

Original Article

Variation in Oil, Protein Content and Fatty Acid Composition of Twelve Turkish Opium Poppy (*Papaver somniferum* L.) Lines

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Abstract

Opium poppy (*Papaver somniferum* L.) has two major products: alkaloids in the capsules and the seeds. The seed contains oil, protein, carbohydrate, moisture and mineral matters. The seed oil is rich in unsaturated fatty acids, particularly linoleic and oleic acid. Remaining meals after oil extraction are the important source for animal diets. The United Nations recognize Turkey and India as traditional poppy producing countries. The aim of this study was to evaluate the seeds of twelve different Turkish opium poppy lines for their protein, oil and fatty acids percentage. The trial was carried out at the Experimental Fields of the Agronomy Department, Faculty of Agriculture of Ankara University, Turkey during 2009-2010. The materials were collected from opium poppy collections in the department. All seeds were sown on 12 October 2009 and harvested during second week of July 2010. The oil was extracted and determined with hexane by foss soxtec 2055 apparatus. Fatty acids were analyzed by gas chromatography and the protein were determined by kjeldahl protein analyzer. Seed oil, protein and fatty acids percentage of twelve different lines were determined. Oil contents of seed lines ranged 40.96 - 50.88%. The major fatty acid in seed oils was linoleic acid (70.60–76.65%) whereas oleic and palmitic acid contents of seed oils ranged from 12.08 -17,71% and from 7.92 – 8.80%, respectively. Protein content of opium poppy ranged 19.67 – 20.94%. In conclusion, those opium poppy lines can be major source of raw materials such as oil and protein.

Key words: Poppy, Fatty acid, Oil extraction, Alkaloid, Capsule

Introduction

Opium poppy (Papaver somniferum L.) is a member of Papaveracae family. It is an erect, annual herb, 30-170 cm tall; flower buds are ovate, dropping before anthesis (hook stage); the fruit is the capsule that usually contains a high number of very small seeds [26]. The plant seems to be one of the few species which were utilized and cultivated since prehistoric times [33]. The narcotic and nutritive values of its products were recognized by the Greeks, Egypt and Romans. Hippocrates (460-377 BC) was one of the first who emphasized the medical advantages of poppy and its preparations. The nutritive property of the seeds was also recognized by him. Poppy spread over from its Middle Asian gene centre through the Roman Empire, later its cultivation became very common in Europe [7]. The reason of the wide distribution of the poppy can be explained by the well-known narcotic, pharmacological and nutritive value of its products [15]. The plant is a multipurpose crop which is used as a medicinal or ornamental plant, as well as a source for seed and oil [11]. It is known that opium poppy contains alkaloid (morphine, codein, tebaine, noscapine, papaverine) in great quantities. Opiate alkaloids and their synthetic derivatives (oxycodone, hydrocodone, pholcodine) are widely used in medicine and these compounds are produced in hundreds of tons for the medicine industry [14]. When poppy has reached complete maturity, the leaves are dry and the seeds contain a maximum of poppy seed oil. Poppy seed oil has been used for a culinary purpose as salad oil, cooking oil. It is also used as dessicant in drying oil for in fine art, as in perfume, cosmetic, medicinal industries and as a vehicle for various parenteral formulations [9, 21, 34]. Poppy seed oil also might provide low-cost renewable resource of high value-added compounds such as tocopherol and phytochemicals. It is the main source of dietary ingredients related to their fatty acid composition and tocopherol content. The oil rich in unsaturated fatty acids are believed to be beneficial agents, and with high level of tocopherols are now added into infant formula, various food products and available as nutraceutical supplements in many countries [19, 22, 25, 27]. Moreover, not only oil components but also remaining seed cake meals after oil extraction are the important source of protein, carbohydrate and non-nutritive but bioactive compounds such as phenolics [23].

A need arise for new and cheaper proteins as protein sources for animal diets, which are very expensive worldwide. Poppy seed meal is a high-level protein source produced in many region of the world. Poppy seed meal containing about 30-36% crude protein and 1-15% crude oil is a good and cheap alternative for common protein sources for farm animals [18]. The production of opium poppy needs some kind of narcotic control measures. As a consequence, all countries interested in the production of poppy have to take into consideration the UN convention on narcotics, signed in 1988. This convention forced the countries not only for controlling their cultivation methods and patrolling cultivation areas, but to build up a new strategy for developing special varieties [6, 24]. Legal producer countries are Turkey, India, Australia, France, Spain and Hungary and other countries are Czech Republic and China. According to FAO (2009), Turkey is the leading producer of poppy seed in the world with 34.194 tons followed by Czech Republic with 32.692 tons [13]. UN recognized Turkey and India as traditional opium poppy producer [31]. This crop is placed among the important industrial oil plants in Turkey [16, 17]. Poppy seed oil usually range from 34 to 50% according to Turkish poppy seed varieties [4]. Fatty acid composition of poppy seed oil change as poppy seed varieties change. In Indian poppy seed genotypes, the content of linoleic, oleic and palmitic acids are 41.0-68.0%, 13.22- 36.79%, and 8.90 21.48%, respectively. In poppy seed oils from Turkey

the main fatty acids are linoleic (56.4-69.2%), oleic (16.1-19.4%) and palmitic acids (10.6-16.3%) [28]. Poppy seed was reported to contain 22.3-24.4% protein content [12].

