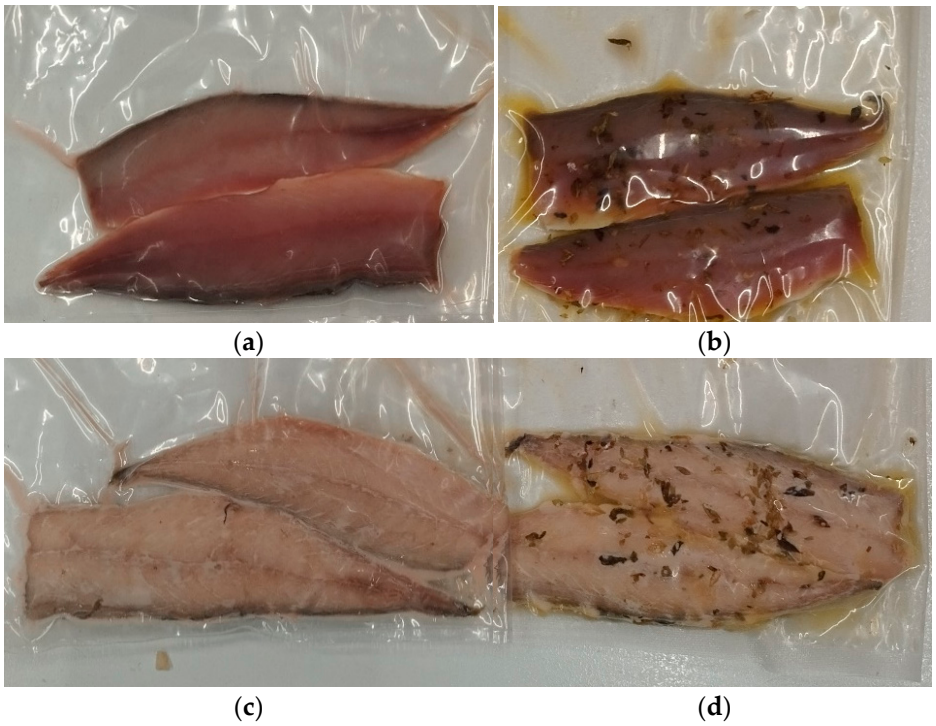

Figure 4. Fillets obtained from different processing methods, divided into four groups and vacuum pre-packed: (**a**) Group A—raw fillets; (**b**) Group B—marinated fillets; (**c**) Group C—sous-vide fillets; (**d**) Group D—marinated sous-vide fillets.

2.4. Collective Interviews

A qualitative research design was adopted to assess the convenience and potential receptiveness of the products developed in this study within collective catering settings in the school context of southern Portugal. The study involved professionals working in public school canteens in the municipality of Faro, as well as in the canteens of the University of Algarve. In total, seven semi-structured group interviews were conducted over a two-month period, following an adapted framework based on Hall [31] and Krueger [32]. The sessions included 69 professionals from 16 educational institutions, comprising operational assistants, coordinators, and procurement managers. Each focus group consisted of 7 to 15 participants and lasted approximately one hour. The interviews were guided by questions designed to elicit opinions grounded in the participants' individual experiences, and active dialogue among participants was encouraged. Both oral and written contributions were systematically recorded by the facilitators, alongside additional written responses provided directly by participants. These were collected using a semi-structured questionnaire, which explored perceptions of product concept and packaging. Key aspects addressed included compatibility with institutional menus, suitability for preparation, versatility, logistical barriers to implementation, and consumer appeal. Participants were also asked to evaluate the appearance, aroma, and texture of the fillets outside the packaging. Finally, they were invited to identify the most appropriate formulations for use in institutional catering. The analysis of responses indicated the following product preferences: A (32.23%), D (30.58%), B (28.10%), and C (9.09%), with some participants selecting more than one option.

Due to their higher levels of acceptance reported during the interviews, products A, B, and D were selected for subsequent storage trial.

2.5. Storage Trial

Based on the products chosen in the first part of this work (A, B, and D), vacuum-package bags with 3 mackerel fillets each were prepared and stored under refrigeration at 4 ± 1 °C. The sampling took place on days 0, 2, 7, 14, 21, 28, 35, 42, and 49 for the evaluation of quality parameters. In each sampling moment, 2 bags of each formulation (1 for physicochemical and chemical analysis and 1 for microbiological analysis) were thawed and opened.

2.5.1. Physicochemical Analyses

Commonly studied quality parameters in seafood products were assessed: color, pH, water activity, moisture, texture, TVB-N, and TBARS.

Color measurements were carried out directly on fish fillets using a trisitumulus colorimeter/spectrophotometer (model PCE-CSM 10, PCE Instruments) according to the CIE Lab color scale (CIE Lab). Measurements of L*, a*, and b*, where L* refers to lightness (0 is black and 100 is white), a* indicates greenness (a < 0) or redness (a > 0), and b* measures the blueness (b < 0) or yellowness (b > 0) of samples, were made at five different points in each fillet. The colorimeter was calibrated using black and white control tiles according to manufacturer instructions [8,16]. As a summary measure, total color change (denoted ΔE) was calculated using $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, where for example $\Delta L^* = (L^*t - L^*0)$ and L* refers to lightness at time *t* (L*t) and time 0 (L*0).

pH was measured three times directly in the fillets using a pH meter (model GLP 21, CRISON Instruments S.A., Allela, Spain) equipped with semi-solids probe No. 52-32 (CRISON) according to Esteves et al. [16].

Water activity (aw) was determined in samples of 2–3 g of minced muscle from each fillet using an aW meter (model HP23-AW-A, Rotronic, Bassersdorf, Switzerland) adjusted to 25 °C. Moisture contents of fillets were determined following the AOAC [33] method (Ref. 950.46). Briefly, 2 g samples per fillet were dried for >16–18 h at 100–102 °C in air oven. After cooling in a desiccator, dried samples were weighted, and the loss in weight was reported as moisture (g).

A Texture Profile Analysis (TPA) was conducted using an LFRA Texture Analyzer (Brookfield Engineering Labs Inc., Middleborough, MA, USA) equipped with a 1.5 kg load cell. The method involved compressing the sample twice with a 12.7 mm-diameter stainless steel spherical probe which approached the sample at a speed of 2 mm/s until 50% of the compression height was reached. A waiting time of 2 s was observed between compressions. Measurements were analyzed using TexturePro Lite v1.1 software (Brookfield Engineering Labs Inc., USA). The texture properties explored in this study were hardness, cohesiveness, chewiness, gumminess, adhesiveness, and springiness. Hardness (in g.f, where 1000 g.f. = 1 kg.f = 9.806 N) is a measure of the force required to deform or break the fish muscle sample. It provides information about the firmness of the muscle and can be used to assess the freshness of the fish. Cohesiveness measures the ability of the fish muscle to stick together after being deformed. It indicates the degree of internal bonding within the muscle and can be used to assess the freshness of the fish. Gumminess (g.f) measures the energy required to disintegrate a semi-solid food to a state ready for swallowing. It reflects the overall texture of the muscle and can be used to assess the freshness of the fish. Springiness (%) measures the ability of the fish muscle to return to its original shape after being compressed. It reflects the elasticity and resilience of the muscle and can be used to assess the freshness of the fish. Chewiness (g.f) represents the effort required to masticate or break down the fish muscle during chewing. It combines the attributes of cohesiveness, hardness, and resilience and can be used to assess the freshness of the fish [34,35].

