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# Stability of bioactives in flaxseed and flaxseed-fortified foods

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A B S T R A C T

Dietary flaxseed is being studied as an alternative or complementary therapy to medications for reducing risk factors related to cardiovascular disease progression. The suggested benefits of alpha-linolenic acid (ALA) as an antihypertensive agent, of secoisolariciresinol diglucoside (SDG) derived enterolignans as antioxidants and estradiol mimetics and dietary fiber for its role in cholesterol lowering are just some of the potential benefits of consuming flaxseed. These studies have progressed from dietary studies involving animal models to large-scale clinical trials with the ultimate goal of adopting its consumption by the general public. To promote adherence in long-term clinical trials and encourage its consumption by the general public, flaxseed in one of its various forms or as its isolated bioactive ingredients is either incorporated into food products, called functional foods, or sprinkled onto foods before consumption. The stability of these bioactives in any of these forms becomes crucial for its acceptance by these populations and in order to optimize its biological actions. The effects of the food matrix, food preparation processes and storage conditions are all factors that can influence bioactive stability and are discussed here.



## 1. Introduction

Cardiovascular diseases (CVDs) are currently the leading cause of death worldwide (World Health Organization, (W. H. O.), 2008). They accounted for ~17.5 million deaths in 2012 which is expected to rise to an estimated 23.3 million by the year 2030 (WHO, 2008). The estimated direct and indirect cost of CVDs in the U.S. in 2010 was 444 billion dollars (Centers for Disease Control and Prevention, 2011). Medications are often prescribed to attenuate risk factors of CVD, yet non-adherence is a reoccurring problem leading to negative cardiac outcomes and death (Kolandaivelu, Leiden, O'Gara, and Bhatt, 2014). With medication prices rising and patients failing to follow drug treatment regimes, alternative management strategies are necessary. Fortunately, management of CV risk factors can proceed through lifestyle modifications, an important one being a healthy diet (Centers for Disease Control and Prevention, 2015).

Bioactive ingredients in food have long been known to have cardioprotective properties (Badimon, Vilahur, and Padro, 2010). Dietary flaxseed is one of these foods that possess an abundance of several bioactive ingredients, the most prominent being alpha-linolenic acid (ALA), the lignan secoisolariciresinol diglucoside (SDG) and dietary fiber. Other less abundant components, yet with suggested CVD-related healthy benefits, include the proteins cysteine, methionine and arginine, phenolic acids, flavonoids and potassium. As randomized clinical trials are lacking on these compounds and due to their low

*Abbreviations:* AA, arachidonic acid; ALA, alpha-linolenic acid; AV, acid value; CD, conjugated diene; CPDFX, crushed partially defatted flaxseed; CGFX, coarse ground flaxseed; CRP, C-reactive protein; CT, conjugated triene; CVD, cardiovascular disease; DBP, diastolic blood pressure; DFX, defatted flaxseed; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; END, enterodiol; ENL, enterolactone; EPA, eicosapentaenoic acid; FA, fatty acid; FFA, free fatty acid; FX, flaxseed; FXO, flaxseed oil; GFM, ground flaxseed meal; HV, headspace volatile; MDA, malondialdehyde; MFX, milled flaxseed; MHFX, milled-hull flaxseed; MUFA, monounsaturated fatty acid; NM, not measured; $O_2$, oxygen; PDFM, defatted flaxseed meal; PUFA, polyunsaturated fatty acid; PV, peroxide value; RH, relative humidity; RT, room temperature; SBP, systolic blood pressure; SDG, secoisolariciresinol diglucoside; SECO, secoisolariciresinol; SFA, saturated fatty acid; SMFX, sieved-milled flaxseed; STMFX, steam-treated milled flaxseed; TBA values, thiobarbituric acid values; TBARS, thiobarbituric acid reactive substances; TFA, total fatty acid; TG, triglyceride; $TxA_2$, thromboxane $A_2$; WFX, whole flaxseed.

