Degradation of Cyanidin 3-Glucoside by Caffeic Acid *o*-Quinone. Determination of the Stoichiometry and Characterization of the Degradation Products

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Caffeic acid *o*-quinone (CQ) was prepared by oxidation of caffeic acid with *o*-chloranil in organic media. The reaction between the purified CQ and cyanidin 3-glucoside (Cy 3-glc, *o*-diphenolic anthocyanin) was monitored by HPLC, and quantitative analyses were performed to establish the stoichiometry of the reaction. The results indicate that Cy 3-glc is degraded by a coupled oxidation mechanism with integration of CQ into the degradation products. The ratio of degraded Cy 3-glc to CQ incorporated into the condensation products was ~2.0. No brown products could be detected, only a slight orange color. Moreover, the addition of purified polyphenol oxidase to the slightly colored media resulted in the disappearance of the caffeic acid formed from the reaction of coupled oxidation (Cy 3-glc/CQ) and the formation of brown polymers. The degradation products were isolated by gel filtration on Sephadex G-25. The UV−vis spectra and chemical analysis (acidic hydrolysis) of the degradation products suggest that they resulted from the condensation of caffeic acid and Cy 3-glc. HPLC analysis showed that the partial purified fraction contained a mixture of complex condensation products.

Keywords: *Caffeic acid o-quinone; anthocyanins; stoichiometry; degradation products; partial characterization*

INTRODUCTION

Browning reactions that occur after fresh blueberries (*Vaccinium corymbosum*) have been crushed are due mainly to polyphenol oxidases (PPOs) (Kader et al., 1997b). Chlorogenic acid (CG) is the major hydroxycinnamic derivative found in blueberries (Kader et al., 1996), and this *o*-diphenolic compound is a good substrate for blueberry PPO (Kader et al., 1997a). The first reaction is the oxidation of chlorogenic acid by PPO to its *o*-quinone (chlorogenoquinone, CGQ), followed by the reaction of the enzymatically generated *o*-quinone with the anthocyanins to form brown polymers (Kader et al., 1997b). Because of the complexity of the blueberry anthocyanin extracts, model systems were useful in the study of the mechanims of anthocyanin degradation by PPO in the presence of CG. The results showed that the enzymatically generated CGQ oxidizes Cy 3-glc (*o*-diphenolic anthocyanin) by a coupled oxidation mechanism with partial regeneration of the CG, which means that part of the CG is incorporated into the degradation products of the Cy 3-glc (Kader et al., 1998). Pelargonidin 3-glucoside (Pg 3-GLC, non-*o*-diphenolic anthocyanin) might react with the quinones or secondary products of oxidation (formed from the CGQ) to form adducts (Kader et al., 1999a). Sarni et al. (1995) studied the degradation of Cy 3-glc and malvidin 3-glucoside (Mv 3-glc) in the presence of caftaric acid and grape PPO. They have shown that the products of anthocyanin (Cy 3-glc and Mv 3-glc) degradation contain both anthocyanin and caftaric acid moieties.

The use of model systems has shown that the enzymatically generated *o*-quinones play an essential role in the process of anthocyanin degradation. However, this approach does not allow determination of the exact mechanisms involved in the degradation of both *o*-diphenolic and non-*o*-diphenolic anthocyanins. Pure solution of quinones should be useful in understanding the mechanisms of anthocyanin degradation. The preparation of pure quinones by chemical oxidation should allow simplification of the model reactions. The reaction between *o*-quinones and anthocyanins could facilitate both the isolation of the reaction products and the study of their structure to understand the mechanism of anthocyanin degradation by PPO. However, the synthesis of *o*-quinones is not easy because of their instability in aqueous media.

