Antioxidant Capacity of Flaxseed Lignans in Two Model Systems

Farah S. Hosseinian[a], Alister D. Muir[b], Neil D. Westcott[b], and Ed S. Krol[a,*]

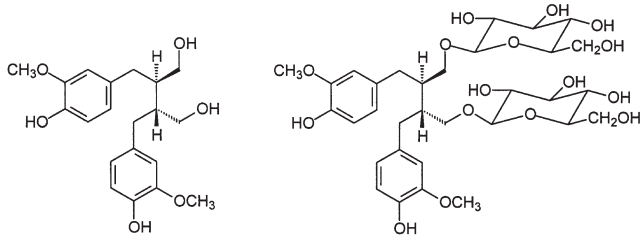
[a]College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, and [b]Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada

ABSTRACT: The flaxseed lignans secoisolariciresinol (SECO) and its diglucoside secoisolariciresinol diglucoside (SDG) are reported to have a number of health benefits associated with their consumption that have in part been attributed to their antioxidant properties. In this study the relative antioxidant capacity of the flaxseed lignans vs. BHT was determined in two model systems. First, an antioxidant stoichiometric value was determined for SECO and SDG in a liposomal system as a mimic of lipid peroxidation. Stoichiometric values for SECO (1.5) and SDG (1.2) vs. BHT (2.0) were measured from the lag time for the formation of conjugated dienes; all values were significantly different ($P < 0.01$). Second, the ability of flaxseed lignans to prevent oxidative degradation of canola oil was determined. Samples were stored at room temperature and analyzed at 30-d intervals over 120 d using a Rancimat™ analyzer. The lignans prevented degradation of canola oil, as measured by induction time, in a concentration-dependent manner. Although SECO demonstrated a trend for better protection than either SDG or polymer containing SDG, they were not significantly different ($P > 0.01$). There was also no significant difference between SECO or SDG and BHT, suggesting flaxseed lignans may be good alternatives to minimize rancidity in oil-based food products.

Paper no. J11306 in *JAOCS 83,* 835–840 (October 2006).

KEY WORDS: Antioxidant food preservative, 2,2′-azobis-(2-amidinopropane) (AAPH), 1,2-dilinoleoyl-sn-glycerol-3-phosphocholine (DLPC), flaxseed lignans, induction time (IT), Rancimat™, secoisolariciresinol (SECO), secoisolariciresinol diglucoside (SDG), SDG polymer, stoichiometry.

Flaxseed consumption has been demonstrated to exhibit potential health benefits including decreased tumor growth, reduced serum cholesterol levels, and decreased formation of breast, prostate, and colon cancers (1–4). These health benefits may be the result of the oil in flaxseed, which has a high content of α-linolenic acid (18:3 ω-3) (approximately 59%), or the polyphenolic lignans that are present in the meal (1,5,6). The major lignan present in flaxseed is secoisolariciresinol (SECO) (Scheme 1) (2). Free SECO is not found in the meal, rather it is present as the diglycoside SDG (7–11) (Scheme 1), which is incorporated into a polymer that is ester-linked *via* 3-hydroxy-3-methylglutaric acid moieties (12,13). Flaxseed is the richest source of food lignans, and can contain 75–800 times more than other food sources (2.0 mg/g based on seed dried weight) (1,14).



SCHEME 1

SECO and SDG are metabolized in the gastrointestinal tract to the mammalian lignans enterodiol (END) and enterolactone (ENL), which also have been associated with beneficial health effects in humans (2,15,16). Several studies have suggested that the antioxidant properties of SECO, SDG, END, and ENL are associated with their beneficial effects (15,17,18,19).

The exposure of vegetable or animal fats to oxygen at ambient temperature can result in autoxidation leading to lipid peroxides (20,21). Lipid peroxidation can result in rancidity of fats and oils, which affects the odor, taste, and nutritional values of these products. The main purpose of using antioxidants in vegetable oils is to prevent or delay the autoxidation process, thus extending the shelf life of the product and minimizing nutritional losses (21). Antioxidant food preservatives that are currently used, phenols such as BHT and BHA, inhibit lipid peroxidation by trapping peroxyl radicals and preventing peroxyl radical chain propagation from occurring (21). Potential liver toxicity from chronic use of these compounds, however, has caused some concerns (22).

