Flaxseeds: Nutritional Potential and Bioactive Compounds

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ABSTRACT

The objective of this work was to study the nutritional and bioactive composition of commercially available flaxseeds with the aim to develop new alternatives for their use as functional and nutraceutical food ingredient. The samples of flaxseed contained 20.86% protein, 31.16% fat, 29.07% crude fiber and 3.75% ash. Essential amino acids represented 34% of total protein. The amino acids profile showed that glutamic acid was the most abundant (3.87 g 100 g^{-1}), followed by arginine (1.93 g 100 g^{-1}) and aspartic acid (1.52 g 100 g^{-1}). Fatty acids analysis indicated that alpha-linolenic acid represents the major fatty acid (54.51% of the total fatty acids). The ratio of unsaturated to saturated fatty acids was 8.67 while the n-3/n-6 PUFA ratio was 3.2. Total phenolics showed average contents of 295.92 mg GAE 100 g^{-1}, of which flavonoids accounted for 25.85 mg QE 100 g^{-1}. The results confirmed that, in addition to being one of the richest sources of alpha-linolenic acid, flaxseed is an essential source of high quality protein, soluble fiber and potent natural antioxidants.

Keywords: *flaxseeds, amino acids, fatty acids, polyphenols, antioxidant activity*

INTRODUCTION

Flax (*Linum usitatissimum* L.) is an annual plant belonging to the *Linaceae* family, widely distributed in the temperate climate zone (Rubilar et al., 2010). Native to West Asia and the Mediterranean, it has been grown and harvested since ancient times for its fiber and seeds (Madhusudhan, 2009). Traditionally, flax is used as the source of linen fibre while flaxseed oil, in the manufacture of paints, varnishes, inks and linoleum, because of its fast polymerization properties (Coskuner and Karababa, 2007; Shim et al., 2014).

Nowadays, flax continues to be widely grown for oil, fiber, and food (Oomah, 2001). It is the third largest natural fiber crop and one of the five major oil crops in the world (Deng et al., 2010), being cultivated in more than 50 countries (Kasote, 2013). Canada is the main flax producer, followed by China, United States and India (Rubilar et al., 2010).

Whole flaxseeds, which have a crisp and chewy texture and a pleasant, nutty taste, are consumed either as diet supplement or as an ingredient in prepared food. They are rich in fat, high-quality protein and dietary fibre, a large proportion of the latter being water-soluble viscous fibers (Kristensen et al., 2012).

Chemical analysis of flaxseed averaged 30–40% fat, 20–25% protein, 20–28% total dietary fibre, 4–8% moisture and 3–4% ash, and the oil contains vitamins A, B, D and E, and minerals. The observed variability in composition is attributed mainly to genotype and environmental parameters

(Coskuner & Karababa, 2007; Shim et al., 2014). Flaxseed is mainly known by its excellent fatty acid profile, which is high in polyunsaturated fatty acids (73% of total fatty acids), moderate in monounsaturated fatty acids (18%), and low in saturated fatty acids (9%). In addition, flaxseed is the seed with the highest omega-3 fatty acid content, as alpha-linolenic acid (ALA) constitutes about 57%, whereas linoleic acid, an omega-6 fatty acid, constitutes about 16% of total fatty acids (Morris, 2001; Ramcharitar et al., 2005; Rubilar et al., 2010).

Flaxseed has considerable potential as a source of various phenolic compounds (Oomah, 2001; Kraushofer and Sontag, 2002) such as lignans, phenolic acids, flavonoids, phenylpropanoids and tannins, with high antioxidant potential (Anwar and Przybylski, 2012; Kasote, 2013). They also accumulates many other biologically active compounds and elements including cyclic peptides, olysaccharides, alkaloids, cyanogenic glycosides, and cadmium (Shim et al., 2014).

