Journal of Food Biosciences and Technology,
Islamic Azad University, Science and Research Branch, Vol. 14, No. 4, 21-42, 2024

Effect of Chitosan Coating Supplemented with Sugar Beet Leaf Extract (*Beta vulgaris L.*) on Quality Attributes of Sevruga Fillets (*Acipenser stellatus*) during Refrigeration

M. Talebi Haghgo [a]***, N. Mooraki*** [b] ****, M. Honarvar*** [c]

[a] M. Sc. Student of the Department of Food Science and Technology, North Tehran Branch, Islamic Azad University, Tehran, Iran.
[b] Associate Professor of Fisheries Science, Department of Marine Science and Technology, North Tehran Branch, Islamic Azad University, Tehran, Iran.
[c] Associate Professor of the Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.

Received: 12 September 2024 Accepted: 12 January 2025

ABSTRACT: This research examines the impact of a chitosan coating infused with sugar beet leaf extract (SBLE), obtained through microwave extraction, on the quality of sevruga fillets during refrigerated storage. The experimental treatments consisted of T_1(0.5%chitosan), T_2(1%chitosan with 0.5% SBLE), T_3(1%chitosan with 1%SBLE), and T_4(2%chitosan with 1.5%SBLE) and C (Pure fillet). The fillets were stored in polythene bags at 4°C for seven days. The findings from the chemical assessment indicated that the control sample exhibited higher levels of pH, free fatty acids, thiobarbituric acid, and peroxide value (PV) compared to the coated samples; however, these differences were not statistically significant ($p > 0.05$). Total volatile basic nitrogen (TVB_N) displayed an upward trend, with the control sample showing significantly elevated levels relative to the other samples ($p < 0.05$). The findings from the microbial assessment indicated a significant increase ($p < 0.05$) in total viable count (TVC) within the control treatment group; however, this increase was not statistically significant for lactic acid bacteria. Additionally, the counts for Enterobacteriaceae and Pseudomonas were recorded at less than 2 Log CFU/g, while *Clostridium perfringens* was found to be below 1 Log CFU/g across all samples. The analysis of sensory attributes utilizing the Principal Component Analysis (PCA) method revealed that the T_2, T_3, T_4 exhibited significant influences from the characteristics of color, taste, aroma, texture hardness, and overall acceptability. In contrast, the control sample and T_1 did not align with these sensory characteristics, with the C sample demonstrating lower levels of acceptability. The primary characteristic of T_2 was its texture hardness, while T_3 was significantly ($p < 0.05$) shaped by the aroma produced and the overall acceptance. In contrast, T_4, which exhibited greater acceptance, was influenced by a combination of attributes, such as taste, color, and overall acceptance, when compared to the other samples.

Keywords: *Aquatic Food Products, Beetroot, Herbal Extracts, Hurdle Effect*

Introduction

In more than two decades, there has been a notable increase in consumer demand for healthy and nutritious food options, resulting in a rise in the consumption of aquatic food products, considering the per capita consumption of 23.3 kg by 2023 (Rathod *et al.*, 2021; Esua

*Corresponding Author: Nargess_Mooraki@Yahoo.com

et al., 2020; FAO, 2018). Fish, recognized as a nutritious source, plays a significant role in a balanced diet (Vieira *et al.*, 2019; Ceylan *et al.*, 2019). Due to its advantageous geographical position, the Caspian Sea serves as a natural habitat for esteemed fish species, including the Sevruga, scientifically classified as *Acipenser stellatus*. This species is recognized as a migratory sturgeon (Khorshidi Sedehi & Shabanipour, 2018). The main factors leading to the decline in fish quality are enzymatic and chemical reactions. In contrast, secondary factors pertain to microbial activity and spoilage that typically arise in the final days of storage (Kalteh *et al.*, 2015). Fish contains a significant amount of unsaturated fats, which can lead to oxidation and chemical degradation of its flesh, a notably pronounced phenomenon in the *Acipenseridae* family (Saini *et al.*, 2021). Fat oxidation primarily yields peroxides, with secondary products including aldehydes, hydroxys, and ketones. Notably, malondialdehyde is the most significant aldehyde produced during the secondary oxidation of fats. This toxic molecule, characterized by its low molecular weight, can react with proteins and organic acids, posing risks for cancer and atherosclerosis (Celi, 2010; Lykkesfeldt, 2001; Del Rio *et al.*, 2005; Lykkesfeldt, 2007). Following the death of a fish, bacteria initiate the breakdown of lactic acid and trimethylamine oxide through the process of glycogenesis. This biochemical activity leads to a decrease in trimethylamine oxide levels and the formation of volatile compounds with low molecular weights, such as ammonia and hydrogen sulfide. Additionally, the oxidation of fats results in the production of ketonic and aldehydic compounds, ultimately contributing to the unpleasant odor associated with fish (Prabhakar *et al.*, 2019; Summers *et al.*, 2017; Burt, 2004; Valipour *et al.*, 2017).

The adoption of innovative preservation techniques, including Hurdel's concept, combining multiple methods to control/eliminate pathogens in food items, guaranteeing their safety for consumption and stability over time, all while preserving their quality, that incorporates films and coatings, is on the rise as a means to enhance food quality and extend shelf life. However, these methods remain underutilized in the context of meat products. Edible films and coatings are formulated using proteins, lipids, and polysaccharides sourced from plant and animal materials, which can be utilised separately or in mixtures on fillets. Furthermore, incorporating minimal emulsifiers can improve food product coatings' effectiveness (Feng *et al.*, 2017; Korkmaz *et al.*, 2019; Sathivel, 2005). Chitosan is an edible coating derived from a polysaccharide structure that includes glucosamine and N-acetylglucosamine, characterized by beta 1,4 linkages. It is extracted from the exoskeletons of crustaceans such as crabs and shrimp. This compound exhibits the capability to inhibit the activity of various Gram-positive and Gram-negative bacteria (Bautista-Banos *et al.*, 2006; Coma *et al.*, 2002; Vasconez *et al.*, 2009). This antimicrobial property may be attributed to the penetration of chitosan into the microbial nucleus, where it interacts with DNA (Sebti *et al.,* 2005). Extensive research has validated the advantageous effects of chitosan coatings containing a variety of extracts and essential oils (Lan *et al.*, 2024; Ucak & Afreen, 2022; Fadiloglu & Coban, 2018; Li *et al.*, 2020; Shoja *et al.*, 2023; Rezaeifar *et al.*, 2020).

Sugar beet, scientifically known as *Beta vulgaris L.*, is a biennial plant belonging to the Chenopodiaceae family. It is utilized in various forms, including fresh, fermented, and cooked, as noted by Latorre *et al.* (2012). Certain types of antioxidants, such as oxalic acid, are present in beet greens. The consumption of beet greens may enhance the functionality of the antioxidant system, including glutathione, and can contribute to the reduction of sugar and fat levels, as well as alleviate some diabetic complications, such as skin issues (Kayashima & Katayama, 2002; Tunali *et al.*, 1998; Sener *et al.*, 2002). The antioxidant properties of *Beta vulgaris L.* extract has been investigated, revealing the presence of nine bioactive polyphenols, including quercetin, sinapic acid, p-coumaric acid, syringic acid, gallic acid, coumarin, caffeic acid, chlorogenic acid, and catechin in the freeze-dried aqueous extract (Indu *et al.*, 2017). The DPPH assay was employed to assess the antioxidant capacity of this extract, which demonstrated an impressive inhibition activity of 97.63% at a concentration of 1000 micrograms per milliliter. Consequently, the application of 600 ppm of antioxidants derived from microwave-extracted sugar beet leaves shows competitive potential against the synthetic antioxidant BHT (Izadi *et al.*, 2021). Research on adding dried beetroot leaves (DBLP) to cookies demonstrated that higher concentrations of DBLP facilitate improvements in both nutritional components and phytonutrient levels. The DBLP-enriched cookies showed significant increases in moisture, protein, total dietary fiber, crude fiber, fat, ash, hardness, phenolic compounds, and antioxidant activity. In contrast, the carbohydrate content was diminished in these cookies (Asadi & Khan, 2020).

This study aims to assess the effectiveness of chitosan-based coatings infused with an extract from beetroot leaves in prolonging the shelf life of Sevruga fillets when stored under refrigeration, focusing on the chemical, microbial, and sensory characteristics of the product.

Materials and Methods

- ***Ingredients***

Sevruga (purchased from the local market in Noor, Mahmmudabad, Mazandaran Province),

The medium molecular weight chitosan (Sigma Co.), Beetroot leaves (purchased from local market in Ahvaz, Khoozestan Province)

- ***Sample Preparation***

Immediately after collection, fresh Sevruga fish with a typical weight of 5 kg were placed in a polystyrene refrigerator with ice weighing three times that of the fish. The samples were washed with tap water, and filleted. Solutions of chitosan were formulated following the methodology outlined by Souza *et al.* (2010), with concentrations of 0.5%, 1%, and 2% prepared individually. Extracts from dried autumn beet leaves were procured utilizing a polar water solvent through a microwave-assisted extraction technique (Samsung, South Korea) conducted at a power setting of 90 watts for a duration of 10 minutes (Talebi Haghgo *et al.*, 2024). In the preparation of coatings incorporating the extract, 0.5% and 1% concentrations of sugar beet leaf extract (SBLE) were individually introduced into a 1% chitosan solution. Additionally, a 1.5% concentration of SBLE was incorporated into a 2% chitosan solution. These mixtures were subjected to magnetic stirring (Heidolph, Germany) for a duration of 10 minutes (Ojagh *et al.*, 2012).

