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Evaluation of Marketed Almond Oils (Prunus dulcis) in Terms of European Pharmacopoeia Criteria

Türkiye Piyasasında Satılan Badem Yağlarının (Prunus dulcis) Avrupa Farmakopesi Kriterleri Açısından Değerlendirilmesi

Short title: Evaluation of Market Almond Oils

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ABSTRACT
INTRODUCTION: Almond oil marketed for health benefits and cosmetic purposes should be in compliance with the European Pharmacopoeia criteria. Therefore, in this study, 17 almond oil samples sold in pharmacies, herbal shops, online and cosmetics stores were analyzed in terms of "Almond Oil" monograph criteria which have been mentioned in European Pharmacopoeia 7.0 (EP 7.0).
METHODS: In this study, 17 almond oil samples sold in pharmacies, herbal, online and cosmetics stores were analyzed in terms of "almond oil" monograph criteria which have been mentioned in European Pharmacopoeia 7.0 (EP 7.0). Appearance, acidity value, and peroxide value were determined and the ingredients were identified with thin layer chromatography. Fatty acids were analyzed by Gas Chromatography method using Flame Ionization Detector.
RESULTS: It was determined that two of the 17 samples were in compliance with the EP 7.0 criteria.
DISCUSSION AND CONCLUSION: Almond oil, which is currently marketed according to the manufacturer's own marketing and quality criteria, is not included in the Turkish Food Codex Standards. Our research has shown that most of the products do not comply with European Pharmacopoeia standards. For this reason, it should be ensured that almond oil is listed in this codex and urgent arrangements should be made for quality control analysis.
Keywords: Fatty acids, Gas Chromatography-Mass Spectrometry, Prunus dulcis, quality control

ÖZ
GİRİŞ ve AMAÇ: Sağlık faydaları ve kozmetik amaçlı pazarlanan badem yağı, Avrupa Farmakopesi kriterlerine uygun olmalıdır. Bu nedenle bu çalışmada eczanelerde, aktar dükkanlarında, internet mağazalarında ve kozmetik mağazalarında satılan 17 badem yağı numunesi Avrupa Farmakopesi 7.0'da (EP 7.0) belirtilen "Badem Yağı" monografi kriterleri açısından incelenmiştir.
YÖNTEM ve GEREÇLER: Bu çalışmada eczanelerde, aktarlarda, internet mağazalarında ve kozmetik mağazalarında satılan 17 badem yağı numunesi, Avrupa Farmakopesi 7.0'da (EP 7.0) belirtilen "badem yağı" monograf kriterleri açısından analiz edilmiştir. Görünüm, asitlik değeri ve peroksit değerleri belirlenmiş ve bileşenler ince tabaka kromatografisi ile tespit edilmiştir. Yağ asitleri, Alev İyonizasyon Detektörü kullanılarak Gaz Kromatografisi yöntemi ile analiz edilmiştir.

BULGULAR: 17 numuneden ikisinin EP 7.0 kriterlerine uygun olduğu belirlenmiştir.
TARTIŞMA ve SONUÇ: Halihazırda üreticinin kendi pazarlama ve kalite kriterlerine göre pazarlanan badem yağı Türk Gıda Kodeksi Standartlarında yer almamaktadır. Araştırmamız, ürünlerin çoğunun Avrupa Farmakopesi standartlarına uymadığını göstermiştir. Bu nedenle badem yağının bu Kodeks içerisinde yer alması sağlanmalı ve kalite kontrol analizi için acil düzenlemeler yapılmalıdır.
Anahtar Kelimeler: Gaz Kromatografisi-Kütle Spektrometresi, kalite kontrol, Prunus dulcis, yağ asitleri

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INTRODUCTION

Turkey, with its diversity of plant species is one of the world's most important gene source. The almond is one among the important species in this gene source, which is grown or cultivated across widespread in Turkey. Almond [*Prunus dulcis* (Mill.) DA Webb, syn. *P. amygdalus* Batsch, and *P. communis* (L.)] are divided into two varieties,[1] pomologically, sweet almond (*Prunus dulcis* var. *dulcis*), and bitter almond (*Prunus dulcis* var. *amara*) (*Prunus amygdalus* Batsch) where all belongs to the family Rosaceae, along with raspberries, peaches, apples and pears. About of 20 species have been reported in Iran while there have been more than 30 wild species in the world.[2]

