applied sciences

MDPI

Article

The Effect of Freezing Raw Material on the Quality Changes and Safety of Salted Anchovies (*Engraulis encrasicolus*, Linnaeus, 1758) at Cold Storage Conditions

Serkan Koral [1], Sevim Köse [2], Matevž Pompe [3] and Drago Kočar [3,*]

[1] Department of Seafood Processing, Faculty of Fisheries, İzmir Katip Çelebi University, İzmir 35620, Turkey; serkan.koral@ikcu.edu.tr
[2] Department of Fisheries Technology Engineering, Faculty of Marine Sciences, Karadeniz Technical University, Trabzon 61530, Turkey; kosesevim@gmail.com
[3] Department of Analytical Chemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, 1000 Ljubljana, Slovenia; matevz.pompe@fkkt.uni-lj.si
* Correspondence: drago.kocar@fkkt.uni-lj.si

Abstract: This study demonstrates the effects of the freezing and frozen storage of anchovies prior to brining and dry-salting on quality changes and food safety during refrigerated storage (4 ± 1 °C). Fresh anchovies were divided into two groups, one of which was used as a control representing fresh raw material; the other was the experimental group and consisted of frozen and thawed anchovies stored at −18 °C for a year. Five different salt concentrations were used for brining (10, 15, 20, 25 and 30%) in addition to the dry-salting method. Microbiological, chemical, physical and sensory analyses were carried out during storage. Salt concentration and salting method had significant effects on the shelf-life of salted anchovy products, with the highest shelf-life corresponding to dry-salted anchovies ($p < 0.05$). The effect of using frozen and thawed raw materials for salting on the shelf-life depended on the processing method since the experimental dry-salted group had a shelf-life one month longer than that of the control group, while the opposite situation occurred for the brined samples, with one exception. Strong correlations were usually found between sensory values and chemical quality parameters (R^2: 0.83–0.99 for the control group and 0.63–0.99 for the experimental group). The results demonstrated that the experimental group, with some exceptions, had better values for most quality and food safety parameters in comparison to the control group, indicating the advantage of using frozen and thawed raw materials before salting to prevent spoilage and enhance food safety. Considering that the experimental group was produced from one-year-stored raw material compared to the control group, the advantage of the freezing and frozen storage of anchovies can be accepted as much higher versus when freezing is not implemented. The positive effect is due to the fact that frozen anchovies absorb salt faster after thawing, especially in groups with high salt concentrations. Therefore, it can be concluded that frozen salted anchovies can be utilized for longer as they have a longer shelf-life, particularly when using either the 30% brining or dry-salting method.

Keywords: anchovy; freezing effect; salting; shelf-life; quality changes; biogenic amines; food safety

Citation: Koral, S.; Köse, S.; Pompe, M.; Kočar, D. The Effect of Freezing Raw Material on the Quality Changes and Safety of Salted Anchovies (*Engraulis encrasicolus*, Linnaeus, 1758) at Cold Storage Conditions. *Appl. Sci.* **2023**, *13*, 6200. https://doi.org/10.3390/app13106200

Academic Editors: Attilio Matera and Francesco Genovese

Received: 23 April 2023
Revised: 9 May 2023
Accepted: 15 May 2023
Published: 18 May 2023

1. Introduction

Anchovies are a pelagic fish species belonging to the *Engraulidae* family [1]. They are among the most captured fish species in world fisheries, having had the highest catch rates for many years. The European anchovy (*Engraulis encrasicolus*) ranks third among common anchovy species, with approximately 514,752 tons produced in 2020 [2]. Anchovies are known ecologically and economically as the most important fish species for the Black Sea ecosystem, as well as for other seas in different countries [1,3,4].

Anchovies are a well-liked and commonly consumed fish in both human and animal diets around the world [2]. Anchovy has a high nutritional value, mainly due to its high polyunsaturated fatty acid content, particularly omega-3 fatty acids [5]. Our previous findings demonstrated that approximately 25 g of European anchovies caught from the Black Sea is enough to comply with the daily recommended n-3 polyunsaturated fatty acid (PUFA) intake for human consumption [5]. Moreover, it was also found that approximately 130 g of edible anchovy meat provides satisfactory levels of eicosapentaenoic acid + docosahexaenoic acid (EPA + DHA) according to recommended weekly requirements from several health authorities [5,6]. However, anchovies have a limited catching season (from October to March), which is usually accompanied by a high production volume. Anchovies are also highly perishable small pelagic fish due to their high fat content and the rapid autolytic degradation, particularly in the abdominal portion, that occurs during storage [7]. Therefore, several preservation techniques are used to distribute high catch rates throughout the year. Among these are freezing, which is most commonly used, followed by salting and marinating. Towards the beginning of new anchovy seasons, frozen anchovies have low consumer demand; therefore, they are sold under value for either human consumption or as animal feed (e.g., tuna feed). Moreover, this species has a better market value and consumer acceptance when marketed as either salted or smoked in comparison to frozen, unprocessed products [8].

Salting is one of the cheapest preserving/processing techniques traditionally applied in the fisheries industry, particularly in undeveloped countries. However, this technique is also now commonly applied around the world, including in developing/developed countries, since it is a method of delaying fish spoilage and preventing food health hazards. It is also used prior to other processing methods such as marinating, drying, fermenting and smoking. Various fish salting methods are known, although the most common ones are dry-salting and brining. The raw materials used for salting can be either fresh or previously frozen. Since fresh anchovies are very susceptible to spoilage, freezing raw materials is commonly applied in the fish processing sector [9]. Moreover, past studies have demonstrated that anchovies are susceptible to parasite infection, particularly with *Anisakis simplex* and *A. pegreffii* [10–12]. As the freezing of fish is one of the methods that has been reported to inactivate the larvae of parasites in seafood products, the European Commission requires the freezing of the fish at a temperature of no more than −20 °C for 24 h prior to such industrial processes for fish products when they are to be consumed raw or semi-raw without cooking [13,14]. In the USA, the FDA (Food and Drug Administration) requires that all fish and shellfish intended for raw or semi-raw (e.g., marinated or partly cooked) consumption be blast-frozen to −35 °C or below for 15 h or be completely frozen to −20 °C or below for 7 days [15,16]. It was claimed that freezing might affect the quality of traditional fish products due to its effect on the texture and protein structure of the fish muscle [11,13,17–19]. Therefore, in order to avoid freezing, some countries, such as Spain, have specified technical salting parameters and curing periods to be able to inactivate *Anisakidae* larvae: salt concentrations in fish above 9% for at least 6 weeks, between 10% and 20% for at least 4 weeks, or more than 20% for at least 3 weeks [20].

On the other hand, different studies have reported some advantages of freezing prior to some traditional fish processing techniques (e.g., salting, marinating and smoking), particularly in terms of a faster salt uptake compared to the fresh use of raw materials, resulting in faster ripening rates, lower biogenic amine contents and longer shelf-life [19,21–23]. However, the frozen raw fish used in past studies relating to this subject were usually stored for less than a month prior to testing (experimental trials). Therefore, the effect of the longer frozen storage period on the quality changes of traditional fish products is not clear. Moreover, there has been no research into the effect of freezing prior to salting on the quality of salted European anchovies. The effect of long-term frozen storage prior to processing on the quality of salted products should be examined in order to allow frozen anchovies to be processed at long intervals outside of the season. Therefore, the purpose of this study was to investigate the effect of freezing the raw materials prior to salting on the

quality of salted anchovies during refrigerated storage. Moreover, it also aimed to identify the changes in food safety parameters during storage at different salt concentrations in comparison to the use of fresh raw materials.

2. Materials and Methods

2.1. Chemicals and Reagents

Salt (rock salt; Billur Tuz, İzmir, Turkey) was obtained from a supermarket. All chemicals and solvents used were analytical and chromatographic grade, respectively. They were purchased from Sigma-Aldrich (Buchs, Switzerland) and Merck (Istanbul, Turkey).

2.2. Sampling Plan and Sample Preparations

The anchovies (*Engraulis encrasicolus*) were caught from the northeast of the Turkish Black Sea by commercial purse-seiners and brought to shore within several hours after the catch. They were purchased from a wholesale market and divided into two groups: the control and experimental groups. The control group contained fresh anchovies, which were immediately brought to the laboratory for processing (brining and dry-salting) in ice at a ratio of 1:1 within an hour. The experimental group was transferred to a commercial fish processing company called POLIFISH, situated 20 min' drive away from our laboratory, in the same conditions. They were frozen immediately at −40 °C in the company's freezer and kept at –18 ± 2 °C in their cold storage units for a year. Then, the frozen fish were transferred to our laboratory via the company's frigofrig system and were defrosted at our laboratory overnight at 4 ± 1 °C in a refrigerator (Arçelik 8810 NF, Trabzon, Turkey). The physical characteristics of the anchovies were determined by measuring the total length and weight of the whole fish. The average size and weight of the fish were 12.48 ± 1.26 cm and 10.21 ± 2.89 g, respectively.

2.3. Salting and Storage Procedures

The anchovies belonging to each group were headed, gutted and washed under running tap water. Then, each group was subdivided into two groups according to their salting methods. The first subgroup was dry-salted using fish:salt ratio of 3:1 (*w*:*w*) in 2 glass jars (8 L each). This was performed layer by layer, starting and ending with salt layers at the bottom and top. The first brine, containing blood that came out of dry-salted products, was replaced with fresh saturated brine after 2 weeks. The second subgroup was brined using salt concentrations of 10, 15, 20, 25 and 30% with a ratio of 1:2 fish:brine (*w*:*w*). Weight was added to the top of the fish in the jar (10 glass jars-8 L) to stop them from floating on the water. The brine solution was changed after the 1st week for the groups relating to salt concentrations of 10, 15 and 20% and after the 2nd week for the groups relating to salt concentrations of 25 and 30%. All groups relating to dry-salting and brining methods were stored in a refrigerator (4 ± 1 °C) for further analysis. The same processing technique was also applied to the frozen and thawed anchovies. The products were stored until they were found to be spoilt depending on their overall sensory evaluation. Sampling was carried out in the first week and then monthly for a year to determine quality changes as well as biogenic amine formation for product safety. Triplicate sampling was carried out from different parts of the raw materials and salted anchovies for each sampling time under sterile conditions in order to avoid microbial contamination. For each sampling, about 500 g of anchovies were weighed and divided into 200 g for sensory analysis, 100 g for microbiological analysis, and the rest for other analyses. The analytical methods are explained below.

2.4. Sensory Analysis

Sensory analyses were performed using a modified method derived from the methods of Karaçam et al. [24], Hernandez-Herrero et al. [25], and Archer [26]. Eight trained panelists judged the texture, appearance, odour, and overall acceptability of the samples

using a 10-point descriptive scale. The criteria used for sensory evaluation are shown in Supplementary Table S1.

2.5. Analysis of Moisture and Salt Contents, a_w and pH

Moisture content was determined by oven-drying 5 g of fish muscle at 105 °C until a constant weight was obtained [27]. The results were expressed as grams of water per 100 g of muscle. The dry matter value was calculated from the results of the moisture contents. The Mohr method was used to determine salt content (NaCl) in fish muscle, as described in Rohani et al. [28]. Water phase salt (WPS) was calculated from the amount of salt in the product relative to the product moisture and salt content using the following equation [29]:

$$\text{WPS\%} = [\text{salt\%}/(\text{salt\%} + \text{moisture\%})] \times 100$$

Water activity (a_w) was measured using an AQUALAB TE3 model water activity meter according to the principles described in Minegishi et al. [30]. The pH measurements were taken with a digital pH meter (Jenco 6230N, San Bernardino, CA, USA) by placing the electrode into the samples where 5 g of fish flesh had been homogenized with 10 mL of distilled water. Readings were carried out for both a_w and pH in triplicate.

2.6. Analysis of Chemical Spoilage Parameters

Total volatile base nitrogen (TVB-N) content was determined according to the method described by Goulas and Kontominas [31]. Thiobarbituric acid (TBA) values, expressed in mg malonaldehyde (MA/kg), were estimated using the method described by Smith et al. [32]. The method of Boland and Paige [33] was used to conduct trimethylamine (TMA) analysis.

2.7. Analysis of Biogenic Amines

Biogenic amines were analyzed using the high-performance liquid chromatography (HPLC) method of Köse et al. [34]. The HPLC equipment used was Shimadzu Prominence LC-20 AT series (Japan) HPLC with an autosampler (SıL20AC, Shimadzu, Japan), a diode array detector (SPD-M20A, Shimadzu, Japan), and an Inertsil column (GL Sciences, ODS-3, 5 µm, 4.6 × 250 mm). This method originated from EU-suggested methods [14]. To extract biogenic amines, 10 mL of 0.4 M perchloric acid was added to a 5 g sample, and the mixture was homogenized by an ultra-turrax homogenizer (IKA T 25, Digital, Taufkirchen, Germany) in an ice bath and centrifuged (MPW 350 R. MPW Med. Instruments., Warsaw, Poland) at 2440 g at 4 °C for 10 min. The supernatant was collected, and the residue was extracted again with 10 mL of a 0.4 M perchloric acid solution. Both supernatants were combined and filtered through Whatman paper (No. 42). The final volume was adjusted to 25 mL with 0.4 M perchloric acid. Each sample extract and diluted standard solutions of 0.5 mL were mixed with 100 µL of 2 N sodium hydroxide and 150 µL of saturated sodium bicarbonate. One milliliter of a dansyl chloride solution (10 mg/mL) prepared in acetone was added to the mixture. This was mixed well, and incubated at 40 °C for 45 min and cooled down to room temperature in 10 min. Subsequently, the residual dansyl chloride was removed by the addition of 50 µL 25% ammonia solution. After 30 min of incubation at room temperature, the extract was adjusted to 5 mL with the ammonium acetate: acetonitrile mixture (1:1 *v*/*v*) and mixed well with a vortex (Nüve NM 110, Ankara, Turkey). The extract was filtered through filters with 0.45 µm pore sizes (Millipore Co., Bedford, MA, USA) and injected into HPLC. The gradient elution system contained 0.1 M ammonium acetate as solvent A and acetonitrile as solvent B. The gradient elution was initiated with 50% A and 50% B, and terminated in 19 min with 90% B, with a run time of 20 min. The system was equilibrated for 8 min before the next run. The flow rate was 0.9 mL/min, and a 20 µL sample was injected into the column. The column temperature was 40 °C, and amines were detected at 254 nm. Triplicate sampling was carried out and performed separately per group at each sampling point.

2.8. Microbiological Analysis

Twenty-five grams of samples were aseptically weighed into a sterile stomacher bag containing 225 mL of sterile physiological saline (0.85%) and homogenized using a stomacher (Mayo, HG400V, Italy) for 4 min at the highest speed. Further decimal dilutions were prepared in physiological saline (0.85%). Total volatile aerobic mesophilic bacteria (TVAMB) were counted using a plate-count agar incubated at 37 °C for 48 h. Histamine-forming bacteria (HFB) were determined according to the methods used by Köse [35] and Niven et al. [36]. The total HFB isolation agar contained 0.5% tryptone, 0.5% yeast extract, 2.35% L-histidine-HCl, 0.5% NaCl, 0.06% bromocresol purple, 0.1% $CaC0_3$, and 2% agar (pH 6.5) [36]. Mesophilic HFB was determined using the same incubation conditions applied to TVAMB. Halophilic bacteria (HB) counts were carried out according to the work of Gürgün and Halkman [37]. Microbial counts were carried out in duplicate and expressed as log CFU g^{-1}.

