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Marzeh Khuzistani essential oil as a natural antioxidant in canola oil under forced conditions

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Abstract

The effects of ultrasound treatment (30 kHz, 100 W, 30 s), Ultraviolet (UV) irradiation (30 min), and marzeh khuzistani essential oil (0.08%) extracted by ohmic ultrasonic extractor on oxidation of canola oil were studied. GC-MS analyses of marzeh khuzistani essential oil (EO) revealed that carvacrol (88.6%) was the major component of EO. Peroxide value (PV), Anisidine value (AnV), Thiobarbituric acid (TBA) value, free fatty acid (FFA), iodine value (IV), and induction period (IP) were measured in canola oil and showed that EO had an antioxidant effect in comparison to BHT in sample oils treated or no treated by ultrasound and UV. In addition, EO was able to reduce the stable 2, 2-diphenyl-1-picrylhydrazyl free radical (DPPH) with a 50% inhibition concentration (IC_{50}) of 29 ± 0.06 µg/mL. The results indicate that ultrasound and UV treatment increase oil oxidation but EO can act as antioxidant and increase oil stability. Therefore, EO could be used as a natural antioxidant in oils for food uses.

Introduction

Lipid oxidation is a challenging problem in the production, processing and storage of vegetable oil. It causes problems such as Unpleasant tastes, odors and color of products and decrease in nutritional quality and safety by the formation of secondary toxic compounds. Their oxidative stability depends on many factors, including the un-saturation of their fatty acids, composition of minor components, use of antioxidants and environment conditions like temperature, UV and exists of free radicals (Keszler *et al*., 2000).

Ultrasound and UV radiation are a type of alternative non-thermal technology for pasteurization and sterilization processing in food industry. Ultrasound is defined as mechanical waves with frequencies higher than threshold for human. It's simplest method for peeling, acceleration or inactivation enzyme reaction, extraction of essential oil and bioactive principles from herbs (Dolatowski *et al*., 2007; Hashemi *et al*., 2011a).

Satureja khuzestanica Jamzad, a member of Laminaceae family, known as marzeh khuzistani, a plant native to Iran. It is a small shrub, branched stem ± 30 cm high, densely leafy, broadly ovaiate-orbicular covered with short white hairs. Extracts and essential oil of this spice plant have been shown to have antiseptic, anti-inflammatory, anti-nociceptive and antimicrobial properties (Hadian *et al.*, 2011a). In this paper, effects of ultrasound, UV treatment and EO on oxidation of canola oil during storage were investigated.

Materials and Methods

Plant materials and chemicals

Aerial parts of marzeh khuzistani (26% initial moisture) were collected from the wild growing plants at the full flowering stage in September to October 2012. The plants were then dried under ambient conditions (30–40˚C) for three days on a large screened tray. They were then kept in a dark and cold room until used shortly after that for the experiments. Voucher specimen of the species (MPH-1582) was deposited at the Herbarium of Medicinal Plants and Drugs Research Institute (MPH), Shahid Beheshti University, Tehran, Iran. Commercial canola oil with no additives was obtained from Savola Behshahr Co., Tehran. Iran. Chemicals such as methanol, acetic acid, chloroform, sodium iodide, sodium thiosulfate, iso-octane, potassium hydroxide, thiobarbituric acid, 1-butanol and p-anisidine were obtained from Merck (Darmstadt, Germany). BHT and DPPH were

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purchased from Sigma Chemical Company (Sigma-Aldrich GmbH, Sternheim, Germany).

Extraction of EO

Extraction of essential oil from marzeh khuzistani was performed with the newly designed ohmic ultrasonic extraction (distillation). The extractor unit consisted of a cylindrical chamber (0.07 m internal diameter and 0.25 m length) made with Teflon. It was equipped with two Titanium electrodes. The system was fully automated for which the voltage (0–300 V) and current (0–16 A) and temperature could be controlled, monitored and recorded to a data sheet throughout the experiment. A Hielscher ultrasonic device (UP100H, 100 W, 30 kHz) with a titanium sonotrode (tip diameter10 mm) was used to sonicate the sample containing the plant materials. The extraction unit was also equipped with an all-glass clevenger-type apparatus. For each experimental run, 19 g (9% moisture) of the plant material was charged in to the chamber together with 500 mL brine (NaCl) solution (0.3% w/v). (Sodium chloride will provide sufficient electrical conductivity between two electrodes for the heat up process to be swift.) Prior to heating process, the plant materials which were fully immersed in brine solution was sonicated for 3 min in order to improve the EO release from the cell. The ohmic system was then switched on. A constant voltage of 150 V was applied between the two electrodes to increase the solution temperature from initial value of 20.8°C right up to boiling. The temperature rise was recorded at about 19.8°C/min. The extraction of EO was continued for 18 min. The EO was collected, dried under anhydrous sodium sulphate and stored in sealed vials at 4°C until used.

