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Research Article

A new alternative animal feed by lipid extraction from fish by-products enriched with algae extracts

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ABSTRACT- The current study investigated the effect of hot water extracts from *Sargassum* sp. and *Padina* sp. macroalgae in preventing the oxidation of oil extracted from rainbow trout waste over 42 days at 30 °C. The treatments were fish oil without preservatives (control: C), fish oil containing 100 ppm butylated hydroxytoluene (BHT), fish oil with 1000 ppm *Sargassum* sp. extract, and fish oil with 1000 ppm *Padina* sp. extract. The extraction yields, DPPH radical scavenging activity, and total phenolic content of Sargassum extract were higher than those of Padina extract by 10.4%, 86%, and 28%, respectively. On the 35th day, the peroxide values (PV) of the fish oil treated with Sargassum and Padina extracts were similar to the control group, with values of 33.7 and 34.3 meq O_2/kg, respectively. However, p-anisidine values in the control samples increased significantly ($P < 0.05$) over the 42 days compared to the other groups. Total oxidation (TOTOX) and free fatty acid (FFA, expressed as oleic acid) values of the fish oils treated with Sargassum and Padina extracts were significantly lower than those of the control samples ($P < 0.05$). Additionally, UV_{232} absorbance values were higher in the control group compared to the samples treated with macroalgae extracts. Color difference (ΔE) and whiteness index (WI) values remained within acceptable ranges for all samples. These findings, particularly the promising effects of Sargassum extract, suggest its potential as a natural preservative in fish oil, offering valuable insights for future studies.

INTRODUCTION

In fish processing, a significant amount of waste is generated, comprising skin, guts, entrails, heads, fins, and other parts, which accounts for 20–50% of the total fish weight, depending on the final product and processing method (Fouda, 2018; Mousavipour et al., 2021$_a$). These by-products have substantial potential for reuse in various industries, including animal feed and food industry (Annett et al., 2009; Lee et al., 2019; Ghaly et al., 2013; Mousavipour et al., 2021$_a$). Fish and fish oil are primary sources of polyunsaturated fatty acids (PUFAs) and omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are essential for human health (Raeisi et al., 2021). These fatty acids are critical for retinal and brain development and play a vital role in human growth (Wang et al., 2011). Additionally, high-value fatty acids in the diet can reduce the risk of cardiovascular diseases, certain types of cancer, and immune system impairments (Ceylan et al., 2018; Kolanowski, 2008). They also enhance stress resistance and regulate gene expression (Wang et al., 2011). Therefore, utilizing marine by-products, including their oils, can reintroduce these materials into the human food chain, as well as livestock and aquatic feed, offering economic and environmental benefits (Ciriminna et al., 2019).

However, unsaturated fatty acids in fish oil are prone to oxidation during storage, processing, and heat treatment due to exposure to light, high temperatures, oxygen, and prooxidants (Lee et al., 2019; Yue et al., 2008). Oxidative and hydrolytic reactions produce volatile compounds, such as hydroperoxides, aldehydes, ketones, and fatty acids, which alter key qualities, including smell, taste, color, texture, and nutritional value. These changes reduce consumer acceptance (Bora et al., 2018). To mitigate these effects, synthetic antioxidants such as BHT, BHA, chelating agents, and antimicrobial compounds are often used to improve product quality and shelf life (Li et al., 2007).

However, concerns about the adverse effects of synthetic antioxidants, including mutagenicity, toxicity, and carcinogenicity, have driven interest in natural alternatives, such as plant-derived polyphenolic compounds (Yue et al., 2008). Natural antioxidants,

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including vitamins, cofactors, phenolic acids, and carotenoids, are widely recognized for their efficacy (Carocho and Ferreira, 2013). Phenolic compounds, such as gallic acid, catechin, myricetin, and caffeic acid, are particularly significant, with applications in food, medicine, and cosmetics (Ebrahimi et al., 2021). Among algae, brown algae exhibit higher antioxidant activity than green or red algae (Park et al., 2004). Compounds isolated from aqueous extracts of Sargassum brown algae are particularly effective in free radical scavenging (Haq et al., 2019). For instance, aqueous and ethanolic extracts of *Sargassum boveanum* contain various phenolic compounds (Rafiee et al., 2007).

Research has also demonstrated the antioxidant potential of microencapsulated extracts of Sargassum, Padina, and Caulerpa algae, as well as free extracts of Caulerpa, in extending the shelf life of fish waste oil (*Oncorhynchus mykiss*) (Mousavipour et al., 2021b; Mousavipour et al., 2021a). Similarly, macroalgae species such as *Champia* sp. and *Porphyra* sp. from New Zealand coasts have been studied for their role in preserving fish oil at 60 °C for 12 days (Kindleysides et al., 2012). These antioxidative properties are also critical for producing animal feed. Once parameters for delaying oxidation in fish oil are established, such materials can be readily adapted for animal feeding applications, as evidenced by prior studies (Mnari et al., 2007; Nakajima et al., 2009; Varona et al., 2021).

The primary aim of this study was to extract fish oil from the by-products of rainbow trout (*Oncorhynchus mykiss*) and evaluate the potential antioxidant effects of aqueous extracts from brown macroalgae (*Sargassum* sp. and *Padina* sp.) on the fish oil. Additionally, this study sought to transcend these findings into practical applications for the animal feed industry and to contribute to advancements in food science research. A key objective was to provide an effective analysis of oxidation parameters, such as TOTOX and FFA, to inform and engage potential readers in these fields.

