

EVALUATION OF FATTY ACIDS PROFILE AND THE CONTENT OF SOME SECONDARY METABOLITES IN SEEDS OF DIFFERENT FLAX CULTIVARS (*LINUM USITATISSIMUM* L.)

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Summary. Flax seed oils currently sold on the worldwide market are not governed by specific standards or specifications. Consumption of flax (*Linum usitatissimum*) seeds is beneficial for human health. Flax seeds, containing about 36-40 % oil are the richest (among crop plants) source of polyunsaturated fatty acids (PUFA) essential in the human diet. PUFA are highly susceptible to oxidation. Five flax cultivars (Sakha 1, Sakha 2, Giza 8, Lithuania and Aryana) were studied to determine protein profile and contents of fatty acids, unsaponifiable matter, total lipids, total tocopherols, total phenolics and total flavonoids in order to assess the nutritional value of flax seeds. No differences between flax cultivars were noticed in the protein profile, fatty acids and unsaponifiable matter content. In contrary, significant differences were established in total tocopherols, total phenolics and total flavonoids. Cvs. Giza 8 and Lithuania showed the highest levels of total tocopherols, total phenolics and total flavonoids among all flaxseed cultivars. Thus, our results suggest that these two cultivars are suitable for commercial use of edible oil.

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Key words: flaxseed, fatty acid, unsaponifiable matter, total tocopherols, total phenolics, total flavonoids.

INTRODUCTION

Flax (*Linum usitatissimum* L.) is a multi-purpose crop. Its seeds containing about 36 to 40 % of oil, have long been used in human and animal diets and in industry as a source of oil and as the basic component or additive of various paints or polymers. Recently there has been a growing interest in the probiotic properties of flax and in its beneficial effects on coronary heart disease, some kinds of cancer and neurological and hormonal disorders (Huang and Milles, 1996; Huang and Ziboh, 2001; Simopoulos, 2002). The beneficial effects are mostly due to flax lipids. Flax oil is the richest plant source of linoleic (omega-6) and linolenic (omega-3) polyunsaturated fatty acids (PUFA), which are essential for humans since they cannot be synthesized in the organism and must be ingested in food. Flaxseed oil is qualitatively different from the more common vegetable oils with high PUFA proportions, such as soya oil, sunflower oil, rape oil, olive oil, etc. Flax oil is a rich source of the following unsaturated fatty acids: oleic (C18, 16–24 %), linoleic (C18, 18–24 %), and linolenic acid (C18, 36–50 %) (Flachowsky et al., 1997) and it has a relatively low glucosinolate content (Schuster and Friedt, 1998). The protein and fiber content in the seed are also important nutritional parameters: the crude protein content in the seed ranges from 25 % to 45 %, while the crude fiber content is about 10 % (Korsrud et al., 1978). The results of the analyses of flax oil have been reported elsewhere (Fofana et al., 2004 and Lukaszewicz et al., 2004). Unsaponifiable lipid constituents of seed oils naturally contain hydrocarbons, terpene alcohols, sterols, tocopherols and other phenolic compounds which may act as oxidation inhibitors under a range of conditions (Bosku and Morton, 1976). The effectiveness of lipid unsaponifiable matters in retarding oil deterioration has been demonstrated by many investigators (Rady and Awatif, 1991; Awatif et al., 1996). In flax grains, lipids are protected against oxidation by various mechanisms, for example, the presence of antioxidants such as lignans, phenols, tocopherols

(vitamin E) and flavonoids (Kitts et al., 1999 and Ahmed et al., 2005). In addition to preventing fat rancidity, these antioxidants could increase commercial value of food products and have beneficial effects on human health. When consumed together with essential unsaturated fatty acids, they can reduce the risk of various diseases (Romieu and Trenga, 2001). The antioxidant ability of phenols, tocopherols (vitamin E) and flavonoids is related to the presence of OH groups which may directly bind to free radicals and chelate metals (Halliwell and Gutteridge, 1999).

The present study was focused on assays of protein profile, fatty acids, unsaponifiable matter, total lipids, total tocopherols, phenolics and flavonoids in seeds of different flax cultivars (*Linum usitatissimum* L.) in order to assess their nutritional value.