The total volatile basic nitrogen (TVB-N) content was analyzed according to the microdiffusion process followed by acid–base titration described by Conway and Byrne [36], which the Portuguese standard NP 2930 [37] adopts and details for quality control and is a routine, scientifically recognized alternative for checking TVB-N limits in fish based on Regulation CE 2074/2005 [38]. In essence, the Conway microdiffusion method determines TVB-N in fish by volatilizing basic nitrogenous compounds under alkaline conditions (extraction and alkalization), capturing them in an acid solution (microdiffusion), and quantifying them through titration. The method uses a specialized piece of glassware called a Conway dish. The muscle content in TVB-N was expressed as mg N/100 g.

The thiobarbituric acid reactive substances (TBARS) content was measured through the spectrophotometric method adapted from the Portuguese Standard NP 3356 [39], using Butylated Hydroxytoluene (BHT) as an antioxidant to prevent further lipid oxidation during sample preparation [40]. Briefly, fish fillet muscle tissue (2 g) was homogenized with BHT and trichloroacetic acid (TCA), filtered, and then reacted with thiobarbituric acid (TBA); the absorbance was measured at 532 nm using a Hitachi U-2000 spectrophotometer (Tokyo, Japan). A standard curve was prepared daily. The TBARS value was expressed as mg MDA/kg fish.

2.5.2. Microbiological Analysis

Microbiological quality was assessed using standard methods described by the Nordic Committee on Food Analysis No. 184:2006 [41], which include the aerobic count of specific spoilage organisms such as sulfite-reducing bacteria (incubated for 72 ± 6 h at 25 ± 1 °C in Iron Agar medium), psychrotrophic bacteria, and mesophilic bacteria (respectively incubated at 5 ± 1 °C and 15 ± 1 °C, for 5 days in Long & Hammer agar medium). In addition, mesophilic lactic acid bacteria (incubated for 72 ± 6 h at 25 ± 1 °C in Man, Rogosa and Sharpe medium) were enumerated according to ISO 15214:1998 [42]. All plates were visually inspected to identify characteristic colony types and morphological features specific to each culture medium. The microbiological counts, expressed as colony-forming units per gram (CFU/g), were converted to their logarithmic values before analysis.

2.6. Statistical Analysis

Results are presented as means ± standard deviations. Two-way ANOVA was conducted with the factors storage time (days) and type of processing applied to the product (different combinations of marination and sous-vide) for each quality parameter assessed during the storage trial.

The eventual significant effects of main factors were compared using the post-hoc multiple comparisons method of Tukey HSD. Whenever the interaction of factors was significant, estimated marginal means were compared pairwise with the correction of Bonferroni for the adjustment to the confidence intervals and significance [43].

The statistical analyses were carried out using IBM SPSS Statistics (version 29.0.2.0) at a significance level of 0.05.

3. Results and Discussion

This study primarily assessed changes in freshness and quality indicators, both physicochemical and microbiological, throughout a refrigerated storage trial (at 4 °C), to determine the shelf-life of the selected products evaluated during the collective interviews: A–raw, B–marinated, and D–marinated and sous-vide.

The storage trial was ended after different periods of time per treatment depending on the combination of bad results obtained in the quality parameters, namely those that are

regulated and/or have established/recommended limits: 28 days for A–raw, 35 days for B–marinated, and 49 days for D–marinated and sous-vide.

There were significant changes (ANOVA, $p < 0.05$) in several of the quality parameters assessed, namely among the processing methods (control/raw, marinated, and sous-vide marinated) and storage times sampled (Table 1). In the case of color, pH, TVBN, some of the textural parameters, and some of the microbiological indicators assessed, there were also significant effects of the interaction of the factors (ANOVA, $p < 0.05$).

Table 1. Results of two-way ANOVAs per quality parameter considering the factors processing methods (control/raw, marinated, and sous-vide marinated) and storage time (days).

Quality Parameters	Factors	F	*p*-Value
a_W	Processing	1.366	0.266
	Day	18.625	**<0.001**
	Processing × Day	0.694	0.737
Moisture content	Processing	13.854	**<0.001**
	Day	4.833	**<0.001**
	Processing × Day	1.274	0.271
L*	Processing	424.312	**<0.001**
	Day	90.937	**<0.001**
	Processing × Day	2.59	**0.012**
a*	Processing	17.935	**<0.001**
	Day	22.343	**<0.001**
	Processing × Day	3.264	**0.002**
b*	Processing	255.371	**<0.001**
	Day	3.822	**0.002**
	Processing × Day	5.581	**<0.001**
ΔE	Processing	4.493	**0.018**
	Day	63.266	**<0.001**
	Processing × Day	7.407	**<0.001**
pH	Processing	14.966	**<0.001**
	Day	2.491	**0.025**
	Processing × Day	2.528	**0.014**
TBARS (mg MDA/kg)	Processing	6.314	**0.004**
	Day	9.658	**<0.001**
	Processing × Day	1.265	0.276
TVB-N (mg N/100 g)	Processing	40.971	**<0.001**
	Day	54.418	**<0.001**
	Processing × Day	11.769	**<0.001**
Hardness (g.f)	Processing	5.513	**0.007**
	Day	1.617	0.147
	Processing × Day	3.198	**0.003**
Cohesiveness	Processing	8.928	**<0.001**
	Day	3.161	**0.006**
	Processing × Day	1.614	0.128
Gumminess (g.f)	Processing	4.200	**0.021**
	Day	2.244	**0.042**
	Processing × Day	4.764	**<0.001**
Springiness (%)	Processing	0.36	0.700
	Day	2.561	**0.022**
	Processing × Day	1.681	0.110

Table 1. *Cont.*

Quality Parameters	Factors	F	*p*-Value
Chewiness (g.f)	Processing	4.438	**0.018**
	Day	2.907	**0.011**
	Processing × Day	4.230	**<0.001**
Mesophiles (log CFU/g)	Processing	113.308	**<0.001**
	Day	107.195	**<0.001**
	Processing × Day	4.220	**0.001**
Psychrophiles (log CFU/g)	Processing	45.602	**<0.001**
	Day	211.972	**<0.001**
	Processing × Day	11.303	**<0.001**
Sulfide-reducing bacteria (log CFU/g)	Processing	93.37	**<0.001**
	Day	85.989	**<0.001**
	Processing × Day	0.767	0.634
Lactic acid bacteria (log CFU/g)	Processing	3.115	0.091
	Day	58.309	**<0.001**
	Processing × Day	2.495	0.060

3.1. Physicochemical Parameters

Throughout the storage trial, the physicochemical indicators of freshness and quality exhibited variable trends, contingent upon the processing methodologies, storage duration, and sometimes their interactions.

