Flaxseed Lignans: Clinical, Industrial, Food Applications with a Major Focus on the Different Extraction Methodologies to Isolate Lignans Enriched with SDG (Secoisolariciresinol Diglucoside)

Jasmeet Kour[1]*, D.C. Saxena[2] and Sukhcharan Singh[3]

[1,2,3]Department of Food Engineering and Technology, Sant Longowal Institute of Engineering and Technology, Longowal, 148 106, District Sangrur, Punjab.
*E-mail: *eru.jassi@gmail.com*

Abstract—*Due to the ever increasing market demand for healthy foods with elevated levels of fiber and various other biologically active substances, the incorporation or the inclusion of such health promising ingredients have played a very pivotal role in the form of various food formulations. Out of all these nutraceutical components, one of the most promising ones are the lignans. These are mainly present as aglycones, glycosides, esterified glycosides or as bio-oligomers. Flaxseeds are the most significant source of lignans as its content is 800 times higher than the other food sources and it provides lignan precursor such as SDG (Secoisolariciresinol diglucoside). Lignans primarily, SDG interfere with the development of different types of diseases like cardio-vascular, diabetic, lupus nephritis, bone, kidney, menopause, mental stress, immunity, aetherosclerosis, hemopoietic, liver necrosis and urinary disorders. Lignans have proven to be prominent anti-inflammatory, antioxidant, antimutagenic, antimicrobial, antiobesity agents. Owing to its pharmacological properties, the lignans especially isolated from flaxseeds have been increasingly used by the food and other industries. The anti-estrogenic potential of flaxseed based lignans has been very well postulated to protect against hormone sensitive cancers such as breast, prostate and colon cancer. The utilization of lignans has been well documented in food applications also as they have been proven to be quite effective in replacing synthetic antioxidants like butylated hydroxyl toluene, butylated hydroxyl anisole to inhibit peroxidation by trapping peroxyl radical chain propagation. Different extraction and purification techniques have been employed for the isolation of lignans from flaxseed. The chief aim of this paper is to explore and emphasize the clinical, industrial and food applications of these nutraceutical ingredients and major significance has been laid on the extraction methodologies of these pivotal health ingredients known as lignans enriched with the principal component, SDG (Secoisolariciresinol diglucoside). In this work, two different methods have been employed for the isolation of lignan extract from flaxseed and the extracts so isolated was subjected to HPLC quantification technique which reported SDG to the levels of 30.92±1.6 (mg/g) and 20.43 ±1.3 (mg/g) of lignan extract respectively. Hence, it can be easily concluded that flaxseeds have a promising future in the food as well as pharmacological sector thus acting as a great boon for these industries as well as for human health.*

Keywords*: Lignans, structure, applications, extraction.*

1. INTRODUCTION

Over the years, there has been a tremendous emphasis on the relation between diet and the prevelance of several chronic degenerative diseases. One of the emerging fields in food science is functional foods owing to their increasing popularity among health conscious consumers. In older days, flaxseed has been just primarily grown for its oil as flaxseed oil is an excellent source of omega-3 fatty acid, linolenic acid. The utilization of flaxseed has been employed in the diet and disease research owing to the tremendous potential health benefits which are attributed to the high content of α-linolenic acid(ALA) and lignan component mainly Secoisolariciresinol diglucoside (SDG). Infact, SDG is the principal and prospective bio active lignan in flaxseed (9). ALA content in flaxseed oil is 55-60%, whereas, the lignan content in flaxseed is 13mg/g of flaxseed(20). Several researchers have demonstrated that flaxseed and its lignans are capable of preventing or slowing down the prevelance of chronic diseases like cancer. Lignans are defined as a group of phenyl propanoid dimmers, in which the phenyl propane units are linked by the central carbon(C_8) of their propyl side chains(16). Lignans are basically phytoesterogens present in seeds, vegetable oils, cereals, legumes, fruits and vegetables as aglycones, glycosides, esterified glycosides or as bio-oligomers(2). The consumption of lignans especially secoisolariciresinol diglucoside have believed to have positive effects on human health. Infact, the increasing popularity of flaxseed is because of the rich content of lignan, principally SDG, omega-3-fatty acids and dietary fiber as well. It is the hull portion of the flaxseed which comprise of about 40% of the seed and is enriched with SDG (4). The prominent lignans present in flaxseed are secoisolariciresinol(SECO), mataresinol(MATA), pinoresinol(PINO), lariciresinol(LARI), isolariciresinol(ISO) and secoisolariciresinol diglucoside(SDG). These lignans have proven to be prominent anti-inflammatory, antioxidative, antimutagenic, antimicrobial and antiobesity agents. These lignans also have immense pharmacological properties, these lignans isolated from flaxseed have been increasingly used by the food as well as other industries. Several studies have reported the successful incorporation of SDG extracts in the form of powder directly into various food products like dressings, spreads, icecreams, cream alternatives, health bars, soups, fillings and coatings as well.