EO analysis

The EO was analyzed by GC-MS. The analysis was carried out on a Thermoquest- Finnigan Trace GC-MS instrument equipped with a DB-5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 µm). The oven temperature was programmed to increase from 60 to 250°C at a rate of 4°C/min and finally held for 10 min; transfer line temperature was 250°C. Helium was used as the carrier gas at a flow rate of 1.1 ml/min with a split ratio equal to 1/50. The quadrupole mass spectrometer was scanned over the 35–465 amu with an ionising voltage of 70 eV and an ionisation current of 150 mA.

GC-FID analyses of the oil were conducted using a Thermoquest-Finnigan instrument equipped with a DB-5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 µm). Nitrogen was used as the carrier gas at the continuous flow of 1.1 mL/min; the split ratio was the same as for GC-MS. The oven temperature was raised from 60 to 250°C at a rate of 4°C/min and held for 10 min. The injector and detector (FID) temperatures were kept at 250 and 280°C, respectively. Semi quantitative data were obtained from FID area percentages without the use of correction factors.

Retention indices (RI) were calculated by using retention times of n-alkanes (C6–C24) that were injected after the oil at the same temperature and conditions. Compounds were identified by comparison of their RI with those reported in the literature (Adams 2007) and their mass spectrum was compared with the Wiley Library (Wiley 7.0).

DPPH radical scavenging activity

The radical scavenging capacity of EO for DPPH was monitored according to the method described by Burits and Bucar (2000). Fifty microlitres of different concentrations of the essential oil samples in methanol (15, 25, 35, 45 and 55 µg/mL) were added to 5 mL of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature under dark condition, the absorbance of the samples was read against a blank at 517 nm. Inhibition of free radical DPPH in percent (I%) was calculated in following way:

$$I\% = (A_{blank} - A_{sample}) / A_{blank} \times 100,$$

where A_{blank} is the absorbance of the control reaction (containing all reagent except the test compound), and A_{sample} is the absorbance of the test compound. EO concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against EO concentration. BHT was used as a control and all tests were carried out in triplicates.

Oil sample preparation

Samples (control, canola oil with EO at 0.08%, and canola oil with BHT at 0.02%) are divided into two groups; the first group treated with ultrasound and UV treatment while the second group prepared with no ultrasound or UV treatment. Then the PET bottles were poured with oils up to 250 mL volume of container, and stored in a laboratory oven under dark condition at 25 ± 1°C during 60 days. The temperature were controlled and the data recorded by data logger (LASCAR, England).

Ultrasonic and UV treatment

The canola oil (control, with EO or BHT) was treated without heating using a fixed Hielscher

ultrasonic device (UP100H, 100 W, 30 kHz) with a titanium sonotrode (tip diameter 10 mm) in a cooled jacket flask (250 mL). Approximately 100 mL of oil was placed in the flask and treated for 30 s with ultrasonic irradiation. In addition, oil samples irradiated with UV rays for 30 min (1.8 kW UV lamp, EMA, Sverdlovsk, Russia).

Chemical tests

PV was measured by treating a solution of oil (5 ± 0.05 g) in 30 mL acetic acid–chloroform with 0.5 mL saturated potassium iodide solution and titration with 0.1 N sodium thiosulfate. Determination of AnV was done by reading the absorbance of a solution of oil (0.5–4 ± 0.001 g) in 25 mL isooctane, treated with 1 mL p-anisidine reagent at 350 nm using solvent with *p*-anisidine reagent as blank in the reference cuvette. Measurement of TBA value was done by heating a 5 mL aliquot of a solution of sample (50–200 mg) in 25 mL 1-butanol with 5 mL TBA reagent at 95°C for 120 min and reading the absorbance at 530 nm using distilled water in the reference cuvette (AOCS, 1998). For determination of FFA, (15 ± 0.01 g) of each oil sample was placed into a 250 mL Erlenmeyer flask and dissolved in 70 mL reagent grade alcohol containing phenolphthalein indicator and then each oil solution was subsequently titrated with the potassium hydroxide solution (AOCS, 1999). The Wijs method was used for the determination of the IV. Iodine chloride was used for double-bond saturation analysis, and the consumed iodine was measured by titration with 0.1 M standard sodium thiosulfate solution (Firestone, 1994).

