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Antioxidant properties of the unsaponifiable matter isolated from tomato seeds, oat grains and wheat germ oil

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Abstract

The resistance of edible fats and oils against oxidation depends on their fatty acid pattern and on the composition of the unsaponifiable matter (USM). The effect of the addition of unsaponifiables of tomato seeds, oat grains and wheat germ oils on the stability of refined rapeseed oil was studied and their antioxidant effectiveness was compared to that of butylhydroxyanisole. The experiments carried out proved all the additives to inhibit oxidation of rapeseed oil, the highest protective effect being observed in samples containing unsaponifiables isolated from tomato seeds oil.

Keywords: Antioxidants; Nonglyceride fraction; Oat grains oil; Tomato seeds oil; Unsaponifiables; Wheat germ oil

1. Introduction

Edible fats and oils as well as fatty products are susceptible to external factors such as temperature, radiation and oxygen, which stimulate the course of undesired processes, among others oxidation. The spontaneous chain reaction of atmospheric oxygen with organic compounds (autooxidation) leads to a number of deteriorative changes that reduce the lifetime of many food products causing the deterioration of lipids and other nutrients. In addition to the undesirable sensory characteristics of autooxidized fats and fat containing foods, question have been raised regarding the safety of oxidation products of lipids—hydroperoxides, epoxides, carbonyl compounds, polymers (Burns & Spector, 1994; Carell, 1984; Yagi, 1991).

Although in oily raw materials natural components occur which inhibit oxidation, they are removed to a great part from raw oil during processing.

The refining process which is necessary to adapt most of vegetables oils for consumption, on one hand eliminates undesired compounds such as peroxides, degradation products, pigments, and on the other hand it causes losses of valuable nutrient substances and natural antioxidants (Gutfinger & Letan, 1974; Johansson, 1979; Lanzon, Albi, Cert, & Gracian, 1994). Removal of natural antioxidants from oils as a result of refining increases their susceptibility to enzymatic and autooxidation. Synthetic antioxidants are widely used as food additives for protecting lipids from being rancid, but they are not always considered as safe. Because of the concern over the safety of synthetic antioxidants in recent years, an interest in antioxidants from natural sources developed.

Unsaponifiable matter (USM) consists of minor components accompanying triacylglycerols (sterols, tocopherols, lipopigments and hydrocarbons) and makes up 0.5–2.5%, exceptionally 5–6% of vegetable oils (Bockisch, 1993; Elmadfa, 1995; Niewiadomski, 1984). The addition of USM isolated from wheat germ, corn or olive oil was found to retard oxidation in vegetable oils and model lipids subjected to heating (Sims, Fioriti, & Kanuk, 1972). The antioxidant activity of tocopherols is well documented (Bauernfeind, 1980; Schuler, 1990; Yung & Min, 1990). Other unsaponifiable components including sterols, carotenoids and squalene may protect the unsaturated fatty acids from being rancid as well (Boskow & Morton, 1975; Gordon & Magos, 1983; Małecka, 1994; Sims et al., 1972).

Tomato seeds make up 0.5–0.6% of fruit and contain 18–33% of fat in dry matter (Niewiadomski, 1984;

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Takasova, Drdak, & Minarovicova, 1995). They are considered as a waste in the production of tomato preserves and juices. Because of the great amount of tomatoes processed in Poland, tomato seeds may be important as a waste raw material.

The present study deals with the elucidating the effect of the unsaponifiable components of tomato seed oil, oat grain oil and wheat germ oil on the oxidation of refined rapeseed oil and at comparing their oxidative effectiveness to that of common synthetic antioxidant—butylhydroxyanizole (BHA).

2. Materials and methods

Seeds were separated from the waste of tomato processing (ZPOW Pudliszki, Poland), dried, comminuted and extracted threefold using chloroform–methanol [2:1 (v/v)]. After evaporation of the solvent, oil samples underwent saponification and unsaponifiables were extracted using diethyl ether according to the AOAC, procedure 933.08 (1990). After removing of the solvent using a vacuum rotary evaporator, yields were solubilized in chloroform. The same steps were applied for isolation of the unsaponifiables from wheat germ and oat grains.

The samples of refined rapeseed oil (low erucic acid) produced by ZT "Kruszwica" S.A., Poland, with the addition of 0.3% (w/w) of the unsaponifiable matter extracted from tomato seeds oil, wheat germ oil, oat grains oil and without any additives (controls) were heated in an oven in open glass vials in the dark at 60 °C for 12 days. Duplicate heating was conducted for each sample of rapeseed oil.

Peroxide value (PV) determined according to AOAC, procedure 965.33 (1990) was used as a measure of the extent of oxidation in heated samples. The slopes of the rise of peroxide values in rapeseed oil samples during storage at 60 °C were calculated.

