

## Enhancing the Shelf Life and Sensory Properties of Rainbow Trout Fillets through Sodium Alginate Coating Containing *Eryngium campestre* Extract at 4°C

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### ABSTRACT

Fresh fish is a highly perishable food item and spoils easily. In this research, after investigating the antioxidant properties of the *Eryngium Campestre* extract (Ece), its effect along with the sodium alginate coating was evaluated on the shelf-life of rainbow trout under refrigerated conditions (4°C) for 12 days. To assess the antioxidant properties of the extract, tests such as DPPH, total phenolics, reducing power, and ABTS were performed. Subsequently, samples treated with an Ece containing alginate coating applied via spray method were analyzed for chemical parameters (TBA, TVN, and pH), microbiological parameters (total psychrotrophic and mesophilic bacteria), and sensory evaluations at four day intervals up to 12 days. The results indicated that Ece possessed significant antioxidant properties. Furthermore, treatments that included the extract combined with the sodium alginate coating significantly reduced pH, TVN, and TBA levels compared to the control sample ( $P < 0.05$ ). Microbial tests indicated that all treated samples inhibited bacterial growth, with a reduction of approximately 3 log CFU g<sup>-1</sup> compared to the control group. In the sensory evaluation, treatments containing Ece and sodium alginate yielded more favorable results than those of the control group. Accordingly, coating the samples with sodium alginate and Ece improved the microbial, chemical, and sensory properties and shelf life of rainbow trout in refrigerator conditions by about four days.

**Keywords:** DPPH, Fresh fish, Microbiological parameters, Sensory properties, TBA, TVN.

### INTRODUCTION

Fish is a vital source of protein and omega-3 fatty acids ( $\omega$ -3 PUFAs), which are important for a healthy diet. Since the body cannot produce omega-3s, they must be obtained from food. These fatty acids offer numerous health benefits, including improved heart health and reduced inflammation (Zarandi *et al.*, 2022). Rainbow trout (*Oncorhynchus mykiss*) is one of the most extensively farmed freshwater fish species worldwide and is preferred by consumers for its high nutritional value. The chemical composition of trout fillets can differ based on various factors such as age,

gender, season, water temperature, and dietary conditions (Foromandi and Khani, 2023). Typically, trout fillets contain approximately 73% water, 20% protein, 5% fat, and 1.5% minerals. This nutritional composition, along with significant levels of essential amino acids and polyunsaturated fatty acids, categorizes rainbow trout as highly perishable food (Popelka *et al.*, 2014).

The shelf life and overall quality of fish can be affected by enzymatic and microbial processes, along with the oxidation of fats (Mazandrani *et al.*, 2016). Additionally, there are safety concerns related to lipid oxidation and microbial growth (Shakour *et*

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al., 2021). Various strategies have been investigated to preserve fish products and extend their shelf life, including the incorporation of plant extracts as natural additives (Charoenphun *et al.*, 2023).

Plant extracts have antibacterial and antioxidant properties (Rathod *et al.*, 2021). To reduce the negative effects of chemical preservatives and meet consumer demand for natural products, plant extracts and edible coatings are used to extend the shelf life and prevent spoilage of fresh fish (Fadiloğlu and Emir Çoban, 2018). These properties are essential as they can control the growth of spoilage microorganisms and shield the fish from oxidative damage. Nevertheless, their application is often restricted due to costs and potential toxicity.

Edible coatings derived from alginate, a biodegradable hydrocolloid, have been implemented to preserve fish fillets. Alginate coatings provide a physical barrier while also improving the overall quality and acceptability of the fish by preserving moisture and minimizing lipid oxidation (Urbonavičiūtė *et al.*, 2023).

*Eryngium campestre* L., part of the *Apiaceae* family, is a perennial plant that grows in Asia, Europe, and Africa. This plant is widely used in traditional medicine to treat various conditions, including coughs, urinary infections, increased urination, kidney dysfunction, and the removal of kidney and bladder stones (Azizkhani and Sodanlo, 2021). *E. campestre* is abundant in phenolic compounds, which contribute to its antioxidant and antimicrobial properties (Kartal *et al.*, 2006). This study examines the impact of *E. campestre* extract in edible and biodegradable coatings, made from sodium alginate, on the quality and shelf life of rainbow trout fillets stored at 4°C. The incorporation of *E. campestre* extract is intended to enhance the preservative properties of these coatings, thereby maintaining the fish's sensory characteristics and nutritional value during extended storage. The research aimed to support sustainable practices in the seafood industry

by promoting natural preservation methods over synthetic chemicals.

## MATERIALS AND METHODS

### Preparation of Ethanolic Extract of *E. campestre*

The maceration method was used to prepare the ethanolic extract from the *E. campestre*. This plant was picked from the forest areas of Amol City. The leafy part and stem were dried in the shade and ground into a powder, passed through a sieve with a 60 µm mesh size. Then, 200 g of powder were mixed with 1 L of pure ethanol and placed in a shaker incubator at 150 rpm for 24 hours at 42°C. To remove most of the solvent, the mixture was placed in a rotary evaporator (Heidolph, Laborota 400 efficient, Germany) at 50°C and 100 rpm under vacuum conditions. To determine the concentration, it was placed in an oven at 45°C and, finally, lyophilized at -50°C for 24 hours. Then, it was refrigerated in a closed container (Alizadeh Amoli *et al.*, 2019).

