Research Article

Quality evaluation of cold-pressed edible oils from Macedonia

Sanja Kostadinović Veličkovska[1], Ludger Brühl[1], Saša Mitrev[2], Hamed Mirhosseini[3] and Bertrand Matthäus[1]

[1] Max Rubner-Institut, Working Group for Lipid Research, Detmold, Germany
[2] Faculty of Agriculture, University "Goce Delčev", Štip, Macedonia
[3] Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, Selangor, Malaysia

The chemical composition and quality of eight pure cold-pressed oils and two blend oils from Macedonia were examined in this work. The highest level of oleic acid was determined in apricot kernel oil and rapeseed oil with abundance of 70.9 and 59.2%, respectively. The highest level of polyunsaturated fatty acids was seen for flaxseed and hemp seed oil with abundance of α-linolenic acid of 55.1% for flaxseed oil and linoleic acid of 57.4% for hemp seed oil. Apricot kernel oil and roasted sesame seed oil had the highest oxidative stability with induction times of 7.6 and 10.9 h. Apricot oil, hemp seed oil, and flaxseed oil had the highest level of total vitamin-E-active compounds with 58.8, 58.1, and 69.7 per 100 g of oil with predomination of γ-tocopherol. The highest peroxide number was detected in blend oils (oil 2 and oil 8) with values over 10 meq O_2/kg of oil. The highest FFA was detected in rapeseed oil (1.57%). Roasted and unroasted sesame seed oils had relatively high specific extinction (K_{232} values 3.55 and 3.33, respectively). However, the highest UV extinction had a blend of pumpkin seed oil and sunflower oil with a value of 3.84.

Practical applications: The results of this study can be applied for determination of the most important major and minor components responsible for quality evaluation of cold-pressed oils. Statistical results indicated a strong relationship between the level of monounsaturated fatty acids in the oil and their oxidative stability. The level of tocopherols and other vitamin-E-related compounds was in strong relationship with the antioxidant activity of the oils measured by the DPPH method. Phytosterols, as minor compounds present in the oils, did not contribute significantly to the total antioxidant potential of the oils but, their levels in particular oils, together with fatty acids, can be useful and reliable markers for the purity of the oils and determination of the composition of blends.

Keywords: Fatty acid profile / Fila oils / Macedonian oils / Quality control / Phytosterols / Tocopherols

Received: December 3, 2014 / Revised: March 1, 2015 / Accepted: March 27, 2015

DOI: 10.1002/ejlt.201400623

1 Introduction

Virgin oils are very popular as 100% natural products with a deep color, typical taste, and smell, produced only with cold-pressing and without any step of refining [1]. On international scale the Codex Alimentarius Standard for Named Vegetable Oils differentiates between virgin and cold-pressed oils. According to the international definition, every virgin oil is cold-pressed oil but not every cold-pressed oil is virgin because roasting of the seeds before cold-pressing is not allowed during production of virgin oils. Edible oils produced this way are good sources of tocopherols, carotenoids and chlorophylls, polyphenols, and other antioxidants with positive impact on human health [2].

Vegetable oils take a very popular place in human nutrition due to the high level of polyunsaturated fatty acids and tocopherols [3]. A positive relation between vegetable oil consumption and reduced risk of coronary heart diseases,

Correspondence: Sanja Kostadinović Veličkovska, Faculty of Agriculture, University "Goce Delcev", 2000 Stip, Macedonia
E-mail: sanja.kostadinovik@ugd.edu.mk

Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; **DPPH,** (diphenyl)-(2,4,6-trinitrophenyl) iminoazanium ion; **FA,** fatty acids; **GC,** gas chromatography; **GLC,** gas liquid chromatography; **PCA,** principal component analyses; **TEAC,** Trolox Equivalent Antioxidant Capacity; **TLC,** thin layer chromatography

level of LDL, degenerative diseases, and cancer is very well known [4].

The most important virgin oil in the western part of Europe, after olive oil, is rapeseed oil. High levels of monounsaturated fatty acids and lower levels of polyunsaturated fatty acids (below 30%) make this virgin oil suitable for cooking and another with range of applications [5].

Cold-pressed apricot oil obtained by screw pressing of the apricot kernels is a very interesting niche oil which has a good potential for thermal processing of food [6]. This oil is usually classified as cold-pressed and not as virgin because usually the kernels of the apricots are roasted before pressing. Significant high levels of monounsaturated oleic acid (higher than 60%) and low levels of polyunsaturated linoleic acid (less than 30%) make this oil popular for cooking and deep-frying of food [7, 8]. The nutritional quality of this oil was tested in the period of 13 wk with albino rats and the results did not indicate any toxic effect of amygdalin which on hydrolysis liberates toxic hydrogen cyanide [9].

The most used cooking oil in traditional cuisine of Asian counties is sesame oil [10]. Regarding the incredible thermal stability of this oil, the effect of sesamol, sesamin, and sesamolin was studied by Lee et al. [11, 12]. According to their results, the degree of degradation of those lignins is lower during the heating time in comparison to the degree of degradation of α-tocopherol. Final conclusion was that those lignins can be used as stronger and more effective antioxidants during deep frying in comparison to α-tocopherol [13, 14]. In the work of Konsoula et al., the extract from unroasted coated sesame seeds showed significant degree of inhibition of oxidation of other oils which can be connected to the high level of lignins in the sesame seeds [15]. This is the reason why we examined two sesame seed oils produced from the same plant material but one oil can be classified as virgin and the other was cold-pressed sesame seed oil with roasting of the seeds before cold-pressing.

Traditional Balkan cuisine as well as cuisines of South-East European countries mainly uses cold pressed and refined sunflower oil. The highest nutritional value of this seed oil is in relationship with significant levels of α-tocopherol [16].

The effect of roasting on the chemical composition and oxidative stability of pumpkin seed oil was examined by Vujasinovic et al. Results from their experiments showed that roasting at 90, 110, and 130°C increased the content of phospholipids, total phenolics, and total tocopherols in oils which resulted in increased induction period from 4.50 to 12.93 h [17].

Oils, better known for medicinal use than as cooking oils, are flaxseed oil, black cumin oil, and hemp seed oil. However, production of cold-pressed flaxseed oil with high quality is not simple due to the very high content of polyunsaturated fatty acids. More than 50% of the total fatty acids in flaxseed oil represent α-linolenic acid. That is the reason of oil instability and fast oxidation [18].

The content of thymoquinone, fatty acid profile, oxidative stability, and antioxidant potential of six different batches of black cumin oil was examined by Lutterodt et al. According to their findings, the oxidative stability index (OSI) of these oils was in the range of 76–155 h and the thymoquinon content of 3.48 to 8.73 mg/g [19]. Regarding the sterol composition of black cumin oil, Cheikh-Rouhou et al. reported β-sitosterol as major sterol in the oil with 44% and 54% followed by stigmasterol with 16.57% and 20.92% [20]. In the work of Ramadan and Morsel, the levels of polar lipids in crude oils correlate with the oxidative stability [21].

Matthäus and Brühl reported more than 80% polyunsaturated fatty acids in virgin hemp seed oil and tocopherol contents between 800 and 1100 mg/L [22]. Latif and Anwar published similar results for the tocopherol content in hemp seed oil produced with enzyme assisted cold-pressing. According to their findings, the level of tocopherols was between 724.4 and 788.8 mg/kg oil and induction times by the Rancimat method were found between 1.35 and 1.71 h [23].

It is obvious that there are already many published studies on the chemical composition and antioxidant potential of edible oils, but, till now, to the best of our knowledge there are no published results for the quality of the most consumable edible oils in Macedonia. Furthermore, some of the plants such sesame, black cumin, and hemp are cultivated for the first time in Macedonia for oil production in order to evaluate the possibility of production as well as the quality of the oils under climatic conditions in South-East region of Macedonia.

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 oxidative stability and antioxidant activity of the most used cold-pressed edible oils from Macedonia.

The second object of this study was to determine parameters which can serve as markers for the purity and the detection of adulterations of oils.

2 Materials and methods

2.1 Harvesting and selection of plant material

Seeds from sunflower (*Helianthus annuus*, *L*.) were collected in September 2012 from the sown fields of the Ovče Pole and Štip valleys. The flaxseed (*Linum usitatissimum*, *L*.) was collected at beginning of October 2012 from the sown fields of Povardarje valley. The kernel seeds from sweet and bitter apricot were collected in June 2012 from the region of Prespa lake. The seeds from hemp (*Cannabis sativa*, L.) and pumpkin (*Cucurbita pepo L*,) were collected 2012 from the region of Strumica. The seeds from black cumin (*Nigella*

sativa L.) and rapeseed (*Brassica napus L.*) were selected from the experimental valley of the Faculty of Agriculture in the region of Štip.