2.9. Statistical Analysis

The data obtained were analyzed via an analysis of variance (One-way ANOVA) and, when significant differences were found, comparisons among means were carried out via a Tukey and Mann–Whitney U test (data not provided in the normality of assumptions) under the program called JMP 5.0.1 (SAS Institute, Inc., Cary, NC, USA) and SPSS (SPSS Inc., Chicago, IL, USA) [38]. A significance level of 95% ($p < 0.05$) was used throughout the analysis. Linear regression analysis was calculated using Microsoft EXCEL, 2003.

3. Results and Discussion

Figure 1 demonstrates sensory values corresponding to salted anchovies from both fresh (control group) and frozen and thawed raw materials (experimental group) stored at refrigerated temperatures. Salt concentration had a significant effect on the shelf-lives of the products ($p < 0.05$), as also supported by previous studies [25,39]. The best shelf-life was obtained with the dry-salted product for over a year in the experimental group. Products brined at 10, 15, 20, 25, and 30% salt concentrations had shelf-lives for the control group of 2 weeks, 2 months, 4 months, 5 months, and 9 months, respectively. Lower shelf-lives (one month for each subgroup depending on salt concentration) occurred for the experimental group for the brined samples except at 10%, which had the same storage life. An opposite situation occurred for the dry-salted products, as freezing or frozen storage of raw materials prior to salting extended the shelf-life of products in refrigerated storage for a month. Therefore, this study demonstrates that salting methods affect the curing of fish depending on the conditions of the raw material used. In the case of using frozen and defrosted raw materials before salting anchovies, it is advised to use dry-salting instead of brining.

Differences in the effect of freezing fish prior to dry-salting and brining were reported by different studies on fish quality and maturation rates [22,23,40]. Similar results were obtained by our earlier study with dry-salted Atlantic bonito (*lakerda*) using fish that had previously been frozen and thawed for a month [23]. The previous study showed that the sensory scores of *lakerda* processed from previously frozen A. bonito were higher than those of freshly processed fish. Faster salt uptake was also obtained. Moreover, Mendes et al. [22] investigated the effect of freezing on the changes in free amino acids and biogenic amines during the ripening of fresh and frozen sardines. They found that freezing had a positive effect on the final quality of ripened sardines as it decreased the bacterial activity responsible for biogenic amine formation. Besteiro et al. [40] reported that frozen and thawed anchovies were successfully ripened, giving a final product with fully acceptable organoleptic characteristics despite the fact that freezing prior to processing slowed ripening.

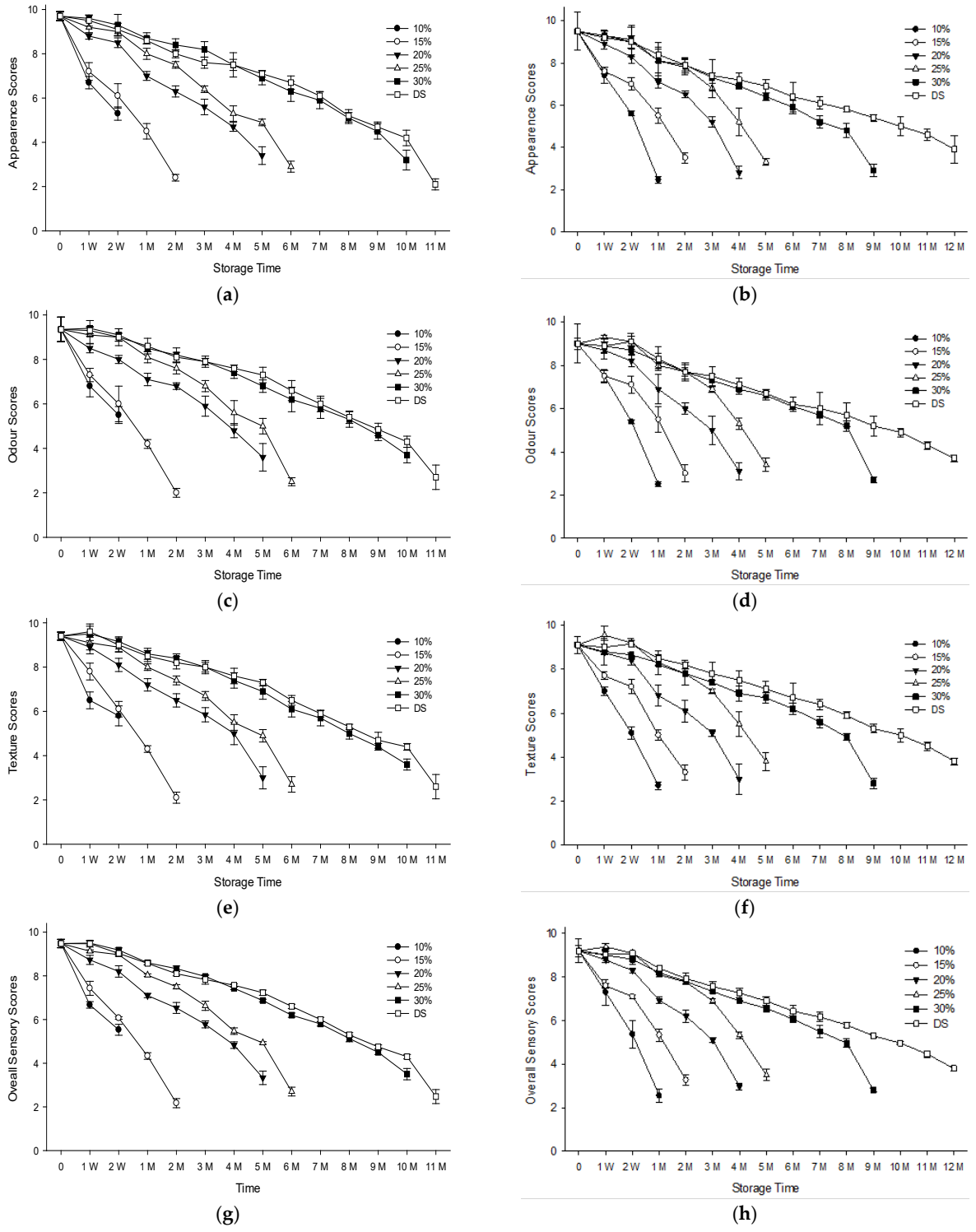

Figure 1. The changes in the sensory values of dry-salted and brined anchovies prepared from fresh and previously frozen and thawed raw materials during refrigerated storage (4 ± 1 °C). DS: dry-salted anchovies, (**a**): appearance scores of the control group, (**b**): appearance scores of the experimental group (prepared from frozen material), (**c**): odour scores of the control group, (**d**): odour scores of the experimental group (prepared from frozen material), I: texture scores of the control group, (**f**): texture scores of the experimental group (prepared from frozen material), (**g**): overall sensory scores results of the control group, (**h**): overall sensory scores results of the experimental group (prepared from frozen material).

The effect of freezing the raw material on the quality changes of salted fish products can vary according to fish species, the type of products, and the time and temperature used for freezing and frozen storage. In fact, Szymczak [18,19] demonstrated that although previously frozen herrings had higher salt uptake when marinating compared to fresh herrings, they had lower sensory values. Sigurgisladottir et al. [17] investigated the changes in microstructure and texture during the smoking of fresh and frozen/thawed Atlantic salmon. They found that the muscle fibers from the frozen and thawed fish shrank, and the extracellular space increased compared to the fresh muscle.

The changes In the moisture contents during the ripening of anchovies stored at refrigerated temperatures for both groups are represented in Figure 2. The main purpose of salting fish is to decrease its moisture content, leading to a reduced a_w of the products. The moisture contents of brined samples significantly dropped in parallel to increasing salt concentration levels in brine, except at 10% ($p < 0.05$). There were strong negative correlations between salt and moisture contents, and moisture content and a_w values for all subgroups of the control with the correlation coefficient results varied in R^2: 0.73–0.99 and R^2: 0.70–0.99, respectively, with the lowest correlations represented by samples brined at 10% (Table 1 and Supplementary Tables S2 and S3). The freezing and frozen storage of raw materials prior to processing significantly affected the salt uptake, and also decreased the moisture contents and a_w ($p < 0.05$). However, the correlation was slightly weaker for the experimental group, standing at R^2: 0.69–0.97 and R^2: 0.78–0.98, in the same respect (Table 1 and Supplementary Tables S2 and S3).

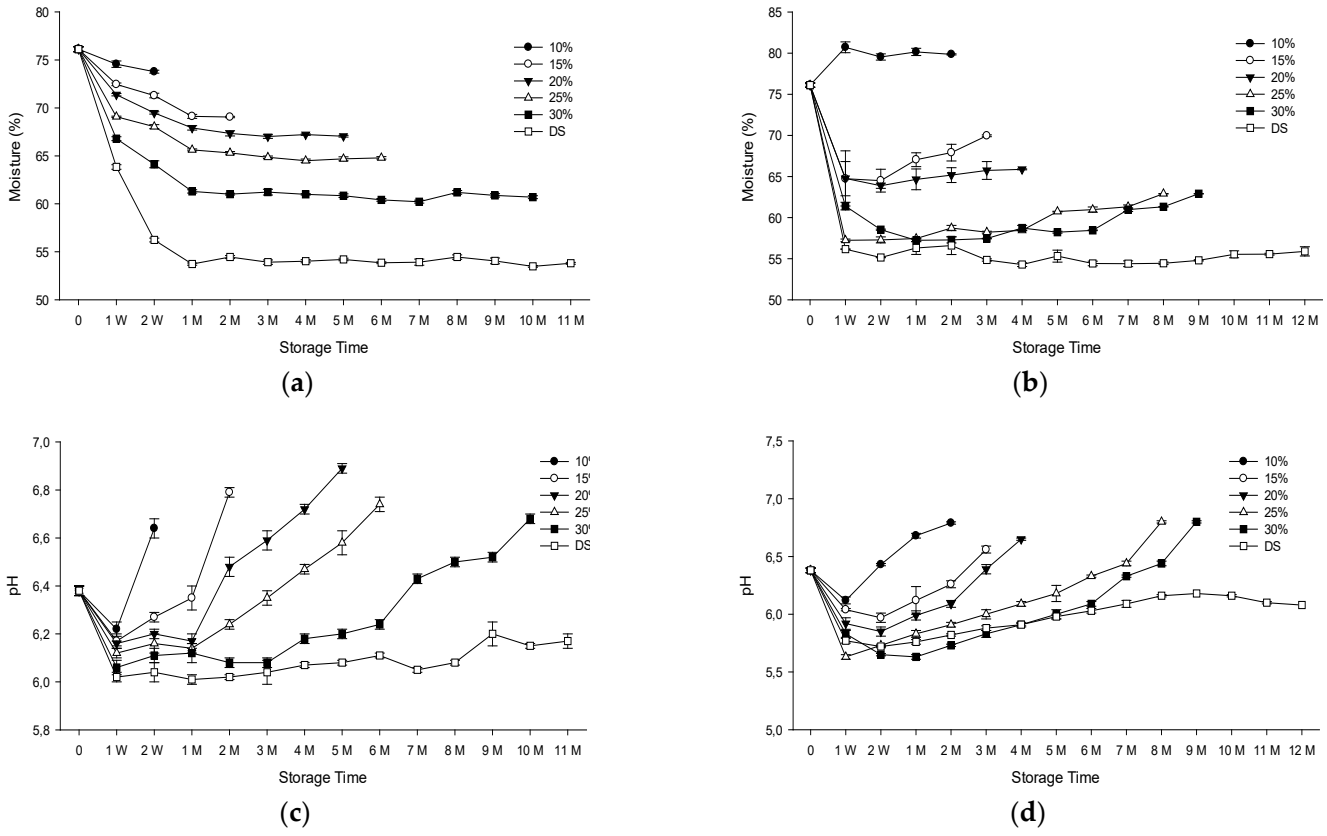

Figure 2. *Cont.*

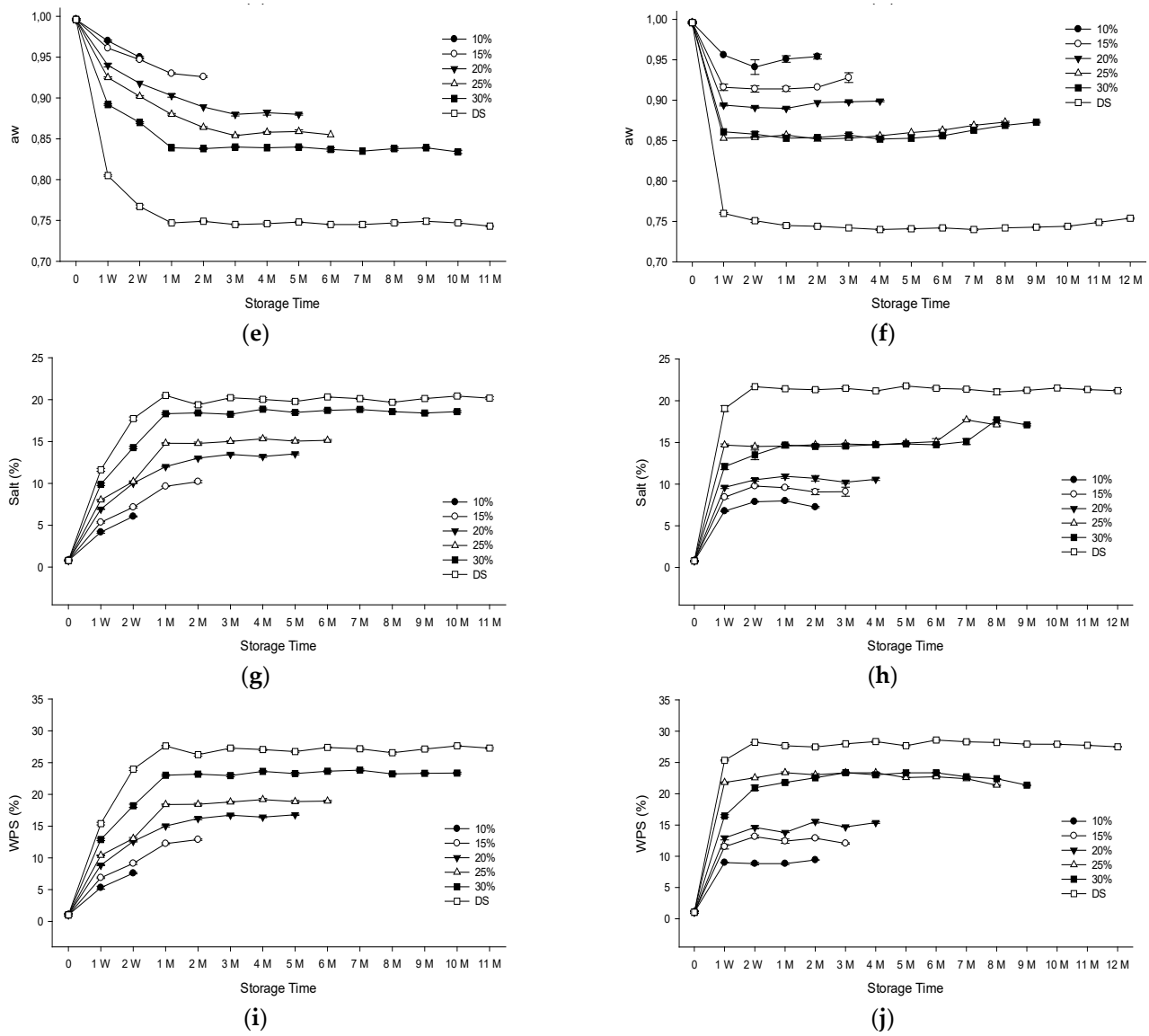

Figure 2. The results for moisture, pH, a_w, salt (%), and WPS (%) of dry-salted and brined anchovies during storage. (**a**) Moisture results of the control group; (**b**) Moisture results of the experimental group (prepared from frozen material); (**c**) pH results of the control group; (**d**) pH results of the experimental group (prepared from frozen material); I a_w results of the control group; (**f**) a_w results of the experimental group (prepared from frozen material); (**g**) salt (%) results of the control group; (**h**) salt (%) results of the experimental group (prepared from frozen material); (**i**) WPS (%) results of the control group; (**j**) WPS (%) results of the experimental group (prepared from frozen material).

