JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 11081–11089 11081
DOI:10.1021/jf102287h

Synthesis, Radical Scavenging Activity, Protection during Storage, and Frying by Novel Antioxidants

Yohann Catel, Felix Aladedunye, and Roman Przybylski*

Department of Chemistry and Biochemistry, University of Lethbridge, Lethbridge, Alberta, Canada T1K3M4

Novel antioxidants, derivatives of trolox, and selected phenolic acids have been prepared in good yields and fully characterized by ^{1}H NMR, ^{13}C NMR, and MS. Their antioxidant activities have been assessed by DPPH and ORAC assays, and during frying and accelerated storage tests. Novel phenolic compounds exhibited higher radical scavenging activities than both trolox and α-tocopherol. Trolox hydroxybenzoate showed a significantly higher protection than α-tocopherol under storage conditions. All new antioxidants performed better than α-tocopherol under frying conditions. Moreover, their outstanding thermal stability makes them more valuable than α-tocopherol for frying applications.

KEYWORDS: Antioxidant; synthesis; frying; phenolics; radical scavenging activity; trolox; storage stability; frying performance

INTRODUCTION

Free radicals are involved in inflammatory and cardiovascular diseases and cancer, and stimulate aging (*1*−*7*). In food, rancidity is one of the major concerns and is mainly related to oxidative degradation of polyunsaturated fatty acids. For years, antioxidants have been used to prevent the degradation of food (*8*). Phenolic derivatives are one of the most effective and commonly used antioxidants. These derivatives slow down the degradation of food ingredients by inhibiting their oxidation (*9*, *10*). Among this family of compounds, both synthetic antioxidants such as BHT, BHA, TBHQ, and natural ones such as tocopherols, phenolic acids, and herbal extracts are used to protect against oxidative degradation. Although synthetic antioxidants have shown good efficiency, their use has been limited because of their possible detrimental effect on human health (*8*). As a consequence, there is a growing interest in the development of new antioxidants that are based on natural components and exhibit low toxicity.

Among the natural antioxidants, α-tocopherol (vitamin E) is the most effective; however, its activity is affected by the environment and the conditions in which these compounds operate (*11*). Distribution and type of substitutes on the chromanol ring are mainly responsible for its effectiveness. Indeed, the methyl groups activate the aromatic ring, and the geometry adopted by the heterocyclic ring results in a stabilization of the phenoxyl radical (*11*). Natural phenolic acids are components of food, and their antioxidative activity has been used for food protection. Besides, some are also known for health stimulating properties. For example, gallic acid and its derivatives have been shown to exhibit the following health affecting properties: cardioprotectiveness (*12*), neuroprotectiveness (*13*), anti-inflammatory properties (*14*), antimutagenic properties (*15*), and anticancerogenic properties (*16*).

The objective of this work was the synthesis of new phenolic compounds with higher antioxidant activities than the common natural antioxidants and improved stability during storage and frying. Given that very often antioxidants are assessed for their free radical scavenging and this assessment has a limited value in predicting antioxidant effectiveness in real food systems (*17*), we applied accelerated storage and frying as measures of their efficacy.

MATERIALS AND METHODS

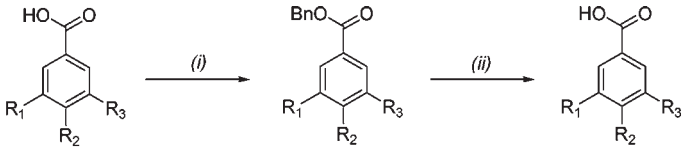
General Procedures. Column chromatography was performed using EMD silica gel Si 60 (40−63 μm). ^{1}H NMR and ^{13}C NMR were recorded on a 300 MHz Bruker Avance II spectrometer (Bruker BioSpin Corporation, Billerica, MA) with TMS as an internal standard. NMR data are presented in the following order: the chemical shift in ppm, multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet), coupling constant in Hertz, and assignment broad band in ^{1}H decoupling. Melting points (Mp) were measured with Electrothermal MEL-TEMP 3.0 (Barnstead). Analyses of residual antioxidants after frying and storage tests were performed on a Finnigan Surveyor LC (Thermo Electron Corp., Waltham, MA, USA) with a Finnigan Surveyor Autosampler Plus and Finnigan Surveyor FL Plus fluorescence detector. The column was a normal phase Diol column (5 μm; 250 × 4.6 mm; Monochrom, Varian, CA). A Beckman DU-65 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) was used in the DPPH assay as well as for the determination of PV. For the ORAC assay, fluorescence was measured on a Cary Eclipse fluorescence spectrophotometer (Varian, Palo Alto, CA). High-resolution mass spectra were obtained with a QSTAR Elite mass spectrometer (AB SCIEX, Concord, ON, Canada) equipped with an electrospray source operated in positive ion mode.

Chemicals. 2,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH), *N*,*N*′-dicyclohexylcarbodiimide (DCC), dimethylaminopyridine (DMAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), fluorescein disodium salt, trolox, and other phenolic acids were purchased from Sigma-Aldrich (St. Louis, MO).

*To whom correspondence should be addressed. Tel: (403) 317-5055. Fax: (403) 329-2057. E-mail: roman.przybylski@uleth.ca.

Published on Web 10/05/2010

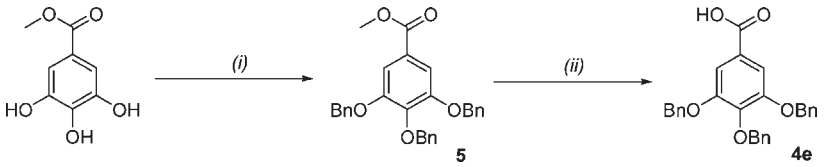
Scheme 1. Reactions, Reagents, and Conditions for the Syntheses of Benzoic Acid Derivatives[a]



2a: $R_1 = R_3 = H$; $R_2 = OH$ **3a**: $R_1 = R_3 = H$; $R_2 = OBn$ **4a** :$R_1 = R_3 = H$; $R_2 = OBn$
2b: $R_1 = OMe$; $R_2 = OH$; $R_3 = H$ **3b**: $R_1 = OMe$; $R_2 = OBn$; $R_3 = H$ **4b**: $R_1 = OMe$; $R_2 = OBn$; $R_3 = H$
2c: $R_1 = R_3 = OMe$; $R_2 = OH$ **3c**: $R_1 = R_3 = OMe$; $R_2 = OBn$ **4c**: $R_1 = R_3 = OMe$; $R_2 = OBn$
2d: $R_1 = R_2 = OH$; $R_3 = H$ **3d**: $R_1 = R_2 = OBn$; $R_3 = H$ **4d**: $R_1 = R_2 = OBn$; $R_3 = H$

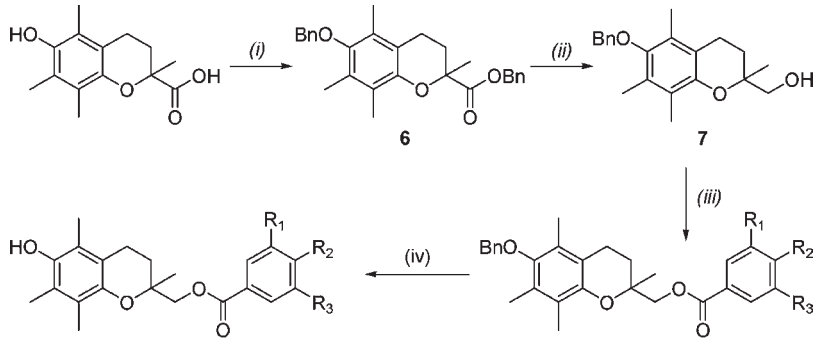
[a] (i) BnBr, K_2CO_3, DMF; (ii) KOH, H_2O/MeOH, reflux.

Scheme 2. Reactions, Reagents, and Conditions for the Synthesis of Benzylated Esters[a]



[a] (i) BnBr, K_2CO_3, DMF; (ii) KOH, H_2O/MeOH, reflux. For details, see the text.

Scheme 3. Reactions, Reagents, and Conditions for the Syntheses of Antioxidants **1** and **8**[a]



1a: $R_1 = R_3 = H$; $R_2 = OH$ **8a** :$R_1 = R_3 = H$; $R_2 = OBn$
1b: $R_1 = OMe$; $R_2 = OH$; $R_3 = H$ **8b**: $R_1 = OMe$; $R_2 = OBn$; $R_3 = H$
1c: $R_1 = R_3 = OMe$; $R_2 = OH$ **8c**: $R_1 = R_3 = OMe$; $R_2 = OBn$
1d: $R_1 = R_2 = OH$; $R_3 = H$ **8d**: $R_1 = R_2 = OBn$; $R_3 = H$
1e: $R_1 = R_2 = R_3 = OH$ **8e**: $R_1 = R_2 = R_3 = OBn$

[a] (i) BnBr, K_2CO_3, DMF; (ii) $LiAlH_4$, THF, 0 °C; (iii) **4a**−**4e**, DCC, DMAP, DCM; (iv) H_2, Pd/C 10%, THF. For details, see the text.

Tetrahydrofuran (THF) and dichloromethane (DCM) were purified using MBraun Solvent Purification System (M. Braun Incorporated, Stratham, NH). Dimethylformamide (DMF) and other solvents used in this work were of HPLC grade and were obtained from VWR (Edmonton, Canada).

Syntheses. Compounds **3a**, **3d, 4a**, **4d**, **4e**, **5**, and **6** were synthesized according to a method described by Tranchimand et al. (*18*), while compound **7** was synthesized according to a procedure described by Muller et al. (*19*).

