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# Effect of plant extracts on the oxidative stability of sunflower oil and emulsion

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**Abstract**

The antioxidative activities of six plant extracts (catnip, hyssop, lemon balm, oregano, sage and thyme) were evaluated in sunflower oil and its 20% oil-in-water emulsion in the dark at 60°C. The oxidation process was followed by measuring the formation of primary (conjugated diene hydroperoxides) and secondary (volatile compounds) oxidation products. Sage extracts (600 and 1200 ppm) effectively inhibited the formation of conjugated dienes and volatile compounds (hexanal and pentanal) in oil and emulsion and showed the highest antioxidative activity compared with 300 ppm BHT. Thyme and lemon balm extracts inhibited hexanal generation more than formation of conjugated dienes in both oil and emulsion. Oregano extract was more active in oil than in emulsion. Catnip and hyssop extracts (600 ppm) showed prooxidative action to sunflower oil at 60°C. These two extracts increased the formation of conjugated dienes compared with the control oil. In emulsions, catnip extract (600 ppm) was active and significantly inhibited the formation of conjugated dienes more than BHT (300 ppm) during additional incubation. 

## 1. Introduction

During storage of oils, fats and other fat containing foods, lipid oxidation is still a major cause of food quality deterioration in spite of a wide use of several antioxidants. The most widely used synthetic antioxidants BHA and BHT are quite volatile and decompose easily at high temperatures (Branen, 1975). There are some serious problems concerning the safety and toxicity of BHA, BHT and TBHQ related to their metabolism and possible absorption and accumulation in body organs and tissues (Linderschmidt et al., 1986; Tappel, 1995). Therefore, the search for preparations of useful natural antioxidants is highly desirable.

The antioxidant properties of many herbs and spices are reported to be effective in retarding the development of rancidity in oils and fatty foods (Bracco et al., 1981). It is known that a number of natural extracts from selected herbs, spices and some vegetables are stable to autoxidation due to the presence of natural phenolic compounds (Ramarathnam et al., 1995). The antioxidant activity of these extracts depends on the type and polarity of extraction solvent, the isolation procedures, purity and identity of antioxidant active components from the raw materials (Chang et al., 1971, Vekiari et al., 1993; Cuvelier et al., 1996).

The use of synergistic mixtures of antioxidants allows a reduction in the concentration of each substrate and also increases the antioxidative effectiveness as compared with the activity of each separate compound. The antioxidant activities of different natural extracts of plant origin such as rosemary, sage, oregano and thyme (Takacsova et al., 1995; Frankel et al., 1996) were studied in various media.

The aim of this work is to evaluate the antioxidant effectiveness of selected plant extracts during oxidation of sunflower oil and emulsion by measuring both primary (hydroperoxides) and secondary (volatile compounds) oxidation products.

## 2. Materials and methods

### 2.1. Materials

Traditional sunflower oil containing 570 ppm natural $\alpha$-tocopherol, and without addition of any synthetic

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antioxidants, was obtained from Cargill, Amsterdam, The Netherlands. Initial quality was checked by determining peroxide value (1.0 meq. $O_2\,kg^{-1}$ oil) and conjugated diene hydroperoxides (absorbance at 234 nm was 0.042 per 50 mg oil dissolved in 5 ml cyclohexane). Fatty acid composition was determined by gas chromatography (HRGC, Carlo Erba Intruments, Milan, Italy) giving 6.6% C16:0, 3.9% C18:0, 21.4% C18:1 and 68.1% C18:2.

Six selected plant materials (catnip, *Nepeta cataria* L.; hyssop, *Hyssopus officinalis* L.; lemon balm, *Melissa officinalis* L.; oregano, *Origanum vulgare* L.; sage, *Salvia officinalis* L., and thyme, *Thymus vulgaris* L.) were collected at the Lithuania Institute of Horticulture, Babtai, and extracted by acetone (Bracco et al., 1981) at the Department of Food Technology, Kaunas University of Technology, Kaunas, Lithuania. Fresh herbs were dried in a ventilated oven at 30 ± 2°C. Dried leaves and flowering parts were ground (max. particle size 0.32 mm) and extracted with acetone in a soxhlet apparatus during 6 h. The solvent was evaporated in a vacuum rotavapour and the residues were finally dried in a vacuum oven at 25 ± 2°C. Then extracts were stored at −18°C until further use.

Butylated hydroxytoluene (BHT) was purchased from Fluka Chemical Co., Amsterdam, The Netherlands, while Tween 80 was from Merck, Darmstadt, Germany.

