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INVITED REVIEW

Flaxseed lignan: Metabolism, extraction and isolation techniques, potential health benefits and applications in dairy foods

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Abstract: Flaxseed lignan has been recognized as a potent phytoestrogen for the treatment of health problems such as cancer, diabetes, hyperlipidaemia, cardiovascular diseases, postmenopausal related diseases and so on. Existing literature suggests that higher dietary or supplementary intakes of lignans and other phytoestrogens have been linked to improved cognitive performance in middle-aged and older people. The lignan secoisolariciresinol diglucoside is found in abundance in flaxseed. Flaxseed lignan metabolites may offer health benefits due to their weak estrogenic or anti-estrogenic effects, antioxidant activity or other yet-to-be-identified mechanisms. The current review explores the metabolism of flaxseed lignan by gut microbiota, its bio accessibility, health benefits of flaxseed lignan in humans and the possible mechanisms, with data from animal and clinical studies in the last few years to back up that assertion, application in food and dairy industry and makes recommendations for future research.

Key words: *Linum usitatissimum* L.; Lignan; Secoisolariciresinol diglucoside; Enterodiol; Enterolactone; Menopause; Functional property; Food applications

Introduction

The health and therapeutic advantages of flaxseed (*Linum usitatissimum* L.) and its derivatives (ground flax, flax oil, defatted flax, flax fiber, and lignan extract) have been proven. Flaxseed includes polyunsaturated oil, soluble and insoluble (dietary) fiber, and the plant lignan secoisolariciresinol diglucoside (SDG), which may all help with disease prevention and health promotion (Raole and Raole, 2022). Some of the possible advantages of flaxseed include alpha-linolenic acid (ALA) as an antihypertensive agent (Verma et al. 2020), enterolignans generated from SDG, enterolactone (ENL) and enterodiol (END) as antioxidants and 17-β estradiol mimetics (Albuquerque et al. 2020), and dietary fiber for cholesterol reduction (Prasad et al. 2020). SDG is a phytoestrogen, or plant hormone (Zare et al. 2022) and flaxseed has been determined to be the greatest source of SDG among diverse plant foods, with almost 1000 times the amount of SDG found in sesame seed, pumpkin seed, wheat, lentils, soybeans, pears, prunes, garlic, asparagus and carrot (Ebrahimi et al. 2021). SDG levels in defatted flaxseed powder have been reported to vary between 6 to 29 g/kg (Kaur and Sharma, 2021) equivalent to 3.4 to 14.40 mg/100g secoisolariciresinol (SECO) (Hyvärinen et al. 2006a). Matairesinol (0.002 g/kg), pinoresinol (0.007 to 0.248 g/kg), lariciresinol (0.028 to 0.033 g/kg), and isolariciresinol (0.102 g/kg) are other lignans identified in flaxseed, although their concentrations are low in comparison to SECO (Edel et al. 2015). Through its mammalian metabolites, enterodiol and enterolactone, SDG is thought to have phytoestrogenic properties (Hu et al. 2007). SDG is initially converted to END in the intestines by microflora, which can subsequently be further metabolized to ENL. SDG is deglycosylated into mammalian lignans by the activity of β-glycosidases, which assures lignan bioavailability and peripheral circulation in humans (Braune and Blaut, 2016). It has also been claimed that flaxseed flour and defatted meal produced the greatest output of END and ENL *in vitro*, up to 800 times that of others (Akter et al. 2021). Other lignans in flaxseed can also be converted to END and/or ENL, although their overall impact on enterolignan concentration is considerably lower than SDG's. *In vivo*, these enterolignans undergo enterohepatic circulation as they get conjugated in the liver following intestinal absorption and are discharged in bile or urine, where they are reabsorbed and repackaged as β-glucuronide or sulphate conjugates (Edel et al. 2015).

Flaxseed contains lignans in the secondary wall of the sclerite cells of the seed's outer integument (Chhillar et al. 2021). It is stored as a hydroxymethyl glutaryl ester-linked complex (SDG-HMG) (Sainvitu et al. 2012). SDG has emerged as a possible dietary

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component for health promotion being a vital substance in plant foods with functional value (Rodríguez-García et al. 2019). *Linum* spp. lignans have been found to be of substantial functional value and are thus intensively investigated. *Linum album*, which is a rich source of the anticancer drug podophyllotoxin (PTOX), has emerged as a paradigm for lignan-based research in other *Linum* species. Arylnaphthalene lignans were found in many different *Linum* species, including *Linum altaicum* (purple flax) and *Linum glaucum* (Chhillar et al. 2021).

Earlier investigations have revealed that SDG is metabolized by bacteria in the intestinal tract of humans and animals to enterodiol (END) and enterolactone (ENL) (Taibi et al. 2021), which have beneficial effects against osteoporosis, cardiovascular disease, hyperlipemia, breast cancer, colon cancer, prostate cancer and menopausal syndromes (Kezimana et al. 2018).

Mechanism of conversion of SDG into END and ENL

Plant lignans may be degraded and transformed to enterolignans (Li et al. 2022) (entero- from Greek *enteron* meaning "intestine") by bacteria in the intestine (Borriello et al. 1985). Enterolactone (ENL) and enterodiol (END) are the two main enterolignans or mammalian lignans generated by mammalian gut bacteria. Following consumption of SDG or similarly glycated lignans, such as pinoresinol diglucoside or sesaminol triglucoside (STG), the sugar moieties are hydrolyzed by *O*-linked deglycosylation in the large intestine, resulting in SECO and the other aglycones (Li et al. 2022). Four reactions must occur while converting SDG to ENL (Senizza et al. 2020). SDG is first transformed to SECO by *O*-linked deglycosylation, and then SECO is converted to the intermediate dihydroxyenterodiol (DHEND) via *O*-linked demethylation (Seyed-Hameed et al. 2020). From here, DHEND may be transformed to END by dehydroxylation, and then to ENL via END dehydrogenation. Alternatively, DHEND can be dehydrogenated to create a lactone ring, resulting in the formation of a second intermediate dihydroxyenterolactone (DHENL), which can subsequently be dehydroxylated to generate ENL (Ruiz de la Bastida et al. 2021; Yoder et al. 2015). ENL, the primary mammalian enterolignan generated in the rumen, is transported into physiological fluids, possibly benefiting human health in terms of menopausal symptoms, hormone-dependent malignancies, cardiovascular disease, osteoporosis, and diabetes (Schogor et al. 2014; Seyed-Hameed et al. 2020). Kuijsten et al. (2006) evaluated the enterolignan pharmacokinetics in healthy men and women taking a single dose of purified SDG (1.31 µmol/kg body weight) and reported that enterolignans were detected in plasma 8 to 10 hours following intake of pure SDG. END and ENL had a maximum plasma concentration after 14.8 h and 19.7 h of SDG ingestion, respectively and the mean elimination half-life of END (4.4 h) was less than that of enterolactone (12.6 h). The pharmacokinetics of the SDG supplemented through different food systems are yet to be identified.

