



Determination of Oil Ratio and Fatty Acid Composition in the Seeds of Some Flax (*Linum usitatissimum* L.) Varieties

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Abstract: In this study, oil ratios and fatty acid components in the seeds of 17 different flax varieties (Sarı-85, Atalanta, Florinda, Floriana, Flanders, Lirina, Mureş, Midin, Mikael, Mc Groger, Antares, Olin, Norman, Linda, Barbara, Dakota, and Fluin) grown under the ecological conditions of Kazova, Tokat, Turkey are determined. For analysis purposes, oil was extracted from seeds by means of cold extraction. Following solvent removal, amounts were calculated. In order to perform fatty acid analysis, first fatty acid methyl esters were formed. Then, gas chromatography method was employed using a FID detector. As the findings of the study revealed, the ratio of oil contained in linseeds ranged from 31.8% to 38.9%. The flax variety having the highest oil ratio was Floriana (38.9%). In the flax varieties analyzed, both the difference between oil ratios and the difference between fatty acid components were found to be statistically significant ($p<0.01$). The rate of unsaturated fatty acids (85.8-89.0%) were higher than the rate of saturated fatty acids (11.0-14.2%) in the seeds of the flax varieties analyzed. The rates of linolenic acid (omega-3), which is the most significant unsaturated fatty acid for flax, varied between 50.32% and 58.28%.

Keywords: Fatty acids, Flax, *Linum usitatissimum* L., Oil ratio, Omega-3

Bazı Keten (*Linum usitatissimum* L.) Çeşitlerine Ait Tohumlarda Yağ Oranı ve Yağ Asitlerinin Belirlenmesi

Öz: Bu çalışmada, Tokat-Kazova şartlarında yetiştirilen 17 farklı keten çeşidinin (Sarı-85, Atalanta, Florinda, Floriana, Flanders, Lirina, Mureş, Midin, Mikael, Mc Groger, Antares, Olin, Norman, Linda, Barbara, Dakota ve Fluin) tohumlarındaki yağ oranı ve yağ asidi bileşenleri belirlenmiştir. Tohumlardaki yağ analizi soğuk ekstraksiyon metodu ile yapılmış ve çözücü uzaklaştırıldıktan sonra miktarları hesaplanmıştır. Yağ asidi analizi elde edilen yağın, yağ asidi metil esterleri oluşturulduktan sonra FID dedektörü kullanılarak gaz kromatografisi cihazıyla yapılmıştır. Çalışmada keten tohumlarındaki yağ oranlarının %31,8-38,9 arasında değiştiği belirlenmiştir. En yüksek yağ oranına sahip keten çeşidi Floriana (%38,9) olmuş ve çeşitlerin yağ oranları arasındaki farklılıklar istatistiksel olarak ($p<0,01$) önemli bulunmuştur. Analiz edilen keten çeşitlerindeki yağ asidi bileşenleri arasındaki farklılıklar çeşitlere göre istatistiksel yönden önemli ($p<0,01$) bulunmuştur. İncelenen çeşitlerin tohumlarındaki doymamış yağ asitleri oranı (%85,8-89,0) doymuş yağ asitleri oranından (%11,0-14,2) daha yüksek bulunmuştur. Doymamış yağ asitlerinden keten için en önemlisi olan linolenik asit (omega-3) oranları %50,32-58,28 arasında değişmiştir.

Anahtar kelimeler: Keten, *Linum usitatissimum* L., Omega-3, Yağ asitleri, Yağ oranı

1. Introduction

Flax (*Linum usitatissimum* L.) is one of the major industrial crops that is long-known and produced in our country. The amount of linseed production is 2.1 million tons in the world. However, it is approximately 3 tons in Turkey (Anonymous 2014). Flax is an agricultural

product used in numerous industries such as food, oil, fibre, and cellulose both in Turkey and in the world. Due to the rapidly emerging artificial fibre industry and its incapability to compete with cotton fibre, flax has been produced mainly for its seeds (Bjelova et al 2012; Büyük 1993).

