JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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

HELENA K. HYVÄRINEN,† JUHA-MATTI PIHLAVA,‡ JAAKKO A. HIIDENHOVI,† VELI HIETANIEMI,‡ HANNU J. T. KORHONEN,† AND EEVA-LIISA RYHÄNEN*,†

Food Research and Chemistry Laboratory, MTT Agrifood Research Finland, FIN-31600 Jokioinen, Finland

The study focused on the effects of processing and storage on the stability of flaxseed-derived secoisolariciresinol diglucoside (SDG) added to various bakery products. The SDG concentration of doughs, baked rye breads, graham buns, and muffins was analyzed by high-performance liquid chromatography−diode array detection; the baked products were analyzed immediately after baking and upon storage at room temperature for 1 week and at −25 °C for 1 and 2 months, respectively. Added SDG was found to withstand normal baking temperatures in all bakery products. SDG also was a relatively stable compound during storage. Similarly, the content of SDG in flax buns containing fat-free flaxseed meal was unaffected by storage. We conclude that cereal-based bakery products can be supplemented with flaxseed-derived SDG.

KEYWORDS: Flaxseed; lignan; secoisolariciresinol diglucoside; SDG; processing; bread; muffin; bun; storage

INTRODUCTION

Lignans, which are diphenolic compounds containing a 2,3-dibenzylbutane skeleton (*1*), are found in higher plants such as flax and other oilseeds, cereals, vegetables, berries, and fruits, as well as tea and wine (*2*−*6*). Flaxseed (*Linum usitatissimum* L.) is one of the richest sources of lignans used in the human diet. The major lignan in flaxseed is secoisolariciresinol, which is present as a diglucoside linked to oligomers by 3-hydroxy-3-methyl glutaryl esters (*7*, *8*). The secoisolariciresinol diglugoside (SDG) content in flaxseeds has been reported to vary from 6100 to 13300 mg/kg (*9*), which corresponds to a secoisolariciresinol content of 3400−7400 mg/kg. Flaxseed also contains small amounts of matairesinol, pinoresinol, lariciresinol, and isolariciresinol (*10*−*12*).

Intestinal bacteria convert plant lignans, e.g., matairesinol, secoisolariciresinol, lariciresinol, and pinoresinol, in the colon into the mammalian lignans enterodiol and enterolactone, which exhibit weak estrogenic activity (*1*, *13*, *14*). Lignans are of great interest because many in vitro and animal studies have shown that they may have a protective role against several diseases that are predominant in the Western countries, such as breast, prostate, and colon cancers (*15*−*17*), as well as against cardiovascular diseases and diabetes (*18*−*21*). The research on flaxseed lignans in disease prevention has been recently reviewed by Westcott and Muir (*22*). Also, several reviews considering health effects of phytoestrogens have been published in the past few years (*23*−*25*).

Flaxseed can be incorporated into various food products to increase the intake of lignans. Traditionally, this has been done by adding flaxseed to bread either as whole seeds or in the form of ground flaxseed meal. However, the characteristic flavor of flaxseed may limit its other applications in foods. More recently, the development of dehulling techniques has made it possible to separate a lignan-rich hull fraction from flaxseed (*26*−*28*), and several hull preparations are now commercially available. On the other hand, the possible presence of harmful substances such as cyanogenic glycosides (*29*) and cadmium (*30*) in flax has to be taken into account if the use of flaxseed in our diet is to be increased. Supplementation of bakery products with SDG isolated or enriched from flaxseed, thus, offers an attractive approach to be investigated. So far, the stability of lignans during processing has been evaluated only in few studies. Muir and Westcott studied the stability of SDG during the bread-making process (*31*). In another study, Liukkonen et al. studied the lignans of sourdough rye bread during fermentation and baking (*32*).

This study examines the effects of processing and storage on the stability of added SDG, isolated from flaxseed or added in the form of defatted flaxseed meal, using several different bakery products as a model.

MATERIALS AND METHODS

Chemicals and Reagents. All solvents and reagents used in the study were of analytical grade. The graham flour (Kotisämpylä, Kemiön Mylly, Finland) for buns, the rye flour mixture (Myllärin ruisleipäaines,

* To whom correspondence should be addressed. Tel: +358 3 4188 3273. Fax: +358 3 4188 3244. E-mail: eeva-liisa.ryhanen@mtt.fi.
† Food Research.
‡ Chemistry Laboratory.

