COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

Flaxseed Lignans: Source, Biosynthesis, Metabolism, Antioxidant Activity, Bio-Active Components, and Health Benefits

Alhassane Touré and Xu Xueming

ABSTRACT: Lignans are compounds found in a variety of plant materials including flaxseed, pumpkin seed, sesame seed, soybean, broccoli, and some berries. The major lignan in flaxseed is called secoisolariciresinol diglucoside (SDG). Once ingested, SDG is converted in the colon into active mammalian lignans, enterodiol, and entero-lactone, which have shown promise in reducing growth of cancerous tumors, especially hormone-sensitive ones such as those of the breast, endometrium, and prostate. Known for their hydrogen-donating antioxidant activity as well as their ability to complex divalent transition metal cations, lignans are propitious to human health. The extraction methods vary from simple to complex depending on extraction, separation, fractionation, identification, and detection of the analytes. Flax lignan is also a source of useful biologically active components found in plant foods, such as phytochemicals, and it is considered a functional food. The safety issues in flaxseed are also briefly discussed.

Introduction

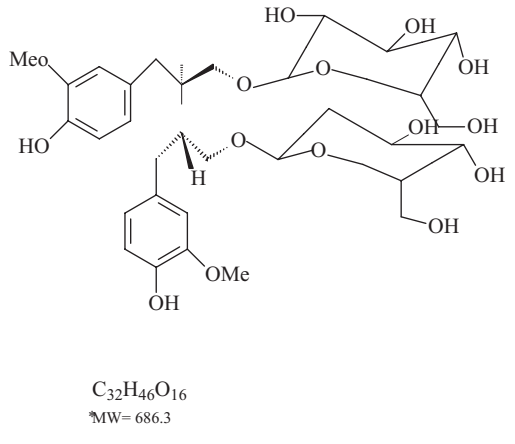
Flax (*Linum usitatisimum* L.) is grown as either an oil crop or as a fiber crop, with fiber (for linen) derived from the stem of fiber varieties and oil from the seed of linseed varieties (Diederichsen and Richards 2003; Vaisey-Genser and Morris 2003). Freeman (1995) reported that the seed of flax is flat and oval with a pointed tip, and varies in color from dark brown to yellow. Depending on the cultivar and growing conditions, flaxseed contains 40% to 50% oil and meal, comprised of 23% to 34% protein, 4% ash, 5% viscous fiber (mucilage), and lignan precursors (9 to 30 mg/g of defatted meal) (Muir and others 1996; Muir and Westcott 2003). Annual world production of flax was 3.06 million metric tons in 1999 to 2000 with Canada the world's largest producer of flax (about 38% of total production) (Anonymous 2000). Flax is currently the 2nd-most important oilseed crop in Western Canada and is grown primarily in the prairie provinces of Saskatchewan (70%), Manitoba (26%), and Alberta (4%) (Anonymous 2000).

Flax is making its mark in the world's food supply as a functional food. It delivers a health boost beyond what might be expected from their traditional nutrient content. Flax fits this description perfectly, being rich in alpha-linolenic acid (ALA), the essential omega-3 fatty acid, and phytochemicals such as lignans (Morris 2003).

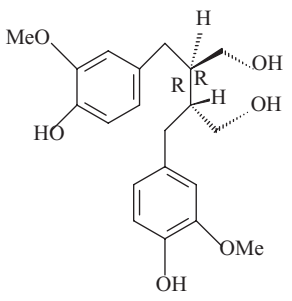
Flaxseed has been the focus of increased interest in the field of diet and disease research due to the potential health benefits associated with some of its biologically active components: oil containing approximately 59% a-linolenic acid) and the presence of plant lignan secoisolariciresinol diglycoside (SDG;).

Lignans are found in most fiber-rich plants, including grains such as wheat, barley, and oats; legumes such as beans, lentils, and soybeans; and vegetables such as garlic, asparagus, broccoli, and carrots (Tham and others 1998; Murphy and Hendrich 2002). Plant lignans are phenolic compounds (Harris and

MS 20090839 Submitted 8/30/2009, Accepted 11/10/2009. Authors are with Chair of Food Technology, Dept. of Chemical Engineering, Inst. Polytechnic, UG, Guinea. Author Touré is also with State Key Laboratory of the School of Food Science and Technology, 1800 Lihu Rd., Wuxi 214122, P.R. China and School of Food Science and Technology, Jiangnan Univ., Wuxi, Jiangsu. Direct inquiries to author Xueming (E-mail: xmxu@jiangnan.edu.cn).



$C_{32}H_{46}O_{16}$
*MW= 686.3



$C_{20}H_{26}O_6$
*MW= 362.3

Figure 1—Structure of SDG and SECO (adapted from Muir and Westcott 2003). *Molecular weight.

Haggerty 1993). Flax is a particularly rich source of a lignan called secoisolariciresinol diglycoside (SDG). SDG is a plant lignan that is converted by bacteria in the colon of humans (and other animal also) to mammalian lignans known as enterodiol (ED) and enterolactone (EL).

We live in a world where free radicals can come from many sources and contribute to the deterioration of health. Sources of free radicals include pollutants, drugs, metal ions, radiation, and high intakes of polyunsaturated fatty acids, and also strenuous exercise, mitochondrial dysfunction, and smoking. These may result in damage to membrane lipids, proteins, nucleic acids, and carbohydrates, which can result in cancer, neurological diseases, lung diseases, diabetes, vascular diseases, autoimmune diseases, premature aging, and eye diseases (Lachance and others 2001).

Lignans are found in many cereals and grains, with the highest amounts occurring in flaxseed. Despite their more widespread occurrence in foods and their greater consumption in Western populations the lignans have received comparatively little attention.

In the United States, flaxseed (FS) and flaxseed meal (FLM, partially defatted FS) have found market acceptability as a component in some cereals, specialty breads, cookies, and salad dressings (Carter 1993; Nesbitt and Thompson 1997). Its growing popularity is due to food components that may provide health benefits beyond basic nutrition (Hasler and others 2000). Among the reported potential health benefits associated with FS and/or FLM are decreased risk of cardiovascular disease (Craig 1999; Jenkins and others 1999), decreased risk of cancer, particularly of the mammary and prostate gland (Craig 1999), antiinflammatory activity (Ranich and others 2001), laxative effect, and alleviation of menopausal symptoms and osteoporosis (Kurzer and Xu 1997).

Phytoestrogen supplementation with flaxseed or soy flour have been reported to increase vaginal cell maturation, an indication of estrogen activity in postmenopausal women (Wilson and others 1990), and to significantly reduce menopause symptom scores, particularly hot flashes and vaginal dryness (Brzezinski and Debi 1999). Dietary studies indicate substantial reduction in breast cancer risk among women with high urinary excretion of phytoestrogens, particularly the isoflavones equol and lignan entero-lactone (Ingram and others 1997). The lower incidence of prostate cancer in Asian men compared to men from North America and Europe has also been speculated to be due to higher dietary intake of isoflavones and lignans (Adlercreutz 1990; Morton and others 1997).

Recent research has demonstrated the ability of SDG to scavenge hydroxyl radicals and shown that SDG have potent antioxidant activity. They are biologically active phytochemicals with apparent anticancer and antioxidant potential. It stands to reason that a review is in order on the extraction, synthesis, metabolism, and antioxidant potentiality of flaxseed lignan, a naturally occurring compound because people everywhere have started to think more about health issues and have taken an interest in natural antioxidant from foods.

Lignans

Sources and structures. Lignans are diphenolic compounds of higher plants formed by the coupling of two coniferyl alcohol residues that are present in the plant cell wall (Jenab and others 1999; Westcott and Muir 2003). Bakke and Klosterman (1956) isolated SDG (Figure 1) from a fat-free extract of linseed meal with a 3% yield. SDG was found to be very soluble in water and alcohol. The same authors then isolated SECO [2,3-di-(methoxy-4-hydroxybenzyl) butane-1,4-diol] (Figure 1) by acid hydrolysis of SDG (Bakke and Klosterman 1956). SECO is the aglycone (nonsugar) portion of SDG. Both SDG and SECO have a UV absorption maximum at 280 nm, which is characteristic for lignans. Flax also contains small amounts of the lignans matairesinol (MAT) (11 μg/g of full fat flaxseed), pinoresinol, pinoresinol diglucoside, isolariciresinol, and a diastereomer of SDG (Whiting 1987; Mazur and others 1996).

