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Evaluation of bioactive compounds and of antioxidant properties in some oils obtained from food industry by-products

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Abstract

The goal of this study was the extraction of oil from apricot and plum kernels harvested in 2004-2006 in the western Romania, for determination of β-carotene, total polyphenolics and total antioxidant capacity. The β-carotene content was determined using a spectrophotometric assay. Tocopherols were analyzed by HPLC method and the content of total polyphenolics was evaluated by Folin-Ciocalteu colorimetric method. The antioxidant activity was measured using the FRAP assay. Results showed that the plum kernel oil is richer in β-carotene (mean = 188 μg/g oil) than apricot kernel oil (in average 61.05 μg/g oil). The oil from apricot and plum kernels has low tocopherols content. The results show that the antioxidant capacity for plum and apricot kernel oil were situated in the range of 0.423-1.895 mM Fe^{2+}/L and 0.86-1.33 mM Fe^{2+}/L respectively. The values of polyphenols content for investigated oils were situated in the range 0.605-2.85 mM gallic acid/L.

Keywords: plum kernel oil, apricot kernel oil, β-carotene, tocopherols, antioxidant capacity, polyphenols

Introduction

Plum and apricot kernels are a valuable by-product derived from the fruit processing industry that can be exploited in order to oil extraction. This oil, besides the lipid fraction, contains many different bioactive compounds such as β-carotene and tocopherols. β-carotene, known as provitamin A, has a important role in wound healing, increase body resistance to toxins and prevent and fight cancer [1, 2].

Tocopherols together with phytosterols and squalene are components present in the unsaponifiable lipid fraction of fruit kernels oil. Tocopherols are fat soluble antioxidants that protect lipids and other membrane components by physically quenching and reacting chemically with singlet oxygen [3].

Antioxidants from seeds and fruit kernels oil are able to neutralize free radicals created during the aging process and have a potential role in preventing the onset of some chronic diseases such as cardiovascular disease, some neurological disorders or certain inflammatory processes. These natural antioxidants are important lipid oxidation inhibitors in food and biological systems and are found in oil seeds in four different forms: α-tocopherol (α -T), β-tocopherol (β-T), γ-tocopherol (γ-T), and δ-tocopherol (δ-T) [4].

The tocopherols may protect against atherogenesis by blocking oxidation of low-density lipoprotein cholesterol and by favorably influencing plaque stability, vasomotor function, and tendency for thrombosis [5]. In addition, tocopherols, due to their ability to

quench free radicals in cell membranes, protecting polyunsaturated fatty acids from damage, play a significant role in prevention of Alzheimer's disease and cancer [6]. Phenolic compounds from crude fruit kernels oil have an important role for the oxidative stability of the polyunsaturated fatty acids of this oil. This protective role is due to their antioxidant properties [7]. The fruit kernel oil can be used in the food industry and cosmetics for their nutritional qualities and as source of bioactive compounds [8]. The composition of this oil depends on the fruit variety, origin place, harvest year and agrotechnical measures aplied to fruit-tree culture [9].

The objective of this study were to investigate the antioxidant properties and evaluated of some bioactive compounds as β-carotene, tocopherols and total phenolics in crude oil obtained from plum and apricot kernels.

Materials and methods

Samples. The oil samples were obtained by petroleum ether extraction in Soxhlet apparatus from apricot and plum kernels harvested in years 2004-2006 in the western Romania.

Spectrophotometric assay of β-carotene from oil. About 2 g of samples were saponified with 25 mL of 8% alcoholic KOH solution in the dark overnight [10]. Subsequently, the samples were centrifuged (2000 rot/min) for 4 minutes and the supernatant was concentrated under vacuum for alcohol removal. The extracts were washed repeatedly with water and petroleum ether. Combined ether extracts, free of alkali, were concentrated under vacuum at 40°C to remove the solvent. The carotenoidic extracts for spectrophotometric determinations were first diluted to a 3 mL with petroleum ether, followed by absorption measuring at λ=453 nm [11]. Based on the absorption values, the amounts of β-carotene were determined as μg/g oil.

