Original Article

Effect of Methanolic Extract from the Multicellular Alga *Caulerpa sertularoides* on Qualitative and Sensory Properties of Rainbow Trout Minced Meat at 4 ± °C

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Abstract

This study aimed to determine the percentage of antioxidant activity and to evaluate a methanolic extract from the multicellular alga *Caulerpa sertularoides* and its application on the qualitative and sensory characteristics of *Oncorhynchus mykiss* minced meat at 4 ± °C. According to the percentage of antioxidant activity of the algal methanolic extract, five groups of *O. mykiss* minced meat were prepared including a control group and samples containing 0.5 and 1% of the algal extract and then tested on days 1, 7, and 14. Results showed that pH levels decreased significantly in the control sample over time ($P < 0.05$) and the samples containing the algal extract showed the highest stability in pH on day 7, which significantly prevented pH changes on day 14 ($P < 0.05$). Similar to pH, the extract effects were also assessed on changes in acidity. The use of 1% *C. sertularoides* extracts significantly inhibited peroxide production on days 7 and 14. The amount of TVB-N showed no significant changes between experimental groups, but its numerical value was lower (1%) in the sample containing the algal extract. The TBARS index increased significantly in the control sample compared to the treatment groups ($P < 0.05$). The values of crude fat and moisture content in the samples did not show significant changes in the experimental days ($P > 0.05$). Considering the benefits of natural antioxidants, the availability, and the low cost of containing sources, as well as the proven disadvantages of synthetic antioxidants, it is recommended to take necessary measures to replace natural antioxidants.

Keywords: Antioxidant, *Caulerpa sertularoides*, *Oncorhynchus mykiss* minced meat, Methanolic extract

Introduction

The production of various fish meat products is rapidly growing and gaining a high diversity in many countries. Reasons for the rapid growth of fish consumption can be attributed to increased people's awareness of the nutritional value of aquatic animals as health foods, increasing attention to the use of ready-to-eat or ready-to-cook foods in homes in terms of saving time, developing technology, possible production of ready-to-eat foods according to the tastes of consumers, and encouraging them to use such processed foods. Also, the access to low-cost raw material, the use of suitable formulation, and modern equipment have provided consumers with possible production of foods with high taste and nutritional value [1]. Along with the efforts of experts and officials of the fisheries sector to increase aquaculture production and achieve comparable success in this field in recent years, there has been no significant and appropriate growth for the production of desirable foods and improvement of methods for aquaculture product consumption in Iran.

One of the problems that always occur during the storage of fish and its products is enzymatic and non-enzymatic rancidity, acting as one of the main factors affecting the shelf life of seafood and their spoilage. This is called rancidity and Cold Storage Flavors in fatty fish and low-fat fish, respectively [2].

The main reasons for this issue are the high levels of long-chain polyunsaturated fatty acids (PUFAs) in the fat structure of marine organisms and the molecules intensifying oxidation in the muscles of these species. The unsaturated bonds in these products, and generally the unsaturated bonds of all fats and oils, form active centers that may react with oxygen. This reaction leads to the formation of primary and secondary oxidation products such as aldehydes, organic acids,

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ketoglycerides, and hydroxyglycerides, which may make fat or high-fat foods unfavorable to consumers. Due to the tendency of consumers to use foods without preservatives and chemical additives, researchers are seeking ways to improve the quality and shelf life of fishery products without adding such substances. In this regard, many efforts have been made to find natural antioxidants from plant sources to replace synthetic antioxidants (e.g. BHA, BHT, or TBHQ) to delay the oxidation of fats. One of these strategies is to use the extracts and essential oils of marine and medicinal plants in fishery products, especially fish meat. Some natural food preservatives are extracts or essences, known as essential oils, as antioxidant additives. An extract is a substance obtained from a compound in a plant or fruit through distillation or squeezing or boiling with the help of a solvent such as water or alcohol. An extract is a substance containing active plant ingredients that creates healing properties in medicinal plants.

The development of seafood production requires paying attention to required technical knowledge and training skilled manpower on the one hand, and taking account of product quality control by producers and the supervision of government organizations to gain the trust of consumers for the use of these products. In recent years, natural antioxidants have generally gained a high interest due to human health, which not only lacks the side effects of synthetic antioxidants such as those remaining in products, but their consumption can maintain and ensure more health for humans.

Therefore, there is increasing attention to the use of medicinal plants as valuable sources of natural antioxidants. Natural antioxidants can be used in boneless fish meat to increase the shelf life and optimize sensory properties through the reduction of fat oxidation.