10.1021/jf0507590 CCC: $33.50
Published on Web 12/09/2005

Table 1. Basic Composition of SDG Buns, Flax Buns, Rye Bread, and Muffins (on a Fresh Weight Basis)

	energy (kJ/100 g)[a]	protein (g/100 g)	carbohydrates (kJ/100 g)[a]	crude fat (g/100 g)	moisture (g/100 g)	ash (g/100 g)
buns	1053	10.1	48.4	1.56	40.1	1.08
flax buns	1010	11.0	45.2	1.46	42.0	1.40
rye bread	968	6.62	47.5	1.30	44.0	1.68
muffins	1913	5.32	68.1	18.0	8.28	0.57

[a] Calculated values.

Helsingin Mylly Oy, Finland) for sour rye loaves, and the fine wheat flour (Sunnuntai, Raisio, Finland) for muffins were purchased from a local grocery store. The rye flour mixture contained freeze-dried sour rye dough and yeast to provide a starter culture for leavening. The original SDG used as a reference was a gift from Dr. Neil Westcott (Agriculture and Agri-Food Canada, Saskatoon, Canada).

Isolation and Purification of SDG from Flaxseed. Ground flaxseed meal from the cultivar Helmi (Elixi Oil Oy, Somero, Finland), containing about 15% of residual fat, was defatted by pilot-scale supercritical extraction (Chematur Engineering, Karlskoga, Sweden). The extraction was conducted at a pressure of 450 atm at 70 °C for 5 h, followed by extraction for 2 h with supercritical carbon dioxide modified with ethanol. After extraction, the fat-free crush was ground to a flour (sieve < 0.55 mm).

An amount of 100 g of fat-free flaxseed powder was hydrolyzed by 2 L of 1 M sodium hydroxide in absolute methanol overnight with a magnetic stirrer at room temperature. After hydrolysis, the pH was adjusted to slightly acidic with concentrated hydrogen chloride, and the slurry was centrifuged at 5000*g* for 5 min. The supernatant was evaporated to near dryness with a rotary evaporator. Next, 25 g of preparative C18 bulk material (Waters C18, 125 Å, Milford, MA) and 25 g of Celite 577 (Fluka Chemie, Buchs, Switzerland) were added to the residual, and the residual solvent was evaporated. A number of such batches were prepared and combined.

Thereafter, 130 g of solid sample was injected into a Biotage 75 flash chromatograph (Biotage, Charlottesville, VA) via a solid injection module. The cleanup was achieved with a Biotage C18-cartridge (75 mm × 300 mm) using 10 L of 3% methanol−water and followed by 5 L of 40% methanol−water as eluents. Several fractions were collected and analyzed by high-performance liquid chromatography (HPLC). The fractions containing SDG were evaporated to near dryness in a rotary evaporator and finally freeze-dried to obtain a slightly yellowish powder. The purity of isolated SDG was at least 95%.

Quantitation of SDG. The analytical HPLC instrument used for SDG identification and quantitation was an HP series 1100 system (Hewlett-Packard, Waldbronn, Germany) equipped with a diode array detector. Nova Pak C18 (3.9 mm × 150 mm, 4 μm, Waters) was used as the analytical column with 0.05 M phosphate buffer (A), pH 2.4, and methanol (B) as a mobile phase (5−90% B in 25 min, hold at 90% for 2 min, and finally 90−5% in 3 min) at 0.9 mL/min. SDG was quantitated at 280 nm. For identification purposes, the UV/vis spectra were recorded at 190−400 nm.

Preparation of Bakery Products. *Graham Buns.* Three graham flour-based bun batches were made for the testing of stability using the same basic recipe. Also, one batch of SDG buns was made for the testing of the effect of proofing using the same recipe.The control buns were baked with graham flour only. One of the batches was baked with added SDG (SDG buns) and one with 10% of the graham flour replaced by fat-free ground flaxseed flour (flax buns). To make the graham buns, 50 g of yeast, 250 mg of SDG, and 2 tsp of salt were dissolved in 5 dL of lukewarm water, followed by the addition of 800 g of flour. The dough was mixed and then proofed for 1 h and shaped into 40 small buns. Before baking at 225 °C for 15 min, the buns were proofed for an additional half hour. When cooled, the buns were packed into plastic bags. Samples were taken from the dough and from the buns after baking, after storage for 1 week at room temperature, and after storage for 1 and 2 months at −25 °C.

