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Effect of Essential Oil and Extracts From Oregano (*Origanum onites* L.) Leaves on the Oxidative Stability of Refined Sunflower Oil

BEKIR SAMI KUNDURACI†, ALI BAYRAK and MUSTAFA KIRALAN*
Department of Food Engineering, Faculty of Engineering,
Ankara University, Diskapi, 06110 Ankara, Turkey
Fax: (90)(312)3178711; Tel: (90)(312)5961433
E-mail: mustafakiralan@yahoo.com

The antioxidant activity of oregano essential oil, ethanol extract, hexane extract and deodorized ethanol extract, deodorized hexane extract from oregano (*Origanum onites* L.) leaves were evaluated in refined sunflower oil using by Schaal oven and Rancimat method. The main component in oregano essential oil, carvacrol was also used evaluated. In Schaal oven test, peroxide value and specific absorption at 232 nm were used in determination of oxidation at 60 ºC. Rancimat apparatus monitored the oxidation of oils at 110 °C to get information about effects of added extracts and oregano essential oil at elevated temperature. Among extracts, hexane extract at 0.1 % concentration exhibited stronger antioxidant activity than deodorized ethanol extract (0.1 %), deodorized hexane extract (0.1 %) and ethanol extract (0.1 %) showed in Schaal oven test at 60 ºC. The effect of the extracts was less significant in Ransimat test. Only hexane and ethanol extracts showed activity, however the other extracts didn't show any activty in Ransimat test. Oregano essential oil and carvacrol didn't influence oxidation stability of refined sunflower oil in tests used.

Key Words: Oregano, Hexane extract, Ethanol extract, Distillation, Sunflower oil, Antioxidant activity.

INTRODUCTION

Lipid oxidation is a major problem in spoilage of vegetable oils and fats and produce free radicals which are known for health hazards. The effective protection method from oxidation is applying antioxidants. Recently, commonly used synthetic antioxidants may cause health problems so importance of natural antioxidants has been increasing. One of the main sources of natural antioxidants is herbs and spices[1,2].

†Ministry of Agriculture and Rural Affairs General Directorate of Protection and Control, Çankaya, Ankara, Turkey.

Oregano is an important group of Lamiaceae (Labiatae) family especially Turkish and Greek *Origanum* types are the well-known oregano spices[3]. In Turkey it is known Izmir kekigi and has an important place in Turkey's export[1].

Oregano is used almost all the world as a spice for unique flavour. It is used in meat and meat products, pizza, salads, stewings, dressings and soups. Oregano oil and oregano resin are used alcoholic and non-alcoholic beverages and also in cosmetics. Turkish people traditionally used its oregano essential oil as a traditional medicine. It has also antioxidant activity[1,3]. The ground spice or an ethanol-derived extract was added to the cottonseed oil before frying and improved oxidative stability of oil[4]. Acetone extract has an affect in sunflower oil and oil-water emulsion[5] at 60 ºC. Methanol extract was determined as strong free radical scavanger[6]. Essential oil retarded lipid oxidation in raw and cooked chicken[7]. Oils showed strong antioxidant avtivity[8] at low concentration (100 ppm).

Herb and spices have an important roles in oxidation of vegetable oils but most of them change the flavour. Distillation may be absolute necessity. Changes in antioxidant activity of herb and spices after distillation must be determined. The aim of the present study was to examine and compare the antioxidant properties of oregano essential oil and extracts (ethanolic, hexane, deodorized ethanol and deodorized hexane extracts) obtained from pre- and post-distillation of oregano in sunflower oil.