General Procedure for the Synthesis of Benzoic Acid Esters (***3b*** *and* ***3c***). Benzoic esters **3b** and **3c** were prepared according to a procedure described by Tranchimand et al. (*18*). Briefly, potassium carbonate (77.2 mmol, 3.0 equiv), and benzyl bromide (77.2 mmol, 3.0 equiv) were added to a solution of the desired benzoic acid derivative (25.7 mmol) dissolved in 100 mL of dry DMF under an argon blanket. The mixture was stirred for 15 h and transferred into distilled water (150 mL). The compound of interest was extracted thrice with diethyl ether (100 mL). The combined extracts were washed with distilled water (100 mL), dried on magnesium sulfate, and concentrated under vacuum using a rotary evaporator.

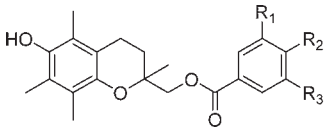
Data for Benzyl 3-Methoxy-4-benzyloxybenzoate (***3b***; ***Scheme 1***). The crude benzoic ester was purified by recrystallization in hexanes. Aspect: white solids. Yield = 89%. The spectral results were in agreement with the published data (*20*).

Data for Benzyl 3,5-Dimethoxy-4-benzyloxybenzoate (***3c***; ***Scheme 1***). The crude benzoic ester was purified by hexane recrystallization. Aspect: white solids. Yield = 76%. ^{1}H NMR (300.0 MHz, $CDCl_3$): δ: 3.73 (s, 6H, OCH_3); 4.96 (s, 2H, OCH_2Ph); 5.23 (s, 2H, OCH_2Ph); 7.18−7.35 (m, 12H, CH_{Ar}). ^{13}C NMR (75.0 MHz, $CDCl_3$): δ 56.2 (OCH_3); 66.8 ($COOCH_2Ph$); 75.9 (OCH_2Ph); 106.9 (C_{Ar}); 125.3 (C_{Ar}); 128.0 (C_{Ar}); 128.2 (C_{Ar}); 128.3 (C_{Ar}); 128.4 (C_{Ar}); 128.5 (C_{Ar}); 128.6 (C_{Ar}); 136.1 (C_{Ar}); 137.3 (C_{Ar}); 141.1 (C_{Ar}); 153.3 (C_{Ar}); 166.2 (C=O).

General Procedure for the Preparation of Carboxylic Acids (***4b*** *and* ***4c***; ***Scheme 1***). Carboxylic acids were prepared according to the procedure described by Tranchimand et al. (*18*). Briefly, potassium hydroxide (72.0 mmol, 5.0 equiv) was added to a solution of the corresponding benzoic ester **3b** or **3c** (14.4 mmol) in a mixture of distilled water (53 mL) and ethanol (210 mL). The mixture was refluxed for 2 h and the solvent evaporated under a vacuum using a rotary evaporator. The residue obtained was dissolved in distilled water (200 mL). The aqueous solution was extracted twice with diethyl ether (50 mL) and acidified with concentrated sulfuric acid until white solids were formed. The suspension was then extracted three times with ethyl acetate (200 mL). The combined extracts were washed with distilled water and dried on magnesium sulfate and finally concentrated under a vacuum using a rotary evaporator.

Data for 3-Methoxy-4-benzyloxybenzoic Acid (***4b***; ***Scheme 1***). Aspect: white solids. Yield = 87%. The spectral results matched the published data (*21*).

Data for 3,5-Dimethoxy-4-benzyloxybenzoic Acid (***4c***; ***Scheme 1***). Aspect: white solids. Yield = 90%. ^{1}H NMR (300.0 MHz, DMSO-d_6): δ 3.84 (s, 6H, OCH_3); 5.00 (s, 2H, OCH_2Ph); 7.25 (s, 2H, CH_{Ar}); 7.27−7.47 (m, 5H, CH_{Ar}); 12.96 (s, 1H, COOH). ^{13}C NMR (75.0 MHz, DMSO-d_6): δ



1a: $R_1 = R_3 = H$; $R_2 = OH$
1b: $R_1 = OMe$; $R_2 = OH$; $R_3 = H$
1c: $R_1 = R_3 = OMe$; $R_2 = OH$
1d: $R_1 = R_2 = OH$; $R_3 = H$
1e: $R_1 = R_2 = R_3 = OH$

Figure 1. Structure of novel antioxidants **1a**−**1e.** For details, see the text.

56.4 (OCH_3); 74.4 (OCH_2Ph); 106.9 (C_{Ar}); 126.5 (C_{Ar}); 128.3 (C_{Ar}); 128.5 (C_{Ar}); 128.6 (C_{Ar}); 138.0 (C_{Ar}); 140.6 (C_{Ar}); 153.3 (C_{Ar}); 167.4 (C═O).

*Procedure for the Synthesis of Esters **8a**−**8e***. DCC (3.06 mmol, 2.0 equiv) and DMAP (0.23 mmol, 0.15 equiv) were added under an argon blanket to an alcoholic solution of compound **7** (1.53 mmol) and the desired benzoic acid derivative (3.06 mmol, 2.0 equiv) in dry DCM (40 mL). The mixture was stirred for 15 h at room temperature, and distilled water (50 mL) was added. The organic layer was removed and washed once more with distilled water (20 mL), then dried over anhydrous magnesium sulfate, and concentrated. Finally, the crude product was purified by flash column chromatography with silica gel, and the solvents used for elution are described for the particular compounds.

*Data for (6′-Benzyloxy-2′,5′,7′,8′-tetramethylchroman-2′-yl) Methyl 4-Benzyloxybenzoate (**8a**; **Scheme 3**)*. Eluent = DCM/hexanes (7:3 v/v). Aspect: highly viscous oil. Yield = 70%. ^{1}H NMR (300.0 MHz, $CDCl_3$): δ 1.40 (s, 3H, CH_3); 1.81−2.10 (m, 2H, C═C−CH_2−CH_2); 2.11 (s, 3H, CH_3); 2.18 (s, 3H, CH_3); 2.22 (s, 3H, CH_3); 2.67 (t, J = 6.9 Hz, 2H, C═C−CH_2); 4.29 (d, J = 11.4 Hz, 1H, CH_2OCO); 4.38 (d, J = 11.4 Hz, 1H, CH_2OCO); 4.69 (s, 2H, OCH_2Ph); 5.13 (s, 2H, OCH_2Ph); 7.00 (d, J = 8.7 Hz, 1H, CH_{Ar}); 7.28−7.53 (m, 10H, CH_{Ar}); 8.00 (d, J = 8.7 Hz, 1H, CH_{Ar}). ^{13}C NMR (75.0 MHz, $CDCl_3$): δ 11.9 (CH_3); 12.0 (CH_3); 12.9 (CH_3); 20.3 (C═C-CH_2); 22.4 (CH_3); 28.8 (C═C−CH_2-CH_2); 68.9 (CH_2OCO); 70.1 (OCH_2Ph); 73 0.8 ($OCCH_2OCO$); 74.8 (OCH_2Ph); 114.5 (C_{Ar}); 117.3 (C_{Ar}); 122.8 (C_{Ar}); 123.2 (C_{Ar}); 126.1 (C_{Ar}); 127.5 (C_{Ar}); 127.7 (C_{Ar}); 127.8 (C_{Ar}); 128.2 (C_{Ar}); 128.3 (C_{Ar}); 128.5 (C_{Ar}); 128.7 (C_{Ar}); 131.7 (C_{Ar}); 136.2 (C_{Ar}); 137.9 (C_{Ar}); 147.4 (C_{Ar}); 148.6 (C_{Ar}); 162.6 (C_{Ar}); 166.1 (C═O). MS (*m*/*z*): calculated for $C_{35}H_{36}O_5$ = 537.2636; found 537.2637 $[M + H]^+$.

*Data for (6′-Benzyloxy-2′,5′,7′,8′-tetramethylchroman-2′-yl) Methyl 3-Methoxy-4-benzyl-oxybenzoate (**8b**; **Scheme 3**)*. Eluent = ethyl acetate/hexanes (25:75 v/v). Aspect: highly viscous oil. Yield = 75%. ^{1}H NMR (300.0 MHz, $CDCl_3$): δ 1.39 (s, 3H, CH_3); 1.81−2.09 (m, 2H, C═C−CH_2−CH_2); 2.11 (s, 3H, CH_3); 2.17 (s, 3H, CH_3); 2.22 (s, 3H, CH_3); 2.67 (t, J = 6.9 Hz, 2H, C═C−CH_2); 3.94 (s, 3H, OCH_3); 4.26 (d, J = 11.4 Hz, 1H, CH_2OCO); 4.39 (d, J = 11.4 Hz, 1H, CH_2OCO); 4.69 (s, 2H, OCH_2Ph); 5.23 (s, 2H, OCH_2Ph); 6.90 (d, J = 8.10 Hz, 1H, CH_{Ar}); 7.27−7.65 (m, 12H, CH_{Ar}). ^{13}C NMR (75.0 MHz, $CDCl_3$): δ 11.9 (CH_3); 12.0 (CH_3); 12.9 (CH_3); 20.3 (C═C-CH_2); 22.4 (CH_3); 28.8 (C═C−CH_2-CH_2); 56.1 (OCH_3); 68.9 (CH_2OCO); 70.8 (OCH_2Ph); 73.8 ($OCCH_2OCO$); 74.8 (OCH_2Ph); 112.5 (C_{Ar}); 112.6 (C_{Ar}); 117.4 (C_{Ar}); 122.9 (C_{Ar}); 123.2 (C_{Ar}); 123.5 (C_{Ar}); 126.1 (C_{Ar}); 127.2 (C_{Ar}); 127.8 (C_{Ar}); 127.9 (C_{Ar}); 128.1 (C_{Ar}); 128.3 (C_{Ar}); 128.5 (C_{Ar}); 128.7 (C_{Ar}); 136.4 (C_{Ar}); 137.9 (C_{Ar}); 147.4 (C_{Ar}); 148.6 (C_{Ar}); 149.2 (C_{Ar}); 152.2 (C_{Ar}); 166.2 (C═O). MS (*m*/*z*): calculated for $C_{36}H_{38}O_6$ = 567.2741; found 567.2743 $[M + H]^+$.

