1   **Enhancing the Shelf Life and Sensory Properties of Rainbow Trout Fillets**
2   **through Sodium Alginate Coating Containing *Eryngium campestre* Extract at**
3   **4°C**
4

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6   **Abstract**

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

24 **Keywords:** Sodium alginate , *Eryngium campestre*, Shelf life, Extract, Rainbow trout.

25
26 **1. INTRODUCTION**

27 Fish is a vital source of protein and omega-3 fatty acids (ω-3 PUFAs), which are important for a
28 healthy diet. Since the body cannot produce omega-3s, they must be obtained from food. These
29 fatty acids offer numerous health benefits, including improved heart health and reduced

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30 inflammation (Zarandi et al., 2022). Rainbow trout (*Oncorhynchus mykiss*) is one of the most

31 extensively farmed freshwater fish species worldwide and is preferred by consumers for its high

32 nutritional value. The chemical composition of trout fillets can differ based on various factors such

33 as age, gender, season, water temperature, and dietary conditions (Foromandi & Khani, 2023).

34 Typically, trout fillets contain approximately 73% water, 20% protein, 5% fat, and 1.5% minerals.

35 This nutritional composition, along with significant levels of essential amino acids and

36 polyunsaturated fatty acids, categorizes rainbow trout as highly perishable food (Popelka et al.,

37 2014).

38   The shelf life and overall quality of fish can be affected by enzymatic and microbial processes,

39 along with the oxidation of fats (Mazandrani et al., 2016). Additionally, there are safety concerns

40 related to lipid oxidation and microbial growth (Shakour et al., 2021). Various strategies have been

41 investigated to preserve fish products and extend their shelf life, including the incorporation of

42 plant extracts as natural additives (Charoenphun et al., 2023).

43   Plant extracts have antibacterial and antioxidant properties (Rathod et al., 2021). To reduce the

44 negative effects of chemical preservatives and meet consumer demand for natural products, plant

45 extracts and edible coatings are used to extend the shelf life and prevent spoilage of fresh fish

46 (Fadıloğlu & Emir Çoban, 2018). These properties are essential as they can control the growth of

47 spoilage microorganisms and shield the fish from oxidative damage. Nevertheless, their application

48 is often restricted due to costs and potential toxicity.

49   Edible coatings derived from alginate, a biodegradable hydrocolloid, have been implemented to

50 preserve fish fillets. Alginate coatings provide a physical barrier while also improving the overall

51 quality and acceptability of the fish by preserving moisture and minimizing lipid oxidation

52 (Urbonavičiūtė et al., 2023).

53   *Eryngium campestre L.*, part of the *Apiaceae* family, is a perennial plant that grows in Asia,

54 Europe, and Africa. This plant is widely used in traditional medicine to treat various conditions,

55 including coughs, urinary infections, increased urination, kidney dysfunction, and the removal of

56 kidney and bladder stones (Azizkhani & Sodanlo, 2021). *E. campestre* is abundant in phenolic

57 compounds, which contribute to its antioxidant and antimicrobial properties (Kartal et al., 2006).

58 This study examines the impact of *E. campestre* extract in edible and biodegradable coatings made

59 from sodium alginate on the quality and shelf life of rainbow trout fillets stored at 4 °C. The

60 incorporation of *E. campestre* extract is intended to enhance the preservative properties of these

61   coatings, thereby maintaining the fish's sensory characteristics and nutritional value during

62   extended storage. The research aims to support sustainable practices in the seafood industry by

63   promoting natural preservation methods over synthetic chemicals.

64

65   **2. MATERIALS AND METHODS**

66   **2.1 Preparation of ethanolic extract of *E. campestre***

67   The maceration method was used to prepare the ethanolic extract from the *E. campestre*. This

68   plant picked from the forest areas of Amol city. The leafy part and stem were dried in the shade

69   and ground into a powder, passed through a sieve with a 60 μm mesh size. 200 grams of powder

70   were mixed with 1 liter of pure ethanol and placed in a shaker incubator at 150 rpm for 24 hours at

71   42°C. To remove most of the solvent, the mixture was placed in a rotary evaporator (Heidolph,

72   laborota 400 efficient, Germany) at 50°C and 100 rpm under vacuum conditions. For concentration,

73   it was placed in an oven at 45°C and finally lyophilized at -50°C for 24 hours, then refrigerated in

74   a closed container (Alizadeh Amoli et al., 2019).

75

76   **2.2 GC-MS analysis of *E. campestre* extract**

77   GC-MS analysis was performed using an Agilent 7890A GC device equipped with an HP-5MS

78   column and a 5975 mass spectrometer (Agilent Technologies, USA) (Mishra & Patnaik, 2020).