SECO is the major lignan present in flaxseed, which is found as the conjugate diglycoside SDG (Ford and others 1999). Early studies have demonstrated that SDG is part of a larger complex (Bakke and Klosterman 1956). An oligomer of SDG, which is ester-linked via 3-hydroxy-3-methylglutaric acid (HMGA), has been identified (Muir and others 2000a, 2000b; Ford and others 2001; Westcott and Muir 2003). A straight chain oligomeric structure composed of 5 SDG residues interconnected by 4 HMGA residues (molecular weight of about 4000 Da) was also reported (Kamal-Eldin and others 2001). SDG may comprise greater than 35% (by weight or mole %) of the polymer (Kamal-Eldin and others 2001; Westcott and Paton 2001). Among foods, flaxseed is the richest source of SDG (7 mg/g or 3.7 mg SECO 2/g). It contains 75 to 800 times more SDG than any other foods (Mazur and others 1996; Westcott and Muir 1996). Variation in flaxseed lignan concentrations depend on the variety, location, and crop year (Westcott and Muir 1996).

Whole seed and ground flax typically contain between 0.7% and 1.9% SDG, which is approximately 77 to 209 mg SDG/tbsp of whole seed or 56 to 152 mg SDG/tbsp of ground flaxseed (Morris

Table 1—Levels of lignans in different food plant products.

Plant source	Lignan	Level (mg/kg; dry weight)	Reference
Flaxseed	SECO[a]	3699	Mazur and others (1996)
	Matairesinol	10.9	
	Matairesinol	7 to 28.5[b]	Kraushofer and Sontag (2002)
	SDG	11900 to 25900[C]	Eliasson and others (2003)
Sesame seed	Sesamin	1547 to 8852[d]	Namiki (1995)
	Sesamolin	1235 to 4765[d]	
Cereals	SECO	0.1 to 1.3	Mazur and Adlercreutz (1998)
	Matairesinol	0 to 1.7	
Vegetables	SECO	0.1 to 38.7	Mazur and Adlercreutz (1998)
	Matairesinol	Trace-0.2	
Legumes	SECO	0 to 15.9	Mazur and others (1998a)
	Matairesinol	0 to 2.6	
Fruits	SECO	Trace-30.4	Mazur (1998);
	Matairesinol	0 to 0.2	Mazur and Adlercreutz (1998)
Berries	SECO	1.4 to 37.2	Mazur and others (2000)
	Matairesinol	0 to 0.8	
Tea	SECO	15.9 to 81.9	Mazur and others (1998b)
	Matairesinol	1.6 to 11.5	

[a]Obtained by enzymatic or acid hydrolysis.
[b]Not reported whether it is of fresh weight or dry weight.
[c]Obtained by alkaline hydrolysis.
[d]In oil.

2004). Flax oil containing added lignans has been available for several years. One such product contains 0.1% SDG or about 14 mg SDG/tbsp flax oil (Morris 2004).

Other food sources of lignans are seeds, legumes, cereals, vegetables, berries, seaweed, tea, and alcoholic beverages (Thompson and others 1991; Namiki 1995; Mazur and others 1996, 1998a, 1998b, 2000; Mazur 1998; Liggins and others 2000; Nurmi and others 2003). Quantitative data on some lignans in food are presented in Table 1.

Biosynthesis of lignans. The biosynthesis of lignan has recently been revised based on the discovery of the dirigent proteins that guide phenolic radical coupling (Davin and others 1997; Davin and Lewis 2000). Lignans are derived mainly via differential partitioning of the monolignol, coniferyl alcohol, to yield the lignan pinoresinol, which in turn serves as the precursor of both secoisolariciresinol and matairesinol (Figure 2). Two genes encoding the corresponding protein involved in the formation of pinoresinol and lariciresinol have been obtained from developing flaxseed (Ford and others 2001).

Elucidation of the pathway leading to the cancer chemopreventive agent secoisolariciresinol (SDG) was first accomplished using *Forsythia intermedia*, a plant that both produces and further enantiometrically metabolizes pure pinoresinol, a dimeric lignan formed from (E)-coniferyl alcohol. It involves the region and stereoselective intermolecular phenoxy radical coupling of 2 molecules of (E)-coniferyl alcohol by a pinoresinol dirigent protein to yield pinoresinol (Ford and others 2001). Sequential enantiospecific reduction of this intermediate then occurs by a reductase to consequently generate lariciresinol and then SDG. Glycosilation of SDG is accomplished by secoisolariciresinol diglucosyl transferase that appears to be localized mainly in the seed (Ford and others 2001). Elucidation of the lignan biosynthetic pathway leads to the development of strategies for enhancing the levels of SDG in staple dietary foodstuffs and maximizing yields of lignans used to treat or protect against human disease (Jung and others 2000).

Analysis of phenolic compounds in flaxseed. Extraction methods vary widely depending on the sample and the phytoestrogen of interest. Following the discovery of SDG by Bakke and Klosterman (1956) and its connection to the mammalian lignans, several methods for the analysis of lignans and other constituents of the fat free portion of flaxseed have been developed, although only a few quantitative ones. The polymeric powder obtained by ethanol : dioxane extraction of defatted flaxseed flour (DFF) was found to release hydroxymethyl glutaric acid (HMGA; Figure 3), 4-O-β-D-glucopyranosyl coumaric acid, and SDG upon alkaline hydrolysis (Klosterman and Smith 1954; Klosterman and others 1955; Bakke and Klosterman 1956), suggesting that these compounds are bound in ester-linked polymeric structure(s) in flaxseed. The compounds may also be released from the polymeric material as aglycones by enzyme or acid hydrolysis (Mazur and others 1996).

Extractions of flaxseed phenols have usually been carried out with organic solvents (Bakke and Klosterman 1956; Bambagiotti-Alberti and others 1994; Rickard and others 1996; Chimichi and others 1999) sometimes mixed with water (Axelson and others 1982; Kozlowska and others 1983; Dabrowski and Sosulski 1984; Amarowicz and others 1994; Westcott and Muir 1996; Meagher and others 1999; Ford and others 2001; Charlet and others 2002; Degenhardt and others 2002; Sicilia and others 2003), but the use of supercritical fluid (SCF) extraction has also been reported (Harris and Haggerty 1993). SDG and cinnamic acids absorb light in the UV-region and have been detected and quantified by column chromatography, HPLC, GC, and NMR techniques. The differences in efficiency of different modes of extractions and hydrolyses have resulted in a wide variation of yields. Methods for the quantification of SDG in flaxseed have been compiled in Table 2.

Hydrolysis of glycosidic bonds of lignans. Two basic approaches have been used to hydrolyze the glycosidic bonds between plant lignans and carbohydrates, and both methods have been used to produce analytical results on lignan concentrations in various foods (Thompson and others 1991; Mazur and others 1996). The Thompson method employs anaerobic *in vitro* fermentation of food containing plant lignans with gut bacteria producing entero-lactone and enterodiol, the concentration of which is subsequently measured by GC and flame ionization detection. The 2nd method, described by Mazur and others (1996), employs hot acid to break the glycoside bond and is a multistepped process using both enzyme and hot acid to hydrolytically remove the

