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Physicochemical characterization and quality of cold-pressed peanut oil obtained from organically produced peanuts from Macedonian "Virginia" variety

S. Kostadinović Veličkovska✉, S. Mitrev and L. Mihajlov

Faculty of Agriculture, University "Goce Delčev", Krste Misirkov bb, 2000 Štip, Macedonia

✉Corresponding author: sanja.kostadinovik@ugd.edu.mk

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SUMMARY: The physicochemical characterization and quality of cold pressed peanut edible oil from the "Virginia" variety, organically produced from the region of Macedonia, were examined in this work for the first time. The fatty acid composition of the oil showed almost equal levels of oleic and linoleic acids with an abundance of 34.19±0.01 and 36.13±0.01%, respectively. The most dominant saturated fatty acid was palmitic acid with a level of 10.06±0.00%. The level of tocopherols and other vitamin-E-related compounds was in strong agreement with the antioxidant activity of the oils measured by the DPPH assay. Almost equal amounts of α and γ tocopherols indicated an antioxidant potential of 288.63±59.78 mg·L^{-1} α-tocopherol. Phytosterols, as minor compounds present in the oils, can be additional antioxidants responsible for the health benefits of this oil in human nutrition. The four major pytosterols were β-sitosterol (1812.21±22.17 mg·kg^{-1} oil), champesterol (320.55±17.07 mg·kg^{-1} oil), Δ5-avenasterol (236.16±14.18 mg·kg^{-1}) and stigmasterol (133.12±12.51 mg·kg^{-1} oil). Induction time, Peroxide number, FFA and specific extinction (K_{232} and K_{270}, values 1.82 and 0.22) gave us an indication of the oxidative stability of cold pressed peanut oil.

KEYWORDS: *Cold pressed peanut oil; DPPH assay; Fatty acid profile; "Filla" oil; Pytosterols; Vitamin-E-active compounds*

RESUMEN: ***Caracterización físico-química y calidad del aceite de cacahuete prensado en frío obtenido de nuez de Macedonia variedad "Virginia" producida orgánicamente***. Se ha abordado por primera vez, en este trabajo, la caracterización físico-química y la calidad de aceite comestible de maní prensado en frío de la variedad "Virginia", de producción ecológica y procedente de la región de Macedonia La composición de ácidos grasos del aceite mostró casi los mismos niveles de los ácidos oleico y linoleico: 34,19±0,01 y 36,13±0,01%, respectivamente. El ácido graso saturado dominante fué el palmítico, 10,06±0,00%. El nivel de tocoferoles y otros compuestos relacionados con la vitamina-E estaba en estrecha relación con la actividad antioxidante de los aceites medidos mediante el ensayo de DPPH. Casi igual cantidad de α y γ tocoferol indicó un potencial antioxidante de 288,63±59,78 mg·L^{-1} de α-tocoferol. Los fitoesteroles, compuestos menores presentes en los aceites, pueden ser antioxidantes adicionales con beneficios para la salud y la nutrición humana. Los cuatro fitosteroles principales fueron β-sitosterol (1812,21±22,17 mg·kg^{-1}), campesterol (320,55±17,07 mg·kg^{-1}), Δ5-avenasterol (236,16±14,18 mg·kg^{-1}) y estigmasterol (133,12±12,51 mg·kg^{-1}). El periodo de inducción, índice de peróxidos, FFA y extinción específica (K232 y K270 con valores de 1,82 y 0,22) nos indican la estabilidad oxidativa del aceite de cacahuete prensado en frío.

PALABRAS CLAVE: *Aceite de cacahuete; Ensayo DPPH; Fitosteroles; Perfil de ácidos grasos; Prensado en frío; Vitamina-E-activa*

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1. INTRODUCTION

Current trends in the consumption of cold-pressed edible oils, in particular, peanut oil make it necessary to research the quality, stability and nutritional impact on human health (Zock *et al*., 1995). The conventional processing of peanuts in the food industry involves mechanical pressing during which the temperature does not surpass 40 °C. This process yields cold-pressed peanut oil and low-value peanut cake. Peanut seeds are a good source of protein, lipids and fatty acids. Peanut seeds contain 45–50% oil (Grosso *et al*., 1999).

