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Dietary Phenolic Acids and Derivatives. Evaluation of the Antioxidant Activity of Sinapic Acid and Its Alkyl Esters

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The action of sinapic acid and its alkyl esters as potential antioxidants has been investigated. For this purpose, a series of sinapic acid ester derivatives was synthesized and their antioxidant activities were evaluated using distinctive analytical methods, namely, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and FRAP UV−vis methods and differential scanning calorimetry. The electron-donating activity and lipophilicity of these phenolic compounds were also evaluated. From the overall results it was concluded that alkyl ester sinapates (linear alkyl esters) present almost the same antioxidant activity, albeit slightly lower, exhibited by the parent compound (sinapic acid). Furthermore, the addition of an alkyl ester side chain has a positive effect on the partition coefficient of sinapic acid, improving its utility as an antioxidant in a more lipophilic medium. The data on the antioxidant activity obtained by different analytical methods correlated well with each other and have revealed interesting antioxidant data of alkyl esters of sinapic acid.

KEYWORDS: Sinapic acid; alkyl esters; antioxidant activity; electrochemistry; DSC

INTRODUCTION

Polyphenols are a complex group of bioactive compounds widely spread in the plant kingdom (*1*). Nowadays it is believed that these compounds are involved in the defense process against deleterious oxidative damage due to, at least in part, their antioxidant properties. Among polyphenolic compounds, hydroxycinnamic acids and derivatives are a well-known group of phytochemicals, which are found in cereals, roasted coffee, asparagus, green vegetables, and many other plants (*2*). In addition to their primary antioxidant activity, this type of compound also possesses other significant biological functions such as anti-inflammatory, anticarcinogenic, and antimicrobial effects (*3*−*5*). In fact, recent data demonstrate the beneficial effects of these compounds as preventive and/or therapeutic agents in several diseases, such as atherosclerosis, inflammatory injury, and cancer (*6*, *7*). Because of their hydrophilic nature, hydroxycinnamic acids cannot be properly used in oil-based processes, which is an important issue in industrial applications, or be effective as antioxidants in biological systems. Recently, some structural modifications have been performed on the phenolic acid skeleton to improve their application outline (e.g., to increase the lipophilicity) (*8*, *9*). Although cinnamic esters, in particular, were shown to display remarkable growth inhibition properties toward some human cancer cell lines, the mechanisms underlying their action are not yet completely understood. Some data point out that it could be intrinsically linked to their antioxidant activity and in turn strongly dependent on their structural characteristics (*10*, *11*).

Sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid; **Figure 1**) is a hydroxycinnamic acid widely distributed in nature and present in many foods, edible plants, and fruits (*12*−*14*). Rapeseed particularly contains sinapic acid as the predominant phenolic compound (*14*). Sinapic acid has been proposed as a potent antioxidant, its effectiveness being described as higher than that of ferulic acid (3-methoxy-4-hydroxycinnamic acid) and sometimes comparable to that of caffeic acid (3,4-dihydroxycinnamic acid) (*15*−*17*). The relevance of sinapic acid in cell protection and in oxidative-related diseases was already reported owing to its peroxynitrite ($ONOO^-$) scavenging activity (*18*, *19*). In addition, anxiolytic and anti-inflammatory properties were also ascribed to this compound (*20*, *21*).

Although a huge number of investigations have been carried out on the study of the influence of esterification on the antioxidant efficiency of phenolic acids, namely, in cinnamic analogues, for example, ferulic acid (*22*, *23*), the information reported about the activity of sinapic acid and its alkyl esters is scarce. Accordingly, an interactive project was developed aiming at accomplishing a more reliable understanding of the antioxidant activity of sinapic acid and its alkyl esters derivatives. Therefore, the synthesis of a new set of lipophilic phenolic antioxidants (**Figure 1**) was realized and their antioxidant profile determined

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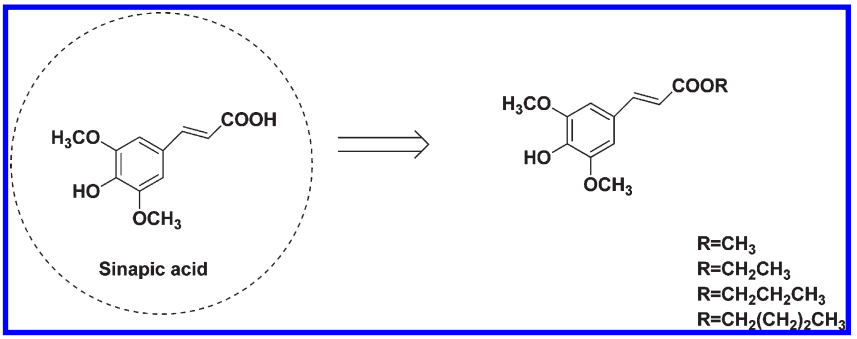

Figure 1. Sinapic acid and its alkyl esters under study.

using different analytical methods. In addition, the redox potentials and partition coefficients of sinapic acid and its derivatives properties were also acquired to test their influence in the antioxidant performance of the phenolic compounds.

