Antioxidant Activity of Rapeseed Phenolics and Their Interactions with Tocopherols During Lipid Oxidation

U. Thiyam*, H. Stöckmann, and K. Schwarz

Institute of Human Nutrition and Food Science, Christian-Albrechts University Kiel, 24118 Kiel, Germany

ABSTRACT: Commercial rapeseed press cakes are rich sources of phenolic compounds, namely, sinapic acid derivatives, which can be extracted as free sinapic acid and its bound forms (such as sinapine, the choline ester of sinapic acid). Fractionated rapeseed extracts rich in sinapic acid and sinapine were compared for their capacity to inhibit the formation of lipid oxidation products. Oxidation at 40°C was monitored by the formation of hydroperoxides (indicating primary oxidation products) and propanal (secondary oxidation products). The 70% methanolic extract of rapeseed meal, added as an equivalent of 500 μmol/kg oil (based on sinapic acid equivalent for sinapic acid-rich extracts or sinapine equivalent for sinapine-rich extracts) showed good antioxidative activity compared with the addition of 500 μmol/kg oil sinapic acid. Apart from this, the interaction between a mixture of α-/γ-tocopherol and sinapic acid was investigated using response surface methodology for the experimental design. The experiments indicated that the addition of sinapic acid (concentration dependent) caused inhibition of peroxide formation, complementing further lower endogenous tocopherol concentration in oils.

Paper no. J11211 in *JAOCS 83*, 523–528 (June 2006).

KEY WORDS: Rapeseed meal, response surface methodology, sinapic acid, sinapine.

Rapeseed and mustardseed are processed into oil, leaving a by-product normally referred to as meals. Meals (press cakes) contain, after the extraction of oil, large amounts of phenolic compounds. The interest in extraction of phenolic compounds from the by-product of rapeseed oil processing arises because they are a source of a significant amount of endogenous antioxidative compounds. However, these antioxidative phenolic compounds contribute to the dark color, bitter taste, and astringency of rapeseed meals. They may also interact with amino acids, enzymes, and other food components, thus influencing the nutritional significance of the meal (1,2). On the other hand, the phenolic compounds can be extracted with pure or aqueous solvents such as methanol, isopropanol, and ethanol and used as natural antioxidants to complement the main antioxidant component of rapeseed oil, namely, the tocopherols. Sinapic acid (SA), the main phenolic compound of rapeseed, constitutes over 73% of free phenolic acids and about 80–99% of the total phenolic acids, mainly occurring as esters and glucosides (3,4). Sinapine, the choline ester of SA, is the main phenolic ester in rapeseed meals. The most active antioxidative components of press cake from canola (which is also a member of the Brassicacae) and of the polar fraction of rapeseed oil have been identified as 1-O-β-D-glucopyranosyl sinapate, an SA derivative (5), and vinylsyringol, a decarboxylation product of SA (6).

SA is a potent radical scavenger and antioxidant in several lipid-containing systems (7). In addition, SA has peroxynitrite ($ONOO^-$) scavenging activity and can be used for protection of cellular defense activity against ONOO—involved diseases (8). When compared with other phenolic acids such as caffeic acid, ferulic acid, *p*-coumaric acid, and reference antioxidants such as BHA, Trolox, and α-tocopherol, SA at concentrations of 500 μmol/kg oil was effective in inhibiting the formation of conjugated diene (CD) hydroperoxides (9). However, aspects of extraction of the main phenolic compound SA in the free or esterified form, namely sinapine, for stabilization of rapeseed oil, needs to be investigated to understand whether it complements the tocopherols, the main antioxidative compound of rapeseed oil. Furthermore, the antioxidative capacity of the individual phenolic compounds as related to their concentration and form (free or bound) needs to be studied. Currently, refined rapeseed and cold-pressed rapeseed oils are common in the edible oil markets. However, the rapeseed oils are subjected to high temperatures during extraction and processing, which removes many phenolics, including SA derivatives. The amounts of phenolics were greatest in crude expelled rapeseed oil, i.e., after pressing, and decreased with an increasing degree of refining (6). To maintain a high content of phenolic compounds in the oil after refining, phenols extracted from the rapeseed meal could be added back to the refined oil, resulting in a value-added rapeseed oil. At present, food manufacturers use food-grade commercial antioxidants to prevent quality deterioration and to maintain the nutritional value of different food products including oils and products containing oil. The objective of the study was to investigate the antioxidative capacity of individual phenolic compounds of rapeseed meal with respect to their concentration and form (free or bound) independent of and together with the tocopherols, the main antioxidative compound of rapeseed oil.

