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Effect of Processing and Storage on the Stability of Flaxseed Lignan Added to Dairy Products

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This study investigated the effects of processing and storage on the stability of purified, flaxseed-derived secoisolariciresinol diglucoside (SDG) added to milk prior to the manufacture of different dairy products. We analyzed the effect of high-temperature pasteurization, fermentation, and milk renneting as well as storage on the stability of SDG added to milk, yogurt, and cheese. Also, the stability of SDG in whey-based drinks was studied. Added SDG was found to withstand the studied processes well. In edam cheese manufacture, most of the added SDG was retained in the whey fraction and 6% was found in the cheese curd. SDG was also relatively stable in edam cheese during ripening of 6 weeks at 9 °C and in yogurt during storage of 21 days at 4 °C. Up to 25% of added SDG was lost in whey-based drinks during storage of 6 months at 8 °C. We conclude that SDG can be successfully supplemented in dairy-based products.

KEYWORDS: Flaxseed; lignan; secoisolariciresinol diglucoside; SDG; processing; storage; stability; milk; yogurt; cheese; whey; dairy products; drinks

INTRODUCTION

Lignans are a group of polyphenolic compounds containing the 2,3-dibenzylbutane skeleton (*1*). They are widely distributed in plants and woody species. Flaxseed (*Linum usitatissimum* L.) is one of the richest sources of the plant lignan precursor secoisolariciresinol diglucoside (SDG), which is found in flaxseed linked to oligomers by 3-hydroxy-3-methylglutaryl esters (*2*, *3*). The content of SDG in flaxseed has been reported to vary from 6.1 to 25.9 mg/g (*4*, *5*), corresponding to a secoisolariciresinol (SECO) content of 3.4−14.4 mg/g. Flaxseed also contains small amounts of other lignans such as matairesinol, pinoresinol, lariciresinol, and isolariciresinol (*6*−*10*).

Plant lignans are metabolized to the mammalian lignans enterodiol (END) and enterolactone (ENL) in the colon by the resident microflora (*11*−*13*). END is further oxidized to ENL, resulting in ENL being the predominating circulating mammalian lignan (*14*). Epidemiological and intervention studies with human subjects have shown increased serum, plasma, and urine concentrations of ENL after consumption of lignan-rich diets (*15*−*17*). Lignans exert many biological properties such as estrogenic, antiestrogenic, antioxidative, and anticarcinogenic properties (*13*). Thus, they are of great interest because of their potential protective role against several diseases predominant in Western countries. A great number of animal model and human studies suggest that a higher intake of lignans reduces the risk of many chronic diseases including hormone-based cancers, for example, breast, prostate, and colon cancer, cardiovascular diseases, and adult diabetes. These studies have been reviewed in many recent articles (*18*−*22*).

Flaxseeds have traditionally been added to bakery products in the form of whole seeds or as mechanically ground flaxmeal. However, the characteristic flavor of flaxseed is regarded as a limiting factor for its wider use in other foodstuffs. Also, the current knowledge about the effects of food processing on the stability of plant lignans is limited. Such studies have mostly been conducted with ground flax or flaxmeal (*23*, *24*). In our previous study (*25*), we demonstrated that isolated and purified SDG remains stable through the breadmaking process. These results are in agreement with respective studies carried out by Nesbitt and Thompson (*26*) and Muir and Westcott (*27*).

Milk has proven a successful raw material and matrix for developing health-promoting functional foods (*28*). An increasing variety of probiotic products, such as yogurts, milk drinks, and cheeses, have been developed and launched on the market (*29*, *30*). Also, dairy products supplemented with other bioactive components, such as plant sterols, peptides, and omega-3-fatty acids, have been introduced commercially (*31*, *32*). Flaxseed-derived purified SDG is available as a nutraceutical on the world market, but food applications have not been introduced so far. The development of functional dairy products based on supplemented SDG is, therefore, an interesting approach.

The aim of this work was to investigate the stability of added purified SDG during the manufacture and storage of different dairy products. We focus on the effects of pasteurization and

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fermentation and the various cheese manufacturing phases using several model products.

MATERIALS AND METHODS

Isolation and Purification of SDG from Flaxseed. SDG was isolated and purified as described by Hyvärinen et al. (*25*). This method has been developed and patented by MTT Agrifood Research Finland (*33*).

