Phenolic Compounds: Their Role During Olive Oil Extraction and in Flaxseed – Transfer and Antioxidant Function

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2.7 FLAXSEED ANTIOXIDANT SYSTEM

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FLAXSEED ANTIOXIDANT SYSTEM

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Abstract:

Flaxseed is grown mostly for industrial processes. Some cultures have used the oil for medicinal purposes and as a source of edible oil. Flaxseed oil contains the highest level of α-linolenic acid (ALA) in comparison to the other oilseeds. The recently concern about the main responsible antioxidant system of both whole and ground flax was the base of this study. The results showed that flax antioxidant system is a water-soluble system. Hull had no effect on the antioxidant properties of the meal, suggesting that SDG, mainly present in the hull, was not implicated in the flax antioxidant system. After alkaline hydrolysis of an ethanol extract, SDG ferulic and *p*-coumaric acid glycosides were found. Water extracts of flax meals showed some antioxidant properties but their effect was minimal compared to the antioxidant properties of the non-extracted meal. This suggested that the main flaxseed antioxidant system is water-soluble. Flax phenolic compounds were found to have some antioxidant properties but they are not the main flaxseed antioxidant system.

Keywords: flaxseed, solin, antioxidation, SDG (Secoisolariciresinol diglucoside),

Introduction

For many years, flaxseed (*Linum usitatissimum*) has been used mainly for industrial purposes to produce paint or linoleum. However, nowadays, there is an increase of the use of flaxseed food because of the nutritional effects of flaxseed on human or animal health [1-4].

The phenolic content of flaxseed is constituted for phenolic acids and lignans where SDG (Secoisolariciresinol diglucoside) is the major compound [5]. Lignans may have antioxidant actions and may help protect against certain cancers. Flaxseed oil contains the highest level of α-linolenic acid (ALA) when compare to the other oilseeds. This fatty acid is an essential fatty acid with beneficial effects for heart disease, inflammatory bowel disease and arthritis. Solin is a form of linum, whose fatty acid composition has been modified to contain less than 5% ALA compared to more than 50% in flaxseed oil, producing a light oil suitable for cooking. Lipid oxidation is responsible most for flavors deterioration in high fat content foods. In seeds, several parameters are implicated in deterioration (mould, bacteria); in oilseeds this usually translated as an increase in the oxidation of the seed lipids. Both whole flax and ground flax were stable a room temperature for several days [6,7].

The study involving the flaxseed system responded to a lately concern about the mechanism of the stability of samples of both whole and ground flax.

Materials and Methods

Samples. Flax and solin were obtained from the Canadian Grain Commission harvest survey. Dehulled flaxseed and flax hull were obtained from Natunola Health (Nepean, ON, Canada). Petroleum ether, acetonitrile, acetic acid and ethanol (ACS reagent) were purchased from Fischer Scientific. Commercial flax oil was purchased from a local health food store. Aldehyde and peroxide test kits were purchased from SafTest Inc. (Tempe, AZ, USA). The Folin-Ciocalteu reagent, ferulic acid and caffeic acid were purchased from Sigma-Aldrich (MI, USA). SDG was purchased from Chromadex (Santa Ana, CA, USA).

Peroxide and Aldehyde Value. Aldehyde and peroxide values were measured using

SafTest Inc test kits [8]. Oil samples were diluted with a preparation reagent (peroxide-free iso-propanol) and then mixed with reagents specific to each assay and incubated at room temperature. Peroxide values were determined using the Peroxysafe assay and aldehyde values are measured using the Alkalsafe test.

Deffating. Cleaned seed samples were ground in a Retch mill, using a 1 mm sieve. Ground seed (approximately 4 g per tube) was placed into stainless steel tubes, with 35 ml organic solvent (petroleum ether) and three stainless steel ball bearings (16mm). Tubes were capped with fluorosilicone stoppers and shaken for one hour. Samples were filtered into beakers through Whatman 25 cm folded filter paper. The extraction was repeated; the filtrates pooled and then evaporated using a rotary evaporator at room temperature. The meals were left at room temperature in the fume-hood for 12 h to allow complete evaporation of the solvent. Oils and meals were stored at −18 °C until further use.