This paper was initially presented at the 95th AOCS Annual Meeting and Expo in Cincinnati, Ohio, May 1–4, 2004.

*To whom correspondence should be addressed at Institute of Human Nutrition and Food Science, Heinrich Hecht Platz 10, Christian-Albrechts University Kiel, 24118 Kiel, Germany. E-mail: thiyam@ivv.fraunhofer.de

MATERIALS AND METHODS

Materials. Commercial rapeseed meal was procured from Teutoburger Ölmühle GmbH (Ibbenbüren, Germany). Refined rapeseed oil for stripping was purchased from a German rapeseed oil company, Ölmühle Ditzingen GmbH (Ditzingen, Germany). All antioxidants including SA were from either Sigma (Diesenhofen, Germany) or Merck (Darmstadt, Germany). All solvents used were of analytical grade. Sinapine as thiocyanite salt was used for calibrations related to sinapine.

Preparation of fractionated and nonfractionated extracts from rapeseed meal. Defatted meal (1 g with negligible oil content) was extracted three times with 9 mL each of extraction solvent (70% methanol, i.e., methanol/water 70:30) using ultrasonication (50 s each time) followed by centrifugation under refrigerated conditions (10 min at 5000 × *g*) and filtration. The extract was made up to a total volume of 25 mL. This extract was added as a nonfractionated extract in rapeseed TG based on sinapine content. Meal extracts were fractionated into free phenolics and esterified phenolics according to the method of Krygier *et al*. (3). Briefly, defatted rapeseed meal was extracted with 70% methanolic solution (6 × ~20 mL), then the total extract was concentrated to ~20 mL. Further, diethyl ether was used to extract the free SA-containing fraction from the concentrated extract, denoted as free extract. The aqueous fraction was the bound extract. All the fractions were redissolved in a known amount of methanol.

Characterization of main phenolic compounds in rapeseed meal extract. Fractionated rapeseed meal extracts were analyzed by HPLC (Agilent 1100 series instrument; Agilent, Waldbronn, Germany) for SA derivatives at 330 nm and for vinylsyringol, a decarboxylated product of SA, at 275 nm. A gradient elution was performed using water/methanol (90:10) with 1.25% *o*-phosphoric acid as solvent A and methanol (100%) with 0.1% *o*-phosphoric acid as solvent B on a C-8 Chrospher column (250 × 4 mm; Knauer GmbH, Berlin, Germany) at 0, 7, 20, 25, and 28 min with 10, 20, 45, 70, and 100% B with a post-run of 3 min. Peaks were identified by comparing their relative retention times with those of authentic standards of SA, sinapine, and sinapoyl-glucose. For vinylsyringol (275 nm), the identification was carried out by comparing the order of retention times with those in the literature.

Incubation with added antioxidants. Rapeseed oils were purified according to the adsorption chromatography method of Lampi *et al*. (10) to strip the rapeseed oils of natural antioxidants, trace metals, and FFA. Rapeseed TG (~20 g) were incubated at 40°C and monitored for the formation of oxidation products. Antioxidants and extracts were added to the oil in aliquots of 50–200 μL methanol or ethanol. The fractions containing free phenolic compounds (free phenolic fraction), bound phenolic compounds (bound-phenolic fraction), and the nonfractionated extract (70% methanolic extract) were added to stripped rapeseed oil at a concentration that was equivalent to 500 μmol/kg oil of phenolic compounds (SA equivalent for SA-rich fraction and sinapine equivalent for sinapine-rich fraction as quantified by HPLC, 330 nm).

