Assessment and comparative analysis of the antioxidant capacity of some food waste for fish oils

E.S. Karabayır* and M. Öğütcü**

*Department of Food Engineering, Faculty of Engineering, Çanakkale Onsekiz Mart University, 17020, Çanakkale, Turkiye
**Corresponding author: mogutcu@comu.edu.tr; mustafaogutcu@gmail.com

Submitted: 11 September 2023; Accepted: 21 February 2024; Published: 02 July 2024

SUMMARY: The aim of the present study was to compare the antioxidant capacities of butylated hydroxy anisole and tocopherol with polar extracts obtained from some food waste such as date seed, walnut shell, sesame hull, spent coffee and spent black tea. In pursuit of this objective, crude fish oil was employed as a control sample, with all extracts added at a concentration of 200 ppm, equivalent to BHA. The acid, peroxide, conjugated dien, viscosity, total chlorophyll and carotenoid contents in the fish oil were monitored during the storage period (60 days) at 25 °C and 35 °C. The date seed and walnut extracts presented higher antioxidants and oxidative stability than the others at the end of the storage period at both temperatures. The findings of the present study showed that the extracts obtained from food wastes/by-products could be evaluated as a natural source of antioxidants, especially for oils which are highly susceptible to oxidation.

KEYWORDS: Antioxidants; Fish oil; Food waste; Oxidation; Storage.

RESUMEN: Evaluación y análisis comparativo de la capacidad antioxidante de algunos residuos de alimentos para aceites de pescado. El objetivo del presente estudio fue comparar la capacidad antioxidante del butilhidroxianisol y el tocoferol con extractos polares obtenidos de algunos residuos de alimentos como semillas de dátil, cáscara de nuez, cáscara de sésamo, desechos de café y desechos de té negro. Para lograr este objetivo, se empleó aceite de pescado crudo como muestra de control, agregándose todos los extractos a una concentración de 200 ppm, equivalente a BHA. Se controlaron los contenedores de peróxidos, dienos conjugados, viscosidad, clorofila total y carotenoides del aceite de pescado durante el período de almacenamiento (60 días) a 25°C y 35°C. Los extractos de semilla de dátil y nuez tuvieron mayores antioxidantes y estabilidad oxidativa que los demás al final del período de almacenamiento a ambas temperaturas. Los hallazgos del presente estudio mostraron que los extractos obtenidos de desechos/subproductos de alimentos podrían evaluarse como una fuente natural de antioxidantes, especialmente para aceites altamente susceptibles y propensos a la oxidación.

PALABRAS CLAVE: Aceite de pescado; Almacenamiento; Desechos alimentarios; Oxidación.


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1. INTRODUCTION

Fish oil (FO) holds a significant position within the realm of edible oils, primarily owing to its rich contents of essential omega fatty acids, specifically docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). These omega fatty acids, EPA and DHA, play a pivotal role in promoting human health by serving as crucial components in the prevention of cardiovascular diseases and certain types of cancers (Hrebień-Filisińska, 2021). Their deficiency has been linked to the onset of immunological disorders, and depression, as well as neurodegenerative conditions such as Parkinson’s and Alzheimer’s diseases (Hrebień-Filisińska, 2021). On the other hand, “FO” is not only used in human nutrition, but also as a source of oil for animal feed, especially aquaculture. One significant drawback associated with the utilization of fish oil (FO) is its pronounced susceptibility to oxidation, resulting in lower storage stability compared to other oils (Tian and Dasgupta, 1999; Örnek et al., 2021).

This sensitivity to oxidation not only limits its shelf life but also causes a gradual decrease in its price. The rising demand for fish oil in both human and animal diets has placed additional pressure on its availability, and production has struggled to adequately meet this growing demand (Misund et al., 2017; Örnek et al., 2021). Artificial antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), gallates, and tert-butylhydroquinone (TBHQ), are used to increase the oxidative stability and shelf life of fish oil. However, the mentioned antioxidants both increase the cost and are harmful to human health; therefore, the use of these additives is gradually decreasing (Hasdemir et al., 2023).

