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ORIGINAL RESEARCH PAPER

COMPOSITION AND PHYSICOCHEMICAL PROPERTIES OF SEED OIL OF RARELY GROWN VARIETIES OF GRAPES

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Abstract: The composition of grape seeds oil from a mixture of two varieties of red grape (Sangiovese and Pinot noir) from Varna region was investigated. Different analytical techniques have been used to determine the content of biologically active compounds, fatty acids, sterols and elemental compositions. It has been found that the oxidation stability of the oil is relatively low - about 4.5 hours. The fluorescence spectrum analyzed at an excitation wavelength of 350 nm contains peaks that are attributed to pigments, oxidizing products and vitamins. Transmission spectra were used to determine the content of *β*-carotene, chlorophyll and color characteristics in the CIELab colorimetric system. Infrared spectroscopic experiments (ATR and permeability) were used for confirmation of the fatty acid profile of the analyzed oil. The results show that this oil is a good source of various healthy nutrients.

Keywords: *chemical composition, color characteristics, fatty acids, sterols, fluorescence spectra, grape seed oil, IR spectra*

INTRODUCTION

Grape seeds are agricultural waste but contain significant amounts of oil, protein, carbohydrates and others, which make them a valuable raw material for oil production [1 – 5]. These seeds are one of the waste products produced during winemaking. The dry seeds contain between 10 and 20 % total fat and that depends mainly on the grape varieties [6, 7]. The grape seed oil has high content of the unsaturated fatty acids - linoleic (63.0 - 73.1 %) and oleic acid (15.4 - 23.0 %) [8, 9]. Sabir *et al*. [10] have found the amount of oleic acid was higher (16.2 - 31.2 %). Grape seed oil contains biologically active compounds such as sterols and phospholipids [11, 12]. The seeds and grape seed oils contain significant amounts of essential macro- and microelements [6, 13]. As grape seed oil has an interesting greenish yellow color and a neutral flavor it can be used in a wide range of food applications – for salads, for marinates and dressings [8]. It may also be used for blending with other more expensive oils i.e.: walnut oil, olive oil or hazelnut oil and other food products such as mayonnaises due its neutral flavor and color. It is a good source of polyunsaturated fatty acids and can be included as an ingredient in different products in pharmaceutical and cosmetic industry. The oil is used for the prevention of a variety of diseases such as thrombosis, cardiovascular diseases, reduction of cholesterol in serum, dilation of blood vessels [2, 14].
The aim of the present study is to evaluate the lipid composition and physicochemical properties of grape oil from rarely grown varieties of grape in Bulgaria and compare them with other oils, extracted from foreign and Bulgarian varieties of grape.

MATERIALS AND METHODS

Chemicals

All solvents and reagents were analytical grade and were used without additional purification. Reference fatty acid methyl esters are purchased from Supelco (USA 37 comp. FAME mix). The standard mixture of sterols contained cholesterol (purity 95 %, New Jersey, USA), stigmasterol (Sigma-Aldrich, purity 95 %, St. Louis, MO, USA) and *β*-sitosterol (with ca 10 % campesterol, ca 75 % *β*-sitosterol, New Jersey, USA). Thin-layer chromatography (TLC) plates were prepared in the laboratory using Silica gel 60 G (Merck, Darmstadt, Germany). Multi-element standard solution 5 for ICP (Merck, Darmstadt, Germany), 1000 $mg \cdot L^{-1}$ Se and 1000 $mg \cdot L^{-1}$ As (Merck, Darmstadt, Germany) were used for the preparation of diluted working standard solutions for calibration for ICP-MS and ICP-OES measurements.

Grape oil seed

Oil obtained from a mixture of seeds of the two red grape varieties in ratio 1:1 (Sangiovese and Pinot noir) was used in this study. The oil tested is from a manufacturer who uses the waste seeds from a winery from the Varna region.