2.2 Purification and cold pressing

The purification process of the plant material started with removal of broken or damaged seeds. This step was necessary because unpurified plant material can negatively affect the quality of cold-pressed oils. After purification, the next step before pressing was preconditioning. During this step, the seeds were heated below 50°C for better extraction of oil and better cake formation. Process of cold pressing was performed by a Komet single press (IBG Monforts Oecotec, Germany). During the process of cold pressing the temperature did not exceed 45°C to avoid an increase of the content of chlorophyll, phosphorus, and free fatty acids which are responsible for a higher rate of oxidation. After pressing, the fresh cloudy oil was purified from solid impurities in the tanks by sedimentation within 17 days.

The quantities of plant material were collected only for needs of this experiment and yield of each cold-pressed oil was lower than 500 g with exception for sesame seeds because half of the quantity of the seeds was used for roasting.

After sedimentation, the collected oils were filtrated by using a protection filter and bottled in dark 250 mL bottle. All types of cold pressed oils were stored at temperatures below 15°C in the dark. Nine samples of experimental oils were 100% pure and two of the samples (oil 2 and oil 8) were blends of pumpkin seed oil–sunflower oil and apricot oil–flaxseed oil–hemp seed oil, respectively (Table 1). These blends were prepared in order to test the significance of chemical composition for the determination of adulterations of pumpkin seed oil with sunflower oil and hemp seed oil with flaxseed and apricot oil. The ratio of blend oil 2 was 80% pumpkin seed oil and 20% sunflower oil and the ratio of blend oil 8 was 40% flaxseed oil, 40% hemp seed oil, and 20% apricot kernel oil.

Table 1. Samples of cold-pressed oils

Samples	Cold-pressed edible oils from Macedonia
Oil 1	Apricot kernel oil
Oil 2	Blend of pumpkin seed oil and sunflower oil (80%:20%)
Oil 3	Sunflower oil
Oil 4	Flaxseed oil
Oil 5	Sesame seed oil
Oil 6	Hemp seed oil
Oil 7	Roasted sesame oil
Oil 8	Blend of apricot kernel oil, hemp seed oil, and flaxseed oil (20%:40%:40%)
Oil 9	Black cumin oil
Oil 10	Rapeseed oil

2.3 Analytical methods

2.3.1 Determination of the fatty acid composition

The fatty acid composition of Macedonian cold pressed edible oils was determined using gas chromatography equipped with flame ionization detector (FID). The esters were prepared using two drops of each oil dissolved in 1 mL of heptane. After addition of 50 μL of sodium methylate with concentration of 2 mol/L, the samples were homogenized. After homogenization, 100 μL of distilled water was added to each sample. Samples were centrifuged and the lower phase was removed while to the upper phase 50 μL of 1 M HCl was added. After second homogenization, sodium sulphate anhydride was added to remove water traces. Finally, the upper phase was transferred in 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. Analyzes were performed on Agilent 6890 equipped with KAS4Plus and FID. The oven temperature was programmed to increase from 150 to 240°C with a rate of 1.5°C/min and maintained isotherm 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. The retention times of separated peaks were confirmed by FAME standards.

2.3.2 Determination of tocopherols

One hundred milligram of each sample of virgin oils was dissolved in 1 mL of heptane. Determination of tocopherols was performed with an HPLC instrument equipped with a L6000 pump, a Merck–Hitachi F-1000 fluorescence detector with excitation wavelength on 295 nm and emission wavelength on 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 and the injection volume 20 μL. The mobile phase was a mixture of heptane and TBME in ratio 95:5.

2.3.3 Rancimat test

For the determination of the oxidative stability of Macedonian oils 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. For each sample of oil, the OSI index was determined in two portions by measuring eight 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 determination of peroxide value, DGF standard method C-VI 6a–Part 1 [05] according to Wheeler was used. In brief, for titration of the oils a standard solution of sodium thiosulfate was prepared with a concentration of 0.01 mol/L. 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 of 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 of 500 μL of saturated solution of potassium iodide the potentiometric titration was performed by using the automatic titrator, Methrom 888 Titrando (Methrom).

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 oils. 10.0 g of oil was dissolved in this mixture and titration was performed by potassium hydroxide with a concentration of 0.1 mol/L. Potentiometric titration was performed by using the automatic titrator, Methrom 888 Titrando (Methrom).

2.3.6 Determination of ultraviolet absorbance expressed as specific UV extinction

This method was equivalent with ISO 3656:2012. For this purpose, 1.0 g of oil was dissolved into 100-mL flask by iso-octane. The mixture was shacked and the solution was transferred in the rectangular quartz cells, with covers, having an optical length of 1 cm and determinations were done 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 oils.

2.3.7 Determination of phytosterols

The sterol composition of the oils was determined following ISO/FIDS 12228:1999 (E). In brief, 250 mg of oil was 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) on which fatty acid anions were retained and sterols passed through. The sterol fraction was separated from other unsaponifiable matter by thin-layer chromatography (Merck), re-extracted from the TLC material, and afterward, the composition of the sterol fraction was determined by GLC using betulin as internal standard. The compounds were separated on a 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, 245–260 °C at 5 °C/min. 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 the retention time.

2.3.8 Determination of total phenolic compounds and total flavonoids

In order to extract the phenolic compounds and flavonoids, 30 mL of hexane was added to 10 g of oil and triple extraction was performed with aqueous methanol (80% v/v). After extraction, the solvent was evaporated with a vacuum rotary evaporator at 40°C. Furthermore, the extracts were centrifuged (3000 rpm for 10 min.) and freezed on −20 prior to analyses.

For determination of total phenolic compounds, 0.5 mL of the methanol extracts were dissolved in 5 mL distilled water and mixed with 0.5 mL of 10 times diluted Folin-Ciocalteu's reagent (Sigma Chemical Co., St. Louis, MO, USA). One milliliter of saturated sodium carbonate (37%) was added to the mixture and it was topped up to 10 mL distillated water. After 3 h, the total phenolics were measured spectrophotometrically at 725 nm. Gallic acid as standard for the preparation of a calibration curve was used (Table 2) in the range of 30 to 300 mg/L.

Determination of flavonoids in the oils was performed by the method of Oomah et al. (1996). Methanolic extract (1 mL) was diluted in 3 mL of distillated water. Furthermore, 200 μL of diphenylboric acid 2-aminoethyl ester solution

Table 2. Calibration curves for total phenolic compounds, total flavonoids, TEAC, and DPPH assays

Total phenolic content	Calibration curve with gallic acid at 725 nm	$y = 0.1487x + 0.0589$ $R^2 = 0.9934$
Total flavonoids	Calibration curve with luteolin at 404 nm	$y = 0.4454x - 1.7234$ $R^2 = 0.9881$
Trolox equivalent antioxidant capacity (TEAC) assay	Calibration curve with trolox at 734 nm	$y = 9.6207x + 3.6883$ $R^2 = 0.9905$
DPPH antioxidant assay	Calibration curve with α-tocopherol at 517 nm	$y = 9.613x + 3.7693$ $R^2 = 0.9903$

(1%, v/v; Sigma Chemical Co.) was added to the mixture and the solution was measured at 404 nm. Luteolin as standard for the preparation of a calibration curve was used (Table 2) in the range from 10 to 100 mg/L.

2.3.9 Determination of antioxidant activity by DPPH and TEAC assays

The antioxidant activity of edible oils was estimated spectrophotometrically by using DPPH and TEAC assay.

For the DPPH assay, the antioxidant activities of the oils were expressed as percentage of decolorization of a solution of the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl radical) at 517 nm. DPPH reagent was dissolved in hexane and 0.25 mL of the stock solution with a concentration of 0.5 M was used for the determination of the antioxidant activity. α-Tocopherol with different concentrations (100–1000 mg/L) was dissolved in hexane and was used as standard for the preparation of the calibration curve (Table 2). The measurements for the oils were performed by direct incorporation of 10 μL of pure oil to 490 μL of DPPH reagent.

The Trolox equivalent antioxidant assay (TEAC) employed in this study gives a measure of the antioxidant activity of methanol extracts of the oils under study. For this purpose 10 mL of ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) solution was prepared from 39.23 mg of ABTS and 7.17 mg of $K_2S_2O_2$ dissolved in Nanopure water to volume. For the calibration curve with Trolox, four standard solutions were prepared in the range from 10 to 500 mg/L (Table 2).

