Quantification of Sterol Contents in Almond (*Prunus amygdalus* L.) Oils

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ABSTRACT: *In this study, the sterol contents of almond kernel oils collected from naturally growing almond trees in Mersin province were determined. Generally, the sterol contents of almond oil samples varied depending on almond types. The major sterols in almond oils wereß-sitosterol, 5-avenasterol and campesterol, followed by 5,24-stigmastadienol, stigmasterol, sitostanol, and cholesterol. While β-sitosterol contents of almond oils varied between 1986 mg/kg (T26) and 3908 mg/kg (T16), 5-avenasterol contents of almond oils samples were in the range between 215.9 mg/kg (T31) and 581.7 mg/kg (T16). In addition, campesterol contents of oils were found from 75.8 mg/kg (T31) to 172.3 mg/kg (T16). Interestingly, all sterol contents (except cholesterol, brassicasterol and 7-campesterol) of T16 almond oil were found higher than those of the other almond types. The current study showed that almond kernels of the investigated almond types from Turkey are potential sources of valuable oil which might be used as edible oil or industrial applications.*

KEYWORDS: *Almond; Type; Kernel oil; Sterol; GC and GC-MS.*

INTRODUCTION

Almond (*Prunus amygdalus* L.) is belonging to the Rosaceae family and genus *Prunus* grows in temperate zones in most countries of the world [1,2]. In many parts of the World, almond is an important oil crop and also an essential dietary component, acting as energy and functional compound sources. In addition, nut oils have been widely enjoyed for food applications due to their particular flavor [3,4]. Also, nuts such as almond and walnut kernels are used as an ingredient in many snacks and other processed foods. On the other hand, content and components of lipids in the confectionery industry are so important, because the high content of the oil reduces the water absorption by almond paste. Sterols are components of the unsaponifiable fractions of oils [5-8]. In vegetable oils, the main constituents are triacylglycerols, making up about 95-98% of the oils. The remaining a non-glycerol fraction consists of different compound classes such as sterols, tocopherols, and hydrocarbons, respectively. Extensive studies have shown that plant sterol and stanols reduce total and LDL-cholesterol levels in humans [9]. According to *Dulf et al.* [10], the richest natural sources of plant sterols in the human diet

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are unrefined vegetable oils, seeds, nuts, legumes and vegetables. Limited studies were conducted on sterol contents of several almond kernel oils. The aim of this study was to determine sterol contents of almond types naturally grown in Mersin district in Turkey.

EXPERIMENTAL SECTION

Material

Almond fruits were harvested in Mersin (Büyükeceli-Gülnar) province in Turkey in 2017. For almond kernels, three trees were determined as replicates. 25 kg were collected from the tree. The fruits were dried at 70 ºC in an oven till reaching dry weight. Then the kernels from each tree were ground to form a homogeneous sample, and stored in brown-color bottles. Samples were kept at refrigerator until analysis.

Methods

Oil content

After sample powder (about 2g) was extracted in a Soxhlet apparatus for 6 h., the solvent was removed at 40 °C in a rotary evaporator. The oil obtained was kept at +4 °C until use.

Sterols

About 250 mg of almond oil was saponified with a solution of ethanolic potassium hydroxide by boiling under reflux. For column separation, silica (70–200 mesh) from Aldrich was used. The glass column (20 cm long, 1.5 cm i.d.) was first filled with 1 g of sodium sulphate and then 1 g of silica. The previously saponified mixture (ca. 5 mL) was loaded onto 3 g of silica, evaporated to dryness, and transferred into the column as the third layer. For the elution of unsaponifiable matter the column was flushed with 20 mL of a mixture of ethyl ether and ethyl acetate (1:1). The unsaponifiable matter was isolated by solid-phase extraction on an aluminium oxide column (Merck, Darmstadt, Düren, Germany) on which fatty acid anions were retained and sterols passed through. For TLC silica plates (Alugran UV 254, Macherey-Nagel, Germany) were used. The sterol fraction was separated from unsaponifiable matter by thin-layer chromatograph re-extracted from the TLC material, and afterwards, the composition of the sterol fraction was determined by GLC using betulin as internal standard. The GC analysis was performed on a HP5890 instrument, using a SE 54 CB fused silica capillary column (Macherey-Nagel, Düren, Germany; 50 m long, 0.32 mm ID, 0.25 µm film thickness). Further parameters were as follows: hydrogen as carrier gas, injection and detection temperature adjusted to 320 °C. The oven temperature was programmed from 245 °C to 260 °C with a 5 °C/min heating rate and maintained at 340°C. The scan range and rate were 100-600 AMU and 2 scan/s, respectively. 1µl of the sample solution was injected into the GC-MS by an autosampler [11].

Statistical analysis

All analyses were carried out three times and the results are given as mean±standard deviation (MSTAT C;UK) of independent almond samples [12].

RESULTS AND DISCUSSION

The sterol contents of almond kernel oils collected from naturally growing almond trees in Mersin province are given in Table 1. Generally, sterol contents of almond oil samples varied depending on almond types with total amounts between 2608 mg/kg (T26) and 5114 mg/kg (T16). The major sterols in almond oils were β-sitosterol, δ5-avenasterol and campesterol, followed by 5,24-stigmastadienol, stigmasterol, sitostanol and chlerosterol. While β-sitosterol contents of almond oils varied between 1986 mg/kg (T26) and 3908 mg/kg (T16), δ5-avenasterol contents of almond oil samples were found between 215.9 mg/kg (T31) and 581.7 mg/kg (T16). This amount of δ5-avenasterol is high in comparison to other vegetable oils such as rapeseed or sunflower oil and interesting for the further application of almond oil as edible oil. δ5-avenasterol is known to act as an antioxidant and as an antipolymerization agent in frying oils due to the structural element of an ethylidene group in the side chain. Some authors described these compounds as most effective as antioxidants with a synergistic effect on other antioxidants during frying [13, 14]. In addition, campesterol contents of oils were found in the range from 75.8 mg/kg (T31) to 172.3 mg/kg (T16).