Sevruga fillets, weighing 250 grams, underwent a coating procedure involved immersion in a series of chitosan solutions. The initial treatment consisted of soaking the fillets in a 0.5% chitosan solution (T_1), followed by a 1% chitosan solution containing 0.5% SBLE (T_2), and subsequently in a 1% chitosan solution with a 1% SBLE concentration (T_3). The fourth group (T_4) was immersed in a solution with 1.5% SBLE, while the control group (C) was prepared without chitosan or extract treatment. After a brief exposure to air for one minute, the fillets were re-immersed in the respective solutions for an additional two minutes. To ensure the development of a consistent coating, the fillets were allowed to remain in the air for two hours. The coated and uncoated control fillets were stored in zippered bags and refrigerated at 4°C for seven days. Chemical, microbial, and sensory evaluations of the fillets were performed on days 0, 3, 5, and 7. All experiments were performed in triplicate.

- ***Chemical Analysis***

pH (Metrohm, Germany) was measured following the approach outlined by Kavitha and Mofi (2006), while acidity was determined through titration based on lactic acid as described by Shelef and Jay (1970). The peroxide value was assessed via titration with Sodium Thiosulfate according to the AOAC (2002) methodology. Additionally, the free fatty acid content was calculated as a percentage of oleic acid using the technique developed by Woyewoda *et al.* (1986). The determination of volatile nitrogenous bases was performed using Kjeldahl's method as per AOAC (1995). Furthermore, the quantity of thiobarbituric acid was measured following the protocol established by Namulema *et al.* (1999), and the absorbance of the resulting solution was recorded using a spectrophotometer (Thermo, Germany) at wavelength of 560 nanometer.

- ***Microbial Analysis***

In the process of microbial testing, 10 grams of samples from each experimental group were combined and homogenized using a Heidolph mixer (Germany) with 90 ml of a 0.85% NaCl solution, all performed under sterile conditions. Subsequently, the necessary dilutions were prepared. The identification of lactic acid bacteria was conducted utilizing MRS agar medium, which was incubated at 37°C for a duration of 48 hours. Clostridium perfringens was cultivated on SPS agar medium and incubated at 37°C for a period ranging from 48 to 72 hours. Pseudomonas was propagated on Pseudomonas agar, enhanced with a CFC selective supplement, and incubated at 37°C for 24 hours. Total viable counts (TVC) were assessed on TSA medium after incubation at 35°C for 48 hours, while Enterobacteriaceae was cultured on VRBG agar, incubated at 37°C for 24 hours, in accordance with the protocols established by Olatunde et al. (2019), Yetim et al. (2006), Hamedi et al. (2017), and Öz and Ucak (2023).

- ***Sensory evaluation***

A sensory analysis was conducted with a cohort of 40 untrained participants, randomly selected, whose palates were attuned to aquatic food products. Notably, the evaluators were not informed about the composition of the samples they were assessing. Prior to frying in canola oil, the fillets were seasoned with 1.5% salt. Every four days, the participants received a scoring questionnaire to evaluate the samples. The sensory evaluation employed a five-point hedonic scale to assess various attributes, including color, flavor, taste, hardness, and overall acceptance. This structured scale allowed participants to rate their preferences, with scores ranging from 1, indicating extreme dislike, to 5, representing extreme liking, and including intermediate options for nuanced responses. It is important to highlight that the control sample was excluded from sensory evaluation owing to safety and health concerns regarding the evaluators, particularly in relation to ensuring confidence in terms of microbial load (Nirmal & Benjakul, 2011).

- ***Statistical Analysis***

For the purpose of statistical analysis, SPSS software version 26 was utilized alongside the K-S test, with a significance criterion established at $P < 0.05$. One-Way Analysis of Variance and Duncan's post hoc test were employed when the data conformed to a normal distribution, maintaining the significance level at $P < 0.05$. In cases where the data did not meet the normality assumption, the Kruskal-Wallis test was utilized to evaluate differences across the experimental groups. Furthermore, principal component analysis (PCA) and Spearman correlation matrix were conducted on the sensory evaluation data using XLSTAT.

Results and Discussion

- ***Chemical Analysis***
- ***pH***

pH measurements and their comparative evaluations were performed among distinct experimental groups. The data presented in Figure 1 reveal that no significant differences were detected among the samples (p=0.625). In general, the levels of this factor showed a decline in the treatment groups on both the third and fifth days relative to the first day of incremental production. Additionally, by the seventh day, a further decrease was noted, with the control sample retaining the highest concentration after a week of storage.

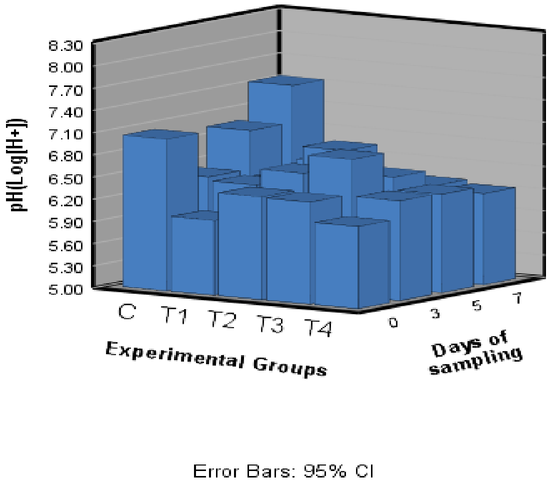

Fig.1. pH value of experimental groups (significance indicated by superscripts at P<0.05).

- ***Acidity***

According to the data depicted in Figure 2, it is evident that the acidity levels among the experimental groups do not differ significantly (p=0.241). The trend observed in all samples shows a gradual increase from the initial day of production to the seventh day of storage, with the first and second treatments demonstrating a more substantial rise in acidity on the seventh day compared to the remaining groups.

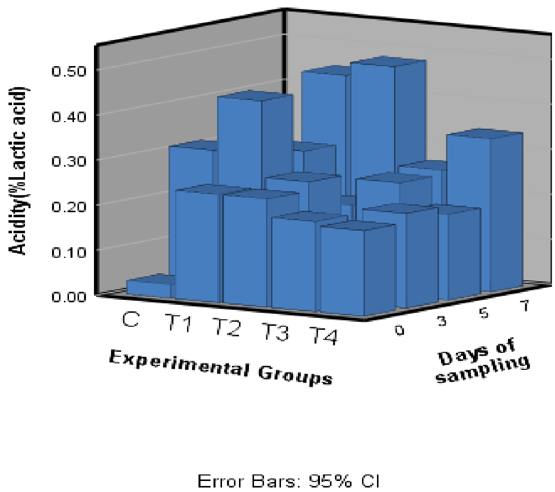

Fig. 2. Acidity value of experimental groups (significance indicated by superscripts at P<0.05).

- ***Peroxide Value (PV)***

Utilizing a one-way analysis of variance, the peroxide index was assessed among twenty experimental groups, maintaining a significance level of P<0.05. The results demonstrated a progressive increase in the peroxide index from day one to day seven (p=0.469) across all groups. The maximum index value was noted on the seventh day for groups C and T_3, whereas T_4 exhibited the minimum value on that day (Figure 3).

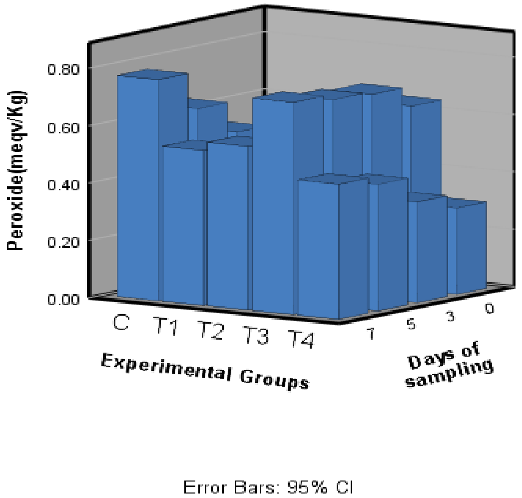

Fig. 3. Peroxide index of experimental groups (significance indicated by superscripts at P<0.05).

- ***Free Fatty Acids Index***

A comparative analysis of free fatty acid levels, based on oleic acid, was conducted between treatment and control samples across the experimental groups. The findings depicted in Figure 4 reveal that the differences in free fatty acid concentrations among the samples were not statistically significant (p=0.103). In general, there was a progressive increase in this parameter from the first day of production in all groups, with the highest levels observed in the control group and the first treatment, whereas the fourth treatment exhibited the lowest levels on the seventh day.

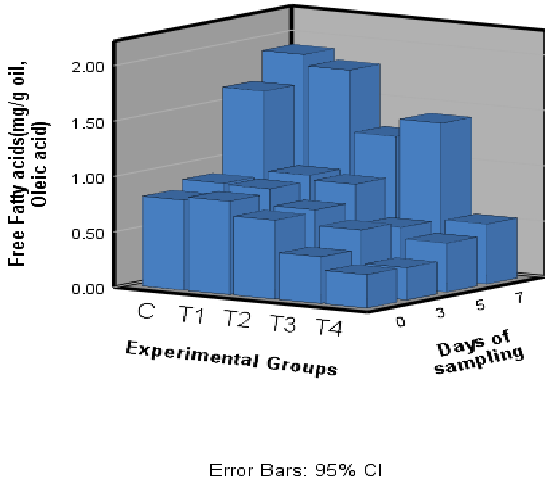

Fig. 4. Free fatty acids content of experimental groups (significance indicated by superscripts at P<0.05).