The carcinogenic effect was reported for BHA, BHT, gallates (propyl, octyl, dodecyl) and TBHQ; hence, TBHQ is banned in certain European Union (EU) countries and Japan (Hrebień-Filisińska, 2021). Another example was Ethoxyquin, which was used as a synthetic antioxidant against the lipid oxidation in EU, but it was banned (Regulation EC 2017/962) due to the adverse effects on human and animal health (Hasdemir et al., 2023). This situation has led to the search for new additives which are natural, inexpensive, easily available, and as effective as their artificial counterparts against oxidation. In this case, food waste/by-products which are rich in phenolic compounds; particularly those obtained from industrial processes come to fore as a natural antioxidant source (Amado et al., 2014; Sindhu et al., 2019).

In the existing literature, it is evident that the utilization of various natural antioxidants derived from diverse food wastes and by-products in fish oils is very limited. Instead, the majority of studies predominantly focused on various extracts sourced from herbs, leaves, spices, and roots as potential antioxidants for fish oils (Hrebień-Filisińska, 2021). Fruit peels (Sekhon-Loodu et al., 2013; Topuz et al., 2015), seeds (Luther et al., 2007; Pazos et al., 2008) and pomace (Pazos et al., 2005) were used as antioxidants for the oxidative stabilization of fish oil. Recently, Hwang et al. (2019) reported the antioxidant activity of spent coffee extracts in fish oil.

The primary objective of this study was to assess the antioxidant capacities of food waste materials, specifically walnut shells, sesame hulls, date seeds, spent coffee, and spent black tea, and compare them with synthetic antioxidants. Additionally, the secondary goal was twofold: to enhance the shelf life of fish oil and to align with the principles of a zero-waste world.

2. MATERIALS AND METHODS

2.1. Materials

Crude fish oil was obtained from the Dardanel Company, located in Çanakkale, Turkey. The walnut shell, date seed, spent coffee and spent black tea were provided from local patisseries and cafes in Çanakkale, Turkey. The sesame hull was obtained from local sesame paste manufacturers (Özen Baharat & Tohum Ltd. Co.) in Çanakkale, Turkey. All chemicals utilized in this study were of analytical grade and were procured from Sigma (USA) and Merck (Darmstadt, Germany).

2.2. Extraction of polar compounds

Various food waste materials, including date seeds, spent coffee, and spent black tea (excluding sesame hulls and walnut shells), were initially dried in a vacuum oven at 45 °C and subsequently ground using a laboratory grinder. The grinding process was not applied to the sesame hull, spent coffee, or spent...
black tea since particle sizes are sufficient for extraction. To extract polar phenolic compounds, 10±0.2 g of the samples were weighed into an Erlenmeyer flask, and 100 ml of ethanol were added. The sesame hull, walnut shell and date seed were stirred at 24 h, while the spent coffee and spent black tea were stirred at 4 h. The stirring process was carried out at room temperature and in darkness without repetition. Following the extraction process, the mixture was filtered through filter paper, and the filtrate was centrifuged at 5000 rpm for 10 minutes. After centrifugation, the clear upper liquid phase was collected. Subsequently, at least 90% of the solvent was evaporated using a rotary evaporator at 45 °C under vacuum conditions. The remaining portion was then reconstituted to a final volume of 10 ml with ethanol, and this solution was used as a stock solution, which was stored at -18 °C.

2.3. Determination of total phenolic content and antioxidant capacity

The total polar phenolic content (TPC) and antioxidant capacity of the extracts obtained from different food wastes were determined according to the Folin-Ciocalteu method (Singleton and Rossi, 1965) and the radical-α, α-diphenyl-β-picrylhydrazyl (DPPH) assay (Benvenuti et al., 2004), respectively. The total phenolic contents and DPPH assay results (50% inhibition concentration, $IC_{50}$) of the fish oil samples are given in Table 1. Both analyses were used to determine the BHA equivalents of the polar extracts for the addition level of the fish oil.

2.4. Preparation of fish oil samples

The fish oil samples, both without additives and with the addition of BHA and the BHA:Tocopherol (TCL) mixture (BHA:TCL), served as control samples. It needs to be noted that the permissible limit for BHA in edible oils, as defined by the Codex Alimentarius (General Standard for Food Additives CODEX STAN 192-1995, 2017), is set at 200 ppm. For this reason, the $IC_{50}$ values of 200 ppm BHA were measured, and the amount of polar extracts that provided inhibition equivalent to the $IC_{50}$ value of 200 ppm BHA was calculated. The addition levels are presented in Table 1. The prepared fish oil samples were stored at temperatures of 25 and 35 °C for a duration of 60 days to assess the impact of the added extracts on the oxidative stability of the oil.