79

80   **2.3 Antioxidant activity analyses**

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

82   extract.

83

84   **2.3.1 DPPH test (1,1-diphenyl-2-picrylhydrazyl)**

85   The study tested the antioxidant activity of an extract by diluting it to 0.25 mg/ml and adding it

86   to a DPPH solution. Absorbance was measured at 517 nm with spectrophotometer (Pharmacia

87   Biotech, Sweden) after 30 minutes in the dark. The free radical inhibition was calculated using the

88   formula: AC * 100 / (AC - AS), where AC is the absorbance of the control, and AS is the

89   absorbance of the sample. BHT was used as a positive control at 1 mg/ml (Ebrahimi & Larypoor,

90   2022).

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94 **2.3.2 Determination of total phenolic content**

95 The total phenolic content of the plant was measured using the Folin-Ciocalteu method. Extract

96 dilutions were mixed with Folin's reagent, gallic acid, and sodium carbonate, then kept in the dark

97 for specified times. Absorbance was measured at 760 nm, and the total phenolic content was

98 expressed as mg of gallic acid per gram of material(Gharedaghi et al., 2020)

99

100 **2.3.3 The method of determining the reducing power**

101 Extracts were mixed with sodium phosphate, potassium ferricyanide, and incubated at 50°C for

102 20 minutes. After adding trichloroacetic acid and centrifuging, distilled water and iron chloride

103 were added to the supernatant. Absorbance was measured at 700 nm to assess the results

104 (Merghache et al., 2014).

105

106 **2.3.4 ABTS radical cation method**

107 The antioxidant capacity was assessed using a modified ABTS method. ABTS•+ radical was

108 generated by mixing ABTS and potassium persulfate, then incubated for 16 hours. The solution

109 was diluted to achieve an absorbance of 0.7 at 734 nm. Extracts or BHT were added to the ABTS•+

110 solution, and after 6 minutes, absorbance was measured. Inhibition percentage was calculated using

111 the formula: $(Ablank - Asample) / Ablank \times 100$, and results were expressed as antioxidant capacity

112 equivalent to ascorbic acid (Kikowska & Thiem, 2021).

113

114 **2.4 Preparation of fish samples**

115 Rainbow trout, weighing approximately 600 ± 50 grams, was procured from a fish sales center in

116 Urmia city. After procurement, the fish was properly examined, and its internal organs were

117 removed. The body of the fish was thoroughly rinsed with potable water to eliminate any residual

118 blood. The dimensions of the fish fillet were measured at approximately 15 cm in length, 10 cm in

119 width, 3 cm in height, and its weight was approximately 100 grams. Subsequently, the fish was

120 transported to the Food Hygiene Laboratory at the Faculty of Veterinary Medicine, Urmia

121 University, within 25 minutes to ensure sample integrity and minimize the risk of microbial

122 contamination.

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2.5 Preparation of treatments

The sodium alginate solution was prepared by dissolving 1.5 grams 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:

- Fish fillet without coating and extract
- Fish fillet with alginate spray coating and without extract
- Fish fillet with alginate 1.5% spray coating containing 0.5% *E. campestre* extract
- Fish fillet 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 quality and stability.

2.6 Chemical analyses of treated fish fillets

2.7.1 pH values

For this purpose, 5 gr of each fish fillet sample was placed in 10 mL of distilled water and homogenized using a homogenizer 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).

2.7.2 Total volatile base nitrogen (TVB-N)

To determine total volatile basic nitrogen (TVB-N), 10 grams 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 milligrams of TVB-N per 100 grams of fish fillets (Ghasemi et al., 2023).

158 **2.7.3 Thiobarbituric acid reactive substance**

159   To measure malonaldehyde content (TBARS), 10 grams of fish fillet was homogenized with 5%

160   TCA (Trichloroacetic acid) and BHT, filtered, and the filtrate adjusted to 50 ml. TBA reagent was

161   added to the filtrate, heated at 100°C for 1 hour, and absorbance at 532 nm was measured. The

162   TBARS value, indicating malonaldehyde, was calculated and expressed as milligrams per kilogram

163   of fish meat (Ghasemi et al., 2023).

164
165 **2.7 Microbiological analyses**

166 **2.8.1 Total mesophilic bacterial count**

167   After diluting the samples from each dilution tube, 100 microliters of each sample were

168   inoculated onto plates containing PCA culture medium and spread uniformly using a Pasteur

169   pipette. Subsequently, the plates were incubated upside down at 37°C for 48 hours. After

170   incubation, the colonies were counted and reported as CFU/g (Muñoz-Tebar et al., 2023).

