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Chemical composition, antiradical and antimicrobial activity of extracts and cold-pressed edible oils from Macedonian nutty fruits

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Introduction

Edible oils are important foodstuff due to their high level of energy, essential polyunsaturated fatty acids and vitamin-E-active compounds. The daily intake of edible oils decreases the risk of coronary heart diseases, degenerative diseases and cancer. In addition to essential fatty acids and vitamin E, the chemical composition of edible oils from different plants can also include minor amounts of polar components such phenolic components as powerful antioxidants responsible for human health benefits. Although they have a remarkable antioxidant activity, phenolic compounds usually are removed from edible oils during the refining process as thermally unstable compounds [1]. This study aims to investigate bioactive components of the oils in terms of fatty acid composition, vitamin-E-active compounds, phytosterols and total phenolic content.

Table 1. Fatty acid composition of cold pressed edible oils (%).

Oil type	Fatty Acid						
	Saturated fatty acid (SFA)						
	C14:0	C16:0	C17:0	C18:0	C20:0	C22:0	C24:0
Poppy seed oil	0.03±0.03[a]	8.51±0.03[b]	0.05±0.00[a]	2.37±0.01[a]	0.12±0.00[a]	ND[a]	ND[a]
Walnut oil	ND[a]	5.93±0.02[a]	0.05±0.00[a]	2.34±0.01[a]	0.10±0.00[a]	ND[a]	ND[a]
Almond oil	ND[a]	6.38±0.01[a]	0.06±0.00[a]	2.82±0.00[ab]	0.15±0.00[a]	0.03±0.00[a]	ND[a]
Wheat germ oil	0.04±0.01[a]	9.29±0.03[b]	0.09±0.00[b]	3.98±0.01[b]	1.04±0.01[b]	1.79±0.01[b]	1.04±0.00[b]

Oil type	Monounsaturated fatty acid (MUFA)								
	C16:1D9	C17:1	C18:1 trans 2	C18:1 trans 1	C18:1 D9	C18:1 D11	C20:1 11	C20:1	C22:1 13
Poppy seed oil	0.13±0.01[a]	ND[a]	14.35±0.02[b]	ND[a]	14.35±0.02[a]	1.06±0.01[b]	ND[a]	0.08±0.01[a]	ND[a]
Walnut oil	0.07±0.00[a]	ND[a]	ND[a]	ND[a]	17.89±0.01[a]	0.78±0.00[a]	ND[a]	0.20±0.00[a]	ND[a]
Almond oil	0.42±0.01[b]	0.09±0.00[a]	ND[a]	ND[a]	67.57±0.02[c]	1.04±0.00[b]	ND[a]	0.07±0.00[a]	ND[a]
Wheat germ oil	0.08±0.00[a]	0.06±0.00[a]	0.11±0.00[a]	0.05±0.00[a]	38.14±0.04[b]	0.97±0.00[ab]	0.04±0.00[a]	0.82±0.00[b]	0.07±0.00[a]

Oil type	Polyunsaturated fatty acid (PUFA)							
	C18:2	C18:3	C18:2 trans 1	C18:3 trans 2	C18:3 trans 1	C20:2 5,11	C20:2 11,14	C20:3 5,11,14
Poppy seed oil	72.28±0.06[d]	0.89±0.01[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Walnut oil	60.73±0.01[c]	11.74±0.01[c]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Almond oil	20.96±0.01[a]	0.39±0.00[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Wheat germ oil	37.71±0.01[b]	2.23±0.00[b]	0.64±0.01[a]	0.11±0.00[a]	0.54±0.00[a]	0.10±0.00[a]	0.07±0.00[a]	0.06±0.01[a]

Table 2. Total phenolic compounds, DPPH assay for the pure oils, DPPH for methanolic oil extracts and TEAC assay for methanolic oil extract.

Samples	TPC assay (mg/L GAE)	DPPH assay for (mg of α-tocopherol/L oil)	DPPH assay for methanol extracts (mg Trolox/L oil)	TEAC assay for methanol extracts (mg of Trolox/L oil)
Almond oil	558.82 ± 10.335[c]	952.93 ± 34.09[b]	160.30±7.10[c]	173.98 ± 1.565[c]
Walnut oil	524.78 ± 18.246[c]	1191.41 ± 19.89[c]	66.69±1.03[b]	138.83 ± 2.209[b]
Poppy seed oil	368.23 ± 17.717[b]	792.57 ± 4.06[a]	56.47±3.43[b]	126.46 ± 4.938[b]
Wheat germ oil	61.57 ± 3.816[a]	1418.92 ± 16.01[d]	27.89±13.61[a]	86.71 ± 21.069[a]

Table 3. Antibacterial and antifungal activity (inhibition zone expressed in mm) a of four investigated oils.

