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Nonenzymic Autoxidative Phenolic Browning Reactions in a Caffeic Acid Model System

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Nonenzymic oxidation of caffeic acid was studied at pH 4.0–8.0, at various concentrations (1–10 mM) and at temperatures 5, 20, and 35 °C. The products of caffeic acid oxidation were investigated by high-performance liquid chromatography (HPLC) with diode array detection. The proportion of different oxidation products formed from the caffeic acid was affected by the conditions, but the major products are formed in each case and give a constant chromatographic profile under a given set of conditions. Later products appeared to derive by further reactions. The rate of the reaction is increased by increasing pH (164×10^{-5} h^{-1} at pH 4.0 and 6020×10^{-5} h^{-1} at pH 8.0; both at 35 °C) and temperature (pH 8.0: 5 °C, 764×10^{-5} h^{-1}; 35 °C, 6020×10^{-5} h^{-1}. pH 5.0: 5 °C, 50×10^{-5} h^{-1}; 35 °C, 825×10^{-5} h^{-1}). The Arrhenius activation energy is 49.0 ± 6.4 kJ mol^{-1} at pH 8.0 for caffeic acid oxidation. The intensity of brown produced at 35 °C in 168 h was highest at pH 6, followed by pH 7 and then pH 8. Brown (420-nm) generation correlated well ($P \leq 0.001$) with caffeic acid consumption at all pH's and temperatures. Some of the oxidation products were formed at equal maximum concentrations independent of the pH but at rates that were highest at high pH. Others were dependent on pH, and the highest concentrations and rates were found at high pH. The controlling factor in the rate of autoxidation is indicated to be phenolate anion concentrations.

Polyphenols are widely distributed in the plant kingdom and are therefore present in all plant-derived food systems and most diets (Deshpande et al., 1984; Singleton, 1981). When fruits or vegetables are damaged by bruising or cutting, polyphenols are very much involved in enzymic browning reactions (Ozo and Caygill, 1986). Hydroxycinnamates such as chlorogenic and caftaric acids as well as flavonoids such as catechin serve as the principal substrates for the phenolase complex responsible for these reactions. However, processed foods with the phenolase destroyed are also susceptible to nonenzymic browning due to autoxidation reactions (Schusler et al., 1985; Singleton, 1987). These nonenzymic reactions have been much less studied, and phenolic browning has tended to be incorrectly equated only with enzymic oxidation.

Initial oxidation of compounds like caffeic acid results in formation of its quinone (Demmin et al., 1981; Fisher and Henderson, 1985). Once the quinone is formed, its strong electrophilic nature makes it very susceptible to nucleophilic attack (Cabanes et al., 1987; Macomber, 1982). After the quinones are formed, the rest of the reaction is the same whether the quinone was produced enzymically or chemically. Therefore, the study of chemical oxidation products is universally applicable to many different types of food products. *o*-Quinones formed may react further with amino acids (Wang et al., 1985) or proteins (Kalyanaraman et al., 1987) or polymerize (Takizawa et al., 1985; White and Que, 1985). Other nutrients may also be directly or indirectly affected via oxidation coupled to that of phenols (Igarashi and Yasui, 1985; Rigo et al., 1983; Scully and Hoigne, 1987; Suzuki and Itoh, 1986; Wildenradt and Singleton, 1974). Food processing that includes alkaline treatment leads to rapid phenol oxidation tying up amino acids and protein nitrogens and thus resulting in decreased nutritional value (Hurrel et al., 1982).

The same reaction takes place at an appreciable rate under acid conditions especially if metal catalysts are present (Hocking and Intihar, 1986; Speier, 1986). Chemical oxidations can even mimic enzymic reactions (Pandell, 1976, 1983). Trace elements naturally present or acquired during processing or from storage containers can catalyze these undesirable changes (in color, flavor, odor, consistency, and solubility) in the food product and

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decrease shelf life and nutritional value.

Hydroxycinnamates, especially caffeic acid derivatives, are common metabolites in the shikimic acid metabolic pathway and are widely distributed throughout the plant kingdom. They are thus present in most foods derived from plants. In fruit juices they are present because of their easy extractability in relatively high concentrations. In some instances, e.g., white grape juice, they are about the only phenols. In these products they are the crucial substrates for oxidative changes. Caffeic acid consumes oxygen rapidly under alkaline conditions (Tulyathan, 1983). In the process, color is also rapidly produced. This phenomenon was further investigated with caffeic acid as a phenol model for cinnamate derivatives to study the oxidation effects of naturally occurring esters, chlorogenic acid and caftaric acid.

Under acidic conditions the nonenzymic reaction is slow, taking weeks and months (Oszmianski et al., 1985), but can occur rapidly (minutes) under alkaline conditions (Rossi and Singleton, 1966; Tulyathan, 1983). This valuable phenomenon may aid in studying these reactions. To utilize this, the alkaline and acid conditions need to be correlated by means of their respective oxidation products.