Flaxseed is the richest source of plant lignans (Ganorkar and Jain, 2013), which are phytoestrogens and serves as precursors in the production of mammalian lignans. Secoisolariciresinol diglucoside (SDG) is the predominant lignan in flaxseed with minor amount of pinoresinol and matairesinol (MAT). Prasad (2005) suggested that lignans may act to prevent oxygen radical production, thus effectively reducing atherosclerosis. They have antioxidant activity and thus may contribute to the anticancer activity of flaxseed.

Flaxseed has recently gained recognition as a functional food ingredient for human nutrition as its consumption has been demonstrated to provide health benefits including decreasing rate of tumor growth, reducing serum cholesterol level, decreasing risk of cardiovascular disease and cancer, particularly of the mammary, prostrate gland and colon cancers, antiinflammatory activity, laxative effect, and alleviation of menopausal symptoms and osteoporosis (Muir and Westcott, 2003; Hemmings et al., 2004; Hosseinian et al., 2006; Toure and Xueming, 2010). In addition to these effects, flaxseed confers beneficial renal function, mediates bone health and exerts strong phytoestrogenic and therapeutic effect in reducing the risk of hormone related cancers.

These health benefits are mainly attributed to the high content of essential omega-3 fatty acid, alpha-linolenic acid, dietary fiber and biologically active components such as lignans, phenolic acids and flavonoids (Westcott and Paton, 2001; Tarpila et al., 2005; Hosseinian et al., 2006).

The growing popularity of flaxseeds as functional food has led to the increase in consumer demand for flax-based products. Nowadays, they are incorporated in multigrain breads, ready-to-eat breakfast cereals, breakfast drinks, salad dressings, biscuits, crackers, soups, cakes, and organic products (Coskuner and Karababa, 2007; Ayelign and Alemu, 2016).

Flaxseed is also added to animal feed to improve animal reproductive performance and health (Heimbach, 2009; Turner et al., 2014). Recent studies have demonstrated that n-3 PUFA-enriched eggs can be produced by adding linseed oil or flaxseed in the hens diets. Feeding flaxseed increased linolenic acid in the egg yolk about 30-fold, and docosahexaenoic acid (DHA) increased nearly fourfold (Lewis et al., 2000; Novak and Scheideler, 2001; Surai and Sparks, 2001).

The objective of this study was to analyze the compositional characteristics, and to assess the nutritional quality (proximate composition, amino and fatty acid profiles), the content of bioactive compunds and the antioxidant activity of flaxseeds available on the market, in order to use them in various health-food and feed applications.

MATERIALS AND METHODS

Plant material

Flaxseeds

Three commercial samples of brown flaxseed (*Linum usitatissimum* L.) were purchased from local markets. Flaxseeds samples were finely ground and analyzed to determine dry matter, crude protein, crude fat, crude fiber, ash, total phenolics, total flavonoids content and antioxidant activity. Amino acids and fatty acids profile was assessed using chromatographic methods.

Chemicals and reagents

Methanol, acetonitril (HPLC grade), formic acid, acetone, hexane (analytical grade), Folin-Ciocalteu reagent (2 N), hydrochloric acid (37%), hydrogen peroxide (30%), potassium chloride, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from

Merck (Germany). Gallic acid (99% purity), anhydrous sodium carbonate (99% purity), aluminium nitrate, potassium acetate, disodium phosphate, sodium citrate, boric acid, sodium disulphite (analytical grade), quercetin and 2,2-diphenyl-1-picrylhydrazyl (DPPH, 90% purity) were procured from Sigma-Aldrich (Germany). Standards of amino acids and fatty acids were purchased also from Sigma-Aldrich (Germany). Derivatization materials: orto-phtaldehyde (OPA), mercapto-propionic acid (AMP) and 9-fluorenylmethyl chloroformate (FMOC), formic acid and thiodiethanol (analytical grade) were supplied by Merck (Germany). The water was purified in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Proximate composition

The chemical composition of flaxseeds was determined according to standard methods: dry matter by the gravimetric method according to ISO 6496:2001, crude protein by the semiautomatic Kjeldahl method according to ISO 5983-2:2009 using a Kjeltec 2300 analyzer unit (Tecator, Sweden), crude fat by ether extraction (SR ISO 6492:2001) using a Soxtec 2055 extraction unit (Tecator, Sweden), crude fiber by digestion with acid and alkali according to ISO 6865:2002 using an automatic analyser (Fibertec 2010, Tecator, Sweden), and ash according to ISO 2171:2010 using a Caloris CL 1206 oven (Romania).

