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RESEARCH ARTICLE (Open Access)

A comparison of antioxidant properties of extracts from defatted and non-defatted flax (*Linum usitatissimum*) seeds

KATARZYNA BRODOWSKA[1*], RIK CATTHOOR[2], AGNIESZKA JOANNA BRODOWSKA[3], MARZENA SYMONOWICZ[4], ELŻBIETA ŁODYGA-CHRUŚCIŃSKA[5]

[1]Ph.D. Student in Bioinorganic Chemistry and Environmental Analysis, Institute of General Food Chemistry, Department of Biotechnology and Food Sciences, Lodz University of Technology, Lodz, Poland.
[2]B.Sc. Student in Bioscience Engineering, Department of Sustainable Organic Chemistryand Technology, Ghent University, Ghent, Belgium.
[3]Ph.D. Student in Bioorganic chemistry and raw cosmetic materials, Institute of General Food Chemistry, Department of Biotechnology and Food Sciences, Lodz University of Technology, Lodz, Poland.
[4]Ph.D. Student in Bioinorganic Chemistry and Environmental Analysis, Institute of General Food Chemistry, Department of Biotechnology and Food Sciences, Lodz University of Technology, Lodz, Poland.
[5]Professor, Institute of General Food Chemistry, Department of Biotechnology and Food Sciences, Lodz University of Technology, Lodz, Poland.

Abstract

Flax seeds are an abundant source of lignans, which have antioxidant properties and significantly reduce the effects of free radicals. This paper presents total polyphenol content (TPC), free radical scavenging activity, and ferric reducing antioxidant power (FRAP) in extracts from defatted and non-defatted flax seeds. The obtained results have revealed that the ability of defatted flaxseed extracts to scavenge the DPPH radical was at the level from 19.7 to 76.1 %, while the scavenging capacity of non-deffated flaxseed extracts ranged from 25.7 to 76.3 %. Among the defatted and non-defatted flaxseed extracts, the second one has shown stronger ferric ion reducing activity with 0.062 ± 0.007 mmol TE/g of flaxseed extract. Studies on the total polyphenol content of flaxseed extracts demonstrate a higher amount of polyphenols in defatted flaxseed extracts (98.8 mg/100g). It was observed that non-defatted flaxseed extracts contain nearly one-third less polyphenols (61.3 mg/100g) compared to defatted flaxseed extracts.

Keywords: flax; antioxidant activity; total phenolics; FRAP

1. Introduction

Flax, *Linum usitatissimum*, is an annual plant and member of the Linaceae family [1]. It can be grown in every country with moderate climate. Flax becomes up to 1.2 meter tall. From June till August, flax plants carry flowers consisting of 5 petals, coloured red or blue. The main flax producing countries are the USA, Canada and Europe. In the USA and Canada, flax is grown for linseed oil and in European countries flax is mostly used as a source of fiber [2, 3].

The search for novel high-quality, as well as cheap sources of proteins and energy-rich compounds has gained popularity due to the problem of hunger and malnutrition in developing countries. Flax seeds, containing a relatively high protein content (about 20 per 100 grams of dried grain), could help to improve the quality of nutrition in large populations [4]. Flax is rich in fats (41 %), proteins (20 %) and dietary fibre (28 %). The composition of flax can depend on growing conditions, seed processing, as well as genetics. Flax seeds with a lower protein content generally have a higher amount of oil [5]. Seeds of flax are the richest source of alpha-linolenic acid, lignans and other nutritional components [6]. Flax does not contain gluten and consequently can be applied in gluten-free diets [4].

Flaxseed oil contains bioactive compounds other than the fats [7]. Tocopherols, vitamin E analogs, anticancer and anti-cardiovascular disease agents, the polyphenolic compounds and flavonoids exhibit therapeutic properties such as anti-microbial, anti-inflammatory, anti-thrombotic, anti-allergenic, anti-atherogenic, anti-cardiovascular disease and vasodilatory effects [8]. Moreover, lignans, phenolic acids and flavonoids are preventative in the decreasing rate of tumor growth and the decreasing incidence of breast, prostrate, and colon cancers [1, 9].