Table 1. Correlation results among the values of salt %, moisture % and water activity for the control and experimental groups during storage in the refrigerator (4 ± 1 °C).

Sample Type	Salt%—a_w		Salt%—Moisture%		Moisture%—a_w	
	C	FM	C	FM	C	FM
10%	R^2 = 0.99	R^2 = 0.96	R^2 = 0.73	R^2 = 0.86	R^2 = 0.70	R^2 = 0.78
15%	R^2 = 0.99	R^2 = 0.96	R^2 = 0.99	R^2 = 0.76	R^2 = 0.97	R^2 = 0.86
20%	R^2 = 0.99	R^2 = 0.98	R^2 = 0.93	R^2 = 0.96	R^2 = 0.92	R^2 = 0.80
25%	R^2 = 0.98	R^2 = 0.85	R^2 = 0.93	R^2 = 0.71	R^2 = 0.93	R^2 = 0.82
30%	R^2 = 0.98	R^2 = 0.85	R^2 = 0.98	R^2 = 0.69	R^2 = 0.99	R^2 = 0.80
DS	R^2 = 0.99	R^2 = 0.99	R^2 = 0.90	R^2 = 0.97	R^2 = 0.92	R^2 = 0.98

C: control group; FM: experimental group; a_w: water activity; DS: dry-salted.

There were significant differences in moisture contents after the treatments using different salt concentrations and salting methods ($p < 0.05$). Moreover, the reduction in the percentage of moisture was significantly faster for dry-salted fish than for brined fish ($p < 0.05$). The changes in moisture contents of brined samples were usually insignificant after the 2nd week for the control group relating to 20, 25 and 30% salt concentrations, with some exceptions. The changes in the brined samples for the experimental group were usually slower and stabilized after the 1st week, with some exceptions. The changes in the levels of dry-salted samples were found to be insignificant throughout the storage after the 1st week for the experimental group and after the 2nd month for the control group. The values of salt and a_w closely supported these findings (Figure 2).

The lowest moisture content for the dry-salted control group was found on the 10th day at 53.48%, while the lowest moisture value for the previously frozen and thawed group was obtained in the 4th month at 54.28%. Czerner and Yeannes [41] obtained moisture contents between 48.59% and 53.83% for salted-ripened anchovies (*E. anchoita*). In our earlier study with commercial salted anchovies from different countries, varying moisture contents were obtained, although the levels were usually under/around 50% for dry-salted products [42]. Therefore, the values determined in this study were close to the values of previous findings.

Significant differences were also obtained in the levels of salt content between the control and experimental groups ($p < 0.05$). Salt uptake was significantly higher for the experimental group in comparison to the control group, with the exception of the 20 and 30% salt concentrations ($p < 0.05$). Similar trends were usually obtained with WPS%, which was calculated from the moisture and salt contents of the products (Figure 2). The salt uptake rapidly increased up to the 2nd week of storage for both groups. However, the increase was found to be significantly faster for the experimental group compared to the control group, with two exceptions ($p < 0.05$). The changes were usually insignificant after the 2nd week of storage. Moreover, salt concentration significantly affected the salt uptake in the brined products ($p < 0.05$). The fastest salt uptake was represented by the dry-salted products for both groups (Figure 2). Anastasio et al. [11] demonstrated that a dry-salting process with a salt concentration of 21% in all parts of the anchovy fillets devitalized *Anisakis pegreffii* larvae in a 15-day period. In the present study, the salt content of previously frozen dry-salted anchovies supported this finding, as the salt content reached the same level in the 1st month. Additionally, freezing and frozen storage also killed the most parasites, indicating the advantage of using previously raw materials during salting since salted products are used to marinate fish that is consumed without cooking; therefore, such products represent parasite health risks [43]. The salt concentrations of various commercially brined and dry-salted anchovies from various countries were determined to be within the safety limits [42].

The salt contents of the products closely affected their a_w since the highest salt content resulted in the lowest a_w levels (Figure 2). The initial a_w level was found to be 0.996 for both groups and dropped at the end of the 2nd week, followed by fluctuations in the levels for each group during storage ($p < 0.05$). Starting from the 1st week of brined anchovies at concentrations of 15 and 20% and starting from the 1st month with other concentrations for the experimental group, with several exceptions, the changes in the a_w values were found to be insignificant during storage. The changes in the a_w of the control group also supported these results, mainly for 30% salt concentration and dry-salted anchovies. Significant differences were obtained among the concentrations of brine for both groups ($p < 0.05$). Moreover, using fresh raw materials instead of previously frozen and thawed anchovies significantly affected the levels of a_w values ($p < 0.05$). The a_w levels dropped significantly faster for the experimental group than the control group, which demonstrated the opposite trend with the salt uptake. A very strong negative correlation was obtained between salt and a_w contents for the control group, with R^2 values ranging from 0.98 to 0.99, while a strong correlation also occurred for the experimental group, with R^2 values ranging from 0.85 to 0.99 (Table 1 and Supplementary Tables S2 and S3). The higher the salt

concentrations of brined samples were, the lower the a_w values became for both the control and experimental groups. The lowest values corresponded to the dry-salted samples for each group.

Water activity is a growth-limiting factor for microorganisms. According to FDA guidelines [16], the minimum a_w to allow the growth of *Staphylococcus aureus* is 0.83, and the minimum level to allow toxin formation using salt is 0.85. The a_w values for dry-salted anchovies of both groups were usually found to be within the safety limit on the 1st week, as suggested by the FDA in order to prevent bacteria growth or toxin formation. The a_w levels of brined anchovies dropped to the safety level for the control group on the 1st and 3rd months for the salt concentration groups of 30 and 25%, respectively. This level was reached earlier for the experimental group on the 1st and 2nd weeks for 25 and 30% salt concentrations, respectively. The levels were found to be above 0.85 for the other salt concentration levels in both groups. Therefore, this study demonstrates the benefits of using previously frozen and thawed raw materials in the salting of anchovies in terms of food safety. The results of Anastasio et al. [11] and Koral and Köse [23] also supported our findings. Therefore, these results imply that using salt concentrations above 25% during brining and dry-salting methods could easily prevent the food safety of salted anchovies during refrigerated storage. Moreover, using previously frozen and thawed raw materials has an advantage over using unfrozen raw materials in terms of the food safety of salted anchovies.

The pH of the salted anchovies significantly dropped from the initial value and then increased with the significant changes for both groups ($p < 0.05$). The pH levels of the experimental group decreased faster than those of the control group, with the lowest value being 5.63. Significant differences occurred between the two groups as well as among the salting methods and salt concentrations ($p < 0.05$). Although the pH value of the product is essential both for its degree of spoilage and food safety, it is commonly used to evaluate a product's safety. The pH of fish immediately after being caught was reported to be between 6.0 and 6.5. Fresh fish is acceptable up to a pH of 6.8, but is considered to be spoiled above a pH of 7.0 [44]. However, Martinez and Gildberg [7] pointed out that at pH 6.5, which is the post-mortem pH of the tissue, the basic proteases play an essential role in the degradation of myofibrillar protein and the solubilization of connective tissue. Therefore, this parameter is used in combination with other spoilage parameters, especially for processed fish products. The pH values of all groups were found to be within acceptable levels at their rejection by sensory evaluation despite rising pH during storage. Moreover, Besteiro et al. [45] pointed out that muscle enzymes such as cathepsins play a role in the ripening process. This is particularly true for cathepsin C, which shows activity at pH 5.5–6.0. Therefore, the pH values of dry-salted and brined anchovies prepared from previously frozen and thawed raw materials are usually superior to those of products obtained from unfrozen raw materials. It was also reported that a pH below 5 prevents most pathogenic bacteria growth or toxin formation [16,43]. The levels of pH obtained for all groups were above 5, indicating that this parameter cannot be used to judge the product safety of salted anchovies. Similar results have been demonstrated by our earlier study of salted A. bonito, which we prepared from both fresh and previously frozen and thawed raw materials and stored at ambient and refrigerated temperatures [23].

The results of TVB-N, TMA, and TBA are shown in Figure 3. The values of all three parameters increased significantly ($p < 0.05$) for both groups throughout the storage, and significant differences ($p < 0.05$) were also observed among different salting and brine concentration subgroups, with some exceptions. The influence of brine concentrations on the values of these parameters was also reported by earlier studies of anchovies prepared from fresh raw material [23,24,46]. The higher TBA amounts found with some samples could be because of dry-salting, which may cause samples to be more susceptible to oil oxidation than brined samples. The increase in TVB-N and TMA levels was significantly faster for the experimental group in comparison to the control ($p < 0.05$), while the differences between

these groups were usually found to be insignificant for TBA results, except for 10 and 25% brined anchovies.

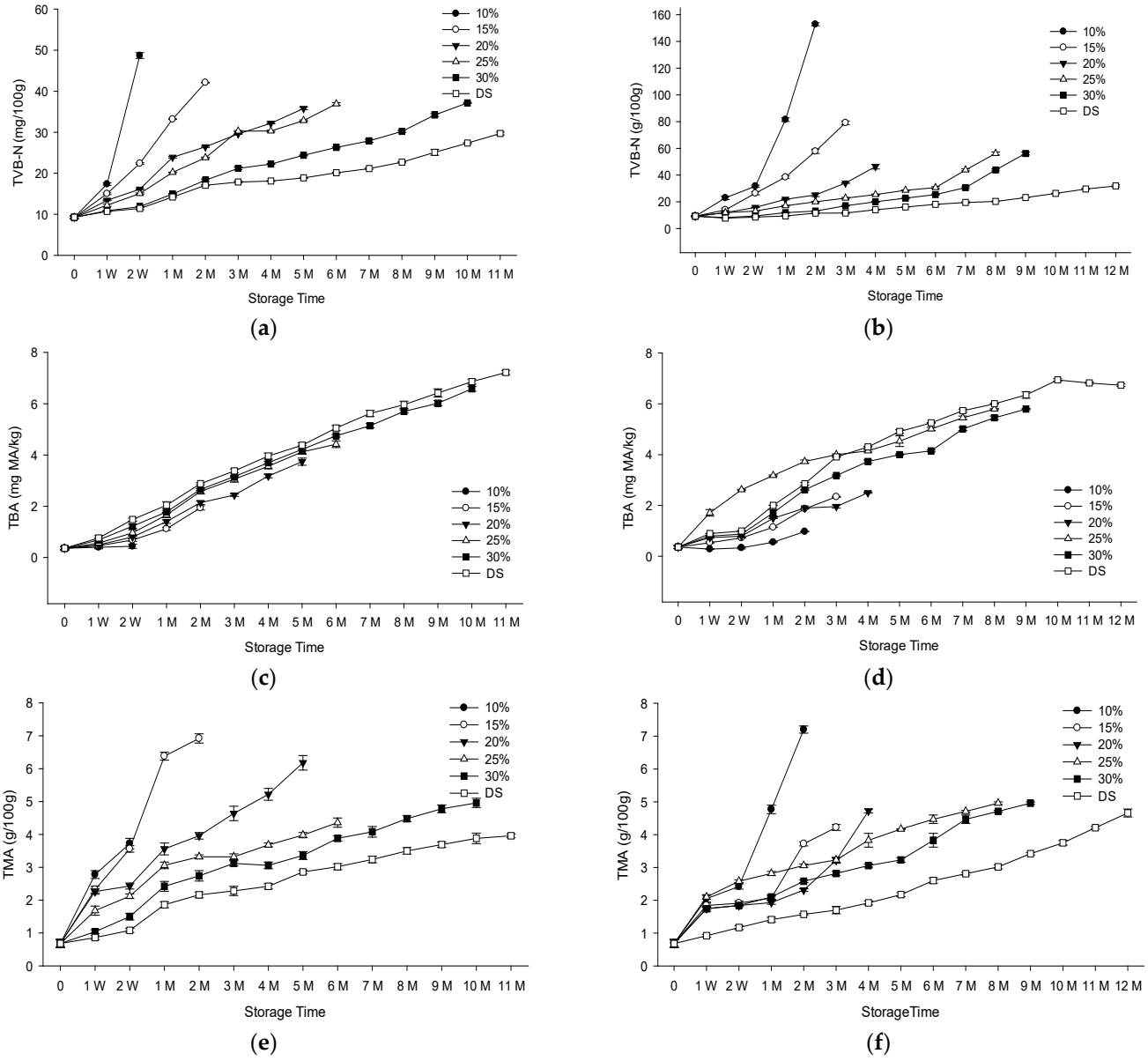

Figure 3. The changes in the TVB-N, TBA, and TMA of dry-salted and brined anchovies prepared from fresh raw material (control group) in comparison to frozen and thawed raw material (experimental group) during refrigerated storage at 4 ± 1 °C. TVB-N: total volatile base nitrogen; TBA: thiobarbituric acid; TMA: trimethylamine; DS: dry-salted; MA: malonaldehyde. (**a**) TVB-N results of the control group; (**b**) TVB-N results of the experimental group (prepared from frozen material); (**c**) TBA results of the control group, (**d**) TBA results of the experimental group (prepared from frozen material), (**e**) TMA results of the control group; (**f**) TMA results of the experimental group (prepared from frozen material).

The European Union sets varying TVB-N limits of 25–35 mg/100 g for unprocessed fishery products for certain fish species that are regarded as unfit for human consumption in cases where organoleptic assessment has raised doubts as to their freshness [14,47]. However, anchovies are not included in EU regulations. Therefore, TVB-N levels can be used only in support of sensory values. The present study showed that TVB-N values supported sensory results obtained for the control group (fresh raw material), with one

exception, for both dried and brined samples. In comparison with frozen and thawed salted anchovies, only brined samples with concentrations of 10, 15, and 20% were in compliance with sensory results. However, although the values of all groups were within acceptable levels with respect to sensory evaluation (Figure 3), there was a very strong negative correlation between TVB-N and sensory values for all groups, with the correlation coefficient results varying in R^2 from 0.90 to 0.98 for the control group and in R^2 from 0.84 to 0.99 for the experimental group (Table 2 and Supplementary Tables S2 and S3).

