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Effect of pH on the Stability of Plant Phenolic Compounds

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It is not uncommon to treat plant-derived foods and feeds with alkali. Such exposure to high pH is being used to recover proteins from cereals and legumes, to induce the formation of fiber-forming meat analogue vegetable protein, for preparing peeled fruits and vegetables, and for destroying microorganisms. In addition to their profound effects on functional and nutritional properties in such foods, such treatments may also cause other side reactions, including the destruction of natural polyphenolic compounds. Because plants contain a large number of structurally different antioxidant, anticarcinogenic, and antimicrobial polyphenolic compounds, it is of interest to know whether such compounds are stable to heat and to high pH. In this model study, the stability of the following natural polyphenols to pH in the range 3−11 was studied with the aid of ultraviolet spectroscopy: caffeic acid, (−)-catechin, chlorogenic acid, ferulic acid, gallic acid, (−)-epigallocatechin, rutin, and the nonphenolic compound *trans*-cinnamic acid. This study demonstrates that caffeic, chlorogenic, and gallic acids are not stable to high pH and that the pH- and time-dependent spectral transformations are not reversible. By contrast, chlorogenic acid is stable to acid pH, to heat, and to storage when added to apple juice. (−)-Catechin, (−)-epigallocatechin, ferulic acid, rutin, and *trans*-cinnamic acid resisted major pH-induced degradation. The results are rationalized in terms of relative resonance stabilization of phenoxide ions and quinone oxidation intermediates. The possible significance of these findings to food chemistry and microbiology is discussed.

Keywords: *Caffeic acid; catechin; cinnamic acid; chlorogenic acid; epigallocatechin; ferulic acid; gallic acid; rutin; plant phenols; pH stability; ultraviolet spectra*

INTRODUCTION

Phenolic compounds are secondary plant metabolites found in all fruits and vegetables, including apples, coffee beans, grapes, potatoes, prunes, and tea leaves (Baron et al., 1997; Balentine et al., 1997; Dao and Friedman, 1994; Donovan et al., 1998; Friedman, 1997; Griffiths and Bain, 1997; Lathia and Frenzen, 1980; Lu and Foo, 1999; Spanos et al., 1990). They seem to be involved in the defense of plants against invading pathogens, including insects, bacteria, fungi, and viruses. The question arises whether these naturally occurring compounds would also be effective antimicrobial compounds against human pathogens such as *Escherichia coli* and *Campylobacter jejuni*. Exploratory studies in this laboratory have shown that this is indeed the case.

As part of a program designed to discover new naturally occurring antimicrobials against human pathogens, we would like to incorporate such compounds into foods to prevent multiplication of bacteria and/or to inactivate the bacteria already present. Because foods are generally cooked, baked, fried, and exposed to high pH to enhance digestibility and nutritional quality, studies are needed to find out whether the natural antimicrobial compounds are stable under food-processing conditions. The objective of this model study was to examine the stability of a series of structurally different polyphenolic compounds dissolved in buffers in the pH range from 3 to 11. Experimentally, we examined the change in the ultraviolet absorption spectra of the phenolic compounds in buffered solutions for several time periods up to 72 h. The results suggest that the stability of the phenolic compounds strongly depended not only on the pH of the buffers and storage time but also on the structure of the phenolic compound. Exploratory studies were also done to assess the stability of chlorogenic acid in the acid pH of ∼3.5−4 of apple juice and cider.

For this study, we selected the following structurally different compounds present in coffee, tea, and fruits and vegetables: caffeic acid, chlorogenic acid, ferulic acid, gallic acid, epicatechin, gallocatechin, rutin, and the nonphenolic compound *trans*-cinnamic acid.

MATERIALS AND METHODS

Materials. The following compounds were obtained from Sigma Chemical Co., St. Louis, MO: caffeic acid, (−)-catechin, chlorogenic acid, (−)-epigallocatechin, ferulic acid, gallic acid, and rutin. *trans*-Cinnamic acid was purchased from Eastman Kodak Co., Rochester, NY. All other compounds used were of reagent grade.

Apple juice and apple cider were bought at local stores. The filters were used for this study came from Schleicher & Schuell, Keene, NH: Spartan-25 filter, pore size = 0.45 μm, nylon membrane, size = 25 mm. The fitting 10 cm^3 syringe is a B-D product, Becton Dickinson & Co., Franklin Lakes, NJ.

Instrumentation. A Sartorius balance, accurate to 0.001 mg, was used for weighing the samples. pH measurements were made with an Orion model 410A pH meter (Orion Research Inc., Boston, MA). The UV and visible spectra were recorded on a Perkin-Elmer Lambda 6 UV−vis spectropho-

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tometer (Norwalk, CT) with a wavelength accuracy of 0.3 nm. The spectrometer cells came from Fisher Scientific Co., Fair Lawn, NJ. The data were collected and analyzed by Lamda Series PECSS software version 3.2 (Perkin-Elmer). To investigate the effect of heat, a Magni Whirl constant-temperature bath was used (Blue M Electric Co., Blue Island, IL).

UV spectra were measured at wavelengths ranging from 190 and 720 nm in 1 nm increments immediately after final dilutions were made. pH values were generally determined immediately before and after each experiment. The computer-generated data and spectra were used to calculate the molar extinction coefficients (ϵ) by the following equation: $\epsilon = A/dc$, where A = absorbance or optical density; d = 1 cm, the thickness of the UV cell; and c = molar concentration of the test compound, moles/L (M); λ = wavelength at which the measurement was made (Freifelder, 1982).

Stock Solutions. The following aqueous solutions were prepared by stirring the samples for 30 min on a magnetic stirrer until clear solutions were obtained: caffeic acid, 1 mg/mL (5.5×10^{-3} M); chlorogenic acid, 1 mg/mL (2.8×10^{-3} M); (−)-catechin, 0.1 mg/mL (3.4×10^{-4} M); (−)-epigallocatechin, 0.1 mg/mL (3.3×10^{-4} M); ferulic acid, 0.05 mg /mL (2.57×10^{-4} M); gallic acid, 1 mg/mL (5.88×10^{-3} M); rutin, 0.125 mg/mL (2.05×10^{-4} M); *trans*-cinnamic acid, 0.148 mg/mL (1 mM).

Buffers. Solutions (0.01 M) of $(NH_4)_3PO_4$ and H_3PO_4 were used to make the buffers at pH 3 and 4.5. They were adjusted to the desired pH with 0.25 and 2.0 N HCl. Solutions (0.1 M) of sodium acetate (8.203 g/L) and glacial acetic acid (6 mL/L) were used to make the buffers at pH 3, 4, 5, and 6. They were adjusted to the desired pH by adding sodium acetate to acetic acid. Solutions (0.01 M) of sodium borate (3.8137 g/L), boric acid (0.6184 g/L), and 1 or 0.25 N NaOH were mixed in various ratios to prepare buffers at pH 7, 8, 9, 9.5, 10, 10.5, and 11. For the pH 7 buffers, the sodium borate solution was added to 100 mL of the boric acid solution until the desired pH was reached.

The stock solutions were then diluted with H_2O or with one of the buffers to the following final concentrations: caffeic acid, 5.5×10^{-5} M; (−)-catechin, 3.4×10^{-5} M; chlorogenic acid, 2.8×10^{-5} M; (−)-epigallocatechin, 6.5×10^{-5} M; ferulic acid, 5.15×10^{-5} M; gallic acid, 5.88×10^{-5} M; rutin, 2.05×10^{-5} M; *trans*-cinnamic acid, 4.0×10^{-5} M.