- ***Thiobarbituric acid reactive substances (TBARs)***

A one-way ANOVA test was employed to compare the experimental groups, with a significance threshold set at P<0.05. The data indicated that the index values exhibited an upward trend across all groups from the first to the seventh day of maintenance (Figure 5). Notably, the control group demonstrated the most substantial increase, while T_4 showed the least. However, statistical analysis revealed no significant differences among the groups (p=0.180).

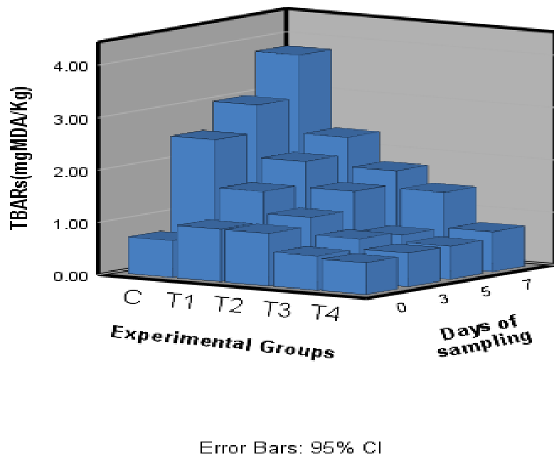

Fig. 5. Thiobarbituric acid reactive substances of experimental groups (significance indicated by superscripts at P<0.05).

- ***Total Volatile Basic-Nitrogen (TVB-N)***

A comparative analysis was conducted on the total content of volatile nitrogenous bases between the treated and control samples within the experimental groups. The findings illustrated in Figure 6 indicate a statistically significant difference in this parameter among the samples (p=0.01). Notably, the levels of this index exhibited an upward trend across all groups from the first to the seventh day of observation (Figure 6), with the control group demonstrating the most substantial increase, while the T_4 showed the least.

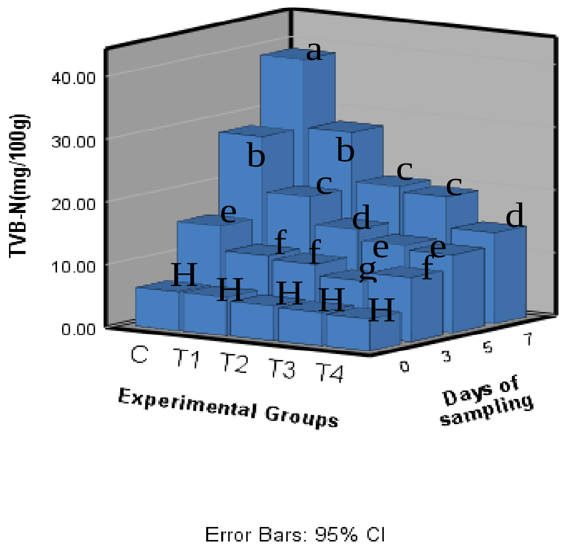

Fig. 6. TVB-N content of experimental groups groups (significance indicated by superscripts at P<0.05).

- ***Microbiological assessment***

An increasing trend in the TVC was noted from the first to the seventh day of storage across experimental groups. The data presented in Figure 7 indicate a significant difference (P<0.05) in the overall bacterial counts among the samples, which can be linked to the growth in microbial populations from the first day of production to the seventh day of storage. The control group experienced the most pronounced increase, whereas the T_4 showed the least variation (P>0.05). Throughout the storage period, from the first to the seventh day, there was a notable upward trend in the population of lactic acid bacteria across all groups. The data presented in Figure 8 indicates that the overall counts of these bacteria among the samples showed no significant variation (P>0.05). During the seven-day examination, the quantification of Enterobacteriaceae bacterial colonies in both control and treatment samples at less than 2 Log CFU/g. Likewise, the Pseudomonas bacterial colonies in all samples, including both control and treatment, were measured at less than 1 Log CFU/g during this period. Additionally, the counts of bacterial colonies in all samples remained below 1 Log CFU/g after the seven days of analysis.

- ***Sensory evaluation results***

PCA analytical technique allowed for generating a lower-dimensional data representation by identifying the most relevant principal components. Table 1 demonstrates a consistent correlation between color, taste, texture, hardness and overall acceptance. The distribution of variance explained by four principal components is presented in Table 2, highlighting that F1 and F2 contribute a considerable share of the total variation in the dataset, exceeding the threshold of 1 and elucidating 91.84% of the variance.

The eigenvalues associated with each principal component are depicted in Figure 9, which visually represents the variance accounted for by the four components. Notably, the "elbow" point, indicating where eigenvalues begin to decrease markedly, is found beyond F2. A review of the scree plot suggests that two components should be preserved for further examination.

Table 3 illustrates the association of each attribute with the principal components. Notably, color, taste, hardness, and overall acceptance are highly correlated with F1, while flavor exhibits a strong correlation with F2. Figure 10 demonstrates that following a storage duration of 7 days, sample T_3 was predominantly distinguished by its potentially appealing flavor and general acceptability. In contrast, samples T_4 and T_2 exhibited low ratings in terms of taste, color, and texture hardness. Meanwhile, T_1 and the control sample were primarily recognized for their less desirable sensory characteristics.

The pH level of muscle tissue in live fish is approximately 7, depending on the fish species, fishing condition and environmental factors. Following the death of the fish, this pH can fluctuate between 6 and 7, influenced by several factors including the species of fish and the season. A pH exceeding 7 is indicative of spoilage in fish (Ozogul *et al.*, 2006; Jamali, 2023; Ozogul & Ucar, 2013). The

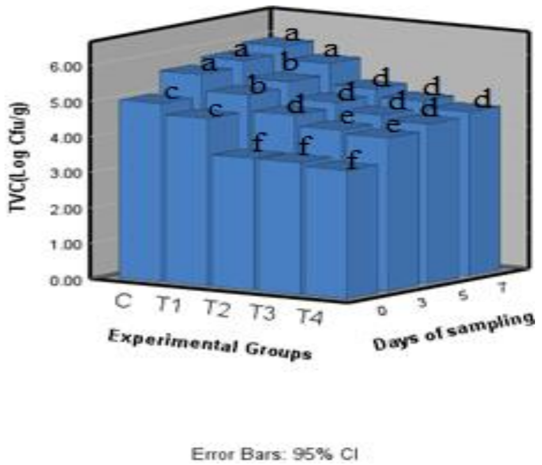

Fig. 7. Changes in the total viable count in the experimental groups (significance indicated by superscripts at P<0.05).

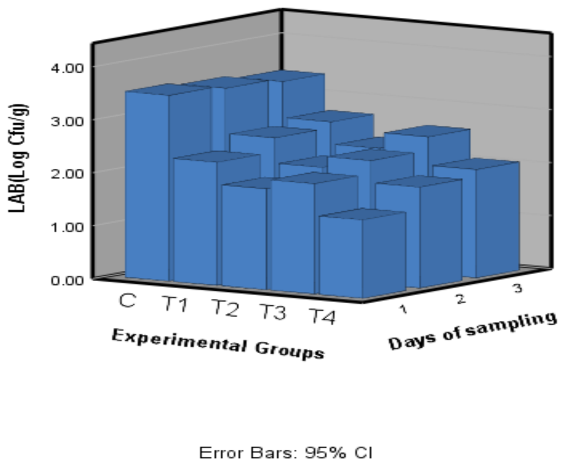

Fig. 8. Changes in the total Lactic acid bacteria count in the experimental groups (significance indicated by superscripts at P<0.05).

Table 1. Spearman correlation matrix indicates a statistically significant relationship among the sensory characteristics of the samples, with a p-value of less than 0.05

Variables	Color	Flavor	Taste	Hardness	Overall acceptance
Color	1	0.256	0.712	0.725	0.912
Flavor	0.256	1	0.340	0.117	0.297
Taste	0/712	0.340	1	0.957	0.921
Hardness	0.725	0.117	0.957	1	0.886
Overall acceptance	0.912	0.297	0.921	0.886	1

Values in bold are different from 0 with a significance level alpha=0.05.

Table 2. The computed eigenvalues within the principal component analysis process framework

	F1	F2
Eigenvalue	3.658	0.935
Variability (%)	73.152	18.697
Cumulative %	73.152	91.849

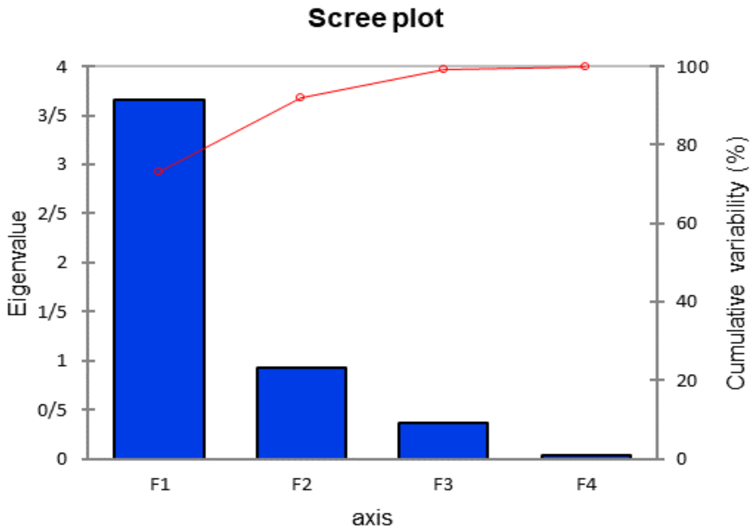

Fig. 9. Presentation of the computed eigenvalues pertaining to the sensory attributes of sevruga fillet samples.

