Plant Archives Vol. 16 No. 1, 2016 pp. 12-17 ***ISSN 0972-5210***

Review Article

"LIGNAN" - ANTIOXIDANT OF LINSEED

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Abstract

One of the most interesting characteristics of flaxseed is its content of complex phenols, such as lignans. The most remarkable one is secoisolariciresinol (SDG), although isolariciresinol, pinoresinol, mataresinol and other derivatives of ferulic acid are also present. Lignan consumption reduces cardiovascular risk and inhibits the development of some types of diabetes. Health benefits of flax 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 SDG is related to the suppression of the oxidant conditions due to oxygen species. SDG 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. The food and cosmetic fields could also potentially exploit their antioxidant activity. This review provides a better understanding of the flaxseed antioxidant activities and suggests that flaxseed lignans may be used as natural antioxidants.

Key words : Linseed, lignan, SDG, SECO, phytoestrogen.

Introduction

Flaxseed (*Linum usitatissimum* L.), one of the oldest cultivated crops, continues to be widely grown for oil, fiber and food (Oomah, 2001). 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 Westcott, 2003). 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. 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 *et al.*, 2001). 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). Lignan consumption reduces cardiovascular risk and inhibits the development of some types of diabetes (Mueller *et al.*, 2010). Health benefits of flax 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 SDG is related to the suppression of the oxidant conditions due to oxygen species. SDG diglycoside and its aglycone, secoisola-riciresinol display a very high antioxidant capacity and act as protectors against damage to DNA and liposomes especially in the epithelial cells of the colon

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exposed to these compounds during the metabolism of colon bacteria which transform them into mammal lignans (Rajesha *et al*., 2006 and Hu *et al*., 2007).

What is lignan

Lignans are phenolic compounds present in edible plants. Secoisolariciresinol (SECO) is present in flaxseed as a glycoside. Secoisolariciresinol diglucoside (SDG) is the most abundant lignan in flaxseed, which can be metabolized to the mammalian lignans, enterodiol and enterolactona by human intestinal microflora (Charlet *et al*., 2002). The flaxseed lignan and its mammalian metabolites are known to have a number of potential health benefits, including decreased formation of breast, prostate and colon cancers attributed to anti-estrogenic and antioxidant properties (Kitts *et al*., 1999). Phytoestrogens are plant chemicals that can have estrogen-like actions in humans and animals. The main phytoestrogens are isoflavones, coumestans, flavonoids and lignans. Flax contains other lignans as well-namely, matairesinol, pinoresinol, lariciresinol, isolariciresinol and secoisolariciresinol. Flaxseed oil Lignans occur in a number of plant foods, but flaxseed is a particularly rich source (lignans are not to be confused with lignins, a type of insoluble fiber). Lignans, along with isoflavones and coumestans, comprise the three major classes of phytoestrogens. Lignans are widely distributed in the plant kingdom, play a role in plant growth and act as antioxidants in human metabolism. In fact, the main lignan in flax and the forms derived from it are all antioxidants. The lignans are related to lignins, which are structural elements in plants. Lignans are being studied for possible use in cancer prevention, particularly breast cancer. Like other phytoestrogens, they hook onto the same spots on cells where estrogen attaches. If there is little estrogen in the body (after menopause) for example, lignans may act like weak estrogen, but when natural estrogen is abundant in the body, lignans may instead reduce estrogen's effects by displacing it from cells. This displacement of the hormone may help prevent those cancers, such as breast cancer, that depend on estrogen to start and develop. In addition, at least one test tube study suggests that lignans may help prevent cancer in ways that are unrelated to estrogen. The richest source of lignans is flaxseed, containing more than 100 times the amount found in other foods, Flaxseed oil, however, does not contain appreciable amounts of lignans. When plant lignans are consumed, intestinal bacteria convert some into two mammalian lignans, enterolactone and enterodiol. These compounds are absorbed from the digestive tract, circulate and are excreted in the urine. Flax seed is one of the best dietary sources of lignans. The main lignan in flax seed is secoisolariciresinol diglucoside (SDG), which is present in large quantities. The lignans are generally cinnamic acid dimmers containing a dibenzylbutane skeleton. When part of the human diet, contain lignans are believed to be converted into mammalian lignans known as enterolactone and enterodiol (Thompson *et al*., 1991).