Reversibility. To establish whether the spectral changes observed at high pH are reversible after reduction to neutral pH, a solution of pH 10.5 was prepared from H_2O and 1.0 N NaOH. To neutralize the solutions to pH ~7, 1.0 and 0.25 N HCl were used. The stock solutions of caffeic, chlorogenic, and gallic acids and (−)-catechin were used. Chlorogenic and gallic acids were first diluted 1:9 with 1 part of the phenol solution and 9 parts of H_2O. Before the measurement, each of the four solutions was diluted 1:9 with the pH 10.5 solution to its final concentration: caffeic acid (5.5×10^{-5} M), (−)-catechin (3.4×10^{-5} M), chlorogenic acid (2.8×10^{-5} M), and gallic acid (5.88×10^{-5} M).

For ferulic acid, a solution of pH 11 (5.15×10^{-5} M) was prepared from H_2O and 1.0 N NaOH. After 24 h, the pH 11 solution was then reduced with 1.0 and 0.25 N HCl to pH 7 and 9.

Optical absorbance was measured immediately after the dilution with the pH 10.5 solution, after 1 or 2 h (chlorogenic acid), and then immediately after neutralization to pH 7. For caffeic acid, additional measurements at pH 7 were made after 2 and 24 h.

pH—Time Study. Measurement were made, in the first 6 h, every 30 min and then after 24, 48, and 72 h. Because of the frequent measurements during the first 6 h, the solutions and the buffers were stored in cuvettes at room temperature and room light. After the first 6 h, they were stored in the refrigerator (7 °C) sealed with Parafilm (American National Can, Chicago, IL). The determinations were made at pH 9.5, 10, and 11 against buffers.

Solubility and Stability of Chlorogenic Acid Added to Apple Juice. To determine the solubility and storage stability of chlorogenic acid, 500 mg of the compound was dissolved in 25 mL of pure apple juice (5.6×10^{-2} M or 2%). The suspension was stirred with constant agitation on a magnetic stirrer for ~150 min until a clear solution was obtained. The solution was used to start two series of experiments. Each series (A and B) started with 10 mL of the 20 mg/mL chlorogenic acid/juice mixture and 10 mL of pure juice, measured with 10 mL volume pipets. The concentrations were halved four times to give final concentrations of 20, 10, 5, 2.5, and 1.25 mg/mL. After the dilutions were completed, all solutions were diluted three times with a 1:9 juice/water mixture; the final dilutions were made immediately before each measurement. The final concentrations ranged from 5.6×10^{-5} to 3.5×10^{-6} M.

The following experiments were carried out to assess the storage stability of chlorogenic acid added to apple juice and cider. A solution of 100 mg of chlorogenic acid in 10 mL (2.8×10^{-2} M or 10 mg/mL or 1%) of apple juice was prepared. The following mixture was made twice: 1 mL of the chlorogenic acid solution plus 9 mL of pure apple juice (2.8×10^{-3} M). One flask of each of these stock mixtures was stored either at room temperature (23° C) or in the refrigerator (7° C). A 1:9 juice/water mixture was prepared to dilute the solutions to 2.8×10^{-5} M. Identical solutions were made of chlorogenic acid and apple cider.

The absorption was determined immediately after the dilution with the 1:9 juice or cider/water mixtures and then at different times up to 432 h. The stock solutions were diluted with fresh diluted 1:9 juice or cider/water mixtures for each measurement. After 48 h, the cider stored at room temperature became cloudy and needed to be filtered. The same happened to the cider stored in the refrigerator after 120 h and with both juice samples on the 13th day of storage. The samples were first diluted to the desired concentration and then filtered.

RESULTS

Figure 1 illustrates the structure of the phenolic compounds evaluated in this study. Figures 2−9 depict the absorption spectra of the eight compounds under various conditions used to assess their stability. Table 1 lists in alphabetical order the calculated ϵ values of the test compounds dissolved in water and in buffers in the pH range 7−11 evaluated in this study. The spectral data in Tables 2−7 provide additional information on the reproducibility of the absorption spectra measurements and stabilities of the test compounds. The observed replicate variabilities based on separate experiments should be taken into account in the evaluation of the cited spectral shifts. Because we believe this to be a model study that should serve as a guide for future studies with other phenolic compounds, we offer the following detailed description of the observed changes in the spectra of structurally different phenolic compounds as a result of pH treatment:

***trans*-Cinnamic Acid.** Figure 2 shows the spectral changes of *trans*-cinnamic acid (4.0×10^{-5} M) in the pH range 7−11 at 0 time. pH 7: The λ_{max} is at 269 nm, 0.789 (the number after the wavelength = absorbance, A, at the indicated concentration); also, a double peak is observed at 204 nm, 0.725; and 213 nm, 0.714. pH 8: The λ_{max} appears at 269 nm, 0.783, and the double peak at 204 nm, 0.670; and 213 nm, 0.695. pH 9: The λ_{max} is also at 269 nm, 0.785. The other peaks shifted to 205 nm, 0.667, and 212 nm, 0.668. pH 9.5: The λ_{max} is at 269 nm, 0.778; 205 nm, 0.659; and 214 nm, 0.669. pH 10: λ_{max} is at 269 nm, 0.781; 205 nm, 0.639; and 214 nm, 0.656. pH 10.5: λ_{max} is at 269 nm, 0.795. The A values of the double peak decreased to 0.657 at 206 nm and 0.682 at 214 nm. pH 11: The λ_{max} remains at 269 nm, 0.781. The first peak of the double peak disappeared, and the second peak is at 214 nm, 0.625.