This research utilized a model caffeic acid system to develop a better understanding of the involvement of cinnamate derivatives during oxidation in food systems under practical conditions. The nonenzymic oxidation of caffeic acid was studied at temperatures 5, 20, and 35 °C and at different pH's. These pH's were chosen to represent possible food storage conditions (usually mildly acidic conditions, pH 4 and 6) and processing of foods sometimes done at higher pH's (7 or 8 and even higher if alkaline treatments are involved).

MATERIALS AND METHODS

HPLC. A Hewlett-Packard (Santa Clara, CA) 1090M HPLC with a diode array detector was used for analytical separation. It was connected to a Hewlett-Packard 9000 series 300 ChemStation for data handling. Three wavelengths (200, 280, 320 nm) were monitored simultaneously, and scanning was done from 200 to 400 nm with the acquisition wavelength 200 nm. A Microsorb (Rainin Instrument Co. Inc., Woburn, MA) column (10 × 0.46 cm) packed with 3-μm C_{18} reversed-phase packing material was used for analysis. All separations were carried out at 40 °C. For HPLC all chemicals and HPLC solvents were from Fisher Scientific (Pittsburgh, PA) except where indicated otherwise. The water used was HPLC grade (18 Mohm) and was obtained from a Milli-Q (Millipore, Milford, MA) water purification system. The mobile phase was filtered with 0.45-μm (Gelman Sciences Inc., Ann Arbor, MI) membrane filters and degassed with helium. The mobile phase used was a gradient with starting mobile phase A as 0.05 M ammonium phosphate containing 0.001 M triethylamine. The pH was adjusted to 2.6 with 85% phosphoric acid. Mobile phase B was 80% acetonitrile and 20% mobile phase A. A linear gradient was run from 0% to 40% mobile phase B in 30 min. The column was then washed with 100% mobile phase B and the column equilibrated with mobile phase A before injecting 20 μL of the next sample.

A calibration curve was obtained by injecting 20 μL each of eight standards containing different concentrations of caffeic acid ranging from 0.025 to 4.0 mM. Injection by the autoinjector was also tested by injecting eight different volumes of 1 mM caffeic acid ranging from 0.2 to 25 μL. The calibration curves were repeated periodically to ensure satisfactory quantitation.

Oxidation of Caffeic Acid. Caffeic acid (6 mM) from Fluka (Ronkonkoma, NY) was dissolved in water by heating on a steam bath. After the mixture was cooled to room temperature, 100 mL was added to a 500-mL Erlenmeyer flask and the pH carefully adjusted with 10% KOH (AR, Mallinckrodt, St. Louis, MO) with use of a pH meter calibrated with standard buffers (pH 4.01, 7.00, and 10.00 from Mallinckrodt). The caffeic acid solution was then sparged with 100% oxygen with a sintered glass fitting. To ascertain saturation with oxygen of the solution and headspace in the Erlenmeyer flask, a Clark electrode was calibrated with 100% O_2 and sodium hydrosulfite (sodium dithionite) (Lee and Tsao, 1979). The flasks were kept at the indicated temperature with a water bath with cooling and heating temperature control (ca. ±0.1 °C maximum deviation). Stirring was accomplished in the bath with use of turbine-driven magnetic stirrers (Thomas Scientific, Swedesboro, NJ). To give similar diffusion rates of oxygen, the stirrers were connected in series to give a similar rate of stirring with the same size magnetic stirring bars.

The oxidations were carried out at pH 4.0, 5.0, 6.0, 7.0, and 8.0 at temperatures 5, 20, and 35 °C. Different concentrations of caffeic acid at 10.0, 6.0, 4.0, 2.0, and 1.0 mM were also oxidized at 20 °C and pH 7.0.

Sampling. The reaction was terminated by acidification of an aliquot in a sample vial. This was done by adding 0.5 μL of concentrated sulfuric acid to a 0.75-mL sample. To prevent precipitation and any loss of the oxidation products, 0.75 mL of 100% acetonitrile was added as the organic phase and the vial sealed. Since these two solvents prevented any precipitation in the model samples, no final filtration was necessary. All samples were analyzed shortly after sampling. Sampling was done at 0, 6, 12, 24, 48, 72, 96, 120, 144, and 168 h or at longer time intervals as indicated, especially with the 5 °C reactions.

Browning. Color change was measured at 420 nm with a Perkin-Elmer (Norwalk, CT) Lambda Array 3840 UV/vis diode array spectrophotometer connected to a Perkin-Elmer 7500 laboratory computer. Samples that were visibly dark brown were measured with 0.1-cm and the rest with 1.0-cm path length.

RESULTS AND DISCUSSION

For the quantitation of caffeic acid, linear calibration curves at 320 nm of $y = 11546x + 71$ ($r = 0.999$), at 280 nm of $y = 6975x + 46$ ($r = 0.999$), and at 200 nm of $y = 7446x + 55$ ($r = 0.999$) were obtained. Calibration curves with $r = 0.999$ were also linear for the eight different volumes injected and monitored at the different wavelengths. In this paper most values are expressed as relative peak area units at 200 nm. The use of 200 nm as opposed to 320 nm (maximum for cinnamate derivatives) enabled us to observe both cinnamate derivatives and other oxidation products that have no absorbance maximum at 320 nm. With only carboxylic acids present and the benzene ring or side chain double-bond conjugation removed, these compounds could still be monitored with sufficient sensitivity at 200 nm.