Samples	Inhibition zone in diameter (mm)				
	Staphylococcus aureus	*Listeria monocytogenes*	*Salmonella enteritidis*	*Escherichia coli*	*Candida albicans*
Almond oil	–	–	–	–	+
Walnut oil	–	–	–	–	+
Poppy seed oil	–	+	–	–	++
Wheat germ oil	–	–	–	–	–

Materials and Methods

Determination of the fatty acid composition

Capillary gas chromatography was used for determination of fatty acid composition. The procedure of preparation of fatty acid methyl esters included dissolving of 2 drops of each oil dissolved in 1 ml of heptane. Furthermore, 50 µL of sodium methylate with concentration of 2 mol/L was added and solution was homogenizedThe upper phase of solution was inserted in GC vials and fatty acid methyl esters were analyzed by CP7420 Select FAME column, 100 m x 0.25 mm internal diameter with 0.25 µm film thickness. Analyzes were performed on Agilent 6890 equipped with KAS4Plus and FID.

Total phenolic compounds (TPC)

The total phenolic content of oil extracts was determined with Folin–Ciocalteu reagent. The Folin–Ciocalteu reagent was prepared as described by Tamokou et al. For each sample, 50 µL of diluted (1:5) oil extract were added to 750 µL water and 50 µL Folin–Ciocalteu reagent, the solutions with total volume of 850 µL were incubated in the dark for 5 min.

Antimicrobial assays

The samples of four cold-pressed oils were investigated for their "in vitro" antbacterial and antifungal properties using a disk-diffusion method, in Petri dishes. The four oils were tested against antibacterial activity against two Gram-positive bacterial strains: *Listeria monocytogenes* (ATCC 13076), and *Staphylococcus aureus* (ATCC 49444), and against two Gram-negative bacterial strains: *Salmonella enteritidis* (ATCC 13076), *Escherichia coli* (ATCC 25922), and against antifungal activity using: *Candida albicans* (ATCC 10231).

Results and discussion

Fatty acid composition

The fatty acid composition of examined oil is presented at Table 1. The most dominant saturated fatty acid in four samples of oils was palmitic acid with levels between 5.93±0.02 and 9.29±0.03%. Regarding unsaturated fatty acid, the most dominant was monounsaturated oleic acid in roasted almond oil with level of 67.57±0.02% and polyunsaturated linoleic acid with level of 20.96±0.01. Although walnut oil was cold-pressed oil obtained from the same family of nuts, the level of unsaturated fatty acid was significantly different. The polyunsaturated linoleic acid was the most dominant in this oil with abundance of 60.73±0.01% and oleic acid was present with level of 17.89±0.01%. The results obtained from this oil was in excellent agreement with those published in the work of Tapia et al. According to their findings, the fatty acid composition of four varieties of walnuts was almost identical to fatty acid composition of walnut oil from Macedonia with linoleic acid as the most abundant fatty acid with percentages between 59 and 60.6%. The second most abundant was oleic acid with level between 12.3 and 16.9%. Poppy seed oil had very similar fatty acid composition with 72.28±0.06% of linoleic and 14.35±0.02% of oleic acid. However, wheat germ oil consisted almost equal abundance of oleic and linoleic acid with 38.14±0.04 and 37.71±0.01% respectively. The significant amount of α-linolenic acid (ALA) was present only in walnut oil with level of 11.74±0.01%.

Total phenolic content (TPC) and antiradical potential of cold-pressed oils determinated by DPPH and ABTS radicals

There is significant impact of total phenolic content on antiradical activity of the examined oils. The methanolic extracts of roasted almond and walnut oil contained the highest amounts of phenolic compounds (558.82±10.33 and 524.78±18.24 mg/L, respectively). Significantly lower quantity was detected for poppy seed oil (368.23±17.71 mg/L) and the lowest total phenolic contents was determinate for wheat germ oil (61.57±3.81 mg/L) (Table 2). The antiradical activity of methanol extracts estimated by ABTS radical was in a good correlation with the amount of total phenolic contents (R^2 = 0.8789). As can be seen from the Table 5, the highest scavenger ability had the methanol extracts of roasted almond and walnut oil (173.98 ± 1.56 mg and 138.83 ± 2.20 mg Trolox/L of oil, respectively). Following the trend of the results estimated using TPC assay, lower scavenger ability than almond and walnut oils had poppy seed oil with 126.46 ± 4.93 mg Trolox/L of oil. The lowest antiradical capacity had wheat germ oil of 86.71 ± 21.06 mg Trolox/L of oil. However, if we compare the data from Table 5, we can notice that although poppy seed oil had approximately 30% less total phenolic content in comparison to walnut oil, the antiradical activity of their methanolic extracts by two radicals is very similar.

Antibacterial and antifungal tests

The results from antibacterial and antifungal activity of the oils are presented in Table 3. Although cold-pressed poppy seed oil had the lowest level of Vitamin-E-active compounds and the lowest antiradical activity measured by DPPH assay, it looks that this oil is the most interested as microbiologically active oil which indicated low antibacterial activity against gram positive bacteria *Listeria monocytogenes* and moderate antifungal activity against *Candida albicans*. Furthermore, cold-pressed almond and walnut oil showed low antifungal activity against *Candida albicans*. None of four cold pressed oils showed activity against gram-positive bacterial strain *Staphylococcus aureus* (ATCC 49444), and two gram-negative bacterial *strains Salmonella enteritidis* (ATCC 13076) and *Escherichia coli* (ATCC 25922).

Reference

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