Sarni-Manchado et al. (1997) studied the reaction of Mv 3-glc with caftaric acid *o*-quinone. Liquid chromatography with ion spray mass spectrometry indicates that the products of degradation are adducts of Mv 3-glc and caftaric acid. The results show also that the hemiacetal forms of the pigment are more reactive than the flavylium form. Recently, we investigated the reaction between anthocyanins and *o*-chloranil (Kader et al., 1999b) and demonstrated that the reaction proceeds in two steps. The first is characterized by the discoloriza-

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tion of the anthocyanin solution accompanied by the formation of three products of combination (anthocyanin−chloranil). Whatever the pigment studied (*o*-diphenolic anthocyanin or non-*o*-diphenolic anthocyanin), this step is characterized by a ratio of degraded anthocyanin to *o*-chloranil of close to 1. The results showed that the carbinol−pseudobase form reacts with *o*-chloranil. During the second step of the reaction (for *o*-chloranil to anthocyanins ratios >1) the colorless combinations react readily with *o*-chloranil to yield brown condensation products. Chloranil was chosen because (i) this *o*-quinone is stable in aqueous media, (ii) it is available commercially, (iii) it has a high redox potential, and (iv) it is totally substituted, which impedes the effect of water (nucleophilic addition). However, the results suggested that the reaction mechanism is different from those described for the PPO-catalyzed oxidation of anthocyanins in the presence of *o*-diphenolic substrates such as chlorogenic and caftaric acids (Kader et al., 1998, 1999a; Sarni-Manchado et al., 1997; Sarni et al., 1995).

The purpose of this work was to study the reactions between *o*-quinones (synthesized from natural phenolic compounds) and anthocyanins. The CQ was prepared from caffeic acid by oxidation with *o*-chloranil and purified by HPLC on the semipreparative scale. The reaction between CQ and Cy 3-glc was monitored by HPLC to determine the stoichiometry of the reaction. The products of the reaction were separated by gel filtration and characterized by their UV−vis spectra.

MATERIALS AND METHODS

Chemicals. Cy 3-glc (Kuromanin) was of HPLC grade from Extrasynthèse (Genay, France). Sephadex G-25 (fine, particule size = 20−80 μm) was from Pharmacia (Uppsala, Sweden). *o*-Chloranil was obtained from Aldrich (Strasbourg, France). Caffeic acid, ascorbic acid, trifluoroacetic acid (99.5% of purity), orthophosphoric acid (minimum 85% purity), and benzenesulfinic acid were obtained from Sigma Chemicals (St. Quentin Fallavier, France). Methanol (HPLC grade), acetic acid (99.5% purity), chloroform (99.5% purity), ethyl acetate (99.5% purity), propan-2-ol (analytical grade), and all other chemicals were of reagent grade from Merck (Darmstadt, Germany). Blueberry PPO (0.194 nkat mL^{-1}) was obtained as described by Kader et al. (1997a). Cy 3-glc (2 mM) was dissolved in McIlvaine buffer (pH 3.5). This buffer was prepared from 0.1 M citric acid adjusted to the correct pH by adding 0.2 M dibasic potassium phosphate.

Preparation and Purification of the CQ. Caffeic acid and *o*-chloranil solutions were prepared in anhydrous methanol. CQ was prepared from caffeic acid by oxidation with *o*-chloranil at 25 °C. The reaction mixture contained 0.2 mL of 50 mM caffeic acid, 0.2 mL of 100 mM *o*-chloranil, and 1.6 mL of chloroform. The reaction was initiated by adding the *o*-chloranil solution. The CQ formed was purified by HPLC. After 2.5 min of reaction, 1.0 mL of the oxidized solution was analyzed by HPLC on the semipreparative scale. The HPLC apparatus was a Spectra Physics system including an SP 8000 ternary HPLC pump, a manual injector (Reodyne, Model 7010 sample injection valve), and a Spectra 100 variable-wavelength detector set at 400 nm and connected to a Chromojet integrator. The constituents of the reaction mixture were separated on a Nucleosil C_{18} (10 μm packing), 250 × 7.5 mm, column protected by a guard cartridge of the same packing. Elution conditions: solvent A, 2.5% acetic acid in water (v/v); solvent B, 80% acetonitrile in solvent A (v/v); flow rate, 1.5 mL min^{-1}. The gradient profile was 0−5 min, 5% B; 5−20 min, 5−20% B; 20−35 min, 20−40% B; 35−45 min, 40−100% B. The CQ was collected and used immediately.