A few samples were chosen and analyzed to determine protein, oil contents and fatty acid composition of poppy seeds. The aim of this study was to evaluate the seeds of twelve different Turkish opium poppy lines for their protein and oil content and fatty acid composition.

Materials and Methods

The trial was carried out at the experimental fields of the Agronomy Department, Faculty of Agriculture of Ankara University, Turkey during 2009-2010. The climatic data of the experimental city (Table 1) and soil analysis results of the experimental soil samples in the field (Table 2) are shown. Twelve Turkish opium poppy lines were used in this study. The materials were token from opium poppy collections in the Agronomy Department Faculty of Agriculture, Ankara University, Ankara. Some charachteristics of varieties are summarized in Table 3.

All materials were sown in 4 rows as autumn planting in 12 October 2009. Sowing was performed as 30×10 cm of plant spacing. Each plot was $1.2 \text{ m} \times 4 \text{ m} = 4.8$ m². Normally fertilization and irrigation were applied to the experiment. Capsules were harvested in the plots during second week of july 2010. The study evaluated based on the percentage of oil, protein, nitrogen, fatty acids, unsaturated fatty acids and saturated fatty acids of seeds.

Table 1 The long term and (2009-2010) outdoors climatic data of the experimental city*

Months	Rainfall (mm)	-	Temperature (°C	<u>C)</u>	Humidity		
Wolluis	Long term	(2009-2010)	Long term	(2009-2010)	Long term	(2009-2010)	
September	17.3	10.3	30.0	18.3	46.4	49.5	
October	26.0	13.7	30.2	16.7	59.1	49.8	
November	32.1	43.1	21.2	7.3	72.1	75.0	
December	45.9	68.0	9.4	5.4	78.0	79.6	
January	39.0	63.0	4.6	3.1	76.5	78.3	
February	35.5	65.1	7.2	6.5	73.1	70.8	
March	36.8	44.6	17.7	8.3	63.0	60.1	
April	43.9	37.5	27.8	12.0	57.8	55.8	
May	52.0	31.0	31.0	17.8	56.6	47.1	
June	34.2	57.8	30.0	21.3	50.5	56.2	
July	15.1	25.7	31.0	25.7	45.9	46.6	
August	11.3	0.4	31.0	28.1	46.5	32.8	

^{*} The government meteorological association of Turkey

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Table 2 Soil analyses results of the experimental soil samples in the field before sowing

Depth	0 – 20 (cm)	20 – 40 (cm)	
pН	7.33	7.26	
CaCo3 %	9.00	10.0	
Organic Material%	1.14	1.02	
Salt %	0.063	0.076	
Sand %	39.99	35.31	
Clay %	22.18	24.35	
Loam %	37.83	40.24	
P_2O_5 Kg/da	5.3	4.5	
K ₂ O Kg/da	140	122	

Table 3 Some identification charachteristics of poppy seeds

Line No.	Seed Color	Flower Color
L1	Grey	Violet
L2	Grey	Violet
L3	Pink	Violet
L4	Grey	Violet
L5	White	White
L6	Grey	Violet
L7	Pink	Violet
L8	Yellow	White
L9	Yellow	White
L10	Grey	Violet
L11	Grey	Violet
L12	Grey	Violet

Oil and Fatty Acids Analyze

The seeds (~1 g) were ground and extracted with hexane by foss soxtec 2055 apparatus. Fatty acid methyl esters were prepared according the AOAC method [1] and analyzed by Shimadzu (Kyoto, Japan) gas chromatography equipped with db 23 capillary column (30mx0.25mm film thickness 0.25µm) and fid (flame ionization detector). Helium at a flow rate of 1.0ml/min was used as a carrier gas. Injector and detector temperatures were 230 and 240°c, respectively. Column temperature was kept at 190°c for 30 min. A sample of 1 µl was injected by the autosampler with a split mode (split ratio of 1:80). The fatty acid identification was based on the comparison of their relative retention times with the corresponding fatty acid methyl ester standards. Individual reference methyl ester standards (myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), gadoleic acid (C20:1), behenic acid (C22:0) and lignoceric acid (C24:0) and as well as fatty acid methyl ester mix (37 components fame mix) were purchased from sigma chemical co. (Sigma-Aldrıch Gmbh, Sternheim, Germany).