3.1.1. Moisture Content and Water Activity

Typically, fresh fish naturally have a very high moisture content, often ranging from 60–80% (0.60–0.80) or even higher. Herein, moisture content was significantly higher in the control (raw) compared to marinated and sous-vide samples (0.7389 ± 0.0123 vs. 0.7078 ± 0.0289 and 0.7210 ± 0.0197), as confirmed via Tukey's test ($p < 0.001$). Over time, moisture decreased steadily, up to day 21, across all treatments (less clearly in the case of the control) ($p < 0.001$). Fillets subjected to marination and to marination and sous-vide were the lowest moisture group (respectively, 0.6900 ± 0.0173 on day 28 and 0.6967 ± 0.0153 on day 35), which aligns with cooking-induced water loss due to the thermal processing of sous-vide affecting the structural integrity of muscle proteins [44]. Thus, the storage duration and processing influenced moisture differently but additively, with no interaction effects. On the other hand, the a_W in fresh fish is typically high (0.94–0.98), making it highly perishable [45]. Overall, a_W remained relatively high and stable for all processing methods, at $a_W > 0.98$, during the storage trial except for a drop at day 49 in sous-vide samples (to ca. 0.96). These results were reflected by the significant effect of storage time (day) ($p < 0.001$), but no significant effect of the processing method ($p = 0.266$) or the interaction between the method and day ($p = 0.737$) in the two-way ANOVA. Results suggests high water retention until late storage, especially in samples that preserve their muscle protein structure [46]. However, at these values of a_W, most spoilage bacteria and pathogenic bacteria can thrive, making the products still highly susceptible to rapid microbial spoilage [47,48].

Water activity and moisture content are both critical factors in determining the freshness, safety, and shelf-life of fish and seafood. While they are related, they measure different properties: moisture content is the total amount of water present, while a_W reflects the availability of that water for microbial growth and chemical reactions. Water activity is more directly linked to spoilage and microbial safety than total moisture content. The combination of very high moisture content and a very high a_W provides an ideal environment

for rapid microbial proliferation and enzymatic degradation and thus limits the shelf-life of the products.

3.1.2. Color Parameters

In terms of changes in color (Figure 5), results for L* (lightness) showed highly significant effects of the processing method, storage time, and their interaction (ANOVA, all $p < 0.001$), indicating that both factors and their combination influenced lightness. Post-hoc Tukey tests revealed that sous-vide samples had much higher L* values (70.92) than marinated and control fillets (46.91 and 43.92), reflecting a paler appearance due to protein denaturation caused by structural changes in muscle proteins, such as a reduction in the α-helix content and an increase in β-sheet structures [49]. An increase in the L* value in fish fillets suggests alterations such as those observed in heme proteins and/or loss channels (dripping) [16]. The storage time also increased L*, particularly from day 7 onward, with a pronounced shift and then a plateau, which was more apparent in sous-vide samples.

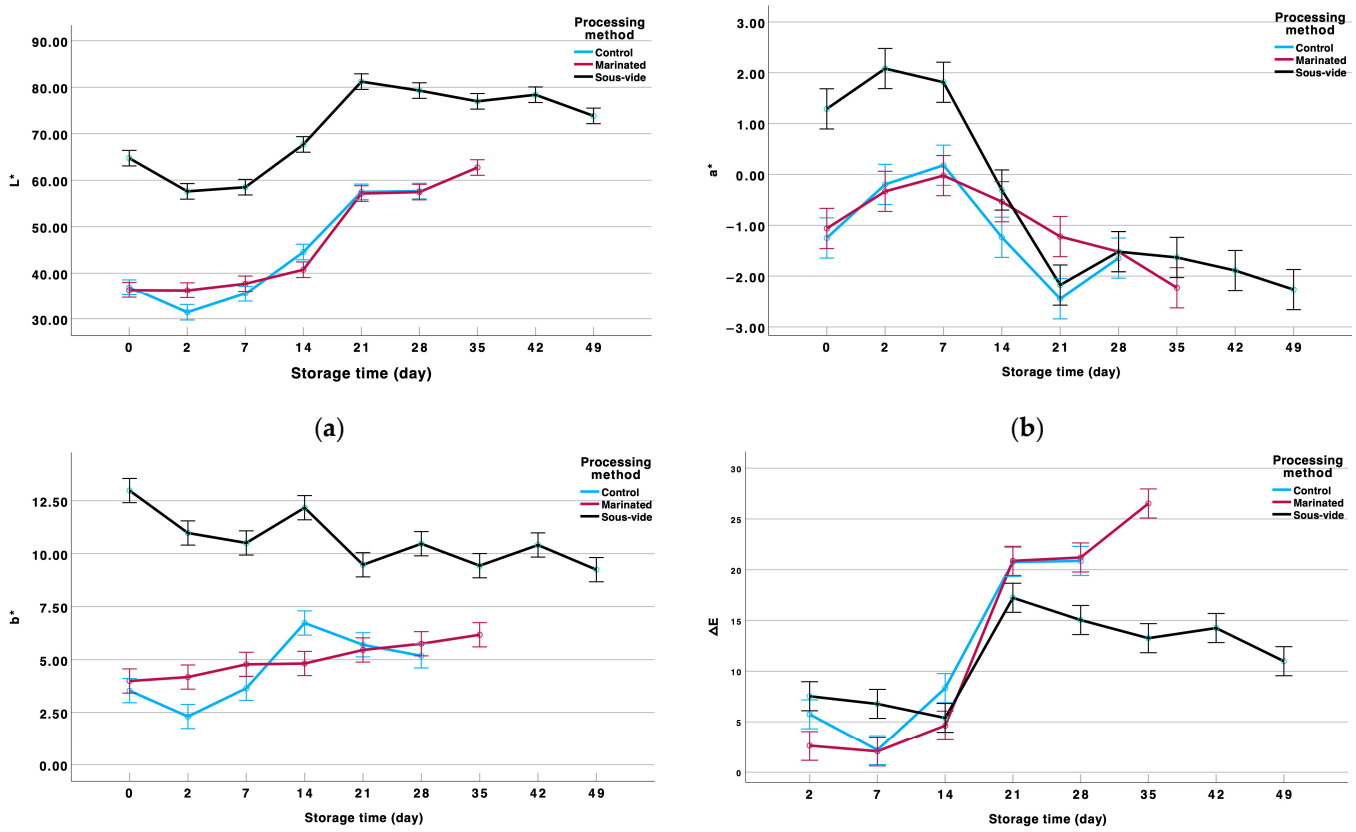

Figure 5. Color parameters: (**a**) L* (lightness); (**b**) a* (greenness/redness); (**c**) b* (blueness/yellowness); and (**d**) total color change ΔE according to the Commission Internationale de l'Èclairage (CIE) Lab scale of vacuum-packaged mackerel fillets (mean ± standard error, $n = 3$).