Table 3. The relationship between the examined sensory attributes and their corresponding eigenvalues is represented by correlation values.

	F1	F2
Color	0.882	-0.47
Flavor	0.358	0.932
Taste	0.958	0.14
Hardness	0.929	-0.246
Overall acceptance	0.984	-0.051

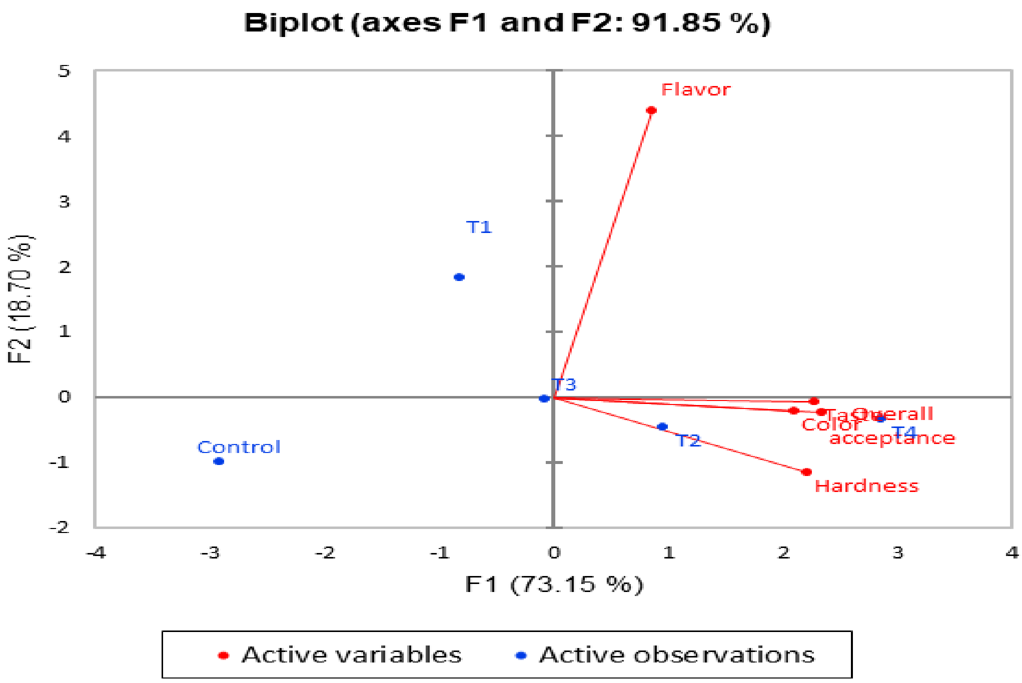

Fig. 10. A biplot demonstrating the distinct separation of samples as a result of evaluator influence.

data reveal that the intended factor rose on the third and fifth days relative to the first day. The lowest pH measurement was noted for T_1 on the first day at 6.01, while the highest pH value was recorded in the control sample on the seventh day at 7.45. Over the course of seven days of refrigerated storage of fish fillets, only the control group's pH exceeded the spoilage limit, the pH level of fish flesh rises from 6.2 to 7.5 with extended storage duration (Kim *et al.,* 2023), on the seventh day, with the pH levels in the other treated groups remaining below this threshold. The investigation by Berizi *et al.* (2018) focused on the effects of chitosan coatings containing pomegranate peel extract on the microbial, chemical, textural, and sensory properties of rainbow trout over a six-month storage period. The highest recorded pH was 7.29 in the uncoated samples. In comparison, the fish fillets that received coatings with various proportions of chitosan and pomegranate peel extract displayed a lower pH, which is consistent with the outcomes of the current study. According to the findings of Abdullahi *et al.* (2014), the silver carp sample that was coated with a chitosan-clay bio nanocomposite activated by rosemary essential oil demonstrated a markedly reduced pH level in contrast to the control sample. The analysis revealed that acidity levels increased throughout the storage duration. The maximum acidity observed in T_2 and T_1 was 0.48% and 0.45% of lactic acid, respectively. This increase in acidity can be explained by the transformation of glucose into organic acids by lactic acid bacteria (Hernandez-Herrero *et al.*, 1999; Fraqueza *et al.*, 2008). According to the findings of Barbosa *et al.* (2023), all treatments exhibited an increase in acidity up to the eighth day of storage, after which a decrease was observed. The research conducted by Salami *et al.* (2021) revealed that the fish marinade with 10% khandel plant extract exhibited the highest acidity levels. The control sample followed this, while an increase in the concentration of the extract resulted in a decrease in acidity. Additionally, the sugar beet leaf extract treatment demonstrated greater acidity than all other treatments, including the control. Another investigation's outcomes suggested that prolonged refrigeration of minced meat resulted in a reduction of its acidity. The control group recorded the minimum acidity on the sixth day, in contrast to the sevruga-treated fillets, which did not exhibit this trend. Among the various treatments, the uncoated fillets had the lowest acidity. In contrast, minced meat with 5% ginger powder displayed the highest acidity levels during storage, decreasing from 1.22 on the initial day to 0.98 by the sixth day (Terefe, 2017). The measurement of peroxide content, which serves as the primary product of fat oxidation, indicates an acceptable range up to 10 milliequivalents per kilogram of fat (Kirk & Sawyer, 1991; Martinsdottir *et al.*, 2001). Findings from this study revealed that the peroxide levels across all tested groups remained within the acceptable threshold throughout the duration of the maintenance period. Chitosan coatings infused with sugar beet leaf extract, particularly the T_4, demonstrated superior efficacy in inhibiting peroxide accumulation. Antioxidants play a crucial role in mitigating fat rancidity by either eliminating peroxides or diminishing the activity of singlet oxygen (Kucukgulmez, 2012). Consistent with the current study's findings, previous research has shown that uncoated samples exhibited significantly higher peroxide levels than their coated counterparts (Naghibi *et al.*, 2016; Nowzari *et al.*, 2013). The proliferation and metabolic activity of certain spoilage bacteria, particularly *Pseudomonas* species, contribute to an elevation in the levels of free fatty acids within food products. This phenomenon is attributed to the enzymatic hydrolysis of phospholipids by phospholipases and the breakdown of triglycerides by lipases (Nirmal & Benjakul, 2011; Nowzari *et al.*, 2013). In the present investigation, sevruga fish samples exhibited an increase in free fatty acid content; however, this increase remained below the 5% threshold. On the seventh day of storage, the control group exhibited the highest concentration of oleic acid at 1.91 mg/g, while the fourth treatment group recorded the lowest concentration at 0.54 mg/g. The observed increase in this parameter within fish fillets can be attributed to the catalytic action of internal enzymes on fats, as well as the influence of spoilage bacteria (Kykkidou *et al.*, 2009). Consistent with the current study's findings, Pourkargar and Rafati (2020) also reported a rising trend in free fatty acids, noting that the fish sample with this index coverage was lower than that of the control sample. Thiobarbituric acid serves as an indicator for assessing the extent of fat oxidation in fish, specifically by quantifying the aldehydes generated during this oxidative process. The acceptable threshold for this parameter in fish intended for consumption is established at 1 to 2 mg of malondialdehyde per kilogram of fish flesh (Kim *et al.*, 2006; Connell, 1990). Observations revealed a steady rise in this measurement throughout all samples from the first to the seventh day. Notably, the control group demonstrated the highest concentration on the seventh day, measuring 3.81 mg MDA/kg, whereas the fourth treatment exhibited the lowest concentration at 0.76 mg MDA/kg, likely attributable to the specific treatment conditions applied to the fillets; treatments incorporating sugar beet leaf extract displayed a variable range throughout the storage duration. The observed reduction in thiobarbituric acid levels on certain days can be attributed to a decline in malondialdehyde levels, which occurs due to its interaction with amino acids and proteins. The overall rise in this parameter during the storage period is linked to the partial dehydration of the fish, an increase in the oxidation of unsaturated fatty acids, and the subsequent formation of aldehydes resulting from hydroperoxide decomposition (Pezeshk *et al.*, 2011; Estaca *et al.*, 2007). According to the findings of Sabu *et al.* (2020), at the conclusion of the tenth day of storage for yellow tuna meat, the control group exhibited a TBARs value of 3.03 mg MDA/kg. In the current investigation, the highest concentration of thiobarbituric acid in the control group was recorded on the final day of storage. Both studies indicated that the TBARs levels in the control group surpassed 3 mg MDA/kg, exceeding the acceptable consumption threshold. Notably, the lowest thiobarbituric acid concentration on the tenth day was observed in the group treated with 2% chitosan and lemon peel extract. Furthermore, research by Sadeghi *et al.* (2020) demonstrated that chitosan coatings infused with Paneerbad extract, as well as the work of Pirouz Zarrin *et al.* (2023) involving chitosan coatings with *Dunaliella* algae extract, effectively mitigated the rise of TBARs in fish fillets compared to untreated samples. TVB-N is a critical parameter for assessing fish quality, as it quantifies non-protein nitrogenous compounds produced through bacteria's proteolytic degradation of proteins and nucleic acids. A 25 mg or less measurement per 100 grams of fish flesh indicates superior quality, while values under 30 mg are considered acceptable.