Table 2. Correlation results between sensory values and the values of TVB-N, TBA, and TMA in the control and experimental groups during storage in the refrigerator (4 ± 1 °C).

Sample Type	TVB-N—Sensory		TBA—Sensory		TMA—Sensory	
	C	FM	C	FM	C	FM
10%	$R^2 = 0.90$	$R^2 = 0.84$	$R^2 = 0.99$	$R^2 = 0.68$	$R^2 = 0.95$	$R^2 = 0.91$
15%	$R^2 = 0.97$	$R^2 = 0.97$	$R^2 = 0.87$	$R^2 = 0.96$	$R^2 = 0.95$	$R^2 = 0.91$
20%	$R^2 = 0.97$	$R^2 = 0.99$	$R^2 = 0.96$	$R^2 = 0.94$	$R^2 = 0.99$	$R^2 = 0.92$
25%	$R^2 = 0.92$	$R^2 = 0.90$	$R^2 = 0.92$	$R^2 = 0.63$	$R^2 = 0.83$	$R^2 = 0.69$
30%	$R^2 = 0.98$	$R^2 = 0.95$	$R^2 = 0.97$	$R^2 = 0.90$	$R^2 = 0.93$	$R^2 = 0.90$
DS	$R^2 = 0.97$	$R^2 = 0.97$	$R^2 = 0.93$	$R^2 = 0.93$	$R^2 = 0.90$	$R^2 = 0.98$

C: control group; FM: experimental group; TVB-N: total volatile base nitrogen; TBA: thiobarbituric acid; TMA: trimethylamine; DS: dry-salted.

TBA is also used as a quality parameter, particularly relating to lipid oxidation. It has been suggested that TBA values less than 5 mg MA/kg are indicative of the good quality of the fish, with 8 mg MA/kg being the limit value for consumption [44]. There was a strong negative correlation between TBA results and sensory values, with R^2 ranging within 0.87–0.99 for the control group, while a weaker correlation was obtained for the experimental group in a range of R^2: 0.63–0.96 (Table 2 and Supplementary Tables S2 and S3). However, the determined values did not reach the limit value of TBA for consumption. Brined samples of all concentrations for both groups showed good quality, depending on TBA values. It was reported that TBA values may not reveal the actual rate of lipid oxidation since malonaldehyde may interact with other fish components, such as the end products of lipid oxidation. Additionally, this interaction varies with fish species [48]. In this study, the highest TBA values were obtained for the dry-salted anchovies for both experimental and control groups at 6.94 and 7.22 mg MA/kg, respectively, towards the end of the storage trial. Our previous study [23] demonstrated that high salt content can accelerate lipid oxidation, while freezing and frozen storage prior to salting can retard oxidative changes in A. bonito products. The present study supported these findings, with the exception that freezing did not retard TBA development for all salt concentration groups. This might be explained by the differences in salting methods and fish species as well as the initial lipid contents. Hernandez-Herrero et al. [25] obtained higher TBA values for dry-salted anchovies. The differences can be attributed to the higher initial TBA contents.

TMA is generated in fresh marine fish by the reduction of trimethylamine oxide (TMAO) by specific spoilage bacteria [41,44]. Connell [49] has fixed a limit of 15 mg/100 g for fresh fish. In the present study, TMA values were well below the suggested limit, with the highest values obtained at 6.92 and 7.2 mg/100 g for the brined control and experimental groups on the 2nd month of storage (for 15 and 10% brine, respectively). There was a very strong negative correlation between TMA and sensory values for all groups, with R^2 values ranging within 0.83–0.99, with the exception of a 25% brined sample of the experimental group, which showed an R^2 value of 0.63 (Table 2 and Supplementary Tables S2 and S3). The higher the salt concentrations were, the lower the TMA contents became, indicating that TMA development can be retarded by the increase in salt concentration for both groups. The rise in levels was found to be higher for the control group in comparison to the experimental group, with several exceptions. This result indicates the advantage of freezing and thawing raw materials over unfrozen raw materials prior to salting. An opposite situation was obtained by our earlier study using A. bonito that had previously

been frozen and thawed for a month [23]. Shiriskar et al. [50] obtained an increase in TMA values during the storage of salted and pressed anchovies (*Stolephorus sp.),* and the levels reached up to 15.3 mg/100 g on the 5th week at ambient temperature.

Table 3 represents the microbial counts of both groups during refrigerated storage. The initial TVAMB and HFB loads of fresh anchovies prior to processing and freezing were found to be 2.74 and 2.12 log cfu/g, respectively. Salt concentration had a significant effect on the bacteria growth ($p < 0.05$), as was also reported by previous studies [24,51]. The lower the concentrations were, the higher the bacterial load became. Therefore, the lowest bacteria count corresponded to the dry-salted samples for both groups, followed by the 30% brined anchovies. The control group usually had better bacterial quality compared to the experimental group, with significant differences between various storage times and brine concentrations ($p < 0.05$). While the TVAMB counts of samples with 10, 15, and 20% brine concentrations increased significantly throughout the storage, the opposite situation was observed for other concentrations and dry-salted products for both control and experimental groups, with the exception of a 25% brine concentration for the frozen and thawed group ($p < 0.05$). A similar pattern occurred for total HFB counts, with some exceptions relating to 20 and 25% brined samples that had fluctuations in the levels. Limited growth was observed for HB in the first week of storage for both groups. Then significant increases were observed for samples relating to 10–25% of brine concentrations ($p < 0.05$). However, HB counts were usually found at $\leq$1.47 log cfu/g for 30% brine and dry-salted products for both groups. No bacterial growth was observed for the control and experimental groups for samples brined with 30% brine and dry-salted starting from the 1st and 2nd months, respectively.

Table 3. Change in the bacteria counts of dry-salted and brined anchovies prepared from fresh raw material (control group) in comparison to frozen and thawed raw material (experimental group) during refrigerated storage at 4 ± 1 °C (n = 3).

Storage Time	Salt Concentration	Sample Type	Microbial Counts (log CFU/g)		
			TVAMB	HB	HFB
1 Week	10%	C	$3.04 \pm 0.24^{a,A,1}$	$2.14 \pm 0.15^{a,A}$	$2.86 \pm 0.12^{a,A,1}$
		FM	$6.64 \pm 0.46^{a,A,2}$	<1.47	$2,92 \pm 0.10^{a,A,1}$
	15%	C	$2.68 \pm 0.08^{a,B,1}$	$2.05 \pm 0.08^{a,A}$	$2.46 \pm 0.14^{a,B,1}$
		FM	$5.88 \pm 0.50^{a,B,2}$	<1.47	$2,78 \pm 0.06^{a,A,2}$
	20%	C	$2.36 \pm 0.10^{a,C,1}$	$1.98 \pm 0.14^{a,A}$	$2.26 \pm 0.09^{a,C,1}$
		FM	$5.49 \pm 0.42^{a,B,2}$	<1.47	$2,58 \pm 0.10^{a,B,2}$
	25%	C	$2.20 \pm 0.09^{a,C,1}$	<1.47	$1.89 \pm 0.10^{a,D,1}$
		FM	$4.00 \pm 0.36^{a,C,2}$	<1,47	$1,96 \pm 0.08^{a,C,1}$
	30%	C	$2.06 \pm 0.14^{a,C,1}$	<1.47	$1.66 \pm 0.06^{a,E,1}$
		FM	$2.48 \pm 0.28^{a,D,1}$	<1.47	$1,82 \pm 0.14^{a,C,2}$
	DS	C	$1.64 \pm 0.08^{D,1}$	<1.47	<1.47
		FM	$2.40 \pm 0.22^{a,D,2}$	<1.47	<1.47
1 Month	10%	C	$4.49 \pm 0.18^{b,A,1}$	$3.06 \pm 0.12^{b,A,1}$	$4.44 \pm 0.12^{b,A,1}$
		FM	$5.82 \pm 0.36^{b,A,2}$	$5.08 \pm 0.28^{b,A,2}$	$6.04 \pm 0.20^{b,A,2}$
	15%	C	$2.96 \pm 0.20^{b,B,1}$	$2.77 \pm 0.14^{b,B,1}$	$2.42 \pm 0.09^{a,B,1}$
		FM	$4.45 \pm 0.28^{b,B,2}$	$4.40 \pm 0.18^{a,B,2}$	$4.79 \pm 0.16^{b,B,2}$
	20%	C	$2.07 \pm 0.10^{b,C,1}$	$2.30 \pm 0.18^{b,C}$	$2.88 \pm 0.14^{b,C,1}$
		FM	$4.26 \pm 0.26^{b,B,2}$	NO	$4.45 \pm 0.22^{b,C,2}$
	25%	C	$1.80 \pm 0.07^{b,D,1}$	$1.77 \pm 0.09^{a,D}$	$1.56 \pm 0.14^{b,D,1}$
		FM	$4.32 \pm 0.28^{a,B,2}$	NO	$4.95 \pm 0.18^{b,B,2}$
	30%	C	$1.72 \pm 0.06^{b,1}$	1.51 ± 0.08^{E}	<1.47
		FM	$3.62 \pm 0.22^{a,C,2}$	NO	NO
	DS	C	<1.47	<1.47	<1.47
		FM	$3.41 \pm 0.30^{b,C}$	NO	NO
2 Months	15%	C	$3.12 \pm 0.18^{b,A,1}$	$3.41 \pm 0.09^{c,A,1}$	$2.86 \pm 0.10^{b,A,1}$
		FM	$6.36 \pm 0.60^{c,A,2}$	$6.43 \pm 0.36^{b,A,2}$	$6.45 \pm 0.34^{c,A,2}$
	20%	C	$3.04 \pm 0.18^{c,A,1}$	$2.48 \pm 0.09^{b,B,1}$	$2.64 \pm 0.12^{b,A,1}$
		FM	$4.45 \pm 0.26^{b,B,2}$	$4.32 \pm 0.30^{a,B,2}$	$4.23 \pm 0.26^{b,B,2}$
	25%	C	<1.47	$2.06 \pm 0.14^{b,C,1}$	<1.47
		FM	$4.20 \pm 0.10^{a,B}$	$4.11 \pm 0.18^{a,B,2}$	$4.28 \pm 0.20^{c,B}$
	30%	C	<1.47	<1.47	<1.47
		FM	$2.65 \pm 0.42^{b,B}$	NO	NO
	DS	C	NO	NO	NO
		FM	NO	NO	NO

Table 3. *Cont.*

Storage Time	Salt Concentration	Sample Type	Microbial Counts (log CFU/g)		
			TVAMB	HB	HFB
3 Months	20%	C	$3.36 \pm 0.16^{d,1}$	$2.55 \pm 0.14^{b,A,1}$	$2.86 \pm 0.09^{b,1}$
		FM	$5.68 \pm 0.42^{a,A,2}$	$4.84 \pm 0.28^{b,A,2}$	$7.60 \pm 0.30^{c,A,2}$
	25%	C	<1.47	$2.20 \pm 0.12^{b,B,1}$	<1.47
		FM	$4.32 \pm 0.22^{a,B}$	$4.38 \pm 0.20^{a,B,2}$	$4.23 \pm 0.16^{c,B}$
	30%	C	<1.47	<1.47	<1.47
		FM	NO	NO	NO
	DS	C	NO	NO	NO
		FM	NO	NO	NO
4 Months	20%	C	$3.12 \pm 0.18^{c,1}$	$2.86 \pm 0.15^{c,A,1}$	$2.92 \pm 0.08^{b,1}$
		FM	$5.60 \pm 0.40^{a,A,2}$	$4.92 \pm 0.26^{b,A,2}$	$4.60 \pm 0.14^{a,A,2}$
	25%	C	<1.47	$2.48 \pm 0.10^{c,B,1}$	<1.47
		FM	$4.00 \pm 0.20^{a,B}$	$3.85 \pm 0.18^{b,B,2}$	$4.18 \pm 0.16^{c,B}$
	30%	C	<1.47	<1.47	<1.47
		FM	NO	NO	NO
	DS	C	NO	NO	NO
		FM	NO	NO	NO
5 Months	25%	C	<1.47	$2.64 \pm 0.09^{d,1}$	<1.47
		FM	4.51 ± 0.26^{b}	$4.48 \pm 0.28^{a,2}$	4.28 ± 0.20^{c}
	30%	C	<1.47	<1.47	<1.47
		FM	NO	NO	NO
	DS	C	NO	NO	NO
		FM	NO	NO	NO
6 Months	30%	C	<1.47	<1.47	<1.47
		FM	NO	NO	NO
	DS	C	NO	NO	NO
		FM	NO	NO	NO
7 Months	30%	C	<1.47	<1.47	<1.47
		FM	NO	NO	NO
	DS	C	NO	NO	NO
		FM	NO	NO	NO
8 Months	30%	C	<1.47	<1.47	<1.47
		FM	NO	NO	NO
	DS	C	NO	NO	NO
		FM	NO	NO	NO
10 Months	30%	C	<1.47	<1.47	<1.47
		FM	NO	NO	NO
	DS	C	NO	NO	NO
		FM	NO	NO	NO
11 Months	DS	C	NO	NO	NO
	DS	FM	NO	NO	NO
12 Months	DS	FM	NO	NO	NO

The different lowercase letters (^{a,b,c}, etc.) within the same column represent statistical differences depending on time spent within the same brine concentration and the dry-salting method for each group (control and experimental groups). The different uppercase letters (^{A,B,C}, etc.) within the same column represent statistical differences among the different brine concentrations within the same brine concentration and dry-salting method for each group (control and experimental). The different superscript numbers (^{1,2}) in the front data within the same column represent statistical differences between control and experimental groups for the same brine concentration and dry-salting method for the same storage time. TVAMB: total viable aerobic mesophilic bacteria; HB: halophilic bacteria; HFB: histamine-forming bacteria; M: month; C: control group; FM: prepared from frozen raw material (experimental group); NO: no microbial growth; NA: not analyzed; <1.47: The petri dish contains less than 30 bacteria.

According to the International Commission for Microbiological Standards of Foods [52], initial counts on fish may vary from 10^2 to 10^7 cfu/g, a total aerobic count that can only be used as an indication of time–temperature conditions during storage if sufficient data are known about a particular fish species. These values can change during handling and processing. The highest TVAMB counts corresponded to the lowest brine concentration (10%) of the experimental group on the 1st week of ripening and significantly decreased during storage ($p < 0.05$) (Table 3). Various researchers reported low bacterial loads during different stages of ripened anchovies [51,53]. In our previous study with commercially brined and dry-salted products, TVAMB counts varied between 2.17 and 2.75 log cfu/g and between undetectable and 3.08 log cfu/g, respectively [42].

The spoilage of salted and dried fish during storage is mainly due to the activity of microorganisms, especially the salt-tolerant halophilic bacteria (HB). Past studies demonstrated low or absent HB counts for these types of products at the beginning of the ripening period, with counts usually increasing during storage [50], which was also supported

by our findings in this study for brined samples relating to 10–25% brine concentrations with few exceptions. High salt concentrations and low storage temperatures inhibited HB bacteria growth, which was demonstrated in this study and in previous works [24]. Similarly, the results of the bacteria count closely supported the values of a_w levels, as the lower the a_w became, the lower the microbial counts observed. Koral et al. [42] obtained total HB counts within the 1.39–2.83 and 1.17–3.17 log cfu/g ranges in the commercial brined and dry-salted products, respectively. For HFB counts, they obtained values varying from undetectable to 2.62 log cfu/g and from undetectable to 2.90 in the same respect [42].