This study investigates the antioxidant properties of flaxseed lignans to determine whether they might be suitable alternatives to BHT in terms of food preservation. Flaxseed lignans are natural products with no known toxicity (23), and they may also have potential health benefits associated with their consumption. This study aims to determine the relative abilities of SECO, SDG, SDG polymer, and BHT to prevent lipid peroxidation. Two model systems were used to measure these properties. First, a peroxyl radical-mediated liposomal oxidation system was used to calculate an antioxidant stoichiometric value, which measures the number of radicals consumed per molecule of antioxidant (15). Second, the ability of each antioxidant to inhibit

*To whom correspondence should be addressed at College of Pharmacy and Nutrition, University of Saskatchewan, 110 Science Pl., Saskatoon, Saskatchewan S7N 5C9 Canada. E-mail: ed.krol@usask.ca

rancidity in a commercially available vegetable oil *via* measurement of lipid oxidation levels using a Rancimat™ analyzer was determined (24,25). This study suggests flaxseed lignans, especially SECO, may be suitable alternatives to BHT for the prevention of lipid oxidation.

EXPERIMENTAL PROCEDURES

Materials. Canola oil (Crisco™; J.M. Smucker Co., Orrville, OH) was purchased from a commercial source in Saskatoon. The oil was free of added antioxidants or preservatives. SECO, SDG, and SDG polymer were obtained from Agriculture and Agri-Food Canada (Saskatoon, Canada). SECO and SDG were ≥99% pure by HPLC and are present as the (*R,R*) (+)-enantiomer as determined by optical rotation using a Jasco P-1010 polarimeter (glass cell; length: 100 mm) (Jasco Corp., Tokyo, Japan). The optical rotation was determined to be: SECO $[\alpha]^{22}_D$ = +35 (*c* 0.20, CH_3OH) and SDG $[\alpha]^{22}_D$ = +0.02 (*c* 0.20, CH_3OH). BHT and Tris-HCl were purchased from Sigma (St. Louis, MO), 2,2′-azobis(2-amidinopropane) (AAPH) was from Monomer-Polymer & Dajac Labs, Inc. (Feasterville, PA) and 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine (DLPC) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). All solvents were HPLC grade. Water was purified using a Millipore Super Q water system with one carbon cartridge followed by two ion exchange cartridges (Bedford, MA). UV spectra were recorded using quartz cuvettes (VWR Canada) on an Agilent 8453 UV/vis photodiode array (PDA) spectrophotometer with ChemStation software (Agilent Technologies Canada Inc., Mississauga, ON).

Antioxidant stoichiometry. Antioxidant stoichiometry was measured using a modification of the method of Liebler and Burr (26). A liposomal oxidation mixture was prepared, using BHT, AAPH, and DLPC. The stoichiometric ratio of SECO and SDG was measured by comparison of the lag time for conjugated diene formation (at 240 nm) in the presence of SECO and SDG vs. the lag time for conjugated diene formation in the presence of a standard antioxidant (BHT). The stoichiometric ratio (*n*) for peroxyl radical trapping was calculated from the following equation: $n = R_i\,\tau/[\mathrm{ArOH}]$, where R_i (mM min^{-1}) is the rate of chain initiation, [ArOH] is the concentration of the antioxidant, and τ is the length of induction time (min) (26).