EXPERIMENTAL

Oregano (*Origanum onites* L.) was collected in Mugla city in Turkey in May 2006. The plant material consisted of flowered tops and stalks. They were dried in sunlight and the leaves of plant were separated from the stem and ground in a grinder (Sinbo, Turkey). All reagents and solvents used were of analytical grade and purchased from Fluka (Buchs, Switzerland) and Merck (Darmstadt, Germany). The sunflower oil was obtained from Biryag (Turkey). Its initial peroxide value was 4.05 meq O_2/kg oil and initial diene value was 4.96. Fatty acid methyl ester (FAME) from sunflower oil according to Anonymous[9] method. Fatty acid methyl esters were analyzed using a Shimadzu GC-2010 (Kyoto, Japan) fitted with a DB 23 capillary column (Agilent J&W 60 m long, 0.25 mm internal diameter and 0.25 μm film thickness) equipped with a flame-ionization detector. The injector and detector temperatures were set at 250 and 240 °C, respectively; oven temperature was maintained 210 °C. Helium was used as a carrier gas at a flow rate of 0.71 mL/min. The sample of 0.5 μL was injected by auto-injector and in the split mode (1:100). The peaks were identified by comparison of their retention times with those of the reference standards. The content (percentage by weight) of fatty acids was calculated from their corresponding integration data. Fatty acid composition (in %) is presented in Table-1.

Determination of essential oil contents of plant material and compositions: The leaves of plants collected were distillate for 4 h by a Clevenger-type apparatus

TABLE-1
FATTY ACID COMPOSITION OF SUNFLOWER OIL

Fatty acid name	Concentration (%)
Myristic acid (C14:0)	0.06
Palmitic acid (C16:0)	5.83
Palmitoleic acid (C16:1)	0.14
Margaric acid (C17:0)	0.04
Margaroleic acid (C17:1)	0.03
Stearic acid (C18:0)	3.65
Elaidic acid (*trans*-oleic acid) (C18:1)	0.05
Oleic acid (C18:1)	33.06
Trans form of linoleic acid (C18:2)	0.20
Linoleic acid (C18:2)	55.84
Linolenic acid (C18:3)	0.05
Arachidic acid (C20:0)	0.25
Gadoleic acid (C20:1)	0.14
Behenic acid (C22:0)	0.66

(yield 3.2 % v/w). The obtained oregano essential oil was dried over anhydrous sodium sulphate and after filtration, stored at +4 ºC until tested and analyzed. The oils were analyzed by GC-MS. The analysis was performed using a Hewlett Packard 6890 N GC, equipped with HP-5 MS (Hewlett-Packard) capillary column (30 m long, 0.25 mm internal diameter, 0.25 µm film thickness) and HP5973 mass selective detector in the electron impact mode (70 eV). Helium was used as carrier gas at flow rate of mL/min. Injector and MS trasfer line temperatures were held at 220 and 290 ºC, respectively. The temperature was programmed as below procedure. 1 µL of sample (diluted 1:100 in acetone, v/v) was injected in the splitless mode and quantities represented as relative area % as derived from the intergrator. Individual components were identified by spectrometric analysis using computer library.

Column temperature program: (1) 50 ºC for 3 min, (2) 50-150 ºC at 3 ºC/min (3) 150 ºC for 10 min (4) 150-250 ºC at 10 ºC/min.

Preparation of the extracts: The wastes left after distillation were dried in oven at 40 ºC. Dried and ground leaves (10-15 g) were extracted successively with 350 mL of hexane and ethanol by using a Soxhlet extractor for 7 h at a temperature not exceeding the boiling point of the solvent. The extracts were filtered and then concentrated *in vacuo* at 35 ºC using a rotary evaporator (Buchi R110, Switzerland). The dried extracts dissolved in solvents to get soluble extract in sunflower oil. The solvent and extract were filtred and dried above conditions mentioned. 5 mL of used solvents in extraction were added and the extracts were stored at -18 ºC until further use.

Determination the effects of extracts and essential oil in sunflower oil: Sunflower oil was used as a lipid substrate to evaluate the effects of extracts because of its prone to oxidation. The extracts, oregano essential oil of 1000 ppm and carvacrol

of 750 ppm were added to oil and then mixed with vortex to get uniform distribution of extracts in oil. Blank samples were prepared under the same conditions without any additives. Oxidation was determined by Schaal Oven and Rancimat test at 60 and 110 ºC, respectively. Carvacrol was comprised of 75 % of oregano essential oil so added as 750 ppm. The aim of carvacrol added is to determine posssible effects of oxidative stability of sunflower oil as well as get information connecting between oregano essential oil and carvacrol. Experiment design was planned as two groups. First, extracts and secondly, oregano essential oil and carvacrol's effects observed in sunflower oil during 12 days. The experiments carried out in duplicate.

