# Antioxidant Activity of Phytosterols, Oryzanol, and Other Phytosterol Conjugates

**Tong Wang[a,*], Kevin B. Hicks[b], and Robert Moreau[b]**

[a]Department of Food Science and Human Nutrition, Iowa State University, Ames, IA 50011, and [b]Eastern Regional Research Center, ARS, USDA, Wyndmoor, Pennsylvania 19038

**ABSTRACT:** Antioxidant activity of phytosterols, oryzanol, ferulic acid ester of sterols, corn fiber oil, and rice bran oil was investigated. Commercial soybean oil and distilled soybean oil FAME were used as substrates for both oxidative stability determination and viscosity analysis after the oil was oxidized. At low concentration, these materials did not improve the oxidative stability of the oil substrates, although the viscosity tended to be reduced slightly. The antipolymerization activity of steryl ferulate was higher at higher concentration than at lower concentration, and steryl ferulate was more effective than oryzanol. Rice bran oil showed very good antioxidant and antipolymerization activities.

Paper no. J10247 in *JAOCS 79*, 1201–1206 (December 2002).

**KEY WORDS:** Antioxidant, antipolymerization, corn fiber oil, ferulic acid ester of phytosterol, phytosterols, rice bran oil.

Phytosterols contained in vegetable oils are hypocholesterolemic (1–3). These phytochemicals and their derivatives may also be potent antioxidants (4–7). The avenasterol of rice bran oil acts as an antioxidant at elevated or frying temperatures owing to the ethylidene group on the side chain of the molecule. Its antioxidant activity has been attributed to the formation of an allylic free radical and its isomerization to other relatively stable free radicals (7). Oryzanol, a group of ferulic acid esters of triterpene alcohols present in rice bran oil, also has been shown to possess strong stabilization effects during frying applications (6). The antioxidant effects of ferulates can similarly be explained by the formation of several resonance-stabilized structures.

Corn fiber oil is an unusual oil in that it contains a very high concentration of total sterols, up to ~14% of the crude oil, compared with less than 1% in most refined vegetable oils (8–12), and it has been shown to lower serum cholesterol levels in several animal models (11,12). In addition to free phytosterols and phytosterol fatty acyl esters, which have been granted a rare health claim by the U.S. Food and Drug Administration for lowering both LDL-cholesterol levels and the risk of heart disease, corn fiber oil also contains high levels of sitostanyl-ferulate derivatives. These sitostanyl-ferulates also were very effective in lowering cholesterol in an animal model in a preliminary study (13). If these steryl ferulate derivatives are both biologically active and chemically suitable as antioxidants at high temperature, a multifunctional food ingredient or nutraceutical could be developed. The objective of this research was to investigate the antioxidant and antipolymerization activities of phytosterols/phytostanols and their ferulic acid esters.

## EXPERIMENTAL PROCEDURES

*Antioxidant activity of various natural and synthetic agents.* Sitostanyl ferulate was synthesized (14) at a purity of >99%. Sitostanol was obtained from Research Plus (South Plainfield, NJ). Soy sterols, obtained from Archer Daniels Midland (Decatur, IL), is a mixture of naturally occurring free sterols, consisting of β-sitosterol (45.7%), campesterol (27.3%), stigmasterol (15.3%), and brassicasterol (4.4%). It has a purity of 95%. γ-Oryzanol was obtained from CTC Organics (Atlanta, GA). Crude corn fiber oil was extracted with hexane from corn fiber (obtained *via* conventional wet-milling, air-drying, and grinding to 20 mesh), and it contained 5.9% FA ester of sterols, 2.1% free sterols, 5.4% ferulic acid ester of sterols (9), and low levels (<1%) of tocopherols, since the fiber was not heated prior to extraction (15). Crude rice bran oil, obtained from Riceland Foods (Stuttgart, AR), contained about 1.86% oryzanols and 0.3% avenasterol (6). Ferulic acid, gallic acid, TBHQ, and δ-tocopherol were purchased from Sigma Chemical (St. Louis, MO). δ-Tocopherol was chosen as one of the controls because it is generally considered as the most active isomer among the other tocopherols.