Decreasing total viable bacteria counts and slight and moderate halophilic counts were also obtained by Hernandez-Herrero et al. [25] during the ripening period. However, they also obtained increasing levels of extreme HB. Although high levels of HB are usually related to spoilage, some halophiles, particularly moderate halophiles and extremely halophilic archaea, are also related to the improving ripening quality of anchovies [41,54,55]. Yeannes et al. [56] reported that the extremely halofilic bacteria require between 15 and 30% concentrations of NaCl for growth. Czerner and Yeannes [41] demonstrated that the ripening process of salted anchovies was dominated by moderate HB. Moreover, they reported that many of the isolated strains showed proteolytic, lipolytic, and TMAO reductase activities. They claimed that these activities contributed to the development of the typical flavor of this product and to the increase in total volatile bases observed during ripening. Therefore, the HB load obtained in this study during storage may help with the flavor development of the brined anchovies.

Histamine is formed by the decarboxylating activity of HFB, mainly members of the genera *Klebsiella*, *Morganella*, *Vibrio*, *Photobacterium*, and others [43,57]. However, Hernandez-Herrero et al. [25] demonstrated that halotolerant and halophilic histamine-producing bacteria isolated during the ripening of salted anchovies generally belong to the *Staphylococcus* genus, possessing powerful histamine-forming activity in the presence of 3% and 10% NaCl. During the ripening of salted anchovies, important proteolysis is observed, with the liberation of peptides and free amino acids. When free histidine is found in sufficient quantities, it can be degraded by microorganisms or their enzymes. Consequently, histamine may be formed at this time and eventually reach toxic levels [25].

In the present study, the products corresponding to 10 and 15% brine concentrations of the control group, and 10, 15, and 20% of the experimental group, contained a salt content of $\leq$10%, which is not safe for histamine development during the ripening process. However, the brine concentrations over these values were within the safety levels for the control group starting from the 2nd week and 1st month for 30% and 20 and 25% brine concentrations, respectively. The salt contents of the experimental group were found to be lower than the suggested value starting from the 1st week of brining for other brine concentrations. The salt contents of dry-salted products were above 10% throughout the storage, starting from the 1st week. The histamine contents obtained in this study were usually found to be under the detection limits, with several exceptions for both groups (Table 4). Therefore, these findings imply that the levels of salt content in the products were within the safety levels for both groups required to prevent HFB activity, which retards histamine formation during storage. Initial HFB in fish is important since previously formed histamine decarboxylases can continue to decarboxylate histidine to histamine even when histamine decarboxylase-positive bacteria are no longer viable [43]. Moreover, Karaçam et al. [24] demonstrated that histamine can develop during the ripening of brined anchovies at ambient temperature but that, conversely, histamine does not develop during refrigerated storage. Therefore, the present study also supports these findings, as salting combined with cold storage can also prevent histidine decarboxylation activity. Similar findings were also obtained by El Filali et al. [58], with the exception of one batch. The effects of salt concentration and the use of previously frozen raw materials on histamine development were not clear in the previous study due to the low levels of histamine obtained in the samples.

Table 4. The changes in the biogenic amines of dry-salted and brined anchovies prepared from fresh raw material (control group) in comparison to frozen and thawed raw material (experimental group) during refrigerated storage at 4 ± 1 °C (n = 3).

Str. Time	Salt Cons.	Sam. Type	Biogenic Amines (ppm)							
			Tryptamine	Phenylethylamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine
2nd Week	10%	C	4.78 ± 0.06 ^{a,A,1}	6.34 ± 0.15 ^{a,A,1}	28.26 ± 0.80 ^{a,A,1}	186.28 ± 3.02 ^{a,A,1}	<0.86	46.72 ± 1.86 ^{a,A,1}	8.12 ± 0.32 ^{a,A,1}	<0.71
		FM	5.05 ± 0.10 ^{a,A,2}	1.54 ± 0.15 ^{a,A,2}	36.03 ± 1.76 ^{a,A,2}	287.22 ± 10.72 ^{a,A,2}	<0.86	78.15 ± 4.25 ^{a,A,2}	9.10 ± 0.43 ^{a,A,2}	<0.71
	15%	C	4.34 ± 0.08 ^{a,B,1}	5.30 ± 0.08 ^{a,B,1}	0.92 ± 0.15 ^{a,B,1}	6.40 ± 0.10 ^{a,B,1}	<0.86	4.72 ± 0.09 ^{a,B,1}	16.12 ± 0.68 ^{a,B,1}	1.40 ± 0.10 ^{a,A,1}
		FM	4.87 ± 0.10 ^{a,B,2}	5.90 ± 1.08 ^{a,B,1}	0.76 ± 0.25 ^{a,B,1}	7.54 ± 0.60 ^{a,B,2}	<0.86	4.12 ± 0.08 ^{a,B,2}	17.42 ± 1.68 ^{a,B,1}	1.59 ± 0.16 ^{a,A,1}
	20%	C	4.78 ± 0.12 ^{a,A,1}	5.68 ± 0.26 ^{a,B,1}	<0.56	5.48 ± 0.17 ^{a,C,1}	<0.86	2.68 ± 0.14 ^{a,C,1}	15.42 ± 0.13 ^{a,B,1}	1.62 ± 0.16 ^{a,B,1}
		FM	4.91 ± 0.01 ^{a,B,1}	5.81 ± 0.57 ^{a,B,1}	0.16 ± 0.07 ^{a,C}	5.22 ± 0.37 ^{a,C,1}	<0.86	2.88 ± 0.04 ^{a,C,1}	16.52 ± 0.23 ^{a,B,2}	1.53 ± 0.09 ^{a,A,1}
	25%	C	4.54 ± 0.18 ^{a,B,1}	9.68 ± 0.26 ^{a,C,1}	<0.56	7.36 ± 0.78 ^{a,D,1}	<0.86	3.50 ± 0.18 ^{a,D,1}	24.68 ± 0.28 ^{a,C,1}	1.78 ± 0.12 ^{a,B,1}
		FM	4.54 ± 0.08 ^{a,B,2}	9.51 ± 1.07 ^{a,C,1}	0.09 ± 0.04 ^{a,C}	7.80 ± 1.84 ^{a,B,1}	<0.86	3.52 ± 0.28 ^{a,D,1}	25.02 ± 0.58 ^{a,C,1}	1.71 ± 0.08 ^{a,A,1}
	30%	C	4.48 ± 0.72 ^{a,B,1}	11.46 ± 0.84 ^{a,D,1}	<0.56	5.66 ± 0.56 ^{a,C,1}	<0.86	3.78 ± 0.60 ^{a,D,1}	26.02 ± 1.25 ^{a,C,1}	1.78 ± 0.28 ^{a,B,1}
		FM	4.69 ± 0.18 ^{a,B,1}	10.57 ± 1.24 ^{a,C,1}	0.13 ± 0.01 ^{a,C}	5.82 ± 0.17 ^{a,C,1}	<0.86	3.46 ± 0.33 ^{a,D,1}	28.31 ± 0.75 ^{a,D,1}	1.89 ± 0.19 ^{a,A,1}
	DS	C	4.76 ± 0.46 ^{a,A,1}	12.32 ± 0.58 ^{a,D,1}	0.98 ± 0.04 ^{a,B,1}	2.98 ± 0.15 ^{a,D,1}	<0.86	3.80 ± 0.20 ^{a,D,1}	14.30 ± 0.14 ^{a,D,1}	1.84 ± 0.08 ^{a,B,1}
		FM	4.68 ± 0.20 ^{a,B,1}	9.82 ± 0,20 ^{a,C,1}	0.15 ± 0.02 ^{a,C,2}	2.80 ± 0.10 ^{a,D,1}	<0.86	3.20 ± 0.20 ^{a,D,2}	7.20 ± 0.43 ^{a,E,2}	1.44 ± 0.14 ^{a,A,2}
1st Month	10%	C	5.90 ± 0.08 ^{b,A,1}	2.54 ± 0.35 ^{b,A,1}	47.03 ± 1.90 ^{b,A,1}	302.26 ± 13.72 ^{b,A,1}	<0.86	81.65 ± 2.65 ^{b,A,1}	11.12 ± 0.43 ^{b,A,1}	<0.71
		FM	12.25 ± 0.25 ^{b,A,2}	1.19 ± 0.13 ^{b,A,2}	197.74 ± 1.27 ^{b,A,2}	369.48 ± 2.10 ^{b,A,2}	4.60 ± 0.08	284.00 ± 1.60 ^{b,A,2}	2.62 ± 0.62 ^{b,A,2}	1.76 ± 0.16 ^{A}
	15%	C	4.86 ± 0.10 ^{b,B,1}	7.12 ± 0.12 ^{b,B,1}	<0.56	6.89 ± 0.08 ^{b,B,1}	<0.86	3.40 ± 0.16 ^{b,B,1}	24.24 ± 0.22 ^{b,B,1}	1.28 ± 0.05 ^{a,A,1}
		FM	4.69 ± 0.11 ^{a,B,1}	7.49 ± 0.13 ^{b,B,2}	0.48 ± 0.01 ^{b,B}	6.63 ± 1.08 ^{a,B,1}	<0.86	3.72 ± 0.06 ^{b,B,2}	27.34 ± 0.50 ^{b,B,2}	1.12 ± 0.01 ^{b,B,1}
	20%	C	4.26 ± 0.21 ^{b,C,1}	7.48 ± 0.34 ^{b,B,1}	0.98 ± 0.09 ^{a,B,1}	5.94 ± 0.42 ^{a,C,1}	<0.86	3.46 ± 0.16 ^{b,B,1}	23.38 ± 0.46 ^{b,B,1}	1.56 ± 0.15 ^{a,B,1}
		FM	4.70 ± 0.01 ^{a,B,2}	7.08 ± 0.54 ^{b,B,1}	0.95 ± 0.04 ^{b,C,2}	5.76 ± 1.00 ^{a,B,1}	<0.86	3.15 ± 0.27 ^{a,C,1}	22.09 ± 1.99 ^{b,C,1}	1.45 ± 0.15 ^{a,C,1}
	25%	C	5.78 ± 0.16 ^{b,A,1}	11.68 ± 0.34 ^{b,C,1}	0.72 ± 0.18 ^{a,B,1}	22.98 ± 0.24 ^{b,D,1}	<0.86	4.38 ± 0.14 ^{b,C,1}	34.12 ± 0.76 ^{b,C,1}	1.78 ± 0.08 ^{a,B,1}
		FM	5.41 ± 0.10 ^{b,C,1}	11.21 ± 0.39 ^{b,C,1}	0.64 ± 0.08 ^{b,B,1}	20.93 ± 1.24 ^{b,C,1}	<0.86	4.09 ± 0.04 ^{b,B,1}	32.23 ± 0.80 ^{b,D,1}	1.87 ± 0.09 ^{a,A,1}
	30%	C	5.18 ± 0.22 ^{b,D,1}	16.34 ± 0.78 ^{b,D,1}	<0.56	9.50 ± 0.50 ^{b,E,1}	<0.86	4.88 ± 0.64 ^{b,C,1}	33.78 ± 1.04 ^{b,C,1}	2.58 ± 0.68 ^{b,C,1}
		FM	5.35 ± 0.42 ^{b,C,1}	14.21 ± 1.01 ^{b,D,1}	0.53 ± 0.19 ^{b,B}	8.60 ± 0.75 ^{b,B,1}	<0.86	4.08 ± 0.04 ^{b,B,2}	32.33 ± 0.11 ^{b,D,1}	2.01 ± 0.01 ^{a,D,2}
	DS	C	5.06 ± 0.16 ^{a,D,1}	12.02 ± 0.28 ^{a,E,1}	1.08 ± 0.14 ^{a,B,1}	3.78 ± 0.10 ^{b,F,1}	<0.86	3.98 ± 0.26 ^{a,C,1}	8.78 ± 0.24 ^{b,D,1}	1.68 ± 0.18 ^{a,B,1}
		FM	4.96 ± 0.11 ^{a,C,1}	10.32 ± 1.77 ^{a,D,1}	1.04 ± 0.04 ^{b,C,1}	3.01 ± 0.14 ^{a,D,2}	<0.86	2.92 ± 0.43 ^{a,C,2}	7.93 ± 0.34 ^{a,E,1}	1.58 ± 0.11 ^{a,A,1}
2nd Month	15%	C	4.82 ± 0.16 ^{b,A,1}	9.03 ± 0.10 ^{c,A,1}	1.36 ± 0.09 ^{b,A,1}	4.28 ± 0.13 ^{c,A,1}	<0.86	3.65 ± 0.08 ^{b,A,1}	34.20 ± 0.68 ^{c,A,1}	1.24 ± 0.12 ^{a,A,1}
		FM	4.92 ± 0.06 ^{a,A,1}	8.23 ± 0.16 ^{c,A,2}	1.07 ± 0.23 ^{b,A,1}	4.08 ± 0.43 ^{c,A,1}	<0.86	3.85 ± 0.11 ^{b,A,1}	32.80 ± 0.88 ^{c,A,1}	1.04 ± 0.33 ^{b,A,1}
	20%	C	4.68 ± 0.10 ^{a,A,1}	9.78 ± 0.37 ^{c,B,1}	1.72 ± 0.13 ^{b,B,1}	6.66 ± 0.32 ^{b,B,1}	<0.86	3.45 ± 0.08 ^{b,B,1}	24.28 ± 0.36 ^{b,B,1}	1.52 ± 0.10 ^{a,B,1}
		FM	4.80 ± 0.11 ^{a,A,1}	9.49 ± 0.57 ^{c,B,1}	1.92 ± 0.23 ^{c,B,1}	6.11 ± 0.41 ^{a,B,1}	<0.86	3.51 ± 0.08 ^{b,A,1}	23.59 ± 0.66 ^{b,B,1}	1.40 ± 0.11 ^{a,A,1}
	25%	C	6.32 ± 0.36 ^{c,B,1}	7.56 ± 0.36 ^{c,C,1}	2.86 ± 0.20 ^{b,C,1}	24.22 ± 0.38 ^{b,C,1}	<0.86	4.62 ± 0.16 ^{b,C,1}	36.44 ± 1.36 ^{b,A,1}	1.84 ± 0.18 ^{a,C,1}
		FM	6.01 ± 0.16 ^{c,B,1}	7.23 ± 0.76 ^{c,C,1}	2.92 ± 0.21 ^{c,C,1}	25.70 ± 0.59 ^{c,C,1}	<0.86	4.32 ± 0.06 ^{b,B,1}	34.28 ± 0.85 ^{b,A,1}	1.74 ± 0.08 ^{a,B,1}
	30%	C	5.68 ± 0.72 ^{b,C,1}	14.26 ± 0.86 ^{c,D,1}	<0.56	4.78 ± 0.50 ^{c,A,1}	<0.86	4.46 ± 0.52 ^{b,C,1}	32.02 ± 1.26 ^{b,A,1}	2.12 ± 0.32 ^{c,C,1}
		FM	5.21 ± 0.58 ^{b,A,1}	12.84 ± 1.13 ^{c,D,1}	0.56 ± 0.16 ^{b,D}	4.89 ± 0.71 ^{a,A,1}	<0.86	4.51 ± 0.49 ^{b,B,1}	33.21 ± 0.37 ^{b,A,1}	2.01 ± 0.02 ^{a,C,1}
	DS	C	5.24 ± 0.46 ^{s,C,1}	10.16 ± 0.28 ^{b,B,1}	1.78 ± 0.08 ^{b,B,1}	5.34 ± 0.68 ^{c,A,1}	<0.86	2.86 ± 0.16 ^{b,D,1}	11.78 ± 0.42 ^{dcC,1}	1.72 ± 0.16 ^{a,C,1}
		FM	5.17 ± 0.17 ^{a,A,1}	11.76 ± 0.08 ^{b,D,1}	1.64 ± 0.08 ^{c,E,1}	5.85 ± 0.83 ^{b,B,1}	<0.86	2.79 ± 0.15 ^{a,C,1}	10.60 ± 0.57 ^{b,C,1}	1.66 ± 0.06 ^{a,B,1}