The basic fatty acids in linseed oil are linolenic, linoleic, oleic, stearic and palmitic fatty acids. The fatty acid composition of linseed oil differs depending on various factors. However, it is in general 50% linolenic, 20% oleic, 15% linoleic, 6% palmitic and 5% stearic acids. Of the said fatty acids, linoleic, linolenic and oleic acids are classified as unsaturated fatty acids, while palmitic and stearic acids are saturated fatty acids (Duke 1983; Kurt 1996).

Linseed oil is rich in polyunsaturated fatty acids as one of the vegetable sources having the highest rate of omega-3. Playing a crucial role in lowering cholesterol, omega-3 fatty acids have a positive effect on both repairing nervous system and slowing and even preventing abnormal cell growth in some cancer types (Bloedon and Szapary 2004; Thompson et al 2000). Moreover, by means of the proteins and soluble fibres it contains, omega-3 helps to prevent some non-communicable diseases (Rubilar et al 2010). In their study Vijaimohan et al (2006), indicate that linseed oil regulates cholesterol metabolism and fat concentration. On the other hand Tzang et al (2009), in their study, analyze the effect of butter, palm oil and linseed oil on mice and state that linseed oil lower cholesterol in mice and that triglyceride and cholesterol levels are lower in the mice that consume linseed oil.

Linseed has been used in traditional medicine for long years for the purpose of relieving pain and healing injuries and skin diseases (Taylor et al 1999; Johnsson et al 2002). It is reported that, in recent years, linseed is used in animal nutrition so as to make foods rich in omega-3 and omega-6 (Hasanoğlu 2007). In addition, use of linseed in bakery products at certain rates is recommended because linseed contains a considerable amount of dietary fibers (Anil 2002).

Flax, which has recently increased in importance as a source of omega-3 in human nutrition, is analyzed in this study. The purpose of this study is to determine fixed oil and fatty acid rates in the seeds of 17 flax varieties that have been adapted to the region. In selecting the varieties suitable for the region, not only seed yield but also quality were taken into

consideration with the intention of enabling people to consume higher-quality linseeds.

2. Materials and Methods

2.1. Materials

The material of this study was taken from a study conducted by Yılmaz and Kınay on 17 different flax varieties under the ecological conditions of Kazova, Tokat in 2010. In the test, seeds of Sarı-85, Atalanta, Florinda, Floriana, Flanders, Lirina, Mureş, Midin, Mikael, Mc Groger, Antares, Olin, Norman, Linda, Barbara, Dakota, and Fluin were used. The seeds were harvested during the physiological maturity period of flax crops. Following blending, the seeds were not processed in any manner.

2.2. Methods

2.2.1. Oil Extraction

For each flax variety, approximately 50 grams of linseeds were ground by a laboratory blender. Of the said 50 grams of ground linseeds, 10 grams were extracted with 30 mL hexane for 24 hours at room temperature. Following filtering by a filter paper and transferring to a tared flask, the solvent of the filtrate was removed under low pressure at 40°C. The total amount of oil was calculated based on 0% humidity, taking weight loss into consideration. For fatty acid analysis, the oil obtained was put into amber glass containers and kept at +4°C.

2.2.2. Preparation of Fatty Acid Methyl Esters

For fatty acid analysis, fatty acid methyl esters were formed. To do so, 30 milligrams of extracted oil was solved in 3 mL hexane. After adding 3 M KOH solution prepared in 3 mL methanol, it was vortexed for 3 minutes. Then, it was kept waiting for 5-10 minutes at room temperature for phasing. 0,5 mL hexane containing methyl esters was put into vials and analyzed by GC-FID. The amount of each fatty acid was determined in percentages according to the rate of peak areas. In the determination of fatty acids, standard fatty acid mixture (Supelco 37 Component FAME mix 47885-U) was used (Demirtaş et al 2011).