Two substrates—commercially refined, bleached, and deodorized (RBD) soybean oil and distilled soybean oil methyl esters (FAME)—were used to test the antioxidant activity of these phytosterol compounds. A short-path molecular still (Pope Scientific, Saukville, WI) was used to distill FAME in order to obtain tocopherol-free substrate. Two concentrations of various compounds, one at 6 μmol/5 g substrate and another at 12 μmol/5 g, were tested. The concentration of 6 μmol/5 g was equivalent to the regulated application of TBHQ, which is 0.02% of the oil. The concentrations of antioxidants contained in corn fiber oil and rice bran oil were prepared by adding the calculated amount of oils that would

*To whom correspondence should be addressed at 2312 Food Sciences Bldg., Iowa State University, Ames, IA 50011.
E-mail: tongwang@iastate.edu

give thecorrect sterol concentrations. The average M.W. of the three major oryzanols (in γ-oryzanol) was estimated to be 580. Oxidative stability index (OSI) was measured according to AOCS standard method Cd 12b-92 (16) with the Omnion OSI instrument (Omnion, Rockland, MA). Viscosity of oil or FAME was measured using a Brookfield viscometer (Stoughton, MA) after the reaction had reached the OSI end point.

*Antioxidant activity of selected compounds at various concentration levels*. Commercial RBD soybean oil was used as substrate to test antioxidant and antipolymerization activities of the selected compounds at various concentration levels. Four concentrations, i.e., 12, 24, 36, and 60 μmol/5 g substrate, were used to test the antioxidant activity of sitostanyl ferulate, oryzanol, soybean sterols, rice bran oil, and ferulic acid. The reason for using various concentrations of an antioxidant is that, although a positive dose–response relationship usually is present, such relationships may be different for different antioxidants. Examining the dose–response relationship is important so the optimal antioxidant concentration can be determined.

*Statistical analysis.* The general linear model of the SAS program (17) was used for the ANOVA. To examine the effect of type of compound and concentration on antioxidant activity, a two-factor factorial design was used, with the type and concentration as two factors. Least significant differences (LSD) were calculated ($P = 0.05$) to compare treatment means.

## RESULTS AND DISCUSSION

*Antioxidant activity of various natural and synthetic compounds.* The values of OSI and viscosity of oils and FAME with various compounds at two concentration levels are shown in Table 1. The results of statistical analysis are shown in Table 2.

Different compounds had significantly different OSI values in both FAME and oil substrates. FAME were oxidized much faster than oil because the natural antioxidant was removed by distillation purification of the FAME. Compared with blank FAME that did not contain any added chemical, the oxidative stability of FAME was significantly improved by adding TBHQ and δ-tocopherol. Rice bran oil improved FAME stability considerably as well. Oil stability was improved by adding gallic acid, TBHQ, and rice bran oil, but δ-tocopherol did not influence stability as much as expected. Various sterols and the sterol-rich corn fiber oil did not seem to improve stability. In addition, higher concentration of the sterols did not significantly improve the stability of either system.

The effect of various compounds on polymerization reaction and viscosity development in both FAME and oil substrates was significant. TBHQ and δ-tocopherol were the most effective compounds in preventing polymerization of the FAME. Although gallic acid was quite effective in improving OSI, it did not prevent viscosity development to a similar degree. Various sterols generally lowered viscosity slightly, particularly at the higher concentration (12 μmol/5 g).

**TABLE 1**
**Antioxidant Activity of Various Natural and Synthetic Agents Tested in Distilled FAME and Commercial Soybean Oil[a]**