Rye Breads. Rye bread was made with the following recipe. The rye loaves, referred to as SDG bread, were made by dissolving 250 mg of SDG in 9 dL of water followed by the addition of 900 g of rye flour mixture. After careful mixing, the dough was proofed for 1 h, shaped into three loaves, and subjected to a second proofing for 40 min. The loaves were baked at 250 °C for 25 min. The bread was packed in plastic bags. Samples were taken from the dough and from the breads right after baking, after storage for 1 week at room temperature, and after storage for 2 months at −25 °C. The control breads were made similarly but without the addition of SDG.

Muffins. Two batches of muffins were made using the same basic recipe with and without the addition of SDG. For one batch, two eggs and 240 g of sugar were whipped thoroughly, followed by the addition of 100 g of melted margarine. After premixing 150 g of wheat flour, 1 tsp of baking powder, and 300 mg of SDG, this mixture was added to form a dough. The dough was poured into 12 cup muffin pans and baked at 175 °C for 23 min. The muffins were packed into plastic bags. Samples were taken from the dough and from the muffins right after baking and after 2 months of storage at −25 °C (batch 1) and from the dough and from the muffins after 1 week of storage at room temperature and after 2 months of storage at −25 °C (batch 2).

Analysis of SDG in Doughs, Breads, and Muffins. Prior to SDG extraction, the freeze-dried muffin samples (2.5 g) were defatted by hexane extraction (40 mL). The other bakery product samples were analyzed without fat extraction.

SDG was extracted from 2.5 g of freeze-dried samples with 50 mL of methanol−water (3:2 v/v) in a magnetic stirrer. Before the addition of methanol, the dough samples were first mixed with water. After 30 min, the extract was carefully decanted through a filter paper into a round-bottom flask. The solids were reextracted, and the extracts were combined. The sample was then evaporated to dryness in a rotary evaporator. For analysis, the sample was redissolved into 2 mL of methanol or, in the case of muffins, into 4 mL of methanol. The graham buns containing 10% of flaxseed flour were analyzed according to the method of Muir and Westcott (*31*). SDG was analyzed by HPLC as described earlier.

Basic Composition Analysis. *Moisture.* The moisture content was determined by weighing the samples before and after freeze drying. The residual moisture was determined by drying at 105 °C overnight (17 h) (*33*, *34*).

Ash. The ash content was analyzed by weighing the samples before and after burning at 500 °C overnight (17 h) (Inhouse method, MTT, Chemistry laboratory).

Nitrogen. The nitrogen content was determined using a Kjeltec Auto 1030 analyzer according to the Association of Official Analytical Chemists' (AOAC) method (*35*, *36*).

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

Crude Fat. The fat content was determined by the Twisselman method, using diethyl ether as a solvent (*37*−*39*).

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) (*40*).

Energy. The energy content was calculated by the formula: energy (kJ/100 g FW) = 17 × total carbohydrate % FW + 17 × protein % FW + 37 × crude fat % FW (*41*).

Total Dietary Fiber. The total dietary fiber was determined according to Lee et al. (*42*) and Rabe (*43*).

