Fatty Acid Composition, Tocopherol and Sterol Contents in Linseed (*Linum usitatissimum* L.) Varieties

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ABSTRACT: *The oil contents of linseeds were determined between 39.9% (Antares) and 43.3% (Railinus). The oleic acid contents of seed oils varied between 0.8% (Maroc) and 24.8 (Railinus). The linoleic acid contents of linseed oil change between 10.2% (Avangard) and 48.2% (Maroc), while the palmitic acid of oils ranged from 4.8% (Antares) to 6.1% (Maroc), stearic acid contents ranged between 4.7% (Avangard) to 23.5% (Maroc). Also, linolenic acid contents of Avangard oil was higher (59.9%) than those of results of other linseed oils. The P8 contents of linseed oil change between 1.1 mg/kg (Railinus) and 16.4 mg/kg (Avangard). Also, the δ-tocopherol content of Railinus oil was also higher (262.7 mg/kg) than those of other linseed oils (0.3-0.4 mg/kg). The total tocopherol in Railinus seed oil was higher (437.4 mg/100 g) ($p<0.05$) than that of Maroc seed oil (30.3 mg/100 g).*

KEYWORDS: *Linseed; Genotypes; Oil; Fatty acid; Tocopherol; Sterol; GC; HPLC.*

INTRODUCTION

Linseed (*Linum usitatissimum* L.) is a plant widely cultivated in Europe for fiber or oil for industrial use [1]. Linseed with its high oil content (30-45 %) can be used in edible and non-edible industrial oil production. The quality of oil depends upon its fatty acid composition which ultimately determines its utilization in industry. Due to the high iodine number, it is used in making varnish, varnishes, linoleum, linoleum, printing ink, soap, leather, mac, resin manufacturing, paint and coatings industry [2,3]. The vegetable oils are dietary sources of unsaturated fatty acids, vitamin E and sterols. The fatty acid composition and an unsaponifiable substances content in oilseeds depend on the plant species, degree of ripening seeds and the climatic conditions [4]. It has been noted that linseed oil prevents the development and spread of tumor, suppresses thrombosis and allergic reactions and eliminates the deficiency of vitamin E against malaria parasites [5]. Data on fatty acid, tocopherol and sterol contents in linseed oils is limited. However, the aim of present study was to determine oil contents, the fatty acid composition, tocopherols and sterol content in several linseed oils.

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EXPERIMENTAL SECTION

Material

As material, Sarı - 85 Avangard, Antares, Maroc and Railinus linseed genotypes (*Linum usitatissimum* L.) were used. The linseed samples were provided from in seed collection herbarium in Çumra High Vocational College, Selçuk University in Konya in Turkey. Raulinus, Maroc, Avangard, and Antares are German-originated oil linseed genotypes. The seeds were transported to laboratory in paper bags. Seeds were dried and cleaned by a combination of manual and mechanical procedures to remove all foreign matter and crushed or immature fruits, and were kept in refrigenator by using.

Reagents

Petroleum ether (40-60 °C) was of analytical grade (Merck, Darmstadt, Germany). Heptane and tert-butyl methyl ether were of HPLC grade (Merck, Darmstadt, Germany). Tocopherol and tocotrienol standard compounds were purchased from CalBiochem (Darmstadt, Germany). Betulin, β-sitosterol, campesterol, and stigmasterol were obtained from Aldrich (Munich, Germany).

Oil content

About 3 g of the seeds were ground in a ball mill and extracted with petroleum ether in a Soxhlet apparatus for 6 h. The solvent was removed by a rotary evaporator at 40 °C and 25 Torr. The oil was dried by a stream of nitrogen and stored at – 20 °C until used.

