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CHARACTERIZATION OF FATTY ACID PROFILE, POLYPHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF COLD PRESSED AND REFINED EDIBLE OILS FROM MACEDONIA

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A B S T R A C T

Fatty acid profile, polyphenolic content and resulting antioxidant activity of the most consumed edible oils on Macedonian market were studied. The levels of total polyphenolics were estimated spectrophotometically by extraction with 60 % aqueous methanol using Folin-Ciocalteau reagent. A common method for determination of the antioxidant activity of edible oils is the 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$) method. This method was used for testing the oils (six cold pressed oils from olive, sunflower, pumpkin, rapeseed, flaxseed and four commercial refined sunflower oils available on Macedonian market). The levels of total polyphenolic of cold pressed oils were in the range from 1.82 to 34.21 mg per 10 g of oil and corresponding antioxidant activity from 0.01 to 4.12 expressed as mmol Trolox per liter of oil. Furthermore, GC-MS analyses were performed for determination of fatty acid profile of the oils. The percentage of 9, 12 linoleinic acid was predominant in comparison with other fatty acids in examined oils.

Keywords: Edible oils, Polyphenolics, Antioxidant activity, Fatty acid.

INTRODUCTION

Polyunsaturated fatty acids and tocopherols are essential foodstuff in human nutrition. Cold pressed edible oils are the most important nutrition products due to the high concentration of essential fatty acids and vitamin E (Butinar *et al*. 2011). However, human intake of saturated fatty acids or unsaturated *trans*-fatty acid increased low density lipoprotein cholesterol associated with increased risk for cardiovascular and cerebrovascular diseases (O'Sullivan *et al*. 2011).

Polyphenolic compounds naturally present in oils have antioxidant activity and protect the organism against cardiovascular and degenerative diseases (Gamazo-Vázquez *et al*. 2003). Polyphenols, as thermally unstable compounds, are usually removed from edible oils during various refining processes. The antioxidant potential of polyphenols depends of the type of polyphenol compounds and their ability to give an hydrogen atom from hydroxyl groups and stabilize the phenoxy radical formed by delocalization of free electron within aromatic structure. The most common method for determination of antioxidant activity of wines is the 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$) method. The TEAC (Trolox Equivalent Antioxidant Capacity) value which was measured for the methanol extracts of oils, expresses the concentration of a Trolox solution whose antioxidant activity is identical to that of the extract itself. This index is defined as the millimolar concentration of a Trolox solution whose antioxidant capacity is equivalent to a 1.0mM solution of the substance under study (Rice-Evans *et al*. 1996; Kähkönen *et al*. 1999). Different radical scavenging tests and their relationships with phenolic compounds were studied on extra virgin olive oils as well as other seed oils (Quiles *et al*. 2002).

Changes of the quality of cold pressed pumpkin seed from Serbia was examined in the work of Vujasinovic *et al*. (Vujasinović *et al*. 2010). According to the findings, the flavor of the oil was accepted till 12 months of storage after which period deterioration of oil was detected. Oxidative stability measured by Rancimat apparatus decreased in the period of 2 years for 25-40 %. Gorjanović *et al.* studied the antioxidant activity of seed oils from three naked and one hulled (Cucurbita pepo L.) variety of pumpkin and found strict correlation

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between hydrogen peroxide scavenge and radical scavenging capacity against the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Gorjanović et al. 2011). Furthermore, antioxidant and lipoxygenase inhibitory activities of pumpkin seed extracts as well as tocopherols content in 12 cultivars of pumpkin seed oils were elucidated (Xanthopoulou *et al*. 2009; Stevenson *et al*. 2007). Physicochemical properties and bioactive compounds of selected seed oils such as flaxseed oil were object of studies of (Fruhwirth and Hermetter (2007); El-Adawy *et al*. 2001; Nyam *et al*. 2009; El-Beltagi *et al*. 2007; Choo *et al*. 2007). The numerical method to predict the polyphenols content in monovarietal olive oils was propose by Matos *et al*. based on the kinetic equation of the oxidation process in the presence of antioxidants and on Rancimat profiles (Matos *et al*. 2007). Retention and distribution of natural antioxidants (*α*-tocopherol, polyphenols and terpenic acids) after shallow frying of vegetables in virgin olive oil was discussed by Kalogeropoulos *et al*. (Kalogeropoulos et al. 2007).