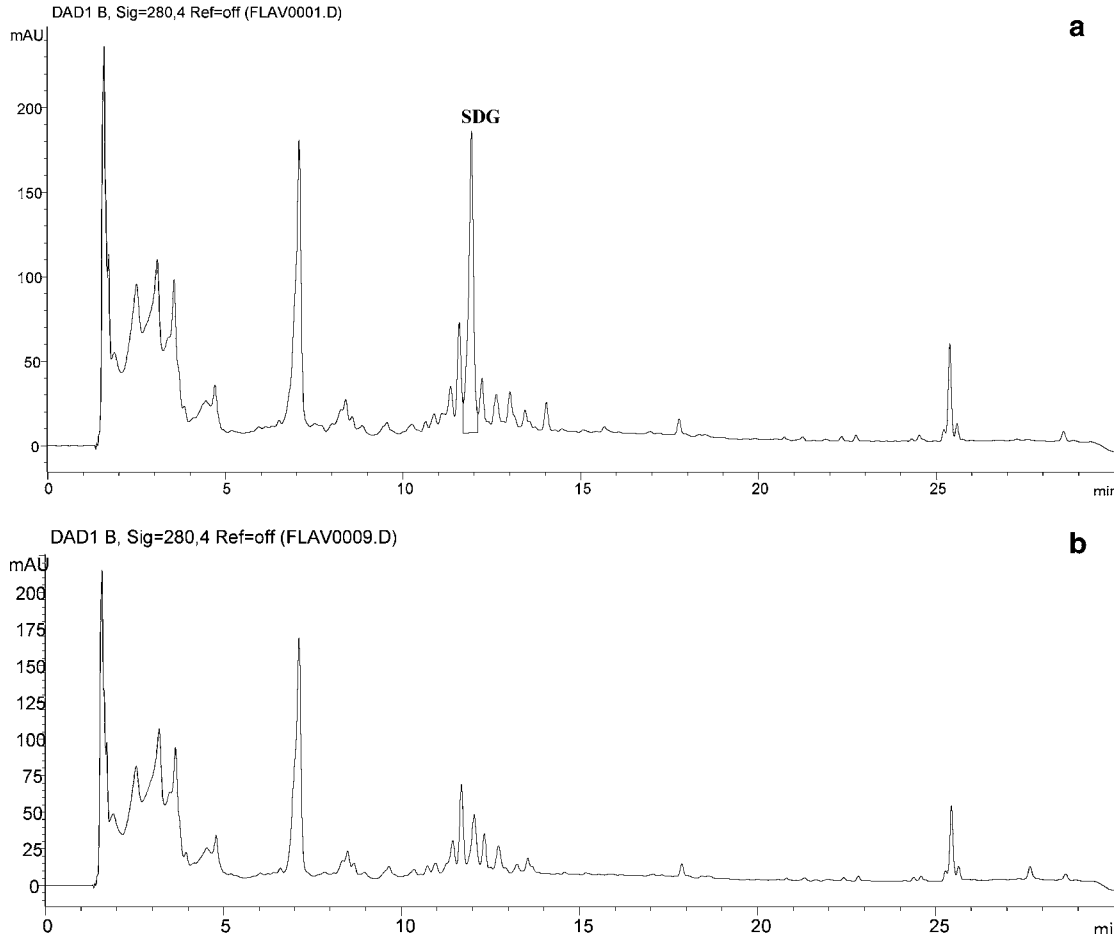

Figure 1. (**a**) HPLC chromatogram of a bun sample containing SDG stored for 1 week at room temperature. (**b**) HPLC chromatogram of a control bun sample stored for 1 week at room temperature.

Table 2. Effect of Proofing on the Content of SDG in SDG Buns (mg/kg dw)

	SDG (mg/kg dw)	SD	*n*
added amount	300		
before proofing	278	52	4
after proofing	246	16	3
after baking	263	7	7

RESULTS AND DISCUSSION

Basic Composition. The basic composition of the SDG buns, flax buns, rye bread, and muffins is presented in **Table 1**. These results agreed well with the published values of the national food database (www.fineli.fi) or the values calculated from the ingredients used.

Stability of SDG. The extraction efficiency was tested with a bun sample containing SDG by using different percentages of methanol. The best result was obtained with 60% methanol (data not shown). The precision of the method was good; the coefficient of variation was below 10% ($n = 7$). The HPLC chromatograms of the control samples showed no peaks interfering with the quantitation of SDG. (**Figure 1a**,**b**). The recovery of SDG added to the control bun samples was 114% ($n = 4$).