### GC-MS Analysis of *E. campestre* Extract

GC-MS analysis was performed using an Agilent 7890A GC device equipped with an HP-5MS column and a 5975 mass spectrometer (Agilent Technologies, USA) (Mishra and Patnaik, 2020).

### Antioxidant Activity Analyses

The following four methods were used to evaluate and check the antioxidant power of the plant extract.

### DPPH Test (1,1-Diphenyl-2-Picrylhydrazyl)

The study tested the antioxidant activity of an extract by diluting it to  $0.25 \text{ mg mL}^{-1}$  and adding it to a DPPH solution. Absorbance was measured at 517 nm with spectrophotometer (Pharmacia Biotech, Sweden) after 30 minutes in the dark. The free radical inhibition was calculated using the formula:  $AC \times 100 / (AC - AS)$ , where AC is the Absorbance of the Control, and AS is the Absorbance of the Sample. BHT was used as a positive control at  $1 \text{ mg mL}^{-1}$  (Ebrahimi and Larypoor, 2022).

### Determination of the Total Phenolic Content

The total phenolic content of the plant was measured using the Folin-Ciocalteu method. Extract dilutions were mixed with Folin's reagent, gallic acid, and sodium carbonate, then, kept in the dark for specified times. Absorbance was measured at 760 nm, and the total phenolic content was expressed as mg of gallic acid per gram of material (Gharedaghi *et al.*, 2020)

### Determination of the Reducing Power

Extracts were mixed with sodium phosphate, potassium ferricyanide, and incubated at  $50^\circ\text{C}$  for 20 minutes. After adding trichloroacetic acid and centrifuging, distilled water and iron chloride were added to the supernatant. Absorbance was measured at 700 nm to assess the results (Merghache *et al.*, 2014).

### ABTS Radical Cation Method

The antioxidant capacity was assessed using a modified ABTS method.  $\text{ABTS}^{\bullet+}$  radical was generated by mixing ABTS and potassium persulfate, and incubated for 16 hours. The solution was diluted to achieve

an absorbance of 0.7 at 734 nm. Extracts or BHT were added to the  $\text{ABTS}^{\bullet+}$  solution and, after 6 minutes, absorbance was measured. Inhibition percentage was calculated using the following formula:

$$(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}} \times 100$$

The results were expressed as antioxidant capacity equivalent to ascorbic acid (Kikowska and Thiem, 2021).

### Preparation of Fish Samples

Rainbow trout, weighing approximately  $600 \pm 50 \text{ g}$ , was procured from a fish sales center in Urmia City. The fish was properly examined, and its internal organs were removed. The body of the fish was thoroughly rinsed with water to eliminate any residual blood. The dimensions of the fish fillet were measured at approximately 15 cm in length, 10 cm in width, and 3 cm in height, and its weight was approximately 100 g. Subsequently, the fish was taken to the Food Hygiene Laboratory at the Faculty of Veterinary Medicine, Urmia University, to ensure sample integrity and minimize the risk of microbial contamination.

### Preparation of Treatments

The sodium alginate solution was prepared by dissolving 1.5 g of sodium alginate in 100 mL of warm distilled water, and the extract of *E. campestre* was added to this solution. The resulting mixture was thoroughly combined to achieve a uniform consistency, transferred to a spray bottle, and shaken well before use. Fish fillets were then coated with sodium alginate solution using a spraying method. Subsequently, the fillets were treated with 2% calcium chloride solution and air-dried at room temperature. Then, the fish fillets were prepared for coating in 3 groups and 1 control sample as follows:

1. Without coating and extract
2. With alginate spray coating and without extract



3. With alginate 1.5% spray coating containing 0.5% *E. campestre* extract
4. With alginate 1.5% spray coating containing 1% *E. campestre* extract.

The control sample and coated fillets were stored in resealable plastic bags in a refrigerator at 4°C. Microbiological, chemical, and sensory evaluations were performed on days 1, 4, 8, and 12 to assess the quality and stability.

### Chemical Analyses of Treated Fish Fillets

#### pH Values

For this purpose, 5 g of each fish fillet sample was placed in 10 mL of distilled water and homogenized for 30 seconds at a speed of 13,500 rpm. Subsequently, the pH of the homogenized sample was measured using a calibrated pH meter. The pH meter had been calibrated prior to measurement using standard buffer solutions with pH values of 4 and 7 to ensure accuracy and reliability in the results (Ojagh *et al.*, 2010).

#### Total Volatile Base Nitrogen (TVB-N)

To determine TVB-N, 10 g of fish fillet was homogenized with distilled water, then, mixed with magnesium oxide or NaOH and heated in a Kjeldahl flask. The distillation vapors were collected in a boric acid solution, and after reaching 50 mL, the solution was titrated with sulfuric acid. The TVB-N value was calculated based on the amount of sulfuric acid consumed, expressed as mg of TVB-N per 100 g of fish fillets (Ghasemi *et al.*, 2023).

#### Thiobarbituric Acid Reactive Substance

To measure malonaldehyde content (TBARS), 10 g of fish fillet was homogenized with 5% TCA (Trichloroacetic

Acid) and BHT, filtered, and the filtrate adjusted to 50 mL. TBA reagent was added to the filtrate, heated at 100°C for 1 hour, and absorbance was measured at 532 nm. The TBARS value, indicating malonaldehyde, was calculated and expressed as mg k<sup>-1</sup> of fish meat (Ghasemi *et al.*, 2023).