For a* (greenness/redness), all effects—processing method, storage time, and the interaction—were also significant (ANOVA, $p < 0.01$). Tukey tests showed that sous-vide fillets were significantly less red than raw or marinated ones ($p < 0.05$), with marinated samples slightly redder overall. During storage time, a* values increased initially (up to day 7)—more pronounced in sous-vide samples—and then decreased in all treatments, suggesting gradual browning or pigment degradation [50].

Also, in the case of b* (blueness/yellowness), processing methods, time, and their interaction were significant factors ($p < 0.001$). Tukey comparisons showed that sous-vide

samples (10.63 ± 1.63) were significantly more yellow than the others ($p < 0.001$) (5.02 ± 0.96 and 4.52 ± 1.64 for marinated and control fillets, respectively), likely due to the Maillard reaction products produced during storage, which contributes to the formation of browning substances and advanced glycation end products, or lipid-oxidation-related pigments [50]. The same intensification of yellowing was observed by Esteves et al. [16] and likely due to lipid oxidation. During the storage time, the values of b* decreased in the case of sous-vide samples but increased gradually in marinated fillets, particularly after day 21, supporting the idea that progressive yellowing in marinated samples and color stability or fading in sous-vide samples reflect different microbial and chemical dynamics during storage [51].

Delta-E (ΔE) quantifies the difference between two colors in the Lab color space and is commonly used to monitor color changes in fish and seafood during storage and spoilage [52]. In terms of ΔE, all the effects—processing method, storage time, and the interaction—were significant (ANOVA, $p < 0.01$). The ΔE progressed similarly across treatments and during the storage time up to day 14, but values were higher for sous-vide fillets (5.39–7.54) than for control (2.18–8.34) or marinated samples (2.05–4.63). On day 21, values soared to ca. 21 in the control and marinated fillets and to 17.2 in the sous-vide samples. Then on, the total color change decreased slowly in the sous-vide fillets (to 11.0). Studies show that ΔE values increase significantly as fish freshness declines, usually correlating with chemical and microbiological spoilage markers [53]. Differences in perceived color can be classified as imperceptible (ΔE < 1.0), small or perceptible through close observation (1 < ΔE < 2), distinct (1.5 < ΔE <3), and very distinct (ΔE > 3) or perceptible at a glance/detectable by the human eye at ΔE values above 5–12 [52–55]. Thus, the estimated changes in color are expected to be readily detectable by consumers.

Overall, sous-vide processing led to paler, less red, and more yellow fillets, with time intensifying these effects, especially after two weeks. However, despite these effects, sous-vide samples retained better microbiological stability, an acceptable texture, and extended shelf-life in terms of TVB-N, which are critical for food service applications (see below).

3.1.3. Changes in pH

pH is widely recognized as a reliable and practical indicator for monitoring fish freshness and spoilage [56]. Herein, the pH of vacuum-packaged mackerel samples ranged from 6.20 to 6.80 during the storage trial (Figure 6a).

The two-way ANOVA for pH showed significant main effects of both the processing method ($p < 0.001$) and storage time ($p = 0.025$), as well as a significant interaction ($p = 0.014$). This interaction indicates that the effect of storage time on pH differed depending on the processing method. Post-hoc Tukey tests revealed that sous-vide samples had significantly higher pH than raw and marinated ones ($p < 0.001$), i.e., 6.55 ± 0.115 vs. 6.43 ± 0.091 and 6.42 ± 0.090, respectively.

A similar difference in initial pH between raw and sous-vide samples was also observed by Pongsetkul et al. [57] in Nile tilapia fillets, with reported values of 6.35 ± 0.03 and 6.50–6.63, respectively. The higher pH observed in sous-vide samples may be attributed to the cleavage of bonds involving various sulphydryl and hydroxyl groups, which occurs more extensively during high-temperature processing [57]. Over storage time, the pH fluctuated between ca. 6.30 and 6.70. The variation was larger in sous-vide fillets than in control/raw and marinated treatments that showed smaller or more stable pH shifts and was significantly different among methods on days 0 and 2 (and then on day 28). These findings seemingly reflect relatively stable proteolytic or microbial activity over time, which was more pronounced in thermally processed (sous-vide) fillets [58]. Contrariwise, as the fish deteriorates, its pH typically rises due to the production of basic compounds from microbial and enzymatic activity. For example, in one study, the pH value of sous-vide

largemouth bass fillets increased after 6 days of storage, presumably due to the volatile alkaline components produced by spoilage bacteria [59]. pH changes in fish muscle are arguably a reliable indicator of quality per se, but herein, they were not related to changes (increases) observed in the TVB-N content and the microbiota (see below), thus not reflecting the breakdown of proteins and the accumulation of basic nitrogenous compounds like ammonia and amines.

3.1.4. TVB-N Content

TVB-N is widely used as a freshness and quality index for fishery products that measures the amount of volatile basic nitrogenous compounds produced as fish spoils, mainly due to the bacterial decomposition of proteins and enzymatic activity (Figure 6b).

In this study, TVB-N contents of fillets at the start of the storage trial were ca. 19 mg N/100 g. The ANOVA revealed strong and significant effects for all factors: processing method, storage time, and their interaction (all $p < 0.001$). This indicates that spoilage-related amine production evolved differently across treatments and storage durations. Tukey tests showed that TVB-N average values were significantly highest in marinated fillets (35.29 mg N/100 g), followed by control/raw (31.70 mg N/100 g), with sous-vide samples showing the lowest values (25.47 mg N/100 g) ($p < 0.05$). Pongsetkul et al. [57] also noted that sous-vide treatments involving higher temperatures and extended processing times are more effective in delaying the formation of basic compounds, such as TVB-N, which result from the activity of endogenous and microbial enzymes. TVB-N increased markedly with time, especially in marinated fillets and control samples, reaching levels suggestive of advanced protein degradation by day 21 (48.01 and 35.59 mg N/100 g in control and marinated fillets, respectively) and day 35 (40.85 mg N/100 g in sous-vide fillets), when the TVB-N accumulation, primarily due to the microbial and enzymatic degradation of fish proteins, exceeded acceptable limits (25–35 mg N/100 g) as stipulated in EU Regulation 1022/2008 that amended Regulation 2074/2005 [60,61]. These findings underline that control and marination did not effectively inhibit microbial or enzymatic spoilage, whereas sous-vide exerted a stronger preservative effect [62].