MATERIALS AND METHODS

General. Linoleic acid (LA, *cis*,*cis*- 9,12-octadecadienoic acid, 99%), 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), quercetin, sinapic acid, and Trolox were purchased from Sigma-Aldrich, St. Louis, MO. 2,4,6-Tripyridyl-*s*-triazine (TPTZ), dimethyl-d_6 sulfoxide (99.8%), and tetramethylsilane (TMS) were obtained from Merck, Darmstadt, Germany. All other reagents and solvents were of pro analysis grade and used without additional purification. Deionized water (conductivity < 0.1 μS cm^{-1}) was used throughout all of the experiments.

Thin-layer chromatography (TLC) was carried out on precoated silica gel 60 F254 (Merck) with a layer thickness of 0.2 mm. For analytical control the following systems were used: ethyl ether/petroleum ether and chloroform/methanol. The spots were visualized under UV detection (254 and 366 nm) and iodine vapor. Normal-phase chromatography was performed using Merck silica gel 60, 0.2−0.5 or 0.040−0.063 mm. Solvents were evaporated in a Büchi Rotavapor (Flawil, Switzerland).

Apparatus. ^{1}H and ^{13}C NMR data were acquired, at room temperature, on a Brüker AMX 300 spectrometer operating at 300.13 and 75.47 MHz, respectively. Dimethyl-d_6 sulfoxide was used as a solvent; chemical shifts are expressed in δ (ppm) values relative to TMS as internal reference; coupling constants (J) are given in hertz. Assignments were also made from distortionless enhancement by polarization transfer (DEPT) (underlined values). Electron impact mass spectra (EI-MS) were carried out on a VG AutoSpec instrument; the data are reported as m/z (% of relative intensity of the most important fragments). Infrared spectra were recorded on an ATI Mattson Genesis series FTIR spectrophotometer using potassium bromide disks; only the most significant absorption bands are reported (ν_{max}, cm^{-1}).

Voltammetric studies were performed using an Autolab PGSTAT 12 potentiostat/galvanostat (Eco-Chemie, The Netherlands) and a one-compartment glass electrochemical cell. Voltammetric curves were recorded at room temperature using a three-electrode system. A glassy carbon working electrode (GCE) ($d = 2$ mm), a platinum wire counter electrode, and an Ag/AgCl saturated KCl reference electrode were used. A Crison pH-meter with glass electrode was used for the pH measurements (Crison, Spain).

Spectrophotometric measurements of the absorbance of DPPH free radical were carried out using a UV−vis spectrophotometer (Bio-Tek, model Uvikon XL). The absorbance of Fe^{2+}/TPTZ complex in the FRAP assay was determined by a microplate reader (Bio-Rad, model 680).

A Netzsch DSC 204 calorimeter (Netzsch, Germany) was employed for the thermoxidation stability measurements. The temperature scale was calibrated using In, Bi, Sn, Zn, and KNO_3, and the enthalpy calibration has been carried out to the heat of fusion of the same standards. The oxidation induction temperature (OIT) of pure and spiked linoleic acid was analyzed by heating the samples at 5 K min^{-1} under constant oxygen flow (50 mL/min). A computer-generated plot of heat flow (W/g) versus temperature was used to graphically determine OIT. OIT was determined by the onset of the oxidation process that is characterized by an exothermic peak in the heat flow−temperature plot.

Synthesis. *General Synthetic Procedure*. The alkyl esters of the phenolic acid were obtained using the following general procedure: Sinapic acid (1.0 g) was dissolved in 75 mL of the corresponding alcohol (methanol, ethanol, *n*-propanol, or butanol) containing 1 mL of H_2SO_4, and the solution was stirred, at room temperature, during ca. 5 days. The solvent was partially evaporated under reduced pressure. The mixture was then extracted with diethyl ether (3 × 75 mL), and the organic phases were combined, washed with 10% Na_2CO_3 solution, and dried over Na_2SO_4. The solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography and then recrystallized.

Methyl sinapate ((*E*)-*methyl 3-(4-hydroxy-3,5-dimethoxyphenyl)propenoate*): yield, 79%; FTIR, 3522, 3460, 3062, 2946, 2845, 1704, 1631, 1604, 1515, 1461, 1428, 1384, 1339, 1287, 1260, 1233, 1181, 1152, 1120; ^{1}H NMR, 8.98 (1 H, s, 4-OH), 7.57 (1H, d, $J = 15.9$, H (β)), 7.03 (2H, s, H(2), H(6)), 6.54 (1H, d, $J = 15.9$, H(α)), 3.80 (6H, s, 2 × OC$\underline{H}_3$), 3.72 (3H, s, C$\underline{H}_3$); ^{13}C NMR, 167.1 (C═O), 148.0 (C(3), C(5)), 145.5 (C(β)), 138.3 (C—OH), 124.4 (C(1)), 114.6 (C (α)), 106.2 (C(2), C(6)), 56.1 (2 × OCH_3), 51.3 (CH_3); EI-MS, 238 (100, M$^{\bullet+}$), 207 (32), 175 (20), 163 (21), 135 (16), 121 (14), 119 (13), 77 (17), 65 (19), 59 (13), 56 (36), 53 (18), 51 (17).