Liposomes were prepared following a modification of the method of Azuma *et al.* (27). Briefly, BHT in hexane (0.80 mM) was added to a screw-capped wide-mouthed 5-mL vial, and the hexane evaporated under nitrogen. To the vial was added 5 mg DLPC in chloroform, and the chloroform was evaporated under nitrogen to obtain a thin-film coating of DLPC. Tris-HCl buffer (0.6 mL), pH 7.0, was added, and the mixture was vortex-mixed for 1 min, resulting in a multilamellar dispersion. The suspension was then transferred into a small LiposoFast™ extrusion device (Avestin Ltd., Ottawa, Canada), outfitted with a polycarbonate membrane (100-nm pore size). The suspension was passed through the membrane 17–20 times to obtain a homogeneous unilamellar liposome. The unilamellar liposome (0.5 mL) was then transferred to a new screw-capped 1.5-mL HPLC vial with a septum on top and diluted with 800 μL of pre-warmed (50°C) Tris-HCl buffer, pH 7.0. The lipid peroxidation reaction was initiated by adding 200 μL of AAPH (0.2 mM in water) solution to a final volume of 1.5 mL to minimize headspace oxidation. The vial was sampled through the septum *via* syringe to minimize the introduction of air to the system. Oxidations were carried out at 50°C in the dark; a control reaction was performed in the absence of BHT. Conjugated diene formation was measured by removing 30-μL aliquots, which were dissolved in 970 μL methanol to a final volume of 1.0 mL. Aliquots were removed at 2-min intervals for 20 min and at 5-min intervals until 30 min. Absorbance was measured at 240 nm using methanol as a blank. SECO and SDG were analyzed using the same concentration as BHT (0.80 mM). Owing to incomplete dissolution of SECO and SDG in hexane, the procedure was modified slightly. To a vial was added 5 mg DLPC in chloroform, and the chloroform was evaporated under nitrogen. To this vial was then added a solution of SDG or SECO dissolved in Tris-HCl buffer (0.6 mL). Other oxidation reaction parameters were the same as those used for BHT. For each analysis, three individual samples were prepared in duplicate, and the data were analyzed statistically by using the Microsoft™ program Excel. Errors were expressed as the SD of the mean.

Rancidity test. The ability of BHT, SECO, SDG, and SDG polymer (comprising mainly HMG-linked SDG) to maintain the shelf life of a commercially available cooking oil was determined by measuring the induction time for autoxidation of canola oil in the absence or presence of an antioxidant using a Rancimat™ analyzer (model 743; Metrohm Ltd., Herisau Switzerland) following AOCS method Cd 12B-92 (28). Two studies were carried out. First, a pilot study compared SECO with BHT at different weight concentrations (10, 20, and 30 mg/100 g oil) of antioxidant or a control reaction containing no antioxidant. BHT is typically present in concentrations of at least 75 mg/kg in foods (24,25). A second study was performed in which samples were treated with different molar concentrations (0.04, 0.06, and 0.08 mmol/100 g oil for BHT, SECO, and SDG; 10, 20, and 30 mg/100 g oil for SDG polymer) of antioxidant or a control reaction containing no antioxidant. Samples were stored at room temperature (23°C) in sealed glass containers in the dark and were analyzed at 30-d intervals over 120 d. For each analysis, 3 g of sample was placed in a clean Rancimat™ measuring tube; the contents of the tube were then heated at 110 ± 2°C and subjected to a stream of air (flow of 20 L/h). The volatile oxidation products bubbled through individual cells containing doubly deionized water, and the conductivity of the water was measured. A change in conductivity was reported as the induction time (IT). Data are the mean of at least two replications.

Analytical HPLC. The products of AAPH-mediated BHT oxidation were determined using an Alliance HPLC (Waters Inc., Milford, MA) on a Symmetry reversed-phase C18 column (3 × 150 mm, 5 μm particle size; Waters Inc.). The mobile phase consisted of 0.05% (vol/vol) trifluoroacetic acid (TFA) in H_2O (solvent A) and 0.05% (vol/vol) TFA in acetonitrile

(solvent B). A gradient elution was performed, using a flow rate of 0.4 mL min^{-1}, with 2% solvent B isocratic for 10.0 min; increased to 40% solvent B over 2 min; isocratic at 40% for 3 min then decreased to 2% over 2 min and isocratic at 2% B for 5 min. Peaks were detected using a 996 UV/vis PDA detector (Waters Inc.) with full spectral scans (200–400 nm).

LC-MS analysis. The reaction products of AAPH-mediated oxidation of BHT in DLPC liposomes were analyzed *via* MS. LC-MS data were determined using the same high-performance liquid chromatograph already indicated, connected to a Quattro-LC (Micromass UK Ltd., Manchester, United Kingdom) equipped with electrospray ionization (ESI)-MS (±) sources (Micromass). HPLC analysis was performed by applying the same gradient as for the analytical HPLC except 0.05% formic acid was used instead of 0.05% TFA.

Statistical analysis. Two-way ANOVA was performed using SAS (Cary, NC). The differences were considered significant when $P < 0.01$.