Cold pressed peanut oil enjoys a very popular place in human nutrition due to the high level of polyunsaturated fatty acids, tocopherols, pytosterols, carotenoids, chlorophylls and polyphenolics (Sullivan *et al*., 2011). A positive correlation between the consumption of polyphenol-enriched cold-pressed oil consumption and a reduced risk of coronary heart diseases, level of "LDL", degenerative diseases and cancer is well known (Varney *et al*., 2011).

Peanuts belong to the genus *Arachis*, a member of the family *Leguminoseae*, and are widely distributed in the tropics and moderate regions of the world. Peanuts make an important contribution to the diet in many countries. Peanut seeds are a good source of protein, lipid and fatty acids for human nutrition. The cold-pressed oil from peanuts is very important due to its significant level of α and γ-tocopherol, as well as significant amount of resveratrol. *Trans*-resveratrol or *trans*-3,5,40-trihidroxystilbene is the most abundant stilbene in peanuts and is responsible for the benefit to human health. The antioxidant and antimicrobial efficiency of resveratrol provides health benefits, such as the prevention of cardiovascular diseases, arteriosclerosis and cancer. Originally, epidemiological studies indicated an inverse relationship between food enriched with resveratrol and the risk of coronary heart disease (Kostadinović *et al*., 2012).

Many researchers have studied the chemical composition and nutritional value of cold-pressed peanut oil. Fatty acid and phytosterol compositions of peanut seeds have been studied by (Grosso *et al*., 1997). Grosso et al. studied the chemical composition of the oil of aboriginal peanut seeds from Uruguay (Grosso *et al*., 1999). Furthermore, Grosso et al. have reported the oil, protein, ash, carbohydrate contents, iodine value and fatty acid composition of some wild peanut specie (Arachis) seeds (Grosso *et al*., 2000). The level of phytosterols in different peanut species from the region of Bolivia and Argentina was the object of study in the work of Grosso *et al*. (1997). The effects of cultivar, location, and their interaction in the fatty acid composition have been investigated by Brown *et al*. (1975). In another study, fatty acid composition, protein levels, amino acid composition and other components have been investigated in peanut seeds (Ahmed and Young, 1982). Lopes et al. published a study for peanuts with an overview of the chemical composition, focusing on secondary metabolites and their biological activity (Lopes *et al*., 2011).

Peanuts are a rich source of oil (45–50%). The oil obtained from the cold pressing of peanuts is pale yellow and has the characteristic "nutty" flavor of roasted peanuts. This characteristic flavor was examined by Chetschik *et al*. (2010). by application of stable isotope dilution analyses (SIDA) and gas chromatography-olfactometry. According to their findings, 26 odor-active compounds were identified and quantified in raw and pan-roasted peanut meal. 3-Isopropyl-2-methoxypyrazine, acetic acid, and 3-(methylthio) propanal showed the highest OAVs in raw peanuts, whereas methanethiol, 2,3-pentanedione, 3-(methylthio) propanal, and 2- and 3-methylbutanal as well as the intensely popcorn-like smelling 2-acetyl-1-pyrroline revealed the highest OAV in the pan-roasted peanut meal.

The fatty acid profile, vitamin-E-active compounds and phytosterol composition of peanut oil from South America was the object of study in the work of Carrín and Carelli. According to their finding, the most dominant fatty acids in peanut oil were palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) acid. According to their findings, palmitic acid was present in the range from 9.3 to 13%, oleic acid from 35.6 to 58.3% and linoleic acid from 20.9 to 43.2% (Carrín and Carelli, 2010). Regarding, the distribution of vitamin E active compounds, the most present were α and γ-tocopherol with levels between 18–57 $mg \cdot kg^{-1}$ oil and 36–78 $mg \cdot kg^{-1}$ oil, respectively. β and δ-tocopherol were not detected nor present in trace amounts till 6 $mg \cdot kg^{-1}$ of oil (Carrín and Carelli, 2010).

It is obvious that there are already many published studies on the chemical composition of cold-pressed

peanut oils, but, until now, to the best of our knowledge, there are no published results on the quality of the cold-pressed peanut oil from the region of Macedonia. The most famous variety of peanuts growing in the region of south-east Macedonia is the "Virginia" variety. The other two main varieties ("Ranner" type and "Valencia" type) are less present in the Macedonian region. The "Virginia" variety of peanuts has the largest kernels and accounts for most of the peanuts roasted in their shell. Therefore, the main object of this study was to give an overview of the chemical composition and general quality parameters including fatty acid profile, content of tocopherols and phytosterols as well as physicochemical evaluation, oxidative stability and antioxidant activity of cold-pressed peanut oil from the Macedonian "Virginia" variety.