Ethyl sinapate ((*E*)-*ethyl 3-(4-hydroxy-3,5-dimethoxyphenyl)propenoate*): yield, 83%; FTIR, 3654, 3496, 3090, 2938, 2841, 1690, 1636, 1600, 1515, 1461, 1425, 1373, 1340, 1287, 1259, 1222, 1187, 1151, 1116; ^{1}H NMR, 8.97 (1 H, s, 4-OH), 7.55 (1H, d, $J = 15.9$, H (β)), 7.02 (2H, s, H(2), H(6)), 6.53 (1H, d, $J = 15.9$, H(α)), 4.16 (2H, q, $J = 7.0$, C$\underline{H}_2$), 3.79 (6H, s, 2 × OC$\underline{H}_3$), 1.24 (3H, t, $J = 7.0$, C$\underline{H}_3$); ^{13}C NMR, 166.6 (C═O), 148.0 (C(3), C(5)), 145.2 (C(β)), 138.2 (C—OH), 124.4 (C(1)), 115.0 (C (α)), 106.2 (C(2), C(6)), 59.7 (CH_2), 56.1 (2 × OCH_3), 14.3 (CH_3); EI-MS, 252 (100, M$^{\bullet+}$), 207 (34), 180 (48), 175 (22), 163 (13), 121 (16), 119 (13), 91 (14), 77 (15), 65 (20), 58 (20), 53 (15), 51 (14).

Propyl sinapate ((*E*)-*propyl 3-(4-hydroxy-3,5-dimethoxyphenyl)propenoate*): yield, 82%; FTIR, 3532, 3425, 2968, 2938, 2880, 2840, 1682, 1634, 1598, 1513, 1459, 1424, 1378, 1340, 1314, 1283, 1222, 1193, 1152, 1118; ^{1}H NMR, 8.95 (1 H, s, 4-OH), 7.55 (1H, d, $J = 15.9$, H (β)), 7.03 (2H, s, H(2), H(6)), 6.53 (1H, d, $J = 15.9$, H(α)), 4.08 (2H, t, $J = 6.6$, C$\underline{H}_2$), 3.79 (6H, s, 2 × OC$\underline{H}_3$), 1.68−1.63 (2H, m, C$\underline{H}_2$), 0.93 (3H, t, $J = 7.4$, C$\underline{H}_3$); ^{13}C NMR, 166.7 (C═O), 148.0 (C(3), C(5)), 145.3 (C(β)), 138.2 (C—OH), 124.4 (C(1)), 114.9 (C (α)), 106.2 (C(2), C(6)), 65.2 (CH_2), 56.1 (2 × OCH_3), 21.7 (CH_2), 10.4 (CH_3); EI-MS, 266 (100, M$^{\bullet+}$), 224 (55), 207 (42), 180 (51), 175 (26), 147 (13), 121 (13), 119 (14), 91 (12), 65 (12).

Butyl sinapate ((*E*)-*butyl 3-(4-hydroxy-3,5-dimethoxyphenyl)propenoate*): yield, 94%; FTIR, 3592, 3511, 2968, 2937, 2895, 2873, 1692, 1636, 1601, 1515, 1456, 1424, 1376, 1343, 1286, 1261, 1223, 1184, 1151, 1117; ^{1}H NMR, 8.94 (1 H, s, 4-OH), 7.55 (1H, d, $J = 15.9$, H (β)), 7.03 (2H, s, H(2), H(6)), 6.53 (1H, d, $J = 15.9$, H(α)), 4.12 (2H, t, $J = 6.4$, C$\underline{H}_2$), 3.80 (6H, s, 2 × OC$\underline{H}_3$), 1.64−1.56 (2H, m, C$\underline{H}_2$), 1.42−1.33 (3H, m, C$\underline{H}_2$), 0.91 (3H, t, $J = 7.3$, C$\underline{H}_3$); ^{13}C NMR, 166.7 (C═O), 148.0 (C(3), C(5)), 145.3 (C(β)), 138.3 (C−OH), 124.4 (C(1)), 115.0 (C(α)), 106.2 (C(2), C(6)), 63.4 (CH_2), 56.1 (2 × OCH_3), 30.4 (CH_2), 18.8 (CH_2), 13.6 (CH_3); EI-MS, 280 (88, M$^{\bullet+}$), 225 (13), 224 (100), 209 (15), 207 (45), 181 (12), 180 (58), 175 (27), 167 (17), 165 (12), 163 (12), 149 (16), 147 (16), 135 (12), 133 (12), 121 (21), 119 (17), 91 (22), 77 (21), 65 (26), 58 (46), 57(13), 55 (15), 53 (19), 51 (16).

Electrochemical Measurements. Stock solutions of the sinapic acid and derivatives (10 mM) were prepared by dissolving an appropriate amount in ethanol. The voltammetric working solutions were prepared, in the electrochemical cell, by diluting 0.1 mL of the stock solution in 10 mL of supporting electrolyte to get a final concentration of 0.1 mM.