*Corresponding author: Katarzyna Brodowska; E-mail: kasia.brodowska88@gmail.com
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As the cotton production reaches its maximum, linen will become more and more important to supply enough textile. Consequently more flax will be grown and thus more flax seeds produced as byproduct. Characterisation and quantification of the useful compounds in flax seeds is therefore highly desired. Flax seeds contain approximately 40% of linseed oil [10]. The demand for linseed oil for human nutrition purposes increases since linseed oil contains polyunsaturated Ω-3 fatty acids such as α-linolenic acid. A western diet typically lacks the appropriate balance between Ω-6 and Ω-3 fatty acids. Usually Ω-6 fatty acids are more abundant than Ω-3. A lower uptake of polyunsaturated fatty acids is connected to cardiovascular diseases, hypertension, inflammatory disorders, and certain disrupted neurological functions [11]. Flaxseed oil is different in respect of quality from most common oils based on plants with high PUFA proportions, such as sunflower oil, olive oil, soya oil, rape oil etc. [12]. The fatty acid composition of linseed oil is presented as follows : oleic (C18, 16-24 %), linoleic (C18, 18-24 %) and linolenic acid (C18, 36-50 %) [13]. Furthermore, it is suggested that natural antioxidants play an important role in slowing down oxidation processes by quenching free radicals, chelating catalytic metals and scavenging oxygen [1]. Polyphenolic compounds are mostly found under glycosylated form [14].

It is well-known that the phenolic acids in plants possess many different functions, such as providing colour, luring bees and other insects for pollination. Flax is also a good source of flavonoids. They inhibit lipid peroxidation, platelet aggregation and capillary permeability and fragility, thus leading to lowered incidence of cardiovascular diseases. Plants lignans are biologically important class of phenolic compounds. The prevailing lignan in seeds of flax is called secoisolariciresinol diglucoside (SDG). SDG has shown a very promising effect in reducing growth of cancerous tumors, mainly breast, prostate and endometrium cancer [5, 15].

In terms of the polyphenol content flax seeds take place in the top ten. Seeds of flax contain almost 1528 mg/100 g, which was determined by chromatography after hydrolysis of the glycosides and esters [16]. Due to a great importance of phenolic compounds in the radical scavenging studies, there is a huge need of determination of antioxidant activity of flax seeds. Seeds of flax constitute a good source of antioxidant compounds, thus can be used as a potential value added ingredient in the food, as well as cosmetic and pharmaceutical industry [17].

DPPH, a stable organic free radical, is very often applied for the evaluation of the antioxidant activity of compounds [1]. Free radicals are molecules possessing an unpaired electron emerging mainly in so-called oxidative stress, which may follow from UV-radiation, medicines taking, air pollution, cigarette smoking and preservatives in food [18]. The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay is widely used in plant biochemistry to evaluate the properties of plant constituents for scavenging free radicals. This method is based on the spectrophotometric measurement of the DPPH concentration change resulting from the reaction with an antioxidant [19]. An alternative assay for identification of antioxidant activity constitute Ferric reducing antioxidant power assay (FRAP) [20]. The main objectives of the present study were the qualification of antioxidant profile of defatted and non-defatted flax seeds using DPPH and FRAP assays, as well as determination of total polyphenol content (TPC).

2. Material and Methods

Samples of defatted milled flax seeds and non-defatted flax seeds (whole grains) were obtained from a local herbs store in Lodz, Poland.

2, 2-Diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's reagent (2 N) and catechin hydrate were obtained from Sigma-Aldrich, Steinheim, Germany. All other chemicals /reagents and solvents used in this study were purchased from Merck as analytical reagent grade materials and applied without subsequent purification.

2.1. Extraction of flax seeds

Non-defatted flax seeds were ground using a coffee grinder in order to get the <0.8 mm particles. Then, the ground material and the defatted milled flax seeds (< 0.8 mm) were extracted with pure methanol, 5 ml methanol for 0.5 g seed material. Extracts were obtained at room temperature using a separator set at 2500 rpm for 5 minutes. The supernatants were decanted and transferred into conical flasks. Freshly prepared extracts were tested at once.

2.2. DPPH radical-scavenging capacity

Free radical-scavenging capacity of defatted and non-defatted flaxseed extracts was determined spectrophotometrically, following the modified procedure described by Hatano et al. [21]. Freshly prepared 0.25 mM DPPH-solution was used for the determination. 2 mL of violet DPPH solution was

pipetted into a centrifuge tube, then 0.5 – 3 mL of sample extract was added and filled with pure methanol till 5 ml. The mixture was incubated in the dark for 30 min at room temperature and afterwards the absorbance was measured using a spectrophotometer Hewlett Packard 8453 at 517 nm. A mixture of 2 mL of DPPH solution and 3 mL of pure methanol was used as a control sample. Radical-scavenging activity was calculated by the following formula:

$$\text{DPPH radical scavenging [\%]} = [(A_0\text{-}A)/A_0] \text{ X } 100$$

where:

A_0 – absorbance of the blank sample at t = 30 min

A – absorbance of the tested extract solution after 30 minutes incubation

2.3. Ferric reducing antioxidant power assay (FRAP)

The ferric-reducing antioxidant power (FRAP) of flaxseed extracts was tested using the assay of Oyaizu with some modifications [20]. The fresh working solution was prepared by mixing 250 mg dissolved TPTZ in 80 mL 40 mM HCl, 800 mL acetate buffer, pH 3.6 and 80 mL $FeCl_3$•6 H_2O. Various concentrations (5 - 50 g/L) of methanolic extracts (0.5 mL) was mixed with 2.5 mL TPTZ. A 50 - µL extract was mixed with 2.5 mL TPTZ. The mixtures were incubated in the dark for 30 min at room temperature. All solutions were used on the day of preparation. The FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored FeII-tripyridyltriazine compound from the colorless oxidized FeIII form by the action of electron donating antioxidants. Standard curve was prepared using different concentrations (25 – 250 mg/mL) of trolox. The results were expressed as mmol trolox equivalent TE/g extract. The obtained calibration curve for trolox was plotted and the linear regression equation calculated: $y = 0.002x – 0.0272$, $R^2 = 0.9972$.

2.4. Determination of total polyphenol content (TPC)

The estimation of the total polyphenol content (TPC) in extracts was done following the Folin-Ciocalteu procedure taking the modification of Singleton & Rossi [22] into account. The extracts were diluted 2-,5- and 10 times. Briefly, 2.5 mL of each diluted extract was transferred into a 25-mL volume flask, which was filled up to the mark with distilled water. Then, to 5 mL of each solution, 0.25 mL of Folin-Ciocalteu reagent and after agitation 0.5 mL of 7 % sodium carbonate solution were added. The mixture was incubated in the dark for 30 minutes at room temperature, after which the absorbance of the samples was measured at λ = 760 nm. Pure methanol was used as a blank. The results were expressed as catechin equivalent (mg/100 g). A result was obtained from each of the three dilutions and the average value was calculated.

2.5. Statistical analysis

The statistical analysis was performed by one-way analysis of variance ANOVA (using OriginPro version 8), followed by Tukey's range test. The results were expressed as means ± standard deviation (SD) of three parallel measurements. There were no significant differences when p = 0.05.

3. Results and Discussion

3.1. DPPH radical-scavenging capacity

The conducted studies showed that the ability of defatted flaxseed extracts to scavenge the DPPH radical was at the level from 19.7 to 76.1 %, while the scavenging capacity of non-deffated flaxseed extracts ranged from 25.7 to 76.3 % (Fig. 3). The highest activity was observed at the concentration of 60 mg/mL for defatted and non-defatted flaxseed extracts. The study also showed that the higher the concentration of extracts (defatted as well as non-defatted seeds of flax), the higher the capacity to scavenge free radicals. Nevertheless, non-defatted flaxseed extracts came out to be more effective scavengers than defatted flaxseed extracts, especially at lower concentrations (Figure 1 and 2). This fact leads to the conclusion that the degreasing process of flax seeds insignificantly lowers its antioxidant activity. According to Fig. 3 the differences between scavening capacity were observed. The 50% scavenging of radicals was noticed in the concentration range 30 – 36 mg/mL.

IC_{50} for defatted flaxseed extracts is presented at the concentration of 35.5 mg/mL and 29.9 mg/mL for non-defatted extracts, which was calculated from linear regression equation : $y = 0.012x + 0.0739$, $R^2 = 0.9901$ and $y = 0.0105x + 0.1859$, $R^2 = 0.9492$, respectively. The IC_{50} value, defined as the concentration of the extract required for 50% scavenging of radicals under experimental condition employed, is a parameter widely used to measure the

free radical scavenging activity. A smaller IC_{50} value corresponds to a higher antioxidant activity [17].

These obtained results have exhibited that flaxseed oil is also a source of antioxidants. Thus, the DPPH scavenging capacity of defatted flaxseed extracts is lower than non-defatted extracts.