Schaal Oven test: The accelerated storage test of oils, Schaal oven was used. Oxidation was accelerated in a forced-draft air oven set at 60 °C. Samples were analyzed during storage for peroxide value[10], diene value[11] to follow the oxidative changes. The inhibition percentage of oil was calculated according to formula below[12]:

$$100\text{-}[(\text{PV increase of sample}/\text{PV increase of control}) \times 100]$$

Absorbance measurements were made using a Hitachi U-2800A UV/VIS spectrophotometer (Tokyo, Japan) at 232 nm. The samples were run in duplicate and averaged.

Rancimat test: Rancimat method (Metrohm model 743, Herisan, Switzerland) at 110 ºC with the air flow rate of 20 L/h according to AOCS Official method[13]. The oxidative stability was expressed as induction period (h). The protection factors (PF) were calculated according to the following formula:

$$PF = IP_{extract}/IP_{control}$$

where $IP_{extract}$ is the induction period of a extraction and $IP_{control}$ is the induction period of a control sample[14]. All measurements were run in duplicate and the mean values were reported in each case.

Statistical analysis: Statistical analysis was carried out using SPSS 11.5 software[15]. One-way ANOVA followed by Duncan's test ANOVA and Duncan tests were used in order to evaluate differences (at 95 % level) between mean values as well as between experiments with and without addition of oregano. Statistical results evaluated changes peroxide values and diene values during storage days in Schaal oven test and evaluation differences determined according to datas obtained from 12 days. Differences between mean values between applications and control also determined based on Rancimat results.

RESULTS AND DISCUSSION

Chemical composition of the essential oils: The results obtained by GC-MS analysis of the essential oils of *O. onites* L. are presented in Table-2. Twenty compounds were identified, representing 89.58 % of the total oil. Carvacrol was determined the main compound in oil. The findings on the major components of *O. onites* oil were in agreement with the previous report[16,17]. However, the amount of carvacrol was higher than previous datas above mentioned. Local, climatic and seasonal factors generally effect essential oils especially the composition of oil.

TABLE-2
CHEMICAL COMPOSITION OF *O. onites* ESSENTIAL OIL

Retention time	Components	Composition (%)	Retention time	Components	Composition (%)
8.62	α-Pinene	0.35	23.75	Carvone	0.15
9.19	Camphene	0.12	24.99	Thymol	0.29
10.35	β-Pinene	0.06	25.83	Carvacrol	73.9
11.07	Myrcene	1.06	30.35	β-Caryophyllene	2.09
12.13	α-Terpinene	1.18	31.71	α-Humulene	0.13
12.52	*p*-Cymene	3.88	32.84	Germacrene-D	0.13
14.13	γ-Terpinene	5.96	34.03	β-Bisabolene	1.66
15.43	Terpinolene	0.18	34.57	δ-Cadinene	0.16
18.99	Isoborneol	1.04	36.64	Spathulenol	0.42
19.57	Terpinene-4-ol	1.29	36.85	Caryophyllene oxide	0.53

Evaluation of antioxidant activity: The Schaal oven test was carried out at 60 ºC. Unfavoured alterations may occur in used extracts such as decomposition, evaporation, *etc.* are less probable[18] at 60 ºC. Peroxide values of sunflower oils treated with extracts, oregano essential oil and carvacrol and control are shown in Figs. 1 and 2.

