

Chemical Kinetics of 5-*o*-Caffeoylquinic Acid in Superheated Steam: Effect of Isomerization on Mate (*Ilex paraguariensis*) Manufacturing

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A set of experiments was carried out to investigate the chemical stability of 5-*o*-caffeoylquinic acid (5-CQA) in the presence of superheated steam. A batch cylindrical reactor made of glass and isothermally operated between 398 and 499 K was used in the experiments. A high-performance liquid chromatograph equipped with a diode array detector was applied to monitor the 5-CQA concentrations. The conversions of 5-CQA were correctly reproduced with a simplified kinetic model represented by a reversible pseudofirst-order reaction of isomerization. The effect of temperature on the forward rate constant was represented by the Arrhenius equation with parameters tuned on experimental data. The heat of isomerization of 5-CQA and the equilibrium constant at 298 K were calculated by involving the integrated form of the van't Hoff equation. The observed reaction was revealed to not be detrimental for the quality of manufactured leaves and branches of mate because the content of total chlorogenic acids was not changed.

KEYWORDS: 5-CQA; isomerization; mate leaves; *Ilex paraguariensis*; chemical kinetics

INTRODUCTION

Dry leaves of mate (*Ilex paraguariensis* Saint Hilaire) are marketed in all of the continents (1–3) to prepare an aqueous infusion similar in taste, color, and aroma (4) to the green tea obtained from dehydrated shoots of *Camellia sinensis*. The commercial importance of mate is supported by the around U.S. \$1 billion involved in the global trade of this commodity (2) and by an expansion rate of world production of approximately 50 tons per year in the past decade (2).

The growing interest for mate on a large-scale is mainly attributed to the bioactive compounds found in their leaves and branches (5–8). Among the several different chemical species already identified in this food product (4), the most common groups with special interest for pharmaceutical purposes are the saponins, purine alkaloids, and chlorogenic acids (CGAs) (5). Saponins and alkaloids have presented antioedematogenic activity on induced edemas (9) and stimulant effects on the central nervous, muscle, and circulatory systems in humans (10), while CGAs have been credited with health benefits against coronary diseases (11, 12) and a potential protective role in the development of cancer (13–15).

The CGAs in mate involve a family of approximately 18 identified esters formed between quinic and *trans*-cinnamic acids (5, 16), whose total content in leaves and branches is in the range from 2 to 13% (dry basis) (5–8). Among these simple phenolic compounds, the caffeoylquinic acids (3-CQA, 4-CQA, and 5-CQA) are the most abundant in mate and represent

approximately 60% of the overall amount of CGAs (8), while the 5-CQA alone is between 15 and 25% (5, 7). The 5-CQA is not only the major component of CGA but is also the single monoester of caffeic acid like CQA commercially available (17). It is important to underline that the IUPAC numbering system is used in the entire manuscript.

The same chemical nature that makes the CGA a reactive species (18, 19) in the human body is also a disadvantage that reduces its stability in the presence of oxygen (20, 21) at a high temperature (22), such as occurs during the stage of enzymatic deactivation and drying of mate leaves and branches (23). This finding has also been supported by a certain number of investigations on the stability of pure 5-CQA in air (24–26).

The main aim of this investigation is to experimentally examine the kinetics of 5-CQA decomposition in superheated steam, which is an oxygen-free drying medium that may reduce chemical (24–26) and enzymatic oxidative reactions with polyphenoloxidases, typically expected by involving the manufacturing of mate leaves with hot air. The experimental results of 5-CQA concentrations were monitored by high-performance liquid chromatography (HPLC) for up to 2 h at isothermal conditions in a range of temperatures from 398 to 499 K to investigate the influence of reaction time and temperature on reaction rates. A simplified kinetic model that indicates isomerization of 5-CQA was proposed to describe the entire set of experimental results.