### 2.1.1. Preparation of oil and emulsion samples

Two concentrations (600 and 1200 ppm) of each plant extract were added to sunflower oil as solutions in absolute ethanol. Afterwards, the samples were heated at 60°C for 1 h in the dark and alcohol was evaporated during stirring. Twenty per cent sunflower oil-in-water emulsions were made with 20 g of sunflower oil containing 600 ppm plant extracts as described before. Tween 80 (1 g) was added and the volume was made up with distilled water in a 100 ml volumetric flask during stirring on a multistirrer (Variomag Multipoint HP, Ika-Labortechnik, München, Germany) for 10 min at 500 rpm. The mixture was transfered to a 250 ml beaker, homogenized by Ultra-Turrax T25 (Janke and Kunkel, Ika-Labortechnik, München, Germany) at 9500 rpm for 2 min and each prepared emulsion was further homogenized using Homogenizer 12705 (ASN.Foss Electronic, Denmark). These oil-in-water emulsions were stored in the dark at 60°C to monitor the stability against oxidation. Oil droplets of these prepared emulsions were examined by microscope with automatic camera attachment (Axiomat Zeiss, Bonn, Germany). Photomicrographs were taken at 100× magnification on Kodak panatonic 400 ASA film.

## 2.2. Methods

### 2.2.1. Oxidation

Oil samples (25 g) and 20% oil-in-water emulsion samples (50 ml) were transferred into screw-capped 380 ml glass bottles covered externally with aluminum foil and subjected to accelerated oxidation in the dark in an oven (Gallenkamp, Germany) at 60°C. Two bottles were prepared from each sample. Oxidative stability was evaluated by measuring conjugated diene hydroperoxides and volatile compounds in oil samples during 25 days of storage and in emulsion samples during eight days of storage.

Table 1
Effect of different plant extracts on the formation of conjugated diene hydroperoxides in sunflower oil during storage in the dark at 60°C (mean ± SD)

| Samples | Storage (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2 | 4 | 7 | 10 | 15 | 20 | 25 |
| Sunflower oil | 0.051 ± 0.004 a | 0.091 ± 0.003 a | 0.209 ± 0.005 a | 0.409 ± 0.002 | 0.644 ± 0.010 a | 0.814 ± 0.006 | 1.064 ± 0.010 a |
| + 300 ppm BHT | 0.043 ± 0.001 b | 0.057 ± 0.001 b | 0.083 ± 0.003 b | 0.115 ± 0.002 a | 0.229 ± 0.002 | 0.371 ± 0.003 | 0.442 ± 0.006 |
| + 600 ppm Catnip | 0.054 ± 0.001 c | 0.109 ± 0.004 c | 0.261 ± 0.003 | 0.422 ± 0.002 | 0.678 ± 0.020 | 0.864 ± 0.009 a | 1.086 ± 0.004 b |
| + 1200 ppm Catnip | 0.053 ± 0.001 c | 0.110 ± 0.003 c | 0.252 ± 0.002 | 0.416 ± 0.003 | 0.666 ± 0.006 | 0.832 ± 0.020 a | 1.080 ± 0.030 ba |
| + 600 ppm Hyssop | 0.053 ± 0.003 ca | 0.095 ± 0.002 a | 0.228 ± 0.002 c | 0.370 ± 0.004 | 0.658 ± 0.006 a | 0.896 ± 0.006 | 1.069 ± 0.003 a |
| + 1200 ppm Hyssop | 0.053 ± 0.001 ca | 0.085 ± 0.002 | 0.226 ± 0.006 c | 0.390 ± 0.003 | 0.638 ± 0.002 a | 0.883 ± 0.003 | 1.045 ± 0.005 |
| + 600 ppm L.balm | 0.054 ± 0.002 ca | 0.094 ± 0.001 a | 0.231 ± 0.002 | 0.340 ± 0.004 | 0.627 ± 0.002 | 0.855 ± 0.008 a | 1.028 ± 0.005 |
| + 1200 ppm L.balm | 0.049 ± 0.003 ca | 0.088 ± 0.002 a | 0.212 ± 0.002 a | 0.327 ± 0.002 | 0.588 ± 0.005 | 0.767 ± 0.003 | 0.984 ± 0.005 |
| + 600 ppm Oregano | 0.057 ± 0.003 | 0.105 ± 0.002 | 0.209 ± 0.004 a | 0.320 ± 0.002 | 0.632 ± 0.009 a | 0.739 ± 0.010 | 0.809 ± 0.030 |
| + 1200 ppm Oregano | 0.048 ± 0.001 a | 0.087 ± 0.001 | 0.203 ± 0.003 | 0.302 ± 0.001 | 0.494 ± 0.002 | 0.553 ± 0.004 | 0.636 ± 0.005 |
| + 600 ppm Sage | 0.044 ± 0.001 b | 0.056 ± 0.001 b | 0.084 ± 0.001 b | 0.116 ± 0.004 a | 0.197 ± 0.006 | 0.234 ± 0.004 | 0.283 ± 0.003 |
| + 1200 ppm Sage | 0.043 ± 0.001 b | 0.054 ± 0.002 | 0.081 ± 0.001 | 0.104 ± 0.001 | 0.162 ± 0.004 | 0.191 ± 0.001 | 0.196 ± 0.002 |
| + 600 ppm Thyme | 0.052 ± 0.001 a | 0.074 ± 0.001 d | 0.158 ± 0.003 | 0.189 ± 0.001 | 0.412 ± 0.003 | 0.673 ± 0.001 | 0.724 ± 0.006 |
| + 1200 ppm Thyme | 0.048 ± 0.001 | 0.073 ± 0.002 d | 0.133 ± 0.002 | 0.182 ± 0.005 | 0.354 ± 0.004 | 0.505 ± 0.005 | 0.615 ± 0.005 |