### Microbiological Analyses

#### Total Mesophilic Bacterial Count

After diluting the samples from each dilution tube, 100 microliters of each sample were inoculated onto plates containing PCA culture medium and spread uniformly using a Pasteur pipette. Subsequently, the plates were incubated upside down at 37°C for 48 hours. Then, the colonies were counted and reported as CFU g<sup>-1</sup> (Muñoz-Tebar *et al.*, 2023).

#### Total Psychrotrophic Count

After diluting the samples from each dilution tube, 100 µL of each sample was inoculated onto plates containing PCA culture medium and spread uniformly across the medium using a Pasteur pipette. The plates were then incubated in an inverted position at 10°C for 7 days. Following incubation, the colonies were counted and reported as CFU g<sup>-1</sup> (Muñoz-Tebar *et al.*, 2023).

### Sensory Evaluation

A trained panel of 10 assessors evaluated the organoleptic properties of the treated fish fillets in two stages. In the first stage, they assessed the taste of cooked fillets, in the second stage, they evaluated refrigerated fillets for texture, aroma, and color on days 1, 4, 8, and 12. Fresh fillets at 4°C were used as the reference for maximum scores, and a 5-point hedonic scale was used for

evaluation (Bazargani-Gilani and Pajohi-Alamoti, 2020).

### Statistical Analysis

After obtaining the data from the tested factors, SPSS version 26 software was used for statistical analysis, including one-way ANOVA for data analysis. Excell version 2022 was used to draw the graphs. Duncan's test was used to classify the samples according to the statistical difference between them and to measure their average. Normality tests (e.g., Shapiro-Wilk and Kolmogorov-Smirnov) were conducted prior to performing ANOVA to ensure the suitability of the parametric test. The results were considered significant at a P-value of < 0.05.

## RESULTS AND DISCUSSION

### GC-MS Results of *E. campestre* Extract

According to Table 1, The GC-MS analysis of *Eryngium campestre* extract identified 32 compounds, with limonene,  $\delta$ -3-carene,  $\beta$ -sesquiphellandrene, and cyclobuta having the highest peak areas. This study's findings align with Fernandes (2013), who identified compounds like germacrene D and  $\alpha$ -cadinol, although there are differences. Variations in chemical profiles are attributed to differences in plant samples, extraction methods, and environmental conditions, underscoring the chemical diversity of *Eryngium campestre* and its potential applications in the pharmaceutical and food industries. (Fernandes, 2013).

### DPPH Antioxidant Test Results

In Figure 1, The DPPH assay results show that, as the concentration of *E. campestre* extract increases, the inhibition of free radicals also increases, indicating a strong

concentration-dependent antioxidant effect. This trend was evident across all concentrations, with the extract demonstrating high antioxidant activity in every dilution. These findings align with the work of Charoenphun *et al.* (2023) and Gharedaghi *et al.* (2020), who observed similar antioxidant effects in plant extracts. Furthermore, Kremer *et al.* (2021) reported comparable results in *E. amethystinum* and *E. alpinum*, confirming the potential of *E. campestre* as a natural antioxidant for applications in food preservation and oxidative stress reduction.

### Total Phenolic Content

Based on the data from Table 2 and Figure 2, the total phenolic content in the *E. campestre* extract was determined using a calibration curve for gallic acid. The study revealed a strong correlation between the high phenolic content and antioxidant activity of the extract. This finding is consistent with Al-Askar *et al.* (2023), who also highlighted significant polyphenolic compounds in *E. campestre* extracts, which are directly linked to antioxidant and antimicrobial properties. These phenolic compounds play a crucial role in scavenging free radicals, supporting their potential in food preservation (Al-Askar *et al.*, 2023)

### The Reduction Power Results

According to Figure 3, the reduction and absorption power at 700 nm for spectrophotometric measurements were observed with 2 mg of *E. campestre* extract (absorbance value of 1.8) and Butylated Hydroxytoluene (BHT) (absorbance value of 3). These results suggest that *E. campestre* extract exhibits significant antioxidant potential, comparable to BHT. The strong reducing power observed in this extract, particularly in its ability to reduce ferric ions, is vital for antioxidant applications. This finding is consistent with Merghache *et*