MATERIALS AND METHODS

Experiments. 5-CQA ($C_{16}H_{18}O_9$, MW = 354.3) used in all of the kinetic experiments was purchased from Sigma-Aldrich (lot 096K1722) with a purity of 98% by sodium hydroxide titration. The same reagent was

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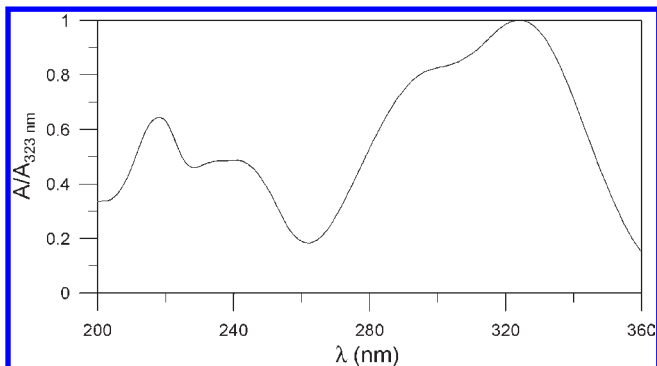


Figure 1. Recorded absorption spectra of an aqueous 5-CQA solution in different wavelengths.

used to prepare standard solutions with ultra pure water used in the kinetic experiments and as reference samples in the analysis by HPLC. To reduce the uncertainty in the concentration values of these solutions, a mass of not less than 10 mg of pure powder 5-CQA was always measured by using a calibrated digital balance (Adventurer, Ohaus) with an uncertainty of ± 0.1 mg.

All of the kinetic experiments were performed in cylindrical batch reactors made of glass. In each of these vessels, a volume of 0.5 mL of standard aqueous solution with an approximately constant concentration of 5-CQA of 0.099–0.101 mg mL⁻¹ was added. Seven to eleven of these reactors were simultaneously placed in an oven equipped with an on-off controller of temperature (Quimis, Q317B242) for up to 5400 s. Temperatures of the samples were also sensed with calibrated K-type thermocouples to identify the initial reaction time, which is characterized by the absence of liquid water and by the presence of superheated steam at the desired temperature. Earlier tests available in the literature (26) have indicated the complete stability of 5-CQA (pure and in foods) in hot water up to the normal boiling point, which assures no decrease of the 5-CQA concentration first of having an atmosphere free of liquid water and oxygen. Vaporization of 5-CQA is also not expected since its melting point (480–482 K) (26) is in the order of the upper limit of temperature adopted in this investigation.

The vessels containing 5-CQA were periodically removed from the oven and immediately refrigerated at 273 K. The residual solid samples were diluted with ultrapure water to have an initial concentration of approximately 0.0564 mol m⁻³ (0.02 mg mL⁻¹) in the hypothesis of no reaction, taken in an ultrasonic bath (Ultraclear 800A, Unique) for 900 s to have complete solubility of 5-CQA, and analyzed. The entire procedure was repeated at 398, 428, 461, and 499 K.

As already mentioned, all of the samples of 5-CQA removed from the reactor were examined with a HPLC apparatus (Agilent HP 1100). It was equipped with a quaternary gradient pump (QualPump G1311A), a degasser (Agilent G1322A), a Rheodyne sampling valve with a 20 μ L sample loop, and a diode array detector (DAD) (Agilent G1315A) that is able to monitor the eluted components in a range of wavelengths from 190 to 400 nm. A 5 μ m C₁₈ packing column (Zorbax ODS, 4.6 cm \times 25 cm) was used. A commercial computational code (Agilent ChemStation A0901) was also applied to automatically operate the HPLC and to promptly calculate the area of the identified peak of 5-CQA at the known retention time of approximately 5 min. Absorption spectra were always recorded at 323 nm, where the investigated compound was proved to present maximum absorbance (see **Figure 1**).

The mobile phase consisted of *A* (ultra pure water at 50 mM of 85% phosphoric acid) and *B* (acetonitrile), which was always pumped at 0.7 mL min⁻¹ by assuming the following linear gradients: 20% *A* for 2 min; 20–50% *B* in 15 min; 50% *B* in 17 min; 50–20% *B* in 19 min, and 20% *B* in 21 min. Quite similar chromatographic conditions, including the composition and elution regime of the mobile phase, were adopted in earlier investigations reported in the literature to separate CGAs (27, 28).

Simplified Kinetic Modeling. A simplified single-step mechanism represented by reaction ¹ was assumed to describe the kinetic experiments. The partial conversion of 5-CQA at equilibrium suggests that when the aqueous solution of 5-CQA was heated above the normal boiling point of

water to have only superheated steam, a reversible isomerization reaction took place.



