fishes

MDPI

Article

Comparative Evaluations to Enhance Chemical and Microbial Quality of Salted Grey Mullet Fish

Ayman M. Khalifah [1], Ahmed N. Badr [2,*], Manal F. El-Khadragy [3], Mohammed G. Shehata [4,5], Sara A. Abdalla [6], Hany M. Yehia [7,8] and Hatem S. Ali [9]

1 Livestock Research Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications (SRTA-City), New Borg El-Arab 21934, Egypt; akhalifah@srtacity.sci.eg
2 Food Toxicology and Contaminants Department, National Research Centre, Cairo 12622, Egypt
3 Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia; mfelkhadragy@pnu.edu.sa
4 Department of Food Technology, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications (SRTA-City), New Borg El-Arab 21934, Egypt; gamalsng@gmail.com
5 Food Research Section, R&D Division, Abu Dhabi Agriculture and Food Safety Authority (ADAFSA), Abu Dhabi 20602, United Arab Emirates
6 Plant Protection and Biomolecular Diagnosis Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications (SRTA-City), New Borg El-Arab 21934, Egypt; abdalla.sara@yahoo.com
7 Food Science and Nutrition Department, College of Food and Agriculture Science, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia; hanyehia@ksu.edu.sa
8 Food Science and Nutrition Department, Faculty of Home Economics, Helwan University, Helwan P.O. Box 11611, Egypt
9 Food Technology Department, National Research Center, Cairo 12622, Egypt; hatem.owyean1@gmail.com
* Correspondence: noohbadr@gmail.com; Tel.: +20-(100)-032-7640

check for updates

Citation: Khalifah, A.M.; Badr, A.N.; El-Khadragy, M.F.; Shehata, M.G.; Abdalla, S.A.; Yehia, H.M.; Ali, H.S. Comparative Evaluations to Enhance Chemical and Microbial Quality of Salted Grey Mullet Fish. *Fishes* **2022**, *7*, 175. https://doi.org/10.3390/fishes7040175

Academic Editors: Shan He and Songlin Li

Received: 17 June 2022
Accepted: 15 July 2022
Published: 19 July 2022

Abstract: Salted fish preparations are popular and widely eaten across the world. Salting is a preservation strategy to enhance shelf life by decreasing water activity in fish muscles. Salted fish demand increases with less regard for preservation characteristics, and novel strategies are required for healthy production. Lysozyme, EDTA, their mixture, and black cumin oil nanoemulsion (BCN) were used for dipping treatments to make preservative films before salting. A medium-sized mullet were classified into six groups: fresh, commercial salted mullet, and four treatments. Stored salted mullet groups were evaluated for chemical composition changes, lipid profile changes, nitrogen profile changes, and microbial contamination. The results were reflected by nonsignificant changes in protein content (21.08 ± 1.05%) of the BCN compared to the fresh (22.41 ± 1.41%), with the lowest salt penetration to the flesh (11.11 ± 0.74%). The lowest value recorded was the BCN for the pH (5.53 ± 0.06) and water activity (0.447 ± 0.019). The changes in lipid profile compared to the fresh were very low, which does not happen in a commercial salted sample. The changes in total volatile and non-protein nitrogen represented very low for the BCN compared to the commercial salted one. *Coliform*, *E. coli*, *Staphylococcus aureus*, *Salmonella*, and *Clostridium* were not detected in the BCN salted mullet. The overall acceptability for the BCN mullet was the best in the test panel list evaluation, followed by the mixture treatment. This research indicated that the BCN treatment before mullet fish salting was optimal, with greater food safety properties and no consumer acceptance alterations.

Keywords: salted mullet; black cumin nanoemulsion; food safety; preservative film; microbial contamination; sensory evaluation; mixture solution; shelf-life extension

1. Introduction

One of the most efficient preservation strategies for preventing rotting and extending the shelf life of seafood is the salting process [1]. Salting techniques can be applied as light salting, with salt content between 10% to 15% of fresh weight, or heavy salting,

between 20% to 25% of fresh weight [2]. The salt gives items an unattractive surface color and an unpleasant flavor with a foul fat smell owing to fat oxidation caused by oxygen interaction [3]. Many researchers believe that during maturation, chemical changes in salted fish begin with protein degradation by proteolytic enzymes and lipid hydrolysis by lipolytic enzymes [3,4]. The maturing phase of salty fish products also depends on microbial activity and the creation of new chemicals that provide an excellent taste [5]. Many different bacteria species are capable of attacking and spoiling salted fish [6,7]. Most of those deteriorative bacteria cause slime, putty fish, and pink and brownish pigmentation on the surface of salted mullet fish.

Microorganisms vary in their tolerance to salt; most normal organisms have a relatively higher salt tolerance and are often inhibited by 5 to 10%. Lactic acid processing bacteria can absorb up to 15% salt, while spore-forming and halotolerant bacteria can tolerate up to 16% salt, showing some growth. These organisms include the micrococci staphylococci. The preservative and antibacterial agents that prevent fish spoilage may include spices and sodium benzoate that can be added to fish during the salting process. The effectiveness of EDTA as a chelating agent and lysozyme as an antibacterial were investigated for seafood such as shrimp [8]. Again, the study by Levin [9] pointed out EDTA as a fish preservative with a concentration of 0.005% of EDTA sodium salt. Although EDTA only slightly affected the bacterial count of fresh fish of Baltic herring, it positively impacted maintaining quality control. It was found that the trimethylamine, hypoxanthine, and volatile essential nitrogen were decreased at a slower rate in EDTA-treated fish than in the untreated controls [9]. Several types of non-traditional oils were recorded previously to have antibacterial and antifungal impacts [10], including jojoba and jatropha [11], bottle gourd oil [12], and the oleoresin oil of pomegranate [13].

Black cumin oil (BCO) is considered a non-traditional oil with high activity as a source of bioactive components [14]. The bioactive components present in this oil type can affect the microbial metabolic pathways and result in an anti-aflatoxigenic impact [14]. Again, the oil was recorded by effective impact as antimicrobial and antifungal [15]. Black cumin essential oil nanoemulsions formed by mixing demonstrated resilience against phase separation and coalescence during room temperature storage [16]. Antimicrobial tests on select pathogen strains revealed that the BCO emulsion was effective against them [14]. Stabilized BCO, on the other hand, demonstrated greater bactericidal efficacy against Gram-positive pathogenic bacteria. The BCO exhibits improved stability, regulated release, and self-assembly with the cell membrane, followed by cellular component elimination [16]. Moreover, Sharif et al. [16] recommended the black cumin oil nanoemulsion (BCN) to create innovative delivery methods with a longer shelf life in dairy and drinks.

Purified lysozyme is presently used in the food industry because of its bacterial action on all Gram-positive bacteria. A mixture of lysozyme and EDTA reduced microbial development and antibacterial activity against microorganisms that cause food spoiling, food poisoning, and foodborne disease, proving that the combination of the chelating agent EDTA and lysozyme could be helpful for the prevention of Clostridium botulinum and Listeriamonocytogenes growth foods.