2.2.3. Gas Chromatography Analysis Program

Fatty acids were analyzed by Perkin Elmer Clarus 500 Series gas chromatography (GC) using a FID (Flame Ionization Detector) detector. Apolar capillary column (TR-FAME 30 m X 0.25 mm X 0.25 μ m I.D) was employed. Split rate was set to 50:1. As the carrier gas, helium was used at a flow rate of 0.5 ml/min. Injection temperature and detector temperature were set to 250°C and 260°C, respectively. Being originally 100°C, column furnace temperature was increased for 2°C per minute and fixed to 220°C.

2.2.4. Statistical Analysis

The results obtained were subjected to variance analyses according to Randomized Block Design. Averages were compared in accordance with Duncan Test (Feixiang et al 2015).

3. Results and Discussion

The ratio of oil contained in the flax varieties used in the study ranged from 31.80% to 38.90. The differences between flax varieties in terms of fat contents were found to be statistically significant ($p < 0.01$). The flax variety having the highest oil ratio was Floriana (38.90%). On the other hand, Midin (31.80%) and Dakota (31.80%) were found to be the flax varieties having the lowest oil ratios. Registered in Turkey, Sarı-85 was ranked as the second with an oil ratio of 38.60% (Table 1).

Table 1. Oil ratio of different flax variety (%)
Çizelge 1. Keten çeşitlerinin yağ oranları (%)

Variety	Oil ratio (%)**	Variety	Oil ratio (%)**
Floriana	38.90 a	Barbara	34.20 fgh
Sarı-85	38.60 ab	Flanders	34.10 fgh
Lirina	38.10 bc	Antares	33.80 gh
Linda	37.50 cd	Mc Gregor	33.60 h
Florinda	37.30 d	Norman	32.70 ı
Fluin	37.30 d	Mureş	32.30 ij
Olin	35.50 e	Dakota	31.80 j
Mikael	34.60 f	Midin	31.80 j
Atalanta	34.50 fg	Average of variety	35.09

** $p < 0.01$. In the table, the same letter was not significantly different

The fatty acid analysis of the seeds from 17 different flax varieties used in the study was performed by gas chromatography. Their amounts were calculated in percentages (%) (Table 2). The fatty acids of palmitic, stearic, oleic, linoleic and linolenic had statistically significant ($p < 0.01$) differences by varieties. By variety averages, linolenic, oleic, linoleic, palmitic and stearic acid rates were 53.38%, 19.44%, 14.53%, 6.07% and 5.67%, respectively (Table 2).

Numerous studies demonstrate that linolenic fatty acids prevent heart diseases (Hu et al 1999; Mori et al 2000; Mozoffarian 2005), cancer (Narisawa 1994; Williams et al 2007), neurodegenerative and inflammatory diseases (Joshi et al 2006; Zhao 2007) and lower blood glucose and cholesterol levels (Pellizzon et al 2007; Pan et al 2009). The present study revealed that linseed contained high levels of linolenic fatty acids, which play an essential part in human nutrition. The importance of linseed in medical and balanced nutrition is demonstrated by the fact that, among all vegetable sources that contain omega-3 (linolenic acid), the rate is 11-18% in walnut (Martinez et al 2006), 4.05% in purslane (Simopoulos 2004) but more than 50% in linseed.

Antares had the lowest rate of palmitic fatty acid, which is a saturated fatty acid (5.47%). On the other hand, Olin had the highest rate of palmitic fatty acid (7.13%). In terms of stearic fatty acid, the lowest rate and the highest rate were detected in Norman (4.77%) and Barbara (6.76%), respectively. The rate of oleic acid, which is an unsaturated fatty acid, was highest in Linda (21.25%) and lowest in Mc Gregor (16.40%). As another unsaturated fatty acid, linoleic acid was highest in Mc Gregor (18.54%) and lowest in Sarı-85 (11.19%). In terms of linolenic acid, which is one of the most important components of linseed oil, Mureş (58.28%) and Sarı-85 (58.21%) had the highest rates, while Fluin (50.32%) had the lowest rate (Table 2).