Initially the disappearance of caffeic acid at high pH was monitored by HPLC. However, when a gradient was used, several oxidation products from caffeic acid were detected. Figure 1 is representative of a typical chromatographic profile of caffeic acid (peak 3) and its oxidation products. No products were produced if oxygen was replaced with nitrogen and caffeic acid remained unchanged. All oxidation products except peak 2 and the solvent peak 1 have chromatographic elution times longer than that of caffeic acid. This would indicate that these oxidation products are less polar than caffeic acid. They are probably not ring-opened products, since ring opening of caffeic acid to carboxy derivatives would increase polarity and thereby decrease retention times in the reversed-phase system. This particular chromatogram was obtained after oxidizing caffeic acid at pH 7 and 20 °C for 96 h. It shows major oxidation products that were always present (peaks 6, 9, and 11–13) and several minor ones. Repeated HPLC on the same or replicated reaction mixtures gave essentially identical chromatograms.

The influence of pH on caffeic acid consumption throughout the experiment at 35 °C is shown in Figure 2. After 6 h, at 35 °C and pH 8.0, caffeic acid consumption was very rapid and 76% had already disappeared (as calculated by relative HPLC peak area units at 200 nm). At pH 4.0, only 1% of the caffeic acid was consumed in the 6-h period. The caffeic acid consumption rate was very

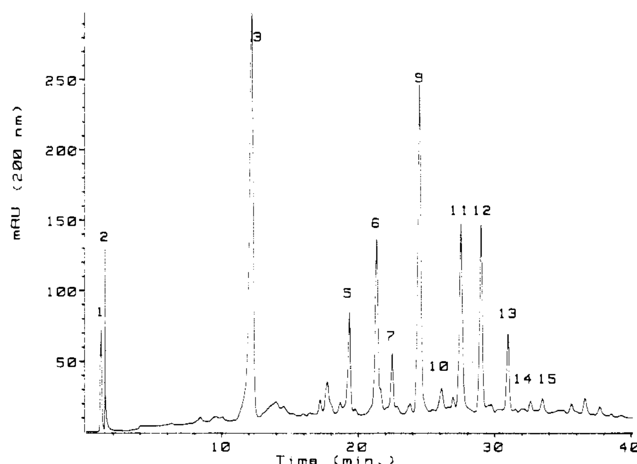

Figure 1. Oxidation of caffeic acid (6 mM) at pH 7.0 at 20 °C after 96 h in 100% oxygen. Analysis of a 20-μL sample by C_{18} reversed-phase HPLC using mobile phase A, ammonium phosphate (0.05 M, pH 2.6) containing 0.001 M triethylamine, and mobile phase B, 80% acetonitrile in mobile phase A. Detection at 200 nm. A linear gradient was applied from 0 to 40% mobile phase B in 30 min. Peak 3 = caffeic acid. The rest of the peaks are oxidation products.

Table I. Rate Constants ($\times 10^{-5}$ h^{-1}) for the First-Order Reaction of Caffeic Acid Oxidation at 5, 20, and 35 °C at pH 4.0–8.0[a]

	35 °C		20 °C		5 °C	
pH	r (n)	rate	r (n)	rate	r (n)	rate
4	0.995 (10)	164	0.940 (10)	50		
5	0.977 (10)	825	0.996 (10)	90	0.939 (15)	50
6	0.984 (10)	2280	0.982 (10)	360	0.999 (15)	332
7	0.982 (8)	3090	0.995 (10)	1150	0.990 (15)	456
8	0.935 (6)	6020	0.985 (10)	2220	0.964 (15)	757

[a] Key: r = correlation coefficient of the curve fitted on the data; (n) = number of data points used in calculating the first-order rate constant; rate = first-order rate constant value in table × 10^{-5}.

rapid at pH 8 (first-order reaction rate 0.060 20 h^{-1}) but much slower at pH 4 (rate 0.001 64 h^{-1}) even though 100% oxygen was present in both cases. These reaction rates are given in Table I. With air these rates would of course be one-fifth as rapid. The large effects of pH and temperature on reaction rate can be seen. This pH dependency indicates the involvement of the reactive phenolate ion in this reaction. The correlation coefficient of the curve fitted to the ln (A/A_0) data is given and the number of data points used (only those where caffeic acid was still present). Even though zero-order curves could fit the data at the slower reacting low pH's and temperatures, they were calculated as first-order reactions since it is so difficult to distinguish between the two reaction orders if the reaction did not utilize much more than 50% of the substrate. The Arrhenius activation energy (kJ mol^{-1}) for caffeic acid oxidation was 49.0 ± 6.4 (r = 0.999) at pH 8.0, 45.1 ± 29 (r = 0.999) at pH 7.0, 44.4* at pH 6.0 (r = 0.864), and 65.8* at pH 5.0 (r = 0.939; asterisk indicates $P \leq 0.05$). At pH 4.0 with the two available reaction rate constants, an activation energy of 59 kJ mol^{-1} is calculated. These constant activation energies at all pH's indicate the same mechanism of normal temperature-dependent autoxidation of caffeic acid. Another factor apparently limiting this reaction is the phenolate ion concentration which is low at low pH.