Several strategies have been applied to increase the shelf life of salted fish, mainly mullet fish. However, the safety characteristics of salting fish still need more studies to perform the best production conditions. This investigation targeted innovative applications of the treatment utilized before the salting process, keeping the sensory evaluation at the customer's demand and increasing the final product's shelf life, safety, and health properties. The main focus of the study was to evaluate the effect of adding different precoated materials such as dipping in lysozyme, EDTA, their combination mixture, and the BCN on the quality characteristics of salted mullet fish.

2. Materials and Methods

2.1. Materials

Fresh mullet (Mugilcephalus) was purchased in one batch (20 kg; 75 fish) from Maryout fish farm. The fish characteristics were 250–320 g in weight, 3.2–4.0 cm in thickness, and 20–24 cm in length. Black cumin oil (BCO) was obtained by cold press extraction of Ethiopian seeds and stored refrigerated till the application. The applied salt (Grade A) was purchased from El-Nasr Saline Company, Alexandria, where the salt purity was 99.6%, with traces of calcium and magnesium (0.02% and 0.04%, respectively).

2.2. Methods

2.2.1. Preparation of Treatment Solutions

Four solutions were applied to enhance the safety and quality of salted mullet. These solutions are EDTA (1 nM), lysozyme (100 ppm), and black cumin nanoemulsion (BCN). The mix treatment was applied using EDTA (1 nM) to lysozyme (100 ppm) by a 1:1 mixing ratio. Nanoemulsion of the BCN was prepared according to the methodology described before [16], with few modifications. Briefly, the emulsion was made by dissolving 3% (w/v) carboxymethylcellulose (CMC-food grade) in double-distilled water and mechanically stirring (6 h; 40 °C). The phase of black cumin oil (BCO) was adjusted towards the CMC powder at a 4:6 (w/w) ratio. The homogenization process was used for the preparation of coarse emulsion. The BCO was added to the prepared CMC solution (3% v/v) during a high-speed homogenizer (Ultra-Turrax T25, IKA Janke & Kunkle, GmbH Co., Staufen im Breisgau, Germany). This step was done at 18,000 rpm for 5 min, followed by a microfluidizer treatment (100 MPa; 5 cycles) (Microfluidizer apparatus Model 101, Microfluidics, Newton, MA, USA).

2.2.2. The Experimental Trials

A commercial salted mullet (feseekh) was purchased from the Alexandria market. The scaled and gutted mullet was divided into six groups to evaluate the impact of the applied treatments. These groups were: the control group (G1), EDTA solution treatment (G2), Lysozyme solution treatment (G3), mix solution treatment (G4), the BCN (G5), and commercial sample (G6).

2.2.3. Determination of Nanoemulsion Characterization

Nanoemulsion was evaluated for the characterization as particle size (PSz), zeta potential (ZP), poly dispersing index (PDI), entrapment efficiency (EE), and loading efficiency (LE) as the same methodology described by Malik et al. [17]. The Malvern apparatus (Nano-S90, Zetasizer, MalvernPanalytical Ltd., Enigma Business Park, Grovewood Road, United Kingdom) was utilized to estimate the PSz, ZP, and PDI values.

However, the accurate concentration of encapsulated BCO in microspheres was measured using a UV-Vis spectrophotometer (Shimadzu/UV-1700, Kyoto, Japan) with the same procedures described before [18]. The oil emulsion was measured to calculate the concentration against a blank emulsion sample without oil existence. Subjection to the absorbance value obtained from the standard curve determined the actual concentration of oil. The % EE and the % LE of oil in particles were calculated utilizing the following equations:

$$\% \, EE = (Mc \div Tc) * 100$$

$$\% \, LE = \frac{BCO \; content}{Total \; BCO \; weight} * 100$$

where *EE*: entrapment efficiency for oil.

Mc: the oil concentration that calculates by measuring.

Tc: the total oil concentration added initially

and % *LE*: loading efficiency for the oil applied.

BCO: black cumin oil.

2.2.4. Salting of Grey Mullet (Mugilcephalus)

The fresh mullet is salted after removing the gills and gut (20% dry salt). Mullet salting was performed in glass jars (30 × 20 × 20 cm) with a glass cover fixed with silicon during the salting period. A layer of salt was poured on the bottom of the salting tank (glass jar), and the prepared fresh fish (control and treatments) were rolled in the salt layer by layer while salt was entered into the emptiness of the gill and gut. The salting process was done at room temperature (20 ± 2 °C) for six weeks until the salted fish matured.

2.2.5. Determination of the Chemical Composition

The fish samples were chemically analyzed to evaluate their proximate analysis before and after the salting process following the AOAC [19]. Moisture content was investigated by drying the samples at 105 °C until constant weight (AOAC method 950.46B). The lipid content was determined following Bligh and Dyers' procedure [20]. The protein content was determined using the Kjeldahl methodology (AOAC method 955.04) [21]. The Electric Hygrometer measured the Aw at 25 °C (Hygrodynamics, Inc., Jessup, MD, USA). The ash content was determined by burning a sample in a muffle furnace at 550 °C (AOAC method 920.153) [19]. Five times (w/v), deionized water was added to the sample to evaluate salinity. The filtrate collected after stirring and centrifugation was measured using a salinity meter (PAL-ES, ATAGO, Tokyo, Japan).

2.2.6. Determination of Lipid Oxidation Changes

The acid value (AV), peroxide value (PV), and thiobarbituric acid (TBA) of samples were estimated to evaluate the potential lipid oxidation. Pearsons' approach [22] was followed to determine the AV amounts. In brief, extracted oil (1 g) was dissolved in equal portions of diethyl ether and ethanol, and 1% phenolphthalein solution was added as an indicator and titrated versus 0.1 mol/L sodium hydroxide, where the AV was calculated [23].

Regarding the PV values estimation, the samples were crushed to dust, and 0.5 g was blended with a 25 mL solution of acetic acid: chloroform (3:2, v/v), and 1 mL of saturated potassium iodide was then added after dark storage (10 min), followed by distilled water addition (30 mL) and starch solution (1 mL; 1% w/v). After the sample titrations until the colorless appearance, sodium thiosulfate (0.01 N) solution was used. The PVs were calculated as milliequivalents peroxide oxygen per kilogram of a sample (m eq/kg).

The methodology of Faustman et al. [24] was applied with a modification to measure TBA levels. Samples were homogenized (15 s) in distilled water (50 mL), followed by a trichloroacetic acid (10 mL; 15%) before being centrifuged (12,440× g force/30 min). The supernatant was filtered utilizing Whatman filter paper (No. 1). To eight milliliters of the collected solution, 2 mL of thiobarbituric acid (0.06 mol/L) was added. The mixture was vortexed (15 s) second before being heated (95 °C/1 h) before cooling on ice. The absorbance was measured at 532 nm using the UV-Vis spectrophotometer. The values were stated as mg malondialdehyde (MDA) equivalent/kg of a sample (mg MDA eq/kg).