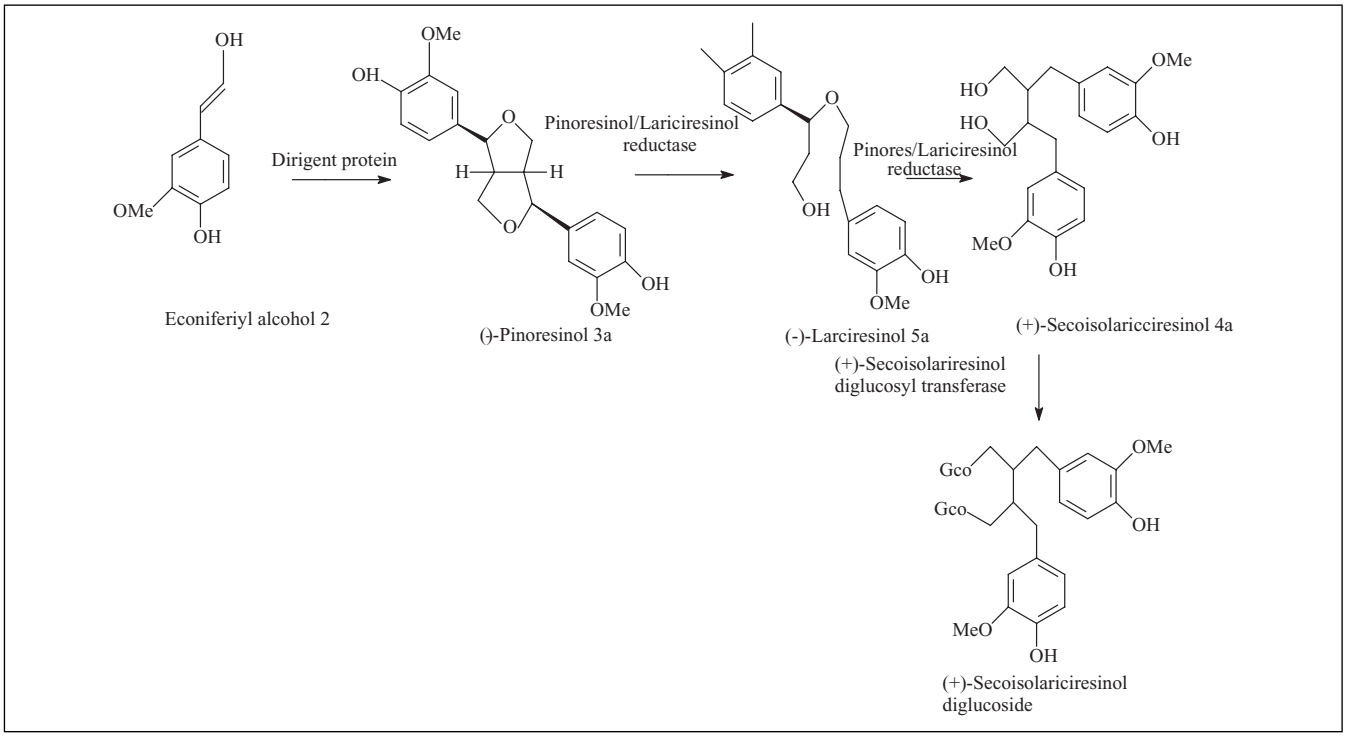


Figure 2—Biosynthesis of lignan (SDG) in flaxseed (adapted from Ford and others 2000).

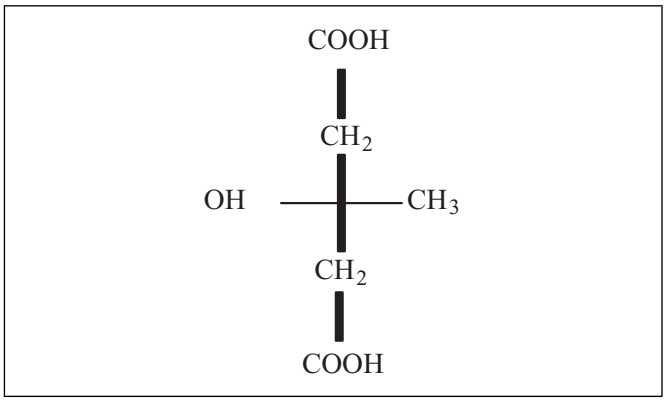


Figure 3—Hydroxymethyl glutaric acid.

carbohydrate component of lignan in food. In this procedure, the food extract is first subjected to enzyme hydrolysis, liberating the lignan from their respective glycosides.

Since the lignans are not completely hydrolyzed by enzymes, hot acid is used to liberate the aglycones that are partitioned off with organic solvents. The organic fractions of lignan aglycones are then purified, derivatized, and analyzed by gas chromatography-mass spectrometry (GC-MS).

However, the stability of products from acid hydrolysis has recently been questioned (Liggins and others 2000). It appears that a naturally occurring lignan called shonanin (3,4-divanillyltetrahydrofuran) is also liberated from its glycosides alongside SDG as a result of acid hydrolysis. To account for the presence of shonanin and its hydrolytic product enterofuran in foods, Liggins and others (2000) modified the procedure of Mazur and others (1996) and simplified it for the quantification of lignans, SDG, matairesinol, and shonanin in food after hydrolytic removal of any conjugated carbohydrate. The modification includes acid hydrolysis of food samples for 1, 2, and 3 h, followed by neutralization with sodium hydroxide, separation of the aglycones from aqueous to organic phase, and quantification of the aglycones by GC-MS after the formation of trimethylsilyl derivatives of the lignans.

The content of lignans in reference foods is reported as the combined concentrations of SDG and shonanin, alongside the concentration of matairesinol after 1, 2, and 3 h of hydrolysis, since optimum time of hydrolysis for the maximum yield of lignans varies between foodstuffs.

Metabolism of flax lignans

Conversion of plant lignans to mammalian lignans. Studies in which flaxseed was fed to rats, monkeys, or humans have found that the urinary excretion of the lignans enterodiol (END) and enterolactone (ENL) are significantly increased (Axelson and others 1982; Westcott and Muir 1997). Excretion of END and ENL increased 3- to 285-times after flaxseed consumption (5 to 10 g daily for 6 wk) in the urine of 18 healthy young women, 31 healthy postmenopausal women, and 6 healthy young men (Lampe and others 1994; Hutchins and others 2000).

The first mammalian lignans, END (MW = 302) and ENL (MW = 298), were identified in humans and animals by Setchell and others (1980). Mammalian lignans are formed in the human body in the gastrointestinal (GI) tract, where GI bacteria hydrolyze the sugar moiety of SDG to release SECO (Muir and others 1996, 1997, 2000b; Ford and others 1999; Thompson, 1999). This is followed by dehydroxylation and demethylation by the colonic microflora to give the mammalian lignan END (Figure 4). END is presumed to be oxidized by the GI microbial flora to give ENL. ENL may also be formed directly from matairesinol, although this

Table 2—Extraction systems for lignan isolation from flaxseed and flaxseed containing foods.

Extraction	Hydrolysis	Purification	Level SDG (μmol/g seed)	References
MeOH-dioxane (1:1) 24 h	Ba methoxide	Cellulose column	3.15	Bakke and Klosterman (1956)
In vitro Fermentation	Na methoxide	Silical gel ($CHCl_3$-MeOH-H_2O)	0.96 to 3.15	Thompson and others (1991)
β-Glucuronidase	β-Glucuronidase	C_{18} SPE	1.19 to 1.97	Obermeyer and others (1995)
β-Glucuronidase	β-Glucuronidase 2 M HCl, 2.5 h, 100°C	Ether extraction/DEAE-Sephadex OH^-QAE-SephadexAC$^-$	9.05 to 10.21	Mazur and Adlercreutz (1998)
Reflux, 80% MeOH, 2 h	β-Glucuronidase	C_{18} SPE + Lipophilic gel chromatography	0.22 to 3.41	Setchel and others (1999)
70% Aqueous alcohol	NaOH	C_{18} SPE	5.24 to 15.74	Westcott and Muir (1998)
95% EtOH-dioxane (1:1) 8 h	nr	nr	0.001 to 0.004	Harris and others (1994)
SCO_2 + THF-H_2O (1:1)	nr	nr	7.15	Wilson and others (1993)
Shaker, 80% MeOH, 4 h, 55°C	1 M HCl, 1 h 100°C	EtOAC-hexane (1:1)	nr	Meagher and others (1999)

nr = not reported.
Source: Adapted from Muir and others (2000a).