The 1.5 h proofing step did not affect the content of SDG in the graham buns, as can be seen from the data in **Table 2**. The SDG content in graham buns and rye breads is presented in **Table 3**. Added SDG was found to remain stable during baking at 225 °C for 15 min. Even longer and hotter baking of 25 min at 250 °C did not decompose SDG. SDG was also found to be a relatively stable compound during storage. After 2 months of storage at −25 °C, there was an increase in SDG content in some cases. This increase could have been due to changes in starch granules leading to more efficient extraction of the SDG

Table 3. SDG Contents (mg/kg dw) of Buns and Breads during Baking and Storage

	SDG buns			flax buns			rye bread		
	SDG (mg/kg dw)	SD	*n*	SDG (mg/kg dw)	SD	*n*	SDG (mg/kg dw)	SD	*n*
dough	260[c]	1	2	2419[d]	169		167	11	2
after baking	272	30	5	2033	148	5	161	2	2
storage 1 week[a]	273	22	5	1916	125	6	153	4	2
storage 1 month[b]	304	13	3	2088	96	3	NA[e]		
storage 2 months[b]	342	11	3	1990	85	3	173	2	3

[a] At room temperature. [b] At −25 °C. [c] SDG conjugate. [d] Calculated amount from the flaxseed flour. [e] NA, not available.

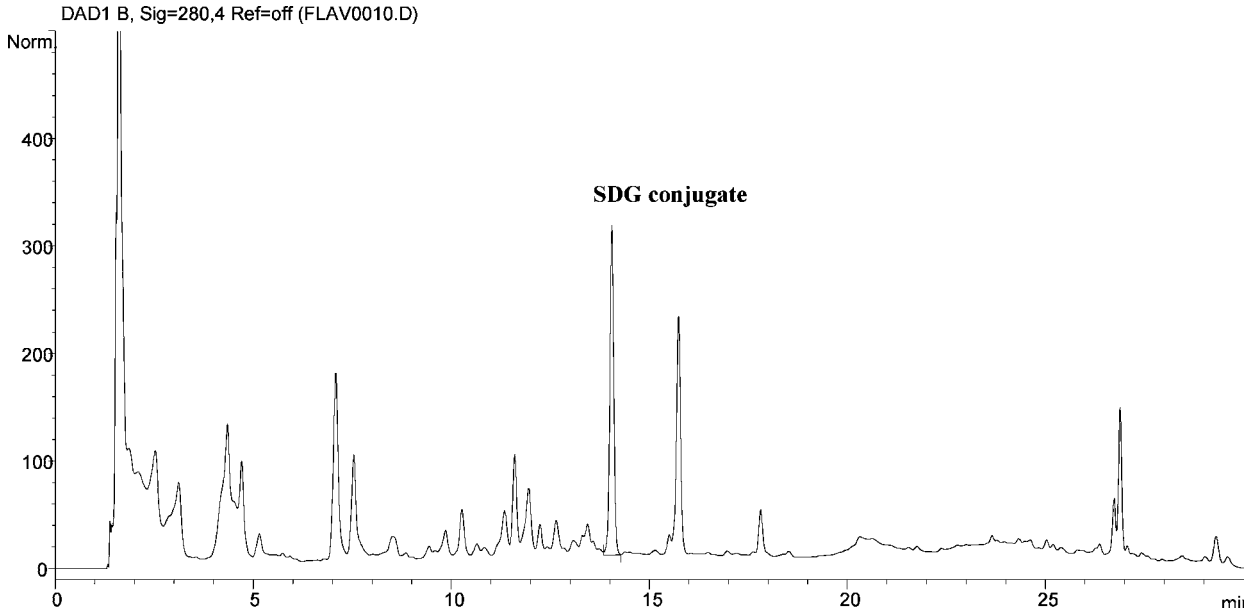

Figure 2. HPLC chromatogram of a bun dough containing SDG in conjugated form.

trapped into the dough matrix. Direct saponification or saponification of the methanol extract resulted in the same SDG content as without saponification (data not shown), indicating that the added SDG stayed in a free form. In one experiment, an unknown conjugate of SDG was seen in the HPLC chromatograms of the bun dough with no free SDG present (**Figure 2**). The conjugate had similar UV spectra as free SDG (**Figure 3**). Interestingly, this conjugate decomposed during baking so that, again, only free SDG was observed in the buns. Because the conjugate was not found in the other doughs prepared similarly but at different times, it is possible that the conjugate was due to the activity of an unknown yeast. The recoveries of added SDG from rye breads were about 40% lower than their calculated theoretical value, which was calculated on the basis of the dry components used for breads. Although the decomposition of SDG cannot be completely excluded, it is more likely that SDG is bound to the bread matrix and is not easily extractable.