Model System of the Degradation of Cy 3-glc by CQ. The reaction mixture contained 0.1 mL of 1 mM Cy 3-glc, 0.250 mL of 0.235 mM CQ, and 0.650 mL of McIlvaine buffer (pH 3.5). To determine the CQ concentration, the reaction mixture contained 0.250 mL of CQ, 0.050 mL of ascorbic acid or sodium benzenesulfinate (20 mM in McIlvaine buffer, pH 3.5), and 0.7 mL of McIlvaine buffer (pH 3.5). After 5 min of reaction, 0.1 mL of the reaction mixture was analyzed by HPLC (Merck-Hitachi L-6200 Intelligent pump equipped with a diode array detector Merck-Hitachi L-3000 connected to a Chromojet integrator). The constituents of the medium were separated on a Lichrosorb 100 RP-18 reversed phase column (250 × 4 mm i.d., 5-μm packing) (Merck, Darmstadt, Germany) protected by a guard cartridge of the same material. The mobile phases were the same as described above. The gradient profile was 0−5 min, 5% B; 5−20 min, 5−40% B; 20−35 min, 40−80% B; 35−45 min, 80−100% B. Elution was performed at a flow rate of 1.0 mL min^{-1}, and 0.1 mL of the reaction mixture was injected using a Basic$^+$ Marathon automatic injector (Spark Holland). Caffeic acid and Cy 3-glc were detected at 280 nm.

The CQ was determined in equivalents of caffeic acid by reduction of the quinone by ascorbic acid. Caffeic acid and Cy 3-glc concentrations were determined by HPLC as described above using a calibration curve ranging from 0.01 to 0.1 mM. For each assay two analyses were conducted on duplicate experimentations. Each data point is therefore the mean of four measurements.

Purification of the Reaction Products. The reaction mixture contained 0.4 mL of McIlvaine buffer (pH 3.5), 0.4 mL of 1 mM Cy 3-glc, and 2.0 mL of 0.2 mM CQ. The reaction was started by adding the CQ solution. After a reaction time of 5 min, the solvent was removed under reduced pressure using a rotary evaporator at 25 °C. The resulting solution was applied onto a Sephadex G-25 column (15 × 3 cm i.d.), previously equilibrated with distilled water, at a flow rate of 15 mL h^{-1}. The reaction products were eluted with distilled water, and the absorbances at 280 and 325 nm were recorded on each 3-mL fraction.

Characterization of the Reaction Products. The UV−vis spectra were recorded from 220 to 600 nm using a Shimadzu UV-260 spectrophotometer. The reaction products (0.5 mg) occurring in the fractions C1 were hydrolyzed by heating at 85 °C in 3.0 mL of 2 M trifluoroacetic acid in methanol under nitrogen for 45 min. The hydrolyzed solutions were cooled, and 2.0 mL of water was added. Methanol and trifluoroacetic acid were removed under vacuum at 35 °C. The residual aqueous solution was extracted four times with ethyl acetate (1:1, v/v). The aqueous phase was concentrated in a rotary evaporator under vacuum at 35 °C. The resulting solution (0.2 mL) was chromatographed to identify glucose on a silica gel plate (Merck, ref 5553) with the propan-2-ol/ethyl acetate/water (50:40:10, v/v/v) system. The glucose was located on TLC plates by spraying with 2% (w/v) naphthoresorcinol solution in acetone and 9% orthophosphoric acid (5:1, v/v) followed by heating in an oven at 105 °C for 10 min.

HPLC of the Partially Purified Products. Fraction C1 was analyzed by HPLC using the same apparatus and column as described for the degradation of Cy 3-glc by CQ. The elution conditions were as follows: solvent A, 1% acetic acid in distilled water (v/v); solvent B, methanol; flow rate, 1.0 mL min^{-1}; linear gradients from 0 to 100% B in 50 min; 0.2 mL of the reaction mixture was injected using a Basic$^+$ Marathon automatic injector (Spark, Holland). The reaction products were detected at 280 nm.