2.2.7. Determination of the Nitrogen Profile Changes

According to Gharibzahedi and Mohammadnabi's [25] microtitration methodology, total volatile nitrogen (TVN) values were determined for the fresh, commercial, and treatments. In brief, a high-speed homogenizer (IKA-T25) was utilized to homogenize 5 g of sample in distilled water (50 mL). The mixture was centrifuged (12,000× g force/4 °C/5 min). A quantity of 5 mL supernatant was mixed with 5 mL of the MgO (10 g/L) and distilled using a Kjeldahl nitrogen apparatus (KN-520, Alva instrument). As a mixed indication, 20 mL of boric acid (0.02 g/L) containing methyl red (1 g/L) and methylene blue (1 g/L) in ethanol was used to generate the distillate. The combined solution was titrated with 0.01 mol/L HCl solution, and 5 mL of distilled water was used as a blank test instead of

the sample. The TVB-N value was determined and represented as mg nitrogen per 100 g sample (mg N/100 g) using the following equation based on HCl consumption:

$$TVN\ (\text{mg}/100\ \text{g}) = \frac{14 * C * (V1 - V2)}{0.1 * Sw}$$

where *C*: the HCl concentration (mol/L). *V*1: volume of the HCl (mL) consumed by the sample. *V*2: volume of the HCl (mL) consumed by the blank. *Sw*: sample weight (g).

Non-protein nitrogen (NPN) was determined using the percent TCA method described by Jacobs [26], and the results were expressed in mg nitrogen per gram of fish sample (mg N/g).

2.2.8. Microbial Analysis of Tested Samples

Microbial analysis was performed on the fish samples before and after salting. The microbial growth was measured by aseptically extracting 25 g from each fish flesh and immersing this in a sterile flask with 250 mL of sterile % peptone diluent. The flask was shacked (150 rpm/25 °C/5 min), and the aerobic plate counts existence was investigated using the standard method agar (SMA), in which pour plates were cultured (4 days/22 °C) [27]. The specific media utilized for assaying each organism separately (*Coliform*, *E coli*, *Staph. aureus*, *Clostridium*, and *Salmonella*) was similar to the aerobic count assay. Using MacConky broth, *coliform* and *E coli* counts were determined. The media were incubated on agar for 24 and 48 h at 37 and 44 °C., respectively. The *Staph. aureus* bacteria were identified using Baird-Parker agar streak plates incubated (37 °C/24 h). For the *Clostridium* sp., the media of reinforced clostridial medium (CM0149, Oxoid limited, Thermo Fisher, London, UK) was performed in the investigation.

Pathogen strains have been plated directly into the media indicated. In addition, a duplicate sample was placed into enrichment media for *Staph aureus* and salmonella (AOAC, 1990) according to the description by Reynolds et al. [28]. Enumeration was achieved by the streak plate and the most probable number methods [29]. On the other hand, isolates had been confirmed biochemically according to the procedures described by Collee et al. [30], using biochemical kits (HiMedia Laboratories Pvt., Ltd., Swastik Disha Business Park, Mumbai, India).

2.2.9. Estimation of Sensory Evaluations

The chromaticity was measured using a Spectro Colorimeter (Tristimulus Color Machine) and the CIF lab color scale; color parameters (L*, a*, and b*) of the control and treated mullet were determined (Hunter, Lab Scan XE, Germany) for a muscular sample part, and the L* value (lightness: L* = 0 for black, L* = 100 for white), a* value (red/green: +a* = redness, −a* = greenness), and b* value (yellow/blue: +b* = yellowness, −b* = blueness) were recorded. Each group of samples was measured five times, and the mean values were obtained.

A test panel evaluation was performed according to the previous methodology [21]. The sensory assessment of snack bars was done following a reference assay (UNI, 10957: 2003) by the same methodology and condition described before [21] using 40 panelists ranging in age from 20 to 40 years old, including 20 males and 20 women. The panel discussion was held at the Food Industries and Nutrition Institute of the National Research Centre (NRC). The mullet groups were presented in random order. The panel members were asked to record their decisions about the sensory attributes of the samples (appearance, color, odor, taste, texture, and overall acceptability). The panelist evaluation was done using a 9-point hedonic scale (1 = non-preferable product, 5 = non-change product, 10 = favorite product).

2.2.10. Statistical Analysis

All tests were carried out in triplicate, and the results were given as means of standard deviation. The data were analyzed using the statistical software for the social sciences (SPSS

V.16, IBM company, Chicago, IL, USA). Duncans' multiple range test (p = 0.05) was used to determine if there was a significant difference between the mean values using analysis of variance (ANOVA).

3. Results

3.1. Characterization of Nanoemulsion

The prepared nanoemulsion, formed by the BCO, was evaluated for PSz, ZP, and PDI values. The Malvern apparatus recorded the values of oil particles within emulsion as 250 ± 5.7 nm by the Malvern apparatus, while these particles ranged between 221–274 nm using the transmitted electron microscope. Moreover, the ZP value was recorded as (−24.0 ± 5.77 mV). The PDI was reported at 0.282, while the % EE and % LE were recorded at 88.41 ± 0.56% and 6.18 ± 0.21%, respectively. These results indicated perfect properties of the prepared emulsion, which refer to an expected outcome with more safety properties.

3.2. Chemical Composition of Mullet

The chemical composition analyses indicated significant changes in moisture, protein, lipid, pH, and aW values in the commercial salted mullet (Table 1). The salt absorbing quantities are normal and expected to decrease the humidity after the salting procedure. The decrease appeared with the lowest percentage in the case of BCN film coating, but despite the decline being varied for the rest treatments, their values were not far from each other. The results indicated a significant deterioration of the total protein values of commercially salted fish, but this deterioration was recorded with the lowest changes for the remnant treatments.

Table 1. Chemical composition of investigated mullet samples.

	Chemical Composition and Mullet Characteristics						
	Moisture (%)	**Protein (%)**	**Lipid (%)**	**Flesh-Salt (%)**	**pH**	**Water Activity (aW)**	**Ash (%)**
Raw	72.27 ± 0.87 ^{a}	22.41 ± 1.41 ^{a}	6.81 ± 0.37 ^{a}	-	6.24 ± 0.01 ^{a}	0.779 ± 0.021 ^{a}	24.51 ± 0.87 ^{a}
Salted (C)	52.81 ± 1.74 ^{d}	14.47 ± 1.27 ^{c}	5.14 ± 0.31 ^{d}	14.43 ± 0.45 ^{a}	6.15 ± 0.05 ^{b}	0.508 ± 0.015 ^{b}	83.22 ±3.21 ^{a}
EDTA	53.24 ± 0.84 ^{c}	20.27 ± 0.94 ^{b}	6.18 ± 0.22 ^{b}	14.27 ± 0.71 ^{a}	5.64 ± 0.02 ^{c}	0.455 ± 0.018 ^{c}	44.53 ± 1.74 ^{c}
Lysozyme	52.42 ± 0.43 ^{d}	20.25 ± 0.81 ^{b}	5.87 ± 0.19 ^{c}	12.31 ± 0.43 ^{b}	5.47 ± 0.09 ^{d}	0.461 ± 0.017 ^{c}	50.42 ± 1.18 ^{d}
Mix	53.21 ± 0.88 ^{c}	20.45 ± 0.84 ^{b}	5.81 ± 0.11 ^{c}	12.05 ± 0.66 ^{b}	5.53 ± 0.06 ^{d}	0.447 ± 0.019 ^{c}	40.89 ± 1.22 ^{b}
BCN	54.94 ± 0.47 ^{b}	21.08 ± 1.05 ^{a}	5.98 ± 0.11 ^{b}	11.11 ± 0.74 ^{c}	5.31 ± 0.02 ^{e}	0.424 ± 0.008 ^{d}	40.55 ± 1.29 ^{b}

-The data were expressed as means ± SD (where n = 3). -Values with different superscript letters are significantly different. -The values of each parameter were calculated as ratio percentages.