*Data for (6′-Benzyloxy-2′,5′,7′,8′-tetramethylchroman-2′-yl) Methyl 3,5-Dimethoxy-4-benzyl-oxybenzoate (**8c**; **Scheme 3**)*. Eluent = DCM/hexanes (4:1 v/v). Aspect: highly viscous oil. Yield = 71%. ^{1}H NMR (300.0 MHz, $CDCl_3$): δ 1.40 (s, 3H, CH_3); 1.84−2.11 (m, 2H, C═C−CH_2−CH_2); 2.13 (s, 3H, CH_3); 2.18 (s, 3H, CH_3); 2.22 (s, 3H, CH_3); 2.68 (t, J = 6.9 Hz, 2H, C═C−CH_2); 3.87 (s, 6H, OCH_3); 4.28 (d, J = 11.4 Hz, 1H, CH_2OCO); 4.43 (d, J = 11.4 Hz, 1H, CH_2OCO); 4.69 (s, 2H, OCH_2Ph); 5.09 (s, 2H, OCH_2Ph); 7.25−7.53 (m, 12H, CH_{Ar}). ^{13}C NMR (75.0 MHz, $CDCl_3$): δ 11.8 (CH_3); 12.0 (CH_3); 12.9 (CH_3); 20.2 (C═C-CH_2); 22.3 (CH_3); 28.9 (C═C−CH_2-CH_2); 56.2 (OCH_3); 69.2 (CH_2OCO); 73.8 ($OCCH_2OCO$); 74.8 (OCH_2Ph); 75.0 (OCH_2Ph); 106.9 (C_{Ar}); 117.3 (C_{Ar}); 123.1 (C_{Ar}); 125.3 (C_{Ar}); 126.1 (C_{Ar}); 127.7 (C_{Ar}); 127.9 (C_{Ar}); 128.0 (C_{Ar}); 128.2 (C_{Ar}); 128.4 (C_{Ar}); 128.5 (C_{Ar}); 128.6 (C_{Ar}); 137.4 (C_{Ar}); 137.9 (C_{Ar}); 141.2 (C_{Ar}); 147.3 (C_{Ar}); 148.7 (C_{Ar}); 153.3 (C_{Ar}); 166.1 (C═O). MS (*m*/*z*): calculated for $C_{37}H_{40}O_7$ = 597.2847; found 597.2831 $[M + H]^+$.

*Data for (6′-Benzyloxy-2′,5′,7′,8′-tetramethylchroman-2′-yl) Methyl 3,4-Dibenzyloxy-benzoate (**8d**; **Scheme 3**)*. Eluent = ethyl acetate/hexanes (1:4 v/v). Aspect: highly viscous oil. Yield = 78%. ^{1}H NMR (300.0 MHz, $CDCl_3$): δ 1.41 (s, 3H, CH_3); 1.83−2.13 (m, 2H, C═C−CH_2−CH_2); 2.15 (s, 3H, CH_3); 2.22 (s, 3H, CH_3); 2.26 (s, 3H, CH_3); 2.70 (t, J = 6.9 Hz, 2H, C═C−CH_2); 4.30 (d, J = 11.4 Hz, 1H, CH_2OCO); 4.39 (d, J = 11.4 Hz, 1H, CH_2OCO); 4.74 (s, 2H, OCH_2Ph); 5.25 (s, 2H, OCH_2Ph); 5.27 (s, 2H, OCH_2Ph); 6.98 (d, J = 8.7 Hz, 1H, CH_{Ar}); 7.29−7.70 (m, 17H, CH_{Ar}). ^{13}C NMR (75.0 MHz, $CDCl_3$): δ 11.9 (CH_3); 12.0 (CH_3); 12.9 (CH_3); 20.3 (C═C-CH_2); 22.4 (CH_3); 28.8 (C═C−CH_2-CH_2); 68.9 (CH_2OCO); 70.8 (OCH_2Ph); 71.2 (OCH_2Ph); 73.8 ($OCCH_2OCO$); 74.8 (OCH_2Ph); 113.2 (C_{Ar}); 115.5 (C_{Ar}); 117.4 (C_{Ar}); 123.0 (C_{Ar}); 123.2 (C_{Ar}); 124.1 (C_{Ar}); 126.1 (C_{Ar}); 127.1 (C_{Ar}); 127.4 (C_{Ar}); 127.8 (C_{Ar}); 127.9 (C_{Ar}); 128.0 (C_{Ar}); 128.1 (C_{Ar}); 128.3 (C_{Ar}); 128.5 (C_{Ar}); 128.6 (C_{Ar}); 128.7 (C_{Ar}); 136.6 (C_{Ar}); 136.9 (C_{Ar}); 137.9 (C_{Ar}); 147.4 (C_{Ar}); 148.3 (C_{Ar}); 148.6 (C_{Ar}); 153.0 (C_{Ar}); 166.0 (C═O). MS (*m*/*z*): calculated for $C_{42}H_{42}O_6$ = 643.3054; found 643.3087 $[M + H]^+$.

*Data for (6′-Benzyloxy-2′,5′,7′,8′-tetramethylchroman-2′-yl) Methyl 3,4,5-Tribenzyloxy-benzoate (**8e**; **Scheme 3**)*. Eluent = DCM/hexanes (4:1 v/v). Aspect: light yellow solids. Yield = 80%. ^{1}H NMR (300.0 MHz, $CDCl_3$): δ 1.36 (s, 3H, CH_3); 1.77−2.06 (m, 2H, C═C−CH_2−CH_2); 2.12 (s, 3H, CH_3); 2.19 (s, 3H, CH_3); 2.23 (s, 3H, CH_3); 2.66 (t, J = 6.6 Hz, 2H, C═C−CH_2); 4.26 (d, J = 11.4 Hz, 1H, CH_2OCO); 4.36 (d, J = 11.4 Hz, 1H, CH_2OCO); 4.70 (s, 2H, OCH_2Ph); 5.14 (s, 6H, OCH_2Ph); 7.22−7.55 (m, 22H, CH_{Ar}). ^{13}C NMR (75.0 MHz, $CDCl_3$): δ 11.9 (CH_3); 12.1 (CH_3); 12.9 (CH_3); 20.2 (C═C-CH_2); 22.4 (CH_3); 28.7 (C═C−CH_2-CH_2); 69.1 (CH_2OCO); 71.2 (OCH_2Ph); 73.7 ($OCCH_2OCO$); 74.8 (OCH_2Ph); 75.2 (OCH_2Ph); 109.1 (C_{Ar}); 117.3 (C_{Ar}); 123.1 (C_{Ar}); 125.1 (C_{Ar}); 126.1 (C_{Ar}); 127.5 (C_{Ar}); 127.6 (C_{Ar}); 127.8 (C_{Ar}); 127.9 (C_{Ar}); 128.0 (C_{Ar}); 128.1 (C_{Ar}); 128.2 (C_{Ar}); 128.4 (C_{Ar}); 128.5 (C_{Ar}); 128.6 (C_{Ar}); 136.7 (C_{Ar}); 137.4 (C_{Ar}); 137.9 (C_{Ar}); 142.5 (C_{Ar}); 147.3 (C_{Ar}); 148.6 (C_{Ar}); 152.5 (C_{Ar}); 165.9 (C═O). MS (*m*/*z*): calculated for $C_{49}H_{48}O_7$ = 749.3473; found 749.3485 $[M + H]^+$.

*General Procedure for the Preparation of Antioxidants **1a**−**1e** (**Figure 1**)*. Palladium on charcoal (10% w/w) was added to a solution of the desired benzylated compound (0.7 mmol in 8 mL of dry THF). The mixture was stirred at room temperature under an atmosphere of hydrogen for 24 h, then filtrated on Celite, and concentrated on a rotary evaporator under reduced pressure. The residue was purified by flash column chromatography with silica gel, and the solvents used for elution are described for each individual component below.

*Data for (6′-Hydroxy-2′,5′,7′,8′-tetramethylchroman-2′-yl) Methyl 4-Hydroxybenzoate (**1a**)*. Eluent = ethyl acetate/hexanes (2:3 v/v). Aspect: white solids. Yield = 90%. Mp = 139−140 °C. ^{1}H NMR (300.0 MHz, $CDCl_3$): δ 1.42 (s, 3H, CH_3); 1.80−2.09 (m, 2H, C═C−CH_2−CH_2); 2.10 (s, 3H, CH_3); 2.11 (s, 3H, CH_3); 2.15 (s, 3H, CH_3); 2.67 (t, J = 6.6 Hz, 2H, C═C−CH_2); 4.21 (s, 1H, OH); 4.27 (d, J = 11.4 Hz, 1H, CH_2OCO); 4.34 (d, J = 11.4 Hz, 1H, CH_2OCO); 5.40 (s, 1H, OH); 6.84 (d, J = 8.7 Hz, 1H, CH_{Ar}); 7.95 (d, J = 8.7 Hz, 1H, CH_{Ar}). ^{13}C NMR (75.0 MHz, $CDCl_3$): δ 11.3 (CH_3); 11.8 (CH_3); 12.2 (CH_3); 20.3 (C═C-CH_2); 22.2 (CH_3); 28.9 (C═C−CH_2-CH_2); 68.9 (CH_2OCO); 73.5 ($OCCH_2OCO$); 115.3 (C_{Ar}); 117.1 (C_{Ar}); 118.6 (C_{Ar}); 121.3 (C_{Ar}); 122.5 (C_{Ar}); 122.8 (C_{Ar}); 132.0 (C_{Ar}); 145.0 (C_{Ar}); 160.0 (C_{Ar}); 166.3 (C═O). MS (*m*/*z*): calculated for $C_{21}H_{24}O_5$ = 357.1697; found 357.1696 $[M + H]^+$.