2.3.10 Statistical analyses

Principal component analyses (PCA) was performed to gain an overview of how the samples were correlated to each other with regard to fatty acid profile, induction time, peroxide value, FFA, specific extinction, DPPH assay, vitamin-E-active compounds, total phenolic compounds, and total flavonoids as well as phytosterols. Correlation matrix was applied in multivariate analyses with Minitab software 17 (Minitab Inc., USA).

Furthermore, 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 $P = 0.05$. The level of significance of differences between the percentages of fatty acids, level of tocopherols, level of phytosterols, total phenolic content and total flavonoids, and values of antioxidant activity measured by DPPH and TEAC assays 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.

The ANOVA results were classified using letters (different letters mean significant differences among results). The letters are a, b, c, d, e, f, g, h, i, and j according to the decrease of the result values.

3 Results and discussion

The type of the seeds and blends of the 10 studied cold-pressed oils are summarized in Table 1. As we can see from Table 1, oil 2 is a blend from cold-pressed pumpkin seed oil and sunflower oil since 100% pure pumpkin seed oil is very seldom used on Macedonian market due to its high price. Oil 8 is a blend of cold pressed apricot, hempseed, and flaxseed oil. All other samples were pure oils obtained from 100% native plant material.

3.1 Fatty acid composition

The fatty acid composition of the oils is presented in Table 3. Regarding the saturated fatty acids, black cumin oil had the highest percentage of palmitic acid (12.2%). Roasted and unroasted sesame seed oils had the same abundance of 9.3% of palmitic acid. Oleic acid as monounsaturated fatty acid was most dominant (70.90%) in cold-pressed apricot kernel oil while hemp seed oil had the lowest level of this monounsaturated fatty acid with only 13.2%. Comparing the results from the fatty acid composition of apricot kernel oil with those published in the work of Tian et al. shows very different results for the two main fatty acids: oleic and linoleic acid [6]. In the sample of Macedonian apricot kernel oil, the main fatty acid was oleic acid with abundance of 70.9%. On the other hand, apricot kernel oil which was examined by Tian et al. had 40.9% oleic acid and 49.3% linoleic acid which can induce lower oxidative stability in comparison to Macedonian apricot kernel oil. The fatty acid profile of Macedonian apricot kernel oil was almost identical to the results published for Turkish oil from *Prunus spp*. in the work of Matthäus and Özcan [8].

A blend of sunflower oil and pumpkin seed oil (oil 2), sunflower oil (oil 3), hemp seed oil (oil 6), and black cumin oil (oil 9) were the richest sources of linoleic acid. Comparing the fatty acid profile of oil 2 and oil 3 reveals very similar percentages of all determined fatty acids. Therefore it seems to be impossible to calculate the percentage of sunflower oil in pumpkin seed oil on the basis of the fatty acid composition, only.

The fatty acid composition of hemp seed oil from Macedonia was in very good agreement with results published for hemp seed oil from New Zealand [24]. Results showed 57.4% linoleic acid and 17.1% α-linolenic acid and were very similar to those published from Matthäus et al. [22], and Latif et al. [23].

Flaxseed oil was the richest source of α-linolenic acid (ALA) with 55.1% of total abundance. Comparing the fatty acid composition of pure apricot kernel oil, flaxseed oil, and hemp seed oil with the fatty acid composition of oil 8 which is

Table 3. Fatty acid composition of cold pressed edible oils (%)

	C16:0	C18:0	C18:1D9	C18:1D11	C18:2	C18:3	C20:0	C20:1	C20:2 11, 14	C22:0
Oil 1	4.25 ± 0.00^{e}	1.36 ± 0.00^{j}	**70.90 ± 0.00^{a}**	1.21 ± 0.00^{b}	20.93 ± 0.00^{h}	0.74 ± 0.00^{e}	–	–	–	–
Oil 2	7.13 ± 0.01^{c}	3.88 ± 0.00^{d}	26.92 ± 0.01^{f}	0.67 ± 0.00^{j}	**60.30 ± 0.00^{a}**	0.18 ± 0.00^{j}	0.28 ± 0.00^{e}	–	–	0.60 ± 0.00^{b}
Oil 3	6.29 ± 0.02^{d}	3.85 ± 0.00^{e}	31.86 ± 0.02^{e}	0.63 ± 0.00^{j}	**56.30 ± 0.03^{d}**	0.27 ± 0.00^{h}	0.27 ± 0.00^{e}	–	–	0.76 ± 0.01^{a}
Oil 4	5.59 ± 0.01^{d}	4.35 ± 0.00^{c}	20.72 ± 0.00h	0.81 ± 0.00^{f}	13.37 ± 0.00^{j}	**55.14 ± 0.01^{a}**	–	–	–	–
Oil 5	**9.28 ± 0.02^{b}**	5.64 ± 0.00^{b}	40.57 ± 0.02^{c}	0.82 ± 0.00^{e}	42.72 ± 0.00^{f}	0.31 ± 0.00^{g}	0.63 ± 0.00^{b}	–	–	–
Oil 6	6.55 ± 0.03^{d}	3.09 ± 0.01^{g}	**13.20 ± 0.01^{j}**	0.71 ± 0.00^{h}	**57.40 ± 0.00^{c}**	**17.06 ± 0.00^{c}**	0.74 ± 0.00^{a}	–	–	0.36 ± 0.00^{c}
Oil 7	**9.28 ± 0.05^{b}**	5.77 ± 0.01^{a}	39.96 ± 0.02^{d}	0.83 ± 0.00^{d}	**43.17 ± 0.01^{e}**	0.34 ± 0.00^{f}	0.63 ± 0.00^{b}	–	–	–
Oil 8	6.06 ± 0.06^{d}	3.63 ± 0.01^{f}	**18.86 ± 0.01^{i}**	0.80 ± 0.00^{g}	**34.97 ± 0.03^{g}**	**35.00 ± 0.01^{b}**	0.36 ± 0.00^{d}	–	–	–
Oil 9	**12.19 ± 0.07^{a}**	3.01 ± 0.01^{h}	22.91 ± 0.02^{g}	1.04 ± 0.00^{c}	**58.17 ± 0.05^{b}**	–	–	–	**2.68 ± 0.01^{a}**	–
Oil 10	4.84 ± 0.04^{e}	1.71 ± 0.00^{i}	**59.16 ± 0.02^{b}**	2.95 ± 0.00^{a}	20.19 ± 0.01^{i}	9.29 ± 0.00^{d}	0.55 ± 0.00^{c}	**1.28 ± 0.02^{a}**	–	–

Fatty acid analyses were performed in duplicate and the variation between duplicates was less than 1%. (cont'd) The different letters mean significant differences among results. The letters are a, b, c, d, e, f, g, h, i, and j according to the decrease of the result values.

blend from these three oils, almost equal levels of linoleic and linolenic acid can be found. Furthermore, a very low level of oleic acid indicates that around 20% of apricot kernel oil was added to the mixture of equal amounts of hemp seed oil and flaxseed oil.

The fatty acid profiles of unroasted and roasted sesame oil show the results from the same raw material but different pretreatments of the seeds. Both sesame seed oils consist of a similar amount of oleic acid and linoleic acid and a very small amount of α-linolenic acid (with abundance of around 0.3%). The results are in very good agreement with those published in the work of Park et al. who used the fatty acid profile for the discrimination of blended sesame seed oil with soybean oil [25]. Black cumin oil was high in linoleic acid and the level of α-linolenic acid was almost identical to of the results from Lutterodt et al. Rapeseed oil also had a favored fatty acid profile with 59.2% of oleic acid [19].

3.2 Oxidative stability of oils

The results from the fatty acid composition in Table 3 and the oxidative stability of the same oils in Table 4 show a very good correlation. As we can see from Table 4, cold-pressed apricot kernel oil had a very good oxidative stability index (OSI) of 7.6 h. The relatively high oxidative stability of this oil can be explained by the high content of oleic acid with abundance of over 70%. However, cold pressed apricot kernel oil is not the most stable cold pressed oil.

