



Article Lipid Composition and Physicochemical Parameters of Flaxseed Oil (Linum usitatissimum L.) from Bulgaria

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Abstract: Flaxseed oil is a prevalent food supplement. On one hand, vegetable oil is used in the food industry and pharmacy due to its health benefits; on the other hand, it has an application as a lubricant oil. The fatty acid composition of the investigated oil was determined as follows: the main fatty acids were α -linolenic (57.5%), oleic (17.5%), linoleic (12.5%), palmitic (6.0%), and stearic acid (4.3%). The content of unsaponifiable matter was 1.4%. The total sterol content was determined (0.5%), with identified β -sitosterol (79.7%) as a main component, followed by stigmasterol. The content of tocopherols was found to be 243 mg/kg. The compound γ -tocopherol predominated (68%) in the tocopherol fraction, followed by γ -tocotrienol (32%). Some physicochemical indicators were also determined—density, surface tension, and dynamic and kinematic viscosity—at the following temperatures: 20, 30, 40, 50, 60, 70, and 80 °C. The increase in temperature led to a decrease in all indexes and good linear dependence was observed. The determined physicochemical indicators provided information about the stability of flaxseed oil, which is very important considering its use in food and technical products.

Keywords: flaxseed; composition; physicochemical properties

1. Introduction

Flax (*Linum usitatissimum* L.) is an annual plant from the family Linaceae. There is no exact information on the origin of flax, since there are many wild species in many areas of the globe. Cultivated species are widely distributed almost everywhere with suitable conditions for their cultivation. The flax crop is grown under different conditions on various continents. In Europe, it is distributed in broad areas, but in the Alps, it reaches a height equal to that of rye. The leading producers are located in Northern and Southern Europe, Eastern Asia, Egypt, Algeria, Morocco, and Brazil.

Flax seeds are a good source of a dietary fibers (about 28 g/100 g), soluble polysaccharides, lignans, phenolic compounds, vitamins (A, C, F, and E), and different mineral elements (phosphorus, magnesium, potassium, iron, copper, manganese, zinc, etc.). They are rich in proteins (about 20–30 g/100 g), lipids (about 40 g/100 g), and carbohydrates (about 29 g/100 g). According to some reports, the amount of vegetable oil in flaxseeds has been found to be (25–45%), respectively [1–4].

The composition and quantities of fatty acids can vary and depend on growing conditions. α -Linolenic acid predominates (34–65%), followed by oleic (16–24%) and linoleic (12.5–17%), while the quantities of saturated palmitic (3.0–6.0%) and stearic acid (3.0–7.1%) are lower [1–8].



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Flaxseed oil is rich in biologically active substances—sterols and tocopherols. They are present in the unsaponifiable fraction, which contains terpenic (sterols, tocopherols, tocotrienols, carotenoids, etc.) and aliphatic (fatty alcohols, saturated and unsaturated hydrocarbons) compounds. They are significant for determining the nutritional value of the oil. According to Gunstone [9] and Piłat and Zadernowski [10], the amount of phytosterols in oils is low (0.2–0.4%). β -sitosterol is a main component of the sterol fraction (more than 50.0%), followed by campesterol, stigmasterol, and Δ^5 -avenasterol [11–13]. The reported amount of tocopherols is between 300 mg/kg and 700 mg/kg as γ -tocopherol predominates, and α -tocopherol in minimal quantity can also be detected in the oil [9,12,14].

In turn, the extracted flaxseed oil is one of the most popular vegetable oils due to its multiple uses in medicine; in the industrial production of various products, such as paints, linoleum, varnishes, inks, and cosmetics, in the past [15–17]; and for nutritional purposes regarding the human diet.

Flaxseed oil is a rich source of the essential fatty acids (EFAs) linoleic acid (ω -6) and α -linolenic acid (ω -3), which regulate prostaglandins synthesis, and hence, induce the wound healing process. Deficiency in EFAs results in phrynoderma or toad skin, horny eruptions on the limbs, poor wound healing, etc. [1,4,8,18,19].

