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Research Article

Comparing Antioxidant Activity of Ethanolic Olive Cake Extract with some Synthetic Antioxidants on Oxidative Stability of Soybean Oil -

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ABSTRACT

Lipid oxidation is one of the main problems in the storage and utilization of edible oils and fats. The use of antioxidants is one of the most effective ways to delay oxidation. In this study, Total Phenolic Compounds (TPC) of olive cake extract (OCE) was evaluated. Then antioxidant activity of OCE and synthetic antioxidant on oxidative stability of soybean oil were compared. For this purpose, peroxide index, anisidine index, and Oxidative Stability Index (OSI) were studied during 4 week. The results revealed that the soybean oil containing 150 ppm OCE had the highest 150 ppm OCE had the highest oxidative stability (OS) during storage period, so that, OS of this oil after 4 weeks (6.9 h) was more than OS of control oil on the first week of storage period (6.26). As well as, OCE at concentration of 150 ppm had more Antioxidant Activity (AA) than synthetic antioxidants in soybean oil.

Keywords: Olive cake extract; Antioxidant activity; Oxidative stability; Soybean oil

INTRODUCTION

Fats and oils play important role in the human diet and provide the main part of needed energy. Lipid oxidation is one of the main reasons for food spoilage through adverse effects on flavor, color, nutritional value and also producing toxic compounds during processing, storage and distribution [1].

The use of antioxidants is one of the most effective ways to prevent oxidation. Antioxidants are classified into two major groups, natural and synthetic types, which have used to prevent oil oxidation. Giving to harmful effects of synthetic antioxidants such as carcinogenesis, natural antioxidants have attracted interest of consumers [2].

Phenolic compounds form the main part of bioactive compounds in olive (*Olea europaea* L.) and processed olive products. Olive oil is one of the oldest natural oils used by human in all over the world [3]. Olive cake and olive mill wastewater are the main by-products of olive oil industry. The control of these by-products is critical for related industries, as these by-products constitute the main part of olive oil industry (440 L wastewater and 35 kg olive cake per 100 kg olive).

Olive cake is known as a cheap and available source of phenolic compounds in the northern provinces of Iran. However, by-products of olive oil industry threaten environmental problems for these regions [4]. Thus, the finding a suitable solution to reduce this environmental problem, and at the same time have economic efficiency is important. So far, animal feed, fuel, and fertilizer are industrial applications of olive cake [5].

Oxidative stability (OS) is a major factor on study the quality of edible oils, which significantly affected by fatty acid composition and other components such as phenolic compounds and tocopherols. Oil oxidation process of degrade polyunsaturated fatty acids (PUFA) and generate free radicals, which these produced products decrease nutritional value and functional properties [6]. Tocopherols and phenolic compounds are the main natural antioxidants that stabilize unsaturated fatty acids (USFA) and these compounds have vital potential for effective protection against oxidative stress in the human body [7].

Soybean oil is one of the sensitive oils to oxidative changes. The sensitivity is related to fatty acid composition and especially because of the high USFA content. Addition of natural antioxidant to edible oils is one of the methods to improve the stability of oils in storage period and thermal processes such as frying [8].

Nowadays researches are ongoing to find natural antioxidant sources and use them to reduce the oxidation of oils, for example rosemary and sage extracts in palm oil [9], Teaw (*Cratoxylum formosum* Dyer) extract in soybean oil [10], various plant extracts in sunflower oil [11], and garlic extract in sunflower oil [12]. However, there is no study in the literature about the use of olive cake extract (OCE) as natural antioxidant to increase oxidative stability of soybean oil. Therefore, this research was aimed to increase oxidative stability of soybean oil using OCE and compare antioxidant activity of OCE with synthetic antioxidants (BHA and BHT).

MATERIALS AND METHODS

Material

Olive cake was obtained from Sina Ebtekare Samen plant (*Bandar-e Gaz, Iran*). Olive cake was dried at 42 ˚C and powdered. After sieving, the powder was defatted using petroleum by Soxhlet method. All chemicals were analytical grade and purchased from Sigma-Aldrich (USA) and Merck (Germany) companies.