Table 4. *Cont.*

Str. Time	Salt Cons.	Sam. Type	Biogenic Amines (ppm)							
			Tryptamine	Phenylethylamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine
3rd Month	15%	FM	$5.07 \pm 0.13^{a,A}$	$10.05 \pm 0.10^{d,A}$	$1.89 \pm 0.04^{c,A}$	$5.20 \pm 0.49^{a,A}$	<0.86	$3.92 \pm 0.06^{b,A}$	$35.05 \pm 1.32^{d,A}$	$1.39 \pm 0.04^{a,A}$
	20%	C	$4.92 \pm 0.46^{c,A,1}$	$9.50 \pm 0.28^{c,A,1}$	$1.86 \pm 0.20^{b,A,1}$	$6.80 \pm 0.26^{b,A,1}$	<0.86	$3.60 \pm 0.23^{b,A,1}$	$23.52 \pm 0.32^{b,A,1}$	$1.59 \pm 0.12^{a,A,1}$
		FM	$4.72 \pm 0.06^{a,B,1}$	$9.10 \pm 0.39^{c,B,1}$	$1.70 \pm 0.11^{c,A,1}$	$5.84 \pm 0.16^{a,A,2}$	<0.86	$3.70 \pm 0.03^{b,A,1}$	$22.49 \pm 0.52^{b,B,1}$	$1.45 \pm 0.10^{a,A,1}$
	25%	C	$5.96 \pm 0.28^{b,B,1}$	$8.78 \pm 0.62^{a,A,1}$	$6.78 \pm 0.86^{c,B,1}$	$33.12 \pm 1.02^{c,B,1}$	<0.86	$4.98 \pm 0.24^{c,B,1}$	$34.50 \pm 0.46^{b,B,1}$	$1.72 \pm 0.18^{a,A,1}$
		FM	$5.82 \pm 0.08^{c,C,1}$	$8.57 \pm 0.35^{c,B,1}$	$6.12 \pm 0.75^{d,B,1}$	$31.79 \pm 0.92^{d,B,1}$	<0.86	$4.69 \pm 0.04^{b,B,1}$	$32.51 \pm 0.53^{b,C,1}$	$1.62 \pm 0.08^{a,A,1}$
	30%	C	$5.56 \pm 0.28^{b,B,1}$	$12.84 \pm 0.94^{a,B,1}$	$1.46 \pm 0.22^{a,A,1}$	$5.68 \pm 0.26^{a,C,1}$	<0.86	$4.14 \pm 0.36^{b,C,1}$	$24.30 \pm 0.88^{a,A,1}$	$1.86 \pm 0.25^{a,A,1}$
		FM	$5.62 \pm 0.06^{b,C,1}$	$11.76 \pm 0.54^{c,C,1}$	$1.30 \pm 0.11^{c,C,1}$	$5.36 \pm 0.05^{a,A,1}$	<0.86	$4.28 \pm 0.06^{b,C,1}$	$33.20 \pm 0.07^{b,C,2}$	$1.77 \pm 0.07^{a,A,1}$
	DS	C	$5.16 \pm 0.09^{a,B,1}$	$15.40 \pm 0.32^{d,C,1}$	$1.58 \pm 0.22^{b,A,1}$	$5.12 \pm 0.18^{c,C,1}$	<0.86	$2.98 \pm 0.10^{b,D,1}$	$16.56 \pm 0.60^{d,C,1}$	$2.56 \pm 0.08^{b,B,1}$
		FM	$5.56 \pm 0.13^{b,C,2}$	$14.07 \pm 0.43^{c,D,2}$	$1.48 \pm 0.12^{d,C,1}$	$5.30 \pm 0.08^{b,A,1}$	<0.86	$2.91 \pm 0.01^{a,D,1}$	$17.87 \pm 0.06^{c,D,2}$	$2.51 \pm 0.28^{b,B,1}$
4th Month	20%	C	$4.86 \pm 0.12^{c,A,1}$	$6.68 \pm 0.16^{d,A,1}$	$0.86 \pm 0.16^{a,A,1}$	$6.48 \pm 0.55^{b,A,1}$	<0.86	$3.86 \pm 0.25^{b,A,1}$	$24.86 \pm 0.24^{b,A,1}$	$1.59 \pm 0.18^{a,A,1}$
		FM	$4.77 \pm 0.22^{a,A,1}$	$6.23 \pm 0.06^{a,A,1}$	$0.81 \pm 0.17^{b,A,1}$	$6.18 \pm 0.95^{a,A,1}$	<0.86	$3.74 \pm 0.05^{b,A,1}$	$22.75 \pm 0.89^{b,A,1}$	$1.49 \pm 0.15^{a,A,1}$
	25%	C	$5.86 \pm 0.52^{b,B,1}$	$4.76 \pm 0.78^{d,B,1}$	$1.46 \pm 0.64^{d,B,1}$	$36.32 \pm 1.24^{c,B,1}$	<0.86	$4.80 \pm 0.28^{c,B,1}$	$32.68 \pm 1.12^{b,B,1}$	$1.64 \pm 0.46^{a,A,1}$
		FM	$5.65 \pm 0.02^{b,B,1}$	$4.30 \pm 1.11^{d,B,1}$	$1.24 \pm 0.14^{e,B,1}$	$37.15 \pm 0.92^{e,B,1}$	<0.86	$4.83 \pm 0.11^{b,B,1}$	$30.41 \pm 2.04^{b,B,1}$	$1.43 \pm 0.13^{b,A,1}$
	30%	C	$6.12 \pm 0.29^{c,C,1}$	$8.12 \pm 0.18^{d,C,1}$	$3.52 \pm 0.22^{b,C,1}$	$4.88 \pm 0.34^{c,C,1}$	<0.86	$4.58 \pm 0.24^{b,B,1}$	$33.18 \pm 0.38^{b,B,1}$	$1.58 \pm 0.16^{a,A,1}$
		FM	$6.09 \pm 1.29^{c,B,1}$	$8.71 \pm 1.18^{d,C,1}$	$3.36 \pm 0.52^{d,C,1}$	$4.93 \pm 0.21^{a,C,1}$	<0.86	$4.21 \pm 0.04^{b,C,1}$	$32.28 \pm 1.38^{b,B,1}$	$1.47 \pm 0.06^{b,A,1}$
	DS	C	$5.72 \pm 0.28^{a,B,1}$	$9.02 \pm 0.22^{d,D,1}$	$2.76 \pm 0.10^{c,D,1}$	$5.58 \pm 0.28^{c,D,1}$	<0.86	$3.20 \pm 0.16^{b,C,1}$	$12.18 \pm 0.26^{c,C,1}$	$2.08 \pm 0.14^{c,B,1}$
		FM	$5.52 \pm 0.08^{b,B,1}$	$9.62 \pm 1.02^{d,C,1}$	$2.56 \pm 0.11^{e,D,1}$	$5.70 \pm 0.20^{b,A,1}$	<0.86	$3.29 \pm 0.10^{b,D,1}$	$13.11 \pm 0.21^{d,C,1}$	$2.22 \pm 0.08^{b,B,1}$
5th Month	25%	C	$7.68 \pm 0.56^{d,A,1}$	$1.78 \pm 0.22^{e,A,1}$	$3.85 \pm 0.38^{e,A,1}$	$34.98 \pm 0.86^{c,A,1}$	<0.86	$3.48 \pm 0.72^{a,A,1}$	$36.56 \pm 1.34^{b,A,1}$	$1.48 \pm 0.58^{a,A,1}$
		FM	$7.51 \pm 0.05^{d,A,1}$	$1.66 \pm 0.02^{e,A,1}$	$3.55 \pm 0.06^{f,A,1}$	$35.78 \pm 0.16^{e,A,1}$	<0.86	$3.22 \pm 1.00^{a,A,1}$	$34.10 \pm 2.21^{b,A,1}$	$1.19 \pm 0.08^{c,A,1}$
	30%	C	$6.34 \pm 0.35^{c,B,1}$	$12.08 \pm 0.34^{a,B,1}$	$1.68 \pm 0.28^{a,B,1}$	$7.22 \pm 0.30^{d,B,1}$	<0.86	$2.88 \pm 0.24^{c,B,1}$	$32.42 \pm 0.78^{b,B,1}$	$1.70 \pm 0.28^{a,B,1}$
		FM	$6.63 \pm 0.05^{d,B,1}$	$12.58 \pm 0.21^{d,B,1}$	$1.77 \pm 0.63^{c,B,1}$	$7.46 \pm 0.31^{b,B,1}$	<0.86	$2.40 \pm 0.03^{c,B,2}$	$33.66 \pm 1.58^{b,A,1}$	$1.71 \pm 0.04^{a,B,1}$
	DS	C	$5.02 \pm 0.34^{a,C,1}$	$8.80 \pm 0.16^{d,C,1}$	$3.48 \pm 0.20^{d,A,1}$	$5.84 \pm 0.56^{c,C,1}$	<0.86	$3.46 \pm 0.26^{a,A,1}$	$10.88 \pm 0.14^{e,C,1}$	$1.68 \pm 0.17^{a,B,1}$
		FM	$5.29 \pm 0.23^{b,C,1}$	$8.71 \pm 1.18^{d,C,1}$	$3.36 \pm 0.12^{f,C,1}$	$5.68 \pm 0.63^{b,C,1}$	<0.86	$3.22 \pm 0.06^{b,A,1}$	$8.40 \pm 0.84^{a,B,2}$	$1.41 \pm 0.19^{a,C,1}$
6th Month	25%	C	$5.86 \pm 0.88^{b,A,1}$	$1.48 \pm 0.24^{e,A,1}$	$2.56 \pm 0.68^{f,A,1}$	$38.20 \pm 0.86^{d,A,1}$	<0.86	$3.78 \pm 0.48^{a,A,1}$	$18.78 \pm 0.88^{c,A,1}$	$1.88 \pm 0.34^{a,A,1}$
		FM	$6.81 \pm 0.18^{e,A,2}$	$0.95 \pm 0.04^{e,A,2}$	$2.29 \pm 0.13^{c,A,1}$	$36.96 \pm 1.98^{e,A,1}$	<0.86	$3.52 \pm 0.08^{b,A,1}$	$17.87 \pm 1.45^{c,A,1}$	$0.99 \pm 0.04^{d,A,2}$
	30%	C	$7.48 \pm 0.28^{d,B,1}$	$8.46 \pm 0.18^{d,B,1}$	$0.96 \pm 0.28^{c,B,1}$	$7.68 \pm 0.38^{d,B,1}$	<0.86	$3.88 \pm 0.25^{b,A,1}$	$19.26 \pm 0.42^{c,A,1}$	$1.78 \pm 0.34^{a,A,1}$
		FM	$7.01 \pm 0.16^{e,A,1}$	$8.67 \pm 0.03^{d,B,1}$	$0.90 \pm 0.16^{f,B,1}$	$7.41 \pm 0.40^{b,A,1}$	<0.86	$3.56 \pm 0.05^{a,A,1}$	$18.01 \pm 0.22^{c,A,1}$	$1.82 \pm 0.04^{a,B,1}$
	DS	C	$5.38 \pm 0.15^{a,A,1}$	$8.20 \pm 0.24^{b,B,1}$	$3.00 \pm 0.17^{e,A,1}$	$5.24 \pm 0.22^{c,C,1}$	<0.86	$2.80 \pm 0.10^{b,B,1}$	$10.96 \pm 0.12^{e,B,1}$	$1.70 \pm 0.10^{a,A,1}$
		FM	$5.09 \pm 0.05^{a,B,1}$	$10.25 \pm 0.31^{d,C,2}$	$3.07 \pm 0.07^{b,C,1}$	$5.01 \pm 0.60^{b,A,1}$	<0.86	$2.89 \pm 0.13^{a,B,1}$	$10.87 \pm 0.42^{b,B,1}$	$1.79 \pm 0.15^{a,B,1}$
7th Month	30%	C	$7.28 \pm 0.48^{d,A,1}$	$10.22 \pm 0.46^{a,A,1}$	$1.46 \pm 0.46^{a,A,1}$	$9.46 \pm 0.46^{b,A,1}$	<0.86	$4.02 \pm 0.26^{b,A,1}$	$22.88 \pm 0.68^{a,A,1}$	$2.14 \pm 0.20^{c,A,1}$
		FM	$7.12 \pm 0.28^{e,A,1}$	$10.52 \pm 0.16^{c,A,1}$	$1.30 \pm 0.10^{c,A,1}$	$9.12 \pm 0.20^{b,A,1}$	<0.86	$4.38 \pm 0.09^{b,A,1}$	$23.38 \pm 0.16^{d,A,1}$	$2.05 \pm 0.09^{a,A,1}$
	DS	C	$4.76 \pm 0.16^{a,B,1}$	$12.36 \pm 0.32^{b,B,1}$	$1.78 \pm 0.17^{b,B,1}$	$4.78 \pm 0.21^{d,B,1}$	<0.86	$2.66 \pm 0.15^{b,B,1}$	$9.98 \pm 0.22^{e,B,1}$	$2.12 \pm 0.09^{c,A,1}$
		FM	$4.82 \pm 0.03^{a,B,1}$	$12.95 \pm 0.52^{b,A,1}$	$1.81 \pm 0.07^{b,B,1}$	$4.80 \pm 0.23^{b,B,1}$	<0.86	$2.72 \pm 0.05^{a,B,1}$	$9.66 \pm 0.45^{b,B,1}$	$2.01 \pm 0.11^{a,A,1}$

