Effects of ultrasound treatment and zenyan essential oil on lipid oxidation of blended vegetable oil

[1]Hashemi, S. M. B., [2,3]*Mousavi Khaneghah, A. and [4]Akbarirad, H.

[1]*Food Science and Technology Department, College of Agriculture, Fasa University, Fasa, Iran*
[2]*Department of Food Science and Technology, Islamic Azad University, Science and Research Branch, Tehran, Iran*
[3]*Current address:Department of Food Science, Faculty of Food Engineering, University of Campinas (UNICAMP), Campinas, São Paulo, Brazil*
[4]*Department of Food Science and Technology, Mazandaran Science and Research Branch, Islamic Azad University, Mazandaran, Iran*

Abstract

Effects of ultrasound treatment (30 kHz, 100 W, 30 s) and zenyan essential oil (0.05% and 0.07%) extracted by ohmic ultrasonic extractor on oxidation of commercial blended oil (contains sunflower, soybean and cotton seed oils) were studied. GC/MS analyses of zenyan essential oil (EO) revealed that thymol (50.07±1.56%) was the major component of EO. Peroxide value (PV), Anisidine value (AnV), free acidity (FFA), iodine value (IV), and induction period (IP) was measured in blended oil and showed that EO in all concentrations had an antioxidant effect in comparison to Butylated hydroxyltoluene BHT) in sample oils treated or no treated by ultrasound. In addition, the fatty acid composition was obtained by the GC equipped with flame ionization detector (FID). Slightly changes were observed in fatty acid composition of all samples during storage. Results showed that ultrasound treatment increase oil oxidation but EO can act as antioxidant and increase oil stability.

Introduction

The problem of lipid oxidation is the greatest economic significance in the production of food lipids. Oxidation of unsaturated lipids not only produces unpleasant flavors and toxic compounds but also can decrease the nutritional value by formation of secondary reaction products in lipids following processing. Many factors could affect lipid oxidation, such as: temperature, ultraviolet light and exist of free radicals (Hashemi *et al*., 2011b; Hashemi *et al*., 2012).

Ultrasound is a type of energy which produces by sound waves of frequencies that are too high to be discerned by human ear. The use of ultrasound in food processing has increased lately. Using of ultrasound in processing creates new and remarkable methodologies which are often matching to classical techniques (Dolatowski *et al*., 2007). It has been used for a diversity of aims e.g. extraction of essential oil from herbs (Hashemi *et al*., 2011a). Sterilization, pasteurization and cleaning (Chemat *et al*., 2004; Dolatowski *et al*., 2007).

The seeds of *Carum copticum* Benth & Hook known as zenyan in Persian, and is mostly grown and used in Iran, Egypt, India and many other countries as a spice, flavoring agent and a condiment. Raw zenyan smells almost like thyme, because it contains thymol. Extracts and essential oil of this spice plant have been shown antiseptic, antispasmodic, anaesthetic, antioxidant and antimicrobial properties (Hashemi *et al*., 2014). In this paper, effects of ultrasound treatment and EO on oxidation and modification of commercial blended vegetable oil during storage were investigated.

Materials and Methods

Plant materials and chemicals

Zenyan (26% initial moisture content) was collected from the suburb of Kazerun city, Fars province, Iran. The species was identified and authenticated by A.R. Khosravi, a plant taxonomist, at Shiraz University, Herbarium, Shiraz, Iran. Voucher specimen (no. 24985) has been deposited in the herbarium. The seeds were then dried under ambient conditions (30–40˚C) for three days on a large screened tray. Plant seeds were then kept in a dark and cold room until used shortly after that for the experiments.

Commercial blended oil contains sunflower, soybean and cotton seed oils with no additives were

*Corresponding author.
Email: *mousavi.amin@srbiau.ac.ir*
Tel: +55(19) 3521-0091; Fax: +55(19) 3521-2153

obtained from Savola Behshahr Co., Tehran. Iran. Chemicals such as methanol, acetic acid, chloroform, sodium iodide, sodium thiosulfate, iso-octane, potassium hydroxide, and p-anisidine were obtained from Merck (Darmstadt, Germany). BHT and DPPH were purchased from Sigma Chemical Company (Sigma-Aldrich GmbH, Sternheim, Germany).

Extraction of EO

Extraction of essential oil from zenyan 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 diameter 10 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, 15 g (10% moisture) of the plant material was charged into 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 23.6°C right up to boiling. The temperature rise was recorded at about 18.8°C/min. The extraction of EO was continued for 17 min. The EO was collected, dried under anhydrous sodium sulphate and stored in sealed vials at 4°C until used.