171
172 **2.8.2 Total Psychrotrophic count**

173   After diluting the samples from each dilution tube, 100 microliters of each sample was inoculated

174   onto plates containing PCA culture medium and spread uniformly across the medium using a

175   Pasteur pipette. The plates were then incubated in an inverted position at 10°C for 7 days.

176   Following incubation, the colonies were counted and reported as CFU/g (Muñoz-Tebar et al.,

177   2023).

178
179 **2.9 Sensory evaluation**

180   A trained panel of 10 assessors evaluated the organoleptic properties of treated fish fillets in two

181   stages. In the first stage, they assessed the taste of cooked fillets, and in the second stage, they

182   evaluated refrigerated fillets for texture, aroma, and color on days 1, 4, 8, and 12. Fresh fillets at

183   4°C were used as the reference for maximum scores, and a 5-point hedonic scale used for

184   evaluation (Bazargani-Gilani & Pajohi-Alamoti, 2020).

185
186 **2.10 Statistical Analysis**

187   After obtaining the data from the tested factors, SPSS version 26 software was used for statistical

188   analysis, including one-way ANOVA for data analysis. Excell version 2022 was used to draw the

189   graphs. Duncan's test was used to classify the samples according to the statistical difference

190 between them and to measure their average. Normality tests (e.g., Shapiro-Wilk and Kolmogorov-

191 Smirnov) were conducted prior to performing ANOVA to ensure the suitability of the parametric

192 test. The results were considered significant at a p-value of < 0.05.

193

194 **3. RESULTS AND DISCUSSION**

195 **3.1 GC-MS results of *E. campestre* extract**

196 According to Table 1, The GC-MS analysis of *Eryngium campestre* extract identified 32

197 compounds, with limonene, δ-3-carene, β-sesquiphellandrene, and cyclobuta having the highest

198 peak areas. This study's findings align with Fernandes (2013), which identified compounds like

199 germacrene D and α-cadinol, although there are differences. Variations in chemical profiles are

200 attributed to differences in plant samples, extraction methods, and environmental conditions,

201 underscoring the chemical diversity of *Eryngium campestre* and its potential applications in the

202 pharmaceutical and food industries. (Fernandes, 2013).

203

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

No.	Compounds	RT	persentage
1	Heptanal	11.23	0.15
2	α-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	δ-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	β-Elemene	33.74	0.21
21	α-cis-Bergamotene	35.84	0.72
22	α-Acoradiene	37.17	0.46
23	E-β-Ionone	38.02	1.23
24	Z-α-Bisabolene	38.65	2.57
25	β-Bisabolene	38.94	1.84
26	Myristicin	39.45	0.17
27	β-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-Hexadecanoic acid	59.65	2.69
Total Identified			99.54

205

206

3.2 DPPH antioxidant test results

208   In Figure 1, The DPPH assay results show that as the concentration of *E. campestre* extract

209   increases, the inhibition of free radicals also increases, indicating a strong concentration-dependent

210 antioxidant effect. This trend was evident across all concentrations, with the extract demonstrating

211 high antioxidant activity in every dilution. These findings align with the work of Charoenphun et

212 al (2023) and Gharedaghi et al. (2020), who observed similar antioxidant effects in plant extracts.

213 Furthermore, Kremer et al. (2021) reported comparable results in *E. amethystinum* and *E. alpinum*,

214 confirming the potential of *E. campestre* as a natural antioxidant for applications in food

215 preservation and oxidative stress reduction.

216
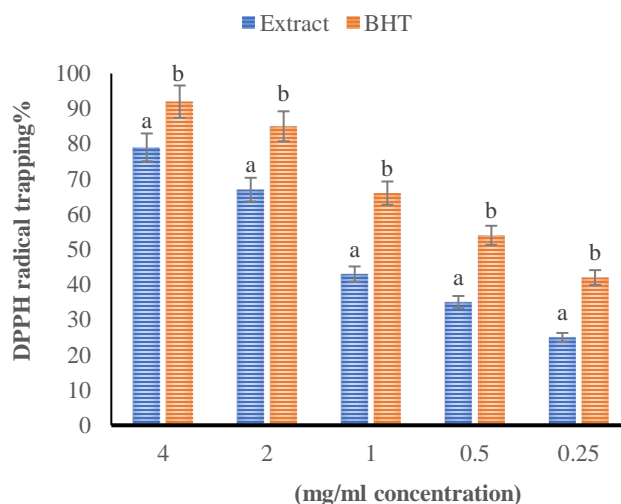
217 **Figure 1.** DPPH radical scavenging rate of different concentrations of *E. campestre* ethanol extract
218 compared to BHT. a-b: Different letters in each concentration indicate a significant difference ($p <$
219 0.05).