Measurement of oxidation products. CD were measured according to the method of Stöckmann *et al*. (11). Aliquots (~10 mg) of oil were dissolved in 5 mL isopropanol, vortexed, and directly measured at 234 nm (DU-530 UV/vis spectrophotometer; Beckman, Fullerton, CA) and calculated as mmol hydroperoxides/kg of oil as previously described by Stöckmann *et al*. (11).

Hydroperoxides were measured using the standardized ferric thiocyanite method according to Stöckmann *et al*. (11). Aliquots (10 mg) of oxidized oil were dissolved in 5 mL of isopropanol and vortexed for 30 s; then50 μL ferrous solution and 50 μL thiocyanite solution were added and the solution was vortexed again for 10 s. After incubating in a water bath at 60°C for 30 min and then cooling to ambient temperature, the absorbances of the samples were measured at 485 nm against a blank without oil samples. Results were expressed as mmol hydroperoxides/kg of sample.

Propanal was measured as an indicator for secondary oxidation products using static headspace GC according to the method of Frankel (12). Approximately 1000 mg of lipids was weighed into headspace vials, hermetically closed immediately, and incubated at 70°C for 15 min.

Central composite design (CCD) for testing oxidative stability of rapeseed triglycerides with added natural antioxidants. The response-surface method (RSM) for analyzing these experiments was based on a CCD generated using the software design expert v6.0.10 (Stat-Ease, Inc. Minneapolis, MN).

Terminology in the context of RSM (13). Calculation is made of the adjusted R^2, i.e., adjusted for the number of terms in the model relative to the number of points in the design, as follows:

$$\text{adjusted } R^2 = \frac{\text{SSresidual/DFresidual}}{(\text{SSresidual}+\text{SSmodel})/(\text{DFresidual}+\text{DFmodel})} \quad [1]$$

where degrees of freedom (DF) denotes the number of independent comparisons available to estimate a parameter (the number of model parameters minus 1) and sum of squares (SS) denotes the total of the sum of squares for the terms in the model, as reported in the "model screen" for RSM designs.

SA was used at a concentration of 50 (low, −1) and 500 μmol/kg oil (high, +1), while the tocopherol mixture (ratio of γ/α 3.5:1) was used at a concentration of 50 (low, −1) and 1000 μmol/kg oil (high, +1) as presented in Table 1. The responses used were the protection factor (PF) estimated to reach a CD value of 25 mmol/kg oil (response 1), a hydroperoxides value of 50 mmol/kg oil (response 2), and propanal formation (response 3) at the fifth week of the incubation study (Table 2).

Protection factor (PF) was calculated as:

$$\text{PF (d)} = (S - C)/C \quad [2]$$

where C = days for the control sample to reach a hydroperoxides value of 50 mmol/kg oil or a CD value of 25 and S = days

TABLE 1
Central Composite Design for Testing Optimal Concentration of Sinapic Acid and Tocopherol Content in Rapeseed Oil

Independent variable	Levels			
	Low-actual (μmol/kg oil)	Low-coded	High-actual (μmol/kg oil)	High-coded
Sinapic acid (SA)	50	−1	500	+1
Tocopherol mixture (Toco-mix)	50	−1	1000	+1

for the oxidized sample to reach a hydroperoxides value of 50 mmol/kg oil or a CD value of 25.

RESULTS AND DISCUSSION

Antioxidant activity of extracts, SA, and sinapine. The extraction of phenolic compounds from commercial rapeseed meal was carried out with aqueous alcohol (70:30 methanol/water) as this extraction solvent showed the best efficiency with respect to phenolic concentration (9).

The fractions containing free-SA (free phenolic fraction), bound-SA, or sinapine (bound phenolic fraction) non-fractionated extract (70% methanolic extract) were compared with SA, and sinapine.