Sample Reconstitution. Oil was added to the sample to reconstitute it to 40% oil content. Briefly, for 1.2 g of meal, 0.8 g of commercial oil was added (reconstitution to 40% oil content), petroleum ether was added and the sample was mixed by manual stirring with a stainless steel spatula until complete evaporation of the petroleum ether. Samples were stored in open glass jars under fluorescent light up to 2 weeks. The added oil was extracted with petroleum ether by mixing 5 ml of petroleum ether with the reconstituted sample. The mixture was filtered through filter paper (VWR Scientific Products), the organic solvent was evaporated under nitrogen and the recovered oil was stored at −20 °C until further analysis.

Extraction of phenolic compounds. Ground samples (1 g, 0.25g hull) were extracted with three times with 80% aqueous ethanol (10 mL/g of seed per extraction) containing 400 ppm sodium metabisulfite by triple extraction using an electric homogenizer. The ethanol extracts obtained by centrifugation (3 min, 1500g) were concentrated to dryness by rotary evaporation (30 °C in a water bath) and the resulting residue resuspended in methanol (2 mL/g of seed extracted) by sonication. These suspensions were stored at -20 °C and were thoroughly re-suspended prior to sampling for further experimentation. Total phenolics were determined using the Folin method immediately after extraction.

A portion of the ethanol extract (0.5 mL) was evaporated to dryness with nitrogen gas and subjected to alkaline hydrolysis by addition of 1mL sodium hydroxide (2M) at room temperature for 24 h. The hydrolysis was terminated with the addition of 0.3 mL hydrochloric acid (6 M). Hydrolyzed extracts were also analyzed by HPLC.

Folin-Ciocalteau assay for total phenolics. The assay was performed by combining 7 mL of deionized water, 0.5 mL of Folin-Ciocalteu's reagent and 50 µL of phenolic extract in a 10 mL volumetric flask. Each sample was mixed and allowed to stand for three minutes before addition of 1 mL of saturated sodium carbonate and dilution to 10.0 mL with deionized water. Samples were mixed and placed in the dark for 60 min prior to analysis. Absorbance at 725 nm was determined on a Cary Biospec 100 (Varian Inc., CA, USA). Samples were quantified against a caffeic acid (≥ 99% purity) calibration curve and the results expressed as equivalent mass of caffeic acid (mg) per Kg of sample.

HPLC Analysis. Analysis was conducted on an Agilent 1100 series HPLC equipped with a column thermostat, diode array detector (DAD), fluorescence detector and an Inertsil ODS3 column (4.6 mm × 250 mm, 5 µm particle size) (GL Sciences, Japan) coupled with an Inertsil ODS2 guard column (4.6 mm × 7.5 mm, 5 µm particle size) (Alltech, PA, USA). The mobile phase components were acetonitrile (A) and 2% aqueous acetic acid (B). The mobile phase program was a linear gradient from 5% A to 66.5% A over 30 min at 1 mL/min. The mobile phase program also contained a cleaning step using 100% acetone at 1 mL/min for 10 min, followed by a 10 min re-equilibration to initial gradient conditions.

Calculation and statistical analysis. The statistical analyses of the results were done using Origin® 6.0 (Microcal Software Inc., Northampton, MA, USA).

Results and Discussion

There is a need in addressing the special needs of seed oxidation. The amount of unsaturation in the oil has an important effect on the oil oxidation; C18:3 fatty acid oxidized about 50 times faster than C18:2 [9]. To be able to compare the different matrices, the oil was removed by cold extraction and the samples were reconstituted with a known oil content and composition.

The meals of reconstituted brown and golden flax and solin samples exhibited very strong antioxidant effects and there was no real difference between the three types of flaxseed (Figure 1). The peroxide content of the oils decreased during the two weeks storage for flax, solin and golden flax. Usually, during the oxidation process of the oil, peroxide and aldehyde contents of the oil increase since peroxides are the sources of aldehydes. The re-extracted oils were not highly oxidized (peroxide <2 mEq/kg); good quality oils usually have peroxide values below 2 mEq/kg. The aldehyde content of the solin oils did not change during the two week storage, whereas there was an increase in aldehyde in the oils from golden flax and flax. There was no statistical difference in total phenolic compounds between solin and flax when expressed as g of caffeic acid/kg (Table 1). However, this did not mean that each sample had the same content in individual phenolic compounds.