The SDG content in flax buns containing fat-free flaxseed meal was also unaffected by storage in a freezer, as shown in **Table 3**. SDG content was also unaffected by the action of yeast and lactobacilli in the sour rye dough during proofing for 1 h and 40 min (**Table 2**). The stability of SDG against lactobacilli, although only for a relatively short time during proofing, is in agreement with our experiments on the stability of SDG in fermented milk products (unpublished data). Intestinal bacteria have been reported to convert SDG to secoisolariciresinol and further to enetrolactone (*44*). The starter cultures used in bread making in the present study did not exhibit such an ability, but one reason for this may be the short incubation time.

The fate of SDG in the muffins is presented in **Table 4**. The measured values of SDG are about 40% lower than the calculated theoretical values of added SDG. This could be explained by analytical difficulties with the muffin matrix. However, it is clear that SDG remained stable during baking at 175 °C for 23 min. SDG was also a relatively stable compound during storage.

The effect of processing on the stability of lignans has been evaluated in relatively few studies. Namiki et al. (*45*) found that sesamin, the predominant lignan in sesame seeds, was very stable during roasting and showed hardly any change even at 200 °C, whereas sesamolin decomposed especially as a result

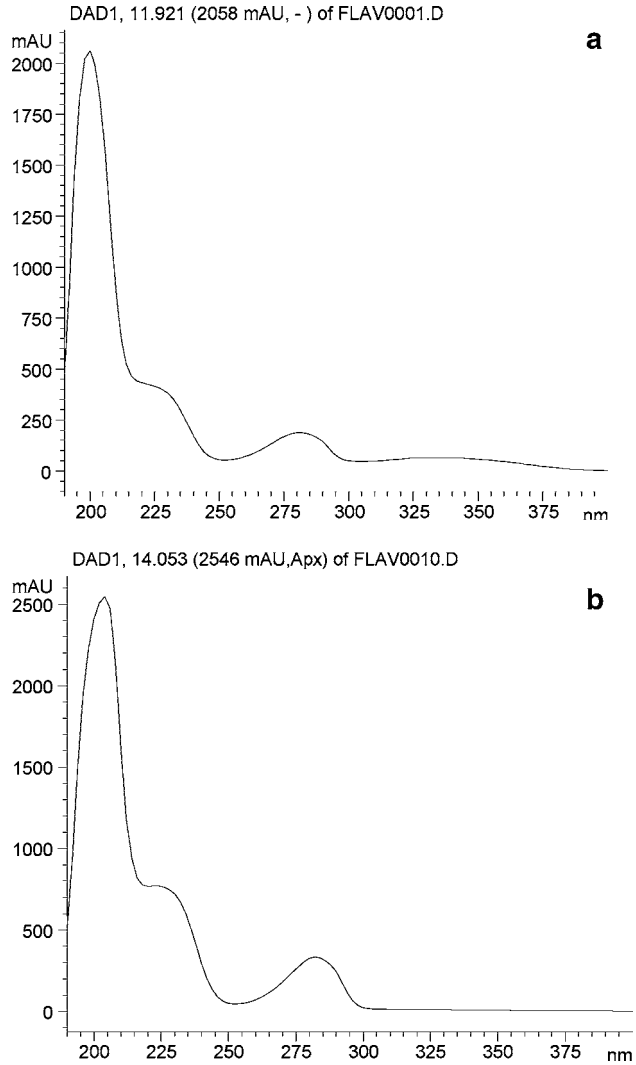

Figure 3. UV spectra of (**a**) SDG in bun sample and (**b**) SDG conjugate in one bun dough batch.

of deep frying. In a commercial virgin oil, lignans were found to be the most stable phenolic compounds during 12 months of storage (*46*). Muir and Westcott (*31*) reported that SDG

Table 4. SDG Content (mg/kg dw) of Muffins during Baking and Storage

	SDG (mg/kg dw)	SD	*n*
	batch 1		
dough	480	28	3
after baking	494	25	3
storage 2 month[b]	442	16	3
	batch 2		
dough	449	0	2
storage 1 week[a]	500	5	3
storage 2 month[b]	479	80	3

[a] At room temperature. [b] At −25 °C.

remained stable during the bread-making process and could be quantitatively recovered when SDG had been added to the bread mix. The lignans of sourdough rye bread have also been found to remain stable during fermentation and baking (*32*).