In the presence of an excess of superheated steam, the rate of isomerization of 5-CQA was promptly reduced to a simplified expression that was typically applied to represent elementary reversible pseudofirst-order reactions.

$$\frac{d[5\text{-CQA}]}{dt} = -k_1[5\text{-CQA}] + k_2[4\text{-CQA}] \quad (2)$$

The integral method of analysis of rate data applied in this investigation required the analytical solution of the above first-order ordinary differential equation (ODE). This procedure was extensively reported in the literature (29) and will not be repeated in detailed in this investigation. However, it basically involved an expression to correlate the disappearance of 5-CQA and production of 4-CQA that was obtained from the extent of reaction or by mass balance. A separable ODE, where the reverse rate constant was usually expressed as the ratio of forward to equilibrium rate constant ($k_2 = k_1/K$) and K were related to the concentrations at equilibrium, was obtained. After rearranging and integrating it with respect to time in terms of conversion of 5-CQA, a solution represented by eq ³ was finally written.

$$-\ln \left[1 - \frac{X_{5\text{-CQA}}}{X_{\text{eq}}} \right] = \frac{k_1 t}{X_{\text{eq}}} \quad (3)$$

A good agreement between experimental results and a straight line in a plot of the left-hand side of eq ³ against reaction time revealed the suitability of the suggested simplified kinetic model for representing the isomerization of 5-CQA in the presence of superheated steam. An arithmetic mean based on experimental results of concentration of 5-CQA for reaction times higher or equal than approximately 1800 s was applied to represent the conversion of 5-CQA at equilibrium. In this circumstance, no significant statistical effect of reaction time on conversions of 5-CQA was noticed. Because an expression for X_{eq} in terms of K was easily proposed and because the dependence of the equilibrium constant on temperature by the integrated form of the van't Hoff equation was well-recognized, the influence of temperature on conversion of 5-CQA at equilibrium was immediately estimated based on eq ⁴. The constants K_{298} and ΔH_{298} were tuned on experimental results of equilibrium conversion of 5-CQA in the temperature range of 398–499 K.

$$X_{\text{eq}} = \frac{K}{1+K} = \frac{K_{298} \exp \left[\frac{-\Delta H_{298}}{R} \left(\frac{1}{T} - \frac{1}{298} \right) \right]}{1 + K_{298} \exp \left[\frac{-\Delta H_{298}}{R} \left(\frac{1}{T} - \frac{1}{298} \right) \right]} \quad (4)$$

As usually reported in the literature, the forward rate constant and temperature were related by the Arrhenius expression. The rate constants and the Arrhenius model parameters were tuned on experimental results by involving the least-squares method of optimization.

RESULTS AND DISCUSSION

Figure 2 shows experimental measurements of absorbance of standard solutions of 5-CQA with a purity of 98% at 323 nm. A coefficient of determination (R^2) close to unity confirms the reliability of the obtained calibration curve. As expected, the straight line also reveals that the well-known Beer–Lambert model (30) would be able to correctly describe the absorption of light at different 5-CQA aqueous concentrations at the investigated wavelength.

Figure 3a shows a typical chromatogram obtained by examining a sample of 5-CQA kept in the reactor at high temperature (398–499 K) in the presence of superheated steam. In almost all of the experiments, only one product was formed, but negligible amounts of unknown compounds eluted at 6.94 ± 0.02 and

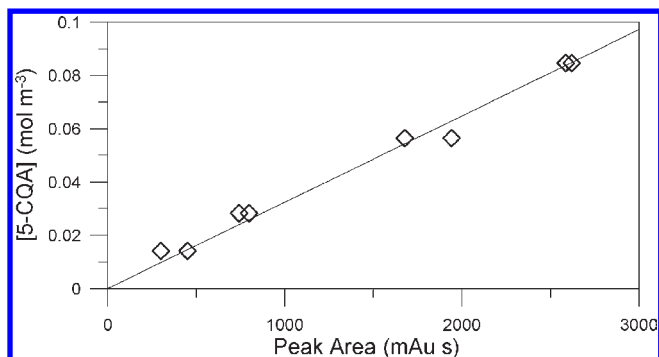


Figure 2. Standard curve for 5-CQA based on detection at 323 nm.