Profiles of the oxidation products are relatively similar at all pH's, especially pH 5.0–8.0, although proportionate amounts and ratios varied. The same observation was made at different temperatures and replicated many times

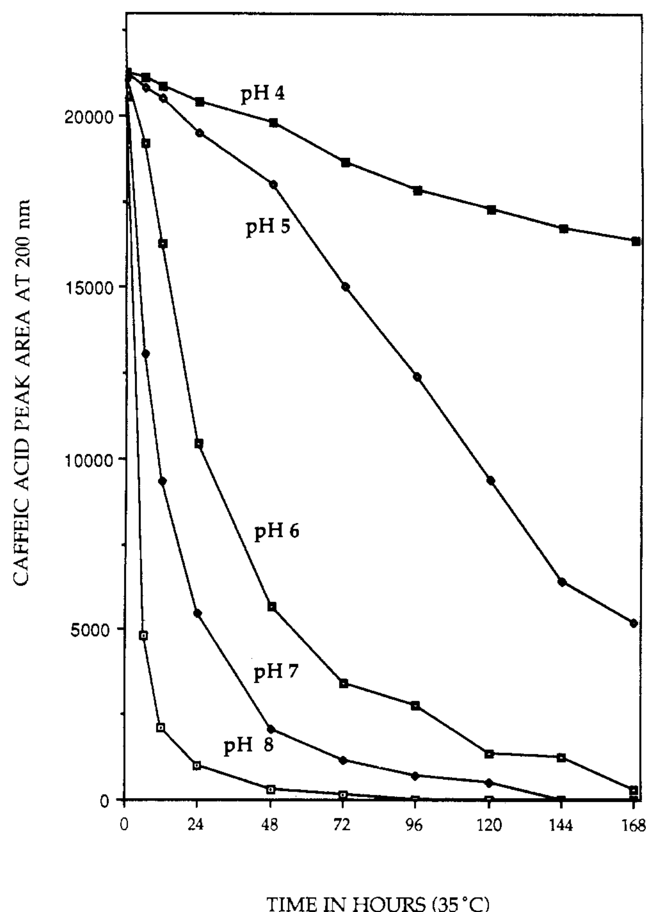

Figure 2. Oxidation of caffeic acid (6 mM) at pH 4.0, 5.0, 6.0, 7.0, and 8.0. The oxidation was carried out at 35 °C over a 1-week period in a solution saturated with 100% oxygen. The data points were obtained by HPLC separation and detection at 200 nm. The HPLC conditions are described in Figure 1.

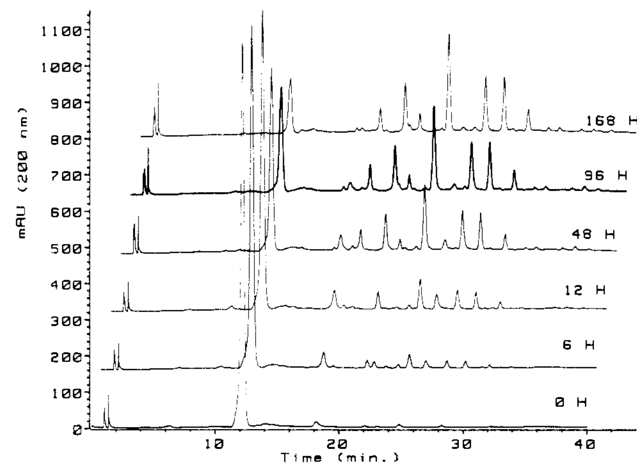

Figure 3. Oxidation of caffeic acid (6 mM) with respect to time. Stacked plot with chromatograms of oxidized caffeic acid sampled at the indicated time intervals. The oxidation was carried out at pH 7.0 and 35 °C in 100% oxygen. The HPLC conditions are described in Figure 1.

by both repeated injection of the same sample and replicated samples from different experiments. Again, the oxidative reaction and the production of these products are slowed as pH is lowered and when oxygen is decreased.

The oxidation of caffeic acid (6 mM) as it progressed in the 168-h period at pH 7.0 and 20 °C is presented by stacked plots in Figure 3. The times of sampling are indicated on the plot. In this figure the decreases in caffeic