We conclude that flaxseed-derived SDG can be added to various bakery matrices that require heating or baking without significant losses during processing or subsequent storage at room temperature or in deep-frozen form.

ACKNOWLEDGMENT

We thank Heli Hiden as well as Tuula Kurtelius, Outi Kurri, Merja Uusitupa, and Anne Sinisalo for their skillful technical assistance.

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) Thompson, L.; Robb, P.; Serraino, M.; Cheung, F. Mammalian lignan production from various foods. *Nutr. Cancer* **1991**, *16*, 43−52.

(3) Mazur, W. Phytoestrogen content in food. In *Bailiere's Clinical Endocrinology and Metabolism*; Adlercreutz, H., Ed.; Bailliere Tindall: London, United Kingdom, 1998; Vol. 12, pp 729−742.

(4) Mazur, W.; Uehara, M.; Wähälä, K.; Adlercreutz, H. Phyto-oestrogen content of berries and plasma concentrations and urinary excreation of enterolactone after a single strawberry-meal in human subjects. *Br. J. Nutr.* **2000**, *83*, 381−387.

(5) Meagher, L. P.; Beecher, G. R. Assessment of data on the lignan content of foods. *J. Food Compos. Anal.* **2000**, *13*, 935−947.

(6) Nurmi, T.; Heinonen, S.; Mazur, W.; Deyama, T.; Nishibe, S.; Adlercreutz, H. Lignans in selected wines. *Food Chem.* **2003**, *83*, 303−309.

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

(8) Kamal-Eldin, A.; Peerlkamp, N.; Johnsson, P.; Andersson, R.; Andersson, R. E.; Lundgren, L. N.; Åman, P. An oligomer from flaxseed composed of secoisolariciresinolglucoside and 3-hydroxy-3-methyl glutaric acid residues. *Phytochemistry* **2001**, *58*, 587−590.

(9) 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.

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

(11) 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.

(12) 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.

(13) 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.

(14) 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.

(15) Bowen, P. E. Evaluating the health claim of flaxseed and cancer prevention. *Nutr. Today* **2001**, *36*, 144−158.

(16) Demark-Wahnefried, W.; Price, D. T.; Polascik, T. J.; Robertson, C. N.; Anderson, E. E.; Paulson, D. F.; Walther, P. J.; Gannon, M.; Vollmer, R. T. Pilot study of dietary fat restricton and flaxseed supplementation in men with prostate cancer before surgery: Exploring the effects on hormonal levels, prostate-specific antigen, and histopathologic features. *Urology* **2001**, *58*, 47−52.

(17) Yang, C. S.; Landau, J. M.; Huang, M.-T.; Newmark, H. L. Inhibition of carcinogenesis by dietary polyphenolic compouns. *Annu. Rev. Nutr.* **2001**, *21*, 381−406.

(18) Prasad, K. Reduction of serum cholesterol and hypercholesterolemic antherosclerosis in rabbits by secoisolariciresinol diglucoside (SDG) isolated from flaxseed. *Circulation* **1999**, *99*, 1355−1362.

(19) Prasad, K. Oxidative stress as a mechanism of diabetes in diabetic BB prone rats: Effect of secoisolariciresinol diglucoside (SDG). *Mol. Cell. Biochem.* **2000**, *209*, 89−96.

(20) Prasad, K. Secoisolariciresinol diglucoside from flaxseed delays the development of type 2 diabetes in Zucker rat. *J. Lab. Clin. Med.* **2001**, *138*, 32−39.

(21) Prasad, K.; Mantha, S. V.; Muir, A. D.; Westcott, N. D. Protective effect of secoisolariciresinol diglucoside against streptozotocin-induced diabetes and its mechanism. *Mol. Cell. Biochem.* **2000**, *206*, 141−149.

(22) Westcott, N. D.; Muir, A. D. Flax seed lignan in disease prevention and health promotion. *Phytochem. Rev.* **2003**, *2*, 401−417.

(23) Wang, L.-Q. Mammalian phytoestrogens: Enterodiol and enterolactone. *J. Chromatogr. B* **2002**, *777*, 289−309.