MATERIALS AND METHODS

Materials

The laboratory chemicals used in this research were dichloromethane, butylated hydroxytoluene (BHT), phenolphthalein, ethanol (96%), Folin-ciocalteu, sodium carbonate, dichloroethane, potassium ferricyanide, *p*-anisidine reagent, glacial acetic acid, NaOH, gallic acid, and $CaCl_2$. All chemicals and standards were obtained from Sigma-Aldrich (St. Louis, MO), Zakaria Tajhiz Parseh (Shiraz, Iran), and Merck (Darmstadt, Germany).

Algae extract

The brown macroalgae (*Sargassum* sp. and *Padina* sp.) were obtained from the Abdf company-algae bank, Shiraz, Iran. Macroalgae were initially cleaned and then washed several times using fresh water. After washing, all macroalgae samples were dried in an oven (KM23S, Fara Azma, Iran) at 36-37 °C, grinded, and stored at -20 °C. To prepare the hot water extract, macroalgae was mixed with distilled water (1:20 w:v) and was autoclaved at 121 °C for 20 min. After cooling, the mixture was filtrated, dried using a freeze-dryer, and stored at -20 °C for further analysis (Ebrahimi et al., 2021). The extraction yield was calculated using the equation given below:

Extraction yield (%) = (Weight of dry extract g / Weight of dry algae) × 100 Eq. (1)

DPPH free radical scavenging activity of algae extract

One mL of various concentrations (0.0001 to 5.5 mg/mL) of macroalgae extract was prepared, and then 1 mL of the DPPH solution was added to each sample. After being placed in the dark for 30 min, the absorbance values of the samples were read at 517 nm using a spectrophotometer (T7, PG Instruments Limited, Spain) (n = 3) (Wang et al., 2009). The percentage of inhibition (%) was obtained according to the below equation:

Inhibitory percentage (%) = [(Absorbance of control – Absorbance of sample) / Absorbance of control] × 100 Eq. (3)

Total phenolic content (TPC) of algae extract

To measure the TPC, 100 µL of different concentrations of the extracts were mixed with Folin-Ciocalteu reagent (750 µL) in distilled water and 750 µL of sodium carbonate solution (2%, w:v). After being placed in the dark for 45 min, the absorbance was read at 765 nm by a spectrophotometer. The results were expressed as mg GAE/ mg DW algae extract (Farvin and Jacobsen, 2013).

Treatments

Fish oil was extracted from rainbow trout viscera waste using the solvent-based method outlined by Bligh and Dyer (1959). The treatments included control (fish oil without any antioxidant), BHT (fish oil supplemented with 100 mg BHT/kg oil), Padina extract (fish oil with 1000 mg extract from Padina/kg oil), and Sargassum extract (fish oil with 1000 mg extract from Sargassum/kg oil). The prepared samples were stored in dark glass tubes and incubated at 30 °C for 42 days using an incubator (503, Arian Azmateb, Iran), following the method described by Mousavipour et al. (2021a).

Evaluation of fish oil quality indices

p-Anisidine value (AV) was specified using AOCS Cd 18-90 (AOCS, 2000). Free fatty acids content (FFAs) as a percentage of oleic acid was evaluated following the AOCS Ca 5a-406 protocol. Peroxide value (PV) as milliequivalents of active oxygen per kg of oil was determined based on AOCS Cd 8-53. The TOTOX value was calculated by adding AV to twice the PV (Deepika et al., 2014). UV_{232} and UV_{270} spectrum analyses of the rainbow trout oil samples were performed using a UV-visible spectrophotometer (T7, PG Instruments Limited, United Kingdom).

Color measurement in fish oil

Color properties of treated oil samples were evaluated with a colorimeter (MAT 2000, Iranian Teb Barez, Shiraz, Iran) according to L*, a*, and b* systems. The L* value defined lightness from black to white between 0

and 100. The a* value ranged from (+) red to (-) green, and the b* value from (+) yellow to (-) blue. Total color changes (ΔE) and whiteness index (WI) were calculated using the following equations (Vardizadeh et al., 2021):

$\Delta E = [(L^*_1-L^*_2)^2+(a^*_1-a^*_2)^2+(b^*_1-b^*_2)^2]^{1/2}$ Eq. (3)

$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$ Eq. (4)

Statistical analysis

All parameters were tested in triplicate. Data analysis was carried out using one-way ANOVA with Duncan's test ($P < 0.05$) using SPSS statistical software. One sample t-test was used for comparing the fish oil quality index in each treatment with the standard limit.

RESULTS AND DISCUSSION

Aqueous extract yield, free radical scavenging activity, and total phenolic content

The results of extraction yield, DPPH radical scavenging activity, and TPC of Sargassum and Padina macroalgae extract are shown in Table 1. The efficiency of the aqueous extract of Sargassum algae were higher than that of Padina ($P < 0.05$).

Table 1. Extraction yields, DPPH radical scavenging activity, and TPC of seaweed extract

Algae	Extraction yield (%)	DPPH radical scavenging (%)	TPC (mg GAE/ g dw extract)
Sargassum extract	10.4 ± 0.6	86.1 ± 3.6	28.0 ± 2.5
Padina extract	6.5 ± 0.5	84.2 ± 0.4	20.0 ± 3.8

Table 2. Effect of aqueous extracts of *Sargassum* sp. and *Padina* sp. on the oxidation indices of rainbow trout oil at 30 °C. ($P < 0.05$). Mean ± SD.