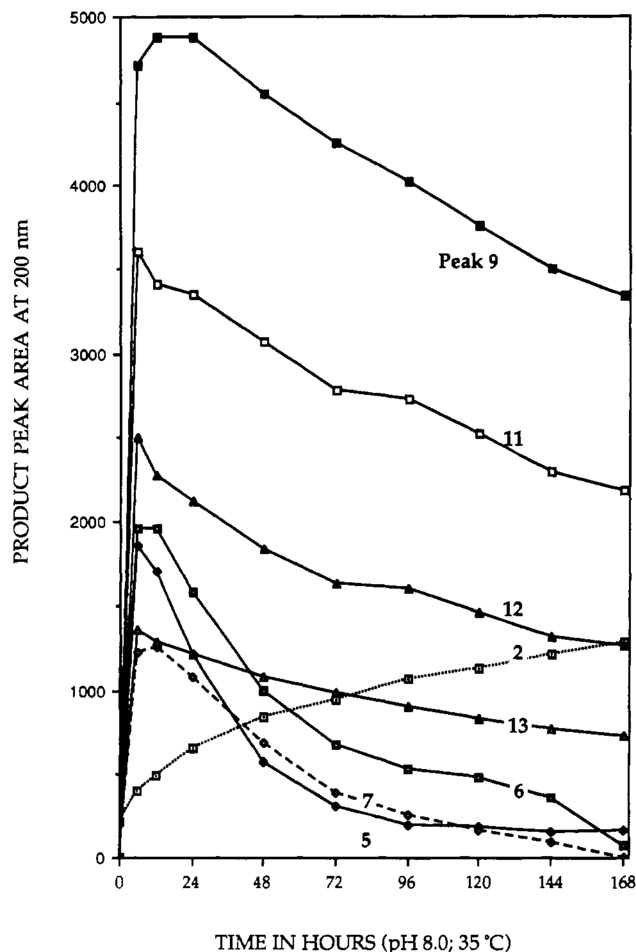

Figure 4. Oxidation products formed from the oxidation of caffeic acid (6 mM) at pH 8.0 and 35 °C. Detection was done at 200 nm, and the HPLC conditions are described in Figure 1.

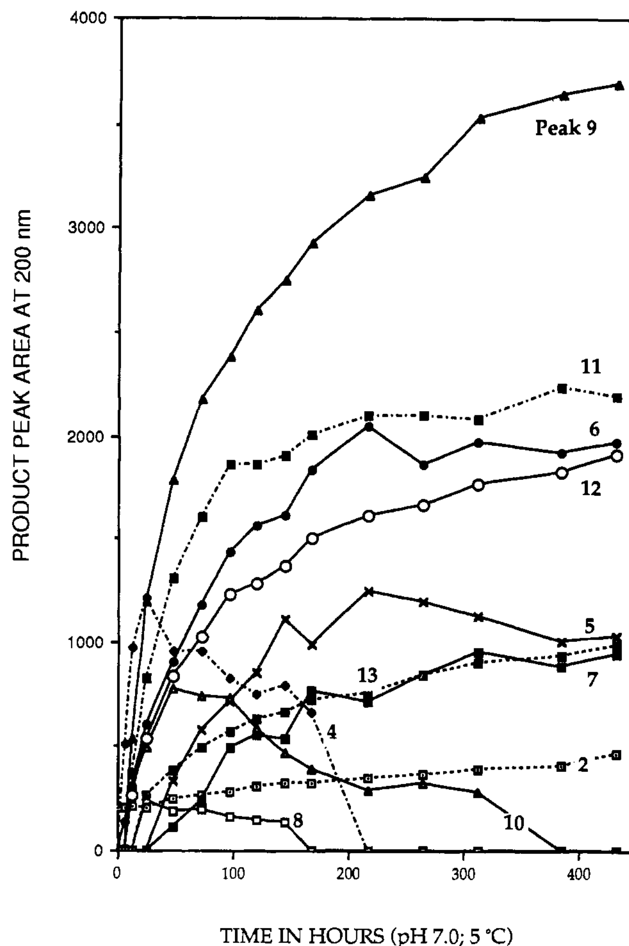

Figure 5. Oxidation products formed from the oxidation of caffeic acid (6 mM) at pH 7.0 and 5 °C. Detection was done at 200 nm, and the HPLC conditions are described in Figure 1. The reaction was followed for about 2.6 weeks (432 h) and sampled every 24 h.

acid with time and increases in oxidation products as well as the disappearance of certain intermediates are evident. The consistency in the chromatographic profile is typical.

The data from an oxidation carried out at pH 8.0 and 35 °C are quantified and presented in Figure 4. This graph shows the rapid appearance of the oxidation products in the early stages of oxidation. At the first data point obtained after 6 h, 76% of the caffeic acid was already oxidized. After 95% of the caffeic acid was oxidized at 24 h, the oxidation products also started to decrease. Presumably the net disappearance results from conversion to additional products not identified as new peaks. All these product peaks decrease except for peak 2, which continuously increased as oxidation progressed. This might be indicative of the formation of a final oxidation product, which might involve ring opening, especially if the high polarity of peak 2 is considered.

Data presented in Figure 5 were obtained from oxidation of caffeic acid at pH 7 and 5 °C. It should be noted that caffeic acid remained even after 432 h of oxidation in 100% oxygen. These conditions would represent an intermediate reaction rate where caffeic acid is consumed over a period of several days. The high correlation coefficients ($P \leq 0.001$) at all pH's and temperatures indicate the direct involvement of caffeic acid in the formation of peaks 6, 9, and 11–13. The initial products are produced in constant ratios. Some of these products, however, are derived by further reactions, e.g. peaks 2, 14, and 15, and only give higher concentrations at the later stages of oxidation. Peaks 4, 8, and 10 become high relatively early and then disappear.