Protein and Nitrogen Analyze

First the seeds were ground. Two jar tablets, 20 ml H_2So_4 and (~1 g) grinded seeds were leyed into tube.

The tubes were burnt in 420°c for 2 hours. Protein measurements of burnt materials were carried out by the Kjeldahl method using a Kjeltec protein analyzer (K-370, Buchi Inc., Flawil, Switzerland).

Results and Discussion

The oil contents of seeds are presented in Table-4. the oil concentration was the highest (50.88%) in L_5 sample whereas L_{11} sample had the lowest oil yield (40.96%). Azcan and *et al.* [4] reported the oil yields of yellow, white and blue seeds as 49.2, 36.8 and 33.6%, respectively. Some studies conducted in Turkey showed that oil content of poppy seeds ranged 35.38 to 47.95% [32], 45-50% [30] and 38.86 -53.39% [3]. This was in agreement with Indian poppy seed varieties, where oil content changed between 40.6-49.1% [28] and 26-52% [5].

The protein and nitrogen contents of seeds are presented in Table 4. the protein concentration was the highest (20.94%) in L_9 sample whereas L_5 sample had the lowest protein yield (19.67%). the nitrogen concentration was the highest (3.35%) in L_9 sample whereas L_5 sample had the lowest nitrogen percentage (3.15%). Srinivas and Narasinga [29] reported 21.5-23.5% crude protein in poppy seed. Özcan and Atalay [26] investigated the crude protein of seven Turkish poppy varieties and their results were between 11.94-13.58%.

Table 4 Oil, protein and nitrogen content of poppy seeds

Line No.	Oil (%)	Protein (%)	Nitrogen (%)	
L1	43.82	20.22	3.23	
L2	42.10	20.41	3.26	
L3	44.44	20.24	3.27	
L4	44.05	19.89	3.18	
L5	50.88	19.67	3.15	
L6	44.77	20.17	3.23	
L7	45.18	22.16	3.55	
L8	49.16	20.39	3.26	
L9	44.36	20.94	3.35	
L10	47.25	19.78	3.16	
L11	40.96	20.48	3.28	
L12	43.51	20.91	3.25	

Fatty acid composition of poppy seed oils is presented in Table-5. The linoleic, oleic and palmitic acids have been reported as major fatty acids in the poppy seed oil [28, 30, 5, 8]. Poppy seed oils contained 88,80% (L₄) to 89.83% (L₇) unsaturated fatty acids made up mainly linoleic acid (C18:2). Rahimi and et al. [32] reported that unsaturated fatty acids in registered Turkish opium poppy cultivars ranged 87.59-89.78%. Major fatty acid linoleic acid is polyunsaturated fatty acid and is essential for human diet, ranging 70.60% (L6) - 76.65% (L8). Linoleic acid is responsible for the biosynthesis of arachidonic acid and some prostaglandins. Oleic acid (C18:1) ranged from 12.08 %(L_3) - 17.71% (L_{12}) is the second most abundant unsaturated fatty acid and also major MUFA in poppy seed oils. Marin et al. [20] reported that linoleic and oleic acid contents were about 79.3% and 9.0% in Papaver somniferum L. oil, respectively. Rahimi and et al. [32] reported that linoleic and oleic acid content of poppy oil ranged 68.76-74.22% and 13.30-17.80% respectively depending on cultivars. Azcan et al. [4] found concentrations of linoleic and oleic acids as 56.4 - 69.2% and 16.1 - 19.4% respectively depending on the color of the seeds. Şener et al. [30] reported that linoleic and oleic acid content of poppy seeds from different locations changed between 32.63 - 74.31% and 10.38 -27.04%, respectively. Linoleic and oleic acid contents of Indian poppy seed oils were reported as 41.0-68.0% and 13.22-36.79% respectively [28]. Linoleic acid contents in most of samples was higher compared to data of [4, 30] and lower than [20]. Oleic acid contents were similar to the data of [4] and [28] and lower than results of [20]. Most of the results with respect to linoleic and oleic acid content are consistent with [30]. These samples were obtained from different locations in Turkey so these differences may due to samples obtained from different locations. Linolenic acid (C18:3) is the third fatty acid and the second PUFA in poppy seed oils and its content ranged 0.44% (L_{12}) - 0.62% (L_{8}). High linolenic content is undesirable for vegetable oils as it