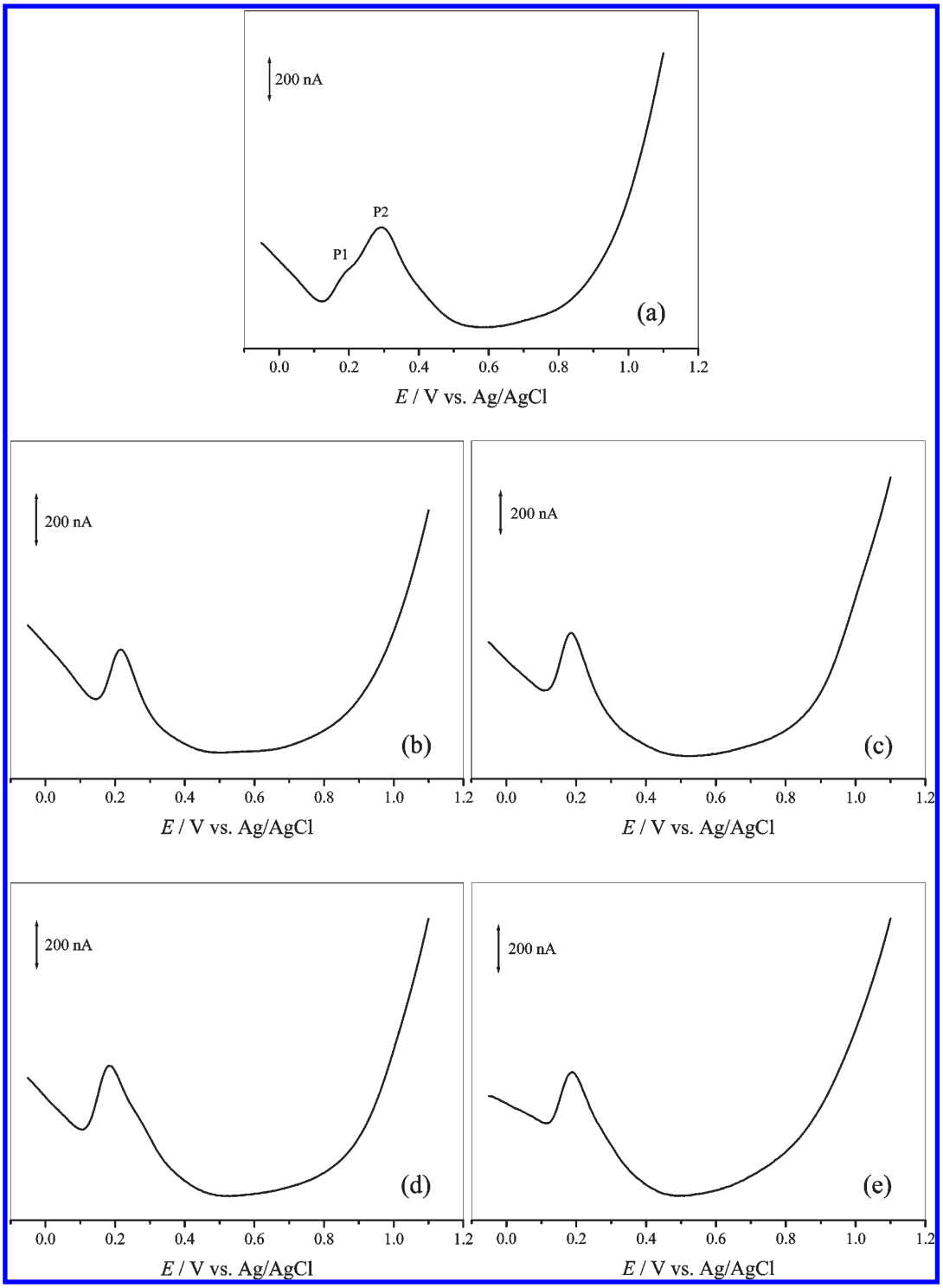

Figure 2. Differential pulse voltammograms for 0.1 mM solutions of (**a**) sinapic acid, (**b**) methyl sinapate, (**c**) ethyl sinapate, (**d**) propyl sinapate, and (**e**) butyl sinapate, in physiological pH 7.3 supporting electrolyte. Scan rate = 5 mV s^{-1}.

The pH 7.3 supporting electrolyte used in the voltammetric determinations was prepared by dilution to 100 mL of 6.2 mL of 0.2 M dipotassium hydrogen phosphate and 43.8 mL of 0.2 M potassium dihydrogen phosphate.

DSC Measurements. Samples of linoleic acid (2.5−3.0 mg) were placed in standard aluminum pans. An appropriate amount of each tested antioxidant was dissolved in methanol to produce a 1 mM solution. Linoleic acid was then spiked, in the aluminum pan, with 10 μL of the antioxidant solution. Small variations in sample size in this weight range had no noticeable effect on the oxidation induction temperature (OIT) measured. Some experiments were performed to check whether the methanol had any effect upon the OIT results. The OIT of linoleic acid was measured with or without methanol, and no significant variation was seen between the two measurements. Samples were kept at room temperature for a time period to allow the removal of excess of solvent. Each sample test was run in triplicate.

Calculation of Partition Coefficient (Log*P*). Calculation of the log *P* values, simulating partitioning of tested compounds in an *n*-octanol/water (1:1, v/v) system, was based on Crippen's fragmentation method (*24*) and was accomplished using the Molinspiration program (*25*).