Determination of total phenolic content

The total phenolic content was quantified spectrophotometrically using the Folin-Ciocalteu's phenol reagent according to the method of Singleton and Rossi (1965). For extraction, 0.3 grams of ground flaxseeds were mixed with 5 mL of methanol and sonicated during 50 min at room temperature. The extracts were centrifuged for 5 min at 4200 rpm and supernatants were filtered through 0.45 µm polyamide membranes. One hundred microliters of each flaxseed methanolic extract were mixed with 5 mL of distilled water and 500 µL of Folin-Ciocalteu reagent. After a 5 min reaction time, 1.5 mL of 20% sodium carbonate solution was added. The reaction mixture was diluted with distilled water to a final volume of 10 mL. The same procedure was also applied to the standard solutions of gallic acid. The absorbance at 765 nm of each mixture was measured on a Varian Cary 50 UV-Vis spectrophotometer (Varian Co., USA) after incubation for 30 min at 40 °C. Results were expressed in mg of gallic acid equivalents (GAE) per 100 g.

Determination of total flavonoid content

The flavonoid content of flaxseeds was determined spectrophotometrically by using the aluminium nitrate method as described by Mohammadzadeh et al. (2007). Briefly, 0.5 mL of flaxseeds methanolic extract was added to a test tube containing 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 M aqueous potassium acetate and 4.3 mL methanol. After 40 min reaction time at room temperature, the absorbance of the mixture was measured at 415 nm using an Evolution 600 UV-Vis spectrophotometer (Thermo Scientific, USA). Quercetin was used as standard and results were expressed in milligrams of quercetin equivalents (QE) per 100 g.

Determination of DPPH radical scavenging activity

The free radical scavenging activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay as described by Oliveira et al. (2008). Each methanol flaxseeds extract (50 µL) was mixed with 3 mL 0.004% (v/v) DPPH in methanol. The mixture was shaken vigorously and kept in the dark for 30 min. The absorbance was then read at 517 nm using an Evolution 600 UV-Vis spectrophotometer (Thermo Scientific, USA). The radical scavenging activity was calculated as a percentage of DPPH inhibition using the following formula: DPPH scavenging activity (%) = $[1 - A_S/A_{DPPH}] \times 100$ where A_S represents the absorbance of the sample extract with DPPH and A_{DPPH} is the absorbance of the DPPH solution without sample. Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic) was used as a standard and 80% methanol was used as a blank. Results were expressed in mmol Trolox per 100 g.

Determination of fatty acids

Fatty acids content was assessed by fatty acid methyl ester (FAME)/gas chromatography according to ISO/TS 17764-2 (2008). Fatty acids from the total lipid extracts were converted to their methyl esters by transesterification in methanol containing 3% concentrated sulfuric acid at 80 °C for 4 h. Methyl esters of fatty acids were analyzed in a Perkin Elmer-Clarus 500 chromatograph equipped with flame ionization detector (FID) and fitted with a BPX70 capillary column (60m x 0.25mm i.d., 0.25µm film thickness). Column temperature was programmed at 5 °C min^{-1} from

Tab. 1. Proximate composition of flaxseeds

Component	Flaxseeds
Dry matter (%)	94.46±1.0
Crude protein (%)	20.86±0.8
Crude fat (%)	31.16±1.4
Crude fiber (%)	29.07±1.2
Ash (%)	3.75 ± 0.36

180 °C to 220 °C. The carrier gas was hydrogen (35 cm s^{-1} linear velocity at 180 °C) and the spliting ratio was 1:100. The injector and detector temperatures were 250 and 260 °C, respectively. FAME identification was done by comparison with retention times of the known standards. The content of each FAME was expressed as weight percentage of total FAME present.