Accordingly, this study aimed to determine the percentage of antioxidant activity and the effect of methanolic extract from the multicellular alga *C. sertularoides* to assess its application on the qualitative and sensory properties of *O. mykiss* minced meat at 4 ± 1 °C.

Materials and Methods

The green alga *Caulerpa sertularoides* collected from its abundance depths of up to 6 m in the southern coasts of the Persian Gulf region (Bushehr province) were purchased from the Fars Algae Reserves Co.

Experimental groups were a control sample (C) containing minced meat with no extract, sample A with minced meat (100 g) + 1 g of dried algal extract, and sample B with minced meat (100 g) + 0.5 g of algal extract. Samples were prepared in three replications, placed in sterile plates, and then refrigerated at 4 ± 1 °C for 14 days. For oxidative analyses, samples were harvested based on the time table. To add the extract, a certain amount of the extract was dissolved in a given amount of distilled water (weight ratio of water to minced meat: 1 to 10) and homogenized completely with 100 g of sample meat; only distilled water was added to samples in the control group. The above process was performed under GMP (Good Manufacturing Production) conditions, that is, production in hygienic conditions with hazard analysis and critical control points (HACCP).

Chemical tests on the experimental samples

1. Measurement of pH (H^+ concentration) was performed according to the national standard of Iran (No. 1028-1386) [3].
2. The percentage of acidity was measured by the titration method.
3. The amount of peroxide (mEq per kg of fat) was calculated according to the following equation:

$$\boldsymbol{PV} = \frac{\boldsymbol{S}\times\boldsymbol{N}\times\mathbf{1000}}{\boldsymbol{W}} \qquad (1)$$

where S is consumed volume of thiosulfate, N is sodium thiosulfate normality, and W is sample weight.

4. The TVB-N value is calculated after the titration

$$x = \frac{(V1-V2)*C*A}{m*5/100} \times 100 \qquad (2)$$

5. In this study, TBARS was measured by the colorimetry method and calculated based on the following equation (National Standard of Iran, No. 10494-1383) [4]:

$$\boldsymbol{TBARS} = \frac{\textbf{(Sample absorption}-\textbf{blank absorption)}\times\mathbf{500}}{\mathbf{200}} \qquad (3)$$

6. Soxhlet method was used to measure fat content.
7. Moisture content was calculated according to the following formula:

$$\text{Moisture content } (\%) = \frac{M1-M2}{M0} \times 100 \qquad (4)$$

8. Sensory evaluation was performed based on measuring the acceptance of samples using 5-point forms (National Standard of Iran, No. 3580-1374) [5]. The prepared samples were evaluated separately by seven relatively trained assessors (lovers of aquatic animal taste) in terms of odor, taste, appearance, texture, and color indices.

Antioxidant activity of algae extracts

Antioxidant activity (AA) of algae extracts was assessed based on the inhibitory activity of DPPH (2,2-diphenyl-1-picril hydazyl) free radical.

$$\text{Free radical neuralization } (\%) = \frac{\textbf{(Sample absorption)}}{\textbf{Blank absorption}} \times \mathbf{100} \qquad (5)$$

The percentage of antioxidant activity was determined using 5, 10, and 25 mg/ml from the algae separately.

Statistical analysis

After measuring the variables, data in groups were recorded and drawn over time. The normal distribution of data was determined using the Kolmogorov-Simonov test. One-way ANOVA was used to compare average values at a significance level of $P \leq 0.05$. Changes between groups and times were determined by the LSD post hoc test at a minimum significance level of $\alpha = 0.05$. The charts were plotted using Excel software.

Results

In the present study, experimental groups were a control sample (C) containing minced meat with no extract, sample A with minced meat (100 g) + 1 g of dried algal extract, and sample B with minced meat (100 g) + 0.5 g of algal extract. Also, the studied days were the first (D1), the seventh (D7), and the fourteenth (D14) days, respectively.

Chemical tests

Evaluation of the antioxidant activity of extracts

As shown in Table 2, the highest percentage of antioxidant activity is observed at 25 µg/ml, while ascorbic acid as a standard had 40% antioxidant activity at 10 µg/ml.

Table 2: Antioxidant activity (mean ± standard deviation) of algal extracts *in vitro*

Marine alga (µg/ml)	Antioxidant activity (%)		
	5	10	25
Caulerpa sertularioides	27.4±4.2	25.2±4.1	33.0±1.5

- pH

To compare the data obtained from pH measurement in the tested groups, normal distribution of data was determined using the Kolmogorov-Simonov test at the level of the graph function. Therefore, pH values were compared one-way ANOVA at $\alpha = 0.55$ and LSD post hoc test.