2.5. Physicochemical features

The acid (Ca 5a-40), K₂32 (Ti 1a-64) and peroxide (Cd 8-53) values were measured according to AOCS (Firestone, 2004). The viscosity values were determined using Brookfield viscosimeter (model DV II Pro with Rheocalc software, Brookfield Eng. Lab., Inc., MA, USA) equipped with a LV-SC4-18 spindle at 25 °C, and the results were expressed as (cP). The total carotenoid and chlorophyll values were determined according to the method detailed by Minguez-Mosquera et al. (1991). In accordance with these methods, 7.5±0.1 g of oil samples were dispensed into tubes and subsequently topped up to 25 ml with cyclohexane. The resulting mixture was

Table 1. Total phenolic contents and $IC_{50}$ values for the extracts and addition levels to fish oil.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total Phenol Content (mg/L)</th>
<th>$IC_{50}$</th>
<th>Addition Level (µL)</th>
<th>Addition Level* (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHA</td>
<td>135.63±4.42f</td>
<td>166.20±2.30b</td>
<td>200</td>
<td>0.2</td>
</tr>
<tr>
<td>BHA:TCL</td>
<td>84.38±0.00g</td>
<td>272.21±12.43a</td>
<td>330</td>
<td>0.33</td>
</tr>
<tr>
<td>TCL</td>
<td>25.31±3.42h</td>
<td>Nd</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>DS</td>
<td>2156.00±21.2a</td>
<td>6.64±0.186f</td>
<td>8</td>
<td>0.008</td>
</tr>
<tr>
<td>SC</td>
<td>369.10±1.41c</td>
<td>43.99±3.32e</td>
<td>50</td>
<td>0.05</td>
</tr>
<tr>
<td>SH</td>
<td>658.60±3.54e</td>
<td>123.30±0.806c</td>
<td>150</td>
<td>0.15</td>
</tr>
<tr>
<td>ST</td>
<td>200.60±0.707d</td>
<td>79.75±2.87d</td>
<td>100</td>
<td>0.1</td>
</tr>
<tr>
<td>WN</td>
<td>1306.00±7.07b</td>
<td>8.33±0.379f</td>
<td>10</td>
<td>0.01</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± standard deviation (n = 8). The lowercase letters indicate differences among the samples in the same column (p ≤ 0.05, one-way ANOVA with Tukey’s test).


*Addition levels equivalent to 200 ppm BHA.
then subjected to spectrophotometric measurements at wavelengths of 470 and 670 nm using a spectrophotometer (Shimadzu UV-1800; Japan). Throughout the storage period, various parameters, including K$_{232}$ (Conjugated Dien, CD), acid value, peroxide value, and viscosity, were monitored. Additionally, the assessment of total carotenoid and chlorophyll content was conducted on both freshly prepared and stored samples.

2.6. Thermal measurements

The thermal behavior of the fish oil samples was determined by thermogravimetric analysis (TGA) using a thermogravimetric analyzer (TGA 4000, Perkin-Elmer, USA). For thermogravimetry and derivative thermogravimetry (TG/DTG) measurements, the samples weighing 5-10 mg were placed into the TG pan. The samples were then heated from 20 to 700 °C at a rate of 10 °C/min under dry air, and the weight loss and deterioration temperatures were determined using the TGA software (Pyris Manager).

2.7. Statistical analysis

The present study was planned duplicate, and all analyses were performed in triplicate. The data obtained from the samples were presented as “Mean±Standard Deviation (SD)”. The data were evaluated with the MINITAB (Minitab 16v) statistical software using Analysis of Variance (ANOVA), similarities and differences among samples were determined according to Tukey’s multiple test at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Total phenolic contents and antioxidant activity

The total phenolic content (TPC) and 50% inhibition concentration (IC$_{50}$) values for the additives used in the fish oil are shown in Table 1. The TPC of the extracts obtained from natural waste/by-products ranged from 200.60 to 2156.00 mg/L. The date seed extracts showed the highest TPC, while the spent tea extracts presented the lowest value. The TPC for samples containing 200 ppm of BHA, BHA:TCL (1:1; w:w), and TCL were measured at 135.63 mg/L, 84.38 mg/L, and 25.31 mg/L, respectively. The IC$_{50}$ values for the natural extracts ranged from 6.64 to 123.30 µg/mL, with their antioxidant activity ranked in descending order as follows: date seed > walnut shell > spent coffee > spent black tea > sesame hull. In comparison, the IC$_{50}$ values for 200 ppm of BHA and the BHA:TCL (1:1; w:w) solution were determined as 166.20 µg/mL and 272.21 µg/mL, respectively. These results demonstrate that all extracts derived from natural waste and by-products exhibited higher TPC and antioxidant activity when compared to both 200 ppm of BHA, BHA:TCL (1:1; w:w), and TCL.