Olive cake extracts (OCE) preparation: OCE was prepared using Soxhlet method and ethanol solvent at 50 ˚C. The extract was filtered by Whatman filter paper (No.1), and then vacuum drying in the 1410D-2E vacuum *oven (Shel Lab, USA)*. The prepared powder was stored in dark container at 4 ˚C until analyses.

Total Phenolic Compound (TPC) determination: Total Phenolic Compound (TPC) of OCE was measured by Folin Ciocalteu method [13]. OCE was diluted in distilled water, and then, 1 ml of this solution, 7.5 ml of distilled water and 0.5 ml Folin-Ciocalteu reagent were mixed in test tube. After 5 min, 1 ml Na_2CO_3 (5%) was added to this test tube and mixed. After 1 h, the absorbance was spectrophotometrically measured by UV-160A spectrophotometer (Shimadzu, Japan) at 725 nm. The TPC was reported as mg of gallic acid equivalents per g of dry olive cake. Standard calibration curve was plotted using gallic acid.

Preparation of OCE and soybean oil blend: OCE was added to refined and without antioxidant soybean oil at concentrations of 0, 50, 100, 150, 200, 250, 300, 350 and 400 ppm. These blends were stored in an oven (Memert, unb 400 model, Germany) at 90˚C for 4 weeks. Chemical analyses including Peroxide Value (PV), Anisidine Value (AV), and Oxidative Stability Index (OSI) were done at time interval of 7 days. As well as, synthetic antioxidant were added to soybean oil at concentration of 75 ppm.

Chemical analysis

Peroxide (Cd 8-53) and anisidine (Cd 18-90) values were determined according to AOCS methods (AOCS, 1997).

Oxidative stability index: Determination of oxidative stability index (OSI) was carried out according to [6] using a Metrohm Rancimat model 743 (Herisau, Switzerland). The tests were done with 3 g of samples at 120 °C and airflow rate of 15 L/h.

Statistical analysis

All measurements were done in triplicate, and obtained data

were subjected to Analysis of Variance (ANOVA) at completely randomized design. Significant differences between means of data were determined by Duncan test; $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Total phenolic compounds of OCE

The TPC of ethanolic OCE was 64.49 mg gallic acid per g extract, which indicates that the TPC extracted from Iranian OCE is in very good level. This TPC content was remarkably more than previous studies [14,15].

Effects of OCE on PV of soybean oil

PV of soybean oil containing OCE and the control oil (soybean oil without OCE) was increased during storage period. The soybean oil containing 150 ppm OCE had the lowest PV over 4 weeks, even compared to the oil containing synthetic antioxidant, which this difference was statistical significant ($p < 0.05$). The PV was decreased by increasing OCE up to 150 ppm, but remarkably increased at high concentrations of OCE, as is shown in Table 1. This PV decreasing is probably due to peroxidation activity of OCE at high concentrations. It is believed that phenolic compounds can have peroxidation activity at high concentrations [15,16], reported that grapefruit seed extract increased oxidative stability of anchovy oil.

Effects of OCE on AV of soybean oil

Similarly to PV, AV of soybean oil containing OCE was increased by extending storage period. The lowest AV (5.23) was observed for soybean oil containing 150 ppm OCE at the first week (Table 2). As well as, OCE at concentration of 200 ppm had more antioxidant activity from syntactic antioxidants after the first and second weeks; however, after third and fourth weeks, this activity was more for syntactic antioxidants (Table 2). At the concentrations higher than 150 ppm, amount of secondary oxidation products was increased by increasing OCE concentration, and therefore the AV increased. The AV increasing represents development of spontaneous reactions and an increase in secondary products derived from the decomposition of hydro peroxides and carbonyl compounds during storage time [1].

Effects of OCE on oxidative stability of soybean oil

As is shown in Table 3, the soybean oil containing 150 ppm OCE had the highest oxidative stability during storage period, so that, OS of this oil after 4 weeks (6.9 h) was more than OS of control oil on the first week of storage period (6.26). OS of soybean oil containing BHA was more than the soybean oil containing BHT, which this is attributed to more AA of BHA (Table 3). The results of this study are in accordance with observations of [17], which reported that OS of edible vegetable oils was significantly increasing by adding polyphenols derived from olive leaf extract.