Tocopherols determination by RP-HPLC method. This protocol consists in extraction of tocopherols from oil using a mixture of acetonitrile:methanol (50:50, v/v), followed by separation through a column Alltima RP C-18 (250mm x 4.6 mm, 5μm) at 40°C (C.T. MATEA et al. 12]). A mixture of acetonitrile: methanol (50:50, v/v) was used as mobile phase with a flow rate of 1.0 mL/min and an absorbance detector 290-325 nm. For chromatographic analysis a Shimadzu VP Series HPLC with fluorescence detector RF-10 AXL was used. Quantification of the tocopherols was made by preparing calibration curves using pure standards (α-, γ- and δ- from Merck and Sigma) in the chromatographic conditions described above. For the calibration curves four concentrations of tocopherols: 0.1; 0.25; 0.5 and 1 μg/mL were used. For each concentration 6 injections were made for an average calculated. Concerning tocopherol analysis, reverse phase chromatography does not distinguish between β and γ-isomers of tocopherol, thus the sum of these isomers is shown throughout as β+γ-tocopherol.

Total antioxidant capacity was evaluated by FRAP (*ferric reducing antioxidant power*) assay. The FRAP method consists in the reduction of Fe^{+3} ions to Fe^{+2}, which forms a blue-colored complex with 2,4,6-tripirydylo-s-triazine (TPTZ). This reduction was monitored by absorption change measuring at 595 nm. The intensity of the color depends on the antioxidant concentration [13]. The antioxidant compounds from the investigated oil were extracted using a mixture of methanol: distilled water (4:1, v/v) at 20°C for 1 hour. The supernatant obtained after centrifugation was used for analysis. Results were expressed as mmol Fe^{2+}/g oil.

Total polyphenols content was measured by a modified Folin-Ciocalteu assay ([14]. For total polyphenols content determinations the supernatants obtained previously in the case of total antioxidant capacity investigations were used. The samples were maintained for 2h in the

dark at room temperature before measuring the absorbance at 750 nm, (Analytic Jena Specord 205). The quantification of the data was based on a calibration curve using gallic acid as the standard and the results were expressed as mM of gallic acid/ g oil.

Statistical analysis. All data were reported as means ± standard deviation of three (n = 3) samples.Statistical analysis was done using the Student's test. The results were considered significant only if the p value was less than 0.05.

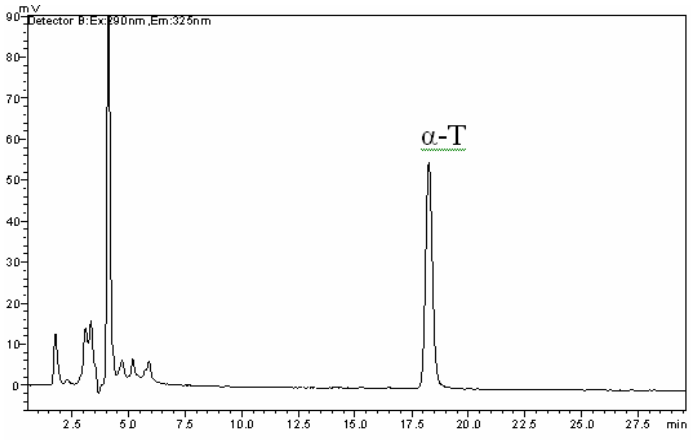
Results and discussion

Evaluation of β-carotene content from analyzed oil. Results obtained for β-carotene content of investigated oil samples are reported in the table 1. The experimental data obtained shows that the β-carotene content depends on the harvest year and fruit species. It can be observed that the plums kernel oil is richer in β-carotene (mean = 188 μg/g oil) than apricot kernel oil (in average 61.05 μg/g oil).