Determination of amino acids

For the amino acids determination, the flaxseed samples were prepared according to the method described by Varzaru et al. (2013) that involves the acid hydrolysis for the release of amino acids from the protein molecules, preceded by oxidation with performic acid for the sulphur amino acids. The chromatographic separation was performed in a HPLC Finningan Surveyor Plus system (Thermo-Electron Corporation, Waltham, MA) equipped with a diode array detector (DAD) and a Hypersil BDS C18 column (250 x 4.6 mm, particle size 5 µm) (Thermo-Electron Corporation, Waltham, MA). The column operated at 45 °C with a flow rate of 1.7 mL min^{-1} using 50 mM phosphate buffer (pH 7.5) as eluent A and water/acetonitrile/methanol (20/20/60) as eluent B. The injection volume was 20 µL. Amino acids were separated with the following linear gradient elution conditions: 2 min step at 0% solvent B; 23 min step that raised solvent B at 57%; 1 min step that raised solvent B at 100%; 3 min step at 100% solvent B; 1 min step that decreased solvent B at 0% and 5 min step at 0% solvent B. The DAD was set at 338 nm to monitor the derivatised amino acids. Stock solution of the standard amino acid mixture was prepared in hydrochloric acid (0.1 M) and contained 500 µg mL^{-1} for each amino acid. Quantitation was based on the external standard method using calibration curves fitted by linear regression analysis. Data were acquired and processed with ChromQuest software.

Statistical analysis

All the experiments were replicated three times for each flaxseed sample and the results were expressed as mean value ± standard deviation. Statistical analysis was performed using Statgraphic Centurion XVI software (StatPoint Technologies, Warrenton, VA, USA).

RESULTS AND DISCUSSIONS

Proximate composition

Table 1 shows the chemical composition of flaxseed. The average moisture content found in our samples was 5.54% while the ash content was 3.75%, values in good agreement with previously reported data (Coskuner and Karababa, 2007). The proximate composition revealed that flaxseeds contained 20.86% protein, which is comparable to the average crude protein level (22%) found by Rubilar *et al.* (2010) but lower than the result reported by Mueller et al. (2010) (23.4%). Shim et al. (2014) pointed out that in previous studies on flaxseed, the reported protein content varied widely from 10 to 31%.

The fat content found in our samples (31.16%) fall well in the range 30-40% indicated by Coskuner and Karababa (2007) but was much lower than the data reported in other studies. Thus, Rubilar et al. (2010) and Mueller et al. (2010) reported in flaxseed a fat content of 40% and 45.2%, respectively.

The average level of crude fiber content found in our samples (29.07%) is comparable to the total dietary fiber content found by the Canadian Grain Commission in brown Canadian flaxseed (28%) but outside the range reported in previous investigations (20-25%) (Coskuner and Karababa, 2007).

Many authors consider that these differences could be attributted to genetic and environmental factors, seed processing and method of analysis (Rubilar et al., 2010; Coskuner and Karababa, 2007; Shim et al., 2014).

Tab. 2. Amino acids content of flaxseeds (g 100 g^{-1})

Amino acids	Flaxseeds
Aspartic acid	1.528±0.03
Glutamic acid	3.871±0.14
Serine	0.872±0.03
Glycine	0.752±0.03
Threonine	0.933±0.03
Arginine	1.937±0.08
Alanine	0.994±0.03
Tyrosine	0.626±0.03
Valine	0.724±0.04
Phenylalanine	0.844±0.05
Isoleucine	0.787±0.03
Leucine	1.199±0.07
Lysine	0.826±0.03
Cystine	0.281±0.02
Methionine	0.322±0.03

Amino acids content

The results regarding the amino acids content of flaxseeds are listed in Table 2. In the present study seven essential amino acids were determined in flaxseeds, namely leucine, isoleucine, lysine, methionine, phenylalanine, threonine, and valine, representing together 34% of total protein. Leucine (1.199 g 100 g^{-1}), and threonine (0.933 g 100 g^{-1}) dominated among essential amino acids while methionine (0.322 g 100 g^{-1}) was the most limiting.