However, values approaching 35 mg warrant careful consideration regarding fish consumption and are associated with spoilage (Jouki *et al.*, 2014; Ozyurt *et al.*, 2009). The research outcomes revealed that the level of TVB-N in the fourth treatment during a seven-day storage period was recorded at less than 20 mg per 100 grams of fish fillet. Conversely, the control group exceeded the allowable limit, with a nitrogen concentration of 38.88 mg/100 grams on the seventh day, categorizing it within the spoilage range. The fourth treatment exhibited the lowest value for this metric on the last day of storage, measuring 14.46 mg of nitrogen/100 grams. Furthermore, various studies have similarly reported an increase in volatile nitrogenous bases in uncoated fish samples compared to coated ones (Jafari *et al.*, 2023; Farsanipour *et al.*, 2020).

The diversity of microorganisms isolated from marine products is influenced by several factors, including the fishing technique employed, the species of fish, and the temperature at which they are stored (Gram & Dalgaard, 2002). According to the International Commission on Microbiological Specifications for Foods (ICMSF, 1986), the acceptable limit for this index in food intended for human consumption is set at 7 Log CFU/g. In the present study, a significant increase in the total viable bacterial population was observed across all experimental groups; however, this increase remained within the acceptable threshold until the conclusion of the seventh day. Notably, on the seventh day, the control sample exhibited the highest bacterial count at 5.95 Log CFU/g, while the T_4 sample recorded the lowest at 4.60 Log CFU/g. The binding of chitosan to bacterial membranes creates an impermeable barrier, which effectively eliminates vital nutrients required for bacterial proliferation. Increasing the concentration of chitosan within edible coatings enhances their antimicrobial characteristics by elevating the positive charge of amino groups, thereby fostering electrostatic interactions. This robust connection between chitosan and the microbial cell wall is further reinforced (Knorr, 1991; Helander *et al.*, 2001; Kong *et al.*, 2008). In this context, research conducted by Sharafati Chaleshtori *et al.* (2015) demonstrated that salmon samples coated with chitosan and lemon essential oil showed a marked decrease in bacterial populations, with higher essential oil concentrations leading to enhanced antibacterial effects. Additionally, control samples exhibited significantly greater total viable counts (TVC) compared to the coated samples (Pilmal *et al.*, 2018; Tingting *et al.*, 2013). Lactic acid bacteria constitute a significant component of the anaerobic microbial flora found in meat. Certain strains of these bacteria play a role in the spoilage of meat products (Jay *et al.*, 2005). The findings from the study indicated that the population of lactic acid bacteria increased during the refrigeration storage of fish, particularly in the control sample. Conversely, fish fillets treated with chitosan and sugar beet leaf extract exhibited a reduced presence of lactic acid bacteria. Notably, the control group recorded the highest concentration of these bacteria on the seventh day of storage, measuring 3.38 Log CFU/g, while the fourth treatment demonstrated the lowest concentration at 2.04 Log CFU/g. Remya *et al.* (2017) demonstrated that the combination of chitosan film, ginger essential oil, and an oxygen absorber effectively suppresses the proliferation of lactic acid bacteria in fish fillets (*Rachycentron canadum*) stored at 2°C. In the current investigation, the chitosan coating enriched with the highest concentration of extract exhibited superior efficacy in inhibiting the growth of these bacteria in sevruga fish fillets. The presence of Enterobacteriaceae in fishery products necessitates thorough examination, as this bacterial family encompasses pathogens like Salmonella, which pose significant health risks to consumers. Studies have indicated that fishing in contaminated waters, along with secondary pollution, contributes to the aquatic environment's contamination with Enterobacteriaceae (Shakhawat *et al.*, 2006; Papadopoulos *et al.*, 2003; Jeevanandam *et al.*, 2001). Analysis of the samples revealed that the levels of Enterobacteriaceae were relatively low in both the coated and control groups; however, the control group exhibited a significantly higher concentration. On the seventh day, end of the storage period, the lowest concentration of this bacterium was observed in the fourth treatment (T_4), while the control group recorded the highest level (1.60 Log CFU/g) on the same day. Research conducted by Nagarajan *et al.* (2021) demonstrated that the application of a chitosan-gelatin coating combined with *Lansium domesticum* (langsat) extract on black tiger shrimp stored at refrigeration temperatures for 20 days effectively reduces the proliferation of *Enterobacteriaceae*. The coated samples consistently showed lower bacterial counts compared to the uncoated samples, with none of the treatments exceeding 2 Log CFU/g. Furthermore, an increase in the extract concentration within the coating correlated with enhanced inhibition of bacterial growth. In this investigation, the quantification of *Pseudomonas* bacteria remained below 1 Log CFU/g throughout the storage duration across all experimental treatments. However, a noticeable increase in bacterial counts was recorded in all samples up to the seventh day. Notably, treatments incorporating sugar beet leaf extract exhibited lower *Pseudomonas* bacteria levels than the control group, which measured 0.84 Log CFU/g. Additionally, research by Jasour *et al.* (2014) assessed the impact of chitosan coating and the combination of chitosan with lactoperoxidase on the quality of rainbow trout. Their findings indicated that over a 16-day storage period, higher counts of *Pseudomonas* fluorescens were detected in samples lacking the chitosan coating. The findings indicate that sevruga fillets with coatings exhibit less *Pseudomonas* bacteria than their uncoated counterparts. Furthermore, the incorporating chitosan into the coatings enhances their antibacterial properties, effectively suppressing the proliferation of *Pseudomonas* in the fish fillets throughout the entire duration of refrigerated storage. *Clostridium perfringens* is not a naturally occurring bacterium in aquatic environments or organisms. The potential for contamination arises post-capture and during storage of aquatic products. This bacterium can be transmitted through aquatic animals exposed to sewage, leading to foodborne illnesses and diarrhea in humans (Hill *et al.*, 1996; Chattopadhyay, 2000). In the samples analyzed in this study, the concentration of *Clostridium perfringens* was found to be below 1 Log CFU/g, likely due to effective preservation methods employed after the fish were caught, which mitigated the risk of secondary contamination. Consistent with findings from similar studies, *Clostridium* species were either undetected or present in minimal quantities in the samples tested. A study involving 652 raw marine and cultured shrimp revealed that none of the samples contained sulfitereducing *Clostridium* (Mohammadi Golrang, 2004).

The sensory evaluation data analysis demonstrated that the coating applied to the sevruga fillets, combined with storage at 4°C, significantly affected the overall acceptance, color, texture hardness, flavor, and taste of the fillet samples on both the first and seventh days after production. Additionally, the findings suggested that neither the control sample nor the first treatment could be effectively described regarding color, flavor, taste, texture hardness, and overall acceptance, while the second, third, and fourth treatments exhibited notable variations influenced by these sensory attributes. The alterations in the color, taste, and flavor of meat are influenced by bacterial activity, oxidation processes, and the formation of volatile compounds. These modifications vary across different meat types and are attributed to multiple factors (Brannan & Mah, 2007). Its texture's hardness negatively impacted the second treatment, while the third treatment was significantly shaped by the aroma it produced, resulting in a favorable overall acceptance. Compared to other samples, the fourth treatment was influenced by a combination of attributes, i.e. taste, color, and overall acceptance, positioning it in second place following the third treatment sample. Numerous studies have demonstrated that applying chitosan coatings and chitosan in conjunction with various extracts and essential oils has enhanced sensory characteristics compared to control groups (Shokri *et al.*, 2020; Chamanara *et al.*, 2015). The influence of the trigeminal gland on assessors' evaluations of specimens is significant, as color may affect both flavor perception and overall acceptance. This consideration should be integrated into the treatment of samples and the application of hurdle techniques.

Conclusion

While no significant differences were observed in the measurements of pH, acidity, peroxide value (PV), Free fatty acids (FFA), and thiobarbituric acid (TBARs), it was noted that the uncoated samples exhibited higher levels of these parameters when compared to the fillets treated with chitosan and sugar beet leaf extract. On the final day of storage, the fillets coated with chitosan that contained the highest concentration of extract (T_4) showed greater effectiveness in reducing the increase in most of the evaluated parameters, with the exception of the pH index, which was lower in the second treatment. Importantly, T_4 significantly improved the control of total volatile basic nitrogen (TVB-N) levels in comparison to the other experimental groups; moreover, the bacterial count was also lower in this group, suggesting its efficacy in inhibiting bacterial proliferation. Additionally, T_4 received higher sensory acceptance ratings from evaluators than the other treatments.

References

Abdollahi, M., Rezaei, M. & Farzi, G. (2014). Influence of chitosan/clay functional Bionano-composite activated with rosemary essential oil on the shelf life of fresh silver carp. International Journal of Food Science and Technology, 49(3), 811–818. http://dx.doi.org/10.1111/ijfs.12369

Asadi, Z. & Khan, M. A. (2021). The effect of beetroot *(Beta vulgaris L.)* leaves powder on nutritional, textural, sensorial and antioxidant properties of cookies. Journal of Culinary Science & Technology, 19(5), 1-5. http://dx.doi.org/10.1080/15428052.2020.1787285

Barbosa, C., Vilarinho, F., Andrade, M., Sanches Silva, A. & Fernando, A. L. (2023). The influence of cultivated cardoon and globe artichoke ethanolic leaf extracts on the shelf life of poultry meat. 3rd Food Chemistry Conference: Shaping a healthy and sustainable food chain through knowledge. 10-12. http://hdl.handle.net/10400.18/9159

Bautista-Banos, S., Hernandez-Lauzardo, A. N., Velazquez-del Valle, M. G., Hernandez-Lopez, M., Ait Barka, E., Bosquez-Molina, E. & Wilson, C. L. (2006). Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. Crop Protection, 25, 108-118. https://doi.org/10.1016/j.cropro.2005.03.010

Berizi, E., Hosseinzadeh, S., Shekarforoush, S. Sh. & Barbieri, G. (2018). Microbial, chemical, texturnal and sensory peroperties of coated rainbow trout by chitosan combined with pomegranate peel extract during frozen storage. Int.J. Biol. Macromol, 106, 1004 - 1013. http://dx.doi.org/10.1016/j.ijbiomac.2017.08.099

Brannan, R. G. & Mah, E. (2007). Grape seed extract inhibits lipid oxidation in muscle form different species during refrigerated and frozen strage and oxidation catalyzed by peroxynitrite and iron/ ascorbate a pyrogallol red model system. Meat Science, 77, 540-546. https://doi.org/10.1016/j.meatsci.2007.05.001

Burt, S. (2004). Essential oils: their antibacterial properties and potential aplications in foods-a review. International journal of food microbiology, 94(3), 223-253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Celi, P. (2010). The role of oxidative stress in small ruminants' health and production. Revista Brasileria De Zootecnia, 39, 348-363. http://dx.doi.org/10.1590/S1516-35982010001300038

Ceylan, Z., Uslu, E., Ispirli, H., Meral, R., Gavgali, M., Yilmaz, M. T. & Dertli, E. (2019).

