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Empirical Kinetic Modelling of the Effect of L-Ascorbic Acid on the Cu(II)-Induced Oxidation of Quercetin

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Abstract: This study aimed at investigating the effect of L-ascorbic acid on the Cu²⁺-induced oxidation of quercetin, within a pH range from 6.7 to 8.3 and temperatures varying from 53 to 87 °C. Initial examinations showed that quercetin degradation obeyed apparent first-order kinetics and it was significantly affected by temperature. Modelling of the effect of L-ascorbic acid by implementing response surface methodology suggested that L-ascorbic acid did not impact quercetin oxidation significantly ($p < 0.05$) and led to an empirical kinetic model based on temperature (T) and pH. Liquid chromatography–diode array–mass spectrometry analyses revealed the presence of typical quercetin degradation and oxidation products, including protocatechuic acid and 2-(hydroxybenzoyl)-2-hydroxybenzofuran-3(2H)-one. It was concluded that the formation of L-ascorbyl or other radicals (superoxide anion) may be involved in quercetin oxidation and this fact merits further attention to illuminate the possible beneficial or adverse nutritional consequences of such reactions in foods.

Keywords: L-ascorbic acid; copper(II); oxidation kinetics; quercetin; response surface methodology

1. Introduction

Quercetin is a ubiquitous flavonoid that belongs to the subclass of flavonols and may be present in several edible fruits and vegetables [1]. Quercetin is a well-studied molecule owed to its wide range of bioactivities, including chemopreventive action and beneficial effects against other cardiovascular degenerative diseases [2]. This particular flavonoid is also notorious for its powerful antioxidant properties, which, along with other dietary antioxidants such as vitamin C, vitamin E and carotenoids, may provide effective protection against oxidative stress [3].

Quercetin is prone to oxidation, which may be brought about either chemically or enzymatically and, depending on the conditions, quercetin oxidation could yield a spectrum of products, arising from dimerization, skeleton alteration and decomposition [4]. In any case, modification of the structural features of the parent molecule unavoidably leads to modification of the biological properties of quercetin, but this does not always entail complete abolishment of its potency. For example, some degradation products formed following quercetin oxidative cleavage were demonstrated to possess inferior antioxidant activity compared with quercetin itself [5]. Furthermore, a quercetin dimer, generated through quercetin oxidation was also shown to be a less effective antioxidant [6]. To the contrary, another quercetin oxidation product [2-(hydroxybenzoyl)-2-hydroxybenzofuran-3(2H)-one]

was shown to exhibit improved antioxidant characteristics [7]. More recent data on this particular product were in the same line, revealing a 200-fold higher cytoprotective potency [8].

L-Ascorbic acid (vitamin C) is a well-known food antioxidant that may eliminate a spectrum of oxidising species, such as superoxide anion radical ($O_2^{\bullet-}$), hydroxyl radical (OH^{\bullet}) and singlet oxygen, due to its ease of oxidation by either a one- or two-electron transfer [9,10]. Ascorbic acid may also lower the oxidation state of metals, such as iron and copper, and their valence, thus affecting oxidation catalysis. These unique features make L-ascorbic acid a powerful antioxidant, capable to protect a variety of substrates from oxidation. L-Ascorbic acid may be rapidly oxidised in the presence of bivalent copper (Cu^{2+}), but the extent of oxidation may be limited in the presence of metal-chelating flavonoids, such as quercetin [11,12].

However, L-ascorbic acid may also act as an antioxidant in such a system, protecting quercetin from Cu^{2+} -induced oxidative degradation [5]. Therefore, depending on the conditions (pH, temperature, the presence of other redox species, relevant amounts of redox species implicated), in a system containing L-ascorbic acid, quercetin and Cu^{2+} , one antioxidant may be sacrificed at the expense of the other, yet a prediction regarding the eventual shift of such a cascade of reactions would be rather difficult, due to the high complexity. This being the case, this study aimed at modelling the effect of L-ascorbic acid on quercetin stability in the presence of Cu^{2+} , considering the kinetic behaviour of quercetin degradation as a function of pH and temperature. Following a preliminary investigation, the interactions between L-ascorbic acid and quercetin were modelled by deploying response surface methodology. Tentative characterisation of major quercetin oxidation products using liquid chromatography–mass spectrometry enabled the identification of putative oxidation pathways.

2. Materials and Methods

2.1. Chemicals

Quercetin dihydrate, L-ascorbic acid, copper(II) chloride dihydrate and citric acid were from Sigma-Aldrich (St. Louis, MO, USA). Di-sodium hydrogen phosphate dihydrate was from Merck (Darmstadt, Germany). Stock solution of quercetin (20 mM) was prepared in acetone and stored at $-20\text{ }^{\circ}\text{C}$. Stock solution of copper(II) chloride (20 mM) was prepared in 0.05 M HCl and stored at $4\text{ }^{\circ}\text{C}$. Stock solution of L-ascorbic acid (5 mM) was prepared exactly before used in double-distilled water. All solvents used for liquid chromatography–mass spectrometry were of HPLC grade.