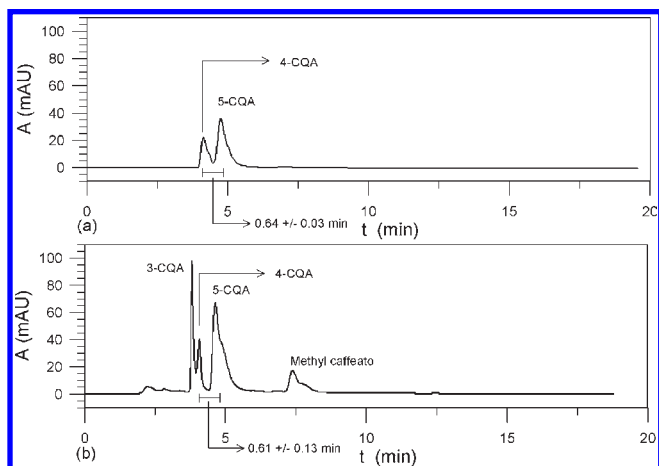


Figure 3. Typical chromatogram of the aqueous solutions of 5-CQA after the reaction in the presence of superheated steam (a) and treatment with TMAH (b) ($\lambda = 323$ nm).

4.38 ± 0.02 min were occasionally observed. This finding explains the option for a simplified kinetic model and reveals the difficulty of a full description of the experimental results with a detailed kinetic mechanism.

According to **Figure 3a**, the single product presents a lower affinity for the stationary phase, and for this reason, it first leaves the chromatographic column. The supposed formation of 3-*o*-caffeoylquinic or 4-*o*-caffeoylquinic acid was based on data of relative retention time of these compounds available in the literature. In particular, when the studies involve separation of mixtures of monoester of caffeic acid like CQA by HPLC-UV at conditions similar to those involved in this investigation (8, 31).

In the absence of LC-MS, a more simple but reliable procedure reported in the literature (32) was adopted to unambiguously reveal whether 3-CQA or 4-CQA was formed. It involved a reaction of 2000 μL of an aqueous solution of 5-CQA ($100 \mu\text{g mL}^{-1}$) with 200 μL of tetramethylammonium hydroxide (TMAH) (20% aqueous). These reactants were well-mixed in vials, where the yellow color was immediately observed, and left to react for 1–5 min. Four hundred microliters of acetic acid (3.5 M) was added in the vessels to terminate the reaction. The chromatogram of the mixture kept in the reactor for 4 min is shown in **Figure 3b**.

It is well-known that 3-CQA is much more hydrophilic than either 4-CQA or 5-CQA and elutes appreciably earlier from reversed phase columns (33). Treatment of pure 5-CQA with TMAH for 1–5 min produced methyl caffeate and two products that eluted before 5-CQA (see **Figure 3b**). The effect of increasing the reaction time was to reduce the content of the more strongly

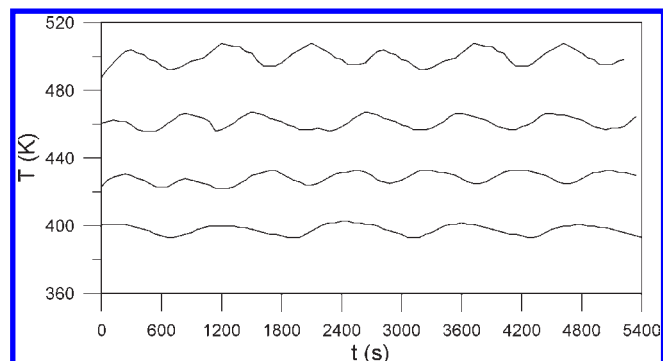


Figure 4. Temperature in the cylindrical glass batch reactor during the reaction.

retained of these products, whereas the more hydrophilic was increased.

Accordingly, the first formed product is assigned as 4-CQA and the more hydrophilic as 3-CQA as previously reported by Clifford et al. (32), and it is concluded that 4-CQA is the major product from the isomerization of 5-CQA when mate is treated with superheated steam. In fact, 4-CQA is the logical first product of 5-CQA isomerization, seeing that the migration of the caffeoyl group from the 5- to the 4-acyl position is the least energetic path. Such transformations have been observed during the early stages of coffee roasting and when green coffee beans are dewaxed by steam treatment (34, 35).