Microorganisms involved in the conversion of SDG to END and ENL

Several researchers have assessed the ability of different gut bacteria to carry out the processes required for the conversion of glycosylated lignans to enterolignans (Senizza et al. 2020; Taibi et al. 2021). *Bacteroides fragilis*, *Bacteroides ovatus*, *Clostridium cocleatum*, *Clostridium saccharogumia*, *Clostridium ramosum*, and *Bacteroides distasonis* have all been found to be capable of doing *O*-deglycosylation, with the first four able to completely deglycosylate SDG within 20 hours of the experiment (Yoder et al. 2015). Deglycosylated SECO then undergo demethylation to create its intermediary DHEND. *Butyribacterium methylotrophicum*, *Eubacterium callanderi*, *Eubacterium limosum*, *Clostridiaceae bacterium* END-2 (commonly known as 'strain END-2') and *Blautia producta* were shown to be capable of catalysing this process (Clavel et al. 2006a; Jin and Hattori, 2010). Following demethylation, *Eggerthella lenta* and *Clostridium scindens* can act on the intermediate to produce END by removing a hydroxyl group from each aromatic ring (Clavel et al. 2006b). *Lactonifactor longoviformis* and the above mentioned 'strain END-2' finish ENL synthesis by forming the lactone ring (Jin and Hattori, 2010). Alternatively, following SECO demethylation, *L. longoviformis* may produce the lactone ring, in which case END does not develop but rather a second intermediate, DHENL, which may then go on to make ENL (Yoder et al. 2015).

Figure 1 depicts the potential metabolism for converting SDG to END and ENL, as well as the microorganisms engaged in these processes. Bacteria generate both END and ENL enantiomers, and human exposure to the enantiomers arises from the interplay between the initial type of substrate and the makeup of the bacterial consortia (de Silva and Alcorn, 2019). When individuals consume their normal diets, (-) ENL predominates in serum, but when supplemented with flaxseed, (+) ENL increases significantly while (-) ENL increases very little. Furthermore, the types of END and ENL extracted after incubating SDG with gut bacteria was (+) END and (+) ENL (Saarinen et al. 2010). McCann et al. (2021) explored the connections between gut microbiota and lignan metabolism. For six weeks, 252 healthy postmenopausal women were given ten grams of ground flaxseed every day. Microbial colonies were discovered in urine and stool samples. There were *Slackia*, *Senegalimassilia*, *Klebsiella* and *Lactobacillus* present, all of which were linked to ENL production. Bacteria previously associated to colorectal cancer and cardiovascular disease such as *Pyramidobacter*, *Odoribacter* and *Fusobacteria*, were shown to be significantly reduced in the FS intervention.

Extraction and Isolation of SDG from Flaxseed

SDG is further polymerized (or oligomerized) in flaxseed, where it exists as part of a bigger complex comprised of five SDG residues linked by ester linkages to four 3-hydroxy-3-methylglutaric acids (Dauwe et al. 2021). SDG (35%), cinnamic acid glycosides, and

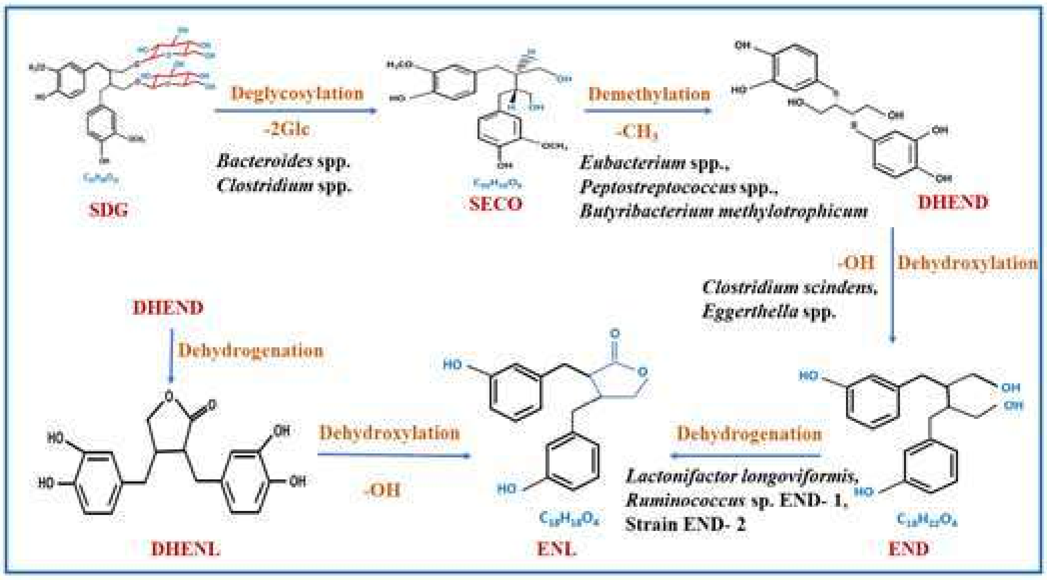

Fig. 1 Potential metabolism for converting SDG to END and ENL and the microorganisms engaged in these processes (Plotted according to the data obtained from Yoder et al. (2015)

hydroxymethyl glutaric acid (HMGA) are common components of this lignan complex (Hosseinian and Beta, 2009). The structure of SDG oligomer is illustrated in Figure 2.

As reported by Eliasson et al. (2003) these ester linkages may be readily and selectively broken by alkaline hydrolysis to produce SDG. To separate additional lignans from flaxseed, the glycosidic link of SDG must be broken.To separate this lignan complex from flaxseed, a simple method is usually required, while further procedures are required to isolate SDG from the complex. The lignans and gums (viscous soluble fibre) in flaxseed are mostly found in the hulls that surround the seeds, whereas the bulk of the proteins and lipids are found in the kernel/embryo. Carbohydrates (48.3 %), proteins (16.8 %), crude oil (26.5 %), moisture (5.0 %), and ash (3.5 %) are the chemical constituents in flaxseed hulls, while, on a dry basis the embryo fraction includes 22.0 % carbohydrates, 23.9 % proteins, 47.7 % crude oil, 3.6 % moisture, and 3.8 % ash (Hosseinian and Beta, 2009). The hull is divided into two distinct fractions, the mucilage fraction and the fibre fraction. The outer layers of hull comprises the mucilage portion and is rich in water soluble carbohydrates whereas the inner layers of hull forms the fibre fraction and is particularly rich in insoluble fibres and lignans. SDG is a component of mucilage fraction. The methods for the extraction of lignan complex make use of SDG's solubility in alcohol and water. The solvent-to-meal ratio during extraction ranges from 5:1 to 7:1 and from 12:1 to 16:1 (Hosseinian and Beta, 2009). For base hydrolysis in water or alcohol to release SDG, sodium hydroxide or calcium hydroxide are typically used (Zhuang et al. 2021). SDG must be released from its polymeric lignan precursor by breaking the ester-linkages in the complex. The SDG concentrates produced with calcium hydroxide easily separate from insoluble calcium salts, yielding a non-hygroscopic and relatively pure product. The base is generally used at a concentration of about 1 normal, and it is preferably used at a concentration of approximately 3-7 percent w/v (Hosseinian and Beta, 2009). The hydrolysis process is