2. MATERIALS AND METHODS

2.1. Harvesting and selection of plant material

The peanuts (*Arachis hypogea*, *L.*) were cultivated in the valleys of Strumica and Gevgelija regions with an average yield of 1200 kg·ha^{-1}. The peanuts collected for the experimental purpose of this study belong to the "Virginia" variety.

The peanuts from the Macedonian "Virginia" variety were collected in mid-October from the valleys of Gevgelia and Strumica, 2012. The selected plant from *Arachis hypogea*, *L.*, was hand-picked and dried for 7–10 days. After drying, the peanut seeds were separated from plant material and sorted according to their quality and maturity. Furthermore, additional dying at 40 °C was performed in drying ovens.

2.2. Purification and cold pressing

The purification process of the plant material started with the removal of broken or damaged peanuts. This step was necessary because unpurified plant material can negatively affect the quality of cold-pressed oil. After purification, the next step before pressing was peeling and roasting. After peeling, the peanuts were roasted at 120 °C for 5 min. The process of cold pressing was performed using a Komet single press (IBG Monforts Oecotec, Germany). After pressing, the fresh cloudy oil was purified from solid impurities in tanks by sedimentation over 17 days.

The quantities of plant material were collected only for the needs of this experiment and the yield of cold-pressed oil was lower than 1 kg.

After sedimentation, the collected oil was filtrated by using a protection filter and bottled in dark 250 mL bottles. The cold pressed oil was stored at temperatures below 15 °C in the dark.

2.3. Analytical methods

2.3.1. Determination of the fatty acid composition

The fatty acid composition of Macedonian cold-pressed peanut oil was determined using gas chromatography equipped with a flame ionization detector (FID). The esters were prepared using 2 drops of each oil dissolved in 1 mL of heptane. After the addition of 50 μL of sodium methylate with a concentration of 2 mol·L^{-1}, the samples were homogenized. After homogenization, 100 μL of distilled water were added to each sample. Samples were centrifuged and the lower phase was removed: while 50 μL of 1 M HCl were added to the upper phase. After a second homogenization, sodium sulphate anhydride was added to remove water traces. Finally, the upper phase was transferred to GC vials and fatty acid methyl esters were analyzed using a capillary GC equipped with a CP7420 Select FAME column, 100 m×0.25 mm internal diameter with 0.25 μm film thickness. The analyses were performed on an Agilent 6890 equipped with KAS4Plus and FID. The oven temperature was programmed to increase from 150 °C to 240 °C at a rate of 1.5 °C·min^{-1} with the isotherm kept at 240 °C for 20 min. The injector and detector temperature were both 260 °C. Hydrogen was used as the carrier gas at an average velocity of 25 mL·min^{-1}. The retention times of separated peaks were confirmed by FAME standards.

2.3.2. Determination of tocopherols

100 mg of cold pressed peanut oil were dissolved in 1 mL of heptane. The determination of tocopherols was performed with an HPLC instrument equipped with an L6000 pump, a Merck-Hitachi F-1000 fluorescence detector with excitation wavelength on 295 nm and emission wavelength of 330 nm and a Diol phase HPLC column 25 cm×4.6 mm ID (Merck, Darmstadt, Germany). The flow rate was 1.3 mL·min^{-1} and the injection volume 20 μL. The mobile phase was a mixture of heptane and TBME at a ratio 95:5.

2.3.3. Rancimat test

For the determination of the oxidative stability of Macedonian peanut oil, a Metrohm Rancimat model 743 (Herisau, Switzerland) was used. In order to determine the oil stability index (OSI), a stream of purified air was passed through 3.6 g of oil at 120 °C at a flow rate of 20 L·h^{-1}. For each sample of oil, the OSI index was determined in two portions by measuring two samples in the apparatus simultaneously. The induction time in hours was automatically recorded and taken as the break point of the plotted curves (the intersection point of the two extrapolated parts of the curve).