EO analysis

The essential oil was analyzed by GC-MS. The analysis was carried out with 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 x 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 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 using 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 and their mass spectrum was compared with the Wiley Library (Wiley 7.0).

Antioxidant activity of EO

The radical scavenging capacity of EO for DPPH was monitored according to the method described by Burits and Bucar (Burits and Bucar, 2000). Fifty microliters 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 equation:

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

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.

Sample preparation

Samples (control, blended oil with EO at 0.05%, blended oil with EO at 0.0 7% and blended oil with BHT at 0.02%) are divided into two groups; the first group treated with ultrasound, and the second group prepared with no ultrasound treatment. Then the polyethylene terephthalate (PET) bottles were poured with oils up to 250 mL volume of container, and stored in a laboratory oven under dark condition at 27 ± 1°C during 60 days. The temperature were controlled and the data recorded by data logger

(LASCAR, England).

Ultrasonic treatment

The blended oil was treated without heating using a fixed Hielscher ultrasonic device (UP100H, 100 W, 30 kHz) with a titanium sonotrode (tip diameter10 mm) in a cooled jacket flask (250 mL). Approximately 100 mL of oil was poured in the flask and treated for 30 sec with ultrasonic irradiation.

Determination of fatty acids profile

Determination of fatty acids profile was done with transmethylation technique followed by GC-FID as a practice method (AOAC, 2000). The gas chromatograph system (Agilent Technologies model 6890N, Germany) equipped with flame ionization detector (FID) and HP88 column with the specifications of 100 m×250 mm×0.2 m was used. Temperature of the column has been raised from 170 to 190°C in 5 minutes, 0.5°C/min, and remained in this temperature for 20 minutes, the detector temperature was at 250°C, the carrier gas was helium at 0.7 mL/min, the pressure was 10 PSI and the amount of sample injection was 1 μL (AOAC, 2000; AOCS, 1999).

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 (AOCS, 1999). 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 (AOAC, 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 (AOCS, 1999).

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 (air flow rate of 15 L/h). 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 (AOCS, 1999).

Statistical analysis

The results were analyzed using one-way ANOVA, and significant differences between groups were determined by the Duncan's multiple range test. All statistical analyses were performed using the SPSS (SPSS 16.0 for Windows; SPSS Inc., Chicago, IL, USA) and Minitab ver. 11.12 (Minitab Inc., USA).

Results

EO composition

The yield of the essential oil of zenyan extracted by ohmic ultrasounic extractor was 21 ±1.4% (v/w). GC-MS analysis of the EO indicated in the identifications of 18 compounds of the oil. EO components percentages were quantified by peak area using the FID detector. Percentages of components of the EO are summarized in Table 1. As the results show, the main components of the EO are thymol (50.07±1.56%), γ- Terpinene (23.92±0.89%) and p-Cymene (22.9±1.03%).

Antioxidant activity of EO assessed by DPPH method

The hydrogen atom or electrons donating ability of some of the corresponding pure compounds were determined by using the bleaching of purple-coloured methanol solution of DPPH (Burits and Bucar, 2000). In this study, the free radical scavenging activity of EO was 23 ±1.2 μg/mL whereas IC_{50} value of BHT was 18.2 ± 0.6 μg/mL. In the DPPH assay, EO exhibited remarkable antioxidant activity. The activity of EO could be associated with high contents of thymol. The DPPH test often related poorly with the capability of compounds to prevent lipid oxidation because this experiment does not account factors such as environmental conditions and the physical situation of antioxidant (Burits and Bucar, 2000; Hashemi *et al.*, 2011b; Hashemi *et al.*, 2012; Hashemi *et al.*, 2014).