The temperature history of samples in the reactors is presented in **Figure 4**. It confirms the successful heat transfer from surrounding air in the oven to the reactor but also shows that the kinetic experiments were indeed carried out in a pseudo isothermal condition. In particular, this deviation from an ideal state of constant temperature is more apparent at higher temperatures, where the on–off control was revealed as less efficient. The increase of the standard deviation of mean temperature from approximately 3 to 5 K when considering the extremes of the range of investigated temperature is clear evidence of this behavior. In summary, it means that experiments were performed at 398 ± 3 , 428 ± 3 , 461 ± 3.5 , and 499 ± 5 K. Although the absence of forced heat convection in the oven has negatively contributed to this scenario, it might be a positive point because it contributes to the excess of superheated steam in the reactor. On the contrary, mass transfer of superheated steam from the reactor to surrounding air would be increased at the top of the reactor, and the hypothesis of a pseudo first-order reaction would be weakened.

The analysis of kinetic data with the integral method by involving eq ³ promptly reveals the need to obtaining the conversions of 5-CQA at equilibrium as a primary step to calculate k_1 in the investigated range of temperature. With this purpose, the experimental results for reaction times higher than or equal to 1800 s (see the data in **Figures 7** and **8**), where equilibrium seems established, were immediately used to calculate a mean conversion for each different temperature. On the basis of these data, **Figure 5** shows that a straight line represented by eq ³ was able to correctly describe the set of kinetic experimental results up to 900 s at the different superheated steam temperatures. In all of these cases, the model estimates approximately more than 90% of the variance on the concentration of 5-CQA due to changes on residence time. In these plots, the angular coefficients times the conversion of 5-CQA at equilibrium are the forward rate constants times the superheated steam concentration in the reactor (k_1) at different temperatures.

As earlier explained, the parameters of the Arrhenius equation, which was applied to represent the influence of temperature on k_1 ,

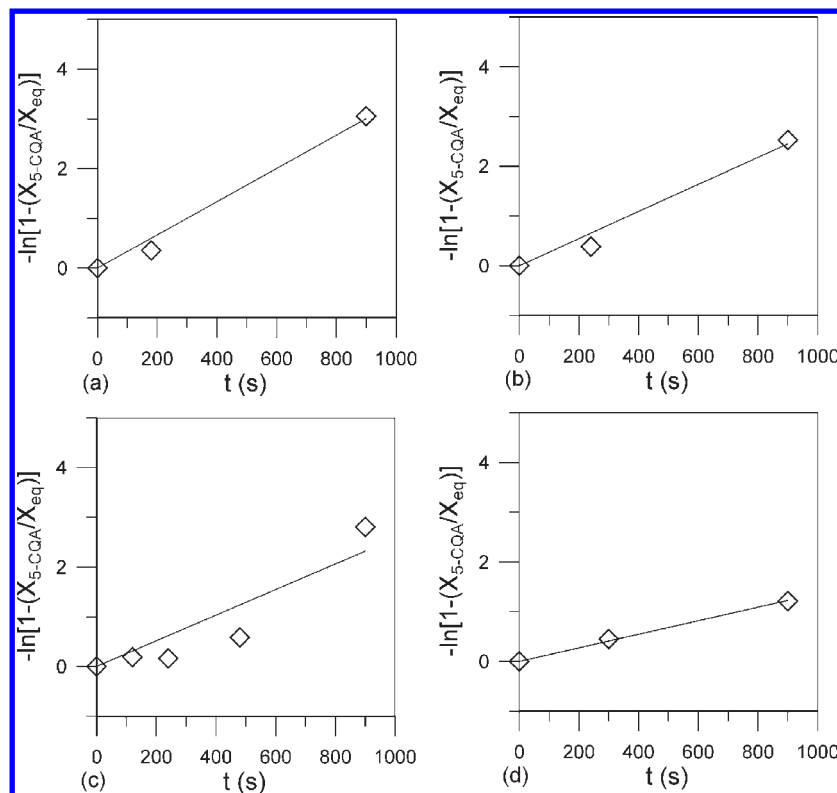


Figure 5. Determination of rate constants at 398 (a), 428 (b), 461 (c), and 499 K (d) based on a tuning procedure by involving eq 3 and X_{eq} experimentally obtained.

were tuned on the found set of forward rate constants times $[H_2O]$. Although the classical diagram of the logarithm of k_1 against the inverse of the temperature is not currently reported, a coefficient of determination (R^2) close to unity confirms the reliability of the Arrhenius model to represent these results. From this analysis emerges a frequency factor times the superheated steam concentration ($A \times [H_2O]$) equal to $4.88 \times 10^{-6} s^{-1}$ and a value of $-E_a/R = 2284$ K, which reveals that an apparent activation energy was indeed obtained. This behavior is attributed to a significant and not accounted effect of heat and mass transport resistances from the surface to the bulk of the solid particles of 5-CQA during reaction. Moreover, it also indicates that the reaction is not strictly an elementary step and the mechanism probably changes with temperature (29), which was confirmed by the presence of additional very small peaks (for species different from 5-CQA and 4-CQA) in the chromatograms at different temperature.