Yang et al. (2014) reported that walnut shell extract ed using different solvents (water, chloroform, methanol, ethanol, ethyl acetate, and n-butanol) and the highest TPC were observed in the sample extracted with ethyl acetate (200.40 mg GAE/g). Afifi et al. (2017) reported that the TPC of date seed powder ranged from 7.63 to 71.72 mg GAE/100 g, depending on the extraction conditions. In a study conducted by Hwang et al. (2019), the TPC values for acetone and ethanolic extracts of spent coffee were reported as 51.79 and 80.36 mg GAE/g extract, respectively. The same study reported % inhibition values for 50, 100, and 200 ppm concentrations of spent coffee extracts, ranging from 22.06 to 91.66, while the % inhibition values for BHT varied between 79 and 96.44 at the same concentrations, respectively. Bravo et al. (2013) reported the antioxidant capacities of the spent coffee extracts obtained from different extraction process measured with ABTS and DPPH method to range from 15.31-152.64 and 5.02-82.40 µmol Trolox/gdm. These variations between both literature findings and our own results can be explained by the different extraction conditions, solvents and type of waste materials used. Another reason for the difference between the findings can be the different antioxidant capacity measurements used such as radical scavenging activity method (DPPH), total phenolic compound (TPC), trolox equivalent antioxidant capacity determination (TEAC) using ABTS radical cation, cupric ion reduction capacity (CUPRAC), ferric-reducing antioxidant power (FRAP) or oxygen radical absorbance capacity (ORAC) (Shahidi and Zhong, 2015). These findings in the literature align closely with our own results, especially regarding the % inhibition obtained from the DPPH assay.

3.2. Physicochemical properties

The acid (AV) and peroxide values (PV) for both the control and antioxidant-added groups of fish oil, stored at both 25 and 35 °C for a duration of 60 days are presented in Figures 1 and 2, respective-
Figure 1. Acid values for the fish oil samples during 60 days at 25 and 35 °C.

CFO: Fish oil without additives (control), FBH: fish oil with Butylated Hydroxy Anisole, FBT: fish oil with Butylated Hydroxy Anisole: Tocopherol, FDS: fish oil with date seed extract, FSC: fish oil spent coffee extract, FSH: fish oil with sesame hull extract, FST: fish oil with spent black tea extract, FWS: fish oil with walnut shell extract.

The results are expressed as mean ± standard deviation (n = 8). Different letters represent significant differences between fresh and stored samples in the same bar while different letters in different bars indicate differences among the samples (p < 0.05, General Linear Model-ANOVA with Tukey’s test). Interactions among the “storage time*additives” and “storage temperature*additives” were found to be significant (p ≤ 0.05).

All additive levels of the extracts from natural sources were determined as the amount equivalent to 200-ppm BHA activity, which is the permissible amount for edible oils according to Codex Alimentarius (2017) (Table 1). The AV of the control samples stored at 25 and 35 °C ranged between 4.42-8.15 and 4.56-8.57 mg KOH/g, respectively. The AV of all fish oils increased during the storage period, while the antioxidant-added samples showed lower AV (Figure 1). The samples stored at 25 °C exhibited slightly lower acid values (AV) than those stored at 35 °C. However, it is important to note that these differences were determined to be statistically significant. The samples supplemented with date seed and walnut shell extracts consistently displayed lower acid values (AV) compared to the other samples at the conclusion of the storage period, and this trend was observed at both temperatures. There were statistically significant differences among the samples in terms of AV (p ≤ 0.05). The differences between the extracted fish oil samples may be explained by the different AV values of the added extracts. As is well known, the lipolysis and hydrolysis reactions of triglycerides cause an increased amount of free fatty acid as well as the acid values of the oils. In addition, a synergistic effect between oxidation and hydrolysis reactions occurred. The extraction process, treatments (refining, frying etc.) and storage conditions affect the free fatty acid and peroxide values of the oils (O’Brien, 2008). Therefore, another reason for
Figure 2. Peroxide values for the fish oil samples during 60 days at 25 and 35 °C.