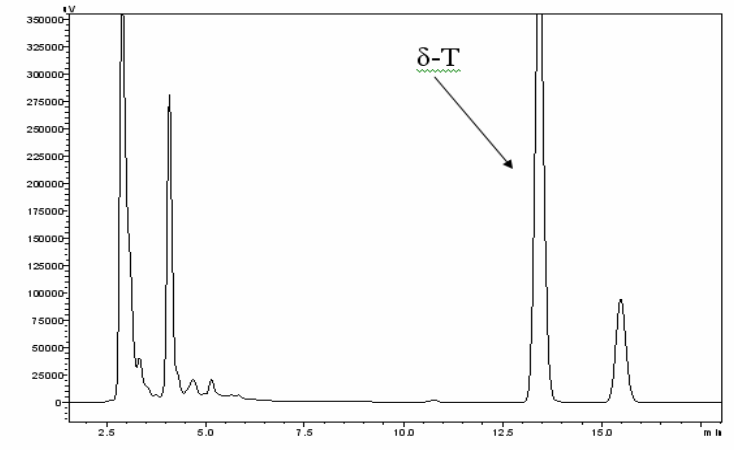
Tocopherols evaluation by HPLC fruit kernels oil. Figure 1 shows the HPLC chromatograms of standard α –tocopherol (a), δ-tocopherol (b) and γ-tocopherol (c). The investigated fruits kernels oil revealed the presence of significant amounts of tocopherols (tables of Fig. 2 and Fig. 3). Tocopherol values reported in this paper are lower than the values obtained in similar studies [4]. One reason of the low content of tocopherols found in the obtained oil can be that the analysis was performed after three months of oil extraction. There are numerous studies about the tocopherols in vegetable oils obtained from other fruits and seeds (as olive, rapeseed, sunflower, peanut, soybean, corn and grape seed) but few studies have been conducted on the tocopherols present in oil obtained from fruit kernels. The obtained data show that the content of isomeric forms of tocopherols depends on the harvest year and fruit species. Fractions of β+γ-tocopherol and δ-tocopherol were identified in all investigated oil samples, while the fraction of α-tocopherol was not detected in apricot kernels oil (2004 harvest year) and plum kernels oil (harvest year 2004 and 2006). The major tocopherol fraction in the both oils was represented by β+γ-tocopherol. For apricot kernels oil, the β+γ-tocopherol fraction accounted between 73.4 and 94.4% of the total tocopherols and in the situation of plum kernels oil, the sum of isomers β+γ was located between 85.9 and 88.9% of the total tocopherols.

Evaluation of antioxidant properties. Antioxidant profile of the oil samples was expressed as total antioxidant capacity and total polyphenols content (table 2). Antioxidant capacity of plum and apricot kernels oil recorded values between 0.42-1.90 mM Fe^2/L and 0.86-1.33 mM Fe^2/L respectively. Values obtained for polyphenol content were located between 0.61 and 2.85 mM gallic acid/L for both investigated oils. These results show that the fruit kernels oil possesses significant antioxidant properties that depends strongly the species and the harvest year. For apricot kernel oil, the lowest polyphenol content was obtained from sample from 2006 (0.88 mM gallic acid/L) and for plum kernel oil for sample from 2005 (0.61 mM gallic acid/L). For plum kernel oil, the highest polyphenol content was obtained for sample from 2006 (2.85 mM gallic acid /L) and for apricot kernel oil in the case of sample from 2005 (1.21 mM gallic acid/L). The maximum and minimum values registered for total antioxidant capacity of oil samples correspond to the maximum and minimum values of polyphenols content of these samples.

In this study, the correlations established between total antioxidant activity and polyphenols content were evaluated. Simple regression models were applied using the Origin 4.1 software program. The correlation between total antioxidant activity and total phenolic content had a correlation coefficient R=0.89949 (Fig. 4). It may be noted that total polyphenols content is a potential candidate as a selection criterion for antioxidant activity in fruit kernels oil, but antioxidant activity of these oils is not limited to phenolics compounds.