Analysis of fatty acids

The fatty acid composition of oil was determined by gas chromatography (GC) after transmethylation of the respective sample with 20 $g \cdot kg^{-1}$ H_2SO_4 in absolute CH_3OH at

50 ºC [15]. Fatty acid methyl esters (FAME) were purified by thin-layer chromatography on 20 × 20 cm plates covered with 0.2 mm silica gel 60 g (Merck, Darmstadt, Germany) layer with mobile phase hexane : diethyl ether (97 : 3, v/v). GC was performed on a HP 5890 series II (Hewlett Packard GesmbH, Vienna, Austria) gas chromatograph equipped with a 60 m × 0.25 mm × 25 µm capillary DB - 23 column (Agilent J&W advanced, Agilent Technology, USA) and a flame ionization detector. The column temperature was programmed from 130 °C (1 min), at 6.5 °C·min^{-1} to 170 °C (0 min), at 3 °C·min^{-1} to 215 °C (9 min) and at 40 °C·min^{-1} to 230 (1 min); the injector and detector temperatures were kept at 250 °C and 270 °C. Hydrogen was the carrier gas at a flow rate 0.8 mL·min^{-1}; split was 1 : 50. Identification of fatty acids was performed by comparison of retention times with those of a standard mixture of fatty acids subjected to GC under identical experimental conditions [16].

Determination of sterols

The oil (sample size of 5 g) was hydrolyzed with 50 mL 2 N ethanolic KOH [17] for one hour. The unsaponifiable lipids were extracted with hexane. The solvent was removed by rotary evaporator (Ika RV3 Eco, Staufen, Germany). Unsaponifiables were dried in an oven at 105 °C for 30 min. The sterol fraction from the unsaponifiable matter is separated by thin-layer chromatography on silica gel 60 G in the mobile phase diethyl ether: hexane (1 : 1, v/v). The qualitative and quantitative composition of the sterol fraction was determined on HP 5890 series II gas chromatograph (Hewlett Packard GesmbH, Vienna, Austria) equipped with 25 m × 0.25 mm (I.D.) × 25 µm (film thickness) DB–5 capillary column (Agilent Technologies, J&W Scientific products Proudly, Santa Clara CA, USA) and flame ionization detector [18].

Phospolipid content

The quantification of phospholipids was carried out spectrophotometrically by measuring the phosphorous content at 700 nm after mineralization of the oil with a mixture of perchloric acid and sulphuric acid (1 : 1, v/v) [19].

Oxidative stability

The oxidative stability of the oil was determined by measuring the induction period using conductometric detection of volatile compounds. A Rancimat Methrom 679 (Herisau, Switzerland) apparatus was used at 100 °C and an airflow rate of 20 L·h^{-1} [20].

Colour parameters

CIELab color parameters (The CIELAB color space, also known as CIE L*a*b* or sometimes incorrectly abbreviated as simply "Lab" color space) is a color space defined by the International Commission on Illumination (CIE) are measured directly by using spectrophotometer (Lovibond Tintometer PFX 195, Solar Way, Solstice Park, UK) [21]. The content of β-carotene and chlorophyll are determined using special software from transmission and absorption spectra. All measurements have been carried out at room temperature immediately after opening the oil bottle. Color coordinates, color parameters a, b and brightness L of tested sample have been measured.

Fluorescence spectra measurements

The sources used to measure the fluorescence spectra are 390 nm, 400 nm, 420 nm light emitting diodes (LEDs). A fiber optic spectrometer Brolight, Hamamatsu Company China with sensitivity in the 200-1100 nm range and a resolution of about 8 nm was used to measure the fluorescence spectra. The oil sample was placed in a cuvette 10 × 10 mm and irradiated by LEDs

FT-IR spectroscopy

Infrared spectra were recorded on a Thermo Fischer Nicolet iS50 FT-IR instrument. The analyzed sample of 200 µL was placed between two KBr disks and the transmittance spectrum was recorded.

