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Antioxidative activity of extracts from selected species of the family *Lamiaceae* in sunflower oil

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The kinetics of peroxide accumulation during oxidation of sunflower oil at 100°C in the presence of different concentrations of hexane, ethyl acetate and ethanol extracts of *Melissa officinalis* L., *Mentha piperita* L., *Mentha spicata* L., *Ocimum basilicum* L., *Origanum vulgare* L. and *Saturejae hortensis* L. have been studied. It has been established that the extracts from *Ocimum basilicum* L. and *Origanum vulgare* L. do not improve the oxidation stability of sunflower oil. The ethanol extracts from the other four spices have proved to be the most active in retarding the autoxidation process. The strongest action has been exhibited by the ethanol extracts from *Saturejae hortensis* L., followed by the ethanol extracts from *Mentha piperita* L. and *Melissa officinalis* L. The stabilization factor F for the ethanol extracts (0.1–0.5%) from *Saturejae hortensis* L. is 1.8–2.3. It is higher than F for 0.02% butylated hydroxytoluene BHT ($F = 1.2$). From a practical point of view (yield and stabilization factor), the direct ethanol extract from *Saturejae hortensis* L. should be recommended as the most suitable antioxidant for the stabilization of sunflower oil. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Health protection and economic reasons have necessitated investigations aimed at enhancing the oxidation stability of lipids and lipid-containing products. There is an increasing trend towards adding suitable harmless natural antioxidants to these products (Dugan, 1980; Pokorny, 1991; Evans & Reyhout, 1992) and, in particular, an increasing interest in herbs and spices as sources of natural antioxidants (Gordon & Weng, 1992; Kim *et al.*, 1994; Kikuzaki & Nakatani, 1994; Cuvelier *et al.*, 1994).

Sunflower oil is widely used in nutrition and is highly appreciated as a source of the essential linoleic (9-cis, 12-cis-octadecadienoic) acid. In a previous communication (Yanishlieva & Marinova, 1995) we have reported our results on the stabilizing effect of selected species of the family *Lamiaceae* grown in Bulgaria during the autoxidation of kinetically pure triacylglycerols of sunflower oil (TGSO) (freed from pro- and antioxidants and trace metals by adsorption chromatography (Popov *et al.*, 1968)). The following species were investigated: *Saturejae hortensis* L. (summer savory), *Mentha piperita* L. (peppermint), *Melissa officinalis* L. (common balm), *Mentha spicata* L. (spearmint), *Ocimum basilicum* L. (common basil) and *Origanum vulgare* L. (oregano). It was established that the extracts from *Saturejae hortensis* L. exhibit the most powerful antioxidative action followed by the extracts from *Mentha piperita* L., *Mentha spicata* L. and *Melissa officinalis* L. It was also found that the ethanol extracts are the most effective antioxidants in TGSO.

The aim of the present investigation is to clarify the antioxidative effect of the same spices in natural sunflower oil which contains a series of microcomponents, such as antioxidants (mainly tocopherols), free fatty acids, partial acylglycerols, sterols, fatty alcohols, dyes, metals, primary and secondary autoxidation products. These components may participate in and contribute to the autoxidation process and affect the inhibiting action of the added antioxidants (Popov & Yanishlieva, 1976; Pokorny, 1981; Kortenska *et al.*, 1991). The problem is important for industrial practice with a view to increasing the oxidation stability of the sunflower oil (and related oils) by using harmless natural antioxidants.

MATERIALS AND METHODS

A commercially available sample of sunflower oil was used. Air-dried powdered leaves of commercially purchased species *Saturejae hortensis* L., *Mentha piperita* L., *Melissa officinalis* L., *Mentha spicata* L., *Ocimum basilicum* L. and *Origanum vulgare* L. were used. Every spice (100 g) was extracted three times for 3 days at room temperature with 500 ml hexane (ethyl acetate or

ethanol, respectively). The ethanol and ethyl acetate extracts were prepared after removing the hexane-soluble fraction. Direct extractions of the plant material with ethanol, as well as extractions with ethanol after treatment with hexane and ethyl acetate, were also performed. After filtration, the solvents were evaporated to dryness under reduced pressure.