Sunflower oil containing 570 ppm $\alpha$-tocopherol. BHT, butylated hydroxytoluene.
Conjugated diene values expressed as absorbances at 234 nm of 50 mg sunflower oil.
Values within each column followed by a different letter or without a letter are significantly different ($p < 0.05$).

*2.2.2. Measurement of conjugated diene hydroperoxides*

Fifty milligrammes of oil (two replicates per bottle) of each sample was dissolved in 5 ml cyclohexane and the conjugated diene absorbance was measured at 234 nm using spectrophotometer 2000 Series Spectro. The absorbance was corrected for background absorbance at 260 nm as described by Roozen et al. (1994). Samples of oil-in-water emulsions (2 ml) were extracted with 2 ml dichloromethane, mixed for 1 min (Vortex), then 0.5 g salt was added and mixed again for 1 min. Dichloro methane extract was evaporated to dryness under nitrogen, then conjugated diene absorbance was measured in 50 mg of extracted oil (two replicates per extract) as described before.

*2.2.3. Measurement of volatile compounds*

Volatile compounds of samples were analyzed periodically by static headspace capillary gas chromatography. One millilitre of each sample (two replicates) was transferred into a special headspace bottle sealed with silicon teflon cap. The bottles were inserted into the headspace sampler (HS 800 Fison instrument) connected to a Mega Series gas chromatograph (HRGC 5300 Carlo Erba instrument). Fifty microlitres of headspace was injected on a capillary DB-wax column (30 m× 0.542 mm, 1 $\mu$m thickness, Chrompack). The programme used was 5 min isothermal at 60°C, 3°C $min^{-1}$ to 110°C, 4°C $min^{-1}$ to 170°C and 3 min isothermal. Volatiles were detected by a flame ionization detector (FID). Standards of pentanal and hexanal were analyzed to confirm the identity of volatile compounds by their retention times.

*2.2.4. Statistical analyses*

Student's *t*-test (O'Mahony, 1986) was used to determine significant differences between average values of oxidation products formed in oils and emulsions with different plant extracts ($p < 0.05$).

Relative prooxidative and antioxidative effect (%) was calculated as follows:

$$\{(\text{control} - \text{sample with plant extract})/(\text{control} - \text{sample with BHT})\} \times 100$$

## 3. Results and discussion

### *3.1. Effect of plant extracts on sunflower oil oxidation*

Six different selected plant materials were extracted by acetone. The effects of individual plant extracts on the oxidative stability of traditional sunflower oil, which is very rich in linoleic triglycerides, were evaluated by measuring the formation of both conjugated dienes and volatile compounds.

*3.1.1. Formation of hydroperoxides*

The rate of hydroperoxide formation increased rapidly in all samples after an induction period of approximately 4 days (Table 1). The formation of hydroperoxides increased significantly more in sunflower oils with catnip and hyssop extracts than in control oil during 25 days of incubation at 60°C (Table 1). Sage followed by thyme, oregano and lemon balm extracts inhibited the formation of hydroperoxides in sunflower oil during the incubation period. The antioxidative activity of sage extracts increased significantly more than BHT (300 ppm) during the last 15 days of incubation. The inhibition of the formation of hydroperoxides was improved by increasing the level of each extract to 1200 ppm.

*3.1.2. Formation of volatile compounds*

Two volatile compounds were selected for monitoring the secondary oxidation process: hexanal (high conc.) and pentanal (low conc.). Hexanal is one of the important volatile products, which has proved to be a useful analytical marker for the oxidative decomposition of n-6 PUFAs (Frankel, 1982).

The rate of hexanal formation increased sharply in the control oil during incubation (Table 2). Hexanal formation was detected earlier in oils with catnip, sage and oregano extracts than in oils with lemon balm, hyssop and thyme. In the presence of 600 ppm catnip extract, the rate of hexanal formation increased significantly more than in control oil during oxidation period of 25 days. Thyme followed by lemon balm, sage, and oregano extracts inhibited the formation of hexanal in sunflower oil during oxidation period of 25 days (Fig. 1).