Almonds, which are typically used as snack food or found as an ingredient in many products,[3,4] have considerable economic value with its different usage areas (gastronomy, confectionery *etc.*).[5-9] They are commonly cultivated for its fruit[10] and placed number one among the products of tree nuts.[11] Due to their high nutritional content and their promising effects on human health with their high levels of monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) content, consumption of and demand for almonds stays high.[5-7] The almond oil obtained from almond seeds is used as medicine, pharmaceutical and cosmetic products to treat dry skin disorders such as psoriasis.[12] A number of studies of the biological value and chemical properties of nut proteins and oils have been reported.[13] *In vivo* studies have reported that almond seeds and oils have hepatoprotective, anti-inflammatory, anticancer and immune stimulant effects. Among tocopherol, phytosterols and many other health-promoting micronutrients,[14] almond with its high content of mono-unsaturated fatty acids, can reduce the gastric carbohydrate absorption rate and increase insulin sensitivity,[15] while also helpful for constipation and restless bowel syndrome.[16-18] The importance of almond fruit with rich oleic, linoleic and linolenic acid content has increased due to the positive effects on cholesterol and cardiovascular disease in human,[12] while the observed blood cholesterol lowering effects of nuts were far better than what was predicted according to their dietary fatty acid profiles.[19,20]

The Almond Oil Market was valued at $1 118 million in 2016 and is expected to reach $2 680 million by 2023 while the growth of almond oil market is driven by rise in production of aromatherapy products, increase in preference of customers towards cosmetic products

containing natural ingredients, rapid urbanization, and growth in applications of almond oil in the pharmaceutical industry.[21]
Differences in the major and minor components of the medicinal oils significantly affect their nutritional, health-promoting activities and their organoleptic properties. Therefore in this study, 17 almond oil samples sold in pharmacies, herbal shops, online and cosmetics stores were analyzed in terms of the criteria specified in the "Almond Oil" monograph in the EP 7.0.[22]

MATERIALS AND METHODS

Materials

Seventeen different brands of almond oils were purchased from pharmacies, herbal, online, and cosmetics stores in Ankara/Turkey in 2017, and 2018. All chemicals used were analytical reagent grade. Oksan Co., Ltd. (Ankara, Turkey) provided helium, hydrogen, and dried air gases for Gas Chromatography with 99.99% purity. Deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).
Gas Chromatography (7890A GC System, Agilent Technologies Inc, US), a capillary column Rt-2560 (100 m, 0.25 mm ID, 0.2 µm) (Restek Corporation Bellefonte, US), vial insert, 250 µl, glass with polymer feet, vial, screw top, 2 ml, amber and cap, screw, blue, PTFE/red silicone septa (Agilent Technologies Inc., US) were used.

Methods

Seventeen different brands of almond oils were analyzed according to the criteria of EP 7.0[22] (appearance, identification, acid value, peroxide value, and composition of fatty acids) and results were compared with pharmacopoeia quality of reference standard almond oil from *P. dulcis* (63445-250 ml, Sigma Aldrich, Lot BCBV9057). All the results are given for at least triplicates and the values are given as mean ± SD (standard deviation).

RESULTS

Appearance

All oil samples were placed into glass droppers and their colors were compared with the pharmacopoeia quality of reference almond oil (Table 1).[22]

Identification

Thin Layer Chromatography (TLC) was used to identify the almond oils.[23] C-18 Silica TLC plate (Supelco 10×10 cm, 0.2 mm) was used as stationary phase. An approximately 20 mg of oil sample, and reference solution of almond oil complying with "Almond Oil European Pharmacopoeia" standards were dissolved in 3 ml of CH_2Cl_2 (for Gas Chromatography MSSupraSolv®). TLC plate was first eluted with ether (Sigma Aldrich, Germany) mobile phase up to 0.5 cm. Then the plate removed from the tank and immersed into the other tank containing CH_2Cl_2: glacial acetic acid (Sigma Aldrich, Germany): acetone (Sigma Aldrich, Germany) (2:4:5, by volume) mobile phase, and eluted 8 cm. The plate removed from the tank and dried with air, and then 100 g l^{-1} solution of phosphomolybdic acid (Sigma Aldrich, Germany) in alcohol as revelator was sprayed. TLC plate was heated at 120 °C for 3 min. Then retention times and stains of the commercial almond oils were compared with the retention times and stains of reference almond oil from *P. dulcis* (Fig. 1). Thin layer chromatogram of samples and reference almond oil (Fig. 1) was also compared with reference chromatogram in European Pharmacopoeia 8.0 (Fig. 2).[23]

Acid (I_A) and Peroxide (I_P) value

The acid value is expressed as milligrams of KOH (Sigma Aldrich, Germany) required to neutralize the free acids present in 1 g of the oil. Therefore, about 10 grams of the oil sample was dissolved in 50 ml of 96 % methanol (Merck, Germany) and peroxide-free ether (Merck, Germany, Germany) mixture (1:1, by volume), then titrated with 0.1 M KOH in the presence

of phenolphthalein (Sigma Aldrich, Germany) indicator until the pink color remained stable for at least 15 s. Acid values of samples were compared the value of maximum 2.0 in 5.0 g oil sample (Table 1).[22,24]