Glutamic acid was the most abundant in flaxseeds (3.87 g 100 g^{-1}), followed by arginine (1.93 g 100 g^{-1}) and aspartic acid (1.52 g 100 g^{-1}) while the limiting amino acids were cystine, methionine, tyrosine, valine and lysine. Previous studies reported also that flax protein is relatively rich in arginine, aspartic acid and glutamic acid, while the limiting aminoacids are lysine, methionine and cysteine (Chung et al., 2005; Shim et al., 2014). Arginine and glutamine are known to have strong effects on the immune functions of the human body while the low lysine/arginine ratio (0.42) suggest that flaxseed protein is less lipidemic and atherogenic than other vegetable proteins (Oomah, 2001).

Fatty Acids Content

Fatty acid methyl esters (FAME) were identified by gas chromatography and their concentration was expressed as weight percentage of total FAME present (Table 3). The results showed that alpha-linolenic acid represents the major fatty acid (54.51% of total FAME) followed by oleic (17.38%) and linoleic (16.13%) acids while palmitic acid was the main saturated acid (5.95%). These results are in good agreement with published values on flaxseeds (Shim et al., 2014). The ratio of unsaturated fatty acids to saturated fatty acids was 8.67 while El-Beltagi et al. (2007) reported between 6.9 and 9.8 in flaxseeds of five cultivars. Flaxseed is known as one of the richest plant sources of n-3 fatty acids, especially alpha linolenic acid, a precursor for the synthesis of very long chain polyunsaturated fatty acids (VLCPUFA), eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6) (Jhala and Hall, 2010). Many clinical and epidemiologic studies have shown positive roles of n-3 fatty acids in infant development, cancer, cardiovascular diseases and more recently, in various mental illnesses (Riediger et al., 2009).

In our study it was found a n-3/n-6 PUFA ratio of 3.2, in good agreement with the findings of Choo et al. (2007) who reported ratios of n-3/n-6 fatty acids in the range 3.28-3.72 in various cold-pressed flaxseed oils sold on the worldwide market.

Nutritional guidelines on lipid composition advocate reducing the n-6/n-3 ratio and increasing n-3 polyunsaturated fatty acid (PUFAs) intake (Weill et al., 2002) for the prevention and

Tab. 3. Fatty acids profile of flaxseeds (% of total FAME)

Fatty acids		Flaxseeds
Myristic	C 14:0	0.08
Pentadecanoic	C 15:0	-
Pentadecenoic	C 15:1	-
Palmitic	C 16:0	5.95
Palmitoleic	C 16:1	0.14
Heptadecanoic	C 17:0	-
Heptadecenoic	C 17:1	-
Stearic	C 18:0	4.15
Oleic cis	C 18:1	17.38
Linoleic cis	C 18:2n6	16.13
Linolenic γ	C 18:3n6	0.23
Linolenic α	C 18:3n3	54.51
Octadecatetraenoic C18:4n3	C18:4n3	0.15
Arachidic		0.12
Eicosadienoic	C20(2n6)	0.11
Eicosatrienoic	C20(3n6)	0.07
Docosadienoic	C22(2n6)	0.28
Docosatrienoic	C22(3n6)	0.25
Docosatrienoic	C22(3n3)	0.11
Eicosapentaenoic	C20(5n3)	-
Lignoceric	C 24:0	-
Other fatty acids		0.34
Fatty acids profile		
Saturated fatty acids (SFA)		10.29
Monounsaturated fatty acids (MUFA)		17.52
Polyunsaturated fatty acids (PUFA), of which:		71.84
▪ n-3		54.77
▪ n-6		17.07
n-3/n-6		3.2

management of chronic diseases (Simopoulos, 2006).

Flaxseed has been shown to be protective against atherosclerosis, vascular dysfunction and ischemia-induced arrhythmias in animal models of cardiovascular disease and part of these effects has been suggested to have been achieved through the high ALA content (Austria et al., 2008).