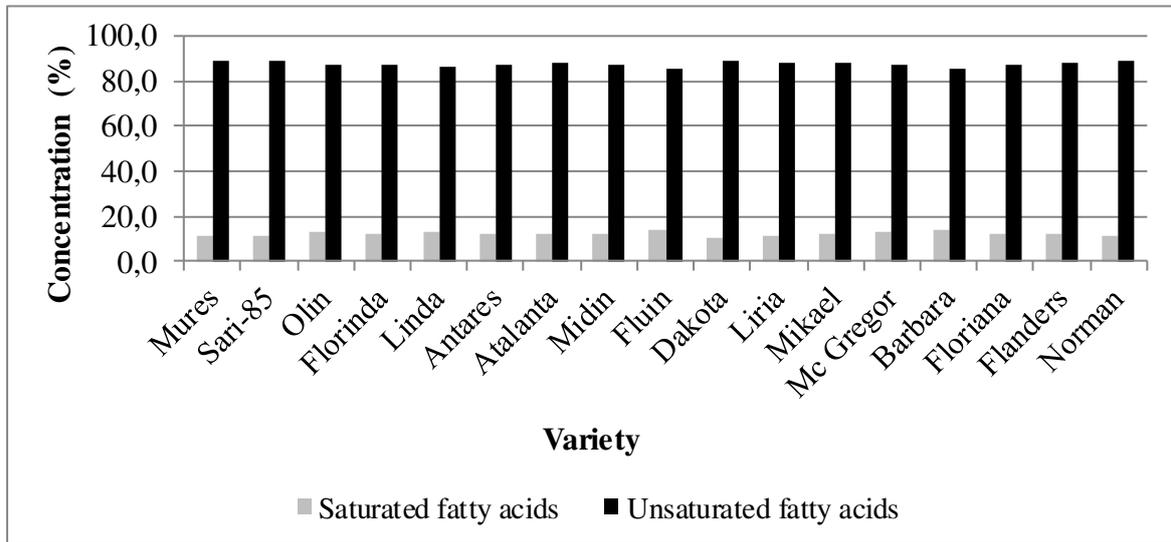
Table 2. Percentage of fatty acids of different flax variety of linseed (%)**Çizelge 2.** Farklı keten çeşitleri tohumlarının yağ asitleri oranı (%)

Variety	Palmitic acid**	Stearic acid**	Oleic acid**	Linoleic acid**	Linolenic acid**
Mureş	5.59 e	5.04 b	19.07 c	11.22 f	58.28 a
Sarı-85	5.68 e	5.00 b	19.04 c	11.19 f	58.21 a
Olin	7.13 a	5.31 b	19.64 c	15.58 b	51.43 j
Florinda	5.80 e	6.40 a	17.94 e	15.86 b	53.46 e
Linda	6.39 bc	6.39 a	21.25 a	13.34 e	51.66 i
Antares	5.47 e	6.40 a	19.95 c	14.11 c	52.95 g
Atalanta	6.03 cd	5.55 b	18.17 d	13.68 e	55.69 b
Midin	6.36 bc	5.71 b	21.19 a	13.18 e	52.60 g
Fluin	6.94 ab	6.49 a	19.91 c	15.33 b	50.32 l
Dakota	5.74 e	4.78 c	17.78 e	15.65 b	55.18 c
Lirina	5.54 e	5.11 b	20.54 b	15.28 b	52.43 h
Mikael	6.35 bc	5.64 b	21.05 a	13.96 d	52.09 h
Mc Gregor	6.57 bc	5.40 b	16.40 f	18.54 a	52.13 h
Barbara	6.47 bc	6.76 a	20.01 b	14.31 c	51.32 k
Floriana	5.58 e	6.17 a	19.42 c	15.77 b	52.07 h
Flanders	5.53 e	5.39 b	18.55 d	15.22 b	54.37 d
Norman	5.92 e	4.77 c	20.46 b	14.76 c	53.25 f
Average of variety	6.07	5.67	19.44	14.53	53.38

