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Comparison of antioxidative and synergistic effects of rosemary extract with α-tocopherol, ascorbyl palmitate and citric acid in sunflower oil

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Abstract

The antioxidative activities of four natural antioxidants: rosemary extract (ROS.CON), α-tocopherol (TOC), ascorbyl palmitate (AP) and citric acid (CA) were studied in sunflower oil stored at 60° C. Among them, rosemary extract (ROS.CON) exhibited the best antioxidative activity, as determined by peroxide and anisidine value measurements. α-Tocopherol showed a prooxidative effect on stability of sunflower oil at tested conditions. The synergistic effects of α-tocopherol (TOC), ascorbyl palmitate (AP) and citric acid (CA) on rosemary extract (ROS.CON) were investigated. When combined with citric acid and especially ascorbyl palmitate, the rosemary extract showed an additive antioxidative effect, while by mixture with α-tocopherol a negative synergism was observed.

1. Introduction

One of the principal causes of food quality deterioration is the oxidation of unsaturated lipids initiated by free radicals. When lipids are exposed to environmental factors such as air, light and temperature, oxidation reactions start to produce undesirable flavours, rancid odours, discoloration and other forms of spoilage. The primary autoxidation products are hydroperoxides, that have no taste and flavour, but their degradation products (aldehydes, ketones ...) are very potent taste and flavour modifiers (Gordon, 1991).

To retard or prevent the oxidative deterioration, the antioxidants are added in food. The added antioxidants then maintain the quality and extend the shelf-life of many food products.

The antioxidants can be of synthetic or natural origin. The use of synthetic antioxidants is restricted in several countries, because of their possible undesirable effects on human health (Branen, 1975; Chen, Shi & Ho, 1992; Kahl & Kappus, 1993). As a result, there is a great interest in obtaining and utilizing the antioxidants from natural sources because they are presumed to be safe since they occur in plants.

The technologically most important natural antioxidants today are: ascorbic and citric acid and their salts, tocopherols and spice extracts (Weinreich, 1998).

Naturally-occurring antioxidants from herbs and spices have been extensively studied for their antioxidative activity (Che Man & Tan, 1999; Chipault, Mizuno, Hawkins & Lundberg, 1952; Economou, Oreopoulou & Thomopoulos, 1991; Madsen, Sorensen, Skibsted & Bertelsen, 1998).

The greatest level of attention among herbs and spices have been focussed on rosemary. Many studies have been made to examine the antioxidative activities of crude rosemary and different rosemary extracts. Parallel with the evaluation of antioxidative activity of rosemary extracts, research was also focused on isolation and identification of antioxidative compounds. The compounds responsible for antioxidative activity of rosemary are mainly phenolic diterpenes such as carnosic acid, carnosol, rosmanol, epirosmanol, isorosmanol, methyl carnosate (Cuvelier, Berset & Richard, 1994; Shwarz & Ternes, 1992) and other phenolic acids, such as rosmarinic acid (Frankel, Huang, Aeschbach & Prior, 1996). Many

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rosemary extracts, for use in food systems, are today available in the market (Bauman, Hadolin, Rižner Hraš & Knez, 1999).

The α-, β-, γ- and δ-tocopherols occur as mixtures in vegetable oils and are the main natural antioxidants in fats (Gordon, 1991). The most abundant and the most biologically active tocopherol in food is α-tocopherol (White & Xing, 1997).

Ascorbyl palmitate is a synthetically-derived oil-soluble ester of ascorbic acid that occurs widely in the vegetable world.

Citric acid is found in almost all plant and animal species. It can chelate metal ions by forming bonds between the metal and the carboxyl or hydroxyl groups of the citric acid molecule (Anon., 1985). Citric acid is very effective in retarding the oxidative deterioration of lipids in foods and is commonly added to vegetable oils after deodorisation (Gordon, 1990).

The mechanisms by which these antioxidants are involved in the control of autoxidation process are different. Rosemary extracts and tocopherol act as radical scavengers, ascorbyl palmitate acts as oxygen scavenger and citric acid is a chelating agent (Kochhar and Rossell, 1990).

Research has evaluated the effectiveness of different natural antioxidants on the stability of a variety of food systems (Pongracz, Weiser & Matzinger, 1995; Richheimer, Bernart, King, Kent & Bailey, 1996).

Considerable evidence has been gathered on the synergistic effects of various compounds in food products. The synergistic effects of rosemary extracts with other antioxidants, especially tocopherols, have been investigated, and conflicting results have been reported (Banias, Oreopoulou & Thomopoulos, 1992; Fang & Wada, 1993; Hopia, Huang, Shwarz, German & Frankel, 1996; Wada & Fang, 1992). Therefore, additional data are needed to fully evaluate the synergistic effects of rosemary extracts with other natural antioxidants.