Flaxseed oil supplementation to human regimens is limited by its high susceptibility to oxidation and polymerization. In addition, uncooked flaxseed contains cyanogenic compounds, which can be toxic to man at high concentration. In order to reduce n-6/n-3 PUFA ratio in human nutrition, many studies have explored the flaxseed or flaxseed oil supplementation to the diet of animals destined for human consumption (Weill et al., 2002; Jhala and Hall, 2010), resulting in healthier food from animal origin. In fact, feeding omega-3 enriched diets by the addition of flaxseed would increase the omega-3 content in eggs and meat and thus enrich the products as the fatty acid profile of the meat and fat is directly affected by the source of fat in diet in swine and poultry (Bernacchia et al., 2014).

Tab. 4. Total phenolics, total flavonoids, and free radical scavenging activity of flaxseeds

Component	Flaxseeds
Total phenolics (mg GAE 100 g^{-1})	295.92±2.3
Total flavonoids (mg QE 100 g^{-1})	25.85±0.4
Antioxidant activity (mmol Trolox 100 g^{-1})	1.45±0.02

Total phenolics, total flavonoids and DPPH radical scavenging activity

Flaxseed has considerable potential as a source of phenolic compounds, as it contains different types of phenolics such as lignans, phenolic acids, flavonoids, phenylpropanoids and tannins (Kasote, 2013). The average total phenolic content found in flaxeed samples was 295.92 mg GAE 100 g^{-1} (table 4), in good agreement with the results reported by Anwar and Przybylski (2012) (300 mg GAE 100 g^{-1}) or by El-Beltagi et al. (2007) who found between 162 and 362 mg GAE 100 g^{-1} in different flaxsees cultivars. The differences of data from literature have been attributed to the differing varieties of flaxseed, extracting solvent, extraction temperature and technique employed. It was found that phenolic acids from flaxseed are mainly composed of trans and cis-sinapic, o-coumaric, p-droxybenzoic, trans-p-coumaric and vanillic acids (Kasote et al. 2013).

The total flavonoid content found in our study (25.85 mg QE 100 g^{-1}) is inside the range reported by Anwar and Przybylski (2012) (20-60 mg CE 100 g^{-1}) but lower than the range of 35 to 71 mg CE 100 g^{-1} reported by Oomah et al. (1996). According previous studies, flavonoids are in the form of their glucoside such as herbacetin 3, 8-O-diglucopynanoside, herbacetin 3, 7-O-dimethyl ether, and kaempferol 3, 7-O-diglucopyranoside.

Flaxseed samples exhibited good capacity towards scavenging DPPH radicals (1.45 mmol Trolox 100 g^{-1}) that could be explained by the presence of appreciable content of total phenolics and flavonoids.

CONCLUSIONS

This study revealed that flaxseeds contains important quantities of compounds with functional and bioactive properties, such as essential amino acids, polyunsaturated fatty acids, lignans, soluble fibre and protein. Flaxseeds are excellent sources of alpha-linolenic acid, which is a precursor of long chain PUFA metabolically synthesized in the human body. They can be used as flours and/or oils to enrich food products with ALA and thus change the n-6/n-3 ratio in the human diet or to develop n-3-enriched feed mixes in order to increase the n-3 fatty acids content in eggs and meat. Apart from n-3 fatty acids, flaxseeds are rich in phenolic compounds, with high antioxidant activity, which helps increase the functionality of the foods to which they are added and supports their uses as functional and nutraceutical food ingredient.