3.1.5. TBARS Values

TBARS (thiobarbituric acid reactive substances) values are widely used to assess lipid oxidation and freshness in fish. Typical TBARS values in fresh fish are low and increases indicate spoilage or oxidative damage.

Results showed significant effects of the processing method ($p = 0.004$) and storage time ($p < 0.001$), but no significant interaction ($p = 0.276$) upon TBARS, indicating that oxidative changes over time occurred consistently across treatments (Figure 6c). Sous-vide samples had significantly higher TBARS values (11.52 mg MDA/kg) than both marinated and control fillets (8.82 and 6.94 mg MDA/kg) (Tukey post-hoc tests, $p < 0.001$), reflecting increased lipid oxidation likely due to heat [58]. The relatively lower TBARS values observed in both marinated and control fillets may be attributed to the retention of lipid oxidation products at intermediate stages, where primary compounds such as hydroperoxides have not yet fully degraded into secondary products, namely aldehydes and ketones, which are detected by the TBARS assay [57]. Over storage, TBARS increased gradually from values of 3–7 mg MDA/kg to 17–18 mg MDA/kg on day 42–49 in sous-vide samples.

A similar pattern was also observed by Pongsetkul et al. [57]. By day 14, all treatments showed signs of (advanced) oxidation, i.e., values > 8 mg/kg. Even though no regulatory limits exist, good-quality frozen or chilled fish typically show TBARS levels in the range 5–8 mg MDA/kg, while values exceeding 8 mg MDA/kg indicate spoilage [63]. Nunes et al. [64] suggest that high-quality sardines stored in ice have

TBARS values < 5 mg MDA/kg. Moreover, values below 0.58 mg MDA/kg are perceived as not rancid, and values above 1.51 mg MDA/kg are perceived as rancid [63,65] in terms of sensory detection. For example, in the literature, reported values range from less than 2 mg MDA/kg in hake stored for 30 days [66] and up to 17.8 mg MDA/kg in iced sardines by day 9 of a 15-day storage trial [64] and 26.03 ± 8.00 mg MDA/kg in rainbow trout packaged in air on monitoring day 11 [67]. In addition, vacuum-packaging consistently results in lower TBARS values than other methods, both during chilled and frozen storage [65,67,68]. Herein, sous-vide fillets consistently had the highest levels, suggesting that thermal processing and subsequent chilled storage negatively influence oxidative lipid stability in mackerel fillets regarding primary and secondary lipid oxidation products [58]. In contrast, marination contributed to retarding the oxidation of samples. In fact, marination can significantly extend the time a fish remains safe and palatable, especially when combined with refrigeration or vacuum-packaging [69]. For example, Lemos et al. [70] observed an extension of up to 20 days in the shelf-life of marinated and vacuum-packaged salmon, and Saju et al. [71] observed an extension of >20 days in sardine.

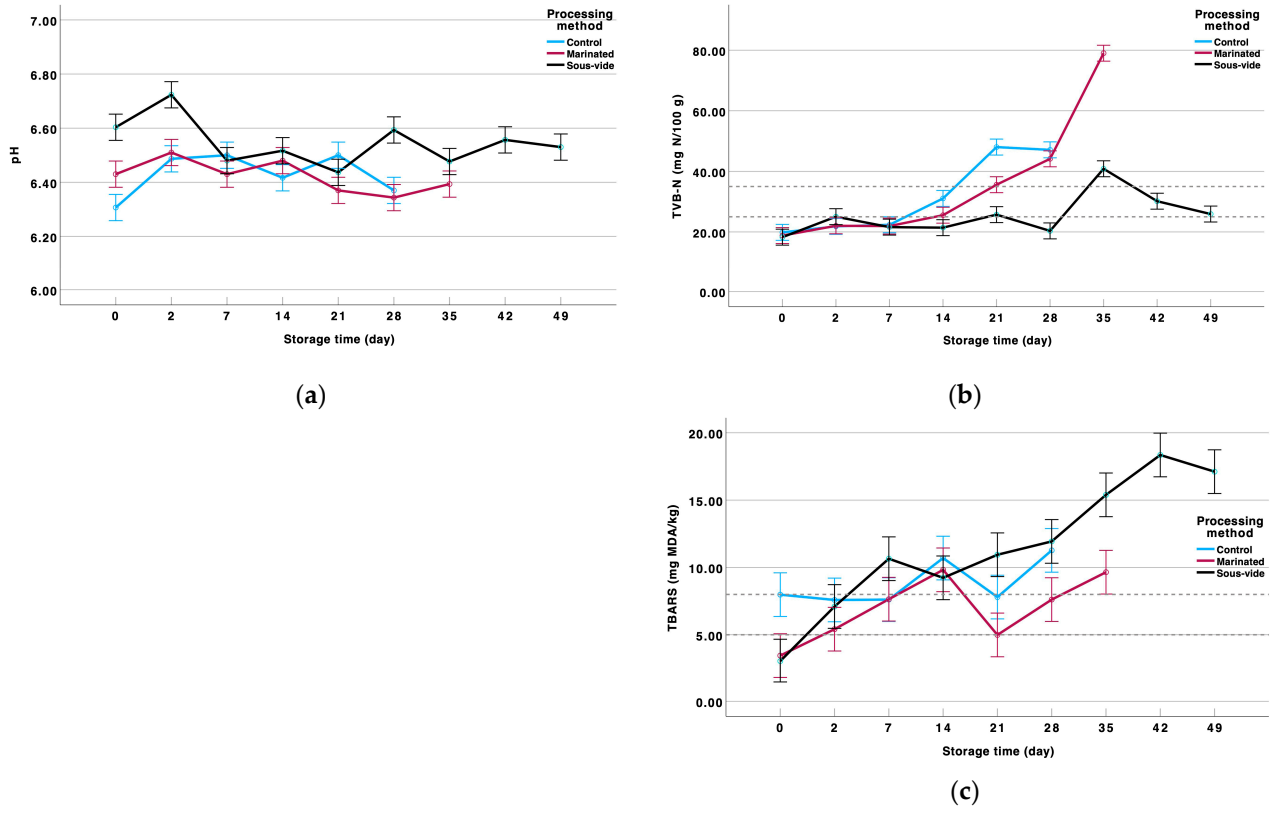

Figure 6. Freshness indicators: (**a**) pH; (**b**) TVB-N (mg N/100 g); and (**c**) TBARS (mg MDA/kg) of vacuum-packaged mackerel fillets (mean ± standard error, n = 3). In the plot of TVBN and TBARS, the parallel dashed, grey lines depict the range of concentrations defined as the threshold [60,61] and acceptable [63].