Protective factors k_o/k_p were used as a measure of the antioxidant effectiveness of added fractions, where k_o—slope of PV for control sample, k_p—slope of PV for inhibited sample.

The unsaponifiable fractions isolated from tomato seeds, oat grain and wheat germ oils were analysed using the gas chromatograph HP 5890 (Hewlett Packard) equipped with the MS detector (MSD 5970), EI, 70 eV and fitted with a capillary column DB-1701 (12 m×0.18 mm×0.4 μm; J&W Scientific). The column temperature was programmed from 260 to 300 °C, injection temperature was set at 280 °C. Helium was the carrier gas at flow rate—0.7 cm^3/min. Identification of peaks was based on retention time of standard substances and MS spectra. Analyses were run in duplicate. Calculations of percent composition of demethylsterol fractions were based on peak area.

3. Results and discussion

The unsaponifiable fractions isolated from tomato seeds, oat grain and wheat germ oils were added to the samples of rapeseed oil and their antioxidative properties studied during 12 days storage period at 60 °C in the dark. Fig. 1 illustrates the effect of unsaponifiables and BHA on peroxide formation in rapeseed oil samples during storage. The slopes of the rise in peroxides for rapeseed oil samples were calculated as an indicator of the rate of oxidation (Table 1). The experiments carried out proved all the additives to inhibit oxidation in rapeseed oil, the highest protective effect being observed in samples containing USM isolated from tomato seeds oil.

A gas chromatogram of the unsaponifiable fraction of tomato seeds oil is presented in Fig. 2. Among the constituents of tomato seeds oil unsaponifiables the following compounds were identified: α- and γ-tocopherols, demethylsterols including brasicasterol, campesterol, stigmasterol, β-sitosterol, Δ-5-avenasterol and 4-methylsterol (citrostadienol). Δ-5-Avenasterol makes up more than 10% of total demethylsterol fraction (Table 2). Δ-5-Avenasterol and citrostadienol are considered as antioxidants because of an ethyliden group in 24,28 position of the side chain which is involved in the

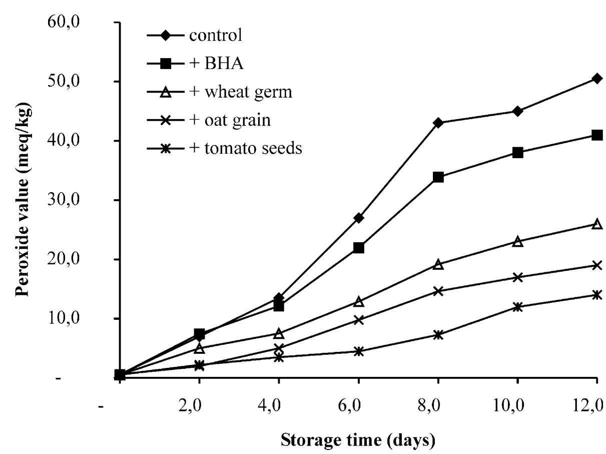

Fig. 1. Peroxide values of rapeseed oil samples with the addition of BHA and unsaponifiables of tomato seeds, oat grain and wheat germ oils during storage at 60 °C.

Table 1
Slopes of peroxide values for rapeseed oil samples during storage at 60 °C and protective factors for each additive

Sample [a]	Slope of PV	Protective factor
Rapeseed oil (control)	4.56	1.00
+ BHA 0.02%	3.65	1.24
+ USM of wheat germ oil 0.3%	2.21	2.06
+ USM of oat grain oil 0.3%	1.69	2.69
+ USM of tomato seeds oil 0.3%	1.14	3.99

[a] Means of four.