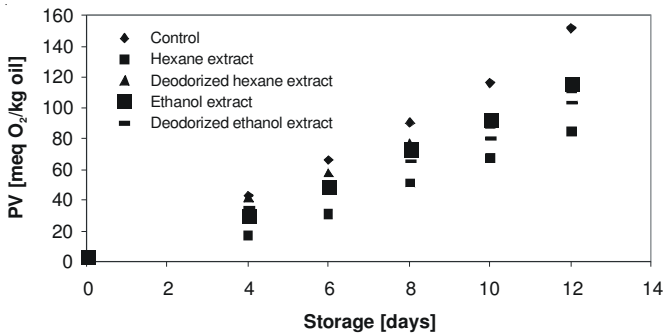

Fig. 1. Changes in peroxide value (meq O_2/kg oil) during storage 12 days in 60 ºC

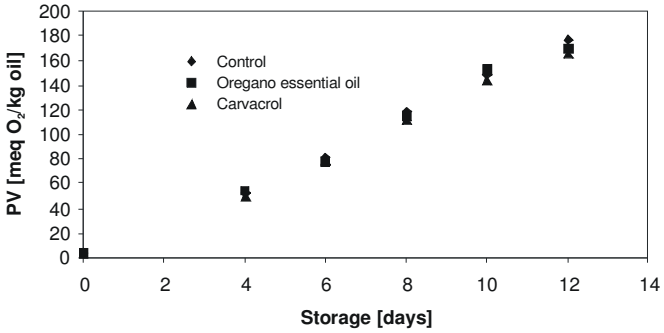

Fig. 2. Changes in peroxide value (meq O_2/kg oil) during storage 12 days in 60 ºC

As seen Fig. 1, end of 12 days of storage, peroxide value increased 151.91 meq O_2/kg oil in control sample. In hexane extract, deodorized hexane extract, ethanol extract and deodorized ethanol extract were 84.35, 112.3, 116.41 and 103.44 meq O_2/kg oil, respectively. As presented in Fig. 2, peroxide value was 176.43 meq O_2/kg oil end of 12 days in control sample. Peroxide values of oregano essential oil and carvacrol was 170.23 and 165.50 meq O_2/kg oil, respectively.

The percentage inhibition was calculated as % after 12 days of storage compared with the control sample. Inhibition percentages of hexane extract, deodorized hexane extract, ethanol extract and deodorized ethanol extract were calculated as 44.47, 26.07, 23.36 and 31.90 %, respectively, after 12 days storage. These results indicated that hexane extract more inhibited oxidation in sunflower oil as compared to all other treatments. Deodorized ethanol extract showed stronger activity than ethanol extract and deodorized hexane extract on sunflower oil's oxidation ($p < 0.05$). Inhibition percentage of oregano essential oil and carvacrol was only calculated as 3.51 and 6.19 %, respectively. Oregano essential oil and carvacrol showed no significant activity in oxidation of sunflower oil ($p > 0.05$).

During the oxidation process, free lipid radicals are formed and two conjugated double bonds deriviated from the original pentadienoic double bond systems. UV absorption is a suitable method to monitor of changes in lipid oxidation[19]. The diene value reached 23.95 from an initial value of 4.96 after 12 days of storage. The data of hexane extract, deodorized hexane extract, ethanol extract and deodorized ethanol extract were 14.65, 18.27, 18.57 and 16.92, respectively, at the end of 12 days (Fig. 3). The statistical results showed that hexane extract exhibited a significantly ($p < 0.05$) stronger antioxidant activity in comparison to that of deodorized hexane extract, ethanol extract and deodorized ethanol extract on control sample. The diene value increased 28.80, diene values of oregano essential oil and carvacrol were 25.67 and 25.70 respectively, after 12 days. Oregano essential oil and carvacrol observed no significantly effect in sunflower oil ($p > 0.05$, Fig. 4).