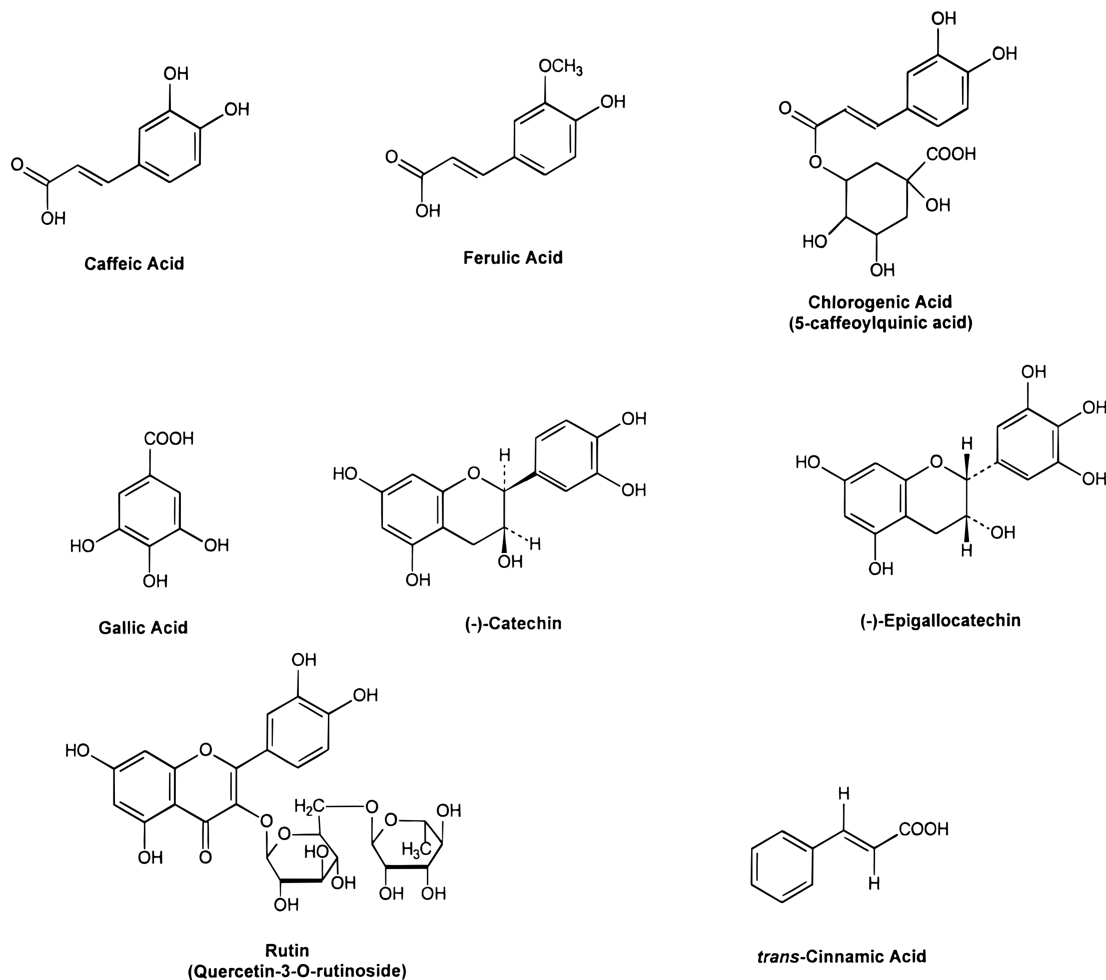

Figure 1. Structures of plant phenolic compounds evaluated in this study.

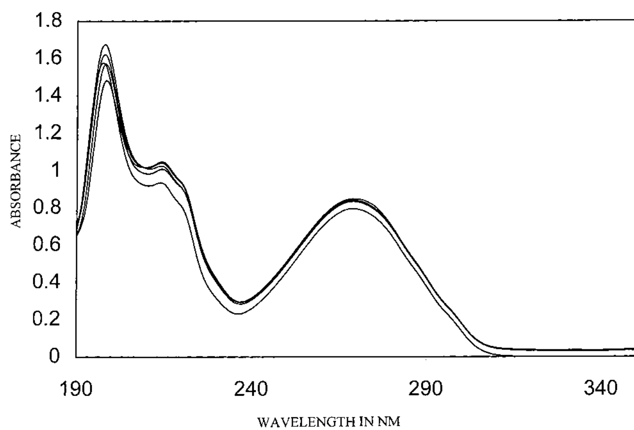

Figure 2. Effect of time on the absorption spectra of *trans*-cinnamic acid at pH 10 (4.0×10^{-5} M) at 0 time and after 1, 24, 48, and 72 h.

Ferulic Acid. The absorption spectra of ferulic acid (5.15×10^{-5} M) in the pH range 7−11 at 0 time are illustrated in Figure 3. pH 7: λ_{max} is at 286 nm and a second peak at 309/310 nm, 0.817. pH 8: The first peak is at 287 nm, and the λ_{max} is at 310 nm, 0.754. pH 9: The λ_{max} appears at 333 nm, 0.743. pH 9.5: The λ_{max} shifted to 344 nm, 1.02. pH 10: The λ_{max} appears at 345 nm, 1.16. pH 10.5: λ_{max} remains at 345 nm, 1.18. pH 11: λ_{max} is at 345 nm, 1.21.

Caffeic Acid. The absorption spectra of caffeic acid (5.5×10^{-5} M) in the pH range 7−11 at 0 time are illustrated in Figure 4. pH 7: The first peak is at 217 nm, 1.05. The other peaks are at 292 nm, 0.677, and 324 nm, 0.849. pH 8: The peaks appeared at 216 nm, 1.12; 293 nm, 0.650; and 328 nm, 0.942. pH 9: The first peak is at 218 nm, 0.958; the other peaks are at 293 nm, 0.633, and 328 nm, 0.947. pH 9.5: The A values of all three peaks decreased. At 216 nm, $A = 0.957$; at 293 nm, 0.619; and at 329 nm, 0.922. pH 10: At 212 nm, A is at 0.964; at 293 nm, 0.567; and at 329 nm, 0.788. pH 10.5: The peaks are at 208 nm, 0.847; 292 nm, 0.509; and 328 nm, 0.560. pH 11: The spectrum has only one peak left at 211 nm, 0.739; both other peaks disappeared.

Chlorogenic Acid. Figure 5 shows the spectral changes of chlorogenic acid (2.8×10^{-5} M) in the pH range 7−11 at 0 time. pH 7: The λ_{max} is at 341 nm, 0.534. pH 8: The λ_{max} appears at 344 nm, 0.551. pH 9: The λ_{max} is at 344 nm, 0.587. pH 9.5: The λ_{max} is shifted to 346 nm, 0.574. pH 10: A new peak appeared at 194 nm, 0.552. At 346 nm, the absorbance decreased to 0.550. pH 10.5: The new peaks have shifted to 197 nm, 0.704, and 347 nm, 0.398. pH 11: The new peaks have shifted to 200 nm, 0.740, and 338 nm, 0.199.

Gallic Acid. The absorption spectra of gallic acid (5.88×10^{-5} M) in the pH range 7−11 at 0 time are illustrated in Figure 6. pH 6: The λ_{max} is at 212 nm, 1.59. The second peak appears at 270 nm, 0.448. pH 8: The λ_{max} appears at 215 nm, 1.46. The other peak is at 276 nm, 0.511. pH 9: The λ_{max} is at 215 nm, 1.41; the second peak is at 277 nm, 0.506. pH 9.5: At 217 nm, $A = 1.37$ and at 277 nm, 0.50. pH 10: The λ_{max} remains

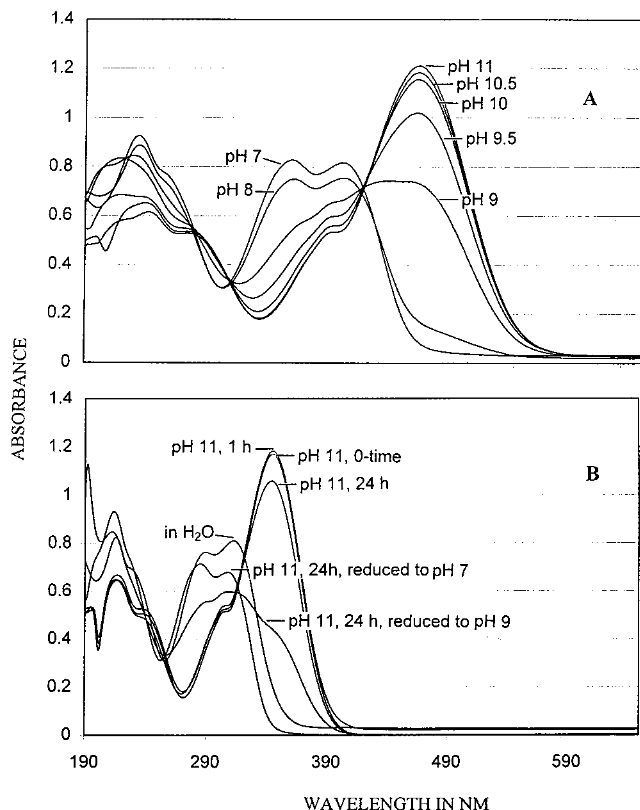

Figure 3. (A) Effect of pH (7−11) on the absorption spectra of ferulic acid (5.15 × 10^{-5} M). The isosbestic point suggests an equilibrium between two absorbing species beginning between pH 8 and 9; λ_{max} at the lower wavelength is associated with the un-ionized molecules and that at the higher wavelength with the ionized molecules. (B) Reversibility of the absorption spectra after lowering of a pH 11 solution to pH 7 or 9.

at 217 nm, 1.23; at 279 nm, 0.480. pH 10.5: At 218 nm, A = 0.893 and at 279 nm, 0.429. pH 11: At 216 nm, A = 0.600 and at 277 nm, 0.362.

