Patented Techniques for the Extraction and Isolation of Secoisolariciresinol Diglucoside from Flaxseed

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Abstract: Plant lignans are phenolic compounds generally containing a dibenzylbutane skeleton. Secoisolariciresinol diglucoside (SDG) is the major lignan found in flaxseed. SDG is known to have antioxidant and anticancer properties. SDG can potentially be used as a natural antioxidant in foods thereby preventing further oxidation reactions and thus enhance the shelf life of foods. This article reviews the patents that are concerned with the extraction of SDG from flaxseed, the richest plant source of lignans. Most of the patented techniques for the extraction, isolation, and purification of SDG are conducted on defatted flaxseed and whole flaxseed. Flaxseed hull is potentially a good starting material. Furthermore, most methods use aliphatic alcohols (methanol, ethanol, isopropanol, butanol) to extract the complexed form of SDG. Combinations of these solvents are commonly used with water. Alkaline hydrolysis liberates SDG from its complexed form. SDG is enriched by a process involving either liquid-liquid partitioning or passing the aqueous phase through anion exchange resins or C18 resins. The SDG is recovered after evaporation of the water. Analytical HPLC coupled with mass spectrometry is performed to determine the quantity and purity of the extracted SDG.

Keywords: Flaxseed, lignans, secoisolariciresinol diglucoside (SDG), SDG polymer, solvent extraction, hydrolysis, liquid chromatography, HPLC.

INTRODUCTION

Lignans & SDG complex in flaxseed: The cells of higher plants contain lignans or diphenolic compounds that are formed by the coupling of two coniferyl alcohol residues [1]. The major lignan in flaxseed is secoisolariciresinol (SECO) Fig. **1** which is present in the form of the diglucoside (i.e. SDG) Fig. (**1**) [2]. In flaxseed, SDG is further polymerized (or oligomerized) existing as part of a larger complex Fig. (**2**) composed of five SDG residues interconnected by ester-linkages to four 3-hydroxy-3-methylglutaric acids [3]. This lignan complex (SDG oligomer/polymer) typically contains SDG (35%), cinnamic acid glycosides and hydroxymethyl glutaric acid (HMGA) [3]. The identity of HMGA present in flaxseed was confirmed by NMR [3]. It has been concluded that HMGA is a substructure of the lignan macromolecule from flaxseed hulls and that it is incorporated in the macromolecule via the same linker-molecule as SDG [3, 4]. A simple procedure is normally required to isolate this lignan complex from flaxseed [3]. Further steps are necessary to liberate SDG from the lignan complex. Besides SDG, the phenolic compounds p-coumaric acid glucoside and ferulic acid glucoside have been isolated [5].

Health benefits of flaxseed lignans: No physiological activities were reported for the lignan secoisolariciresinol diglucoside (SDG) at the time when Bakke and Klosterman [6] described a laboratory process for extracting SDG from flaxseed meal. Investigations on physiological activities of flaxseed lignans started about 30 years ago either on whole flaxseed or purified SDG [7]. Flaxseed lignans including SDG and its aglycone, secoisolariciresinol (SECO) are now known to exhibit a number of health benefits, including

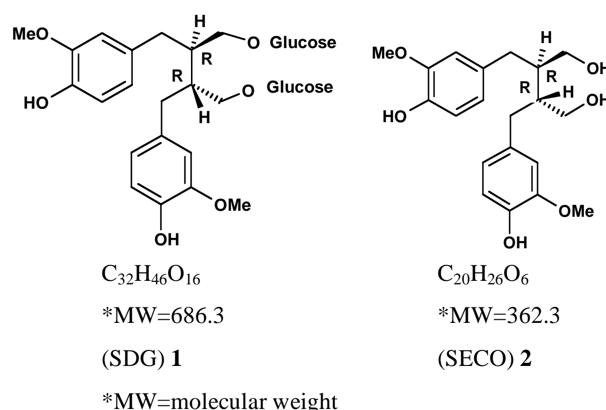

Fig. (1). Structure of SDG and SECO [30].

decreased tumor growth, reduction of serum cholesterol levels and decreased formation of breast, prostate and colon cancers [8-12]. When flaxseed is consumed as part of the human diet, increased levels of enterolactone and enterodiol are found in urine [7]. As precursors of the mammalian lignans enterolactone and enterodiol [13], flaxseed lignans are classified as phytoestrogens [Lampe 2003]. In addition, flaxseed lignans demonstrate antioxidant activities [8, 14, 15]. Thus, SDG could simply be used as a natural antioxidant additive to foods [15] given the growing interest in finding alternative antioxidant food preservatives. In the latter, this includes natural products such as SDG that also have the potential to provide health benefits due to their antioxidant properties [8, 14, 15].