Salt concentration in the flesh of fish treatments was recorded highly for the commercial treatment; however, it was the lowest for the BN treatment. It is a significant point that the BCN has the lowest water activity value, which links to microbial contamination activities. The raised values in ash content of the treatments were known to join with the diffusion of salt content to the flesh, where this salt contained several minerals.

3.3. Lipid Oxidation Changes in Mullet Treatments

The changes recorded for the lipid profile are indicated by the values of PV, AV, TBA, and free fatty acids (FFA) (Table 2). The AV and PV values indicate the oxidation degree that happened to the lipid content of the product. The FFA values were estimated as oleic acid, which reflected the degree of decomposition in fat content due to maturation conditions.

Table 2. The changes in lipid profile of the treated salted mullet samples compared to the commercial sample.

	AV (mg/g Oil Extracted)	PV (meq/Kg)	TBA (mg MDA eq/Kg)	FFA (as % Oleic)
Raw	0.24 ± 0.005^{a}	8.98 ± 1.02^{a}	0.317 ± 0.074^{a}	2.75 ± 0.49^{a}
Salted (C)	2.79 ± 0.214^{f}	35.71 ± 2.43^{f}	2.191 ± 0.227^{d}	7.95 ± 0.54^{e}
EDTA	1.84 ± 0.184^{e}	24.54 ± 1.47^{e}	1.081 ± 0.218^{b}	5.57 ± 0.41^{c}
Lysozyme	1.37 ± 0.055^{d}	20.25 ± 0.81^{c}	1.487 ± 0.192^{c}	6.71 ± 0.38^{d}
Mix	1.09 ± 0.021^{c}	21.45 ± 0.84^{d}	1.211 ± 0.141^{c}	5.64 ± 0.45^{c}
BCN	0.97 ± 0.008^{b}	14.28 ± 1.31^{b}	0.684 ± 0.078^{b}	3.78 ± 0.63^{b}

-The data were expressed as means ± SD (where $n = 3$). -Values with different superscript letters are significantly different. -The values of each parameter were calculated as ratio percentages. -AV: acid value, PV: peroxide value; TBA: thiobarbituric acid; FFA: free fatty acid.

The BCN mullet was the most acceptable product of salted maturate mullet, followed by the mix-coated mullet. The FFA value was very high for the commercial salted mullet; no significant differences were recorded between the BCN, EDTA, and the mix solution (EDTA-lysozyme) treatments. However, the mullet treated with lysozyme or EDTA solution is better than the commercial mullet.

3.4. Total Volatile Nitrogen (TVN) and Non-Protein Nitrogen (NPN)

The commercial sample of salted mullet was recorded with the highest TVN and NPN values. The TVN changes were nonsignificant for the EDTA and mixed coating treatments (Figure 1A). At the same time, it was recorded as nonsignificant for the NPN values in EDTA, lysozyme, and their hybrid solution of coating (Figure 1B). The BCN-coated samples were recorded as much as the fresh mullet with the highest quality. Generally, the TVN and NPN values are linked to the decomposition or deterioration of the fish's flesh. This decomposition may increase the chance of spoilage microorganism growth and cause several harmful consumer impacts.

3.5. Microbial Analysis of Tested Mullet (Raw and Treated)

The microbial flora of commercial and treated samples of the salted mullet fish after the maturation at room temperature (20 ± 2 °C) are shown in Table 3. It was found that the bacterial content of total count, *Coliform*, *E. coli*, and *Staph aureus* represented a moderate level of contamination for the raw mullet. The microbial load was recorded at 2.6×10^5 CFU/g for the total bacterial count, while *Salmonella* sp., and *Clostridium* sp., were not detected in the raw mullet.

The microbial load was changed after storage time for the matured samples, both coated and commercial. For the treated samples, it is clear that lysozyme, EDTA, and combined lysozyme-EDTA solution effectively reduced the number of mesophiles and *Staph aureus*. The combination of EDTA (1 mM) and lysozyme (100 mg/L) had an effective impact on the mesophile bacteria *Staph aureus* more than the impact of the individual application of EDTA or lysozyme. At the same time, the effects of BCN-coating before the salting process reflect the most effective treatment against *Staph aureus*. The commercial sample was recorded as the most contaminated mullet with the existence of pathogenic *Salmonella* sp., (0.02×10^2 CFU/g).