3.1.6. Texture

In terms of fillets' textures, there were significant effects of processing methods, and clear changes were observed over the storage time (Figure 7).

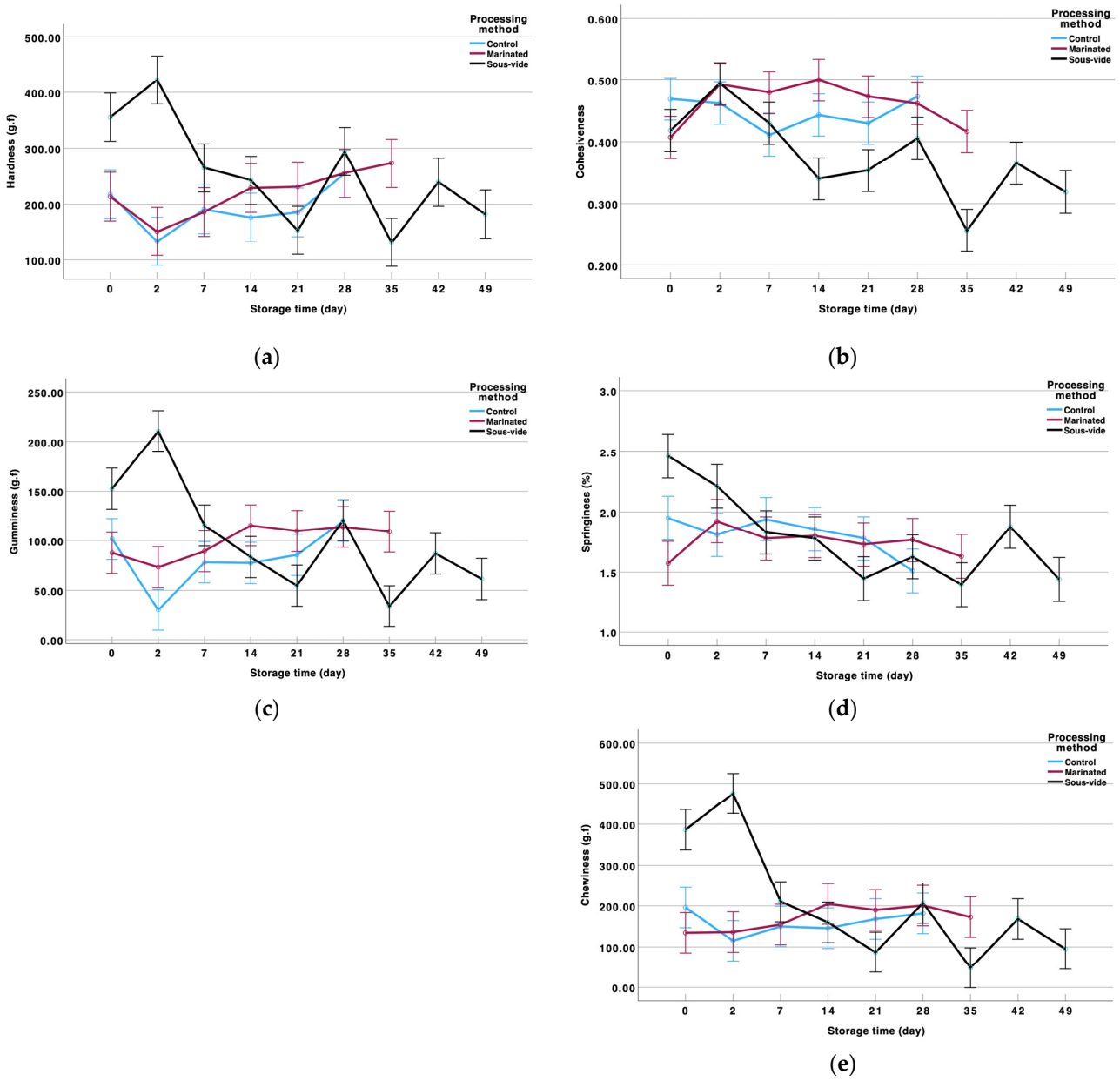

Figure 7. Texture parameters: (**a**) hardness (g.f); (**b**) cohesiveness; (**c**) gumminess (g.f); (**d**) springiness (%); and (**e**) chewiness (g.f) of vacuum-packaged mackerel fillets (mean ± standard error, n = 3).

There are significant effects of the processing method (two-way ANOVA p = 0.007) and the interaction (p = 0.003), but not of storage time (p = 0.147), on the hardness of samples. Changes in hardness were different across treatments and storage durations; seeming to be only statistically significant in sous-vide fillets. Nonetheless, sous-vide fillets were significantly harder than control ones (Tukey post-hoc tests, p = 0.027), while marinated fillets' hardness was intermediate. Across storage, trends in hardness were distinct between treatments (Figure 7a). In sous-vide samples, after a peak on day 7 (ca. 420 g.f), resulting from the marination and heat treatment [23], hardness decreased up to day 21 and fluctuated around 200 g.f until the end of the storage trial. In contrast, the hardness of the control/raw and marinated fillets initially decreased (from about 220 to ca. 150 g.f) and then increased steadily up to 250 g.f on days 28–35.

For cohesiveness (Figure 7b), the processing method and storage time were statistically significant factors (ANOVA, $p < 0.001$), but not their interaction (p = 0.128). Seemingly, changes in cohesiveness over time occurred consistently across treatments. Tukey's test

revealed that sous-vide samples were less cohesive (0.376) than both marinated and control samples (0.462 and 0.448) ($p < 0.001$). The storage time caused a general decline in cohesiveness, most markedly in sous-vide fillets starting on day 14. These changes reflect progressive structural weakening and gel breakdown in muscle tissue during storage [57].

Gumminess (Figure 7c) was slightly but significantly affected by the processing method (ANOVA, $p = 0.021$) and storage time (ANOVA, $p = 0.042$), but more importantly by their interaction (ANOVA, $p < 0.001$). Gumminess followed the pattern of changes observed for hardness (and less closely for cohesiveness) closely, consistent with the cumulative effects of hardness and cohesiveness evolution.

Only storage time had a significant effect on springiness (ANOVA, $p = 0.022$); neither processing method nor the interaction were significant ($p > 0.05$). A decline in springiness with storage was evident (Figure 7d), particularly in control and sous-vide samples (from ca. 2% and 2.5% on day 0 to about 1.5% on days 28 and 49, respectively). The gradual loss of "elasticity" is likely due to muscle matrix fatigue and proteolytic activity.