220

221 **3.3 Total phenolic content results**

222 Based on the data from Table 2 and Figure 2, the total phenolic content in the *E. campestre* extract

223 was determined using a calibration curve for gallic acid. The study revealed a strong correlation

224 between the high phenolic content and antioxidant activity of the extract. This finding is consistent

225 with Al-Askar et al. (2023), who also highlighted significant polyphenolic compounds in *E.*

226 *campestre* extracts, which are directly linked to antioxidant and antimicrobial properties. These

227 phenolic compounds play a crucial role in scavenging free radicals, supporting their potential in
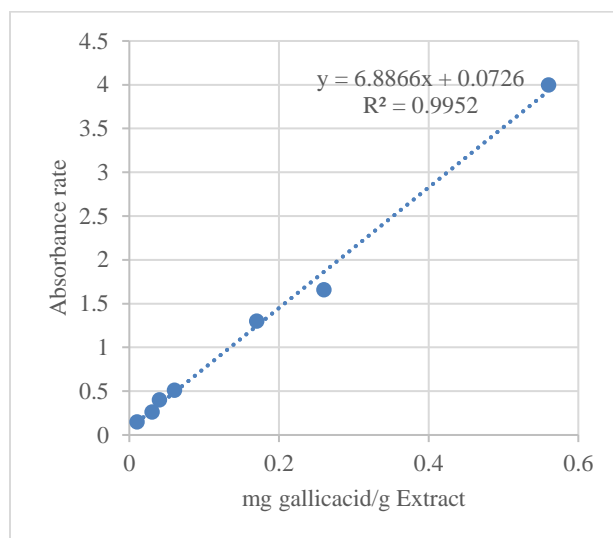
228 food preservation (Al-Askar et al., 2023)

229

230

Figure 2. Gallic acid standard curve.

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

Antioxidant assay by DPPH method			
R	(p) sig	mg gallic acid/g extract	
0.950	0.005[**]	125±9.80	Total phenol in the Alcoholic Extract

233 [**] Correlation is significant at the 0.01 level.

234 **3.4 The reduction power results**

235 According to Figure 3, The reduction and absorption power at 700 nm for spectrophotometric

236 measurements were observed with 2 mg of *E. campestre* extract (absorbance value of 1.8) and

237 butylated hydroxytoluene (BHT) (absorbance value of 3). These results suggest that *E. campestre*

238 extract exhibits significant antioxidant potential, comparable to BHT. The strong reducing power

239 observed in this extract, particularly in its ability to reduce ferric ions, is vital for antioxidant

240 applications. This finding is consistent with Merghache et al. (2014), who noted similar reducing

241 power in *E. tricuspidatum* essential oil, further supporting the potential of *E. campestre* as a natural

242 antioxidant and a promising alternative to synthetic antioxidants in food preservation (Merghache
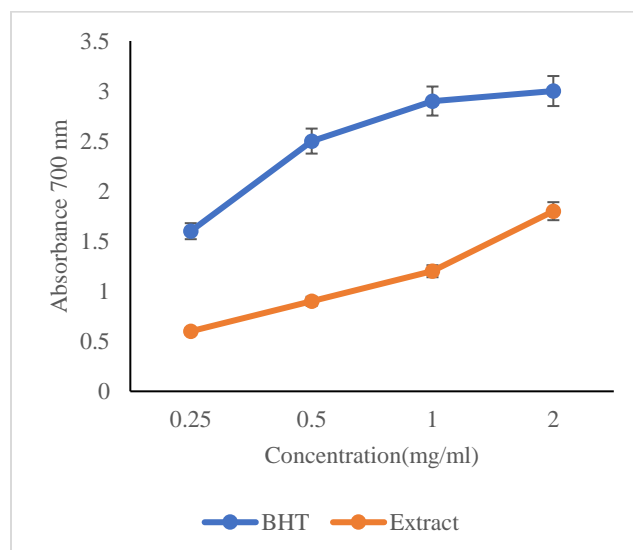
243 et al., 2014).

10

244
245 **Figure 3.** The rejuvenating potency of *E. campestre* extracts compared to BHT.
246 .

247 **3.4 ABTS radical inhibitory test results**

248 In Table 3, *E. campestre* extract showed inhibitory activity at all concentrations, with its

249 performance lower than BHT, except at the highest concentration where it was comparable.

250 Increasing the extract concentration enhanced its free radical scavenging ability, as confirmed by

251 the ABTS test. While its antioxidant activity was somewhat lower than BHT, the improved efficacy

252 with higher concentrations suggests its potential for food preservation, supporting its use as a

253 natural antioxidant to extend shelf life and improve food quality, consistent with previous studies

254 (Kikowska & Thiem, 2021).

255

256 **Table 3**. Inhibition percentage and antioxidant capacity equivalent to ascorbic acid of different
257 concentrations of alcoholic extract and BHT.

