FLAXSEED AND FLAXSEED CAKE AS A SOURCE OF COMPOUNDS FOR FOOD INDUSTRY

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ABSTRACT

Flax (*Linum usitatissimum*) has been used for centuries as a source for oil extraction. In recent years it has attracted considerable interest as a result of studies which attribute potential health benefits to its components, including the prevention of chronic non-communicable diseases. Among these compounds presenting biological activity, alpha-linolenic acid, lignans and soluble fibre are of special interest. Southern Chile has comparative advantages for the cultivation of this crop. Together with its full processing, this crop could strengthen regional industry. The purpose of the present work is to learn how the best use can be made of these compounds, by studying flaxseed and flaxseed cake as sources of compounds of interest for food industry. Oil extracted from flaxseed contained 51.86% of linolenic, 16.34% of linoleic and 20.98% of oleic acid. Fractioning of defatted flaxseed cake produced a polyphenol content of 0.73 mg GAE g^{-1} extract and a protein isolate of considerable purity, 53.15% yield with 0.78 g of albumin equivalent g^{-1} protein isolate. Additionally, a polysaccharide was isolated with low protein content as impurity, 10.71% yield with 1.37 mg of glucose equivalent per gram of polysaccharide. This information will form the basis for assessing the extraction of products of interest for the food industry from flaxseed cake.

Keywords: flaxseed, flaxseed oil, protein, polysaccharide, polyphenols, food industry.

INTRODUCTION

Flax (*Linum usitatissimum*) is an annual species of the *Linaceae* family, growing to a height of 0.3-1. m, which is cultivated for the production of textile fibre, seed and flaxseed (linseed) oil. Until a few years ago, flax was cultivated in Los Lagos Region, Southern Chile, mainly as raw material for textile industry. Today, flax is cultivated in Araucanía Region for oil extraction. Studies have shown that crop yield is higher in this Region, because of its soil and climate characteristics. Flax is best suited for fertile, fine textured, and loamy soils: An important factor is the amount of rainfall during the growing period. Adequate moisture and relatively cool temperatures, particularly during the period from flowering to maturity, seem to favour both oil content and oil quality.

The seed is located in the extremities of the branches in round capsules, each of which contains from one to ten seeds. It is well known that flax seeds are a source of high quality proteins, soluble fibre and a high content of polyunsaturated fatty

acids (Pradhan *et al*., 2010). They present values of approximately 30-40% lipids, 20-25% proteins, 4-8% moisture, 3-4% ash (Coskuner and Karababa, 2007) and 20-25% dietary fibre, of which 10% corresponds to soluble fibre. This chemical composition varies with geographical location and variety.

In recent years flaxseed has become known as a functional food due to its nutritional composition, which has positive effects on disease prevention providing health-beneficial components such as alpha-linolenic acid (Bozan and Temelli, 2008), lignans and polysaccharides (other than starch). Due to their anti-hypercolesterolemic, anti-carcinogenic and glucose metabolism controlling effects, these components may prevent or reduce the risk of various important diseases such as diabetes, lupus nephritis, arteriosclerosis and hormone-dependent types of cancer (Bilek and Turhan, 2009, Williams *et al*., 2007). Likewise, antibacterial and fungistatic activity has been reported in oligosaccharides extracted from this seed (Guilloux *et al*., 2009), which can control the growth of pathogens affecting the agricultural sector, such as *Alternia solani* and *Alternia alternata*, as well as the human pathogen *Candia albicans*; it can also control the deterioration of foodstuffs by the fungi *Penicillium chrysogenum*, *Fusarium graminearum* and *Aspergillus flavus* (Xu *et al*., 2008).

Flaxseed oil, the principal component of this seed, is rich in alpha-linolenic, linoleic and oleic acids, and for years has been the focus of interest in this seed. After extraction of the oil (with a yield of approximately 30%) a large quantity of pressed flaxseed cake remains, which is discarded and is still considered to be waste, or at best a sub-product of no value (Figuerola *et al*., 2008). The flaxseed cake is mainly used as a cattle feed, although flaxseed meal is used as an additive in baking products (Coskuner and Karababa, 2007).