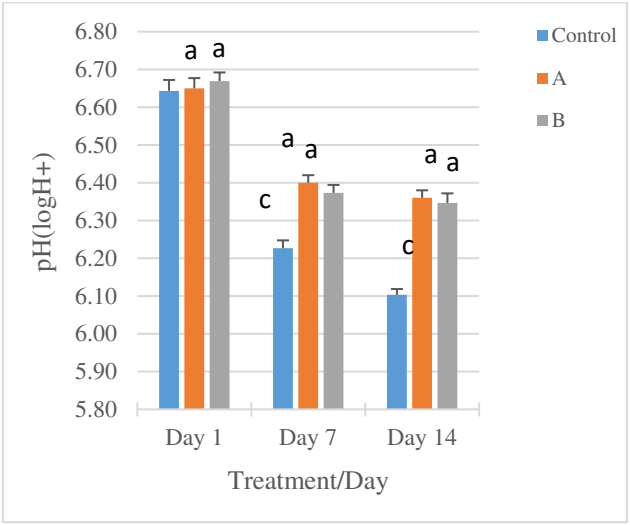

Figure 1. Changes in the pH of samples of rainbow trout minced meat during the test period. *Subscript letters indicate significant differences between the experimental groups at $p < 0.05$.

As shown in Figure 1, a pH of 6 was recorded for the samples on the first day, but pH levels decreased significantly in all samples over time ($P < 0.05$). The pH values of samples were not significantly different, ranging from 6.64 to 6.71 on the first experimental day, but significant differences occurred in pH values of 6.23 (control) to 6.40 (sample A) on the seventh day ($P < 0.05$). On day 14, minimum and maximum pH values were measured in the control (6.10) and sample A (6.36), respectively. In general, samples A and B were not significantly different on all the test days ($P > 0.05$).

Acidity

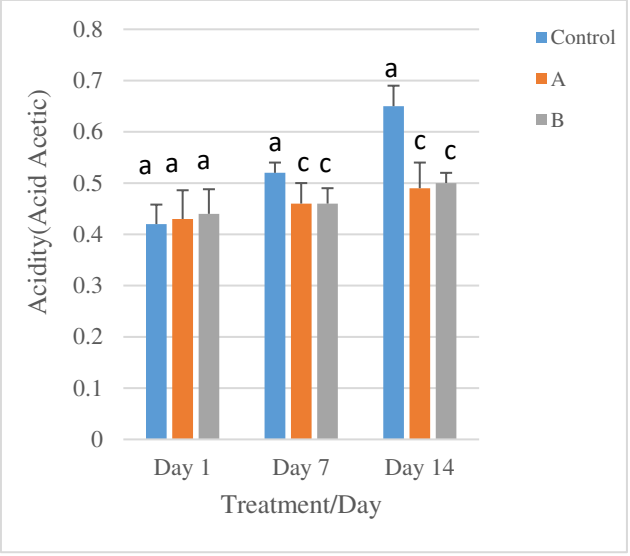

Figure 2. Changes in the acidity of samples of rainbow trout minced meat during the test period. *Subscript letters indicate significant differences between the experimental groups at $p < 0.05$.

Figure 2 indicates that the total average acidity (0.14) is uppermost in group A among the experimental samples containing 0.5% and 1% extract of *C. sertularoides* compared to the control group over time.

Peroxide

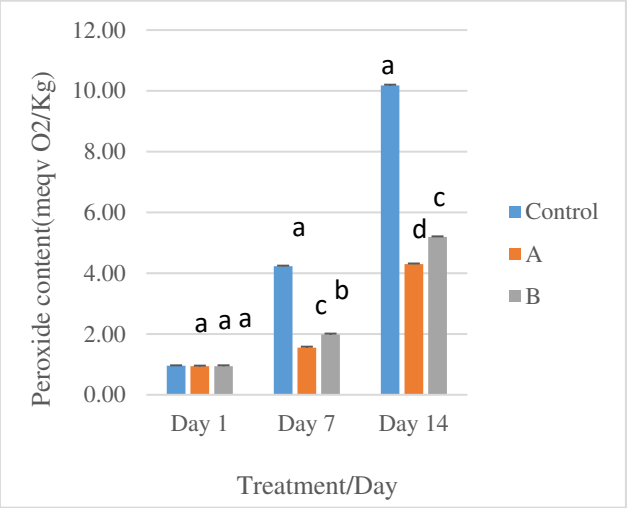

Figure 3. Changes in peroxide levels of rainbow trout minced meat samples during the test period. *Subscript letters indicate significant differences between the experimental groups at p < 0.05.