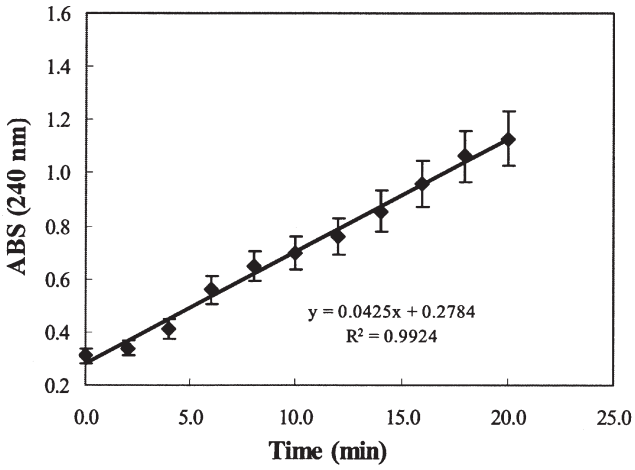


FIG. 1. Conjugated diene formation as a measure of AAPH-mediated peroxidation of DLPC liposomes (pH 7.4, 50°C). ABS, absorbance.

RESULTS AND DISCUSSION

Stoichiometric ratio (n) determination. BHT ($n = 2$) was used as a standard for antioxidant activity to determine an R_i value (0.09 ± 0.01 mM min^{-1}) from AAPH-mediated lipid peroxidation of DLPC liposomes at 50°C ($\tau = 17.8 \pm 0.04$ min) (26). An n of 2 was confirmed for BHT by analyzing products of the BHT/AAPH/DLPC reaction. LC-MS data showed that BHT formed one major oxidation product (retention time = 2.6 min, ESI (*m/z* 337 $[M - H]^-$ and *m/z* 335 $[M + H]^+$), λ_{max} 350 nm) consistent with a cyclohexadiene-peroxyl radical adduct (26). The R_i value was then used to calculate n for each antioxidant (Table 1). HPLC analyses of a control mixture of BHT, AAPH, and DLPC at room temperature indicate no interaction occurred between the components in the absence of heat.

A plot of absorption (240 nm) vs. time (min) for a control reaction in the absence of antioxidant was linear for conjugated diene formation over 20 min (Fig. 1). In the presence of antioxidant, lag time was determined from the intercept of two lines representing the delay prior to the onset of lipid peroxidation and the oxidation phase, when the antioxidant (BHT, SECO, or SDG) had been consumed. This is demonstrated in Figure 2, where the line representing delay ($y = 0.0035x + 0.3621$) and the line representing the onset of lipid peroxidation ($y = 0.0174x + 0.1762$) in the presence of 0.80 mM SECO are shown. SECO delayed AAPH-induced lipid autoxidation by 13.3 min. The stoichiometric ratios (n) for BHT, SECO (1.5 ± 0.05), and SDG (1.2 ± 0.1) are significantly different (Table 1). This suggests that both SECO and SDG are less potent antioxidants than BHT, although SECO is superior to SDG.

Induction time determination. The results for the pilot study comparing SECO with BHT by weight (mg) per 100 g oil are shown in Table 2. There was no significant difference between the induction times at time = 0 for control, BHT, and SECO at all concentrations. Protection against autoxidation was concentration-dependent, as induction times were significantly different for BHT at 10, 20, and 30 mg and for SECO at 10, 20, and 30 mg. Induction time decreased with increasing storage time for all samples. Induction times for both BHT and SECO were

TABLE 1
Stoichiometric Ratio (*n*) for SECO (0.80 mM) and SDG (0.80 mM) Inhibition of AAPH-Mediated Peroxidation of DLPC Liposomes[a], pH 7.4, 50°C

Sample	Lag time (min)	R_i value (mM min^{-1})	Stoichiometric ratio *n*
SECO	13.4 ± 0.1	0.09 ± 0.01	1.5* ± 0.2
SDG	10.8 ± 0.03	0.09 ± 0.01	1.2* ± 0.03

[a]Data are expressed as mean ± SD. *$P < 0.01$ compared with BHT. SECO, secoisolariciresinol; SDG,secoisolariciresinol diglucoside; AAPH, 2,2′-azobis-(2-amidinopropane); DLPC, 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine; R_i, rate of chain initiation.