Like many other seeds, flaxseed has high globulin content, 18.6%, and contains a protein similar to albumin which accounts for 17.7% of total protein. Flaxseed protein is relatively rich in arginine, aspartic acid and glutamic acid, and the limiting aminoacids are lysine, methionine and cysteine (Chung *et al*., 2005). Flaxseed and flax products are among the richest sources of vegetable lignans, particularly the lignan secoisolariciresinol diglycoside (SDG). The health benefits of flaxseed lignans reside in their antioxidant capacity as sequestrators of hydroxyl radicals, and as estrogenic compounds due to their structural similarity to 17-β-estradiol. The antioxidant capacity of flaxseed lignan (SDG) is related to the suppression of the oxidant conditions of the reactive species of oxygen. Secoisolariciresinol diglycoside and its aglycone secoisolariciresinol display a very high antioxidant capacity and act as protectors against damage to DNA and liposomes – especially in the epithelial cells of the colon exposed to these compounds – during the metabolism of colon bacteria which transform them into mammal lignans (Rajesha *et al*., 2006; Hu *et al*., 2007).

Another important component of flaxseed is Mucilage. This is obtained from aqueous extractions and its composition presents a heterogeneous mixture of polysaccharides made up of xylose, glucose, galactose, arabinose, ramnose, fucose and galacturonic acid (75% neutral and 2 acid fractions). The neutral fraction is considered to consist of high molecular weight galacto -arabino-xylan ($Mw = 1.16x10^6$ h mol^{-1}), while the 2 acid fractions are rhamnogalacturan in type (6.5×10^5 g mol^{-1} and 1.7×10^4 g mol^{-1}) (Warrand *et al.*, 2005; Guilloux *et al*., 2009). However, the molar mass of

these fractions may differ according to the genotype and the climatic conditions under which the plant is grown (Goh *et al*., 2006). Some polysaccharides have ramified structures. Furthermore they often have high molecular weights, and tend to form aggregates in solution, which may mask the behaviour of the individual macro-molecules (Yang and Zhang, 2009). The range of polysaccharides which can be extracted from flaxseed has aroused great interest, not only for the obvious health benefits, principally due to the soluble fibre content, but also for the potential application of flaxseed in foods as a functional foodstuff, taking advantage of its physical properties as a thickener and emulsifier. The mucilage obtained from flaxseed cake is very similar to gum arabic in its emulsifying properties (Coskuner and Karababa, 2007) and is comparable to guar gum in its capacity to bind water.

One application sought for flaxseed polysaccharides is to substitute chemical additives for food conservation. Thus some oligosaccharides and polysaccharides have been described as food additives with antimicrobial properties, effective against pathogenous bacteria and fungi. A known group of oligosaccharides used for their antimicrobial effects are Chito-oligosaccharides. Anti-tumour and antioxidant properties have also been described, and the capacity to capture free radicals. Another interesting feature of polysaccharides is that they encourage the growth and development of the gastro-intestinal micro-flora, with a pro-biotic effect being described. For example, galacto-oligosaccharides, fructooligo-saccharides and cyclodextrins are known to be pre-biotic substances.

Thus flaxseed and pressed flaxseed cake still have an immense usable potential in lipids, proteins, soluble fibre and lignans. The objective of the present work was to study flaxseed and pressed flaxseed cake as a resource of compounds which are of interest to the food industry, especially proteins, soluble fibre and polyphenol extract. A chemical and nutritional description was carried out using proximal analysis of flaxseed and flaxseed cake acquired in the La Araucanía Region, Chile. The fatty acid profile of the oil was assessed, as well as the contents of mucilage, protein and total polyphenols in the cake.

MATERIALS AND METHODS

Raw material

Flax seeds, a brown variety acquired in the local market in the La Araucanía Region, Chile, and flaxseed cake obtained as waste from cold pressing of flax seeds.

Proximal analysis

The chemical composition (content in moisture, lipids, nitrogen and ash) of the flaxseed cake was analysed according to the AOAC (1995) procedures.