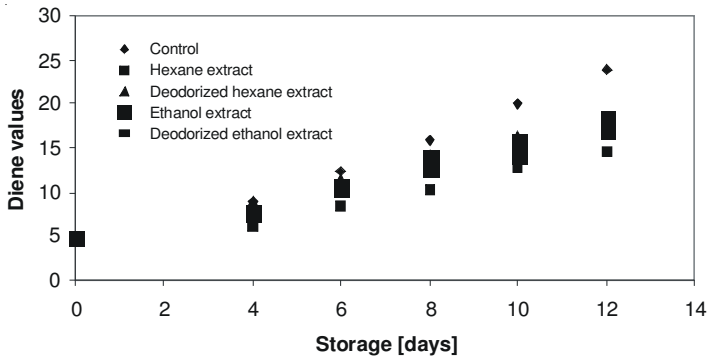

Fig. 3. Effect of several extracts on the formation of conjugated dienes of sunflower oil stored at 60 ºC

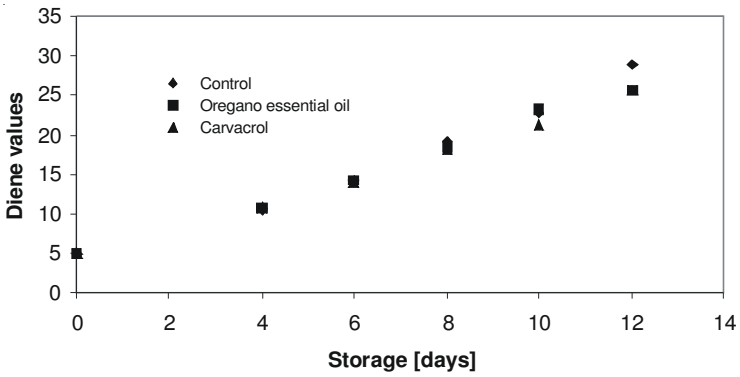

Fig. 4. Effect of oregano essential oil and carvacrol on the formation of conjugated dienes of sunflower oil stored at 60 ºC

Primary products of lipid oxidation are hydroperoxides, which are generally referred to as peroxides. Peroxide value is a measure of hydroperoxides from occuring in lipid oxidation so the results of peroxide value estimation give a clear indication of lipid oxidation. Diene value was also measured to confirm peroxide values. Consequently, the hexane extract of oregano had the best effect and significantly retarded the rate of peroxide values and conjugated dienes formation. However, deodorized hexane extract, ethanol extract and deodorized ethanol extract showed antioxidant activity according to datas obtained from peroxide values and diene values. However, oregano essential oil and carvacrol didn't influence oxidation of sunflower oil.

Previous studies showed that oregano extract had a strong antioxidant effect in lard[20,21]. Abdalla and Roozen[5] confirmed the antioxidant activity of oregano extract in sunflower oil and oil in water emulsion during storage in the dark at 60 ºC. The results of all previous studies on oregano extracts are in agreement with the results of this work. However, essential oil and carvacrol didn't significantly influence oxidation stability of sunflower oil. Kulisic *et al.*[22] showed oregano essential oil, fractions of oil and carvacrol has good antioxidant activity in β-carotene bleaching (BCB) test, the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and the thiobarbituric acid reactive species (TBARS) assay. Another work revealed that carvacrol confirmed to possess the highest antioxidant activity *in vitro* tests[23]. Interpretation of present results about oregano essential oil and carvacrol with other works are difficult. Antioxidant effectiveness of an essential oil or to its main component or components may change because several compounds (either from the sunflower oil or the essential oils) can interact so antagonistic or synergistic effects may occur as well as evaluation methods of antioxidant activity may give different results[24].

It is interesting to note that the deodorized hexane extract was not effective as hexane extract did in the test performed. Opposite to deodorized hexane extract, deodorized ethanol extract observed antioxidant activity not affected process.

Bandoniene *et al.*[25] worked to explain the differences between the effects of extracts with three possibility factors. First, deodorized ethanol extract didn't contain main active compounds such as carvacrol, thymol, γ-terpinene, *p*-cymene found in essential oil[23]. Secondly, volatile and non-volatile compounds can be present in the plants in the form of adjuncts with other molecules and they may show antioxidant and prooxidant properties. They can be released different ways such as hydrolysis or other cleavage processes during hydrodistillation and contribute to oxidation process as antioxidant or pro-oxidant. And finally, heat- and water-induced chemical reactions can also occur and numerous compounds may be formed with different chemical and physical properties and change the activity of a complex extract system. Pizzale *et al.*[21] identified three main compounds (caffeic acid, rosmarinic acid and carvacrol) amounted, on average, to 55 % of the total phenolic compound content of oregano samples. After distillation, most of carvacrol was removed however deodorized ethanol extract had antioxidant activity in sunflower oil. This activity may be originated caffeic acid, rosmarinic acid or the other pheolic compounds.