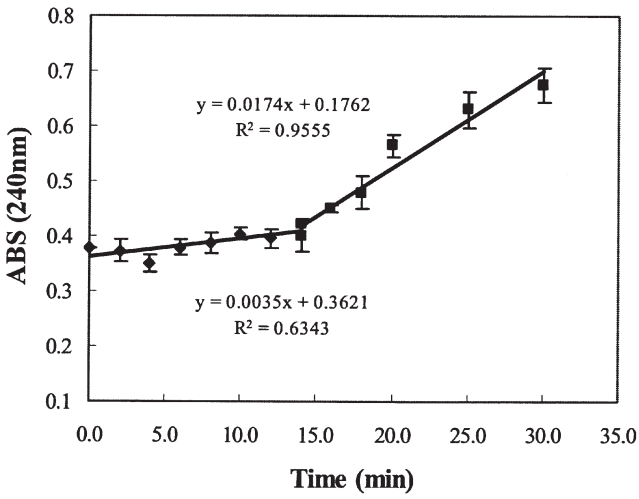


FIG. 2. Conjugated diene formation for the determination of antioxidant-mediated lag time (min) for AAPH-mediated peroxidation of DLPC liposomes at pH 7.4, 50°C, in the presence of 0.80 mM secoisolariciresinol. For abbreviations see Table 1.

TABLE 2
Induction Time (h) Measured Using a Rancimat at 110°C for BHT and SECO at 10, 20, and 30 mg/100 g Oil at Different Storage Times[a] (d)

Storage time (d)	Induction time (h)						
	Control	SECO 2 (10 mg)	SECO 2 (20 mg)	SECO 2 (30 mg)	BHT 17 (10 mg)	BHT 17 (20 mg)	BHT 17 (30 mg)
0	11.29 ± 0.04	11.26 ± 0.06	11.28 ± 0.04	11.25 ± 0.01	11.33 ± 0.01	11.29 ± 0.04	11.36 ± 0.02
30	10.38 ± 0.02	10.68* ± 0.02	10.98* ± 0.01	11.13* ± 0.05	10.61* ± 0.08	11.05* ± 0.06	11.17* ± 0.04
60	9.93 ± 0.02	10.19* ± 0.02	10.44* ± 0.02	10.98* ± 0.03	10.22* ± 0.04	10.86* ± 0.04	11.11* ± 0.05
90	8.62 ± 0.01	9.77* ± 0.04	10.21* ± 0.01	10.62* ± 0.02	9.92* ± 0.01	10.50* ± 0.08	10.82* ± 0.04
120	7.09 ± 0.04	9.01* ± 0.01	9.16* ± 0.02	10.03* ± 0.04	9.05* ± 0.02	9.44* ± 0.01	10.27* ± 0.06

[a]Samples were stored at 23°C. Data are expressed as mean ± SD. * P <0.01 compared with control. For abbreviation see Table 1.

significantly longer than control at all concentrations; however, BHT and SECO were not significantly different from each other. These data suggest that, on a per weight basis, SECO is comparable with BHT for the prevention of canola oil autoxidation. SECO, however, has a M.W. of 362 g/mol whereas BHT has a M.W. of 220 g/mol; therefore, 10 mg of BHT corresponds to 0.045 mmol and 10 mg of SECO corresponds to 0.027 mmol. This suggested that each molecule of SECO demonstrates a capacity for protection against autoxidation in our system nearly 1.6 times greater than BHT.

The results for the second study, comparing the ability of BHT and flaxseed lignans to protect against autoxidation in the model system on a per mole basis, are shown in Table 3. Induction time decreased with increasing storage time for control and all antioxidants. Protection against autoxidation was concentration-dependent, as induction times for BHT, SECO, and SDG were significantly different at 0.04, 0.06, and 0.08 mmol. Antioxidant induction time was significantly different from control at all concentrations; however, BHT, SECO, and SDG were not significantly different from each other. These results suggest that flaxseed lignans should be effective at preventing autoxidation of canola oil at the typical concentrations currently used for BHT in food.