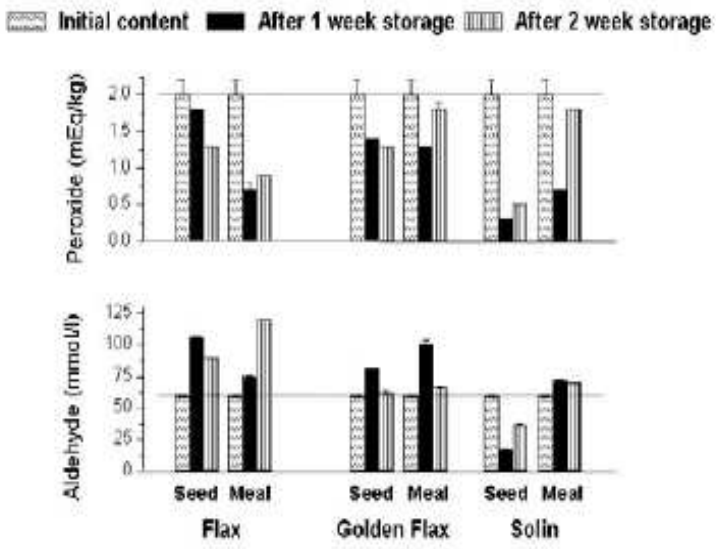

Figure 1. Comparison of flax and solin antioxidant system. All seeds were etracted with petroleum ether (cold extraction)

The HPLC profiles (Figure 2) of the two types of flax (brown flax and solin) used in this oxidation study showed a different phenolic compound composition, even if the total phenolic contents were similar (Table 1). The HPLC chromatogram showed that the peak areas of *p*-coumaric and ferulic acids glycosides were more important than SDG areas, however, the standard curves showed that SDG was the main phenolic found in flaxseed (Table 2). The λmax absorption was found at 208 nm.

Table 1. Total phenolic content by Folin-Ciocalteau (mean + SD, n=3) of flax and solin

		Total phenolic content by Folin-Ciocalteau (g caffeic acid/ kg sample)	
		Ethanol extract	After NaOH hydrolysis
Flax	Seed (n = 6)	3.06 ± 0.13	4.18 ± 0.26
	Meal (n = 6)	2.81 ± 0.18	6.49 ± 0.73
	Oil	0.027	0.027
	Hull 1	9.07 ± 2.01	12.38 ± 1.53
	Hull 2	9.11 ± 0.61	14.33 ± 0.84
	Dehulled meal 1	3.71 ± 0.30	5.28 ± 0.94
	Dehulled meal 2	3.41 ± 0.46	5.24 ± 1.00
	Water Extract	1.19 ± 0.12	2.51 ± 0.09
	Water extracted meal	2.01 ± 0.11	4.60 ± 0.02
Solin	Seed (n = 6)	3.38 ± 0.26	4.75 ± 0.41
	Meal (n = 6)	3.05 ± 0.31	6.54 ± 0.83
	Oil	0.019	0.018
	Water Extract	1.01 ± 0.06	2.38 ± 0.14
	Water extracted meal	1.78 ± 0.10	4.71 ± 0.12

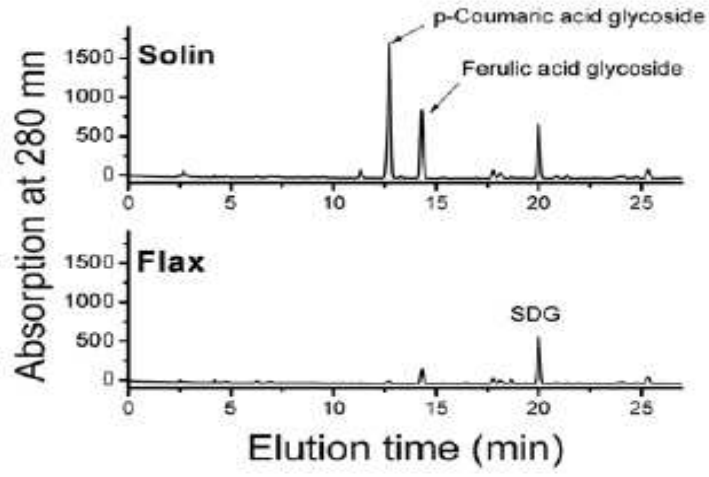

Figure 2. Phenolic compound analysis. HPLC profile of flax and solin after alkaline hydrolysis (2M NaOH for 24 h at room temperature).

Solin seed contained more polar phenolic compounds. Acid and stronger alkaline treatments were not able to free the phenolic acids, it was hypothesized that the phenolics were bound by an ether link to the sugar moiety.