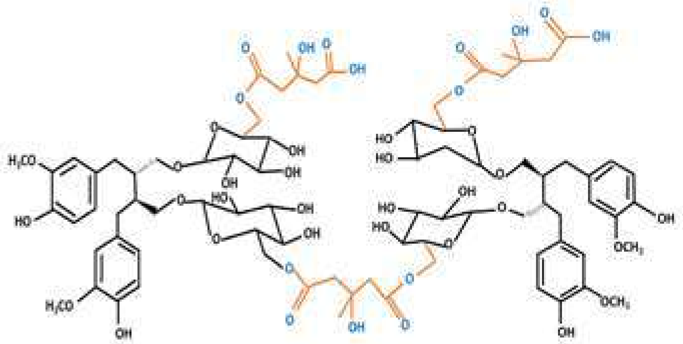

Fig. 2 Structure of SDG oligomer containing SDG and 3 hydroxy-3-methyl-glutaric acid (HMGA) units (shown in different colour)

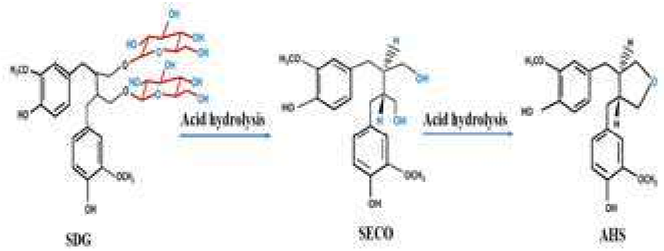

Fig. 3 Conversion of secoisolariciresinol diglucoside (SDG) to anhydrosecoisolariciresinol (ASH)

typically carried out over a period of 4 to 24 hours (Zhuang et al. 2021). This procedure also destroys the cyanogenic glycosides, resulting in an extract free of cyanogenic glycosides or free cyanide. Apart from alkaline hydrolysis, acid and enzymatic hydrolysis are described in the literature for extraction of SDG (Renouard et al. 2010; Sicilia et al. 2003). The stability of acid hydrolysis products has lately been questioned, because under acidic environment SECO can be partly converted to its anhydrous form anhydrosecoisolariciresinol (AHS) which is also called as shonanin (Lehraiki et al. 2010). The conversion of SDG to AHS is shown in Figure 3. The enzymatic technique is a gentle and selective procedure, but it does not provide full lignan hydrolysis since these metabolites concentrate in the seed's highly resistant layer (Lehraiki et al. 2010).

Eliasson et al. (2003) compared two different methods for the extraction of SDG. In the first method the SDG complex was extracted using a mixture of 1,4-dioxane - 95% aq. ethanol (1:1 v/v). The alcoholic extract was then subjected to alkaline hydrolysis using 0.3 *M* aqueous sodium hydroxide in order to isolate SDG from SDG-HMGA complex. In other method defatted flaxseed flour was subjected to direct alkaline hydrolysis using 2 *M* NaOH and distilled water. The authors reported that the direct alkaline hydrolysis resulted in higher yield as compared to the other method. Hosseinian and Beta (2009) attempted to extract SDG with just water followed by direct alkaline hydrolysis in an attempt to eliminate the usage of organic solvents described in prior techniques and to achieve a product free of solvent residues. This technique was claimed to be appropriate for extracting SDG from flaxseed hull in large amounts with high purity for nutritional supplements or nutraceutical applications. Base hydrolysis is generally performed at temperatures above room temperature (50 to 100 °C). A greater temperature is required to separate SDG from bigger molecular weight components like protein and starch residues, which are coagulated and precipitated by heat. The pH range is 10 to 13, with 11.8 to 12.5 being the ideal range. The pH of the solution must be acidified to a pH range between 3 and 8.5 after hydrolysis to prevent the ionisation of any functional groups in the aliphatic and aromatic parts of the SDG molecule. Apart from the traditionally applied methods for SDG extraction, later the efficiency of microwaves and ultrasound for SDG extraction was carried out (Beejmohun et al. 2007; Corbin et al. 2015; Nemes and Orsat, 2011; Zhang and Xu, 2007). Microwave assisted extraction (MAE) for 3 min with 1 *M* sodium hydroxide alkaline treatment was reported to produce the highest quantity of SDG (16.1 mg/g), p-coumaric acid glucoside (3.7 mg/g), and ferulic acid glucoside (4.1 mg/g), regardless of irradiation power proving MAE as an efficient method for SDG extraction in improving SDG yield as well as in saving time and energy (Beejmohun et al. 2007). Similarly Corbin et al. (2015) developed an efficient ultrasound assisted extraction (UAE) method for SDG. The UAE technique has been shown to be extremely effective for reducing mucilage entrapment of flaxseed phenolics because deep modification of the seedcoat ultrastructure and mucilage release occurs during ultrasonic treatment. According to Corbin et al. (2015) the conditions which were found to be optimal for UAE of SDG from flaxseeds include water as solvent supplemented with 0.2 N sodium hydroxide for alkaline hydrolysis of the SDG–HMGA complex, an extraction time of 60 minutes at a temperature of 25 °C, and an ultrasound frequency of 30 kHz. Under these conditions the yield of SDG was reported as 23.6 mg/g on dry basis. In their study, Thomas et al. (2023) assessed the efficacy of various extraction methods, including direct alkaline hydrolysis coupled with magnetic stirring, microwave, and ultrasound, in extracting SDG from defatted flaxseed. The extraction methods employed yielded SDG quantities ranging from 11.74 to 14.30 mg g^{-1} flaxseed on a dry matter basis. The optimal production of SDG was found to be achieved by the utilization of direct alkaline hydrolysis (using a 1 M aqueous NaOH solution) in conjunction with magnetic stirring (at a rate of 400 rpm for 1 hour at a temperature of 60 °C).