The peroxide value is expressed as milliequivalent of active oxygen, the quantity of peroxide contained in 1000 g of the substance. So, about 5 grams of oil was placed in a 250 ml conical flask fitted with a ground-glass stopper. 30 ml of a mixture of chloroform (Merck, Germany) and glacial acetic acid (2:3, by volume) was added. After the oil dissolved, 0.5 ml of saturated potassium iodide (Merck, Germany) solution was added and shaken exactly 1 min, then 30 ml of water was added. It was titrated with 0.01 M sodium thiosulfate (Sigma Aldrich, Germany) until the yellow color is almost discharged. 5 ml of starch solution was added and continued the titration, until the color is discharged. It was carried out a blank test under the same conditions. Peroxide values were compared the value of maximum 15.0 in 5 g oil (Table 1).[22,24]

Preparation of fatty acid methyl esters (FAMEs) standard

All standard solutions were prepared in an ice bath and stored at -20 °C. 0.1 g of FAME37, C4-24 (Sigma Aldrich, Germany) reference standard was dissolved in 250 µl of CH_2Cl_2 (400 mg ml^{-1} FAME37, C4-24), and then, 75 µl of 400 mg ml^{-1} FAME37, C4-24 was diluted to 1.0 ml with CH_2Cl_2 (30 mg ml^{-1} FAME37).

Preparation of FAMEs in almond oils

Each oil was dried at 100-105 °C. 1.0 g of oil was weighed into a 25 ml round-bottomed flask with a ground-glass neck fitted with a reflux condenser and a gas port into the flask. 10 ml of anhydrous methanol, and 0.2 mL of 60 g l^{-1} KOH in methanol were added. The reflux condenser was attached, passed nitrogen through the mixture at a rate of about 50 ml min^{-1}, shacked and heated to boiling. When the solution is clear, it was continued heating for a further 5 min and cooled the flask and transferred the contents to a separating funnel. The flask was rinsed with 5 ml of anhydrous chromatographic quality of heptane, 99 % (Sigma Aldrich, Germany), then the rinsing was transferred to the separating funnel and shacked. 10 ml of a 200 g l^{-1} NaCl (Sigma Aldrich, Germany) solution was added and shacked vigorously. It was allowed to separate two phases and transferred the upper organic layer to a vial containing anhydrous Na_2SO_4 (Sigma Aldrich, Germany), allowed to stand, then filtered.

Analysis of FAMEs with GC-FID

FAME37, C4-24 standard, and fatty acids in almond oil were analyzed by Gas Chromatography (GC) equipped with an auto sampler model Agilent 7693, and flame ionization detector (FID).[25] A capillary column Rt-2560 (100m, 0.25 mm ID, 0.2 µm), vial insert, 250 µl, glass with polymer feet, vial, screw top, 2 ml, amber and cap, screw, blue, PTFE/red silicone septa were used as the column, and sample vial, respectively. The GC oven was programmed to 100 °C, held for 4 min, and then increased by 3 °C min ramp to 240 °C, held for 10 min. The injector and FID detector temperature were 225 °C and 250 °C, respectively. The injection volume was 2 µl with the split ratio 200:1. Helium was used as the carrier gas at 1.2 ml min^{-1}, 20 cm s^{-1} at 175 °C. The FAMEs in oil samples were identified from the chromatogram by comparing their retention times with the standard FAME37, C4-24 and the number of FAMEs in the oil samples was expressed as percentage by weight of all the fatty acid methyl esters (FAMEs) from the total detected fatty acids (Figure 3 and 4). Peak area was used for quantitative analysis of FAMEs. The content of FAMEs in almond oil samples was listed in Table 2a, and 2b.

DISCUSSION

EP 7.0 states that the appearance of almond oil should be clear or pale yellow. The appearance, acid values and the peroxide values of almond oil samples are given in Table I.