Figures 1 and 2 depict the formation of hydroperoxides and propanal, respectively, in stripped rapeseed bulk oils. Whereas SA and the free phenolic fraction (containing over 90% SA) caused marked inhibition of the formation of hydroperoxides and propanal, all other additives showed clearly lower activity or even slight pro-oxidant activity. In the non-fractionated extract, sinapine is the major SA derivative, representing approximately 70% of the phenolic compounds, whereas the SA concentration amounts to approximately 10%. In the bound fraction, the concentration of sinapine is enhanced to 85%. As sinapine showed slight pro-oxidant activity compared with the control sample with respect to hydroperoxide and propanal formation, the low activity of the nonfractionated extract and the bound fraction can be attributed to the content of sinapine. In previous investigations (14), SA and sinapine caused radical-scavenging activity toward the DPPH (diphenylpicrylhydrazyl) radical in methanol solution. To scavenge 25×10^{18} DPPH radicals, the required concentrations (in mM) were 30.8 for SA and 47.5 for sinapine in comparison with 21.5 for Trolox, the water-soluble analog of α-tocopherol (15). Although these results suggested a lower antioxidant activity of sinapine compared with SA, they do not suggest that sinapine is not an active antioxidant in rapeseed oil TG. Also, the structural properties of sinapine suggest a marked antioxidant potential due to its phenolic group. According to the polar paradox, which describes the phenomenon of increasing antioxidant activity of compounds with increasing polarity in bulk oil (16), sinapine, which is more polar than SA, should have exhibited a higher activity. The strong turbidity of samples during the storage experiment containing sinapine and the bound fraction suggests that both additives were only dispersed in the oil phase but not solubilized. According to Uri (17), low solubility of an antioxidant does not limit its activity as long as small quantities of the antioxidants are in solution. In the case of sinapine, however, it can be assumed that its solubility in oil was too low for it to

TABLE 2
Central Composite Design Arrangement and Responses Used for the Design

Run number (random)	Variable levels		Response 1 Conjugated dienes protection factor (PF) (d)	Response 2 Hydroperoxide formation protection factor (hydroperoxide PF) (d)	Response 3 Propanal formation (μmol/ kg oil)
	Sinapic acid (SA) (μmol/kg oil)	Tocopherol mixture (Toco-mix) (μmol/kg oil)			
6	275	525	2.30	2.03	265
16	275	525	2.50	2.29	300
17	275	525	2.20	1.79	325
20	275	525	2.22	1.61	325
3	43.2	525	2.10	1.83	265
15	593.2	525	2.20	2.03	300
11	275	146.7	4.70	4.00	244
18	275	1196.7	0.90	1.18	210
19	50	50	3.00	3.51	375
7	500	50	5.00	5.85	260
9	50	1000	1.20	0.90	230
4	1	1	1.39	1.20	260

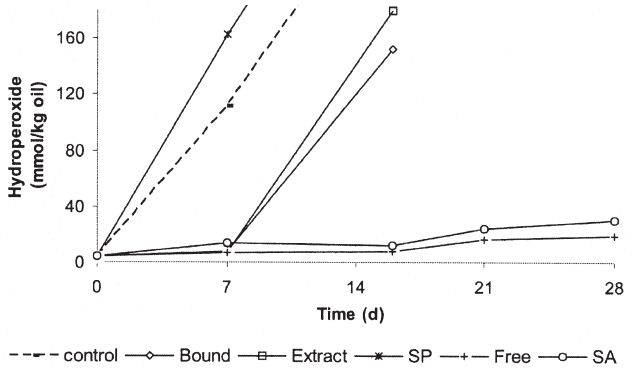


FIG. 1. Effect of fractionated rapeseed meal extracts on the formation of hydroperoxides in purified rapeseed oil at 40°C in the dark. Control, sinapine (SP), sinapic acid (SA), extract nonfractionated 70% methanolic extract (Extract), free phenolic fraction (Free), bound phenolic fraction (Bound), all added to stripped rapeseed oil at a concentration of 500 μmol/kg oil SA or SP equivalent.