Determination of IP of oil samples

The oxidative stability of each oil sample was determined using a Rancimat instrument (Metrohm model 734, Switzerland) at 110°C. The results were expressed as the induction time (h) of each sample, which is the length of time that passes before an oil sample obtains a significant measurable rancidity defined as the time point where an oil sample's oxidation becomes rapidly accelerated (Firestone,1994).

Statistical analysis

Experiments on each of samples were performed at three times. Statistical analyses were calculated at each time, so were analyzed by SPSS (SPSS 16.0 for Windows; SPSS Inc., Chicago, IL, USA) and Minitab ver. 11.12 (Minitab Inc., USA).

Results and Discussion

EO composition

Amount of the essential oil extracted from marzeh khuzistani using ohmic ultrasonic extractor was 16.41% (v/w). GC-MS analysis of the EO resulted in the identifications of 16 compounds. The percentages of each component of EO were quantified by peak area using the FID detector. Percentages of components of the EO are summarized in Table 1. Main components of the EO include: carvacrol (88.6%), thymol (1.7%), and linalool (0.8%).

Antioxidant activity of EO measured by DPPH method

The hydrogen atom or electron donating ability of some the corresponding pure compounds were determined using the bleaching of purple-colored methanol solution of DPPH (Burits and Bucar, 2000). In this study, the free radical scavenging activity of EO was 29 ± 0.06 µg/mL whereas IC_{50} value of BHT was 18.4 ± 0.4 µg/mL. In the DPPH assay, EO exhibited remarkable antioxidant activity. The activity of the EO could be associated with high content of carvacrol (Hashemi *et al*., 2011b, 2012).

Effect of EO, UV and ultrasound treatment on the canola oil oxidation

The PV test is used for detection the initiation stage of the oxidation process. As shown in Figure 1 oil samples were prepared with EO show better efficacy against oxidative reactions in comparison with BHT. UV and ultrasound treatments both have negative effect on oxidative stability of all samples especially in oil without any additives. UV treatment resulted in higher peroxide formation than ultrasound treatment. The findings also confirm that ultrasound treatment combined with UV strongly increased oxidation of the samples so that antioxidant effects of EO and BHT was practically disappeared.

The TBA test is an easy and quick method widely used for the assessment of secondary product of oxidation in which malonaldehyde is derivatized (AOCS, 1998). The effects of EO and synthetic antioxidants (BHT) on TBA values of canola oil at 25°C for 60 days are shown in Figure 2. As it shows, TBA values were considerably higher in the samples with UV and ultrasound treatments, whereas UV was more destructive than ultrasound. However combination of ultrasound and UV had a large impact on the production of secondary oxidation

Table 1. Essential oil composition of Marzeh Khuzistani identified by GC-MS

Component	R_I	Percentage
α-Pinene	939	0.06
Myrcene	983	0.04
β-Pinena	978	0.04
p-Cymene	1018	0.40
Limonene	1026	0.70
γ-Terpinene	1063	0.06
trans-sabinene hydrate	1075	0.30
Linalool	1099	0.80
Borneol	1163	0.50
Terpine-4-ol	1164	0.60
Cuminaldeheyde	1218	0.10
Carvacrol methyl ether	1242	0.30
Thymol	1292	1.70
Carvacrol	1302	88.60
β-Caryophyllene	1432	0.40
β-Bisabolene	1528	0.06
Total		97.00

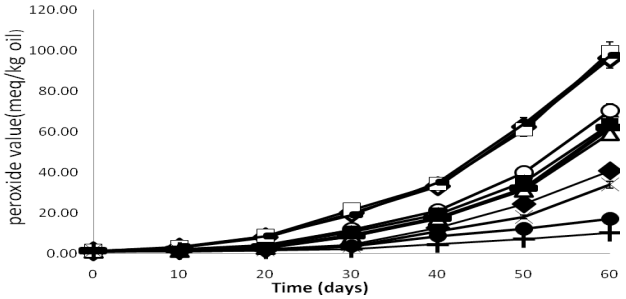

Figure 1. PV of canola oil samples

Control (♦), EO+UV+Ultrasound (◊), BHT+UV (▲), BHT+Ultrasound (Δ), BHT (●), Control+UV (○), Control+Ultrasound (■), Control+UV+Ultrasound (□), EO+UV (—), EO+Ultrasound (×), EO (+), BHT+UV+Ultrasound (-)

