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Degradation Kinetics of Chlorogenic Acid at Various pH Values and Effects of Ascorbic Acid and Epigallocatechin Gallate on Its Stability under Alkaline Conditions

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ABSTRACT: 5-Caffeoylquinic acid (5-CQA) is generally referred to as chlorogenic acid and exhibits various biological activities such as antioxidant activity and porcine pancreas α-amylase inhibitory activities. 5-CQA may be useful as an antioxidant for food and to prevent diabetes and obesity. The degradation of 5-CQA and caffeic acid (CA) in an aqueous solution at 37 °C and pH 5.0−9.0 was studied. The degradation of 5-CQA and CA, demonstrating time and pH dependence (i.e., the rate constant, k, was higher at higher pH), was satisfactorily described by the Weibull equation. The stability of 5-CQA at pH 7.4 and 9.0 was improved by adding (−)-epigallocatechin gallate (EGCG) and ascorbic acid (AA). Moreover, the degradation of 5-CQA in the presence of EGCG or AA could be described by the Weibull equation. The k value in the presence of EGCG or AA was dependent on their concentration.

KEYWORDS: *chlorogenic acid, caffeic acid, (−)-epigallocatechin gallate, degradation kinetics, Weibull equation*

INTRODUCTION

Chlorogenic acids refer to a family of esters formed between quinic acid (QA) and one or multiple cinnamate derivatives such as caffeic (CA), ferulic (FA), and *p*-coumaric acids. The nomenclature for chlorogenic acids is based on the IUPAC numbering system.[1] 5-Caffeoylquinic acid (5-CQA) is generally referred to as chlorogenic acid. 3-Caffeoylquinic acid (3-CQA) and 4-caffeoylquinic acid (4-CQA) differ from 5-CQA with respect to the position of the substitution of the CA moiety on QA. These three CQA isomers are present in many plants such as coffee, yacon, prune, sweet potato, and potato.[2−5] Various biological activities have been reported for CQAs such as antioxidant activity,[6] antimutagenicity,[7] cancer suppression,[8] matrix metalloproteinase inhibition,[9] tyrosinase inhibition,[10] and DNA methylation inhibition.[11]

Isomeric 3-, 4-, and 5-CQAs have similar functions. For example, the antioxidant activity of all three isomers was reported to be approximately equivalent,[2,10] as was the tyrosinase inhibitory activity.[10] We previously reported the α-amylase inhibitory activities of nine different chlorogenic acids, which included the three CQA isomers (3-, 4-, and 5-CQAs), three dicaffeoylquinic acid (diCQA) isomers (3,4-, 3,5-, and 4,5-diCQAs), and three feruloylquinic acid (FQA) isomers (3-, 4-, and 5-FQAs), found in green coffee beans, and their core constituents, namely, CA, FA, and QA, against the porcine pancreas α-amylase isozyme I (PPA-I) in the catalyzed hydrolysis of *p*-nitrophenyl-α-D-maltoside.[12,13] By comparison of the IC_{50} values, the order of inhibitory activity was established as follows: 3,4-diCQA = 4,5-diCQA > 3,5-diCQA > 5-CQA > 4-CQA > 3-CQA > CA > 5-FQA > 4-FQA > 3-FQA > FA ≫ QA.[12,13] All CQAs and FQAs as well as 3,5-diCQA, CA, and FA exhibited mixed-type inhibition, thereby showing stronger binding to the enzyme−substrate complex (ES) than to the enzyme (E) itself. 3,4-DiCQA and 4,5-diCQA exhibited mixed-type inhibition, but, in contrast, apparently bound more strongly to E than to ES.[12,13] The α-amylase inhibition modes of 3-, 4-, and 5-CQAs were the same, and these CQA isomers showed similar IC_{50} values (0.08−0.23 mM) that were relatively different from those of other chlorogenic acids such as diCQAs (0.02−0.03 mM) and FQAs (1.09−2.55 mM).[12,13] It is reported that 3-CQA and 4-CQA are formed when a solution of 5-CQA (pH 8.0 adjusted with a dilute ammonia solution) is heated for 30 min in a boiling water bath.[14] Dawidowicz and Typek reported that 14 compounds, including 3-CQA and 4-CQA, could be formed from 5-CQA by heating an aqueous solution of this derivative at different pH values (4.0−9.0).[15,16] The degradation rate constants and half-lives of 5-CQA at 20 and 37 °C and pH 7.4 and 9.0 were evaluated for a pseudo-first-order reaction.[17]

It is reported that the degradation kinetics of (+)-catechin in an aqueous solution in the absence and presence of ascorbic acid (AA) or octanoyl ascorbate at various pH values and temperatures can be expressed by the Weibull equation.[18] In addition, it is reported that the hydrolysis of 11 different disaccharides (cellobiose, gentiobiose, isomaltose, maltose, trehalose, lactose, leucrose, melibiose, palatinose, sucrose, and turanose) and 5 different monosaccharides (galactose, glucose, mannose, sorbose, and fructose) in subcritical water at 180−260 °C could be expressed by the Weibull equation.[19,20] Knowledge about the degradation kinetics of food materials in an aqueous solution is important for processing, using, and storing foods and beverages because it will be able to set the use-by date of foods and to predict the duration of their