RESULTS AND DISCUSSION

Synthesis and Isolation of the CQ. CQ was prepared by chemical oxidation of caffeic acid by *o*-chloranil in organic media (chloroform). We followed the method described by Davies (1976) for the synthesis of CQ and CQ methyl ester. The CQ was isolated by semipreparative HPLC. The UV−vis spectra of the purified CQ and caffeic acid were recorded from 240 to 500 nm (Figure 1). The CQ exhibited three absorption maxima at 248,

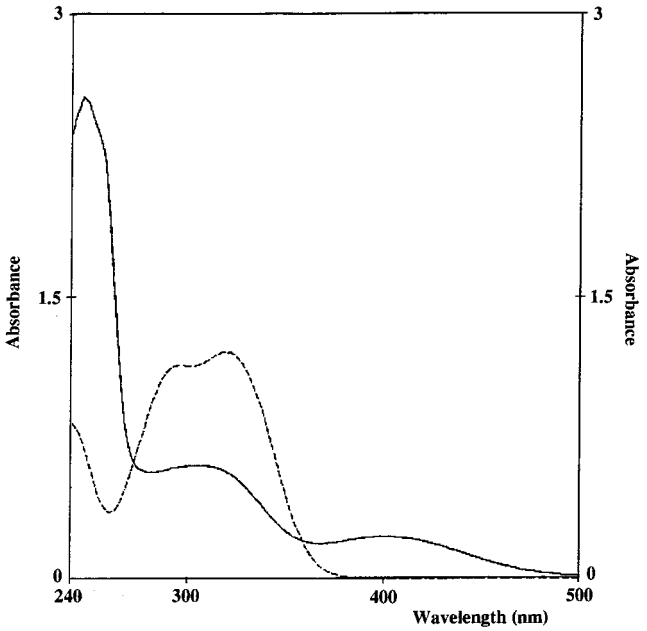

Figure 1. UV−vis spectra of caffeic acid (dashed line) and the purified caffeic acid *o*-quinone (solid line) collected after elution from the HPLC column.

300−330, and 400 nm. The UV−vis spectrum was very close to that published by Cheynier and Moutounet (1992) for the CQ obtained by enzymatic oxidation of caffeic acid at pH 3.6. *o*-Quinones formed by enzymatic and chemical oxidation of *o*-diphenols can also be characterized through the formation of their stable phenylsulfonyl derivatives (reaction 1) (Davies and Pierpoint, 1975). The addition of sodium benzenesulfi-

$$\text{CQ} + \text{benzenesulfinic acid} \rightarrow \text{caffeic acid benzenesulfone} \quad (1)$$

nate to the *o*-quinone solution (yellow coloration) resulted in an instantaneous discoloration of the solution. The *o*-quinone solutions treated with sodium benzenesulfinate were analyzed by HPLC on the analytical scale. The elution profile showed the presence of trace amounts of caffeic acid along with the expected phenylsulfonyl peak. This result suggests that spontaneous reduction of the CQ can take place. The amount of caffeic acid detected in the *o*-quinone solutions is relatively constant, ranging from 2 to 3 μM. Cheynier and Moutounet (1992) observed the same phenomenon after analyzing a solution of CQ by HPLC. In fact, the formation of caffeic acid can probably be explained by dismutation of the CQ in caffeic acid and hydroxycaffeic acid rather than by a process of spontaneous reduction of the CQ (Richard-Forget at al., 1992).