The findings in Figure 3 suggest the control group the highest total average (15.37) of peroxide level between experimental samples containing 0.5% and 1% of *C. sertularoides* extract, as well as among 0.5 and 1% experimental samples impregnated with *Laurencia papillosa* extract compared to the control group.

Total volatile nitrogen bases (TVB-N)

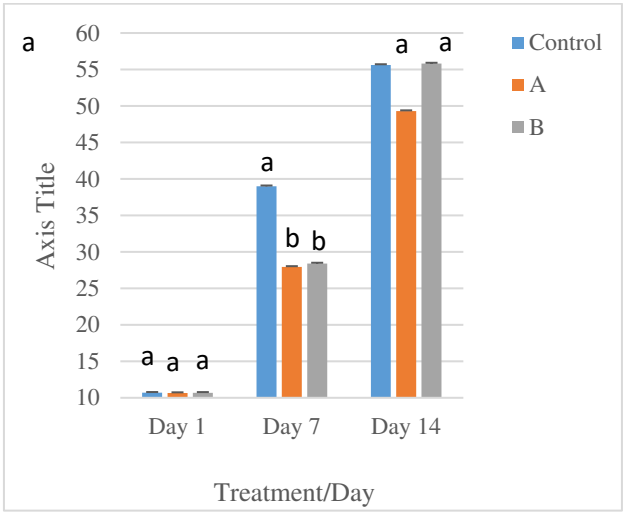

Figure 4. Changes in TVB-N levels of rainbow trout minced meat samples during the test period. *Subscript letters indicate significant differences between the experimental groups at p < 0.05.

As shown in Figure 4, the highest (56.76) and the lowest (10.69) TVB-N levels were recorded in the sample containing 1% of *L. papillosa* extract and that without algal extract after two weeks of experimentation. Overall, the control group contained the highest total average (35.10) of the TVB-N level between the analyzed experimental samples containing 0.5% and 1% of *C. sertularoides* extract over time.

Thiobarbituric acid

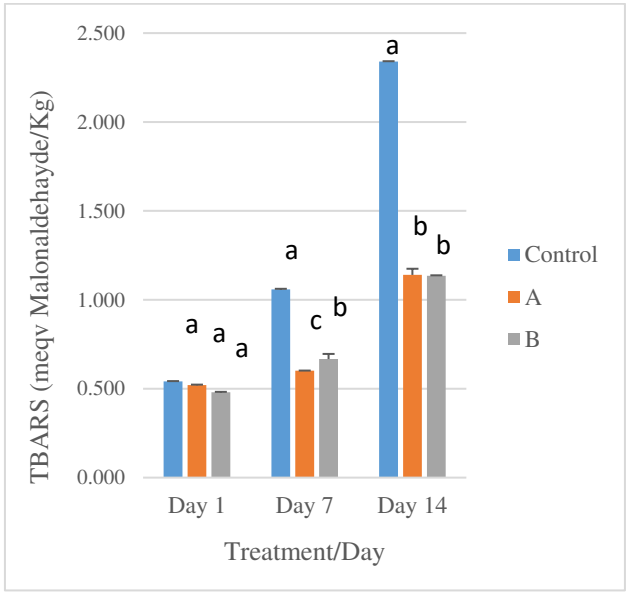

Figure 4. Changes in thiobarbituric acid levels of rainbow trout minced meat samples during the test period. *Subscript letters indicate significant differences between the experimental groups at p < 0.05.

The results showed that all experimental groups increased significantly over time (P < 0.05). In the groups impregnated with 1% *C. sertularoides* algal extract, a significant ascending trend (P < 0.05) was observed until the end of the experiment and also contained less thiobaric acid levels than the control group. In a similar comparison, this quantity had an upward trend among the samples containing 0.5% of the algal extract, with a lower thiobarbituric acid level than the control group. In general, experimental samples containing 0.5% and 1% algal extract of *C. sertularoides* over time.