This study compares the effectiveness of rosemary extract (ROS.CON), α-tocopherol (TOC), ascorbyl palmitate (AP) and citric acid (CA) in sunflower oil. The abilities of α-tocopherol, ascorbyl palmitate and citric acid to improve the antioxidative activity of rosemary extract were also tested under the same experimental conditions. The prepared samples were held at 60°C for 11 days. Primary oxidation products were measured by a iodometric peroxide value method and secondary oxidation products were measured by *p*-anisidine value.

2. Materials and methods

2.1. Materials

The sunflower α-tocopherol-free oil was purchased from Gea, Slovenska Bistrica, Slovenia. Oil was found to be free of tocopherols by high-performance liquid chromatography (Mayne, Taylor & Parker, 1988). The fatty acid composition determined by gas chromatography (GC) of the sunflower oil was as follows: 6.38% 16:0; 4.02% 18:0; 25.70% 18:1; 62.36% 18:2; 0.22% 18:3; 0.38% 20:0; 0.68% 20:1 and 0.26% 22:0.

Rosemary extract (ROS.CON) was obtained from Pinus, Chemical Works, Slovenia. The contents of two major rosemary antioxidative components, carnosic acid and carnosol, were 36.33 and 4.79%, respectively.

Natural α-tocopherol (T 3634) and citric acid (C 0759) were purchased from Sigma, Germany and were used without purification.

All other chemicals and solvents used were of analytical grade.

2.2. Methods

2.2.1. HPLC analysis of rosemary extract

The method was similar to that used by Richeimer et al. (1996). The HPLC system consisted of a constaMetric 3000 (Milton Roy) pump, spectroMonitor 3100 (Milton Roy) UV–VIS detector, HP 3396 integrator (Hewlett Packard) and Rheodyne injector (Cotati, CA). The column was LiChrosorb RP-18, 7 μm (Merck, Germany) and was run at 2 ml/min. The mobile phase was a mixture of acetonitrile and water (65:35) and contained 0.5% phosphoric acid and 1 mM EDTA. The detection wavelength was 230 nm.

2.2.2. Sample preparations

The antioxidants or their mixtures were added to sunflower oil in the following quantities: 0.02 wt.% of rosemary extract (ROS.CON), 0.01 wt.% of α-tocopherol (TOC), 0.01 wt.% of ascorbyl palmitate (AP), 0.01 wt.% of citric acid (CA), 0.02 wt.% of rosemary extract and 0.01 wt.% of α-tocopherol (ROS.CON + TOC), 0.02 wt.% of rosemary extract and 0.01 wt.% of ascorbyl palmitate (ROS.CON + AP), 0.02 wt.% of rosemary extract and 0.01 wt.% of citric acid (ROS.CON + CA). For the control, the sample without added antioxidant was used (control).

After careful mixing, the samples (50.0±0.1 g) were transferred to an oven maintained at 60±1°C for an accelerated storage study for 11 days. Three replicate samples were stored.

Oxidative stability was determined by measuring peroxide and anisidine values every 24 h.

2.2.3. Peroxide value

Primary oxidation products — hydroperoxides — were determined by peroxide value measurements. 1±0.1 g of oil was weighed and subjected to iodometric determination of peroxide value (AOCS, 1990).

The induction period was considered as the number of days needed for the peroxide value of the sample to

become 20 meq O_2/kg of fat (Economou et al, 1991). This is in agreement with a general consideration that oils become rancid at peroxide values higher than 20.

2.2.4. Anisidine value

Formation of secondary oxidation products was measured by *p*-anisidine value (AOCS, 1990). All reagents were free of carbonyl compounds.

2.2.5. Stabilisation factor

The effectiveness of all tested antioxidants and their mixtures was expresed as the stabilization factor (Yanishlieva & Marinova, 1996):

$$F = \frac{IP_{\text{inh}}}{IP_0}$$

where IP_{inh} is the induction period in the presence of an inhibitor, and IP_0 is the induction period of the non-inhibited system.

2.2.6. Synergism

Percent synergism was calculated as follows (Bishov, Masuoka & Kapsalis, 1977):

$$Syn\% = \frac{(IP_{\text{m}} - IP_{\text{c}}) - (IP_1 - IP_{\text{c}}) - (IP_2 - IP_{\text{c}})}{(IP_{\text{m}} - IP_{\text{c}})} \cdot 100$$

where: IP_{m}, IP_{c}, IP_1 and IP_2 are the induction periods of the sample containing the mixture of additives, the control sample, the sample containing the rosemary extract and the sample containing other natural antioxidant, respectively.