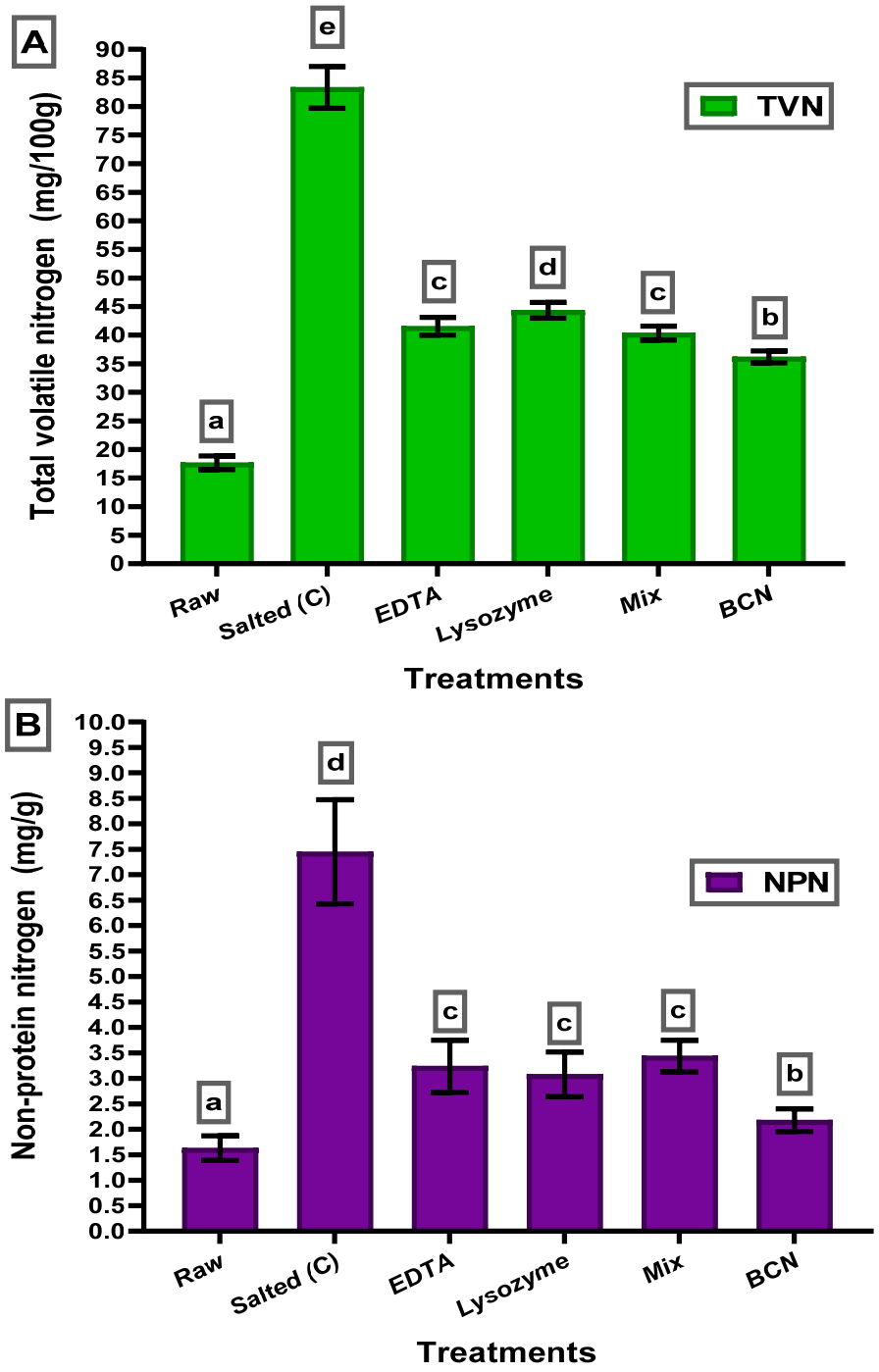

Figure 1. The Protein content changes evaluation results of the salted mullet are enhanced by coating before the salting process. The data are represented as means ± SD values; different letters on a column mean significant differences for each graph. (**A**) represents the total volatile nitrogen value changes according to the treatments. (**B**) represents the non-protein nitrogen value changes according to the treatments. Lysozyme was applied at 100 ppm concentration; the EDTA was used at 1 nM concentration. Mix solution was prepared from lysozyme (100 ppm) and EDTA solution (1 nM).

Table 3. Microbial flora determined for investigated mullet samples after maturation.

	Bacterial Contamination					
	***TPC*/g**	***Coliform Group***	***E. coli***	***Staph. aureus***	***Salmonella* sp.**	***Clostridium* sp.**
Raw	2.6×10^5	2.3×10^3	4.1×10^2	2.3×10^3	ND	ND
Salted (C)	6.13×10^5	0.31×10^3	0.05×10^2	4.54×10^4	0.02×10^2	ND
EDTA	1.24×10^6	0.05×10^2	0.01×10^2	1.37×10^4	ND	ND
Lysozyme	0.61×10^6	ND	ND	1.21×10^4	ND	ND
Mix	0.54×10^6	ND	ND	1.46×10^3	ND	ND
BCN	0.27×10^4	ND	ND	ND	ND	ND

Contamination levels for the samples are expressed in the CFU/g sample.

3.6. Sensory Evaluations of Treated Salted Mullet

Table 4 shows the values of the color attributes for the current investigation of salted mullet compared to raw-fresh mullet. Before the salting process, the (L*) value changes were recorded as not far from the fresh for EDTA, lysozyme, and mix-solution coating. While the (L*) was so close for the fresh mullet and BCN treatment, the changes in values of (L*), (a*), and (b*) for the commercial salting sample were recorded as the highest recorded values, with the highest (ΔE) value. The mixed treatment is a significant point as the second choice after the BCN treatment.

Table 4. The changes in color attributes of commercial and treated coating mullet compared to raw mullet.

	L*	**a***	**b***	**ΔE $_{Value}$**
Raw	30.14 ± 0.02	0.27 ± 0.03	7.84 ± 0.05	-
Salted (C)	57.36 ± 0.03	-1.24 ± 0.08	-16.39 ± 0.07	28.54 ± 0.03
EDTA	40.34 ± 0.06	0.56 ± 0.04	10.21 ± 0.05	10.47 ± 0.01
Lysozyme	36.56 ± 0.07	0.84 ± 0.05	12.66 ± 0.08	8.05 ± 0.03
Mix	34.08 ± 0.05	0.69 ± 0.01	10.44 ± 0.03	3.33 ± 0.01
BCN	32.25 ± 0.02	0.38 ± 0.03	8.27 ± 0.02	2.15 ± 0.01

The data were expressed as means ± SD (where $n = 3$); L* scale represents the lightness; a* scale represents redness; b* scale represents the yellowness. The ΔE $_{values}$ were calculated as the square root of the sum of differences between L*, a*, and b* for each treatment and raw mullet.

The commercial salting or coated-before procedures resulted in significant changes ($p = 0.05$) in the groups' (L*), (a*), and (b*) values. The (L*) values reflect blackness and whiteness, the (a*) integrity redness and greenness, and the b* values represent the degree of yellowness and blueness. The colorimeter's (a*) value was red when the (+) value was higher and green when the (−) value was higher. When the (+) value was displayed, the (b*) value was yellow, and it was blue when the value changed to a negative value. Generally, color measurement is a significant parameter in processed fish products because consumers associate a feature of fish and their products with color. The moderate redness (a*) values for coated samples before salting ranged from 0.38 ± 0.03 to 0.84 ± 0.05. It was consistent with the raw mullet value (0.27 ± 0.03) but far from the commercial value (-1.24 ± 0.08); also, this occurred for the yellowness (b*).

For the panelist evaluation of salted mullet treatments (Figure 2), the first choice was salted-BCN mullet, followed by salted-mix coating. The contrast between the coating uses of the mix-solution and lysozyme coating was not far. The commercial salted mullet was recorded as the bad choice for the panelist compared to the coated treatment before salting.

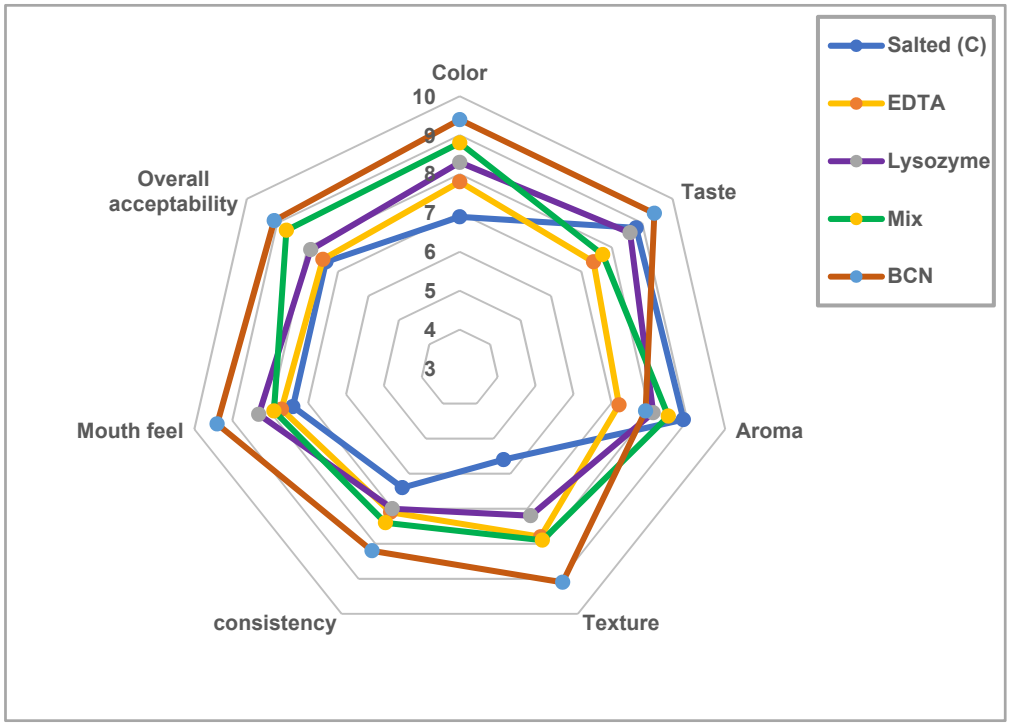

Figure 2. The sensory evaluation results of the salted mullet are enhanced by coating before the salting process. The sensory evaluation data for the salted mullet are represented as means of panelist degrees. Lysozyme was applied at 100 ppm concentration; the EDTA was used at 1 nM concentration. Mix solution was prepared from lysozyme (100 ppm) and EDTA solution (1 nM).