Supercritical carbon dioxide (SC-CO_2) is a non-toxic and cost-effective solvent. It can extract polar phenolic compounds from plant components in conjunction with polar modifiers. This extraction occurs at temperatures and pressures above the critical point of carbon dioxide, which is 31 °C and 7.4 MPa, respectively. Comin et al. (2011) conducted a study to evaluate the effect of the supercritical carbon dioxide method on flaxseed SDG extraction. The analysis revealed that the optimal conditions for extracting SDG were 7.8 mol% ethanol, 45 MPa pressure, and 60 °C temperature. Nevertheless, using SC-CO_2 extraction resulted in substantially less SDG than conventional extraction techniques. Supercritical antisolvent fractionation (SAF), which uses the non-polarity of CO_2 to precipitate certain chemicals from a solution, is another attractive but understudied method. According to Perretti et al. (2013), SAF produced maximum lignan content of 12.96 g L^{-1} when treated for 180 min at a pressure of 30 MPa and a flow rate of 15 kg/h of CO_2.

Pressurized low polarity water (PLPW) extraction, also known as subcritical water extraction, is a technology that modifies the properties of water to improve its extraction ability by heating the water to temperatures of up to 374 °C and maintaining the pressure at a level high enough to keep the water in a liquid state. When water is heated from 25 to 200 °C, its dielectric constant drops from 79 to 35, approaching values close to those for ethanol or methanol. At 25 °C, the polarity of pure water is about the same as that of water-methanol or water-acetonitrile combinations (Cacace and Mazza, 2006). The feasibility of extracting lignans from flaxseed meals using pressurized low-polarity water (PLPW) was evaluated in a fixed bed extraction cell. Maximum lignan extraction was obtained at a high temperature (180 °C), flow rate of 0.6–2 mL/min, high pH (9) and a co-packing ratio of 1:1.5 meal to glass beads (Ho et al. 2008). In another investigation, the maximum yield (12.94 mg/g) and extraction yield (72.57%) were obtained using 5 g of flaxseed meal sticks at 180 °C for 15 minutes, 1.500 pressure, and 40% fresh water (Ozkaynak-Kanmaz and Ova, 2013).

Following extraction, the hydrolysate is concentrated by a rotary evaporator before being subjected to either a liquid/liquid partition, such as an ethyl acetate/water system, or an anion exchange to enrich the lignans further. The resulting lignan-enriched solution is subjected to chromatographic separation to extract lignans with a purity higher than 90%. To separate SDG from other contaminants, the SDG-containing hydrolysate is passed through a glass column filled with Sephadex anion exchange resin or C-18 reverse-phase resin (Hosseinian and Beta,

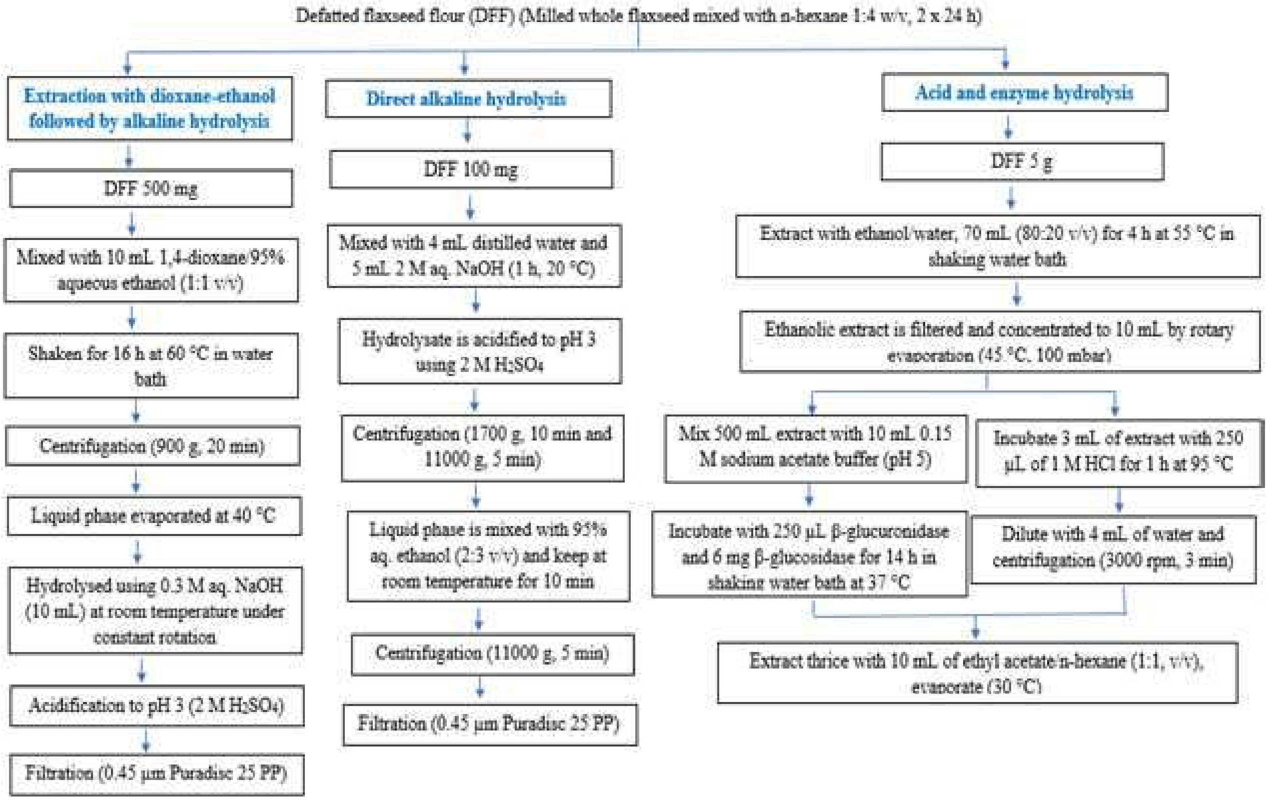

Fig. 4 Different methods for extraction of SDG (Eliasson et al. 2003; Johnsson et al. 2000; Sicilia et al. 2003)