Fatty Acid Composition

A drop oil was dissolved in 1 mL of *n*-heptane, 50 μg of sodium methylate was added. The closed tube was agitated vigorously for 1 min at room temperature. After addition of 100 μL of water, the tube was centrifuged at 4500 g for 10 min and the lower aqueous phase was removed. Then 50 μL of HCl with 1 mol methyl orange was added. The solution was shortly mixed. The lower aqueous phase was rejected. About 20 mg of sodium hydrogen sulphate (monohydrate, extra pure; Merck, Darmstadt, Germany) was added, and after centrifugation at 4500 g for 10 min, the top n-heptane phase was transferred to a vial and injected in a HP-5890A gas chromatograph (Hewlett-Packard, Waldbronn, Germany) with a capillary column, CP-Sil 88 (100 m long, 0.25 mm ID, film thickness 0.2 μm). The temperature program was as follows: from 155 °C; heated to 220 °C (1.5 °C/min), 10 min isotherm; injector 250 °C, detector 250 °C; carrier gas 36 cm/s hydrogen; split ratio 1:50; detector gas 30 mL/min hydrogen; 300 mL/min air and 30 mL/min nitrogen; manual injection volume less than 1 μL [6,7].

Tocopherols

A solution of 250 mg of oil in 25 mL of n-heptane was directly used for the HPLC. The HPLC analysis was conducted using a Merck-Hitachi low-pressure gradient system, fitted with a L-6000 pump, a Merck-Hitachi F-1000 fluorescence spectrophotometer (detector wavelengths for excitation 295 nm, for emission 330 nm), and a D-2500 integration system. The samples in the amount of 20 μL were injected by a Merck 655-A40 autosampler onto a Diol phase HPLC column 25 cm x 4.6 mmID (Merck, Darmstadt, Germany) used with a flow rate of 1.3 mL/min. The mobile phase used was n-heptane/tert-butyl methyl ether (99+1,v/v) [8].

Sterols

About 250 mg of oil was saponified with a solution of ethanolic potassium hydroxide by boiling under reflux. The unsaponifiable matter was isolated by solid-phase extraction on an aluminium oxide column (Merck, Darmstadt, Germany) on which fatty acid anions were retained and sterols passed through. The sterol fraction was separated from unsaponifiable matter by thin-layer chromatograph (Merck, Darmstadt, Germany), re-extracted from the TLC material, and afterward, the composition of the sterol fraction was determined by GLC using betulin as internal standard. The compounds were separated on a SE 54 CB (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, split ratio 1:20, injection and detection temperature adjusted to 320 °C, temperature program, 245 °C to 260 °C at 5 °C/min. Peaks were identified either by standard compounds (β-sitosterol, campesterol, stigmasterol) by a mixture of sterols isolated from rape seed oil (brassicasterol) or by a mixture of sterols isolated from sunflower oil (Δ7-avenasterol, Δ7-stigmasterol, and Δ7-campesterol). All other sterols were identified by GC-MS for the first time and afterward by comparison of the retention time [6].

Table 1: Oil contents and fatty acid compositions of several linseed genotype (%).

Samples	Fatty Acids													
	Oil	Palmitic	Palmitoleic	Heptadecanoic	Stearic	Oleic	Vaccenic	Linoleic	Linolenic	Arachidic	Gondoic	Behenic	Lignoceric	Total
Sarı 85	43.0	5.6	0.1	0.1	5.7	22.2	0.8	11.6	52.2	0.2	0.1	0.2	0.1	99.0
Avangard	42.2	5.6	0.1	0.1	4.7	17.0	0.8	10.2	59.9	0.2	0.1	0.2	0.1	99.1
Antares	39.9	4.8	0.1	0.1	6.0	22.3	0.8	12.5	51.2	0.3	0.2	0.2	0.1	98.7
Maroc	40.9	6.1	0.1	0.1	6.1	23.5	0.8	13.4	48.2	0.2	0.1	0.2	0.1	99.1
Railinus	43.3	5.7	0.1	0.1	6.4	24.8	0.7	12.7	47.8	0.2	0.2	-*	-	89.9
Mean±SD	42.9 ±1.44	5.56 ±0.47	0.1 ±0.0	0.1 ±0.0	5.78 ±0.65	21.96 ±2.96	0.78 ±0.04	12.08 ±1.23	51.9 ±4.9	0.22 ±0.04	0.14 ±0.05	0.16 ±0.09	0.08 ±0.04	97.16 ±4.06

Statistical analyses

The data were analyzed by analysis of variance in MSTAT statistical program according to "Split Plots on Randomized Complete Block" [9].