4. Discussion

Regarding the characteristics of nanoemulsion prepared using the BCN, the values for particle size, zeta potential, and the PDI reflect high stability for the emulsion used for mullet coating before the salting process. However, the PDI was recorded at a value of 0.282, indicating the emulsion stability as it was less than 0.5 in value [31]. The measurement for the emulsion stability (% EE) and the loading efficiency (% LE) was also recorded by valuable content, reaching more than 88%, indicating the encapsulation of most oil amounts into the CMC coating material [32]. This ratio also reflects the availability of oil released during the maturation period [33]. Controlled release and availability of the BCN during the maturation time function against microbial contamination [34].

Changes in the lipid profile of salted mullet fish reflect the deterioration and spoilage degree of the product [35]. The lipid profile parameters for the mullet were determined as the AV, PV, TBA, and FFA values. The value recorded for the AV reflects the highest value of the hydrolytic rancidity of oil was 2.79 $\pm$ 0.214 mg KOH/g oil for commercial salted mullet. In contrast, the AV value was the lowest for the BCN-treated samples (0.97 $\pm$ 0.008 mg KOH/g oil) as a precoated mullet before the fish was salted. Consequently, the FFA percentage had the same manner (7.95% for the commercial sample, and 3.78% for the BCN sample, respectively).

The salting process reduced the moisture content, pH, and crude protein of grey mullet fish [36,37]. These studies observed that the NPN increased by up to 8 mg/gm after the salting period, whereas the TVN increased by up to 90 mg/100 g of flesh. The influence of prepared mullet salted fish with lysozyme, EDTA, and a combination of lysozyme and EDTA on moisture, pH salt, total lipid, and crude protein levels of salted fish is restricted compared to commercial salted mullet. Adding lysozyme and EDTA to salted fish influenced lipid hydrolysis and protein degradation, but the FFA was somewhat lower in treated salted fish than in commercial samples. The NPN in treated salted fish was lower than in commercial samples. The TVN was reduced from 58.3 mg/100 g in untreated

samples to 42.7 mg/100 g when 100 mg/L lysozymes and 1 mM EDTA were used. The TVN level rises according to the autolytic amino acid deamination and microbiological contamination occurrence [38]. This theory is explained by protein denaturation, which could occur at greater brine concentrations just when salt penetrates the flesh [2]. Protein denaturation always happens in salted fish; however, it usually occurs more slowly than salt penetration [39]. The salt ions serve as counterions to negatively and positively charged groups, disrupting the proteins' natural structure. The sarcolemma in intact muscle prevents salt from penetrating the flesh and causing swelling of the myofibrillar matrix [5]. Salt denaturation and pH changes reduce the extractability of fish muscle proteins [39]. In the present investigation, the lower changes in the TVN levels could be joined to the impact of coated dipping layer formed by the treatments. These layers (EDTA, lysozyme, mixture, and BCN) serve a double agent impact, decrease the salt penetration rate, and preserve against microbial contamination.

Several metabolic interactions destroy seafood components regarding microbial contamination, reducing shelf life and losing quality [40]. When the total bacterial count exceeds 5 to 6 log CFU/g, it is termed early spoiling, and values of 7 log CFU/g make the food unsafe for ingestion [41]. Raw fish and its products with a total microbiological count of more than 107 are likewise regarded as undesirable by the international commission for microbiological standards of food (ICMSF) [42]. Microbial contamination of foods may develop due to improper handling during distribution, processing, and storage [43]. These microbial families have previously been identified in various processed fish products and linked to raw materials or contamination during processing [44,45]. When Ekici and Alisarli [46] investigated the microbiological changes in pearl mullets held at 4 °C for 15 days, they discovered that the number of total viable counts on the first day was 8 × 102 CFU/g, rising to 9 × 10^9 CFU/g on the 15th day. Previous research found significant levels of aerobic plate count and total coliforms in sun-dried milkfish with minimal salt content [44].

On the other hand, aerobic plate counts, *E. coli*, and total coliforms were not identifiable in dried fish generated by high salts [47]. The total mesophilic bacteria count was 4.6 × 10^2 cfu/g in the first month after salt processing [48]. In the subsequent months of storage (6 months), mesophilic, psychotropic, coliform bacteria, and fungi did not increase in bonito salted by dry salting and kept at 4 °C. In Samsun, yeast-mold levels of 10^2 CFU/g were found in 38% of ready-to-eat salted anchovy samples [49]. Moreover, the initial microbial contamination of the mullet may affect its quality. On three months of storage, the total aerobic psychrophile bacteria count of frozen pearl mullet was 4.87 log CFU/g in whole samples, 4.78 log CFU/g in cleaned samples, 4.96 logs CFU/g in tested samples, and 4.85 log CFU/g in cleaned samples, which their internal guts were removed [46]. Fish deterioration has been documented when psychrophile microbes reach 107–108 CFU/g [50]. Pseudomonas count of frozen pearl mullet was 3.27 log CFU/g in whole samples, 2.80 log CFU/g in cleaned samples after three months of storage, and 3.23 log CFU/g in cleaned samples after four months of storage [46].

Regarding the sensory characterization, color attributes in both mixture treatment and the BCN are reflected close to the fresh fish color attributes. The shiny appearance was noticed as being present for the BCN treatment, opposite to the commercial salted sample that was deep dark grey. The preferability of the panelists ordered as BCN > Salted C > Mix > Lysozyme > EDTA. In this concern, the BCN could be recommended as an alternative method to prepare the safe and healthy salted mullet.

5. Conclusions

Fish salting is commercial preservation, where this type is widely requested, especially for traditional festivals. Applying several solutions for the dipping treatment of fish before the salting step assists the preservation properties during the storage time. Several contaminations reported in salted fish included chemical composition, nitrogen and lipid profile changes, and microbial contamination. Our findings revealed that the treatment by mixture solution of EDTA and lysozyme before salting was recorded in the following order

after the BCN treatment with good sensory and microbial acceptance properties. The BCN was recorded to have the best result with a salted mullet close to the fresh characterization. The results from this study suggest that the BCN treatment before the mullet fish salting is the best treatment, providing more safety and without changes for consumer acceptance.