**Table 1.** GC-MS analysis of *E. campestre* extract.

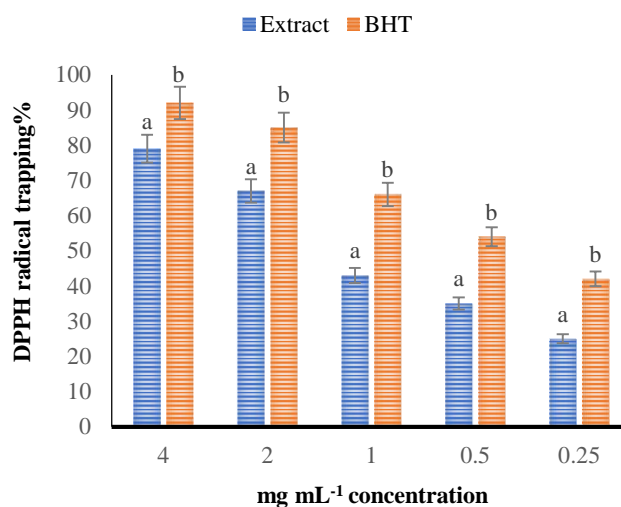
No.	Compounds	RT	percentage
1	Heptanal	11.23	0.15
2	$\alpha$ -Pinene	11.47	1.87
3	n-Heptanol	12.31	0.42
4	Verbenene	13.57	0.84
5	Myrcene	14.54	1.95
6	n-Octanal	14.89	1.53
7	$\delta$ -3-Carene	15.56	6.79
8	p-Cymene	16.55	0.37
9	Limonene	16.69	26.71
10	Benzene acetaldehyde	17.15	0.16
11	n-Octanol	18.47	0.54
12	p-Mentha-2,4(8)-diene	19.65	0.27
13	Linalool	20.08	0.42
14	cis-p-Mentha-2,8-dien-1-ol	21.91	0.18
15	Z-4-Decenal	24.13	0.13
16	trans-Carveol	25.92	0.53
17	Citronellol	27.12	0.38
18	Thymol	29.97	0.23
19	Carvacrol	30.36	0.35
20	$\beta$ -Elemene	33.74	0.21
21	$\alpha$ -cis-Bergamotene	35.84	0.72
22	$\alpha$ -Acoradiene	37.17	0.46
23	E- $\beta$ -Ionone	38.02	1.23
24	Z- $\alpha$ -Bisabolene	38.65	2.57
25	$\beta$ -Bisabolene	38.94	1.84
26	Myristicin	39.45	0.17
27	$\beta$ -Sesquiphellandrene	39.61	15.25
28	Widdrol	41.13	0.83
29	trans-Longipinocarveol	45.87	5.28
30	Cyclobuta	46.16	24.19
31	n-Octadecane	50.87	0.28
32	n-Hexadecenoic acid	59.65	<b>2.69</b>
Total identified			<b>99.54</b>

al. (2014), who noted similar reducing power in *E. tricuspidatum* essential oil, further supporting the potential of *E. campestre* as a natural antioxidant and a promising alternative to synthetic antioxidants in food preservation (Merghache et al., 2014).

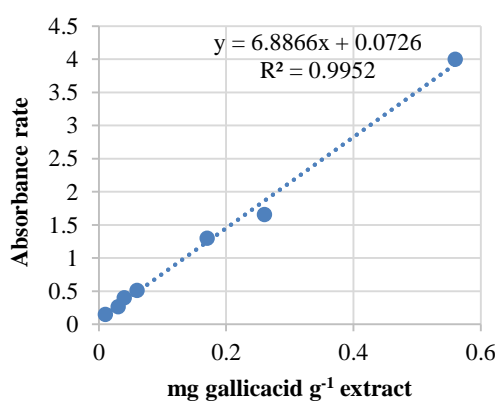
### ABTS Radical Inhibitory Test Results

In Table 3, *E. campestre* extract showed inhibitory activity at all concentrations, with its performance lower than BHT, except at the highest concentration where it was

comparable. Increasing the extract concentration enhanced its free radical scavenging ability, as confirmed by the ABTS test. While its antioxidant activity was somewhat lower than BHT, the improved efficacy with higher concentrations suggests its potential for food preservation, supporting its use as a natural antioxidant to extend shelf life and improve food quality, consistent with previous studies (Kikowska and Thiem, 2021).



**Figure 1.** DPPH radical scavenging rate of different concentrations of *E. campestre* ethanol extract compared to BHT. a-b: Different letters in each concentration indicate a significant difference ( $P < 0.05$ ).

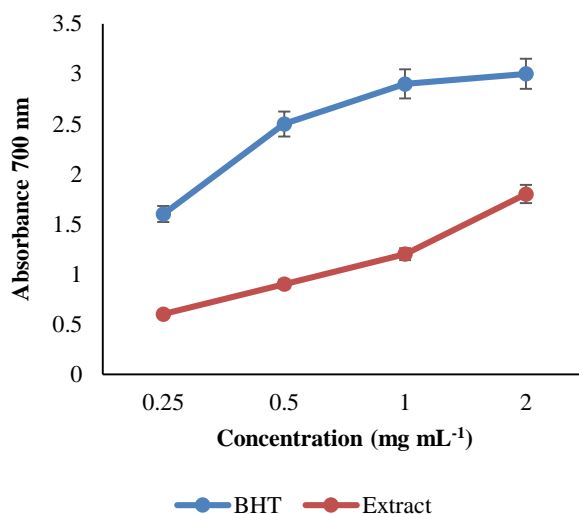


**Figure 2.** Gallic acid standard curve.

**Table 2.** Comparison of total phenol content of the ethanolic extract of *E. campestre* and the correlation between total phenol and antioxidant activity values.

Antioxidant assay by DPPH method			
R	(P) Sig.	mg gallic acid g <sup>-1</sup> extract	Total phenol in the Alcoholic Extract
0.950	0.005**	125±9.80	

\*\* Correlation is significant at the 0.01 level.