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REFERENCES

1. Anwar F., Przybylski, R. (2012). Effect of solvents extraction on total phenolics and antioxidant activity of extracts from flaxseed (*Linum usitatissimum* L.). Acta Scientiarum Polonorum Technologia Alimentaria, 11, 293–301.
2. Austria J.A., Richard M.N., Chahine M.N., Edel A.L., Malcolmson L.J., Dupasquier C.M., Pierce G.N. (2008). Bioavailability of alpha-linolenic acid in subjects after ingestion of three different forms of flaxseed. Journal of the American College of Nutrition, 27, 214–221.
3. Ayelign A., Alemu T. (2016). The functional nutrients of flaxseed and their effect on human health: a review. European Journal of Nutrition and Food Safety, 6(2), 83–92.
4. Bernacchia R, Preti R, Vinci G (2014). Chemical composition and health benefits of flaxseed. Austin Journal of Nutrition and Food Science, 2(8), id 1045.
5. Choo W, Birch J, Dufour J (2007). Physicochemical and quality characteristics of cold-pressed flaxseed oils. Journal of Food Composition and Analysis, 20, 202–211.
6. Chung M., Lei B., Li-Chan E. (2005). Isolation and structural characterization of the major protein fraction from NorMan flaxseed *(Linum usitatissimum* L.). Food Chemistry, 90, 271–279.