To the best of our knowledge there are no published results for the quality of the most consumable edible oils in Macedonia. Therefore, the main object of this study, phenolic content, resulting antioxidant activity and the composition of fatty acid of the most used edible oils on the Macedonian market were examined.

MATERIALS AND METHODS

Samples of oils: The ten, most consumable cold pressed and refined food oils were selected from the biggest food markets in Macedonia.

Chemicals and standards: In the present study 2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) in the crystallized diammonium salt form, horseradish peroxidase type VI-A, hydrogen peroxide 30% (v/v), Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid, a water soluble tocopherol analogue), gallic acid, Folin-Ciocalteu reagent, Na_2CO_3 in salt form, ethanol, methanol and distilled water were obtained from Merck, Germany. *tert*-butyl methyl ester and trimetylsulfonium hydroxide was purchased from Sigma-Aldrich.

Apparatus: For determination of total polyphenolics and antioxidant activity of oils, UV/VIS spectrophotometer Bruker IFS 66 was used for the analyses. The absorbance was measured at room temperature on 765 nm and 734 nm respectively.

Qualitative analysis of fatty acids was performed by using Gas Chromatograph - Mass Spectrometry (GC-MS) HP 6890 with HP5973 mass selective detector. For identification purposes, NIST and Wiley mass spectra databases as well as a homemade database were used. Separation of the compounds was performed using an HP Carbowax column (60 m x 0.25 mm x 0.25 μm) and identification is based on comparison of retention time and mass spectra with those from the libraries. The temperature profile was follow: 60°C (2 min) to 180°C with 5°C temperature grade and from 180°C to 240°C with 20°C. Injector temperature was set to 260 ºC; injection mode split with split ratio 1:5; Helium was used as carrier gas, using 35 kPa (32 cm/s); interface temperature was 280 ºC; acquisition mass range, 40-400 m/z; solvent cut, 2 min.

Determination of fatty acid methyl esters: Preparation of fatty acid methyl esters was performed according to international standard ISO 12966-3:2011. According to this standard, 10.0 g of oil was transferred into the test tube. Furthermore, 500 μL of *tert*-butyl methyl ester (TBME) was dissolved into the test tube and the solution has been warmed and mixed with 250 μL methanolic solution of trimetylsulfonium hydroxide. Glycerides were based-catalysed transesterified and fatty acid methyl esters were formed. During the reaction of esterification, fatty acids were converted into the salts. These salts were decomposed in the injector of GC-MS instrument into the methyl esters and dimethylsulfide. For complete pyrolysis, the temperature of injector was 250°C.

Determination of the percentage of methyl esters of fatty acids present in the sample was analyzed by gas chromatography – mass spectroscopy.

Determination of total polyphenolics content and antioxidant activities of edible oils: The levels of total polyphenols were determined spectrometrically by extraction with 60 % aqueous methanol using Folin-Ciocalteu reagent. The Folin-Ciocalteu method in virgin olive oil samples was reported by Gutfinger (1981). In brief, 10 g of oil was dissolved in 50 mL hexane and extracted 3 times with 60 % of aqueous extract of methanol. The extracts were evaporated and lyophilised and dissolved in 1 ml methanol. The 0.1 ml of methanol extract mixed with 0.4 ml pure methanol was added in volumetric flask of 10 mL containing 0.5 mL of Folin-Ciocalteu's reagent and 1.5 mL 20 % solution of Na_2CO_3. The measurements were performed after 1 h storage at 765 nm and expressed as gallic acid equivalent (GAE, mg/L) based on a calibration curve ($y=0.0121x+0.0938$ $R^2=0.9992$) obtained with standard of gallic acid.