MATERIALS AND METHODS

Plant Material

Five different flax cultivars, Sakha 1, Sakha 2, Giza 8 (Egypt), Lithuania (Lithuania) and Aryana (France) were cultivated under field conditions in 2006/2007 at the National Research Centre, Egypt. After seed maturity, the plants were immediately harvested and seed samples were taken. Part of the harvested seeds were used for oil extraction.

Extraction of total protein fraction

Defatted flax meal was extracted with 0.03 M Tris-HCl (pH 8.0) buffer at room temperature to yield whole buffer extract as described by Iwabuchi and Yamauchi (1987), then centrifuged at 3000 g for 30 min at 15 °C. SDS-PAGE was carried out according to the method of Laemmli (1970). Soluble protein content was estimated according to Bradford (1976).

Lipid Extraction

Total lipid extraction was performed according to Folch (1957). This extraction was used to determine fatty acid and unsaponifiable composition.

The seed oil content was determined using the Soxhlet extraction according to the official method (AOAC, 1960). From each cultivar 50 g of seeds were ground and then extracted with petroleum ether in a Soxhlet apparatus for 6 h [AOAC]. After extraction, the samples were ground again, but more finely, and extracted for 6 h (second extraction). Petroleum ether was evaporated under reduced pressure using a rotavapor. Lipid content was expressed as g/100 g of seed fresh weight.

Separation of fatty acids and unsaponifiables from samples

Lipid material was saponified with methanolic KOH (40 %, w/v) for 24 h at room temperature according to Ahmed et al. (1986). The unsaponifiables were extracted three times with ether. The aqueous layer was acidified with HCl (1:1, v/v) and the liberated fatty acids were extracted three times with ether. The combined extracts of unsaponifiables and fatty acids were washed several times with distilled water until the washings were neutral to phenolphthalein indicator, then dried over anhydrous sodium sulfate.

Methylation of lipid materials

The standard and the sample fatty acids were converted to methyl esters using ethereal solution of diazomethane according to Vogal (1975).

Determination of fatty acid composition by GC–MS

The fatty acid methyl esters were determined by GC–MS using Trace GC Model 2000 series produced by Thermo equipped with Selective Detector Mass Spectroscopy Model SSQ 7000 produced by Finnigan. This equipment was interfaced via HP chemstation version A 02.12 software (Hewlett-Packard, Avondale, PA). The gas chromatography was equipped with DB-5 (5 %-phenyle) Methylpolysiloxane 25 μ capillary column, 50 m x 0.25 mm i.d, 1.5 m thickness. The operating conditions for gas chromatography were as follows: injector temperature 250 °C, carrier gas: helium at 30 cm/sec, measured at 150 °C, oven temperature 50 °C for 4 min, 150 °C for 4 min and held at 250 °C until the chromatogram was completed. The detector

temperature was 280 °C. Mass spectroscopy operating parameters were electron ionization at 70 ev, accelerating voltage 10 kV and scan M/Z range from 50 to 500. Identification of fatty acid constituents way was carried out by comparing retention times with those of authentic reference compounds, or peak – matching library (NIST) Standard Mass Library, Version 2.0 and available literatures.

Determination of unsaponifiable profile by GC–MS

The unsaponifiable fractions were finally collected in ether and taken to dryness under vacuum. The residue was analysed using the gas chromatograph HP 5890 (Hewlett Packard) equipped with the MS detector (MSD 5970), EI, 70 ev and fitted with a capillarycolumn DB-1701 (12 m x 0.18 mm x 0.4 mm; J&W Scientific). The column temperature was programmed from 260 to 300 °C while injection temperature was set at 280 °C. Helium was the carrier gas at a flow rate of 0.7 cm³/min. Identification of peaks was based on the retention time of standard substances and MS spectra. Analyses were run in duplicate. Calculations of percent composition of demethylhydrocarbons and demethylsterol fractions were based on the peak area.