Content of SDG flaxseed versus other sources: Flaxseed has the highest amounts of lignans in the plants used in the human diet, while other sources such as rye, buckwheat, millet, soya, and barley yield 2-5 ug of lignans per g of grain [16-18]. The highest concentrations of lignans (e.g.

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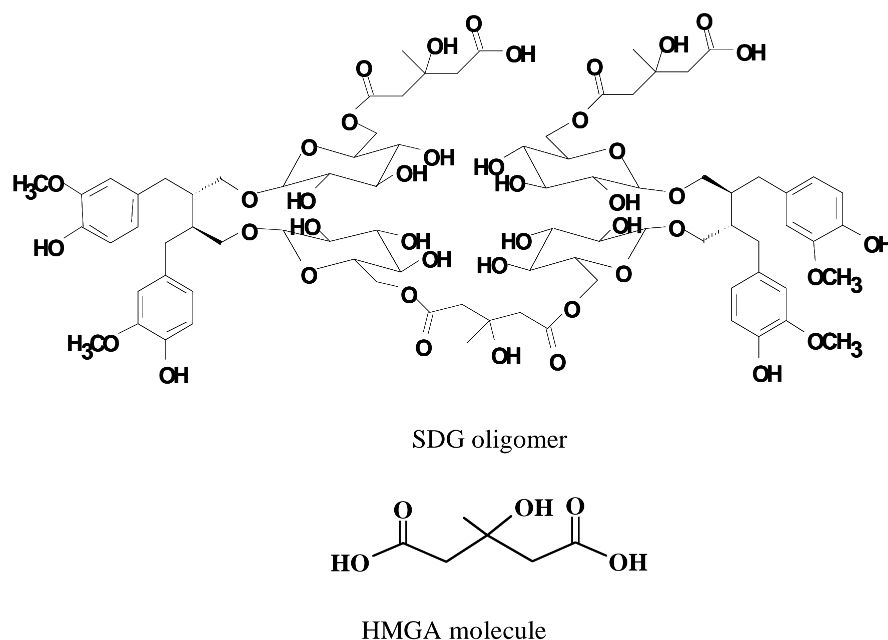

Fig. (2). Structure of SDG oligomer containing SDG and 3 hydroxy-3-methyl-glutaric acid units (HMGA) [15].

hydroxymatairesinol) in the plant kingdom are found in the heartwood of branches and twists and especially in the knots of certain trees; however, the contribution of flaxseed to the overall dietary intake of lignans is rather low, due to the low consumption of flaxseed in the normal diet. Studies have shown that defatted flaxseed contains SDG varying between 9 and 30 mg/g and up to 800 times more lignans than any other foods [2]. The SDG was found in amounts of up to 20 mg per gram of defatted flaxseed [1]. The SDG content in flaxseed hull has been reported to be at least 10% percent by weight [19] whereas in defatted flaxseed [20], it ranges from 0.9% to 3.0% by weight. Since the most of the SDG is distributed in the flax hull, it would be beneficial to provide a process that could separate SDG from the flaxseed hull. The technique may also lead to SDG of a higher yield and purity in comparison to SDG obtained from flaxseed meal [21].