DPPH Radical Scavenging Activity. The radical scavenging activity of sinapic acid and its derivatives against the stable free radical DPPH was measured as described previously, with some modifications (*26*). Briefly, the test compounds were dissolved in dimethyl sulfoxide and incubated

with a methanolic solution of DPPH 100 μM. The test concentrations (in the range of 1−100 μM) were carefully chosen for each compound to produce a suitable dose−response curve. After 30 min of incubation at room temperature in the dark, the absorbance at 517 nm was measured in a spectrophotometer. The percent inhibition of DPPH radical was calculated on the basis of the reduction of light absorbance. The dose−response curve was plotted by using the software SigmaPlot for Windows version 8.0, and IC_{50} values were calculated by the software curve-expert (for Windows, version 1.34). Trolox was used as reference standard.

Ferric Reducing Antioxidant Power (FRAP Assay). The FRAP assay was performed as described (*27*, *28*). Briefly, the FRAP solution was freshly prepared by mixing 10 mL of acetate buffer 300 mM (pH 3.6), 1 mL of ferric chloride hexahydrate 20 mM in distilled water, and 1 mL of TPTZ 10 mM in HCl 40 mM. Ten microliters of the test compound dissolved in dimethyl sulfoxide was mixed with 190 μL of the FRAP solution. Absorbance was determined at 595 nm in a microplate reader, after 30 min of incubation at room temperature. Sinapic acid derivatives were tested at the final concentration of 40 μM, whereas reference compounds, Trolox and quercetin, were tested at the final concentration of 20 μM. Absorbance in the presence of test compounds was compared with absorbance in the presence of quercetin, and the FRAP value for test compounds was expressed as milliequivalents of quercetin per mole of compound.

RESULTS AND DISCUSSION

Chemistry. Sinapic acid derivatives (**Figure 1**) were synthesized straightforwardly by Fisher acid catalysis esterification (*9*). These reactions led to high yields of the desired compounds, which were identified by the following spectroscopic techniques: NMR (^{1}H NMR, ^{13}C NMR), FTIR, and MS-EI.

Electrochemical Measurements. The measurement of the reducing capacity and electrochemical behavior of phenolic compounds can provide important information concerning their free radical scavenging activity. In fact, the antioxidant activity of different species is closely related to their redox properties and, consequently, knowledge of their redox behavior is a very important basis to obtain better explanations of their properties. Thus, the study of the electrochemical properties of sinapic acid and its synthesized ester derivatives was accomplished. The oxidative behavior of the compounds was studied, at physiological pH 7.3, by differential pulse and cyclic voltammetry, using a glassy carbon working electrode.

The differential pulse voltammetric study of sinapic acid (**Figure 1**) revealed the presence of two convolved anodic peaks, P1 and P2, at 183 and 298 mV, respectively (**Figure 2a**; **Table 1**). These oxidation peaks are related with the oxidation of the phenolic group present in the molecular structure. The appearance of the two waves may be interpreted by assuming that the electrochemical oxidation of sinapic acid takes place on the glassy carbon electrode by electron transfer for both free and adsorbed forms. The free form corresponds to the first peak (P1), whereas the strongly adsorbed form is oxidized at a more anodic potential (P2). The occurrence of an adsorption peak has been also described in the literature for voltammetric studies involving related compounds, namely, ferulic acid (*29*, *30*).

For all of the synthesized sinapic alkyl esters only one anodic wave was observed at physiological pH using differential pulse voltammetry (**Figure 2b−e**; **Table 1**). These oxidation waves could also be ascribed to the presence of a phenolic group in the structure of the molecules (**Figure 1**). The appearance of a single wave for the sinapic alkyl esters could be interpreted as a result of a less significant adsorption propensity of these compounds when compared with sinapic acid. In fact, the peak observed for alkyl esters occurs at a potential similar to that obtained for wave P1 attributed to the oxidation of the free form of sinapic acid.

Cyclic voltammograms were recorded at different sweep rates. The cyclic voltammograms obtained for sinapic acid have also shown two convolved anodic peaks (**Figure 3a**). For the alkyl ester derivatives only one anodic wave is seen (**Figure 3b−e**). All of these anodic peaks appear to correspond to irreversible processes.

Table 1. Redox Potentials (E_p) Obtained for Sinapic Acid and Its Alkyl Esters

compound	E_p (mV)	log$P^{a,b}$
sinapic acid	183; 298	1.29/1.26
methyl sinapate	219	1.55/1.88
ethyl sinapate	189	1.89/2.26
propyl sinapate	182	2.38/2.76
butyl sinapate	190	2.79/3.32

[a] Determined with ChemDraw software (ChemDraw Ultra 11.0, Cambridge Soft Corp.). [b] Determined with Molinspiration Calculation service (www.molinspiration.com).

The results found herein for sinapic acid and derivatives corroborate the scarce data available in the literature for sinapic acid (*31*, *32*). Moreover, the voltammetric behavior observed is consistent with the oxidation mechanism suggested in the literature for ferulic acid, a phenolic antioxidant with an analogous molecular structure. Therefore, the oxidation mechanism for sinapic acid and its alkyl esters should involve a one-electron transfer from the phenolate ion followed by one irreversible dimerization process due to a radical−radical coupling reaction between two phenoxyl radicals (*29*, *33*).

The differential pulse voltammetric study of the reference compound Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) revealed the presence of a well-defined anodic peak, E_p = 110 mV, at physiological pH (*9*).

