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Research Article

The Oxidative Stability of Chia Seed Oil Enriched with Oregano (*Origanum vulgare* L.) and Yarrow (*Achillea millefolium*) Extracts

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Oxidative stability of chia seed oil enriched with oregano (*Origanum vulgare* L.) and yarrow (*Achillea millefolium*) extracts at different concentrations (600, 1200, and 1800 ppm) was evaluated under accelerated oxidation conditions for 5 days. The total phenolic compounds and antioxidant activity of oregano extract were higher than the yarrow extract. With decreasing concentrations of extracts and increasing time, the oxidative stability of chia seed oil decreased significantly ($p < 0.05$). At the first day, the acid value of chia seed oil did not show a significant difference, and the highest acid value was related to the control sample at the end of the storage period. The oil containing 1200 and 1800 ppm of oregano extract had the lowest acid, peroxide, anisidine, and Totox values. In the rancimat, the highest oxidative stability index (OSI) was shown in the sample containing 1800 ppm oregano extract, followed by yarrow extract. Our findings showed the potential of oregano and yarrow extracts by improving the oxidative stability of chia seed oil, especially at 1200 and 1800 ppm. At similar concentrations, oregano extract was more successful than yarrow extract in reducing the oxidation rate.

1. Introduction

Chia (*Salvia hispanica*) belongs to the *Lamiaceae* family which contains 15–24 ml/100 mL protein, 26–41 ml/100 mL carbohydrates, and 25–40 ml/100 mL fat [1]. Chia seeds are a good source of vitamins, minerals, and soluble and insoluble fibers [2, 3]. The oil content of chia seed is higher than that of other vegetable oils such as cottonseed, soybean, and sunflower [3]. The most important characteristic of chia seed oil is the presence of high amounts of polyunsaturated fatty acids (PUFA), including 55–60 ml/100 mL linolenic acid (ω-3) and 18–20 ml/100 mL linoleic acid (ω-6). These fatty acids play a very important role in preventing cancer, metabolic diseases, injuries, and cardiovascular diseases [1, 4–8].

Although polyunsaturated fatty acids are nutritionally desirable, their higher levels make chia seed oil sensitive to oxidation [9, 10]. Oxidation is responsible for the unpleasant taste and rancidity of the oil. Therefore, it is necessary to use antioxidants to prevent oxidation [11].

Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butylhydroquinone (TBHQ) have health risks [10, 12]. Therefore, strong antioxidants with fewer side effects are necessary in the food industry [11].

Medicinal plants are rich sources of natural antioxidants. Several studies reported the enrichment of oils with antioxidants from medicinal and aromatic plants [10, 12–17]. Yarrow (*Achillea millefolium*) belongs to the *Asteraceae* family, is one of the most famous and oldest medicinal plants that has been widely used in traditional medicine [18]. The antioxidant activity of yarrow *in vitro* and in food models has been reported by researchers [19, 20]. Oregano (*Origanum vulgare* L.) belongs to the *Lamiaceae* family, which contains vitamins (A, B, and C), minerals, and tannin. Many studies have confirmed its antioxidant and antimicrobial properties [21, 22].

Jung et al. determined the oxidative stability of chia seed oil using rosemary and garlic extracts during accelerated

storage at 65°C for 14 days. The results showed that rosemary extract has higher oxidative stability than garlic extract. γ-tocopherol, linoleic acid, and α-linolenic acid were well preserved in treated oil [23]. Bordón et al. produced edible vegetable oil mixtures rich in ω-3 fatty acids with higher oxidative stability than chia oil and showed that chia oil is a suitable alternative to obtain ω-3 enriched oils with higher oxidative stability [9]. Rodriguez et al. investigated the oxidative stability of microencapsulated chia seed oil enriched with different natural antioxidants. The addition of natural antioxidants, depending on the type and concentration, protected chia oil against lipid oxidation and provided the highest induction time in rancimat and the lowest peroxide values after 90 days [24]. Abad and Shahidi showed that the oxidative stability of chia seed oil was affected by the composition of fatty acids, triacylglycerols, and storage conditions [25].

The aim of this study was to evaluate the effectiveness of oregano (*Origanum vulgare* L.) and yarrow (*Achillea millefolium*) extracts in the oxidative stability of chia seed oil under accelerated oxidation.

2. Materials and Methods

2.1. Materials. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin–Ciocalteu were obtained from Sigma–Aldrich (UK). BHT, gallic acid, p-anisidine, glacial acetic acid, chloroform, starch indicator, phenolphthalein, sodium hydroxide, sodium thiosulfate, and all chemical reagents were purchased from Merck Company (Germany).