**Figure 3.** The rejuvenating potency of *E. campestre* extracts compared to BHT.

**Table 3.** Inhibition percentage and antioxidant capacity equivalent to ascorbic acid of different concentrations of alcoholic extract and BHT.<sup>a</sup>

Concentration (mg mL <sup>-1</sup> )		Antioxidant capacity eq. ascorbic acid (mg mL <sup>-1</sup> )	Inhibition %
0.125	Extract	0.00 ± 0.001 <sup>aA</sup>	9.61 ± 6.51 <sup>aA</sup>
	BHT	0.01 ± 0.001 <sup>bA</sup>	64.85 ± 11.01 <sup>bA</sup>
0.25	Extract	0.006 ± 0.00 <sup>aA</sup>	46.37 ± 12.59 <sup>aB</sup>
	BHT	0.12 ± 0.01 <sup>bB</sup>	91.51 ± 1.67 <sup>bB</sup>
0.5	Extract	0.013 ± 0.02 <sup>aB</sup>	84.21 ± 0.68 <sup>aC</sup>
	BHT	0.18 ± 0.01 <sup>bC</sup>	92.46 ± 4.30 <sup>bC</sup>
1	Extract	0.09 ± 0.01 <sup>aC</sup>	85.26 ± 8.01 <sup>aC</sup>
	BHT	0.18 ± 0.01 <sup>bC</sup>	94.64 ± 4.21 <sup>bD</sup>
2	Extract	0.14 ± 0.01 <sup>aD</sup>	92.29 ± 2.21 <sup>aD</sup>
	BHT	0.18 ± 0.01 <sup>aC</sup>	96.86 ± 1.21 <sup>bE</sup>

<sup>a</sup> In each column, non-identical lowercase letters indicate a significant difference at the P < 0.05 level; between the extract and BHT at the same concentration. Non-identical uppercase letters also indicate a significant difference between different concentrations of the same compound at the P < 0.05 level.

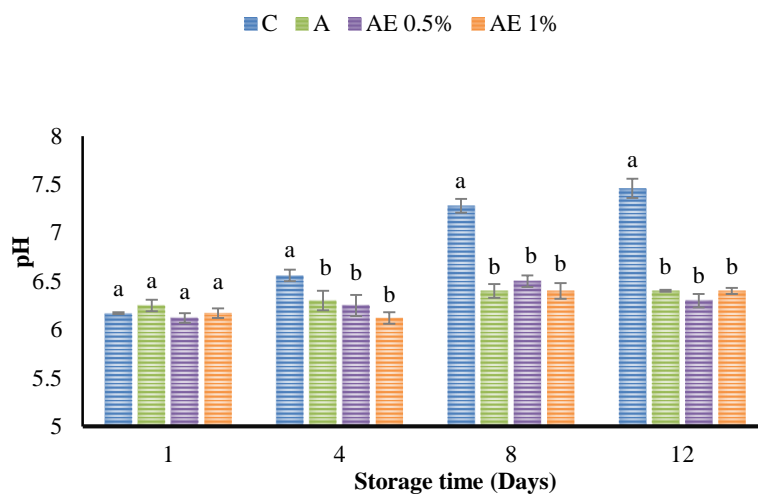
### Chemical Composition of Fish Fillet

According to the analysis carried out on the rainbow trout sample, the approximate

amount of ash, fat, protein, and moisture was presented in Table 4, the obtained results are consistent with the findings of Torabi Delshad *et al.* (2012).

**Table 4.** Chemical composition of rainbow trout.

Composition	Percentage
Moisture	71.5 ± 0.23
Protein	22.16 ± 0.33
Fat	3 ± 0.63
Ash	1.6 ± 0.23



**Figure 4.** Comparison of pH changes in rainbow trout fillet samples during the storage at 4°C. Each day, non-identical lowercase letters indicate a significant difference at the  $P < 0.05$  level. (C: Control, A: Alginate coating, AE 0.5%: Alginate containing 0.5% extract, AE 1%: Alginate containing 1% extract).

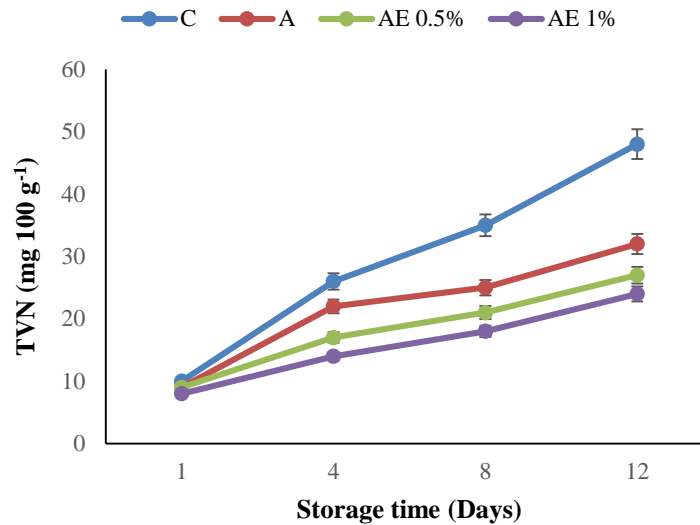
### pH Measurement Results

During refrigerated storage, all rainbow trout samples showed an increase in pH, with the control sample exhibiting a much higher increase, especially on days 8 and 12. As shown in Figure 4, coated fillet samples with the extract maintained a stable pH within the permissible range (6-7), showing no significant increase from day 4 onwards. This aligns with Alizadeh *et al.* (2020), who found that treated samples displayed better pH stability, highlighting the antimicrobial and preservative efficacy of plant-derived bioactive compounds in delaying spoilage and maintaining food quality (Alizadeh Amoli *et al.*, 2019).