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physiological effects in food. Likewise, the results of degradation kinetics of food materials at physiological temperature (37 °C) and pH (7.0 and 7.4) provide us some insights into their stability in the human body. In general, pH ranges of coffee are from 5.0 to 6.0.[21,22] It is reported that chlorogenic acid is stable at acid pH 3−5.[17,23] On the contrary, it is reported that chlorogenic acid is unstable at pH >10.[24,25]

In this study, the degradation of 5-CQA and CA in an aqueous solution at 37 °C and pH 5.0−9.0 was investigated. 5-CQA is included in the above-mentioned plants. Green coffee beans contain 4−14% of chlorogenic acids, and about 50% of the total chlorogenic acids is 5-CQA in green coffee beans.[26,27] CA forms 5-CQA with QA, and it is known that CA is produced with QA by heat treatment of 5-CQA. The degradation was described by the probabilistic Weibull equation, and the kinetic parameters were evaluated. In addition, the degradation kinetics of 5-CQA in an aqueous solution in the presence of either (−)-epigallocatechin gallate (EGCG) or AA was examined.

MATERIALS AND METHODS

Materials. (−)-Epigallocatechin gallate (lot STH4770) was purchased from Wako Pure Chemical (Osaka, Japan). Decaffeinated green coffee bean extract (DGCBE) was obtained from Ominedo Pharmaceutical Industry (Nara, Japan). The DGCBE was produced by extraction with 56% (v/v) aqueous ethanol using green coffee beans (*Coffea canephora* cv. Vietnam) treated with supercritical CO_2 treatment to remove caffeine.[28] The contents of chlorogenic acids and caffeine in DGCBE were 40.5% (w/w dry matter) and 0.003% (w/w dry matter), respectively.[28] Chlorogenic acid hemihydrate (lot M7M4404), caffeic acid (lot M4F5063), D-(−)-quinic acid (lot M5M5664), L-(+)-ascorbic acid (lot M7T9666), and all other chemicals of reagent grade were acquired from Nacalai Tesque (Kyoto, Japan).

Purification of 3-CQA and 4-CQA. 3-CQA and 4-CQA from DGCBE were purified as described in our previous paper.[13]

HPLC Analysis of 3-CQA, 4-CQA, 5-CQA, and CA. HPLC analysis of 3-, 4-, and 5-CQAs and CA was performed according to the procedures[12,13] previously reported with slight modifications. The sample solution was applied to reversed-phase column chromatography in a preparative HPLC 7400 system (GL Science, Tokyo) on a Cadenza CD-C18 [4.6 mm (i.d.) × 15.0 cm] column (Imtakt, Kyoto) at a column temperature of 40 °C. The mobile phase was composed of solvents A (10 mM sodium phosphate) and B (acetonitrile), and the gradient program was as follows: 0−5.0 min, 5−12% (v/v) B; 5.0−10.0 min, 12% (v/v) B; 10.0−15.0 min, 12−15% (v/v) B; 15.0−25.0 min, 15−30% (v/v) B; 25.0−30.0 min, 30% (v/v) B; 30.0−30.1 min, 30−90% (v/v) B; 30.1−35.0 min, 90% (v/v) B; 35.0−35.1 min, 90−5% (v/v) B; 35.1−45.0 min, 5% (v/v) B. The injection volume and the flow rate of the sample solution were 20 μL and 1.0 mL/min, respectively. 3-, 4-, and 5-CQAs and CA were detected at 325 nm (on the spectra of CQAs) by absorption using a photodiode array at 325 nm. The spectra of CQAs exhibit an absorption maximum near 325 nm and a shoulder at 300 nm.[29] 3-, 4-, and 5-CQAs and CA in the samples were identified by comparing the retention times and the UV spectra of nine standard materials, which included 5-CQA and CA purchased from Nacalai Tesque and 3-CQA and 4-CQA purified in our laboratory according to the previously reported method.[13,30] 3-, 4-, and 5-CQAs and CA were detected in the HPLC chromatograms at 8.0, 11.1, 10.6, and 12.6 min, respectively. Linear calibration curves consisting of five points for each compound were obtained in the following ranges for 3-, 4-, and 5-CQAs and CA: from 10 μM to 0.24 mM 3-CQA, from 10 μM to 0.24 mM 4-CQA, from 10 μM to 0.24 mM 5-CQA, from 5 μM to 0.3 mM CA. The regression coefficients (R^2) for the linear calibration curves for 3-, 4-, and 5-CQAs and CA were 0.9975, 0.9979, 0.9974, and 0.9995, respectively. The limit of detection for 3-, 4-, and 5-CQAs and CA was defined as the concentration that produced a signal-to-noise (S/N) ratio >3, and the values were 5, 5, 5, and 2.5 μM, respectively. Their limit of quantification was defined as the concentration that produced an S/N ratio >10, and the values were 10, 10, 10, and 5 μM, respectively.