RESULTS AND DISCUSSION

The oil contents of linseeds were found between 39.9% (Antares) and 43.3% (Railinus) (Table 1.). The findings were found partly low than that of the researchers who stated that the crude oil content ranged from 38.0% to 40.3% [10], from 37.6% to 41.5% [11]. Palmitic, stearic, oleic, linoleic and linolenic acids were found as the most abundant fatty acids in linseed oils (Table 1). The fatty acid compositions of the lineseed oil varied depending on varieties. The linolenic acid content of Avangard oil was higher (59.9%) than those of other linseed oils used in experiment. The findings were lower than that of the researcher who stated that linolenic acid ratio was 65.9% *Kurt et al.* [12] and higher than that of the researchers who stated the linolenic acid rates were 5% *Green & Marshall* [13], 2.6% *Kurt et al.,* [12]. These findings were in compliance with the researchers who stated that the linolenic acid rates ranged from these values; 45.5-64.2% *Green & Marshall* [13], 52.6% *Yazicioglu & Karaali* [14], 50-55% *Röbbelen et al.* [15], 35-59.8% *Salunkhe et al.* [1], 28.3-48.7% *Basbag et al.* [16]

Table 2: Tocopherol contents of several linseed genotype oils (mg/100g).

Tocopherols	Linseed Genotypes				
	Sarı 85	Avangard	Antares	Maroc	Railinus
α-Tocopherol	0.8	0.6	0.6	0.3	1.5
α-Tocotrienol	0.9	0.7	0.6	0.3	0.6
ɣ-Tocopherol	33.9	31.9	30.2	19.6	169.7
Plastochromanol-8	13.9	16.4	15.8	9.7	1.1
ɣ-Tocotrienol	-*	-	-	-	0.3
Δ-Tocopherol	0.4	0.4	0.4	0.3	262.7
Δ-Tocotrienol	-	-	-	-	0.5
Total	49.9	49.9	47.6	30.3	437.4

**nonidentified*

and 46.2-54.6% *Carson & McGregor* [17]. *Rafatowski et al.,* [4] reported that cold pres linseed oil contained 7.0% palmitic, 3.7% stearic, 15.0% oleic and 67.4% linoleic acid. The oleic acid contents of seed oils varied between 0.8% (Maroc) and 24.8 (Railinus). The linoleic acid contents of linseed oil range from 10.2% (Avangard) to 48.2% (Maroc), while the palmitic acid of oils changed between 4.8% (Antares) and 6.1% (Maroc). Also stearic acid contents of oil samples ranged from 4.7% (Avangard) to 23.5% (Maroc). The total fatty acid contents of linseed oils were determined between 98.7% (Antares) and 99.2% (Maroc). These values were found to be lower than the values of 24.4-31.8% *Carson and McGregor* [17], higher than the values of 9% *Yazıcıoglu & Karaali* [14] and these values were in compliance with the values of 13.3-25.2% *Green & Marshall* [13], 15-20% *Röbbelen et al.,* [15], 14.7-39.0% *Salunkhe et al.,* [1], 7.9-22.0% Basbag et al.,[16]. It is important that erucic acid was not found in any oil samples.