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abundance in flaxseed, the forthcoming discussion will be restricted to ALA, SDG and dietary fiber. Recent studies document the potential for dietary flaxseed to reduce two of the leading risk factors of CVD, hypertension and high cholesterol levels (Edel et al., 2015; Rodriguez-Leyva et al., 2013). The bioactive ingredients responsible for each of these actions are very different. Dietary milled flaxseed (MFX) was shown to reduce hypertension through the inhibition of soluble epoxide hydrolase by ALA, and not by either of the metabolites of SDG (Caligiuri et al., 2014). Cholesterol reductions were attributed to the high fiber content of flaxseed and not to circulating concentrations of ALA or SDG metabolites (Edel et al., 2015). Thus, the ingestion of flaxseed as a functional food may yield better health-related effects than consuming separate, isolated bioactives. Understanding how flaxseed and its bioactives are preserved and maintained in foods then becomes critical information for our cardiovascular health.

Flaxseed interventional studies require participants to consume flaxseed in one of its many forms, dependent upon the research scope. In its simplest form, flaxseed can be ingested as whole flaxseed (WFX), MFX, flax oil (FXO), partially defatted flaxseed meal (PDFM), flaxseed hull (FXH) or as ALA or SDG supplements. The latter bioactive compounds are typically consumed in capsule form while the others are either consumed by the spoonful or sprinkled onto yogurt, cereal, margarine, applesauce or salad. An alternative more popular approach, especially when considering adherence in long-term clinical studies, is by incorporating flaxseed into food products that may include bakery items, pasta or dairy products (Austria et al., 2008; Bloedon et al., 2008; Dodin et al., 2008; Rodriguez-Leyva et al., 2013; Taylor, Noto, Stringer, Froese, and Malcolmson, 2010). Meats, such as poultry and beef, have also been fortified with flaxseed, however, these foods are not routinely used in clinical trials due to the higher costs associated with preparing and distributing these food items to participants. Foods prepared specifically with the goal of enriching a bioactive ingredient or providing healthy outcomes are known as functional foods. Preliminary studies on ALA or SDG metabolite bioavailability resulting from dietary intake of equal theoretical amounts of ALA from flaxseed flour or FXO capsules (Cunnane et al., 1993) or SDG from raw flaxseed or flaxseed baked into food products (Nesbitt, Lam, and Thompson, 1999), suggested that food matrix has minimal effect on bioactive content as physiological concentrations were unaffected. An important consideration of nutritional bioactives, which in essence are similar to medications, is the way in which they are consumed. Medications are either prescribed to be taken with or without food and it is well understood that food–drug interactions may exist that could enhance or reduce drug performance (Jáuregui-Garrido and Jáuregui-Lobera, 2012a, 2012b). Therefore, like medications, flaxseed bioactives may be altered by the matrix in which they are contained. Therefore, clinical trial design using nutritional therapies must consider any food matrix/flaxseed-bioactive interactions and all processing conditions that are involved in preparing and storing the fortified-food products to insure that all bioactive ingredients are preserved and not destroyed.

Several key processes are used to prepare flaxseed-fortified foods that are routinely used in clinical investigations that may impact the stability of bioactive compounds. In the context of this review, stability refers to bioactive content (i.e., amounts of ALA or SDG) and to amounts of oxidation by-products that can be measured via analytical assays or by volatile or sensory analysis. Domestic processes that may alter bioactive stability include fermentation, which has active bacterial cultures or yeast for yogurt or bread preparations, respectively, grinding or milling, extrusion for pasta/noodle formation and thermal processing necessary for cooking or baking. Storage conditions may also contribute to compound stability. This may be a combination of temperature variables which include ambient, refrigeration or freezing and/or packaging types which range from vacuum-sealed, open or closed to atmospheric conditions, glass or plastic containers or dark or clear. Each of these storage conditions can play a significant role in the preservation of the bioactive ingredients.