What foods contain lignans ?

Most plant foods contain small amounts of lignans, but flax seeds are by far the best source. Lignans are present in a wide variety of plant foods, including seeds (flax, pumpkin, sunflower, poppy, sesame), whole grains (rye, oats, barley), bran (wheat, oat, rye), beans, fruits (particularly berries) and vegetables. Secoisolariciresinol and matairesinol were the first plant lignans identified in foods. Pinoresinol and laricresinol, two recently identified plant lignans, contribute substantially to total dietary lignan intakes. A survey of 4,660 Dutch men and women during 1997-1998 found that the median total lignan intake was 0.98 mg/day. Lariciresinol and pinoresinol contributed about 75% to the total lignan intake, while secoisolariciresinol and matairesinol contributed only about 25%. Plant lignans are the principal source of phytoestrogens in the diets of people who do not typically consume soy foods. The daily phytoestrogen intake of postmenopausal women in the U.S. was estimated to be less than 1 mg/day, with 80% from lignans and 20% from isoflavones. Flaxseed is by far the richest dietary source of plant lignans, and lignan bioavailability can be improved by crushing or milling flaxseed. Lignans are not associated with the oil fraction of foods, so flaxseed oils do not typically provide lignans unless ground flaxseed has been added to the oil. A variety of factors may affect the lignan contents of plants, including geographic location, climate, maturity, and storage conditions.

Lignan content of flax

Where flax is concerned, the problem of measuring its lignan content is compounded by the structure of the seed. SDG is the major flax lignan. It does not exist in the free form in the seed rather, it exists as a complex composed of five SDG molecules bound together with other molecules in the outer fibre layer of the seed. Extracting SDG from flax is difficult and its incomplete extraction is responsible for some of the variation in SDG values reported in the literature. Some researchers have analyzed the SDG content of flax or estimated its SDG content based on the concentrations of its metabolites (*e.g.*, SECO). Other researchers, such as Thompson and coworkers, analyzed flax samples to quantify the content of individual lignans, including SECO, which is a key end-product of SDG metabolism. Theoretically, the amount of SECO in flax samples reflects the amount of SDG present, provided the conversion is complete and the concentrations of pinoresinol and lariciresinol have been quantified. Whole flax seed contains 1-26 mg SDG/g, which works out to about 11-286 mg SDG/tbsp of whole seed or about 8-208 mg SDG/tbsp of milled flax. Whole flax seed contains about 42 mg of total lignans/tbsp, and milled flax contains about 30 mg of total lignans/tbsp, based on an analysis of four lignans in flax: matairesinol, pinoresinol, lariciresinol and SECO. Flax oil containing added lignans has been available for several years. One such product contained 0.1% SDG or about 14 mg SDG/ tbsp flax oil. The amount of SDG obtained from lignin enriched oil depends on how well the SDG particles mix with the oil. Adding SDG to flax oil is a bit like mixing oil and water because SDG is not soluble in oil, it resists mixing with the oil and tends to settle in the bottom of the container.

Adverse effects of lignan

Women, who are pregnant or breastfeeding should avoid high intake of flaxseed or purified lignans. One study found that pregnant rats, who ate large amounts of flaxseed (5% or 10% of their diet), or a purified lignan present in flaxseed, gave birth to offspring with altered reproductive organs and functions and that lignans were also transferred to the baby rats during nursing. In humans, eating 25 g of flaxseed per day amounts to about 5% of the diet. High intake of lignans may not be safe for women with a history of estrogen-sensitive cancer, such as breast cancer or uterine cancer. A few test tube studies suggest that certain cancer cells can be stimulated by lignans such as those present in flaxseed. Other studies found that lignans inhibit cancer cell growth. As with estrogen, lignans' positive or negative effects on cancer cells may depend on dose, type of cancer cell and levels of hormones in the body. If you have a history of cancer, particularly breast cancer, talk with your doctor before consuming large amounts of flaxseeds.