2. CLINICAL APPLICATIONS OF LIGNANS

One of the principal lignans of flaxseed, SDG was first isolated and identified from flaxseed in 1956, but no biological activity was attributed to the compound at that time. The role of secoisoresinol or its glycoside (SDG) from flaxseed as a precursor for the mammalian lignans was demonstrated by none other than Axelson et al. (1982). Infact, several studies have demonstrated that flaxseed, SDG or its mammalian lignans have been proven to be quite successful in the prevention or reduction in the risk of breast cancer and in the retardation of the development of lupus nephritis (11). Lignans are the phytoesterogens which are esterogenic like structures and can elicit both weak esterogenic and antiesterogenic activities. SDG is metabolized into more biologically active mammalian lignans enterodiol (END) and enterolactone(ENL) by mocrobiota in the colon. Lignans are reported to exhibit effects against hormone-dependant types of cancer (12).

SDG is a recognized nutraceutical. It is present in flaxseed in the form of complex polymeric structures, in which it is covalently bonded via ester linkages to 3-hydroxy-3-methyl glutaryl (HMG). SDG has been shown to reduce the levels of LDL-cholesterol (bad cholesterol) in blood, the risk of diabetes, and hormone related cancer. It has antioxidative, cardioprotective effect and improves the renal function in lupus nephritis patients (14). Lignans mainly exert their health effects by influencing the estrogen metabolism hence they are cateogarized as one of the biggest classes of phytoestrogens. Flaxseed lignans have immense cancer fighting properties and provide numerous health benefits for both men and women primarily improving cardiovascular health, as well as inhibiting the side effects of menopause and in the treatment of cushing disease. Another major breakthrough in the clinical applications of lignans is that they have proven to influence intracellular enzymes as well as protein synthesis and also in reducing the concentration of free hormones in the plasma by stimulating the production of sex hormone-binding globulin in the liver. One of the significant attempts made by (1) involved the study of the effect of combined lignan phytoesterogen and melatonin treatment and the secretion of steroid hormones by adrenal carcinoma cells. This study concluded with this fact that the addition of ENL (lignan enterolactone) and END(lignan enterodiol) with melatonin to cAMP-stimulated cells(treated cells) resulted in significant decrease in estradiol and cortisol concentration at 24 and 48 hrs, compared with the concentration in the cells stimulated with cAMP alone(cAMP control cells). (15) conducted a study to analyse the reduction of serum cholesterol and hypercholesterolemic artherosclerosis in rabbits by secoisolariciresinol diglucoside isolated from flaxseed.

One of the significant achievements in the clinical applications of lignans was in the form of an attempts made by (7). The cytotoxic effect of partial pure lignan on some cell lines including the murine mammary adrenn L2OB and Rhabdomyosarcoma RD,(Rat Embryo Fibroblast REF) was analysed. Lignans behave as great therapeutic agent and antitumorous against many diseases.