HPLC Analysis of QA. HPLC analysis of QA was achieved using a preparative HPLC 7400 system (GL Science, Tokyo, Japan). The column was a combination of four columns [Shodex RSpak KC-811, 8.0 mm (i.d.) × 30.0 cm, Tokyo, Japan] at a column temperature of 60 °C. The injection volume of the sample solution was 20 μL. The UV detector was set at 445 nm. The mobile phase and postcolumn reagent were 3 mM perchloric acid and 0.2 mM bromothymol blue containing 15 mM Na_2HPO_4 and 2 mM NaOH, respectively. The flow rates of the mobile phase and postcolumn reagent were set to 1.0 and 0.5 mL/min, respectively. The reagent stream was combined with the effluent stream by a stainless steel mixing tee that joined the two streams. QA in the samples was identified by comparing the retention times and was detected in the HPLC chromatogram at 27.3 min. Linear calibration curves of five points for QA were obtained in the concentration range from 0.5 to 10 mM QA. R^2 for the linear calibration curves for QA was 0.9991. The limit of detection for QA was 0.1 mM (S/N ≥ 3). The limit of quantification of QA was 0.5 mM (S/N ≥ 10).

Degradation of 5-CQA, CA, and QA at Different pH Values. 5-CQA, CA, and QA were dissolved in distilled water to produce 6, 3, and 6 mM solutions, respectively. The initial concentration of CA was set lower than that of 5-CQA because that the solubility of CA is lower than that of 5-CQA. A portion of each solution (0.8 mL) was combined separately with a buffer (3.2 mL of 0.1 M sodium acetate buffer at pH 5.0, 5.5, and 6.0; 0.1 M sodium phosphate buffer at pH 6.5, 7.0, 7.4, and 8.0; and 0.1 M Tris-HCl buffer at pH 8.5 and 9.0) in an amber vial, and the vials were incubated at 37 °C in a thermostated water bath. A portion of each of the resulting solutions (0.2 mL) was removed from the vials and mixed with 0.8 mL of 0.2 M potassium−HCl buffer (pH 2.0). The amounts of 3-, 4-, and 5-CQAs, CA, and QA in the mixture were measured according to the above-mentioned HPLC method.

Degradation of 5-CQA in the Presence of EGCG or AA at pH 7.4 or 9.0. (−)-EGCG or AA was dissolved in distilled water to prepare solutions with the following concentrations: 0.01, 0.1, 1.0, 3.0, and 6.0 mM. A portion of each solution (0.8 mL) was combined with 5-CQA (0.8 mL, 6 mM) and a buffer (2.4 mL of 0.134 M sodium phosphate buffer at pH 7.4; and 0.134 M Tris-HCl buffer at pH 9.0) in an amber vial, and the vials were incubated at 37 °C in a thermostated water bath. A portion of each of the resulting solutions (0.2 mL) was removed from the vials and mixed with 0.8 mL of 0.2 M potassium−HCl buffer (pH 2.0). The amounts of 3-, 4-, and 5-CQAs in the mixtures were measured according to the above-mentioned HPLC method.

RESULTS

Degradation of 5-CQA at Different pH Values. Figures 1 and 2 show the relationship between the relative amount of the residual 5-CQA ($C_i/C_{0,\ 5\text{-CQA}}$) and incubation time during the incubation of 1.2 mM 5-CQA at 37 °C and pH 5.0−9.0. C_i represents the concentrations of 3-CQA, 4-CQA, or 5-CQA or total CQA, which is a combination of the total amount of 3-, 4-, and 5-CQAs, at a specific incubation time. $C_{0,\ 5\text{-CQA}}$ also represents the concentration of 5-CQA at the initial concentration of 5-CQA. The degradation of 5-CQA was time and pH dependent (Figures 1 and 2). 4-CQA was produced after incubation of a solution of 5-CQA either for 72 h at pH 5.0 or for 24 h at pH 5.5 (Figure 1). When the 5-CQA solution was incubated at 37 °C at pH 6.0−9.0, 4-CQA and 3-CQA were produced (Figures 1 and 2). At pH 5.0−6.5, the amount of 3-CQA and 4-CQA increased depending on the increase in incubation time (Figure 1). At pH 7.0−9.0, the amount of 3-CQA and 4-CQA produced by the incubation of 1.2 mM 5-CQA solution increased with incubation time to