2.2.7. Statistical analysis

All measurements were replicated three times. The results obtained for peroxide and anisidine values were statistically analyzed with the Student's *t*-test using a significance level of $P < 0.05$.

3. Results and discussion

The peroxide values of sunflower oil with added single antioxidants at 60°C are presented in Fig. 1. Rosemary extract (ROS.CON) retarded the hydroperoxide formation significantly ($P < 0.05$). The addition of ROS.CON lowered the final peroxide value after 11 days from 200, as by control sample, to 120 meq/kg. Ascorbyl palmitate (AP) also showed a considerable but not significant ($P < 0.05$) stabilization effect. Citric acid showed a slight antioxidative effect toward the oxidative stability of sunflower oil. α-Tocopherol (TOC) exhibited a prooxidative effect. After 9 days of storage at 60°C, the peroxide value of sample with added α-tocopherol began to decrease. The formation of hydroperoxides is, in that phase, slowed by their decomposition into secondary products.

Secondary oxidation products, determined as anisidine value, showed a similar development (Fig. 2). Formation of secondary products began after lag phases of up to 4 days. The differences are obvious but not statistically significant ($P < 0.05$). The anisidine value of control sample reached 33, and that containing rosemary extract reached 25.

Fig. 3 illustrates peroxide values of sunflower oil with added antioxidative mixtures of rosemary extract with α-tocopherol (ROS.CON + TOC), ascorbyl palmitate (ROS.CON + AP) and citric acid (ROS.CON + CA) at 60°C. The rate of oxidation was reduced by all three tested antioxidative mixtures. The peroxide values of samples with added ROS.CON + CA and ROS.CON + AP are lower than by sample with added ROS.CON. Thus, in sunflower oil at 60°C, combinations of rosemary extract with citric acid, and especially ascorbyl palmitate, have synergistic effects in preventing hydroperoxide

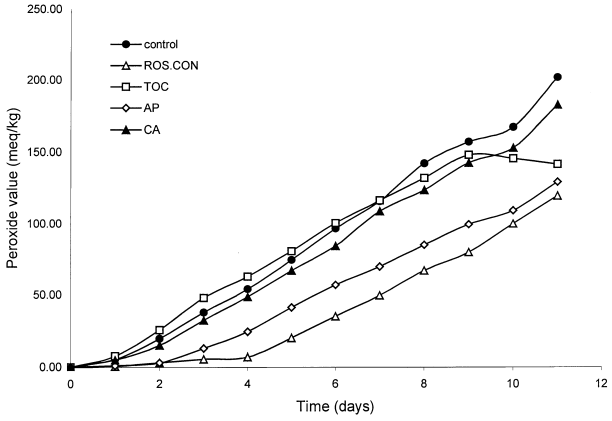

Fig. 1. Peroxide values of sunflower oil without antioxidant and with added rosemary extract (ROS.CON), α-tocopherol (TOC), ascorbyl palmitate (AP) and citric acid (CA) during storage at 60°C.

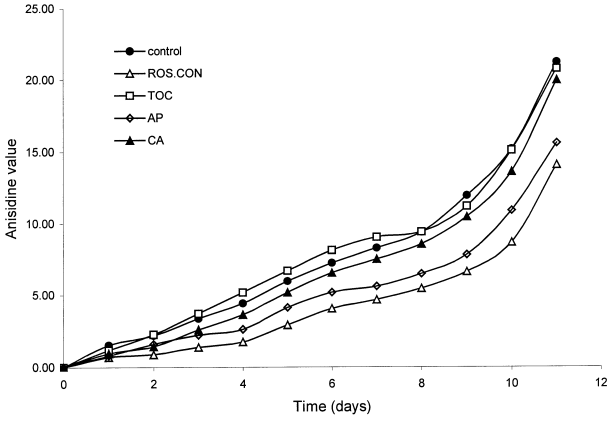

Fig. 2. Anisidine values of sunflower oil without antioxidant and with added rosemary extract (ROS.CON), α-tocopherol (TOC), ascorbyl palmitate (AP) and citric acid (CA) during storage at 60°C.