(−)-Catechin. The changes in absorption spectra of (−)-catechin (3.4 × 10^{-5} M) in the pH range 7−11 at 0 time are illustrated in Figure 7A,B. pH 7: For λ_{max} at 205 nm, 2.36; 282 nm, 0.225. pH 9: For λ_{max} at 207 nm, 2.23. For λ_{max} at 285 nm, 0.286. pH 9.5: The λ_{max} is shifted to 208 nm, 2.05; 285 nm, 0.287. pH 10: For λ_{max} at 209 nm, 1.77; 285 nm, 0.273. pH 10.5: The peak has shifted to 212 nm, 1.71; at 286 nm A increased to 0.301. pH 11: λ_{max} is at 210 nm, 1.89; 287 nm, 0.297.

(−)-Epigallocatechin. For (−)-epigallocatechin (6.5 × 10^{-5} M), only minor changes in the spectra occurred at pH 9.5, 10, and 10.5 at 0 time (Figure 7C). pH 9.5: The λ_{max} was at 216 nm, 2.42. pH 10: The λ_{max} is shifted to 218 nm, 2.35. pH 10.5: λ_{max} is at 218 nm, 2.31.

Rutin. The absorption spectra of rutin (2.05 × 10^{-5} M) in the pH range 7−11 at 0 time are illustrated in Figure 8. pH 7: The peaks are at 203 nm, 0.793; 261 nm, 0.434; and 366 nm, 0.318. pH 8: The peaks are at 199 nm, 0.804; 267 nm, 0.478; and 377 nm, 0.343. pH 9: The peaks are at 203 nm, 0.743; 269 nm, 0.504; and 381 nm, 0.359. pH 9.5: The peaks are at 203 nm, 0.712; 269 nm, 0.498; and 381 nm, 0.354. pH 10: The peaks are at 203 nm, 0.783; 268 nm, 0.491; and 381 nm, 0.350. At pH 10.5: The peaks are at 204 nm, 0.735; 269 nm, 0.484; and 381 nm, 0.346. pH 11: λ_{max} is at 209 nm, 0.67; 269 nm, 0.516; and 384 nm, 0.372. Quercetin, the aglycon of rutin, was not sufficiently soluble in water for inclusion in this study. It readily dissolves in alcoholic media (Friedman and Smith, 1984).

Effect of Time on Absorption Spectra at Different pH Values. Figure 4B shows the time-dependent degradation of caffeic acid at pH 10 after 1 and 24 h. Corresponding changes for chlorogenic acid are shown in Figure 5B and for gallic acid in Figure 6B. The results show extensive degradation of the three compounds even at this intermediate pH value. The spectrum of *trans*-cinnamic acid did not change (results not shown), whereas the corresponding spectra for catechin and rutin (Figures 7B and 8B) were largely unaffected, even after exposure to longer time periods at higher pH values.

Stability of Caffeic and Chlorogenic Acids in Acid pH. Tables 6 and 7 list the A and ϵ values at 314 nm of caffeic and chlorogenic acids in the pH range 3−6.

Reversibility of Spectral Changes of Ferulic Acid at pH 11. Figure 3B shows that reducing a pH 11 solution of ferulic acid to either pH 9 or 7 regenerated the original spectra, taking into account a dilution factor during the neutralization. This result suggests that ferulic acid is stable at the high pH.

Nonreversibility of Spectral Changes of Caffeic, Chlorogenic, and Gallic Acids and (−)-Catechin at pH 10.5. These compounds showed similar results. Compared to solutions of phenols dissolved in H_2O, the spectra of pH 10.5 solutions shifted and decreased immediately at 0 time followed by further decreases over a 2 h incubation period. After the solutions were neutralized to pH ∼7, no further spectral changes were measurable (Figures 4C and 5C). Because the original spectra were not regenerated on neutralization, the spectral changes of the pH 10.5 solutions are nonreversible.

Apple Juice. The absorption of spectra of different amounts of chlorogenic acid dissolved in apple juice are illustrated in Figure 9. A plot of concentration versus absorbance maximum at 325 nm shows a linear fit of the data with 95% confidence intervals. Six separate solubility determinations were carried out with each concentration to demonstrate reproducibility and linearity. These results suggest that UV spectroscopy can be used to determine the amount of chlorogenic acid present in apple juice.

DISCUSSION

Structure−Absorption Spectra Relationships. To place our findings in their proper perspective, we first offer the following brief summary of factors that influence the ultraviolet absorption spectra of phenols as described by Dearden and Forbes (1959). Phenols usually show two bands between 200 and 360 nm. The band at the shorter wavelength is known as the B-band and the one at longer wavelength as the C-band. Both bands are displaced to longer wavelength for phenols dissolved in NaOH solution. The absorption spectra and extinction coefficients of phenols are influenced by the nature of the solvent, electron-withdrawing and electron-donating substitutents in the benzene ring(s), intra- and intermolecular hydrogen-bonding, steric effects, and the pH-dependent formation of resonance forms with altered conjugation compared to the parent compounds.

For example, resonance, tautomers, hydrogen-bonding, and hydrated structures as well as colors of polyphenolic anthocyanins are strongly influenced by