*Data for (6′-Hydroxy-2′,5′,7′,8′-tetramethylchroman-2′-yl) Methyl 3-Methoxy-4-hydroxy-benzoate (**1b**)*. Eluent = ethyl acetate/hexanes (1:1 v/v). Aspect: white solids. Yield = 70%. Mp = 171−172 °C. ^{1}H NMR (300.0 MHz, DMSO-d_6): δ 1.03 (s, 3H, CH_3); 1.43−1.75 (m, 2H, C═C−CH_2−CH_2); 1.75 (s, 3H, CH_3); 1.78 (s, 3H, CH_3); 1.82 (s, 3H, CH_3); 2.25−2.38 (m, 2H, C═C−CH_2); 3.57 (s, 3H, OCH_3); 3.90 (d, J = 11.4 Hz, 1H, CH_2OCO); 3.99 (d, J = 11.4 Hz, 1H, CH_2OCO); 6.17 (s, 1H, OH); 6.58 (d, J = 8.1 Hz, 1H, CH_{Ar}); 7.20 (s, 1H, CH_{Ar}); 7.24 (d, J = 8.1 Hz, 2H, CH_{Ar}); 8.48 (s, 1H, OH). ^{13}C NMR (75.0 MHz, DMSO-d_6): δ 12.1 (CH_3); 12.3 (CH_3); 13.2 (CH_3); 20.2 (C═C-CH_2); 22.2 (CH_3); 28.8 (C═C−CH_2-CH_2); 56.0 (OCH_3); 68.5 (CH_2OCO); 73.7 ($OCCH_2OCO$); 112.9 (C_{Ar}); 115.6 (C_{Ar}); 117.2 (C_{Ar}); 120.8 (C_{Ar}); 120.9 (C_{Ar}); 121.6 (C_{Ar}); 123.2 (C_{Ar}); 123.9 (C_{Ar}); 144.4 (C_{Ar}); 146.0 (C_{Ar}); 147.9 (C_{Ar}); 152.1 (C_{Ar}); 165.8 (C═O). MS: calculated for $C_{22}H_{26}O_6$ = 387.1802; found 387.1781 $[M + H]^+$.

*Data for (6′-Hydroxy-2′,5′,7′,8′-tetramethylchroman-2′-yl) Methyl 3,5-Dimethoxy-4-hydroxybenzoate (**1c**)*. Eluent = ethyl acetate/hexanes (2:3 v/v). Aspect: white solids. Yield = 81%. Mp = 144−145 °C. ^{1}H NMR (300.0 MHz, $CDCl_3$): δ 1.38 (s, 3H, CH_3); 1.79−2.06 (m, 2H,

C=C−CH_2−CH_2); 2.11 (s, 3H, CH_3); 2.12 (s, 3H, CH_3); 2.15 (s, 3H, CH_3); 2.69 (t, J = 6.9 Hz, 2H, C=C−CH_2); 3.93 (s, 6H, OCH_3); 4.22 (s, 1H, OH); 4.27 (d, J = 11.4 Hz, 1H, CH_2OCO); 4.40 (d, J = 11.4 Hz, 1H, CH_2OCO); 5.90 (s, 1H, OH); 7.33 (s, 2H, CH_{Ar}). ^{13}C NMR (75.0 MHz, $CDCl_3$): δ 12.1 (CH_3); 12.3 (CH_3); 13.2 (CH_3); 20.2 (C=C-CH_2); 22.2 (CH_3); 28.9 (C=C−CH_2-CH_2); 56.5 (OCH_3); 68.7 (CH_2OCO); 73.7 ($OCCH_2OCO$); 107.2 (C_{Ar}); 117.2 (C_{Ar}); 119.6 (C_{Ar}); 120.8 (C_{Ar}); 121.6 (C_{Ar}); 123.2 (C_{Ar}); 141.3 (C_{Ar}); 144.4 (C_{Ar}); 146.0 (C_{Ar}); 148.0 (C_{Ar}); 165.8 (C=O). MS: calculated for $C_{23}H_{28}O_7$ = 417.1908; found 417.1902 $[M + H]^+$.

Data for (6′-Hydroxy-2′,5′,7′,8′-tetramethylchroman-2′-yl) Methyl 3,4-Dihydroxybenzoate (***1d***). Eluent = ethyl acetate/hexanes (1:1 v/v). Aspect: white solids. Yield = 76%. Mp = 182−183 °C. ^{1}H NMR (300.0 MHz, $CDCl_3$): δ 1.38 (s, 3H, CH_3); 1.86−2.08 (m, 2H, C=C−CH_2−CH_2); 2.09 (s, 3H, CH_3); 2.11 (s, 3H, CH_3); 2.14 (s, 3H, CH_3); 2.66 (t, J = 6.6 Hz, 2H, C=C−CH_2); 4.31 (s, 2H, CH_2OCO); 4.35 (s, 1H, OH); 6.31 (s, 1H, OH); 6.40 (s, 1H, OH); 6.89 (d, J = 8.4 Hz, 1H, CH_{Ar}); 7.50 (s, 1H, CH_{Ar}); 7.55 (d, J = 8.4 Hz, 1H, CH_{Ar}). ^{13}C NMR (75.0 MHz, $CDCl_3$): δ 11.3 (CH_3); 11.8 (CH_3); 12.2 (CH_3); 20.4 (C=C-CH_2); 22.5 (CH_3); 29.1 (C=C−CH_2-CH_2); 69.3 (CH_2OCO); 73.6 ($OCCH_2OCO$); 114.9 (C_{Ar}); 116.5 (C_{Ar}); 117.4 (C_{Ar}); 118.6 (C_{Ar}); 121.4 (C_{Ar}); 122.4 (C_{Ar}); 122.9 (C_{Ar}); 123.9 (C_{Ar}); 143.0 (C_{Ar}); 144.9 (C_{Ar}); 145.3 (C_{Ar}); 148.9 (C_{Ar}); 166.4 (C=O). MS: calculated for $C_{21}H_{24}O_6$ = 373.1646; found 373.1663 $[M + H]^+$.

Data for (6′-Hydroxy-2′,5′,7′,8′-tetramethylchroman-2′-yl) Methyl 3,4,5-Trihydroxy-benzoate (***1e***). Eluent = ethyl acetate/hexanes (1:1 v/v). Aspect: white solids. Yield = 77%. Mp = 212−213 °C. ^{1}H NMR (300.0 MHz, DMSO-d_6): δ 1.30 (s, 3H, CH_3); 1.73−2.00 (m, 2H, C=C−CH_2−CH_2); 2.00 (s, 3H, CH_3); 2.04 (s, 3H, CH_3); 2.05 (s, 3H, CH_3); 2.59 (t, J = 6.9 Hz, 2H, C=C−CH_2); 4.20 (s, 2H, CH_2OCO); 6.99 (s, 2H, CH_{Ar}); 7.47 (s, 1H, OH); 9.10 (s, 3H, OH). ^{13}C NMR (75.0 MHz, DMSO-d_6): δ 12.2 (CH_3); 12.3 (CH_3); 13.2 (CH_3); 20.2 (C=C-CH_2); 22.0 (CH_3); 28.7 (C=C−CH_2-CH_2); 68.3 (CH_2OCO); 73.7 ($OCCH_2OCO$); 109.0 (C_{Ar}); 117.0 (C_{Ar}); 119.7 (C_{Ar}); 120.8 (C_{Ar}); 121.6 (C_{Ar}); 123.2 (C_{Ar}); 139.0 (C_{Ar}); 144.4 (C_{Ar}); 145.9 (C_{Ar}); 146.0 (C_{Ar}); 166.0 (C=O). MS: calculated for $C_{21}H_{24}O_7$ = 389.1595; found 389.1580 $[M + H]^+$.

DPPH Radicals Scavenging Assay. The DPPH assay was performed according to Nenadis and Tsimidou (*22*). Briefly, to 2960 μL of 0.1 mM ethanolic solution of DPPH, 40 μL of synthesized antioxidant solution in ethanol was added at the following concentrations: 0.37, 0.74, 1.11, 1.85, 3.7, and 5.2 mM, forming the ratios between the molar amounts of antioxidant to the molar amount of DPPH radicals at 0.05, 0.10, 0.15, 0.25, 0.5, and 0.7, respectively. The decrease of absorbance at 516 nm was measured at 25 °C after 20 min of reaction time. The blank solution contained the same amount of DPPH and 40 μL of ethanol. Each test was performed in triplicate. The results are expressed as the %DPPH inhibition calculated according to the following equation:

$$\%\ DPPH = \frac{(Ac - At)100}{Ac}$$

where *Ac* and *At* are the absorbances of the control sample and the test sample, respectively. All standard deviations for DPPH tests were below 3.0%. Both trolox and α-tocopherol were used as references. The IC_{50} represents the concentration of antioxidant required to decrease the initial amount of DPPH by 50%.

ORAC Assay. ORAC assays were performed according to Szydlowska-Czerniak et al. (*23*). Briefly, the fluorescein disodium salt and AAPH solutions were prepared in 75 mM phosphate buffer (pH 7.4). The antioxidant solutions, 1 mM of each compound, were dissolved in methanol, and a specific volume of it dissolved in the buffer to provide the required amount of antioxidant within a range of 3.125−25.00 μM. Four different concentrations were tested for each antioxidant. A solution of fluorescein, 3.0 mL (0.0816 μM), was mixed with 0.5 mL of antioxidant solution directly in a quartz cuvette. The mixture was kept at 37 °C for 10 min and 0.5 mL of the AAPH solution (153.0 μM) added. The fluorescence was measured at 37 °C for 30 min at 30 s intervals. The emission and excitation were set at 525 and 485 nm, respectively. For a blank, phosphate buffer replaced the antioxidant solution. Each antioxidant solution was prepared in duplicate, and three measurements were performed for each sample. A calibration curve was generated using trolox as the reference antioxidant.