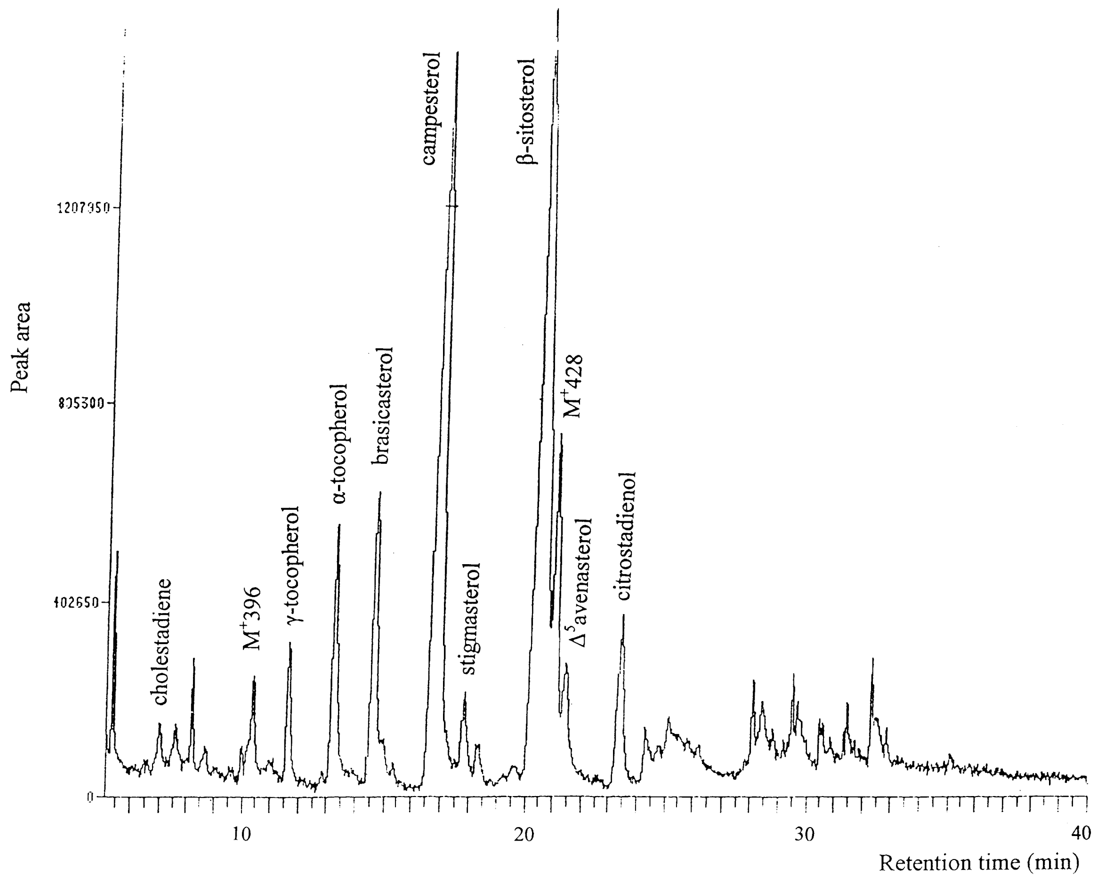

Fig. 2. Gas chromatogram of the unsaponifiable matter isolated from tomato seeds oil.

Table 2
Percent composition of demethylsterol fractions of tomato seeds, oat grains and wheat germ oils

Demethylsterol	Tomato seeds oil[a]	Oat grain oil[a]	Wheat germ oil[b]
brasicasterol	9.8	10.6	–
campesterol	29.5	31.9	22.2
stigmasterol	4.5	1.5	2.0
β-sitosterol	43.2	45.7	67.1
Δ5-avenasterol	10.8	10.2	6.2

[a] Means of two.
[b] According to Barnes (1982).

antioxidant action (Gordon & Magos, 1983). Both compounds as well as tocopherols may contribute to the antioxidant properties of the unsaponifiables of tomato seeds.

The protective effect of oatmeal and extracts from oat bran is well known. Prepared oat extracts with antioxidant activity were patented by Musher (1936). According to Duve and White (1991) Δ-5-avenasterol belongs to the components of oat grain propounding the highest antioxidant effectiveness. It is noteworthy to mention that oat oil is very stable although it contains high amount of polyunsaturated fatty acids.

Antioxidant properties of wheat germ constituents are connected with the presence of tocopherols, which occur in the amount of 1790–3200 mg/kg (Barnes, 1982). According to Sims et al. (1972) Δ-5-avenasterol covers 2–6% of demethylsterols of wheat germ oil and citrostadienol is the main component of its 4-methylsterol fraction. Both compounds possess the ethyliden bound in the side chain, which is believed to be involved in the antioxidant action (Gordon & Magos, 1983). Among hydrocarbons present in wheat germ oil squalene was identified (Barnes, 1982). There is some evidence concerning its antioxidant activity, especially on heating (Małecka, 1994; Owen, Mier, Giacosa, Hull, Spiegelhalder, & Bartsch, 2000; Sims et al., 1972).

There is scant information in the literature concerning antioxidant properties of tomato extracts, however, potential antioxidants including licopene and some phenolic compounds—quercetin, rutin and prunin—were identified (Dugan, 1980; Hirayama, Nakamura, Hamada, & Kobayasi, 1994). Extracts of tomato peels did not exert antioxidant activity in model system β-carotene-linoleic acid (Pratt, 1992). According to the results of El Tanboly and Sayed, (1995) powdered tomato seeds exhibited antioxidant activity in milk fat during heating at 170 °C.

4. Conclusion

The 0.3% addition of the unsaponifiable matter isolated from tomato seeds oil showed a higher antioxidative activity than 0.02% of BHA and USM from oat grain or wheat germ oil. Δ5-Avenasterol and citrostadienol, as well as tocopherols, identified in USM of tomato seeds oil might belong to components exhibiting potential antioxidant activity.

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