Heat Treatment of Milk. The effect of heat treatment, as routinely practiced in yogurt manufacture, was mimicked on a laboratory scale in a batch process. Purified SDG (200 mg) was added to 200 mL of raw milk (fat content approximately 4.3%), mixed, and subjected thereafter to heat treatment at 85 °C for 20 min with continual stirring in a water bath. Milk was then cooled to 4 °C in a water bath and packed in 250 mL glass bottles. The content of SDG was analyzed immediately after processing. Control milk was prepared in the same way but without SDG addition. The heat treatment process was replicated two times (two trials).

Yogurt Preparation. Fat-free milk powder (Valio Ltd., Finland) (4%, w/v) was added to raw milk (fat content approximately 4.3%) and mixed well. Milk was heat treated at 90 °C for 20 min in a water bath. A quantity of 2 × 200 mL per trial was taken for yogurt preparation in glass bottles. The procedure was repeated once ($n = 4$). After the milk was cooled to 43 °C, SDG (100 mg) and 0.02% (w/w) starter culture ABY-1 (Christian Hansen, Hoersholm, Denmark) containing *Streptococcus thermophilus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, and *Bifidobacterium* were added to the milk. Fermentation of milk to yogurt was carried out at 43 °C for 5 h (pH > 4.6). Finally, the yogurt was stirred and packed in plastic cups. The SDG content was analyzed directly after manufacture or after storage at 4 °C for 21 days. After manufacture and storage, the quality of the yogurt was evaluated by determining its pH and visual appearance. Control yogurt was prepared in the same way but without SDG supplementation.

Edam Cheese Manufacture. The experimental cheeses were manufactured at the MTT pilot processing plant. Batches of 10 L of milk (fat content 2.76%) were pasteurized at 72 °C for 15 s, allowed to cool to 32−33 °C, and transferred to a cheese vat for the preparation of cheeses with an average fresh weight of 1 kg. Cheese was produced using a BD-type starter culture containing *Lactococcus lactis* and *Leuconostoc mesenteroides* (Probat 505, Visby, Denmark) and rennet (Standard Premium 225, Chr. Hansen Lab.) added at 1.5% (w/v) and 3.6 mL per 10 L, respectively. SDG was added to cheese milk at a concentration of 1.0 g/10 L and mixed well. This cheese was referred to as SDG cheese. Control cheese was prepared in the same way but without SDG supplementation. The curd was cut for 5 min and stirred further for 15 min, and part of the whey was removed. The temperature of the curd−whey mixture was raised to 38 °C with stirring. After cooking, the whey was discarded and the cheese was pre-pressed for 40 min and molded. The cheeses were pressed for 55 min and brined for 5 h (salt content 21%), vacuum wrapped (cubed blocks, about 1 kg in weight) in plastic film (BK1L Red Bags, Cryovac UK Limited, England), and ripened at 9 °C for 6 weeks.

Preparation of Whey Drinks. Whey-based drinks were prepared using juice concentrates (lingonberry and raspberry, Marli, Turku, Finland), whey, and water. Each juice concentrate was first diluted with water to contain 20% or 40% (v/v) juice. These solutions were further diluted with whey to samples containing either 20% or 40% (v/v) of juice solution. 200 mg of SDG was added to the lingonberry drinks and 150 mg to the raspberry drinks and stirred well. The drinks were diluted to final volume with water (lingonberry drinks 2 L and raspberry drink 1.5 L). The content of SDG in both drinks was 10 mg/100 mL. The drink samples were pasteurized by heating up to 80 °C and packed immediately in 150 mL plastic cups, which were sealed with plastic covers (Pro Ruchti, Uttigen, Switzerland). Samples were taken from the drinks before and after heating, and after 1, 2, 3, and 6 months of storage at 8 °C. Basic composition and microbiological quality were also determined.

Analysis of SDG and Its Metabolites in the Products. To analyze SDG, 1.0 mL of a whey drink and milk sample, respectively, was mixed with 1.0 mL of methanol containing 0.1% acetic acid and left to settle for 15 min. The sample was then centrifuged for 10 min at 1500 rpm (5000*g*), and the supernatant was filtered through a disposable 0.45 μm membrane filter (Acrodisc, Pall, East Hills, NY). Yogurt samples (1.0 g) were extracted with 20 mL of methanol for 15 min on a magnetic stirrer. The sample was left to settle, and the organic phase was pipetted carefully into a round bottle through a filter paper (MN 640 w, Machery Nagel, Germany). The extraction was repeated, and the organic phase was combined into the round bottle. The sample was evaporated to dryness in a rotary evaporator, redissolved in a small amount of methanol, and transferred to a 10 mL volumetric bottle quantitatively with methanol. The sample was then filtered (MN 640 w, Machery Nagel, Germany) for HPLC analysis.