2.2. Treatment, Sampling and UV-Vis Measurements

For the preliminary experiments, an aliquot of 30 mL of phosphate-citrate buffer (50 mM, pH 7.0), was placed in a 50 mL glass reaction vial and spiked with an appropriate volume of a quercetin stock solution and copper(II) chloride stock solution, to give corresponding final concentrations of 33.3 and 66.6 μM . The mixture was under constant magnetic stirring at 300 rpm and heated up at $70\text{ }^{\circ}\text{C}$. Constant temperature was provided by immersing the reaction vial in oil bath, heated by a precisely thermostated hot plate (YellowLine MST Basic C, Richmond, VA, USA). Sampling was accomplished by withdrawing aliquots of 1 mL at regular intervals (5 to 40 min). After withdrawal, the sample was placed in 1.5 mL Eppendorf tube and cooled down with tap water. The cooled sample was transferred immediately to a 1 mL quartz cell and the absorbance of the A-band maximum of quercetin (Figure 1) was obtained for tracing kinetics. Following measurements, the sample was returned to the reaction vial to maintain the initial headspace volume. For the response surface methodology, the pH of the reaction mixtures and the reaction temperature were adjusted as dictated by the experimental design. The concentration of L-ascorbic acid in the reaction mixture, also defined by the experimental design, was adjusted by spiking the mixture with appropriate volume of the L-ascorbic acid stock solution.

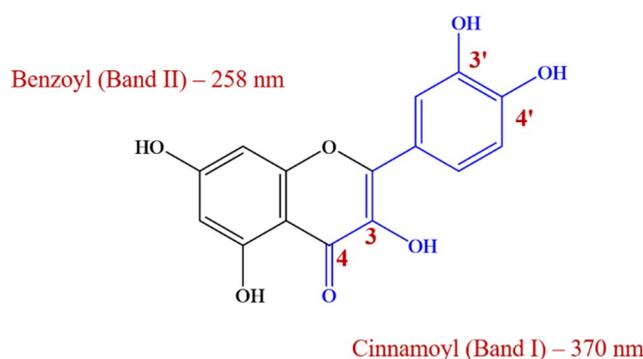


Figure 1. Quercetin structure and assignment of characteristic absorption maxima to specific structural features.

2.3. Large-Scale Treatment and Sample Preparation

A phosphate-citrate buffer solution (50 mM, pH 7.5) was spiked with appropriate volumes of quercetin, Cu^{2+} and L-ascorbic acid to give final concentrations of 333, 666 and 400 μM , respectively. This solution was heated at 87 °C for 40 min and then it was cooled down to ambient temperature with tap water. A volume of 15 mL of the solution was adjusted to pH 1 with 6 N HCl and then extracted in a separatory funnel three times with 15 mL ethyl acetate:diethyl ether (8:2). The extracts were pooled and taken to dryness in a rotavapor (Büchi Rotavapor R-114, Merck), at 40 °C. The residue was dissolved in 1.5 mL methanol and this sample was used for chromatographic analyses.

2.4. Experimental Design

The purpose of the assay was to evaluate the effect of L-ascorbic acid on the stability of quercetin in the presence of Cu^{2+} , as a function of L-ascorbic acid concentration (C_{AA}), temperature (T) and pH. The stability of quercetin was estimated by calculating the apparent first-order decay constant k , by tracing kinetics. Thus, k was considered as the response. To simultaneously assess the influence of the three variables, a central composite design—uniform precision methodology with six central points was deployed. The independent variables were coded between -1.682 (lower limit) and $+1.682$ (upper limit), using the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X_i}, \quad i = 1, 2, 3. \quad (1)$$

Terms x_i and X_i correspond to the dimensionless and the actual value of the independent variable i . The term X_0 is the actual value of the independent variable i at the central point of the design, and ΔX_i the step change of X_i (Table 1). The choice for the range of values for all variables was based on preliminary runs and previous data [5]. ANOVA was carried out to estimate model significance, the significance for each polynomial coefficient, and determine the overall coefficient R^2 for the mathematical model. Statistically non-significant dependent terms ($p > 0.05$) were not considered in the models and were omitted. The models were validated by carrying out runs using the predicted optimal conditions and comparing the predicted values with the actual (measured) ones.

Table 1. Actual and coded values of all three independent variables considered for the experimental design.