Table 1: PV (meq O_2 / Kg oil) of soybean oil containing OCE at different concentrations during 4 weeks.

Concentration of OCE (ppm)	Time (week)			
	1	2	3	4
0	14.10^{a}	20.60^{a}	32.52^{a}	68.44^{a}
50	11.31^{d}	16.08^{b}	26.67^{c}	50.24^{e}
100	10.34^{e}	13.26^{e}	25.16^{d}	47.57^{f}
150	5.53^{j}	8.11^{j}	10.23^{k}	41.34^{k}
200	7.04^{h}	9.00^{i}	24.06^{h}	46.45^{h}
250	7.94^{g}	12.51^{g}	25.61^{g}	47.25^{g}
300	11.31^{d}	14.25^{d}	25.45^{f}	50.44^{d}
350	12.47^{c}	15.70^{c}	26.14^{e}	51.44^{c}
400	12.20^{b}	16.46^{b}	27.87^{b}	52.27^{b}
*	6.54^{i}	10.41^{h}	19.63^{j}	44.44^{j}
**	9.61^{f}	12.82^{f}	20.49^{i}	45.46^{i}

Different letters within each column indicate significant difference ($p < 0.05$)
* BHA, ** BHT

Table 2: AV of soybean oil containing OCE at different concentrations during 4 weeks.

Concentration of OCE (ppm)	Time (week)			
	1	2	3	4
0	8.74^{a}	13.29^{a}	18.62^{a}	24.85^{a}
50	6.57^{c}	11.78^{b}	14.83^{e}	16.89^{e}
100	6.29^{d}	9.82^{f}	12.83^{g}	15.28^{g}
150	5.53^{g}	7.87^{j}	8.54^{j}	10.11^{j}
200	5.73^{f}	8.26^{i}	13.35^{f}	15.86^{f}
250	6.10^{e}	9.33^{g}	13.76^{e}	16.80^{e}
300	6.28^{d}	10.62^{d}	14.34^{d}	17.25^{d}
350	6.65^{c}	10.82^{d}	15.36^{c}	18.87^{c}
400	6.88^{b}	11.56^{c}	16.13^{b}	20.88^{b}
*	5.79^{f}	8.45^{h}	11.09^{i}	12.14^{i}
**	6.14^{e}	11.78^{d}	12.40^{h}	13.75^{h}

Different letters within each column indicate significant difference ($p < 0.05$)
* BHA, ** BHT

Table 3: OSI (h) of soybean oil containing OCE at different concentrations during 4 weeks.

Concentration of OCE (ppm)	Time (week)			
	1	2	3	4
0	6.26^{g}	3.47^{i}	2.84^{j}	2.31^{h}
50	7.56^{e}	5.16^{h}	3.11^{i}	2.45^{g}
100	10.25^{b}	7.08^{e}	6.82^{d}	6.76^{d}
150	12.48^{a}	8.88^{a}	7.89^{a}	6.90^{a}
200	10.55^{b}	8.58^{b}	7.61^{b}	6.20^{b}
250	9.35^{c}	6.81^{f}	6.42^{e}	5.74^{c}
300	8.83^{d}	5.68^{g}	5.35^{f}	4.77^{d}
350	8.21^{e}	5.48^{g}	4.74^{g}	3.28^{e}
400	7.72^{f}	5.22^{h}	4.23^{h}	3.47^{f}
*	10.58^{b}	7.79^{c}	7.09^{c}	6.12^{b}
**	9.41^{c}	7.43^{d}	6.85^{d}	5.82^{c}

Different letters within each column indicate significant difference ($p < 0.05$)
* BHA, ** BHT

CONCLUSIONS

This study revealed that OCE successfully improve OS of soybean oil. OCE at concentration of 150 ppm showed higher antioxidant activity compared to synthetic antioxidants (BHA and BHT) in soybean oil. The soybean oil containing 150 ppm OCE had the highest OS over storage period.

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