Phenolic compounds have been shown to be powerful antioxidants [10]. Flaxseed has been shown to contain SDG and some phenolic acids [11]. SDG is mainly found in the hull of the seeds.

To test if SDG was part of the flaxseed antioxidant system, hulls and dehulled meals from two types of flaxseed were reconstituted with commercial oil and the results are presented in Figure 3.

The flax hull was not able to prevent the oxidation of the commercial flax oil, both peroxide and aldehyde contents of the oil increased during the two week storage for both hull samples. The dehulled meals showed little to no loss of antioxidant effect; the peroxide and aldehyde contents of the oils from the dehulled meals were lower than the peroxide and aldehyde contents of the flax meal and seed. This suggested that SDG, p-coumaric acid glycoside and ferulic acid glycoside might not be implicated in the main flaxseed antioxidant system as expected.

Table 2. Phenolic composition of hull and dehulled meals of flax, water-extracted meals of flax, solin and golden flax (all results are expressed in mg/kg of deffated meal)

		p-coumaric acid glycoside*	Ferulic acid			SDG
			Glycoside form*	Free form	Total	
Brown Flax	Sample 1					
	Hull	556.8	311.8	303.9	615.7	5768
	Dehulled meal	202.1	118.7	26.2	144.9	495
	Sample 2					
	Hull	1138.5	968.0	510.07	1478.7	4875
	Dehulled meal	256.0	180.2	62.4	242.6	956
	Sample 3					
	Water extracted meal	535.8	285.8	112.0	397.8	1803
	Water extract	28.7	21.8	6.7	28.5	214
	Total	564.5	307.6	118.7	426.3	2016
Golden flax	Water extracted meal	805.0	387.1	142.3	529.4	2568
	Water extract	24.2	16.6	39.2	55.8	121
	Total	829.2	403.7	181.5	585.2	2689
Solin	Water extracted meal	455.4	277.8	79.2	356.9	1439
	Water extract	37.0	29.0	18.5	47.5	180
	Total	492.4	306.8	97.7	404.5	1619

*expressed as free phenolic acid form

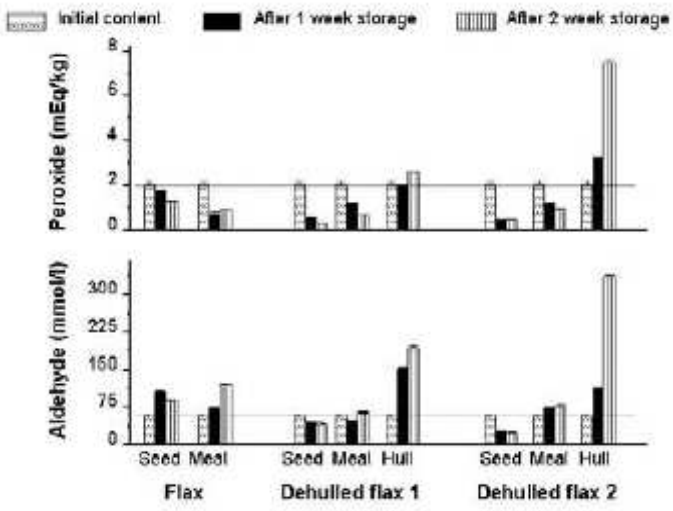

Figure 3. Comparison of antioxidant system in processed flaxseed. Samples extracted with petroleum ether (cold) and reconstituted to 40% oil content with commercial flax oil.

As expected, SDG was mainly found in the hull; *p*-coumaric acid and ferulic acid glycosides were found both in the hull and the dehulled meal (Figure 4, Table 2). The meal was not completely dehulled, intact seeds and part of the hull was found in the sample, this might explain its relatively high content in SDG. A water extract containing phenolic compounds was prepared from defatted flaxseed to see if they had an antioxidant effect, even if they were not implicated in the main flaxseed antioxidant system. The water-extracted meal and the water extract showed different total phenolic content (Table 1) and composition (Table 2). SDG, *p*-coumaric acid glycoside and ferulic acid glycoside were mainly found in the water-extracted meal with little of these found in the water extract (Figure 5). The peroxide and aldehyde contents of the oil from the water-extracted meal increased. The results suggested that watr extract strongly reduced the antioxidant properties of the flax meals.

Although the water extracts contained low level of phenolic compounds, an antioxidant effect was still observed. The peroxide and aldehyde content of the commercial flax oil stored in the same condition were doubled the peroxide and aldehyde contents of the oils supplemented with the water extracts (Figure 6). There was no relationship, however between the amount of extract and the antioxidant effect.