Colorimetric assay of total tocopherols (vitamin E)

Aliquots (10, 15, 20, and 25 mg) of a 10 g/l solution of α -tocopherol in ethanol were transferred to a volumetric flask and the volume was adjusted to 8 ml with ethanol. Each of the solutions and 1 ml of 2,2'-dipyridyl reagent were pipette into a 10-ml volumetric flask and mixed. A 1-ml portion of ferric chloride reagent was added to the 10-ml volumetric flask and the mixture was shaken for 10 s. The absorbance of the mixture was read at 520 nm against ethanol as a blank (Philip et al., 1954). Then, the standard curve was drawn. The above-described procedure was followed by using 10, 20, 30, 40 mg sample solutions. The content of α -tocopherol in the extracts was calculated from the regression equation of the standard curve.

Extraction of flavonoids

Extraction of flavonoids was carried out using the method previously described for olive oil (Vazues et al., 1973). A 10 g aliquot of flaxseed was moved with 50 ml hexane in a separation funnel and flavonoids were extracted with three volumes of 60 % methanol. The methanol phase was then moved to a second separation funnel and washed with 20 ml hexane. The methanol phase was then collected and dried with anhydrous Na_2SO_4 and again dried in a vacuum evaporator at 40 °C. The resultant flavonoids were resuspended in methanol and extracted with three portions of chloroform, each half the volume of the methanol phase. The chloroform was removed and the methanol dried again in the vacuum evaporator at 40 °C. Flavonoids were resuspended in water and extracted with petroleum ether (60–80 ml) until a clear organic phase was obtained. The water phase was saturated with NaCl and extracted with four portions of ethyl acetate (EA), each a third of the water phase volume. The EA fractions were collected and dried with anhydrous Na_2SO_4 . The EA was dried in a vacuum evaporator and flavonoids were resuspended in methanol and kept at – 20 °C.

Determination of total flavonoids

Flavonoids content of different fractions was measured using a colorimetric assay developed by Zhishen et al. (1999). A known volume (0.5 ml) of flavonoid extract or standard solutions of rutin (Sigma) was added to a 10 ml volumetric flask. Distilled water was added to make a volume of 5 ml. At zero time, 0.3 ml of 5 % (w/v) NaNO_2 was added to the flask. After 5 min, 0.6 ml of 10 % (w/v) AlCl_3 was added and after 6 min, 2 ml of 1 M NaOH was added to the mixture followed by the addition of 2.1 ml distilled water. The absorbance was read at 510 nm and flavonoids content was expressed as mg rutin equivalents/100 g FW.

Determination of total phenolics

Total phenolics were determined by the Folin–Ciocalteu method (Sadasivam and Manickam, 1992). Briefly, aliquots of 0.1 g lyophilized

powder of samples were dissolved in 1 ml deionized water. This solution (0.1 ml) was mixed with 2.8 ml of deionized water, 2 ml of 2 % sodium carbonate (Na_2CO_3), and 0.1 ml of 50 % Folin–Ciocalteu reagent. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was read at 750 nm against a deionized water blank. Measurements were calibrated to a standard curve of prepared gallic acid solution, and the total phenolics concentration was expressed as mg gallic acid equivalents /100 g sample on a wet weight basis.

Statistical analysis

The statistical analysis was performed by one-way ANOVA, followed by Dunnett's *t*-test. The results were expressed as the mean \pm SE to show variations in a group. Differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Polypeptide profile

The results presented in Fig. 1 showed that the protein profiles of total soluble protein were represented by 6 major bands with molecular weight of 200, 100, 50, 30, 20 and 10 KDa. The electrophoretic analysis of flax seed protein did not show any changes between the cultivars studied.

Fatty acid profile

Seeds of all analyzed cultivars contained about 36–39 % oil (Table 3) and the composition of flax fatty acids was cultivar-dependent (Table 1). The unique feature of flax is the accumulation of large amounts of linolenic acid, the final product of three desaturation steps. The amount of total unsaturated fatty acids in flaxseed cultivars was 87–91 % while the amount of total saturated fatty acids ranged from 9 to 12 % (Bhatty, 1995; Choo et al., 2007). Fatty acid analysis of the flax seeds of the cultivars grown in Egypt showed 46–50 % linolenic acid whereas the mean values of linoleic acid, oleic acid