Tocopherol contents of oil samples exhibited differences depending on oil samples. The major tocopherol were ɣ-tocopherol and Plastochromanol-8 (P8) in all the varieties of linseed oil (Table 2). The ɣ-tocopherol content of of Railinus oil was higher than those of other linseed oils ($p<0.05$). The P8 contents of linseed oils changed between 1.1 mg/kg (Railinus) and 16.4 mg/kg (Avangard). The δ-tocopherol content of Railinus seed oil was also higher (262.7 mg/kg) than those of other linseed oils (0.3-0.4 mg/kg). The total tocopherol content of Railinus seed oil was higher (437.4 mg/100 g) ($p<0.05$) than that in Maroc seed oil (30.3 mg/100 g). Linseed oil contained 29.84 mg/100 g ɣ-tocopherol and 2.98 mg/100 g α-tocotrienol [18]. The rapeseed oils contained ɣ-tocopherol in the range of 28.16 to 53.58 mg/100 g [19]. Our results were found partly than those of literature values.

The major sterol was β-stosterol in all the varieties of linseed oils (Table 3), and were found between 1517.5 mg/kg (Maroc) to 1902.4 mg/kg (Antares). There was significant difference ($p<0.05$). The stigmasterol contents of linseed oils changed between 334.0 mg/kg (Maroc) and 450.5 mg/kg (Antares). In addition, the contents of campesterol were found between 786.4 mg/kg (Sarı-85) and 1167.1 mg/kg (Antares). Also, 5-avenasterol contents of linseed oils were found between 314.8 mg /kg (Maroc) and 435.1 mg/kg (Antares). In addition, 24-methylene was determined between 83.4 mg/kg (Maroc) and 113.3 mg/kg (Antares). Chlerosterol contents of linseed oil samples were found between 36.8 mg/kg (Maroc) and 48.7 mg/kg (Antares). In addition, while ɣ-and δ-tocotrienols are found in only Railinus oil, 5,23-stigmasterol was not found in all oil sample. In previous study, *Itoh et al.* [18] reported that linseed contained 29% campesterol, 9.0% stigmasterol, 46% β-stosterol, 13% δ5-avenasterol and 2% δ7-stigmasterol. The linseed oils were determined as potential sources of sterols. Sterol contents exhibited differences depending on varieties of linseed plants.

CONCLUSIONS

Thus, the current study showed the linseeds of the investigated species of linseeds from Turkey to be

Table 3: Sterol contents of several linseed genotype oils (mg/kg).

Sterols	Linseed Genotypes				
	Sarı 85		Antares	Maroc	Railinus
Cholesterol	19.7		36.3	24.9	28.7
Brassicasterol	15.6		19.6	24.2	24.3
24-methylene	96.3		113.3	83.4	92.6
Campesterol	786.4		1167.1	885.6	918.6
Campestanol	11.0		12.1	9.2	11.7
Stigmasterol	343.1		450.5	334.0	376.9
7-Campesterol	19.3		42.3	27.3	23.9
Chlerosterol	43.1		48.7	36.8	41.3
β-stosterol	1559.1		1902.4	1517.5	1552.1
Stostanol	19.5		19.8	17.9	18.5
δ5-avenasterol	368.9		435.1	314.8	319.3
5,24-Stigmasterol	47.7		55.4	41.7	50.7
7-Stigmasterol	-*		15.5	15.4	19.8
7-Avenasterol	14.2		26.0	17.9	15.7
Total	3343.9		4344.1	3308.9	3494.1

a potential source of functional valuable oil which might be used for food and most industrial applications. It was established some differences among results. These differences of bioactive constituents of linseed cultivars may be due to genetic factors, soil properties, growth conditions and analytical procedures. Among the tested oils, all linseed oil has the highest level of linolenic acid, which is usually in sufficient amounts in diet and the lowest ratio of linoleic acid to linolenic acid. Tocopherol levels in this oil appear to be adequate to stabilize its unsaturated fatty acids. These results emphasize important differences in the relative ratios of oleic to linoleic acids in both family seed oils, and compare similarly the most literature value.

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