Elemental composition

About 0.3 g of oil were weighted on an analytical balance (Mettler Toledo XPE 205) in a PTFE vessel. 6 mL 65 % HNO_3 and 2 mL 30 % H_2O_2 were added. Five samples with three blanks were digested in a microwave furnace at 200 °C for 20 min. Samples were diluted to 50 mL final volume for analysis. Inductively coupled plasma-mass spectrometer ("X SERIES 2"– Thermo Scientific, USA) and inductively coupled plasma optical emission spectrometer (ICP-OES - Jobin Yvon, Ultima 2, France) were used for the determination of elements.

RESULTS AND DISCUSSION

The content of phospholipids and sterols of grape seed oil have been investigated. The content of phospholipids and sterols in the oil were 1.1 % and 0.2 %, respectively. Individual sterol composition of the investigated sample is given in Table 1.

Table 1. *Sterol composition of grape seed oil*

Sterols	**%**
Cholesterol	0.5 ± 0.01
Campesterol	9.9 ± 0.04
Stigmasterol	8.0 ± 0.2
β-Sitosterol	80.6 ± 1.1
Δ5-Avenasterol	0.1 ± 0.01
Δ7-Stigmasterol	0.9 ± 0.06

*Means ± SD of three determinations

β-Sitosterol (80.6 %) was the main component in the sterol fraction, followed by campesterol (9.9 %) and stigmasterol (8.0 %). Content of *β*-sitosterol is similar to grape oil from other Bulgarian varieties from two white grape varieties – Bolgar (70 %), and Super ran Bolgar (72.1 %), and from two red grape varieties – Mavroud (70.4 %) and Shiroka melnishka loza (72.1 %) [1]. The content of *β*-sitosterol in Bulgarian grape seed oil is higher than the oils from the varieties Garnacha Tintorera, Petit Verdot and Syrah (between 66 – 67 %), reported from Pardo *et al.* [22].

The fatty acid composition of the grape seed oil from varieties Sandjoveze and Pinot Noir is presented in Table 2.

Nine fatty acids were identified in the glyceride oil. Linoleic acid (64.7 %) predominates in the grape oil followed by oleic (19.0 %) and palmitic acid (12.3 %). The stearic acid content (3.3 %) was found to be similar to other vegetable oils, where it is about 3 % [23]. The other fatty acids were detected in negligible quantities. These results are close to data reported earlier by other authors [5, 24]. There are slight variations in the percentage of fatty acids between grape varieties because fatty acid composition depends mainly on variety and the geographic region.

Table 2. *Fatty acid composition of grape seed oil*

Fatty acids	**%**
Caprylic $C_{8:0}$	0.1 ± 0.01
Capric $C_{10:0}$	0.1 ± 0.01
Myristic $C_{14:0}$	0.1 ± 0.01
Palmitic $C_{16:0}$	12.3 ± 0.5
Palmitoleic $C_{16:1}$	0.3 ± 0.02
Margarinic $C_{17:0}$	0.1 ± 0.01
Stearic $C_{18:0}$	3.3 ± 0.5
Oleic $C_{18:1}$	19.0 ± 1.1
Linoleic $C_{18:2}$	64.7 ± 1.7
Saturated fatty acids, %	16.0
Unsaturated fatty acids, %	84.0
Monounsaturated fatty acids, %	19.3
Poliunsaturated fatty acids, %	64.7

The oxidative stability of grape seed oil is relatively low; it has a value of approximately 4.5 h. This can be explained by the high content of polyunsaturated fatty acids and lacking presence of tocopherols, which are natural antioxidants. It is two times lower than that for grape seed oils, obtained from varieties Garnacha Tintorera (8.14 h), Petit Verdot (8.07 h), Syrah (7.90 h). The oxidative stability of Bulgarian grape seed oil is close to this from Monastrell (6.38 h), obtained from Pardo *et al.* [22].
The chemical composition and fatty acid profile were further investigated by ATR-IR measurement of the oil sample. Characteristic peaks for C=C double bond (1456 cm^{-1}), C=O group (1742 cm^{-1}) and aliphatic CH_2 groups (2853 and 2922 cm^{-1}) corresponding to the mono- and polyunsaturated fatty acids are worth mentioning (Figure 1). The band at 1742 cm^{-1} corresponding to stretching vibrations of C=O bonds from the carbonyl groups, does not depend on the length of fatty acid chains or their degree of unsaturation. The signal around 3008 cm^{-1} is formed by C-H stretching vibrations of unsaturated parts of fatty acid chains. Another band at 914 cm^{-1} is known to be formed by the vibrations of the C-C bonds between CH_2 groups and C=C double bonds [25]. These observations are in accordance with the composition of the oil outlined in Table 2. The spectrum shows similar peaks compared to results already published in the literature [26].