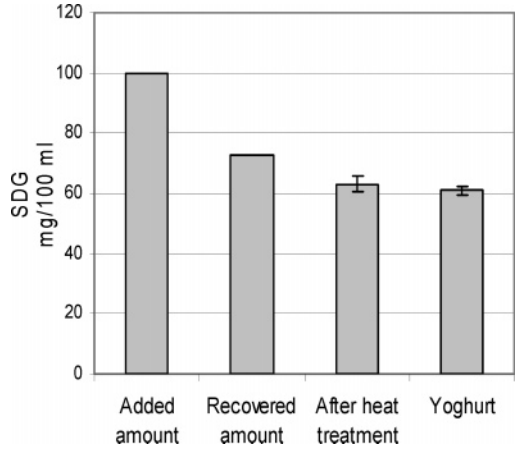

Figure 1. Stability of added SDG in milk during heat treatment and manufacture of yogurt ($n = 4$).

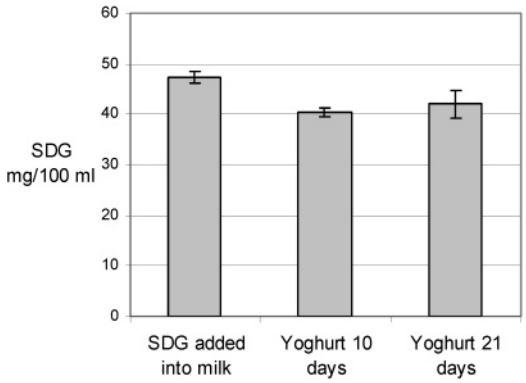

Figure 2. Stability of SDG during storage of yogurt ($n = 4$), stored at 4 °C.

Cheese samples (10.0 g) were weighed into plastic jars and homogenized with 50 mL of 80% methanol using a mixer (Bamix, Switzerland) for 1 min. The blade was washed with 25 mL of 80% methanol combining the washing with the homogenized sample. The sample jar was flushed carefully with argon, sealed with a lid, and stirred overnight on a magnetic stirrer. After extraction, the sample was filtered through a filter paper (MN 640 w, Machery Nagel, Germany) into a separation funnel, and the solid residue in the filter paper was washed with 20 mL of 80% methanol. Fat was removed from the methanol phase using liquid−liquid partitioning with 100 mL of hexane. After separation of the phases, the lower methanolic phase was transferred to a round bottle. The sample was then evaporated to dryness in a rotary evaporator, redissolved carefully in a small amount of methanol in an ultrasound bath, and transferred to a graduated 15 mL test tube quantitatively with a few milliliters of methanol. The sample was evaporated down to 3−4 mL with a stream of nitrogen and adjusted to the final volume of 4 mL with methanol and filtered (MN 640 w, Machery Nagel, Germany) for HPLC analysis.

The identification and quantitation of SDG and some of its possible metabolites, such as SECO, END, and ENL, was performed with the HP series 1100 system (Hewlett-Packard, Waldbronn, Germany) equipped with a diode array detector. Nova Pak C18 (3.9 × 150 mm,

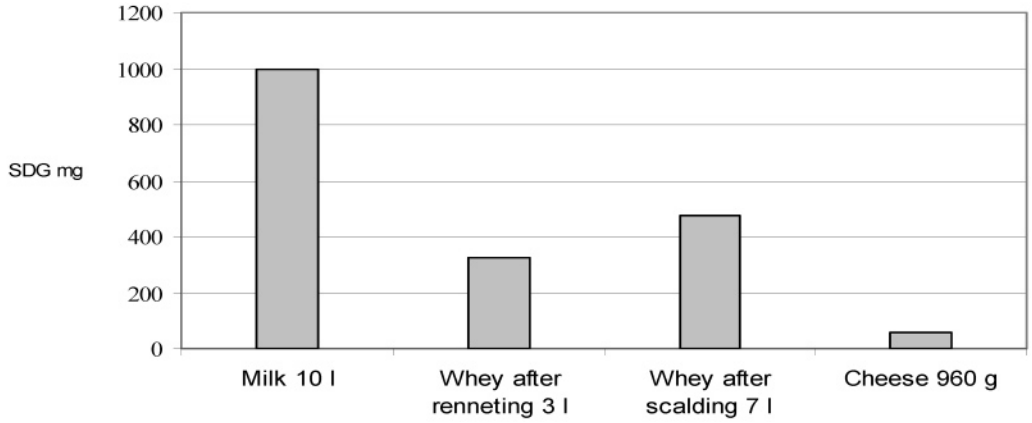

Figure 3. Distribution of absolute amount of SDG added into milk prior to cheese manufacture. dw = dry weight, * at 9 °C.