The antioxidant activity of oils was determinate using the $ABTS^{\bullet+}$ method for the screening of antioxidant activity as a decolorization assay applicable to both lipophilic and hydrophilic antioxidant, including flavonoids, hydroxycinnamates, carotenoids and plasma antioxidants (Kostadinović *et al*. 2012).

The technique involves the direct production of the blue /green $ABTS^{\bullet+}$ chromophore trough the reaction between $ABTS^{\bullet+}$ and potassium persulfate. This has absorption maxima at 414, 645, 734 and 815 nm.

For this purpose 10 ml of solution of ABTS was prepared. The ABTS solution was consist from 38.43 g of ABTS and 6.90 mg of $K_2S_2O_2$, and filled with nanopure water.

For calibration curve 5 ml solution of 12,52 mg of Trolox standard is diluted with 97 % of ethanol and four standard solutions were prepared for calibration curve with concentration of 250, 500, 750 and 1000 mmol/5ml respectively. The molar extinction coefficient at 734 nm was 250,32 g/mol. Calibration curve of Trolox is y=0.2523+0.0453 and R=0.9996.

RESULTS AND DISCUCCION

The values obtained from total polyphenolic content (range from 1.82 to 34.2 mg per 10 g) and corresponding antioxidant activity (range from 0.01 to 4.12 expressed as mmol Trolox per liter) favored cold pressed in comparison to refined oils. This behaviour can be explained by refining process during which very high temperature is usually applying and the polyphenolic compounds were destroyed. During the whole process of cold pressing, the temperature do not exceed 40 °C and the total activity of polyophenolic compounds (especially tocopherols) enables higher values for antioxidant potential.

According to the results presented on Table 1, the extra virgin olive oil showed highest antioxidant activity (4.12 mmol Trolox) and the highest value for total polyphenolics (34.21 mg/10g of extracted oil expressed as mg of gallic acid equivalent). The antioxidant potential of cold pressed rapeseed oil with value of 1.88 mmol Trolox and the concentration of total phenolic content of 15.59 mg of gallic acid equivalent were significantly high which indicated high quality of this edible oil. The cold pressed olive pomace oil had higher antioxidant activity than sunflower seed but lower than virgin olive oil. Olive promace oil is produced from the promace as by-product of olive oil production. This edible oil, obtained by cold pressing of pomace, usually consist low levels of natural antioxidants which make shorter shelf life. Because of lower quantity of tocopherols, process of oxidation naturally goes faster in pomace oil with resulted lower antioxidant potential. As we can concluded from the Table 1, olive oil extracted from pomace still consisted 15,42 mg of polyphenolic compounds per 10 g of pure oil which can lead to the conclusion that olives in comparison with other plant seeds had oil with very good quality.

Also, the difference in antioxidant activity of oils from various oilseeds can be resulted by different levels of α, β and γ-tocopherols. According to the findings of Stevenson *et al*., γ- and β-tocopherols are more effective in trapping the hydroperoxide intermediate and stooping the autooxidation of the oil in comparison to α-tocopherol (Stevenson et al. 2007). In comparison with oils obtained from cold pressing of the sunflower seeds where the antioxidant activity is supported to the high concentration of α-tocopherol which consist 95% of all tocopherols in the oil, the antioxidant activity of pumpkin seed oil only 41% is resulted by tocopherols and 59 % to the polar phenolic compounds (Xanthopoulou *et al*. 2009).

If we compare results from cold pressed and refined sunflower oils we can noted higher value for total polyphenolics and antioxidant activity for sunflower oil obtained by cold pressing. In fact, during the process of refining and desodoration of the oils, the temperature is very high and thermal process destroyed the molecule of phenolic compounds (Raß *et al*. 2008).