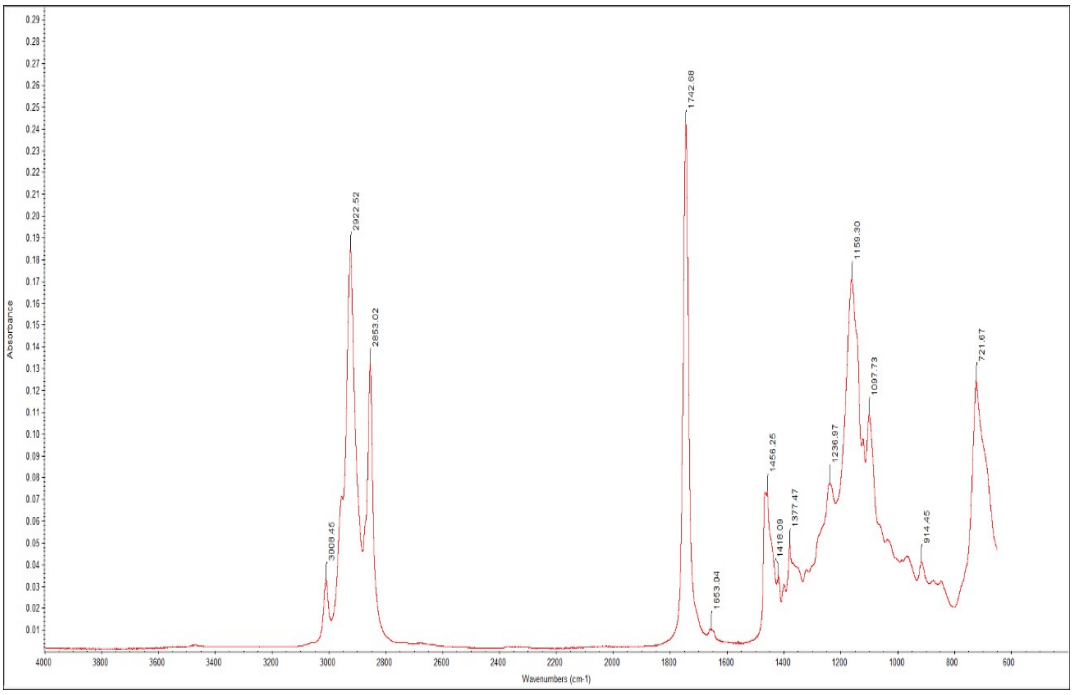

Figure 1. *IR spectra for grape seed oil*

The fluorescence spectra (Figure 2) in the visible region of the investigated sample is obtained for excitation wavelength respectively λ_{ex} = 390 nm, 400 nm and 420 nm. The best ration for fluorescence emission vs. excitation intensity is found at λ_{ex} = 390 nm (Figure 2).