During the heat treatment of vegetable oils, undesirable processes such as oxidation, polymerization, etc., occur, which leads to the deterioration of their taste and nutritional qualities. Because flaxseed oil's unsaturated fatty acid composition is easily oxidized, it should be stabilized with different antioxidants, for example, butylhydroxytoluene or other synthetic products or plant extracts [8], or encapsulated [20]. The oil is rich in polyunsaturated fatty acids—the most important being linolenic (ω -3) and linoleic (ω -6) as the main ones. They have a good effect on health as food supplements and for preventing coronary heart disease [5,21–26].

Liquid components, including vegetable oils, are characterized by various physicochemical parameters, such as pH, solubility, density, viscosity, surface tension, etc., and their determination is essential for the oil's application, storage and transportation. They are influenced by various factors—oil composition, temperature, pressure, etc.

The saturated-to-unsaturated fatty acid ratio determines vegetable oils' viscosity. Its value is between 0.041 mPa.s and 3.115 mPa.s and depends on their type [27]. Regarding the viscosity values and other physicochemical indicators related to the stability of vegetable oils, such as density, surface tension, etc., temperature change also has an impact [28,29].

Very often, vegetable oils are included in food products, under different technological conditions, such as temperature, pressure, etc., which can change their physicochemical indicators, and therefore, the quality of the food product. High temperatures often provoke various processes in oils and foods, affecting people's health.

For that reason, the aim of this study was to establish the lipid composition of flaxseed oil (through determination of the biologically active substances—unsaponifiable matter, sterols, tocopherols, and fatty acids). On the other hand, it is imperative to determine some physicochemical characteristics at different temperatures related to its stability and application in the food industry.

2. Materials and Methods

2.1. Materials

The analyzed flaxseed oil was purchased from the commercial network of the city of Plovdiv, in 2023. The oil was obtained as cold-pressed flaxseed oil.

2.2. Methods

2.2.1. Determination of Lipid Composition

Composition of fatty acids

Gas chromatography (GC) was used for determination of the fatty acid composition of the oil. Briefly, the vegetable oil (100 mg) was pre-esterified with 5 mL methanol in the presence of sulfuric acid in order to obtain fatty acid methyl esters (FAMEs) [30,31]. The determination of FAMEs was carried out using an Agilent 8860 gas chromatograph (Santa Clara, CA, USA) equipped with a capillary column DB-FastFAME (30 m \times 0.25 mm \times 0.25 µm (film thickness)) and a flame ionization detector (FID). Nitrogen was the carrier gas. The temperature of the inlet was 270 °C and that of the detector was 300 °C. The temperature program of the column was as follows: 70 °C (1 min), followed by increasing of the temperature up to 180 °C at a rate of 6 °C/min, and then, at a rate of 5 °C/min, increasing of the temperature up to 250 °C. The volume of the sample injected in the system was 1 µL. For the identification of the FAMEs, we used a standard mixture from Supelco, USA (FAME mix 37 components, Supelco, Bellefonte, PA, USA).

• Content of sterols

Unsaponifiables were determined according to the ISO standard [32]. A total of 5 g of the oil was subjected to saponification with 50 mL of 2N KOH, and then, the unsaponifiable matter was separated from the solution through extraction with 50 mL of *n*-hexane. The extraction was repeated five times, and then, the solvent was removed using a rotaryvacuum evaporator. The rest of the unsaponifiables were dried at 105 °C to a constant weight and their content was determined gravimetrically. The sterols were isolated from the unsaponifiable matter via thin-layer chromatography (TLC) [33] and their total content was determined spectrophotometrically at a wavelength of 597 nm. Individual sterol composition was determined using an HP 5890 gas chromatograph (Santa Clara, CA, USA) equipped with a DB-5 capillary column (25 m \times 0.25 mm) (Santa Clara, CA, USA) and FID. The carrier gas was hydrogen and the temperatures of the inlet and the detector were as follows: 300 °C and 320 °C. The temperature program of the column was 90 °C (3 min), increasing up to 290 °C at a rate of 15 °C/min, and then, increasing at a rate of 4 °C/min up to 310 °C (5 min). The volume of the sample injected was 1 μ L. Identification was established by comparing the retention times with those of a standard sterol mixture (Acros Organics, New Jersey, USA) [34].