Main properties

SECO is known to have many physiological properties and health benefits. Indeed, SECO is converted into enterolignans (enterodiol (END) and enterolactones (ENL)) by the anaerobic intestinal microflora (Wang *et al*., 2000). Three other components of flaxseed, matairesinol (MATA), lariciresinol (LARI) and pinoresinol (PINO) are also converted into enterolignans. These four coumpounds are mammalian oestrogens precursors, also called phyto-oestrogens (Raffaelli *et al*., 2002). mammalian oestrogens, these compounds are potentially interesting for combating some hormone-dependent cancers. Over the past ten years, this topic has been extensively studied and reviewed (Adlercreutz, 1995; Thompson *et al*., 1996; Wang L.Q., 2002; Duncan *et al*., 2003; Boccardo *et al*., 2006; Albertazzi *et al*., 2008). Some epidemiologic investigations have shown that the risk of breast, prostate and colon cancers is lower in countries or regions in which the diet is particularly rich in lignans. However, others were considered conclusively "negative", *i.e.* failed to demonstrate any protective effect on carcinogenesis (Boccardo *et al*., 2006). Several cell culture and animal experiments have also shown the positive effect of enterolignans against these cancers. Phyto-oestrogens were also suggested to play a role in protection against diabetes and cardiovascular diseases (Duncan *et al*., 2003 and Albertazzi *et al*., 2008).

Extraction method of lignan

The choice of method for the extraction of lignans depends on their molecular structure. Less polar lignans can be extracted by hexane but in contrast, SECO, with a higher polarity can be extracted by polar solvents such as aqueous methanol or ethanol. SDG and SECO were first identified in flaxseed by Bakke *et al.* (1956). Westcott *et al*. (1998) patented an optimized extraction and purification method of flaxseed lignans. Their general extraction steps for SECO are schematized in following grinding, the flour obtained is usually defatted with organic non polar solvents (*e.g.* hexane) to facilitate alcoholic extraction of SDG. After defatting, the lignans are extracted by a primary alcohol (aqueous methanol or ethanol, with an alcohol content of 55 to 75% v/v being preferred). The crude extract undergoes an alkaline treatment (sodium or potassium hydroxide 1N, 3-7% w/v) which breaks the SDG-HMG link and releases free SDG. An extraction yield of about 30 mg SDG per gram of defatted flaxseed flour (DFF) is obtained with a mixture of ethanol and water (65/35, v/v). To obtain the aglycone form of the SECO, SDG must undergo an acidic or an enzymatic treatment in order to break the glycosidic link (Bakke *et al*., 1956 and Schwartz *et al*., 2006). For safety and environmental issues, replacement of the organic solvents is a growing requirement. Supercritical fluids, such as supercritical carbon dioxide (SC-CO2), which have gas-like diffusivity and liquid-like density have been used successfully as greener alternatives to non-polar solvents such as hexane to defat flaxseed flour (Bozan *et al*., 2002). Pressurized low polarity water (PLPW) extraction (also named subcritical water extraction) has also been applied to obtain lignans such as SDG from flaxseed (Cacace *et al*., 2006). The defatted flaxseed flour (DFF) undergoes a high temperature (*circa* 140°C) treatment under high pressure (*circa* 5.2 MPa) to ensure liquid state of water. In this subcritical state, water's dielectric constant and polarity decrease and reach similar values to those of a methanol-water mixture. Toxicity of this method is lower than a methanolic extraction but the effect of the temperature on extracts must be taken into account. SDG extraction yield is quite good (10 mg SDG per gram of seeds) but some phenolic compounds may be damaged. Real usefulness of organic solvents to extract SDG from flaxseed is not established. For example, a direct alkaline hydrolysis process that does not require the use of organic solvent has provided an extraction yield of 14 to 31 $mg.g^{-1}$ dry DFF depending on the origin of the flaxseeds (Eliasson *et al*., 2003). This process can be microwave-assisted (Nemes *et al*., 2011). Different parameters were studied to optimize the microwave-assisted extraction such as the microwave (MW) power level (between 30 and 360 W), the time of residence in the MW cavity (between 1 and 25 min), the molar concentration of sodium hydroxide (between 0.25 and 1 M) and the time of MW power application (30 or 60 $s{\cdot}min^{-1}$). The yields obtained with the optimized MW-assisted extraction method (0.5 M NaOH for 3 min with a MW power level of 135 W and MW power application of 30 $s{\cdot}min^{-1}$) were 6% higher than those obtained with the Elianson's reference hydrolysis method which is much longer (1 M NaOH for 1 h at room temperature) and were 10% higher than the control method (0.5 M NaOH for 3 min at room temperature). This organic solvent and corrosive product free process is a promising green method to obtain SDG from flaxseed.

