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Oxidative stability and stabilization of grape seed oil

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Oxidative stability of vegetable oil recovered from grape seeds was investigated. The Induction period (IP) of the oil determined by Rancimat method at 100ºC was found to be 7 h. Different natural antioxidant mixtures and individual pure compounds were examined for stabilization of the oil. The use of vegetable extracts, in concentration 0.3% has insignificant effect on the stabilization (1.1 - 1.3 times). The addition of some individual pure antioxidants in concentration 0.05% has a better effect. The best results were established using butyl gallate – Induction period was 26.2 h and rosemary – Ip was 18.7 h. The oxidative stability increased gradually with the increasing of concentration of butyl gallate and was highest at 0.2%- Induction period was found to be 42.1 h.

Key words: Grape seed oil, oxidative stability, stabilization, antioxidants

INTRODUCTION

The fruits of grape (Vitis vinifera L.) find application as food for direct consumption, as well as source for the production of wine. Grape seeds are waste product after separation of the wine and have recently been utilized for obtaining of glyceride oil [1-3]. The grape seed oil presents interest as functional food with therapeutical effect because it contains a high quantity of polyunsaturated fatty acids in particular linoleic acid [4]. Moreover glyceride oil is used as salad oil and for preparing cosmetic products. Besides triacylglycerols, the glyceride oil contains micro components valuable biologically active substances as tocopherols, phytosterols, carotenoids which play a significant role for the estimation of food value and increasing oxidative stability [5]. On the other hand, the presence of high content of unsaturated fatty acids makes the oil very unstable towards oxidation, the term of storage decreases and the oil deteriorated of food value.

Lipid oxidation of grape seed oil is an important problem and leads to a decrease of quality, safety and nutritional value. For stabilization and prolongation of the term for conservation of the oil many different antioxidants in food chemistry are used for stabilization and prolongation safety of the oil. Natural antioxidants come from plant leafs; stems or seeds are in high demand for food application because of their safety compared to synthetic antioxidants [6, 7].

In Bulgaria, there are has also significant quantities of grape seeds as waste products which are used for obtaining of glyceride oil. So far, the composition and oxidative stability of the oil have not been investigated. In this connection the aim of this work is to investigate the composition of the oil, its oxidative stability and possibilities for stabilization of the oil by different natural and synthetic antioxidants for prolongation of the term for preservation of its food value.

EXPERIMENTAL

All solvents and reagents were with analytical grade of purity and were used without additional purification. Reference phospholipids and fatty acid methyl esters were purchased from Fluka (Chemie Gmbh, Switzerland). Reference tocopherols isomers and individual sterols were purchased from Merck (Darmstadt, Germany).

Sample: Grape seed oil. The oil was purchased from a local market and was used directly for the investigation.

Determination of bioactive compounds

*Phospholipids.*The quantification was carried out spectrophotometrically against a standard curve by measuring the phosphorous content at 700 nm after and mineralization of the substance with a mixture of perchloric acid and sulphuric acid, 1:1 by volume. Etalon – 10 μl/cm^3 water solution of KH_2PO_4 as phosphorus [8].

Sterols. The oil was hydrolized with ethanolic KOH [9], sterols were extracted with light

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petroleum ether and purified by thin layer chromatography.

Total sterol content was determined spectrophotometrically [10] at 597 nm.

Tocopherols. Tocopherols were determined directly in the oil by high performance liquid chromatography (HPLC) [11] on a "Merck-Hitachi" (Merck, Darmstadt, Germany) instrument equipped with 250 mm x 4 mm Nucleosil Si 50 – 5 column (Merck, Darmstadt, Germany) and fluorescent detector "Merck-Hitachi" F 1000.

*Fatty acids.*The fatty acid composition was determined by gas chromatography after transmethylation of the respective sample with 2% methanolic H_2SO_4 at 50°C according to Christie [9]. GC was performed on a HP 5890 (Hewlett Packard GmbH, Austria) gas chromatograph equipped with a 60 m x 0.25 mm (I.D.) capillary DB – 23 column (Hewlett Packard GmbH, Austria) and a FID [12].