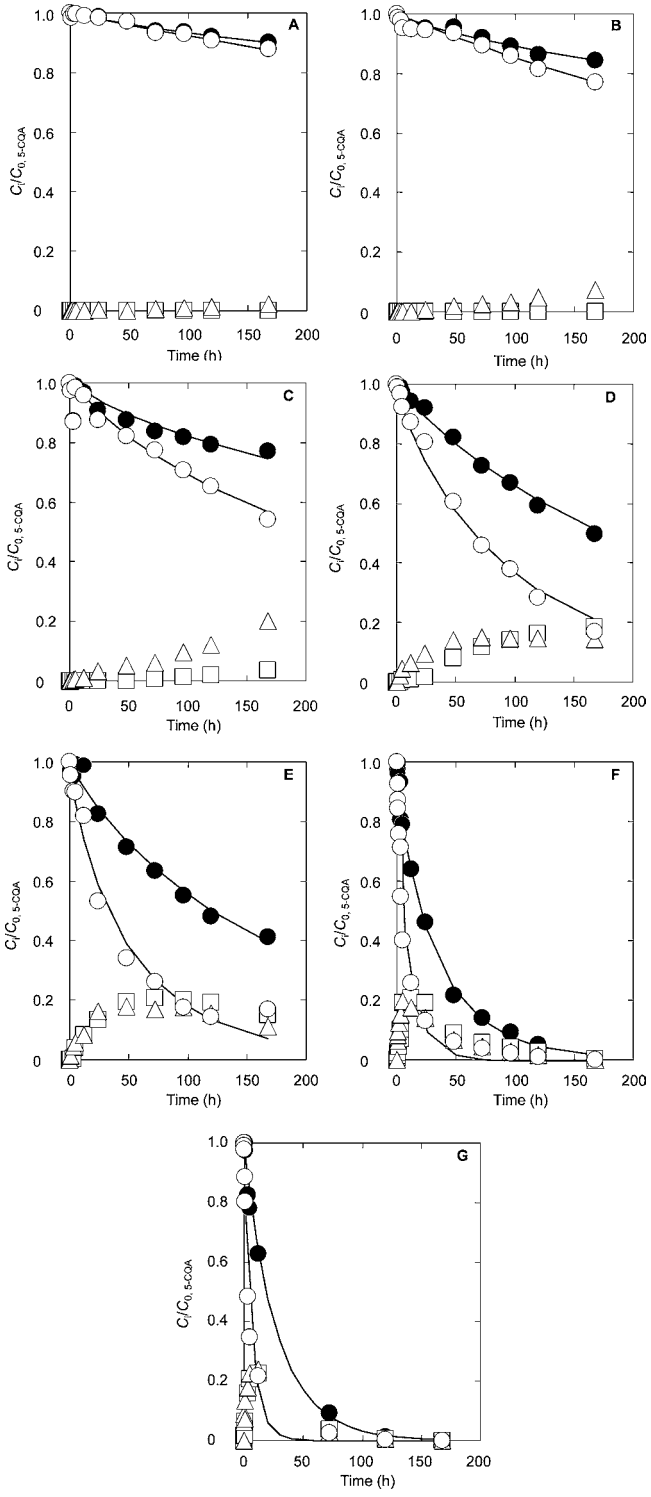

Figure 1. Degradation of 1.2 mM 5-CQA at 37 °C and the following pH values: (A) 5.0; (B) pH 5.5; (C) pH 6.0; (D) pH 6.5; (E) pH 7.0; (F) pH 8.0; (G) pH 8.5. C_i represents the concentration of 3-, 4-, or 5-CQA or total CQA at the indicated incubation time. $C_{0,\ 5\text{-CQA}}$ represents the initial concentration of 5-CQA. The solid curves were calculated using the estimated kinetic parameters of the Weibull model. The symbols are 5-CQA (open circles), 4-CQA (open triangles), 3-CQA (open squares), and total CQA (solid circles).

reach the maximum concentration of 0.25 mM after 48 h at pH 7.0, 12 h at pH 7.4, or 5 h at pH 8.0−9.0, and the ratios $C_{3\text{-CQA}}/C_{0,\ 5\text{-CQA}}$ and $C_{4\text{-CQA}}/C_{0,\ 5\text{-CQA}}$ were both equal to 0.2 (Figures 1 and 2). Subsequently, 3-CQA and 4-CQA production decreased after reaching the maximum level of 0.25 mM at pH 7.0−9.0 (Figures 1 and 2).

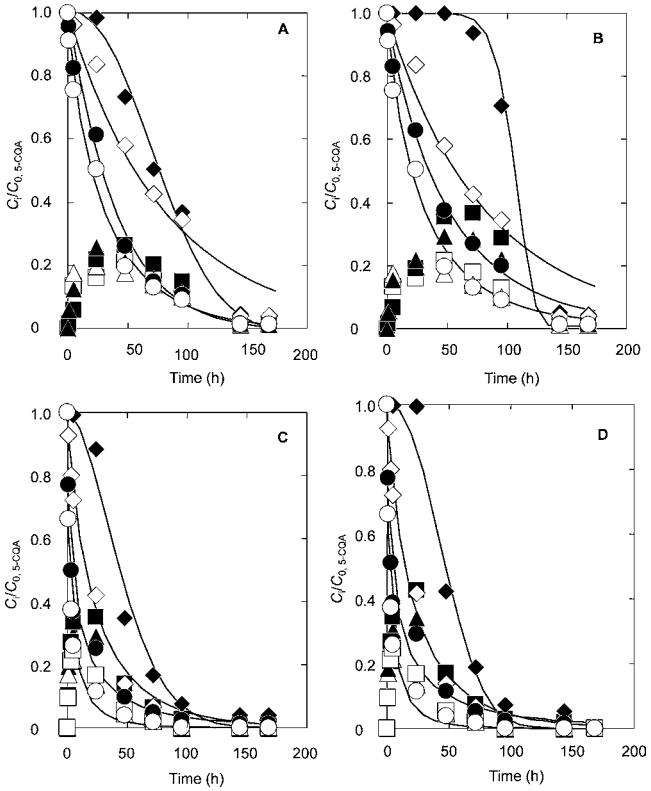

Figure 2. Degradation of 1.2 mM 5-CQA in the absence and presence of 1.2 mM EGCG or 1.2 mM AA at 37 °C and pH 7.4 and 9.0: (A) degradation in the absence and presence of 1.2 mM EGCG at 37 °C and pH 7.4; (B) degradation in the absence and presence of 1.2 mM AA at 37 °C and pH 7.4; (C) degradation in the absence and presence of 1.2 mM EGCG at 37 °C and pH 9.0; (D) degradation in the absence and presence of 1.2 mM AA at 37 °C and pH 9.0. The symbols open circles, solid circles, open triangles, solid triangles, open squares, solid squares, open diamonds, and solid diamonds represent 5-CQA, 5-CQA in the presence of EGCG or AA, 4-CQA, 4-CQA in the presence of EGCG or AA, 3-CQA, 3-CQA in the presence of EGCG or AA, total CQA, and total CQA in the presence of EGCG or AA, respectively. The solid curves were calculated using the estimated kinetic parameters of the Weibull model. See the caption of Figure 1 regarding C_i and $C_{0,\ 5\text{-CQA}}$.