In the presence of excess reducing agents such as ascorbic acid, *o*-quinones are reduced to their original *o*-diphenols (reaction 2). The reaction is stoichiometric

$$\text{CQ} + \text{ascorbate} \rightarrow \text{caffeic acid} + \text{dehydroascorbate} \quad (2)$$

with ascorbic acid (Rouet-Mayer et al., 1990), and consequently this property can be used to determine the CQ concentration. This was achieved by taking samples in duplicate and stabilizing one of them by adding ascorbic acid (1 mM) to reduce the CQ back to the corresponding hydroquinone (caffeic acid) and adding sodium benzenesulfinate (1.0 mM) to the other to trap

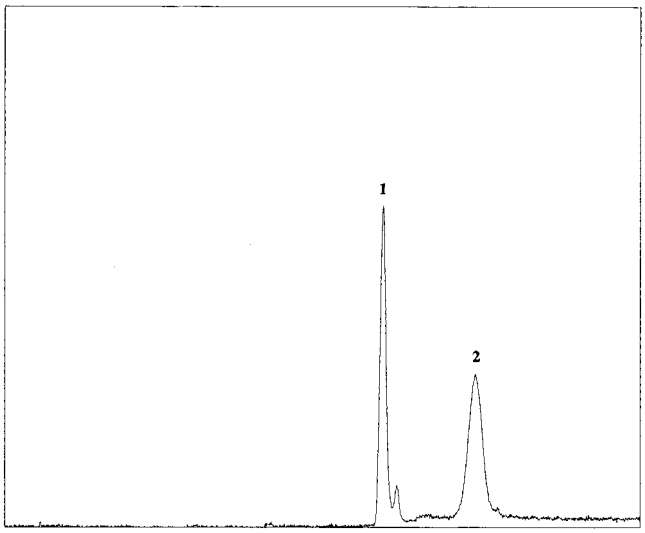

Figure 2. HPLC elution profile at 280 nm of a solution of Cy 3-glc (0.1 mM) incubated for 5 min with the purified CQ (0.06 mM) in McIlvaine buffer (pH 3.5). Peak 1, caffeic acid; peak 2, remaining Cy 3-glc.

CQ as the corresponding phenyl sulfone (Cheynier and Ricardo da Silva, 1991). The amount of CQ was then calculated as the difference between the caffeic acid concentrations in the ascorbic acid and benzenesulfinate samples.

Degradation of Cy 3-glc by CQ in McIlvaine Buffer (pH 3.5). The addition of CQ to a Cy 3-glc solution resulted in the red color disappearing and the concomitant formation of a slight orange color. The reaction mixture was analyzed by HPLC at 280 nm (Figure 2). The chromatogram shows that part of the pigment has disappeared and that a new product (peak 1) has formed with an elution time shorter than that of Cy 3-glc (peak 2). This new peak has been identified as caffeic acid on the basis of its retention time and UV−vis spectrum (Figure 1). This result shows that Cy 3-glc is oxidized by CQ, leading to the formation of caffeic acid according to reaction 3. It is highly probable that

$$\text{CQ} + \text{Cy 3-glc} \rightarrow \text{caffeic acid} + \text{Cy 3-glc o-Q} \quad (3)$$

Cy 3-glc degradation proceeds by a mechanism of coupled oxidation as proposed by Kader et al. (1998) and Sarni et al. (1995). Additional experiments were carried out to characterize the Cy 3-glc *o*-Q formed during this reaction process (reaction 3). Thus, sodium benzenesulfinate was added to the discolored reaction mixture to trap the Cy 3-glc *o*-Q, which can then be detected as the corresponding phenyl sulfone by HPLC. However, no Cy 3-glc phenyl sulfone was detected in the reaction mixture, which means that Cy 3-glc *o*-quinone is very reactive and proceeds readily to condensation products, as mentioned by Sarni et al. (1995). No brown color was observed in the reaction mixture, which means that the degradation products of the Cy 3-glc are not involved in the formation of brown polymers as observed previously by Kader et al. (1998). However, the addition of blueberry PPO induced the formation of brown polymers. The HPLC analysis under the same conditions showed that the caffeic acid had disappeared from the reaction mixture. This could indicate that the browning reaction is mainly due to the condensation products of caffeic acid oxidation (PPO). On the other hand, the degradation products of Cy 3-glc are slightly colored.