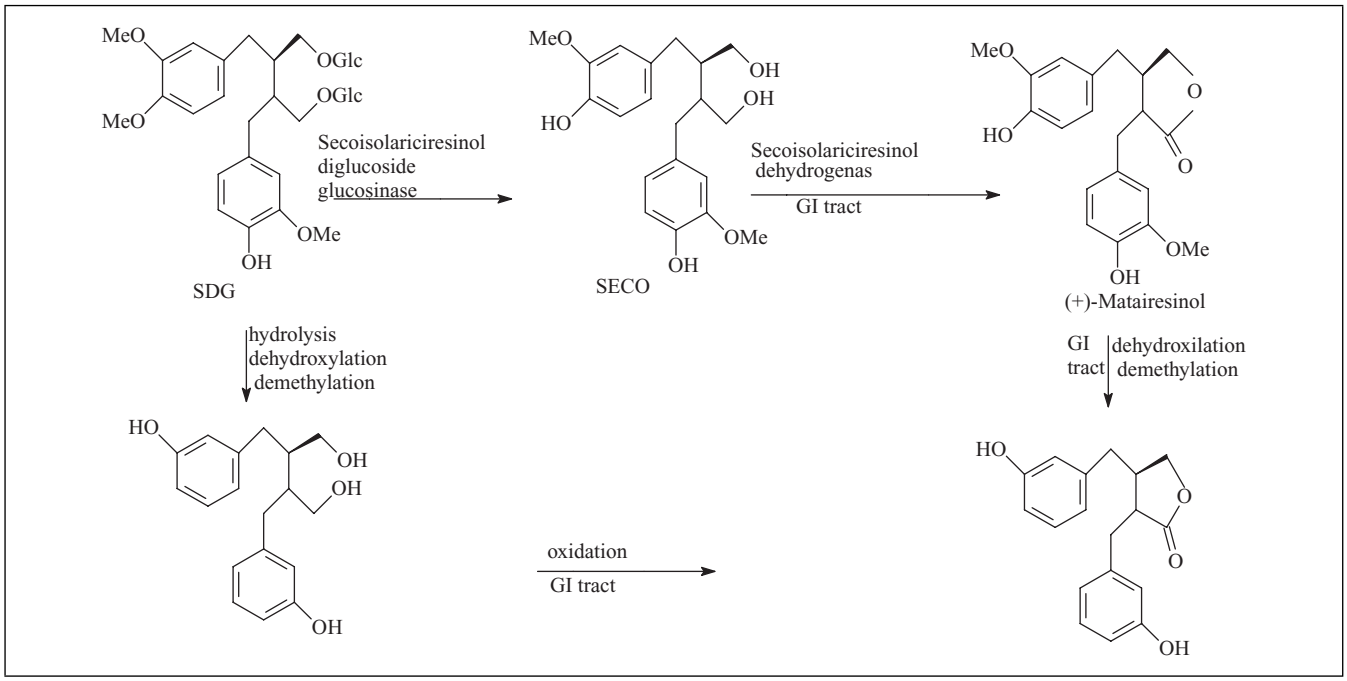


Figure 4—Biosynthesis pathway of flaxseed lignans SDG, SECO, and their corresponding mammalian lignans END and ENL (adapted from Ford and others 2001).

is likely to be a minor metabolic route if other lignans are present in the diet (Setchell and others 1980; Borriello and others 1985; Westcott and Muir 1997; Muir and others 1996, 2000a). The mammalian lignans differ from plant lignans in that mammalian lignans have hydroxyl groups at the 3′ position while plant lignans have their oxygenated substituents at the 3′ and 4′ positions (Axelson and Setchell 1981; Thompson 1999; Muir and Westcott 2003). A scheme outlining the proposed biosynthetic pathway of END and ENL from the flaxseed lignans SECO and SDG is shown in Figure 4. Concentrations of mammalian lignans in urine are typically greater than in plasma, thus most analytical methods target the measurement of urinary lignan levels (Muir and Westcott 2003).

Role of gut flora in the oxidation of plant lignans to mammalian lignans. Incubation of flaxseed by bacteria present in stools, at a concentration of 10^3 to 10^4 bacteria/g of stool resulted in the formation of END and ENL (Borriello and others 1985). This study showed that plant lignans are converted into END and ENL and the conversion is not reversible, thus END and ENL cannot convert to plant lignans *in vivo*. Furthermore, incubation of flaxseed in a human fecal bacterial culture at 1000 to 10000 bacteria/g converted END to ENL, but sterile fecal cultures could not (Setchell and others 1980). These results suggest that human gut floras are responsible for the conversion of plant lignans to mammalian lignans. A time course study of the metabolism of SDG by human fecal cultures shows initial demethylation (Figure 5), occurs prior (20 to 30 h) to dehydroxylation (48 h) (Wang and others 2000). Urinary excretion of END and ENL has been used as an index of plant lignan intake (Thompson 1998).

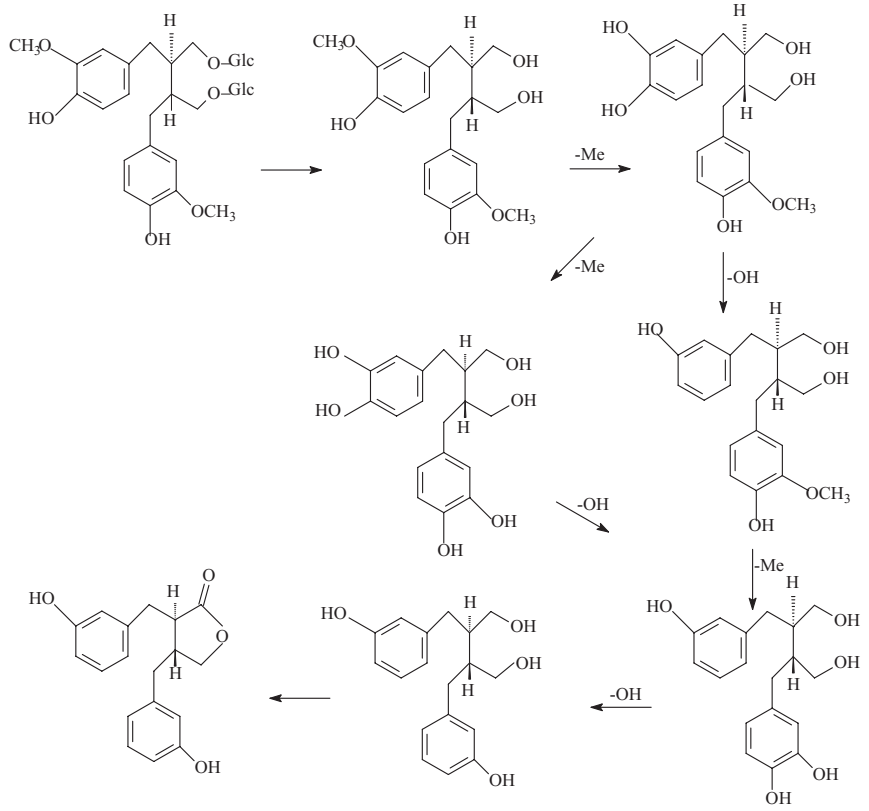


Figure 5—Metabolism of SDG by human fecal bacteria (Muir and Westcott 2003) -OH = dehydroxylation, -Me = demethylation.

Antioxidant scavenging activity of lignans. Natural antioxidants can be classified as primary (chain-breaking) antioxidants, which can react directly with lipid radicals and convert them into stable products, or as secondary (preventive) antioxidants, which can lower the rate of oxidation by different mechanisms (Decker and others 2005). Primary antioxidants most often act by donating a hydrogen atom, while secondary antioxidants may act by binding metal ions able to catalyze oxidative processes, by scavenging oxygen, by absorbing UV radiation, by inhibiting enzymes or by decomposing hydroperoxides (Schwarz and others 2001). It is known that different natural phenolic compounds function as both primary and secondary antioxidants by different mechanisms. Monitoring of either the decrease of the radical or the antioxidant, or the formation of products can be used for assessing the antioxidant activity (Decker and others 2005).

The antioxidant activity of SDG was lower than END and ENL in both lipid and aqueous *in vitro* model systems (Kitts and others 1999). The antioxidant activity was monitored by hydroxyl and peroxyl radical scavenging activity of SDG, END and ENL using a lipid emulsion system and inhibition of deoxyribose (Kitts and others 1999). In this study, the deoxyribose assay was used to evaluate the nonsite-specific and sitespecific Fenton reactant-induced ·OH-scavenging activity (Kitts and others 1999). The degree of oxidation of Fe^{2+} to Fe^{3+} (in the Fenton reaction) by peroxyl radicals can be measured by the ammonium thiocyanate assay (Kitts and others 1999).

Beneficial effects of SDG in cancer and lupus nephritis showed that these beneficial effects could be due to the ability of SDG to scavenge ·OH radicals (Prasad 1997).

The antioxidant activity of the flaxseed lignans and metabolites have been to exert protective effects against AAPH-induced oxidation, particularly in the recent literature (Hosseinian and others 2006, 2007; Chun and others 2007), evaluating the efficacy of these plant and mammalian lignans in protecting against AAPH peroxyl radical-induced damage and studies with DPPH (Eklund and others 2005).