Crude fat

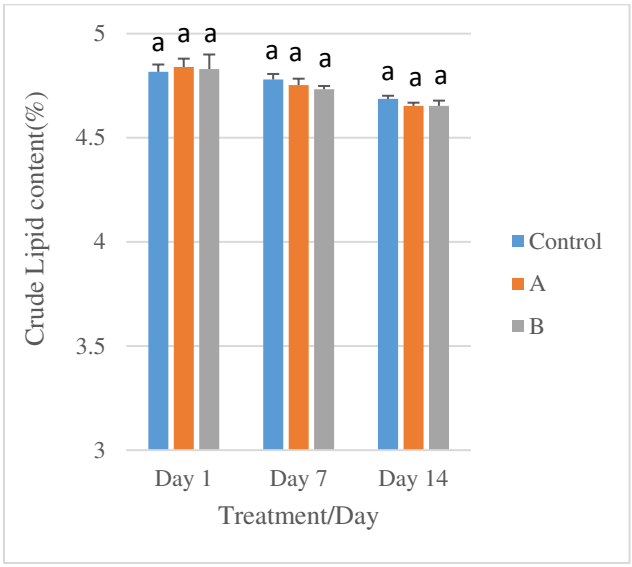

Figure 6. Changes in crude fat levels of rainbow trout minced meat samples during the test period. *Subscript letters indicate significant differences between the experimental groups at $p < 0.05$.

The results indicate that the experimental groups were not significantly different over time ($P > 0.05$), with descending and ascending trends at the beginning and the end of the experiment. In experimental samples impregnated in 1% *C. sertularoides* algal extract, this quantity had an ascending trend over time. This group also contained higher crude fat than the control group at the experiment onset, but it had less content to the end of the experiment.

Humidity

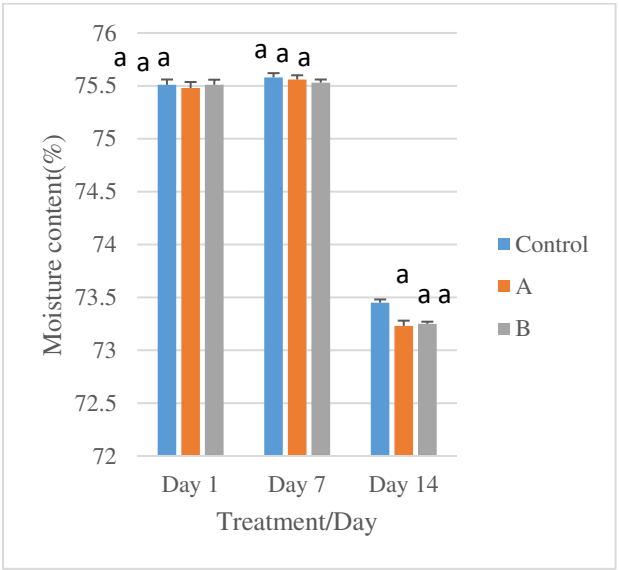

Figure 7. Changes in moisture contents of rainbow trout minced meat samples during the test period. *Subscript letters indicate significant differences between the experimental groups at $p < 0.05$.

Figure 7 represents the findings of moisture content that was generally descending and then ascending among all experimental groups over time. Moisture content increased initially in samples impregnated with 1% *C. sertularoides* algal extract and then decreased over time from the first week onwards, but it was consistently lower than the control group.

Evaluation of organoleptic characteristics

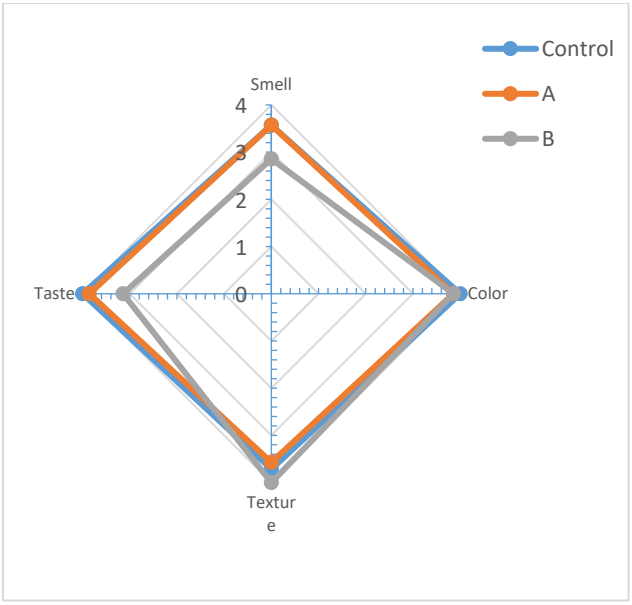

Figure 8. Evaluation of organoleptic characteristics of rainbow trout minced meat samples in the first week of the experiment.

In the sensory evaluation of the product in the first week of the experiment (Fig. 8), samples in A, control, D, C, and B groups are ordered respectively in terms of the odor factor, indicating the acceptance of sample A containing 1% of *C. sertularoides* extract, which was of better quality from the panelists' point of view due to the higher level of extract than sample B.