A novel perspective for lactobacillus reuteri: nanoencapsulation to obtain functional fish fillets. LWL-Food Scitechnol, 115, 108427. http://doi.org/10.1016/j.lwt.2019.108427

Chamanara, V., Farhoudi, A. & Ahmadi, A. (2015). Effects of chitosan coating on the quality of rainbow trout fillet during storage in refrigerator. Persian Journal of Seafood Science and Technology, 1, 12-15. https://www.fd-science.com

Chattopadhyay, P. (2000). Fish–catching and handling, In: Robinson R.K. (ed.): Encyclopedia of Food Microbiology. 2, Academic Press, London, 1547.

Coma, V., Martial-Gros, A., Garreau, S., Copinet, A., Salin, E. & Deschamps, A. (2002). Edible antimicrobial films based on chitosan matrix. Journal of Food Science, 67(3), 1162-1169. https://doi.org/10.1111/j.1365-2621.2002.tb09470.x

Connell, J. J., editor. (1990). Methods of assessing and selecting for quality, in: Connel, J. J., Control of fish quality. 3rd edition, Oxford: Fishing news books, 50-122.

Del Rio, D., Stewart, A. J. & Pellegrini, N. (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutrition, Metabolism and Cardiovascular Diseases, 15(4), 316-328. https://doi.org/10.1016/j.numecd.2005.05.003

Estaca, J. G., Montero, P., Gimenez, B. & Guillen, M. C. G. (2007). Effect of functional Edible films and high pressure processing on Microbial and oxidative spoilage in cold-smoked sardine *(Sardina*

Pilchardus). food chemistry, 105(2), 511-520. http://dx.doi.org/10.1016/j.foodchem.2007.04.006

Esua, O. J., Cheng, J. H. & Sun, D.W. (2021). Functionalization of water as a nonthermal approach for ensuring safety and quality of meat and seafood products. Critical Reviews in Food Science and Nutrition, 61(3), 431-449. https://doi.org/10.1080/10408398.2020.1735297

Fadiloglu, E. E. & Coban, O. E. (2018). Effect of chitosan edible coating enriched with sumac on the quality and the shelf life of rainbow trout *(Oncorhynchus mykiss, Walbaum, 1792)* fillets. Journal of Food Safety, 38(2). http://dx.doi.org/10.1111/jfs.12545

FAO. (2018). FAO yearbook. Rome: Fishery and Aquaculture Statistics 2016. http://www.fao.org/fishery/static/Yearbook/YB2016_USBcard/index.htm

Farsanipour, A., Khodanazary, A. & Hosseini, S. M. (2020). Effect of chitosan-whey protein whey protein isolated coatings incorporated with tarragon Artemisia dracunculus essential oil on the quality of *Scomberoides commersonnianus* fillets at refrigerated condition. International Journal of Biological Macromolecules, 155, 766-771. https://doi.org/10.1016/j.ijbiomac.2020.03.228

Feng, X., N. g, V. K., Mikš-Krajnik, M. & Yang, H. (2017), Effects of fish gelatin and tea polyphenol coating on the spoilage and degradation of myofibril in fish fillet during cold storage, Food and Bioprocess Technology, 10(1), 89-102. https://link.springer.com/article/10.1007/s11947-016-1798-7

Fraqueza, M. J., Ferreira, M. C. & Barreto, A. S. (2008). Spoilage of light (PSE-like) and dark turkey meat under aerobic or modified atmosphere package: Microbial indicators and their relationship with total volatile basic nitrogen. British Poultry Science, 49(1), 12–20. http://dx.doi.org/10.1080/00071660701821675

Gram, L. & Delgaard, P. (2002). Fish spoilage bacteria-problems and solutions. Current opinion in biotechnology, 13(3), 262-266. https://doi.org/10.1016/S0958-1669(02)00309-9

Hamedi, H., Kargozari, M., Mahasti-shotorbani, P., Moghadam, N. B. & Fahidmanesh, M. (2017). A novel bioactive edible coating based on sodium alginate and galbanum gum incorporated with essential oil of Ziziphora persica: The antioxidant and antimicrobial activity, and application in food model. Food Hydrocolloids, 72, 35-46. http://dx.doi.org/10.1016/j.foodhyd.2017.05.014

Helander, I. M., Nurmiaho-Lassila, E. L., Ahvenainen, R., Rhoades, J. & Roller, S. (2001). Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria. International J Food Microbiology, 71(2-3), 235-244. https://doi.org/10.1016/s0168-1605(01)00609-2

Hernández-Herrero, M. M., Roig-Sagués, A. X., López-Sabater, E. I., Rodríguez-Jerez, J.J. & MoraVentura, M.T. (1999). Influence of storage temperature on the quality of beef liver; pH as a reliable indicator of beef liver spoilage. Journal of the Science of Food and Agriculture, 79(14), 2035–2039. https://doi.org/10.1002/(SICI)1097-0010(199911)79:14%3C2035

Hill, R. T., Straube, W. L., Palmisano, A. C., Gibson, S. L. & Colwell, R. R. (1996). Distribution of sewage indicated by *Clostridium perfringens* at a deep-water disposal site after cessation of sewage disposal. Applied and Environmental Microbiology, 62(5), 1741–1746.

https://doi.org/10.1128/aem.62.5.1741-1746.1996

Indu, R., Adhikari, A., Ray, M., K.Hazra, A., K.Sur, T. & Kumar Das, A. (2017). Antioxidant properties of polyphenolic rich HPLC standardized extract of *Beta vulgaris L.* roots. Journal of Research, 6(3), 2619-2624. http://dx.doi.org/10.21276/IJRDPL.2278-0238.2017.6(3).2619-2624

International Commission on Microbiological Specifications for Foods. (1986). Sampling Plans for Fish and Shellfish. In: Microorganisms in Foods. Sampling for Microbiological Analysis: Principles and Scientific Applications. 2: 2nd Edition, University of Toronto Press, Toronto, 181-196.

Izadi, S., Honarvar, M. & Mirzaei, H. (2021). Investigation of adding antioxidant compounds extracted from sugar beet leaves by ultrasonic method on oxidative stability of soybean oil. FSCT, 18(118), 285-296. http://fsct.modares.ac.ir/article-7-52843-fa.html

Jafari, A., Hosseini, S. M. & Khodanazary, A. (2023). Effect of chitosan coating and galicacid on quality properties of common carp *(Cyprinus carpio)* fillet during storage at refrigerator. Journal of Oceanography, 13(52), 121-131. http://dorl.net/dor/20.1001.1.15621057.1401.13.52.9.4

Jamali, S., Pajohi-Alamoti, M., Sari, A. & Aghajani, N. (2023). Use of Aloe Vera-based Edible Coating Containing Nanoemulsion of Ginger Essential Oil to Extend Trout Fillet Shelf-Life. Iranian Journal of Nutrition Sciences & Food Technology, 18(1), 93-108. http://dorl.net/dor/20.1001.1.17357756.1402.18.1.8.5

Jasour, M. S., Ehsani, A., Mehryarc, L. & Naghibi, S. S. (2014). Chitosan coating incorporated with the lactoperoxidase system, an active edible coating for fish preservation. Journal of the Science of Food and Agriculture, 95(6), 1373-1378. https://doi.org/10.1002/jsfa.6838

Jay, J. M., Loessner, M. J. & Golden, D. A. (2005). Modern Food Microbiology. New York: NY, Springer, 63-91. https://rdcu.be/dVh8Q

Jeevanandam, K., Kakatkar, A., Doke, S. N., Bongirwar, D. R. & Venugopal, V. (2001). Influence of salting and gamma irradiation on the shelf-life extension of threadfin bream in ice. J.Food Res, INT, 34(8), 739-746. http://dx.doi.org/10.1016/S0963-9969(01)00100-4

Jouki, M., Yazdi, F. T., Mortazavi, S. A., Koocheki, A. & Khazaei, N. (2014). Effect of quince seed mucilage edible films incorporated with oregano or thyme essential oil on shelf life extension of refrigerated rainbow trout fillets. International Journal of Food Microbiology, 174, 88-97. https://doi.org/10.1016/j.ijfoodmicro.2014.01.001

Kalteh, S., Alizadeh doughikollaee, E. & Yousef elahi, M. (2015). Effect of edible gelatin coating on the quality of fish finger of *Hypophthalmichthys molitrix* during refrigerated storage. FSCT, 212(48), 79-88. http://fsct.modares.ac.ir/article-7-879-en.html