| | | OSI (h) | | | | Viscosity (cP) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Conc. | FAME | | Soybean oil | | FAME | | Soybean oil | |
| | (μmol/5 g) | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Sitostanol-ferulate | 6 | 5.48 | 0.60 | 11.30 | 0.35 | 29.60 | 2.97 | 166.70 | 21.92 |
| | 12 | 6.28 | 1.10 | 11.70 | 0.07 | 25.15 | 0.49 | 508.00 | 12.73 |
| Sitostanol | 6 | 4.80 | 0.35 | 11.53 | 1.38 | 36.45 | 7.28 | 293.45 | 46.46 |
| | 12 | 4.90 | 0.71 | 11.05 | 0.42 | 28.55 | 1.63 | 957.90 | 154.29 |
| Soy sterols | 6 | 5.08 | 1.03 | 12.43 | 0.53 | 33.90 | 4.10 | 190.10 | 12.02 |
| | 12 | 4.80 | 0.92 | 11.28 | 0.46 | 30.30 | 2.97 | 808.20 | 105.50 |
| Oryzanol | 6 | 6.05 | 0.21 | 12.18 | 0.32 | 28.65 | 1.63 | 217.95 | 31.04 |
| | 12 | 6.28 | 0.60 | 11.53 | 0.11 | 27.45 | 0.21 | 875.30 | 0.71 |
| Corn fiber oil | 6 | 5.23 | 0.39 | 10.75 | 0.57 | 32.95 | 1.20 | 323.90 | 22.06 |
| | 12 | 5.45 | 1.20 | 10.30 | 0.42 | 33.45 | 2.76 | 1538.50 | 440.53 |
| Rice bran oil | 6 | 9.18 | 0.39 | 13.78 | 0.04 | 29.90 | 5.09 | 136.85 | 39.81 |
| | 12 | 11.95 | 0.64 | 14.68 | 1.03 | 20.10 | 3.82 | 521.25 | 29.49 |
| δ-Tocopherol | 6 | 12.40 | 2.62 | 12.25 | 0.42 | 13.65 | 6.15 | 196.55 | 55.65 |
| | 12 | 17.78 | 0.95 | 12.55 | 0.35 | 7.69 | 0.81 | 638.20 | 2.40 |
| Ferulic acid | 6 | 6.60 | 1.13 | 12.55 | 0.42 | 28.45 | 2.33 | 169.40 | 39.32 |
| | 12 | 7.05 | 1.48 | 11.88 | 0.74 | 26.35 | 5.02 | 816.65 | 48.01 |
| Gallic acid | 6 | 35.55 | 0.21 | 29.40 | 2.62 | 24.20 | 1.27 | 1327.50[b] | 142.13 |
| TBHQ | 6 | 21.98 | 2.93 | 35.30 | 0.49 | 9.92 | 1.53 | 300.75[b] | 116.46 |
| Blank | | 4.69 | 0.35 | 11.33 | 0.19 | 34.93 | 2.69 | 208.60 | 46.39 |
| | | | | | | | | 860.45[c] | 292.11 |

[a]Heating time for FAME conc. of 6 μmol/5 g = 17.5 h, FAME conc. 12 μmol/5 g = 18 h, oil conc. of 6 μmol/5 g = 20 h, and oil conc. of 12 μmol/5 g = 25 h. FAME heating was at 90°C, and oil heating was at 100°C due to the oils' high oxidative stability index (OSI) values.
[b]Heating time was 48 h.
[c]Heating time was 25 h, as for the 12 μmol/5 g concentration treatment for oil.

**TABLE 2**
**Summary of Statistical Analysis of Various Compounds as Antioxidants in FAME and Soybean Oil Systems[a]**

| | OSI | | | | Viscosity | | | |
|---|---|---|---|---|---|---|---|---|
| | FAME | | Oil | | FAME | | Oil | |
| | *P* value at 5% | LSD value | *P* value at 5% | LSD value | *P* value at 5% | LSD value | *P* value at 5% | LSD value |
| Compounds | <0.0001 | 1.445 | <0.0001 | 1.199 | <0.0001 | 4.72 | <0.0001 | 151.8 |
| Concentration | 0.0750 | 0.588 | 0.3470 | 0.490 | 0.0060 | 1.93 | <0.0001 | 62.0 |
| Replication | 0.0570 | 0.588 | 0.0090 | 0.490 | 0.0430 | 1.93 | <0.0001 | 62.0 |
| Cpds × concentration | <0.0001 | | 0.8940 | | 0.3270 | | <0.0001 | |

[a]LSD, least significant difference; Cpds, compounds; for other abbreviation see Table 1.