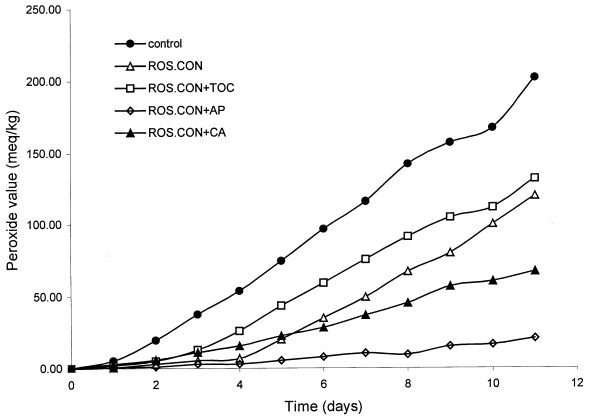

Fig. 3. Peroxide values of sunflower oil without antioxidant and with added antioxidant mixtures of rosemary extract + α-tocopherol (ROS.CON + TOC), rosemary extract + ascorbyl palmitate (ROS.CON + AP) and rosemary extract + citric acid (ROS.CON + CA) during storage at 60°C.

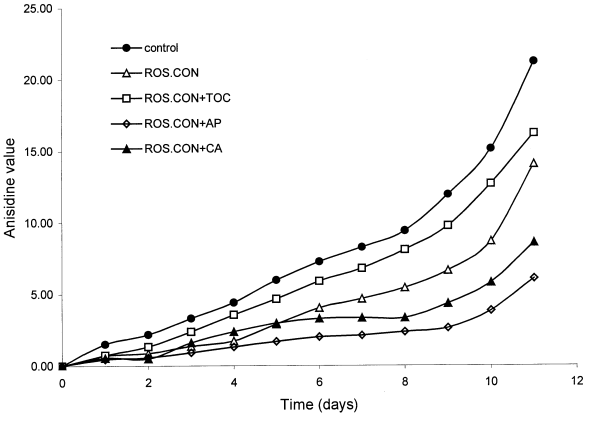

Fig. 4. Anisidine values of sunflower oil without antioxidant and with added antioxidant mixtures of rosemary extract + α-tocopherol (ROS.CON + TOC), rosemary extract + ascorbyl palmitate (ROS.CON + AP) and rosemary extract + citric acid (ROS.CON + CA) during storage at 60°C.

formation, compared to rosemary extract. The calculated synergism is: 2.61% for ROS.CON + CA and 56.0% for ROS.CON + AP. The effect of ROS.CON + AP was significantly ($P < 0.05$) better compared to the effect of ROS.CON. A mixture of rosemary extract with citric acid exhibited a weak synergistic activity. In comparison with the control sample, ROS.CON, ROS.CON + AP and ROS.CON + CA reduced the hydroperoxide formation significantly ($P < 0.05$). On the other hand, the calculated synergism of ROS.CON + TOC is −0.21%. α-Tocopherol reduced the antioxidant effect of rosemary extract, however rosemary extract increased the stability of α-tocopherol. This is in agreement with findings of Hopia et al. (1996) who found that α-tocopherol decreased the oxidative stability of the two main rosemary constituents — carnosol and carnosic acid. Banias et al. (1992) also reported that α-tocopherol showed a strong negative effect with different plant extracts.

Table 1
Stabilisation factor (F) for tested antioxidants and their mixtures in sunflower oil at 60°C

Additive	F
ROS.CON	2.14
AP	1.84
CA	1.16
TOC	0.86
ROS.CON + AP	5.50
ROS.CON + CA	2.34
ROS.CON + TOC	1.80

Results of anisidine value measurements are very similar to results of peroxide value measurements, as seen in Fig. 4. A mixture of rosemary extract and citric acid showed up to day 5 of storage at 60°C, higher formation of secondary products, and after day 5 that formation is slowed by rosemary extract. The sunflower oil, with mixtures of ROS.CON + CA and ROS.CON + AP added, had a significantly ($P < 0.05$) lower level of secondary products compared to the control sample.

In Table 1, the stabilisation factors for all tested antioxidants and their mixtures are presented. The highest stabilisation factor was observed by sample with an added mixture of rosemary extract and ascorbyl palmitate (ROS.CON + AP) and the lowest by sample with added α-tocopherol (TOC).

The highest antioxidative activity, among all tested antioxidants and their mixtures, was exhibited by a mixture of rosemary extract and ascorbyl palmitate (0.02 wt.% ROS.CON + 0.01 wt.% AP). This mixture extended the induction period to reach a peroxide value of 20 meq/kg in a sunflower oil under tested conditions to over 330 h as opposed to rosemary extract alone (128 h) or ascorbyl palmitate alone (110 h), indicating a strong synergism.

On the basis of peroxide and anisidine value measurements and stabilization factor, the order of antioxidative activity was: ROS.CON + AP > ROS.CON + CA > ROS.CON > AP > ROS.CON + TOC > CA > TOC.

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