Isolation of SDG from flaxseed: Flaxseed contains 40-50% oil. The defatted flaxseed (meal) contains 23-34% protein, 4% ash, 5% viscous fibre (mucilage) and lignan precursors [1, 2, 20]. Most techniques for the extraction, isolation, and purification of secoisolariciresinol diglucoside (SDG) are conducted on defatted flaxseed, whole flaxseed, and SDG oligomer/polymer [2, 5]. Furthermore, several methods use a mixture of ethanol, methanol, or some other chemical mixture as a solvent to extract SDG [1, 6]. Defatted flaxseed contains SDG in a form bound to the HMGA resulting in the SDG oligomer/polymer. Alkaline hydrolysis is used to liberate the SDG from the precursor complex [3]. About 20 patents on lignan and/or SDG extraction from flaxseed have been published in recent years starting from the mid-1990s. This article will review important patents mainly concerned with the extraction of flaxseed lignans in various forms including SDG oligomer/polymer, SDG and SECO.

EXTRACTION OF SDG

Constituents of flaxseed hull and kernel: Flaxseed lignans and gums (viscous soluble fibre) are located mainly in the hulls enclosing the seeds while the majority of the proteins and oils are in the kernel/embryo [21]. Hulls and kernels constitute 22.6% and 72.2%, respectively of whole flaxseed which contain carbohydrates, crude oil, protein, moisture, and minerals [2, 21, 22]. Both the flaxseed hull, which is rich in SDG, and the kernel/embryo rich in oil with high α-linolenic acid content, are of interest for food and non-food uses [22-24]. Table **1** shows the major compositions of flaxseed hull and flaxseed kernel [19, 21].

Table 1. Composition of Flaxseed Hull and Flaxseed Kernel [2, 19]

Composition (%)	Hull	Kernel/Embryo
Carbohydrates	48.3	22.0
Proteins	16.8	23.9
Crude oil	26.5	47.7
Minerals	3.5	3.8
Moisture	5.0	3.6

Hulling: Separation of flaxseed hulls from kernels is typically performed by an air classification and sieving procedure [19]. A dry process for dehulling has been developed in order to utilize all components of flaxseed [19]. The dehulling process is composed of three steps: first the flaxseed is dried, then broken (milled) and fractionated by air classification to produce a hull fraction and a kernel (embryo) fraction. The chemical constituents of hulls include carbohydrates (48.3%), proteins (16.8%), crude oil (26.5%),

moisture (5.0%) and ash (3.5%). The embryo fraction contains carbohydrates (22.0%), proteins (23.9%), crude oil (47.7%), moisture (3.6%) and ash (3.8%) on dry basis [19]. Flaxseed lignans and gums are extracted sequentially from the hulls while the kernels, purified by sieving, are used in food and/or feed formulations. Figure **3** illustrates the dehulling process of flaxseed to separate hull from kernel [19]. In other methods, flaxseed was abraded and the hull was removed from whole flaxseed in a mill equipped with millstones [19, 21, 24, 25]. As the flaxseed passes over the abrasive rotator, contact with rotator separates the flaxseed components into hulls and kernels [26]. The hull is subsequently divided into two fractions including the mucilage fraction and the fibre fraction [26]. The mucilage fraction comprises the outer layers of hull and is rich in water soluble carbohydrates. SDG is part of mucilage fraction. The fibre fraction comprises the inner layers of hull and is particularly rich in insoluble fibres and lignans [19, 21, 25]. High lignan hull and high fibre hull are commercially available. One patent describes high lignan (3% to 5% or greater) flaxseed products obtained using air classification technique and sieves, wherein the lighter fraction contains the valuable high lignan concentrate [23]. The high lignan flaxseed meal can include approximately 40-50% insoluble dietary fibre and 50-60% soluble dietary fibre instead of the typical 60-70% insoluble dietary fiber and 30-40% soluble dietary fibre found in flaxseed meal [23].

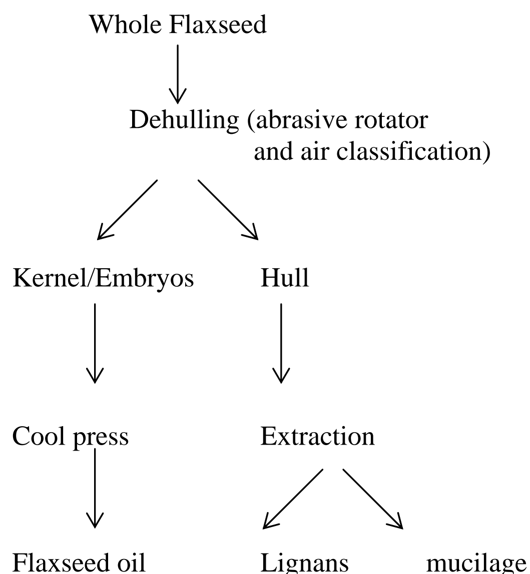

Fig. (3). Dehulling process of flaxseed [19].