Index	Treatments	Time (day)						
		0	7	14	21	28	35	42
PV (meq O_2/ kg)	Control	8.0 ± 0.6 ^{Ae}	15.7 ± 1.2 ^{Acd}	18.0 ± 3.8 ^{Ac}	24.0 ± 1.2 ^{Ab}	35.0 ± 1.2 ^{Aa}	14.7 ± 0.0 ^{Ccd}	14.0 ± 2.9 ^{Cd}
	BHT	8.7 ± 0.6 ^{Ad}	11.7 ± 1.2 ^{Bd}	11.3 ± 0.6 ^{Bd}	12.0 ± 2.1 ^{Cd}	19.3 ± 4.0 ^{Bc}	25.3 ± 2.3 ^{Bb}	30.3 ± 0.6 ^{Ba}
	Sargassum extract	9.3 ± 0.9 ^{Ae}	10.0 ± 1.0 ^{Bde}	13.0 ± 1.5 ^{Bde}	13.7 ± 1.7 ^{Cd}	20.3 ± 2.9 ^{Bc}	33.7 ± 2.6 ^{Aa}	28.3 ± 2.5 ^{Bb}
	Padina extract	9.0 ± 0.6 ^{Ad}	10.3 ± 1.2 ^{Bd}	17.3 ± 0.6 ^{Ac}	20.0 ± 2.5 ^{Bc}	20.7 ± 1.7 ^{Bc}	34.3 ± 2.5 ^{Ab}	42.7 ± 2.7 ^{Aa}
p-Anisidine value	Control	8.8 ± 0.6 ^{Ad}	9.9 ± 0.3 ^{Acd}	10.9 ± 0.8 ^{Acd}	12.8 ± 1.0 ^{Abc}	15.2 ± 0.8 ^{Ab}	21.3 ± 3.0 ^{Aa}	22.8 ± 2.4 ^{Aa}
	BHT	8.3 ± 0.36 ^{Aa}	8.4 ± 2.8 ^{Aa}	8.4 ± 1.6 ^{Aa}	9.5 ± 1.2 ^{Ba}	10.0 ± 6.0 ^{Aa}	10.3 ± 0.7 ^{Ba}	11.0 ± 3.6 ^{Ba}
	Sargassum extract	8.4 ± 0.5 ^{Aa}	8.3 ± 3.2 ^{Aa}	8.7 ± 2.9 ^{Aa}	8.9 ± 2.5 ^{Ba}	10.0 ± 2.1 ^{Aa}	10.6 ± 1.0 ^{Ba}	10.7 ± 3.6 ^{Ba}
	Padina extract	8.3 ± 0.7 ^{Aa}	8.2 ± 4.0 ^{Aa}	9.0 ± 0.1 ^{Aa}	9.6 ± 1.3 ^{Ba}	10.0 ± 6.5 ^{Aa}	10.8 ± 2.4 ^{Ba}	11.7 ± 1.4 ^{Ba}
TOTOX value	Control	26.1 ± 3.6 ^{Ae}	41.3 ± 2.1 ^{Ad}	46.9 ± 7.8 ^{Acd}	60.9 ± 2.7 ^{Ab}	85.2 ± 1.7 ^{Aa}	50.7 ± 3.9 ^{Cc}	50.9 ± 4.4 ^{Cc}
	BHT	25.3 ± 2.5 ^{Ad}	31.7 ± 3.4 ^{Bd}	31.1 ± 0.5 ^{Cd}	33.5 ± 4.0 ^{Cd}	48.6 ± 10.8 ^{Bc}	61.0 ± 4.0 ^{Bb}	71.7 ± 4.7 ^{Ba}
	Sargassum extract	27.0 ± 1.8 ^{Ae}	28.3 ± 2.5 ^{Bde}	34.7 ± 5.9 ^{BCde}	36.2 ± 3.0 ^{Cd}	50.7 ± 7.6 ^{Bc}	78.0 ± 4.6 ^{Aa}	76.4 ± 3.0 ^{Bb}
	Padina extract	26.3 ± 1.3 ^{Ad}	28.9 ± 6.3 ^{Bd}	43.7 ± 1.1 ^{ABc}	49.6 ± 5.0 ^{Bc}	51.3 ± 6.0 ^{Bc}	79.5 ± 5.2 ^{Ab}	97.0 ± 4.1 ^{Aa}
FFA (Oleic acid, %)	Control	3.4 ± 0.1 ^{Ad}	4.4 ± 0.5 ^{Ac}	4.6 ± 0.1 ^{Abc}	4.8 ± 0.3 ^{Abc}	5.4 ± 0.9 ^{Abc}	5.6 ± 1.2 ^{Ab}	6.8 ± 0.4 ^{Aa}
	BHT	2.9 ± 0.0 ^{Bc}	3.5 ± 0.2 ^{BCbc}	3.5 ± 0.2 ^{Cbc}	3.9 ± 0.4 ^{Bab}	4.0 ± 0.2 ^{Bab}	3.6 ± 0.4 ^{Bab}	4.2 ± 0.6 ^{Ba}
	Sargassum extract	2.6 ± 0.1 ^{Cc}	2.9 ± 0.2 ^{CEc}	3.7 ± 0.4 ^{BCb}	3.7 ± 0.0 ^{Bb}	4.0 ± 0.4 ^{Bab}	4.1 ± 0.1 ^{Bab}	4.2 ± 0.1 ^{Ba}
	Padina extract	3.1 ± 0.1 ^{Bb}	3.8 ± 0.4 ^{ABab}	3.9 ± 1.0 ^{BCab}	4.4 ± 0.1 ^{Aa}	4.5 ± 0.2 ^{Ba}	4.5 ± 0.7 ^{ABa}	4.7 ± 0.6 ^{Ba}
UV_{232}	Control	2.4 ± 0.0 ^{Bd}	3.5 ± 0.0 ^{Ac}	4.3 ± 0.0 ^{Ab}	5.4 ± 0.5 ^{Aa}	5.0 ± 0.0 ^{Aa}	4.0 ± 0.0 ^{Abc}	4.4 ± 0.6 ^{Ab}
	BHT	2.2 ± 0.0 ^{Cb}	2.7 ± 0.3 ^{Bb}	2.8 ± 0.4 ^{Cb}	2.8 ± 0.1 ^{Cb}	2.8 ± 0.0 ^{Cb}	2.8 ± 0.0 ^{Bb}	4.2 ± 0.0 ^{Aa}
	Sargassum extract	2.1 ± 0.0 ^{Dc}	2.4 ± 0.1 ^{Bc}	2.4 ± 0.1 ^{Dc}	2.5 ± 0.1 ^{Cc}	2.5 ± 0.1 ^{Dc}	3.7 ± 0.5 ^{Ab}	4.3 ± 0.7 ^{Aa}
	Padina extract	2.6 ± 0.0 ^{Ad}	3.4 ± 0.1 ^{Ac}	3.2 ± 0.1 ^{Bc}	3.4 ± 0.1 ^{Bc}	3.4 ± 0.1 ^{Bc}	4.1 ± 0.0 ^{Ab}	4.6 ± 0.43 ^{Aa}
UV_{270}	Control	0.4 ± 0.0 ^{Ad}	0.5 ± 0.0 ^{Bc}	0.6 ± 0.0 ^{Ab}	0.7 ± 0.1 ^{Ab}	0.7 ± 0.1 ^{Ab}	0.8 ± 0.0 ^{Aa}	0.9 ± 0.1 ^{Aa}
	BHT	0.3 ± 0.0 ^{Ab}	0.3 ± 1.0 ^{Db}	0.4 ± 0.1 ^{Bb}	0.4 ± 0.0 ^{Cb}	0.5 ± 0.1 ^{Bb}	0.4 ± 0.1 ^{Cb}	0.6 ± 0.2 ^{Ba}
	Sargassum extract	0.3 ± 0.0 ^{Ad}	0.4 ± 0.0 ^{Cc}	0.5 ± 0.0 ^{ABbc}	0.5 ± 0.0 ^{Bb}	0.6 ± 0.1 ^{ABb}	0.6 ± 0.0 ^{Bb}	0.7 ± 0.0 ^{ABa}
	Padina extract	0.3 ± 0.1 ^{Ac}	0.6 ± 0.0 ^{Ab}	0.6 ± 1.1 ^{Ab}	0.6 ± 0.0 ^{Bb}	0.6 ± 0.0 ^{Ab}	0.6 ± 0.1 ^{Bb}	0.9 ± 1.1 ^{Aa}