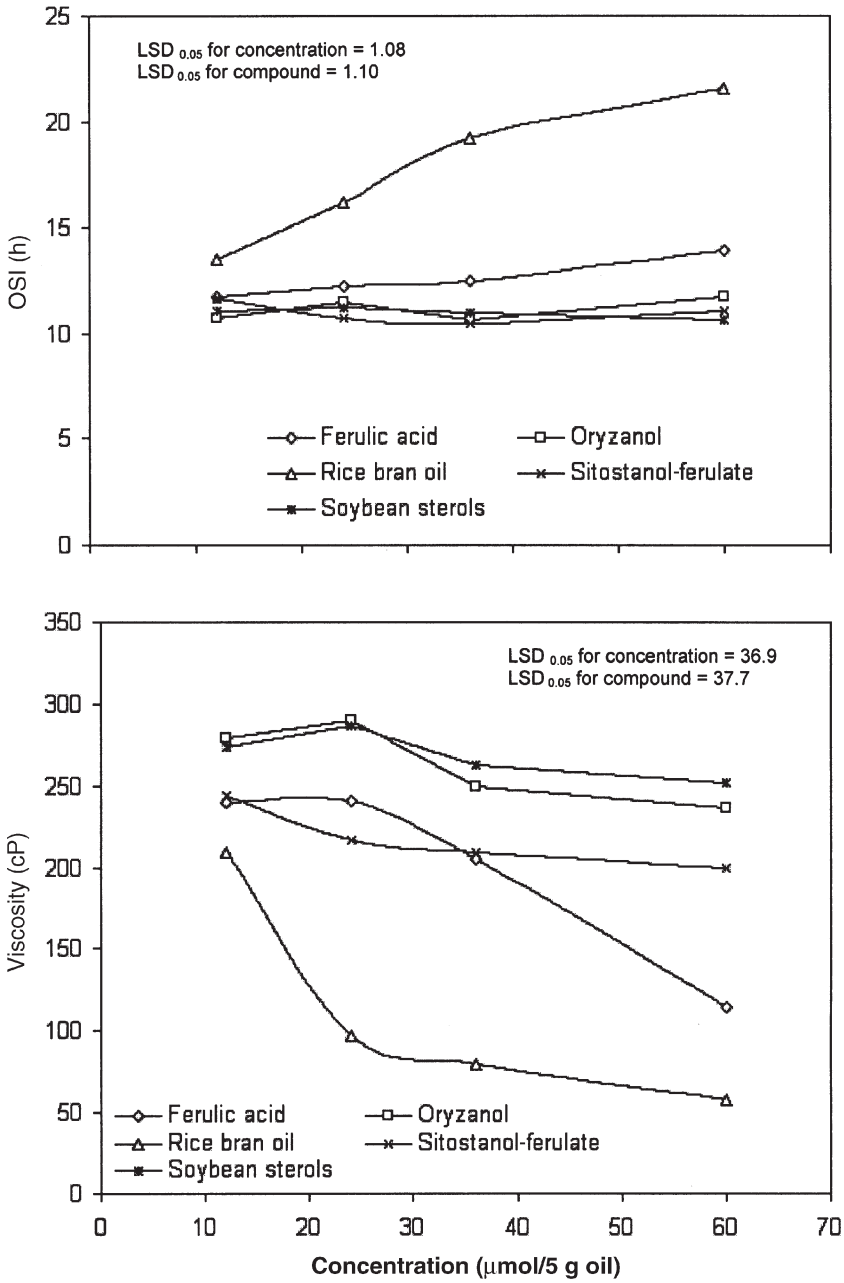


**FIG. 1.** Antioxidant activity of selected agents at various concentrations in soybean oils. LSD, least significant difference.

The viscosity developed in oil was much higher than for FAME because of its TAG structure, compared with the single acyl chain in FAME. We also noticed that the higher-concentration samples had much higher viscosity than the lower-concentration samples, owing to the difference in heating time. The high-concentration oil was heated for 25 h, the low-concentration oils for 20 h. Therefore, the expected viscosity reduction at the higher concentration was not seen. This observation indicated that once the oil is oxidized, the polymerization reaction can proceed very rapidly. TBHQ again showed its significant anti-polymerization effect in oil compared with gallic acid.

*Antioxidant activity of selected compounds at various concentration levels*. The effect of type and concentration of selected compounds is shown in Figure 1. The interaction between type and concentration was significant for OSI but not for viscosity at the 5% probability level. Therefore, the main effects of type and concentration on viscosity were examined (Fig. 2).