The lowest antioxidant activity for flaxseed oil was expectable due of the highest amount of α-linolenic acid (Table 2). α-linoleinic acid consist 3 double bonds in its structure. Stability of edible oils which consist high percentage of free and bond unsaturated fatty acids is very problematic due to their ability for fast oxidation. Consequently, the lowest level of oxidative stability of flexseed oil was observed. The percentage of the same fatty acid in cold pressed rapeseed oil was significant lower, only 2,73 % which can be indicator of higher stability of rapeseed in comparison with flexseed oil (Fruhwirth and Hermetter (2007); El-Adawy *et al*. 2001; Nyam *et al*. 2009).

Although pumpkin seed oil did not have high value of total phenolic content (6.84 mg of gallic acid equivalent) and value of 0,34 mmol Trolox Equivalent Antioxidant Activity (TEAC), the oil did not have low nutritional value. Pumpkin seed oil is very reach with lignins, chlorophyll and ß-carotene and in the same time have very pleasant characteristic flavor which ranking the oil

in the range of the most expensive edible oils (Vujasinović *et al*. 2010; Gorjanović et al. 2011).

Although cold pressed sunflower oil had significantly higher antioxidant activity in comparison with refined sunflower oils (which predominantly comes from α-tocopherol as main antioxidant in this plant oil), refined oils also had higher concentrations of fatty acids but, low levels of resins which oxidized very fast. From the last, we can conclude that cold pressed sunflower oil has higher antioxidant potential but lower shelf life in comparison to refined sunflower oil due to the high level of resins. Because of very high temperature and similar process of rafination, most of the naturally present antioxidant is destroyed and there is no significant difference between antioxidant activities of refined sunflower oils from different producers (Table 1).

Table 1. Total polyphenolics and resulting antioxidant activity of cold pressed and refined edible oils.

Sample of oil	**Type of edible oil**	**Total phenolic content (TIC) mg of GAE per 10 g of oil**	**Trolox Equivalent Antioxidant activity (TEAC)** /mmol Trolox
1.	Cold pressed rapeseed oil	15.59 ± 0.09	1.88 ± 0.05
2.	Cold pressed flaxseed oil	2.85 ± 0.01	0.01 ± 0.005
3.	Cold pressed pumpkin seed oil	6.84 ± 0.03	0.34 ± 0.05
4.	Cold pressed sunflower oil	19.28 ± 0.11	0.98 ± 0.07
5.	Refined sunflower oil	2.14 ± 0.05	0.18 ± 0.04
6.	Refined sunflower oil	5.48 ± 0.12	0.21 ± 0.06
7.	Refined sunflower oil	1.82 ± 0.01	0.01 ± 0.003
8.	Refined sunflower oil	2.01 ± 0.05	0.01 ± 0.001
9.	Extra virgin olive oil	34.21 ± 0.06	4.12 ± 0.27
10.	Olive pomace oil	15.42 ± 0.14	1.20 ± 0.12

Table 2. Amount (in %) of esterified fatty acids in cold pressed and refined edible oils.

Esters of fatty acids	**Rt. (min)**	**Cold pressed sunflower oil**	**Cold pressed rapeseed oil**	**Cold pressed flaxseed oil**	**Cold pressed pumpkin seed oil**	**Extra virgin olive oil**
Palmitic acid C16:0	38.40	3.93 ± 0.12	2.49 ± 0.07	3.50 ± 0.08	6.64 ± 0.79	8.55 ± 1.33
Stearic acid C 18:0	44.20	1.86 ± 0.07	-	1.10 ± 0.16	1.82 ± 0.14	0.65 ± 0.25
Oleic acid C18:1	44.93	17.7 ± 0.03	43.8 ± 12.5	12.8 ± 1.44	38.1 ± 12.1	43.90 ± 12.8
Linoleinic acid C18:3	46.30	39.8 ± 3.25	10.0 ± 0.19	23.6 ± 3.99	16.3 ± 4.28	5.82 ± 0.58
α-linolenic acid	47.78	-	2.73 ± 0.12	5.22 ± 0.12	-	-
Esters of fatty acids	**Rt. (min)**	**Refined sunflower oil**	**Refined sunflower oil**	**Refined sunflower oil**	**Refined sunflower oil**	**Olive pomace oil**
Palmitic acid C16:0	38.40	-	3.80 ± 0.99	3.71 ± 0.13	3.75 ± 0.54	8.55 ± 1.74
Stearic acid C18:0	44.20	-	0.52 ± 0.11	0.67 ± 0.06	0.65 ± 0.36	0.65 ± 0.11
Oleic acid C 18:3	44.93	4.32 ± 0.89	19.3 ± 8.11	13.3 ± 0.18	23.9 ±0.94	43.9 ± 13.55
Linoleinic acid C18:3	46.30	10.40 ± 9.16	38.4 ± 1.48	32.0 ± 5.19	29.9 ± 3.58	5.82 ± 9.27