In the evaluations made, it was observed that the samples coded s7, s9, s10, s14 and s17 do not have this appearance and their colors are pale green or dark yellow (Table I).
Calculation of acid value in fixed oils is an important quality criterion. It also gives an idea of whether the oil has exceeded its shelf life. The increase in the amount of free fatty acid is an indication that there will be a decrease in the stability of the oil against oxidation. This situation is defined as the oil becoming bitter. According to the EP 7.0, it is reported that the acid value in the almond oil should be "maximum 2.0 in 5 g oil". When evaluated in terms of this criterion; acid values of the samples coded s3, s7, s10 and s14 (I_A= 3.30 to 9.18) were found to be well above the criteria reported (Table I).
The peroxide content of the oil samples indicates that an oil has started to oxidize. Peroxidation process occurs due to high temperature and light exposure. Contact with metal surfaces can also cause the oil to oxidize faster. In addition, oxygen breaks down unsaturated fatty acids, resulting in smaller aldehyde molecules such as malondialdehyde. The lower the peroxide values, the longer the shelf life of the oil. A high peroxide value usually indicates poor processing and poor oil quality. According to the EP 7.0, it is reported that the peroxide values in almond oil should be "maximum 15.0 in 5 g oil". When the results are investigated, the samples, s2 (I_P = 17.30) and s17 (I_P = 19.59) were found to have high peroxide values compared to the maximum values (Table I).
The fatty acids in commercial almond oils were identified by comparing TLC with the reference almond oil from *P. dulcis* at the same condition (Fig. 1). Results of the fatty oils reported in the EP8.0 (Fig. 2)[23] were also used identification of fatty acids of samples. TLC obtained from samples s5, s6, s8, s9, s11, s12, and s13 is similar to the reference almond oil chromatogram and the corresponding pharmacopeia chromatogram that were shown in Fig. 2. Nevertheless, the general profile of samples s1, s2, s3, s4, s7, s10, s14, s15 and s16 coded expressions are not suitable.
Quality of the oil is related to the contents, types and the number of fatty acids. In the monograph EP 7.0 Almond oil, defined as "*Amygdalae oleum raffinatum*" and its fatty oil was obtained from the ripe seeds of *P. dulcis* var. *dulcis* or *P. dulcis* var. *amara* or a mixture of both varieties by cold expression. It is then refined. A suitable antioxidant may be added. Another almond oil registered in the EP 7.0 is "Almond oil, virgin, "*Amygdalae oleum virginale*", and its fatty oils were obtained by cold expression from the ripe seeds of *P. dulcis* var. *dulcis* or *P. dulcis* var. *amara* or a mixture of both varieties.
According to EP 7.0, the fatty acid composition of refined almond oil and virgin almond oil are given as; palmitic acid (4.0-9.0 %), palmitoleic acid (0.8 % max), margaric acid (not more than 0.2 %), stearic acid (3.0 % max), oleic acid (62-86 %), linoleic acid (20-30 %), linolenic acid (0.4 % max), arachidic acid (not more than 0.2 %), eicosenoic acid (0.3 % max), behenic acid (not more than 0.2 %), erucic acid (0.1 % max).[22] Supelco FAME37mix, C4-24 was used as standard reference material to detect the fatty acids in almond oil with GC-FID.
Fatty acids are defined as the organic compounds formed by a hydrocarbonated chain and a carboxylic acid group which are normally bounded with glycerol-forming acylglycerides (mono-, di- or triglycerides).[26] While α-linolenic acid~~,~~ and linoleic acids~~, and oleic acid~~ are the essential fatty acids, which the human body cannot produce, the unsaturated fatty acids in almond oil (50–81 % oleic, and 6–37 % linoleic acid) become more desirable for the physicochemical and nutritional properties and its significant role in human diet.[27,28,29,30,31] ~~The ratio of these two fatty acids (oleic/linoleic) is considered an important criterion for establishing the quality and stability of the oil.[32]~~ Their fatty acid profiles depend on the oil's variety and origin, which affects their stability against rancidity during transport, storage and directly affecting their products and also influencing their price.[33] Oil content and composition of almond seed and the fatty acid are usually referred to as the quality characteristic of almond conditions,[32] while it depends on the genotype, climatic conditions,

agriculture and harvest. The amounts of oleic acid, oleic/linoleic acid ratio and tocopherol concentration, all are used as quality indicators while the oleic/linoleic acid ratio is significant in determining the quality of the kernel due to its preventive effect on lipid oxidation.[32] When the fatty acid contents of the almond oil samples analyzed are evaluated according to the EP 8.0,[25] it is seen that the oleic acid (C18:1) amounts of samples with s1, s2, s4, s5, s6, s8,and s9 codes are lower than the reference value and the linoleic acid (C18:2) amounts are higher than the reference value (Table III). This difference may also be due to the production of gums, as well as the possibility of oxidation of oleic acid ($C_{18}H_{34}O_2$) to linoleic acid ($C_{18}H_{32}O_2$) depending on the production methods (refined, infiltration, cold press etc.), storage conditions (heat, light, etc.), antioxidant addition and amount. However, the samples with s7, s10, s11 and s12 codes were found to meet the criteria required by the EP 7.0.[22] When all the results are evaluated; among the 17 almond oil samples sold in the pharmacy, only 2 (s11 and s12) of them were found to meet the EP 7.0[22] criteria for quality (Table IV).