SDG polymer was also included in this study (7,12). A specific M.W. cannot be assigned to the polymer, although a lower limit of 35 wt% SDG in the polymer (7,12) allows an estimate of SDG equivalents such that 10, 20, and 30 mg/100 g oil correspond to SDG concentrations of 0.0044, 0.0087, and 0.0131 mmol/100 g oil, respectively. Therefore, the results for SDG polymer in this study cannot be compared directly with the other antioxidants. It is important to note that other phenolics, including cinnamates, are present in unknown amounts in SDG polymer, and these may contribute to the antioxidant effects (7). SDG polymer showed significant concentration-dependent increases in induction time vs. control (Table 3). A tentative comparison of induction times for the SECO results at 120 d from our pilot study (Table 2) suggests that 10 mg of SDG polymer/100 g oil (9.10 h) compares favorably with 10 mg of SECO/100 g oil (9.01 h). SDG polymer may be the most economically feasible form of flaxseed lignans to use in the role of food preservative since SDG polymer requires fewer processing steps and is likely to be less expensive to isolate than SECO or SDG.

The stoichiometric ratio study shows that SECO is a better antioxidant than SDG; the induction time study shows a similar trend, although the results are not statistically significant.

TABLE 3
Induction Time (h) Measured Using a Rancimat at 110°C for BHT, SECO, and SDG at 0.04, 0.06, and 0.08 mmol/100 g Oil and SDG Polymer at 10, 20, and 30 mg/100 g Oil at Different Storage Times[a] (d)

Storage time (d)	Induction time (h)						
	Control	SECO (0.04 mM)	SECO (0.06 mM)	SECO (0.08 mM)	BHT (0.04 mM)	BHT (0.06 mM)	BHT (0.08 mM)
0	11.43 ± 0.02	11.45 ± 0.15	11.60 ± 0.02	11.61 ± 0.06	11.45 ± 0.06	11.60 ± 0.08	11.63 ± 0.13
30	10.06 ± 0.01	11.12* ± 0.18	11.46* ± 0.07	11.27* ± 0.07	11.22* ± 0.07	11.40* ± 0.04	11.59* ± 0.06
60	8.90 ± 0.02	10.52* ± 0.24	10.93* ± 0.03	11.16* ± 0.02	10.72* ± 0.08	11.16* ± 0.13	11.51* ± 0.06
90	7.74 ± 0.03	9.93* ± 0.05	10.42* ± 0.07	10.65* ± 0.03	10.14* ± 0.19	10.68* ± 0.06	11.07* ± 0.13
120	6.76 ± 0.02	9.37* ± 0.61	9.95* ± 0.14	10.23* ± 0.04	9.46* ± 0.19	10.39* ± 0.21	10.65* ± 0.13
Storage time (d)	Control	SDG (0.04 mM)	SDG (0.06 mM)	SDG (0.08 mM)	SDG polymer (10 mg)	SDG polymer (20 mg)	SDG polymer (30 mg)
0	11.43 ± 0.02	10.96 ± 0.14	11.25 ± 0.07	11.35 ± 0.13	11.41 ± 0.08	11.50 ± 0.13	11.50 ± 0.02
30	10.06 ± 0.01	10.63* ± 0.17	10.84* ± 0.10	11.10* ± 0.08	10.82* ± 0.12	11.02* ± 0.08	11.24* ± 0.22
60	8.90 ± 0.02	10.00* ± 0.18	10.70* ± 0.06	10.78* ± 0.12	10.31* ± 0.09	10.60* ± 0.03	10.91* ± 0.11
90	7.74 ± 0.03	9.36* ± 0.05	10.36* ± 0.12	10.17* ± 0.12	9.70* ± 0.13	10.19* ± 0.05	10.58* ± 0.04
120	6.76 ± 0.02	8.86* ± 0.12	9.25* ± 0.42	9.80* ± 0.02	9.10* ± 0.12	9.70* ± 0.02	10.18* ± 0.04

[a]Samples were stored at 23°C. Data are expressed as mean ± SD. *P < 0.01 compared with control. For abbreviations see Table 1.

This trend, i.e., that SECO may confer better protection than SDG, may be due to one of two reasons. First, the alcohol groups on SECO, which are connected to glucose in SDG, may be an important contributor to antioxidant activity. Second, the glucose moieties on SDG and SDG polymer cause them to be less lipid soluble than SECO; thus, SDG and SDG polymer may not incorporate into the oil as well as SECO or BHT.