Chewiness showed significant main effects of the processing method, storage time, and their interaction (all $p < 0.05$). While Tukey tests did not reveal statistically significant pairwise differences between processing methods ($p > 0.2$), chewiness was highest in sous-vide samples (204.27 g.f vs. 170.52 g.f and 159.46 in marinated and control samples), particularly during the initial 7 days (Figure 7e). The interaction effect reflects that chewiness trajectories varied depending on the method.

Overall, texture deteriorated with time regardless of the treatment. Sous-vide samples were harder (firmer), gummier, and chewier, but less cohesive. Apparently, marination alone preserved structural qualities better over storage. Both marinated and sous-vide vacuum-packaged mackerel fillets might correspond to consumer preferences. Consumers strongly prefer fresh fish and seafood over processed options. Fresh products are perceived as having better texture and quality. Also, a firm(er) texture is often associated with freshness and quality in fish products. In the case of cooked fish, a flaky (less cohesive) texture is generally desirable, as it indicates proper cooking and good quality [72,73]. Sensory studies, e.g., [16,34], confirm that packaging interventions such as vacuum-packaging lead to measurable changes in organoleptic (sensory) texture attributes over storage, with appropriately chosen methods helping to maintain consumer-preferred qualities such as freshness, firmness, and moistness.

3.2. Microbiological Parameters

In terms of microbiological quality, there were clear differences among the processing methods, and significant changes over the storage time were observed.

There were significant effects of the processing methods, storage time, and more importantly their interaction (two-way ANOVA, all $p < 0.001$) on the abundance of mesophilic bacteria (Figure 8a). Storage time had a strong influence, with counts increasing steadily across all treatments, from ca. 4 log CFU/g at the start up to 9–10 log CFU/g at the end of the storage trial. The low initial counts indicate very good fish quality (values of ca. 4 log CFU/g are common for many fish species; [74]). The increase occurred much earlier in the case of control and marinated samples and reached microbiologically critical levels (i.e., 7 log CFU/g) [75] by day 7 for control and marinated samples and day 21 for sous-vide fillets.

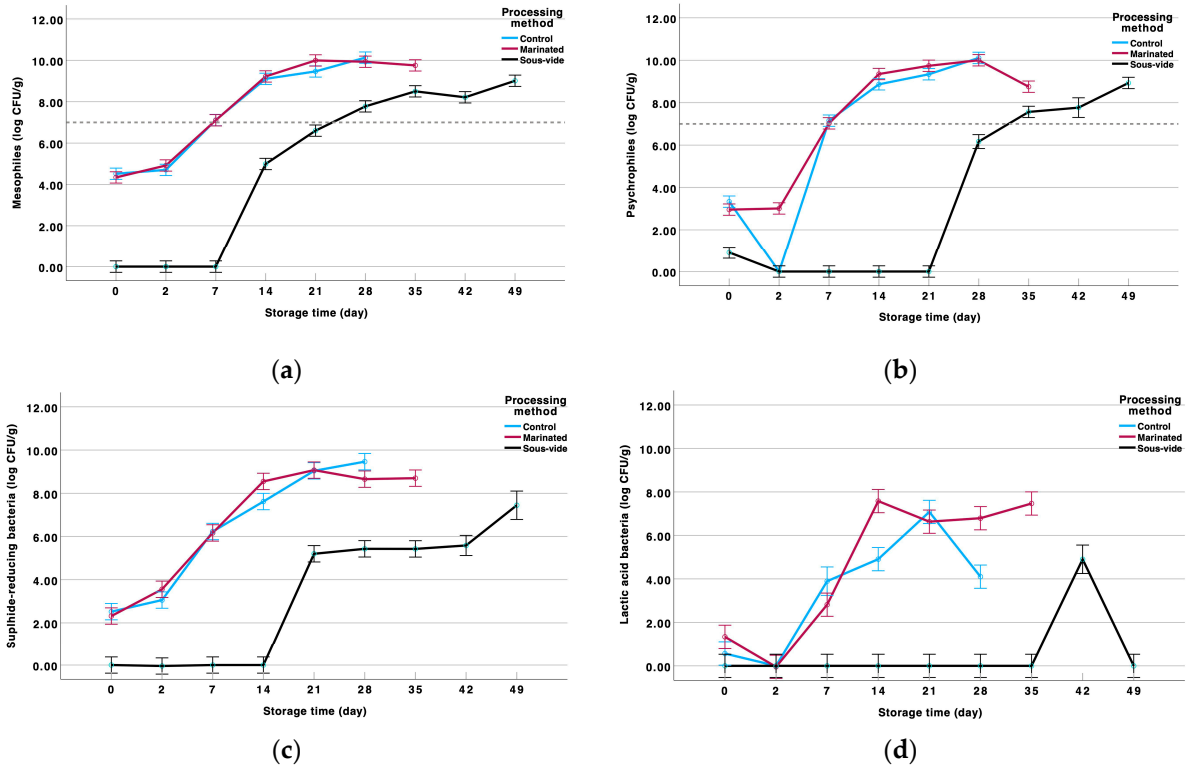

Figure 8. Microbiological indicators: (**a**) mesophilic (aerobic); (**b**) psychotrophic counts; (**c**) sulfide-reducing bacteria; and (**d**) lactic acid bacteria (mean ± SD) in log CFU/g. Each point represents an average of three samples. Dashed lines in the upper plots (**a**,**b**) correspond to the microbiologically critical level of 7 log CFU/g [75].

Psychrophilic bacteria also showed significant main and interaction effects ($p < 0.001$). The lowest final psychrotrophic counts were found in sous-vide fillets, suggesting some suppressive effect from heat treatment (Figure 8b). Nonetheless, all treatments showed strong time-related increases, starting at ca. 3 log CFU/g on day 0 and reaching 7 log CFU/g after 7 days in the case of control and marinated samples and post-day-28 in sous-vide samples. Our results are in line with previous studies. The initial TVC and psychrophic bacteria counts in chub mackerel stored at +4 °C were 4.5 and <3 log CFU/g, respectively [76]. The initial TVC of fresh mackerel packaged in a vacuum and kept in a chill room maintained at 0–2 °C was 4.14 log CFU/g, and it increased steadily, crossing the limit of 7 log CFU/g on day 16 [77]. Pongsetkul et al. [57] found that both mesophilic and psychrophilic bacteria in sous-vide vacuum-packaged tilapia fillets gradually increase with the storage time. In their study, mesophilic and psychrophilic bacterial counts were higher than 6 log CFU/g after 6 weeks (ca. 42 days).