As we can see from the Table 4, roasted sesame seed oil has the highest oxidative stability index with 10.9 h. Although this oil consists of 9.3% saturated palmitic acid and 43.1% of monounsaturated oleic acid, the fatty acid profile is not the reason for this high stability. The highest impact on the oxidative stability of this oil has the roasting process of the seeds of sesame. Roasting of sesame seeds is essential because of two reasons. The first and less important reason is the deactivation of enzymes which usually react with triacylglycerols (TAG) affecting the oil quality for instance by hydrolysis or production of phospholipids [26]. However, the fatty acid composition of roasted and unroasted sesame oils show almost the identical profile, but roasted sesame seed oil had more than double the stability of unroasted sesame seed oil which leads to conclusion that deactivation of enzymes as lipase and lipoxygenase by heat pre-treatment can not significantly improve the oxidative stability of the oil. This improved stability is most probably provided by Maillard Reaction Products (MRPs) formed during roasting, which are products of the reactions between reducing sugars and amino acids at elevated temperatures and low moisture. Durmaz et al. explained that MRPs obtained from model systems could also retard the oxidative deterioration of oils. According to their findings during the process of roasting of apricot kernel seeds, degradation of naturally occurring antioxidants, and formation of antioxidant MRPs occurs together. Under severe roasting conditions, the degradation

Table 4. Oil stability index (OSI) from Rancimat test, peroxide number, free fatty acids, specific extinction, and DPPH assay

Cold pressed virgin oils	Induction time at 120°C (h)	Peroxide number (meq O_2/kg of oil)	FFA (%)	Specific extinction K_{232}	Specific extinction K_{268}	Consumption of DPPH after 15 min at 517 nm (equivalent as mg/L α-tocopherol)
Oil 1	**7.6 ± 0.3^{b}**	7.1 ± 0.1^{b}	0.24 ± 0.00^{f}	1.99 ± 0.00^{f}	0.13 ± 0.00^{h}	**547.58 ± 59.78^{a,b}**
Oil 2	2.3 ± 0.1^{f}	**10.6 ± 0.1^{a}**	0.98 ± 0.00^{b}	**3.84 ± 0.03^{a}**	**1.25 ± 0.00^{b}**	**294.42 ± 115.18^{e}**
Oil 3	2.6 ± 0.3^{f}	2.6 ± 0.3^{d}	0.91 ± 0.00^{c}	2.42 ± 0.01^{d,e}	0.16 ± 0.00^{g}	231.84 ± 138.88^{g}
Oil 4	**1.5 ± 0.0^{g}**	1.1 ± 0.1^{e}	0.10 ± 0.00^{g}	2.38 ± 0.00^{e}	0.23 ± 0.01^{f}	**616.77 ± 73.91^{a}**
Oil 5	4.2 ± 0.1^{d}	4.8 ± 0.1^{c}	0.20 ± 0.01^{g}	**3.33 ± 0.11^{c}**	**0.72 ± 0.00^{c}**	559.77 ± 129.83^{b}
Oil 6	**1.5 ± 0.1^{g}**	7.7 ± 0.2^{b}	0.91 ± 0.03^{c}	2.48 ± 0.00^{d}	0.33 ± 0.00^{e}	**508.94 ± 212.16^{b}**
Oil 7	**10.9 ± 0.3^{a}**	**0.7 ± 0.0^{f}**	**0.37 ± 0.00^{e}**	**3.55 ± 0.02^{b}**	**1.58 ± 0.01^{a}**	579.97 ± 172.61^{a,b}
Oil 8	**0.4 ± 0.0^{h}**	**10.3 ± 0.4^{a}**	0.93 ± 0.00^{c}	2.45 ± 0.02^{d}	0.26 ± 0.01^{f}	276.04 ± 76.65^{f}
Oil 9	**4.28 ± 0.0^{c}**	705 ± 0.2^{b}	0.85 ± 0.00^{d}	2.05 ± 0.03^{e}	0.54 ± 0.02^{d}	325.05 ± 25.92^{d}
Oil 10	3.1 ± 0.0^{e}	4.7 ± 0.5^{c}	**1.57 ± 0.00^{a}**	1.86 ± 0.01^{g}	0.11 ± 0.00^{h}	433.69 ± 136.92^{c}

All analyses were performed in duplicate. The different letters mean significant differences among results. The letters are a, b, c, d, e, f, g, and h according to the decrease of the result values.

rate might have been higher than the formation of MRPs and the total antioxidant capacity could be reduced [27].

Furthermore, the susceptibility against oxidative deterioration of hemp seed oil, flaxseed oil, and oil blend 8 can be explained also by the fatty acid composition. In terms of fatty acids, hemp seed oil had very high abundance of polyunsaturated fatty acid for instance α-linolenic acid and a very low level of monounsaturated fatty acids e.g., oleic acid. Flaxseed oil, with over 50% of α-linolenic acid is also a very unstable oil and blending these two oils with apricot oil did not really improve the oxidative stability. According to the findings of Brühl et al., samples from different varieties of hemp seeds, had identical fatty acid composition with significant high levels α-linolenic acid of about 47–55%.

Although it might be that a higher content of thymoquinone can improve the oxidative stability of black cumin oil, this oil had a lower oxidative stability in comparison to published data [19]. Possibly this can be explained by the lowest level of vitamin-E-active compounds (24.8 mg/100 g oil) found in the present work. The lowest oxidative stability showed the blend of pumpkin seed oil and sunflower oil (oil 2) with an induction period of 2.3 h. This strongly indicated that seeds of pumpkin were not roasted at elevated temperature forming antioxidant active MRPs.

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

In Table 4 results for parameters describing the quality of the different oils are presented. 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 used raw material.

The lowest peroxide value was found for roasted sesame seed oil with 0.7 meq O_2/kg of oil and flaxseed oil with 1.1 meq O_2/kg of oil, while the other oils showed significant higher peroxide values from 2.6 to 10.6 meq O_2/kg of oil. None of the oils exceeded the limit of 15 meq O_2/kg of oil given by the Codex Standard for Named Vegetable Oils for cold-pressed and virgin oils. The quality of the roasted sesame oil was also confirmed by the lowest percentage of free fatty acid (0.37%) and the highest oxidative stability (10.9 h). On the other hand, oil blend 8 had a relatively high peroxide value (10.3 meq O_2/kg) which was in good correlation with the lowest OSI (0.4 h). Both indices are indications for the instability of the oil blend. Although the Rancimat test did not show a very low OSI value blend 2 had the highest peroxide value of 10.6 meq O_2/kg of oil, similar to blend 8 which indicates a weak oxidative state of this oil.

For all oils the content of free fatty acids was significant below the limit defined for cold-pressed and virgin oils by the Codex Standard for Named Vegetable Oils as 2.0%. Except of rapeseed oil for all other oils the content of free fatty acids was below 1%. This was an indication for the use of high quality raw material for the preparation of the different oils.

Results for specific extinction presented in Table 4 showed the highest level for oil blend 2 and unroasted and roasted sesame oil which is an indication for the presence of conjugate diene and triene systems.

Only poor correlations below $R = 0.25$ were found between oxidative stability and peroxide value and antioxidant activity, respectively, showing that the contribution of the peroxide value and the antioxidant activity to the oxidative stability of the oils was only weak in comparison to other parameters such as fatty acid composition.

3.4 Vitamin-E-active compounds

Fatty acid profile of the oils is not the only indicator for the identity of oils. 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 5 shows that the lowest amount of tocopherols and tocotrienols was detected for black cumin oil (24.8 mg/100 g of oil) and the richest source of vitamin-E-active compounds was hemp seed oil (69.7 mg/100 g of oil). Hempseed oil had a significant level of γ-tocopherol (64.9 mg/100 g of oil) comprising of about 93% of the total vitamin-E-active compounds. It was reported that the high degree of unsaturation of fatty acids in flaxseed oil correlates with the high γ-tocopherol content. Kamal-Eldin et al. suggested a positive correlation between content of C18:3 and γ-tocopherol [28]. Although blend oil 8 contained a high amount of γ-tocopherol, the other parameters indicated a low stability. It is obvious that the level of total vitamin-E-active compounds of 63.2 mg/100 g of oil was insufficient to protect the oil from oxidation.

Generally speaking, apricot kernel oil and hemp seed oil contained the highest amount of γ-tocopherol while in sunflower oil the highest content of α-tocopherol was found. The high content of α-tocopherol in comparison to other tocopherols can be used as marker for the purity of sunflower oil. However, the content of 12.4 mg of α-tocopherol/100 g in blend 2 can not be a reliable proof to identify 20% of sunflower oil in pumpkin seed oil. The results obtained for apricot kernel oil were in good agreement with the finding of Matthäus and Özcan [8]. Their results showed γ-tocopherol as dominant in all *Prunus* varieties.

β-Tocotrienol was detected in higher amounts in black cumin oil and in traces in apricot kernel oil. A significant content of plastochromanol-8 was detected in flaxseed oil. On the other hand, hemp seed oil was the richest in δ-tocopherol. Seven point nine milligram per hundred gram of plastochromanol-8 in blend oil 8 indicated the presence of flaxseed oil in apricot oil, but 1.4 mg/100 g of δ-tocopherol can not be an indication for the presence of hemp seed oil in apricot oil because apricot oil is a natural source of this tocopherol.