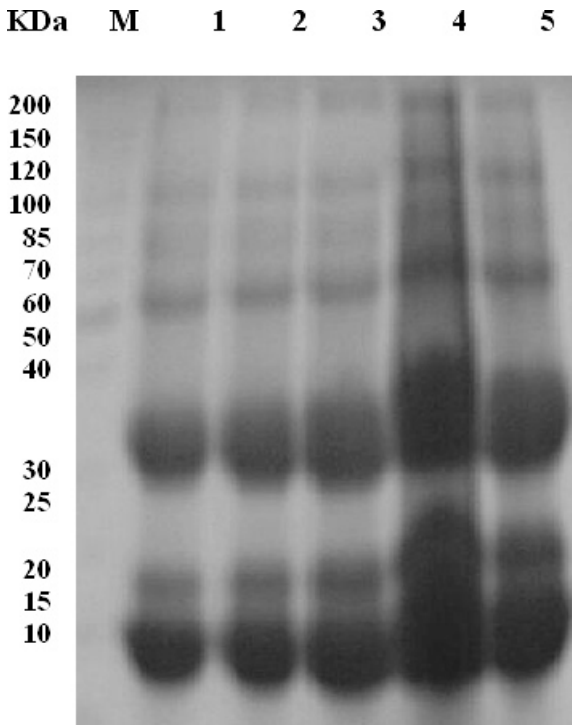


Fig. 1. Protein profile of flax seeds of five cultivars after separation by SDS-PAGE. M-molecular marker proteins, lane 1-Sakha 1, lane 2-Sakha 2, lane 3-Giza 8, lane 4-Lithuania, lane 5-Aryana.

and stearic acid were 18.2, 22 and 3.7 %. Our results were in agreement with Lukaszewicz et al. (2004) and Choo et al. (2007). According to the current knowledge, most of plant fatty acids arise in plastids and usually contain up to 16–18 carbon atoms in their aliphatic chains. They are subsequently transported to the cytosol as saturated FA or, after the modification by specific desaturase, as monounsaturated FA. Further modifications (desaturations) resulting in the formation of PUFA (polyunsaturated fatty acids, such as linoleic and linolenic acids) take

place in the cytosol. It is supposed that oleic acid is synthesized mainly through stearic acid. PUFA from flax oil are essential for human diet and lower the risk of diseases related to cholesterol oxidation. Consumption of oleic, linoleic and linolenic acids lowers the level of LDL in human blood. Unfortunately, flax oil with high PUFA content is readily oxidized and thus has a minor role in human diet.

Unsaponifiable matter profile

GC of trimethylsilated unsaponifiable fractions readily separated hydrocarbons and phytosterols (Table 2) showing qualitative and quantitative

Table 1. Relative percent of fatty acid profile in flax seeds of five cultivars.

| Fatty acid | Relative % of fatty acid in seeds of flax (<i>Linum usitatissimum</i>) cultivars | | | | | |
|-------------------------|--|---------|--------|-----------|--------|------------|
| | Sakha 1 | Sakha 2 | Giza 8 | Lithuania | Aryana | Means |
| C14:0 | 0.8 | 0.6 | 0.7 | 0.7 | 0.5 | 0.7 ± 0.1 |
| C16:0 | 7.4 | 5.6 | 7.6 | 7.7 | 7.2 | 7.1 ± 0.9 |
| C18:0 | 3.6 | 3.1 | 3.4 | 4.2 | 4.4 | 3.7 ± 0.6 |
| C18:1 | 21.5 | 22.1 | 20.9 | 21.2 | 24.4 | 22 ± 1.4 |
| C18:2 | 19.2 | 18 | 18.7 | 18.1 | 17.4 | 18.3 ± 0.7 |
| C18:3 | 47.4 | 50.7 | 48.7 | 48.2 | 46.1 | 48.2 ± 1.7 |
| ¹TS | 11.8 | 9.3 | 11.7 | 12.6 | 12.1 | 11.5 ± 1.3 |
| ²TUS | 88.1 | 90.8 | 88.3 | 87.5 | 87.9 | 88.5 ± 1.3 |
| ³US/S | 7.5 | 9.8 | 7.6 | 6.9 | 7.3 | 7.8 ± 1.1 |