2.3.4. Peroxide value

For the determination of peroxide value, the DGF standard method C-VI 6a – Part 1 (05) according to Wheeler was used. In brief, for titration of the oil, a standard solution of sodium thiosulfate was prepared with a concentration of 0.01 mol·L^{-1}. A mixture of glacial acetic acid and isooctane was prepared in the ratio of 60:40 and was used as extracting agent for the oils. A saturated solution of potassium iodide was prepared by dissolving 10 g potassium iodide in 5 mL of boiled Millipore water. Analyses were performed by dissolving of 5.0 g of oil in 50 mL of extracting agent and 100 ml of Millipore water. After adding 500 µL of the saturated solution of potassium iodide, the potentiometric titration was performed by using the automatic titrator, Methrom 888 Titrando (Methrom, Herisau, Switzerland).

2.3.5. Determination of free fatty acid content (Acidity)

For determination of the content of free fatty acids the DGF standard method C-V 2 (06) was used. In brief, a mixture of ethanol and light petroleum was prepared in the ratio of 50:50 and was used as extracting agent for the oil. 10.0 g of oil was dissolved in this mixture and titration was performed using potassium hydroxide at a concentration of 0.1 mol·L^{-1}. Potentiometric titration was performed using the automatic titrator, Methrom 888 Titrando (Methrom, Herisau, Switzerland).

2.3.6. Determination of ultraviolet absorbance expressed as specific UV extinction

This method was equivalent to ISO 3656:2012. For this purpose, 1.0 g of oil was dissolved into a 100-mL flask by iso-octane. The mixture was shaken and the solution was transferred into rectangular quartz cells with covers, having an optical length of 1 cm and determinations were made at 232 and 268 nm. The absorption at the wavelengths specified in the method is due to the presence of conjugated diene and triene in the oil.

2.3.7. Determination of phytosterols

The sterol composition of the cold-pressed peanut oil was determined following ISO/FIDS 12228:1999 (E). In brief, 250 mg of oil were saponified with a solution of ethanolic potassium hydroxide by boiling under reflux. The unsaponifiable matter was isolated by solid-phase extraction on an aluminium oxide column (Merck, Darmstadt, Germany) onto which fatty acid anions were retained and sterols passed through. The sterol fraction was separated from other unsaponifiable matter by thin-layer chromatography (Merck, Darmstadt, Germany), re-extracted from the TLC material, and afterwards, the composition of the sterol fraction was determined by GLC using betulin as internal standard. The compounds were separated on an SE 54 CB (50 m long, 0.32 mm ID, 0.25 µm film thickness) (Macherey-Nagel, Düren, Germany). Further parameters were as follows: hydrogen as carrier gas, split ratio 1:20, injection and detection temperature adjusted to 320 °C, temperature program, 245 °C to 260 °C at 5 °C·min^{-1}. Peaks were identified either by standard compounds (β-sitosterol, campesterol, stigmasterol), by a mixture of sterols isolated from rape seed oil (brassicasterol) or by a mixture of sterols isolated from sunflower oil (Δ7-avenasterol, Δ7-stigmasterol, and Δ7-campesterol). All other sterols were firstly identified by GC-MS and afterward analyzed by comparison of retention times.

2.3.8. Determination of antioxidant activity by DPPH assay

The antioxidant activity of cold-pressed peanut oil was estimated spectro-photometrically using the DPPH assay.

For this purpose, the antioxidant activity of the oil was expressed as percentage of de-colorization of a solution of the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl radical) at 517 nm. The DPPH reagent was dissolved in hexane and the stock solution of 0.25 mL with a concentration of 0.5 M was used for the determination of the antioxidant activity. α-tocopherol at different concentrations (100–1000 mg·L^{-1}) was dissolved in hexane and used as standard for the preparation of the calibration curve.

2.3.9. Statistical analyses

A one-way ANOVA was used to examine the level of every particular minor and major compound by considering the type of oil. The significance level of all statistical analyses was set at 0.05. The level of significance of difference between the percentages of fatty acids, level of tocopherols, level of phytosterols, and values of antioxidant activity measured by the DPPH assay mean values was determined at 5% by a one-way ANOVA using Tukey's test. This treatment was carried out using SPSS v.16.0 software, IBM Corporation, USA.