CONCLUSION

Many vegetable oils are sold with health-promoting claims or statements that they are beneficial against diseases while almond oils are one of them, as they are sold in “natural”, “organic products”, “local products” shops, cosmetics store chains and pharmacies. Almond oil, which is marketed for health benefits and cosmetics, must meet the European pharmacopoeia criteria. Currently these almond oils are marketed as fixed oils with the producer’s own marketing and quality criteria, while our study shows that most of the products are off-limits of Pharmacopeias. A pharmacopoeia’s core mission is to protect public health by creating and making available public standards to help ensure the quality of products while the user or procurer can make an independent judgement regarding quality, thus safeguarding the health of the public. To establish the necessary quality criteria whereas show no harm to user, the almond oil (if for human use) needs to be encouruaged to be pharmacopoeia compliance. Additionally, currently, almond oil is not included in the Turkish Food Codex Standards. For this reason, it should be ensured that almond oil is listed in this codex and urgent arrangements should be made for quality control analysis.

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Table 1. Appearance, acid value and, peroxide value of almond oils

Sample No	Appearance	I_A[a] ± SD[b] mg KOH g^{-1} oil	I_P[c] ± SD[b] ml g^{-1}
s1	Clear liquid	0.34 ± 0.04	10.61 ± 0.86
s2	Pale yellow	0.48 ± 0.01	17.30 ± 0.87
s3	Pale yellow	3.30 ± 0.01	4.85 ± 0.99
s4	Clear liquid	0.36 ± 0.04	10.94 ± 1.60
s5	Clear liquid	0,80 ± 0,03	9.40 ± 0.82
s6	Clear liquid	0.32 ± 0.001	8.16 ± 0.77
s7	Dark yellow	9.18 ± 0.09	3.06 ± 0.77
s8	Clear liquid	0.36 ± 0.01	3.76 ± 0.14
s9	Pale green	1.22 ± 0.02	5.40 ± 0.18
s10	Yellowish green	8.65 ± 0.32	4.46 ± 0.32
s11	Clear liquid	0.18 ± 0.01	7.14 ± 0.07
s12	Pale yellow	1.24 ± 0.06	7.68 ± 0.28

s13	Clear liquid	0.35 ± 0.04	9.52 ± 3.30
s14	Dark yellow	6.04 ± 0.16	6.10 ± 0.28
s15	Pale yellow	0.32 ± 0.02	2.43 ± 0.10
s16	Clear liquid	0.35 ± 0.01	19.59 ± 0.41
s17	Dark yellow	0.38 ± 0.03	8.98 ± 0.09
Almond oil (Pharmacopoeia quality)	Pale yellow	0.44 ± 0.02	3.46 ± 0.29

[a] Reference acid value is maximum 2.0, determined on 5.0 g; [b] SD; Standard deviation, n = 3; [c] Reference peroxide value is maximum 15.0

Table 2A. Content of FAMEs in almond oil samples 1-9

FAME	CONTENT, % IN ALMOND OIL ± SD[a]								
	s1	s2	s3	s4	s5	s6	s7	s8	s9
C14:0	0.1 ± 0.0	0.1 ± 0.0	-	-	-	-	0.1 ± 0.0	-	-
C16:0	6.2 ± 0.5	10.2 ± 0.3[b]	4.9 ± 0.3	5.7 ± 0.3	5.5 ± 0.3	5.7 ± 0.3	6.5 ± 0.3	5.5 ± 0.3	5.7 ± 0.3
C16:1	-	-	0.3 ±0.0	-	-	-	0.4 ± 0.1	0.2 ± 0.0	-
C18:0	3.4 ± 0.1[b]	4.6 ± 0.1[b]	1.2 ± 0.2	3.8 ± 0.1[b]	3.9 ± 0.1[b]	3.6 ± 0.1[b]	1.2 ± 0.2	3.5 ± 0.2[b]	2.8 ± 0.2
C18:1	29.3 ± 0.4[b]	25.9 ± 0.5[b]	66.1 ± 0.6	30.5 ± 0.6[b]	29.9 ± 0.5[b]	30.4 ± 0.5[b]	64.2 ± 1.0	33.8 ± 0.3[b]	36.4 ± 0.5[b]
C18:2	57.8 ± 1.3[b]	51.4 ± 0.3[b]	20.6 ± 0.1	57.6 ± 0.6[b]	58.3 ± 0.6[b]	57.9 ± 0.6	24.9 ± 1.5	54.3 ± 1.2[b]	52.9 ± 1.2[b]
C20:0	0.3 ± 0.0[b]	0.6 ± 0.1[b]	0.4 ± 0.0[b]	0.3 ± 0.0[b]	0.3 ± 0.0[b]	0.3 ± 0.0[b]	-	0.3 ± 0.0[b]	-
C18:3	0.3 ± 0.0	0.6 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
C20:1	-	5.2 ± 0.3[b]	5.5 ± 0.3[b]	0.1 ± 0.0	-	-	-	-	-
C18:3	0.3 ± 0.0	0.1 ± 0.0	-	-	0.1 ± 0.0	0.1 ± 0.0	-	-	-
C20:2	0.9 ± 0.2	0.6 ± 0.1	0.3 ± 0.0	0.9 ±0.1	0.9 ± 0.1	0.9 ± 0.1	-	0.9 ± 0.1	0.8 ± 0.1
C24:0	-	-	-	0.4 ± 0.0	-	-	-	-	-
C20:5	0.4 ± 0.2	-	-	-	0.4 ± 0.0	0.4 ± 0.0	-	0.5 ± 0.0	-

