Ajowan as a Source of Natural Lipid Antioxidant†

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The methanolic extracts of ajowan seeds (*Carum coptimum*) were tested as a source of natural antioxidants. A storage test and a heating test of soybean oil treated with methanolic extracts were carried out. Results showed a marked decrease in oxidation of the oil, which was measured by using peroxide values, conjugated diene values, and gas chromatography of oxidized fatty acid methyl esters 18:2, 18:3, and 16:0. The formation of primary and secondary oxidation products of oxidized soybean oil was significantly lower for ajowan extracts than for the control. Thus, a methanolic extract of ajowan exhibits good antioxidative activity and is recommended as a potential source of natural antioxidant.

Keywords: *Ajowan, natural antioxidant*

INTRODUCTION

From 5 to 10 lb of synthetic food additives (used to increase shelf life, prevent spoilage by bacteria, and retain nutrients) are consumed each year by the average U.S. citizen (Specchio, 1992). However, synthetic antioxidants are used at legal limits to prevent food from deterioration during storage, transportation, and processing to reduce deterioration, rancidity, and discoloration from oxidation (Dziezak, 1986). Utilized synthetic antioxidants prevent formation of toxic substances in food, and they are effective and less expensive than natural antioxidants. However, as consumers have become cautious about nutritional quality and safety regarding their food and its chemical additives, food scientists are trying to identify antioxidants that are safe, effective, and mostly of natural origin. A significant number of natural antioxidants have been identified (Shahidi et al., 1992), among them rosemary (Shai et al., 1985) and vitamin E have commercial significance. β-Carotene (Burton and Ingold, 1984), ginger rhizome (Lee et al., 1986), and many spices and cereal extracts have been found as promising sources. The need for widely utilized and easily available natural antioxidants continues to exist.

The present work was conducted with ajowan seeds (*Carum coptimum*) to determine the antioxidative power of different extracts in soybean oil. Ajowan is cultivated around the Mediterranean Sea and in southern Asian countries. It is utilized as a flavoring substance. This commodity was tested in our research as its oil has been utilized for a long time as a principal source of thymol and other phenolic substances.

MATERIALS AND METHODS

Ajowan seeds (cleaned and dried) were obtained from GSCSC Ltd., Gandhinagar, Gujarat, India. Refined, bleached, and deodorized (RBD) soybean oil, without additives or citric acid, was collected from the cooler of the deodorization section of an edible oil refinery at Kraft Food Ingredients, Memphis, TN, and was put immediately under nitrogen in amber bottles and stored at −25 °C. All experimental work was carried out in glass equipment, immersed in EDTA (0.5% w/v) for at least 24 h, rinsed several times with deionized water, and sterilized and dried at 150 °C to avoid metal contamination.

Analytical Procedure. The procedures of Duve and White (1991) and Farag et al. (1989) were followed for extraction and identification and testing of antioxidative activity of the extracts.

Extraction. Cleaned ajowan seeds were finely ground (1-mm screen) in a mill. All solvents utilized were distilled prior to use. One hundred grams of each group sample was extracted in a Soxhlet extractor with 250 mL of solvents for 6 days. After air-drying, the residues were re-extracted with the solvent combination shown in Figure 1. Crude, viscous, concentrated extracts were obtained after rotary evaporation at 45 °C. All extracts were stored under nitrogen at −18 °C until tested.

Rapid Evaluation of Antioxidative Activity among Extracts by Thin-Layer Chromatography (TLC). In this procedure (Daniels and Martin, 1967; Pratt and Miller, 1984; Duve and White, 1991), after activation at 100 °C for 1 h, thin-layer chromatography (TLC) plates (0.25 mm) precoated with silica gel G (Fisher Scientific, Itasca, IL) were streaked with 200 μL of extract and developed by a mixture of solvents of chloroform/ethanol/acetic acid (98:2:2). Plates were sprayed with a solution (9 mg of β-carotene dissolved in 30 mL of chloroform, to which 2 drops of linoleic acid and 60 mL of ethanol were added) and exposed to daylight for 6 h. The intensity of the resulting orange color corresponded to the relative antioxidant activity of the extracts (Marco, 1968; Taga et al., 1984).

Antioxidant Dispersion. Methanolic extracts of ajowan (MEA), which showed the highest antioxidative activity among all extracts in the TLC test, at levels of 0.02%, 0.05%, 0.1%, and 0.2%, and BHT and TBHQ at 0.02% were added to RBD soybean oil. The mixture was stirred for 30 min at 50 °C. A control sample was prepared each time under the same conditions without the addition of any antioxidant/extract.