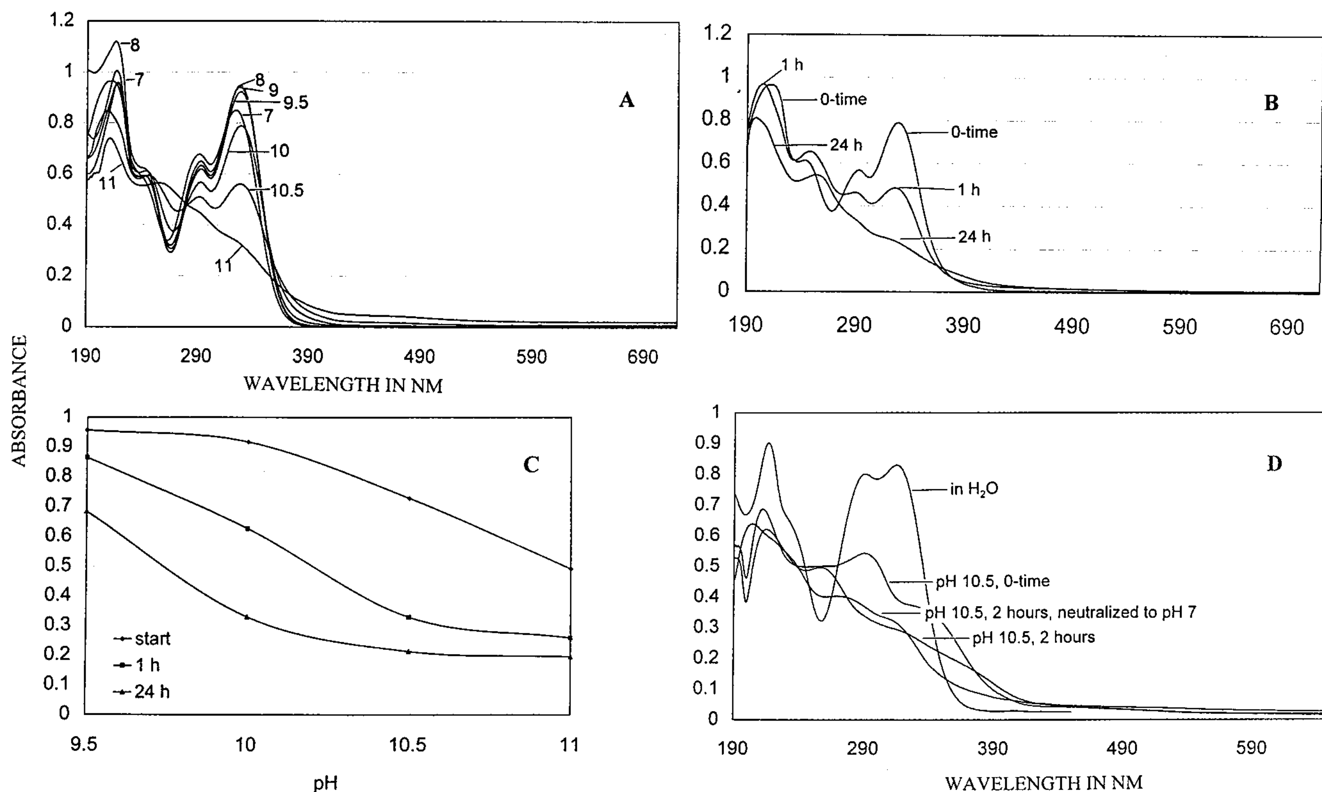

Figure 4. Effect of pH (7−11) and time on the absorption spectra of caffeic acid: (A) pH 7−11 at 0 time; (B) pH 10 at 0 time, 1 h, and 24 h; (C) pH effect on absorbance at 325 nm at 0 time, 1 h, and 24 h; (D) effect of neutralizing a pH 10.5 solution (5.5×10^{-5} M) to pH 7 (nonreversibility of the pH effect).

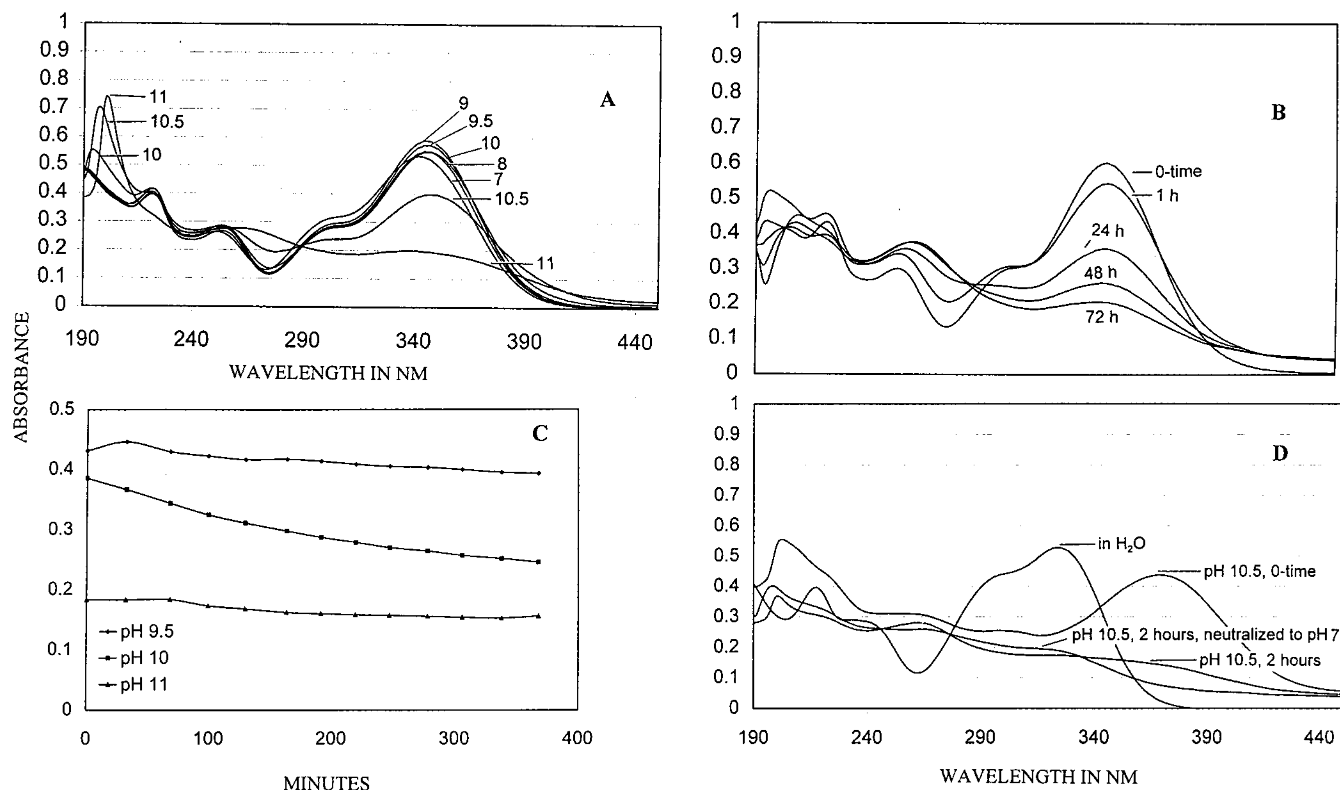

Figure 5. Effect of pH (7−11) and time on the absorption spectra of chlorogenic acid: (A) pH 7−11 at 0 time; (B) pH 10 at 0 time and 1, 24, 48, and 72 h; (C) pH effect on absorbance at 325 nm; (D) effect of neutralizing a pH 10.5 solution (2.8×10^{-5} M) to pH 7 (nonreversibility of the pH effect).

pH. Thus, the red wine pigment malvidin 3-glucoside is red at pH 1 (flavylium form), colorless at pH 4−5 (carbinol pseudo-base), purple at pH 6−7 (quinoidal base), and yellow at pH 7−8 (keto−enol tautomers). At pH 8−10, anthocyanins form highly colored ionized anhydro bases. These hydrolyze rapidly at pH 12 to

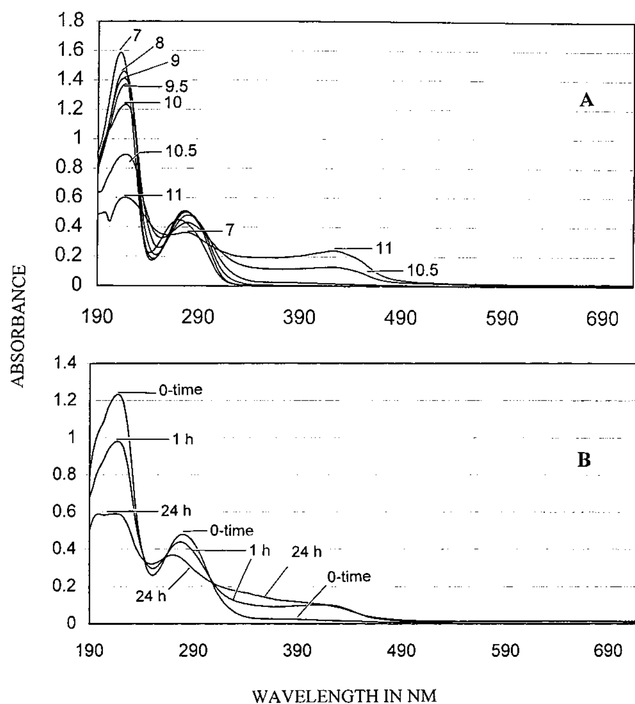

Figure 6. Effect of pH (7−11) and time on the absorption spectra of gallic acid (5.88×10^{-5} M): (A) pH 7−11 at 0 time; (B) pH 10 at 0 time and after 24 h.