Content of tocopherols

Individual tocopherols were determined using a high-performance liquid chromatograph (HPLC) from Merck-Hitachi (Merck, Darmstadt, Germany). The column was a Nucleosil Si 50-5 (250 mm × 4 mm). Fluorescence detection was used (excitement at 295 nm and emission at 330 nm). The mobile phase was *n*-hexane:dioxane, 96:4 (v/v) and the flow rate was set at 1 mL/min. The content of the tocopherols was determined after diluting 30 mg of the oil with *n*-hexane (2% solution) and injecting 20 µg of the solution into the system under the described conditions. The identification was carried out by comparing the retention times of the peaks with those of a standard solution of tocopherols [35].

2.2.2. Determination of Physicochemical Parameters

The physicochemical parameters of the oil were determined at seven temperatures (20, 30, 40, 50, 60, 70, and 80 °C), the selection of which was carried out according to a variety of technological regimes in which they were included in various food products. The investigated parameters were as follows:

Surface tension was determined using Equation (1) [36]:

$$\gamma = \frac{rg}{2} (\Delta H \rho_0 - h\rho) \tag{1}$$

where *r* is the radius of the capillary, m; $g = 9.8 \text{ m/s}^2$ —the acceleration of gravity; ΔH —the maximum difference in the two gauges of the gauge, m; *h*—liquid level, m; ρ_0 , ρ —the density of the water and another liquid, kg/m³.

 Viscosity. Rheological analysis was performed using a vertical falling ball viscometer. Spindle number 5 was used to determine the ball speeds. For each speed, the dynamic viscosity (Pa.s) was calculated using the experimentally determined density and Stokes' law, Equation (2):

$$\eta = \frac{2}{9} \frac{(\rho_l - \rho_b)gr^2}{v} \text{ (vertical fall down)}$$
(2)

where *g* is the acceleration of gravity, m/s^2 ; ρ_l —density of the liquid, kg/m³; ρ_b —density of the ball, kg/m³; *r*—radius of the ball, m; *v*—speed with uniform movement determined by the road per unit time.

There is a relationship between dynamic and kinematic viscosity. It can be represented by the density and measured viscosity.

Kinematic viscosity was determined according to Equation (3):

$$=\frac{\eta}{\rho}$$
(3)

where η is the dynamic viscosity, Pa.s; ρ —density, kg/m³.

The ball density was taken from standard literary data according to the material it was made of.

v

Density was determined according to Equation (4):

$$p = \frac{m_1 - m}{V} \tag{4}$$

where ρ —density of the test liquid, kg/m³; *m*—mass of the pycnometer, kg; *m*₁—mass of the pycnometer with liquid, kg; *V*—volume, m³.

2.2.3. Statistical Analysis

All measurements were performed in triplicate and the results are presented as the mean value of the individual measurements with the corresponding standard deviation (SD), using Microsoft Excel.

3. Results and Discussion

Flaxseed oil is a yellow liquid with a characteristic smell and taste.

3.1. Lipid Composition

The fatty acid composition of the investigated flaxseed oil was established. The analysis of flaxseed oil revealed the presence of 20 fatty acids (100% of the total composition). The primary fatty acids were α -linolenic acid (57.5%) followed by oleic (17.5%), linoleic (12.5%), palmitic (6.0%), and stearic acid (4.3%). The results of the fatty acid composition of flaxseed oil are presented in Table 1 and Figure 1.

Flaxseed oil is distinguished by a unique fatty acid composition, which is predominated by the presence of polyunsaturated α -linolenic acid. The quantity of α -linolenic acid (57.5%) is in agreement with the data presented earlier by Bera et al. [37], where it is between 50.0% and 60.0%. The higher content of α -linolenic acid was at the expense of the lower quantity of oleic acid (17.5%). The results concerning the amount of oleic acid correspond with the data from the literature [5,9–11,38–42], reporting it to be between 15.0% and 24.0%. In the investigated flaxseed oil, the position isomer of oleic acid (C_{11–18:1}) was not detected, which was determined earlier in other studies [43].