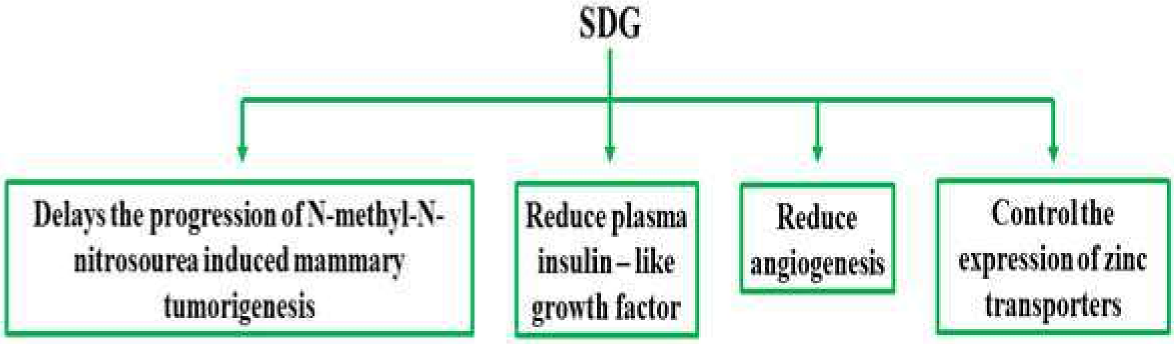

Fig. 5 Potential impact of SDG in breast cancer prevention

2009). The SDG is recovered by evaporation through freeze, spray, or vacuum drying. In a study, SDG was predicted to be recovered using lyophilization at a purity of 31% and yield of 3.2% by weight from defatted flaxseed with a 90% recovery rate (Dobbins and Wiley, 2004). Steps for extracting SDG from whole flaxseed by direct alkaline hydrolysis, acid and enzyme hydrolysis and extraction with dioxane ethanol followed by alkaline hydrolysis are illustrated in Figure 4.

Health benefits with consumption of the flax lignan secoisolariciresinol diglucoside

In human clinical trials and animal models, research on the health effects of whole flaxseed or flaxseed products has shown positive changes in blood lipid profiles, diabetes, and inflammation, as well as protection against certain types of cancer (Hu et al. 2022). At least three health-promoting components are present in flaxseed: soluble fibers or mucilage (approximately 6% of dry weight); high levels of polyunsaturated fatty acids (73% of the total fatty acid), especially α-linolenic acid, ω-3 PUFA (approximately 20% of dry weight); and the plant lignan secoisolariciresinol diglucoside. Many of the health benefits of flaxseed are primarily attributable to lignan, specifically SDG.

Cancer

Lignans are phytoestrogens found in nature and have health benefits for many diseases, including cancer. Several *in vivo* studies (Chikara et al. 2017; Ren et al. 2016; Scherbakov et al. 2021) have linked the anticancer effects of flaxseed to its main lignan, secoisolariciresinol diglucoside. *In vitro* investigations of breast, colon, and prostate cancers demonstrate that ENL inhibits tumor growth. Several studies suggest that ENL's antiproliferative capability results from its effects on the cell cycle and cell death induction (Tannous et al. 2020; Xiong et al. 2015). The cytotoxic effects of ENL appear to be specific to cancer cells. ENL (25–75 mM for 24 and 48 hours) inhibited the proliferation of human prostate cancer cells without affecting the vitality of healthy prostate epithelial cells (Chen et al. 2007).

Dietary components like SDG can increase mammary gland differentiation early and prevent breast cancer (Kezimana et al. 2018). SDG concepts on breast cancer prevention are shown in Figure 5. The advancement of N-methyl-N-nitrosourea-induced mammary tumorigenesis causes cancer. SDG slows the progression of N-methyl-N-nitrosourea by altering terminal end-bud differentiation (Rickard et al. 2009; Tan et al. 2004). SDG decreases plasma insulin-like growth factor I, decreasing breast cancer risk (Rickard et al. 2000). Zinc is abundant in breast cancer tissues. SDG can regulate the expression of Zn transporters (Zhang et al. 2008a). Finally, the vascular endothelial growth factor increases angiogenesis, which helps cancer development. END and ENL may prevent breast cancer by decreasing angiogenesis (Jungeström et al. 2007). Numerous *in vivo* and *in vitro* studies show that cancer cells, particularly breast cancer cells, express P-glycoprotein (P-gp) (Zhang et al. 2021). Morsy et al. (2020) mitigated P-gp-induced cancer using secoisolariciresinol (SECO). SECO, and secoisolariciresinol-4',4"-diacetate, derivatives of SDG suppressed breast cancer cell growth (Scherbakov et al. 2021). Also, SDG decreased mouse tumor volume by inhibiting nuclear factor-kappa B (Bowers et al. 2019). Chen et al. (2009) examined the effects of whole flaxseed (100 g/kg diet) and SDG (1 g/kg diet) on breast cancers in athymic postmenopausal mice. Whole flaxseed and SDG reduced palpable tumour size by enhancing apoptosis. Shah and Patel (2016) found that male Sprague-Dawley rats given 500 mg/kg SDG-rich *L. usitatissimum* extract for 18 weeks protected type 2 diabetes-related colon cancer.

Cell cycle regulatory genes in lung tissue are dramatically altered by lignan-rich flaxseed (Lim et al. 2021). In animal models, flaxseed protects the lungs against oxidative damage and inflammation, two significant factors in lung cancer growth and propagation. Chikara et al. (2017) found that ENL is a promising adjuvant therapy for lung cancer (Figure 6). ENL stops lung cancer cell proliferation in the G1 phase, the first of four cell cycle stages in eukaryotic cells (Tannous et al. 2020).

Lipid profile and cardiovascular health

Estrogen and phytoestrogens regulate cardiovascular disease during postmenopause (Rietjens et al. 2016). According to the third National Cholesterol Education Program report, 20–30 g of fibre and 5–10 g of soluble fraction daily lowers saturated fat and cholesterol absorption (Prim et al. 2012). Aqueous flaxseed extract was tested for anti-hepatotoxicity in Albino rats. One hundred grams of ground seeds were suspended in 500 mL of distilled water and filtered after 24 hours. The study used the concentrated extract after desiccating it in an oven at 30 °C for 24 hours. Supplementing 400 mg/kg of defatted flaxseed aqueous extract for 30 days enhanced liver function markers (Mushatet and Jawad, 2020). Okhti et al. (2016) studied the effects of dietary flaxseed-derived lignan on fatty liver disease in rabbits. 40 mg/kg/day of extracted pure flax lignan for 14 days reduced inflammatory cells in clogged blood vessels and sinusoids and moderate fibrosis in rabbit liver tissue's portal region around the bile ducts.

Inflammation

SDG helps to prevent oxidative stress and inflammation in preclinical models of diabetes and heart disease (Parikh et al. 2019). Pietrofesa et al. (2016) tested flaxseed lignan for asbestos-induced acute inflammation in mice. Asbestos-exposed mice fed a control diet had acute inflammation with increased peritoneal lavage fluid (PLF), WBCs, and pro-inflammatory cytokines, but those fed flaxseed lignans had a significant decrease. SDG reduced systemic inflammatory response-induced blood-brain barrier leakage and leukocyte adhesion and motility in aseptic encephalitis. SDG directly suppresses inflammatory cell-blood-brain barrier interactions and leukocyte inflammation (Rom et al. 2018).