The antioxidant activities of SECO, SDG, END, and ENL have also been suggested to contribute in reduction of hypercholesterolemia, atherosclerosis, and diabetes (Prasad 2000a, b). The antioxidant activity of SECO, END, and ENL was investigated by Prasad (2000b) using chemiluminescence (CL) of zymosan-activated polymorphonuclear leukocytes (PMNL). This study demonstrated that SDG, SECO, END, ENL, and vitamin E, at a concentration of 2.5 mg/mL, produced a reduction of zymosan-activated PMNL by 23.8%, 91.2%, 94.2%, 81.6%, and 18.7%, respectively (Prasad 2000b). The antioxidant activity was highest with SECO and END and lowest with vitamin E. The relative antioxidant potency of SECO, END, ENL, and SDG was 4.9, 5, 4.3, and 1.3, respectively, as compared to vitamin E (Prasad 2000b). This study suggested SECO, END, and ENL are, respectively, 3.82, 3.95, and 3.43 times more potent than SDG (Prasad 2000b). Activation of PMNL is known to generate oxygen-free-radicals. Oxygen-free radical-producing activity of PMNL was monitored by measuring luminol-dependent CL. The reduction of PMNL, therefore, reflects the antioxidant activity of the compounds studied (Prasad 2000b). Zymosan is a polysaccharide that is capable of stimulating inflammatory cytokine production and PMNL. Zymosan may serve as a model

for the study of innate immune responses (Prasad and others 1991).

Health benefits

Lignans. The health benefits of flaxseed lignans are thought to be due to antioxidant activity, primarily as hydroxyl radical scavengers (Prasad 1997; Kitts and others 1999), and also as estrogenic and antiestrogenic compounds due, in part, to the structural similarity to 17-b-estradiol (Waters and Knowler 1982; Adlercreutz and others 1992). The behavior of the lignans depends on the biological levels of estradiol. At normal estradiol levels, the lignans act as estrogen antagonists, but in postmenopausal women (at low estradiol levels) they can act as weak estrogens (Rickard and Thompson 1997; Hutchins and Slavin 2003). Other activities related to estrogen include the *in vivo* synthesis of 2-hydroxy estrogen, a compound that may protect against cancer (Haggans and others 1999) and inhibit the binding of estrogen and testosterone to receptors on sex-binding globulin (Martin and others 1996).

The presence of the oxidized metabolites is unique and may provide additional reasons for the health benefits of lignans. Classical antioxidant mechanisms show that the addition of an ortho-hydroxyl group to a monophenol enhances the antioxidant activity of the original monophenol. Thus, some of the mammalian lignan metabolites may actually have greater or different activity than the parent lignan. Kitts and others (1999) reported that enterolactone and enterodiol had greater antioxidant activity than the parent.

Role of flaxseed lignan in cancer prevention

Breast cancer. Lignans could be a significant part of a treatment regimen for cancer based on the large number of small-scale studies. The presence of flaxseed lignans in MCF-7 tumors and the observed lignan binding to ER suggests that the lignan function may be ER-mediated (Adlercreutz and others 1992; Saarinen and others 2000). Although the lignans have been shown to be protective against breast cancer, minor structural alterations may influence overall activity (Saarinen and others 2005). Thus, many of the aforementioned benefits might be the results of specific structural features needed for lignans to bind to ER.

Flaxseed was among the best food sources in the prevention of *in vivo* spontaneous chromosomal damage in mice (Trentin and others 2004). The exact reason for the chromosomal damage prevention has not seen identified; however, the mechanism may be related to the antioxidant function of flaxseed components.

Lignans have antioxidant activity and thus may contribute to the anticancer activity of flaxseed (Prasad 1997; Kitts and others 1999; Yuan and others 1999; Kangas and others 2002).

Prostate cancer. Lignan, enterodiol, and enterolactone were believed to be partly responsible for the growth inhibition of 3 human prostate cancer cell lines (Lin and others 2001). Morton and others (1997) reported that higher enterolactone levels in prostatic fluid were associated with populations with a low risk of prostate cancer. In a small clinical study, prostate cancer cell proliferation decreased and apoptosis increased in men fed 30 g of flaxseed per day (Demark-Wahnefried and others 2001). A significant factor which may have influenced their study was that the subjects were on a low-fat diet.

A subsequent study by those authors further supported the role of flaxseed in combination with a low-fat diet as a means to control prostate growth (Demark-Wahnefried and others 2004). In this study, prostate-specific antigen level and cell proliferation both decreased from baseline after only 6 mo on the dietary regime.

Colon and skin cancer. Although not extensively evaluated, flaxseed has been shown to inhibit colon and skin cancers in cell cultures and in animal studies as reviewed by Thompson (2003) and Morris (2003).

Danbara and others (2005) reported that a 10 mg/kg dose of enterolactone, by subcutaneous injection 3 times per week, reduced the expression of colon 201 human colon cancer cells in athymic mice. Using various testing protocols, Danbara and others (2005) concluded that the tumor suppression was due to apoptosis and decreased cell proliferation. In general, flaxseed may be a valuable tool in the fight against various cancers. Further research is needed in clinical settings to support the role of flaxseed in cancer prevention in human populations.

Diabetes prevention. Low-glycemic-index foods containing soluble fiber may not only prevent certain metabolic ramifications of insulin resistance, but also reduce insulin resistance (Reaven and others 1993). Soluble fiber and other components of flaxseed fractions could potentially affect insulin secretion and its mechanisms of action in maintaining plasma glucose homeostasis. Flaxseed was shown to reduce the postprandial blood glucose response in humans (Cunnane and others 1993; Jenkins and others 1999). A consumption of 50 g/d ground flaxseed by young females over a 4-wk period caused a reduction in blood glucose levels (Cunnane and others 1993). Similar findings were observed in postmenopausal women fed a 40 g/d flaxseed fortification diet (Lemay and others 2002). Bread containing 25% flaxseed gave a glycemic response that was 28% lower than the control (no flaxseed) bread (Jenkins and others 1999).

Prasad and others (2000) reported that rats fed 22 mg SDG/kg and treated with the diabetes-promoting chemical streptozotocin had 75% lower incidence of type-1 diabetes than the streptozotocin-treated control group. However, the serum glucose of the SDG plus streptozotocin-treated rats had significantly higher serum glucose levels than the streptozotocin-treated control group.

Antinutrients. Flaxseed has several compounds that may negatively influence health and well-being. In some cases, the negative impact might simply be an assumption based on literature reports of similar compounds from other foods. The 2 components that have been questioned most frequently are the cyanogenic glycosides and linatine, an antipyridoxine factor.

Cyanogenic glycosides are not exclusive to flaxseed. These compounds can be found in a number of plants including brassica vegetables and especially cassava. Many of the health concerns regarding cyanogenic glycosides stem from studies showing that cassava was toxic to animals and humans (McMahon and others 1995). However, cassava contains significantly more cyanogenic glycosides than flaxseed. Furthermore, the release of hydrogen cyanide from flaxseed would be minimal and below the toxic or lethal dose. At the recommend daily intake of about 1 to 2 tablespoons, approximately 5 to 10 mg of hydrogen cyanide is released from flaxseed, which is well below the estimated acute toxic dose for an adult of 50 to 60 mg inorganic cyanide and below the 30 to 100 mg/d humans can routinely detoxify (Roseling 1994). Daun and others (2003) reported that a person would have to consume 8 cups (1 kg) of ground flaxseed to achieve acute cyanide toxicity.

In addition to cyanogenic glycosides, trypsin inhibitor, linatine, and phytic acid are other antinutrients contained in flaxseed. Trypsin inhibitor activity (TIA) in flaxseed was low when compared to those in soybean and canola seeds. Bhatty (1993) reported laboratory-prepared flaxseed meals containing 42 to 51 units of TIA, which was slightly higher than 10 to 30 units observed by Madusudhan and Singh (1983) and commercially obtained flaxseed meal (14 to 37 units). The contents of phytic acid were significantly different among cultivars. AC Linora has the lowest phytic acid content of 2280 mg/100 g and low ALA yellow-seeded cultivar Linola 947 has the highest content

(3250 mg/100 g seed) among the 8 cultivars examined (Oomah and others 1996).

Kratzer (1946) reported that pyridoxine supplementation in chicks on diets containing linseed meal was necessary to counteract their vitamin B6 deficiency. Klosterman and others (1967) identified the antipyridoxine factor linatine. Although linatine is a problem in chicks, flaxseed has not been associated with a vitamin B6 deficiency in humans. In fact, no effect on serum pyridoxine levels in subjects consuming 45 grams of flaxseed per day over 5 wk has been observed (Dieken 1992).