Uppercase letters indicate a significant difference in the column and lowercase letters indicate a significant difference in the row ($P < 0.05$). Mean ± SD. FFA: Free fatty acids; BHT: Butylated hydroxytoluene.

Research by Ebrahimi et al. (2021) demonstrated that aqueous extraction yielded higher amounts of algae extract compared to other solvents. Similarly, Vardizadeh et al. (2021) reported that sulfated polysaccharide (SPS) yields obtained using the aqueous method were higher in Sargassum algae than in Padina. The extraction efficiency was 23% for *Sargassum muticum* (Sabeena et al., 2013), 22.22% for *S. siliquastrum* (Cho et al., 2007), and 19.93% for *Padina boergesenii* (Kumar and Sudha, 2012). Echegaray et al. (2021) and Ceylan et al. (2022) emphasized the importance of DPPH analysis in evaluating the quality of animal feed (such as pig feed) and food industry applications. DPPH analysis measures free radical scavenging activity, with the purple color of the DPPH radical changing to yellow when scavenged (Do et al., 2014). The aqueous extracts of Sargassum and Padina exhibited a high free radical inhibitory capacity of approximately 85%. A comparative study of various seaweeds revealed that the brown algae *Cystoseira myrica* exhibited the highest antioxidant activity, while the green algae *Enteromorpha intestinalis* showed the lowest. Similar antioxidant properties were observed in SPS extracted from the cell walls of Sargassum and Padina. Vardizadeh et al. (2021) also found high antioxidant activity in green Caulerpa algae. Chew et al. (2008) attributed the superior free radical scavenging ability of brown algae such as *Padina antillarum* compared to green and red algae to their phlorotannin content. Consistent with these findings, Yangthong et al. (2009) demonstrated that aqueous and autoclaved extracts of Sargassum had higher antioxidant activity. The TPC expressed as mg of gallic acid equivalent (GAE) per gram of dry weight (DW) of extract, was found in Table 1. The TPC was higher in the Sargassum extract (28.0 ± 2.5 mg GAE/g dw) than in Padina extract, consistent with findings from Babakhani et al. (2012). In addition to algae, other food materials like olive leaves also contain significant phenolic content, which plays a critical role in determining feed quality. Studies have highlighted the potential role of phenolic-rich by-products and herbs on improving the nutritional value of animal feed (Boudhrioua et al., 2009; Saeed et al., 2019). Phenolic compounds are naturally inclined toward aqueous solvents; however, they may also exist as fat-soluble compounds, insoluble forms, or bound to cell walls (Punampalam et al., 2018). The TPC in various algae species has been quantified as follows: *Fucus vesiculosus* (35.1 µg gallic acid), *Fucus evanescent* (25.8 µg gallic acid), *Dictyosiphon foeniculaceus* (11 µg gallic acid), *Laminaria digitata* (9.4 µg gallic acid), and *Fucus serratus* (50.3 µg gallic acid) per mg of SPS in dry weight (Bittkau et al., 2020). These phenolic compounds play a critical role in delaying the onset of fat oxidation and the subsequent decomposition of hydroperoxides in food products. Differences in the structure and number of hydroxyl groups in phenolic compounds derived from algae result in varying antioxidant activities. Furthermore, the antioxidant efficacy of these phenolic compounds in different food systems is influenced by their polarity and molecular characteristics (Wang et al., 2009).