autoxidation causing off-flavor compounds in oils [28]. Linolenic acid content appears to be similar to the material analyzed by [4] and [28]. However, linolenic acid was not identified by Marin et al. [20] in their samples. Cowan et al. [10] found that relation between flavor perception and oils relates to the linolenic acid contents in oil. A taste panel showed best scores for cottonseed oil (0%) followed by copper-reduced (0%) and nickel-reduced soybean oils (3%). The amounts of the other unsaturated fatty acids were below 0.29%. (C16:1, 0.14-0.28%), heptadecenoic palmitoleic (C17:1, 0.02-0.03%) and gadoleic acid (C20:1, 0.03-0.06%) were MUFA found in trace amounts in all oil samples. Palmitoleic, heptadecanoic and gadoleic acids were not identified by Azcan et al. [4], Singh and et al. [28], Sener et al. [30] and Marin et al. [20]. Palmitic (C16:0) and stearic acid (C18:0) were found as major saturated fatty acids in all seed oils. The percentage of palmitic acid in seed oils ranged 7.92% (L_7) - 8.80% (L_2) . Stearic acid varied between 1.88% (L_{12}) and 2.30% (L_{10}) . Rahimi and et al. [32] reported that palmitic and stearic acids depending on registered cultivars changed beetwen 7.96% to 10.19% and 1.84% to 2.40% respectively. Palmitic acid and stearic acids were found in Indian poppy seed oils as 8.90- 21.48% and 1.40-10.80%, respectively [28]. Azcan et al. [4] reported that palmitic and stearic acids changed depending on the color of seeds and their percentage ranged 10.0-13.0% and 2.5-3.2%, respectively. Şener et al. [30] determined palmitic and stearic acid contents as 8.33-23.00% and 0-4.30%, respectively. Palmitic and stearic acids were found as 9.6% and 1.9% by Marin et al. [20]. Our results with respect to palmitic and stearic acid concentrations are consistent with Azcan et al. [4], Singh et al. [28] and Marin et al. [20]. The other three saturated fatty acid in poppy seed oils, myristic (C14:0), heptadecanoic (C17:0) and arachidic acid (C20:0) were also present in small concentrations usually accounting for less than 0.09% of the oil composition.

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Table 5 Fatty acid composition of poppy seeds (%)

Line NO.	C14:0	C16:0	C17:0	C18:0	C20:0	S.*	C16:1	C17:1	C18:1	C18:2	C18:3	C20:1	U.S.*
L1	0.04	8.73	0.05	2.07	0.06	10.95	0.15	0.03	15.33	72.98	0.52	0.04	89.05
L2	0.04	8.80	0.05	2.13	0.07	11.09	0.15	0.03	16.92	71.25	0.52	0.05	88.91
L3	0.04	8.42	0.05	2.02	0.07	10.60	0.16	0.02	12.08	76.52	0.58	0.04	89.40
L4	0.04	8.77	0.05	2.27	0.06	11.20	0.15	0.02	14.55	73.53	0.51	0.04	88.80
L5	0.04	8.43	0.06	2.12	0.06	10.72	0.16	0.02	13.80	74.76	0.50	0.04	89.28
L6	0.05	8.70	0.04	2.22	0.07	11.08	0.15	0.03	17.50	70.60	0.59	0.06	88.92
L7	0.04	7.92	0.05	2.09	0.06	10.17	0.16	0.03	13.22	75.80	0.57	0.05	89.83
L8	0.04	8.08	0.05	1.96	0.08	10.22	0.14	0.02	12.31	76.65	0.62	0.04	89.78
L9	0.04	8.19	0.05	2.05	0.07	10.40	0.16	0.03	13.44	75.35	0.57	0.05	89.60
L10	0.05	8.70	0.05	2.30	0.06	11.16	0.15	0.03	15.79	72.29	0.52	0.05	88.84
L11	0.04	8.68	0.05	2.06	0.04	10.87	0.16	0.02	15.75	72.68	0.47	0.04	89.13
L12	0.05	8.48	0.04	1.88	0.02	10.47	0.28	0.02	17.71	71.06	0.44	0.03	89.53

*S.: saturated fatty acids and *U.S.:unsaturated fatty acids

Conclusion

In conclusion, opium poppy seeds are major source of raw materials such as oil and protein with potential application as nutraceuticals and functional foods. The major fatty acids in the seed oil were linoleic, oleic and palmitic acids. The seed oils had a balanced fatty acid distribution having a high content of unsaturated fatty acids. Linoleic and oleic acids are two important unsaturated fatty acids in human diet. Increase in their concentration relates to the quality of related oil. With a balanced fatty acid composition, the seeds could be used in some foods to improve their nutritional value. Legal opium poppy production is allowed under the rules of the United Nations and Turkey is one of legal producer. Protection of traditional poppy producing position for Turkey is very important. Industrial evaluation of poppy seed and producing oil and protein can protect this position of Turkey.

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