The examined oil contained one of the essential components, ω -6 linoleic acid (12.5%). These results are analogous to those reported by several other authors (between 10.0% and 20.0%) [11,12,37,38,40–42,44]. The results of the palmitic acid content analysis in our study are in agreement with those reported by some other authors, where it is 5.0–7.0% [10–12,37,38,40–42,44,45]. The content of stearic acid was insignificantly different from that reported in other studies, where it is in a range between 3.0% and 6.0% [9,10,12,42]. The differences in the values of fatty acids probably depend on environmental factors and different solvents used to extract the oils. The remaining fatty acids in the examined oil were present in minimal amounts.



Table 1. Fatty acid composition of flaxseed oil ¹.

Figure 1. GC chromatogram of fatty acid methyl esters (FAME) of flaxseed oil.

The content of saturated, unsaturated, mono- and polyunsaturated fatty acids is presented in Figure 2.

The studied oil was rich in unsaturated fatty acids (88.7%), with the majority being polyunsaturated (70.7%). The composition of the unsaturated fatty acids included linolenic, oleic, and linoleic acids, which play a significant role in the higher amounts of this fraction. The content of the monounsaturated fatty acids was about 18.0%. Saturated fatty acids (11.3%) are not typical for flaxseed oils. The ratio of saturated to unsaturated fatty acids was about 1:8. According to all previous studies by other authors, unsaturated fatty acids predominate in flaxseed oil.

Biologically active components in lipids (unsaponifiable matter, sterols, and tocopherols) were determined in the oil, as well. Data on the content of the main biologically active components in the investigated oil are shown in Table 2 and Figure 3, and in Sup-



plementary Materials Figure S1. Data are scarce regarding the unsaponifiable matter of flaxseed oil. Its content in the oil was 1.4%.



Biologically Active Components	Content, %	
Unsaponifiable matter, % of the oil	1.4 ± 0.01	
Sterols, % of the oil	0.5 ± 0.0	
Sterol composition, % of the sterol fraction		
Cholesterol	0.2 ± 0.0	
Brassicasterol	5.9 ± 0.05	
Campesterol	5.9 ± 0.05	
Stigmasterol	7.2 ± 0.07	
Δ^7 -campesterol	4.2 ± 0.04	
β -sitosterol	75.5 ± 0.70	
Δ^{5} -avenasterol	1.1 ± 0.01	
Tocopherols, mg/kg in the oil	243 ± 0.0	
γ -tocopherol, % of total tocopherols	68 ± 0.60	
γ -tocotrienol, % of total tocopherols	32 ± 0.30	
1 - Mean + SD (n = 3).		

Table 2. Total content of unsaponifiable matter, sterols, and tocopherols in flaxseed oil ¹.

The obtained results for the unsaponifiable matter were higher than those reported for peanut oil (1.0%) and palm kernel oil (1.0%). On the other hand, its quantity was lower than that of corn (2.8%), rapeseed (2.0%), and sesame oil (2.0%). The content of unsaponifiable matter in the surveyed flaxseed oil was similar to sunflower (1.5%), soybean (1.5%), and mustard seed oil (1.5%) [46].

A significant part of unsaponifiable matter consists of sterols. The sterol content of the oil was found to be 0.5%, which differed from the literary data (0.42%) [47]. According to Piłat and Zadernowski [10], the sterol content is twice as low—0.23%. The total sterol content depends on the temperature of the environment—an increase in temperature leads to a rise in the sterol content.

The individual composition of the sterol fraction was established, where β -sitosterol and stigmasterol were the major components. The main compound of the sterol fraction of flaxseed oil was β -sitosterol. The amount of β -sitosterol in the oil was 75.5%, considerably different from other reports. According to other authors, it is 20.6% [13], 33.6% [11], 40.6% [48], and 50.0% [12]. The quantities of brassicasterol and campesterol were equal. Cholesterol was present in minimal amounts (0.2%) in the oil. The presence of cholesterol

results from the same biosynthetic pathway as that typical for phytosterols (i.e., via cycloartenol as a key intermediate). Since numerous studies demonstrate that phytosterols from the lipid fraction lower total and LDL cholesterol levels, sterols from the lipid fractions from flaxseed oil could constitute a sanogenic ingredient in the prevention and treatment of hypercholesterolemia.