Oil and cyanogenic sugar removal: Several methods make use of defatted flaxseed meal as the starting material. The meal is obtained first by crushing whole flaxseed using a screw press and followed by oil removal using organic solvents such as hexane [1]. While oil is removed the flaxseed meal still contains an oil residue of about 2-4% by weight [2, 19]. The solid residue is used mainly as an animal feed. In one invention supercritical CO_2 modified with ethanol is used to remove fat from the cold-pressed crushed flaxseed [20].

One of the major problems associated with using flax in foods is the toxicity associated with cyanogenic glycosides present in flaxseed. Cyanogenic glycosides are nitrogenous secondary plant metabolites which if consumed in excess over a long period of time can result in goitrogenic problems to human [4]. The major cyanogenic glycosides present in flaxseed are linustatin, neolinustatin and linamarin with linustatin accounting for 54-76% of the total cyanogenic glycoside content. Defatting of flaxseed meal with hexane is known to produce an enrichment of all individual cyanogenic glycosides on equal weight basis in the meal [4]. Ultrafiltration can be used to remove cyanogenic sugars present in flaxseed. In one study, lignan from flaxseed is first extracted with organic solvents; the separated liquid fraction containing the lignan product is then subjected to ultrafiltration to remove cyanogenic sugars and other impurities [4].

Extraction of lignan complex: Bakke and Klosterman (1956) were among the earliest workers to report a laboratory process for extracting SDG from defatted flaxseed (meal) using equal parts of 95% ethanol and 1,4-dioxane [6]. Other solvent extraction methods have since been reported in the past 12 years. Table **2** shows different extraction methods used for SDG isolation from flaxseed. A variety of organic solvents including methanol, ethanol, 1,4-dioxane, acetone, isopropanol, butanol or mixtures are used [1, 2, 4, 6, 19, 27]. Aqueous solvents are also employed [1, 2]. The extraction methods take advantage of the solubility of SDG in alcohol and water [6]. For example, defatted flaxseed meal is extracted with a mixture of acetone and water [2], while another method uses a mixture of methanol or ethanol with water [1]. During extraction, the solvent to meal ratio ranges from 5:1 to 7:1 [1, 4] and from 12:1 to 16:1 [2]. An even higher ratio of 20:1 is used in one invention where 1M sodium hydroxide in methanol (1:20 w/v) for simultaneous extraction and hydrolysis [21]. The length of extraction is 24 hours for some of the techniques [1, 21]. One invention relates to compositions extracted from plants such as soy, flax, tea, and cocoa for their phytochemicals, including saponogenins and saponins, catechins, lignans, phenolic acids, and isoflavones [27]. In the invention, lignans are extracted from defatted flaxseed meal using 85% ethanol at 40°C for 10 minutes. The ethanol is then evaporated and fractionation of lignans performed using XAD-4 resin column.

Hydrolysis to liberate SDG: In order to liberate SDG, sodium hydroxide or calcium hydroxide are commonly employed for base hydrolysis in water or alcohol (Table **2**). SDG must be liberated from its polymeric lignan precursor by breaking the ester-linkages present in the complex [3, 4]. In one method, calcium hydroxide is used to liberate free SDG in the solution from the complex after acetone is removed [2]. The SDG concentrates obtained using calcium hydroxide separate easily from insoluble calcium salts to provide a product which is non-hygroscopic and of relatively high purity [2]. Sodium hydroxide is a base of choice for several inventions [1, 19, 20]. Other bases include ammonium hydroxide and potassium hydroxide [1]. The concentration of the base is typically about 1 normal and it is preferably used in an amount of about 3-7% w/v. The hydrolysis is normally carried out for a period of about 4 to