Conclusions

Phytoestrogens, such as lignans, which act as either estrogen agonists or antagonists have generated interest because of their potential use in hormone replacement therapy and cancer prevention. Mammalian lignans are produced by the action of gut microflora on precursors such as the plant lignan SDG. Flaxseed lignans have potential antioxidant advantages in that they are natural antioxidants with potential health benefits. This review provides a better understanding of the flaxseed antioxidant activities and suggests that flaxseed lignans may be used as natural antioxidants. More *in vivo* studies are needed to ascertain the propitious effects of lignans secoisolariciresinol and to see if there are any dangers in possible overdoses.

References

Adlercreutz H. 1990. Western diet and western diseases: some hormonal and biochemical mechanisms and associations. Scandinavian J of Clinic and Labort Investig Supplem 201:3–23.

Adlercreutz H, Mousavi Y, Clark J, Hockerstedt K, Hämälainen E, Wähälä K, Mäkelaä T, Hase T. 1992. Dietary phytoestrogens and cancer: *in vitro* and *in vivo* studies. J Steroid Biochem Mol Biol 41:331–7.

Amarowicz R, Wanasundara PKJPD, Shahidi F. 1994. Chromatographic separation of flaxseed phenolics. Die Nahrung 38:520–6.

Anonymous. 2000. Oil world statistics update. Oil World 31:9–10.

Axelson M, Setchell KD. 1981. The excretion of lignans in rats: evidence for an intestinal bacterial source for this new group of compounds. FEBS Lett 123:337–42.

Axelson M, Sjövall J, Gustafsson BE, Setchell KDR. 1982. Origin of lignans in mammals and identification of a precursor from plants. Nature 298:659–60.

Bambagiotti-Alberti M, Coran SA, Ghiara C, Giannelini V, Raffaelli A.1994. Revealing the mammalian lignan precursor secoisolariciresinol diglucoside in flaxseed by ionspray mass spectrometry. Rapid Commun Mass Spectrom 8:595–8.

Bhatty RS. 1993. Further compositional analyses of flax: mucilage, trypsin inhibitors and hydrocyanic acid. J Am Oil Chem Soc. 70:899–904.

Bakke JE, Klosterman HJ. 1956. A new diglucoside from flaxseed. Proceedings of the North Dakota Academy of Science, July, Grand Forks, North Dak. Volume X. p 18–21.

Borriello SP, Setchell KD, Axelson M, Lawson AM. 1985. Production and metabolism of lignans by the human fecal flora. J Bacteriol 58:37–43.

Brzezinski A, Debi A. 1999. Phytoestrogens: the "naturel'' selective estrogens receptor modulators. Eur J Obstet Gynecol 85:47–51.

Carter JF. 1993. Potential of flaxseed and flaxseed oil in baked goods and other products in human nutrition. Cereal Foods World 38:753–9.

Charlet S, Bylund A, Zhang JX, Bergh A, Damber JE, Widmark A, Johansson A, Bensaddek L, Raynaud S, Gillet F, Mesnard F, Fliniaux MA. 2002. An HPLC method for the quantification of anhydrosecoisolariciresinol. Application to the evaluation of flax lignan content. Plant Physiol Biochem 40:225–9.

Chimichi S, Bambagiotti-Alberti M, Coran SA, Giannellini V, Biddau B. 1999. Complete assignment of the 1H and 13C NMR spectra of secoisolariciresinol diglucoside, a mammalian lignan precursor isolated from *Linum usitatissimum*. Magnet Resonan Chem 37:860–3.

Chun H, Yvonne VY, David DK. 2007. Antioxidant activities of the flaxseed lignan secoisolariciresinol diglucoside, its aglycone secoisolariciresinol and the mammalian lignans enterodiol and enterolactone *in vitro*. Food Chem Toxicol 45:2219–2227.

Cunnane SC, Ganguli S, Menard C. 1993. High α-linolenic acid flaxseed (*Linum usitatissimum*): some nutritional properties in humans. Br J Nutr 69:443–53.

Craig WJ. 1999. Health-promoting properties of common herbs. Am J Clin Nutrit 70:491S–9S

Dabrowski K, Sosulski F. 1984. Composition of free and hydrolysable phenolic acids in defatted flours of ten oilseeds. J Agric Food Chem 32:128–30.

Danbara N, Yuri T, Tsujita-Kyutoku M, Tsukamoto R, Uehara N, Tsubura A. 2005. Enterolactone induces apoptosis and inhibits growth of colon 201 human colon cancer cells both *in vitro* and *in vivo*. Anticancer Res 25:2269–76.

Daun JK, Barthet JV, Chornick TL, Duguid S. 2003. Structure, composition, and variety development of flaxseed. In: Cunnane SC Thompson LU, editors. Flaxseed in human nutrition. 2nd ed. Champaign, Ill.: AOCS Press. p 1–40.

Davin BL, Lewis NG. 2000. Dirigent proteins and dirigent sites explain the mystery of specificity of radical precursor coupling in lignan and lignan biosynthesis. Plant Physiol 123:453–61.

Davin BL, Wang HB, Crowell AL, Bedgar DL, Martin DM, Sarkenen S, Lewis NG. 1997. Stereoselective biomolecular phenoxy radical coupling by an auxiliary (dirigent) protein without an active center. Science 75:362–366.

Degenhardt A, Habben S, Winterhalter P. 2002. Isolation of the lignan secoisolariciresinol diglucoside from flaxseed (*Linum usitatissimum* L.) by high-speed counter-current chromatography. J Chromatogr A 943:299–302.

Decker EA, Warner K, Richards MP, Shahidi F. 2005. Measuring antioxidant effectiveness in food. J Agric Food Chem 53:4303–10.

Demark-Wahnefried W, Price DT, Polascik TJ, Robertson CN, Anderson EE, Paulson DF, Walther PJ, Gannon M, Vollmer RT. 2001. Pilot study of dietary fat restriction and flaxseed supplementation in men with prostate cancer before surgery: exploring the effects on hormonal levels, prostate-specific antigen, and histopathologic features. Urology 58:47–52.

Demark-Wahnefried W, Robertson CN, Walther PJ, Polascik TJ, Paulson DF, Vollmer RT. 2004. Pilot study to explore effects of low-fat, flaxseed-supplemented diet on proliferation of benign prostatic epithelium and prostate-specific antigen. Urolog 63:900–4.

Diederichsen A, Richards K. 2003. Cultivated flax and the genus *Linnum* L: taxonomy and gerplasm conservation. In: Muirand AD, Westcott ND, editors. Flax, the genus Linum. London: Taylor & Francis. p 22–54.

Dieken HA. 1992. Use of flaxseed as a source of omega-3 fatty acids in human nutrition. In the 54th proceeding of Flax Inst. of United States. p 1–4.

Eklund PC, Langvik OK, Warna JP, Salmi TO, Willfor SM, Sjoholm RE. 2005. Chemical studies on antioxidant mechanisms and free radical scavenging properties of lignans. Organic Biomolec Chem 3:3336–47.

Eliasson C, Kamal-Eldin A, Andersson R, Åman P. 2003. High-performance liquid chromatographic analysis of secoisolariciresinol diglucoside and hydroxycinnamic acid glucosides in flaxseed by alkaline extraction. J Chromatogr A 1012:151–9.

Ford JD, Davin LB, Lewis NG. 1999. Plant lignans and health: cancer chemoprevention and biotechnological opportunities. Basic Life Sci 66:675–94.

Ford TD, Davin LB, Lewis NG. 2000. Chemopreventive flaxseed lignans: toward bioengineering the metabolic pathways. Proceedings of the 58th Flax Institute of United States; March 23–25; Fargo, N.Dak. p 39–48.

Ford JD, Huang KS, Wang HB, Davin LB, Lewis NG. 2001. Biosynthetic pathway to the cancer chemopreventive secoisolariciresinol diglucoside-hydroxymethylglutaryl ester-linked lignan oligomers in flax seeds (*Linumusitatissimum*). J Natural Prod 64:1388–97.

Freeman SC. 1995. Structure of flaxseed. In: Cunnane LU, editor. Flaxseed in human nutrition. Champaign, Ill.: AOCS Press. p 11–21.

Haggans CJ, Hutchins AM, Olson BA. 1999. Effect of flaxseed consumption on urinary estrogen metabolites in postmenopausal women. Nutr Cancer 33:188–95.

Harris RK, Haggerty WJ. 1993. Assays for potentially anticarcinogenic phytochemicals in flaxseed. Cer Foods World 38:147–51.