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

258 In each column, non-identical lowercase letters indicate a significant difference at the $p<0.05$ level between the extract
259 and BHT at the same concentration. Non-identical uppercase letters also indicate a significant difference between
260 different concentrations of the same compound at the $p<0.05$ level.
261

262 **3.5.1 Chemical composition of fish fillet**

263 According to the analysis carried out on the rainbow trout sample, the approximate amount of ash,

264 fat, protein, and moisture was presented in the table 4, The obtained results are consistent with the

265 findings of Torabi Delshad et al. (2012).

266 **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

270

271 **3.5.2 pH measurement results**.

272 During refrigerated storage, all rainbow trout samples showed an increase in pH, with the control

273 sample exhibiting a much higher increase, especially on days 8 and 12. As shown in Figure 4,

274 Coated fillet samples with the extract maintained a stable pH within the permissible range (6-7),

275 showing no significant increase from day 4 onwards. This aligns with Alizadeh et al. (2021), who

276 found that treated samples displayed better pH stability, highlighting the antimicrobial and

277 preservative efficacy of plant-derived bioactive compounds in delaying spoilage and maintaining

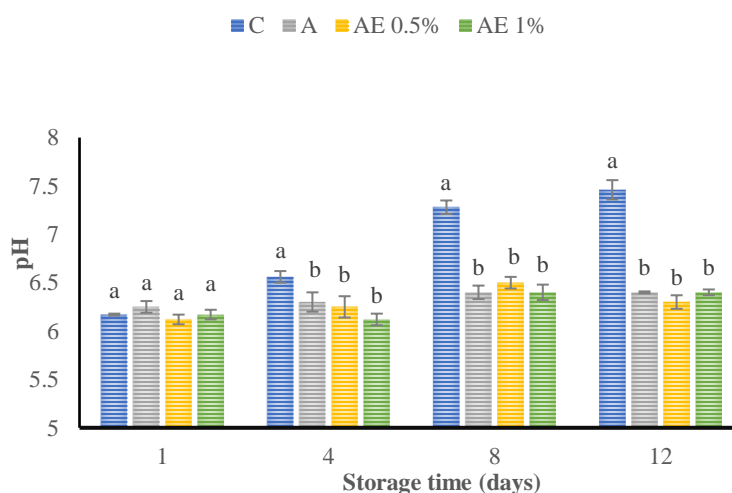278 food quality (Alizadeh et al., 2019).

279

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

284

285 **3.5.3 TVB-N results**

286 According to Figure 5, our study revealed an upward trend in TVB-N levels in all samples during

287 storage. However, the bioactive coating effectively kept these levels below the permissible limit

288 (25 mg/100 g) throughout the storage period. In contrast, the control sample exceeded this limit

289 after 4 days, and the alginate-coated sample did so after 12 days. These results align with previous

290 studies (Ojagh et al., 2010), which found that chitosan-based coating combined with plant

291 compounds, significantly reduced TVB-N levels in fish samples, and maintaining them below the

292 acceptable limit for 16 days. Similarly, Öz (2018) demonstrated that garllic supplementation

293 inhibited microbial growth, and lowering TVB-N levels during frozen storag. Ozogul et al. (2017)

294 reported that nanoemulsions with essential oils like rosemary and thyme effectively reduced

295 spoilage, while Öz et al. (2017) highlighted the role of black cumin oil in slowing TVB-N increases

296 in fish fillets at 2°C. These findings are inconsistent with ours, underscore the potential of bioactive

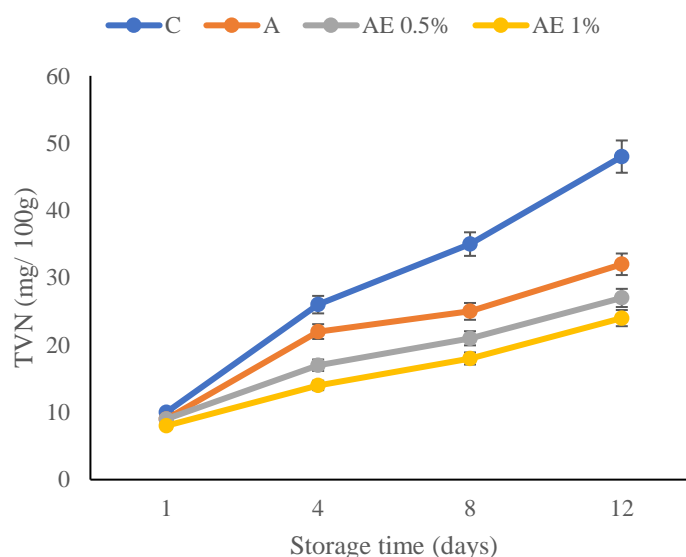297 coatings in preserving fish quality.