Pentanal was detected first in oils with 600 and 1200 ppm catnip extracts (after 2 and 4 days) and in other samples after 7 and 15 days of oxidation (Table 3). The rate of pentanal formation increased significantly more in oil with 600 ppm catnip extract than in control oil from 7 days till 20 days of oxidation. Oregano, sage and thyme extracts had the most inhibited pentanal formation at the end of oxidation (Fig. 1). The formation of both hexanal and pentanal decreased significantly more in samples with 1200 ppm extracts than in samples with 600 ppm. These results indicate that sage extract has a strong antioxidative effect during initial and final steps of oxidation followed by thyme, oregano and lemon balm. Catnip extract (600 ppm) showed a prooxidative effect during oxidation of traditional sunflower oil.

Previous studies showed that sage extract had a strong antioxidant effect in sunflower oil (Pokorny et al., 1997). The study of Banias et al. (1992) showed that oregano and thyme extracts have strong antioxidant effects in stabilizing lard. The antioxidative effects of sage, thyme and oregano are confirmed by Takacsova et

Table 2
Effect of different plant extracts on the formation of hexanal in sunflower oil during storage in the dark at 60°C (mean ± SD)

| Samples | Storage (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2 | 4 | 7 | 10 | 15 | 20 | 25 |
| Sunflower oil | 0.52 ± 0.1 a | 6.78 ± 0.6 | 8.24 ± 1.1 | 22.83 ± 2.1 | 31.75 ± 2.9 | 32.60 ± 2.6 | 36.48 ± 3.3 |
| + 300 ppm BHT | BD | BD | BD | 1.04 ± 0.2 | 1.79 ± 0.3 a | 1.90 ± 0.2 | 3.11 ± 0.3 |
| + 600 ppm Catnip | 0.73 ± 0.1 | 8.51 ± 0.5 | 15.47 ± 1.8 | 30.24 ± 2.8 | 37.69 ± 4.2 | 36.89 ± 2.8 | 39.88 ± 3.8 |
| + 1200 ppm Catnip | BD | 3.39 ± 0.3 | 3.90 ± 0.4 | 8.45 ± 1.1 | 13.13 ± 1.2 | 13.87 ± 1.1 | 25.89 ± 2.2 a |
| + 600 ppm Hyssop | BD | BD | 0.85 ± 0.1 | 2.42 ± 0.4 | 11.20 ± 1.1 b | 11.98 ± 1.0 a | 24.38 ± 2.3 a |
| + 1200 ppm Hyssop | BD | BD | BD | 1.50 ± 0.4 a | 10.29 ± 1.0 b | 10.47 ± 1.0 | 18.99 ± 1.6 b |
| + 600 ppm L.balm | BD | BD | BD | 1.60 ± 0.5 a | 4.90 ± 0.5 c | 6.48 ± 0.6 | 11.83 ± 1.1 c |
| + 1200 ppm L.balm | BD | BD | BD | BD | 3.71 ± 0.4 | 4.93 ± 0.4 | 9.89 ± 1.2 d |
| + 600 ppm Oregano | BD | 1.02 ± 0.2 | 1.19 ± 0.2 a | 3.30 ± 0.6 b | 10.64 ± 1.0 b | 12.06 ± 1.1 a | 20.32 ± 2.2 b |
| + 1200 ppm Oregano | BD | BD | 1.36 ± 0.2 a | 3.00 ± 0.6 b | 6.51 ± 0.6 | 8.89 ± 0.9 b | 9.03 ± 1.1 d |
| + 600 ppm Sage | 0.51 ± 0.1a | 4.30 ± 0.5 | 6.40 ± 1.0 | 10.50 ± 1.1 | 15.80 ± 1.3 | 16.30 ± 1.4 | 16.40 ± 1.5 |
| + 1200 ppm Sage | BD | 2.12 ± 0.3 | 2.70 ± 0.4 | 4.10 ± 0.6 | 5.10 ± 0.5 c | 5.73 ± 0.4 | 6.60 ± 0.7 e |
| + 600 ppm Thyme | BD | BD | BD | 1.80 ± 0.3 a | 5.80 ± 0.5 | 9.17 ± 1.1 b | 11.78 ± 0.9 c |
| + 1200 ppm Thyme | BD | BD | BD | BD | 1.85 ± 0.2 a | 2.65 ± 0.3 | 6.28 ± 0.5 e |

Hexanal expressed as FID peak area (Vs) in static headspace GC. BD is below detection level.
Values within each column followed by a different letter or without a letter are significantly different ($p < 0.05$).

al. (1995) in rapeseed oil during storage at 60°C. Yanishlieva and Marinova (1995) reported that the extract of *Melissa officinalis* L. (lemon balm) exhibited antioxidative action. The results of all previous studies on sage, thyme, oregano and lemon balm are in agreement with the results of this work. No published papers were found on catnip or hyssop.