Metabolism of lignans

The lignans SDG, SECO, pinoresinol, lariciresinol and matairesinol in flax are converted by bacteria in the colon to the mammalian lignans, enterodiol and enterolactone. Enterodiol and enterolactone are called mammalian lignans or enterolignans because they are produced in the gut of humans and other mammals; they are not found in plants. Enterodiol can be converted to enterolactone. The biologic activity of flax and other plant lignans depends on the presence of certain bacteria in the gut. Some humans appear to lack either the right type or a sufficient number of gut bacteria to convert SDG and other lignans to mammalian lignans, and taking antibiotics virtually stops the production of enterodiol and enterolactone in the gut for several weeks. Enterodiol and enterolactone have three metabolic fates: 1) They can be excreted directly in the feces, 2) They can be taken up by epithelial cells lining the human colon, conjugated with glucuronic acid or sulfate and excreted in the feces or enter the circulation or 3) They can be absorbed from the gut and transported to the liver, where free forms are conjugated before being released into the bloodstream. Eventually, they undergo enterohepatic circulation that is, they are secreted into bile and reabsorbed from the intestine and are excreted in the urine in conjugated form. Based on a kinetic study involving 12 healthy adults, the mammalian lignans appear to be absorbed from the colon about 8-10 hours after the plant lignans are eaten and reach a maximum concentration in the bloodstream about 7-10 hours later. The concentration of enterodiol and enterolactone in the feces, blood and urine is related to the concentration of plants lignans in the diet large intakes of plant lignans result in large amounts of these mammalian lignans in biological fluids. Eating flax or flax-containing food products increases the blood levels of mammalian lignans and the excretion of mammalian lignans and/or total lignans in feces and urine. Consuming a diet supplemented with a lignin /SDG complex derived from flax also increases mammalian lignan excretion in urine. The bioavailability of the mammalian lignans can be enhanced by crushing and milling flax. Lignan metabolism is far more complex than originally thought. Plant lignans are not metabolized completely to mammalian lignans, and some plant lignans such as SECO can be detected in plasma. Furthermore, lignan metabolism may not stop at enterodiol and enterolactone, there being additional metabolites derived from these mammalian lignans. These new findings raise questions about which lignan is the most important and most biologically active form.

Biological effects of lignans

Flax lignans and the mammalian lignans (enterodiol and enterolactone) are biologically active. Lignans have anticancer and antiviral effects, influence gene expression and may protect against estrogen-related diseases such as osteoporosis. Diets high in lignans may help maintain good cognitive function in postmenopausal women reduce the risk of uterine fibroids in middle-aged women, reduce breast cancer risk in women and reduce the risk of acute fatal coronary events and prostate cancer in men.