Oil extraction

Flaxseed oil was obtained by pressing the seeds in a disc press and subsequent filtration (filter press).

Identification of fatty acids

The fatty acid composition was determined from the hydrolysis of 300 mL of flaxseed oil with a solution of KOH-MeOH and boiling in a reflux condenser for 5 min. The hydrolyzed sample was esterified by the addition of HCl-MeOH and it was carried to a boil for 15 min. After cooling, 12 mL of distilled water and 18 mL of n-heptane were added to the mixture, then it was

transferred to a funnel and after phase separation 10 mL of the ether solution were recovered. The solution was filtered through Na_2SO_4 and the solvent was evaporated by N_2 stream. Finally, the sample was dissolved in 3 mL of chloroform and the fatty acid profile was analyzed in a Hewlett Packard gas chromatograph, model HP6890 GC System, equipped with a FID detector and a reversed phase capillary column SP2380 (30 m long, 0.25 mm internal diameter, 0.20 µm film thickness), injector temperature: 250°C, Split 1:100, injection volume 1 µL, using Helium as the transporter gas at a flow of 33 psi. The fatty acids were identified by reference to the Supelco-37 standard (for 37 fatty acids) and quantified using the HPCHEM Stations software, being expressed as a percentage of area, according to the total of the fatty acids identified.

Defatting

The flaxseed cake was defatted using Soxhlet extraction equipment with petroleum ether until the sample was exhausted.

Extraction of mucilage, polyphenols and protein

To extract the mucilage the defatted flax cake was mixed with distilled H_2O (ratio 1:60) adjusting to pH 12 with sodium hydroxide 1 N. The mixture was stirred in a shaker at 200 rpm for 30 min at 25ºC, and then centrifuged for 15 min at 4000 rpm. The supernatant was separated and the procedure was repeated twice more with the precipitate. The exhausted precipitate was used for the extraction of polyphenol compounds with aqueous ethanol at 50%, solid-liquid ratio (1:60), in a shaker for 30 min at 200 rpm and 25ºC. The solution was centrifuged at 6500 rpm for 15 min at 4ºC and the supernatant containing the polyphenol extract (EPf) was concentrated in a rotary vacuum evaporating system at 60ºC.

To separate the protein, the supernatant was acidified to the isoelectric point of the protein (pH 4.4), the neutralised protein was left to settle for 20 min to ensure the precipitation of the whole protein mass, then the protein isolate (APr) was centrifuged for 30 min at 6000 rpm and 4ºC, and the precipitate was separated, frozen and lyophilized.

The supernatant containing the dissolved polysaccharide, which was purified with trichloroacetic acid for 12 hours at 4ºC to precipitate the still dissolved proteins, was centrifuged for 30 min at 6000 rpm at 4ºC. The supernatant was precipitated with an equal volume of ethanol for 12 hours at -18ºC (Cerning, 1990). The polymer was recovered by centrifuging at 7000 rpm for 30 min at 4°C. The pellet was dissolved in hot water and neutralizedwith NaOH 1 M.

The clear liquid obtained by centrifuging was dialysed for three days in water using a cut-off membrane of 6000 - 8000 Da and changing the water twice daily. The dialysed solution was lyophilized to obtain the polysaccharide (Pol). The complete process diagram is shown in Figure 1.

Analysis of total sugars using the method of Dubois et al. (1958)

400 µL of phenol (5 % w v^{-1}) and 2 mL concentrated H_2SO_4 were added to 400 µL of a sample solution (5 g L^{-1}). This was left to settle for 10 min at ambient temperature. It was shaken and kept at 27°C for 20 min. The absorbance of the solution was read at 476 nm.