It is reported that 3-, 4-, and 5-CQAs have similar biological functions such as antioxidant activity because of their similar structures.[2,10] Their structures differ only in the substitution position of the CA substructure on QA. Therefore, we evaluated the degradation of total CQA (Figures 1 and 2). Moreover, the degradation of total CQA was also time and pH dependent (Figures 1 and 2).

The following Weibull equation is known to be applicable:[18,31,32]

$$C/C_0 = \exp[-(kt)^n] \quad (1)$$

k is the rate constant (the inverse of which is called the scale parameter), and n is the shape constant. The kinetic parameters k and n were calculated from eq 1 using nonlinear least-squares regression with Solver of Microsoft Office Excel 2003.[33,34] Figure 3A shows the relationship betweenthe rate constant and pH. The respective n values for 5-CQA at pH 5.0, 5.5, 6.0, 6.5,

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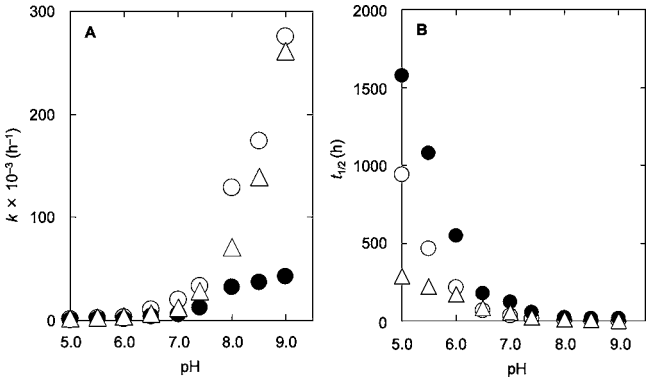

Figure 3. (A) Relationship between the k values of 5-CQA (open circles), total CQA (solid circles), and CA (open triangles) and pH. (B) Relationship between the $t_{1/2}$ values of 5-CQA (open circles), total CQA (solid circles), and CA (open triangles) and pH.

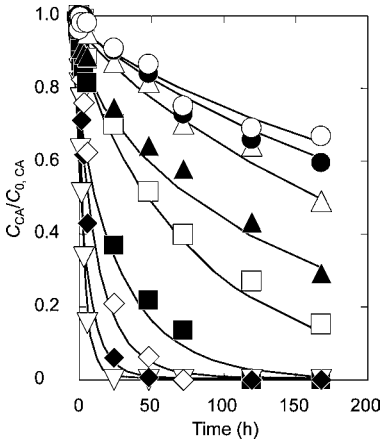

Figure 4. Degradation of 0.6 mM CA at 37 °C at pH 5.0 (open circles), pH 5.5 (solid circles), pH 6.0 (open triangles), pH 6.5 (solid triangles), pH 7.0 (open squares), pH 7.4 (solid squares), pH 8.0 (open diamonds), pH 8.5 (solid diamonds), and pH 9.0 (inverted triangles). C_{CA} represents the concentration of CA at the indicated incubation time. $C_{0, CA}$ represents the initial concentration of CA. The solid curves were calculated using the estimated kinetic parameters of the Weibull model.

7.0, 7.4, 8.0, 8.5, and 9.0 were 0.97, 0.96, 0.79, 0.85, 0.83, 0.80, 0.76, 0.84, and 0.54, and the values for total CQA at pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.4, 8.0, 8.5, and 9.0 were determined to be 0.85, 0.76, 0.73, 0.90, 0.88, 0.97, 0.84, 0.94, and 0.78. The curves in Figures 1 and 2 were drawn using the k and n values. The correlation coefficients (R) for 5-CQA at pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.4, 8.0, 8.5, and 9.0 were 0.986, 0.979, 0.958, 0.997, 0.991, 0.997, 0.997, 0.996, and 0.999, respectively. The R values for total CQA at pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.4, 8.0, 8.5, and 9.0 were 0.979, 0.948, 0.988, 0.996, 0.989, 0.966, 0.998, 0.996, and 0.998, respectively. The k values of 5-CQA and total CQA increased with pH (Figure 3A). The n values of 5-CQA and total CQA were 0.5−1.0 and exhibited no tendency associated with pH variation. The half-lives (time to reduce the concentration by half, i.e., $t_{1/2}$) of 5-CQA and total CQA were calculated from eq 2.[35]

$$t_{1/2} = (0.693)^{1/n}/k \quad (2)$$

The $t_{1/2}$ values of 5-CQA and total CQA decreased with increasing pH (Figure 3B). At pH 5.0, $t_{1/2}$ values of 5-CQA, CA, and total CQA were 940.7, 289.2, and 1572.7, respectively. At pH 7.5−9.0, the $t_{1/2}$ values were substantially zero (very rapid degradation) (Figure 3B).