In most studies of antioxidant activity, lipid oxidation has been regarded as a single step process. Since lipid oxidation is a complex multistep process (Huang et al., 1994), it is important to study the ability of antioxidants to interfere with the various steps of the oxidation process. Only limited work has been reported on the effects of sage extracts on both hydroperoxide formation and hydroperoxide decomposition.This is the first study on the effects of catnip, hyssop, lemon balm, oregano and thyme extracts on the formation of conjugated diene hydroperoxides and volatile compounds during oxidation of sunflower oil in the dark at 60°C.

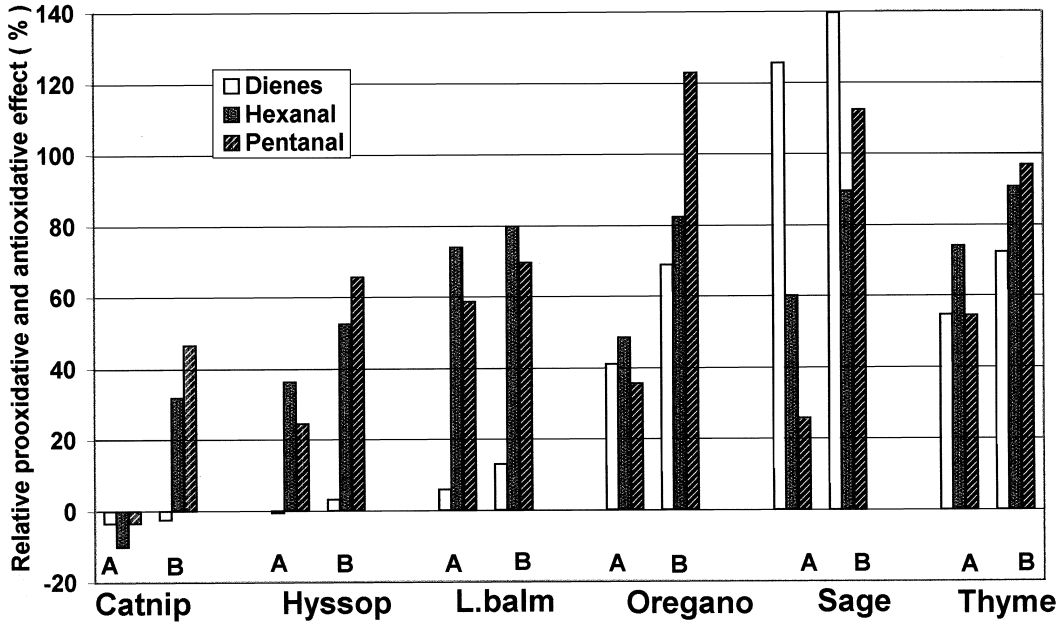


Fig. 1. Relative effects of different plant extracts (A, 600 ppm; B, 1200 ppm) on the primary and secondary oxidation products of sunflower oil after 25 days of storage in the dark at 60°C.

Table 3
Effect of different plant extracts on the formation of pentanal in sunflower oil during storage in the dark at 60°C (mean ± SD)

| Samples | Storage (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2 | 4 | 7 | 10 | 15 | 20 | 25 |
| Sunflower oil | 0.26 ± 0.1 | 1.22 ± 0.2 a | 1.60 ± 0.2 | 3.80 ± 0.4 | 5.73 ± 0.4 | 5.94 ± 0.4 | 6.58 ± 0.6 a |
| + 300 ppm BHT | BD | BD | BD | BD | 1.80 ± 0.2 a | 2.22 ± 0.2 a | 2.04 ± 0.3 b |
| + 600 ppm Catnip | 0.14 ± 0.1 | 1.21 ± 0.3 a | 2.54 ± 0.3 | 5.15 ± 0.5 | 6.50 ± 0.7 | 6.84 ± 0.7 | 6.74 ± 0.7 a |
| + 1200 ppm Catnip | BD | 0.74 ± 0.2 | 0.70 ± 0.1 a | 2.02 ± 0.2 a | 3.05 ± 0.2 | 2.96 ± 0.2 b | 4.47 ± 0.4 c |
| + 600 ppm Hyssop | BD | BD | 0.43 ± 0.1 b | 0.89 ± 0.2 b | 3.66 ± 0.4 | 0.39 ± 0.1 | 5.47 ± 0.5 |
| + 1200 ppm Hyssop | BD | BD | BD | BD | 2.35 ± 0.2 | 2.62 ± 0.3 b | 3.60 ± 0.3 d |
| + 600 ppm L.balm | BD | BD | BD | 0.90 ± 0.2 b | 1.75 ± 0.3 a | 2.82 ± 0.3 b | 3.92 ± 0.4 ed |
| + 1200 ppm L.balm | BD | BD | BD | BD | 1.20 ± 0.2 b | 2.39 ± 0.2 a | 3.42 ± 0.3 ed |
| + 600 ppm Oregano | BD | BD | 0.50 ± 0.1 b | 1.00 ± 0.2 c | 2.12 ± 0.2 | 3.23 ± 0.3 c | 4.97 ± 0.5 |
| + 1200 ppm Oregano | BD | BD | BD | BD | 1.10 ± 0.2 b | 1.00 ± 0.1 d | 1.00 ± 0.2 |
| + 600 ppm Sage | BD | BD | 1.20 ± 0.1 | 1.85 ± 0.2 a | 4.32 ± 0.6 | 4.40 ± 0.5 | 5.42 ± 0.5 |
| + 1200 ppm Sage | BD | BD | 0.57 ± 0.1 | 0.92 ± 0.1 b | 2.68 ± 0.2 | 1.21 ± 0.1 d | 1.48 ± 0.1 |
| + 600 ppm Thyme | BD | BD | 0.92 ± 0.1 | 1.50 ± 0.1 | 2.84 ± 0.3 | 3.24 ± 0.3 c | 4.21 ± 0.4 c |
| + 1200 ppm Thyme | BD | BD | 0.76 ± 0.1 a | 1.20 ± 0.1 c | 1.65 ± 0.2 a | 1.83 ± 0.2 | 2.19 ± 0.3 b |