Table 1. Oxidation of Cy 3-glc by CQ in McIlvaine Buffer (pH 3.5) in Different Reaction Conditions[a]

expt	initial amount of Cy 3-glc, μmol	initial amount of CQ, μmol	amount of caffeic acid formed, μmol	amount of remaining Cy 3-glc, μmol	theoretical amount of Cy 3-glc *o*-Q formed, μmol
I	0.1 ± 0.005	0.047 ± 0.003	0.033 ± 0.003	0.067 ± 0.006	0.033
II	0.075 ± 0.004	0.0525 ± 0.004	0.039 ± 0.004	0.036 ± 0.003	0.039
III	0.095 ± 0.006	0.042 ± 0.003	0.027 ± 0.002	0.062 ± 0.005	0.033

[a] All compounds were determined by HPLC using calibration curves. For each assay two analyses were conducted on duplicate experimentations. Each data point is therefore the mean of four measurements.

Quantitative analyses were carried out to determine the stoichiometry of the reaction between Cy 3-glc and CQ (Table 1). The reaction was monitored by HPLC (analytical scale). The CQ concentration was determined as indicated above in the presence of ascorbic acid and sodium benzenesulfinate (reactions 1 and 2). Cy 3-glc and caffeic acid concentrations were determined by HPLC using calibration curves that were constructed by injection (100 μL) of 10 standards containing different concentrations of caffeic acid or Cy 3-glc ranging from 0.01 to 0.1 mM. In the experiments, the initial amount of Cy 3-glc was always higher than that of CQ. Under these experimental conditions, after 5 min of reaction, the residual CQ content was almost zero, which means that all of the CQ reacted. The results (Table 1) show that the amount of caffeic acid formed is equivalent to the amount of Cy 3-glc which has reacted. Moreover, the amount of caffeic acid formed was much lower than that expected from the initial content of CQ. This result indicates that part of the CQ is integrated into the degradation products of the pigment. In experiment I (Table 1), 0.033 μmol of Cy 3-glc reacted with 0.033 μmol of CQ, leading to 0.033 μmol of caffeic acid and 0.033 μmol of Cy 3-glc *o*-Q. The latter then reacted readily with 0.0165 μmol of CQ, leading to the formation of several combinations with a global stoichiometry corresponding to [2 Cy 3-glc *o*-Q/1 caffeic acid *o*-Q]. To explain our results, the following pathway of Cy 3-glc degradation could be proposed (reactions 4−6). Such a pathway has already been proposed by Kader et al. (1998).

$$2\ \text{Cy 3-glc} + 2\ \text{CQ} \rightarrow 2\ \text{caffeic acid} + 2\ \text{Cy 3-glc } o\text{-Q} \quad (4)$$

$$2\ \text{Cy 3-glc } o\text{-Q} + 1\ \text{CQ} \rightarrow [2\ \text{Cy 3-glc } o\text{-Q/1 CQ}] \quad (5)$$

$$2\ \text{Cy 3-glc} + 3\ \text{CQ} \rightarrow 2\ \text{caffeic acid} + [2\ \text{Cy 3-glc } o\text{-Q/1 CQ}] \quad (6)$$

The total amount of CQ that reacted was 0.0495 μmol (0.033 + 0.0165). This value was close to the initial amount of CQ (0.047 μmol). The ratio of degraded Cy 3-glc to the CQ integrated into the reaction products was close to 2:1. The same ratio value has been reported by Kader et al. (1998) and Sarni et al. (1995).

Partial Purification of the Reaction Products. HPLC analysis did not detect the reaction products. This result prompted us to develop a method to isolate the condensation products arising from the reactions between anthocyanins and CQ to characterize them. For this purpose, the reaction products were separated on a Sephadex G-25 column eluted with distilled water (Figure 3). This gel filtration allowed the separation of the condensation products from (i) the caffeic acid and the remaining anthocyanin used and (ii) the salts of the buffer solution. The reaction products obtained after 5 min of reaction between the Cy 3-glc and CQ were chromatographed on a Sephadex G-25 column. Under these conditions, three peaks were obtained: a small peak (C1) that absorbed at 280 and 330 nm, followed by two large peaks (C2 and C3) that were shown to be caffeic acid and Cy 3-glc, respectively, by HPLC analysis of the corresponding fractions.