2.2. Preparation of Plant Samples. Oregano (*Origanum vulgare* L.) and yarrow (*Achillea millefolium*) were obtained from medicinal plant centers (Isfahan, Iran) and approved by the botanical herbarium. Their flowers were crushed by a grinder. The powders were kept in glass containers at 4°C.

2.3. Extract Preparation. In the maceration method, 100 g of dried powder was mixed with 500 ml of 80% methanol and kept at room temperature (22°C) for 24 h. The methanolic extract was concentrated using a rotary evaporator (IKA, RV 10, and China) at 40°C, dried in an incubator (Behdad, Iran), and stored in the refrigerator [23].

2.4. Total Phenolic Compounds (TPC). The total phenolic compounds were analyzed by the Folin–Ciocalteu method. Briefly, 0.5 ml of the extract was mixed with 5 ml of Folin–Ciocalteu reagent and 4 ml of 1 M sodium carbonate solution. The mixture was kept at room temperature for 15 min. Then, the absorption of the solution was read by a spectrophotometer (Thechnicon, Italy) at 765 nm. The total phenolic compounds were expressed using a standard curve based on gallic acid and expressed as mg gallic acid/g extract [26].

2.5. Antioxidant Activity. The antioxidant activity was determined using the DPPH assay. Briefly, 1 ml of DPPH methanolic solution was added to 3 ml of extract solutions and mixed for 30 min in a dark place by a shaker (VWR Orbital, Model 1000, and China). The absorption was measured at 517 nm using a UV-Vis spectrophotometer (Thechnicon, Italy), and DPPH inhibition was calculated according to the following equation [19]:

$$\%\ \text{inhibition of DPPH} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs sample}}\right) \times 100. \quad (1)$$

2.6. Treatment of Chia Seed Oil. Chia seed oil was extracted using a cold press and was treated with different levels of oregano and yarrow extracts (600, 1200, and 1800 ppm) and BHT (200 ppm) and packed in glass containers. All the tests were done after oil heating in the oven at 90 ± 5°C for 5 days. The control sample was produced without an antioxidant.

2.7. Oxidative Stability. The acid, peroxide, and anisidine values were determined according to the AOCS Cd 3-63, Cd 8-53, and Cd18-90 methods by titration with sodium hydroxide and sodium thiosulfate and spectrometrically, respectively [27–29]. Totox was calculated based on 2 (PV) + pAV [23]. The oxidative stability index (OSI) was determined by rancimat according to the AOCS Cd12b-92 standard at 110°C and an airflow rate of 20 L/h. Oxidative stability was expressed as the induction period (h) or oxidative stability index [30].

2.8. Statistical Analysis. All experiments were performed in 3 replicates. Analysis of variance (ANOVA) was used using SPSS software (Ver. 22) at 5% level ($p < 0.05$).

3. Results and Discussion

3.1. TPC and Antioxidant Activities. The phenolic compounds and antioxidant activity of oregano and yarrow extracts are shown in Table 1. As it is known, oregano extract had significantly higher total phenolic compounds and antioxidant activity compared to yarrow extract ($p < 0.05$).

Chia contains large amounts of natural antioxidants such as phenolic compounds [5, 6, 31, 32]. High amounts of TPC in the extracts are responsible for their antioxidant effects. The phenolic compounds have antioxidant activity and play an important role in preventing oil autoxidation [33]. The antioxidant properties of oregano have been confirmed previously [21, 22, 34]. Yarrow is also a good source of phenolic compounds that inhibit-free radicals [19, 20, 34–37].

3.2. Oxidative Stability. The oxidative stability of treated chia seed oil samples is shown in Figure 1. As can be seen in Figure 1(a), at the first day of storage, the acid value (AV) did not show a significant difference ($p > 0.05$) in different groups (0.03–0.05 mg KOH/g oil). AV in the control group was significantly higher than that in the other groups ($p < 0.05$) and reached 0.38 mg KOH/g oil at the end of the

Table 1: TPC and antioxidant activities of plant materials.

Plant material	TPC (mg gallic acid/g)	Antioxidant activity (%)
Oregano	30.19 ± 0.01^{a}	81.24 ± 0.01^{a}
Yarrow	12.82 ± 0.01^{b}	72.23 ± 0.01^{b}

The mean ± SD (standard deviation) within columns with different letters differs significantly ($p < 0.05$).