Protein analysis using Bradford's method (1976)

500 µL of Comassie reagent and 1.2 mL of distilled water were added to 50 µL of

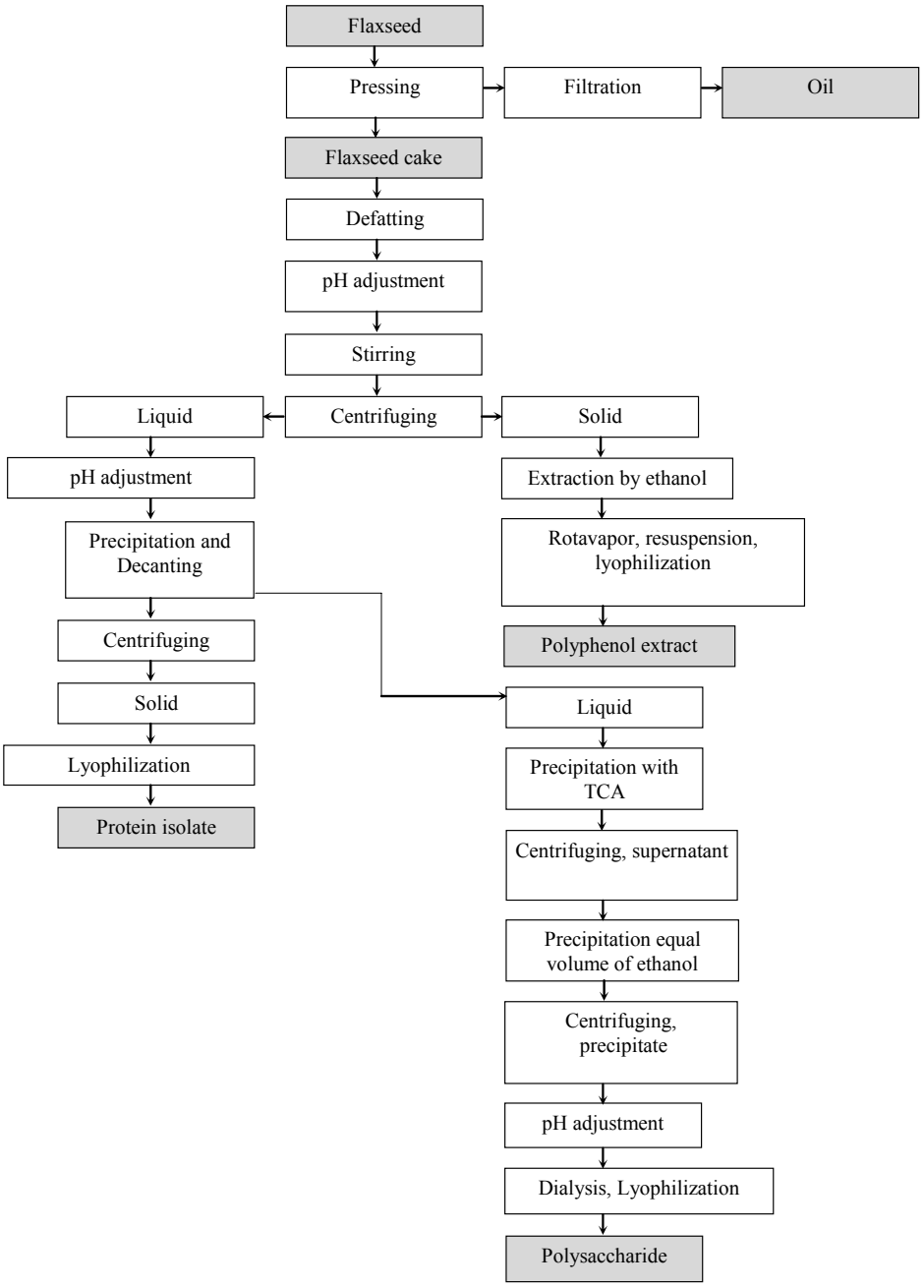

Figure 1. Obtaining polysaccharides, polyphenols and proteins from flaxseed cake.

the sample. The absorbance was read at 595 nm. The concentration of proteins in the solution was obtained by interpolation in the calibration curve:

$$C\ (g\ L^{-1}) = 0.9705\ Abs595 - 0.4875 \quad (1)$$

Determining the concentration of polyphenol compound

The total content of polyphenols was measured using the Folin-Ciocalteu method (Singleton and Rossi, 1965). A mixture of 2.5 ml of Folin-Ciocalteu reagent, in 10 times dilution, 2.5 mL of a solution of sodium carbonate at 7.5% (w v^{-1}) and 0.5 mL of phenol extract was prepared. The mixture was kept at 45ºC for 15 min, and the absorbance was then measured at 765 nm.