The amount of the other sterols was significant lower. For sigmasterol (37.6 mg/kg (T6) - 99.1 mg/kg (T16)), sitostanol (35.8 mg/kg (T25) – 94.8 mg/kg (T16)) 5,24-stigmastadienol (17.9 mg/kg (T25) - 47.5 mg/kg (T6)) and chlerosterol (26.1 mg/kg (T26) – 56.0 mg/kg (T16)) medium amounts were found, while the average amount

Table 1: Sterol contents of several almond types (mg/kg oil).

Sterols	Almond Types										Mean±SD*
	T6	T16	T19	T24	T25	T26	T28	T29	T30	T31	
Cholesterol	5.9	8.6	3.7	5.3	17.9	3.7	4.8	6.9	4.3	3.8	6.50±4.4
Brassica sterol	9.9	4.3	3.7	5.3	10.6	11.2	4.8	15.3	4.3	3.8	7.32±4.10
24-methylencholesterol	5.9	8.6	7.5	5.3	-**	3.7	4.8	4.7	4.3	3.8	5.40±1.7
Campesterol	104.8	172.3	115.7	137.3	143.3	78.3	116.3	114.6	85.0	75.8	114.34±30.7
Campestanol	4.0	4.3	2.9	-	-	3.2	-	-	-	-	3.60±0.7
Stigmasterol	37.6	99.1	41.1	47.5	71.6	59.6	72.7	53.5	42.5	41.7	56.7±19.4
7-campesterol	7.9	8.6	7.5	5.3	16.3	14.9	9.7	15.3	8.5	7.6	10.2±3.9
5,23-stigmastadienol	1.3	17.2	-	-	-	-	-	-	2.6	3.8	6.23±7.4
Chlerosterol	35.6	56.0	33.6	37.0	35.8	26.1	33.9	30.6	34.0	30.3	35.3±8.00
β-sitosterol	2390	3908	2351	2657	2360	1986	2297	2567	2240	2209	2496.5±529.3
Sitostanol	47.5	94.8	37.3	37.0	35.8	37.3	38.8	53.5	46.8	45.5	47.43±17.7
5-avenasterol	263.0	581.7	272.5	285.2	304.5	328.0	363.4	282.7	242.3	215.9	313.92±102.9
5,24-stigmastadienol	47.5	47.4	29.9	26.4	17.9	44.7	43.6	22.9	46.8	45.5	37.3±11.6
7-stigmasterol	4.0	73.3	3.7	5.3	-	3.7	4.8	7.6	4.3	7.6	12.70±22.8
7-Avenasterol	17.8	30.2	3.7	10.6	-	7.5	4.0	7.6	12.8	15.2	12.2±8.3
Total amount	2983	5114	2914	3264	3018	2608	2998	3183	2778	2709	3156.90±716.7

for the other sterols was below12 mg/kg. Interesting is the high content of total sterols in sample T16 with 5114 mg/kg, which is about 60% more than the average value of the total content. The variation between the other samples is small with 195.4 mg/kg in comparison to 699.0 mg/kg for all samples.

Matthäus et al. [6] reported that bioactive properties of almond and walnut kernels were investigated in developing almond and walnut kernels at 10 days intervals. The β-sitosterol contents of both almond and walnut oils were found between 1957 and 2558 mg/kg, and 1192 and 4426 mg/kg, respectively [6]. *Fernandes et al.* [7] reported that almond oil contained 70.9 mg/kg campesterol, 24.1 mg/kg stigmasterol, 2163 mg/kg ß-sitosterol and 278.5 mg/kg δ5-avenasterol. In another study, Dulf et al. [10] determined 6.3 mg/100 g campesterol, 3.8 mg/100 g campestanol, 6.0 mg/100 g stigmasterol, 152.7 mg/100 g β-sitosterol, 4.9 mg/100 g sitostanol, 20.1 mg/100 g δ5-avenasterol in sweet almond oil. Madawala et al. [15] found in almond oil 134 µg/g campesterol, 55 µg/g stigmasterol, 580 µg/g β-sitosterol, 32 µg/g δ5-avenasterol. *Philips et al.* [16] determined 143.4 mg/100 g β-sitosterol, 5 mg/100 g stigmasterol, 4.9 mg/100 g campesterol, 3.2 mg/100 g sitostanol, 19.7 mg/100 g δ5-avenasterol and 3.3 mg/100 g campestanol in almond oil.

There were some differences between the sterol contents of the different almond oils from this study, compared to the literature. These differences can be probably due to genetic variation, geographical factors, harvest time and analytic conditions [17]. Although the genotypes showed partly similar findings to related references with respect to oil content, some genotypes contained more oil than identified almond varieties or genotypes [5]. Type, genetic factors, ecological conditions and different ripening, harvest time generally effect the chemical composition of almond kernel [18,19].

CONCLUSIONS

Almond oil is characterized by the high amount of sterols mainly consisting β-sitosterol and δ5-avenasterol. These findings may be useful for dietary information, which requires prior knowledge of the nutritional composition of almond kernel oils. In addition the high content of δ5-avenasterol may result in a higher oxidative stability during heating or storage. The current study shows that almond kernels of the investigated almond types from Turkey are a potential source of valuable oil which might be used for edible purposes and industrial applications.

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