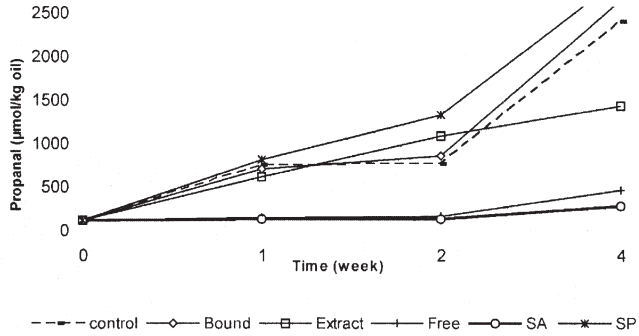


FIG. 2. Effect of fractionated rapeseed meal extracts on the formation of propanal in rapeseed oil at 40°C in the dark. Control (no antioxidants), sinapine (SP), sinapic acid (SA), extract (nonfractionated 70% methanolic extract), free (free phenols fraction), bound (bound phenols fraction), all added to stripped rapeseed oil at a concentration of 500 μmol/kg oil.

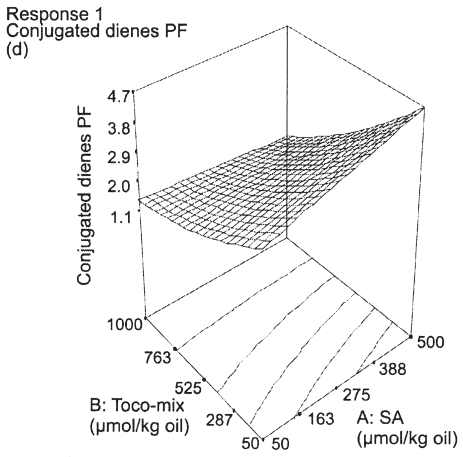
function as an antioxidant when added alone. The slight prooxidant effect may be attributable to the additional interfaces formed by the dispersed sinapine.

Interaction of tocopherols and SA. As rapeseed meal extracts can be hydrolyzed to yield SA, the potent component effective against lipid oxidation in bulk lipids, it was of further interest to understand the interactions of SA with tocopherols, the main antioxidants in refined rapeseed oils. The CCD of the experiment was done to test the hypothesis that the interaction of SA and tocopherols depends on their respective concentrations and to predict the interaction between both antioxdants within a wide range of concentrations. The range of concentration for SA (50 to 500 μmol/kg oil, corresponding to –1and +1) was based on previous experimental results (9). The low (−1) concentration for tocopherol was 50 μmol/kg whereas the high (+1) concentration of 1000 μmol/kg oil is similar to that found in edible rapeseed oils (18). Tocopherol was investigated in a mixture of the α- and γ-isomers in a ratio of 1:3.5, representing their natural composition in rapeseed oil (18). Responses desired were maximal values for response 1 (PF for CD) and 2 (PF for hydroperoxides) and minimal values for response 3 (formation of propanal). At week 5 the control (no added antioxidants) sample of the rapeseed TG showed a propanal value of over 2600 μmol/kg oil.

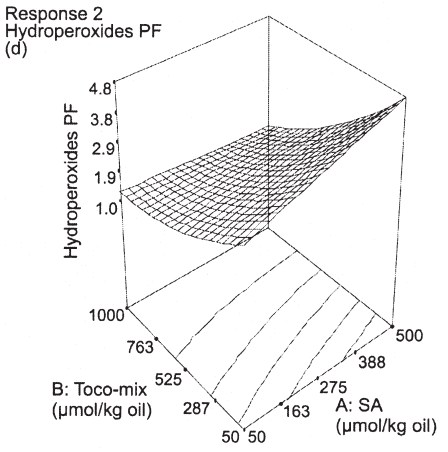
Figures 3a and 3b show that the inhibition of primary oxidation products (CD and hydroperoxides) was highest for the lowest tocopherol concentration (50 μmol/kg oil) and the highest SA concentration (500 μmol/kg oil). Increasing tocopherol concentration caused a reduction of the PF (responses 1 and 2) at all SA concentrations. On the other hand, increasing the SA concentration caused the strongest increase of the PF at low tocopherol concentration whereas the lowest effects for SA can be expected at the highest tocopherol concentration. The influence of SA on the activity of tocopherol can be described as additive at low tocopherol concentration and as neutral at high tocopherol concentration. It can be assumed that SA functions as an antioxidant parallel to α- and γ-tocopherol at low tocopherol concentration. At higher tocopherol concentration SA may lose its activity due to reaction with increasing amounts of tocopheryl radicals. Increasing amounts of tocopheryl radicals, which rapidly decompose, were attributed by other authors to the reduced activity of α-tocopherol at higher concentration (19,20).