Storage Test and Heating Test. Six replications of each treatment along with controls were carried out. Samples were stored at controlled temperatures of 32 and 60 °C in the storage test. They were also kept at 180 °C for 10 h every day for 14 days for the heating test. Samples were taken every 5 days for 80 days at 32 °C, every 2 days for 20 days at 60 °C, and every 2 days for 14 days at 180 °C and checked for peroxide values (AOCS, 1987) with storage test and tested for conjugated diene values (AOCS, 1987) in the heating test. After each specified interval, oil samples also were placed in vials, flushed with nitrogen, and frozen at −25 °C for later testing of fatty acid compositions by the GC method (Slover and Lanza, 1979) for both storage and heating tests.

Statistical Analyses. The experimental design was completely randomized, and six replications per treatment were randomly and independently processed. Data analysis and graphic plotting were used with SAS programs (SAS, 1985). Differences were determined by comparing treatment means using the least significant difference multiple comparison method and then the procedure of Student, Newman, and Keuls (Newman, 1939; Keuls, 1952). Analysis of variance and regression analysis were also used to analyze the data (Steel and Torrie, 1980).

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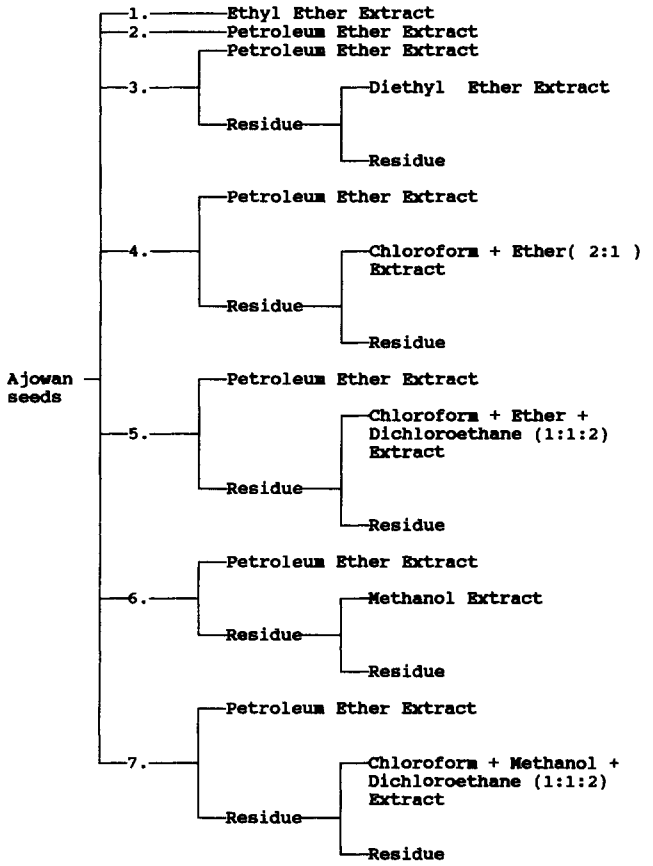

Figure 1. Scheme for extraction of antioxidants from ajowan seeds.

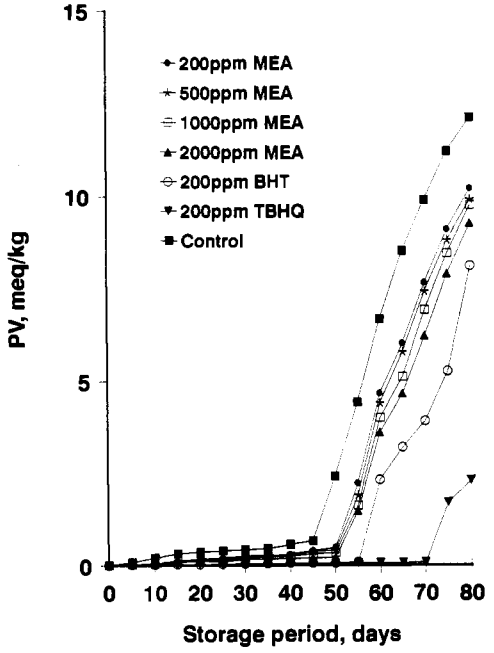

Figure 2. Oxidation of soybean oil treated with methanolic extracts of ajowan (MEA), BHT, and TBHQ during storage at 32 °C as measured by peroxide values (PV).