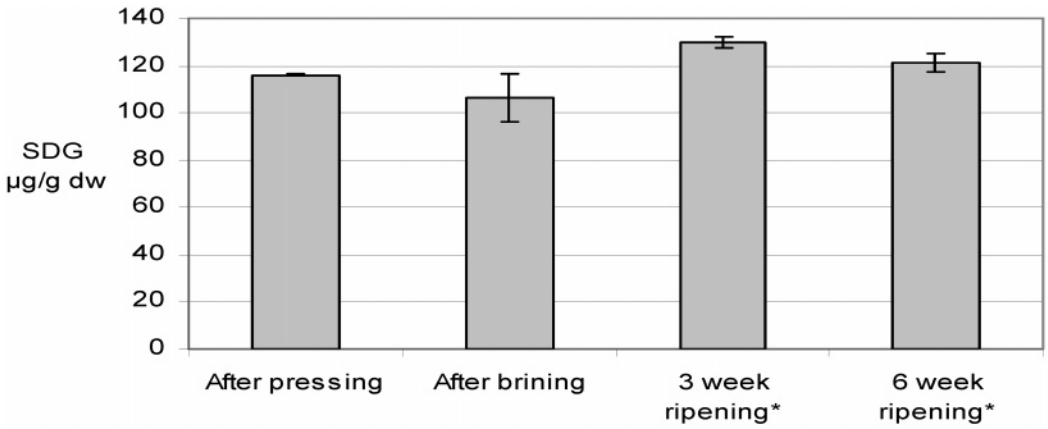

Figure 4. SDG content of edam cheese after manufacture and ripening.

4 μm, Waters, Milford, USA) was used as the analytical column. For milk, a mobile phase of 0.05 M phosphate buffer (A), pH 2.4, and methanol (B) was used with a gradient profile as follows: 5−58% B in 58 min, 58−90% B in 32 min, then hold at 90% for 12 min, and finally 90−5% in 3 min at 0.9 mL/min. Later, a faster gradient system was used in the analysis of SDG in cheese, yogurt, whey, and whey-based products as described by Hyvärinen et al. (*46*). The original SDG used as a reference compound was a gift from Dr. Neil Westcott (Agriculture and Agri-Food Canada, Saskatoon, Canada). SECO was purchased from ArboNova (Turku, Finland), and END and ENL were from Sigma-Aldrich (St. Louis, MO).

Basic Composition Analysis. *Moisture.* Moisture content was analyzed by weighing the samples before and after freeze-drying. The residual moisture was determined by drying at 105 °C overnight (17 h) (*34*, *35*).

Ash. Ash content was analyzed by weighing the samples before and after burning at 500 °C overnight (17 h) (in-house method, MTT, Laboratories).

Nitrogen. Nitrogen content was determined using a Kjeltec Auto 1030 analyzer according to the Association of Official Analytical Chemists (AOAC) method (*36*, *37*).

Protein. Protein content was calculated by the following formula: 6.25 × nitrogen.

Crude Fat. Fat content was determined by the Twisselman method, using diethyl ether as a solvent (*38*−*40*).

Total Carbohydrates. The content of total carbohydrates was calculated by the following formula: total carbohydrates (g/100 g FW) = 100 − moisture (%) − protein content (%/FW) − crude fat (%/FW) − ash (%/FW) (*41*).

Energy. Energy content was calculated as follows: energy (kJ/100 g FW) = 17 × total carbohydrate% FW + 17 × protein % FW + 37 × crude fat % FW (*42*).

Microbiological Analysis. Coliform bacteria were determined in VRB agar (Difco, Le Pont de Claix, France) incubated at 30 °C for 24 h (*43*).

Total counts of bacteria were determined in Plate Count Agar (PCA) (Difco, Le Pont de Claix, France), which were incubated at 30 °C for 72 h (*44*).

Aerobic spore-forming bacteria were determined in PCA agar incubated at 30 °C for 72 h. The sample (5 mL) was pasteurized at 80 °C for 10 min before plating (*45*).