From the voltammetric data it could be concluded that the introduction of an alkyl ester group in the ethylenic side chain of sinapic acid did not significantly change the oxidation potentials, when compared to the precursor. The results obtained strongly suggest that the alkyl groups have modest or even no effect on the electron density of the phenolic group. Hence, the introduction of an alkyl group did not significantly influence the energy of the electron transfer process as can be deduced from the similarity of the E_p values for these ester derivatives.

DSC Measurements. Lipid peroxidation has long been recognized as a problem either in the food industry or in biological systems (*34*, *35*). Unsaturated fatty acids may undergo oxidation processes that in turn result in the formation of a wide range of products. Biological systems with a higher concentration of polyunsaturated fatty acids will, of course, oxidize more quickly and, as a result, lead to a rapid development of rancid off-flavors. To minimize oxidative rancidity, antioxidants can be added. To deal with some of these consequences, one must recognize the mechanisms by which antioxidants of either natural or synthetic origin control oxidation (*36*).

Many techniques have been developed and used to detect and measure the extent of oxidation in fats and fats-containing systems and to ascertain their resistance to oxidative deterioration. As the transfer of an oxygen molecule to an unsaturated fatty acid requires energy, the oxidative stability of lipid systems can be established by using thermal analysis. Of all the thermal analytical methods available, differential scanning calorimetry (DSC) is one of the most versatile and is suitable for a vast range of applications. A simple method to compare the efficiency of several antioxidants and/or stabilizing systems is the determination of the OIT. Particularly for polyolefins, OIT tests are well established for quality control purposes as a quick screening method to check the activity of the stabilization systems (*9*, *37*).

Therefore, the antioxidant activity of sinapic acid and its alkyl esters was evaluated by DSC using pure linoleic acid (LA) as a lipid model system. Extrapolated OITs were obtained from the

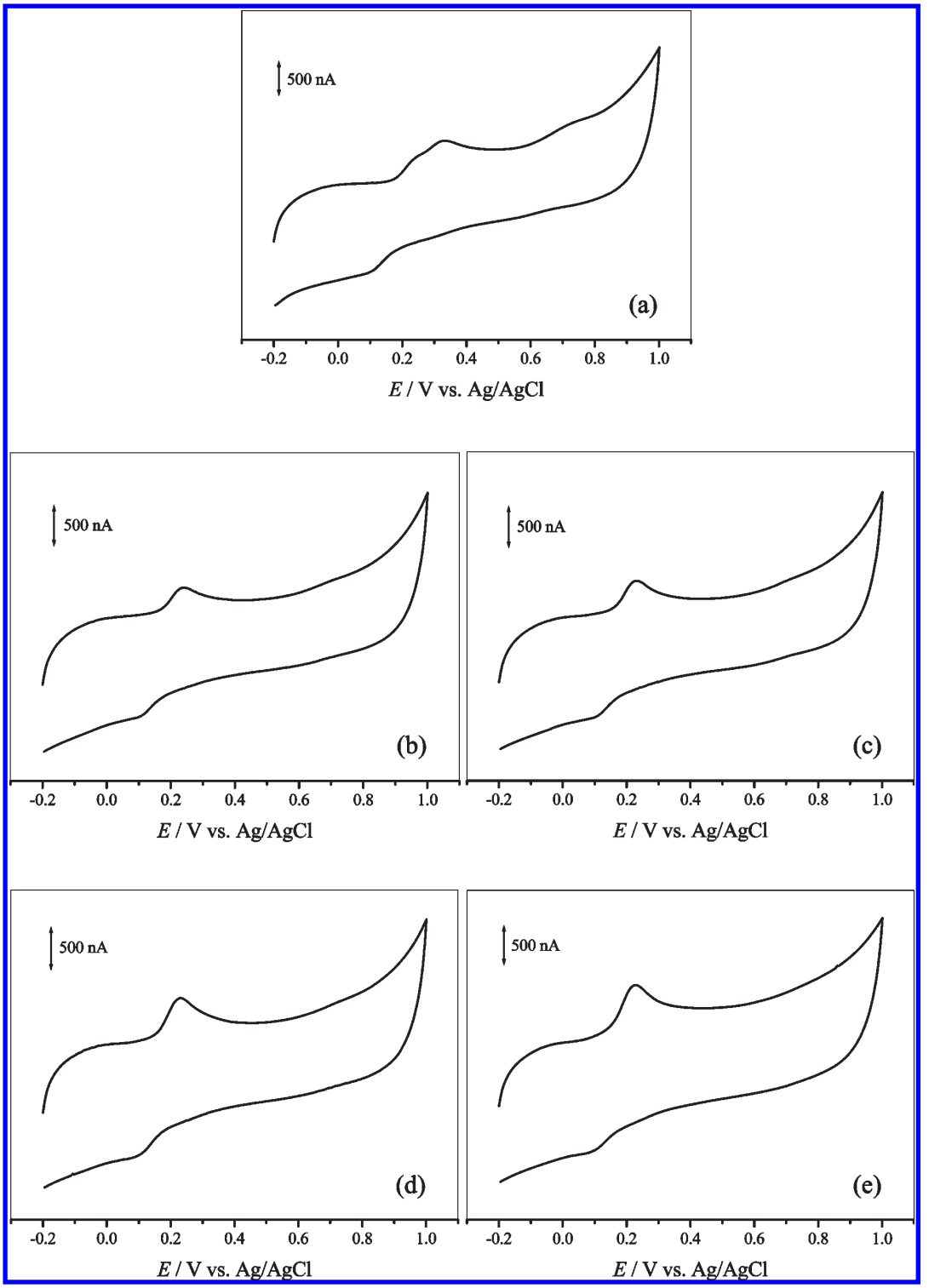

Figure 3. Cyclic voltammograms for 0.1 mM solutions of (**a**) sinapic acid, (**b**) methyl sinapate, (**c**) ethyl sinapate, (**d**) propyl sinapate, and (**e**) butyl sinapate, in physiological pH 7.3 supporting electrolyte. Scan rate = 50 mV s^{-1}.