Values are given as mean ± SD. CFO: Fish oil without additives (control), FBH: fish oil with Butylated Hydroxy Anisole, FBT: fish oil with Butylated Hydroxy Anisole: Tocopherol, FDS: fish oil with date seed extract, FSC: fish oil spent coffee extract, FSH: fish oil with sesamum hull extract, FST: fish oil with spent black tea extract, FWS: fish oil with walnut shell extract.

The results are expressed as mean ± standard deviation (n = 8). Different letters represent significant differences between fresh and stored samples in the same bar while different letters in different bars indicate differences among the samples (p < 0.05, General Linear Model-ANOVA with Tukey’s test). Interactions among the “storage time*additives” and “storage temperature*additives” were found to be significant (p ≤ 0.05).

the difference between acid values can be the differences between the oxidative stability of the fish oil samples. In other words, this situation can be explained by the different resistance of the added antioxidants against oxidation.

One of the most important oxidation indicators of edible oils is peroxide value, and PV shows initial oxidation level as well as providing information about the storage conditions of oils (Iqbal et al., 2008). The PV values for the control samples stored at 25 and 35 °C ranged between 5.46-35.21 and 10.89-44.49 meq O₂/kg, respectively. The PV of BHA-added samples stored at 25 °C ranged from 10.88 to 17.22, while that of the BHA:TCL-added fish oils ranged from 12.26 to 28.82 meq O₂/kg during the storage period. Similar to the AV of the fish oils, the PV values increased during the storage period. As expected, the PV values of the fish oils stored at 35 °C were higher than the samples stored at 25 °C (Figure 2). Furthermore, the fish oil containing date seed extract exhibited lower PV at both storage temperatures. When comparing the control samples and those supplemented with tocopherol to the natural antioxidant extracts obtained from food waste and/or by-products, it became evident that all of the natural extracts provided superior protec-
tion against oxidation compared to both the control samples and those with added tocopherol. However, in comparison to the natural extracts added to fish oils, it was observed that the samples with BHA exhibited greater protection against oxidation than the spent coffee and black tea extracts at both storage temperatures. Additionally, sesame hull extracts showed very similar PV results to BHA at both temperatures. However, the most remarkable findings were that fish oils added with walnut shell and date seed extracts had lower peroxide values than those with BHA addition at both temperatures. As is well known, lipid oxidation is a complex chain reaction basically consisting of beginning, propagation and termination. Temperature, light, moisture, presence of oxygen, physical and chemical properties of the substrate, and the presence of oxidation initiators or catalysts all affect the course of oxidation (O’brien, 2008). Antioxidants are defined as agents that delay and/or prevent the oxidation reaction. There are two main groups—primary and secondary antioxidants. The primary antioxidant group comprises free radical scavengers and chain-breaking agents; while the secondary group is involved in the deactivation of metals, inhibition of the breakdown of lipid hydroperoxides to unwanted volatile products, and the regeneration of primary antioxidants (Koleva et al., 2002). Therefore, the differences among the samples mentioned above can be explained by the fact that antioxidants themselves work out their protective properties at different stages of the oxidation process and through different mechanisms.

Figure 3 presents the conjugated diene (CD) values, and Figure 4 presents the viscosity values of the fish oil samples. The CD value is a significant parameter for assessing the degree of degradation in oils and evaluating the effectiveness of the antioxidants employed (Iqbal et al., 2008). The average of the CD values for the fresh samples was 2.27. Consistent with the AV and PV findings, the CD values showed

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**Figure 3.** Conjugated diene of the fish oil samples during 60 days at 25 and 35 °C.