Degradation of CA at Different pH Values. Figure 4 shows the relationship between the residual CA, $C_{CA}/C_{0, CA}$, and incubation time during the incubation of 0.6 mM CA at 37 °C at pH 5.0−9.0. The degradation of CA was time and pH dependent (Figure 4). The n values for CA at pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.4, 8.0, 8.5, and 9.0 were 0.81, 0.85, 0.89, 0.70, 0.89, 0.85, 0.87, 0.86, and 0.62, respectively. The k and $t_{1/2}$ values of CA at 37 °C at pH 5.0−9.0 were calculated according to the aforementioned methods (Figure 3). The curves in Figure 4 were drawn using the k and n values. The R values for CA at pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.4, 8.0, 8.5, and 9.0 were 0.974, 0.996, 0.990, 0.993, 0.993, 0.997, 0.999, 0.999, and 0.999, respectively. The k values of CA increased with pH (Figure 3A). The n values of CA were 0.7−0.9 and exhibited no tendency associated with pH variation. The $t_{1/2}$ values of CA decreased with increasing pH (Figure 3B). QA remained at 100% concentration when it was incubated at 37 °C and pH 5.0−9.0 for 48 h (data not shown).

Effects of EGCG and AA on the Stability of 5-CQA at pH 7.4 and 9.0. It is reported that AA increases the stability of four epicatechin derivatives, including (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and (−)-EGCG, in phosphate buffer at pH 7.4 and 37 °C.[36] The 1.2 mM 5-CQA solution was incubated in the absence and presence of 2 μM, 20 μM, 0.2 mM, 0.6 mM, and 1.2 mM EGCG or AA at 37 °C and pH 7.4 or 9.0, and the amounts of 3-, 4-, and 5-CQAs and total CQA were measured in comparison to incubation time in this study. Figure 2 shows the change in the amounts of 3-, 4-, and 5-CQAs and total CQA versus incubation time when the 1.2 mM 5-CQA solution was incubated with 1.2 mM EGCG or 1.2 mM AA at 37 °C and pH 7.4 or 9.0. The k, n, and $t_{1/2}$ values of 5-CQA in the presence of EGCG or AA at 37 °C and pH 7.4 and 9.0 were calculated according to the above-mentioned methods (Table 1). The curves in Figure 2 were drawn using the k and n values from Table 1. The R values for 5-CQA and total CQA in the presence of EGCG or AA at the different pH values are summarized in Table 1. The k values of 5-CQA in the presence of AA or EGCG at pH 7.4 and 9.0 decreased with an increasing concentration of AA or EGCG (Table 1). In contrast, both the n and $t_{1/2}$ values of 5-CQA in the presence of AA or EGCG at pH 7.4 and 9.0 increased with the concentration of AA or EGCG (Table 1).

■ DISCUSSION

5-CQA may be useful as an antioxidant for food and in the prevention of diabetes and obesity.[10,12,13] This study examined the stability of 5-CQA in the absence and presence of EGCG or AA at 37 °C at pH 5.0−9.0. The degradation of 5-CQA was dependent on the pH, and the positional isomerization of 5-CQA occurred during degradation (Figures 1 and 2). It was reported that the stability of 5-CQA decreased in a pH-dependent manner (i.e., the lower the pH, the greater the stability) and that 3-CQA and 4-CQA, which are isomers of 5-CQA, were produced during degradation.[17] This finding agrees with the results obtained in the current study. In this study, 4-CQA was produced during the incubation of the 5-CQA solution at 37 °C and pH 5.0 and 5.5 (Figure 1). When the 5-CQA solution was incubated at 37 °C and pH 6.0, 6.5, and 7.0, 3-CQA and 4-CQA were produced (Figure 1). However,

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Table 1. Rate Constants, Shape Constants, Half-Lives, and Correlation Coefficients of 5-CQA and Total CQA for the Incubation of 5-CQA in the Presence of EGCG or AA at 37 °C and pH 7.4 or 9.0

		5-CQA (at pH 7.4)				total CQA (at pH 7.4)				5-CQA (at pH 9.0)				total CQA (at pH 9.0)			
compd	compd (mM)	$k \times 10^{-3}$ (h^{-1})	n	$t_{1/2}$ (h)	R	$k \times 10^{-3}$ (h^{-1})	n	$t_{1/2}$ (h)	R	$k \times 10^{-3}$ (h^{-1})	n	$t_{1/2}$ (h)	R	$k \times 10^{-3}$ (h^{-1})	n	$t_{1/2}$ (h)	R
EGCG	0.002	33.5	0.81	18.9	0.997	12.4	1.52	63.1	0.997	243.0	0.50	2.0	0.993	34.5	0.83	18.6	0.994
EGCG	0.02	30.8	0.85	21.1	0.995	12.3	1.90	66.8	0.980	218.5	0.52	2.3	0.992	34.2	0.91	19.6	0.997
EGCG	0.2	28.8	0.89	23.0	0.995	11.7	1.96	71.2	0.986	127.2	0.48	3.7	0.991	27.1	1.16	26.9	0.997
EGCG	0.6	27.4	1.07	25.9	0.982	11.7	1.98	71.3	0.987	119.9	0.50	4.0	0.991	19.1	1.73	42.3	0.996
EGCG	1.2	25.3	1.03	27.6	0.995	11.5	2.29	74.1	0.991	108.4	0.55	4.7	0.994	19.5	2.15	43.3	0.997
AA	0.002	29.5	0.88	22.4	0.989	12.4	1.90	66.3	0.986	234.0	0.48	2.0	0.992	36.2	0.90	18.4	0.990
AA	0.02	25.7	1.03	27.2	0.994	11.5	2.47	75.1	0.981	230.6	0.58	2.3	0.994	32.8	0.83	19.6	0.998
AA	0.2	22.6	1.03	31.1	0.994	10.3	3.71	88.1	0.987	141.5	0.54	3.6	0.998	25.2	1.69	32.0	0.992
AA	0.6	21.2	0.99	32.6	0.994	9.7	5.69	97.0	0.989	145.1	0.48	3.2	0.993	21.1	2.01	39.5	0.995
AA	1.2	20.2	0.91	33.1	0.994	9.3	9.09	103.0	0.997	112.9	0.47	4.1	0.990	17.4	2.27	48.9	0.992