There has been no reported evidence of SECO, SDG, or SDG polymer-induced toxicity. *In vitro* studies have found SECO to be nongenotoxic to microtubule stability, induction of mitotic arrest, micronuclei induction, and mutagenicity of the hypoxanthine guanine phosphoribosyltransferase gene locus in cultured Chinese hamster V79 cells and to cell-free microtubule formation (23). This indicates that flaxseed lignans may be useful as alternative natural antioxidant preservatives and may be applicable in this role in vegetable oils and possibly in other foods. It may also be attractive to include flaxseed lignans as a preservative in vegetable oils because of the potential to provide additional health benefits as antioxidant or antiestrogenic compounds. The requirement for increased amounts of flaxseed lignans for this purpose could also provide a value-added economic benefit to flaxseed growers.

ACKNOWLEDGMENTS

This work was funded by a Health Services Utilization and Research Commission (HSURC) and a Saskatchewan Flax Development Commission (SFDC) grant and supported by Agriculture and Agri-Food Canada (Saskatoon Research Centre). The authors also thank Dr. Martin Reaney (Saskatoon Research Centre) for providing use of the Rancimat™ apparatus.

REFERENCES

1. Muir, A.D., and N.D. Westcott, Flaxseed Constituents and Human Health, in *Flax, the Genus Linum* (A.D. Muir and N.D. Westcott, eds.), Taylor & Francis, London, 2003, pp. 243–251.
2. Westcott, N.D. and A.D. Muir, Flax Seed Lignan in Disease Prevention and Health Promotion, *Phytochem. Rev. 2*:401–417 (2003).
3. Lucas, E.A., S.A. Lightfoot, L.J. Hammond, L. Devareddy, D.A. Khalil, B.P. Daggy, B.J. Smith, N. Westcott, V. Mocanu, Soung do Y, and B.H. Arjmandi, Flaxseed Reduces Plasma Cholesterol and Atherosclerotic Lesion Formation in Ovariectomized Golden Syrian Hamsters, *Atherosclerosis 173*:223–229 (2004).
4. Hemmings, S.J., N. Westcott, A. Muir, and D. Czechowicz, The Effects of Dietary Flaxseed on the Fischer 344 Rat: II. Liver Gamma-Glutamyltranspeptidase Activity, *Cell. Biochem. Func. 22*:225–231 (2004).
5. Westcott, N.D., and A.D. Muir, Process for Extracting Lignans from Flaxseed, U.S. Patent 5,705,618 (1998).
6. Oomah, B., Processing of Flaxseed Fiber, Oil, Protein, and Lignan, in *Flaxseed in Human Nutrition* (L. Thompson and S. Cunnane, eds.), AOCS Press, Champaign, IL, 2003, pp. 363–386.
7. Westcott, N.D., and A.D. Muir, Chemical Studies on the Constituents of *Linum* spp., in *Flax, the Genus Linum* (A.D. Muir and N.D. Westcott, eds.), Taylor & Francis, London, 2003, pp. 55–73.
8. Ford, J.D., K.S. Huang, H.B. Wang, L.B. Davin, and N.G. Lewis, Biosynthetic Pathway to the Cancer Chemopreventive Secoisolariciresinol Diglucoside-Hydroxymethyl Glutaryl Ester-Linked Lignan Oligomers in Flax (*Linum usitatissimum*) Seed, *J. Nat. Prod. 64*:1388–1397 (2001).
9. Clark, W.F., A.D. Muir, N.D. Westcott, and A. Parbtani, A Novel Treatment for Lupus Nephritis: Lignan Precursor Derived from Flax, *Lupus 9*:429–436 (2000).
10. Prasad, K., Secoisolariciresinol Diglucoside from Flaxseed Delays the Development of Type 2 Diabetes in Zucker Rat, *J. Lab. Clin. Med. 138*:32–39 (2001).
11. Muir, A.D., N.D. Westcott, K. Ballantyne, and S. Northrup, Flax Lignans—Recent Developments in the Analysis of Lignans in Plant and Animal Tissues, in *Proceedings of the 58th Flax Institute of the USA*, Flax Institute of the USA, Fargo, ND, 2000, pp. 23–32.