298

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

302

303 **3.5.4 TBARS value results**

304 In alignment with Mehdizadeh et al. (2019), our data indicated that all treatments, except the

305 control group, maintained Thiobarbituric Acid (TBA) levels within the revised permissible range

306   of 1-2 mg MDA/kg throughout the 12-day storage period, as delineated0 in Table 5. A more

307   granular analysis revealed a markedly attenuated rate of TBA increase in the coated samples,

308   particularly evident from day 4 onwards. During this interval, coated samples consistently

309   exhibited demonstrably lower TBA values than the control, a trend that persisted until day 8.

310   Subsequently, a gradual elevation in TBA levels was observed from day 12 onwards, primarily

311   attributed to the progressive nature of lipid oxidation. This body of evidence underscores the

312   efficacy of the coatings in providing a robust protective barrier against lipid peroxidation,

313   effectively retarding the formation of malonaldehyde a critical marker of lipid degradation and

314   concomitant quality decline in fish products. This refined permissible range accentuates the subtle

315   yet significant protective influence of our coatings. Alginate and extract coatings slowed TBA

316   increases more effectively than control or alginate alone samples, demonstrating a statistically

317   significant impact on oxidation. Similarly, Gharehdaghi et al. (2020) noted alginate coatings delay

318   lipid oxidation by acting as oxygen barriers, with enriched coatings further reducing oxidation and

319   microbial growth in fish.

320

321   **Table5**. Changes in the index of thiobarbituric acid (mg MDA/kg) in rainbow trout fillets during
322   storage at 4°C)

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

323   (C: Control, A: Alginate coating, AE 0.5%: Alginate containing 0.5% extract, AE 1%: Alginate
324   containing 1% extract)
325   Small letters in each column and non-identical capital letters in each row indicate a significant
326   difference in the level (p<0.05).
327

3.6 Microbiological results

3.6.1 Total mesophilic bacterial count

330   Table 6 shows the data related to the total microbial load of fish samples stored in the refrigerator

331   during 12 days. According to it, the microbial load increased with increasing storage time in all

332   samples. In this study, the total microbial load in the control sample on day 8 and other treatments

333   on day 12 exceeded the permissible limit (7 log CFU/g). The highest total microbial load was for

334   the control sample, which exceeded the limit on all days of study days 1 and 4. The results are

335   consistent with the research of Bazargani-Gilani (2018) , The initial TVC in both studies was

336 approximately 4 log CFU/g. In the control group of the cited study, TVC reached 7.46 log CFU/g

337 by day 6, exceeding the permissible limit, whereas in our study, this threshold was surpassed on

338 day 8. Consistent with the cited study, extract-treated samples in our research exhibited lower

339 microbial loads, remaining below the 7 log CFU/g limit, highlighting the effective antimicrobial

340 properties of the extracts.

341

342 **Table6.**Total mesophilic bacterial counts (log CFU/g) of rainbow trout fillets during storage at

343 4°C.

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

344 Different letters in each day indicate significant differences ($p < 0.05$). (C: Control, A: Alginate coating, AE 0.5%:
345 Alginate containing 0.5% extract, AE 1%: Alginate containing 1% extract)

346

347 **3.6.2 Total psychrotrophic bacterial count**

348 According to Table 7, the total count of psychrotrophic bacteria in rainbow trout fillets exceeded

349 the established 7 log CFU/g limit by day 4 in the control group, whereas the alginate-coated and

350 extract-coated samples reached this limit on days 8 and 12, respectively. These results are

351 consistent with the findings of Raeisi et al. (2020), who also observed a significant antimicrobial

352 effect of plant extracts in extending the shelf life of fish during cold storage. On day 12, the

353 psychrotrophic bacterial load reached 7.3 log CFU/g, aligning with our findings and indicating a

354 progressive increase in microbial load as storage time increased. In contrast, Sallam (2007)

355 reported a similar trend, with the control group exceeding the 7 log CFU/g limit by day 12.

356 However, our study underscores the effectiveness of the *E. campestre* extract, which inhibited

357 psychrotrophic bacterial growth earlier, by day 4, highlighting its potential in preserving fish

358 quality during refrigerated storage. The antimicrobial effects of *E. campestre* extract were

359 significant, especially against mesophilic and psychrotrophic bacteria, delaying microbial spoilage

360 during refrigeration. This finding aligns with Ebrahimi & Larypoor (2022), who observed similar

361 effects of plant extracts on refrigerated fish. Extract-treated samples showed lower microbial load

362 than controls, with levels remaining below permissible limits, highlighting its potential in delaying

363 spoilage.