Author Contributions: Data curation, A.M.K., M.F.E.-K., S.A.A., H.M.Y. and H.S.A.; formal analysis, A.N.B. and M.G.S.; funding acquisition, M.F.E.-K. and H.M.Y.; investigation, A.M.K., A.N.B., M.G.S. and H.S.A.; methodology, A.N.B. and S.A.A.; project administration, A.N.B., M.F.E.-K., H.M.Y. and H.S.A.; resources, A.M.K., M.G.S., S.A.A. and H.S.A.; software, M.F.E.-K., M.G.S., S.A.A., H.M.Y. and H.S.A.; supervision, H.M.Y.; validation, A.N.B.; writing—original draft, M.G.S.; writing—review and editing, A.N.B. All authors have read and agreed to the published version of the manuscript.

Funding: The Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia, project number PNURSP2022R23.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data regarding this work are represented in the manuscript.

Acknowledgments: The authors appreciate the Researcher Support Program (PNURSP2022R23), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

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

References

1. Farag, M.A.; Zain, A.E.; Hariri, M.L.; El Aaasar, R.; Khalifa, I.; Elmetwally, F. Potential food safety hazards in fermented and salted fish in Egypt (Feseekh, Renga, Moloha) as case studies and controlling their manufacture using HACCP system. *J. Food Saf.* **2022**, *42*, e12973. [CrossRef]
2. Barat, J.M.; Rodríguez-Barona, S.; Andrés, A.; Fito, P. Cod salting manufacturing analysis. *Food Res. Int.* **2003**, *36*, 447–453. [CrossRef]
3. Getu, A.; Misganaw, K.; Bazezew, M. Post-harvesting and major related problems of fish production. *Fish. Aquac. J.* **2015**, *6*, 1000154. [CrossRef]
4. Qiu, L.; Zhang, M.; Bhandari, B.; Yang, C. Shelf life extension of aquatic products by applying nanotechnology: A review. *Crit. Rev. Food Sci. Nutr.* **2022**, *62*, 1521–1535. [CrossRef]
5. Ghaly, A.E.; Dave, D.; Budge, S.; Brooks, M.S. Fish spoilage mechanisms and preservation techniques. *Am. J. Appl. Sci.* **2010**, *7*, 859. [CrossRef]
6. Gram, L.; Dalgaard, P. Fish spoilage bacteria–problems and solutions. *Curr. Opin. Biotechnol.* **2002**, *13*, 262–266. [CrossRef]
7. Lehane, L.; Olley, J. Histamine fish poisoning revisited. *Int. J. Food Microbiol.* **2000**, *58*, 1–37. [CrossRef]
8. Chander, R.; Lewis, N.F. Effect of lysozyme and sodium EDTA on shrimp microflora. *Eur. J. Appl. Microbiol. Biotechnol.* **1980**, *10*, 253–258. [CrossRef]
9. Levin, R.E. The effectiveness of EDTA as a fish preservative. *J. Milk Food Technol.* **1967**, *30*, 277–283. [CrossRef]
10. Badr, A.N.; Youssef, M.; Abdel-Razek, A.G.; Shehata, M.G.; Hassanien, M.M.; Amra, H. Natural Antioxidants: Preservation Roles and Mycotoxicological Safety of Food. *Egypt. J. Chem.* **2021**, *64*, 285–298. [CrossRef]
11. Badr, A.N.; Shehata, M.G.; Abdel-Razek, A.G. Antioxidant activities and potential impacts to reduce aflatoxins utilizing jojoba and jatropha oils and extracts. *Int. J. Pharmacol.* **2017**, *13*, 1103–1114. [CrossRef]
12. Abdel-Razek, A.G.; Badr, A.N.; Alharthi, S.S.; Selim, K.A. Efficacy of Bottle Gourd Seeds' Extracts in Chemical Hazard Reduction Secreted as Toxigenic Fungi Metabolites. *Toxins* **2021**, *13*, 789. [CrossRef] [PubMed]
13. Badr, A.N.; Ali, H.S.; Abdel-Razek, A.G.; Shehata, M.G.; Albaridi, N.A. Bioactive Components of Pomegranate Oil and Their Influence on Mycotoxin Secretion. *Toxins* **2020**, *12*, 748. [CrossRef] [PubMed]
14. Abdel-Razek, A.G.; Badr, A.N.; El-Messery, T.M.; El-Said, M.M.; Hussein, A.M.S. Micro-nano encapsulation of black seed oil ameliorate its characteristics and its mycotoxin inhibition. *Biosci. Res.* **2018**, *15*, 2591–2601.
15. Abdel-Salam, A.M.; Badr, A.N.; Zaghloul, A.H.; Farrag, A.R.H. Functional yogurt aims to protect against the aflatoxin B1 toxicity in rats. *Toxicol. Rep.* **2020**, *7*, 1412–1420. [CrossRef]
16. Sharif, H.R.; Abbas, S.; Majeed, H.; Safdar, W.; Shamoon, M.; Khan, M.A.; Shoaib, M.; Raza, H.; Haider, J. Formulation, characterization and antimicrobial properties of black cumin essential oil nanoemulsions stabilized by OSA starch. *J. Food Sci. Technol.* **2017**, *54*, 3358–3365. [CrossRef]
17. Malik, M.R.; Al-Harbi, F.F.; Nawaz, A.; Amin, A.; Farid, A.; Mohaini, M.A.; Alsalman, A.J.; Hawaj, M.A.A.; Alhashem, Y.N. Formulation and Characterization of Chitosan-Decorated Multiple Nanoemulsion for Topical Delivery In Vitro and Ex Vivo. *Molecules* **2022**, *27*, 3183. [CrossRef]