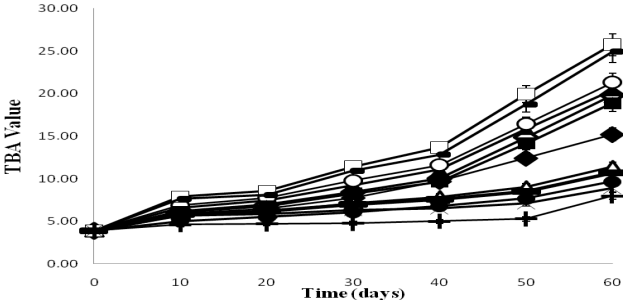

Figure 2. TBA value of canola oil samples

Control (♦), EO+UV+Ultrasound (◊), BHT+UV (▲), BHT+Ultrasound (Δ), BHT (●), Control+UV (○), Control+Ultrasound (■), Control+UV+Ultrasound (□), EO+UV (—), EO+Ultrasound (×), EO (+),BHT+UV+Ultrasound (-)

but comparison of samples were prepared with EO to samples with BHT showed that TBA values in oil with EO were less than oil with BHT.

AnV is a test for aldehydes and it plays a significant role in the oxidation process of edible oil and fats. Increases in AnV indicated the rapid rate of

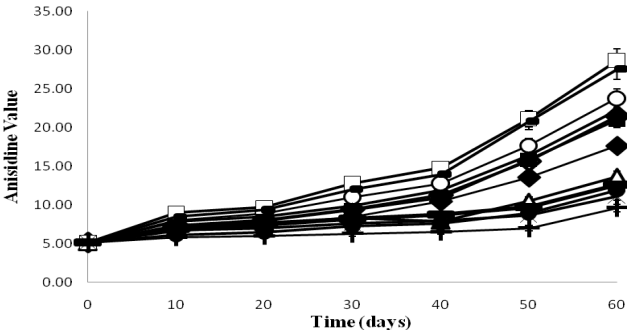

Figure 3. AnV of canola oil samples

Control (♦), EO+UV+Ultrasound (◊), BHT+UV (▲), BHT+Ultrasound (Δ), BHT (●), Control+UV (○), Control+Ultrasound (■), Control+UV+Ultrasound (□), EO+UV (—), EO+Ultrasound (×), EO (+), BHT+UV+Ultrasound (-)

secondary oxidation (Figure 3). As shown in Figures 2 and 3, results of AnV value measurements are very similar to results of TBA values measurements. AnV results also show samples added with EO were more stable than samples contain 0.02% BHT.

The presence of free fatty acid is an important measure of rancidity of oils. FFAs are formed as a result of hydrolysis reaction of triacylglycerol and increased by reaction of oil with moisture (Shahidi and Zhong, 2010; Frega *et al*., 1999). Results (Figure 4) show that using of EO in preventing the formations of free fatty acids were more effective than the BHT. Destructive effects of UV and ultrasound in formation of free fatty acids were also observed.

Iodine value (IV) is a measurement that estimates the degree of unsaturation present in fats and oils. A decrease in the IV is consistent with the increase of lipid oxidation (Naz *et al*., 2004). Changes in IV were presented in Figure 5. As seen in Figures 4 and 5 results of IV and FFA were almost the same and IV value in samples treated with EO was more compared to the sample treated with BHT. Results show that there were no significant differences between UV treatment and combination of it with ultrasound treatment. The evaluation of antioxidant effectiveness regularly fits to an extension of the IP due to the addition of the antioxidant compound. According to the results which are shown in Figure 6, the treated samples with EO were more stable against oxidation than treated samples with BHT. In addition, EO has been able to reduce the damaging effects of UV and ultrasound.

In canola oil samples treated with UV and ultrasound which stored for 60 days, a cloudy medium appeared within the oil. In common conditions, degradation of vegetable oils generates only unpleasant flavor after months of storage. This formed medium inside the bulk canola oil is because of polymer formation after degradation of triglycerides. In this study, polymer and oxidation compounds in UV and ultrasound treatment oil appeared more