first exothermal peak of nonisothermal oxidation of LA (**Figure 4**) (*9*, *38*). The DSC curve shapes are similar to that obtained in the thermogram for the uninhibited LA oxidation (**Figure 4A**). Under the described experimental conditions the OIT obtained for pure LA was 108.1 °C. The addition of sinapic acid modifies the OIT of linoleic acid from 108.1 to 150.3 °C (**Table 2**). This phenolic acid acts as an antioxidant, slowing, significantly, linoleic acid oxidation. This behavior is consistent with the data found in the literature for related phenolic acids (*9*, *39*).

The sinapic alkyl esters also exhibit antioxidant capacity toward LA oxidation as shown by the increase observed in OIT (**Figure 4A**; **Table 2**). From the results it can be concluded that the alkyl esters display an antioxidant ability correspondent to that ascribed to sinapic acid. The same stabilization pattern is also reported in the literature for other alkyl ester derivatives of phenolic antioxidants (*9*, *39*).

DSC first-order derivative thermograms were also evaluated to provide sharper and better defined peaks facilitating OIT data reading and acquisition (**Figure 4B**; **Table 2**).

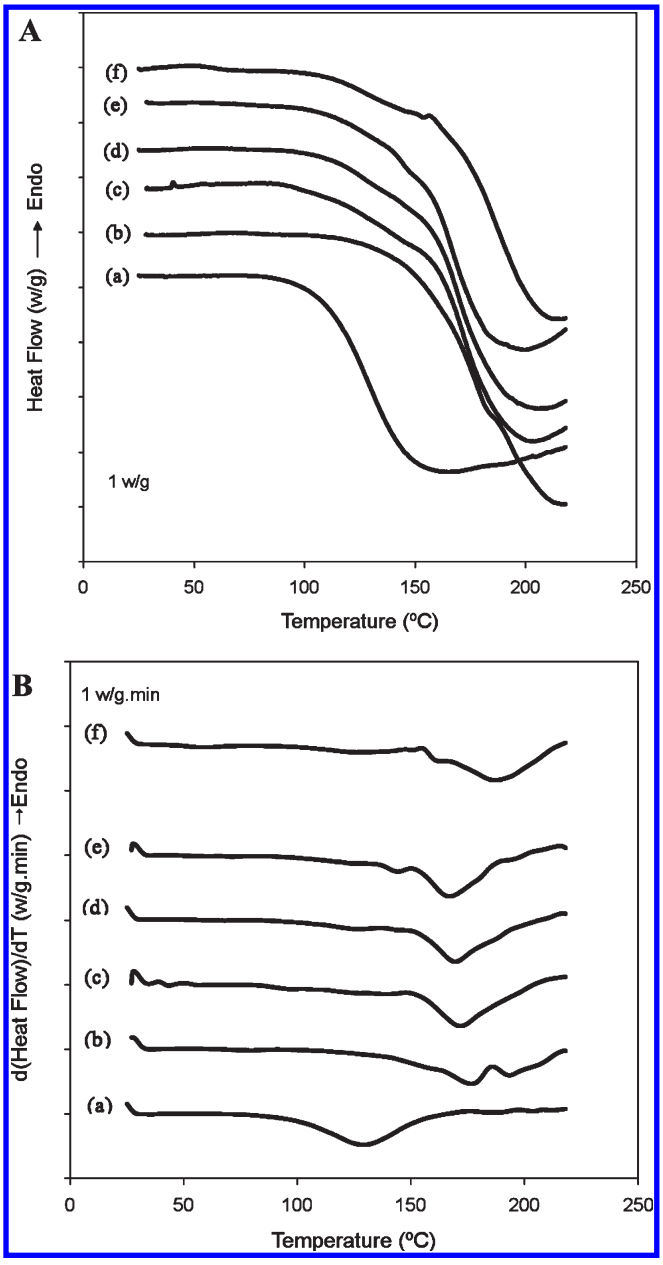

Figure 4. (**A**) DSC and (**B**) first-order derivative curves of nonisothermal oxidation of (a) linoleic acid (LA), (b) LA + sinapic acid, (c) LA + methyl sinapate, (d) LA + ethyl sinapate, (e) LA + propyl sinapate, and (f) LA + butyl sinapate.

Table 2. Oxidation Induction Temperature (OIT) and Peak Temperature of the Derivative Curve (PTDC) Obtained for Pure and Inhibited Linoleic Acid (LA)

compound	OIT (°C)	PTDC (°C)
pure LA	108.1	128.7
LA + sinapic acid	150.3	176.2
LA + methyl sinapate	161.1	171.7
LA + ethyl sinapate	159.4	169.4
LA + propyl sinapate	159.3	167.0
LA + butyl sinapate	162.2	177.1
LA + Trolox	156.7	151.1

The OIT for linoleic acid stabilized with the reference compound, Trolox, has also been measured. For the LA inhibition with Trolox an increase of the OIT to higher values was observed, evidencing a clear delay of lipid oxidation (**Table 2**).