Harris RK, Greaves J, Alexander D, Wilson T, Haggerty WJ.1994. Development of stability-indicating analytical methods for flaxseed lignans and their precursors. In: Osawa CT, Huang MT, Rosen RT, editors. Food phytochemicals II: teas spices, and herb. Washington, D.C.: Am Chem Soc. p 295–305.

Hasler CM, Kundrat S, Wool D. 2000. Functional foods and cardiovascular disease. Curr Atheroscl Rep 2:467–75.

Hosseinian FS, Muir AD, Westcott ND, Krol ES. 2006. Antioxidant capacity of flaxseed lignans in two model systems. J Am Oil Chem Soc 83:835–40.

Hosseinian FS, Muir AD, Westcott ND, Krol ES. 2007. AAPH mediated antioxidant reactions of secoisolariciresinol and SDG. Organ Biomolec Chem 5:644–54.

Hutchins AM, Slavin JL. 2003. EVects of flaxseed on sex hormone metabolism. In: Thompson LU, Cunnane SC, editors. Flaxseed in human nutrition. 2nd ed. Champaign, Ill.: AOCS Press. p 126–49.

Hutchins AM, Martini MC, Olson BA, Thomas W, Slavin JL. 2000. Flaxseed influences urinary lignan excretion in a dose-dependent manner in postmenopausal women. Cancer Epidem Biomark Prev 9:1113–8.

Ingram D, Sanders K, Kolybaba M, Lopez D. 1997. Case-control study of phytoestrogens and breast cancer. Lancet 350:990–4.

Jenab M, Rickard SE, Orcheson LJ, Thompson LU. 1999. Flaxseed and lignans increase cecal beta-glucuronidase activity in rats. Nutrit Cancer 33:154–8.

Jenkins DJA, Kendall CW, Vidgen E, Agarwal S, Rao AV, Rosenberg RS, Diamandis EP, Novokmet R, Mehling CC, Perera T, Griffin LC, Cunnane SC. 1999. Health aspects of partially defatted flaxseed, including effects on serum lipids, oxidative measures, and *ex vivo* androgen and progestin activity: a controlled crossover trial. Am J Clin Nutr 69:395–402.

Jung W, Yu O, Lau SMC, O'Keefe DP, Odell J, Fatder G, McGonigle B. 2000. Identification and expression of isoflavone synthase, the key enzyme for biosynthesis in legumes. Nutr Biotechnol 18:208–12.

Kamal-Eldin A, Peerlkamp N, Johnsson P, Andersson R, Andersson RE, Lundgren LN, Aman P. 2001. An oligomer from flaxseed composed of secoisolariciresinol diglucoside and 3-hydroxy-3-methyl glutaric acid residues. Photochem 58:587–90.

Kangas L, Saarinen N, Mutanen M. 2002. Antioxidant and antitumor effects of Hydroxymatairesinol (HM-3000, HMR), a lignan isolated from the knots of spruce. Eur J Cancer Prev 11:S48–57.

Kitts DD, Yuan YV, Wijewickreme AN, Thompson LU. 1999. Antioxidant activity of the flaxseed lignan secoisolariciresinol diglycoside and its mammalian lignan metabolites enterodiol and enterolactone. Molecul Cell Biochem 202: 91–100.

Kraushofer T, Sontag G. 2002. Determination of matairesinol in flax seed by HPLC with coulometric electrode array detection. J Chromatogr B 777:61–6.

Klosterman HJ, Smith F. 1954. The isolation of beta-hydroxy-beta methylglutaric acidfrom the seed of flax (*Linum usitatissimum*). J Am Chem Soc 76(5):1229–30.

Klosterman HJ, Smith F, Clagett CO. 1955. The constitution of linocinnamarin. J Am Chem Soc 77(2):420–1.

Klosterman HJ, Lamoureux GL, Parsons JL. 1967. Isolation, characterization, and synthesis of linatine. Avitamin B6 antagonist from flaxseed (Linum ustitatissimum). Biochemist 6:170–7.

Kozlowska H, Zadernowski R, Sosulski FW. 1983. Phenolic acids in oilseed flours. Nahrung 27:449–53.

Kratzer FH. 1946. The treatment of linseed meal to improve its feeding value for chicks. Poult Sci. 25:541–2.

Kurzer MS, Xu X. 1997. Dietary phytoestrogens. Ann Rev Nutr 17:353–81.