### TVB-N Results

According to Figure 5, our study revealed an upward trend in TVB-N levels in all samples during storage. However, the

bioactive coating effectively kept these levels below the permissible limit ( $25 \text{ mg } 100 \text{ g}^{-1}$ ) throughout the storage period. In contrast, the control sample exceeded this limit after 4 days, and the alginate-coated sample did so after 12 days. These results align with previous studies (Ojagh *et al.*, 2010), which found that chitosan-based coating combined with plant compounds significantly reduced TVB-N levels in fish samples and maintained them below the acceptable limit for 16 days. Similarly, Öz (2018) demonstrated that garlic supplementation inhibited microbial growth, and lowering TVB-N levels during frozen storage. Ozogul *et al.* (2017) reported that nanoemulsions with essential oils like rosemary and thyme effectively reduced spoilage, while Öz *et al.* (2017) highlighted the role of black cumin oil in slowing TVB-N increases in fish fillets at 2°C. These findings are inconsistent with ours, and underscore the potential of bioactive coatings in preserving fish quality.



**Figure 5.** Changes of TVB-N in rainbow trout fillet during the storage period at 4°C. (C: Control, A: Alginate coating, AE 0.5%: Alginate containing 0.5% extract, AE 1%: Alginate containing 1% extract).

**Table 5.** Changes in the index of thiobarbituric acid (mg MDA kg<sup>-1</sup>) in rainbow trout fillets during storage at 4 °C).

Treatments	Storage days			
	1	4	8	12
C	0.42 ± 0.01 <sup>Aa</sup>	2.02 ± 0.11 <sup>Ba</sup>	2.72 ± 0.04 <sup>Ca</sup>	3.12 ± 0.02 <sup>Da</sup>
A	0.40 ± 0.00 <sup>Aa</sup>	1.7 ± 0.06 <sup>Bb</sup>	2.42 ± 0.01 <sup>Cb</sup>	2.62 ± 0.02 <sup>Db</sup>
AE 0.5%	0.41 ± 0.01 <sup>Aa</sup>	0.89 ± 0.04 <sup>Bc</sup>	1.62 ± 0.02 <sup>Cc</sup>	2.40 ± 0.06 <sup>Dc</sup>
AE 1%	0.32 ± 0.01 <sup>Aa</sup>	0.78 ± 0.06 <sup>Bd</sup>	1.48 ± 0.01 <sup>Cd</sup>	2.22 ± 0.06 <sup>Dd</sup>

(C: Control, A: Alginate coating, AE 0.5%: Alginate containing 0.5% extract, AE 1%: Alginate containing 1% extract). Small letters in each column and non-identical capital letters in each row indicate a significant difference in the level ( $p < 0.05$ ).

### TBARS Value Results

In agreement with Mehdizadeh *et al.* (2019), our data indicated that all treatments, except the control group, maintained TBA levels within the revised permissible range of 1-2 mg MDA kg<sup>-1</sup> throughout the 12-day storage period, as delineated 0 in Table 5. A more granular analysis revealed a markedly attenuated rate of TBA increase in the coated samples, particularly evident from day 4 onwards. During this interval, coated samples consistently exhibited demonstrably lower TBA values than the control, a trend that persisted until day 8. Subsequently, a gradual elevation in TBA levels was observed from day 12 onwards, primarily attributed to the

progressive nature of lipid oxidation. This body of evidence underscores the efficacy of the coatings in providing a robust protective barrier against lipid peroxidation, effectively retarding the formation of malonaldehyde, a critical marker of lipid degradation and concomitant quality decline in the fish products. This refined permissible range accentuates the subtle yet significant protective influence of our coatings. Alginate and extract coatings slowed TBA increases more effectively than the control or alginate alone samples, demonstrating a statistically significant impact on oxidation. Similarly, Gharehdaghi *et al.* (2020) noted alginate coatings delayed lipid oxidation by acting as oxygen barriers, with enriched coatings further

reducing oxidation and microbial growth in fish.

## Microbiological Results

### Total Mesophilic Bacterial Count

Table 6 shows the data related to the total microbial load of fish samples stored in the refrigerator during 12 days. According to it, the microbial load increased with increasing storage time in all samples. In this study, the total microbial load in the control sample on

threshold was surpassed on day 8. Consistent with the cited study, extract-treated samples in our research exhibited lower microbial loads, remaining below the 7 log CFU g<sup>-1</sup> limit, highlighting the effective antimicrobial properties of the extracts.

### Total Psychrotrophic Bacterial Count

According to Table 7, the total count of psychrotrophic bacteria in rainbow trout fillets exceeded the established 7 log CFU g<sup>-1</sup> limit by day 4 in the control group, whereas

**Table 6.** Total mesophilic bacterial counts (log CFU g<sup>-1</sup>) of rainbow trout fillets during storage at 4°C.<sup>a</sup>

Treatments	Storage days			
	1	4	8	12
C	4.42 ± 0.12 <sup>a</sup>	6.02 ± 0.11 <sup>a</sup>	7.8 ± 0.04 <sup>a</sup>	9.12 ± 0.02 <sup>a</sup>
A	3.40 ± 0.10 <sup>b</sup>	5.7 ± 0.06 <sup>b</sup>	7.42 ± 0.11 <sup>a</sup>	7.95 ± 0.02 <sup>b</sup>
AE 0.5%	2.41 ± 0.11 <sup>c</sup>	3.89 ± 0.04 <sup>c</sup>	5.62 ± 0.02 <sup>b</sup>	7.40 ± 0.06 <sup>b</sup>
AE 1%	2.32 ± 0.16 <sup>d</sup>	3.78 ± 0.19 <sup>d</sup>	5.48 ± 0.26 <sup>b</sup>	7.22 ± 0.06 <sup>c</sup>

<sup>a</sup> Different letters in each day indicate significant differences (P < 0.05). C: Control, A: Alginate coating, AE 0.5%: Alginate containing 0.5% extract, AE 1%: Alginate containing 1% extract.