3. RESULTS AND DISCUSSION

3.1. Fatty acid composition

Fatty acids are very important to human nutrition and in vegetable oils they are manly presented as triacylglycerols (TAG). Furthermore, they are classified as saturated, monounsaturated (MUFA) and polyunsaturated fatty acid (PUFA). The unsaturated fatty acids are classified into well-known series, such

as omega-9, omega-6 and omega-3. Omega-9 fatty acids are considered as non-essential for the human diet and omega-6 and omega-3 are essential since these fatty acids cannot be synthetized by mammals and they must be obtained from the diet.

The fatty acid composition of the cold pressed peanut oil from the "Virginia" variety is presented in Table 1. Regarding saturated fatty acids, the cold-pressed oil from peanuts had the highest percentage of palmitic acid (10.06±0.00%). Stearic acid was presented in significantly lower levels (4.40±0.01%). Oleic acid and linoleic acids as unsaturated fatty acids were the most dominant with levels of 34.19±0.01 and 36.13±0.01%, respectively. Lignoceric acid was abundant at a level of 3.93±0.00%. Paullinic (Omega -7) fatty acid was detected at the level of 1.37±0.00% in the sample of peanut oil organically produced from the territory of Macedonia. The fatty acid profile was in good agreement with data published by Özcan and Seven who stated that palmitic and stearic acids were the most abundant saturated fatty acids in a Turkish variety of peanut oils (in the range of 8.70% to 13.03% for palmitic acid and 3.77% to 4.53% for stearic acid) (Özcan and Seven, 2003). However, Carrín and Carelli (2010) stated that the degree of saturation in peanuts oil is dependent on temperature, genotype, seed maturity, and growth location as well as the interaction of all these factors. More precisely, seed development in regions with lower temperatures usually produces oil with higher levels of unsaturated fatty acid. According to the results published by Carrín and Carelli (2010), Macedonian peanut oil belongs to the relatively high linoleic acid oil category (36.13%). As can be seen from table 1, the highest abundance with a percentage of 37.05±0.03% was attributed to monounsaturated fatty acids (MUFA). Oleic acid was the most dominant monounsaturated fatty acid. Furthermore, polyunsaturated fatty acid (PUFA) was present at the level of 36.62±0.01% with linoleic acid as the most dominant fatty acid. Finally, the lowest percentage belonged to saturated fatty acids (SFA) with 18.49±0.01% of the total fatty acid composition. The total fatty acids dominated 92% whereas the rest belonged to traces below 0.05% each.

Table 1. Fatty acid composition of cold-pressed peanut oil (%). Fatty acid analyses were performed in duplicate and the variation between duplicates was less than 1%

Fatty acids	(%)
C16:0	**10.06±0.00^{b}**
C18:0	**4.40±0.01^{c}**
C18:1, trans 1	0.51±0.00^{e}
C18:1, trans 2	0.25±0.00^{e}
C18:1, trans 3	0.16±0.01^{e}
C18:1 D9	**34.19±0.01^{a}**
C18:1 D11	0.57±0.01^{e}
C18:2	**36.13±0.01^{a}**
C18:3	0.33±0.00^{e}
C20:0	0.10±0.00^{e}
C20:1	**1.37±0.00^{d}**
C20:2, 11, 14	0.16±0.00^{e}
C24:0	**3.93±0.00^{c}**
SFA	**18.49±0.01**
MUFA	**37.05±0.03**
PUFA	**36.62±0.01**
Total	**92.16±0.05**

GC analyses were performed in duplicate. The different letters mean significant differences ($p<0.05$) among results. The letters are a, b, c, d and e according to the decrease in the result values.

3.2. Oxidative stability

Regarding the results from Table 2, the cold-pressed peanut oil from Macedonian "Virginia" variety had a very good oxidative stability index (OSI) of 6.3±0.3 h which corresponded to over 18% of saturated fatty acids in this oil. Furthermore, the relatively high oxidative stability of this oil can be explained by the high content of monounsaturated oleic acid with an abundance of 34.19%. On the other hand, the remarkable stability of this oil can be explained by the Maillard Reaction Products (MRPs) formed during roasting, which are products of the reactions among reducing sugars and amino acids at elevated temperatures and low moisture. Durmaz *et al.* (2010) explained that the MRPs obtained from model systems could also retard the oxidative deterioration of oils. According to their findings, during the process of roasting apricot kernel seeds, the degradation of naturally occurring antioxidants and formation of antioxidant MRPs occured together. Under severe roasting conditions, the degradation rate might have been higher than the formation of MRPs and the total antioxidant capacity could be reduced. According to the findings of O' Keefe et al., high oleic peanut oils with 75.6% oleic acid had better oxidative stability than normal oil (O' Keefe *et al*., 1993). Almost equal amounts of oleic and linoleic acid and a significant amount of Vitamin-E-active compounds resulted in oxidative stability for over 6 h.