Table 2. Summary of Patented Methods to Obtain SDG from Flaxseed

Inventor/date	Patent Type/Patent Number	Source	Interests	Method	Results
Pizzey, G. R. 05/23/2006 [23]	United States Patent 20067048960	Flaxseed meal	Mechanical method for the production of high lignan flaxseed meal	Milling and seiving system using aspirator to separate lighter density portion (high in lignan) from coarser portion	Increasing the lignan content of processed flaxseed product by 3-7%
Westcott, N.D. Muir, A. D. (Saskatoon, CA) - 01/06/1998 [1]	United States Patent 5705618	Flaxseed meal	Chemical method for the extraction of SDG and cinnamic acid derivative	Mixtures of aliphatic alcohols including methanol, ethanol, isopropanol, or butanol with water, alcohol-to-water ratios ranging from 1.85:1 to 3:1; separating residual solids from the phenolic-rich alcohol solvent; base hydrolysis to liberate SDG and cinnamic acid derivatives from its oligomeric form	Up to 20 mg per gram of SDG (purity 90%)
Westcott, N.D. (Saskatoon, CA) Paton, D. (Saskatoon, CA) - 07/24/2001 [3]	United States Patent 20016264853	Flaxseed meal	Chemical method for the extraction of SDG oligomer/ polymer	Alcoholic extraction followed by ultrafiltration; low molecular weight species remain with a filtrate and higher molecular weight oligomer/polymer are retained on the ultrafiltration membrane	370 mg/g solids of SDG, 160 mg/g solids of cinnamic acid glucoside (measured as methyl ester), 50 mg/g solids of ferulic acid glucoside (measured as methyl ester) and 96 mg/g solids of HMGA (measured as its dimethyl ester)
Myllymäki,O. (Espoo, FI) - 08/27/2002 [26]	United States Patent 20026440479	Whole flaxseed	Mechanical method for the production of fiber fraction rich in lignans	Removing flaxseed hull from flaxseed endosperm by abrasion, wherein a firstly removed outer portion of the husk is separated as a mucilage fraction and then a secondly removed inner portion is separated as a fibre fraction	800-1480 mg/100g of total lignans
Shukla, R. (Decatur, IL) Hilaly, A. K. (Springfield, IL) Moore, K.M. (Mount Zion, IL) - 07/27/2004 [4]	United States Patent 20046767565	Plant materials including flaxseed	Chemical method for the production of the lignan complex removing cyanogenic sugars;reducing microbial component	Solvent extraction to obtain SDG polymer; ultrafiltration (while simultaneously adding solvent solution) to remove cyanogenic sugars and to reduce microbial content component	Lignan complex : 1.9 g/L (purity 11.8%); ultrafiltration: retentate (0.9 g/L) with 23.3% purity; permeate (0.2 g/L) with 8.1% purity
Dobbins, Thomas A. (Howard, OH) Wiley, David B. (Warsaw, OH) - 10/19/2004 [2]	United States Patent 20046806356	Flaxseed meal	Chemical method for the extraction isolation, and purification of SDG	A continues extraction method; solvent comprising acetone 45% acetone/55% water, solvent to feedstock ratio: 12:1 to 16:1and water (35: 65 v/v) to extract SDG; separating residual solids from the SDG-containing extract	19.3 grams of a light-colored, fluffy hygroscopic solid containing 31% by wt. SDG with recovery of 90%

(Table 2) Contd…..