18. Azad, A.K.; Al-mahmood, S.M.A.; Doolaanea, A.A. Development and Validation of UV-Vis Spectrophotometric Method for Estimation of Black Seeds and Peppermint Oil in Emulsion. *Anal. Chem. Lett.* **2021**, *11*, 607–617. [CrossRef]
19. AOAC. *Official Methods of Analysis*, 19th ed.; Association of Official Analytical Chemists: Arlington, VA, USA, 2012.
20. Bligh, E.G.; Dyer, W.J. A rapid method of total lipid extraction and purification. *Can J. Biochem. Physiol.* **1959**, *37*, 911–917. [CrossRef]
21. Khojah, E.Y.; Badr, A.N.; Mohamed, D.A.; Abdel-Razek, A.G. Bioactives of Pomegranate By-Products and Barley Malt Grass Engage in Cereal Composite Bar to Achieve Antimycotic and Anti-Aflatoxigenic Attributes. *Foods* **2022**, *11*, 119. [CrossRef]
22. Pearson, F.J., Jr.; Hanshaw, B.B. Sources of Dissolved Carbonate Species in Ground Water and Their Effects on Carbon-14 Dating. Available online: https://inis.iaea.org/collection/NCLCollectionStore/_Public/01/001/1001624.pdf (accessed on 10 July 2022).
23. Egan, H.; Cox, H.E.; Pearson, D. *Pearson's Chemical Analysis of Foods*; Churchill livingstone: London, UK, 1981.
24. Faustman, C.; Specht, S.M.; Malkus, L.A.; Kinsman, D.M. Pigment oxidation in ground veal: Influence of lipid oxidation, iron and zinc. *Meat Sci.* **1992**, *31*, 351–362. [CrossRef]
25. Gharibzahedi, S.M.T.; Mohammadnabi, S. Effect of novel bioactive edible coatings based on jujube gum and nettle oil-loaded nanoemulsions on the shelf-life of Beluga sturgeon fillets. *Int. J. Biol. Macromol.* **2017**, *95*, 769–777. [CrossRef]
26. Jacobs, S. The determination of amino acids in food hydrolyzates with a stabilized indanetrione hydrate reagent. *Microchem. J.* **1965**, *9*, 87–397. [CrossRef]
27. Anderson, M.E.; Marshall, R.T. Reducing microbial populations on beef tissues: Concentration and temperature of lactic acid. *J. Food Saf.* **1989**, *10*, 181–190. [CrossRef]
28. Reynolds, A.E.; Harrison, M.A.; Rose-Morrow, R.; Lyon, C.E. Validation of dry cured ham process for control of pathogens. *J. Food Sci.* **2001**, *66*, 1373–1379. [CrossRef]
29. Imai, I.; Ishida, Y.; Hata, Y. Killing of marine phytoplankton by a gliding bacterium *Cytophaga* sp., isolated from the coastal sea of Japan. *Mar. Biol.* **1993**, *116*, 527–532. [CrossRef]
30. Collee, J.G.; Mackie, T.J.; McCartney, J.E. *Mackie & McCartney Practical Medical Microbiology*; Harcourt Health Sciences: New York, NY, USA, 1996.
31. Eid, A.M.M.; Elmarzugi, N.; El-Enshasy, H.A. Preparation and evaluation of olive oil nanoemulsion using sucrose monoester. *Int. J. Pharm. Pharm. Sci.* **2013**, *5* (Suppl. 3), 434–440.
32. Pando, D.; Beltrán, M.; Gerone, I.; Matos, M.; Pazos, C. Resveratrol entrapped niosomes as yoghurt additive. *Food Chem.* **2015**, *170*, 281–287. [CrossRef]
33. Yue, P.-F.; Lu, X.-Y.; Zhang, Z.-Z.; Yuan, H.-L.; Zhu, W.-F.; Zheng, Q.; Yang, M. The study on the entrapment efficiency and in vitro release of puerarin submicron emulsion. *AAPS PharmSciTech* **2009**, *10*, 376–383. [CrossRef]
34. Badr, A.N.; El-Said, M.M.; El-Messery, T.M.; Abdel-Razek, A.G. Non-traditional oils encapsulation as novel food additive enhanced yogurt safety against aflatoxins. *Pak. J. Biol. Sci.* **2019**, *22*, 51–58. [CrossRef]
35. Rosa, A.; Scano, P.; Atzeri, A.; Deiana, M.; Mereu, S.; Dessì, M.A. Effect of Storage Conditions on Lipid Components and Color of Mugil cephalus Processed Roes. *J. Food Sci.* **2012**, *77*, C107–C114. [CrossRef] [PubMed]
36. Talab, A.S.; Ghanem, M.H. Effects of different salt concentrations on the quality alterations and shelf-life of the grey mullet fish. *Egypt. J. Aquat. Biol. Fish.* **2021**, *25*, 583–595. [CrossRef]
37. El-Sherif, S.A.; Abd El-Ghafour, S. Investigation of the quality properties and nutritional values of four fish species from Lake Qaroun, Egypt. *Int. J. Chem. Tech. Res.* **2016**, *9*, 16–26.
38. Hansen, L.T.; Gill, T.; Røntved, S.D.; Huss, H.H. Importance of autolysis and microbiological activity on quality of cold-smoked salmon. *Food Res. Int.* **1996**, *29*, 181–188. [CrossRef]
39. Ali, M. Shelf life determination of the brined golden mullet Liza aurata during vacuum refrigerated storage using some quality aspect. *Acta Sci. Pol. Technol. Aliment.* **2012**, *11*, 37–43. [PubMed]
40. Akonor, P.T.; Ofori, H.; Dziedzoave, N.T.; Kortei, N.K. Drying Characteristics and Physical and Nutritional Properties of Shrimp Meat as Affected by Different Traditional Drying Techniques. *Int. J. Food Sci.* **2016**, *2016*, 7879097. [CrossRef]
41. Lee, S.H.; Moon, W.S.; Park, K.N. Antimicrobial activity of *Caesalpina sappan* L. extracts and its effect on preservation of ground meats. *J.-Korean Soc. Food Sci. Nutr.* **2000**, *29*, 888–892.
42. Gould, G.W. Micro-Organisms in Foods 4. Application of the Hazard Analysis Critical Control Point (HACCP) System to Ensure Microbiological Safety and Quality: ICMSF, Blackwell Scientific Publications, London, 1988, 357 pp., ISBN 0-632-02181-0. *Food Control* **1990**, *1*, 246–247.
43. Hashem, M.; Alamri, S. Contamination of common spices in Saudi Arabia markets with potential mycotoxin-producing fungi. *Saudi J. Biol. Sci.* **2010**, *17*, 167–175. [CrossRef]
44. Hsu, H.-H.; Chuang, T.-C.; Lin, H.-C.; Huang, Y.-R.; Lin, C.-M.; Kung, H.-F.; Tsai, Y.-H. Histamine content and histamine-forming bacteria in dried milkfish (*Chanos chanos*) products. *Food Chem.* **2009**, *114*, 933–938. [CrossRef]
45. Kung, H.-F.; Chien, L.-T.; Liao, H.-J.; Lin, C.-S.; Liaw, E.-T.; Chen, W.-C.; Tsai, Y.-H. Chemical characterisation and histamine-forming bacteria in salted mullet roe products. *Food Chem.* **2008**, *110*, 480–485. [CrossRef]
46. Ekici, K.; Alisarli, M. Histamine formation and microbiological changes in endemic Chalcalburnus tarichi Pallas 1811 (Inci Kefali) stored at 4 C. *Arch. Med. Vet.* **2008**, *40*, 95–98. [CrossRef]

47. Hwang, C.-C.; Lin, C.-M.; Kung, H.-F.; Huang, Y.-L.; Hwang, D.-F.; Su, Y.-C.; Tsai, Y.-H. Effect of salt concentrations and drying methods on the quality and formation of histamine in dried milkfish (*Chanos chanos*). *Food Chem.* **2012**, *135*, 839–844. [CrossRef] [PubMed]
48. Turan, H.; Kaya, Y.; Erkoyuncu, İ.; SÖNmez, G. Chemical and microbiological qualities of dry-salted (lakerda) bonito (sarda sarda, bloch 1793). *J. Food Qual.* **2006**, *29*, 470–478. [CrossRef]
49. Ogur, S.; Erkan, N. Microbiological and chemical quality of different types of salted pearl mullet (*Chalcalburnus tarichi Pallas*, 1811). *J. Food Saf.* **2020**, *40*, e12717. [CrossRef]
50. Gram, L.; Trolle, G.; Huss, H.H. Detection of specific spoilage bacteria from fish stored at low (0 C) and high (20 C) temperatures. *Int. J. Food Microbiol.* **1987**, *4*, 65–72. [CrossRef]