3.5 Total phenolic compounds and total flavonoids

The results of the total content of phenolic compounds and flavonoids are presented in Table 6. The highest content of total phenolic compounds was determined in black cumin oil with a level of 295.8 mg/kg oil which is in excellent agreement with results published by Tauseef Sultan et al. who found 310.26 mg/kg of phenolic compounds [29]. The oils from sesame seeds were also relatively rich in phenolic compounds, whereas sesame seed oil from unroasted seeds showed a higher level (133.42 mg/kg oil) in comparison to the oil from roasted seeds (128.98 mg of gallic acid per kg oil) since the heat treatment affects the extractability of phenolic compounds. The values were higher in comparison to those published in the work of Konsoula and Liakopoulou-Kyriakides [15], because these sesame seed oils were obtained by hexane extraction instead of cold-pressing in the present work. The extraction process by solvent preserve leaves most of the phenolic acids in sesame seed cake [15]. Furthermore, results for the content of phenolic compounds in crude rapeseed oil were ten times less than results

Table 5. Vitamin E active compounds in cold pressed oils (mg/100 g of oil)

	α-Tocopherol	β-Tocopherol	γ-Tocopherol	β-Tocotrienol	Plastochromanol 8	δ-Tocopherol	Total
Oil 1	–	–	**55.0 ± 0.2^{b}**	0.4 ± 0.0^{b}	–	**1.9 ± 0.0^{b}**	**58.8 ± 2.1**
Oil 2	12.4 ± 0.1^{b}	1.7 ± 0.1^{c}	10.7 ± 0.1^{f}	–	0.3 ± 0.0^{f}	0.4 ± 0.0^{f}	25.6 ± 1.7
Oil 3	**20.7 ± 0.1^{a}**	2.1 ± 0.1^{b}	5.1 ± 0.0^{g}	–	0.4 ± 0.0^{e}	0.2 ± 0.0^{g}	28.5 ± 1.1
Oil 4	0.2 ± 0.0^{f}	–	40.4 ± 0.2^{d}	–	**17.1 ± 1.1^{a}**	0.4 ± 0.0^{f}	**58.1 ± 3.9**
Oil 5	–	–	51.6 ± 0.1^{c}	–	–	0.5 ± 0.0^{e}	52.1 ± 1.0
Oil 6	1.7 ± 0.0^{d}	0.2 ± 0.0^{d}	**64.9 ± 4.3^{a}**	–	0.4 ± 0.0^{e}	**2.5 ± 0.1^{a}**	**69.7 ± 4.4**
Oil 7	–	–	51.9 ± 1.1^{c}	–	0.6 ± 0.1^{d}	0.5 ± 0.2e	53.1 ± 1.1
Oil 8	0.9 ± 0.0^{e}	–	53.0 ± 0.3^{c}	–	**7.9 ± 0.4^{b}**	**1.4 ± 0.0^{c}**	**63.2 ± 0.7**
Oil 9	0.1 ± 0.0^{g}	**2.3 ± 0.0^{a}**	2.1 ± 0.0^{h}	20.2 ± 0.1^{a}	–	–	**24.8 ± 0.1**
Oil 10	7.4 ± 0.1^{c}	–	33.8 ± 0.3^{e}	–	2.9 ± 0.2^{c}	0.7 ± 0.1^{d}	44.8 ± 0.7

HPLC analyses were performed in duplicate. The different letters mean significant differences among results. The letters are a, b, c, d, e, f, g, and h according to the decrease of the result values.

Table 6. Total phenolc compounds, total flavonoids and TEAC assay

Samples	mg of gallic acid/kg oil	mg of luteolin/kg oil	mg of Trolox/kg oil
Oil 1	37.95 ± 4.81^{h}	19.17 ± 3.98^{e}	44.55 ± 9.11^{h}
Oil 2	48.51 ± 1.12^{g}	18.14 ± 4.32^{f}	57.11 ± 2.99^{e}
Oil 3	60.82 ± 14.32^{e}	12.09 ± 1.14^{g}	52.81 ± 9.33^{g}
Oil 4	72.54 ± 21.19^{d}	$\mathbf{45.12 \pm 9.22^{b}}$	55.14 ± 12.42^{f}
Oil 5	$\mathbf{133.42 \pm 22.51^{b}}$	22.82 ± 5.12^{d}	$\mathbf{157.82 \pm 22.89^{b}}$
Oil 6	64.19 ± 11.12^{e}	14.55 ± 1.76g	52.11 ± 11.98^{g}
Oil 7	$\mathbf{128.98 \pm 33.98^{c}}$	19.12 ± 3.67e	$\mathbf{114.92 \pm 13.81^{c}}$
Oil 8	55.91 ± 8.19^{f}	39.12 ± 4.29^{c}	57.88 ± 8.31^{e}
Oil 9	$\mathbf{295.8 \pm 12.8^{a}}$	$\mathbf{69.18 \pm 1.55^{a}}$	$\mathbf{396.12 \pm 23.89^{a}}$
Oil 10	74.13 ± 4.19^{d}	11.13 ± 2.11^{h}	92.43 ± 17.31^{d}

All analyses were perfomed in duplicate. The different letters mean significant differences among results. The letters are a, b, c, d, e, f, g, and h according to the decrease of the result values.

published by Zheng et al. [30]. According to their findings, the level of polar phenolic compounds in samples of rapeseed oils determined by Folin Ciocalteu's reagent were in the range of 10.17 - to 80.63 mg/100 g of oil [30]. Koski et al. published even higher levels of total phenolic compounds in rapeseed oil in the range from 439 to 1066 mg/kg expressed as caffeic acid equivalents [31]. In the sample of Macedonian rapeseed oil, 74.13 mg/kg of total phenolic compounds expressed as gallic acid equivalent was determined.

The quantities of total flavonoids were significantly lower in comparison to the content of total phenolic compounds. The level of total flavonoids in flaxseed oil was approximately four times less than the levels published by Teh and Birch. The similar tendency was observed for cold pressed hemp seed oil and rapeseed oil [24]. Except black cumin oil and hemp seed oil with 69.18 and 45.12 mg luteolin/kg of oil, the other examined oils had levels of total flavonoids below 40 mg luteolin/kg of oil.

3.6 Antioxidant activity by DPPH and TEAC assays

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 the 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, sterols, and phenolic compounds and lead to the formation of active radicals able to initiate new oxidation reaction chains. Kinetic studies on the oxidation rate data are useful tools to interprete differences in the antioxidant effect of α- and γ-tocopherol. Turan et al. described that the antioxidant is effectively consumed in reactions with peroxyl radicals, when the induction period is linearly dependent on the initial concentration of the antioxidant, but it is an indication that the antioxidant participates in side reactions when this relation is not linear [32].

Results presented in Table 4 showed a good correlation between antioxidant activity of the oils related to α-tocopherol and the sum of total tocopherols and tocotrienols presented in Table 5. Flaxseed oil and hemp seed oil had the highest total level of tocopherols and these oils showed the highest antioxidant capacity by the DPPH method. Since, the level of total phytosterols were similar in both oils (Table 7), we can conclude that vitamin-E-active compounds had stronger impact on the total antioxidant activity in comparison to the impact of phytosterols.

The lowest antioxidant potential was found for sunflower oil because of the lower level of tocopherols and domination of α-tocopherol instead of γ-tocopherol. A comparison of the levels of vitamin-E-active compounds and the results from DPPH assay from all ten oils, shows that oils with higher levels of γ-tocopherol had higher antioxidant potential. It might be that γ-tocopherol had the strongest antioxidant potential in comparison with α-tocopherol and all other vitamin-E-active compounds [33].

The antioxidant activity of the methanolic extracts of the oils determined by Trolox equivalent antioxidant capacity (TEAC) assay was in very good correlation with content of total phenolic compounds ($r = 0.9856$). As can be seen from Table 6, the highest scavenger activity had the methanolic extract of black cumin oil with an antioxidant activity equivalent to 396.12 mg of Trolox/kg of oil. Furthermore, unroasted and roasted sesame seed oils indicated antioxidant activity equal to 157.82 mg and 114.92 mg of Trolox/kg of oil, respectively. The lowest antioxidant potential had apricot kernel oil with an antioxidant activity of 44.55 mg of Trolox/kg of oil.