It can be assumed that α-tocopherol determines the trend of the concentration dependency of the tocopherol mixture and overwhelms the activity of γ-tocopherol. The decreasing activity of α-/γ-tocopherol mixtures (1:1) and soybean tocopherols (α/β/γ/δ 12:1:58:19) was also reported by Huang *et al*. (20). However, they carried out their investigation in stripped corn oil and found an increase in activity from 250 to 500 ppm followed by a decrease at concentrations from 750 to 3000 ppm. In the present study SA was not able to change the trend of decreasing activity of tocopherols.

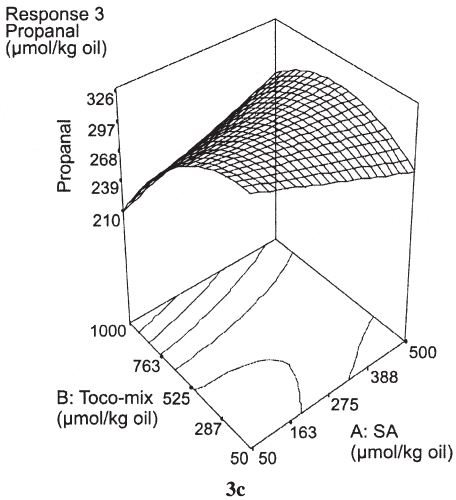
With respect to the formation of secondary oxidation products (propanal formation) the pattern (Fig. 3c) indicated a different concentration-dependent interaction between SA and tocopherol. The lowest propanal formation can be expected at the highest tocopherol concentration combined with the lowest SA concentration and vice versa at the highest SA concentration combined with the lowest tocopherol concentration. Further, SA displayed an opposite behavior with respect to the tocopherol concentration. At low tocopherol concentration the increase in SA resulted in lower propanal formation whereas at high tocopherol concentration an increase in SA caused a higher propanal formation. However, the effect of SA according to the model (Table 3) was not significant. Overall, the formation of propanal was strongly retarded at all concentrations. The control sample without antioxidants reached a concentration of over 2600 μmol/kg oil after 5 wk, and the addition of antioxidants inhibited the formation by 87.5 to 92%. By contrast, the effect on primary oxidation products differed more strongly with PF varying from 0.9 to 5.85 (PF hydroperoxides).



3a



3b



3c

FIG. 3. Variation of the (a) Response 1 (conjugated dienes PF, %), (b) Response 2 (hydroperoxides PF, %), and (c) Response 3 (propanal, µmol/kg oil) as related to concentration of independent factors: Tocopherol-mixture (Toco-mix) and sinapic acid (SA). PF, protection factor.

With respect to the activity of tocopherols, the results are in good agreement with those of Huang *et al.* (20). At all concentrations (250–3000 ppm) for tocopherol mixtures containing α- and γ-tocopherols in different ratios the authors found strong inhibition (over 90%) of hexanal formation in stripped corn oil at a hexanal concentration of approximately 1350 µmol/kg oil. Hexanal is the major volatile secondary oxidation product from linoleic acid, whereas propanal is a characteristic degradation product from linolenic acid.

Diagnostic checking of fitted model. Polynomial equations were fitted to the set of experimental data. Regression analysis of the fitted models for all three responses indicated that the fitted quadratic models were significant with "Prob > *F*" less than 0.05. The final equations in terms of actual factors for the three responses were as depicted in Table 3. The fit of the model as evaluated by determinant coefficients (R^2) and adjusted R^2 (adj R^2) were in agreement (Table 3). Further, the ANOVA of the fitted models indicated the models are significant. Lack of fit was nonsignificant for the models.