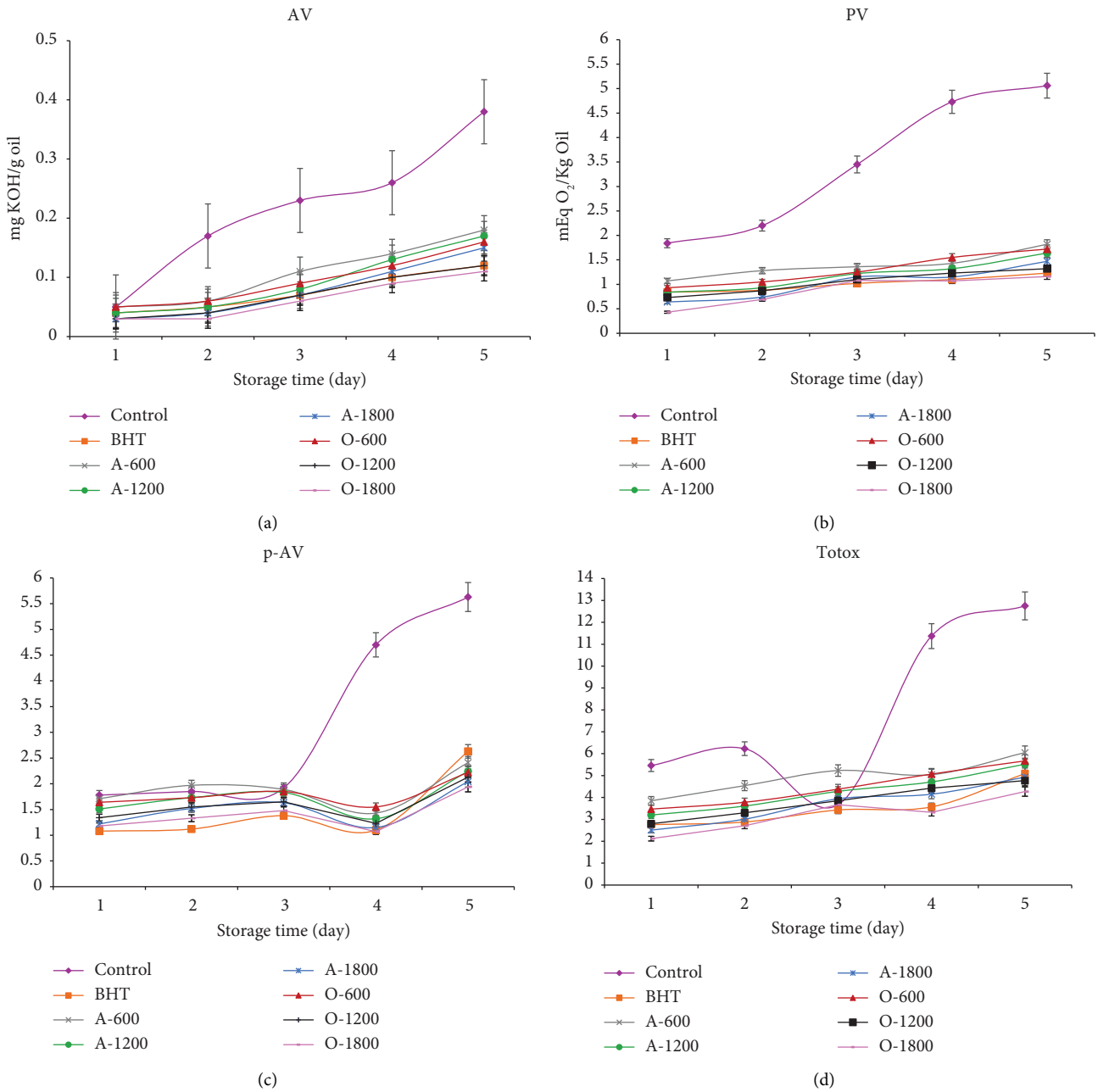

Figure 1: Continued.