364

365 **Table7.** Total psychrotrophic bacterial counts (log CFU/g) of rainbow trout fillets during storage
366 at 4°C

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

367 Different letters in each day indicate significant differences ($p < 0.05$). (C: Control, A: Alginate coating, AE 0.5%:
368 Alginate containing 0.5% extract, AE 1%: Alginate containing 1% extract).
369

370 **3.7 Sensory evaluation results**

371 Figure 6 presents the sensory evaluation of texture, color, and smell. The results of all three

372 parameters were measured and presented as for each treatment. The results demonstrating that fish

373 fillets treated with *E. campestre* extract maintained a superior overall quality compared to untreated

374 samples throughout storage. As these were the only sensory attributes assessed in this study, all

375 relevant findings were included. The observed delay in sensory deterioration aligns with the

376 findings of Foromandi and Khani (2023), who reported that chitosan coatings enriched with garlic

377 extract and coriander essential oil enhance the quality of fish fillets. This highlights the dual

378 advantage of the *E. campestre* extract in preserving both antioxidant and sensory properties,

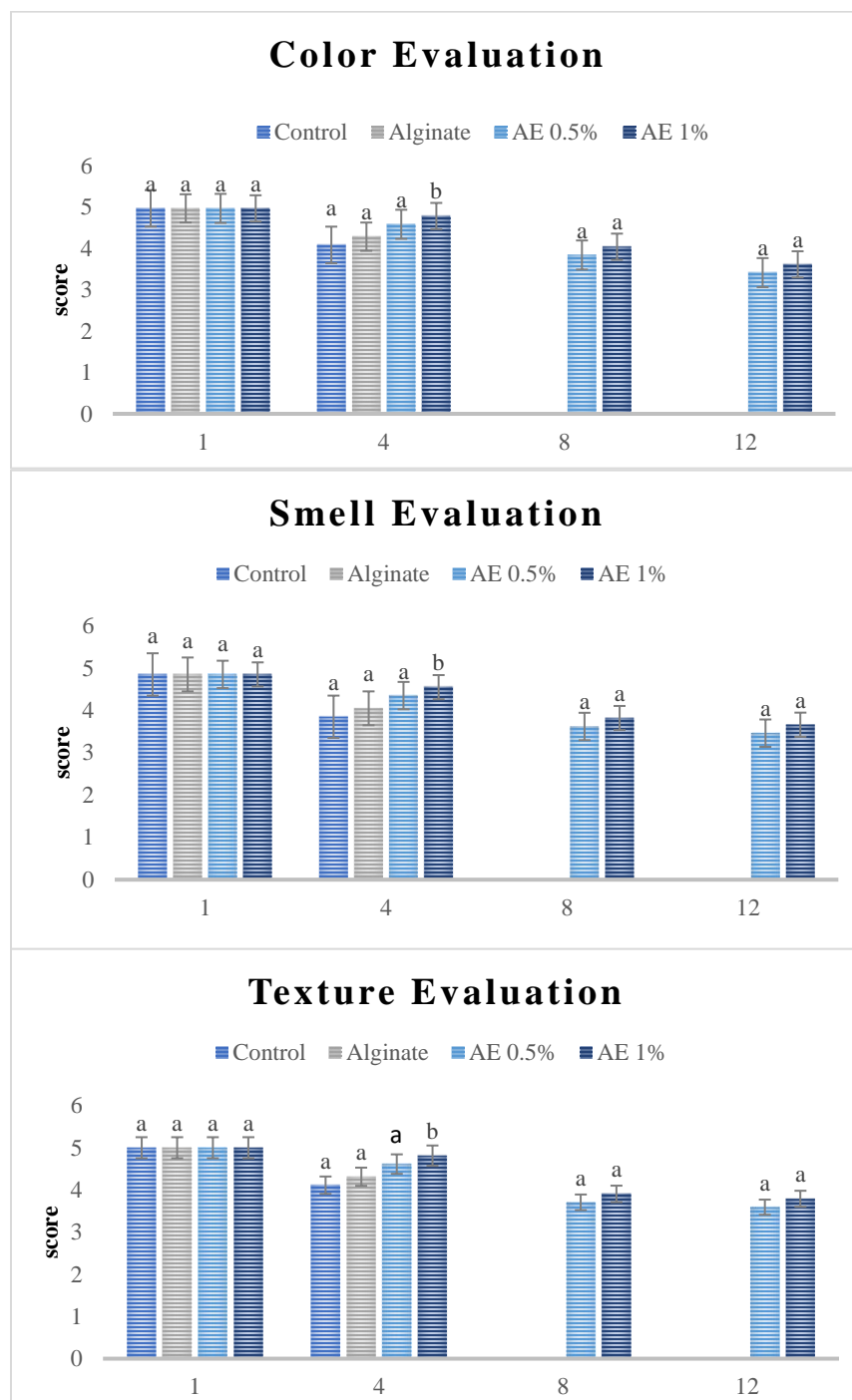379 underscoring its potential as a valuable option for the food industry