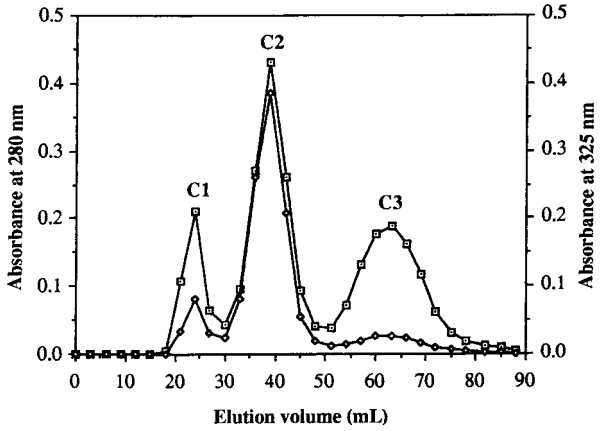

Figure 3. Elution profile on Sephadex G-25 of a solution of Cy 3-glc (0.133 mM) incubated for 5 min with the purified CQ (0.133 mM) at pH 3.5. Absorbance at 280 nm (□); absorbance at 335 nm (◇).

Examining the elution profile (Figure 3) reveals that the Sephadex G-25 gel has separated the Cy 3-glc (MW = 484.85) from the caffeic acid (MW = 180.2). As expected from the fractional domain (1000−5000 Da), Cy 3-glc and caffeic acid should be eluted in a single peak. The chromatographic behavior of Cy 3-glc in the Sephadex G-25 gel could be explained by the existence of interactions (hydrogen bonds) between the pigment and the gel. Consequently, calibrating the gel to estimate the molecular weight of the different fractions would appear to be difficult.

The fractions corresponding to peak C1 were pooled and concentrated under vacuum in a rotary evaporator. The UV−vis spectra were recorded between 220 and 600 nm (Figure 4). The spectrum of C1 showed a maximum at 266 nm and a shoulder at 335−345 nm. The absorption at 335−345 nm could suggest the incorporation of the caffeic moiety into the degradation products of the pigment. The degradation products occurring in peaks C1 were hydrolyzed by heating at 85 °C in 2 M trifluoroacetic acid/methanol (v/v) for 45 min. The fractions obtained after acidic hydrolysis were analyzed by TLC (see Materials and Methods). After spraying the developed chromatogram, we identified glucose, which is linked at the C-3- position of the cyanidin. The overall results suggest that the products of Cy 3-glc degradation contain both caffeic acid and Cy 3-glc moieties. This is consistent with previous papers on the oxidative degradation of Cy 3-glc and Mv 3-glc in the presence of caffeoyltartaric acid and grape PPO (Sarni-Manchado et al., 1997; Sarni et al., 1995), which reported that the degradation products of Cy 3-glc and Mv 3-glc contained both caffeoyltartaric acid and anthocyanin moieties. Moreover, these degradation products were gradually

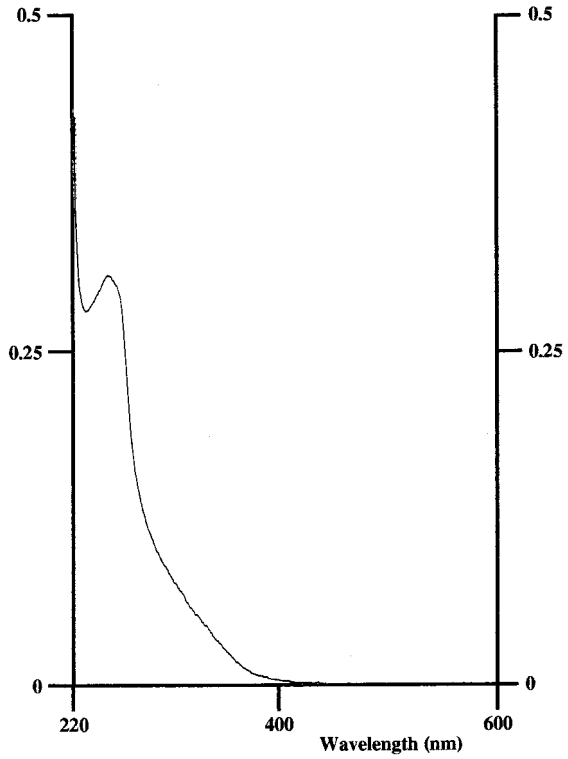

Figure 4. UV−vis spectrum of the concentrated fractions corresponding to peak C1 obtained from the fractionation on Sephadex G-25 of the degraded Cy 3-glc (C1). Spectra were in distilled water.