Pentanal expressed as FID peak area (Vs) in static headspace GC. BD is below detection level.
Values within each column followed by a different letter or without a letter are significantly different ($p < 0.05$).

### 3.2. Effect of plant extracts in sunflower oil-in-water emulsion

Twenty per cent sunflower oil-in-water emulsions were prepared from sunflower oil containing 570 ppm natural $\alpha$-tocopherol. These prepared emulsions were physically stable during eight days of oxidation at 60°C, and the particle sizes averaged between 0.1 and 5 $\mu$m (Fig. 2). This photomicrograph of prepared emulsion is shown to illustrate the influence of droplet size on the release of volatiles (Lee, 1986). The initial pH of emulsions with and without plant extracts ranged between 4.0 and 4.1 and final pH of stored emulsions between 3.8 and 3.9.

#### 3.2.1. Formation of hydroperoxides

Oxidation was more rapid in sunflower oil-in-water emulsions than in sunflower oils. In all emulsion samples, the rate of hydroperoxide formation increased sharply after one day of oxidation (Table 4). During the first two days of incubation, the rate of hydroperoxide formation increased significantly more in the presence of 600 ppm of lemon balm or oregano than in the control emulsion. Catnip and sage extracts inhibited hydroperoxide formation and showed high antioxidative activity even more than BHT, during the last five days of incubation. Thyme extract showed similar antioxidative activity in emulsion as in oil. Hyssop extract showed a low antioxidative effect in both emulsion and oil.

#### 3.2.2. Formation of volatile compounds

Pentanal was formed in control emulsion after two days (0.20 as FID peak area (Vs) in static headspace GC) and in all emulsions with plant extracts after six days (ranged between 0.40 and 2.20 as FID peak area (Vs)). Sage and lemon balm extracts inhibited pentanal formation more than the others (results not shown).

Hexanal formation was detected earlier in all emulsions than in oils. The rate of hexanal formation increased significantly more in emulsion with 600 ppm catnip extract than in control emulsion during oxidation (Table 5). Lemon balm followed by sage and thyme extracts inhibited hexanal formation more than hyssop and oregano (Fig. 3). These results show that sage extract was the most active natural antioxidant in sunflower oil-in-water emulsion as well as in oils during initial and final steps of lipid oxidation. Catnip extract seems to be antioxidative in the primary reaction and neutral or even prooxidative in the secondary reactions.

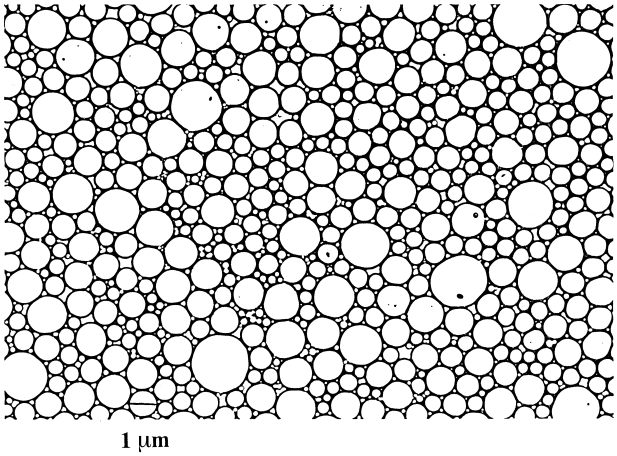


Fig. 2. Photomicrograph of 20% sunflower oil-in-water emulsion prepared at room temperature with 1% Tween 80.