Table 4. *Cont.*

Str. Time	Salt Cons.	Sam. Type	Biogenic Amines (ppm)							
			Tryptamine	**Phenylethylamine**	**Putrescine**	**Cadaverine**	**Histamine**	**Tyramine**	**Spermidine**	**Spermine**
8th Month	30%	C	$7.96 \pm 0.46^{d,A,1}$	$14.88 \pm 0.80^{c,A,1}$	$1.86 \pm 0.64^{a,A,1}$	$10.22 \pm 0.55^{b,A,1}$	<0.86	$5.58 \pm 0.44^{d,A,1}$	$28.56 \pm 0.24^{a,A,1}$	$1.88 \pm 0.12^{a,A,1}$
		FM	$7.80 \pm 0.36^{f,A,1}$	$14.34 \pm 0.26^{b,A,1}$	$1.90 \pm 0.24^{e,A,1}$	$10.78 \pm 0.36^{c,A,1}$	<0.86	$5.16 \pm 0.15^{d,B,1}$	$27.64 \pm 0.12^{a,A,1}$	$1.96 \pm 0.12^{a,A,1}$
	DS	C	$5.28 \pm 0.25^{a,B,1}$	$12.86 \pm 0.12^{b,B,1}$	$1.90 \pm 0.20^{b,B,1}$	$4.35 \pm 0.41^{d,B,1}$	<0.86	$3.02 \pm 0.05^{b,B,1}$	$8.60 \pm 0.14^{b,B,1}$	$1.98 \pm 0.06^{a,A,1}$
		FM	$5.12 \pm 0.13^{a,B,1}$	$12.95 \pm 0.16^{b,B,1}$	$1.96 \pm 0.02^{d,A,1}$	$4.76 \pm 0.04^{b,B,1}$	<0.86	$3.12 \pm 0.15^{b,A,1}$	$8.66 \pm 0.25^{e,B,1}$	$1.92 \pm 0.06^{a,A,1}$
9th Month	30%	C	$7.88 \pm 0.38^{d,A,1}$	$14.02 \pm 0.24^{c,A,1}$	$2.28 \pm 0.18^{d,,A,1}$	$9.02 \pm 0.14^{b,A,1}$	$<0.86^{1}$	$4.82 \pm 0.18^{b,A,1}$	$33.26 \pm 1.12^{b,A,1}$	$1.66 \pm 0.22^{a,A,1}$
		FM	$7.56 \pm 0.18^{f,A,1}$	$12.88 \pm 0.34^{d,A,2}$	$2.48 \pm 0.08^{f,A,1}$	$9.38 \pm 0.24^{b,A,1}$	1.43 ± 0.21^{2}	$4.96 \pm 0.11^{e,A,1}$	$32.82 \pm 0.42^{b,A,1}$	$1.76 \pm 0.05^{a,A,1}$
	DS	C	$5.38 \pm 0.08^{a,B,1}$	$15.78 \pm 0.14^{b,B,1}$	$2.10 \pm 0.09^{b,B,1}$	$4.28 \pm 0.08^{d,B,1}$	<0.86	$3.42 \pm 0.10^{a,B,1}$	$7.68 \pm 0.24^{f,B,1}$	$2.28 \pm 0.15^{c,B,1}$
		FM	$5.52 \pm 0.08^{a,B,1}$	$15.25 \pm 0.34^{b,B,1}$	$2.11 \pm 0.04^{e,A,1}$	$4.10 \pm 0.13^{b,B,1}$	<0.86	$3.52 \pm 0.04^{c,B,1}$	$7.43 \pm 0.14^{a,B,1}$	$2.11 \pm 0.05^{b,B,1}$
10th Month	DS	C	$4.86 \pm 0.09^{a,1}$	$10.86 \pm 0.36^{b,1}$	$2.52 \pm 0.06^{c,1}$	$4.28 \pm 0.12^{d,1}$	<0.86	$3.50 \pm 0.32^{a,1}$	$9.48 \pm 0.06^{e,1}$	$2.02 \pm 0.10^{a,1}$
		FM	$4.76 \pm 0.06^{c,1}$	$11.12 \pm 0.16^{b,1}$	$2.46 \pm 0.16^{e,1}$	$4.48 \pm 0.32^{b,1}$	<0.86	$3.72 \pm 0.22^{c,1}$	$9.16 \pm 0.16^{a,1}$	$2.32 \pm 0.01^{b,2}$
11th Month	DS	C	$5.56 \pm 0.20^{a,1}$	$9.86 \pm 0.18^{a,1}$	$3.98 \pm 0.07^{f,1}$	$4.78 \pm 0.19^{d,1}$	<0.86	$3.62 \pm 0.08^{a,1}$	$9.98 \pm 0.11^{e,1}$	$1.98 \pm 0.12^{a,1}$
		FM	$5.82 \pm 0.04^{d,1}$	$9.75 \pm 0.44^{c,1}$	$3.81 \pm 0.17^{d,1}$	$4.89 \pm 0.09^{b,1}$	<0.86	$3.22 \pm 0.12^{b,2}$	$9.76 \pm 0.21^{b,1}$	$1.92 \pm 0.10^{a,1}$
12th Month	DS	FM	5.32 ± 0.10^{a}	10.45 ± 0.12^{c}	2.78 ± 0.13^{e}	5.25 ± 0.21^{b}	1.12 ± 0.03	2.92 ± 0.08^{a}	10.89 ± 0.34^{b}	2.21 ± 0.09^{b}

The different lowercase letters (^{a,b,c}, etc.) within the same column represent statistical differences depending on time spent within the same brine concentration and dry-salting method for each group (control and experimental groups). The different uppercase letters (^{A,B,C}, etc.) within the same column represent statistical differences amongst the different brine concentrations within the same brine concentration and dry-salting method for each group (control and experimental). The different superscript numbers (^{1,2}) in the front data within the same column represent statistical differences between control and experimental groups for the same brine concentration and dry-salting method for the same storage time. ±: standard deviation; <0.56: limit of detection of putrescine; <0.86: limit of detection of histamine; <0.71: limit of detection of spermine; DS: dry-salted; C: control group; FM: experimental group (raw materials previously frozen and thawed).

The current European regulations set a maximum average histamine value of 200 mg/kg for ripened anchovies, which is twice the maximum value allowed for fresh or frozen fish [14,47]. The Food and Drug Administration of the USA [16] set a stricter upper allowable limit for histamine at 50 ppm for fish species, as histamine is generally not uniformly distributed in decomposed fish and numerous outbreaks have been caused by this amount. Low levels of histamine formation during the ripening of anchovies and other fish species were also reported by other studies, despite an increased concentration trend in some studies [23,25,59]. However, Rodriguez-Jerez et al. [60] pointed out that the accumulation of high histamine concentrations in salted fish could occur due to poor quality of the raw material, inadequate handling, or other causes encountered during its shelf-life. Therefore, previously formed histamine in the raw material cannot be destroyed by the salting process [43], despite some degradation in histamine activity being suggested by Tapingkae et al. [61]. Various researchers reported high histamine levels for several commercial brined and dry-salted anchovies above the permitted levels set by either the FDA or the European Commission [42,62]. High levels of histamine can be attributed to the poor quality of the anchovies used for processing. In fact, Veciana-Nogués et al. [63] demonstrated that ripening had little influence on the formation of amines, and therefore the amount of amines in the final products depends primarily on the levels of these substances in the raw material. Later on, the authors showed a good correlation to exist between the development of histamine and tyramine on the one hand and alternative spoilage parameters (TVB-N, TMA, hypoxanthine, pH) on the other during storage at both ambient and refrigerated temperatures [64]. According to the European Commission [65], about 26 notifications have been made relating to histamine health risks caused by anchovy products since 2015, with some histamine levels reaching over 3000 ppm. The majority of these notifications were made for salted, brined, and marinated anchovies (almost half of them packed in oil). Rodriguez-Jerez et al. [60] also investigated histamine development during the ripening of semi-preserved anchovies at ambient temperatures. They obtained high histamine levels since the temperature and low salt content of <15% affected histamine development.

The presence of biogenic amines other than histamine is also important in terms of food safety and quality. In the case of histamine toxicity, the potentiating effect of other biogenic amines present in foods such as tyramine, putrescine, and cadaverine was reported due to their competition with histamine-metabolizing enzymes. Moreover, quantities of about 100–800 mg/kg of tyramine and 30 mg/kg of phenylethylamine have been reported to be toxic doses in foods. Several studies tried to link different levels of biogenic amines with the spoilage of fish species [66]. The levels of biogenic amines were under 40 ppm, mainly below 10 ppm, with the exception of samples corresponding to 10% brine concentrations in relation to putrescine, cadaverine and tyramine. Although the groups had statistical differences, the levels fluctuated throughout the storage, with inconsistent changes recorded. The values of putrescine, cadaverine and tyramine in the 10% brined samples increased significantly from the 2nd week to the 1st month, reaching 47.03, 302.26, and 81.65 ppm, respectively, for the control group and 197.74, 369.48, and 284.0 ppm in the same respect for the experimental group. Then the samples for both groups relating to this brine concentration were spoiled according to sensory results. Since the levels of tyramine were found to be over 100 ppm for the 10% brined sample of the experimental group, this concentration was considered unsuitable for the brining of previously frozen raw anchovies. The levels of phenylethylamine were well below the suggested upper limit. Low values of biogenic amines other than histamine were also reported for commercially salted anchovies during the storage period of 14 months [67].

4. Conclusions

The results of this study demonstrated that the freezing of raw materials prior to processing had significant effects on the quality and safety parameters of dry-salted and brined anchovies in comparison to freshly used raw materials ($p < 0.05$). The experimental

group generally had better values for the majority of quality and food safety parameters in comparison to the control group, particularly for the samples with higher salt concentrations and dry salting. Moreover, the salt concentration and salting method had significant effects on the shelf-life of salted anchovy products, with the highest shelf-life corresponding to dry-salted samples produced from previously frozen and thawed anchovies ($p < 0.05$). Strong correlations were usually found between sensory values and chemical quality parameters. Considering that the experimental group is produced from a one-year-old raw material compared to the control, the advantage of freezing and frozen storage of anchovies can be asserted to be much greater than it appears. The positive effects occur due to the fact that frozen anchovies absorb salt faster after thawing. Therefore, this study indicates that frozen anchovies can be further salted for a longer shelf-life, using in particular either the 30% brining or dry-salting method. The results also demonstrate the advantage of utilizing frozen anchovies to increase the valorization of this product.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/app13106200/s1, Table S1: The criteria used to determine sensory changes during refrigerated storage of the control and experimental groups of brined and dry-salted anchovies. Table S2: The results of regression analysis between sensory values and chemical quality parameters (TVB-N, TBA and TMA) for the experimental group stored in a refrigerator. Table S3: The results of regression analysis between sensory values and chemical quality parameters (TVB-N, TBA and TMA) for the control group stored in a refrigerator. Table S4: The results of regression analysis among the values of salt %, moisture % and water activity for the control group during storage in a refrigerator (4 ± 1 °C). Table S5: The results of regression analysis among the values of salt %, moisture % and water activity for the experimental group during storage in a refrigerator (4 ± 1 °C). Table S6: The results of statistical analysis for Figures 1–3 relating to sensory values, chemical quality parameters (TVB-N, TBA, TMA), pH, aw, moisture, salt and WPS.

Author Contributions: Conceptualization, S.K. (Sevim Köse), S.K. (Serkan Koral) and D.K. (Drago Kočar); methodology, S.K. (Sevim Köse), S.K. (Serkan Koral) and D.K.; formal analysis, S.K. (Sevim Köse), S.K. (Serkan Koral) and D.K., investigation, S.K. (Serkan Koral), D.K., S.K. (Sevim Köse), and M.P. (Matevz Pompe); resources, S.K. (Sevim Köse) and M.P.; data curation, S.K. (Sevim Köse) and S.K. (Serkan Koral); writing—original draft preparation, S.K. (Sevim Köse), D.K. and S.K. (Serkan Koral); writing—review and editing, S.K. (Sevim Köse), S.K. (Serkan Koral) and D.K.; visualization, S.K. (Sevim Köse), S.K. (Serkan Koral); supervision, S.K. (Sevim Köse) and M.P.; project administration, S.K. (Sevim Köse), D.K. and M.P.; funding acquisition, S.K. (Sevim Köse), M.P. and D.K. All authors have read and agreed to the published version of the manuscript.

Funding: The research was funded by the TÜBITAK (Turkey) (Grant No. TOVAG 107O327) Slovenian research agency (ARRS, Grant number P1–153), KTU-BAP project No. 2006.117.001.4 and EU TRUEFOOD project No. FOOD-CT-2006-016264.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Acknowledgments: We acknowledge the support of TÜBITAK (Turkey), Slovenian Research Agency (ARSS), the European Union, Karadeniz Technical University, and Ljubljana University. We also thank Bekir Tufan of Karadeniz Technical University for his help in transporting fish samples. We also appreciate the help of the seafood company POLIFISH for their support in this study during the freezing, storing, and transporting of the fish samples.