Different concentrations of caffeic acid ranging from 1 to 10 mM were chosen to represent concentrations found in food samples. Typically 1 mM caftaric acid would be present in grape juice and 1 mM chlorogenic acid in other food products. The 10 mM would represent the highest concentrations of cinnamate-type phenols found in food systems such as coffee. Another purpose of this experiment was to establish whether the same types of oxidation products are still formed at higher concentrations or whether other mechanisms of polymerization occur. To illustrate the effect of concentration on the formation and proportion of oxidation products, data from the 1–10 mM concentrations of caffeic acid were analyzed after they were normalized. This is done by expressing all the data as 1 mM, that is to divide the data by the particular concentration used. The five data points calculated from the five different concentrations were then pooled and the mean, standard error, and the coefficient of variation calculated and presented in Table II.

The pH was monitored at each sampling and was found not to change in the 70 h. This indicates that little open-ring products or carboxylic acids were formed through oxidation under these conditions. This also eliminated the need to buffer the solutions. It was also found that even with the concentration varying over 1 order of magnitude, the same types of oxidation products were still formed. The coefficients of variation over this concentration range were typically in the order of 10% as indicated in Table II. They did vary more for certain peaks, but no new peaks were observed and the qualitative

Table II. Effect of Various Concentrations of Caffeic Acid on Oxidation Carried Out at 20 °C and pH 7.0 (All Data Values Normalized to 1 mM)[a]

	6 h	12 h	24 h	48 h	72 h
caffeic	3130 (55) [4]	2674 (78) [7]	2115 (94) [10]	1483 (85) [13]	1005 (79) [17]
peak 9	198 (17) [11]	301 (20) [15]	483 (23) [12]	580 (34) [13]	677 (36) [12]
peak 11	138 (12) [20]	230 (19) [18]	335 (15) [10]	412 (24) [12]	449 (30) [15]
peak 12	107 (4) [9]	150 (5) [8]	217 (10) [11]	281 (15) [12]	344 (20) [14]
peak 13	85 (4) [12]	106 (9) [19]	145 (17) [26]	179 (23) [28]	208 (27) [29]
sum	662 (21) [8]	925 (33) [8]	1329 (63) [11]	1683 (85) [12]	1946 (104) [12]
brown	0.05 (0.001) [5]	0.08 (0.004) [12]	0.15 (0.01) [15]	0.26 (0.01) [12]	0.35 (0.01) [9]

[a] The mean values are given with the standard error in parentheses and the coefficient of variation in brackets. In all cases $n = 5$ data points that were pooled. Sum of products = total of peak areas of products (excluding any remaining caffeic acid).

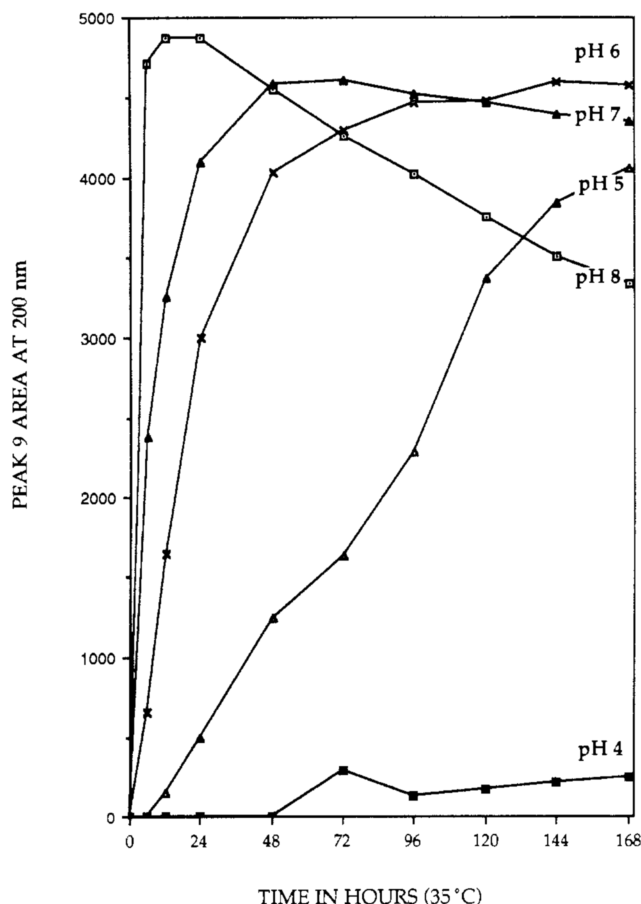

Figure 6. Effect of pH 4.0–8.0 on the formation of oxidation product peak 9 at 35 °C. Detection was done at 200 nm, and the data of samples taken at different times are presented.