3.3. Peroxide value, free fatty acids (acidity) and specific extinction

Peroxide value and specific extinction at 232 nm summarize the oxidative state, while the amount of free fatty acids reveals some information about the quality of the raw material used. The content of free fatty acids in Macedonian peanut oil was significantly below the limit defined for cold-pressed and Virgin oils by the Codex Standard for Named Vegetable Oils as 2.0%. This was an indication of

TABLE 2. Oil stability index (OSI) from the Rancimat test, peroxide number, free fatty acids (FFA), specific extinction and DPPH assay

Cold- pressed peanut oil	Induction time at 120 °C (h)	Peroxide number (meq O_2·kg^{-1} oil)	FFA (%)	Specific extinction		Consumption of DPPH after 15 min. at 517 nm (equivalent as mg·L^{-1} α-tocopherol)
				K_{232}	K_{268}	
Sample	6.3±0.3[b]	5.1±0.1[b]	0.55±0.00[c]	1.82±0.00[c]	0.22±0.00[d]	288.63±59.78[a,b]

All analyses were performed in duplicate. The different letters mean significant differences ($p<0.05$) among results. The letters are a, b, c and d according to the decrease in the result values.

the use of high quality raw material for the preparation of cold-pressed peanut oil.

3.4. Vitamin-E-active compounds

The fatty acid profile of the oil is not the only indicator for the identity and quality of an oil. Vitamin-E-active compounds such as tocopherols and tocotrienols are very important minor compounds responsible for the oxidative stability of the oil and its antioxidant activity.

Table 3 shows almost equal amounts of α and γ tocopherols (14.38±0.20 and 14.51±0.20 mg·100 g^{-1} of oil, respectively). The total level of Vitamin-E-active compounds in Macedonian peanut oil was similar to the results published by Jonnala et al. According to their findings, the peanut oil from the variety "Tamrun 96" had the highest tocopherol content with 32.2 mg·100 g^{-1} oil (Jonnala *et al*., 2006). Macedonian peanut oil from the "Virginia" variety had 29.56±0.4 mg·100 g^{-1} of oil of total Vitamin-E-active compounds. The levels of β-tocotienol (0.35±0.00 mg·kg^{-1} of oil) and δ-tocopherol (0.31±0.00 mg·kg^{-1} of oil) were in good agreement with the published results of Carrín and Carelli (Carrín and Carelli, 2010).

3.5. Antioxidant activity by DPPH assay

Oxidative stability and antioxidant activity are two parameters which explain the resistance of oils against oxidative deterioration by oxygen during heating and storage. Antioxidant effectiveness of oil is dependent on the extent to which the antioxidant participates in side reactions, such reactions with species other than peroxyl radicals. These side reactions will decrease the level of antioxidant active compounds such as tocopherols, phytosterols and phenolic compounds and lead to the formation of active radicals able to initiate new oxidation reaction chains.

Statements in our work are very similar to those made by Tuberoso *et al*. (2007); Kostadinović Veličkovska and Mitrev (2013) and Kostadinović Veličkovska *et al*. (2015) who concluded that free radical scavenging was mainly influenced by the tocopherol content and polyunsaturated fatty acids in oil.

3.6. Sterol composition in oils

Pytosterols are minor usaponifiable compounds which are predominant in cold-pressed oils and almost absent in refined oils. Their presence in the oils is frequently connected to higher antioxidant activity (Grosso *et al*., 1997). The published results for the total level of pytosterols in peanut oil were in the range of 900–4344 mg·kg^{-1} of oil (Carrín and Carelli, 2010).