If we compare the results obatined from Total Phenolic Content (TPC) and Trolox Equavalent Antioxidant Activity (TEAC) very good correlation can be found as shown in Fig. 1.

Although the main natural antioxidant in edible oils are α-tocopherol (Vitamnin E), this correlation shows that polar phenolic compunds also contribute to the overall antioxidant activity of the oils.

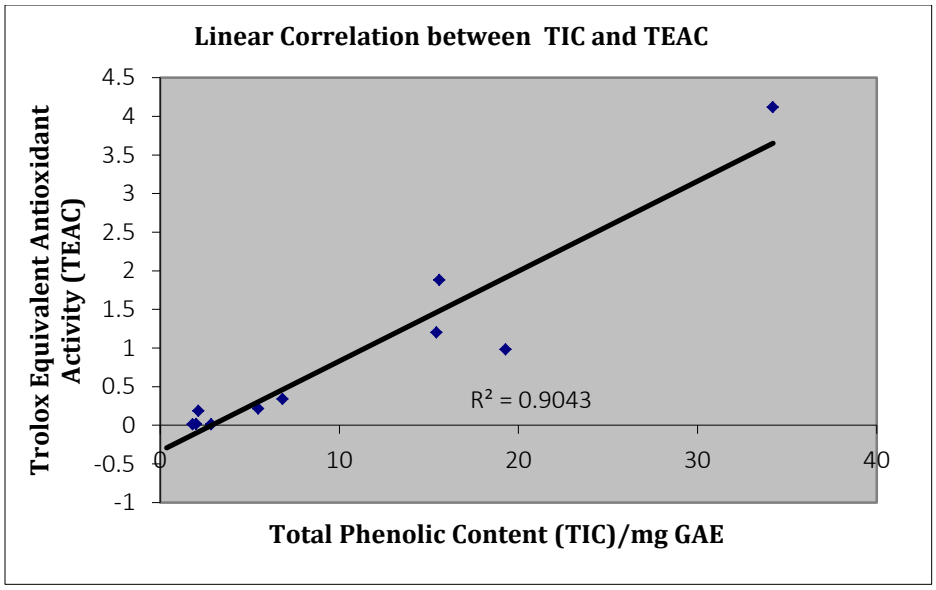

Fig. 1. Linear correlation between the total phenolic and antioxidant capacity (R2 = 0,9043).

CONCLUSION

Extra virgin olive oil had the highest antioxidant activity in comparison with all other analyzed oils. Cold pressed rapeseed oil had also high level of polyphenolic content and significant antioxidant activity. Generally speaking, cold pressed oils showed greater amount of polyphenolic compounds but lower stability than corresponding refined oils.

Results obtained from GC-MS analyses indicated higher amount of fatty acids in refined sunflower than corresponding cold pressed oils. All refined sunflower oils had similar values of antioxidant activity which indicate similar process of rafination. Difference between the antioxidant activity of virgin olive and pomace oil indicate importance of the starting plant material. The lowest value of antioxidant activity had cold pressed flaxseed oil. This value, which is comparable with refined oils, was resulted of very high abundance of unsaturated fatty acid with three double bonds which accelerated the oxidation process of oil.

In conclusion, apart from the type of plant or seeds from which the oils are obtained, results from total polyphenolic content, antioxidant activity and GC-MS analysis proved the way of production as crucial for the quality of edible oils.

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