In terms of color factor, almost all samples were scored similarly, and the use of the algal extract in minced meat did not affect compared to the control sample.

Also, the appearance factor was not affected by the application of algal extract. However, the control, D, B, C, and A samples, respectively, had the highest scores in terms of overall acceptance.

Discussion and Conclusion

Compounds with antioxidant activity increasingly become important as the formation of active oxygen species (ROS) and active nitrogen species (RNS) are associated with the pathology of various diseases such as diabetes, cardiovascular inflammation, neurodegenerative disorders, and certain types of cancer.

Algae are of great interest owing to the possession of active antioxidant molecules such as ascorbate and glutathione (GSH) in a fresh state, as well as secondary metabolites including carotenoids (α and β-carotene), fucogsanthin, astaxanthin, similar amino acids, and catechins, gallate, fluoroutanines, eckol, and tocopherols.

Dorulong et al. (2013) found that *C. sertularoides* ethyl acetate extract contained the highest total phenol content (123.87 ± 2.67 mg GAE/g extract), while *L. tronoi* had the lowest total phenol content (592 mg GAE/g)., although the highest free radical activity (82.62 ± 1.5 %) was reported in *L. tronoi* hexane extract. However, the reduction power of *C. sertularoides* ethyl acetate extract was found to be higher than that of *Laurencia* [6].

Nursial et al. (2016) examined the percentage of antioxidant activity in 20 different algal species [7]. They found that total phenol content was uppermost in *Padina australis*, *C. sertularoides*, and *Hypnea* sp., respectively, while the antioxidant activity measurement by FRAP ionic reduction revealed the highest antioxidant activities in *Chaetomorpha* sp., *C. sertularoides* and *C. racemose*, respectively. However, the use of the DPPH method showed that *P. australis, Sargassum echinocarpum, Hypnea* sp., and *C. sertula*, respectively, contained the highest antioxidant activities. Hence, it is not possible to determine the antioxidant activity of the extracts and algal species.

Changes in pH

One of the primary chemical changes in fish meat is pH changes. Due to variable pH values of fish meat depending on the species, it can be stated that pH is not an accurate indicator for determining the freshness and quality of most aquaculture animals, but it is used as a complementary indicator for other parameters. The value of pH is among the factors affecting microbial growth and food spoilage, the amount of which is generally 6.7-7 in live fish.

Research by Dejban et al. (2013) showed the effect of time on the storage of samples containing thyme and rosemary extracts, with a combination of both natural antioxidants and compared it with control treatment on frozen minced meat of silver carp. The obtained pH values decreased in all treatments at the end of the storage period, with the greatest (5.62 ± 0.34) and the lowest (5.62 ± 0.32) mean reductions in the control treatment and treatment 3 (containing rosemary antioxidant), respectively, after 6 months; a comparison between treatments showed a significant difference ($p < 0.05$) during the experiment.

Aubourg et al. (2004) tried to minimize the spread of product rancidity and maintain its quality by the effect of two antioxidants, citric acid, and ascorbic acid, on frozen mackerel fillets in a pH range of 3.6-9.6, which is in the range (6.10-6.71) of the present study [8].

Adnani et al. (2016) studied the effect of propolis extract on the biochemical and microbiological properties of *O. mykiss* fillet [9]. They reported that fish storage in vacuum packaging led to the activity of lactic acid bacteria, acid production, and decreased pH, with a good growth of lactic acid bacteria up to day 16 due to a good environmental pH. However, their results showed that the production of lactic acid in the control samples was lower in the last days of storage due to lower pH than the desired pH of bacterial growth. This decrease in pH is in line with that in the present study.

Acidity

The acidity of live fish muscle is close to 7, but after death, it changes significantly depending on the season, species, and other factors. The above results generally indicate that the control group on the 14th day and both the control group and group C on the first day had the highest (0.65) and lowest (0.42) slope of the trend, respectively, compared to the whole experimental groups. Examination of the control group and the experimental treatments on days 1, 7, and 14 suggests that the lowest acidity levels were recorded in the control and group C (0.42), groups A and B (0.46), and group B (0.5). It is noteworthy that a significant upward trend was observed in the experimental groups in the first and second weeks of the experiment.

An increase in acidity observed by Ojagh et al. (2010) regarding the effect of chitosan coatings enriched with cinnamon essential oil on the quality of cold rainbow trout is similar to that in the present study [10]. Recent research suggests that increased acidity may be attributed to the production of volatile basic compounds, such as ammonia and trimethylamine (TMA), due to an interaction of internal enzymes with microbial enzymes.