Lipid samples, containing 0.1 and 0.5% of the extracts, were prepared by adding aliquots of the extract solutions in purified acetone or ethanol, followed by evaporation of the solvents in nitrogen.

Oxidation was carried out at 100°C (±0.2°C) by blowing air through the samples (5 g) in the dark at a rate of 100 ml min^{-1}. The process was followed by withdrawing samples (about 0.1g) at definite time intervals and subjecting them to iodometric determination of the peroxide concentration, i.e. the peroxide value PV (Yanishlieva *et al.*, 1978). The antioxidative effectiveness of the extracts was estimated on the basis of the induction period IP, which was determined by the method of the tangents to the two parts of the kinetic curve (Yanishlieva & Popov, 1971; Le Tutour & Guedon, 1992). All kinetic curves were the average result of three independent experiments.

The effectiveness was expressed as a stabilization factor *F*:

$$F = IP_x/IP_0$$

where IP_x is the induction period in the presence of the extract, and IP_0 is the induction period in the absence of the additive.

RESULTS AND DISCUSSION

It was found that the oxidation stability of the sunflower oil, IP_0, during its oxidation at 100°C was 5.2 h (Fig. 1, curve 0). An addition of 5% powdered air-dried leaves of the investigated *Lamiaceae* species did not increase the IP of the oil (data not shown). As already established (Yanishlieva & Marinova, 1995), the oxidation stability of pure TGSO increases from 3.6– to 14.8–fold in the presence of the same additives.

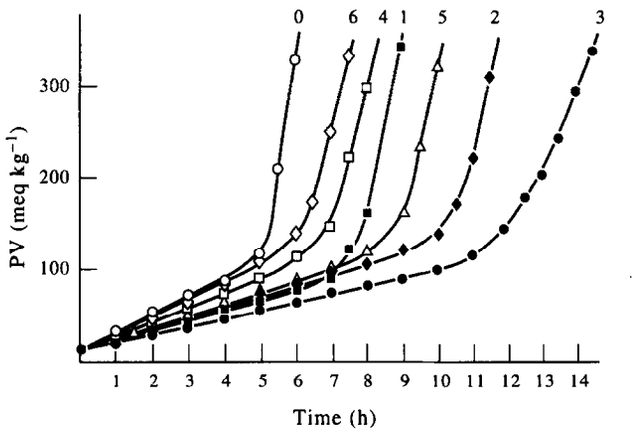

Fig. 1. Kinetic curves of peroxide accumulation during oxidation of sunflower oil (0) at 100°C in the presence of 0.5% extracts from *Saurejae hortensis* L., obtained by extraction with different solvents: 1–hexane, 2–ethanol, 3–ethanol (after extraction with hexane), 4–ethyl acetate (after extraction with hexane), 5–ethanol (after extraction with hexane and ethyl acetate). Comparison with 0.02% BHT (6).

These results should be explained with the optimal concentration of natural antioxidants in sunflower oil, mainly tocopherols, which may hinder the inhibiting action of the additives (the powdered leaves).

The yield of the different extracts from the species is presented in Table 1. Figure 1 shows, by way of example, the kinetic curves of peroxide accumulation during oxidation of the sunflower oil in the presence of 0.5% extracts from *Saturejae hortensis* L., obtained by extraction with different solvents (see Table 1). For comparison, the kinetic curve of autoxidation in the presence of 0.02% of the widely used synthetic antioxidant BHT (curve 6 in Fig. 1) is also given.

After processing all the kinetic curves obtained, the values for the stabilization factor F were determined (Table 2). The stabilization factor F for 0.02% BHT was 1.2. It should be noted that *F* for BHT found at the experimental conditions used is possibly reduced by the volatility of the antioxidant (Gordon & Mursi, 1994).