**Table 7.** Total psychrotrophic bacterial counts (log CFU g<sup>-1</sup>) of rainbow trout fillets during storage at 4°C.<sup>a</sup>

Treatments	Storage days			
	1	4	8	12
C	5.32 ± 0.25 <sup>a</sup>	7.02 ± 0.11 <sup>a</sup>	7.72 ± 0.04 <sup>a</sup>	9.12 ± 0.02 <sup>a</sup>
A	4.40 ± 0.10 <sup>b</sup>	5.40 ± 0.16 <sup>b</sup>	7.32 ± 0.11 <sup>b</sup>	7.52 ± 0.12 <sup>b</sup>
AE 0.5%	2.48 ± 0.01 <sup>c</sup>	4.88 ± 0.14 <sup>c</sup>	6.85 ± 0.02 <sup>c</sup>	7.40 ± 0.05 <sup>c</sup>
AE 1%	2.32 ± 0.01 <sup>c</sup>	4.58 ± 0.19 <sup>c</sup>	6.38 ± 0.26 <sup>d</sup>	7.22 ± 0.06 <sup>d</sup>

<sup>a</sup> Different letters in each day indicate significant differences (P < 0.05). C: Control, A: Alginate coating, AE 0.5%: Alginate containing 0.5% extract, AE 1%: Alginate containing 1% extract.

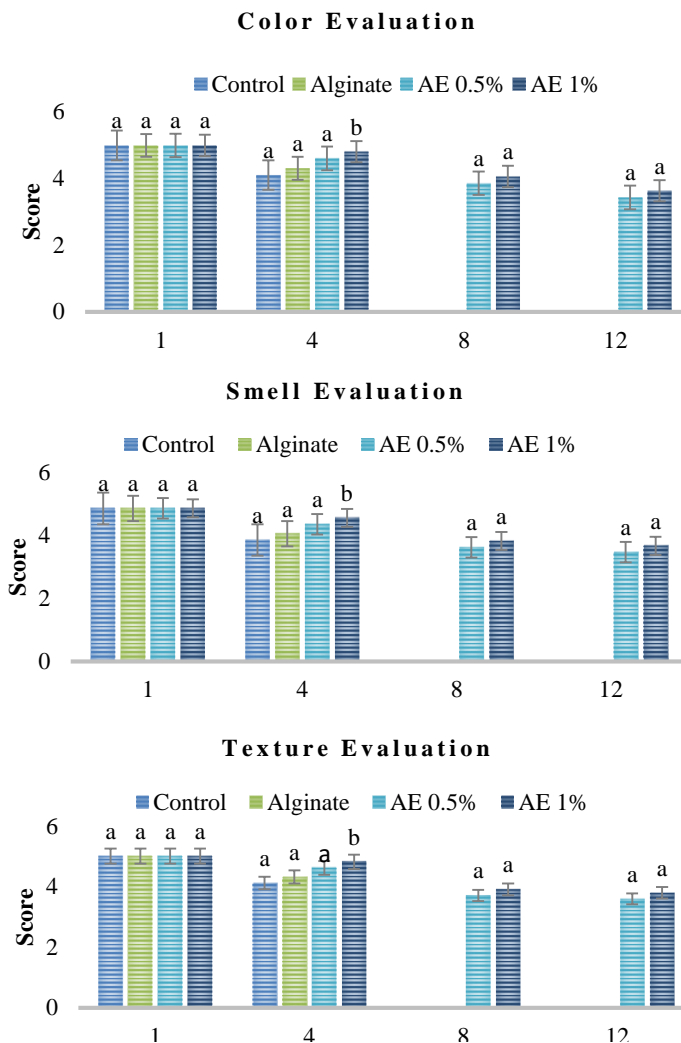
day 8, and other treatments on day 12, exceeded the permissible limit (7 log CFU g<sup>-1</sup>). The highest total microbial load was for the control sample, which exceeded the limit on all days of study days 1 and 4. The results are consistent with the research of Bazargani-Gilani (2018). The initial TVC in both studies was approximately 4 log CFU g<sup>-1</sup>. In the control group of the cited study, TVC reached 7.46 log CFU g<sup>-1</sup> by day 6, exceeding the permissible limit, whereas in our study, this

the alginate-coated and extract-coated samples reached this limit on days 8 and 12, respectively. These results are consistent with the findings of Raeisi *et al.* (2020), who also observed a significant antimicrobial effect of plant extracts in extending the shelf life of fish during cold storage. On day 12, the psychrotrophic bacterial load reached 7.3 log CFU g<sup>-1</sup>, aligning with our findings and indicating a progressive increase in microbial load as storage time increased. In