Inventor/date	Patent Type/Patent Number	Source	Interests	Method	Results
Empie, M. (Forsyth, IL, US) Gugger, E. (Latham, IL, US) - 05/31/2005 [27]	United States Patent 20056900240	Flaxseed meal other vegetable matter including soy tea, and cocoa	Chemical method for isolation of phytochemicals, including saponogenins and saponins, catechins, lignans, phenolic acids, and isoflavones	Ethanolic extraction; dissolving in water; ultrafiltration; freeze-drying	Initial weight: 978 g of defatted flaxseed meal; SECO (18.2 mg/g):
Cui, W. (Guelph, CA) Han, N. F. (Brampton, CA) - 04/04/2006 [19]	United States Patent 20067022363	Flaxseed hulls and lignan-rich flaxseed products for applications as ingredients for nutraceuticals, functional foods, feeds and other food and non-food products	Mechanical method using dehulling to obtain lignan rich flaxseed products	Continuous dehulling process, fractionation of the dehulled products, comparison with traditional extraction method with methanol: 1, 4 dioxane at 60° C for 36 hr.; centrifugation Supernatant hydrolysis with 0.5 M NaOH at room temperature for 24 h acidification of the hydrolyzate with 2M H_2SO_4 to pH 3 C_{18} resin using water to remove sugars; eluting SDG with methanol	The SDG content in defatted flaxseed ranged from 0.9% to 3.0% by weight whereas in flaxseed hull, it was at least 10% percent by weight; It was found that the extraction efficiency of the lignan with alcohol was low, and the method was time consuming
Pihlava, J. (Rusko, FI) Hyvarinen, H. (Jokioinen, FI) Ryhanen, E. (Helsinki, FI) Hietantemi, V. (Jokioinen, FI) - 02/12/2004 [20]	United States Patent 20040030108	Crushed flaxseed	Chemical method for the isolation of SDG	Supercritical carbon dioxide extraction 1-5 hours, pressure 300-450 atm and temperature 50-80°C alkaline hydrolysis (1M NaOH: MeOH, 1: 20 (w/v). to obtain SDG separation and purification with glass column chromatography using C18 as packing material	SDG with the particle size <5 mm with 90% purity
Kankaanpaa-Anttila, B. Anttila, M. 1999 [22]	United States Patent 5925401	Whole flaxseed	Chemical method for producing a product containing flax proteins and flax mucilage	Cold and/or hot pressed to separate oil; alkaline extraction followed by acid precipitation	Flax protein product containing flax mucilage

24 hours [1]. An additional benefit of this process is the destruction of the cyanogenic glycosides yielding an extract free of cyanogenic glycosides or free cyanide. In one invention, SDG was extracted using only water followed by direct alkaline hydrolysis in an attempt to obtain a product free of solvent residues and thus avoiding the use of organic solvents reported in previous methods Fig. (**4**) [28]. The method is considered suitable for extracting SDG from flaxseed hull in large quantities with purity highly suitable for nutritional supplements or nutraceutical purposes [28]. Temperatures above room temperature (50 to 100°C) are typically used for base hydrolysis. A higher temperature is needed to separate SDG from larger molecular weight compounds such as protein and starch residues which are coagulated and precipitated by the heat [2]. The pH ranges from 10 to 13 although 11.8 to 12.5 is the preferred range. After hydrolysis, the pH of the solution need to be acidified in order to prevent the ionization of any functional groups in the aliphatic and aromatic part of the SDG molecule [2, 6]. The base hydrolyzate is lowered to a pH range between 3 and 8.5 [1, 2].

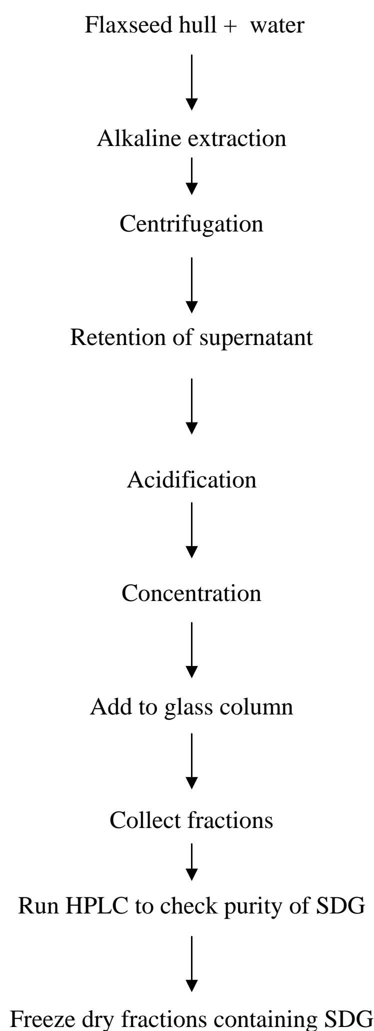

Fig. (4). Aqueous extraction method of SDG from flaxseed hull [30].