[a] SD; Standard deviation, n=3; [b] out of limit

Table 2B. Content of FAMEs in almond oils samples 10-17 and reference almond oil

FAME	CONTENT, % IN ALMOND OIL ± SD[a]								
	s10	s11	s12	s13	s14	s15	s16	s17	Reference almond oil
C16:0	5.8 ± 0.0	5.7 ± 0.2	5.5± 0.2	3.9 ± 0.1[b]	6.3 ± 0.3	6.9 ± 0.3	5.7 ± 0.2	5.7 ± 0.1	5.5 ± 0.2
C16:1	-	0.4 ± 0.1	0.4 ± 0.1	-	0.4 ± 0.1	-	0.4 ±0.1	0.2 ± 0.0	-
C18:0	1.2 ± 0.0	0.8 ± 0.2	0.5 ± 0.2	2.5 ± 0.5	1.0 ± 0.2	4.3 ± 0.4[b]	3.9 ± 0.2[b]	1.9 ± 0.3	0.9 ± 0.1
C18:1	69.8 ± 1.5	67.6 ± 0.5	68.8 ± 0.9	67.8 ± 1.3	65.6 ± 0.6	23.2 ± 0.6[b]	31.3 ± 0.5[b]	16.7 ± 0.7[b]	68.3 ± 0.1
C18:2	23.2 ± 0.3	24.5 ± 0.7	23.3 ± 0.6	22.7 ± 0.9	25.3 ± 0.6	28.5 ± 0.2	20.2 ± 0.2	74.0 ± 1.6[b]	24.7 ± 0.4
C20:0	-	-	-	0.3 ± 0.0[b]	-	3.7 ± 0.9[b]	4.2 ± 0.3[b]	0.5 ± 0.0[b]	-
C18:3	-	-	-	0.3 ± 0.0	0.3 ± 0.0	2.7 ± 0.0	2.8 ± 0.0	0.3 ± 0.1	-
C20:1	-	-	-	-	-	31.3 ± 0.1[b]	32.9 ± 0.4[b]	-	-
C20:2	-	-	-	1.6 ± 0.1	-	-	-	0.6 ± 0.0	-
C22:0	-	-	-	-	-	0.2 ± 0.1	0.2 ± 0.1	-	-
C24:0	-	-	-	0.9 ± 0.0	-	-	-	-	-

[a] SD; Standard deviation, n=3; [b] out of limit

Table 3. Comparison of FAMEs in almond oils according to the EP 7.0

FAMEs	s1	s2	s3	s4	s5	s6	s7	s8	s9	s10	s11	s12	s13	s14	s15	s16	s17
C16:0	R	↑	R	R	R	R	R	R	R	R	R	R	↓	R	R	R	R
C16:1	-	-	R	-	_	-	R	R	-	-	R	R	-	R	-	R	R
C17:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C18:0	↑	↑	R	↑	↑	↑	R	↑	R	R	R	R	R	R	↑	↑	R
C18:1	↓	↓	R	↓	↓	↓	R	↓	↓	R	R	R	R	↓	↓	↓	R
C18:2	↑	↑	R	↑	↑	↑	R	↑	↑	R	R	R	R	R	R	R	↑
C18:3	R	R	-	-	R	R	-	-	-	-	-	-	-	-	-	-	-
C20:0	↑	↑	↑	↑	↑	↑	-	↑	-	-	-	-	↑	-	↑	↑	↑
C20:1	-	↑	↑	R	-	-	-	-	-	-	-	-	-	-	↑	↑	_
C22:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑	↑	-
C22:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