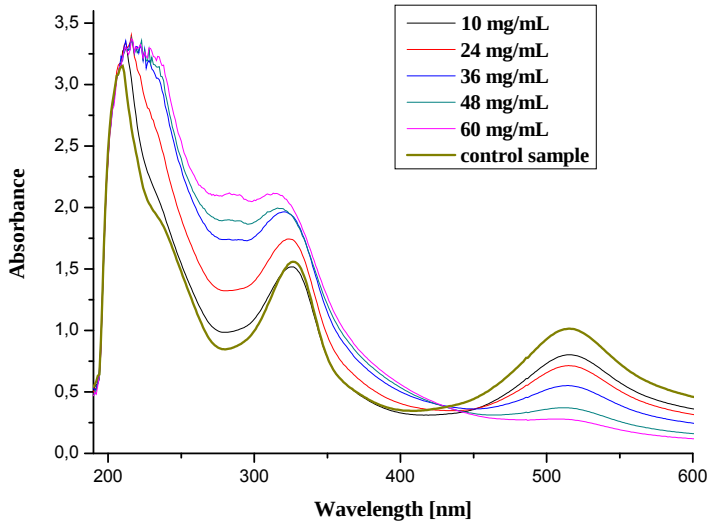

Figure 1. The UV-VIS spectra of antioxidant activity for defatted flaxseed extracts

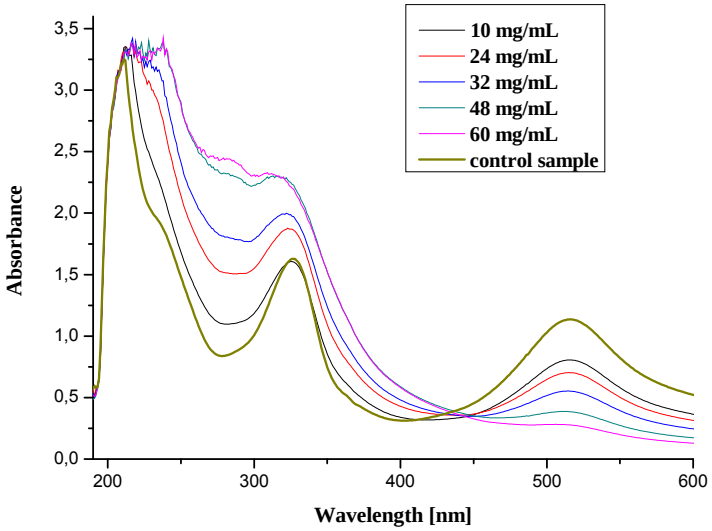

Figure 2. The UV-VIS spectra of antioxidant activity for non-defatted flaxseed extracts

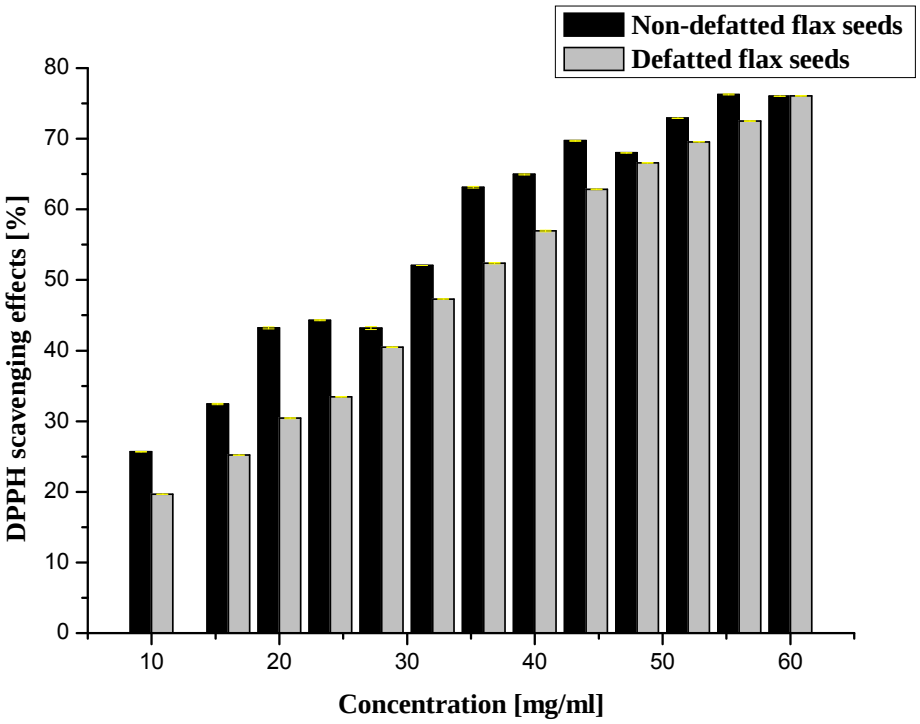

Figure 3. The graphical comparison scavenging capacity of defatted and non-defatted flaxseed extracts

According to studies of Amin & Thakur, percentage scavenging of DPPH radical of non-defatted flax seeds is presented as follows: the lowest concentration 10 mg/ml corresponded to 32.33 ± 0.31424 %, while for the highest concentration 50 mg/ml was 82.53 ± 0.17954 %. The IC_{50} value of ethanolic extract of flax seeds was 25.63 mg/ml, which means that this concentration of ethanol extract of flax seeds was required to cause 50% scavenging activity of DPPH radical [23].