Figure 3. GC chromatogram of the individual sterol composition of flaxseed oil: a—cholesterol; b—brassicasterol; c—campesterol; d—stigmasterol; e— Δ^7 -campesterol; f— β -sitosterol; g— Δ^5 -avenasterol.

Tocopherols and their unsaturated derivatives (tocotrienols) are the primary natural antioxidants that accompany all vegetable oils. They are effective against oxidative processes. Tocopherol content in the examined flaxseed oil was 243 mg/kg, which differs from the reports by Herchi et al. [44], where it was between 400 mg/kg and 500 mg/kg, and Bozan and Temelli [14], where it was 794 mg/kg. According to Bozan and Temelli [14], the quantity and individual composition of tocopherol are very specific and depend on environmental and agrometeorological conditions. The compounds γ -tocopherol and γ to cotrienol were present in the to copherol fraction, with γ -to copherol being the dominant one. All the obtained results are in agreement with those by Teneva et al. [43], who reported the highest content of γ -tocopherol (67.5%), followed by γ -tocotrienol, respectively (31.0%), for oils from other Bulgarian flaxseed varieties. It is probable that the higher γ -tocotrienol content was at the expense of γ -tocopherol. The values of the total tocopherols and γ -tocopherols are comparable with others found in the literature. The main to copherol present in the to copherol fraction was γ -to copherol, followed by α -to copherol. The tocopherol fraction differed in composition from other commonly used oils, such as sunflower (where α -tocopherol predominated), soybean (where γ - and δ -tocopherols were the main components), and corn oils (where γ -tocopherol prevailed, but there were also traces of α - and δ -tocopherols), and was somewhat similar to those of sesame oil (where γ -tocopherol was also the major one) [46].

3.2. Physicochemical Parameters

The density and surface tension values of flaxseed oil are presented in Table 3.

The observed values for density were between 0.938 g/cm³ at 20 °C and 0.901 g/cm³ at 80 °C. According to [17], the density value is 0.9319 g/cm³ at 15 °C, while in our study, at the lowest temperature of 20 °C, the measured density was 0.938 g/cm³. This value does not differ from the data of other authors ($\rho = 0.933 \text{ kg/m}^3$) [20]. These data indicate that as the temperature increases, the density values tend to decrease, which has also been found for other vegetable oils [29,49,50].

Temperature, °C	Density, g/cm ³	Surface Tension, mN/m
20	0.938 ± 0.008	44.124 ± 0.107
30	0.930 ± 0.005	41.924 ± 0.114
40	0.924 ± 0.003	39.886 ± 0.173
50	0.921 ± 0.008	37.908 ± 0.088
60	0.914 ± 0.004	36.450 ± 0.113
70	0.908 ± 0.001	34.170 ± 0174
80	0.901 ± 0.001	31.787 ± 0.095

Table 3. Density and surface tension of flaxseed oil ¹.

 $\overline{1-Mean \pm SD (n = 3)}$.

It is known that the physical, physicochemical, and thermodynamic properties of vegetable oils depend on various factors, such as fatty acid composition, the content of other biologically active substances, temperature, etc. [27–29].

Surface tension and viscosity are used as measures to determine the stability of a system. The smaller the surface tension compared to that of water (72.75 mN/m, 20 °C), the more stable the system. Surface tension in liquids is caused by unchanged intermolecular bonding and an increase in viscosity. Any solution with a higher viscosity than water has a more minor surface tension than water. As temperature increases, viscosity and surface tension decrease.

The surface tension results show values between 44.124 mN/m and 31.787 mN/m at different temperatures in our study. The values published in the literature, ranging from 23.2 to 56.1 mJ/m² [51], 12.79 dyne/cm [27] and 31.5 mN/m [17], differed from those reported in our study. The data from Table 3 show that the increase in temperature leads to a decrease in the surface tension, which confirms the findings in other studies [52].