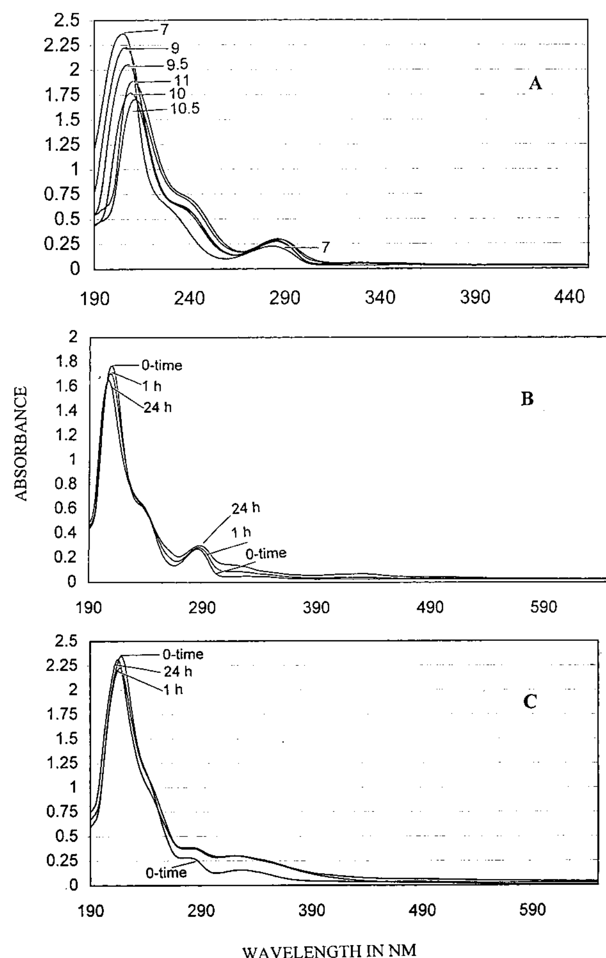

Figure 7. Effect of pH (7−11) and time on the absorption spectra of (−)-catechin (2.8×10^{-5} M) and (−)-epigallocatechin (6.5×10^{-5} M): (A) (−)-catechin at pH 7−11 and 0 time; (B) (−)-catechin at pH 10 at 0 time, 1 h, and 24 h; (C) (−)-epigallocatechin at pH 10 at 0 time, 1 h, and 24 h.

ionized chalcones (Brouillard et al., 1982; Hoshino and Tamura, 1999; Jurd, 1964; Pataky, 1965; Wong, 1989). Although the various forms probably vary in their susceptibility to oxidative degradation and complex formation and reactions with other phenols, amino acids, proteins, and metal ions, all of the forms transformed in the pH range 1−8 retained their antioxidative activity (Lapidot et al., 1999).

The results of this study demonstrate that the susceptibility of structurally different plant phenolic compounds to pH strongly depends on the structure of the phenol. The following discussion attempts to relate the observed changes in the absorption spectra to the structures of the eight compounds we studied.

First, the compound *trans*-cinnamic acid is not a phenol because it does not have any OH groups located on the benzene ring. The absorption spectrum of this compound was not changed significantly under the influence of pH. This observation suggests that conjugated nonphenolic aromatic acids such as *trans*-cinnamic acid are stable at high pH. Second, ferulic acid with a single OH group (the compound is identical to caffeic acid except one of the two OH groups is methylated) also appears to be stable at high pH at 0 time and after 1 and 24 h (Figure 3B), presumably because it cannot form quinone oxidation products (see below). The figure also shows that neutralizing a pH 11 solution of ferulic acid to 7 or 9 regenerates the original spectra. This is not the case with three other compounds discussed below.

In contrast, the spectrum of caffeic acid, which has a structure identical to that of cinnamic acid, except that caffeic acid has two phenolic OH groups, is dramatically changed with pH. The extent of the spectral changes are directly related to pH in the range 7−11. This observation suggests that the two adjacent phenolic OH groups which are attached to the benzene ring of caffeic acid, are responsible for the observed changes. It is also important to note that the changes are time-dependent and nonreversible. Changing the pH of the buffer from 10 back to 7 did not regenerate the original spectrum.

The results with chlorogenic acid, a caffeic acid ester, are very similar to those observed with caffeic acid. The spectral changes observed at high pH are also irreversible when the pH is reduced from 10 to 7. These observations suggest that esterification of the carboxyl group of caffeic acid with quinic acid to form chlorogenic acid does not change the susceptibility of the molecule to the effects of pH on the absorption spectra.

Gallic acid was also unstable at high pH. Gallic acid has three phenolic OH groups but no carbon−carbon double bond conjugated to the benzene ring, as in the case with caffeic and chlorogenic acids, and our results suggest that the phenolic OH groups may be primarily responsible for the observed spectral changes for the three unstable compounds, caffeic, chlorogenic, and gallic acids.

Except for pH-induced shifts, the absorption maxima of other phenolic compounds such catechin, epigallocatechin, and rutin were much less pronounced at high pH. Although these compounds all have phenolic OH groups, their multiring aromatic structures are more complex than the monoring phenolic compounds, which are susceptible to the effects of pH. Evidently, their ionized and resonance forms are more resistant to degradation by pH than are the monocyclic polyphenolic compounds. Related studies showed that (a) both the absorption

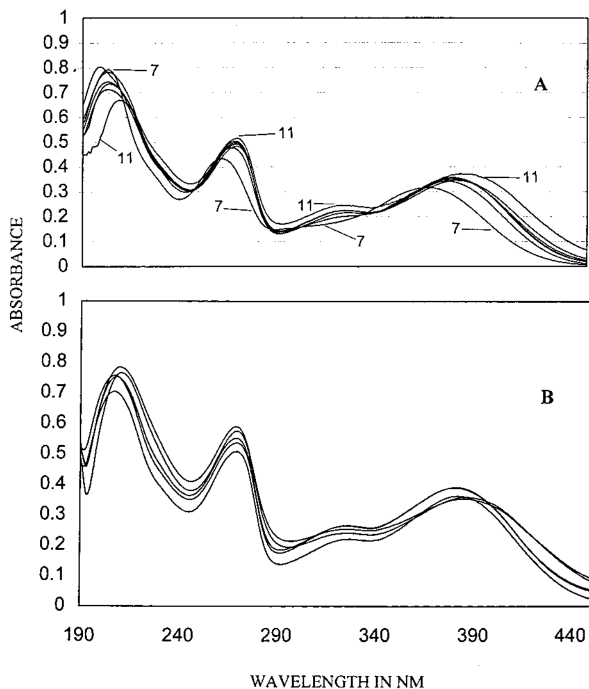

Figure 8. Effect of pH (7−11) and time on the absorption spectra of rutin (2.05×10^{-5} M): (A) pH 7−11 and 0 time; (B) pH 10 at 0 time and 1, 24, 48, and 72 h.

maximum and mutagenic activity of quercetin, the aglycon of rutin (Figure 1), were reduced after exposure of an alcoholic solution to high pH and oxygen (Friedman and Smith, 1984); and (b) UV spectroscopy can be used to follow the polyphenol oxidase-catalyzed oxidation of quercetin (Jimenez amd Garcia-Carmona, 1999) and iron chelation by chlorogenic acid (Kono et al., 1998).