3. INDUSTRIAL APPLICATIONS OF LIGNANS

The utilization of flaxseed finds its prevelance in the industrial world as well as in the health world to a considerable extent. There are several components of flaxseeds which provide a number of health benefits in the form of fiber, lignans and fatty acids (14). Flaxseeds are the most effective source of lignans as their content is 800 times higher than the other food sources (14). Flaxseeed has been a part of human diet for thousands of years in Asia, Europe, Africa, North America and more recently in Australia (4). Due to the potential health attributes associated with the bioactive components, flaxseed has been the focus of ever-increasing interest in the field of diet and disease. It is one of the emerging functional foods because of its high content of lignans and fiber. Lignans have proven to be potent anti- carcinogenic compounds. Infact, flaxseed is an oilseed which is utilized in both industrial and natural health products(17).

Flaxseed has been a part of human diet for thousands of years in Asia, Europe, Africa, North America and more recently in Australia. In one of the significant works carried out by [14], flaxseed cake was subjected to extraction and acidic hydrolysis to obtain extracts rich in polyphenols and lignans. The identification of secoisoresinol, mataresinol and lariciresinol in the flaxseed extracts was accomplished using HPLC quantification technique. Both the crude as well as hydrolysed extracts exhibited potent anti bacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli.* This was a very pivotal step in order to evaluate the utilization of flaxseed extracts in food and cosmetics industries.

4. APPLICATIONS OF LIGNANS IN FOOD INDUSTRY

A considerable interest has been generated in the incorporation of flaxseed into several food products due to the presence of bioactive ingredients which provide enormous health benefits. One of the pivotal bioactive ingredients in flaxseed is the SDG (Secoisolariciresinol diglucoside) and α-linolenic acid (ALA). The usage of flaxseed meal was introduced as a fodder for lots of farm animals(for milk and eggs) and fish as well as enrich their products with omega-3 fatty acids. Food manufacturers have started increasing the use of polyunsaturated fatty acids (PUFA), rich in omega-3-fatty acids in their formulations. But due to their susceptibility towards oxidation reactions that lead to the development of undesirable off-flavours and other toxic products. Hence due to these several antioxidants like butylated hydroxytoulene(BHT) and butylated hydroxyanisole(BHA) have been added in order to curb peroxidation by trapping peroxyl radicals and inhibiting peroxyl radical chain propagation. Hence, instead of using these synthetic antioxidants, some natural antioxidants like polyphenolic compounds were thought to be effective in curbing the various degenerative oxidative processes. In one such study by(8) , commercially available SDG extract was incorporated to the formulation of dairy beverages enriched with flaxseed oil. (10) incorporated flaxseed flour at four different concentrations (5, 15, 30 and 45%) as a replacement for wheat flour in the production of cakes in order to evaluate the nutritional value. Cakes made with 5, 15 and 30 % of flaxseed flour were most accepted by the consumers and revealed the high dietary fiber levels ranging from 3.5-6.2g and linolenic acid ranging from 445-2500 mg/100g respectively.

In another work, the value addition of convenience foods like pasta was carried out by utilizing strengthful ingredients like flaxseed flour or its defatted portion by none other than (3). Functional bread was also produced by the addition of flaxseed flour. Hence, it can be easily concluded that flaxseed can be easily exploited as a promising tool in the optimization of foods rich in functional ingredients.

5. EXTRACTION OF LIGNAN CONCENTRATE FROM DEFATTED FLAXSEED POWDER

5. 1. Method I

1. *Collection and authentication of plant:* Seeds of *Linum usitatissimum* were procured from SKUAST, Jammu. Before processing, these flaxseeds were stored at cold temperature.

2. *Sample preparation*: Flaxseeds were milled in a coffee grinder. Then it was subjected to defatting using n- hexane in a Soxhlet apparatus for seven hours. Residual oil was also removed by magnetic stirring of the milled flaxseed with n-hexane (1:4 w/v) at ambient temperature for two hours.

3. *Chemicals*: 1M Sodium Hydroxide solution, absolute ethanol (99.99 %), 1 M Hydrochloric acid solutions were procured from Sigma Aldrich Chemicals Ltd., India. All chemicals used were of analytical grade.