RESULTS

The chemical and nutritional composition of the flax seed presented a high content of oil and protein (43.90% and 21.34% respectively) (Table 1). These proportions coincide with those reported by Mueller *et al*. (2010a): 45.2% lipids and 23.4% protein.

Table 1. Chemical composition of flaxseed

Flaxseed		**Reference****
Humidity	8.30%	7.4%
Protein*	21.34%	23.4%
Lipids	43.90%	45.2%
Fibre	6.21%	--
Ash	2.66%	3.5%
Non nitrogenated extract	17.59	27.8%

* Conversion factor used, 6.25
** Mueller *et al.*, 2010a

For oil extraction, the values obtained by various authors reflect a wide range of extract yield, due principally to the different extraction processes used. However, many authors agree on the contents of linoleic acid (n-6) and alpha-linolenic acid (n-3) found in flax oil: for n-3 a range between 50.0 and 57.0% is reported and for n-6 a range between 13.96 and 15% (Williams *et al*., 2007; Bozan and Temelli, 2008; Pradhan *et al*., 2010) of the total fatty acids extracted.

Furthermore, the content of oleic acid (n-9) coincides with the 21% found by Guillevic *et al*. (2009) and is close to the 15.07% reported by Bozan and Temelli (2008). The results of the fatty acids profile in flax oil are presented in Table 2. The used oil presented a free acidity of 1.3 % oleic acid and a peroxide index of 0.17 meq O_2 kg^{-1} of fat.

The flaxseed cake (prior to defatting) presented 21.78% of NNE, 29.37% lipids and 27.78% protein (Table 3). The protein value is close to the 29% reported by Goh *et al.* (2006) for pressed flaxseed cake. However, the composition differs from the values reported by Mueller *et al*., 2010a. In principle, the difference may be attributed to the low yield of flaxseed oil extracted in this study.

The results of the chemical composition of the flaxseed cake indicate that it may be expected to be an important source of protein isolate, polyphenols and soluble fibre. Prior to fractioning these compounds, a defatting process was applied to the cake to prevent the lipids becoming rancid.

Table 4 shows the results for protein yield g^{-1} defatted flaxseed cake and the content of proteins and impurities in the protein isolate. Table 5 shows the polysaccharide yield g^{-1} defatted flaxseed cake and the content of total sugars and impurities in the protein isolate.

Table 2. Fatty acid profile for flaxseed oil

Fatty acid	% methyl ester	Fatty acid	% methyl ester	Fatty acid	% methyl ester
C4:0 Butyric	ND	C18:0 Stearic	5.12	C22:1 E rucic	ND
C6:0 Caproic	ND	C20:0 Eicosanoic	ND	C24:1 Tetracosaenoic	ND
C8:0 Caprylic	ND	C22:0 Docosanoic	ND	C18:2 Linoleic	16.34
C10:0 Capric	ND	C24:0 Tetracosanoic	ND	C18:3 Linolenic	51.86
C11:0 Undecanoic	ND	C10:1 Decaenoic	ND	C20:2 Eicosadienoic	ND
C12:0 Lauric	ND	C14:1 Myristoleic	ND	C20:3 Eicosatrienoic	ND
C13:0 Tridecanoic	ND	C15:1 Pentadecenoic	ND	C20:4 Eicosatetraenoic	ND
C14:0 Myristic	0.04	C16:1 Palmitoleic	0.05	C20:5 Eicosapentaenoic	ND
C15:0 Pentadecanoic	0.01	C17:1 Heptadecenoic	ND	C22:2 Docosadienoic	ND
C16:0 Palmitic	5.53	C18:1 oleic	20.98	C22:5 Docosapentaenoic	ND
C17:0 Heptadecanoic	0.08	C20:1 Eicoseanoic	ND	C22:6 Docosahexaenoic	ND