Values are given as mean SD. CFO: Fish oil without additives (control), FBH: fish oil with Butylated Hydroxy Anisole, FBT: fish oil with Butylated Hydroxy Anisole: Tocopherol, FDS: fish oil with spent coffee extract, FSC: fish oil with sesame hull extract, FST: fish oil with spent black tea extract, FSH: fish oil with walnut shell extract. The results are expressed as mean ± standard deviation (n = 8). Different letters represent significant differences between fresh and stored samples in the same bar while different letters in different bars indicate differences among the samples (p < 0.05, General Linear Model-ANOVA with Tukey’s test). Interactions among the “storage time*additives” and “storage temperature*additives” were found to be significant (p ≤ 0.05).
an increase during the storage period at both temperatures. As expected, the fish oil without any antioxidants showed the highest CD value at the end of the storage period, surpassing both the BHA: TCL- and BHA-supplemented samples. Furthermore, in line with the BHA: TCL- and BHA-added fish oils, the fish oils enriched with natural extracts displayed lower CD values than the control samples (those without antioxidants) (Figure 3). These observations align with previous research. For instance, Luther et al. (2007) reported that black raspberry seed extracts significantly reduced the degradation of n-3 polyunsaturated fatty acids, akin to our results. Similarly, Uçak (2018) found that fish oil samples containing propolis (at 500 and 1000 ppm concentrations) had lower PV and CD values compared to both control samples and those supplemented with BHT, which is consistent with our findings. Hwang et al. (2019) reported that the CD and PV values for fish oils with different concentrations added with spent coffee extracts varied between 0.51-4.87 mmol/L and 6.02-8.37 meq O$_2$/kg during a 14-day storage period. Additionally, the fish oils treated with walnut shell and date seed extracts exhibited lower CD values than those treated with both the control samples and those added with BHA or BHA:TCL. Furthermore, the fish oils treated with sesame hull extracts displayed CD values which are similar to those treated with BHA at both storage temperatures. The AV, PV and CD results proved that sesame hull, walnut shell and date seed extracts could be used as natural antioxidants instead of BHA and tocopherol against oil oxidation. The fish oils supplemented with spent coffee and black tea extracts demonstrated effectiveness in preventing oil oxidation, although their efficacy was slightly less pronounced compared to the other extracts. These variations in effectiveness may stem from differences in the efficiency of the extraction process. Factors such as extraction temperature, duration, and the choice of solvent can significantly influence the antioxidant activity of the extracts. Con-

Figure 4. Viscosity values for the fish oil samples during 60 days at 25 and 35 °C.

Values are given as mean ± SD. CFO: Fish oil without additives (control), FBH: fish oil with Butylated Hydroxy Anisole, FBT: fish oil with Butylated Hydroxy Anisole: Tocopherol, FDS: fish oil with date seed extract, FSC: fish oil spent coffee extract, FSH: fish oil with sesame hull extract, FST: fish oil with spent black tea extract, FWS: fish oil with walnut shell extract.

The results are expressed as mean ± standard deviation (n = 8). Different letters represent significant differences between fresh and stored samples in the same bar while different letters in different bars indicate differences among the samples (p < 0.05, General Linear Model-ANOVA with Tukey’s test). Interactions among the “storage time*additives” and “storage temperature*additives” were found to be significant (p ≤ 0.05).
sequently, it is possible that optimizing these extraction parameters could further enhance the antioxidant activities of sesame hull, walnut shell, and date seed extracts. Recently, in a study conducted by Hwang et al. (2019), the antioxidant activity of spent coffee extracts was evaluated in comparison to BHT in soybean and fish oils. Their research revealed that acetone extracts exhibited greater antioxidant activity when contrasted with ethanolic extracts, and that an escalation in the addition levels of acetone extracts correlated with improved antioxidant activity. Furthermore, the researchers posited that spent coffee extracts had the potential to serve as natural antioxidants for omega-3 oils and fish oils within the same study. These findings in the literature corroborate the outcomes of our own investigation.

The viscosity values of all fish oils were significantly influenced by the additives used, storage time, and temperature, with statistical significance at the p ≤ 0.05 level. These viscosity values are presented in Figure 4. Notably, the control and fish oils supplemented with BHA: TCL were more susceptible to changes in viscosity compared to fish oils with BHA and natural extracts at both storage temperatures. Conversely, the fish oils treated with sesame hull extracts exhibited viscosity levels which were similar to those treated with BHA, while fish oils treated with walnut shell and date seed extracts demonstrated lower viscosity values. The observations mentioned above underscore that natural extracts derived from sesame hull, walnut shell, and date seed were at least as effective as BHA in preserving oil viscosity, in addition to mitigating oxidation.