4. Isolation of SDG: The defatted flaxseed meal (200 g) was subjected to alkaline hydrolysis using 1 M NaOH for 1 hour with constant stirring. This was followed by ethanolic extraction (75%). Filtrate was acidified to pH 3.5 using 1 M HCl. The acidified filtrate was subsequently concentrated using rotary evaporator. The concentrated filtrate was then dried in a tray drier at a temperature of 50^{o}C. The dried lignan concentrate was stored at 4^{o}C till further analysis. 5. *Identification and Quantification of SDG (Secoisolariciresinol diglucoside) by HPLC* Chromatographic technique

The analytical HPLC instrument used for the identification and quantification of active ingredient (SDG) in flaxseed lignan concentrate was HPLC Agilent 1260 series equipped with a UV detector. The analytical column used was RP C18, 150 mmx4.6 mm, 5μ particle size and the mobile phase was methanol: Acetic acid (70:30 v/v). Injection volume used was 20μL. Temperature of the detector was 30^{0} C. SDG content in the lignan concentrate came out to be 30.92±1.6 (mg/g) of the extract as shown by the chromatographic analysis in **Fig. 1.**

5. 2. Method II

1. *Collection and authentication of plant:* Seeds of *Linum usitatissimum* were procured from SKUAST, Jammu. Before processing, these flaxseeds were stored at cold temperature.

2. *Sample preparation*: Flaxseeds were milled in a coffee grinder. Then it was subjected to defatting using n- hexane in a Soxhlet apparatus for ten hours. Residual oil was also removed by magnetic stirring of the milled flaxseed with n-hexane (1:6 w/v) at ambient temperature for two-three hours.

3. Chemicals: Ethanol (55-75%), 1N NaOH/ 1 N KOH. All chemicals were of analytical grade and were procured from Sigma Aldrich Chemicals Ltd., India.

4. Isolation of SDG: The defatted flaxseed powder obtained was extracted using a primary alcohol (aqueous methanol or ethanol having an alcoholic content of 55-75%). The crude extract so obtained was subjected to alkaline treatment (1 N KOH/NaOH, 4 % w/v).

5. *Identification and Quantification of SDG (Secoisolariciresinol diglucoside) by HPLC* Chromatographic techniqu

The analytical HPLC instrument used for the identification and quantification of active ingredient (SDG) in flaxseed lignan concentrate was HPLC Agilent 1260 series equipped with a UV detector. The analytical column used was RP C18, 150 mmx4.6 mm, 5μ particle size and the mobile phase was methanol: Acetic acid (70:30 v/v). Injection volume used was 20μL. The active ingredient, SDG in the lignan concentrate came out to be 20.43 ±1.3 (mg/g) of lignan extract as shown in **Fig 2**.

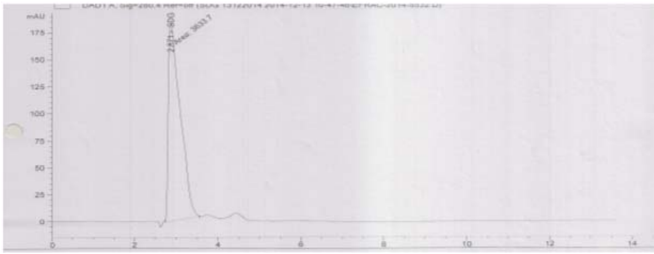

Fig. 2. Quantification of SDG in lignan concentrate by HPLC analysis.

REFERENCES

[1] Fecteau, K. A., Eiler, H., Oliver, J. W. Effect of combined lignan phytoestrogen and melatonin treatment on secretion of steroid hormones by adrenal carcinoma cells, AJVR, 72, 5, May 2011, pp. 675-680.

[2] Gerstenmeyer, E., Reimer, S., Berghofer, E., Schwartz, H. and Sontag, G. Effect of thermal heating on some lignans in flaxseeds, sesame seeds and rye, Food Chemistry, 138, 2013, pp. 1847-1855.

[3] Gupta, R. and Shivhare, U. S. Optimization of Process variables for the Development of Flaxseed and Defatted Flaxseed Based Pasta, Journal of Nutrition and Food Sciences.

[4] Hao, M. and Beta, T. Development of Chinese steamed bread enriched in bioactive compounds from barley hull and flaxseed hull extracts, Food Chemistry, August 2012, 133, pp. 1320-1325.