RESULTS AND DISCUSSION

Heat Treatment and Fermentation of Milk. The effect of heating and fermentation of milk on the stability of SDG is presented in **Figure 1**. SDG content was not markedly affected by heat processing or fermentation. Further, no metabolites of SDG were detected, indicating that no decomposition of SDG occurred during processing. Purified SDG has been reported to remain rather stable during food processing, for example, baking (*25*, *27*). Fermentation for several hours does not seem to degrade or convert SDG to an aglycone form. This result is in agreement with our previous results on a fermentation process in which sourdough rye bread was made (*25*). On the other hand, a recent study by Tsangalis et al. (*46*) showed that fermentation of soymilk with bifidobacteria resulted in a significant increase in the concentration of aglycones, such as equol, which are bioactive forms of soy isoflavones. Also, studies by Hutchins et al. (*47*) indicated that, during the production of tempeh, a soy product fermented with *Rhizopus oligosporus*, isoflavone glycosides are hydrolyzed to unconjugated aglycones. In the present study, the yogurt starter containing lactic acid bacteria and bifidobacteria did not cause any degradation of SDG during fermentation, suggesting that SDG is rather stable to hydrolysis by starter bacteria as compared to soy isoflavones. From a technological viewpoint, it is important to note that SDG does not seem to interfere with the yogurt manufacturing process.

Table 1. SDG Content (μg/mL) of Berry Whey Drinks after Manufacture and Storage ($n = 2$) (Values Are Means ± Standard Deviation (SD))[a]

	LA	LB	LC	LD	RA	RB	RC	RD
pH	3.32	3.66	3.01	3.21	3.81	4.07	3.39	3.65
added	100	100	100	100	100	100	100	100
fresh	98.7 ± 0.0	97.6 ± 4.2	103.0 ± 1.3	109.0 ± 2.8	84.6 ± 0.3	86.2 ± 2.5	83.8 ± 4.7	85.4 ± 5.9
1 month	76.2 ± 8.5	80.3 ± 4.2	86.1 ± 5.4	85.1 ± 0.1	90.6 ± 1.0	90.5 ± 3.0	90.6 ± 1.1	99.2 ± 0.0[b]
2 months	92.6 ± 0.7	87.7 ± 2.3	87.4 ± 1.5	89.7 ± 1.5	90.7 ± 0.1	88.8 ± 5.5	83.5 ± 2.8	88.7 ± 0.3
3 months	91.9 ± 9.9	87.8 ± 5.5	91.0 ± 1.4	94.7 ± 2.2	80.5 ± 7.0	92.6 ± 6.9	89.6 ± 5.7	85.9 ± 11.7
6 months	81.5 ± 0.7[c]	76.6 ± 1.6	75.5 ± 0.5	75.5 ± 0.3	82.5 ± 1.2	82.1 ± 0.5	81.1 ± 0.8	83.8 ± 1.9

[a] L = lingonberry, R = raspberry. A: Drink containing 20% juice concentrate and 20% whey. B: Drink containing 20% juice concentrate and 40% whey. C: Drink containing 40% juice concentrate and 20% whey. D: Drink containing 40% juice concentrate and 40% whey. [b] $n = 1$. [c] $n = 6$.

As for acid production (pH), a similar pattern was observed between the test and the control yogurt.

The stability of SDG was also tested during extended storage of yogurt. As shown in **Figure 2**, SDG was found to be chemically stable in acidic conditions during storage of 10 and 21 days. Studies by Milder et al. (*48*) have shown that matairesinol is sensitive to low pH. The different structure and form of the lignans may contribute to this phenomenon, indicating that SDG is more stable in such processing conditions.

Cheese Manufacture. The effect of various factors in cheese manufacture on the stability of SDG was studied by making experimental edam cheeses. The pH of cheese during drainage was 6.6 and after 1 day 6.5−6.6. The high pH value of fresh cheese may be due to a failure of the starter culture used. However, after maturation of 3 weeks, the pH had decreased to 5.3. Supplementation of SDG in cheese milk did not have an adverse effect on cheese production. SDG was mostly transferred to whey during cheese manufacture (**Figure 3**). However, the recovery of SDG from whey should be improved in the manufacture of fresh (not ripened) cheeses with a high moisture content. In our study, only about 6% of the SDG was recovered in fresh edam cheese (before pressing). Pressing, brining, and ripening at 9 °C for 6 weeks showed no adverse effects on the SDG content of the cheese (**Figure 4**). As in the case of yogurt production, the starter lactic acid bacteria and enzymes present in the cheese did not hydrolyze SDG during manufacture and ripening.

Whey Drinks. According to the national food database (www.fineli.fi), the basic composition of the whey drinks prepared for this study was similar to that of commercial whey-based drinks. Their energy content varied between 155 and 379 kJ/100 g (fineli data in parentheses) (170−220 kJ/100 g), protein 0.06−0.18 g/100 g (0−1.5 g/100 g), carbohydrates 9.1−22.1 g/100 g (9−12 g/100 g), and fat <0.3 g/100 g (0−0.1 g/100 g).