The area under the fluorescence decay curve (AUC) was calculated as follows:

$$AUC = \sum_{t=0.5}^{30} \frac{f_t + f_{(t-0.5)}}{4}$$

where f_t is the fluorescence at time t (min).

The net AUC corresponding to the sample was calculated using the following equation:

$$AUC_{net} = AUC - AUC_{blank}$$

For each antioxidant, a regression between AUC_{net} and the compound concentrations was calculated, and the results were expressed as trolox equivalents (TE).

Canola Oil Triacylglycerides Isolation. Canola oil was stripped of its endogenous minor components including antioxidants via adsorption chromatography, following the procedure described by Lampi et al. (*24*).

Accelerated Storage. The ability of the new antioxidants to protect oil against oxidative degradation was determined using the Schaal Oven test. To 1 g of pure canola triacylglycerols (CTG) 350 ppm of the tested antioxidant was added in a vial (National Scientific Target DP Vials; 2 mL, 12 × 32 mm). The samples were stored in the dark for five days at 60 °C, providing the surface area to volume ratio at 0.78. Samples were examined at 24 h intervals for peroxide value and the residual amounts of antioxidant. The effectiveness of the new compounds was compared with α-tocopherol as natural antioxidant and butylated hydroxytoluene (BHT) as a synthetic antioxidant. Experiments were set up in two repetitions for each tested antioxidant, and samples from each repetition were analyzed in duplicate.

Peroxide Value (PV). To assess PV method published by Hornero-Mendeza et al. and modified by Shanta et al. was used (*25*, *26*). Briefly, 200 mg of oil was dissolved in 5 mL of hexane; to 200 μL of the sample solution, 5 mL of methanol/chloroform/HCL (1:1:0.012, v/v.), then 100 μL of NH_4SCN (30% w/w in water), and 100 μL of ferrous chloride (0.4% in water) were added. After 5 min of incubation at room temperature, the absorbance at 480 nm was measured.

Test Frying. The effectiveness of the developed antioxidants to protect CTG under frying conditions was assessed using a frying test system. CTG (12.0 g), fortified with 500 μg/g of the studied antioxidant, was weighed into a glass beaker (Pyrex, USA). An octagonal stir bar (ThermoFischer Scientific, USA) was placed into the vessel, evolving the final surface-to-volume ratio to 0.42. Then vessel was heated at 185 ± 5 °C for 10 min, and 1.2 g of formulated starch (a mixture of gelatinized potato starch with glucose and silica gel, 4:1:1 w/w) was added. The heating was continued for another 20 min without mixing and then was stirred at 500 rpm. Heating and stirring were afterward maintained for 90 min. About 0.5 g of oil sample was withdrawn at the 60th, 80th, 100th, and 120th minutes of heating. Selected sampling points reflect the frying time based on the amount of polar components formed and correspond to 1, 3, 5, and 7 days of actual frying time using an institutional fryer (General Electric Company, NY, USA). The frying performance of oils was assessed by the measurement of total polar components (TPC) and the amount of retained tocopherol and added antioxidant. Samples from two repetitions of the frying test were analyzed in duplicate for TPC and the residual antioxidant.

Total Polar Compounds. The amounts of polar compounds were determined by a gravimetric procedure following AOAC Method 982.27 with the Schulte modification (*27*, *28*).

Residual Tocopherol and Novel Antioxidant. Tocopherols and the new antioxidants were analyzed according to AOCS Official Method Ce 8−89 (*29*). Briefly, oil samples (50.0 mg) were weighed directly into autosampler vials and dissolved in hexanes (1.0 mL). For tocopherol, the mobile phase consisted of 7% methyl *tert*-butyl ether in hexanes with a flow rate of 0.6 mL/min, and the fluorescence detector was set for excitation at 292 nm and emission at 325 nm. For the new antioxidants, the mobile phase was changed to 65% methyl *tert*-butyl ether in hexane and the fluorescence detector emission set to 394 nm. For each run, 10 μL of sample was injected.

Statistical Analysis. Data were analyzed by single factor analysis of variance (ANOVA) and regression using Minitab 2000 statistical software (Minitab Inc., PA, version 13.2). Significant differences between means

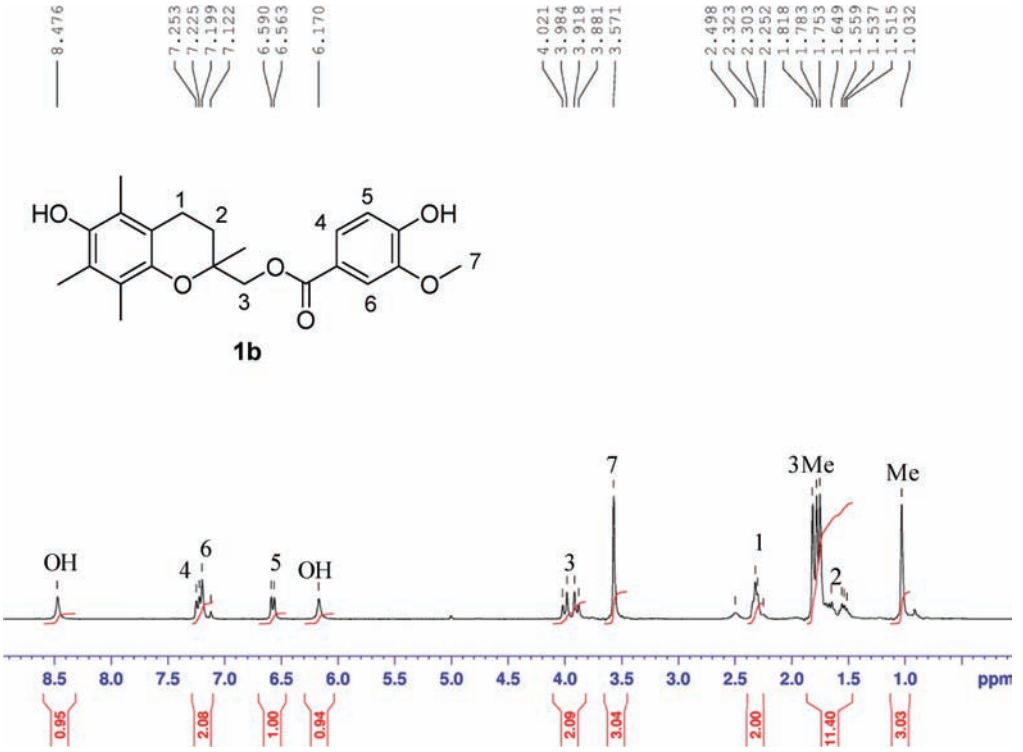


Figure 2. ^{1}H NMR spectrum of novel antioxidant **1b**. For details, see the text.

were determined by Duncan's multiple range tests. Statistically significant differences were determined at the $P < 0.05$ level.

RESULTS AND DISCUSSION

Synthesis. In order to produce the new antioxidants **1a**−**1e** (**Figure 1**), benzoic acids **4a**−**4e** have been prepared in two steps using commercially available derivatives of benzoic acids **2a**−**2d** (**Scheme 1** and **2**). Benzoic derivatives, **2a**−**2d**, have been benzylated using benzyl bromide and potassium carbonate in dimethylformamide (DMF) to produce **3a**−**3d** esters with the following yields: 68% (**3a**), 89% (**3b**), 76% (**3c**), and 94% (**3d**). Saponification of **3a**−**3d** esters with potassium hydroxide in a methanol/water solution has formed the desired benzoic acids derivatives with the yields of 87% (**4a**,**4b**), 90% (**4c**), and 95% (**4d**). Gallic acid derivative **4e** has been synthesized from methyl gallate following the reaction described in **Scheme 2** (*18*). Derivatives of trolox **1a**−**1e** have been prepared in four steps as described in **Scheme 3**. Alcohol **7** has been obtained according to the procedure described by Muller et al. (*19*). A Steglich esterification between compound **7** and benzoic acid derivatives **4a**−**4e** formed esters **8a**−**8e** (*30*). These esters have been purified by flash chromatography and were produced with the following yields: 70% (**8a**), 75% (**8b**), 71% (**8c**), and 80% (**8d**,**8e**). Antioxidants **1a**−**1e** have been obtained by hydrogenation of compounds **8a**−**8e** over palladium catalyst. Isolated antioxidants formed white solids and were produced with the following yields: 90% (**1a**), 70% (**1b**), 81% (**1c**), 76% (**1d**), and 77% (**1e**).

The structures of antioxidants **1a**−**1e** have been confirmed by ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, and MS. As an example, the ^{1}H NMR spectrum of antioxidant **1b** in **Figure 2** is included. The ^{1}H NMR spectrum is characterized by four methyl groups at 1.03 ppm, 1.75 ppm, 1.78 ppm, and 1.82 ppm (s), by methylene groups at 1.43−1.75 ppm (m) and 2.25−2.38 ppm (m), and by a methoxy group at 3.57 ppm (s). The inequivalent H_3 protons next to the ester group are easily identified by two doublets at 3.90 ppm and 3.99 ppm. As for the aromatic protons, they are identified by two doublets at 6.58 ppm and 7.24 ppm and by a singlet at 7.20 ppm. The complete disappearance of the characteristic signals for the benzyl groups of ester **8b** (two singlets at 4.69 ppm and 5.23 ppm as well as a multiplet at 7.27−7.65 ppm) clearly demonstrate that the removal of benzyl protection of the alcohol groups has been completed.