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

References

1. Sahin, C.; Akin, S.; Hacimurtazaoglu, N.; Mutlu, C.; Verep, B. The stock parameter of anchovy (*Engraulis encrasicolus*) population on the coasts of the Eastern Black Sea: Reason and implications in declining of anchovy population during the 2004–2005 and 2005–2006 fishing seasons. *Fresenius Environ. Bull.* **2008**, *17*, 2159–2169.
2. FAO. Fisheries Statistics, Rome, Italy. 2022. Available online: https://www.fao.org/fishery/en/aqspecies/2106/en (accessed on 6 April 2023).
3. TUIK. Turkish Statistical Institute, State Fisheries Statistics. In *Turkey: TUIK.*; 2020. Available online: http://www.tuik.gov.tr (accessed on 6 April 2023).
4. FISHBASE. 2021. Available online: http://www.fishbase.org/search.php (accessed on 6 April 2023).
5. Tufan, B.; Koral, S.; Köse, S. Changes during fishing season in the fat content and fatty acid profile of edible muscle, liver and gonads of anchovy (*Engraulis encrasicolus*) caught in the Turkish Black Sea. *Int. J. Food Sci. Technol.* **2011**, *10*, 439–443. [CrossRef]
6. FAO/WHO. Food and Agriculture Organization of the United Nations. *FAO/INFOODS Global Food Composition Database for Fish and Shellfish Version 1.0-uFiSh1.0. Rome, Italy.* 2016. Available online: https://www.fao.org/3/i6655e/i6655e.pdf (accessed on 7 April 2023).
7. Martinez, A.; Gildberg, A. Autolytic degradation of belly tissue in anchovy (*Engraulis encrasicholus*). *Food Sci. Technol.* **1988**, *23*, 185–194. [CrossRef]
8. Köse, S.; Gökoğlu, N.; Mol Tokay, S.; Baygar, T.; Pembe Özer, N.; Arık Çolakoğlu, F.; Meriç, İ.; Alçiçek, Z. Türkiye'deki su ürünleri işleme sektörünün durumu, sorunları ve çözüm önerileri. In Proceedings of the Ziraat Mühendisleri Odası, VII., Teknik Kongresi, Ankara, Turkey, 1–4 September 2010; pp. 821–852.
9. Montaner, M.I.; Zugarramurdi, A. Influence of anchovy quality on yield and productivity in salting plants. *J. Food Qual.* **1995**, *18*, 69–82. [CrossRef]
10. Rello, F.J.; Adroher, F.J.; Benítez, R.; Valero, A. The fishing area as a possible indicator of the infection by anisakids in anchovies (*Engraulis encrasicolus*) from southwestern Europe. *Int. J. Food Microbiol.* **2009**, *129*, 277–281. [CrossRef]
11. Anastasio, A.; Smaldone, G.; Cacace, D.; Marrone, R.; Voi, A.L.; Santoro, M.; Cringoli, G.; Pozio, E. Inactivation of *Anisakis pegreffii* larvae in anchovies (*Engraulis encrasicolus*) by salting and quality assessment of finished product. *Food Control* **2016**, *64*, 115–119. [CrossRef]
12. Guardone, L.; Nucera, D.M.; Lodora, L.B.; Tinacci, L.; Acutis, P.L.; Guidi, A.; Armani, A. *Anisakis* spp. larvae in different kinds of ready to eat products made of anchovies (*Engraulis encrasicolus*) sold in Italian supermarkets. *Int. J. Food Microbiol.* **2018**, *268*, 10–18. [CrossRef]
13. Sánchez-Alonso, I.; Carballeda-Sangiao, N.; González-Muñoz, M.; Navas, A.; Arcos, S.C.; Mendizábal, A.; Cuesta, F.; Careche, M. Freezing kinetic parameters influence allergenic and infective potential of *Anisakis simplex* L3 present in fish muscle. *Food Control* **2020**, *118*, 107373. [CrossRef]
14. EC Directive. L 338/12 EN Official Journal of the European Union. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on Microbiological Criteria for Foodstuffs. Available online: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32005R2073 (accessed on 8 March 2023).
15. EFSA. Panel on Biological Hazards (BIOHAZ); Scientific Opinion on risk assessment of parasites in fishery products. *EFSA J.* **2010**, *8*, 1543. [CrossRef]
16. FDA. *Fish & Fisheries Products Hazards & Controls Guide*, 2nd ed.; FDA, Office of Seafood: Washington, DC, USA, 2020; p. 276.
17. Sigurgisladottir, S.; Ingvarsdottir, H.; Torrissen, O.J.; Cardinal, M.; Hafsteinsson, H. Effects of freezing/thawing on the microstructure and the texture of smoked Atlantic salmon (*Salmo salar*). *Food Res. Int.* **2000**, *33*, 857–865. [CrossRef]
18. Szymczak, M. Comparison of physicochemical and sensory changes in fresh and frozen herring (*Clupea harrengus* L.) during marinating. *J. Sci. Food Agri.* **2010**, *91*, 68–74. [CrossRef] [PubMed]
19. Szymczak, M.; Kołakowski, E.; Felisiak, K. Influence of salt concentration on properties of marinated meat from fresh and frozen herring (*Clupea harengus* L.). *Int. J. Food Sci. Technol.* **2012**, *47*, 282–289. [CrossRef]
20. Oliver, A.P.; Esteban, M.M.; Pascual, C.C.; Canales, E.T. Informe del Comite Científico de la Agencia Espanola de Seguridad Alimentaria y Nutricion (AESAN) sobre medidas para reducir el riesgo asociado a la presencia de Anisakis. *Rev. Del Com. Científico De La AESAN* **2007**, *6*, 59–66.
21. Deng, J.C. Effect of freezing and frozen storage on salt penetration into fish muscle immersed in brine. *J. Food Sci.* **1977**, *42*, 348–351. [CrossRef]
22. Mendes, R.; Goncalves, A.; Nunes, M.L. Changes in free amino acids and biogenic amines during ripening of fresh and frozen sardine. *J. Food Biochem.* **1999**, *23*, 295–306. [CrossRef]
23. Koral, S.; Köse, S. The effect of using frozen raw material and different salt ratios on the quality changes of dry salted Atlantic bonito (*lakerda*) at two storage conditions. *Food Health* **2018**, *4*, 213–230. [CrossRef]
24. Karaçam, H.; Kutlu, S.; Köse, S. Effect of salt concentrations and temperature on the quality and shelf-life of brined anchovies. *Int. J. Food Sci. Technol.* **2002**, *37*, 19–28. [CrossRef]
25. Hernandez-Herrero, M.M.; Roig-Sagues, A.X.; Lopez-Sabater, E.I.; Rodriguez-Jerez, J.J.; Mora-Ventura, M.T. Influence of raw fish quality on some physicochemical and microbial characteristics as related to ripening of salted anchovies (*Engraulis encrasicholus* L.). *J. Food Sci.* **2002**, *67*, 2631–2640. [CrossRef]

26. Archer, M. *Sensory Assessment Score Sheets for Fish and Shell Fish*; Research & Development Department of Seafish; Torry & QIM: England, UK, 2010; 58p.
27. AOAC. Moisture in Meat and Poultry Products. In *Official Methods of Analysis of AOAC International*; Cunniff, P., Ed.; Official Method 985; AOAC International: Arlington, VA, USA, 1995; p. 14.
28. Rohani, A.C.; Arup, M.J.; Zahrah, T. Brining parameters for the processing of smoked river carp (*Leptobarbus hoevenii*). *J. Sci. Food Agri.* **2010**, *38*, 51–61.
29. Losikoff, M. Clostridium botulinum concerns. In *International Smoked Seafood Conference Proceedings, Proceedings of the International Smoked Seafood Conference, Alaska, AK, USA, 5–7 March 2008*; Kramer, D.E., Brown, L., Eds.; Alaska Sea Grant College Program, University of Alaska Fairbanks: Alaska, AK, USA, 2008.
30. Minegishi, Y.; Tsukamasa, Y.; Miake, K.; Shimasaki, T.; Imai, C.; Sugiyama, M.S.; Hinano, H. Water activity and microflora in commercial vacuum-packed smoked salmons. *Food Hyg. Saf. Sci.* **1995**, *36*, 442–446. [CrossRef]
31. Goulas, A.E.; Kontominas, M.G. Effect of salting and smoking method on the keeping quality of chub mackerel (*Scomber japonicus*): Biochemical and sensory attributes. *Food Chem.* **2005**, *93*, 511–520. [CrossRef]
32. Smith, G.; Hole, M.; Hanson, S.W. Assessment of lipid oxidation in Indonesian salted-dried marine catfish (*Arius thalassinus*). *J. Sci. Food Agric.* **1992**, *51*, 193–205. [CrossRef]
33. Boland, F.E.; Paige, D.D. Collaborative study of a method for the determination of trimethylamine nitrogen in fish. *JAOAC* **1971**, *54*, 725–727. [CrossRef]
34. Köse, S.; Kaklıkkaya, N.; Koral, S.; Tufan, B.; Buruk, K.C.; Aydın, F. Commercial test kits and the determination of histamine in traditional (ethnic) fish products evaluation against an EU accepted HPLC method. *Food Chem.* **2011**, *125*, 1490–1497. [CrossRef]
35. Köse, S. Investigation into Toxins and Pathogens Implicated in Fish Meal Production. Ph.D. Thesis, Loughborough University of Technology, Loughborough, UK, 1993.
36. Niven, C.F., Jr.; Jeffrey, M.B.; Corlett, D.A., Jr. Different plating medium for quantitative detection of histamine-producing bacteria. *Appl. Environ. Microbiol.* **1981**, *41*, 321–322. [CrossRef]
37. Gürgün, V.; Halkman, A.K. *Mikrobiyolojide Sayım Yöntemleri*; Gıda Teknolojisi Derneği: Ankara, Turkey, 1990; 146p.
38. Sokal, R.R.; Rohlf, F.J. *Introduction to Biostatistics*; W.H. Freeman and Company: New York, NY, USA, 1987.
39. Yapar, A. Üç farklı tuz konsantrasyonu kullanılarak hazırlanan tuzlanmis hamsi (*Engraulis encrasicolus*)'lerde kalite değisimi. *Turk. J. Vet. Anim. Sci.* **1999**, *23*, 441–445.
40. Besteiro, I.; Rodrígues, C.J.; Tilvejar, C.; Pascual, C. Selection of attributes for the sensory evaluation of anchovies during the ripening process. *J. Sens. Stud.* **2000**, *15*, 65–77. [CrossRef]
41. Czerner, M.; Yeannes, M.I. Bacterial contribution to salted anchovy (*Engraulis anchoita* Hubbs & Marinni, 1935) ripening process. *J. Aquat. Food Prod. Technol.* **2014**, *23*, 102–114.
42. Koral, S.; Tufan, B.; Ščavničar, A.; Kočar, D.; Pompe, M.; Köse, S. Investigation of the contents of biogenic amines and some food safety parameters of various commercially salted fish products. *Food Control* **2013**, *32*, 597–606. [CrossRef]
43. Köse, S. Evaluation of seafood safety health hazards for traditional fish products: Preventive measures and monitoring issues. *Turk. J. Fish. Aquat. Scien.* **2010**, *10*, 139–160.
44. Huss, H.H. *Fresh Fish Quality and Quality Changes*; FAO Danish International Development Agency: Rome, Italy, 1988; pp. 43–45.
45. Besteiro, I.; Rodrígues, C.J.; Tilvejar, C.; Pascual, C. Chymotrypsin and general proteolytic activities in muscle of *Engraulis encrasicolus* and *Engraulis anchoita* during the ripening process. *Eur. Food Res. Technol.* **2000**, *210*, 414–418. [CrossRef]
46. Del Valle, C.E.; Filsinger, B.E.; Yeannes, M.I.; Soule, C.L. Shelf life of brine refrigerated anchovies (*Engraulis anchoita*) for canning. *J. Food Sci.* **1984**, *49*, 180–182. [CrossRef]
47. EC Directive. Commission Regulation (EU) No 1019/2013 of 23 October 2013 Amending Annex I to Regulation (EC) No 2073/2005 as Regards Histamine. *Off. J. Eur. Union* **2013**, *282*, 46–47. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/%20PDF/?uri=CELEX:32013R1019&from=EN (accessed on 8 April 2023).
48. Massa, A.E.; Manca, E.; Yeannes, M.I. Development of quality index method for anchovy (*Engraulis anchoita*) stored in ice: Assessment of its shelf-life by chemical and sensory methods. *Food Sci. Technol. Int.* **2012**, *18*, 339–351. [CrossRef] [PubMed]
49. Connell, J.J. *Control of Fish Quality*, 4th ed.; Fishing News Books; A Division of Blackwell Science Ltd.: Oxford, UK, 1995; 245p.
50. Shiriskar, D.S.; Khedkar, G.D.; Lior, D. Production of salted and pressed anchovies (*Stolephorus* sp.) and its quality evaluation during storage. *J. Food Sci. Technol.* **2013**, *50*, 1172–1178. [CrossRef] [PubMed]
51. Rodriguez-Jerez, J.J.; Lopez-Sabater, E.I.; Roig-Sagues, A.X.; Mora-Ventura, M.T. Evolution of histidine decarboxylase bacterial groups during the ripening of Spanish semi-preserved anchovies. *J. Vet. Med. Ser. B.* **1993**, *40*, 533–543. [CrossRef]
52. ICMSF (International Commission for the Microbiological Specifications for Foods). *Microorganisms in Foods 6, Microbial Ecology of Food Commodities*, 2nd ed.; Kluwer Academic/Plenum Publishers: New York, NY, USA, 2005; pp. 174–249.
53. Mohamed, S.B.; Mendes, R.; Slama, R.B.; Oliveira, P.; Silva, H.A.; Bakhrouf, A. Changes in bacterial counts and biogenic amines during the ripening of salted anchovy (*Engraulis encrasicholus*). *J. Food Nutr. Res.* **2016**, *4*, 318–326. [CrossRef]
54. Aponte, M.; Blaiotta, G.; Francesca, N.; Moschetti, G. Could halophilic archaea improve the traditional salted anchovies (*Engraulis encrasicholus*) safety and quality? *Lett. Appl. Microbiol.* **2010**, *51*, 697–703. [CrossRef]
55. Alfonzo, A.; Randazzo, W.; Barbera, M.; Sannino, C.; Corona, O.; Settanni, L.; Moschetti, G.; Santulli, A.; Francesca, N. Effect of salt concentration and extremely halophilic archaea on the safety and quality characteristics of traditional salted anchovies. *J. Aquat. Food Prod. Technol.* **2017**, *26*, 620–637. [CrossRef]

56. Yeannes, M.I.; Ameztoy, I.M.; Ramirez, E.E.; Felix, M.M. Culture alternative medium for the growth of extreme halophilic bacteria in fish products. *Ciênc. Tecnol. Aliment., Campinas.* **2011**, *31*, 561–566. [CrossRef]
57. Rodtong, S.; Nawong, S.; Yongsawatdigul, J. Histamine accumulation and histamine-forming bacteria in Indian anchovy (*Stolephorus indicus*). *Food Microbiol.* **2005**, *22*, 475–482. [CrossRef]
58. El Filali, F.; Hanoune, S.; Moukrim, A.; Kaaya, A.; Bou'mhandi, N. Histamine and microbiological changes during storage of semi-preserved anchovies. *Afr. J. Microbiol. Res.* **2016**, *10*, 1455–1459.
59. Pons-Sánchez-Cascado, S.; Veciana-Nogues, M.T.; Bover-Cid, S.; Marine-Font, A.; Vidal-Carou, M.C. Volatile and biogenic amines, microbiological counts, and bacterial amino acid decarboxylase activity throughout the salt-ripening process of anchovies (*Engraulis encrasicholus*). *J. Food Prot.* **2005**, *68*, 1683–1689. [CrossRef] [PubMed]
60. Rodriguez-Jerez, J.J.; Lopez-Sabater, E.I.; Hernandez-Herrero, M.M.; Mora-Ventura, M.T. Histamine, putrescine and cadaverine formation in Spanish salted semipreserved anchovies as affected by time/temperature. *J. Food Sci.* **1994**, *59*, 993–997. [CrossRef]
61. Tapingkae, W.; Tanasupawat, S.; Parkin, K.L.; Benjakul, S.; Visessanguan, W. Degradation of histamine by extremely halophilic archaea isolated from high salt-fermented fishery products. *Enzyme Microb. Technol.* **2010**, *46*, 92–99. [CrossRef]
62. Veciana-Nogués, M.T.; Mariné-Font, A.; Vidal-Carou, M.C. Changes in biogenic amines during the storage of Mediterranean anchovies immersed in oil. *J. Agric.Food Chem.* **1997**, *45*, 1385–1389. [CrossRef]
63. Veciana-Nogués, M.T.; Albala-Hurtado, S.; Mariné-Font, A.; Vidal-Carou, M.C. Changes in biogenic amines during the manufacture and storage of semipreserved anchovies. *J. Food Prot.* **1996**, *9*, 1218–1222. [CrossRef]
64. Veciana-Nogués, M.T.; Vidal-Carou, M.C.; Mariné-Font, A. Histamine and tyramine during storage and spoilage of anchovie, *Engraulis encrasicholus*: Relationships with other fish spoilage indicators. *J. Food Sci.* **2006**, *55*, 1192–1193. [CrossRef]
65. European Commission. RASFF Portal. Available online: https://webgate.ec.europa.eu/rasff-window/portal/?event=searchResultList&StartRow=1 (accessed on 7 May 2023).
66. Kočar, D.; Köse, S.; Koral, S.; Tufan, B.; Ščavničar, A.; Pompe, M. Analysis of biogenic amines using immunoassays, HPLC, and a newly developed IC-MS/MS technique in fish products-A comparative study. *Molecules* **2021**, *26*, 6156. [CrossRef]
67. Dehaut, A.; Himber, C.; Mulak, V.; Grard, T.; Krzewinski, F.; Le Fur, B.; Duflos, G. Evolution of volatile compounds and biogenic amines throughout the shelf-life of marinated and salted anchovies (*Engraulis encrasicolus*). *J. Agric. Food Chem.* **2014**, *62*, 8014–8022. [CrossRef]