Antioxidants. Antioxidants were purchased from Fluka (Chemie Gmbh, Switzerland): butyl gallate, Merck (Darmstadt, Germany) - α-tocopherol, oxynex, Hofman-La Roshe (Switzerland) – ascorbylpalmitat; Koch-Light Laboratories Ltd (Germany) – quercetin and rosemary. The natural antioxidant mixtures are with undefined composition and were obtained by "Ikarov" Ltd.

Stabilization. To the samples (25 g grape seed oil) were added natural and synthetic antioxidants as pure compounds or as mixture of two compounds. The compositions of antioxidants were prepared as follows: The compounds were precisely weighed and solubilized in pure ethanol as 20 g/100 ml solution. Then the solution was added to the oil in order to obtain desired concentration of the antioxidants. The obtained solution was vigorously mixed and than the ethanol was removed by flush with nitrogen. Then the oil was stored in dark bottles at 20°C.

Oxidative stability. The stability of the oil was examined by measurement the change of Induction period using conductometric detection of volatile products of oil degradation-Rancimat method [5]. The oxidative test was used Rancimat apparatus Methrom 679 (Methrom, Herisau, Switzerland). Three milliliters of each sample were weighted into reaction vessel glassware. The heat temperature was set a 100°C; the rate of air flow through the sample was about 20 l/h; the volume of bidistilled water into the trap was 60 ml. All determinations of the oxidative stability were performed in three replicates.

Antioxidative effect (AOE) was calculated by the formula:

$$AOE = \frac{\text{Introducti on period (IP) with additive}}{\text{IP without additive}}.$$

*Statistics.*All data are presented as a mean value of three separate measurements ± standard deviation (SD, at P = 0.05).

RESULTS AND DISCUSSION

General characteristics of the oil were determined, such as: content of total phospholipids, sterols and tocopherols and fatty acid composition. The results are shown in Table 1.

Table 1. Content of bioactive compounds in grape seed oil.

Compounds	Content
Sterols, %	0.2 ± 0.06
Phospholipids, %	1.5 ± 0.6
Tocopherols, mg/kg	46 ± 0.9
Fatty acids, %	
$C_{14:0}$ Myristic	0.1 ± 0.2
$C_{16:0}$ Palmitic	9.4 ± 3.8
$C_{16:1}$ Palmitoleic	0.1 ± 0.5
$C_{17:0}$ Margaric	0.1 ± 0.5
$C_{18:0}$ Stearic	3.7 ± 1.5
$C_{18:1}$ Oleic	17.9 ± 3.6
$C_{18:2}$ Linoleic	67.9 ± 13.6

In comparison with other vegetable oils as sunflower, olive, rapeseed [13], grape seed oil is characterized by a low content of sterols and tocopherols but the quantity of phospholipids is relatively high. Linoleic acid predominates in the triacylglycerols followed by oleic acid. This composition of grape seed oil is close to data reported earlier by other researchers [3, 6, 14-17].

Natural antioxidants and synthetic analogs of natural antioxidants were used for stabilization of the oils. They have some advantages in comparison with synthetic antioxidants as follows: readily accepted by consumers; they are safe additives with nutraceutical value [18].

Antioxidant activity of different natural plant antioxidant mixtures in concentration 0.3% was examined for stabilization of grape seed oil. The results are presented in Table 2.

Table 2. Antioxidant activity of natural plant antioxidant mixtures.

Antioxidant mixture	Induction period, h	Antioxidative effect, times
1. Control sample	7.0 ± 0.3	-
2. Comomile	8.3 ± 0.3	1.2
3. Nettle	7.0 ± 0.1	1.0
4. Marigold	7.0 ± 0.1	1.0
5. Yellow tutsan	5.7 ± 0.2	0.8
6. White milfoil	7.0 ± 0.2	1.0
7. Unsaponifiable of grape seed oil (extract of resveratrol)	8.1 ± 0.3	1.2

The oxidative stability of investigated grape seed oil was found to be higher than values reported earlier (4.8 h), but significantly lower in comparison with other vegetable oils as olive (22.0 h), corn (11.0 h) [19]. These data are result of different content of unsaturated fatty acids and respectively tocopherols and sterols – the main antioxidant and synergist in the oil.

Rancimat test showed the insignificant increasing of the stability (0 – 20%) regardless of relatively high concentration of the added antioxidant mixtures.