Peroxide value (PV)

The PV measures the total hydroperoxide content in a sample. Hydroperoxides, as primary oxidation products, lack taste and smell but rapidly degrade into non-reactive substances such as aldehydes, alcohols, acids, and ketones. These secondary products are the primary contributors to the deterioration of oil quality, causing unpleasant tastes and odors (O'brien, 2008; Jamshidi et al., 2020). During long-term storage, lipid oxidation in feed materials can significantly limit shelf life and negatively impact the physiological state and productivity of farmed fish, as noted by Ponomarev et al. (2022). Thus, controlling oxidation in such materials is critical for maintaining quality. In this study, PV trends for all samples showed an increase during the storage period, followed by a decrease in some treatments, as detailed in Table 2. The control sample exhibited a peak PV earlier (35 mEq O_2/kg oil). For human consumption, PV values should remain below 10 mEq O_2/kg, as outlined by Deepika et al. (2014). In the present work, control samples exceeded this threshold on the 14th day, while samples with Padina, BHT, and fish oil with Sargassum extract remained within limits until the 21st and 28th days, respectively ($P < 0.05$). Various algae extracts have demonstrated antioxidant properties in fish oils over different times and temperatures. For instance, Santoso et al. (2004) reported antioxidative effects of *Caulerpa sertularoides* extract on fish oil within 3 to 24 hours. Vardizadeh et al. (2021) observed similar effects with *Hizikia fusiformis* extract, which exhibited antioxidant activity at 60 °C for 12 days. SPS from *Sargassum boveanum* and *Padina distromatica* provided antioxidative protection for rainbow trout oil stored at 65 °C for 18 days, limiting the formation of secondary metabolites (Vardizadeh et al., 2021). The antioxidant potential of algae extracts can be attributed to the presence of bioactive compounds such as pigments, which inhibit free radicals and delay fat oxidation and hydroperoxide decomposition (Fernando et al., 2016). The results of this study align with findings by Mousavipour et al. (2021b) who explored the relationship between extracts from *Sargassum* sp., *Padina* sp., *Caulerpa* sp., and fish oils stored at 30 °C for 42 days. Furthermore, Kop et al. (2019) demonstrated that PV increased by increasing storage time and temperature, depending on the type of raw oil material used in fish feed. Collectively, these findings suggest that algae-based extracts offer a promising solution for delaying lipid oxidation in fish oil, thereby extending its shelf life and improving its quality for both human and animal applications.

Anisidine number

The anisidine number test is a key method for evaluating the secondary oxidation of lipids (Wang et al., 2018). This parameter is critical not only for consumer safety but also for assessing the quality of animal feed materials. For instance, Yıldırım and Cantas (2020) reported that the p-anisidine values in marine fish feed were measured at 8.08. Factors such as storage time and raw material type significantly influence the stability of oils. In the present study, the anisidine number exhibited a consistent upward trend, reaching 22.85 mg/kg oil by the 42nd day.

While changes in the other samples progressed more gradually and mirrored those in the BHT group, the control sample remained below the standard limit (< 20) only until the 28th day. By contrast, the other samples stayed within the acceptable limit through the 42nd day of storage. Comparable results were reported by Agregán et al. (2016), who found similar anisidine values in brown algae (*Bifurcaria bifurcata*) extracts stored at 60 °C for 16 days. Additionally, Kindleysides et al. (2012) evaluated the extracts of two brown algae (*Ecklonia radiata* and *Macrocystis pyrifera*) and two red algae species (*Champia* sp. and *Porphyra* sp.) during the storage of *Macruronus novaezelandiae* fish oil at 60 °C for 12 days. Their findings indicated that the brown algae exhibited superior antioxidant capacity compared to the red algae, with *E. radiata* extract being particularly effective. These studies demonstrated that the algae extract significantly reduced both primary (PV) and secondary (anisidine number) oxidative spoilage processes, highlighting their potential as natural antioxidants for oil preservation.