The effect of processing and storage of the whey drinks on the content of added SDG is presented in **Table 1**. All whey drinks showed good hygienic quality. No coliform bacteria, total colonies bacteria, or aerobic spore-forming bacteria from pasteurized sample were detected even after 6 months storage at room temperature (data not presented). Added SDG was found to remain relatively stable during pasteurization. However, a slight reduction in SDG content was detected after storage of 1 month. After 6 months, SDG losses of up to 25% were observed. This may have been due to the low pH (3.0−4.1) of the whey drinks. No possible metabolites of SDG were detected, but it must be noted that the analytical method was not optimized for these compounds.

In conclusion, SDG, isolated using a method developed by MTT, was added to milk to test its stability during the manufacture of different dairy products. SDG was found to be relatively stable during high-temperature batch pasteurization of milk and whey and under the conditions applied in customary manufacture of yogurt and ripened cheese. SDG recovery in edam cheese was low, suggesting that supplementation of SDG in the manufacture of semi-hard or hard cheeses is not applicable if SDG is added directly to cheese milk. The purified lignan fraction, SDG, isolated from flaxseeds seems to be technologically and organoleptically suitable to be supplemented especially in liquid and acidic dairy products, in particular.

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LITERATURE CITED

(1) Setchell, K. D. R. Discovery and potential clinical importance of mammalian lignans. In *Flaxseed in Human Nutrition*; Cunnane, S. C., Thompson, L. U., Eds.; AOCS Press: Champaign, IL, 1995; pp 82−98.

(2) Ford, J. D.; Huang, K.-S.; Wang, H.-B.; Davin, L. B.; Lewis, N. G. Biosynthetic pathway to the cancer chemopreventive secoisolariciresinol diglucoside-hydroxymethyl glutaryl ester-linked lignan oligomers in flax (*Linum usitatissimum) seed. J. Nat. Prod.* **2001**, *64*, 1388−1397.

(3) Johnsson, P.; Peerlamp, N.; Kamal-Eldin, A.; Andersson, R. E.; Andersson, R.; Lundgren, L. N.; Åman, P. Polymeric fractions containing phenol glucosides in flaxseed. *Food Chem.* **2002**, *76*, 207−212.

(4) Johnsson, P.; Kamal-Eldin, A.; Lundgren, L. N.; Åman, P. HPLC method for analysis of secoisolariciresinol diglucoside in flaxseeds. *J. Agric. Food Chem.* **2000**, *48*, 5216−5219.

(5) Eliasson, C.; Kamal-Eldin, A.; Andersson, R.; Åman, P. High-performance liquid chromatographic analysis of secoisolariciresinol diglucoside and hydroxycinnamic acid glucosides in flaxseed by alkalinen extraction. *J. Chromatogr., A* **2003**, *1012*, 151−159.

(6) Mazur, W.; Fotsis, T.; Wähälä, K.; Ojala, S.; Salakka, A.; Adlercreutz, H. Isotope dilution gas chromatographic-mass spectrometric method for determination of isoflavonoids, coumestrol and lignans in food samples. *Anal. Biochem.* **1996**, *233*, 169−180.

(7) Qui, S.-X.; Lu, Z.-Z.; Luyengi, L.; Lee, S. K.; Pezzuto, J. M.; Farnsworth, N. R.; Thompson, L. U.; Fong, H. H. S. Isolation and characterization of flaxseed (*Linum Usitatissimum*) constituents. *Pharm. Biol.* **1999**, *37*, 1−7.

(8) Sicilia, T.; Niermeyer, H. B.; Honig, D. M.; Metzler, M. Identification and stereochemical characterization of lignans in flaxseed and pumpkin seed. *J. Agric. Food Chem.* **2003**, *51*, 1181−1188.

(9) Heinonen, S.; Nurmi, T.; Liukkonen, K.; Poutanen, K.; Wähälä, K.; Deyama, T.; Nishibe, S.; Adlercreutz, H. In vitro metabolism of plant lignans: new precursors of mammalian lignans enterolactone and enterodiol. *J. Agric. Food Chem.* **2001**, *49*, 3178−3186.