Comparing the results from total flavonoids and total phenolic compounds with values obtained for TEAC and DPPH assay, it can be conclude that total phenolic compounds affect the values of antioxidant activity determined by TEAC assay. On the other hand, there is weaker correlation between results from total flavonoids and TEAC assay ($r = 0.7199$). In the work of Alu'datt et al., the

Table 7. Determination of the amount of sterols (mg/kg)

	Oil 1	Oil 2	Oil 3	Oil 4	Oil 5	Oil 6	Oil 7	Oil 8	Oil 9	Oil 10
Cholesterol	5.04 ± 0.25^{f}	7.94 ± 3.25^{e}	4.24 ± 0.01^{g}	21.16 ± 4.05^{h}	3.96 ± 0.05^{h}	14.78 ± 7.04^{c}	5.71 ± 3.03^{f}	20.25 ± 0.15^{b}	12.18 ± 5.08^{d}	29.45 ± 7.07^{a}
Brassicasterol	4.20 ± 0.07^{e}	4.13 ± 1.05^{e}	2.27 ± 0.05^{g}	23.76 ± 9.05^{b}	–	1.72 ± 0.05^{h}	8.58 ± 2.15^{d}	11.47 ± 0.09^{c}	3.48 ± 0.08^{f}	**533.39 ± 98.75^{a}**
24-Metylencholesterol	7.98 ± 2.01^{g}	6.36 ± 2.28^{g}	3.24 ± 0.98^{h}	72.58 ± 27.08^{c}	92.24 ± 13.58^{b}	$29.57 \pm 3.09e$	98.92 ± 14.05^{b}	47.92 ± 0.16^{d}	$20.89 \pm 9.68f$	**148.87 ± 45.21^{a}**
Champesterol	**199.17 ± 38.06^{e}**	**222.46 ± 32.01^{d}**	**227.15 ± 48.79^{d}**	**763.95 ± 128.19^{e}**	**973.35 ± 122.25^{b}**	**504.50 ± 27.59**	**966.34 ± 128.14^{b}**	**606.15 ± 128.55^{c}**	**182.80 ± 48.12^{e}**	**2979.80 ± 489.97^{a}**
Champestanol	29.41 ± 9.78^{e}	56.25 ± 12.37^{c}	48.67 ± 12.38^{d}	43.29 ± 12.88^{d}	71.87 ± 32.55^{a}	52.96 ± 12.21	70.33 ± 23.25^{a}	54.34 ± 13.13^{c}	37.08 ± 12.55^{e}	$66.08 \pm 37.58^{a,b}$
Stigmasterol	24.37 ± 4.03^{e}	**246.93 ± 28.17^{c}**	**273.55 ± 88.19^{c}**	**282.21 ± 98.21^{c}**	**$329.92 \pm 97.05^{a,b}$**	90.10 ± 48.24^{d}	**364.24 ± 49.67^{a}**	**172.46 ± 47.17^{c}**	**252.44 ± 48.75^{c}**	21.49 ± 12.55^{e}
Δ7-Champesterol	4.20 ± 2.59^{g}	53.39 ± 14.28^{b}	74.63 ± 25.05^{a}	41.34 ± 12.09^{c}	29.30 ± 17.05^{e}	33.36 ± 24.05^{d}	24.59 ± 12.23^{e}	21.94 ± 11.05^{e}	10.45 ± 1.15^{f}	39.80 ± 22.89^{c}
Δ5,23-Stigmastadienol	–	6.36 ± 0.07^{b}	8.76 ± 1.22^{a}	–	–	–	–	–	1.74 ± 0.00^{c}	–
Chlerosterol	$28.15 \pm 0.^{19c,d}$	20.02 ± 12.09^{d}	**292.05 ± 125.07^{a}**	26.04 ± 7.07^{d}	52.63 ± 21.22^{b}	21.32 ± 1.03^{d}	51.46 ± 27.45^{b}	20.92 ± 9.15^{d}	15.67 ± 4.59^{e}	34.23 ± 12.59^{c}
β-Sitosterol	**3394.37 ± 98.17^{a}**	**1806.37 ± 125.09^{c}**	**1634.36 ± 432.29^{c}**	**1451.73 ± 132.79^{c}**	**3376.72 ± 128.95^{a}**	**2311.35 ± 432.44^{b}**	**2274.05 ± 128.59^{b}**	**$1970.32 \pm 128.79^{b,c}$**	**828.72 ± 358.96^{d}**	**3656.50 ± 198.50^{a}**
Sitostanol	**123.96 ± 25.32^{b}**	34.96 ± 13.43^{d}	18.45 ± 7.07^{e}	28.32 ± 7.44^{e}	76.40 ± 38.11^{c}	52.96 ± 15.28^{d}	**1156.75 ± 459.89^{a}**	45.22 ± 12.19^{d}	68.42 ± 15.98^{c}	53.34 ± 14.89^{d}
Δ5-Avenasterol	**336.16 ± 74.18^{c}**	70.87 ± 25.44^{e}	69.12 ± 33.15^{e}	**399.39 ± 78.29^{b}**	**507.61 ± 128.57^{a}**	**219.06 ± 35.47^{d}**	**518.62 ± 28.89^{a}**	**302.74 ± 57.79^{c}**	**202.48 ± 47.89^{d}**	**268.28 ± 33.97^{d}**
Δ5,24-Stigmastadienol	44.12 ± 7.29^{d}	**145.23 ± 39.42^{a}**	37.97 ± 12.22^{d}	58.59 ± 13.48^{c}	79.23 ± 12.59^{b}	11.35 ± 4.04^{f}	87.48 ± 12.25^{b}	25.31 ± 12.59^{e}	31.34 ± 12.25^{e}	39.80 ± 12.55^{e}
Δ7-Stigmastenol	25.63 ± 12.07^{e}	**370.24 ± 48.45^{b}**	**471.49 ± 98.21^{a}**	22.78 ± 9.58^{e}	$35.65 \pm 24.31^{d,e}$	53.30 ± 13.34^{d}	44.03 ± 17.89^{d}	46.91 ± 21.88^{d}	44.74 ± 14.59^{d}	81.99 ± 34.55^{c}
Δ7-Avenasterol	17.22 ± 5.53^{e}	**139.51 ± 43.92^{a}**	**121.04 ± 29.27^{b}**	28.32 ± 14.09^{d}	50.93 ± 19.91^{c}	28.89 ± 16.68^{d}	49.76 ± 32.25^{c}	29.02 ± 26.68^{d}	33.60 ± 8.77^{d}	13.53 ± 2.59^{e}
Total	**4243.98 ± 279.54**	**3191.02 ± 401.32**	**3286.99 ± 913.95**	**3263.46 ± 554.29**	**5679.81 ± 656.19**	**3425.22 ± 640.55**	**5720.86 ± 939.73**	**3374.97 ± 469.37**	**1746.03 ± 588.44**	**7966.55 ± 1023.66**

All analyses were performed in triplicate. The different letters mean significant differences among results. The letters are a, b, c, d, e, f, g, h, i, and j according to the decrease of the result values.

antioxidant potential of flaxseed was connected to the significant amount of *p*-coumaric acid and ferulic acid with levels of 47.45 ± 2.46 and $23.36 \pm 2.25\%$ from the total phenolic content for full fat flaxseed [34].

Vitamin-E-active compounds had the strongest impact on the antioxidant activity of the oils measured by DPPH assay. This finding in the present work is very similar to the work of Tuberoso et al., who concluded that free radical scavenging was mainly influenced by the tocopherol content and the content of polyunsaturated fatty acids in the oils [35].

3.7 Sterol composition in oils

Phytosterols are plant sterols with structure similar to cholesterol. They are minor constituents in oils and normal constituents of human diet. Phytosterols, primarily β-sitosterol, campesterol, and stigmasterol are membrane constituents of plants that effectively reduce serum LDL cholesterol and atherosclerotic risk. Their presence in the oils frequently is connected to higher antioxidant activity [21].

Table 7 shows the content of total phytosterols with the highest level (7966.55 mg/kg of oil) in rapeseed oil and lowest level in black cumin oil (1746.03 mg/kg of oil). The level of total pytosterols for apricot oil (4243.98 mg/kg) was similar to the level of total pytosterols in apricot kernel oil found from Turan et al., for Hacikiz variety (3761 mg/kg oil) [34]. The total quantity of phytosterols in flaxseed oil from Macedonia (3263.46 mg/kg) was lower in comparison to the results of Ciftci et al. but champesterol was also the next dominant phytosterol after sitosterol [36].