Table 4 shows the level of particular phyrosterols as well as the total content of phytosterols in peanut oil (2658.59±74.82 mg·kg^{-1} of oil). β-sitosterol was the major phytosterol with amounts of 1812.21±22.17 mg·kg^{-1} oil. Champesterol was the second, most dominant phytosterol in cold-pressed peanut oil with a level of 320.55±17.07 mg·kg^{-1} oil. Δ5-avenasterol was found to be the third most abundant sterol with a level of 236.16±14.18 mg·kg^{-1} oil. It was found that Δ5-avenasterol has an antipolymerization effect, which could protect the oil from oxidation during prolonged heating at high temperatures. The significant oxidative stability of cold-pressed peanut oil can be attributed to the presence of higher levels of this phytosterol apart from the fatty acid profile and vitamin-E-related compounds in the oil. Stigmasterol was present in the amount of 133.12±12.51 mg·kg^{-1} oil and all other phytosterols were present in amounts bellow 35 mg·kg^{-1} oil. The results of the phytosterol composition obtained from Macedonian peanut oil from "Virginia" variety were in good agreement with the results of Grosso *et al*. (1997). According to their findings, β-sitosterol was detected at levels of 55.3 to

TABLE 3. Vitamin-E-active compounds in cold-pressed oils (mg·100 g^{-1} of oil). HPLC analyses were performed in duplicate

	α-tocopherol	γ-tocopherol	β-tocotrienol	δ-tocopherol	Total
Cold- pressed peanut oil	**14.39±0.2[a]**	**14.51±0.2[a]**	0.35±0.0[b]	0.31±0.0[b]	**29.56±0.4**

HPLC analyses were performed in duplicate. The different letters mean significant differences ($p<0.05$) among results. The letters are a and b according to the decrease in the result values.

TABLE 4. Determination of the content of pytosterols (mg·kg^{-1})

Phytosterols in cold pressed peanut oil	
Cholesterol	3.78±0.25^{i}
Brassicasterol	7.99±0.09^{h}
24-Methylencholesterol	14.55±0.02^{g}
Champesterol	**320.55±17.07^{b}**
Champestanol	32.55±1.72^{e}
Stigmasterol	**133.12±12.51^{d}**
Δ7-Champesterol	5.78±0.59^{g}
Δ5,23-Stigmastadienol	0.81±0.01^{j}
Chlerosterol	13.81±0.11^{g}
ß-Sitosterol	**1812.21±22.17^{a}**
Sitostanol	29.88±1.33^{e}
Δ5-Avenasterol	**236.16±14.18^{c}**
Δ5,24-Stigmastadienol	14.52±1.28^{g}
Δ7-Stigmastenol	8.63±1.07^{h}
Δ7-Avenasterol	24.25±2.52^{f}
Total	**2658.59±74.82**

GC analyses were performed in duplicate. The different letters mean significant differences ($p<0.05$) among results. The letters are a, b, c, d, e, f, g, h and i according to the decrease in the result values.

61.6% from the total phytosterols in all peanut varieties. The abundance of β-sitosterol in Macedonian peanut oil was 68.16% from the total phytosterol content. Campesterol was present from 13.7 to 17.2% in the samples from Bolivia and Argentina. The content of campesterol in Macedonian peanut oil was 12.06%. However, Macedonian peanut oil from the "Virginia" variety consists of almost double the quantity of Δ5-avenasterol in comparison to stigmasterol. In the samples of peanut oil from Bolivia and Argentina almost equal contents of both phytosterols were detected (Grosso *et al*., 1997).

4. CONCLUSIONS

Cold-pressed peanut oil obtained from organically produced peanuts from the Macedonian "Virginia" variety was investigated for the first time. The analytical methods presented in this study can be used for fast and accurate chemical characterization of major and minor constituents present in vegetable oils.

Results from this study show that the most important class of compounds responsible for the antioxidant activity of peanut oil measured by the DPPH assay was Vitamin-E-active compounds. Almost equal amounts of α and γ-tocopherol indicated peanut oil as a valuable source of Vitamin-E-active compounds with a total amount of 29.56±0.4 mg·100 g^{-1} oil. Phytosterols are the second most important class of compounds which can participate in the antioxidant potential of the oil. Apart from β-sitosterol as the most abundant phytosterol (1812.21±22.17 mg·kg^{-1} oil), the other three phytosterols, Δ5-avenasterol (236.16±14.18 mg·kg^{-1} oil), champesterol (320.55±17.07 mg·kg^{-1} oil), and stigmasterol (133.12±12.51 mg·kg^{-1} oil) can contribute to the antioxidant potential of the oil. However, the antioxidant assay indicated Vitamin-E-active compounds as the most active against the DPPH radical. Finally, oxidative stability is strongly influenced by the degree of unsaturation of fatty acids as well as the effect of roasting.

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