Peroxide

Since peroxides are odorless compounds, they cannot be detected by consumers, but these compounds cause the formation of secondary compounds, such as aldehydes and ketones, which can be used to detect rancidity or oxidation. Therefore, the study of peroxide has a high value in the literature. In the current study, the peroxide number was in the range of 0.93-10.18. Our findings show that the highest and lowest levels of peroxide numbers belonged respectively to the control group in the second week (10.18) and group D (0.93) on the first day of the experiment. Kamani et al. (2014) investigated on fat oxidation of rainbow trout fillets (at 4 ± ℃) using image processing technique and observed a significant incremental trend ($P < 0.05$) [11]. They found the lowest amount of peroxide index (0.83 mEq) on the first day of storage at refrigerated temperature, which reached a maximum value (14.27 mEq) on the 20th day. In this research, an ascending trend was observed among all experimental groups over time, which corresponds to the above study.

According to recent investigations, the maximum permissible amount of peroxide in fish and its products is 10 mEq of

oxygen per kg of fish tissue. According to our results, all treatments did not exceed the permissible peroxide limit during the storage period at 4 ± 1 °C. In a study by Fathi (2012) on rainbow trout fillet, peroxide index was zero at the time of production until the end of the month, which reached less than 3.45 in all treatments at the end of 5 months after production, suggesting that the product retained its quality by the end of the fifth month, similar to the present study [12].

Total volatile base nitrogen (TVB-N)

The level of this variable is usually considered to be 35-40 mg of TVB-N per 100 g of fish muscle as an indicator of spoiled meat [13]. The present results indicate that TVB-N levels of the tested samples did not exceed the recommended maximum allowable limit, hence this product is not problematic for human consumption. A study by Nasiri et al. (2016) concerning the effects of *Myrtus communis* aqueous extract on qualitative changes in rainbow trout revealed that the TVBN index in the treated group was less than the standard level until day 10; however, this amount reached higher than the standard (43.93 mg/100 g) in the control group, which is contrary to the results of this study [14].

Nekooeifard (2016) reported that the values of this variable were significantly different in the two groups except on day zero, and reached its maximum in the control group on the 12th day and in the treatment group on the 18th day [15]. An increase in TVBN was mainly attributed to the bacterial decomposition of fish meat and an increase in bacterial load during the research period. Similarly, minimum amounts of this variable were respectively 3 and 6 for the control group and the treatment group on days zero and 3 in this study. A comparison of the experimental treatments in the first and second weeks suggests that the lowest amount of TVB-N was related to the group containing 1% of *Caulerpa* algal extract. Hector et al. (2013) also compared propolis extract with smoke treatment in kechmaa fish and detected that propolis extract significantly reduced the rate of increasing TVB-N index compared to other treatments. However, they reported no significant differences between different percentages of propolis applications. They attributed this effect of propolis to its antimicrobial properties and the ability to reduce the capacity of bacteria in the oxidative deamination of non-protein nitrogen compounds. According to the present results, the algal extract at 0.5 and 1% could to a higher extent reduce the increasing rate of TVB-N. In general, their studied treatments further reduced the increasing rate of this quantity relative to the control group, which is consistent with the results of this research.

Thiobarbituric acid (TBA)

TBA is widely used as an indicator of secondary fat oxidation and results from the presence of reactants with TBA obtained from the second stage of auto-oxidation, during which peroxides are oxidized to substances such as aldehydes and ketones. Besides, oxidation-produced Malone aldehydes can react with other fish fillet compounds, such as nucleic acids, phospholipids, nucleotides, proteins, amino acids, and other aldehyde compounds, in which case TBA cannot be a good indicator to measure the secondary oxidation of fats.

Our findings showed that the amount of TBA was in the range of 0.3-9.75 during the two weeks of storage. Based on the approximate analysis results of the studied groups, the highest and lowest levels of this variable respectively belonged to group B in the initial days and the control group on day 14 of the experiment. Also, a closer look at the statistical analysis reveals that the addition of 0.5% and 1% of *Caulerpa* and *Laurancia* extracts could positively affect the increasing trend of TBA production.