The effect of the individual pure natural and synthetic antioxidants put in oil in concentration (0.05%) is presented in Table 3.

The highest extension of the stability was established using butyl gallate. Induction period as index for stability was found to be 26.2 h. The other antioxidants increase the stability significant by less (from 7.0 h for control sample to 9.9 – 18.7 h (1.4 – 2.7 times). In this connection the next investigations were performed by butyl gallate only. Since as salad oil grape seed oil is used as a component for manufacturing of cosmetic products where it is possible to put a large quantity of antioxidants (about 0.2%), the investigation was carried out with concentrations of butyl gallate 0.02 – 0.2%.

Table 3. Antioxidant activity of individual pure natural and synthetic antioxidant mixtures.

Antioxidant mixtures	Induction period, h	Antioxidative effect, times
1. Control sample	7.0 ± 0.3	-
2. β-carotene + α-tocopherol	9.9 ± 0.2	1.4
3. Oxinex	11.1 ± 0.4	1.6
4. Rosemary	18.7 ± 0.4	2.7
5. Quercetine	14.1 ± 0.3	2.0
6. Ascorbyl palmitat	11.3 ± 0.5	1.6
7. Butyl gallate	26.2 ± 0.5	3.7

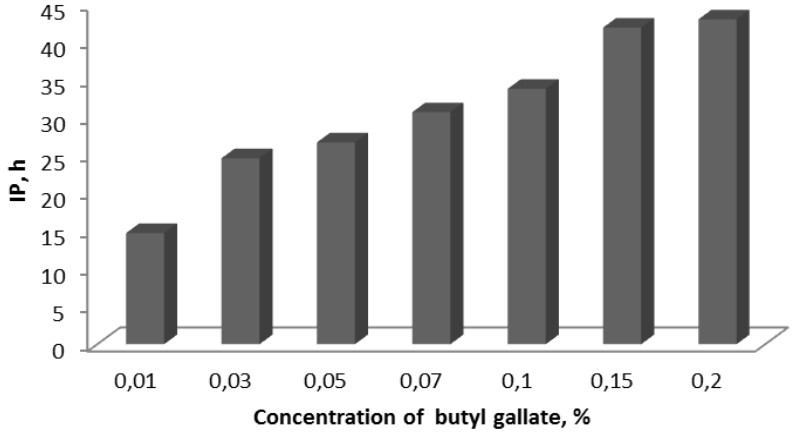

Fig. 1. Antioxidant activity of butyl gallate with different concentrations.

The influence of concentration of butyl gallate over oxidant stability of the oil is presented in Fig. 1.

It was observed good correlation between concentration of the added butyl gallate and increasing of the Rancimat test Induction time. The oxidative stability increased gradually and was highest at 0.2% - Induction period was found to be 43.0 h.

Grape seed oil has very low oxidative stability as a result of the high content of unsaturated fatty acids mainly linoleic. The uses of natural plant antioxidant mixtures do not increase significantly stability of the oil. The best results were obtained using butyl gallate. Quantities about 0.05% added into oil for food purposes increase Induction period more than 2 times. When grape seed oil is used in cosmetics, 0.2% butyl gallate can be put in the oil and Induction period was 6 times more than that of unstabilized oil.

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ОКСИДАНТНА СТАБИЛНОСТ И СТАБИЛИЗИРАНЕ НА ГРОЗДОВО МАСЛО

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(Резюме)

Изследвана е оксидантната стабилност на масло, получено от гроздови семена. Индукционният период, определен чрез метода на Rancimat при 100^{0}C, е 7 часа. За стабилизирането на маслото са използвани различни природни екстракти и индивидуално чисти антиоксиданти. Употребата на растителни екстракти в концентрация 0,3% има незначителен ефект върху стабилизирането (1,1-1,3 пъти). По-добър ефект показаха някои индивидуално чисти антиоксиданти в концентрация 0,05%. Най-добри резултати са постигнати при употребата на бутил галат, при който индукционният период е 26,2 часа и екстракт от розмарин с индукционен период 18,7 часа. Установено е, че оксидантната стабилност нараства значително с увеличаването на концентрацията на бутил галат, като най-добри резултати се постигат при концентрация 0,2%, при която индукционният период е 42,1 часа.