On the other hand, Gaafar *et al.* have reported that different defatted flax seeds cultivars possessed antioxidant scavenging activity in the field from 5.57 ± 0.28 % to 9.05 ± 0.76 % which corresponded to the lowest concentraton of flax seeds 5 µg/ml. The highest concentration of defatted flax seeds 50 µg/ml was characterized by percentage scavenging of DPPH radical ranging from 55.17 ± 0.56 % to 88.56 ± 0.28 %. These differences between flax seeds cultivars may be caused by country of origin of tested seeds of flax [24].

3.2. Ferric reducing antioxidant power assay (FRAP)

Determination of the ferric reducing antioxidant power is a simple direct test of antioxidant capacity. In this study, assay of reducing activity was based on the reduction of ferric to the ferrous form in the presence of reductants (antioxidants) in the tested samples. The antioxidant activity of defatted and non-defatted flaxseed extracts, determined by the FRAP method are summarized in Table 1.

Among the defatted and non-defatted flaxseed extracts, the second one has shown stronger ferric ion reducing activity with 0.062 ± 0.007 mmol TE/g of flaxseed extract. However, there is no significant difference between FRAP in both extracts of Linum usitatissimum. Extract isolated from defatted seeds of flax exhibited insignificantly lower antioxidant potential with 0.058 ± 0.009 mmol TE/g of extract. According to the study conducted by Girma et al. obtained results are comparable to literature data [25]. In this study, extracts from non-defatted seeds of flax possessed lower ferric reducing capacities than its extracts without fat phase.

3.3. Determination of total polyphenol content (TPC)

Studies on the total polyphenol content of flaxseed extracts demonstrate a higher amount of polyphenols in defatted flaxseed extracts (98.8 mg/100g). It was observed that non-defatted flaxseed

extracts contain nearly one-third less polyphenols (61.3 mg/100g) compared to defatted flaxseed extracts (Table 2). These results were compared to literature data [5]. The obtained results are closely the same as described in literature [26].

Table 1. Ferric reducing antioxidant power (FRAP) of the flaxseed extracts (mmol TE/g)

Sample of *Linum usitatissimum*		FRAP assay (mmol TE/g)
Extract	defatted	0.058 ± 0.009
	non-defatted	0.062 ± 0.007

The results obtained were expressed as Mean ± SD with n=3.

FRAP expressed as mmol trolox equivalent /g of sample.

Table 2. Total phenolic content by Folin-Ciocalteau

Research material	Current research mg catechin/100g sample	Oomah et al., 1995 mg/100g seed	Morris, 2007 mg/100g seed
Defatted flax seeds	98.8 ± 0.01	80 - 100	not mentioned
Non-defatted flax seeds	61.3 ± 0.02	not mentioned	64 - 80

Studies reported by Wanasundara & Shahidi showed that total phenolics content of defatted flax seeds ranges from about 130 to 220 mg/100g, as gallic acid equivalents [27]. In turn, El-Beltagi *et al.* conducted research on different flax cultivars from Egypt (Sakha 1, Sakha 2 and Giza 8), Lithuania (Lithuania) and France (Aryana) presented different various total phenolics. For example, Sakha 1, Sakha 2 and Aryana cultivars possessed total phenolics equal to 162. 205 and 185 mg/100g, while Giza 8 and Lithuania had higher total phenolics (362 and 352 mg/100g) [13].Unfortunately, there is a lack of data of the total phenolics content of non-defatted flax seeds.

Phenolic compounds in flaxseed appear in association with fibre in plant cell walls [28]. Flax hosts three types of phenolic compounds: phenolic acids, flavonoids and lignans. The majority of phenolics are believed to exhibit antioxidant and anticancer effects in humans [29]. Flax contains about 8-10 g of total phenolic acids per kilogram of flax. What is more, it contains about 35-70 mg of flavonoids/100g. Flax is a significant source of a lignan called secoisolariciresinol diglucoside (SDG), which can be found in amounts ranging from 1 mg/g of seed to nearly 26 mg/g of seed [5].

4. Conclusions

So far, in most of the studies defatted flax seeds have always been investigated. This study shows that the process of degreasing has an influence on the antioxidant activity. It was confirmed that non-defatted seeds are the richer source of antioxidants. Flax seeds should be explored not only without fat phase, but also as whole seeds. The implementation of full flax seeds in foods should be further investigated and could give rise to novel foods beneficial for human health.

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