Table 3 indicates that for response 1 (CD PF) and 2 (hydroperoxide PF) the factors SA (term A) and tocopherol concentration (term B) are significant along with the interaction of the factors (term AB). In contrast, only tocopherol was significant for response 3 propanal along with the interaction of factor A and B. In describing an optimal combination of tocopherols and SA, the effect on the formation of primary oxidation products seems to be more important, as the effect on the formation of secondary oxidation products (propanal) varied in a narrow range.

Using the models according to Table 3 at concentrations of 500 µmol/kg oil of SA combined with 50 µmol/kg oil of tocopherol, maximal values for responses 1 [CD PF (4.7, d)] and 2 [hydroperoxide PF (4.8, d)] and a minimal value for response 3 propanal (263 µmol/kg oil) formation were obtained.

Rapeseed meal could be extracted to obtain significant amounts of phenolic compounds, namely SA, in the free and esterified form. The study indicates that phenolic compounds derived from rapeseed as free SA effectively prevent lipid oxidation in rapeseed TG. Free phenolic compounds are the most potent fraction, as compared with bound phenolic fractions of rapeseed meal extracts, in effectively preventing lipid oxidation in rapeseed TG. Therefore, extracts rich in SA derived from by-products of rapeseed oil processing could be used to stabilize rapeseed oils especially those with low amounts of endogenous tocopherols. Furthermore, the study also indicates that SA complements the inhibition of hydroperoxide and propanal formation apart from the tocopherols, which are the main antioxidants in rapeseed oils. This concept can be applied to commercial refined rapeseed oils after the processing to stabilize the oils as with antioxidants naturally present in rapeseed.

TABLE 3
Model Relating Responses to the Independent Variables in Coded Form with Desirability[a] for Optimal Response

Response 1 (Desirable*[1] high PF) Conjugated dienes PF*[1]	Response 2 (Desirable *[2] high PF) Hydroperoxide PF*[2]	Response 3 (Desirable*[3] low propanal formation) Propanal
+2.28	+2.05	+299.55
+0.29 * A	+0.37 * A	−26.64 * B
−1.35 * B	−1.41 * B	−31.95 * B2
+0.30 * B2	+0.45 * B2	+31.25 * A * B
−0.45	* A * B	−0.51 * A * B
R^2 = 0.962 adjR^2 =0.94	R^2 = 0.858 adjR^2 = 0.778	R^2 = 0.7 adjR^2 = 0.6
*[1]Protection factor (PF) = (control – sample/control) (using days to reach 25 mmol conjugated dienes/kg oil)	*[2]Protection factor (PF) = (control – sample/control) (using days to reach 50 mmol hydroperoxide/kg oil)	*[3]Propanal (μmol/kg oil) at week 5 when control reached a value of over 2600 μmol/kg oil

[a]coded.

ACKNOWLEDGMENTS

Financial support by the German-Academic-Exchange (DAAD) for Usha Thiyam is gratefully acknowledged. Sinapine thiocyanite was generously contributed by Dr. Alfred Baumert, Leibnitz Institut of Plant Biochemistry, IPB-Halle, Germany.