¹Total saturated fatty acids, ²Total unsaturated fatty acids, ³Ratio of unsaturated fatty acids to saturated fatty acids.

differences among the cultivars. The mean of total hydrocarbons was 86 % while the mean of total sterols was 13.9 %. C26 and C28 hydrocarbons were higher in all cultivars by 14 % and 16.5 %, respectively. The ratio of total hydrocarbons to total sterols (TH/TS) in Sakha 1, Sakha 2, Giza 8, Lithuania and Aryana was 4.928, 5.588, 7.504, 6.66 and 6.704, respectively. This may be due to the different cultivar and origin (Choo et al., 2007).

Total tocopherols content

Tocopherols consist of a polar chromanol ring with a lipophilic prenyl side chain and comprise four homologous forms, α -, β -, γ - and δ -tocopherol, differing only in the number and position of methyl substituents on the chromanol head group (Traber et al., 1996). These different tocopherol forms have varying antioxidative abilities, with α -tocopherol being the

Table 2. Relative percent of unsaponifiable matter in flax seeds of five cultivars.

| Components | Relative % of unsaponifiable matter in seeds of flax (<i>Linum usitatissimum</i>) cultivars | | | | | |
|----------------------------|--|---------|--------|-----------|--------|------------|
| | Sakha 1 | Sakha 2 | Giza 8 | Lithuania | Aryana | Means |
| C16 (Hexadecane) | 2.3 | 2.8 | 2.7 | 3 | 2.5 | 2.6 ± 0.3 |
| C18 (Octadecane) | 4.1 | 3 | 5.6 | 6.2 | 4 | 4.5 ± 1.3 |
| C 20 (Eicosane) | 4.6 | 5.3 | 5.3 | 6.4 | 5.1 | 5.3 ± 0.7 |
| C22 (Docosane) | 4.5 | 5.6 | 7.2 | 6.3 | 4.8 | 5.7 ± 1.1 |
| C24 (Tetraecosane) | 8.6 | 9.2 | 9.3 | 8.2 | 9.9 | 9.0 ± 0.7 |
| C25 (Pentaecosane) | 6.6 | 7.4 | 8.3 | 7.5 | 8.1 | 7.5 ± 0.7 |
| C26 (Hexaecosane) | 14.8 | 13.9 | 13.2 | 13.6 | 15.6 | 14 ± 1.0 |
| C28 (Octaecosane) | 16.4 | 17.3 | 15.7 | 15.1 | 18.1 | 16.5 ± 1.2 |
| Squaline | 3.7 | 2.6 | 2.6 | 2.8 | 2.5 | 2.8 ± 0.5 |
| C30 (Triacontane) | 8.1 | 9.3 | 9.8 | 8.6 | 2.5 | 8.6 ± 1.0 |
| C32 (Dotriacontane) | 9.6 | 8.2 | 8. | 10.4 | 9.3 | 9.2 ± 0.8 |
| Cholesterol | 4.8 | 3.9 | 2.5 | 3 | 3.6 | 3.5 ± 0.9 |
| Campesterol | 3.9 | 3.9 | 3.2 | 3.7 | 3.3 | 3.6 ± 0.3 |
| Stigmasterol | 3.6 | 3.4 | 3.1 | 3.3 | 3 | 3.3 ± 0.2 |
| B-sitosterol | 4.7 | 4 | 2.8 | 3.2 | 3.1 | 3.6 ± 0.8 |
| ¹TH | 83 | 84.8 | 88.4 | 86.8 | 87 | 86 ± 2.1 |
| ²TS | 17 | 15.2 | 11.6 | 13.2 | 13 | 14 ± 2.1 |
| ³TH/TS | 4.9 | 5.6 | 7.6 | 6.6 | 6.7 | 6.3 ± 1.0 |

¹Total hydrocarbons, ²Total sterols, ³Ratio of total hydrocarbons to total sterols.