The oxidative stability of oil was significantly affected by type of compounds tested and their concentration. Rice bran oil had significantly better antioxidant activity than the others, and its activity increased markedly with its concentration

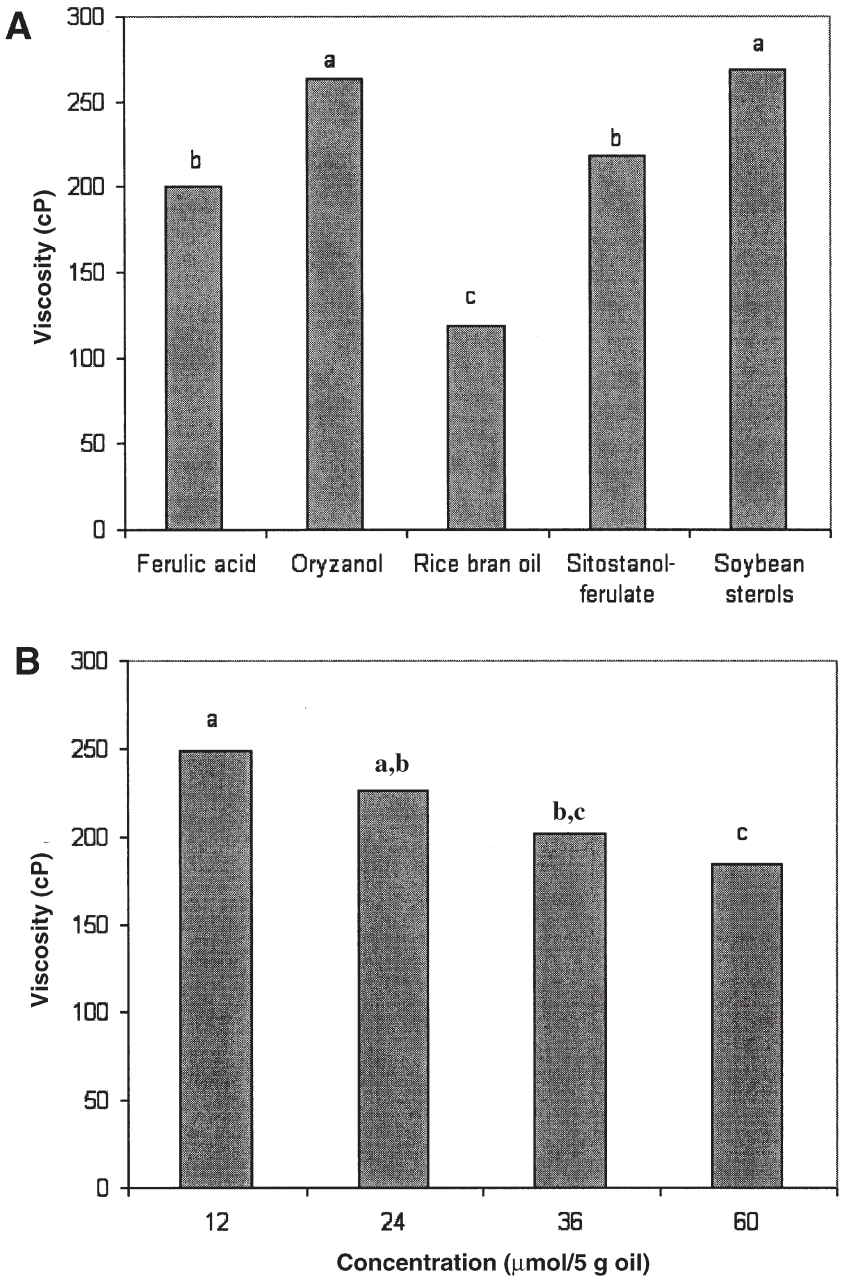


**FIG. 2.** Main effects of antioxidant type and concentration on viscosity of oxidized soybean oil. Note: Different letters at top of bars indicate statistical difference at 5% probability level.

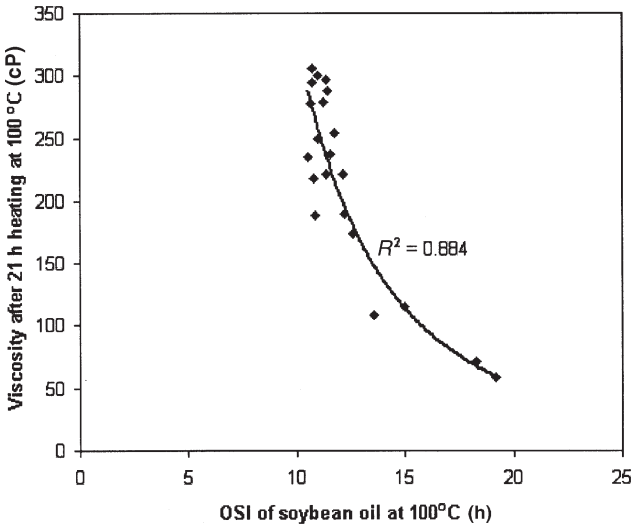


**FIG. 3.** Relationship of oxidative stability and polymerization of soybean oil. OSI, oxidative stability index.

in an oil. Ferulic acid had similar activity compared with others at lower concentration but showed improved activity at higher concentration.