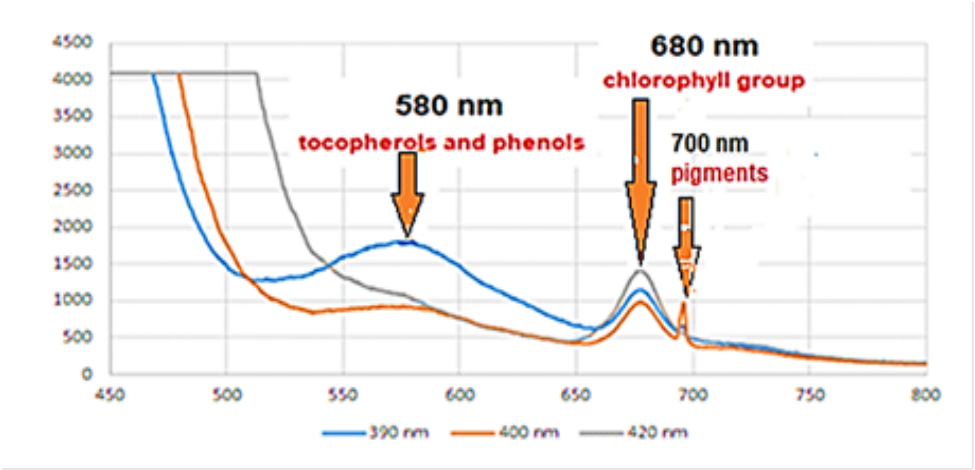

Figure 2. *Fluorescence spectra for grape seed oil*

The fluorescence emission bands of the sample were compared with the characteristic emission bands of natural fluorophores and are clearly distinguishable for the different types of cold pressed oils. It has been shown that emission peaks around 410 nm, 430

nm and 450 nm are associated with oxidation products, peaks around 485 nm, 520 nm, 560 nm are associated with β-carotene content, these about 680 nm and 720 nm are related to the chlorophyll content [27, 28].
In our case there are three fluorescence peaks for investigated sample related to phenol content at about λ= 570-580 nm; chlorophyll at λ = 675-680 nm and nondetermined pigments at λ = 700 nm. The similar maximum as the last one is observed from Sikorska *et al.* for other vegetable oil [29].
In order to determine β-carotene and chlorophyll content it is necessary to conduct spectral analysis – see table 3. So, the maximum at 580 nm is due to β-carotene content at about 70 ppm, and last two peaks correspond to chlorophyll content of 17 ppm.
The color characteristics in two color systems XYZ and CIELab are shown in Table 3. On the base of transmission, spectra in the visible region the pigments chlorophyll and β-carotene are determined. It is evident that color coordinates X and Y are between 0.45 and 0.5. Parameter a* is of negative value while parameter b* is positive, which is characteristic of yellow colors.

Table 3. *Color parameters of grape seed oils.*

Parameter	Grape seed oil
X	44.18
Y	48.97
Z	2.93
x	0.4598
y	0.597
L	75.43
a	-10.61
b	99.26
Clorophyll	17.04 ppm
β-carotene	67.99 ppm

Determination of color parameters for refined oils is important, as their determination is part of the standard for oil certification.
Elemental analysis of the grape oil demonstrated that content of As and Cd is <0.02 $\mu g \cdot g^{-1}$, Pb = 0.07 $\mu g \cdot g^{-1}$, Ni=0.37 $\mu g \cdot g^{-1}$, Mn =0.21 $\mu g \cdot g^{-1}$, Co and Cu 0.04 $\mu g \cdot g^{-1}$ each, Se=0.03 $\mu g\ g^{-1}$. Concentrations of Fe, Mg and Zn are 16.7, 16.7 and 7.78 $mg \cdot kg^{-1}$. Relatively high content of Fe, Ni and Mn support low value of oxidation stability as those elements favor oxidation. RSD for Fe, Mn and Zn concentrations are 3 - 6 % (determined by ICP-OES) and are 5 – 8 % for the other elements (determined by ICP-MS).

CONCLUSIONS

The grape seed oil has a high content of polyunsaturated fatty acids (84 %). Sterol composition of the oil isolated from seeds of Bulgarian grape varieties was different for oils from other countries. β-sitosterol predominated in sterol fraction. The low value of toxic elements shows that the product can be used in food, and cosmetics medical industry. An important problem is to improve the oxidative stability of the grape seed

oil, which could be resolved by the addition of natural antioxidants such as phenolic components.

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