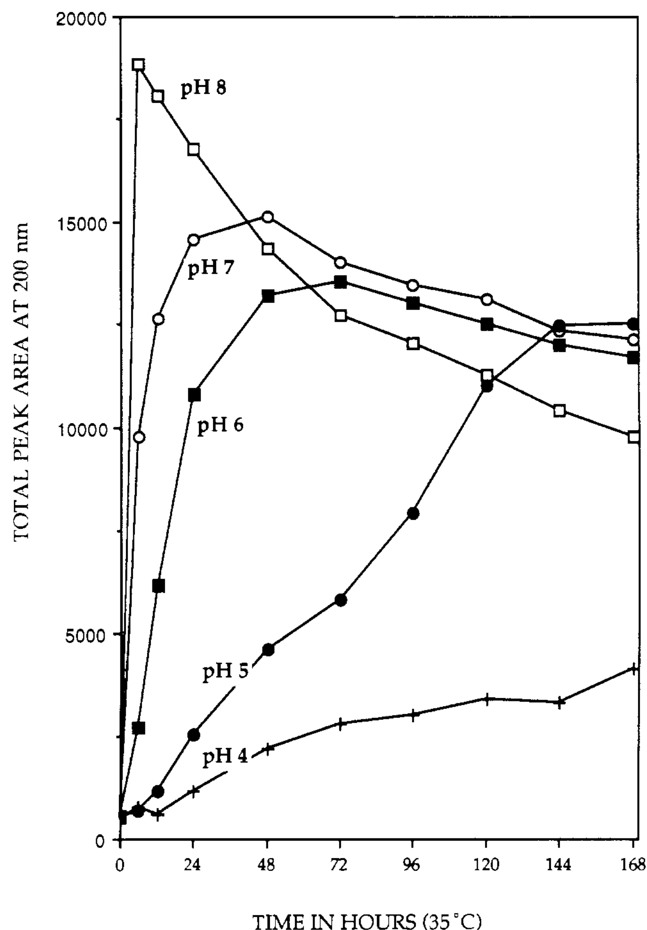

Figure 7. Effect of pH 4.0–8.0 on the peak area sum obtained at 200 nm of all oxidation products formed from caffeic acid with respect to time at 35 °C. The peak area of caffeic acid is excluded from this calculation. The pH values are indicated on the graph.

chromatographic profile remained constant. From Table II it can be concluded that the proportions of oxidation products formed over this concentration range were also constant. When the data were graphed, it was also evident that the changes observed were random and no trend could be seen except for the proportional increase in concentration of the oxidation products, which was dependent on the initial caffeic acid concentration. These data also confirm that the oxygen supply was adequate at all concentrations throughout the experiment. The small differences between the different caffeic acid concentrations are also reflected in the coefficient of variation of ca. 10% in the products' sum total.

The proportion of certain oxidation products varied depending on the oxidation conditions. Peak 9 was formed in highest concentration and produced in equal amounts from pH 5–8 (Figure 6). Only the time varied when the maximum concentration was reached. This rate was pH dependent, and the lower the pH, the longer it took peak 9 to reach its maximum. Similar behavior was found for peak 6. The proportion of peak 7 formed was very dependent on the pH. At pH 8 its highest concentration formed, and all lower pH's gave lower concentrations and slower rates. The pH dependency observed could be indicative of the involvement of the phenolate anions in the formation of these products, which will explain their high concentration at high pH.

When the total HPLC peak areas of oxidation products (at 200 nm excluding caffeic acid) are summed for a particular treatment, the product sum value was obtained. When these values are plotted as shown in Figure 7, it is evident that the total amount of oxidation products formed was also dependent on pH. More formed at higher pH's. Since more products with absorbance at 420 nm are produced at lower pH, it must be concluded that other kinds of oxidation products form more color. These products could be higher molecular weight compounds not shown by the HPLC conditions applied. It is unlikely that these

Table III. Correlation Coefficients (r) of Caffeic Acid Consumed (Peak Area Measured at 200 nm) with Brown (Measured at 420 nm) Formed[a]

pH	temp, °C 5	20	35
4	ppt	0.850+ (10)	0.946* (10)
5	0.873* (15)	0.925* (10)	0.952* (10)
6	0.963* (15)	0.988* (10)	0.972* (10)
7	0.973* (15)	0.985* (10)	0.979* (7)
8	0.946* (9)	0.956* (9)	tf

[a] Key: ppt = caffeic acid precipitated due to the low temperature and low pH, no data points obtained; tf = reaction too fast, not enough data points, all caffeic acid consumed and data not used. Number of data points used for calculating the correlation coefficient are given in parentheses. For these correlation values only data points were used if caffeic acid was still present in excess of 5% of the original concentration. Key: +, $P \leq 0.01$; *, $P \leq 0.001$.

oxidation products have only little UV absorption. It is, however, possible that many different products are produced in small amounts. If one makes the assumption that the products have the same molar extinction as caffeic acid at 200 nm, the initial product sum is slightly greater than the original caffeic acid and then falls to about 75% of the original. This gives confidence that we are accounting for most of the consumed caffeic acid. As the products are isolated and characterized, a better recovery balance will be obtained.