The structures of catechin, epicatechin, and rutin deserve additional comment. The first two compounds consist of two separate aromatic systems; two benzene rings connected by a six-membered, nonaromatic, oxgen-containing ring. The structures of these molecules are therefore not planar, and the π-electrons of the two benzene rings cannot interact with each other via conjugation.

Moreover, because the phenolic OH groups in the first benzene ring are located in the meta-position to each other, they cannot form quinones. Those of the second benzene ring can form quinones because they are situated ortho to each other. By contrast, the aglycon part of rutin has a completely conjugated aromatic system. The cited structural features undoubtedly influence stabilities to pH because the spatial arrangement between an OH group and the π-electron system governs the extent of π-orbital overlap and consequently electronic spectra, ground and excited-state energies, and susceptibilities to chemical change (Mataga and Kubota, 1970).

The fact that the observed changes in the absorption spectra of caffeic, chlorogenic, and gallic acids are not reversible suggests that these compounds were changed chemically under the influence of pH. We do not know the nature of these chemical transformations. One possibility is that the decrease in the absorption maxima with increasing pH may be the result of formation of unstable quinone intermediates, and other resonance forms described elsewhere (Friedman, 1997). These may ultimately oxidize in the presence of air to diketo derivatives or other degradation products. In contrast to the observed stability of an aqueous solution of chlorogenic acid heated for 1 h at 90 °C (Table 5), earlier studies showed that chlorogenic acid in the presence of other food ingredients was partly lost during cooking and baking (Chuda et al., 1998; Friedman and Dao, 1990). It is also worth noting that the decarboxylation of caffeic acid and related polyphenols to vinylphenols during storage and processing of citrus juices adversely affects the quality of the products (Lee and Nagy, 1996).

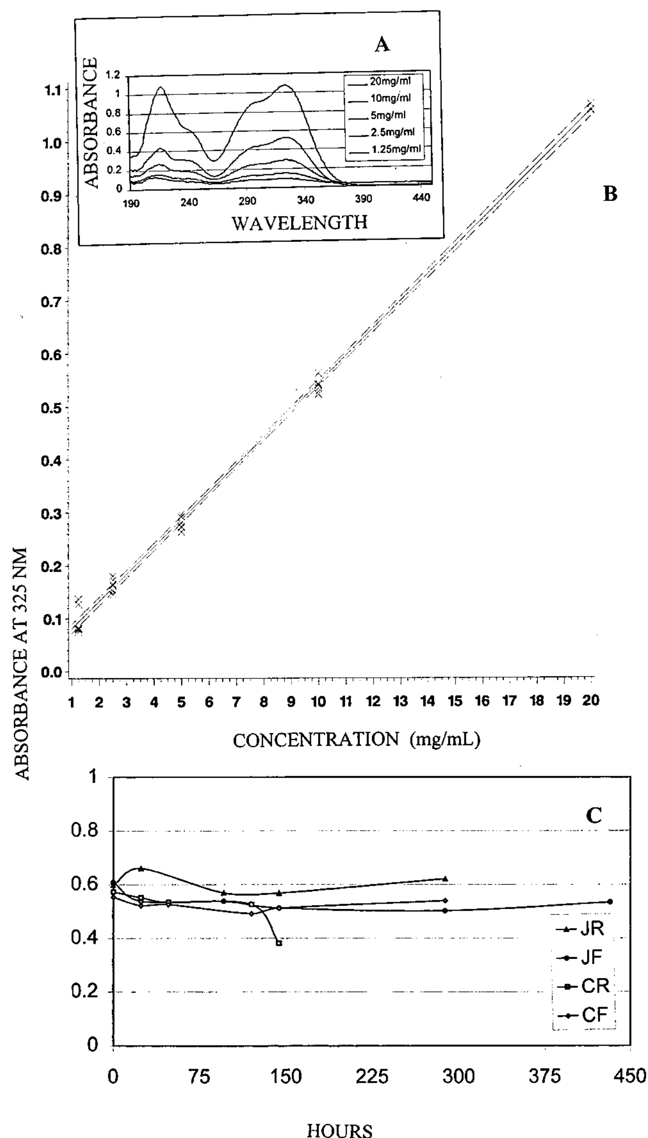

Figure 9. Absorbance and stability of chlorogenic acid added to apple juice: (A) concentration dependence of absorption spectra; (B) linear relationship between concentration and absorbance at 325 nm ($n = 6$); (C) stability in apple juice (J) and cider (C) stored at room temperature (R) and in a refrigerator (F).

Apple Juice. Contamination of apple cider and juice with *Escherichia coli* 0157:H7 and *Salmonella typhimurium*, pathogenic microorganisms that cause foodborne illnesses in humans, may result from using apples that have fallen to the ground, fertilizing apple orchards with cow manure, as well as improperly washing apples before processing (Hancock, 1997). If this hypothesis is correct, it can be assumed that the pathogens adhere to the surface of the apples before entering the food chain.

Table 1. Extinction Coefficients of Polyphenolic Compounds Listed Alphabetically at Different pH Values[a]

compound		pH							
		H_2O	7	8	9	9.5	10	10.5	11
caffeic acid	ϵ_{216} =	16687	18101	20179	17137	17168	17225	14575	12722
	ϵ_{290} =	14443	12158	11531	11205	10954	10079	9156	8183
	ϵ_{314} =	14958	13807	13829	13681	13261	11526	8875	6563
(−)-catechin	ϵ_{202} =	66113	67376		60867	53633	40233	25831	29869
	ϵ_{278} =	4761	6135		7170	7074	6667	7286	7065
chlorogenic acid	ϵ_{324} =	18854	14943	13607	14393	13954	13364	10346	6850
(−)-epigallocatechin	ϵ_{206} =	38441				33042	25993	24359	
ferulic acid	ϵ_{236} =	12376	12563	11947	11487	11093	10654	10240	10384
	ϵ_{322} =	14452	12498	12174	14138	15000	15223	15029	15192
gallic acid	ϵ_{212} =	27537	27008	24493	23219	22758	20560	14936	9992
	ϵ_{262} =	8792	7029	6423	6153	5998	5833	5998	5879
rutin	ϵ_{198} =	44559	36695	39196	34985	33441	36563	33715	24063
	ϵ_{256} =	19549	20091	19554	19202	18826	18704	18284	19598
	ϵ_{352} =	15568	13923	12764	12520	12295	12334	12144	12881
trans-cinnamic acid	ϵ_{204} =	17937	18128	17490	16648	16428	15925	16273	11868
	ϵ_{214} =	17498	17808	17355	16878	16728	16410	17060	15620
	ϵ_{269} =	20128	19713	19583	19633	19478	19553	19865	19525

[a] Calculated at the wavelength of each compound dissolved in H_2O (pH 6.20).