TOTOX

The TOTOX value, a combined measure of peroxide and anisidine numbers (representing primary and secondary oxidation products), provides comprehensive assessments of lipid oxidative damage (Ghaly et al., 2013; Wang et al., 2018). In the present study, the control group exhibited the highest TOTOX value, peaking at 2.85 on the 28th day before showing a downward trend for the remainder of the storage period. The S group samples reached their highest TOTOX value on the 35th day, while the BHT and P samples demonstrated a consistent upward trend throughout the storage period (Table 2). All treatments (TOTOX value < 26) remained within the acceptable limit during the first week of storage, as defined by Šimat et al. (2017) and Deepika et al. (2014). Agregán et al. (2017) noted that control group TOTOX values were five times higher than those of samples treated with algae extracts. Similarly, Mousavipour et al. (2021b) reported that the TOTOX value of control samples reached 85.2 on the 28th day before declining, whereas fish oil samples containing three different algae extracts exhibited TOTOX values of 33.1, 54.5, and 67.3, respectively. In fish feed stored at 4 °C and 24 °C for 45 days, Yıldırım and Cantas (2020) determined TOTOX values of 17.89 and 24.94 (P < 0.05), respectively. Siriwardhana et al. (2004) established a correlation between chemical indicators like TOTOX and the shelf life of food oils, aligning with the findings of the present study. The observed relationship between algae extract treatments and TOTOX values highlights their potential in enhancing the oxidative stability and shelf life of fish oils.

Free fatty acids (FFA)

Lipid hydrolysis is an enzymatic reaction catalyzed by lipase, which produces FFAs during storage (Gokoglu et al., 2012). In the present study, FFA levels in the control and algae extract treatments showed a sharp increase until the 7th day, after which they stabilized for the remainder of the storage period. In the BHT treatment, a trend similar to that observed in the oil containing free Sargassum extract was noted, as shown in Table 2. The permissible range of FFAs in crude fish oil is reported to be 1–7% oleic acid (Ghaly et al., 2013; Vardizadeh et al., 2021). In the control treatment, FFA levels remained significantly below this limit until the 28th day of storage. Conversely, the fish oil with BHT, Sargassum, and Padina extracts maintained FFA levels significantly below the permissible threshold throughout the 42-day period (P < 0.05). Mousavipour et al. (2021b) observed a continuous increase in FFA levels from 3.4% to 6.8% in the control group (without antioxidants) in 42 days (P < 0.05). Additionally, studies by Najafi et al. (2015) and Arabameri et al. (2019) identified a correlation between oxidative stability and factors such as phenolic content and the ratio of monounsaturated to polyunsaturated fatty acids. Shurson et al. (2015) also reported wide variability in FFA content in lipids derived from local feed, ranging from 5.8% to 51.6%. These findings underscore the importance of antioxidant treatments in maintaining FFA levels and oxidative stability in fish oils.

UV_{232} and UV_{270} indices

Absorbance at 232 nm (UV_{232}) indicates intermediate oxidation products, primarily hydroperoxides (conjugated dienes), catalyzed by the lipoxygenase enzyme. Meanwhile, absorbance at 270 nm (UV_{270}) represents secondary oxidation products, such as conjugated trienes. Measuring UV_{232} and UV_{270} serves as a critical index for tracking the progression of lipid oxidation (Topuz et al., 2015; Shahidi and Zhong, 2005). In this study, the trends of UV_{232} and UV_{270} changes mirrored those observed in FFA results and TOTOX values. The control treatment exhibited the highest absorbance values, while the other treatments showed a relatively stable trend during storage, with the BHT and S treatments displaying the lowest absorbance levels (Table 2). An increasing slope in the graphs during storage indicated the accumulation of primary oxidation products. The subsequent decrease in UV_{232} absorbance was attributed to the consumption of these primary products as they transitioned to the secondary oxidation products (Gokoglu et al., 2012; Topuz et al., 2014). The effect of *Punica granatum* (pomegranate peel) extract on lipid oxidation in *Engraulis encrasicholus* (anchovy) fish oil was investigated using UV_{232} and UV_{270} tests by Topuz et al. (2014). The initial UV_{232} value was 2.15, which increased to 3.45 during storage, reflecting the progression of oxidation. However, no significant changes were observed in UV_{270} values for the fish oil samples, as reported by Ucak (2018). In this study, treated samples exhibited lower UV_{232} values (minimum 3.06) compared to the control samples, which reached the highest value of 3.91. This highlights the effectiveness of antioxidant treatments in mitigating lipid oxidation during storage.

Table 3. Effect of aqueous extract of Sargassum and Padina on the color indices of rainbow trout oil at 30 °C.