(10) Milder, I. E. J.; Arts, I. C. W.; van de Putte, B.; Venema, D. P.; Hollman, P. Lignan content of Dutch plant food: a database including lariciresinol, pinoresinol, secoisolariciresinol and matairesinol. *Br. J. Nutr.* **2005**, *93*, 393−402.
(11) Borriello, S. P.; Setchell, K. D.; Axelson, M.; Lawson, A. M. Production and metabolism of lignans by the human faecal flora. *J. Appl. Bacteriol.* **1985**, *58*, 37−43.
(12) Bowey, E.; Adlercreutz, H.; Rowland, I. Metabolism of isoflavones and lignans by the gut microflora: a study in germ-free and human flora associated rats. *Food Chem. Toxicol.* **2003**, *41*, 631−636.
(13) Rowland, I.; Faughnan, M.; Hoey, L.; Wähälä, K.; Williamson, G.; Cassidy, A. Bioavailability of phyto-oestrogens. *Br. J. Nutr.* **2003**, *89*, S45−S58.
(14) Wang, L. Q.; Meselhy, M. R.; Li, Y.; Qin, G. W.; Hattori, M. Human intestinal bacteria capable of transforming secoisolariciresinol diglucoside to mammalian lignans, enterodiol and enterolactone. *Chem. Pharm. Bull.* **2000**, *48*, 1606−1610.
(15) Kilkkinen, A.; Stumpf, K.; Pietinen, P.; Valsta, L. M.; Tapanainen, H.; Adlercreutz, H. Determinants of serum enterolactone concentration. *Am. J. Clin. Nutr.* **2001**, *73*, 1094−1100.
(16) Tarpila, S.; Aro, A.; Salminen, I.; Tarpila, A.; Kleemola, P.; Akkila, J.; Adlercreutz, H. The effect of flaxseed supplementation in processed food on serum fatty acids and enterolactone. *Eur. J. Clin. Nutr.* **2002**, *56*, 157−165.
(17) Vanharanta, M.; Mursu, J.; Nurmi, T.; Voutilainen, S.; Rissanen, T. H.; Salonen, R.; Adlercreutz, H.; Salonen, J. T. Phloem fortification in rye bread elevates serum enterolactone level. *Eur. J. Clin. Nutr.* **2002**, *56*, 952−957.
(18) Westcott, N. D.; Muir, A. D. Flax seed lignan in disease prevention and health promotion. *Phytochem. Rev.* **2003**, *2*, 401−417.
(19) Cornwell, T.; Cohick, W.; Raskin, I. Dietary phytoestrogens and health. *Phytochemistry* **2004**, *65*, 995−1016.
(20) Arts, I. C. V.; Hollman, P. C. H. Polyphenols and disease risk in epidemiologic studies. *Am. J. Clin. Nutr.* **2005**, *81*, S317−S325.
(21) Korhonen, H. J .T.; Ryhänen, E.-L.; Pihlava, J.-M. Flaxseed lignan - bioactive incredient for functional food. *Innovations Food Technol.* **2005**, *28*, 54−57.
(22) McCann, M. J.; Gill, C. I.; McGlynn, H.; Rowland, I. R. Role of mammalian lignans in the prevention and treatment of prostate cancer. *Nutr. Cancer* **2005**, *52*, 1−14.
(23) Meagher, L. P.; Beecher, G. R. Assessment of data on the lignan content of foods. *J. Food Compos. Anal.* **2000**, *13*, 935−947.
(24) Crosby, G. A. Lignans in food and nutrition. *Food Technol.* **2005**, *59*, 32−36.
(25) Hyvärinen, H.; Pihlava, J.-M.; Hiidenhovi, J.; Hietaniemi, V.; Korhonen, H. J. T; Ryhänen, E.-L. Effect of processing and storage on the stability of flaxseed lignan added to bakery products. *J. Agric. Food Chem.* **2006**, *54*, 48−53.
(26) Nesbitt, P. D.; Thompson, L. U. Lignan in homemade and commercial products containing flaxseed. *Nutr. Cancer* **1997**, *29*, 222−227.
(27) Muir, A. D.; Westcott, N. D. Quantitation of the lignan secoisolarisiresinol diglucoside in the baked goods containing flax seed or flax meal. *J. Agric. Food Chem.* **2000**, *48*, 4048−4052.
(28) Rowan, A. M.; Haggarty, N. W.; Ram, S. Milk bioactives: discovery and proof of concept. *Aust. J. Dairy Technol.* **2005**, *60*, 114−120.
(29) Desmond, C.; Corcoran, B. M.; Coakley, M.; Fitzgerald, G. F.; Ross, R. P.; Stanton, C. Development of dairy-based functional foods containing probiotics and prebiotics. *Aust. J. Dairy Technol.* **2005**, *60*, 121−126.
(30) Korpela, R.; Saxelin, M. Functional dairy products- the Nordic perspective. *Aust. J. Dairy Technol.* **2005**, *60*, 170−172.