Viscosity is a measure of the resistance of liquids to their movement, moving parallel layers relative to each other, and can be considered as a measure of fluid friction. Thus, water is defined as a liquid with a low viscosity (1 mPa.s, 20 °C), because its layers move with little resistance during the movement of the fluid. Vegetable oils, however, have a high viscosity because of the large resistance created when the layers move. This is called dynamic viscosity. Systems with higher viscosity are considered more stable. The viscosity of various edible vegetable oils ranged from 0.041 mPa.s to 3.115 mPa.s [27].

The results of the dynamic viscosity analysis are presented in Figure 4. Viscosity decreased with an increase in temperature. The observed values were between 29.195 mPa.s at 20 °C and 14.913 mPa.s at 80 °C. A strong linear relationship between the two parameters for the temperature range between 20 °C and about 45 °C was observed.



Figure 4. Temperature dependence of the dynamic viscosity of flaxseed oil. *—dynamic viscosity values.

According to our findings, a rise in temperature led to the creation of deviations from the linear dependence. A regression analysis of this relationship was performed and a linear Equation (5) with a high correlation coefficient was obtained:

$$y = 33.986 - 0.258*x, R^2 = 0.954$$
(5)

Kinematic viscosity is determined through calculations from the ratio of dynamic viscosity and density, and is represented by the ratio of viscous force to inertial force. The relationship between dynamic and kinematic viscosity is presented in Figure 5.



Figure 5. Relationship between dynamic and kinematic viscosity of flaxseed oil. *—dynamic viscosity values; *—kinematic viscosity values.

The kinematic viscosity was determined to be $31.125 \text{ mm}^2/\text{s}$ (at 20 °C), 28.707 mm²/s (at 30 °C), 26.285 mm²/s (at 40 °C), 21.416 mm²/s (at 50 °C), 18.617 mm²/s (at 60 °C), 17.163 mm²/s (at 70 °C), and 16.552 mm²/s (at 80 °C). The literature reports data for kinematic viscosity of 24.994 mm²/s at 40 °C [17], which is close to the values in our study. The lower slope of the lines for both viscosities indicates that the oil has a relatively low stability, explained by the composition of the lipid fraction.

Similar dependencies of the physicochemical parameters on temperature have been established for other vegetable oils, which is in agreement with the data we obtained.

Boda et al. [53] investigated the kinematic viscosity of liquids, including sunflower, soybean, and coconut oils, at varying temperatures (50, 55, 60, 65, 70, 75, and 80 °C). According to them, the kinematic viscosity of all liquids decreased as their temperature increased.

Hlaváč et al. [54] determined the physical properties, such as density, dynamic and kinematic viscosity, and fluidity, at a temperature range of 5–32 °C. They found that the density dependencies of samples on temperature were characteristic of decreasing linear function within a measured temperature range.

Stanciu [55] determined the dynamic viscosity of sunflower oil at temperatures between 313 K and 373 K and shear between 3.3 s^{-1} and 120 s^{-1} .

The results obtained in this study showed that all investigated physicochemical indicators decrease with an increase in temperature, which is an indication of its influence on the properties of the oils, including their stability. This property should be considered when including flaxseed oil in different food products, depending on the temperature conditions during individual technological operations.

4. Conclusions

• The main fatty acids in the lipid fraction of the flaxseed oil were α -linolenic, oleic, linoleic, palmitic, and stearic acid. β -sitosterol was the major sterol in vegetable oil

and γ -tocopherol prevailed in the tocopherol fraction. The biological components in the lipid fraction define flaxseed oil as a suitable supplement in food.

- Some physicochemical parameters (density, surface tension, and dynamic and kinematic viscosity) were determined at seven different temperatures. Dependence was established between the studied parameters and temperature, as the parameters decreased with a temperature increase.
- With the data obtained on the chemical composition of the lipid fraction and physicochemical parameters of the studied samples, and after the additional determination of some kinetic and thermodynamic indicators, which will be the subject of future research, it will be possible to predict the shelf life of flaxseed oil for the purpose of inclusion in the human diet and various food products with health benefits.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app131810141/s1, Figure S1. GC chromatogram of the individual tocopherol composition of flaxseed oil. $1-\gamma$ -Tocopherol; $2-\gamma$ -Tocotrienol.

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