7. Coskuner Y., Karababa, E. (2007). Some physical properties of flaxseed (*Linum usitatissimum* L.). Journal of Food Engineering, 78(3), 1067–1073.
8. Deng X., Long S., He D., Li X., Wang Y., Liu J., Chen X. (2010). Development and characterization of polymorphic microsatellite markers in *Linum usitatissimum*. Journal of Plant Research, 123, 119–123.
9. El-Beltagi HS, Salama ZA, El-Harir DM (2007). Evaluation of fatty acids profile and the content of some secondary metabolites in seeds of different flax cultivars (*Linum usitatissimum* L.). General and Applied Plant Physiology, 33, 187–202.
10. Ganorkar P.M., Jain R.K. (2013). Flaxseed – a nutritional punch. International Food Research Journal, 20(2), 519–525.
11. Heimbach J.T. (2009). Determination of the generally recognized as safe status of the addition of whole and milled flaxseed to conventional foods and meat and poultry products. In Flax Canada 2015 (pp. 1–178). Port Royal, VA: JHeimbach LLC.
12. Hemmings S.J., Westcott N.D., Muir A.D., Czechowicz D. (2004). The effects of dietary fl axseed on the Fischer 344 rat: II. Liver gamma-glutamyl transpeptidase activity. Cell Biochemistry and Function, 22, 225–231.
13. Hosseinian F.S., Muir A.D., Westcott N.D., Krol E.S. (2006). Antioxidant capacity of fl axseed lignans in two model systems. Journal of the American Oil Chemists' Society, 83, 835–840.
14. Jhala A.J., Hall L.M. (2010). Flax (*Linum usitatissimum* L.): Current uses and future applications. Australian Journal of Basic and Applied Sciences, 4(9), 4304–4312.
15. Kasote D.M. (2013). Flaxseed phenolic as natural antioxidants. International Food Research Journal 20, 1, 27–34.
16. Kraushofer T., Sontag G. (2002). Determination of some phenolic compounds in flax seed and nettle roots by HPLC with coulomertic electrode array detection. European Food Research and Technology, 215, 529–533.
17. Kristensen M., Jensen M.G., Aarestrup J., Petersen K.E., Søndergaard L., Mikkelsen M.S., et al. (2012). Flaxseed dietary fibers lower cholesterol and increase fecal fat excretion, but magnitude of effect depend on food type. Nutrition and Metabolism, 9, 8.
18. Lewis N.M., Seburg, S., Flanagan, N.L. (2000). Enriched eggs as a source of N-3 polyunsaturated fatty acids for humans. Poultry Science, 79, 971–974.
19. Madhusudhan B. (2009). Potential benefits of flaxseed in health and disease – a perspective. Agriculturae Conspectus Scientificus, 74, 67–72.
20. Mohammadzadeh S., Sharriatpanahi, M., Hamedi, M., Amanzadeh, Y., Sadat Ebrahimi, S.E., Ostad, S.N. (2007). Antioxidant power of Iranian propolis extract. Food Chemistry, 103, 729–733.
21. Morris, D.H. (2001). Essential nutrients and other functional compounds in flaxseed. Nutrition Today, 36(3), 159–162.
22. Muir A., Westcott, N. (2003). Flaxseed constituents and human health. In: Muir AD,Westcott ND, editors. Flax: the genus Linum. London: Taylor and Francis, pp. 243–251.
23. Mueller K., Eisner, P., Yoshie-Stark, Y., Nakada, R., Kirchhoff, E. (2010). Functional properties and chemical composition of fractionated brown and yellow linseed meal (*Linum usitatissimum*). Journal of Food Engineering, 98(4), 453–460.
24. Novak C., Scheideler, S.E. (2001). Long term effects of feeding flaxseed-based diets. 1. egg production parameters, components, and eggshell quality in two strains of laying hens. Poultry Science, 80, 1480–1489.
25. Oliveira I., Sousa, A., Ferreira, I.C.F.R., Bento, A., Estevinho, L., Pereira, J.A. (2008). Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. Food and Chemical Toxicology, 46, 2326–2331.
26. Oomah B.D. (2001). Flaxseed as a functional food source. Journal of the Science of Food and Agriculture, 81, 889–894.
27. Prasad K. (2005). Hypocholesterolemic and antiatherosclerotic effect of flax lignan complex isolated from flaxseed. Atherosclerosis, 179(2), 269–275.
28. Ramcharitar A., Badrie N., Mattfeldt-Beman M., Matsuo H., Ridley C. (2005). Consumer acceptability of muffins with flaxseed (*Linum usitatissimum*). Journal of Food Science, 70, S504–S507.
29. Riediger ND, Othman RA, Suh M, Moghadasian MH (2009). A systemic review of the roles of n-3 fatty acids in health and disease. Journal of the American Dietetic Association, 109, 668–679.
30. Rubilar M., Gutiérrez C., Verdugo M., Shene C., Sineiro, J. (2010). Flaxseed as a source of functional ingredients. Journal of Soil Science and plant nutrition, 10, 373–377.
31. Shim Y.Y., Gui B., Arnison, P.G., Wang Y., Reaney, M.J.T. (2014). Flaxseed (*Linum usitatissimum* L.) bioactive compounds and peptide nomenclature: A review. Trends in Food Science and Technology, 38, 5–20.
32. Simopoulos A.P. (2006). Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. Biomedicine and Pharmacotherapy, 60, 502–507.
33. Singleton V.L., Rossi J.A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagent. American Journal of Enology and Viticulture, 16, 144–158.
34. Surai P.F., Sparks N.H.C. (2001). Designer eggs: From improvement of egg composition to functional food. Trends in Food Science and Technology, 12, 7–16.
35. Tarpila A., Wennberg T., Tarpila S. (2005). Flaxseed as a functional food. Current Topics in Nutraceutical Research 3, 167–188.
36. Touré A., Xueming X. (2010). Flaxseed lignans: source, biosynthesis, metabolism, antioxidant activity, bio-active components, and health benefits. Comprehensive Reviews in Food Science and Food Safety, 9(3), 261–269.
37. Turner T.D., Mapiye C., Aalhus, J.L., Beaulieu, A.D., Patience, J.F., Zijlstra, R.T., et al. (2014). Flaxseed fed pork:

n-3 fatty acid enrichment and contribution to dietary recommendations. Meat Science, 96, 541–547.

38. Varzaru I., Untea A.E., Martura T., Olteanu, M., Panaite, T.D., Schitea, M., Van, I. (2013). Development and validation of an RP-HPLC method for methionine, cystine and lysine separation and determination in corn samples. Revista de Chimie - Bucharest, 67(7), 673–679.
39. Weill P., Schmitt B., Chesneau G., Daniel N., Safraou F., Legrand P. (2002). Effects of introducing linseed in livestock diet on blood fatty acid composition of consumers of animal products. Annals of Nutrition and Metabolism 46, 182–191.
40. Westcott N.D., Paton D. (2001). Complex containing lignan, phenolic and aliphatic substances from flax process for preparing. U.S. patent 6,264,853.