In all the oils β-sitosterol was the major phytosterol with amounts in the range between 828.72 mg/kg for black cumin oil and 3656.5 mg/kg for rapeseed oil. Δ5-avenasterol was found to be the second most abundant sterol in almost all examined oils with exception of sunflower oil and blend of pumpkin seed oil and sunflower oil. The range of Δ5-avenasterol was between 268.28 mg/kg for rapeseed oil and 518.62 mg/kg for roasted sesame seed oil. It was reported that Δ5-avenasterol has an antipolymerization effect, which could protect oil from oxidation during prolonged heating at high temperatures [37]. Significant oxidative stability of apricot kernel oil and acceptable oxidative stability of flaxseed oil can be resulted from the presence of higher level of this phytosterol apart from fatty acid profile and vitamin-E-related compounds in these oils.

Sitostanol was present in apricot kernel oil with abundance of 123.96 mg/kg and in roasted sesame oil with 1156.75 mg/kg. Brassicasterol was found exclusively in rapeseed oil usable as marker for a mixture of rapeseed oil to other oils. Δ5,23-Stigmastadienol was absent in almost all oils and it was present only in trace amounts in sunflower oil and in the mixture of pumpkin seed oil and sunflower oil and black cumin oil. Unroasted sesame seed oil had a very similar concentration of total phytosterols to roasted sesame seed oil. Possibly these two oils are the best proof that phytosterols did

not contribute significant to the total oxidative stability of oils and that the key factor for the oxidative stability is the roasting process of the sesame seeds with the formation of MRP(s). The presence of campesterol was lowest in apricot kernel oil (199.17 mg/kg) and highest in rapeseed oil (2979.80 mg/kg). Δ7-Stigmastenol and Δ7-Avenasterol had significant amounts in the blend of sunflower oil and pumpkin seed oil and pure sunflower oil. The level of Δ7-stigmastenol was 471.49 mg/kg determined in blend oil and 370.24 mg/kg in sunflower oil.

Chlerosterol was present in higher level only in sunflower oil with dosage of 292.05 mg/kg.

The percentages of the three main phytosterols sitosterol, champesterol, and stigmasterol in black cumin oil were in good agreement with published results of Cheikh-Rouhou et al. [18]. Percentages of β-sitosterol and champesterol in hemp seed oil were in very good agreement with results of Matthäus and Brühl [23].

Oil 2 (blend of sunflower and pumpkin seed oil) and sunflower oil showed a similar phytosterol composition. Here the similar level of Δ7-stigmastenol in oil 2 can give an indication to the presence of sunflower oil as cheaper oil in pumpkin seed oil.

Finally, a comparison between the antioxidant activity and the phytosterol profiles show that phytosterols are more useful as markers for the purity of oils than for their contribution in the overall antioxidant potential.

3.8 Application of principal component analyses (PCA) for separation and classification of Macedonian cold-pressed oils

As shown in Fig. 1, Principal Component Analyses (PCA) was applied in order to see grouping, separation, and classification of the edible oils by all measured parameters, fatty acid profile, oxidative stability (Rancimat test), peroxide value, FFA, specific extinction, vitamin-E-active compounds, phytosterols, total phenolic compounds, total flavonoids, DPPH, and TEAC assays.

Figure 1 showed a separation of the oils in three groups. The first group in the negative part of PC1 consists of two oils with similar properties: virgin sunflower oil and blend of sunflower and pumpkin seed oil. Figure 1 reveals that similarities between these two oils are due to similar profile of fatty acids, vitamin-E-active compounds, and phytosterols. The other two groups which were classified by PCA due to similar properties of the oils can be found in the positive part of PC1. The first group of oils in the positive part of PC1 consists of apricot oil [1], flaxseed oil [4], hemp seed oil [6], and a mixture of these two oils with apricot kernel oil [8]. The dominant fatty acid in flaxseed oil was ALA with abundance of 55.14% and the dominant fatty acid in hemp seed oil was linoleic acid with 57.40%, followed by significant high levels of γ-tocopherol and very low quantities of saturated fatty acids: palmitic and stearic acid. As could have been expected, the most similar oil to them was blend oil 8 which was mixture of 40% of flaxseed oil, 40% of hempseed oil, and only 20% of apricot kernel oil.

The second group of oils in the positive part of PC1 consists of unroasted [5] and roasted sesame seed oil [7]. Although roasted sesame seed oil had the highest oxidative stability, significantly higher than that of unroasted sesame seed oil, the influence of other parameters describing the composition of the oils and showing that these two oils were obtained from the same plant material led to a grouping of the two oils in the same area of the PCA.

was found in the negative part of PC1 and the positive part of PC2, apart from the other oils. One possible reason

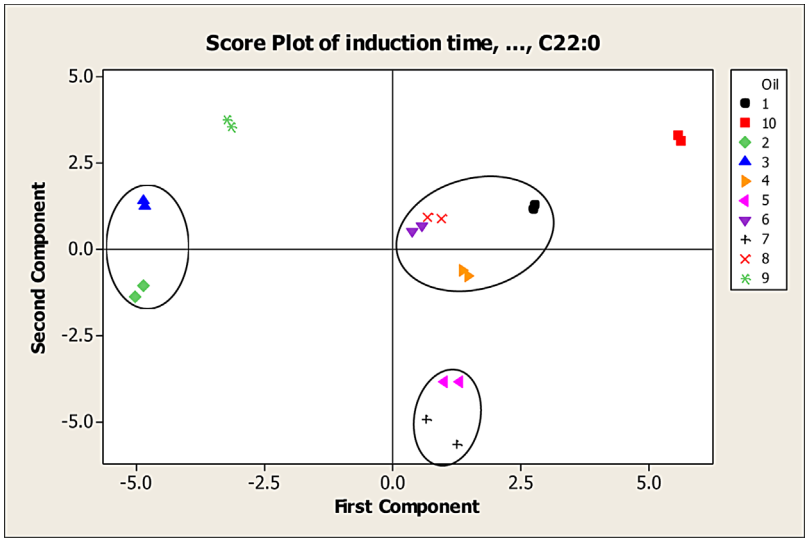

Figure 1. Score plot of fatty acid profile, induction time, peroxide number, FFA, specific extinction, total phenolic compounds, total flavonoids, DPPH assay, vitamin-E-active compounds, and phytosterols.

that this oil did not belong to any of the three groups could be the highest level of total phenolic compounds and total flavonoids as well as significant higher antioxidant potential determined by TEAC assay.

Furthermore, rapeseed oil [10] was found apart from the other groups in the positive part of PC1 and PC2. This oil had significantly higher amount of total phytosterols as well as the highest level of brassicasterol, 24-methylencholesterol, campesterol, and β-sitosterol.

4 Conclusions

Eight cold-pressed oils from Macedonia as well as two blends were investigated regarding the composition of the main constituents and different quality parameters. These results were used to differentiate the cold-pressed oils by Principle Component Analysis. The study has shown that the quality of different types of cold-pressed oils produced in Macedonia were similar to oils from other locations described in literature. The investigation also revealed that it was possible to differentiate different types of oil mainly according to their fatty acid composition, the amount of vitamin-E-active compounds, and phytosterols while the contribution of other quality parameters e.g., describing the oxidative state have only a small effect. As a result of PCA with the different parameters it was possible assign sunflower oil to the blend of sunflower oil and pumpkin seed oil and apricot oil, hemp seed oil and flaxseed oil to a blend of these three oils, respectively. Additionally the two sesame oils, roasted and unroasted were grouped together although both oils showed significant differences with regard to the oxidative stability. The results also revealed that a differentiation of back cumin oil and rapeseed oil from the other oils was possible since they were located apart from the other oils in the PCA plot. It can be concluded that the composition of the fatty acids, vitamin-E-active compounds, and phytosterols are helpful parameters for the characterization and differentiation of different vegetable plant oils or blends of it.

Finally, antioxidant activity of the oils depends on the assay which is applied. The highest impact on the antioxidant activity of the oils determined by DPPH had vitamin-E-active compounds. On the other hand, the methanol extracts from the oils with the highest levels of total phenolic compounds indicated the highest antioxidant activity obtained by (TEAC) assay.

Research grant for University Academics and Scientist from Deutscher Akademischer Austausch Dienst (DAAD) for Sanja Kostadinović Veličkovska is gratefully acknowledged.

The authors have declared no conflict of interest.

References

[1] Matthäus, B., Oil Technology, *Technological Innovations in Major World Oil Crops*. in: SK Gupta (Ed.), Springer, New York 2012, *2*, pp. 23–92.

[2] Matthäus, B., Spener, F., What we know and what we should know about virgin oils—A general introduction. *Eur. J. Lipid Sci. Technol.* 2008, *110*, 597–601.