(31) Korhonen, H.; Pihlanto, A. Bioactive peptides: new challenges and opportunities for the dairy industry. *Aust. J. Dairy Technol.* **2003**, *58*, 129−134.
(32) Sharma, R. Market trends and opportunities for functional dairy beverages. *Aust. J. Dairy Technol.* **2005**, *60*, 195−198.
(33) Pihlava, J.-M.; Hyvärinen, H.; Ryhänen, E.-L.; Hietaniemi, V. Process for isolating and purifying secoisolariciresinol diglucoside (SDG) from flaxseed. Patent WO 02062812, 2002.
(34) AOAC (Association of Official Analytical Chemists) method 7.007, drying at 135 °C. In *Official Methods of Analysis of the Association of Official Analytical Chemists*, 13th ed.; Horwitz, W., Ed.; AOAC: Washington, DC, 1980; p 125.
(35) AOAC (Association of Official Analytical Chemists) method 14.004, air oven method (3) − Official final action. In *Official Methods of Analysis of the Association of Official Analytical Chemists*, 13th ed.; Horwitz, W., Ed.; AOAC: Washington, DC, 1980; p 211.
(36) AOAC (Association of Official Analytical Chemists) method 7.021, Authomated method - Principle. In *Official Methods of Analysis of the Association of Official Analytical Chemists*, 13th ed.; Horwitz, W., Ed.; AOAC: Washington, DC, 1980; p 127.
(37) AOAC (Association of Official Analytical Chemists) method 14.068, Kjeldahl method. In *Official Methods of Analysis of the Association of Official Analytical Chemists*, 13th ed.; Horwitz, W., Ed.; AOAC: Washington, DC, 1980; p 220.
(38) AOAC (Association of Official Analytical Chemists) method 7.003, Drying in vacuo at 95−100 °C. In *Official Methods of Analysis of the Association of Official Analytical Chemists*, 13th ed.; Horwitz, W., Ed.; AOAC: Washington, DC, 1980; p 125.
(39) AOAC (Association of Official Analytical Chemists) method 7.056, Determination. In *Official Methods of Analysis of the Association of Official Analytical Chemists*, 13th ed.; Horwitz, W., Ed.; AOAC: Washington, DC, 1980; p 132.
(40) AOAC (Association of Official Analytical Chemists) method 922.06, Fat in flour, acid hydrolysis method. In *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed.; Helrich, K., Ed.; AOAC: Arlington, VA, 1990; p 780.
(41) *Nutrient Composition of Foods*, 2nd ed.; Rastas, M., Seppänen, R., Knuts, L.-R., Karvetti, R.-L., Varo, P., Eds.; Kansaneläkelaitos: Helsinki, Finland, 1989; p XIX.
(42) *Nutrient Composition of Foods*, 2nd ed.; Rastas, M., Seppänen, R., Knuts, L.-R., Karvetti, R.-L., Varo, P., Eds.; Kansaneläkelaitos: Helsinki, Finland, 1989; p XVII.
(43) International Dairy Federation (IDF) Standard 073B. *Milk and milk products - Enumeration of coliforms*; IDF: Brussels, Belgium, 1998.
(44) International Dairy Federation (IDF) Standard 100B. *Milk and milk products - Enumeration of microorganisms*; IDF: Brussels, Belgium, 1991.
(45) Harrigan, W. F.; McCance, M. E. Enumeration and isolation of Bacillus. In *Laboratory Methods in Food and Dairy Microbiology*, 2nd ed.; Harrigan, W. F., McCance, M. E., Eds.; Academic Press: London, UK, 1976; pp 176−177.
(46) Tsangalis, D.; Ashton, J. F.; McGill, A. E. J.; Shah, N. P. Enzymic transformation of isoflavone phytoestrogens in soymilk by β-glucosidase-producing bifidobacteria. *J. Food Sci.* **2002**, *67*, 3104−3113.
(47) Hutchins, A. M.; Lampe, J. W.; Martini, M. C.; Campbell, D. R.; Slavin, J. L. Vegetables, fruits, and legumes: effect on urinary isoflavonoid phytoestrogen and lignan excretion. *J. Am. Diet Assoc.* **1995**, *95*, 769−774.
(48) Milder, I. E. J.; Arts, I. C. W.; Venema, D. P.; Lasaroms, J. J. P.; Wähälä, K.; Hollman, P. Optimization of a liquid chromatography-tandem mass spectrometry method for quatification of the plant lignans secoisolarisiresinol, matairesinol, lariciresinol and pinoresinol in foods. *J. Agric. Food Chem.* **2004**, *52*, 4643−4651.

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