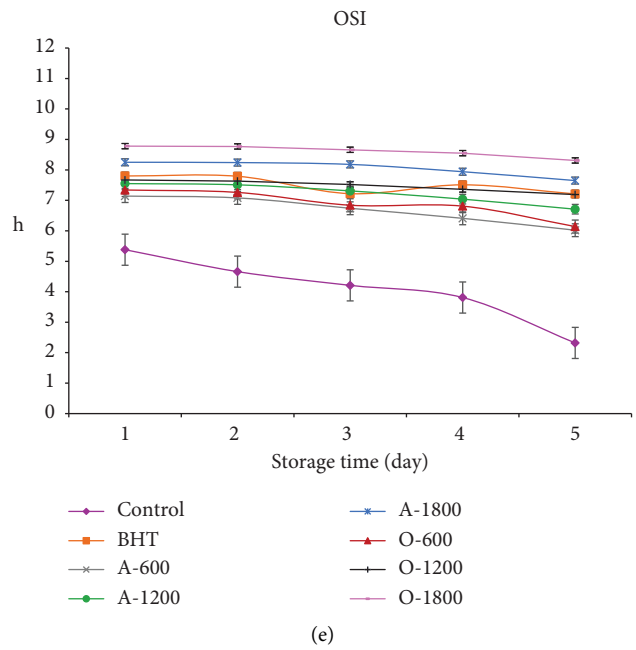

Figure 1: The oxidative stability of chia seed oil during 5 days. O-600, O-1200, and O-1800: the treated oils with 600, 1200, and 1800 ppm oregano extract, respectively. A-600, A-1200, and A-1800: the treated oils with 600, 1200, and 1800 ppm *Achilea* extract, respectively.

storage. BHT, O-1200, and O-1800 groups had the lowest AV at the end of storage. AV of the samples was according to the national standard of Iran No. 4178 (1–4 mg KOH/g oil) during storage, which indicates the good quality of the oils [38].

The highest (5.06 mEq O_2/kg oil) and lowest (1.16 mEq O_2/kg oil) peroxide values (PV) were related to the control and O-1800 groups at the end of the storage (Figure 1(b)). According to the national standard of Iran (No. 4179), the permissible limit of PV is 5 mEq O_2/kg for edible oils [39]. On the other hand, the control group was acceptable until the fourth day of storage. Chia seed oil contains a high amount of unsaturated fatty acids, which increases the oxidation rate [1, 3].

The pattern of anisidine value (p-AV) changes is similar to AV and PV (Figure 1(c)). At the end of the storage, the highest p-AV (5.63) was related to the control group, while according to the national standard of Iran (No. 4093), the standard limit of p-AV is 10 [40]. Therefore, all groups were within the range of the standard. O-1800, A-1800, and O-1200 groups have the lowest p-AV. The increase of p-AV indicates the expansion of the secondary products of oxidation.

As can be seen in Figure 1(d), on the third day, Totox in the A-600 group was higher than in other groups. At the end of the storage, the highest Totox was observed in the control and A-600 groups. The O-1800 and O-1200 groups had the lowest Totox values.

The oxidative stability index (OSI) in the control group was significantly ($p < 0.05$) lower than that in other groups (Figure 1(e)). At the end of the storage, the highest OSI was observed in the O-1800 and A-1800 groups, respectively (8.31 to 7.65 h).

The oxidation rate in the control group was significantly higher than the other groups during storage ($p < 0.05$). It seems that there is a concentration-dependent effect in the oxidation process. By decreasing the concentration of the extracts, the oxidation rate increased and the OSI decreased significantly ($p < 0.05$). At similar concentrations, oregano extract was more effective than yarrow extract in the oxidation reduction and OSI increase, which is probably related to the higher phenol content in this extract (Table 1). The antioxidant activity of oregano and yarrow extracts has been reported previously [19–22].

Similar results have been reported for the use of natural antioxidants such as rosemary and garlic in chia oil [23, 24] and other natural antioxidants in vegetable oils [41–48]. Jung et al. improved the oxidative stability of treated chia seed oil with rosemary (*Rosmarinus officinalis* L.) and garlic (*Allium cepa* L.) extracts from an induction period of 0.43 ± 0.01 h to 1.30 ± 0.06 h. Recently, the methanolic extracts from the petals of 23 edible flower species have been used to enhance the oxidative stability of cold-pressed chia seed and flax oil. The different flower species had different protective effects against oil oxidation, with the flowers with the highest concentration of flavonoids and carotenoids being the most effective. The highest protection was the synergy between flavonoids, tocopherols, and carotenoids [49]. The blends of chia oil with walnut, almond, or sesame oils increased the oxidative stability of the oils subjected to accelerated storage conditions while maintaining a high level of omega-3 fatty acids [9]. The supplementation of chia oil with sesame oil increased the OSI value and the shelf life [50].

4. Conclusion

Oregano (*Origanum vulgare* L.) and yarrow (*Achillea millefolium*) extracts were able to inhibit the DPPH radical, and this activity was higher in oregano extract, which can be attributed to the presence of more TPC. The oxidative stability of chia seed oil under accelerated oxidation

conditions decreased with the decrease in the concentration of extracts. The highest AV, PV, p-AV, and Totox levels were observed in the control group. The O-1800 and O-1200 groups had the lowest oxidation rates. The results of this study confirmed that oregano and yarrow extracts showed better oxidative stability in chia oil than BHT. Therefore, these extracts have a high potential as natural antioxidants in edible oils.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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