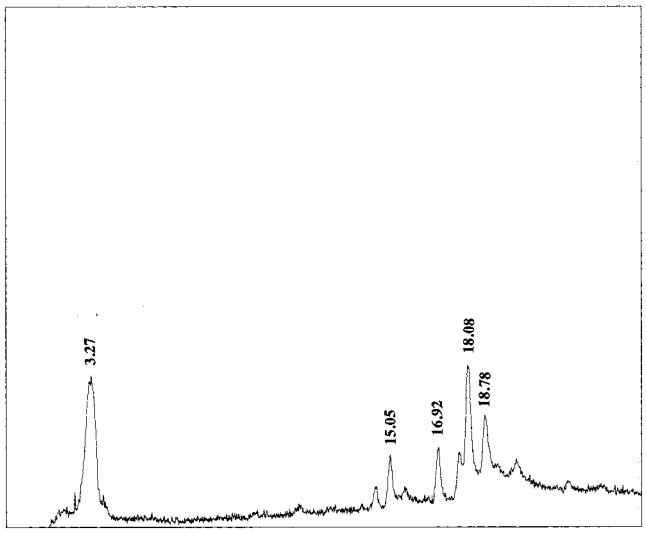

Figure 5. HPLC elution profile at 280 nm of the degradation products eluted in peak C1.

replaced by colorless products as a result of further oxidative degradation.

HPLC analysis of fraction C1 (Figure 5) shows that several products of degradation can be formed from the reaction between Cy 3-glc and CQ, which supports the idea of the complex degradation process mentioned by Kader et al. (1998, 1999a).

A question raised by this work, and one that is to be examined in future work, is how well chemical oxidation mimics enzymatic oxidation. Recently, we studied the mechanism of anthocyanin degradation in model solutions containing CG, blueberry PPO, and purified anthocyanins (Cy 3-glc and Pg 3-glc). Kinetic studies have shown that Cy 3-glc (*o*-diphenolic anthocyanin) is degraded by a mechanism of coupled oxidation involving the CGQ generated by PPO-oxidation of CG. This reaction is accompanied by a partial regeneration of the CG, which means that part of the CG is incorporated into the degradation products of the Cy 3-glc. The overall results suggest the formation of condensation products with the following stoichiometry: [2 Cy 3-glc/1 CGQ].

The use of simpler model systems containing purified anthocyanins and CQ has confirmed most of the results described above. Indeed, Cy 3-glc reacts with the CQ according to a mechanism of coupled oxidation leading to the formation of combinations with the following stoichiometry: [2 Cy 3-glc/1 CQ]. However, the exact mechanism remains to be determined by characterizing the structure of the degradation products. For this reason, the next step in this work will involve both isolating and determining the structure of the resolved condensation products. The combination of gel filtration and HPLC analysis (on the semipreparative scale) could provide the condensation products in sufficient amounts to allow their structural characterization. We are convinced that chemical oxidations will prove to be a valuable contribution to achieving this aim.

ABBREVIATIONS USED

CG, chlorogenic acid; CGQ, chlorogenoquinone; Cy 3-glc, cyanidin 3-glucoside; Cy 3-glc *o*-Q, cyanidin 3-glucoside *o*-quinone; Mv 3-glc, malvidin 3-glucoside; Pg 3-glc, pelargonidin 3-glucoside; CQ, caffeic acid *o*-quinone (caffeic acid *o*-Q); PPO, polyphenol oxidase; UV−vis, ultraviolet−visible.

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