[3] Sullivan, T. A. O'., Ambrosini, G. L., Mori, T. A., Beilin, L. J., Oddy, W. H., Omega-3 index correlates with reduced cardiovascular disease risk factors in adolescent boys. *Lipids* 2011, *46*, 59–67.

[4] Varney, M. E., Buchanan, J. T., Dementieva, Y., Hardman, W. E., Sollars, V. E., A high Omega-3 fatty acid diet has different effects on early and late stage myeloid progenitors. *Lipids* 2001, *46*, 47–57.

[5] Matthäus, B., Brühl, L., Amoneit, F., The DGF Rapeseed Oil Award—A Tool to Improve the Quality of Virgin Edible Rapeseed Oil. *Lipid Technol.* 2008, *20*, 31–34.

[6] Tian, H., Zhang, H., Zhan, P., Tian, F., Composition and antioxidant and antimicrobial activities of white apricot almond (*Amygdalus communis L.*) oil. *Eur. J. Lipid Sci. Technol.* 2011, *113*, 1138–1144.

[7] Tian, H., Zhan, P., Zhang, H., Development of fatty acid fingerprint of white apricot almond oil by gas chromatography-mass spectrometry. *Eur. J. Lipid Sci. Technol.* 2014, *116*, 126–133.

[8] Matthäus, B., Özcan, M. M., Fatty acids and tocopherol contents of some *Prunus*. Spp. kernel oils. *J. Food Lipids* 2009, *16*, 187–199.

[9] Grandhi, V. M., Mulki, M. J., Mukerji, B., Iyer, V. J., Cherian, K. M., Safety evaluation of wild apricot oil. *Food Chem. Toxicol.* 1997, *35*, 583–587.

[10] Wu, W. H., The contents of lignans in commercial sesame oils of Taiwan and their changes during heating. *Food Chem.* 2007, *104*, 341–344.

[11] Lee, J., Lee, Y., Choe, E., Effect of sesamol, sesamin and sesamolin extracted from roasted sesame oil on the thermal oxidation of methyl linoleate. *LWT–Food Sci. Technol.* 2008, *41*, 1871–1875.

[12] Lee, S. W., Jeung, M. K., Park, M. H., Lee, S. Y., Lee, J. H., Effect of roasting conditions of sesame seeds on the oxidative stability of pressed oil during thermal oxidation. *Food Chem.* 2010, *118*, 681–685.

[13] Rangkadilok, N., Pholphana, N., Mahidol, C., Wongyai, W., et al. Variation of sesamin, sesamolin and tocopherols in sesame (*Sesamum indicium L.*) seeds and oil products in Thailand. *Food Chem.* 2010, *122*, 724–730.

[14] Azeez, M., Morakinyo, A., Genetic diversity of fatty acids in sesame and its relatives in Nigeria. *Eur. J. Lipid Sci. Technol.* 2011, *113*, 238–244.

[15] Konsoula, Z., Liakopoulou-Kyriakides, M., Effect of endogenous antioxidants of sesame seeds and sesame oil to the thermal stability of edible vegetable oils. *LWT—Food Sci. Technol.* 2010, *43*, 1379–1386.

[16] Bendini, A., Barbieri, S., Valli, E., Buchecker, K., et al. Quality evaluation of cold pressed sunflower oils by sensory and chemical analysis. *Eur. J. Lipid Sci. Technol.* 2011, *113*, 1375–1384.

[17] Vujasinović, V., Djilas, S., Dimić, E., Basić, Z., Radocaj, O., The effect of roasting on the chemical composition and oxidative stability of pumpkin oil. *Eur. J. Lipid Sci. Technol.* 2012, *114*, 568–574.

[18] Omar, K. A., Shan, L., Wang, Y. L., Wang, X., Stabilizing flaxseed oil with individual antioxidants and their mixtures. *Eur. J. Lipid Sci. Technol.* 2010, *112*, 1003–1011.

[19] Lutterodt, H., Luther, M., Slavin, M., Yin, J. J., et al. Fatty acid profile, thymoquninone content, oxidative stability and antioxidant properties of cold pressed black cumin seed oils. *LTW—Food Sci. Technol.* 2010, *43*, 1409–1413.

[20] Cheikh-Rouhou, S., Besbes, S., Lognay, G., Blecker, C., et al. Sterol composition of black cumin (*Nigella sativa L.*) and Aleppo pine (*Pinus halepensis Mill.*) seed oils. *J Food Compos. Anal.* 2008, *21*, 162–168.

[21] Ramadan, M. F., Mörsel, J. T., Oxidative stability of black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.) and niger *Guizotia abyssinica* Cass.) crude seed oils upon stripping. *Eur. J. Lipid Sci. Technol.* 2004, *106*, 35–43.

[22] Matthäus, B., Brühl, L., Virgin hemp seed oil: An interesting niche product. *Eur. J. Lipid Sci. Technol.* 2008, *110*, 655–661.

[23] Latif, S., Anwar, F., Physicochemical studies of hemp (*Cannabis sativa L.*) seed oil using enzyme-assisted cold pressing. *Eur. J. Lipid Sci. Technol.* 2009, *111*, 1042–1048.

[24] Teh, S. S., Birch, J., Physicochemical and quality characteristics of cold pressed hemp, flax and canola seed oils. *J. Food Compos. Anal.* 2013, *30*, 26–31.

[25] Park, Y. W., Chang, P. S., Lee, J. H., Application of triacylglycerol and fatty acid analyses to discriminate blended sesame oil with soybean oil. *Food Chem.* 2010, *123*, 377–383.

[26] Veldsink, J. W., Muuse, B. G., Meijer, M. M., Petrus Cuperus, T. F., Heat pretreatment of oilseeds: Effect on oil quality. *Fett/Lipid* 1999, *101*, 244–248.

[27] Durmaz, G., Karabulut, I., Topću, A., Asiltürk, M., Kutlu, T., Roasting-Related Changes in Oxidative Stability and Antioxidant Capacity of Apricot Kernel Oil. *J Am Oil Chem Soc.* 2010, *87*, 401–409.

[28] Kemal-Edin, A., Effect of fatty acids and tocopherols on the oxidative stability of vegetable oils. *J. Lipid Sci. Technol.* 2006, *58*, 1051–1061.

[29] Tauseef Sultan, M., Butt, M.S., Anjum, F. M., Safety assessment of black cumin fixed and essential oil in normal Sprague dawley rats: Serological and hematological indices. *Food Chem. Toxicol.* 2009, *47*, 2768–2775.

[30] Zheng, C., Yang, M., Zhou, Q., Liu, C-L., Huang, F-H., Changes in the content of canolol and total phenolics, oxidative stability of rapeseed oil during accelerated storage. *Eur. J. Lipid Sci. Technol.* 2014, *116*, 1675–1684.

[31] Koski, A., Pekkarinen, S., Hopia, A., Wähälä, K., Heinonen, M., Processing of rapeseed oil: Effects on sinapic acid derivative content and oxidative stability. *Eur. Food Res. Technol.* 2003, *217*, 110–114.

[32] Turan, S., Topcu, A., Karabulut, I., Vural, H., Hayaloglu, A. A., Fatty acid, tryacilglicerol, pytosterol and tocopherol variations in kernel oil of Malataya Apricots from Turkey. *J. Agric. Food Chem.* 2007, *55*, 10787–10794.

[33] Huang, S. W., Frankel, E. N., German, J. B., Antioxidant activity of α and γ-tocopherols in bulk oils and in oil-in-water emulsions. *J. Agric. Food Chem.* 1994, *42*, 2108–2114.

[34] Alu'datt, M. H., Rababah, T., Ereifej Alli, K. I., Distribution, antioxidant and characterisation of phenolic compounds in soybeans, flaxseed and olives. *Food. Chem.* 2013, *139*, 93–99.

[35] Tuberoso, C. I. G., Adam Kowalczyk, A., Sarritzu, E., Cabras, P., Determination of antioxidant compounds and antioxidant activity in commercial oilseeds for food use. *Food. Chem.* 2007, *103*, 1494–1501.

[36] Ciftci, O. N., Przybylski, R., Rudzińska, M., Lipid components of flax, perilla, and chia seeds. *Eur. J. Lipid Sci. Technol.* 2012, *114*, 794–800.

[37] Herchi, W., Sakouhi, F., Habib Kallel, S. B., Pepe, C., Changes in Fatty Acids, Tocochromanols, Carotenoids and Chlorophylls Content during Flaxseed Development. *J. Am. Oil. Chem. Soc.* 2011, *88*, 1011–1017.