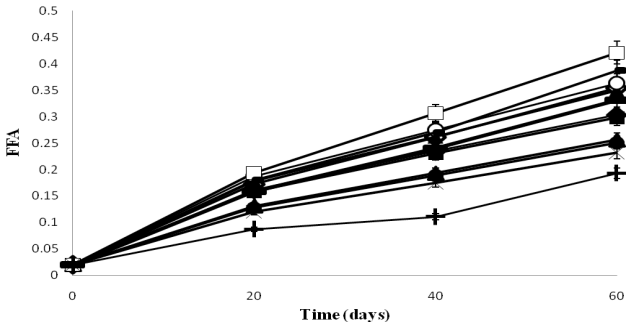

Figure 4. FFA of canola oil samples

Control (♦), EO+UV+Ultrasound (◊), BHT+UV (▲), BHT+Ultrasound (Δ), BHT (●), Control+UV (○), Control+Ultrasound (■), Control+UV+Ultrasound (□), EO+UV (—), EO+Ultrasound (×), EO (+), BHT+UV+Ultrasound (-)

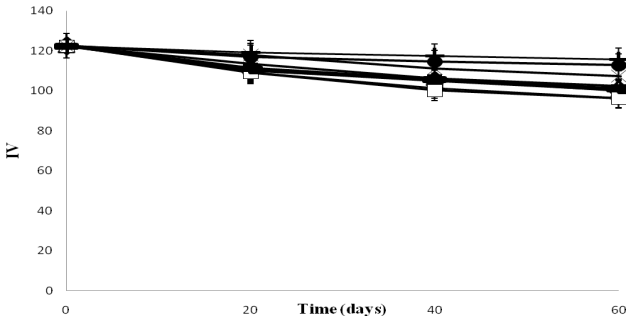

Figure 5. IV of canola oil samples

Control (♦), EO+UV+Ultrasound (◊), BHT+UV (▲), BHT+Ultrasound (Δ), BHT (●), Control+UV (○), Control+Ultrasound (■), Control+UV+Ultrasound (□), EO+UV (—), EO+Ultrasound (×), EO (+), BHT+UV+Ultrasound (-)

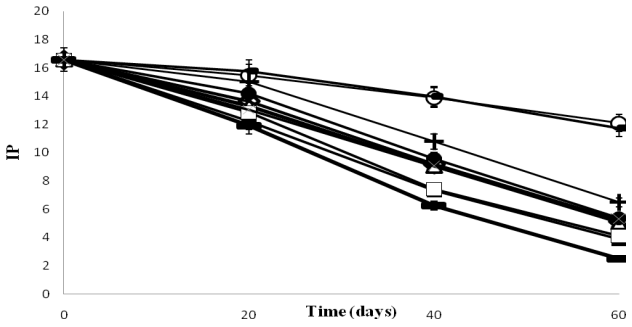

Figure 6. IP of canola oil samples

Control (♦), EO+UV+Ultrasound (◊), BHT+UV (▲), BHT+Ultrasound (Δ), BHT (●), Control+UV (○), Control+Ultrasound (■), Control+UV+Ultrasound (□), EO+UV (—), EO+Ultrasound (×), EO (+), BHT+UV+Ultrasound (-)

rapidly than typical oxidation process. The ultrasound oxidation of sample oil was recognized to cavitation that affects structural and functional components up to the point of lipid deterioration. This phenomenon can cause lipid oxidation by purely thermal, sonolysis and shear forces (Chemat *et al*., 2004). In addition, it should be pointed out that chlorophylls and their decomposition products in vegetable oils are possible photo sensitizers, generating singlet oxygen in the presence of light. Thereby, singlet oxygen quickly reacts with unsaturated fatty acids to generate a blend of nonconjugated and conjugated hydroperooxides that easily decompose to produce adverse flavor compounds (Keszler *et al*., 2000). On the other hand, result of GC-MS shows that main compound of EO is carvacrol and antioxidant activity of it depends on this component. This phenolic monoterpene compound of EO has a hydrogen donating ability which can act as chain breaking antioxidants in free radical chain reactions, converting lipid radicals to more stable products, thus extending the shelf life of canola oil (Shahidi and Zhong, 2010).

Conclusions

In this study we clearly demonstrate that EO is a suitable antioxidant for preserving canola oil against oxidation. The activity of a natural antioxidant such as EO containing components such as carvacrol is observed when applied to canola oil both during autoxidation and oxidation after UV and ultrasound treatment. This activity is more profound during autoxidation and it diminishes following treatment of samples with UV and ultrasound treatment. The EO offered slightly better activity than those provided by an artificial antioxidant such as BHT particularly under forced conditions.

Oil samples added with EO did not show any foreign flavor, foreign odor or toxicity. Therefore, BHA can be substituted with the EO only if the EO is used at higher concentrations.

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