3.3. Total carotenoids and chlorophyll contents

As is known, fish cannot synthesize carotenoids, but can obtain them from natural or commercial feeds containing carotenoids. Carotenoids also affect the oxidative stability of oils due to their antioxidant activities (Selim et al., 2021). The total carotenoid (TCar) contents in the fresh and stored fish oils are given in Table 2. According to Table 2, the TCar contents in the samples stored at 25 °C ranged from 0.38 mg/kg to 0.52 mg/kg, while those of the samples stored at 35 °C ranged from 0.38 mg/kg to 0.50 mg/kg. Selim et al. (2021) reported that Tcar values for mackerel waste oils were 128.34 mg/g and 98.12 mg/g for sardine waste oil. The disparities between the findings in the literature and our own results may be attributed to several factors, including variations in fish species, geographic regions, dietary compositions, and other environmental factors. The fish oils stored at 35 °C showed a higher loss in carotenoid content than the samples stored at 25 °C. The highest loss in carotenoid content during storage was observed in the fish oil without antioxidants at both storage temperatures. Compared to the control samples, it was observed that the loss in carotenoids was less in the fish oils with natural extracts at both temperatures. The total chlorophyll (Tchl) contents in the fresh and stored fish oil samples are given in Table 3. As seen in Table 3, the Tchl values for the fish oils with and without additives ranged from 4.81 mg/kg to 4.50 mg/kg during the 60-day storage period. Similar to our findings, Koning et al. (1999) reported that the Tchl contents of anchovy and pilchard oils ranged from 2.0 to 37.0 mg/l. Both storage time and temperature were significantly effective on the chlorophyll contents in the fish oils (p ≤ 0.05). The highest Tchl loss was observed in the control samples at both temperatures. Although the changes in the treated fish oil samples may have appeared minor, they were statistically significant. Notably, the fish oils treated with natural extracts, especially when compared to BHA-treated fish oils, exhibited higher total carotenoid (Tcar) values at the conclusion of the 35 °C storage period. When both the “Tcar” and

### Table 2. Total carotenoid contents in the fresh and stored fish oil samples.

<table>
<thead>
<tr>
<th>Samples*</th>
<th>Total Carotenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 °C</td>
</tr>
<tr>
<td>CFO</td>
<td>0.50±0.01b*</td>
</tr>
<tr>
<td>FBT</td>
<td>0.49±0.01bc</td>
</tr>
<tr>
<td>FSC</td>
<td>0.52±0.01a</td>
</tr>
<tr>
<td>FDS</td>
<td>0.52±0.01d</td>
</tr>
<tr>
<td>FSH</td>
<td>0.47±0.01d</td>
</tr>
<tr>
<td>FST</td>
<td>0.46±0.01e</td>
</tr>
<tr>
<td>FWS</td>
<td>0.48±0.01d</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± standard deviation (n = 8). The lowercase letters indicate differences among the values in the same column (p ≤ 0.05, one-way ANOVA with Tukey’s test).

* CFO: Crude fish oil without additives (control), FBT: fish oil with Butylated Hydroxy Anisole, FSH: fish oil with Butylated Hydroxy Anisole: Tocopherol, FDS: fish oil with date seed extract, FSC: fish oil spent coffee extract, FSH: fish oil with sesame hull extract, FST: fish oil with spent black tea extract, FWS: fish oil with walnut shell extract.
Table 3. Total chlorophyll contents in the fresh and stored fish oil samples.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total Chlorophyll</th>
<th>25 °C</th>
<th>35 °C</th>
<th>Stored 25 °C</th>
<th>Stored 35 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFO</td>
<td>4.78±0.03ab*</td>
<td>4.76±0.01ab</td>
<td>4.63±0.01h</td>
<td>4.50±0.01h</td>
<td></td>
</tr>
<tr>
<td>FBH</td>
<td>4.80±0.03ab</td>
<td>4.79±0.01ab</td>
<td>4.70±0.01g</td>
<td>4.66±0.01g</td>
<td></td>
</tr>
<tr>
<td>FBT</td>
<td>4.80±0.01ab</td>
<td>4.79±0.01ab</td>
<td>4.81±0.01a</td>
<td>4.67±0.01f</td>
<td></td>
</tr>
<tr>
<td>FDS</td>
<td>4.81±0.01a</td>
<td>4.78±0.01ab</td>
<td>4.73±0.01c</td>
<td>4.70±0.01d</td>
<td></td>
</tr>
<tr>
<td>FSC</td>
<td>4.78±0.01ab</td>
<td>4.76±0.01b</td>
<td>4.70±0.01e</td>
<td>4.68±0.01e</td>
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<tr>
<td>FSH</td>
<td>4.78±0.01ab</td>
<td>4.81±0.01a</td>
<td>4.70±0.01f</td>
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<tr>
<td>FST</td>
<td>4.73±0.01b</td>
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<tr>
<td>FWS</td>
<td>4.80±0.01ab</td>
<td>4.80±0.01ab</td>
<td>4.76±0.01b</td>
<td>4.78±0.01a</td>
<td></td>
</tr>
</tbody>
</table>

The results are expressed as mean ± standard deviation (n = 8). The lowercase letters indicate differences among the samples in the same column (p ≤ 0.05, one-way ANOVA with Tukey’s test).