Table 3. FRAP and DPPH Scavenging Activities of Sinapic Acid and Its Alkyl Esters

compound	DPPH (IC_{50} μM)	FRAP (IC_{50}, μM/mol)[a]
sinapic acid	32.2 ± 6.2	482.6 ± 61.1
methyl sinapate	48.7 ± 7.0	355.5 ± 66.1
ethyl sinapate	51.9 ± 6.3	338.2 ± 53.4
propyl sinapate	50.6 ± 6.1	341.9 ± 46.3
butyl sinapate	50.1 ± 3.8	347.3 ± 75.7
Trolox	38.4 ± 8.0	427.4 ± 86.2

[a] μM quercetin equiv/mol. Values represent the mean of four to seven experiments ± SD.

In conclusion, nonisothermal DSC data show that sinapic acid and its alkyl ester derivatives are good antioxidants regarding the inhibition of lipid oxidation. Moreover, the gathered results allow one to conclude that the sinapic alkyl ester derivatives studied exhibit an antioxidant capacity that closely matches that found for Trolox.

Estimation of Partition Coefficients (Log *P*). In view of better correlation of the overall properties of the antioxidant compounds, the lipophilicity, expressed as the octanol−water partition coefficient and herein called log *P*, was calculated either theoretically, according to Crippen's fragmentation method, or using the Molinspiration property calculation program (see **Table 1**) (*24*, *25*).

The data examination allows one to conclude that the introduction of an alkyl group leads to a significant increase of the sinapic acid lipophilicity (**Table 1**). It must be stressed that the partition coefficients calculated for the sinapic acid derivatives are well correlated with their structural features.

Higher lipophilicity is often required in the antioxidant research field, because it could modify the absorption and distribution properties of hydroxycinnamic antioxidants, increasing their ability to interact with the polar head groups of the membrane or to achieve local concentration at the water−lipid interface, preventing the initial reaction between aqueous radicals and lipids (*40*).

Antioxidant Activity. Antioxidant activity data of sinapic acid and its alkyl esters, measured by DPPH and FRAP assays, are shown in **Table 3**. A good correlation was observed between both methods (r^2 = 0.998, data not shown). From the DPPH and FRAP results one can conclude that sinapic acid itself has a higher activity when compared to that obtained from alkyl esters. All of the sinapic alkyl esters have similar antioxidant activities, a fact that is in accordance with electrochemical and DSC data.

It may be stated that the examined phenolic compounds have significant reducing capacity. The reducing abilities of the alkyl esters are similar and lower than those of sinapic acid and Trolox. Trolox has been shown to possess good reducing capacity in the FRAP assay (*27*), and therefore a reducing ability at the level of Trolox should be considered a remarkable activity.

Insertion of an alkyl ester side chain has been reported to have different effects on the antioxidant activity on phenolic acid systems (*9*, *30*, *40*, *41*). Previous works has shown that caffeic acid (3,4-dihydroxycinnamic acid) alkyl esters have lower DPPH and ABTS radical scavenging activities than caffeic acid itself, data that seem to be dependent on the extension, or type, of the ester side chain (*30*, *40*−*43*). Other investigators have reported that alkyl esterification of ferulic acid (4-hydroxy-3-methoxycinnamic acid) decreases its activity against DPPH (*22*, *44*). From these results one can assume that the effect of the alkyl ester side chain in hydroxycinnamic systems is strongly related to the number of hydroxyl groups and the aromatic substitution pattern. In fact, it seems to be intimately connected with the different

oxidation mechanisms/capacity of stabilization of intermediates associated phenolic acid systems. Caffeic acid and its derivatives operate via quinone intermediate and ferulic and sinapic derivatives via semiquinone intermediates. The last intermediates are additionally stabilized by the inductive and mesomeric effects of methoxyl functions. These overall effects can surpass the mesomeric/inductive influence of the ethylenic side chain.

After the studies performed in this research area, one can say that a general antioxidant relationship statement cannot be performed for phenolic acids derivatives, in general, and, in particular, for hydroxycinnamic acid derivatives: each family is a study case, and each structural modification could affect the activity in a particular way as consequence of the steric, mesomeric, and inductive effects on the system.

From the overall results it can be concluded that alkyl sinapates present almost the same radical scavenging activity as well as reducing capacity. These activities were slightly lower than the activity displayed by the parent compound, sinapic acid. However, the synthesized antioxidants possess higher lipophilicity, which is a considerable advantage for antioxidants that are intended to function as membrane protectors in vivo and may compensate for their slightly lower antioxidant activity.

The electrochemical and DSC data showed that sinapic acid and its alkyl ester derivatives are good antioxidants regarding the inhibition of lipid oxidation. Moreover, the gathered results allow one to conclude that the sinapic alkyl ester derivatives exhibit an antioxidant capacity that closely matches that found for Trolox.

In addition, the overall data could be a useful tool for bioavailability and pharmacokinetics studies because some of these compounds are intrinsic components of the diet.

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