among these, only 4-CQA was observed earlier (Figure 1). Moreover, both 3-CQA and 4-CQA were produced under higher pH conditions (7.4, 8.0, 8.5, and 9.0) (Figures 1 and 2). Although in these cases both products were observed simultaneously, the observed amount of 4-CQA was than that of 3-CQA (Figures 1 and 2). The amounts of 3-, 4-, and 5-CQAs decreased after each of these three isomers reached similar concentrations during the incubation of the 5-CQA solution at 37 °C and pH 7.0−9.0 (Figures 1 and 2). Xie et al. have proposed that 5-CQA is first isomerized to 4-CQA and then to 3-CQA,[17] which is supported by results obtained in the present experiment.

The degradation of 5-CQA and total CQA was described satisfactorily by the Weibull model. The k values of 5-CQA and total CQA increased with pH (Figure 3A). The Weibull model exhibits a sigmoidal pattern for $n > 1$, simple first-order kinetics for $n = 1$, and a steep decrease in C/C_0 during the early stages for $n < 1$.[18−20] The n values of 5-CQA and total CQA at pH 5.0−9.0 were <1, suggesting that the concentrations of 5-CQA and total CQA steeply decrease in the early stage of the incubation of the 5-CQA solution at 37 °C at pH 5.0−9.0. The $t_{1/2}$ values of 5-CQA and total CQA decreased with increasing pH (Figure 3B). In general, pH ranges of coffee are from 5.0 to 6.0.[21,22] The $t_{1/2}$ values of total CQA at pH 5.0, 5.5 and 6.0 were 1572.7, 1074.9, and 547.1 h, respectively, and the values decreased linearly in the pH range 5.0−6.0. The $t_{1/2}$ values of total CQA at pH 7.0 and 7.4 were 119.8 and 54.4 h, respectively, and it was indicated that total CQA is fairly stable in the small intestine (pH 7.0) and blood plasma (pH 7.4). The $t_{1/2}$ values of total CQA at pH 8.0, 8.5, and 9.0 were significantly small (about 1/100) in comparison with the values at pH 5.0 and were 20.2, 18.3, and 14.6 h, respectively. On the other hand, the $t_{1/2}$ values of total CQA in the pH range of 8.0−9.0 showed hardly any difference (up to 1.4 times). Figure 5 shows that the relationship between $t_{1/2,\ 5\text{-CQA}}$ and

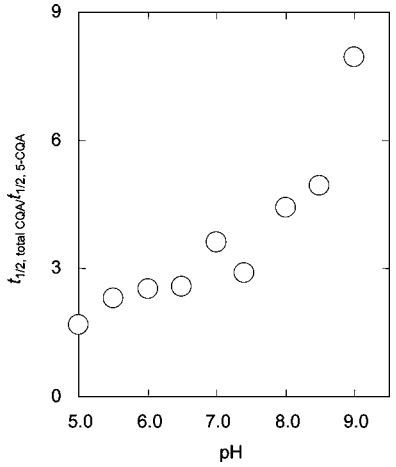

Figure 5. Relationship between $t_{1/2,\ 5\text{-CQA}}$ and $t_{1/2,\ total\ CQA}$ at pH 5.0−9.0.

$t_{1/2,\ total\ CQA}$. The values of $t_{1/2,\ total\ CQA}/t_{1/2,\ 5\text{-CQA}}$ increased with pH, and the maximum value was 7.9 at pH 9.0 (Figure 5). It is reported that various biological activities, such as antioxidant activity, tyrosinase inhibition, α-amylase inhibition, and α-glucosidase inhibition, were identified for 3-, 4-, and 5-CQAs, and the potencies of each of the isomers for these activities are approximately equivalent.[2,10,12,13,37] Therefore, the

various biological effects would be partly derived from 3-CQA and 4-CQA produced when 5-CQA decreases under alkaline conditions. In addition, it was reported that the degradation rate constants (k) and half-lives ($t_{1/2}$) of 5-CQA at 37 °C and pH 7.4 and 9.0 were evaluated for a pseudo-first-order reaction.[17] In the above-mentioned study, the k and $t_{1/2}$ values at 37 °C and pH 7.4 in 100 mM phosphate buffer were 0.063 h^{-1} and 11 h, respectively, and at the same temperature and pH 9.0 in the same buffer, the values were 1.11 h^{-1} and 0.62 h, respectively.[17] In the current study, the k and $t_{1/2}$ values of 5-CQA at 37 °C and pH 7.4 in 100 mM sodium phosphate buffer were 0.033 h^{-1} and 19.2 h, respectively, and at the same temperature and pH 9.0 in 100 mM Tris-HCl buffer were 0.28 h^{-1} and 1.8 h, respectively.