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 $4\pm1°C$) (C: Control, A: Alginate coating, AE 0.5%: Alginate containing 0.5% extract, AE 1%: Alginate containing 1% extract). 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.*

391 According to the Figure 7, no significant difference was observed in the scoring of the taste

392 parameter after cooking the samples. The results of this section are consistent with the findings of

393 Alizadeh Amoli et al. (2019) who did not observe any significant differences between the
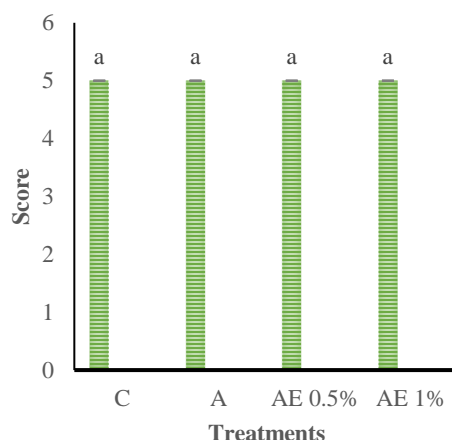
394 treatments after cooking.

395
396 **Figure 7.** The sensory evaluation of taste of different treatments after cooking at first day of storage
397 (C: Control, A: Alginate coating, AE 0.5%: Alginate containing 0.5% extract, AE 1%: Alginate
398 containing 1% extract)
399
400 **4. CONCLUSIONS**

401 The research showed that adding E. campestre extract to alginate coating enhanced antimicrobial

402 and antioxidant properties, and effectively preserving the sensory qualities of the fish fillets. This

403 coating improved smell, texture, color and delayed spoilage, leading to the shelf life extension of

404 rainbow trout by approximately four days, reaching 8 days compared to the control. However,

405 further studies are needed to better understand the efficacy and mechanisms of E. campestre's

406 antimicrobial and antioxidant effects for food preservation.

407

411
412 **5. References**

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516   افزایش ماندگاری و خواص حسی فیله ماهی قزل آلای رنگین کمان از طریق پوشش سدیم آلژینات حاوی

517   عصاره *Eryngium campestre* در دمای 4 درجه سانتی گراد

518

519   محمد عابدی، حسین تاجیک، و تورج مهدی زاده

520   چکیده

521

522   ماهی تازه یک ماده غذایی بسیار فاسد شدنی است و به راحتی فاسد می شود. در این تحقیق، پس از بررسی خواص آنتی

523   اکسیدانی *عصاره Eryngium campestre* (Ece)، اثر آن به همراه پوشش آلژینات سدیم بر ماندگاری ماهی قزل آلای رنگین

524   کمان در شرایط سردخانه (4 درجه سانتیگراد) به مدت 12 روز بررسی شد. برای ارزیابی خواص آنتی اکسیدانی عصاره،

525   آزمایش‌هاتی مانندDPPH ، فنول کل، قدرت کاهشی و ABTS انجام شد. پس از آن، نمونه‌های تیمار شده با پوشش آلژینات

526   حاوی Ece که به روش اسپری اعمال شده بود، از نظر پارامترهای شیمیایی (TBA، TVN و pH) پارامترهای

527   میکروبیولوژیکی (کل باکتری‌های روانگردان و مزوفیل)، و ارزیابی‌های حسی در فواصل چهار روزه تا ۱۲ روز آنالیز

528   شدند. نتایج نشان داد که Ece دارای خواص آنتی اکسیدانی قابل توجهی است. علاوه بر این، تیمار هایی که شامل عصاره همراه

529   با پوشش آلژینات سدیم بودند به طور قابل توجهی سطوح pH، TVN و TBA را نسبت به نمونه شاهد (P< 0/05) کاهش

530   دادند .آزمایش‌های میکروبی نشان داد که تمام نمونه‌های تیمار شده رشد باکتری را در مقایسه با نمونه شاهد، با کاهش تقریباً

531   log 3 CFU/g نسبت به گروه کنترل، مهار کردند. در ارزیابی حسی، تیمار های حاوی Ece و آلژینات سدیم نتایج مطلوب

532   تری نسبت به گروه کنترل به همراه داشت. با توجه به نتایج به‌دست‌آمده، پوشش نمونه‌ها با آلژینات سدیم و Ece باعث بهبود

533   خواص میکروبی، شیمیایی، حسی و ماندگاری ماهی قزل‌آلای رنگین کمان در شرایط یخچال تا حدود چهار روز شد.