Effect of EO and ultrasound treatment on the blended oil oxidation

As mentioned in Table 2, there was no major difference between the untreated and ultrasound-treated oils with or without additives during 60 days of storage. Most notable fatty acids in the blended oil included palmitic acid (9.43±0.34%), oleic acid (23.80±0.69%), linoleic acid (58.00±1.2%) and linolenic acid (2.42±0.06%) that some slight
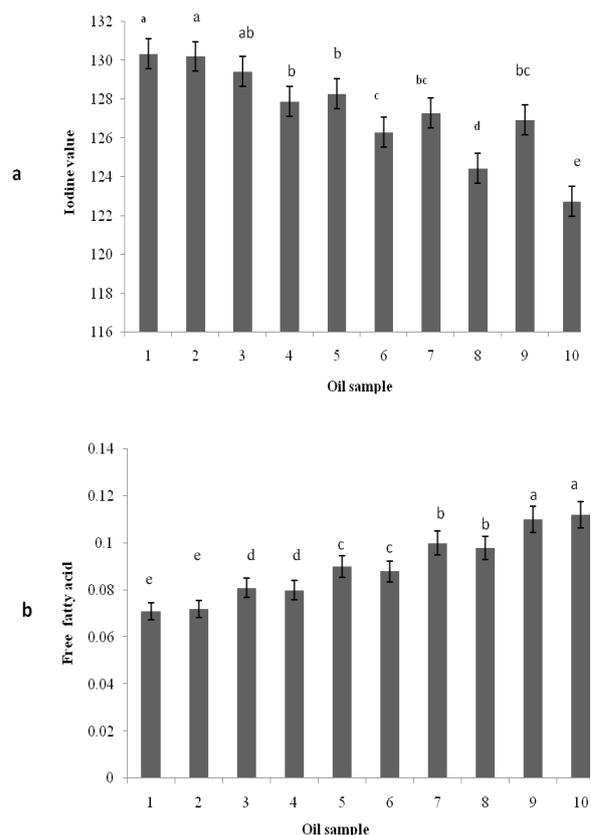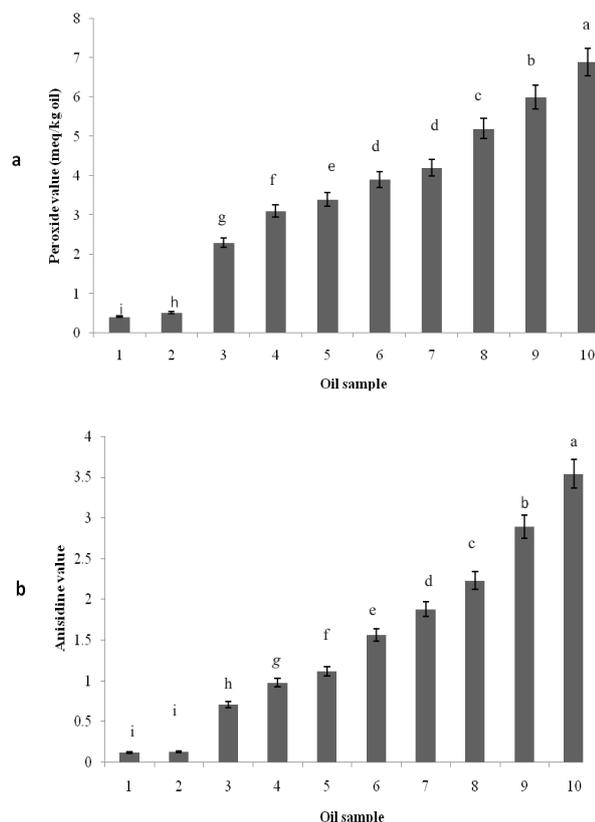
Figure 1a,b. PV (a) and AnV (b) of blended oil samples. Original oil (1), oil treated by ultrasound and no stored (2), oil added with 0.07% EO and no ultrasound treated (3), oil added with 0.07% EO and treated by ultrasound (4), oil added with 0.05% and no ultrasound treated (5), oil added with 0.05% EO and treated by ultrasound (6), oil added with 0.02% BHT and no ultrasound treated (7), oil added with 0.02% BHT and treated by ultrasound (8), oil no treated by ultrasound (9), oil treated by ultrasound (10).

Figure 2a,b. IV (a) and FFA (b) of blended oil samples. Original oil (1), oil treated by ultrasound and no stored (2), oil added with 0.07% EO and no ultrasound treated (3), oil added with 0.07% EO and treated by ultrasound (4), oil added with 0.05% and no ultrasound treated (5), oil added with 0.05% EO and treated by ultrasound (6), oil added with 0.02% BHT and no ultrasound treated (7), oil added with 0.02% BHT and treated by ultrasound (8), oil no treated by ultrasound (9), oil treated by ultrasound (10).

changes were observed after irradiation and storage. Fatty acids composition of oil with no additives and treated by ultrasound indicated fatty acids was changed to palmitic acid (9.77±0.31%), oleic acid (24.55±0.71%), and linoleic acid (57.93±1.39%) after 60 days storage.