Purification of SDG: The hydrolysate is concentrated by rotary evaporation [1, 21, 29] prior to subjecting it to either a liquid/liquid partition, using an ethyl acetate/water system for example, or an anion exchange to further enrich the lignans. The lignan-enriched solution thus obtained is subjected to chromatographic separation to isolate lignans at a purity of greater than 90 percent. The SDG containing hydrolysate is subjected to a glass column packed with Sephadex anion exchange resin or C-18 reverse phase resin in order to isolate SDG from other impurities [1, 20]. The SDG is then recovered after evaporation of the water using freeze-drying, spray-drying, and vacuum drying. In one invention, the SDG is recovered using lyophilization at a purity of 31% and yield of 3.2% by weight from the defatted flaxseed with 90% recovery [2]. A substanially pure chemically bound complex is derived from flax containing secoisolariciresinol diglucoside, cinnamic acid glycosides and hydroxy methyl glutaric acid [3]. The complex (1.9 g/l) is obtained by preparing an aqueous aliphatic alcoholic extract from flax and subjecting this aqueous extract to ultrafiltration whereby low molecular weight species remain with a filtrate and higher molecular weight species comprising the separated complex are retained [3].

SDG analysis: Early studied have suggested a straight-chain oligomeric structure composed of five secoisolari-ciresinoldiglucoside (SDG) residues interconnected by four 3-hydroxy-3-methyl glutaric acid (HMGA) residues (molecular weight ca. 4000 Da) [3, 29]. However, a recent study has shown the hydroxycinnamic acid glucosides and ferulic acid residues to be connected directly to SDG but no linkage between HMGA and the hydroxycinnamic acid glucosides [29]. The composition and content of phenolic glucosides of defatted flaxseed were (+)-SDG (11.9-25.9 mg/g), (-)-SDG (2.2–5.0 mg/g), *p*-coumaric acid glucoside (1.2-8.5 mg/g), and ferulic acid glucoside (1.6-5.0 mg/g) [30]. In most studies, high-performance liquid chromatography (HPLC) coupled with photodiode array detector and mass spectrometric (MS) procedures are used for the quantification and analysis of the purity of SDG obtained [7, 30, 31]. An excellent review is written by Muir (2006) that can provide outstanding information for different analytical methods for SDG analysis and its influence on biological activity [32].

CURRENT & FUTURE DEVELOPMENTS

This review article provided useful information on recent patents on flaxseed lignans and common methods for extraction and purification of lignans including SDG. This is important for better understanding about the chemistry and mechanism of action of flaxseed lignans in biological systems.

Future biological studies require confirmation of SDG and the aglycones SECO as chain breaking antioxidants in prevention of lipid oxidation, protein oxidation and DNA oxidation associated with oxidative stress. Other *in vitro* studies with animal and human hepatic microsomal preparations could use these compounds to investigate their antioxidant actions. Tissue culture of animal and human cancer cells could be a useful way to examine whether these compounds have antitumorigenic properties. Further investigation on how antioxidant properties vary between SDG and SECO (*in vivo*) would be enlightening because most of the glycosides would be converted to aglycones by intestinal microorganisms. In addition, it may be beneficial to treat the flax products in a way that would convert SDG to SECO in order to increase its antioxidant capacity. Specifically, the following questions remain to be elucidated:

1. What extraction procedure is appropriate to yield desired lignans or SDG/SECO-containing fractions for specified functional uses?
2. Is there a need for toxicological and safety profiles for lignans and SDG/SECO?
3. Do lignans as SDG act as preventive antioxidants?
4. Do SECO and SDG exhibit a synergistic effect in *in vitro* and *in vivo* systems?
5. Is the mechanism of the inhibition of oxidation by SECO and SDG in the membranes the same as those in homogeneous solutions?

It is anticipated that appropriate extraction and purification techniques of plant lignans will continue to be developed as the conundrum surrounding the role of SDG and other lignans in the prevention of chronic illnesses is resolved by mounting strong scientific evidence.

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