Viscosity of the oil was also significantly affected by type and concentration of the compounds. Rice bran oil was the most effective agent in preventing polymerization, and its activity increased dramatically with an initial increase in concentration but tended to level off at higher concentration. The effectiveness of ferulic acid as an antipolymerization agent was more evident at higher concentration than at lower concentration. Sitostanyl-ferulate showed significantly more antipolymerization activity than did oryzanol and soybean sterols. It is not clear why sitostanyl ferulate was more effective at the concentration tested than oryzanol, which is a mixture of ferulic acid esters of various triterpene alcohols that are structurally very similar to phytosterols, including cycloartenol, 24-methylene-cycloartenol, and a small amount of campesterol. These observations also suggest that the good antioxidant/antipolymerization activity of rice bran oil may not be due to its oryzanol content alone but to other minor lipid components, such as avenasterols. The phytosterol avenasterol, constituting 32% of the total phytosterols in oats), greatly reduced the degree of deterioration of soybean oil at frying temperature (180°C) (4,5). The content of tocopherol and tocotrienol in rice bran oil is usually relatively low, about 20 times less than its phytosterol content; therefore, its contribution to the oxidative stability of the system may be very small. The quantity of rice bran oil added in the concentration levels in this experiment ranged from 0.17 to 1.7 g in 5 g of oil substrate. Other minor components, in addition to avenasterol, also may have contributed to the antioxidant activity of rice bran oil.

The antioxidant activity of phytosterols also was studied by other researchers. Xu and Godber (18) examined the antioxidant activity of the major components of rice bran oryzanol in a linoleic acid model system, at slightly elevated temperature and by monitoring peroxide formation. They found that the antioxidant activities of various steryl ferulates were lower than those with ferulic acid and α-tocopherol. The concentration levels used in their study (1:100 to 1:500, sterol/substrate, molar ratio) were similar to those used in our study (approximately 1:100 to 1:1000 for oil substrate, and 1:300 to 1:3000 for FAME substrate). At a 1:500 ratio of sterol to substrate, Xu and Godber (18) detected no antioxidant activity. The antioxidant activity of oryzanol also was reported as not significant at concentrations lower than 0.5% (1:300 molar ratio of sterol to oil substrate) (19). If we had used a higher concentration of phytosterols in our study, we might have observed higher antioxidant/antipolymerization activity of the steryl ferulate.

There has been some concern expressed about the high-temperature testing of oxidative stability of oil and antioxidant activity because of the rapid oxidation, polymerization, and decomposition of antioxidant (20). Nevertheless, there is also evidence that the OSI method gives a reliable estimation of oil stability, and it is an AOCS recommended standard method. The OSI method should be particularly useful in evaluating stability of frying oil and the effectiveness of antioxidants used in this type of oil.

Various lipid materials could be used as substrates to test the activities of antioxidants. Methyl esters of vegetable oils should be an ideal system to evaluate antioxidant activity, because they can be purified easily to ensure that no other minor lipid components interfere with the evaluation. Methyl linoleate was successfully used as a model system for antioxidant activity study by OSI (21). The only drawback with soybean FAME was that it did not develop significant viscosity. Therefore, oil may be a better substrate to evaluate the antipolymerization effect of antioxidants.

The relationship of oxidative stability and viscosity development is shown in Figure 3. Oils with similar stabilities as indicated by the OSI value may have quite different viscosities. This observation indicates that different antioxidants may work differently. Some may prevent peroxide formation. Therefore, the subsequent reactions are delayed. Others may not prevent peroxide formation, but they may delay the subsequent polymerization reaction.

## ACKNOWLEDGMENTS

Journal Paper No. J-19634 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 0178, and supported by Hatch Act and State of Iowa funds.