RESULTS AND DISCUSSION

Storage Test at 32 °C. The average peroxide values (PV) of six replications for each treatment were plotted against days of heating as shown in Figure 2. Figure 2 shows a typical pattern in the rise of PV for all treatments at 32 °C storage. The control (without added antioxidants) had the higher PV of all the treatments during storage at 32 °C, indicating the highest intensity of oxidation. The

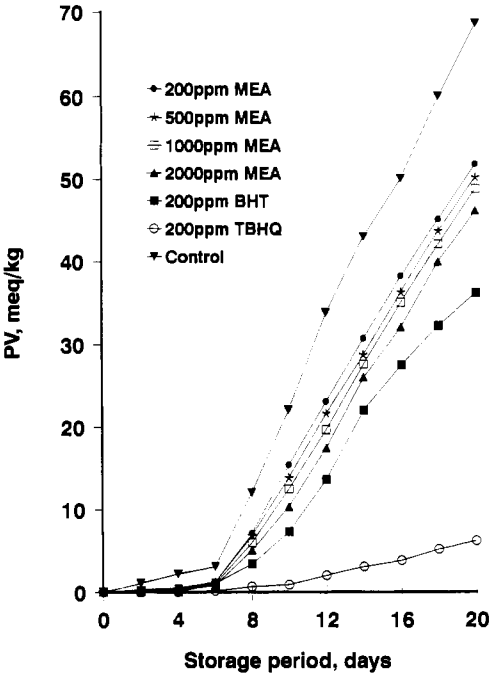

Figure 3. Oxidation of soybean oil treated with methanolic extracts of ajowan (MEA), BHT, and TBHQ during storage at 60 °C as measured by peroxide value (PV).

significantly lower PV ($P < 0.05$) for different concentrations of MEA than for control indicate the antioxidative power of the extracts. TBHQ had the lowest rise in PV. Increasing concentrations of MEA increased the stability of soybean oil by decreasing the rate of oxidation, which is shown by lower PV for each treatment of MEA.

Storage Test at 60 °C. The average PV of six replications of each treatment were plotted against time as shown in Figure 3. The control sample was oxidized at the highest intensity, because it lacked antioxidative substances, indicated by higher PV. Treatments with MEA were more stable than the control during oxidation. As shown in Figure 3, the higher PV was observed for the control and the lowest slope for the treatment with TBHQ. The latter indicated the highest oxidative stability of the system. The PV for treatments with MEA at increasing concentrations were significantly lower than that of the control, which indicates the antioxidative capacity of the extracts.

Heating Tests. For heating at 180 °C, the number of 10-h heating cycles was plotted against averaged conjugated diene (CD) values for each treatment as shown in Figure 4. A typical pattern can be seen in the rise of CD for all treatments. The control had the greatest rise in the CD values. The CD rise for treatments with MEA was significantly lower than that of the control. However, the CD values for MEA treatments were significantly higher than those for the treatments with added BHT and TBHQ, indicating some antioxidative properties of MEA at 180 °C heating.

Fatty Acid Methyl Esters. Fatty acid methyl esters as relative percentage of fatty acids in soybean oil were determined for all treatments before and after 14 days of heating at 180 °C (Table 1). The 18:2/16:0 ratio also was calculated and is shown in the table. According to Augustin et al. (1987), this ratio is a good indicator of the deterioration of heated oil. The 18:2/16:0 ratio was significantly lower for the control, indicating a greater degree of oil deterioration. The least deteriorated oil, according to this parameter, was that with TBHQ. The significantly higher

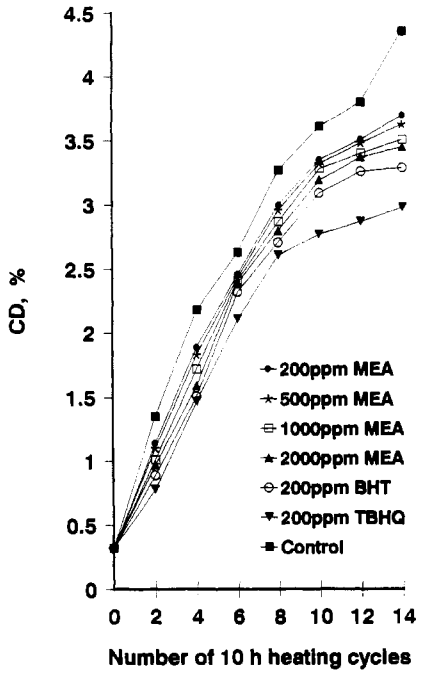

Figure 4. Oxidation of soybean oil with added methanolic extracts of ajowan (MEA), BHT, and TBHQ as measured by conjugated diene (CD) value during heating at 180 °C.

Table 1. Fatty Acid Methyl Esters of Refined, Bleached, and Deodorized Soybean Oil before and after 14 Days of Heating at 180 °C

treatment	16:0	18:0	18:1	18:2	18:3	18:2/16:0[a]
day 0						
fresh oil	10.80	3.10	22.50	55.90	7.80	5.18[a]
day 14						
control	18.50	5.50	33.10	42.90		2.30[b]
MEA[b]						
0.02%	17.20	5.40	32.80	44.75		2.60[c]
0.05%	17.20	5.39	32.75	44.90		2.61[c]
0.1%	17.00	5.38	32.73	45.32		2.66[c]
0.2%	16.80	5.36	32.70	45.64		2.72[d]
BHT, 0.02%	16.40	5.30	32.60	46.20		2.82[e]
TBHQ, 0.02%	16.10	4.70	29.90	49.20		3.06[f]

[a] Means with different letters in the column are significantly different ($P < 0.05$). [b] Methanolic extracts of ajowan.

ratio for treatments with MEA than for the control indicates the antioxidative properties of MEA.