The degradation of CA was time and pH dependent (Figure 4). It is reported that CA is more easily oxidized at higher pH because two protons are released for the two-electron oxidation of CA via the semiquinone.[38,39] It is considered that the degradation of CA is based on oxidization and oxidative polymerization. Moreover, the degradation of CA was satisfactorily described by the Weibull model (Figure 4). The n values of CA at pH 5.0−9.0 were <1. It is suggested that CA rapidly decreases in the early stages during the incubation of CA solution at 37 °C at pH 5.0−9.0. The k values of CA were slightly higher than those of 5-CQA at 37 °C and pH 5.0, 5.5, and 6.0 and slightly lower than the values of 5-CQA at 37 °C at pH 6.5−9.0 (Figure 4A). 5-CQA was actively isomerized to 3-CQA and 4-CQA at a pH >6.5 (Figures 1 and 2). It is considered that the k values of 5-CQA became greater than those of CA because of the concurrent isomerization of 5-CQA in addition to its decomposition at a pH >6.5. Dawidowicz and Typek reported that CA can be formed from 5-CQA by heating (boiling) an aqueous solution of this species at pH 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0, and the amount of CA produced increased with pH.[16] It is reported that the amount of CA formed from 5-CQA during heating of an aqueous solution of this isomer at 100−200 °C depends on heating time and temperature.[16,17] However, CA was not produced during the degradation of 5-CQA at 37 °C and pH 5.0−9.0 in the current study. It is considered that the thermal decomposition of 5-CQA to CA progressed only minimally at 37 °C. Moreover, the possibility of the produced CA rapidly decomposing is considered because there is no significant difference between the k values of 5-CQA and CA (Figure 3A).

The stability of an aqueous of 5-CQA solution at 37 °C and pH 7.4 and 9.0 improved in the presence of EGCG or AA (Figure 2). In addition, the degradation of 5-CQA in the presence of EGCG or AA was satisfactorily described by the Weibull model (Figure 2). It was suggested that the protective effects of EGCG or AA on 5-CQA were dose dependent by comparing the $t_{1/2}$ values at the respective concentrations from this experimental result (Table 1). The $t_{1/2}$ values of 5-CQA and total CQA in the presence of EGCG or AA at pH 7.4 increased about 1.5 times compared to their values in the absence of EGCG or AA at a molar ratio between 5-CQA and EGCG or AA of 1:1 (Table 1). Moreover, their $t_{1/2}$ values in the presence of EGCG or AA at pH 9.0 were about 2.5−3.0 higher than their values in their absence. It was indicated that protective effects of EGCG and AA were stronger at higher pH conditions. The n values of total CQA in the presence of EGCG or AA were >1, and they increased with pH (Table 1). The curves drawn by the Weibull model showed a sigmoidal pattern (Figure 2). Therefore, the degradation of total CQA in the early stages was strongly obstructed. The n values of 5-CQA in the presence of EGCG or AA were independent of pH (Table 1). Therefore, these results suggest that EGCG and AA have minor effects on the isomerization of 5-CQA, but they are effective in preventing the decomposition of 3-, 4-, and 5-CQAs in the early stages. It was reported that AA significantly increased the stability of EC, ECG, EGC, and EGCG in 60 mM sodium phosphate buffer at 37 °C and pH 7.4[36] as well as the stability of (+)-catechin in 0.2 M sodium phosphate buffer at 60 °C and pH 6.0.[18] The stability of these catechins by the addition of AA possibly improved because AA serves as a reducing agent that can protect the catechins and recycle their free radical form.[36] The stability of 5-CQA and total CQA may also be improved by adding EGCG or AA due to similar roles of EGCG and AA.

In conclusion, the degradation of 5-CQA and total CQA and CA during the incubation of 5-CQA and CA, respectively, at 37 °C and pH 5.0−9.0 was satisfactorily described by the Weibull equation. Furthermore, the degradation of 5-CQA and total CQA during the incubation of 5-CQA in the presence of EGCG or AA at 37 °C and pH 7.4 and 9.0 satisfactorily correlated with the Weibull equation. The k, n, and $t_{1/2}$ values were evaluated by the Weibull model. The k values of 5-CQA in the presence of AA or EGCG at pH 7.4 and 9.0 decreased with an increasing concentration of AA or EGCG. The n values of the total CQA in the presence of EGCG or AA were >1 and increased with increasing pH, and the curves drawn by the Weibull model showed a sigmoidal pattern (Figure 2). EGCG and AA strongly obstructed the degradation of total CQA in the early stages of incubation of the 5-CQA solution at 37 °C and pH 7.4 and 9.0. The protective effects of EGCG and AA on 5-CQA indicated the dose dependency of 5-CQA by comparison between $t_{1/2}$ values at each concentration.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

5-CQA,5-caffeoylquinic acid; CA,caffeic acid; QA,quinic acid; EGCG,epigallocatechin gallate; AA,ascorbic acid; DGCBE,decaffeinated green coffee bean extract

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