a



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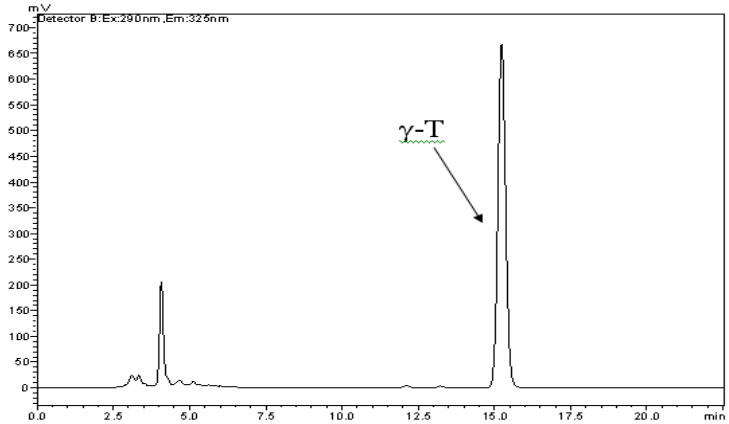
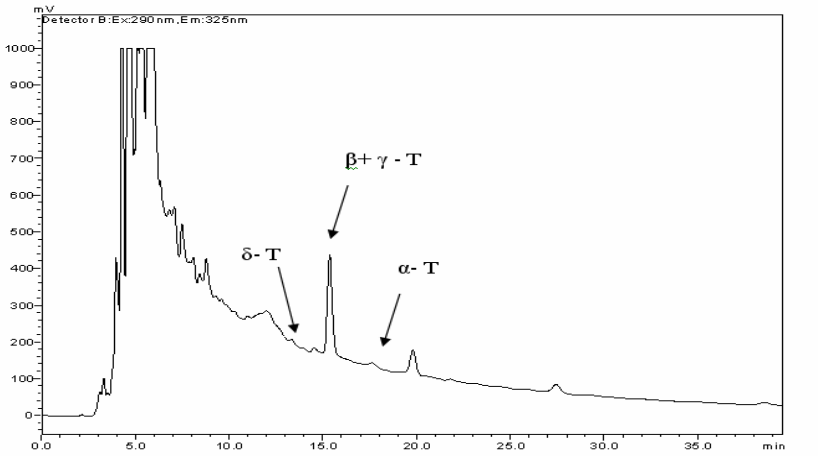


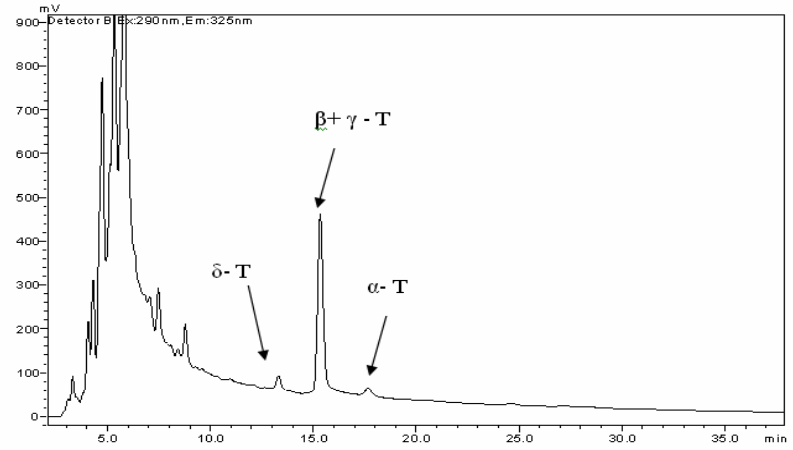
c

Retention time (min.)	Compound
17.571	α-tocoferol
13.318	δ-tocoferol
15.389	γ-tocoferol

Fig. 1. HPLC chromatograms of standard α –tocopherol (a), δ-tocopherol (b) and γ –tocopherol (c)