* CFO: Crude fish oil without additives (control), FBH: fish oil with Butylated Hydroxy Anisole, FBT: fish oil with Butylated Hydroxy Anisole: Tocopherol, FDS: fish oil with date seed extract, FSC: fish oil spent coffee extract, FSH: fish oil with sesame hull extract, FST: fish oil with spent black tea extract, FWS: fish oil with walnut shell extract.

“Tchl” results of the samples are considered together, it becomes evident that natural extracts possess greater antioxidant activity than both BHA and tocopherol. Additionally, these natural extracts are effective in preventing content loss, particularly in oils stored at elevated temperatures.

### 3.4. Thermal features

The thermal decomposition process of the fish oil samples is presented in Figure 5. The initial temperatures for the thermal decomposition (Ton) of the samples obtained from the thermograms were significantly different from each other. The control sample (171.26 °C) presented lower Ton values, while the BHA-added fish oil (196.44 °C) had quite similar Ton to the fish oil added with date seed extract (194.97 °C). Similar results for hoki and tuna oils were reported by Tengku-Rozaina and Birch, (2016). In addition, Dweck and Sampaio (2004) reported that the Ton values of olive...
and corn oils were higher than the fish oils examined in this study. The thermal decomposition of the fish oil samples occurred in three distinct stages (Figure 5). The samples exhibited significant differences in temperature ranges spanning from 300 to 375 °C, 375 to 450 °C, and 450 to 600 °C. However, no thermal changes were observed in any of the fish oils at temperatures of 600 °C and above. Different oils may have different decomposition temperatures and weight loss rates, depending on their fatty acid composition. Nonetheless, the disparities observed among the samples can be attributed to the use of different additives, despite the fact that the oils used in this study were the same. When examining the weight loss of the fish oils, it became evident that the thermal stability of the fish oil samples followed the order: fish oil with BHA > fish oil with BHA: TCL > fish oil (FBH > FBT > FO). Conversely, the fish oils supplemented with natural extracts exhibited higher weight loss temperatures and lower weight loss rates compared to both the control and FBT samples. Nevertheless, the thermal stability of the fish oils treated with natural extracts exhibited a different trend, with the following order observed: fish oil with date seed extract > fish oil with walnut shell extract > fish oil with sesame hull > fish oil with spent black tea > fish oil with spent coffee (FDS > FWS > FSH > FST > FSC). In alignment with the PV and CD results, the TGA results indicated that the fish oil sample treated with date seed extract (FDS) demonstrated the closest antioxidant activity to that of BHA. The outcomes of this study reaffirm that extracts with high phenolic content can enhance the oxidative stability of fish oils. These findings provide further evidence of a linear relationship between the total phenolic component content and antioxidant capacity.

4. CONCLUSIONS

The total phenolic contents and antioxidant capacities of the date seed, sesame hull, spent coffee ground, and spent black tea, were determined successfully as well as the amounts of them equivalent to 200 ppm BHA. All the antioxidant-added fish oils had lower PV values than the control samples. Walnut shell, date seed and sesame hull extracts provided equivalent protection to BHA against oxidation, while they were more protective than the combination of BHA and tocopherol. In conclusion, the findings of the present study demonstrated that natural extracts could be equally effective as artificial antioxidants in combating oil oxidation. Extracts obtained from various food waste sources hold promise as natural antioxidants for the food industry. The optimization of extraction processes for these food waste materials may further enhance the commercialization potential of such products in future studies.

ACKNOWLEDGMENTS

The authors would like to express their special thanks to Dardanel Öntenst Food Industry Inc. (Çanakkale, Türkiye), which provided for the supply of crude fish oil. The authors would like to thank to Özen Baharat & Tohum Ltd. Co., (Çanakkale, Türkiye) for the supply of sesame hull.

DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

FUNDING SOURCES

No funding was received to assist with the preparation of this manuscript.

AUTHORSHIP CONTRIBUTION STATEMENT

E. S. Karabayır: Formal analysis, Investigation, Writing – original draft. M. Öğütcü: Conceptualization, Supervision, Methodology, Writing – original draft, Writing – review & editing.

REFERENCES