Postmenopausal symptoms

As shown in Figure 7, flaxseed lignan inhibits postmenopausal symptoms by mimicking estrogen. However weakly, END and ENL bind estrogen receptors α and β due to their structural similarity to 17-β-estradiol. Mammalian lignans preferentially bind ERα (Sacco et al. 2011). ERα in osteoblasts, osteoclasts, and their progenitors regulates bone turnover genes (Sacco et al. 2011). ENL and 6-hydroxy-ENL prefer ERα over Erβ (Adlercreutz, 2007). Kim et al. (2002) investigated postmenopausal women's urine phytoestrogen excretion and bone mineral density. In 88 postmenopausal women, urine phytoestrogen metabolites were linked with bone mineral density (BMD). BMD favourably correlates with urine enterolactone, the metabolic end product of flaxseed lignan, which inhibits menopausal osteoporosis.

Sturgeon et al. (2011) administered postmenopausal women 15 g of powdered flaxseed daily for six weeks to test its influence on diabetes. Flaxseed supplementation affects diabetes markers IGF-1, IGF-BP3, and C-peptide. Lemay et al. (2002) found that flaxseed

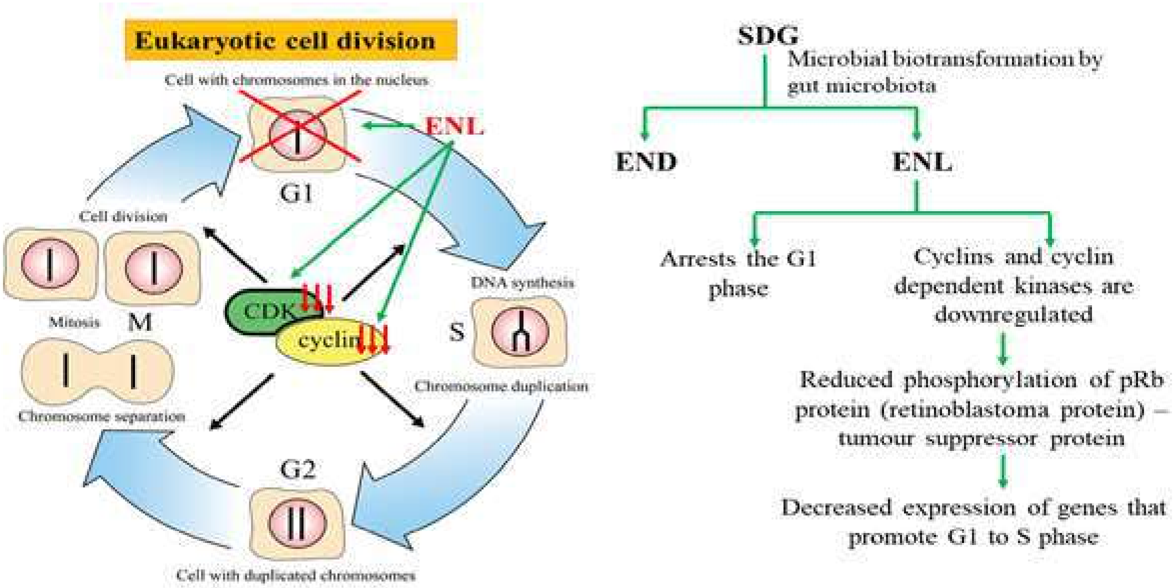

Fig. 6 Possible mechanisms for the anticarcinogenic property of ENL derived from SDG

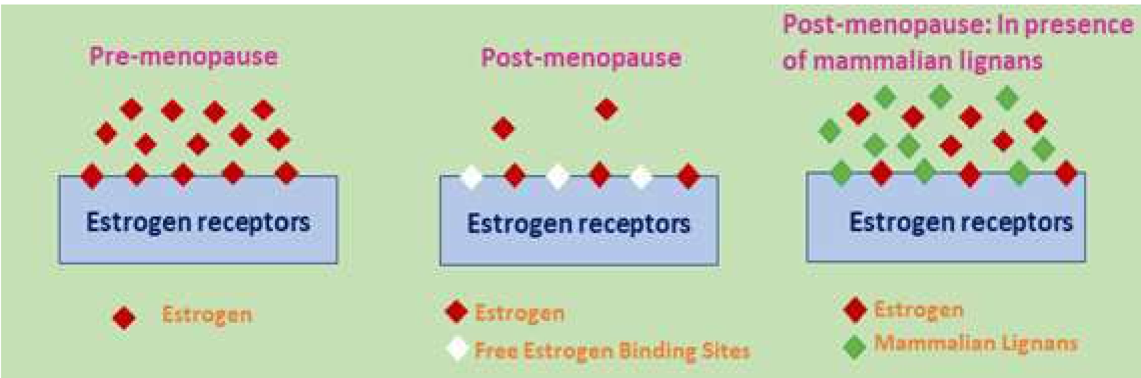

Fig. 7 Binding of mammalian liganans to estrogen receptors in absence of estrogen

reduces glucose and insulin levels and moderate menopausal symptoms. Thirty-eight women were fed bread with 25 grams of ground flaxseed and 46 milligrams of lignan for three months to compare menopausal symptoms. After three months, flaxseed significantly reduced hot flashes and KMI (Simbalista et al. 2010). Hallund et al. (2008) supplemented healthy postmenopausal women's diets with 500 mg of flaxseed lignan complex daily for six weeks to evaluate inflammatory markers. C-reactive protein, a liver-released inflammation marker, fell dramatically in the test individuals after the study. The effect of SDG in alleviating various diseases and disorders are summarized in Table 1.

Application of flaxseed lignan in dairy and bakery products

As whole seeds or flaxseed powder, flaxseeds have long been used in baking and other culinary products, but their use in milk and milk products is restricted due to their distinct flavor. Milk has demonstrated that it is an effective matrix for developing functional foods. Bioactive components, such as phytosterols, peptides, and omega-3 fatty acids, are commonly added to dairy products (Hyvärinen et al. 2006a). Given the health benefits of flaxseed lignans, developing functional dairy products based on flaxseed lignan supplementation will be an exciting strategy. In addition, milk is well known for promoting bone health. The casein-derived bioactive peptide VLPVPQK has been shown to stimulate osteoanabolism *in vitro* and in an ovariectomized rat model of osteoporosis (Mada et al. 2018). Thomas et al. (2023) evaluated the effect of SDG on the growth of probiotic bacteria *Lactiplantibacillus plantarum* for its possible use for the development of SDG enriched fermented milk. A fermentation dynamics investigation was conducted on buffalo milk samples with varying concentrations of lignan (0 to 400 mg of SDG per 100 mL of milk) and co-cultures comprising *Lactiplantibacillus plantarum*, *Streptococcus salivarius* ssp. *thermophilus*, and *Lactobacillus delbrueckii* ssp. *bulgaricus*. The findings of the study indicate that the fermentation dynamics of *Lactiplantibacillus plantarum* and the starter cultures in the milk were not influenced by SDG. They also evaluated the effect of SDG enriched fermented milk in alleviating postmenopausal osteoporosis. A functional set *dahi* with a desirable probiotic (*Lactiplantibacillus plantarum* A5) count of 9.36 log CFU/mL and excellent techno-functional attributes (DPPH: 41.95% RSA, firmness: 485.49 g, sensory overall acceptability: 8.51) was developed to contain 260 mg of SDG in 20 g of *dahi*. The