R: between reference range, ↑: more than reference value, ↓ less than reference value, -: not found

Table 4. Comparison with criteria according to EP 7.0

Sample no	Apperance	TLC	FAMEs	I_A	I_P
s1	+	-	-	+	+
s2	+	-	-	+	-

s3	+	-	-	-	+
s4	+	-	-	+	+
s5	+	+	-	+	+
s6	+	+	-	+	+
s7	-	-	+	-	+
s8	+	+	-	+	+
s9	-	+	-	+	+
s10	-	-	+	-	+
s11	+	+	+	+	+
s12	+	+	+	+	+
s13	+	+	-	+	+
s14	_	-	-	-	+
s15	+	-	-	+	+
s16	+	-	-	+	-
s17	-	+	-	+	+

+: appropriate -: not appropriate

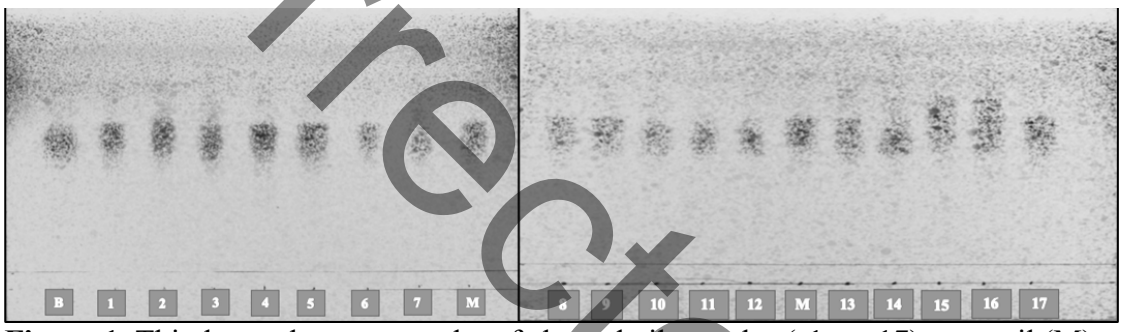

Figure 1. Thin layer chromatography of almond oil samples (s1 to s17), corn oil (M), and reference almond oil obtained from *P. dulcis* (B)

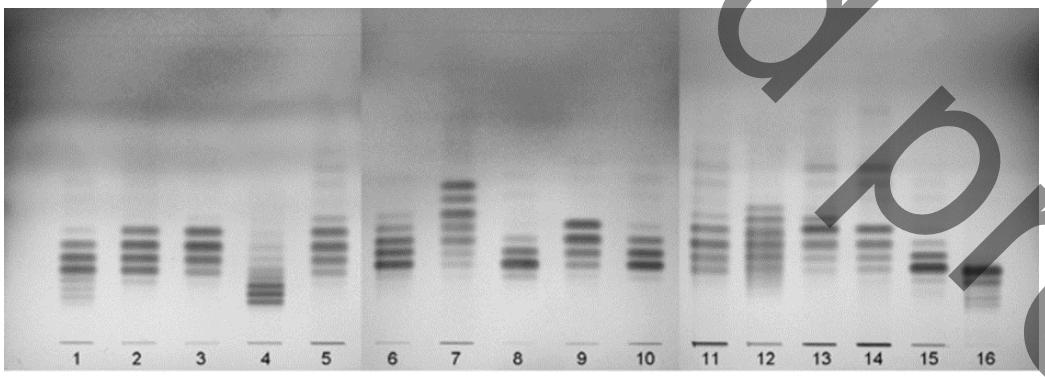

Figure 2. Thin Layer Chromatograms for the identification of fatty acids in European Pharmacopoeia 8.0[23] (1: Arachis oil, 2: Sesame oil, 3: Maize oil, 4: Rapeseed oil, 5: Soya-bean oil, 6: Rapeseed oil (erucic acid-free) 7: Linseed oil, 8: Olive oil, 9: Sunflower oil 10: Almond oil, 11: Wheat-germ oil, 12: Borage oil, 13: Evening primrose oil, 14: Safflower oil (type I), 15: Safflower oil (type II), 16: Hydrogenated arachis oil)