Specific actions of lignans include the following

- The main flax lignan SDG is an antioxidant. It scavenges for certain free radicals like the hydroxyl ion. Our bodies produce free radicals continually as we use (oxidize) fats, proteins, alcohol and some carbohydrates for energy. Free radicals can damage tissues and have been implicated in the pathology of many diseases like atherosclerosis, cancer and Alzheimer disease. In a rat study, feeding flax at levels of 5% and 10% in the diet prior to administering a liver toxin protected against oxidative stress in liver tissue compared with a normal diet not containing flax. The mammalian lignans, enterodiol and enterolactone, also act as antioxidants. Indeed, the antioxidant action of SECO and enterodiol is greater than that of vitamin E.

- The mammalian lignans affect receptors found on the surface of cell membranes. For instance, they activate the pregnane X receptor, which is involved in the metabolism of bile acids, steroid hormones and many drugs. Enterolactone is a moderate activator of the receptor, suggesting it has the ability to affect the metabolism of some drugs. A study conducted in France suggested that some plant lignans, along with enterodiol and enterolactone, affect hormone receptors in breast tissue. Among 58,049 French women who did not eat soy regularly, a high dietary intake of lignans (>1395 µg/day) was associated with a reduced risk of breast cancer. The benefit was limited to women with estrogen receptor positive (ER+) and progesterone receptor positive (PR+) tumours, suggesting that the biologic effects of lignans derive in part from their effects on cell hormone receptors.
- The mammalian lignans stimulate the synthesis of sex hormone binding globulin (SHBG), which binds sex hormones and reduces their circulation in the bloodstream, thus decreasing their biologic activity. In a meta-analysis, higher blood levels of SHBG were associated with an 80% lower risk of type 2 diabetes in women and a 52% lower risk in men. Low blood levels of SHBG have been found in postmenopausal women with breast cancer.

Flax and hormone metabolism

Dietary fibre and fat affect estrogen levels in the body. Specifically, the intake of total fat and saturated fat is positively correlated with plasma concentrations of estradiol and estrone, whereas the intake of dietary fibre is negatively correlated with plasma levels of these hormones. Because flax contains both fat and dietary fibre, some researchers have investigated its effects on hormone metabolism, as described below.

Women

Flax has hormonal effects in women. In 18 premenopausal women with normal menstrual cycles, eating 10 g of flax daily for 3 months lengthened the luteal phase of the women's menstrual cycle. In 25 postmenopausal women, who ate 25 g of milled flax daily for 2 weeks, vaginal cell maturation was stimulated, suggesting an estrogenic effect of flax on women's reproductive tract. However, several clinical studies lasting 2-12 weeks reported no effect of consuming 10-40 g (1+ to 5 tbsp) of milled flax daily on blood levels of estradiol, estrone, follicle-stimulating hormone or luteinizing hormone in young women of reproductive age or in postmenopausal women.

Men

Flax consumption does not appear to affect sex hormone metabolism in men, based on findings of the one study published in this area. Eating 13.5 g milled flax daily for 6 weeks had no effect on plasma concentrations of testosterone, free testosterone or sex-hormone-binding globulin in six healthy young men. It is not known whether sex hormone metabolism in men is affected by long-term flax consumption.

Conclusion

Lignans, including SECO, are abundant in superior plants and present properties particularly interesting to the health sector. The food and cosmetic fields could also potentially exploit their antioxidant activity. The two approaches to obtain SECO are extraction from natural raw materials and chemical synthesis. Organic solvents are often used to extract this lignan from biomass. Greener processes have been developed in the past few years, but further work is needed to improve the extraction yields. The chemical synthesis alternative, allows control of the structure of the desired compounds and some of the purification steps are thus avoided. Another advantage of organic synthesis is that it gives access to a larger range of structures and this may open new applications opportunities. Only a few studies have focused on enzymes applied in SECO synthesis. However, this synthesis pathway has considerable potential. Indeed, the specificity of enzyme activity leads to stereochemically-controlled compounds that should not require tedious purification steps and the process may have a lower environmental footprint.

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