Products having absorbance at 420 nm are produced in the initial stages of oxidation of caffeic acid. Most were not produced toward the end as expected, but throughout the reaction as indicated in Figure 8. Color produced at 420 nm correlated well ($P \leq 0.001$) with caffeic acid consumed at all temperatures and pH's (except at the low pH's and temperatures as indicated in Table III). Correlations were calculated only with data points in which there was still caffeic acid remaining. These correlation coefficients (r) are presented in Table III. The amount of pigmentation eventually produced was higher at pH 7 and even higher at pH 6 than at the pH 8 reaction in which less brown was produced but at a faster rate. Due to the much slower formation of brown at pH 4 and 5, the experiment was stopped before brown production reached a maximum.

Molecules with sufficient conjugation to give absorbance at 420 nm did not form only in the later stages of the reaction. The fact that color at 420 nm correlated so well with caffeic acid consumption at all temperatures and pH's indicates the formation of smaller "polymer" molecules with enough conjugation to produce color. Caffeic acid oxidation is thus directly involved in the formation of brown oxidation products. These results also indicate that more conjugated products form at lower pH's (6 and 7) and slower reaction rates, which leads to more color production. This could be due to a higher degree of polymerization or the formation of products with a higher extinction coefficient.

This research showed that caffeic acid oxidation is very pH dependent and extremely rapid at pH 8.0 and higher. The pH dependency of this reaction and the relatively constant low Arrhenius activation energies at all pH's on the order of 50 kJ also indicate that phenolate ions are involved. Caffeic acid consumption is directly correlated to the formation of color at all pH's and temperatures. Oxidation products that are formed directly from caffeic acid were detected due to their stability at varying conditions and high correlation with caffeic acid consumption. Other products changed through secondary reactions (peaks 2, 4, 8, and 10) as evidenced by disappearance or

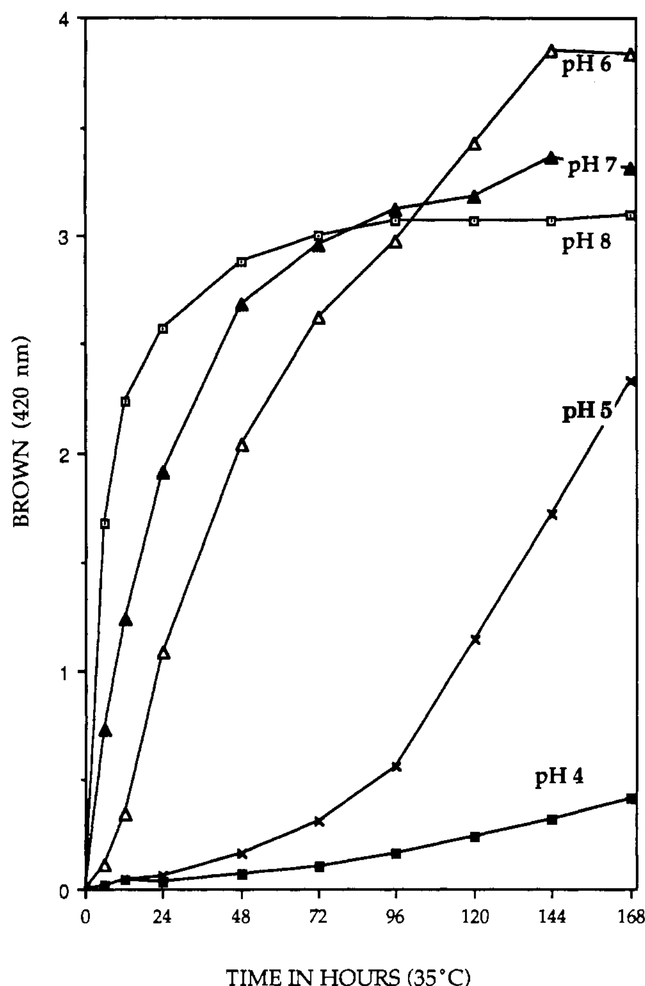

Figure 8. Effect of pH 4.0–8.0 on brown produced with the oxidation of caffeic acid (6 mM) at 35 °C. The brown is measured spectrophotometrically at 420 nm.

late accumulation. The oxidation of caffeic acid at different concentrations, pH's, and temperatures gave similar chromatographic profiles in which proportions and ratios of some of the products varied, but others remained relatively constant (peaks 6, 9, and 11–13). This oxidation reaction, which is slow under acid conditions, as usually encountered in foods and beverages, can thus be conveniently studied at a higher pH for shorter reaction times. Food undergoing processing at a higher pH will be susceptible to phenolic oxidation even if the treatment is just for short periods. Caffeic acid derivatives are also involved in phenolic autoxidative reactions under storage conditions even at low pH's and temperatures. Although this reaction is slowed under acidic conditions, it can be appreciable during prolonged storage of processed food.

Structural characterization of these oxidation products is currently under way by UV/vis spectrophotometry, ^{1}H and ^{13}C NMR, and mass spectrometry. As a next step, numerical analysis will be done on the data obtained at the different pH's and temperatures to provide more kinetic data.

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