Antioxidant Capacity Assays. *DPPH Assay*. The radical scavenging properties of the synthesized antioxidants **1a** to **1e**, trolox (**A1**), and α-tocopherol (**A2**) have been evaluated by the DPPH assay. The results of this study are included in **Table 1**. These results attest that the radical scavenging activities of the new compounds **1d** (IC_{50} = 0.82 mM) and **1e** (IC_{50} = 0.63 mM) were significantly higher than those of the commercially available trolox (IC_{50} = 1.75 mM) and tocopherol (IC_{50} = 1.61 mM). As for antioxidant **1a** (IC_{50} = 1.92 mM), its scavenging activity toward DPPH radicals was the lowest among those synthesized (**Table 1**). Antioxidants **1b** (IC_{50} = 1.69 mM) and **1c** (IC_{50} = 1.64 mM) provided scavenging activities similar to those of trolox and α-tocopherol. In the case of this test, it was established that the number of hydroxyl groups on the aromatic ring define radical scavenging activity. When comparing activities for compounds **1a** (2 OH), **1d** (3 OH), and **1e** (4 OH), where the only structural differences are in the number of hydroxyl groups on the ring, statistically significant differences have been observed in the DPPH radical scavenging potency in the decreasing order: **1e** > **1d** > **1a**. It is well established that phenolic compounds scavenged radicals by proton donation (*10*). Consequently, this article's results are consistent with this observation because a higher number of hydroxyl groups resulted in the higher capacity of proton donation. A dimeric antioxidant containing two hydroxyl groups in fused double chromanol rings had better DPPH radical scavenging activity and provided double reducing power compared to those of α-tocopherol (*31*, *32*). Furthermore, it is a well established fact that the configuration of the hydroxyl group on the ring in *ortho* position increases the radical scavenging activity (*33*). Our results also confirmed that the configuration of

Table 1. DPPH and ORAC Tests Results for Novel Antioxidants[a]

	DPPH test		ORAC test
antioxidant	IC_{50} (mM)	inhibition (%)	trolox equivalents (μM)
1a	1.92 ± 0.01	29.9 ± 1.2	3.78 ± 0.26
1b	1.69 ± 0.06	32.2 ± 2.3	5.10 ± 0.26
1c	1.64 ± 0.04	34.4 ± 1.8	2.81 ± 0.29
1d	0.82 ± 0.01	65.0 ± 2.1	4.48 ± 0.13
1e	0.63 ± 0.01	88.1 ± 2.2	2.26 ± 0.12
trolox[b]	1.75 ± 0.02	31.7 ± 1.9	
α-tocopherol[b]	1.61 ± 0.02	34.6 ± 1.7	
caffeic acid[b]			4.46 ± 0.22

[a] Compounds **1a**−**1e**, novel antioxidants. For their structures, see **Figure 1**. [b] Reference antioxidants.

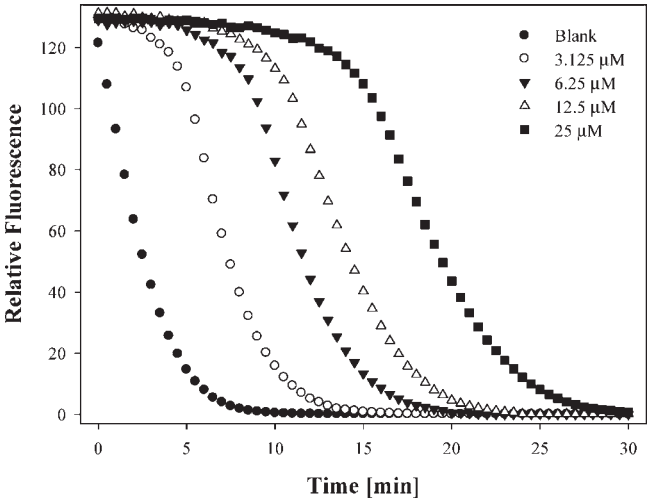


Figure 3. Fluorescence decay profiles induced by AAPH for the new **1b** antioxidant at different concentrations.

methoxy substituents to the hydroxyl group on the ring in *ortho* position increased the radical scavenging activity of the antioxidant (*34*). Indeed, **1b** (1 methoxy group, IC_{50} = 1.69 mM) and **1c** (2 methoxy groups, IC_{50} = 1.64 mM) were more efficient than **1a** (IC_{50} = 1.92 mM). Component **1c** was slightly more effective in radical scavenging activity than **1b**; however, the differences were not statistically significant. This trend can be related to the structure differences discussed above. Similar trends discussed above related to the half-life of DPPH radicals were observed for the inhibition (**Table 1**).

ORAC Assay. The antioxidant capacities of the synthesized antioxidants using caffeic acid as the reference have been assessed by the ORAC assay. **Figure 3** depicts the decay curves for different concentrations (3.125−25.0 μM) of antioxidant **1b**. A regression coefficient above 99% has been achieved for the correlation between the AUC_{net} and the antioxidant concentration for each compound tested (the results are not included). The antioxidant capacities of each compound, expressed in trolox equivalents, are given in **Table 1**. According to these results, all of the synthesized phenolic compounds exhibited a higher antioxidant capacity than trolox. An improved antioxidant activity was reported for a synthesized compound containing hydroxytyrosol attached to the chromanol ring, which inhibited the formation of malondialdehyde in the rat liver microsomal membrane when oxidation was induced by AAPH (*35*). The data obtained for caffeic acid (4.46 TE) was similar to the one published by Gomez-Ruiz et al. (4.52 TE; *36*). Antioxidant **1b** (5.10 TE) was the most effective radical scavenger among the studied compounds, whereas **1e** (2.26 TE) was the least active. It is worth mentioning that the ORAC assessment gives

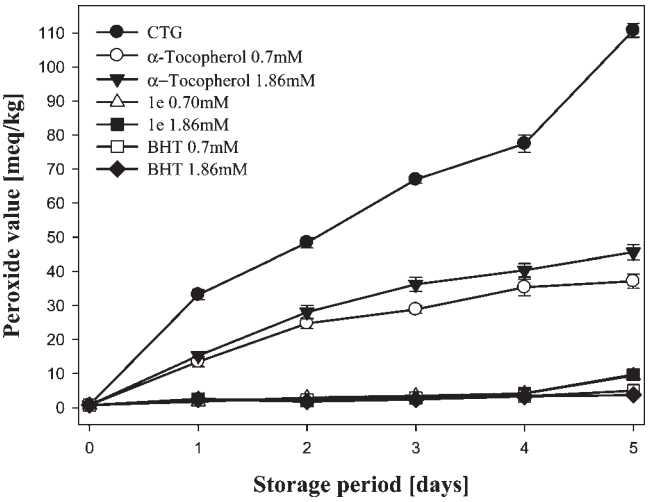


Figure 4. Changes in the peroxide value during accelerated storage of canola triacylglycerols fortified with different amounts of α-tocopherol, BHT, and the novel antioxidant **1e**. CTG, canola triacylglycerides. For details, see the text.

results different from those of the DPPH assay. For example, **1e** was the least efficient radical scavenger in the ORAC assay, whereas it was the most effective scavenger for the DPPH radicals (**Table 1**). Contrary to the DPPH results, no trend has been observed in the ORAC test. There is as well as lack of relationship between the numbers of hydroxyl or methoxy groups on the chromanol ring. Each of the tests utilizes different stable free radicals, and a different mechanism of scavenging is involved, making data not comparable and not transferrable to the food system (*37*−*39*). Both DPPH and ORAC assays confirmed that the new antioxidants **1a**−**1e** bestowed radical scavenging activity similar to or higher than that of some commercially available antioxidants. Therefore, developed phenolic compounds are good candidates to improve food stability.

Accelerated Storage Stability (*Schaal Oven Test*). Since scavenging activity is not directly indicative of antioxidant effectiveness in a food system, we also tested the developed compounds in real food applications (*39*). The ability of the phenolic derivatives **1a**−**1e** to protect CTG from oxidative degradation was assessed under Schaal Oven test (SOT) conditions. In order to determine the amount of antioxidant needed for an optimal protection of the oil, α-tocopherol, BHT, and **1e** have been added to CTG at two concentrations, 0.7 mM and 1.86 mM. The results of the SOT indicated that an antioxidant concentration of 0.7 mM, equivalent to 350 ppm, was sufficient (**Figure 4**). Indeed, when 1.86 mM of antioxidant was added, the PV's were not significantly different from 0.7 mM (**Figure 4**). The results also demonstrated that the new antioxidant **1e** was significantly more effective than α-tocopherol at protecting CTG from oxidative degradation. The amounts of hydroperoxides formed at the end of the storage period were significantly lower for CTG fortified with **1e** (PV = 9.4 meq/kg) than α-tocopherol (PV = 37.1 meq/kg), and the activity was similar to that of BHT at the same concentration (**Figure 4**). In order to compare the efficiency of all novel antioxidants, **1a**−**1e**, each compound has been added to CTG at 0.7 mM and subjected to accelerated storage. All compounds discussed in this article have very good solubility in canola oil. The results showed that all newly developed antioxidants (**1a**−**1e**) significantly inhibited CTG oxidative degradation under accelerated storage conditions compared to the unprotected canola triacylglycerols (**Figure 5**). After 5 days of storage, PV of CTG

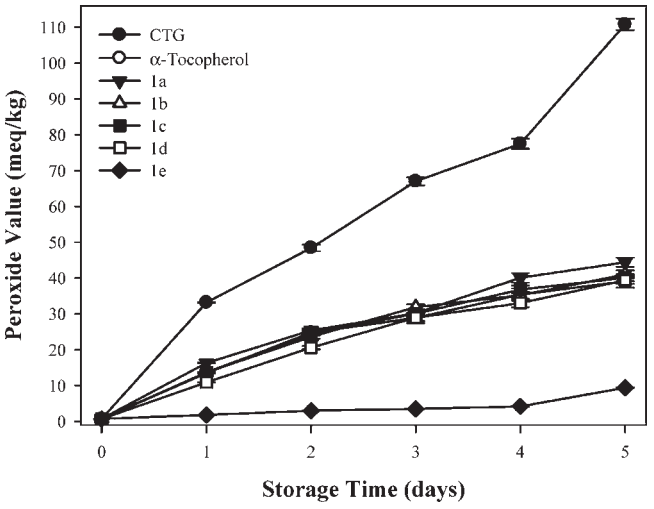


Figure 5. Changes in peroxide formation during the storage of canola triacylglycerols with different antioxidants added at 300 ppm. CTG, canola triacylglycerols. For details, see the text.