Table 1: Functional relevance of SDG

Experiments	Targeted health benefit	Key findings	Reference
Defatted flaxseed aqueous extract 400 mg/kg body weight was administered orally to Albino rats for 30 days	Cholesterol	Administration of defatted flaxseed aqueous extract orally for 30 days increased liver function indicators including Alanine transaminase (ALT), Aspartate transaminase (AST), and Alkaline phosphatase (ALP)	(Mushatet and Jawad, 2020)
Rabbits were administered with 40 mg/kg/day of extracted pure flax lignan for 14 days	Fatty liver disease	The flaxseed lignan lowered inflammatory cells in clogged blood arteries and sinusoids, as well as in mild fibrosis in the portal region around the bile ducts of rabbit liver tissue	(Okhti et al. 2016)
SDG rich extract (500 mg/kg) was administered orally to rats for 18 weeks	Diabetes and colon cancer	SDG-rich extract of *L. usitatissimum* had a chemopreventive impact on colon cancer linked with type 2 diabetes mellitus, which might be mediated by CDK4 inhibition	(Shah and Patel, 2016)
Mice induced with ulcerative colitis were administered with SDG in doses of 100 and 200 mg/kg/day orally	Anti-inflammatory activity	SDG is effective against inflammatory bowel diseases such as ulcerative colitis	(Xu et al. 2016)
Patients aged between 45-75 years were supplemented with 100 mg and 200 mg lignan rich extract of flaxseed hulls (LinumLife EXTRA) for 8 weeks	Benign Prostatic Hyperplasia	Flaxseed hull extract supplementation offered better alleviation in obstructive symptoms of BPH, such as the sensation of incomplete bladder emptying, "stopping and beginning" when urinating, a weak urine stream, and "straining while urinating".	(Simons et al. 2015)
Estimating antidepressant-like effect of SDG (160 mg/kg) in ovariectomized mice subjected to unpredictable chronic stress	Antidepressant activity	• Chronic stress-induced increases in the serum corticosterone and adrenocorticotropic hormone were reversed by treatment with SDG • SDG's behavioural effects in ovariectomized mice may be connected to their regulating effects on the neuroendocrine-immune network and neurotrophin factor expression	(Ma et al. 2013)
Rats were fed with control diet (NC), control diet with 0.02% SDG lignan- enriched flaxseed powder (NCL), high-fructose and fat diet (HFD) and high-fructose and fat diet with 0.02% SDG lignan-enriched flaxseed powder (HFDL) for 12 weeks	Lipid profile, hypertension	• The total cholesterol in mg/dl for NC, NCL, HFD and HFDL were 69, 67, 70 and 63, respectively. The LDL cholesterol in mg/dl for NC, NCL, HFD and HFDL were 10, 11, 17 and 9, respectively and the HDL cholesterol in mg/dl for NC, NCL, HFD and HFDL were 39, 38, 27 and 35, respectively. • SDG-lignan supplementation could lower systolic blood pressure by 45% in the rats fed the HFDL diet compared to the rats on the HFD diet	(Park and Velasquez, 2012)
Feeding of basal diet and basal diet supplemented with 1 g SDG per kg diet for 8 weeks to mice	Breast tumor	• SDG metabolites, enterolactone and enterodiol are structurally similar to human estrogen (17β-estradiol), they have binding affinity to estrogen receptors (ER) and thus may modulate hormone-related diseases such as breast cancer. • SDG have been shown to attenuate tumorigenesis through reduction in cell proliferation and angiogenesis, as well as an increase in apoptosis via modulation of the estrogen receptor and growth factor-signaling pathways	(Truan et al. 2012)

Supplemented 15 g of ground flaxseed per day to postmenopausal women for 6 weeks	Diabetes	Flaxseed supplementation impacted circulating levels of IGF-1, IGF-BP3, or C- peptide which are markers of diabetes	(Sturgeon et al. 2011)
Evaluating the effects of SDG (100 mg/day) intake on hypercholesterolemia and liver disease risk factors in moderately hypercholesterolemic men	Hepatoprotective and anti-cholesterolemic activity	SDG reduces blood- cholesterol levels and also reduces the risk of liver disease	(Fukumitsu et al. 2010)
Antibacterial properties of SDG isolated from Indian flaxseed cultivars were evaluated	Anti-bacterial activity	Inhibition of bacterial diseases caused by *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Agrobacterium tumefaciens*, *Bacillus cereus* and *Escherichia coli*	(Rajesha et al. 2010)
Basal diet of mice in postmenopausal condition was supplemented with SDG (1g/kg diet) for 8 weeks	Breast tumor	SDG significantly reduced the tumor growth primarily through reducing tumor cell proliferation rather than increasing apoptosis.	(Saggar et al. 2010)
38 postmenopausal women were fed with 25 g of ground flaxseed containing about 46 mg lignan for 3 months in the form of bread	Postmenopausal symptoms	Flaxseed significantly reduced hot flashes and Kupperman Menopausal Index (KMI)	(Simbalista et al. 2010)
Compared the effects of whole flaxseed (100 g/kg diet), SDG (1 g/kg diet), and flaxseed hull (18 g/kg diet) on breast tumour development in postmenopausal athymic mice.	Breast tumour	Whole flaxseed and SDG significantly reduced ER- and growth factor-related biomarkers, indicating that down regulation of the ER- and growth factor-mediated cell signalling pathway is the optimal mode of action for both whole flaxseed and SDG	(Chen et al. 2009)
Sixty-two hypercholestrolemic postmenopausal women were fed with 40 g/day of flaxseed powder in baked products	Cholesterol	Both total cholesterol and LDL cholesterol decreased significantly at 5^{th} week by 8% and 11%, respectively	(Bloedon et al. 2008)
Low-fat muffins supplemented with 500 mg SDG were fed for 6 weeks to postmenopausal women	Inflammation	Significant decrease (0.88 to 0.80 mg/L) in C-reactive protein (CRP) was observed in test women	(Hallund et al. 2008)
Administered 30 g flaxseed in bread and muffins to postmenopausal women for 3 months	Cholesterol	Observed reduction in total cholesterol and LDL cholesterol by 7% and 10%, respectively and 22% reduction in lipoprotein (a) which is a predictor of atherosclerosis.	(Patade et al. 2008)
Patients were administered with 300 and 600 mg SDG in the form of a lignan-enriched flaxseed extract over a period of 4 months	Lower urinary tract symptoms	The International Prostate Symptom Score (IPSS) of treatment groups were significantly decreased and were reported to be as -3.67 (control), -7.33 (300 mg SDG) and -6.88 (600 mg SDG). The QOL score (quality of life, a subjective score evaluated as part of LUTSs) was also observed to be improved in treatment groups than control.	(Zhang et al. 2008b)
Determining the efficacy of SDG (20 mg/kg) in a hypercholesterolemic myocardial infarction rat model	Angiogenic and cardioprotective activity	Provides protection against cardiovascular diseases	(Penumathsa et al. 2007)
Investigating the effect SDG (360 mg lignan per day) on glycemic control, lipid profiles and insulin sensitivity in type 2 diabetic patients	Anti-diabetic activity	Daily supplementation of SDG improved glycemic control in type 2 diabetes individuals in a small but statistically significant way, without altering fasting glucose, lipid profiles, or insulin sensitivity	(Pan et al. 2007)