a



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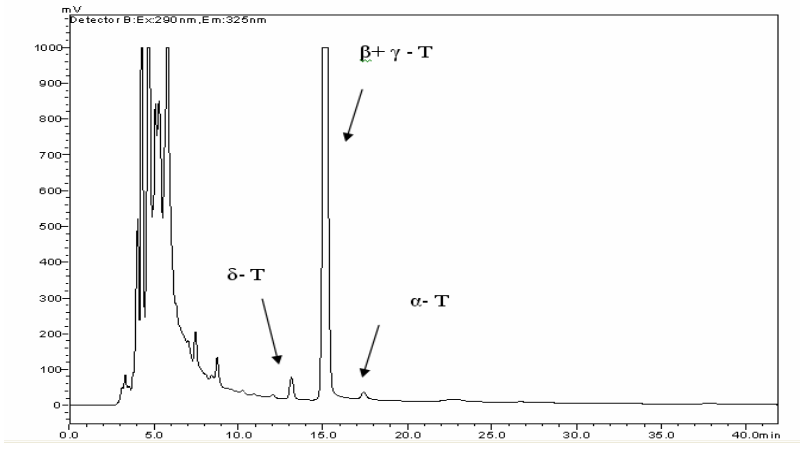
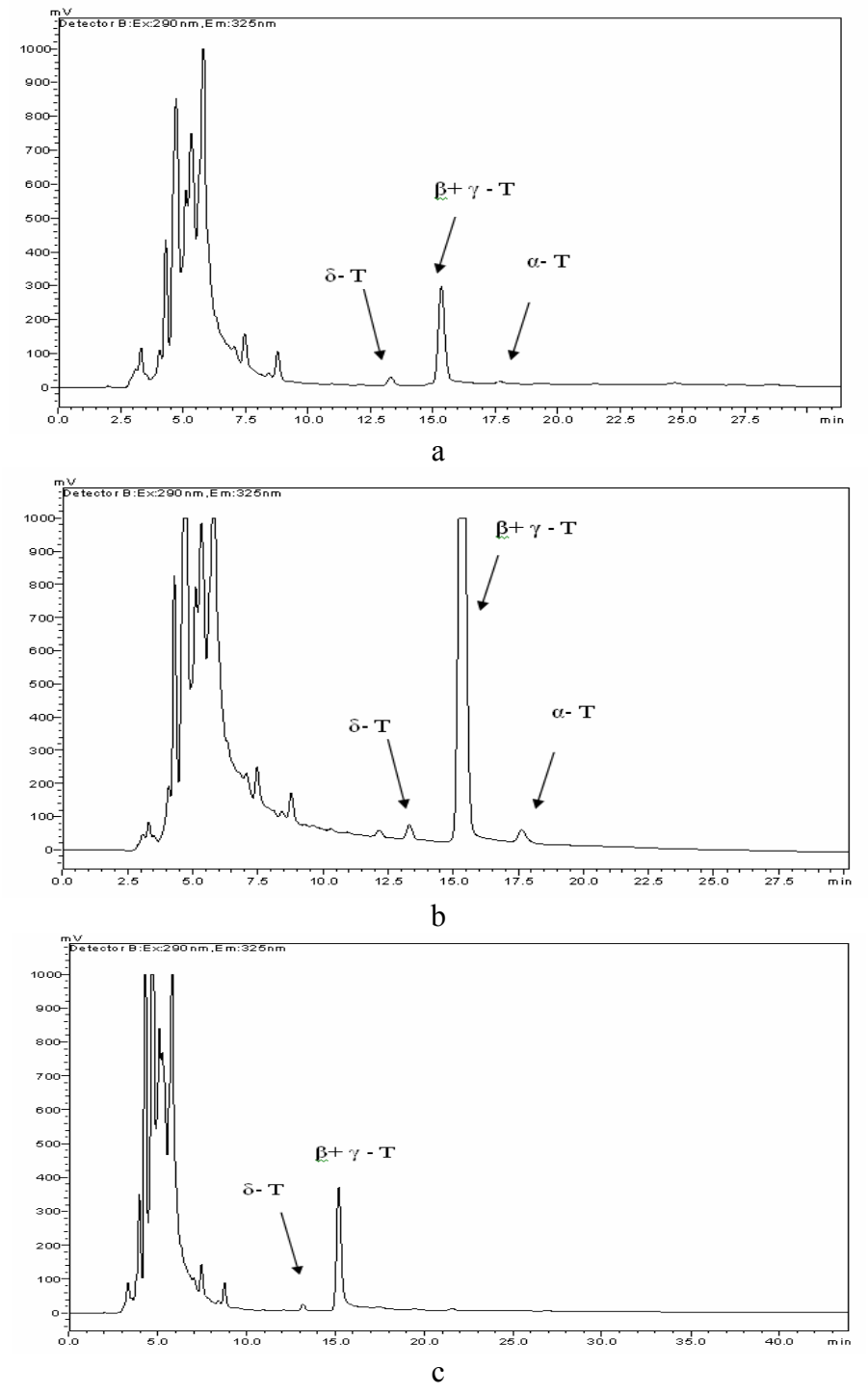