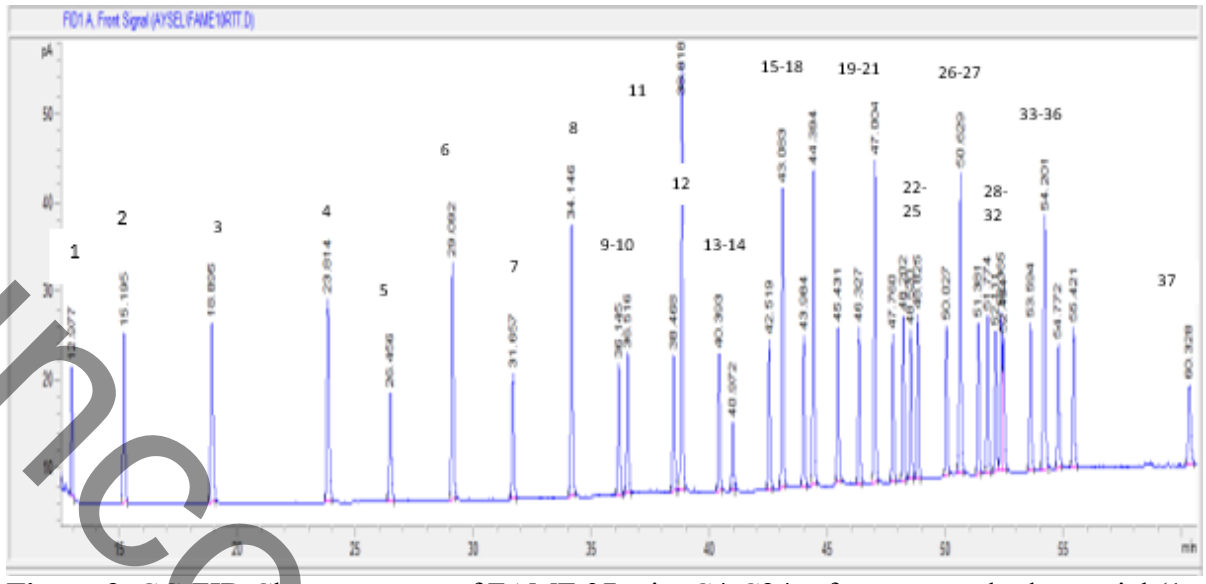

Figure 3. GC-FID Chromatogram of FAME 37 mix, C4-C24 reference standard material (1- C4:0, 2- C6:0, 3- C8:0, 4- C10:0, 5- C11:0, 6- C12:0, 7- C13:0, 8- C14:0, 9- C14:1(*cis*-9), 10- C15:0, 11- C15-1(*cis*-10), 12- C16:0, 13- C16:1(*cis*-9), 14- C17:0, 15- C17:1 (*cis*-10), 16- C18:0, 17- C18:1 (*trans*-9), 18- C18:1 (*cis* -9), 19- C18-2 (*trans*-9,12), 20- C18:2 (*cis*-9,12), 21- C20:0, 22- C18:3 (*cis*-6,9,12), 23- C20:1 (*cis*-11), 24- C18:3 (*cis*-9,12,15), 25- C21:0, 26- C20:2 (*cis*-11,14), 27- C22-:0, 28- C20:3 (cis-8,11,14), 29- C22:1 (*cis*-13), 30- C20:3 (*cis*-11,14,17), 31- C20-4 (*cis*-5,8,11,14), 32- C23:0, 33- C22:2 (*cis*-13,16), 34- C24:0, 35- C20:5 (*cis*-5,8,11,14,17), 36- C24:1 (*cis*-15), 37- C22:6 (*cis*-4,7,10,13,16,19))

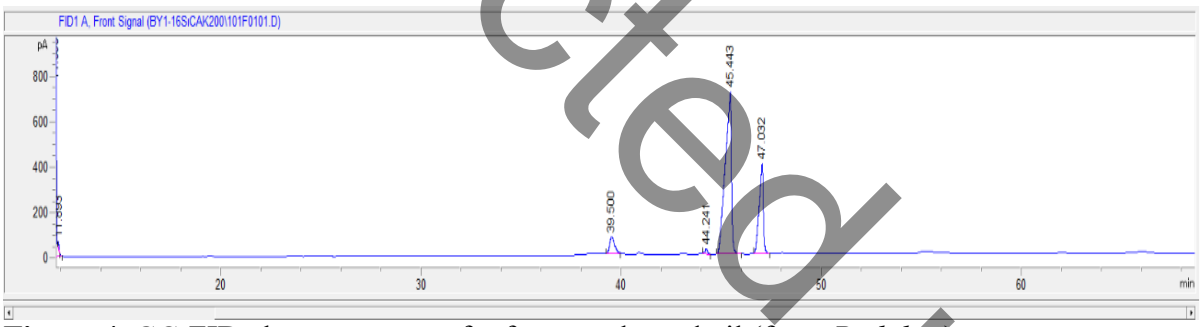

Figure 4. GC-FID chromatogram of reference almond oil (from *P. dulcis*)