## REFERENCES

1. Ling, W.H., and P.J.H. Jones, Dietary Phytosterols: A Review of Metabolism, Benefits, and Side Effects, *Life Sci. 57*:195–206 (1995).
2. Moreau, R.A., R.A. Norton, and K.B. Hicks, Phytosterols and Phytostanols Lower Cholesterol, *inform 10*:572–577 (1999).
3. Law, M.R., Plant Sterol and Stanol Margarines and Health, *West. J. Med. 173*:43–47 (2000).

4. White, P.J., and L.S. Armstrong, Effect of Selected Oat Sterols on the Deterioration of Heated Soybean Oil, *J. Am. Oil Chem. Soc. 63*:525–529 (1986).
5. Tian, L.L., and P.J. White, Antipolymerization Activity of Oat Extract in Soybean and Cottonseed Oils Under Frying Conditions, *Ibid. 71*:1087–1094 (1994).
6. Kochhar, S.P., Stable and Healthful Frying Oil for the 21th Century, *inform 11*:642–647 (2000).
7. Gordon, M.H., and P. Magos, The Effect of Sterols on the Oxidation of Edible Oils, *Food Chem. 10*:141–147 (1983).
8. Moreau, R.A., V. Singh, S.R. Eckhoff, M.J. Powell, K.B. Hicks, and R.A. Norton, Comparison of Yield and Composition of Oil Extracted from Corn Fiber and Corn Bran, *Cereal Chem. 76*:449–451 (1999).
9. Moreau, R.A., M.J. Powell, and K.B. Hicks, Extraction and Quantitative Analysis of Oil from Commercial Corn Fiber, *J. Agric. Food Chem. 44*:2149–2154 (1996).
10. Norton, R.A., R.A. Moreau, and K.B. Hicks, Corn Fiber Oil: Analysis, Composition, and Potential as a Cholesterol-Lowering Food Ingredient, *Abstracts*, *90th AOCS Annual Meeting & Expo*, Orlando, 1999.
11. Wilson, T., A. DeSimone, C. Romano, and R. Nicolosi, Corn Fiber Oil Lowers Plasma Cholesterol Levels and Increases Cholesterol Excretion Greater Than Corn Oil and Similar to Diets Containing Soy Sterols and Soy Stanols in Hamsters, *J. Nutr. Biochem. 11*:443–449 (2000).
12. Ramjiganesh, T., S. Roy, R. Nicolosi, T. Young, J. McIntyre, and M. Fernandez, Corn Husk Oil Lowers Plasma LDL Cholesterol Concentrations by Decreasing Cholesterol Adsorption and Altering Hepatic Cholesterol Metabolism in Guinea Pigs, *Ibid. 11*:358–366 (2000).
13. Nicolosi, R., T. Wilson, and C. Lawton, Corn Fiber Oil: Cholesterol-Lowering Properties and Mechanisms of Action in Animal Models, *Abstracts, 92nd AOCS Annual Meeting & Expo*, Minneapolis, 2001, p. S115.
14. Condo, A.M., Jr., D.C. Baker, R.A. Moreau, and K.B. Hicks, Improved Method for the Synthesis of *trans*-Feruloyl-β-sitostanol, *J. Agric. Food Chem. 49*:4961–4964 (2001).
15. Moreau, R.A., K.B. Hicks, and M.J. Powell, Effect of Heat Pretreatment on the Yield and Composition of Oil Extracted from Corn Fiber, *Ibid. 47*:2869–2871 (1999).
16. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., AOCS Press, Champaign, 1994.
17. *SAS Software, Release 8.1 (1999–2000)*, SAS Institute, Cary, NC.
18. Xu, Z., and J.S. Godber, Antioxidant Activities of Major Components of γ-Ozyzanol from Rice Bran Using a Linoleic Acid Model, *J. Am. Oil Chem. Soc. 78*:645–649 (2001).
19. Fukushi, J., Edible Rice Bran Oil—Part III: Antioxidant Effects of Oryzanol, *Hokkaido-Ritsu Eisei Kenkyushoho 16*:111–114 (1966).
20. Frankel, E.N., In Search of Better Methods to Evaluate Natural Antioxidants and Oxidative Stability in Food Lipids, *Trends Food Sci. Technol. 4*:220–225 (1993).
21. Nakatani, N., Y. Tachibana, and H. Kikuzaki, Establishment of a Model Substrate Oil for Antioxidant Activity Assessment by Oil Stability Index Method, *J. Am. Oil Chem. Soc.78*:19–23 (2001).

[Received February 7, 2002; accepted August 29, 2002]