Therefore, the aim of this review was firstly to identify the most abundant flaxseed bioactives with observed roles in CVD prevention and secondly, to define optimal processing and storage conditions that preserves the content of flaxseed bioactives in both its natural form and when it is fortified in food products.

## 2. Description and morphology of flaxseed

Flaxseed, otherwise known as linseed, is produced in over 50 countries worldwide, with Canada and China leading in global production (FAOSTAT, 2013). Cultivated flaxseed (*Linum usitatissimum*) consists of a tall shoot with peripheral branches each containing a periwinkle blue or white flower with five petals. In addition numerous bolls on the plant encapsulate ~10 seeds in each. Flaxseeds may be brown, a variety typically produced in Canada, or yellow as produced in the USA and known as Omega. Both of these varieties contain similar ALA omega-3 fatty acid content (Morris, 2007). Some genetically modified varieties of flaxseed contain up to 71% ALA (Expert Panel, 2008). Another yellow variety also exists, called Solin, which is low in ALA. Flaxseed is a small seed (3–5 mm), shaped with a pointed apex and a rounded base. The seed coat is smooth, hard and shiny and when consumed has a chewy consistency and a pleasant flavor (Carter, 1996).

Flaxseed contains an inner embryo located at the core of the seed and is enclosed by an ovule with an inner and outer envelope (Diederichsen and Richards, 2003). Approximately seven different cell types comprise a single flaxseed (Gassner, 1951; Rüdiger, 1954). The outer portion of the ovule consists of a single layer of epidermal cells, followed by a thick layer (1 to 5 cells deep) of parenchyma cells also called ring cells. The epidermal cells provide mucilage fiber, composed of polysaccharides, polypeptides and glycoproteins (Heinze and Amelunxen, 1984), and the parenchyma cells may contain tannin-like compounds and/or chlorophyll. The inner portion of the ovule contains 3 cell types: a single layer of sclerenchyma fiber on the outer layer, followed by transversal cells and then pigment cells at the innermost position. An endosperm layer, containing oil and protein, tightly adheres to the seed coat and surrounds the embryo, which contains two large cotyledons. The embryo contains ~57% of the oil and is low in fiber (Wiesenborn, Tostenson, and Kangas, 2003). The hull is the primary location of SDG and lignans (Madhusudhan, Wiesenborn, Schwarz, Tostenson, and Gillespie, 2000) and is a rich source of water-soluble mucilage fiber (Bhatty and Cherdkiatgumchai, 1990).

## 3. Nutritional and phytochemical compositions of flaxseed

Cultivated flaxseed is rich in fat, protein and fiber. Proximate analysis of a Canadian brown flaxseed variety contains 42% fat, 27% dietary fiber, 18% protein, and 7% moisture with minimal vitamin and mineral content (Table 1) (Morris, 2007; United States Department of Agriculture (USDA), Agricultural Research Service, 2015). Whole and ground flaxseed have similar distributions of these nutritional components, however, flax oil in its natural state is 100% fat, 53% of which is ALA, has low protein content and is devoid of carbohydrates and dietary fiber (Morris, 2007; United States Department of Agriculture (USDA), Agricultural Research Service, 2015). Flaxseed is also rich in dietary lignans, the most prominent being SDG (Table 1). SDG content varies with flaxseed cultivar (Spence, Thornton, Muir, and Westcott, 2003), growing region and year (Westcott and Muir, 1996). Other lignans in flaxseed include matairesinol, lariciresinol, isolariciresinol and pinoresinol, but are represented to a much lower extent (Gerstenmeyer, Reimer, Berghofer, Schwartz, and Sontag, 2013; Meagher, Beecher, Flanagan, and Li, 1999; L. U. Thompson, Boucher, Liu, Cotterchio, and Kreiger, 2006).

Several hundred species of the genus *Linum* exist, yet, most studies involving flaxseed phytochemicals focus on the species *L. usitatissimum* as it is the most produced variety. Information regarding the phytonutrient composition of flaxseed can be obtained readily from the United States Department of Agriculture (United States Department of