Khaniki et al. (2015) investigated on the antioxidant effect of pomegranate extract on rainbow trout on days zero, 2, and 4, and observed no significant differences between the type of tested extracts in the reduction process of TBA levels [16]. However, pomegranate extracts contained significantly lower TBA levels due to their phenolic and antioxidant contents than the control sample, which is similar to that of Fun et al. (2008) [13]. The results of the above studies are similar to the present data, as the studied groups containing *Caulerpa* and *Laurancia* extracts due to having phenolic and antioxidant compounds similarly contained less TBA levels in the first and second weeks of the experiment than the control sample. The lower level of this variable in the samples containing the extract than the control sample may be attributed to the presence of phenolic compounds in the extracts [16].

An acceptable TBA level of 1-2 mg malondialdehyde per kg of fat has been suggested in fish. According to the present study, the values of the whole experimental groups did not exceed the limit reported above for 14 days of storage. Decreased levels of TBA on some storage days may be due to decreased hydroperoxides and reactions between malondialdehyde and proteins, amino acids, and glycogen, thereby reducing malondialdehyde and subsequently TBA levels.

Crude fat

According to the results of this study, the amount of crude fat did not change significantly (except in the control group) during storage time ($P > 0.05$), which could have resulted from the antioxidant properties of *Laurencia* and *Caulerpa* algal extracts. The present findings indicated that the crude fat was in the range of 4.65-4.85, with the highest and lowest levels respectively related to group D (first day of the experiment) and groups A and B (second week). Fat content in farmed rainbow trout was reported to be 4.88% [17], which is in the range of that obtained in the study.

Kamani et al. (2014) studied changes in *O. mykiss* carcass composition during storage at refrigerated temperature and reported that fat content increased significantly during storage ($P < 0.05$). The initial level of this index (5.47%) decreased until the tenth day, which was not statistically

significant (P > 0.05), but a significant incremental trend (5.63%) was observed from the tenth day onwards (P < 0.05), which is consistent with the results of the present study, as fat content in the group containing algae extract was higher than the control group at the experiment onset. This difference can be related to factors such as nutrition, culture, and capture season, fish size, and culture conditions [11]. They also reported that the amount of fat showed a statistically significant increase (P < 0.05) during storage time, which does not correspond to that of our study as a downward trend was observed in the experimental groups with increasing test time.

Moisture content

The present results revealed that moisture content did not change significantly in all groups during storage time (P < 0.05). Our measured moisture content was in the range of 73.23-75.58, with the highest and lowest levels respectively in the control (seventh day of the experiment) and A groups (the second week). Water content comprises most of the fish's weight, which is often about 70 percent relative to fillet weight in high-fat fish such as rainbow trout. In the muscles of fresh fish, water is found in free and attached (firmly attached to the protein) forms so that it cannot be removed from the fish body under high pressure [18]. In the present study, a decreasing trend was observed in the moisture content of the studied groups, with the lowest amount of this quantity in the second week of the experiment, so the moisture content from 75.48 on the first day of the experiment reached 73.25 in the group containing 1% of *Caulerpa* algal extract in the second week. In general, the reason for moisture reductions reported in similar studies could be changed in pH and water retention capacity, in which case water leaves the fish body and takes along some water-soluble nutrients, such as B vitamins, from the fish muscles, thereby lowering the nutritional value and quality of fish meat. However, some researches attributed water depletion to storage at poor temperature conditions, which in turn leads to dehydration of meat during storage time [19]. Also, moisture content depends on various factors such as gender, age, size, and other environmental variables [20].

Evaluation of organoleptic properties

Sensory evaluation is used as one of the indicators of fish quality assessment during storage. Despite many efforts to develop laboratory standards for fish, organoleptic testing is still the best way for assessment of freshness. Evaluation of organoleptic properties along with chemical experiments (as a complementary method) is necessary to determine the degree of spoilage and shelf life of fish and its products (Standard 3580, 1995).

The first-week results indicate that samples A, control, and C had the highest scores in terms of the odor factor. After 14 days and in the second week of the experiment, however, sample A and then sample B still gained the highest scores, with the control sample having the least score. Accordingly, the extract of *C. sertularoides* could control the rancid smell of minced meat in the refrigerator for 14 days.

It should be noted that sample C containing the *C. sertularoides* extract could not be stored due to microbial spoilage, possibly due to secondary contamination, and was removed from the experimental system on day 14.

Izei (2011) investigated the qualitative and sensory changes in the fish fingers prepared from *Atheria boyeri* after rapid frying at 181 °C and during refrigeration storage [21]. It was reported that samples in the first phases were excellent to good quality in terms of organoleptic factor evaluations, and the quality dropped to an acceptable level in the final phases. As a result of recent research, it can be concluded that the loss of color, texture, and taste properties with the progression of shelf life under refrigeration can be due to the compounds resulting from the oxidation of fatty acids.

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