REFERENCES

1. Naczk, M., R. Amarowicz, A. Sullivan, and F. Shahidi, Current Research Developments on Polyphenolics of Rapeseed/Canola: A Review, *Food Chem. 62*:489–502 (1998).
2. Kozlowska, H., M. Naczk, F. Shahidi, and R. Zadernowski, Phenolic Acids and Tannins in Rapeseed and Canola, in *Canola and Rapeseed: Production, Chemistry, Nutrition, and Processing Technology*, edited by F. Shahidi, Van Nostrand Reinhold, New York, 1990, pp. 40–45.
3. Krygier, K., F. Sosulski, and L. Hogge, Free, Esterified, and Insoluble-Bound-Phenolic Acids. 2. Composition of Phenolic Acids in Rapeseed Flour and Hulls, *J. Agric. Food Chem. 30*:330–336 (1982).
4. Sosulski, F.W., and K.J. Dabrowski, Composition of Free and Hydrolyzable Phenolic Acids in the Flours and Hulls of Ten Legume Species, *Ibid. 32*:131–133 (1984).
5. Wanasundara, U., R. Amarowicz, and F. Shahidi, Isolation and Identification of an Antioxidative Component in Canola Meal, *Ibid. 42*:1285–1290 (1994).
6. Koski, A., S. Pekkarinen, A. Hopia, K. Wähälä, and M. Heinonen, Processing of Rapeseed Oil: Effects on Sinapic Acid Derivative Content and Oxidative Stability, *Eur. Food Res. Technol. 217*:110–114 (2003).
7. Pekkarinen, S.S., H. Stöckmann, K. Schwarz, M. Heinonen, and A.I. Hopia, Antioxidant Activity and Partitioning of Phenolic Acids in Bulk and Emulsified Methyl Linoleate, *J. Agric. Food Chem. 47*:3036–3043 (1999).
8. Zou, Y., A.R. Kim, J.E. Kim, J.S. Choi, and H.Y. Chung, Peroxynitrite Scavenging Ability of Sinapic Acid Isolated from *Brassica juncea*, *Ibid. 50*:5884–5890 (2002).
9. Thiyam, U., A. Kuhlmann, H. Stöckmann, and K. Schwarz, Prospects of Rapeseed Oil By-products with Antioxidative Potential, *C.R. Chimie 7*:611–616 (2004).
10. Lampi, A.-M., L. Kataja, A. Kamal-Eldin, and P. Vieno, Antioxidant Activities of α- and γ-Tocopherols in the Oxidation of Rapeseed Oil Triacylglycerols, *J. Am. Oil Chem. Soc. 76*:749–755 (1999).
11. Stöckmann, H., K. Schwarz, and T. Huynh-Ba, The Influence of Various Emulsifiers on the Partitioning and Antioxidant Activity of Hydroxybenzoic Acids and Their Derivatives in Oil-in-Water Emulsions, *Ibid. 77*:535–542 (2000).
12. Frankel, E.N., Formation of Headspace Volatiles by Thermal Decomposition of Oxidized Fish Oils vs. Oxidized Vegetable Oils, *Ibid. 70*:767–772 (1993).
13. Design Expert Manual (version 6.0.10), Stat-Ease, Inc., Minneapolis, 2002.
14. Thiyam, U., Antioxidative Compounds of Rapeseed Oil Byproducts for Stabilization of Rapeseed Bulk Oil and Emulsions, Ph.D. Thesis, University of Kiel, Kiel, Germany, 2005.
15. Thiyam, U., H. Stöckmann, T. Zum Felde, and K. Schwarz, Antioxidative Effect of the Main Sinapic Acid Derivatives from Rapeseed and Mustard Oil By-products, *Eur. J. Lipid Sci. Technol. 108*:239–248 (2006).
16. Porter, W.L., Paradoxical Behaviour of Antioxidants in Food and Biological Systems, *Toxicol. Ind. Health 9*:93–122 (1993).
17. Uri, N., Mechanism of Antioxidation, in *Autoxidation and Antioxidants,* edited by W.O. Lundberg, John Wiley & Sons, New York, Vol. 1, 1961, Chapter 4.
18. King, B., J.A. Turrell, and S.A. Zilka, Analysis of Minor Fatty Acid Components by Capillary Column GLC, and of Triglycerides by HPLC, Research Report No. 563, Leatherhead Food Research Association. Leatherhead, Surrey, United Kingdom, 1986.
19. Lambelet, P., and J. Löliger, The Fate of Antioxidant Radicals During Lipid Autoxidation. I. The Tocopheroxyl Radicals, *Chem. Phys. Lipids 35*:185–198 (1984).
20. Huang, S.-W., E.N. Frankel, and B.J. German, Effects of Individual Tocopherols and Tocopherol Mixtures on the Oxidative Stability of Corn Oil Triglycerides, *J. Agric. Food Chem. 43*:2345–2350 (1995).

[Received August 16, 2005; accepted April 6, 2006]