** p<0.01, In each column, the same letter was not significantly different

The rate of unsaturated fatty acids (linolenic, linoleic and oleic acids) of the flax varieties used in the study differed between 85% and 89%,

which can be regarded as low. The rate of unsaturated fatty acids was highest in Dakota and lowest in Fluin (Figure 1).

**Figure 1.** Saturated and unsaturated fatty acids ratio in linseed**Şekil 1.** Keten tohumunda doymuş ve doymamış yağ asitleri

Unsaturated fatty acids are categorized as polyunsaturated fatty acids and monounsaturated fatty acids. In the linseeds used in the study, the rate of polyunsaturated fatty acids (linolenic and

linoleic acids) ranged from 65% to 71%. The highest rate and the lowest rate were detected in Dakota and Barbara, respectively. On the other hand, the rate of monounsaturated fatty acids

(oleic acid) varied between 16% and 21.3%. Linda had the highest rate of monounsaturated fatty acids by 21.3%, while the lowest rate of monounsaturated fatty acids was seen in Mc Gregor by 16% (Figure 2).

The factor that makes flax important is the rate of linolenic fatty acid (omega-3), which is a polyunsaturated fatty acid, it contains (Figure 3). This study demonstrated that the rate of linolenic fatty acids ranged from 50.0% to 58.3% by varieties. One of the two varieties having the highest rates of linolenic fatty acids (Mureş and Sari-85) is Turkey's registered variety called Sari-85. The other variety is known to be uneconomic in terms of seed production because of having low seed yield and being fibrous (Yılmaz et al 2007).

In recent years, consumers have tended to consume healthy foods. Omega-3 fatty acid is also medically advantageous by virtue of solving heart and nervous system problems (Harris 2012; Raatz

2012) and preventing non-chronic communicable diseases (Morais 2011). In developed countries, consumption of foods rich in omega-3 in human nutrition is on the increase. Today, animal products -especially fish- are consumed in general as a source of omega-3. However, linseed is a vegetable source rich in omega-3 and a good choice in terms of sustainability.

17 different flax varieties used in the study were evaluated in terms of oil ratio; Floriana (38.9%) and Midin and Dakota (31.8%) were found to be the flax varieties having the highest oil ratio and the lowest oil ratio, respectively. A study conducted under the ecological conditions of Tokat suggested that Floriana was one of the flax varieties having the highest seed yield per decare with 200 kilograms (Yılmaz et al 2007).

This variety is also advantageous in terms of having a high oil ratio and, accordingly, a high oil yield per decare (approximately 77.8 kg/da).