Table 2. Effect of pH (7−11) on Absorbance of Chlorogenic Acid (2.8 × 10^{-5} M)[a]

	λ_{max} at 324 nm			λ_{max} at 324 nm	
pH	data set a	data set b	pH	data set a	data set b
7	0.418	0.439	10	0.374	0.387
8	0.381	0.436	10.5	0.290	0.350
9	0.403	0.405	11	0.192	0.229
9.5	0.390	0.389			

[a] Data are from two separate experiments.

Table 3. Effect of pH (7−11) on Absorbance of Rutin at Peaks A and B (2.05 × 10^{-5} M)[a]

	absorbance at peak A at 256 nm		absorbance at peak B at 352 nm		ratio A/B	
pH	data set a	data set b	data set a	data set b	a	b
7	0.411	0.398	0.285	0.271	1.44	1.47
8	0.400	0.424	0.261	0.266	1.53	1.59
9	0.393	0.399	0.256	0.251	1.53	1.59
9.5	0.385	0.390	0.252	0.247	1.53	1.58
10	0.383	0.380	0.252	0.242	1.52	1.57
10.5	0.374	0.372	0.249	0.235	1.50	1.61
11	0.401	0.374	0.264	0.239	1.52	1.56

[a] Calculated at the wavelength of rutin dissolved in H_2O (pH 6.42).

Table 4. Effect of pH (7−11) on Absorbance of *trans*-Cinnamic Acid (4.0 × 10^{-5} M)[a,b]

	λ_{max} at 269 nm			λ_{max} at 269 nm	
pH	data set a	data set b	pH	data set a	data set b
7	0.788	0.807	10	0.781	0.796
8	0.783	0.808	10.5	0.795	0.798
9	0.785	0.802	11	0.781	0.793
9.5	0.778	0.802			

[a] Calculated at the wavelength of *trans*-cinnamic acid dissolved in H_2O (pH 6.02). [b] The data are from two separate experiments.

E. coli 0157:H7 is exceptionally tolerant to the acid pH of apple juice or cider (Miller and Kaspar; 1994; Tortorello et al., 1998). This strain of *E. coli* survives in fresh apple cider stored at 20 °C, whereas alcoholic fermentation of fresh cider destroys the pathogen (Semanchek and Golden, 1996). However, most ciders are not fermented.

Table 5. Effect of Heating an H_2O Solution of Chlorogenic Acid at 90 °C for 1 h on Absorbance (2.8 × 10^{-5} M)[a]

chlorogenic acid	λ_{max} at 324 nm	ϵ_{324}
before heating	0.541	19182
after heating	0.513	18172

[a] Calculated at the wavelength of chlorogenic acid dissolved in H_2O (pH 6.20).

Table 6. Effect of Acid pH (3 and 4.5)[a] on Absorbance of Chlorogenic Acid at 324 nm (2.8 × 10^{-5} M)[b]

	λ_{max} at 324 nm		ϵ_{324}	
chlorogenic acid	pH 3	pH 4.5	pH 3	pH 4.5
0 time	0.574	0.515	20330	18254
after 24 h	0.567	0.555	20107	19678

[a] Phosphate buffers. [b] Calculated at the wavelength of chlorogenic acid dissolved in H_2O (pH 6.20).

Table 7. Effect of Acid pH (3, 4, 5, and 6)[a] on Absorbance of Caffeic Acid (5.5 × 10^{-5} M)[b]

	λ_{max} at 314 nm			
caffeic acid	pH 3	pH 4	pH 5	pH 6
0 time	0.895	0.782	0.651	0.586
after 1 h	0.900	0.774	0.667	0.610
after 24 h	0.909	0.790	0.657	0.598

	ϵ_{314}			
caffeic acid	pH 3	pH 4	pH 5	pH 6
0 time	16121	14090	11727	10569
after 1 h	16223	13947	12028	10987
after 24 h	16387	14234	11846	10781

[a] Acetate buffers. [b] Calculated at the wavelength of caffeic acid dissolved in H_2O.

To destroy microorganisms, most manufacturers use heat to pasteurize apple ciders and juices. Because the heat treatment requires energy, it adds to the cost of the juices. In addition, heat treatment can also induce nonenzymatic browning reactions between amino acids, proteins, and carbohydrates present in apple juice to form Maillard browning products (Friedman, 1996; Friedman et al., 1990; Molnar-Perl and Friedman, 1990). Such products are reported to affect the taste, appearance, and quality of foods. Heat and storage are

also reported to diminish both phenolic compounds and vitamins, especially vitamin C (Chaudry et al., 1998; Friedman et al., 1987). For these reasons, a need exists to develop stable new antimicrobial compounds that will protect apple juice against microbial infection.

Exploratory studies in this laboratory showed that certain naturally occurring phenolic compounds including chlorogenic and gallic acids and tea polyphenols possess antimicrobial properties; that is, they can prevent multiplication of and/or kill various microorganisms. In principle, such compounds could be used to protect apple juice and other foods against infection by human pathogens, as has already been demonstrated for milk (Payne et al., 1989; Rosenthal et al., 1997) and with unnatural additives for apple juice (Fisher and Golden, 1998). However, before such studies could be undertaken, it is necessary to establish the solubilities and stabilities of the added phenolic compounds. Our results show that (a) the UV method can be used to measure the concentration of added chlorogenic acid in apple juice; and (b) the added chlorogenic acid appears to be stable in the pH environment of apple juice when stored in a refrigerator for up to two weeks. These observations should facilitate the development of practical processes for the incorporation of chlorogenic acid and related phenolic compounds into apple juice.

CONCLUSIONS

This investigation has demonstrated that some naturally occurring polyphenolic compounds which are part of our diet are damaged when exposed to high pH. The chemical structure of the phenolic compounds appears to have a profound effect on their susceptibility to such destruction as measured by their absorption spectra. The detailed chemical changes in the susceptible phenolic compounds that may be responsible for the observed spectral changes are not known. Although our findings with eight representative compounds need to be extended to many more phenolic compounds, the cited structural features of the susceptible molecules may help to predict the stabilities to pH of other phenolic compounds having structures that are similar to those we studied. It is worth noting that although the high pH used to inactivate antinutrients in cowpeas reduced their polyphenol content by up to 67%, no concurrent formation of lysinoalanine was observed (Uzogara et al., 1990). This lysine derivative is both formed and degraded in food proteins exposed to high pH (Friedman, 1999a). High pH also racemizes L-amino acids to D-isomers (Friedman 1999b).

Our findings may also be useful in future studies designed to assess the anticarcinogenic, antioxidative, and antimicrobial properties of plant polyphenols subjected to food-processing conditions that use heat and high pH. If a specific phenolic compound is found to be unstable under such food-processing conditions, it may not be effective as an antioxidative or antimicrobial compound when present in foods subjected to baking, cooking, frying, microwaving, or high pH. For example, because epigallocatechin but not gallic acid was stable at high pH, expectations are that the tea polyphenol epigallocatechin gallate (a gallic acid ester) will not be stable.

A need also exists to determine the minimum concentration of phenolic and related compounds needed to protect apple juice against bacterial infection. Related studies are also needed to establish the stability and value of adding naturally occurring antioxidative and antimicrobial phenolic and other compounds to various food formulations including meat, poultry, and canned foods subjected to food processing. Such studies should produce new information that will make it possible to improve the quality and safety of foods.

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