| Flax cultivars | * Total lipids g / 100 g | ** Total tocopherols mg / 100 g Oil | *** Total flavonoids (mg rutin eq)/ 100 g) | **** Total phenolics (mg GAE / 100 g) |
|----------------|--------------------------|-------------------------------------|--|---------------------------------------|
| Sakha 1 | 36.2 ± 0.3 ^d | 17.9 ± 0.3 ^c | 16.1 ± 0.3 ^b | 162 ± 9 ^c |
| Sakha 2 | 39.2 ± 0.3 ^a | 21.0 ± 0.7 ^b | 14.4 ± 0.4 ^c | 205 ± 13 ^b |
| Giza 8 | 39.2 ± 0.4 ^a | 24.5 ± 0.3 ^a | 20.0 ± 0.4 ^a | 362 ± 15 ^a |
| Lithuania | 36.7 ± 0.2 ^c | 24.2 ± 0.3 ^a | 19.5 ± 0.3 ^a | 352 ± 15 ^a |
| Aryana | 37.9 ± 0.2 ^b | 17.4 ± 0.4 ^c | 12.9 ± 0.4 ^c | 185 ± 13 ^b |
| LSD (0.05) | 0.52 | 0.83 | 0.66 | 0.46 |

*Total lipid of flaxseed (g/100g FW), **Total tocopherol contents of flaxseed oils (mg/100g), ***Total flavonoid content of flaxseed (mg rutin eq/100g), ****Total phenolic content on a wet weight basis of flaxseeds cultivars calculated as equivalent (mg of Gallic/100 g). Data represent the mean ± SE of three experimental replicates. Values with different letters in the same experiment and the same column are significantly different (P ≤ 0.05).

Table 3. Content of total lipids, total tocopherols, total flavonoids and total phenolics in flax seeds of five cultivars.

most biologically active form. They function as lipid-soluble antioxidants that are able to scavenge oxygen radicals and to quench singlet oxygen (Brigelius-Flohe and Traber, 1999; Ricciarelli et al., 2001). Table 3 shows the tocopherols content of the five cultivars flaxseed oils. Tocopherols are natural lipophilic antioxidants found in vegetable oils. Giza 8 and Lithuania had the highest total tocopherol content among all oils.

Flavonoids content

Flavonoids are a group of natural benzo- γ -pyrane derivatives ubiquitous in the plant kingdom, and exert multiple biological effects including antioxidative activity (Pekkarinen et al., 1999). Flavonoids have been reported by Nieto et al. (1993) and Hudson and Lewis (1983) to have the potential to function as antioxidants in food lipid systems. The total flavonoid contents (Table 3) in oils of Sakha 1, Sakha 2 and Aryana (16.1, 14.43 and 12.94 mg/100 g) are lower than those found in Giza 8 and Lithuania (19.98 and 19.51 mg/100 g). This was due to the hydrophilic nature of flavonoids which were not effectively extracted with the oil. Total flavonoid content of flaxseeds as reported by Oomah et al. (1996) ranged from 30.2 to 83.5 mg/100 g.

Total phenolics content

The phenolic acids family is composed of the cinnamic (C6–C3) and the benzoic (C6–C1) acid derivatives, characterized by a benzene ring substituted with one or more hydroxyl or methoxy groups and a carboxylic group (Larson, 1997). Phenolic acids are natural hydrophilic antioxidants, which occur ubiquitously in fruits, vegetables, spices and aromatic herbs (Silva et al., 2000). The total phenolics content of flax seed meal on a wet weight basis, as reported by Wanasundara and Shahidi (1994) ranges from about 130 to 220 mg/100 g, as gallic acid equivalents. Sakha 1, Sakha 2 and Aryana had lower total phenolics (162, 205 and 185 mg/100 g) (Table 3) than Giza 8 and Lithuania (362 and 352 mg/100 g).

Conclusions

The results of this study showed that seed oil extracted from five flax cultivars was quite similar in protein profile, fatty acids and unsaponifiable matter content, with only a few significant variations. In contrary, significant differences were observed between cultivars concerning total tocopherols, total phenolics and total flavonoids. Giza 8 and Lithuania had the highest levels of total tocopherols, total phenolics and total flavonoids among all oils. Thus, our results suggest that these two cultivars are suitable for commercial use of edible oil.

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