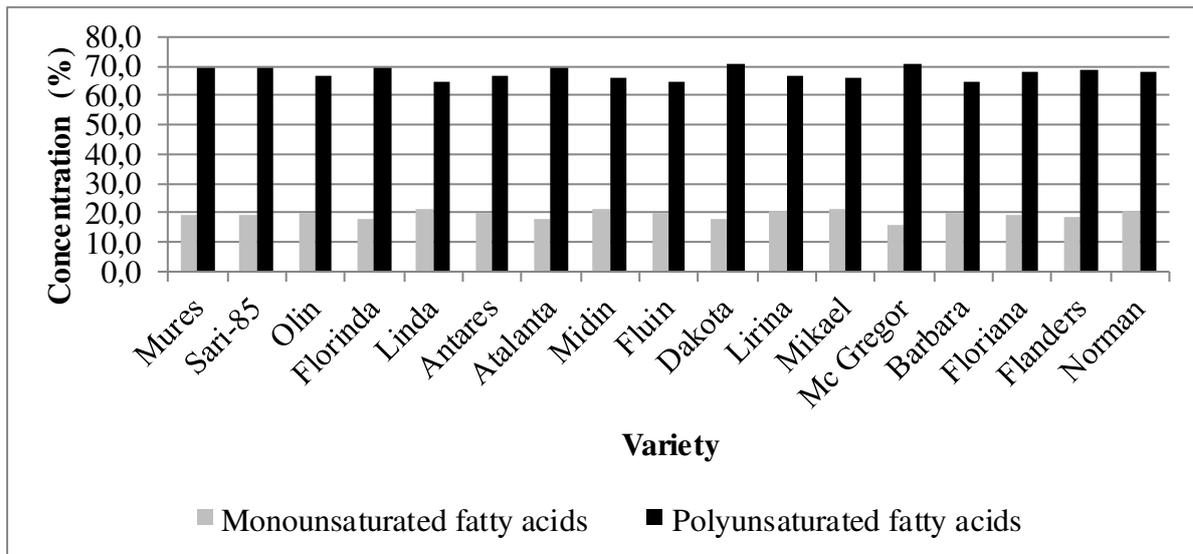


Figure 2. Polyunsaturated and monounsaturated fatty acids ratio in linseed (%)

Şekil 2. Keten tohumunda tekli ve çoklu doymamış yağ asitleri oranı (%)

Compared to other flax varieties, Sari-85 was found to be rich in linolenic acid content. Linseed oil rich in linolenic acids is less favored as edible oil due to rapid oxidation of polyunsaturated fatty acids. Instead, such linseeds can be used directly in human nutrition with foods and poultry rations. In recent years, in addition to being extracted, linseeds have been widely used by being crushed

or as a whole for omega-3. This study demonstrated that Sari-85, which has yellow seeds, and Floriana, which has brown seeds and high seed yield per decare and oil yield, are commercially preferable as a source of omega-3.

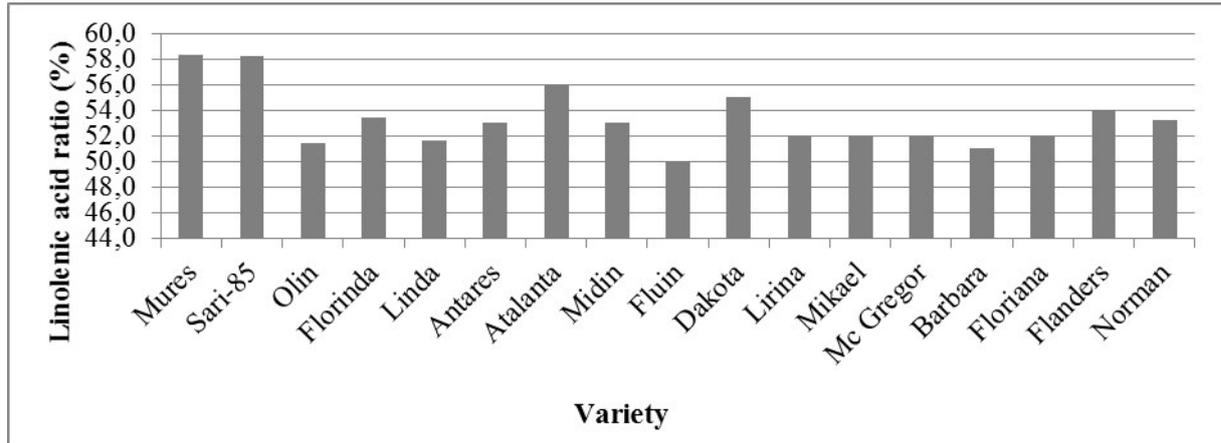


Figure 3. Linolenic acid composition in linseed varieties (%)

Şekil 3. Keten çeşitlerinin linolenik yağ asitleri oranı (%)

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