developed product was administered to ovariectomized (OVX) rats. According to the study ovariectomy decreased serum calcium, estrogen, and bone ash calcium levels by 32.27, 30.95, and 48.46 percent, respectively, compared to control group, while daily administration of SDG-enriched *dahi* (20 g) for eight weeks restored them. The proximal tibial metaphysis and distal femoral epiphysis micro-CT study showed that the ovariectomy lowered bone mineral density (BMD) by 11.06% and 9.18%, respectively, and lowered Trabecular thickness (Tb. Th) by 12.66% and 11.86%, respectively, while increasing Trabecular separation (Tb. Sp.) by 90.69% and 87.70%, respectively, compared to the sham control-group rats. SDG-enriched *dahi* improved BMD by 16.06 and 12.24% and Tb. Th by 35.32 and 19.62%, respectively, and decreased Tb. Sp by 47.04 and 47.22%, respectively, in OVX rats. The results suggest that the developed set *dahi* may help treat postmenopausal osteoporosis (Thomas et al. 2024).

Some studies have examined the effect of flaxseed lignan on the physicochemical and sensory properties of dairy products. In one such study, (Jeong et al. 2017) prepared bioactive *kefir* with added flaxseed extract at 1% to 3%. They reported that the pH and sensory scores were not substantially altered by the addition of flaxseed extract in comparison to control *kefir*. The effect of supplementation with 3.75 percent flax lignan on various properties of *misti dahi* made by substituting 10% honey for sugar was analyzed (Paul et al. 2016). The developed product had the same pH, moisture, acidity, total solids, and whey syneresis values as the control and 20-day shelf life. In addition, they discovered that 100 g/mL of lignan-enriched *misti dahi* inhibited -amylase by 48.41%, compared to 17.54% in the control group. This suggests that flax lignan-enriched *misti dahi* may be an anti-diabetic agent to enhance human health and treat diabetes. In another investigation, Hyvärinen et al. (2006a) investigated the stability of SDG when it was added to cold- and hot-dairy products such as milk, cheese, yoghurt, and whey drinks. SDG was not adversely affected by fermentation, pasteurisation, or chilling.

Manufacturers are increasingly incorporating polyunsaturated fatty acids (PUFA), rich in omega-3 fatty acids, into their formulations to improve the nutritional value of processed foods. Conversely, these components are susceptible to oxidation reactions, which can result in rancidity and potentially toxic substances. To mitigate this issue, Matumoto-Pintro et al. (2011) added flaxseed lignan extract (50–200 mg SDG/kg beverage) to dairy beverages enriched with PUFA, which were then tested for their resistance to light- and heat-induced oxidation. Initial concentrations of propanal and hexanal in dairy beverages containing SDG were reduced by 87% and 58%, respectively, indicating that flaxseed lignan offered protection against light- and heat-induced oxidation during the preparation of PUFA-enriched dairy beverages. According to the report, the optimal SDG concentration to prevent heat- and light-induced lipid oxidation in dairy beverages is less than 50 mg/L.

Popular in Chinese cuisine, the Chinese steamed bun, also known as *Mantou*, is a variety of steamed bun or bread-like items (Laohasongkram et al. 2011). It is made with wheat flour, water, and a leavening agent such as yeast or traditional sourdough. The aromatic compounds, such as aldehydes produced in the product as a result of enzymatic or spontaneous oxidation of the wheat flour fraction during fermentation, are desirable because they affect the overall aroma profile of bread. However, their excessive production during processing can result in off-flavors (Zhang et al. 2016). (Hao and Beta, 2012) analyzed the antioxidative potential of flaxseed hull extract in steamed Chinese bread. The addition of flaxseed hull extract (1 g extract/100 g flour) increased the total phenolic content of the product by 138.34% and the antioxidant activities in terms of DPPH activity and ORAC values by 90.69% and 67.43%, respectively; therefore, flaxseed hull extract can be developed as a functional food ingredient to improve the phytochemical content and antioxidative potential of refined flour products, such as steamed bread. The amount of lignan or flaxseed added to the bread, the type of flaxseed used, and the type of bacterial or yeast cultures used in the leavening process are all factors that influence the amount of SDG in bread. A significant amount of SDG (73-75%) was recovered from the samples, both in free and complex forms, demonstrating that SDG could tolerate heat during the milling, fermentation, and cooking procedures (Muir and Westcott, 2000). Similarly, bread containing whole flaxseed and defatted flaxseed flour preserved a significant level of lignan content following the dough, proofing, baking, and storing steps (Hyvarinen et al. 2006b).

Conclusion

Lignans forms a large group of phytoestrogens with chemical structures characterized by C_6 - C_3 units linked by a β-β' (8-8') carbon bond and multiple mechanisms of action. Lignans are ER ligands that act like estrogens in some tissues, such as mammary, ovary, prostrate, bone, cardiovascular tissue etc. Many estrogenic herbs have been used in Asian countries for thousands of years to treat various diseases and flaxseed lignan is an emerging nutraceutical ingredient capable for the prevention and treatment of diseases like cancer, diabetes, hyperlipidaemia, cardiovascular diseases, postmenopausal related diseases and so on. This review concludes that flaxseed lignan consumption can significantly reduce these diseases to a large extent. Further studies are necessary for exploring low-cost extraction and isolation techniques and also to evaluate the effect of incorporation of flaxseed lignan to different dairy and food products.

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Conflict of interests

The authors declare that they have no conflict of interests.

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