12. Westcott, N.D., and D. Paton, Complex Containing Lignan, Phenolic and Aliphatic Substances from Flax Process for Preparing, U.S. Patent 6,264,853 (2001).
13. Kamal-Eldin, A., N. Peerlkamp, P. Johnsson, R. Andersson, R.E. Andersson, L.N. Lundgren, and P. Aman, An Oligomer from Flaxseed Composed of Secoisolariciresinoldiglucoside and 3-Hydroxy-3-Methyl Glutaric Acid Residues, *Phytochemistry 58*:587–590 (2001).
14. Muir, A.D., and N.D. Westcott, Quantitation of the Lignan Secoisolariciresinol Diglucoside in Baked Goods Containing Flax Seed or Flax Meal, *J. Agric. Food Chem. 48*:4048–4052 (2000).
15. Adlercreutz, H., O. Lapcík, R. Hampl, K. Wähälä, N. Al-Maharik, C.-J. Wang, and H. Mikola, Immunoassay of Phytoestrogens in Human Plasma, *J. Nutra. Fun. Med. Food. 2*:131–133 (1999).
16. Muir, A.D., and N.D. Westcott, Mammalian Metabolism of Flax Lignans, in *Flax, the Genus Linum* (A.D. Muir and N.D. Westcott, eds.), Taylor & Francis, London, 2003, pp. 230–242.
17. Kitts, D.D., Y.V. Yuan, A.N. Wijewickreme, and L.U. Thompson, Antioxidant Activity of the Flaxseed Lignan Secoisolariciresinol Diglycoside and Its Mammalian Lignan Metabolites Enterodiol and Enterolactone, *Mol. Cell. Biochem. 202*:91–100 (1999).
18. Prasad, K., Antioxidant Activity of Secoisolariciresinol Diglucoside-Derived Metabolites, Secoisolariciresinol, Enterodiol, and Enterolactone, *Int. J. Angiol. 9*:220–225 (2000).
19. Jenkins, D.J., C.W. Kendall, E. Vidgen, S. Agarwal, A.V. Rao, R.S. Rosenberg, E.P. Diamandis, R. Novokmet, C.C. Mehling, T. Perera, L.C. Griffin, and S.C. Cunnane, Health Aspects of Partially Defatted Flaxseed, Including Effects on Serum Lipids, Oxidative Measures, and *ex vivo* Androgen and Progestin Activity: A Controlled Crossover Trial, *Am. J. Clin. Nutr. 69*:395–402 (1999).
20. Halliwell, B., The Role of Oxygen Radicals in Human Disease, with Particular Reference to the Vascular System, *Haemostasis 23* (Suppl. 1):118–126 (1993).
21. Shahidi, F., and P.K. Wanasundara, Phenolic Antioxidants, *Crit. Rev .Food Sci. Nutr. 32*:67–103 (1992).
22. Devi, R.S., S. Narayan, K.V. Mohan, K.E. Sabitha, and C.S. Devi, Effect of a Polyherbal Formulation, Ambrex, on Butylated Hydroxy Toluene (BHT) Induced Toxicity in Rats, *Indian J. Exp. Biol. 41*:1294–1299 (2003).
23. Kulling, S.E., E. Jacobs, E. Pfeiffer, and M. Metzler, Studies on the Genotoxicity of the Mammalian Lignans Enterolactone and Enterodiol and Their Metabolic Precursors at Various Endpoints *in vitro*, *Mutat. Res. 416*:115–124 (1998).
24. Murcia, M.A., M. Martinez-Tome, A.M. Jimenez, A.M. Vera,

M. Honrubia, and P. Parras, Antioxidant Activity of Edible Fungi (truffles and mushrooms): Losses During Industrial Processing, *J. Food Prot. 65*:1614–1622 (2002).
25. Martinez-Tome, M., A.M. Jimenez, S. Ruggieri, N. Frega, R. Strabbioli, and M.A. Murcia, Antioxidant Properties of Mediterranean Spices Compared with Common Food Additives, *J. Food Prot. 64*:1412–1419 (2001).
26. Liebler, D.C., and J.A. Burr, Antioxidant Stoichiometry and Oxidative Fate of Vitamin E in Peroxyl Radical Scavenging Reactions, *Lipids 30*:789–793 (1995).
27. Azuma, K., K. Ippoushi, H. Ito, H. Higashio, and J. Terao, Evaluation of Antioxidative Activity of Vegetable Extracts in Linoleic Acid Emulsion and Phospholipid Bilayers, *J. Sci. Food Agric. 79*:2010–2016 (1999).
28. AOCS, Oil Stability Index (OSI), *Official Methods and Recommended Practices of the AOCS,* 5th edn., AOCS Press, Champaign, 1997.

[Received January 4, 2006; accepted June 29, 2006]