Equation ⁴ mathematically describes the effect of temperature on the conversion of 5-CQA at equilibrium. It had initially required the determination of a set of equilibrium constants by rearranging the first two left-side terms in eq ⁴ to have an explicit algebraic expression for K as a function of experimental X_{eq} . In this way, the dependence of the equilibrium constant on temperature by the integrated form of the van't Hoff equation (nominator of eq ⁴) was allowed as presented in **Figure 6**. The intercept and slope of the straight line in this figure represent the equilibrium constant ($K_{298} = 1.68$) and the ratio of heat of reaction to the universal gas constant ($-\Delta H_{298}/R = 1013$ K) at 298 K, respectively. The good agreement between experimental and calculated results supported by a coefficient of determination close to unity and no trend in residuals positively contributes to the reliability of these parameters. The magnitude of the found value of K_{298} supports the reversible nature of the reaction and a tendency of major production of 4-CQA in the presence of

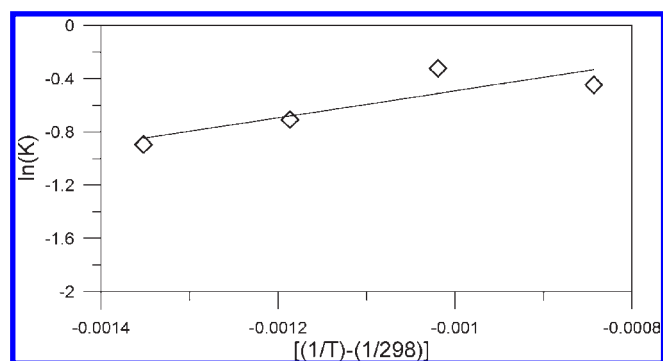


Figure 6. Influence of temperature on equilibrium constant. Symbols: data based on experimental conversions of 5-CQA at equilibrium (X_{eq}) from 398 to 499 K. Curve: van't Hoff equation with parameters ($K_{298} = 1.68$ and $\Delta H_{298} = -8425$ J mol⁻¹) tuned on experimental results.

superheated steam at equilibrium, which is reduced with the increase of temperature. The negative heat of isomerization reveals an exothermic reaction with low surrounding gain of energy from the system and a negative difference of enthalpy between product and reactants. As expected for exothermic reactions, the heat of reaction was greater than the activation energy (29).

Figures 7 and **8** present comparisons between experimental and calculated dimensionless 5-CQA concentrations at the investigated temperatures. In all of the cases, the proposed simplified mechanism of reaction was able to reproduce the experimental results. In spite of the absence of earlier kinetic similar investigations to check the consistency of the current experiments and modeling approach, some experimental evidence reported in the literature supports isomerization of 5-CQA at particular operating conditions (17, 31, 36, 37).

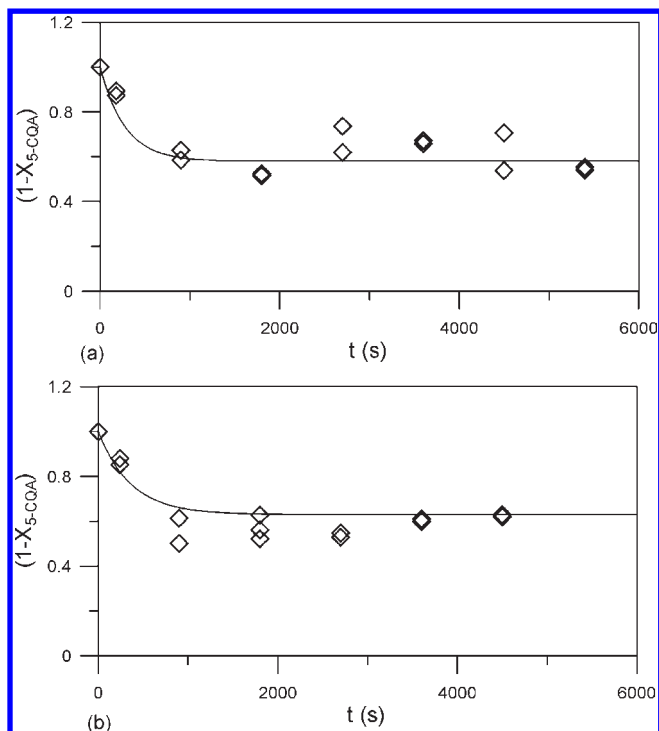


Figure 7. Comparison between experimental (symbols) and calculated (curves) results of 5-CQA isomerization at 398 (a) and 428 K (b).