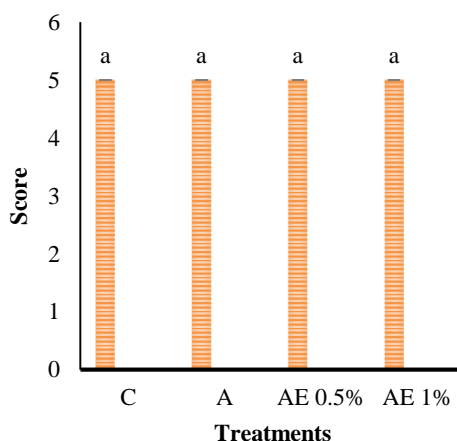


contrast, Sallam (2007) reported a similar trend, with the control group exceeding the 7 log CFU g<sup>-1</sup> limit by day 12. However, our study underscores the effectiveness of the *E. campestre* extract, which inhibited psychrotrophic bacterial growth earlier, by day 4, highlighting its potential in preserving fish quality during refrigerated storage. The antimicrobial effects of *E. campestre* extract were significant, especially against

mesophilic and psychrotrophic bacteria, delaying microbial spoilage during refrigeration. This finding aligns with Ebrahimi and Larypoor (2022), who observed similar effects of plant extracts on refrigerated fish. Extract-treated samples showed lower microbial load than the controls, with levels remaining below permissible limits, highlighting its potential in delaying spoilage.



**Figure 6.** The results of the three parameters (color, smell, texture) of evaluating the sensory properties of different treatments during storage in the refrigerator (temperature 4±1°C). Different letters in each day indicate significant differences (P < 0.05). \*Note: The control and alginate treatments were removed on the 8th day as they did not receive any scores, while the extract and alginate treatments continued to be evaluated and remained in the scoring table until the 12th day.



**Figure 7.** The sensory evaluation of taste of different treatments after cooking at first day of storage. (C: Control, A: Alginate coating, AE 0.5%: Alginate containing 0.5% extract, AE 1%: Alginate containing 1% extract).

### Sensory Evaluation Results

Figure 6 presents the sensory evaluation of texture, color, and smell. The results of all three parameters were measured and presented for each treatment. The results demonstrating that fish fillets treated with *E. campestre* extract maintained a superior overall quality compared to the untreated samples throughout storage. As these were the only sensory attributes assessed in this study, all relevant findings were included. The observed delay in sensory deterioration aligns with the findings of Foromandi and Khani (2023), who reported that chitosan coatings enriched with garlic extract and coriander essential oil enhance the quality of fish fillets. This highlights the dual advantage of the *E. campestre* extract in preserving both antioxidant and sensory properties, underscoring its potential as a valuable option for the food industry.

According to Figure 7, no significant difference was observed in the scoring of the taste parameter after cooking the samples. The results of this section are consistent with the findings of Alizadeh Amoli *et al.* (2019) who did not observe any significant differences between the treatments after cooking.

### CONCLUSIONS

This research showed that adding *E. campestre* extract to alginate coating enhanced antimicrobial and antioxidant properties, and effectively preserved the sensory qualities of the fish fillets. This coating improved smell, texture, color and delayed spoilage, leading to the shelf life extension of rainbow trout by approximately four days, reaching 8 days compared to the control. However, further studies are needed to better understand the efficacy and mechanisms of *E. campestre*'s antimicrobial and antioxidant effects for food preservation.

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## افزایش ماندگاری و خواص حسی فیله ماهی قزل آلاهی رنگین کمان از طریق پوشش سدیم آلژینات حاوی عصاره *Eryngium campestre* در دمای 4 درجه سانتی گراد

محمد عابدی، حسین تاجیک، و تورج مهدی زاده

### چکیده

ماهی تازه یک ماده غذایی بسیار فاسد شدنی است و به راحتی فاسد می شود. در این تحقیق، پس از بررسی خواص آنتی اکسیدانی عصاره *Eryngium campestre* (Ece)، اثر آن به همراه پوشش آلژینات سدیم بر ماندگاری ماهی قزل آلاهی رنگین کمان در شرایط سردخانه (4 درجه سانتیگراد) به مدت 12 روز بررسی شد. برای ارزیابی خواص آنتی اکسیدانی عصاره، آزمایشاتی مانند DPPH، فنول کل، قدرت کاهشی و ABTS انجام شد. پس از آن، نمونه های تیمار شده با پوشش آلژینات حاوی Ece که به روش اسپری اعمال شده بود، از نظر پارامترهای شیمیایی (TBA، TVN و pH) پارامترهای میکروبیولوژیکی (کل باکتری های روان گردان و مزوفیل)، و ارزیابی های حسی در فواصل چهار روزه تا ۱۲ روز آنالیز شدند. نتایج نشان داد که Ece دارای خواص آنتی اکسیدانی قابل توجهی است. علاوه بر این، تیمارهایی که شامل عصاره همراه با پوشش آلژینات سدیم بودند به طور قابل توجهی سطوح pH، TVN و TBA را نسبت به نمونه شاهد ( $P < 0/05$ ) کاهش دادند. آزمایش های میکروبی نشان داد که تمام نمونه های تیمار شده رشد باکتری را در مقایسه با نمونه شاهد، با کاهش تقریباً  $3 \log \text{CFU/g}$  نسبت به گروه کنترل، مهار کردند. در ارزیابی حسی، تیمارهای حاوی Ece و آلژینات سدیم نتایج مطلوب تری نسبت به گروه کنترل به همراه داشت. با توجه به نتایج به دست آمده، پوشش نمونه ها با آلژینات سدیم و Ece باعث بهبود خواص میکروبی، شیمیایی، حسی و ماندگاری ماهی قزل آلاهی رنگین کمان در شرایط یخچال تا حدود چهار روز شد.