The effect of extracts, oregano essential oil and carvacrol on oxidation of sunflower oil was also evaluated at elevated temperature (110 °C) using Rancimat apparatus. The IPs and PFs of them are represented graphically in Fig. 5. As seen Fig. 5, hexane extract had greater activity than the others did in sunflower oil. Furthermore, ethanol extract showed significantly effect in sunflower oil as results of statistical analyses ($p < 0.05$). However, deodorized hexane extract, deodorized ethanol extract, oregano essential oil and carvacrol didn't show effect in Ransimat test ($p > 0.05$).

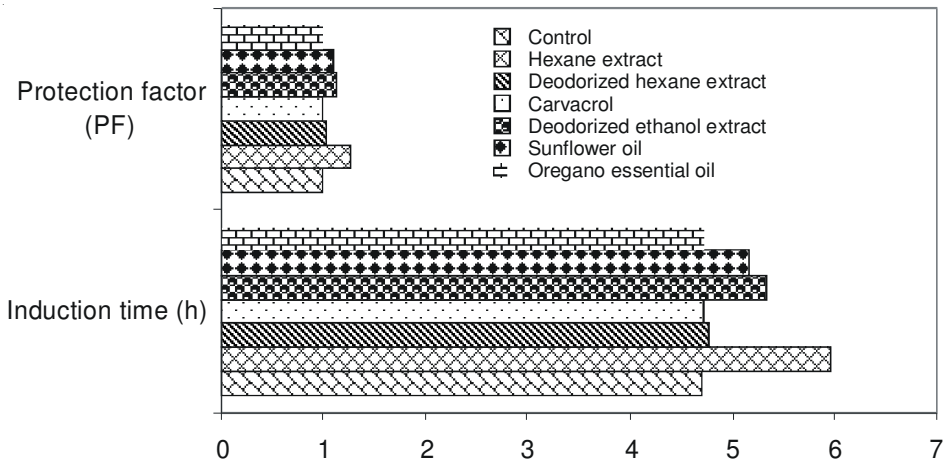

Fig. 5. Effects of extracts, oregano essential oil and carvacrol in sunflower oil during storage at 110 °C (IP-Induction period, determined by the Rancimat method)

In frying temperatures, ethanol and diethyl ether extracts from oregano showed antioxidant activity[4]. Organic solvents of higher polarity are more effective in quantitative recovery of these substances than non-polar solvents due to phenolic compounds have chemical structure[26]. But in above mentioned work which revealed that low polarity extract, diethyl ether extract showed antioxidant activity as high polarity extract, ethanol extract did. In this work, low polarity extract, hexane extract improved oxidative stability of sunflower oil as well as low polarity extract, ethanol extract. However, deodorized hexane extract, deodorized ethanol extract, oregano essential oil and carvacrol observed no significantly activity as results of statistical analyses. Rancimat method may not be suitable for oregano essential oil and carvacrol because of used high temperature so may occur degradation of oregano essential oil[27]. It can be supposed that deodorized hexane extract and deodorized ethanol extract are less effective at higher temperature[25] as well as it may be originated from distillation prosess because deodorized hexane extract and deodorized ethanol extract are products from distillation wastes.

Conclusion

It can be concluded that hexane extract and ethanol extract from *O. onites* L. at concentrations of 0.1 % were found to possess antioxidant, including the Schaal oven and Rancimat tests. Deodorized hexane extract and deodorized ethanol extract showed antioxidant activity only in Schaal oven test at 60 °C. Wastes of distillation may be evaluated and improve oxidation stability of sunflower as well as may not affect flavour of oil because of distillation process.

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