Kayashima, T. & Katayama, T. (2002). Oxalic acid is available as a natural antioxidant in some systems. Biochem Biophys Acta, 10, 1573(1), 1-3. https://doi.org/10.1016/s0304-4165(02)00338-0

Khorshidi Sedehi, S. & Shabanipour, N. (2018). The survey of cellular sequence of the retina structure of the Acipenser stellatus eye. Journal of Animal Research, 31(2), 145-152.

https://dorl.net/dor/20.1001.1.23832614.1397.31.2.3.1

Kim, D. Y., Park, S. W., & Shin, H. S. (2023). Fish Freshness Indicator for Sensing Fish Quality during Storage. Foods, 12(9), 1801. https://doi.org/10.3390/foods12091801

Kirk, R. S. & Sawyer, R. (1991). Pearson's Composition and Chemical Analysis of Foods. 9th edition, Longman Scientific and Technical, Harlow, Essex, UK. https://librarysearch.northumbria.ac.uk/permalink/f/1t01hd3/44UON_ALMA2129278920003181

Kim, S.Y., Jeong, S. M., Park, W. P., Nam, K. C., Ahn, D. U. & Lee, S. C. (2006). Effect of Heating Conditions of Grape Seeds on The Antioxidant Activity of Grape Seed Extracts. Food Chemistry, 97(3), 472-479. http://dx.doi.org/10.1016/j.foodchem.2005.05.027

Knorr, D. R. (1991). Ecovering and utilization of chitin and chitosan in food processing waste management. Journal of Food Technology, 45, 114-122. https://doi.org/10.4236/as.2013.410076

Kong, M., Chen, X. G., Liu, C. S., Yu, L. J., Ji, Q. X., Xue, Y. P., Cha, D. S. & Park, H. (2008). Preparation and antibacterial activity of chitosan microspheres in a solid dispersing system. Journal of Frontiers of Materials Science in China, 2(2), 214-220. http://dx.doi.org/10.1007/s11706-008-0036-2

Korkmaz, F., Kocaman, E. M. & Gonca, A. L. A. K. (2019). Using of quinoa based film to extend the shelf life of rainbow trout fillets under cold storage (4±1 °C) condition. Marine Science and Technology Bulletin, 8(2), 76-84. https://doi.org/10.33714/masteb.651262

Kucukgulmez, A. (2012). Effect of chitosan on the shelf life of marinated sardine *(Sardina pilchardus)* fillets during refrigerated storage. Journal of Italian Animal Science, 11(3), 262-265. http://dx.doi.org/10.4081/ijas.2012.e48

Kykkidou, S., Giatrakou, V., Papavergou, A., Kontominas, M. G. & Savvaidis, I. N. (2009). Effect of thyme essential oil and Packaging treatments on fresh Mediterranean swordfish fillets during storage at 4°C. Food Chemistry, 115, 169 -175. https://doi.org/10.1016/j.foodchem.2008.11.083

Latorre, M. E., Bonelli, P. R., Rojas, A. M. & Gerschenson, L. N. (2012). Microwave inactivation of red beet *(Beta vulgaris L. Varconditive)* peroxidise and polyphenoloxidase and the effect of radiation on vegetable tissue quality. Journal of Food Engineering, 109(4), 676_684. http://dx.doi.org/10.1016/j.jfoodeng.2011.11.026

Lan, W., Zhou, M., Zhang, B., Liu, Sh., Yan, P. & Xie, J. (2024). Effect of chitosan-gentianic acid derivatives on the quality and shelf life of seabass *(Lateolabrax japonicus)* during refrigerated storage. International Journal of biological Macromolecules, 274 (Pt1), 133276. https://doi.org/10.1016/j.ijbiomac.2024.133276

Li, T., Sun, X., Chen, H., He, B., Mei, Y., Wang, D. & Li, J. (2020). Effect of the Combination of Vanillin and Chitosan Coating on the Microbial Diversity and Shelf-Life of Refrigerated Turbot *(Scophthalmus maximus)* Filets. Frontiers in Microbiology, 11, 462. https://doi.org/10.3389%2Ffmicb.2020.00462

Lykkesfeldt, J. (2001). Determination of Malondialdehyde as Dithiobarbituric Acid Adduct in Biological Samples by HPLC with Fluorescence Detection:

Comparison with Ultraviolet-Visible Spectrophotometry. Clinical Chemistry, 47(9), 1725-1727. http://dx.doi.org/10.1093/clinchem/47.9.1725

Lykkesfeldt, J. (2007). Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking. Clinica chimica acta, 380(1-2), 50-58. https://doi.org/10.1016/j.cca.2007.01.028

Martinsdóttir, E., Sveinsdottir, K., Luten, J. & Schelvis-Smit, R. (2001). Sensory evaluation of fish freshness, Grethe Hyldig QIM eurofish.

Mohammadi Golrang, P. (2004). Identification of biological hazards and determination of the critical control points in processing of exported shrimp of Iran. Thesis, School of Veterinary Medicine, Tehran University.

Nagarajan, M., Rajasekaran, B., Benjakul, S. & Venkatachalam., K. (2021). Influence of chitosan-gelatin edible coating incorporated with longkong pericarp extract on refrigerated black tiger Shrimp *(Penaeus monodon)*. Current Research in Food Science, 4, 345-353. https://doi.org/10.1016/j.crfs.2021.05.003

Naghibi, S., Ehsani, A., Tajik, H., Talebi, A. & Delirezh, N. (2016). Effect of chitosan enriched with lycopene coating on fatty acid profile and fat oxidation parameters of rainbow trout fillet during refrigerated storage. Journal of Food Hygiene, 6(21), 29-44.

Nirmal, N. P. & Benjakul, S. (2011). Use of tea extracts for inhibition of polyphenoloxidase and retardation of quality loss of Pacific white shrimp during iced storage. LWT Food Science and Technology, 44(4), 924-932. http://dx.doi.org/10.1016/j.lwt.2010.12.007

Nowzari, F., Shabanpour, B. & Ojagh, S. M. (2013). Comparison of chitosan–gelatin composite and bilayer coating and film effect on the quality of refrigerated rainbow trout. Food Chemistry, 141(3), 1667-1672. http://dx.doi.org/10.1016/j.foodchem.2013.03.022

Ojagh, S. M., Rezaei, M., Razavi, S. H. & Hosseini, S. M. H. (2012). Effect of antimicrobial coating on shelf-life extension of rainbow trout *(Oncorhynchus mykiss)*. FSCT, 9(34), 13-23. http://fsct.modares.ac.ir/article-7-6776-fa.html

Olatunde, O. O., Benjakul, S. & Vongkamjan, K. (2019). Combined effects of high voltage cold atmospheric plasma and antioxidants on the qualities and shelf-life of Asian sea bass slices. Innovative Food Science and Emerging Technologies, 54, 113-122. https://doi.org/10.1016/j.ifset.2019.03.012

Öz, M. & Uçak, İ. (2023). Investigation of quality parameters of common carp (*Cyprinus carpio*) meat marinated with traditional method used in Anatolia during storage at − 18°C. International Journal of Gastronomy and Food Science, 33, p.100755. https://doi.org/10.1016/j.ijgfs.2023.100755

Ozogul, Y., Ozogul, F., Kuley, E., Ozkutuk, A. S., Gokbulut, C. & Kose, S. (2006). Biochemical Sensory and microbiological attributes of wild turbot *(Scophthalmus maximus)*, from the Black sea, During chilled storage. Food chemistry, 99(4), 752-758. http://dx.doi.org/10.1016/j.foodchem.2005.08.053

Ozogul, Y. & Uçar, Y. (2013). The effects of natural extracts on the quality changes of frozen chub mackerel *(Scomber japonicus)* burgers. Food Bioprocess Technology, 6(6), 1550-1560. http://dx.doi.org/10.1007/s11947-012-0794-9

Özyurt, G., Kuley, E., Özkütük, S. & Özogul, F. (2009). Sensory, microbiological and chemical assessment of the freshness of red mullet *(Mullus barbatus)* and goldband goatfish *(Upeneus moluccensis)* during storage in ice. Food chemistry, 114(2), 505-510. https://doi.org/10.1016/j.foodchem.2008.09.078

Papadopoulos, V., Chouliara, L., Badeka, A., Savvaidis, I. N. & Kontominas, M. G. (2003). Effect of gutting on microbiological, and sensory properties of aquacultured sea bass *(Dicentrarchus labrax)* stored in ice. Food Microbiol, 20(4), 411-420. http://dx.doi.org/10.1016/S0740-0020(02)00148-X

Pezeshk, S., Rezaei, M. & Hosseini, H. (2011). Effects of turmeric, shallot extracts, and their combination on quality characteristics of vacuum-packaged rainbow trout stored at 4±1 °C. Journal of food science, 76(6), 387-391. http://dx.doi.org/10.1111/j.1750-3841.2011.02242.x

Pilmal, M., Alizadeh Doughikollaee, E. & Yousef Elahi, M. (2018). Effect of Edible Chitosan Coating Containing Cinnamon Essential Oil on the Shelf Life of Silver Carp Fish Finger during Refrigerated Storage. Journal of Fisheries, 71(3), 294-305. https://doi.org/10.22059/jfisheries.2019.267297.1053

Pirouz Zarrin, Y., Abdolahi Cheleh bary, Z., Khaki Arani, S., Hashemi Kochaksaraei, H., Moulaei, S., Jamshidi Tehraniyan, M. & Latifi, Z. (2023). The effect of chitosan-based edible coating containing *Dunaliella* algae extract on the qualitative and microbial characteristics of Beluga Sturegeon *(Huso huso)* fillet during the storage period. Journal of applied microbiology in food industry, 9(2), 62-78. http://amfi.ir/article-1-60-en.html