(24) Duncan, A. M.; Phipps, W. R.; Kurzer, M. S. Phyto-oestrogens. *Best Pract. Res. Clin. Endocrinol. Metab.* **2003**, *17*, 253−271.

(25) Cornwell, T.; Cohick, W.; Raskin, I. Dietary phytoestrogens and health. *Phytochemistry* **2004**, *65*, 995−1016.

(26) Oomah, D.; Mazza, G.; Keenaschuk, E. Dehulling characteristics of flaxseed. *Lebensm.-Wiss. Technol.* **1996**, *29*, 245−250.

(27) Myllymäki, O. Processing of flaxseed. Patent CA 2304303, 2001.

(28) Madhusudhan, B.; Wiesenborn, D.; Schwartz, J.; Tostenson, K.; Gillespie, J. A dry mechanical method for concentrationf of lignan secoisolariciresinola diglucoside in flaxseed. *Lebensm.-Wiss. Technol.* **2000**, *33*, 268−275.

(29) Muir, A. D.; Westcott, N. D. Flaxseed constituents and human health. In *Flax. The Genus Linum*; Muir, A. D., Westcott, N. D., Eds.; Taylor & Francis: London, United Kingdom, 2003; Chapter 12, pp 243−251.

(30) Li, Y.-M.; Chaney, R. L.; Schneiter, A. A.; Miller, J. F.; Elias, E. M.; Hammond, J. J. Screening for low grain cadmium phenotypes in sunflower, durum wheat and flax. *Euphytica* **1997**, *94*, 23−30.

(31) Muir, A. D.; Westcott, N. D. Quantitation of the lignan secoisolariciresino diglucoside in the baked goods containing flax seed or flax meal. *J. Agric. Food Chem.* **2000**, *48*, 4048−4052.

(32) Liukkonen, K.-H.; Katina, K.; Wilhelmsson, A.; Myllymäki, O.; Lampi, A.-M.; Kariluoto, S.; Piironen, V.; Heinonen, S.-M.; Nurmi, T.; Adlercreutz, H.; Peltoketo, A.; Pihlava, J.-M.; Hietaniemi, V.; Poutanen, K. Process-induced changes on bioactive compounds in wholegrain rye. *Proc. Nutr. Soc.* **2003**, 1−6.

(33) 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.

(34) 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.

(35) 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.

(36) 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.

(37) 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.

(38) 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.

(39) 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.

(40) *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.

(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 XVII.

(42) Lee, S. C.; Prosky, L.; De Vries, J. W. Determination of total soluble and insoluble dietary fibre in foods—Enzymaticgravimetric method, MES-TRIS buffer: collaborative study. *J. AOAC Int.* **1992**, *75*, 395−416.

(43) Rabe, E. Analysis and consumption of dietary fiber in Germany. In *Natural Antioxidants and Food Quality in Atherosclerosis and Cancer Prevention*; Kumpulainen, J. T., Salonen, J. T., Eds.; The Royal Society of Chemistry: Cambridge, United Kingdom, 1996; pp 322−327.

(44) Boriello, S. P.; Setchell, K. D. R.; Axelson, M.; Lawson, A. M. Production and metabolism of lignans by the human faecal flora. *J. Appl. Bacteriol.* **1985**, *58*, 37−43.

(45) Namiki, M.; Fukuda, Y.; Takei, Y.; Namiki, K.; Koizumi, Y. Changes in functional factors of sesame seed and oil during various types of processing. In *Bioactive Compounds in Foods. Effect of Processing and Storage*; Lee, T.-C., Ho, C.-T., Eds.; American Chemical Society: Washington, DC, 2002; Chapter 7, pp 85−104.

(46) Morelló, J.-R.; Motilva, M.-J.; Tovar, M.-J.; Romero, M.-P. Changes in commercial virgin olive oil (cv Arbequina) during storage, with special emphasis on the phenolic fraction. *Food Chem.* **2004**, *85*, 357−364.

Received for review April 4, 2005. Revised manuscript received October 17, 2005. Accepted November 4, 2005. The study was funded by the Ministry of Agriculture and Forestry of Finland, HK-Ruokatalo (Turku, Finland) and MTT Agrifood Research Finland.

JF0507590