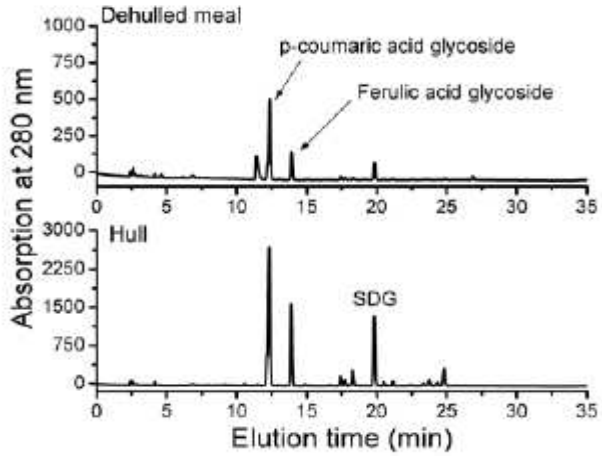

Figure 4. Phenolic compound analysis HPLC profile of flax dulled and dehulled flax meal after alkaline hydrolysis (2M NaOH for 24h at room temperature).

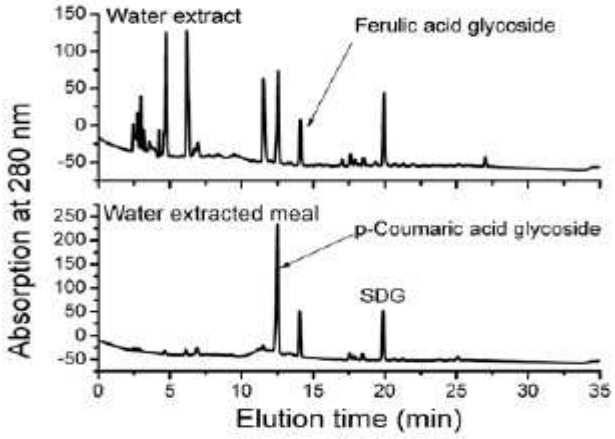

Figure 5. Phenolic compound analysis, HPLC profile of flax water extract and water extracted flax meal after alkaline hydrolysis (2M NaOH for 24h at room temperature).

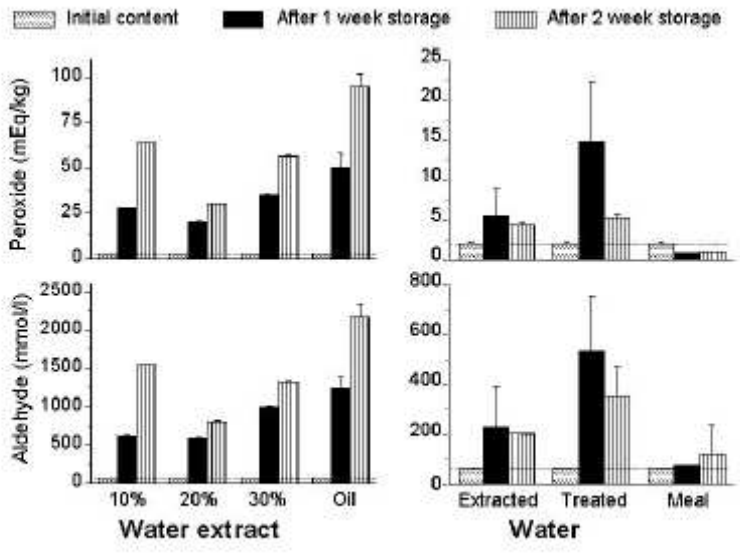

Figure 6. Comparison of antioxidant system after water extraction. Samples reconstituted to 40% oil content with commercial flax oil.

CONCLUSION

The flax antioxidant system appears to be a water-soluble system. Hull had no effect on the antioxidant properties of the meal, suggesting that SDG, mainly present in the hull, was not involved in the flax antioxidant system.

After alkaline hydrolysis of an ethanol extract, SDG ferulic and *p*-coumaric acid glycosides were found. Water extracts of flax meals showed some antioxidant properties but their effect was minimal compared to the antioxidant properties of the non-extracted meal. This suggested that the main flaxseed antioxidant system is water-soluble.

Flax phenolic compounds were found to have some antioxidant properties but they are not the main flaxseed antioxidant system.

ACKNOWLEGEMENTS

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