Table 4
Effect of plant extracts on the formation of conjugated diene hydroperoxides in 20% sunflower oil-in-water emulsions during storage in the dark at 60°C (mean ± SD)

| Samples | Storage (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Emulsion (control) | 0.182 ± 0.003 | 0.305 ± 0.003a | 0.426 ± 0.009 | 0.576 ± 0.002 | 0.621 ± 0.005 | 0.706 ± 0.008 | 0.855 ± 0.006 | 1.168 ± 0.006 |
| + 300 ppm BHT | 0.080 ± 0.002 | 0.149 ± 0.003 | 0.301 ± 0.002 | 0.383 ± 0.004 | 0.425 ± 0.006 | 0.494 ± 0.002 | 0.614 ± 0.005 | 0.635 ± 0.004 |
| + 600 ppm Catnip | 0.154 ± 0.001 | 0.272 ± 0.005 | 0.332 ± 0.005 | 0.359 ± 0.004 | 0.399 ± 0.002 | 0.448 ± 0.004 | 0.608 ± 0.004 | 0.625 ± 0.004 |
| + 600 ppm Hyssop | 0.207 ± 0.002 | 0.301 ± 0.002a | 0.377 ± 0.008 | 0.435 ± 0.003 | 0.488 ± 0.006 | 0.571 ± 0.005 | 0.848 ± 0.009 | 1.014 ± 0.005 |
| + 600 ppm L. balm | 0.279 ± 0.005a | 0.358 ± 0.008 | 0.402 ± 0.002a | 0.488 ± 0.004 | 0.554 ± 0.004 | 0.640 ± 0.004 | 0.864 ± 0.004 | 1.026 ± 0.004 |
| + 600 ppm Oregano | 0.270 ± 0.002a | 0.353 ± 0.006 | 0.403 ± 0.006a | 0.477 ± 0.007 | 0.519 ± 0.005 | 0.586 ± 0.008 | 0.827 ± 0.006 | 0.983 ± 0.005 |
| + 600 ppm Sage | 0.092 ± 0.003 | 0.180 ± 0.005 | 0.308 ± 0.003 | 0.370 ± 0.006 | 0.410 ± 0.009 | 0.479 ± 0.007 | 0.579 ± 0.005 | 0.590 ± 0.003 |
| + 600 ppm Thyme | 0.164 ± 0.003 | 0.285 ± 0.003 | 0.360 ± 0.005 | 0.467 ± 0.008 | 0.483 ± 0.004 | 0.531 ± 0.007 | 0.598 ± 0.022 | 0.767 ± 0.005 |

Conjugated diene values expressed as absorbances at 234 nm of 50 mg extracted oil from emulsion.
20% sunflower oil-in-water emulsions were prepared from sunflower containing 570 ppm natural α-tocopherol.
Values within each column followed by a different letter or without a letter are significantly different ($p < 0.05$).

Table 5
Effect of different plant extracts on the formation of hexanal in 20% sunflower oil-in-water emulsions during storage in the dark at 60°C (mean ± SD)

| Samples | Storage (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Emulsion (control) | 0.44 ± 0.2 a | 2.57 ± 0.7 | 5.16 ± 0.8 | 10.53 ± 1.2 a | 13.12 ± 1.3 a | 23.40 ± 2.2 | 32.15 ± 3.1 a | 50.43 ± 7.5 a |
| + 300 ppm BHT | 0.40 ± 0.1 | 1.78 ± 0.4 a | 2.30 ± 0.2 | 4.16 ± 0.3 | 5.81 ± 0.6 | 8.52 ± 0.9 | 8.92 ± 0.8 | 9.67 ± 1.1 |
| + 600 ppm Catnip | 0.51 ± 0.1 b | 5.02 ± 0.7 | 10.20 ± 1.1 | 16.62 ± 1.5 | 22.10 ± 1.8 | 36.90 ± 3.1 | 41.20 ± 3.6 | 49.60 ± 7.2 a |
| + 600 ppm Hyssop | 0.52 ± 0.1 b | 3.80 ± 0.3 b | 8.50 ± 1.0 | 13.20 ± 1.1 b | 14.10 ± 1.2 b | 15.83 ± 1.3 | 21.20 ± 2.0 | 30.74 ± 2.6 |
| + 600 ppm L.balm | 0.13 ± 0.1 b | 3.11 ± 0.3 c | 7.22 ± 0.8 a | 13.18 ± 1.2 b | 13.60 ± 1.2 ab | 13.64 ± 1.1 | 13.80 ± 1.3 b | 14.20 ± 1.3 |
| + 600 ppm Oregano | 0.45 ± 0.3 a | 3.62 ± 0.4 b | 7.80 ± 0.8 a | 14.12 ± 1.3 | 16.88 ± 1.4 | 20.02 ± 2.1 | 31.12 ± 2.6 a | 43.03 ± 3.9 |
| + 600 ppm Sage | 0.43 ± 0.1 a | 1.84 ± 0.3 a | 4.30 ± 0.4 | 9.72 ± 1.0 a | 10.22 ± 1.1 | 11.20 ± 1.1 | 13.65 ± 1.2 b | 14.50 ± 1.3 |
| + 600 ppm Thyme | 0.64 ± 0.3 | 2.91 ± 0.3 c | 6.50 ± 0.5 | 10.15 ± 1.0 a | 14.24 ± 1.2 | 16.93 ± 2.2 | 17.14 ± 2.1 | 18.99 ± 2.7 |