Conclusion. The antioxidative properties of methanolic extracts of ajowan have been demonstrated in a lipid system. The rate of oxidation of soybean oil was decreased by MEA as a natural antioxidant at both storage and heating temperatures. Samples treated with MEA indicated that MEA acted as an effective antioxidant to decrease the rate of oxidation of lipids. The concentration of antioxidative substances in MEA was substantially lower than in BHT and TBHQ, because MEA is a mixture of methanol-soluble materials, which may include phenolic and hydroxyphenolic antioxidant compounds with acids, alcohols, sugars, or glycerides. Extraction and identification of phenolic and hydroxyphenolic antioxidant compounds are recommended. Thus, ajowan has potential as a source of natural antioxidant to decrease the rate of lipid oxidation.

LITERATURE CITED

AOCS. Cd 8–53 (86), Peroxide Value. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 3rd ed.; AOCS: Champaign, IL, 1987a.

AOCS. Ti la–64 (73), Conjugated Diene. *Official Methods and Recommended Practices of the American Oil* Chemists' *Society*, 3rd ed.; AOCS: Champaign, IL, 1987b.

Augustin, M. A.; Telingai, A.; Heng, L. K. J. Relationship between measurements of fat deterioration during heating and frying in RBD olein. *J. Am. Oil Chem. Soc.* **1987**, *64*, 1670–1675.

Burton, G. W.; Ingold, K. U. Beta carotene: an unusual type of lipid antioxidant. *Science* **1984**, *224*, 569–573.

Daniels, D. G. H.; Martin, H. F. Antioxidants in oats; monoesters of caffeic and ferulic acid. *J. Sci. Food Agric.* **1967**, *18*, 589–595.

Duve, K. J.; White, P. J. Extraction and identification of antioxidants in oats. *J. Am. Oil Chem. Soc.* **1991**, *68*, 365–370.

Dziezak, J. D. Antioxidants: The ultimate answer to oxidation. *Food Technol.* **1986**, *40*, 94–100.

Farag, R. S.; Badei, A. Z. M. A.; Hewedi, F. M.; El-Baroty, G. S. A. Antioxidant activity of spice essential oils on linoleic acid oxidation in aqueous media. *J. Am. Oil Chem. Soc.* **1989**, *66*, 792–799.

Keuls, M. The use of the studentized range in connection with the analysis of variance. *Euphytica* **1952**, *1*, 112–122.

Lee, Y. B.; Kim, Y. S.; Ashmore, C. R. Antioxidant property in ginger rhizome and its application to meat products. *J. Food Sci.* **1986**, *51*, 20–23.

Marco, G. J. A rapid method for evaluation of antioxidants. *J. Am. Oil Chem. Soc.* **1968**, *45*, 594–598.

Newman, D. The distribution of the range in samples from a normal population in terms of an independent estimate of standard deviation. *Biometrika* **1939**, *31*, 20–30.

Pratt, D. E.; Miller, E. E. A flavonoid antioxidants in spanish peanuts. *J. Am. Oil Chem. Soc.* **1984**, *61*, 1064–1067.

SAS. *SAS User's Guide: Statistics*; SAS Institute: Cary, NC, 1985.

Shahidi, F.; Janitha, P. K.; Wanasundara, P. D. Phenolic antioxidants. *Crit. Rev. Food Sci. Nutr.* **1992**, *32* (1), 67–103.

Shai, B.; Josephson, D. B.; Maurer, A. J. Antioxidant properties of rosemary oleoresin in turkey sausage. *J. Food Sci.* **1985**, *50*, 1356–1359.

Slover, T.; Lanza, E. Quantitative analysis of food fatty acids by capillary gas chromatography. *J. Am. Oil Chem. Soc.* **1979**, *56*, 933–943.

Specchio, J. J. Antioxidants. In *Encyclopedia of Food Science and Technology*; Hui, Y. H., Ed.; Wiley: New York, 1992; Vol. 1, p 73.

Steel, R. G. D.; Torrie, J. H. *Principles and Procedures of Statistics*, 2nd ed.; McGraw-Hill Book: New York, 1980.

Taga, M. S.; Miller, E. E.; Pratt, D. E. Chia seeds as a source of natural lipid antioxidants. *J. Am. Oil Chem. Soc.* **1984**, *61*, 928–931.

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