Index	Treatment	Time (day)						
		0	7	14	21	28	35	42
L*	Control	61.0 ±2.9 ^{Aab}	64.2 ± 2.7 ^{Aa}	61.5 ± 2.1 ^{Bab}	59.1 ± 1.5 ^{Bb}	63.3 ± 3.1 ^{Aa}	61.8 ± 3.5 ^{Bab}	61.8 ± 1.5 ^{Aab}
	BHT	62.6 ±1.1 ^{Aab}	65.0 ± 2.9 ^{Aa}	63.3 ± 2.4 ^{ABab}	63.6 ± 2.1 ^{Aab}	63.8 ± 1.6 ^{Aab}	63.5 ± 1.7 ^{Ab}	61.2 ± 1.6 ^{Ab}
	Sargassum extract	63.0 ± 2.7 ^{Aa}	63.4 ± 3.2 ^{Aa}	63.3 ± 3.0 ^{ABa}	62.5 ± 1.9 ^{ABa}	62.3 ± 3.7 ^{Aa}	63.8 ± 2.4 ^{Aa}	61.1 ± 2.9 ^{Aa}
	Padina extract	62.2 ± 1.6 ^{Aabc}	65.4 ± 2. 6 ^{Aa}	64.5 ± 2.7 ^{Aab}	65.0 ± 3.2 ^{Aa}	61.0 ± 4.6 ^{Abc}	63.9 ± 0.7 ^{Aab}	60.2 ± 2.6 ^{Ac}
a*	Control	-7.0 ± 0.4 ^{Bbc}	-7.6 ± 0.6 ^{Bc}	-7.0 ± 0.7 ^{Bbc}	-7.0 ± 0.6 ^{Abc}	-6.3 ± 0.9 ^{ABab}	-5.7 ± 0.6 ^{Aa}	-6.7 ± 0.5 ^{Ab}
	BHT	-6.6 ± 0.4 ^{Aab}	-6.4 ± 0.6 ^{Aa}	-6.3 ± 0.4 ^{ABa}	-6.6 ± 0.5 ^{Aab}	-6.5 ± 0.5 ^{Bab}	-7.4 ± 0.6 ^{Ac}	-7.0 ± 0.4 ^{Abc}
	Sargassum extract	-6.6 ± 0.4 ^{Aa}	-6.4 ± 0.9 ^{Aa}	-5.8 ± 1.0 ^{Aa}	-6.4 ± 0.8 ^{Aa}	-5.9 ± 0.3 ^{ABa}	-5.2 ± 4.5 ^{Aa}	-6.6 ± 0.6 ^{Aa}
	Padina extract	-6.7 ± 0.4 ^{Bbc}	-6.4 ± 0.4 ^{Ab}	-6.8 ± 0.5 ^{ABbc}	-6.6 ± 0.6 ^{Abc}	-5.6 ± 0.5 ^{Aa}	-7.2 ± 0.5 ^{Acd}	-7.7 ± 0.8 ^{Bd}
b*	Control	1.4 ± 0.3 ^{Ac}	1.2 ± 0.6 ^{ABc}	4.4 ± 0.5 ^{Aab}	2.7 ± 0.3 ^{Abc}	3.5 ± 1.1 ^{Aab}	4.3 ± 1.5 ^{ABab}	4.6 ± 1.2 ^{Ba}
	BHT	1.5 ± 0.7 ^{Ab}	1.8 ± 1.1 ^{Ab}	1.3 ± 0.4 ^{Cb}	1.5 ± 0.1 ^{Bb}	2.9 ± 0.1 ^{Aa}	3.0 ± 0.4 ^{Ba}	2.8 ± 0.4 ^{Ca}
	Sargassum extract	1.4 ± 0.5 ^{Ac}	1.9 ± 0.7 ^{Abc}	2.8 ± 0.2 ^{Bb}	2.5 ± 0.3 ^{Ab}	2.2 ± 0.7 ^{Abc}	4.3 ± 0.7 ^{ABa}	2.8 ± 0.8 ^{Cb}
	Padina extract	1.7 ± 0.3 ^{Acd}	1.2 ± 0.1 ^{Ad}	3.1 ± 1.1 ^{Bc}	2.8 ± 0.4 ^{Ac}	2.1 ± 1.3 ^{Acd}	4.9 ± 0.4 ^{Ab}	6.7 ± 1.0 ^{Aa}
ΔE	Control	7.3 ± 1.9 ^{Aa}	4.0 ± 0.3 ^{Ad}	4.6 ± 0.5 ^{Bcd}	7.0 ± 1.3 ^{Aab}	5.4 ± 0.6 ^{ABbcd}	6.2 ± 0.2 ^{Aabc}	4.3 ± 0.4 ^{Ad}
	BHT	6.3 ± 0.3 ^{Aab}	5.0 ± 0.4 ^{Acd}	6.7 ± 0.4 ^{Aa}	6.3 ± 0.1 ^{Aab}	4.9 ± 0.2 ^{Bcd}	4.6 ± 0.5 ^{ABd}	5.6 ± 0.9 ^{Abc}
	Sargassum extract	6.5 ± 0.1 ^{Aa}	5.1 ± 1.0 ^{Aa}	5.9 ± 0.7 ^{ABa}	5.6 ± 0.5 ^{Aa}	6.8 ± 0.6 ^{ABa}	5.4 ± 2.1 ^{Aa}	6.2 ± 1.0 ^{Aa}
	Padina extract	6.4 ± 0.3 ^{Aab}	4.5 ± 0.6 ^{Abc}	5.0 ± 1.4 ^{Bb}	5.7 ± 0.4 ^{Aab}	7.3 ± 1.9 ^{Aa}	2.6 ± 0.5 ^{Bc}	4.8 ± 1.5 ^{Ab}
WI	Control	45.5 ± 1.5^{Aab}	44.5 ± 1.4 ^{Aab}	46.6 ± 1.0 ^{Aa}	43.3 ± 3.1 ^{Aabc}	40.1 ± 1.4 ^{Bc}	45.4 ± 2.7 ^{Aab}	42.8 ± 1.4 ^{Abc}
	BHT	44.8 ± 1.1 ^{Aa}	33.2 ± 10.3 ^{Ba}	45.7 ± 1.6 ^{ABa}	46.2 ± 1.1 ^{Aa}	44.6 ± 0.1 ^{Aa}	46.2 ± 2.3 ^{Aa}	40.9 ± 5.5 ^{Aab}
	Sargassum extract	44.5 ± 2.8^{Aa}	43.2 ± 1.5 ^{Aa}	42.8 ± 2.3 ^{Ba}	44.6 ± 2.2 ^{Aa}	43.5 ± 2.3 ^{ABa}	40.1 ± 6.1 ^{Aa}	43.5 ± 1.7 ^{Aa}
	Padina extract	45.3 ± 1.3^{Aa}	43.8 ± 1.0 ^{Aab}	45.3 ± 1.2 ^{ABa}	45.4 ± 2.7 ^{Aa}	42.0 ± 3.4 ^{ABab}	44.0 ± 0.6 ^{Aab}	41.2 ± 1.5 ^{Ab}

Uppercase letters indicate a significant difference in columns and lowercase letters indicate a significant difference in rows ($P < 0.05$). Mean ± SD.