Hexanal expressed as FID peak area (Vs) in static headspace GC.
Values within each column followed by a different letter or without a letter are significantly different ($p < 0.05$).

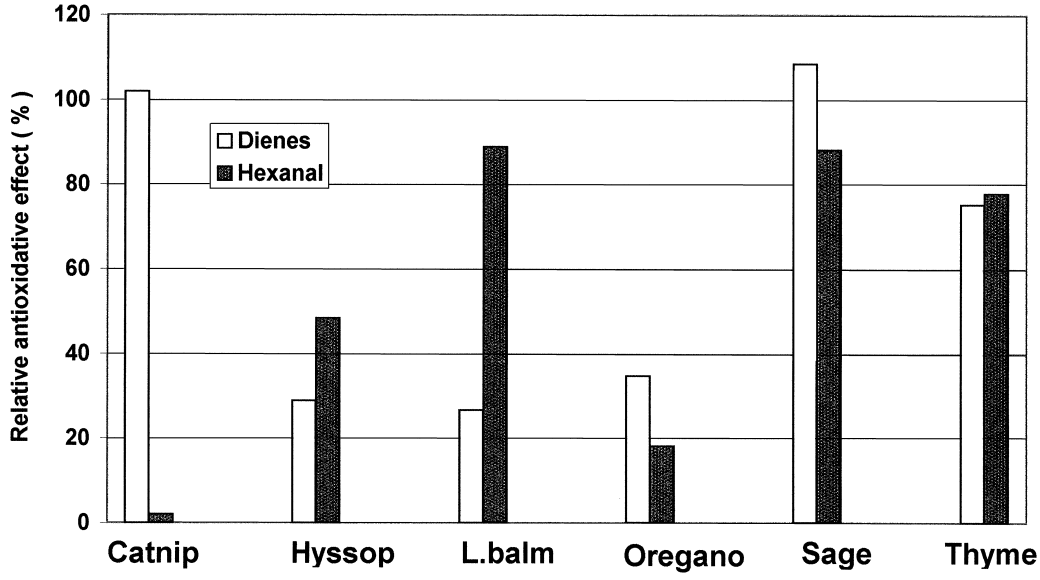


Fig. 3. Relative effects of different plant extracts (600 ppm) on the primary and secondary oxidation products of 20% sunflower oil-in-water emulsion after eight days of storage in the dark at 60°C.

Lemon balm extract demonstrates an opposite effect (Fig. 3).

It is difficult to evaluate natural antioxidants in oils and food emulsions in view of the complex interfacial affinities between air–oil and oil–water interfaces involved. Frankel et al. (1994, 1996) and Huang et al. (1994, 1996) showed that the relative effectiveness of lipophilic and hydrophilic antioxidants was dependent on the lipid substrate, physical state (bulk oil, emulsion), antioxidant concentration, oxidation time and temperature, and the analytical method used to determine the extent and end point of oxidation. Moreover, the hydrophilic antioxidants become diluted in water phase and cannot adeqately protect the oil in the oil–water interface.

## 4. Conclusions

Sage extract shows highest antioxidative activity during primary and secondary oxidation of both traditional sunflower oil containing 570 ppm natural $\alpha$-tocopherol and its oil-in-water emulsion in the dark at 60°C. Thyme and lemon balm extracts inhibited the generation of hexanal and pentanal more than the formation of conjugated dienes in both oil and emulsion. Oregano extract was more active in oil than in its emulsion. Catnip extract showed prooxidative effects in sunflower oil during incubation, however, in emulsion the formation of hydroperoxides was inhibited in the beginning of oxidation.

It will be of interest to purify and identify the active components in various plant extracts and assess their individual contribution to antioxidant activity. Furthermore, an understanding of the mechanisms involved, and the factors influencing the antioxidant activity of these promising natural antioxidants, would be of significant value in development of such natural antioxidants to control lipid oxidation in foods.

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