Increase in PV indicated the rapid rate of primary oxidation, while increase in AnV showed the fast rate of secondary oxidation (Figure 1). Results of primary and secondary oxidation measurement showed EO at two concentrations decreased oil oxidation and EO at 0.07% was the most stable sample. Stored oil sample which treated by ultrasound waves showed the least stability among all samples in term of formation of primary and secondary oxidation products.

Discussion

Samples with added EO at two concentrations in comparison with samples contain 0.02% BHT, were more stable. The other chemical parameter of blended oil also was affected by ultrasound treatment and additives. The IV is decreased during the storage period as shown in Figure 2a. The amount of unsaturated fatty acid has a direct effect on IVs. As a result; existence of poly unsaturated fatty acids in the blended oil, significantly cause to decline in IVs in both treated and untreated blended oil samples (P≤0.05) after 60 days. However, maximum decrease was observed in stored sample which treated by ultrasound. EO was effective in prevention of IV decrease, especially at 0.07% concentration. Sample with EO at 0.05% was not significantly different with sample contain 0.07% EO and treated by ultrasound. Amounts of FFA in oil samples were shown in Figure 2b. It is well accepted that during storage period, partial hydrolysis of oils has taken place, thus FFA content was increased. There was a significant increase (P≤0.05) in all oil samples, while there was not observed any effect of ultrasound on FFA formation. In addition, the sample contained EO at concentration of 0.07% was the most stable
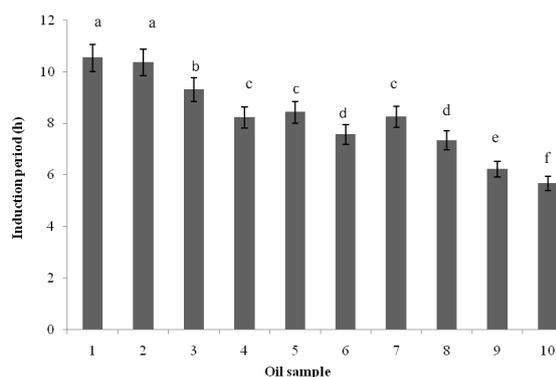
Figure 3. IP of blended oil samples. Original oil (1), oil treated by ultrasound and no stored (2), oil added with 0.07% EO and no ultrasound treated (3), oil added with 0.07% EO and treated by ultrasound (4), oil added with 0.05% and no ultrasound treated (5), oil added with 0.05% EO and treated by ultrasound (6), oil added with 0.02% BHT and no ultrasound treated (7), oil added with 0.02% BHT and treated by ultrasound (8), oil no treated by ultrasound (9), oil treated by ultrasound (10).

sample. According to the results, significant decrease (P≤0.05) was observed at IP in oil samples, as shown in Figure 3. Among the oils, no treated oil samples by ultrasound and with EO at 0.07% have the maximum IP and stability. Treated blended oil with ultrasound, fewer than 30 kHz and 100W for 30 s, had slightly changes in sensory and organoleptic qualities of the oil with no modification in visual characteristics. In the sonicated blended oil stored for 60 days, a cloudy medium appeared within the oil. In common conditions, degradation of commercial edible oils generates only unpleasant flavor after months of storage. This formed medium inside the bulk blended oil is because of polymer formation after degradation of triglycerides. In this study, polymer and oxidation compounds in treat oil appeared more rapidly than typical oxidation process. The oil itself contains metals such as copper and iron, which are catalysts for oxidation. Firstly because contact between the metallic ultrasonic probe and the blended oil which was suspected to be responsible for the oxidation of oil. The ultrasound oxidation of blended oil was then 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 (generated free radicals) and shear forces (Chemat *et al.*, 2004 a,b). Chemat *et al.* (2004a) found that depend on the ultrasound duration and power, some chemical parameters of oils were changed. Effects of EO as antioxidant depend on thymol content. This phenolic compound is a primary antioxidant which either delays or prevents the initiation step by reacting with a lipid-free radical or prevents the propagation step by reacting with the proxy or alkoxy radicals (Hashemi *et al.*, 2011a,b; Hashemi *et al.*, 2012; Hashemi *et al.*, 2014). Therefore, EO can increase lipid stability against oxidation in oil samples treated or not treated by ultrasound.

Conclusions

In this study we clearly demonstrated that EO is a suitable antioxidant to preserving oil against oxidation. The EO offered slightly better activity than those provided by a syntactic antioxidant such as BHT particularly under forced conditions. In addition, ultrasound treatment of oil increases oxidation process. Therefore, the food lipid after ultrasonic treatment will need to be determined to verify (or not) the innocuousness of ultrasound against food products.

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