ND: Not determined

Table 3. Chemical composition of flaxseed cake

Flaxseed cake		Reference**
Humidity	10.65%	9.7%
Protein*	27.78%	43.3%
Lipids	29.37%	1.67
Fibre	7.02%	--
Ash	3.40%	6.4%
Non nitrogenated extract	21.78%	48.7%

* Conversion factor used, 6.25
** Mueller *et al*., 2010a

Fractioning produced a protein and polysaccharide yield g^{-1} defatted flaxseed cake of 63.86% and all the soluble protein and insoluble fibre could be separated.

Mueller *et al*. (2010b) achieved a simplified extraction of soluble fibre:protein, which was 48% of the yield. The amount achieved in the present study shows a greater extraction of the separated compounds. The fact that the compounds could be obtained in isolation facilitates the subsequent study and evaluation of the physical and chemical properties of these products, which will be of use in determining possible applications.

It is important to note that the protein isolate obtained is rich in proteins (Table 4), with 0.782 grams of albumin equivalent g^{-1} protein isolate and with a quantity of 0.027 mg of glucose equivalent g^{-1} protein isolate, being polysaccharide, which has not been separated from the isolate (impurity) (Table 4). The polysaccharide isolate presents a sugar concentration of 1.37 mg of glucose equivalent g^{-1} polysaccharide isolate, a value which is considered to be low, given that the used methodology represents the macro-molecules with the greatest weight as glucose. The of polysaccharide isolate presents 0.053 mg albumin equivalent g^{-1} of polysaccharide isolate; this is protein which was not separated from the polysaccharide (impurity) (Table 5).

Of the polysaccharides extracted in the mucilage, Mueller *et al*. (2010a) tentatively identify the presence of glucose 36.5%, followed by galactose and xylose (17.3 and 17.2% respectively). Guilloux *et al*. (2009) reported that the sugars with the largest volumes are xylose (21.7%), galactose (8.1%), arabinose (7.9%) and ramnose (8.4%).

Table 4. Yield values of protein g^{-1} defatted flaxseed cake, protein content and impurities in protein isolate.

Protein yield	53.15 ± 0.001 % w w^{-1}
Protein content	0.782 ± 0.113 g albumin equivalent g^{-1} protein isolate
Impurities - sugars	0.027 mg glucose equivalent g^{-1} protein isolate

Table 5. Yield values of polysaccharide g^{-1} defatted flaxseed cake, total sugar content and impurities in polysaccharide isolated.

Polysaccharide yield	10.71 ± 0.006 % w w^{-1}
Total sugar content	1.37 mg glucose equivalent g^{-1} polysaccharide
Protein impurity	0.053 ± 0.003 mg albumin equivalent g^{-1} polysaccharide

Finally, the polyphenol extract obtained using aqueous ethanol at 50% presented 0.7275 ± 0.0022 mg GAE g^{-1} extract. This value is low when compared to the 20.8 mg GAE g^{-1} flaxseed cake extract (Ho *et al*., 2007), considering that extraction with aqueous ethanol at 50% showed a greater yield in polyphenol extraction (Shene *et al*., 2009). However the activity of the polyphenol compounds in flaxseed has been extensively studied for example flaxseed polyphenols in their pure form control nephritis lupica in humans. Flaxseed lignans combined with isoflavones and insulin are administered as a drink to treat menopause symptoms in women, while biscuits made of flaxseed lignans combined with genistein have proved useful in combating cancer (Oomah, 2001) showing the importance of these compounds for human health.

CONCLUSIONS

The oil extracted from flaxseed is rich in linolenic, linoleic and oleic acids. Fractioning of the defatted flaxseed cake produced a polyphenol extract, a protein isolate of considerable purity, and a polysaccharide isolate with a low content of protein as an impurity. The information collected will serve as a basis for studies which will be of interest for food industry.

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