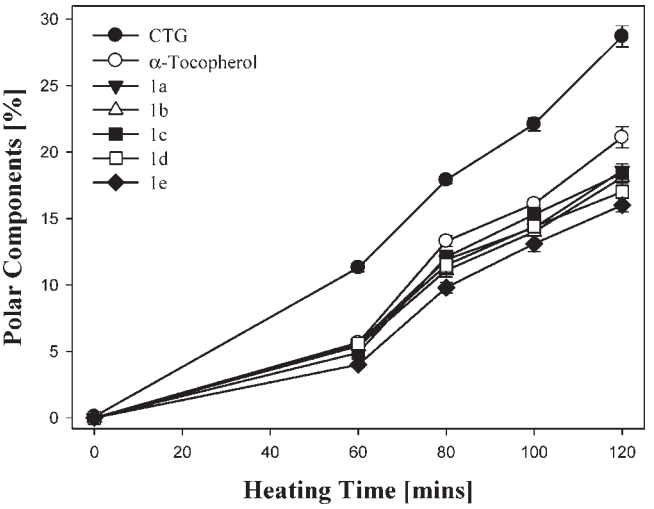


Figure 6. Formation of polar components during canola triacylglyceride test frying with α-tocopherol and novel antioxidants **1a**−**1e** added at 500 ppm. For details, see the text.

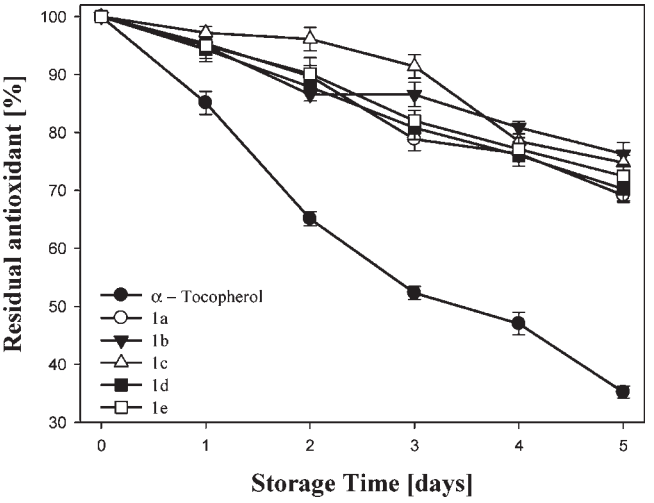


Figure 7. Percentage of remaining antioxidant during the accelerated storage of canola triacylglycerols fortified with α-tocopherol and novel antioxidants **1a**−**1e** added at 300 ppm.

was at 111 meq/kg, whereas for triacylglycerides fortified with antioxidants **1a**−**1e** were between 9.4 and 44.4 meq/kg (**Figure 5**). PV values for oils containing compounds **1b**, **1c**, and **1d** were not statistically different. Hence, the effectiveness to protect CTG from oxidation by **1b**, **1c**, and **1d** was lower than that for **1e**. Among these compounds, **1a** was the least effective in canola triacylglyceride protection against oxidative degradation and was less efficient than α-tocopherol. The phenolic compound **1e** exhibits the highest antioxidant potency among the novel compounds in both accelerated oil storage and in scavenging the DPPH radicals. This observation demonstrates that the DPPH scavenging activity test closely describes the behavior of a particular compound in oil, whereas the ORAC assay did not show any relationship (**Table 1** and **Figure 5**).

Protection during Frying. Although many compounds are known to display antioxidant activities at ambient temperature, the stringent conditions such as high temperature (~185 °C) and prolonged exposure of the oil to oxygen during frying tally an additional demand on antioxidants. Consequently, the antioxidant for institutional and industrial frying should not only be effective at frying conditions but also thermally stable and retain low volatility to prevent its evaporation (*40*). For example, BHT is not efficient under frying conditions due to it evaporative losses at elevated temperatures (*41*). The results from this study demonstrated that all developed antioxidants protected CTG from degradation during frying (**Figure 6**). At the end of the frying operation, the amount of total polar compounds accumulated in CTG was 25.7%, while the amounts accumulated in triacylglycerides fortified with the new antioxidants ranged from 17.0% to 18.8%. Thus, antioxidants **1a**−**1e** were less efficient than BHT in the Schaal Oven test; the opposite occurred under frying conditions. At the initial stage of frying (70th min; 1 day of actual frying), no significant difference was observed in the effectiveness of antioxidants **1a**−**1e** and α-tocopherol (**Figure 6**). However, as frying progressed, CTG fortified with antioxidants **1a**−**1e** accumulated significantly lower amounts of polar compounds than CTG with α-tocopherol, indicating more efficient protection against oxidative degradation. Antioxidants **1a**−**1c** protected triacylglycerols at the same level, whereas **1d** and **1e** were significantly more efficient. Hence, it seems that a higher number of hydroxyl groups on the chromanol aromatic rings tends to improve the ability of the antioxidant to prevent oxidation of the oil during frying. The presence of methoxy substituents in the *ortho* position to the hydroxyl group did not affect the efficiency of the antioxidant.

Antioxidant Stability. In order to investigate the stability of **1a** to **1e**, the amounts of antioxidant remaining at different stages during storage and frying have been measured (**Figures 7** and **8**). All novel antioxidants exhibited significantly higher stability than α-tocopherol. Indeed, under accelerated storage, 69.2% (**1a**), 76.3% (**1b**), 74.8 (**1c**), 70.2% (**1d**), and 72.5% (**1e**) of added antioxidants have been observed at the end of storage time, compared to 35% for CTG containing α-tocopherol (**Figure 7**). α-Tocopherol has been completely depleted at the 80th min of the frying test, whereas 27.0% (**1a**), 37.5% (**1b**), 49.1% (**1c**), 18.1% (**1d**), and 24.5% (**1e**) still remained (**Figure 8**). It is noteworthy that in frying test **1e** exhibited a significantly higher stability than the other compounds. As a consequence, it is expected that significantly higher amounts of antioxidant will be carried over to fried foods when frying in oil containing one of the newly developed phenolic compounds. Hence, foods fried in such oils will possess higher storage stability than foods fried in oils containing α-tocopherol at the same concentration level.

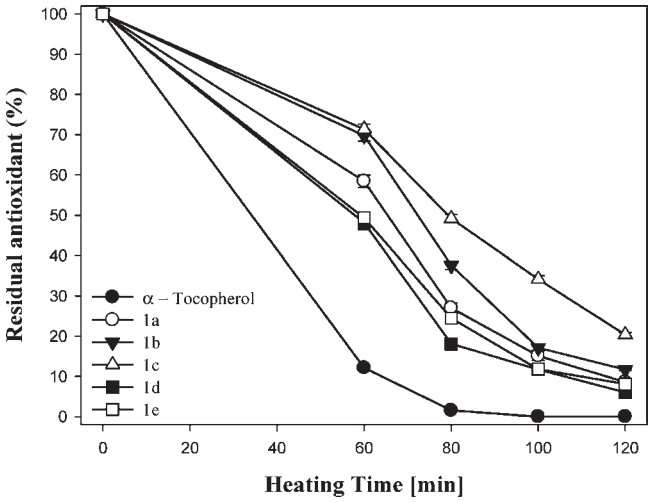


Figure 8. Percentage of remaining antioxidant during canola triacylglycerol test frying fortified with α-tocopherol and novel antioxidants at 500 ppm.

Additionally, fried food containing higher amounts of antioxidants can offer better nutritional quality.

A convenient method has been developed for the synthesis of novel antioxidants. These phenolic components have been prepared in four steps from trolox and phenolic acids and shown to be produced in good yields. The radical scavenging activities of these compounds were different when assessed by DPPH and ORAC assays, indicating the lack of compatibility of these tests in assessing antioxidant activity. These studies have demonstrated that each prepared phenolic antioxidant offered a higher antioxidant activity than trolox and α-tocopherol. Although a trend has not been observed between chemical structure and data from the ORAC assay, the results obtained with the DPPH assay can be explained by the chemical structure of the components. Furthermore, the new antioxidants were better at protecting the oil from oxidation during storage and frying when compared to the standard antioxidants used today. The antioxidant **1e** displayed superior protection of oil under accelerated storage conditions when compared to that of other novel antioxidants and α-tocopherol. Results also indicated that novel antioxidants were significantly better in protecting frying oil than α-tocopherol. Antioxidants **1d** and **1e** were the most effective at frying temperatures. Phenolic compound **1e** was the most efficient antioxidant under storage and frying conditions. The remarkably high thermal stability of novel antioxidants makes them very valuable under frying conditions, and these compounds may also improve the nutritional quality of fried foods.