c

No. crt.	Tocopherols	Retention time (min.)	Amount from apricot kernel oil (µg/100 g)		
			2004	2005	2006
1	α -T	17.571	n.d.	42.40	43.54
2	β+γ -T	15.381	152.00	207.00	1259.40
3	δ - T	13.318	9.04	32.80	60.00

Fig. 2. The chromatograms HPLC of tocopherols for apricot kernels oil (a – 2004, b – 2005, c - 2006)



No. crt.	Tocopherols	Retention time (min.)	Amount from plum kernels oil (µg/100 g)		
			2004	2005	2006
1	α -T	17.571	n.d. (<0.1)	122.80	n.d. (<0.1)
2	β+γ -T	15.381	164.00	1057.20	162.20
3	δ - T	13.318	27.00	44.00	20.20

Fig. 3. The chromatograms HPLC of tocopherols for plum kernels oil (a – 2004, b – 2005, c - 2006)

Table 1. β-Caroten content from fruit kernel oil

Harvest year	β-caroten content of sample (μg/g oil)	
	plum kernel oil	apricot kernel oil
2004	188.65±2.03	61.05±2.08
2005	184.95±1.84	58.35±1.51
2006	191.21±2.13	62.46±2.16

Table 2. Total polyphenols and total antioxidant capacity values for fruit kernel oil

Samples	Total antioxidant capacity (mM Fe^{2+}/L)			Total polyphenols (mM gallic acid /L)		
	2004	2005	2006	2004	2005	2006
apricot kernel oil	1.29±0.11	1.33±0.12	0.86±0.07	1.28±0.14	1.30±0.15	0.88±0.09
plum kernel oil	1.78±0.15	0.42±0.03	1.90±0.16	1.79±0.17	0.61±0.05	2.85±0.24

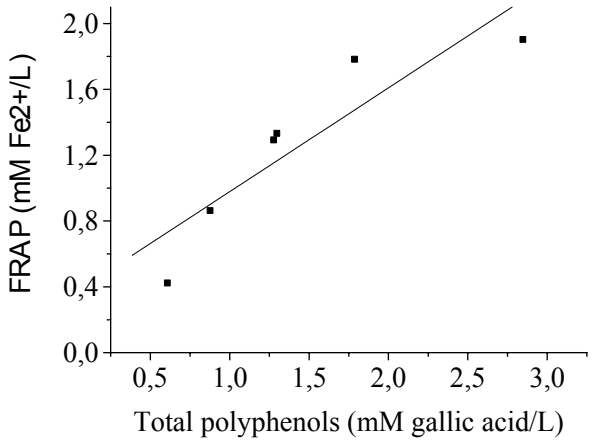


Fig. 4. Correlation between FRAP and polyphenols content from fruit kernel oil, R=0.899

Conclusions

Results of this study highlight that plum kernel oil is a richer source of β-carotene than apricot kernel oil. The content of isomeric forms of tocopherols depends on the harvest year and fruit species. For all analyzed samples, the major tocopherol fraction was represented by β+γ-tocopherol that was located in the range 73.4 and 94.4% of the total tocopherols. The antioxidant capacity of plum kernel oil was situated between 0.42 and 1.90 mM Fe^{2+}/L and between 0.86-1.33 mM Fe^{2+}/L for apricot kernels oil. For investigated oils, the polyphenols content were situated from 0.61 to 2.85 mM gallic acid/L. These data reveal that the oil obtained from plum and apricot kernels shows antioxidant properties similar to those of traditional unrefined vegetable oils. Results show a positive linear correlation between antioxidant activity and total phenolic content. The results of our study demonstrated that fruit kernels oil is a potential source of considerable amounts of tocopherols, carotenes and phenolics compounds. This oil could be utilized into various food products and cosmetics offering health benefits.

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