Lachance PA, Nakat Z Jeong WS. 2001. Antioxidants: an integrative approach. Nutr 17:835–8.
Lampe JW, Martini MC, Kurzer MS, Adlercreutz H, Slavin JL. 1994. Urinary lignan and isoflavonoid excretion in premenopausal women consuming flaxseed powder. Am J Clin Nutr 60:122–8.
Lemay A, Dodin S, Kadri N. 2002. Flaxseed dietary supplement versus hormone replacement therapy in hypercholesterolemic menopausal women. Obstet Gynecol 100:495–504.
Liggins J, Grimwood R, Bingham SA. 2000. Extraction and quantification of lignan phytoestrogens in food and human samples. Analyt Biochem 287:102–9.
Lin X, Switzer BR, Demark-Wahnefried W. 2001. Effect of mammalian lignans on the growth of prostate cancer cell lines. Anticancer Res 21:3995–4000.
Madusudhan KT, Singh N. 1983. Studies on linseed proteins. J Agric Food Chem 31:959–63.
Mazur W. 1998. Phytoestrogen content in foods. Baillière's Clinic Endocrinol Metabol 12:729–42.
Mazur WM, Adlercreutz H. 1998. Naturally occurring oestrogens in food. J Pure Appl Chem 70:1759–76.
Mazur W, Duke JA, Wähälä K, Rasku S, Adlercreutz H. 1998a. Isoflavonoids and lignans in legumes: nutritional and health aspects in humans. Nutr Biochem 9:193–200.
Mazur W, Wähälä K, Salakka A, Hase T, Adlercreutz H. 1998b. Lignan and isoflavonoid concentrations in tea and coffee. Br J Nutr 79:37–45.
Mazur, W, Fotsis T, Wahala K, Ojala S, Salakka A, Adlercreutz H. 1996. Isotope dilution gas chromatographic-mass spectrometric method for the determination of isoflavonoids, coumestrol, and lignans in food samples. Annals Biochem 233:169–80.
Mazur W, Uehara M, Wähälä K, Adlercreutz H. 2000. Phytoestrogen content of berries, and plasma concentrations and urinary excretion of enterolactone after a single strawberry-meal in human subjects. Br J Nutr 83:381–7.
Martin ME, Haourigui M, Pelissero C. 1996. Interactions between phytoestrogens and human sex steroid binding protein. Life Sci 58:429–36.
McMahon JM, White WLB, Sayre RT. 1995. Cyanogenesis in cassava (Manihot esculenta Crantz). J Exp Botany 46:731–41.
Meagher LP, Beecher GR, Flanagan VP, Li BW. 1999. Isolation and characterization of the lignan, isolariciresinol pinoresinol in flaxseed meal. J Agric Food Chem 47:3173–80.
Morris DH. 2004. Flax—a health and nutrition primer. Available from: www.flaxcouncil.ca. Accessed Jan 16, 2004.
Morris DM. 2003. Flax, a health and nutrition primer. Winnipeg, Manitoba, Canada: Flax Council of Canada. p 9–19.
Morton MS, Chan PSF, Cheng C, Blacklock N, Matos-Ferreira A, Abranches-Montero L, Correia R, Lloyd S, GriYths K. 1997. Lignans and isoflavonoids in plasma and prostatic fluid in men: Samples from Portugal, Hong Kong, and the United Kingdom. Prostate 32:122–8.
Muir AD, Westcott ND. 2003. Flaxseed constituents and human health. In: Muir AD, Westcott ND, editors. Flax: the genus Linum. London: Taylor & Francis. p 243–51.
Muir AD, Westcott ND, Aubin AA. 1996. Detection of lignans in α-Linolenic acid enriched eggs. In 11th Ann. meeting Canadian Section. American Oil Chemical. Society, Saskatoon, AOCS, 25.
Muir AD, Westcott ND, Prasad K. 1997. Extraction, purification and animal model testing of an anti-atherosclerotic lignan secoisolariciresinol diglucoside from flaxseed (*Linum usitatissimum*). In Second world congress on medicinal and aromatic plants (WOCMAP II). Mendoza Argentina Acta Horticulturae 245–8.
Muir AD, Westcott ND, Ballantyne K, Northrup S. 2000a. Flax lignans: recent developments in the analysis of lignans in plant and animal tissues. In Proc. Flax Inst. of the USA, 58. Fargo, N. Dak.: Flax Inst. of the USA. p 23–32.
Muir AD, Ballantyne K, Aubin AJ, Yurach DC. 2000b. LC-MS/MS analysis of lignans in plasma and urine: problems and solutions. In biomedical uses of flax conference, Winnipeg, MB, Canada.
Murphy PA, Hendrich S. 2002. Phytoestrogens in foods. Advanc Food Nutr Res 44:195–246
Namiki M. 1995. The chemistry and physiological functions of sesame. Food Rev Int 11:281–329.
Nesbitt PD, Thompson LU. 1997. Lignans in homemade and commercial products containing flaxseed. Nutr Cancer 29:222–7.
Nurmi T, Heinonen S, Mazur W, Deyama T, Nishibe S, Adlercreutz, H. 2003. Lignans in selected wines. Food Chem 83:303–9.
Obermeyer WR, Musser SM, Betz JM, Casey RE, Pohland AE, Page SW. 1995. Chemical studies of phytoestrogens and related compounds in dietary supplements: flax and chaparral. Proc Soc Expo Biol Med 208:6–12.
Oomah BD, Mazza G, Przybylski R. 1996. Comparison of flaxseed meal lipids extracted with different solvents. Lebism Wiss Technol 29:654–8.
Prasad K. 1997. Hydroxyl radical-scavenging property of secoisolariciresinol diglucoside (SDG) isolated from flax-seed. Mol Cell Biochem 168:117–23.
Prasad K. 2000a. Oxidative stress as a mechanism of diabetes in diabetic BB-prone rats: effect of secoisolariciresinol diglucoside (SDG). Molecul Cell Biochem 209:89–96.
Prasad K. 2000b. Antioxidant activity of secoisolariciresinol diglucoside-derived metabolites, secoisolariciresinol, enterodiol, and enterolactone. Int J Angiol 9:220–5.
Prasad K, Chaudhary AK, Kalra J. 1991. Oxygen-derived free radicals producing activity and survival of activated polymorphonuclear leukocytes. Mol Cell Biochem 103:51–62.
Prasad K, Mantha SV, Muir AD, Westcott ND. 2000. Protective effect of secoisolariciresinol diglucoside against streptozotocin-induced diabetes and its mechanism. Mol Cell Biochem 206:141–50.
Ranich T, Bhathena SJ, Velasquez MT. 2001. Protective effects of dietary phytoestrogens in chronic rrenal disease. J Ren Nutr 11:183–93.
Rickard SE, Thompson LU. 1997. Phytoestrogens and lignans: effects on reproduction and chronic disease. In: Shahidi F, editor. Antinutrients and phytochemicals in foods. New York: Oxford Univ. Press. p 273–93.
Rickard SE, Orcheson LJ, Seidl MM, Luyengi L, Fong HS, Thompson LU. 1996. Dose-dependent production of mammalian lignans in rats and *in vitro* from the purified precursor secoisolariciresinol diglycoside in flaxseed. J Nutr 126:2012–9.
Roseling H. 1994. Measuring effects in humans of dietary cyanide exposure to sublethal cyanogens from Cassava in Africa. Acta Hort 375:271–83.
Reaven GM, Brand RJ, Chen YDI, Mathur AK, Goldfine I. 1993. Insulin resistance and insulin secretion are determinants of oral glucose tolerance in normal individuals. Diabetes 42 (9):1324–32.
Saarinen NM, Warri A, Makela SI. 2000. Hydroxymatairesinol, a novel enterolactone precursor with antitumor properties from coniferous tree (*Picea abies*). Nutr Cancer 36:207–16.
Saarinen NM, Penttinen PE, Smeds AI, Hurmerinta TT, Makela SI. 2005. Structural determinatnts of plant lignans for growth of mammary tumors and hormonal responses *in vivo*. J Steroid Biochem Mol Biol 93:209–19.
Schwarz K, Bertelsen G, Nissen LR, Gardner PT, Heinonen MI, Hopia A, Tuong HB, Lambelet P, McPhail D, Skibsted LH, Tijburg L. 2001. Investigation of plant extracts for the protection of processed foods against lipid oxidation. Comparison of antioxidant assays based on radical scavenging, lipid oxidation and analysis of the principal antioxidant compounds. Eur Food Res Technol 212:319–28.
Setchell KD, Lawson AM, Mitchell FL, Adlercreutz H, Kirk DN, Axelson M. 1980. Lignans in man and in animal species. Nature 287:740–2.
Setchel KDR, Childresse C, Zimmer-Nechemias L, Cai J. 1999. Methods of measurement of dietary secoisolariciresinol using HPLC with multichannel electrochemical detection. J Med Food 2:193–8.
Sicilia T, Niemeyer HB, Honig DM, Metzler M. 2003. Identification and stereochemical characterization of lignans in flaxseed and pumpkin seeds. J Agric Food Chem 51:1181–8.
Tham DM, Gardner CD, Haskell WL. 1998. Potential health benefits of dietary phytoestrogens: a review of the clinical, epidemiological, and mechanistic evidence. J Clin Endocrinol Metabol 83:2223–35.
Thompson LU. 1998. Experimental studies on lignans and cancer. Baillieres Clin Endocrinol Metabol 12:691–705.
Thompson LU. 1999. Role of lignans in carcinogeneis, in phytochemicals in human health protection, nutrition, and plant defense. In: Romeo JT, editor. Recent advances in phytochemistry, 33. New York: Kluwer Academic Plenum Publishers. p 51–65.
Thompson LU. 2003. Flaxseed, lignans, and cancer. In: Thompson LU, Cunnane SC, editors. Flaxseed in human nutrition. 2nd ed. Champaign, Ill.: AOCS Press. p 194–218.
Thompson LU, Robb P, Serraino M, Cheng F. 1991. Mammalian lignans production from various foods. Nutr Cancer 16:43–52.
Trentin GA, Moody J, Torous DK, Thompson LU, Heddle, JA. 2004. The influence of dietary flaxseed and other grains, fruits and vegetables on the frequency of spontaneous chromosomal damage in mice. Mutat Res 551:213–22.
Vaisey-Genser M, Morris H. 2003. Introduction: history of the cultivation and uses of flaxseed. In: Muir AD, Westcott ND, editors. Flax: the genus linum. London: Taylor & Francis. p 1–21.
Wang LQ, Meselhy MR, Li Y, Qin GW, Hattori M. 2000. Human intestinal bacteria capable of transforming secoisolariciresinol diglucoside to mammalian lignans, enterodiol and enterolactone. Chem Pharmaceut Bull 48:1606–10.
Waters AP, Knowler JT. 1982. Effect of a lignan (HPMF) on RNA synthesis in the rat uterus. J Rep Fertil 66:379–81.
Westcott ND, Muir Northrup SF. 1998. Analysis of flax lignans after enzymatic and chemical extraction. Proceedings of the 57th Flax Institute of United States; Fargo, N.Dak. p 27–30.
Wilson TC, Wahlquist ML, Burger HG, Medley G. 1990. Oestrogenic effects of plant food in postmenopausal women. Br Med J 301:905–6.
Wilson TC, Harris RK, Winters TR, Greaves JG, Haggerty WT. 1993. Supercritical fluid extraction of polar materials from animal feeds and plant matrices. 20th ACS Natl. Meeting, Denver, Colo.: Am Chem Soc 33:391–4.
Whiting DA. 1987. Lignans, neolignans and related compounds. Nat Prod Rep 4:499–525.
Westcott ND, Muir AD. 1997. Medicinal lignan from Flaxseed. In prairie medicinal and aromatic plants conference, Brandon, Manitoba, Canada, 71–6.
Westcott ND, Muir AD. 1996. Variation in the concentration of the flax seed lignan concentration with variety, location and year. In Proc 56th Flax Institute of the United States Conference, Fargo, N.Dak.: Flax Inst. of the United States. p 77–80.
Westcott ND, Paton D. 2001. Complex containing lignan, phenolic and aliphatic substances from flax process for preparing. U.S. patent 6,264,853.
Westcott ND, Muir AD. 2003. Chemical studies on the constituents of *Linuum* spp. In: Muir AD, Westcott ND, editors. Flax: the genus Linum. London: Taylor & Francis. p 55–73.
Yuan YV, Rickard SE, Thompson LU. 1999. Short-term feeding of flaxseed or its lignan has minor influence on *in vivo* hepatic antioxidants status in young rats. Nut Res 19:1233–43.