Further investigations are underway to get a better understanding of the relationship between the structure and antioxidant activity of these novel antioxidants. The nature of the degradation products from the new antioxidants will also be studied to better understand what degradation products may form.

ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; CTG, canola triacylglyceride; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DCM, dichloromethane; DMAP, 4-dimethylaminopyridine; DMF, dimethylformamide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; THF, tetrahydrofuran; TPC, total polar components; PV, peroxide value.

LITERATURE CITED

(1) Parthasarathy, S.; Steinberg, D.; Witztum, J. L. The role of oxidized low-density lipoproteins in the pathogenesis of atherosclerosis. *Annu. Rev. Med.* **1992**, *43*, 219−225.

(2) Regnström, J.; Nilsson, J.; Tornwall, P.; Landou, C.; Hamsten, A. Susceptibility to low-density lipoprotein oxidation and coronary atherosclerosis in man. *Lancet* **1992**, *339*, 1183−1186.

(3) Halliwell, B. Free radicals reactive oxygen species and human diseases: A critical evaluation with special reference to atherosclerosis. *Br. J. Exp. Pathol.* **1989**, *70*, 737−757.

(4) Sachidanandame, K.; Fagan, S. C.; Ergul, A. Oxidative stress and cardiovascular disease: antioxidants and unresolved issues. *Cardiovasc. Drug. Rev.* **2005**, *23*, 115−132.

(5) Khandrika, L.; Kumar, B.; Koul, S.; Maroni, P.; Koul, H. K. Oxidative stress in prostate cancer. *Cancer Lett.* **2009**, *282*, 125−136.

(6) Block, G. The data support a role for antioxidants in reducing cancer risk. *Nutr. Rev.* **1992**, *50*, 207−213.

(7) Beckman, K. B.; Ames, B. N. The free radical theory of aging matures. *Physiol. Rev.* **1998**, *78*, 547−581.

(8) Frankel, E. D. *Antioxidants in Food and Biology. Facts and Fiction*; The Oily Press: Bridgwater, England, 2007; p 1.

(9) Silva, F. M.; Borges, F.; Guimaraes, J. J.; Lima, F. C.; Matos, C.; Reis, C. Phenolic acids and derivatives: Studies on the relationship among structure, radical scavenging activity, and physicochemical parameters. *J. Agric. Food Chem.* **2000**, *48*, 2122−2126.

(10) Shahidi, F.; Janitha, P. K.; Wanasundara, P. D. Phenolic antioxidants. *Crit. Rev. Food Sci.* **1992**, *32*, 67−103.

(11) Kamal-Eldin, A; Appelqvist, L. A. The chemistry and antioxidant properties of tocopherols and tocotrienol. *Lipids* **1996**, *31*, 671−701.

(12) Priscilla, D. H.; Prince, P. S. M. Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in Wistar rats. *Chem.-Biol. Interact.* **2009**, *179*, 118−124.

(13) Lu, Z.; Nie, G.; Belton, P. S.; Tang, H.; Zhao, B. Structure−activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives. *Neurochem. Int.* **2006**, *48*, 263−274.

(14) Kroes, B. H.; Van den Berg, A. J.; Quarles van Ufford, H. C.; Van Dijk, H. C.; Labadie, R. P. Anti-inflammatory activity of gallic acid. *Planta Med.* **1992**, *58*, 499−504.

(15) Gichner, T.; Pospisil, F.; Veleminsky, J.; Volkeova, V.; Volke, J. Two types of antimutagenic effects of gallic and tannic acids towards N-nitroso-compounds-induced mutagenicity in the Ames *Salmonella* assay. *Folia Microbiol.* **1987**, *37*, 55−62.

(16) Inoue, M.; Suzuki, R.; Sakaguchi, N.; Li, Z.; Takeda, T.; Ogihara, Y.; Jiang, B. Y.; Chen, Y. Selective induction of cell death in cancer cells by gallic acid. *Biol. Pharm. Bull.* **1995**, *18*, 1526−1530.

(17) Alamed, J.; Chaiyasit, W.; Mc Clements, D. J.; Decker, E. A. Relationships between free radical scavenging and antioxidant activity in foods. *J. Agric. Food Chem.* **2009**, *57*, 2969−2976.

(18) Tranchimand, S.; Tron, T.; Gaudin, C.; Iacazio, G. First chemical synthesis of three natural depsides involved in flavonol catabolism and related to quercetinase catalysis. *Synth. Commun.* **2006**, *36*, 587−597.

(19) Muller, T.; Coowar, D.; Hanbali, M.; Heuschling, P.; Luu, B. Improved synthesis of tocopherol fatty alcohols and analogs: microglial activation modulators. *Tetrahedron* **2006**, *62*, 12025−12040.

(20) Arnott, E. A.; Crosby, J.; Evans, M. C.; Ford, J. G.; Jones, M. F.; Leslie, K. W.; Mcfarlane, I. M.; Sependa, G. J. Process for Preparation of 4-Fluoro-2-methyl-1H-indol-5-ol from Fluorohalonitrobenzenes and Acetoacetate Esters. PCT Int. Appl. 200805322, 2008.

(21) Thurston, D. E.; Murty, V. S.; Langley, D. R.; Jones, G. B. O-Debenzylation of a pyrrolo[2,1-c][1,4]benzodiazepine in the presence of a carbinolamine functionality: synthesis of DC-81. *Synthesis* **1990**, *1*, 81−84.

(22) Nenadis, N.; Tsimidou, M. Observations on the estimation of scavenging activity of phenolic compounds using rapid 1,1-diphenyl-2-picrylhydrazyl (DPPH·) tests. *J. Am. Oil Chem. Soc.* **2002**, *79*, 1191−1195.

(23) Szydlowska-Czerniak, A; Karlovits, G; Dianoczki, C; Recseg, K; Szlyk, E. Comparison of two analytical methods for assessing antioxidant capacity of rapeseed and olive oils. *J. Am. Oil Chem. Soc.* **2008**, *85*, 141−149.

(24) Lampi, A. M.; Kamal-Eldin, A. Effect of α- and γ-tocopherols on thermal polymerization of purified high-oleic sunflower triacylglycerols. *J. Am. Oil Chem. Soc.* **1998**, *75*, 1699−1703.

(25) Hornero-Mendez, D; Perez-Galvez, A; Minguez-Mosquera, M. I. A. Rapid spectrophotometric method for determination of peroxide value in food lipids with high carotenoid content. *JAOCS* **2001**, *78*, 1151−1155.
(26) Shantha, C.; Decker, E. A. Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *J. AOAC Int*. **1994**, *77*, 421−424.
(27) AOAC. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed.; AOAC Inc.: Arlington, VA, 1990; Method 982.27.
(28) Schulte, E. Economical micromethod for determination of polar components in frying fats. *Eur. J. Lipid Sci. Technol*. **2004**, *106*, 772−776.
(29) AOCS. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; AOCS Press: Champaign, IL, 1999; IL Methods Ce 8−89, Ce 1−62.
(30) Neises, B.; Steglish, W. Simple method for the esterification of carboxylic acids. *Angew. Chem., Int. Ed. Engl*. **1978**, *17*, 522−524.
(31) Rosenau, T; Potthast, A; Hofinger, A; Kosma, P. Calixarene-type macrocycles by oxidation of phenols related to vitamin E. *Angew. Chem., Int. Ed*. **2002**, *41*, 1171−1173.
(32) Gregor, W; Grabner, G; Adelwöhrer, C; Rosenau, T; Gille, L. Antioxidant properties of natural and synthetic chromanol derivatives: Study by fast kinetics and electron spin resonance spectroscopy. *J. Org. Chem*. **2005**, *70*, 3472−3483.
(33) Scott, G. Antioxidants. *Chem. Ind*. (*London*) **1963**, *7*, 271−281.
(34) Saito, S; Kawabata, J. Effects of electron-withdrawing substituents on DPPH radical scavenging reactions of protocatechuic acid and its analogues in alcohol solvents. *Tetrahedron* **2005**, *61*, 8101−8108.
(35) Palozza, P; Simone, R; Picci, N; Buzzoni, L; Ciliberti, N; Natangelo, A; Manfredini, S; Vertuani, S. Design, synthesis, and antioxidant potency of novel α-tocopherol analogues in isolated membranes and intact cells. *Free Radical Biol. Med*. **2008**, *44*, 1452−1464.
(36) Gomez-Ruiz, J. A.; Leake, D. S.; Ames, J. M. In vitro antioxidant activity of coffee compounds and their metabolites. *J. Agric. Food Chem*. **2007**, *55*, 6962−6969.
(37) Tabart, J.; Kevers, C.; Pincemail, J.; Defraigne, J.-O.; Dommes, J. Comparative antioxidant capacities of phenolic compounds measured by various tests. *Food Chem*. **2009**, *113*, 1226−1233.
(38) Alamed, J.; Chaiyasit, W.; McClements, D. J.; Decker, E. A. Relationships between free radical scavenging and antioxidant activity in foods. *J. Agr. Food Chem*. **2009**, *57*, 2969−2976.
(39) Frankel, E. N.; Finley, J. W. How to standardize the multiplicity of methods to evaluate natural antioxidants. *J. Agric. Food Chem*. **2008**, *56*, 4901−4908.
(40) Lee, J.; Lee, Y.; Choe, E. Effects of sesamol, sesamin, and sesamolin extracted from roasted sesame oil on the thermal oxidation of methyl linoleate. *LWT-Food Sci. Technol*. **2008**, *41*, 1871−1875.
(41) Augustin, M. A.; Berry, S. K. Efficacy of the antioxidants BHA and BHT in palm olein during heating and frying. *J. Am. Oil Chem. Soc*. **1983**, *60*, 1520−1523.

Received for review June 14, 2010. Revised manuscript received August 26, 2010. Accepted September 3, 2010.