Comparison of the data for *F* in Table 2 with the stabilization factors for kinetically pure TGSO (Yanishlieva & Marinova, 1995) shows that the natural sunflower oil is much more difficult to stabilize than are

Table 1. Yield of the extracts from different species of the family *Lamiaceae* obtained by their treatment with the following solvents: 1-hexane, 2-ethanol, 3-ethanol (after hexane), 4-ethyl acetate (after hexane), 5-ethanol (after hexane and ethyl acetate) from (Yanishlieva & Marinova, 1995)

Species	Yield (%) Solvent				
	1	2	3	4	5
Saturejae hortensis L.	2.8	9.2	7.6	1.4	5.5
Mentha piperita L.	1.4	8.9	7.3	1.8	—
Melissa officinalis L.	1.3	5.4	2.8	1.8	—
Mentha spicata L.	1.7	8.6	7.8	1.5	—
Ocimum basilicum L.	1.4	4.5	3.4	1.5	—
Origanum vulgare L.	1.6	3.6	4.3	1.4	—

Table 2. Stabilization factors F of the extracts from different species of the family *Lamiaceae* obtained by their treatment with the following solvents: 1-hexane, 2-ethanol, 3-ethanol (after hexane), 4-ethyl acetate (after hexane), 5-ethanol (after hexane and ethyl acetate). Oxidation of sunflower oil at 100°C

Species	F									
	Solvent									
	1		2		3		4		5	
	Concentration									
	0.1%	0.5%	0.1%	0.5%	0.1%	0.5%	0.1%	0.5%	0.1%	0.5%
Saturejae *hortensis* L.	1.5	1.5	1.8	2.0	1.9	2.3	1.3	1.3	1.3	1.7
Mentha piperita L.	1.2	1.2	1.3	1.5	1.6	1.9	1.2	1.2	—	—
Melissa officinalis L.	1.1	1.3	1.4	1.9	1.3	1.8	1.0	1.0	—	—
Mentha spicata L.	1.2	1.2	1.5	1.5	1.5	1.5	1.0	1.0	—	—
Ocimum basilicum L.	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	—	—
Origanum vulgare L.	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	—	—

its pure triacylglycerols. This statement is in accordance with the results of many authors which have established, as mentioned above, that the tocopherol concentration in sunflower oil is close to the optimal one for its stabilization (Sedlacek, 1968; Sherwin & Luckadoo, 1970; List *et al.*, 1972; Peredi, 1973; Kahl & Hildebrandt, 1986; Yanishlieva & Marinova, 1988; Ivanov & Davcheva, 1992). The best antioxidative effect has been achieved using a mixture of 0.05% ascorbyl palmitate and 1% lecithin (stabilization factor 2.5) (Yanishlieva & Marinova, 1988). The latter is close to the stabilization factors of the 0.5% ethanol extracts from *Saturejae hortensis* L., followed by the ethanol extracts from *Mentha Piperita* L. and *Melissa officinalis* L. (see Table 2). These extracts have a better stabilization effect, than does 0.02% BHT. As is seen from Table 2, the extracts from *Ocimum basilicum* L. and *Origanum vulgare* L. do not improve the oxidation stability of sunflower oil.

In general, the stabilizing effect of the ethanol extracts from the investigated species of *Labiatae* family in sunflower oil follows the same sequence as does kinetically pure triacylglycerols of sunflower oil (Yanishlieva & Marinova, 1995). The results obtained demonstrate that, from a practical point of view (yield and stabilization factor), the direct ethanol extract from *Saturejae hortensis* L. should be recommended as the most suitable antioxidant for stabilization of sunflower oil.

These investigations were performed at 100°C, a temperature widely used for accelerated stability determination. It would be practically interesting to elucidate the effect of the antioxidant recommended on the oxidation stability of sunflower oil at room temperature, as well as on the changes occuring in the oil at high temperature (180°C) treatment in air. Investigations in this respect are in progress.

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