Pourkargar, H. & Rafati, A. (2020). Effect of edible Chitosan coating containing *Mentha piperita L.* essence on shelf life of Rainbow Trout fillet *(Oncorhynchus mykiss)* during storage in refrigerator temperature (4±1°C). J. Aqua, 10(1), 53-66. https://sid.ir/paper/950215/en

Prabhakar, P. K., Srivastav, P. P. & Pathak, S. S. (2019). Kinetics of total volatile basic nitrogen and trimethylamine formation in stored rohu *(Labeo rohita)* fish. Journal of Aquatic Food Product Technology, 28(5), 452-464. http://dx.doi.org/10.1080/10498850.2019.1604598

Rathod, N. B., Ranveer, R. C., Bhagwat, P. K., Fatih, O., Sottawat, B., Santhosh, P. & Uday Shriramrao, A. (2021). Cold plasma for the preservation of aquatic food products: An overview. Compr Rev Food Sci Food Saf, 1–19. https://doi.org/10.1111/1541-4337.12815

Remya, S., Mohan, C. O., Venkateshwarlu, G., Sivaraman, G. K. & Ravishankar, C. N. (2017). Combined effect of O2 scavenger and antimicrobial film on shelf life of fresh cobia *(Rachycentron canadum)* fish steaks stored at 2 °C. Food Control, 71(2), 71-78. http://dx.doi.org/10.1016/j.foodcont.2016.05.038

Rezaeifar, M., Mehdizadeh, T., Mojaddar Langroodi, A. & Rezaei, F. (2020). Effect of chitosan edible coating enriched with lemon verbena extract and essential oil on the shelf life of vacuum rainbow trout *(oncorhynchus mykiss)*. Journal of Food Safety, 40(3), e12781. https://doi.org/10.1111/jfs.12781

Sabu, S., Ashita, T. & Stephy, S. (2020). Chitosan and lemon peel extract coating on quality and shelf life of yellowfin tuna *(Thunnus albacares)* meat stored under refrigerated condition. Indian J. Fish, 67(1), 114-122. http://dx.doi.org/10.21077/ijf.2019.67.1.91361-15

Sadeghi, M., Arshadi, A., Mirdar Harijani, J. & Haddadi, F. (2020). Antioxidant activity of chitosan edible coating enriched with aqueous extract of Withania coagulans fruit on chemical changes and sensory properties of silver carp *(Hypophthalmichthys molitrix)* stored in refrigerator. Journal of Fisheries, 72(4), 363-374. https://doi.org/10.22059/jfisheries.2020.75190

Saini, R. K., Prasad, P., Sreedhar, R.V., Akhilender Naidu, K., Shang, X. & Keum, Y.S. (2021). Omega 3polyunsaturated fatty acids (PUFAs): Emerging plant and microbial sources, oxidative stability, bioavailability, and health benefits-A review. Antioxidants, 10(10), 1627. https://doi.org/10.3390/antiox10101627

Sathivel, S. (2005). Chitosan and Protein Coatings Affect Yield, Mois ture Loss, and Lipid Oxidation of Pink Salmon *(Oncorhynchus gorbuscha)* Fillets during Frozen Storage. Journal of Food Science, 70(8), e455-e459. https://doi.org/10.1111/j.1365-2621.2005.tb11514.x

Salami, P., Mooraki, N. & Honarvar, M. (2021). Optimizing of *Eruca sativa* (khandal) Extraction by Microwave Method and its Application in Marinated *Huso huso* (Beluga) Fillet. Food Technology & Nutrition, 18(4), 53-70. https://srb.sanad.iau.ir/en/Article/832536

Sebti, I., Martial-Gros, A., Carnet-Pantiez, A., Grelier, S. & Coma, V. (2005). Chitosan polymer as bioactive coating and film against *Aspergillus niger* contamination. Journal of Food Sciences, 70, 100-104. https://doi.org/10.1111/j.1365-2621.2005.tb07098.x

Sener, G., Sacan, O., Yanardag, R. & Ayanoglu-Dulger, G. (2002). Effect of chard *(Beta vulgaris L. var.cicla)* extract on oxidative injury in the aorta and heart of streptozotocin-diabetic rats. J Med Food, 5(1), 37-42. https://doi.org/10.1089/109662002753723205

Shakhawat, H. M., Akter-Uzzaman, M., Afzal-Hossain, M. & Shamsul-Islam, M. (2006). Effect of gamma Irradiation and vacuum packaging on the shelf life extension of beef kebab during refrigerated storage. J.Bangladesh microbial, 23(2), 156-158. http://dx.doi.org/10.3329/bjm.v23i2.883

Sharafati-chaleshtori, R., Taghizadeh, M., Miri, S., Asadi, Z., Abdipor, M. & Shiri, V. (2015). The effect of chitosan coating contained lemon essential oil on microbial quality of rainbow trout. JFM, 2(2), 7-19. https://sid.ir/paper/250674/fa

Shoja, B., Mottalebi, A., Akbari-Adergani, B. & Rokni, N. (2023). The Effect of Chitosan Coating Enriched with Orange Peel *(Citrus Sinensis)* Waste Extract on Extending Preservation and Chemical and Functional Properties of Refrigerated Beluga Sturgeon *(Husohuso)* Fillet. Journal of Veterinary and Marine Research, 3(1), 1-9. https://10.32592/ARI.2024.79.4.711

Shokri, S., Parastouei, K., Taghdir, M. & Abbaszadeh, S. (2020). Application an edible active coating based on chitosan-Ferulago angulata essential oil nanoemulsion to shelf life extension of Rainbow trout fillets stored at 4 °C. International Journal of Biological Macromolecules, 153, 846-854. http://dx.doi.org/10.1016/j.ijbiomac.2020.03.080

Souza, B. W. S., Cerqueira, M. A., Ruiz, H. A., Martins, J. T., Casariego, A., Teixeira, J. A., Vicente, A. A. (2010). Effect of chitosan_based containing on the shelf life of salmon *(Salmo salar)*. J Food Chem, 58(21), 11456-62. https://doi.org/10.1021/jf102366k

Summers, G., Wibisono, R. D., Hedderley, D. I. & Fletcher, G. C. (2017). Trimethylamine oxide content and spoilage potential of NewZealand commercial fish species. NewZealand Journal of Marine and Freshwater Research, 51(3), 393-405. http://dx.doi.org/10.1080/00288330.2016. 1250785

Talebi Haghgo, M., Mooraki, N. & Honarvar, M. (2024). Effect of chitosan coating containing sugar beet leaves *(Beta vulgaris L.)* obtained extraction by Microwave method on chemical, microbial and sensory properties of fish stellate *(Acipenser stellatus)* fillet in refrigerated. M.Sc. Thesis, North Tehran Branch, Islamic Azad University.

Terefe, Z. K. (2017). Effect of ginger *(Zingiber officinale)* powder addition on pH, titratable acidity and total viable bacterial counts of minced meat under refrigerated storage. Inernational Journal of Food Science and Nutrition Engineering, 7(2), 38-42. https://10.5923/j.food.20170702.03

Tingting, L., Jianrong, L., Wenzhong, H. & Xuepeng, L. (2013). Quality enhancement refrigerated red drum *(Sciaenops ocellatus)* fillets using chitosan coating containing natural preservatives. J Food Chem, 138(2-3), 821-826. https://doi.org/10.1016/j.foodchem.2012.1 1.092

Tunali, T., Yarat, A., Yanardag, R., Ozcelik, F., Ozsoy, O., Ergenekon, G. & Emekli, N. (1998). The effect of chard *(Beta vulgaris L. var. cicla)* on the skin of streptozotocin induced diabetic rats. Pharmazie, 53(9), 638-640. https://europepmc.org/article/med/977021 2

Ucak, I. & Afreen, M. (2022). Effect of Chitosan Coating Enriched with Peppermint Essential Oil Emulsion on the Microbiological Quality of Fish Meatballs. Eurasian Journal of Food Science and Technology, 6(1), 60-68. https://orcid.org/0000-0002-9701-0824

Valipour Kootenaie, F., Ariaii, P., Khademi Shurmasti, D. & Nemati, M. (2017). Effect of Chitosan Edible Coating Enriched with Eucalyptus Essential Oil and α-Tocopherol on Silver Carp Fillets Quality During Refrigerated Storage. Journal of Food Safety, 37(1), e12295. https://doi.org/10.1111/jfs.12295

Vasconez, M. B., Flores, S. K., Campos, C. A., Alvarado, J. & Gerschenson, L. N. (2009).

Antimicrobial activity and physical properties of chitosan-tapioca starch based edible films and coatings. Food research international, 42(7), 762-769. http://dx.doi.org/10.1016/j.foodres.2009.0 2.026

Vieira, B. B., Mafra, J. F., Da Rocha Bispo, A. S., Ferreira, M. A., De Lima Silva, F., Rodriguse, A.V. N. & Evangelista-Barreto, N. S. (2019). Combination of chitosan coating and clove essential oil reduces lipid oxidation and microbial growth in frozen stored tambaqui *(Colossoma macropomum)* fillets. LWT, 116, 108546. http://dx.doi.org/10.1016/j.lwt.2019.10854 6

Yetim, H., Kayacier, A., Kesmen, Z. & Sagdic, O. (2006). The effect of nitrite on the survival of *Clostridium sporogenes* and the autoxidation properties of the kavurma. Meat Science, 72(2), 206-210. https://doi.org/10.1016/j.meatsci.2005.07.0 02