The UV_{270} value for the control group was 0.92, while the other samples remained below 0.80. This parameter, closely linked to other oxidative stability indicators, serves as a reliable metric for evaluating fish oil quality, whether for animal feed or human consumption. Notably, the applied treatments effectively limited the rapid increase in both UV_{232} and UV_{270} values, as observed in the present study.

Color indicators

The color of a product can change during oxidative and hydrolytic processes due to the production of compounds associated with spoilage. This change is particularly significant as color plays a critical role in consumer acceptability (Sabzipour et al., 2019). During storage at high temperatures, fat oxidation leads to the formation of compounds such as peroxides, hydroxides, and conjugated dienes, along with the decomposition and polymerization of glycerides, all of which can alter the color of oil (White, 1991). The results of colorimetric indices are summarized in Table 3. Oxidation progression in the control sample led to the increases in total color change (ΔE), browning, and yellowness (b*), while whiteness and lightness (L*) decreased. Regarding the a* index, which measures red-green balance, all treatments showed negative values. Statistically, no significant differences were observed among treatments for this parameter ($P > 0.05$). However, in the control sample, the a* index increased with oxidation, indicating primary oxidation progression. These findings align with previous studies by Sabzipour et al. (2019), Mousavipour et al. (2021a, 2021b). Further comparisons reveal that fish oil used in modern salmon diets, based on colorimetric analyses, exhibited a greener color (lower a*), a more bluish tone (lower b*), and reduced whiteness in Atlantic salmon fillets (Katerina et al., 2020). The total color change (ΔE) reflects the cumulative differences in measured color parameters during storage and serves as a key index for assessing color variations over time (Chung et al., 2014). ΔE values can vary depending on factors like food type and processing temperature. Condón-Abanto et al. (2019) classified meat color changes into three categories: "good" ($\Delta E \leq 7$), "acceptable" ($7 < \Delta E < 9$), and "unacceptable" ($\Delta E \geq 9$). While ΔE provides useful insights, it should not be solely relied upon to define overall quality. Future studies could aim to standardize this parameter. In the current study, the control samples exhibited the highest ΔE value (7.3), while the lowest value among all samples was 6.3. These findings suggest that treatments effectively limited oxidative-induced color changes compared to the control.

The total color change index exhibited a fluctuating trend across all treatments over the 42-day period, with the highest value observed in the control treatment and the lowest change seen in the BHT treatment. The discrepancy between this index and other indicators, such as peroxide, anisidine, and TOTOX, may be attributed to the minimal effect of pigments in the extracts (Valizadeh et al., 2019). In addition to ΔE, the whiteness index (WI) provides insight into the transparency of the oil, which tends to decrease with increased oxidation. The presence of spoilage compounds typically reduces both the transparency and whiteness of the oil (Sabzipour et al., 2019). As shown in Table 3, the brightness of all treatments declined over the storage period, with the S treatment exhibiting the smallest decrease in brightness.

CONCLUSION

Seaweeds are widely used as food supplements and antioxidants in many countries. Research indicates that algae cells possess mechanisms that enable them to quickly adapt to aquatic environments through the production of secondary metabolites, which protect them against oxidative stress and other environmental factors. In the present study, the aqueous extracts of two brown macroalgae, *Sargassum* sp. and *Padina* sp., were evaluated for their natural antioxidant properties in the preservation of rainbow trout waste oil. The results showed that the spoilage indices of fish oil containing algae extracts were significantly lower than those of the control group. This suggests that algae extracts could be used as a replacement for industrial preservatives in fish oil processing and animal feed, aligning with findings from other studies. The peroxide value in the control samples reached 35 meq O_2/kg oil on the 28th day, while the Padina samples exceeded this value only on the 42nd day. The p-anisidine value of the control samples increased from 8.8 to 22.8 by the end of the storage period, while samples with Padina and Sargassum extract showed values of 10.7 and 11.7, respectively. Additionally, the free fatty acid content of the control samples was higher (6.8%) compared to fish oil with Padina and Sargassum extract (4.7% and 4.2%, respectively). The b* value of fish oil treated with Sargassum extract (1.4 to 2.8) remained more stable compared to the control samples (1.4 to 4.6). The promising results observed in this study suggest that fish oil treated with seaweed extracts could be a cost-effective and organic solution for application in various food and animal feed products, including supplements for aquatic, livestock, and poultry feed.

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CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization, Funding acquisition, Investigation: Nesa Mousavipour; Supervision, draft preparation, Validation, Resources, Writing-review, and Editing: Sedigheh Babaei; Project administration, Methodology, resources: Marzieh Moosavi-Nasab; Writing-review and Editing, Software: Zafer Ceylan.

DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest.

ETHICAL STATEMENT

This work is not related to experimental animals or specific human diseases that requires publication and approval of publication ethics.

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