[5] Imran, M., Ahmad, N., Anjum, F. M., Khan, M. K., Mushtaq, Z., Nadeem, M. and Hussain, S. (2015). Potential protective properties of flax lignan secoisolariciresinol diglucoside, Nutritional Journal, 14, July 2015, pp. 1-17.

[6] Ioana, P. A. Characterization and Utilization of flaxseed lignans. University of Agricultural Sciences and Veterinary medicines, Cluj Napoca, pp. 1-46.

[7] Jumaily, E. F. and Mahdi, A. A.In vitro cytotoxic study for partial pure lignan Extract from flaxseed(Linum usitatissimum) on Rhabdomyosarcoma(RD) and Murine Mammary Adenocarcinoma(MMA) cell lines, 3, January 2013, pp. 18-24.

[8] Kasote, D. M. Flaxseed phenolics as natural antioxidants, International Food Research Journal, 20, 1, 2013, pp. 27-34.

[9] Moore, S. S. and Rajesha, J. Secoisolariciresinol Diglucoside: A potent multifarious bioactive phytoestrogen of flaxseed, Research and Reviews in Biomedicine and Biotechnology, 2, 3, May 2011, pp. 1-24.

[10] Moraes, E. A., Dantas, M. I. S., Morais, D. C., Silva, C. O., Castro, F. A. F., Martino, H. S.D. and Ribeiro, S. M. R., Sensory evaluation and nutritional value of cakes prepared with whole flaxseed flour, Cienc. Tecnol. Aliment., 30, 4, 2010, pp. 974-979.

[11] Muir, A. D., Westcott, N. D. and Prasad, K. Extraction, Purification and Animal Model Testing of an Anti-Atherosclerotic lignan secoisilariciresinol diglucoside from flaxseed(Linum usitatissium), Proc. WOCMAP-2, 1999, pp. 245-248.

[12] Nahla, E. A., Thabet, H. A. and Ahmed, H. F. Nature and Science, 11, 5, 2013, pp. 71-80.

[13] Oomah, B. D. Flaxseed as a functional food source, Journal of the science of Food and Agriculture, 81, March 2001, pp. 889-894.

[14] Pag, A. I., Radu, D. G., Draganescu, D., Popa, M. I. and Sirghie, C. Flaxseed Cake- A Sustainable Source of Antioxidant and Antibacterial extracts, Cellulose Chemistry and Technology, 48, 2014, pp. 265-273.

[15] Prasad, K. Reduction of Serum Cholesterol and Hypercholesterolemic Atherosclerosis in Rabbits by Secoisolariciresinol Diglucoside Isolated From Flaxseed. HyperCholesterolemic Atherosclerosis and SDG, 99, 1999, pp. 1355-1362.

[16] Sarajlija, H., Cukeilj, N., Novotni, D., Mrsic, G., Brncic, M. and Curic, D. Preparation of Flaxseed for Lignan Determination by Gas Chromatography- Mass Spectrometry method. Czech J. Food Sci., 30, 1, January 2012, pp. 45-52.

[17] Shim, Y. Y., Gui, B., Arnison, P. G. and Wang, Y and Reaney, J. T. Flaxseed (Linum usitatissimum L.) bioactive compounds and peptide nomenclature: A review, Trends in Food Science and Technology, 38, July 2014, pp. 5-20.

[18] Singh, K. K., Mridula, D. Rehal, J., Barnwal, P. Flaxseed: a potential source of food, feed and fiber. Critical Rev. Food Sci Nutr., 51, 3, March 2011, pp. 210-22.

[19] Toure, A. and Xueming, Xu. Flaxseed Lignans: Source, Biosynthesis, Metabolism, Antioxidant Activity, Bio- Active compounds, and Health Benefits, Comprehensive Reviews in Food Science and Food Safety, 9, April 2010, 261-269.

[20] Zanwar, A. A., Hegde, M. V., Bodhankar, S. L. Cardioprotective activity of flax lignan concentrate extracted from seeds of Linum usitatissimum in isoprenalin induced myocardial necrosis in rats, Interdiscip. Toxicol., 4, 2, July 2011, pp. 90-97.