Abundances of sulfide-reducing bacteria were low at the start of the storage trial, <2.6 log CFU/g (Figure 8c). These low initial abundances indicate good hygienic conditions [16]. ANOVA revealed significant effects of the processing method and storage time ($p < 0.001$), but no interaction ($p = 0.634$), suggesting that changes in the abundance of sulfide-reducing bacteria (SRB) during storage occur consistently across treatments. Sous-vide samples had significantly lower SRB counts (ca. 4.44 log CFU/g) than marinated and control fillets (6.23 and 6.72 log CFU/g) (Tukey tests, $p < 0.001$). This suggests that sous-vide processing, while suppressing psychrophiles, may be effective against anaerobic spoilage bacteria. Over the storage time, SRB levels rose sharply, particularly in control and marinated fillets. The increase in the abundances of SRB in control and marinated

samples occurred ca. 2-weeks earlier and reached levels ca. 2 log CFU/g lower than sous-vide samples.

In the case of lactic acid bacteria (LAB), there was a significant effect of storage time ($p < 0.001$), but not from the processing method or their interaction ($p > 0.6$), on the abundances. Initial LAB abundances were low (Figure 8d). After an early decrease after 2 days of storage (from ca. 2 log CFU/g to ~0.02 log CFU/g), LAB in the control and marinated samples increased sharply to 7–8 log CFU/g by days 14 and 21, respectively. Only after 42 days, did LAB surge in sous-vide samples (ca. 5 log CFU/g). LAB are a diverse group of Gram-positive, non-spore-forming, catalase-negative, and oxidase-negative microorganisms that produce organic acids, primarily lactic acid, as a major end-product of carbohydrate fermentation, which lowers the pH of seafood [78]. This exerts a bio-preservation effect since this acidic environment inhibits the growth of many spoilage bacteria [79]. In this study, the lowered pH derived from the growth of LAB might have been counterbalanced by the increased TVB-N content (see above). In addition, LAB can outcompete other microorganisms for nutrients and space and the formation of lactic acid and bacteriocins, effectively limiting the growth of spoilage and pathogenic bacteria [15]. Herein, this competitive exclusion behavior was not apparent.

The microbial quality indicators revealed that sous-vide processing significantly delayed bacterial growth compared to raw and marinated treatments, highlighting its effectiveness in extending shelf-life by suppressing mesophilic, psychrophilic, sulfide-reducing, and lactic acid bacteria under refrigerated storage. Based on microbial counts reaching the critical threshold of 7 log CFU/g, the estimated shelf-life was approximately 7 days for raw and marinated fillet, and up to 28 days for sous-vide samples.

4. Conclusions

This study successfully developed and evaluated mackerel-based products processed via sous-vide, marination, and vacuum packaging, with a focus on their potential implementation in school canteens in southern Portugal. We assessed changes in the freshness and quality of raw, marinated, and sous-vide marinated vacuum-packaged mackerel fillets during 4 °C refrigerated storage.

Physicochemical and microbiological analyses showed that both the processing method and storage duration had significant effects on product quality. Sous-vide processing notably extended the product's shelf-life to over 21 days, significantly exceeding the 7-day shelf-life of raw and marinated fillets by effectively delaying bacterial growth (e.g., mesophilic bacterial counts of 6.60 log CFU/g at day 21 vs. 9.46 and 9.99 log CFU/g in raw and marinated samples) and maintaining acceptable levels of key attributes such the TVB-N content (only reaching/exceeding acceptable limits of 25–35 mg N/100 g on day 35) and texture. Both marination and sous-vide processing produced vacuum-packaged mackerel fillets with textural attributes consistent with consumer expectations, as firmness is linked to freshness and quality, while a less cohesive texture is desirable in cooked fish). Although sous-vide fillet samples exhibited higher lipid oxidation (average TBARS values of 11.52 mg MDA/kg vs. 8.82 and 6.94 mg MDA/kg in marinated and control fillets), they generally retained overall physicochemical stability and microbiological safety throughout the storage. In contrast, raw and marinated samples showed earlier and more pronounced signs of degradation in both quality indicators and safety thresholds. Overall, these results highlight sous-vide as a highly effective method for preserving mackerel fillet quality and extending its shelf-life under refrigerated conditions, supporting its practical adoption in institutional food services.

Despite these promising results, the study has limitations. We did not investigate changes in protein secondary/tertiary structures or sensory properties such as flavor, which

are important for a comprehensive evaluation of consumer acceptance. Moreover, the work focused on storage at 4 °C under laboratory conditions, which may differ from large-scale canteen operations. Future research should therefore explore the protein structure, sensory profiling, and consumer testing in real food service contexts and optimize sous-vide parameters to minimize oxidative changes while maintaining quality and safety.

Author Contributions: Conceptualization, E.E. and J.A.; methodology, B.S.F., E.E. and J.A.; software, B.S.F., E.E. and J.A.; validation, B.S.F., E.E. and J.A.; formal analysis, B.S.F., E.E. and J.A.; investigation, B.S.F., E.E. and J.A.; resources, B.S.F., E.E. and J.A.; data curation, B.S.F., E.E. and J.A.; writing—original draft preparation, B.S.F., E.E. and J.A.; writing—review and editing, B.S.F., E.E. and J.A.; visualization, B.S.F., E.E. and J.A.; supervision, E.E. and J.A.; project administration, B.S.F. and J.A.; funding acquisition, E.E. and J.A. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the Project REVITALGARVE: Revitalização das zonas rurais (PRR-C05-i03-I-000237), through the F7-PRR-Plano de Recuperação e Resiliência (Rede Rural). This study had the financial support of the Portuguese Foundation for Science and Technology (FCT) to CIMA (UID/0350/2020) and ARNET (LA/P/0069/2020)". ARNET (DOI:10.54499/LA/P/0069/2020); CIMA BASE (DOI:10.54499/UIDB/00350/2020); CIMA PROGRAMÁTICO (DOI:10.54499/UIDP/00350/2020). This study also received Portuguese national funds from FCT to CCMAR through projects UIDB/04326/2020 (DOI:10.54499/UIDB/04326/2020) and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed at the corresponding author.

Acknowledgments: The author would like to acknowledge the contribution of Clarisse Ramalho, Vera Gonçalves, and Neusa Rodrigues for technical support during the laboratory work and the contribution of Chinedu Lucky Eze during sample preparation and visits to the schools' canteens. During the preparation of this manuscript, the authors used Microsoft Copilot (powered by GPT-4) and ChatGPT (GPT-4 Turbo with web access) for the purposes of vocabulary enhancement, grammar and syntax correction, and concise rewriting of complex paragraphs. The authors have reviewed and edited the output and take full responsibility for the content of this publication.

Conflicts of Interest: The authors declare no conflicts of interest.

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