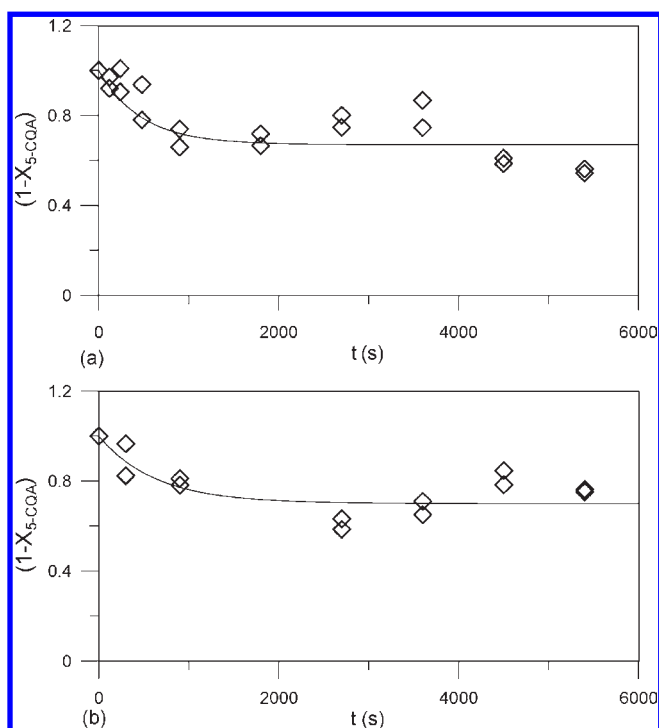


Figure 8. Comparison between experimental (symbols) and calculated (curves) results of 5-CQA isomerization at 461 (a) and 499 K (b).

From a practical point of view, the experimental and modeling results reveal that 5-*o*-caffeoylquinic acid, and probably all of the members of the family of CGAs, found in mate and in other foods rich in CGA manufactured at elevated temperatures, present negligible rates of oxidation when in contact with superheated steam. The reduction of 5-CQA concentration followed by the formation of 4-CQA observed at the investigated experimental conditions is not detrimental for the quality of food products

since the content of total CGA is not changed. The obtained results also expose the need of replacing the operation of drying with hot air typically found in the food industry by an analogous procedure with superheated steam.

ABBREVIATIONS USED

A , absorbance of aqueous solution of 5-CQA (mAu); A_{323} , absorbance of aqueous solution of 5-CQA at 323 nm (mAu); A , frequency factor in the Arrhenius equation for the forward reaction rate constant times the concentration of superheated steam (s^{-1}); CGA, chlorogenic acids; DAD, diode array detector; E_a , apparent activation energy of the forward reaction of isomerization of 5-CQA ($J mol^{-1}$); HPLC, high-performance liquid chromatograph; k_1 , forward reaction rate constant times the concentration of superheated steam (s^{-1}); k_2 , reverse reaction rate constant times the concentration of superheated steam (s^{-1}); K , equilibrium constant for the reaction of isomerization of 5-CQA to 4-CQA; K_{298} , equilibrium constant for the reaction of isomerization of 5-CQA at 298 K; R , universal gas constant ($J mol^{-1} K^{-1}$); R^2 , coefficient of determination; t , time of reaction (s); T , temperature (K); X_{eq} , conversion of 5-CQA at equilibrium; X_{5-CQA} , conversion of 5-CQA; ΔH_{298} , heat of the reaction of isomerization of 5-CQA to 4-CQA at 298 K ($J mol^{-1}$); λ , wavelength (nm); [5-CQA], concentration of 5-*o*-caffeoylquinic acid ($mol m^{-3}$); [4-CQA], concentration of 4-*o*-caffeoylquinic acid ($mol m^{-3}$); [H₂O], concentration of superheated steam ($mol m^{-3}$).

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