Independent Variable	Code	Coded Variable Level				
		-1.682	-1	0	1	1.682
C_{AA} (μM)	X_1	6.4	20	40	60	73.6
pH	X_2	6.7	7	7.5	8	8.3
T (°C)	X_3	53	60	70	80	87

2.5. Liquid Chromatography-Diode Array-Mass Spectrometry (LC-DAD-MS)

A Finnigan (San Jose, CA, USA) MAT Spectra System P4000 pump, coupled with a UV6000LP diode array detector and a Finnigan AQA mass spectrometer was employed. Chromatography was carried out on a Fortis RP-18 column, 150 mm × 2.1 mm, 3 μm, at 40 °C. Mass spectra were acquired with electrospray ionization (ESI) in positive ion mode, with probe temperature 350 °C, acquisition 10 and 50 eV, source voltage 25 V, capillary voltage 4 kV and detector voltage 650 V. The eluents were (A) 1% acetic acid and (B) methanol. The flow rate was 0.2 mL·min⁻¹, and the elution programme used was from 0 to 10% B in 2 min; from 2 to 22 min, 100% B; from 22 to 27 min, 100% B; and then back to initial conditions within 5 min.

2.6. Statistics

Each experiment was carried out at least twice and each measurement at least three times. The values reported are means ± standard deviation. The experimental design and all relevant statistical analyses were performed with JMP™ Pro 13 (SAS, Cary, NC, USA). Curve fitting for estimating kinetic models was performed using SigmaPlot™ 12.5 (Systat Software Inc., San Jose, CA, USA), at least at a 95% significance level ($p < 0.05$).

3. Results and Discussion

3.1. Initial Spectrophotometric Examination

The flavonoid skeleton of quercetin (Figure 1) exhibits a distinct λ_{\max} in methanol, at 370 nm (Band I) [13]. On the other hand, quercetin oxidative degradation leads in the formation of simple phenolics that possess uniformly low absorbance at this region of the spectrum [14]; thus, quercetin degradation may be effectively monitored by recording A_{370} , without perturbation. However, quercetin is an efficient metal chelator and complexation with Cu²⁺ has been shown to provoke a bathochromic shift in λ_{\max} . In methanol, complexation of Cu²⁺ by the 3-OH and the 4-C=O group may lead in a λ_{\max} shift at 436 nm [15], but upon addition of a complexing agent such as EDTA, the initial λ_{\max} is almost completely restored. In a phosphate buffer at pH 7.4, a shift at 460 nm has been reported and attributed to a quercetin: Cu²⁺ 1:1 complex [16].

Quercetin (33.3 μM) in phosphate-citrate buffer at pH 7.5 exhibited λ_{\max} at 372 and 258 nm and upon addition of Cu²⁺ and L-ascorbic acid at a quercetin: Cu²⁺: L-ascorbic acid molar ratio of 1:2:1.8, no bathochromic shift was observed. It should be noted that the lack of bathochromic shift in the UV-vis spectrum of quercetin upon chelation with Cu²⁺ has also been reported by other authors [17]. At this point, the effect of pH should also be considered. While up to pH 7.4 the shift observed in Band I absorption is limited to a few nm, at pH 8.6 this shift may reach up to 25–30 nm, owing to the deprotonation of the B-ring OH groups [18]. Indeed, at pH 8.3 quercetin displayed a bathochromic shift for both Bands I and II, having λ_{\max} at 390 and 269 nm.

3.2. Preliminary Oxidation Kinetics and Temperature Effects

Early studies on quercetin oxidation demonstrated that this reaction obeyed apparent first-order kinetics [19]:

$$u = k[Qt], \quad (2)$$

where k is the experimentally determined reaction rate constant (min⁻¹) and [Qt] the molar concentration of quercetin. The reaction was shown to be pH-dependent within a pH range from 4.1 to 7, exhibiting a linear correlation between k and pH. Such a phenomenon was further confirmed by more recent examinations, which showed a pH-dependent degradation of quercetin within a narrower pH region of 6 to 7.5, at 37 °C, but also a temperature-dependent increase in k , within a range of 37 to 65 °C [20]. In the same line, latter investigations showed that Cu²⁺-induced quercetin oxidation at pH 8 and 97 °C also displayed first-order kinetics, with k being linearly correlated

with $[\text{Cu}^{2+}]$ [14]. Under these conditions, L-ascorbic acid was proven to inhibit quercetin oxidative degradation significantly ($p < 0.05$), when added at a quercetin: L-ascorbic acid molar ratio of 2 [5].

On this ground, preliminary experiments were carried out to draw information for the kinetic behaviour of quercetin degradation in a citrate-phosphate buffer. First, it was tested the effect of temperature by performing kinetic assays at both 50 and 90 °C and pH 7, with a quercetin: Cu^{2+} molar ratio of 2 (Figure 2a). Using SigmaPlot™ 12.5, the model best fitted to experimentally obtained data was pseudo first-order kinetics, described as follows:

$$A_{367(t)} = A_{367(0)}e^{-kt}. \quad (3)$$

$A_{367(t)}$ represents the absorbance at 367 nm at any time t (min), $A_{367(0)}$ the initial absorbance and k (min^{-1}) the apparent first-order decay constant. The absorbance at 367 nm was used because, under the conditions employed, it was found to be the λ_{max} of quercetin Band I. The linearised form of Equation (3) would be:

$$\ln A_{367(t)} = \ln A_{367(0)} - kt. \quad (4)$$

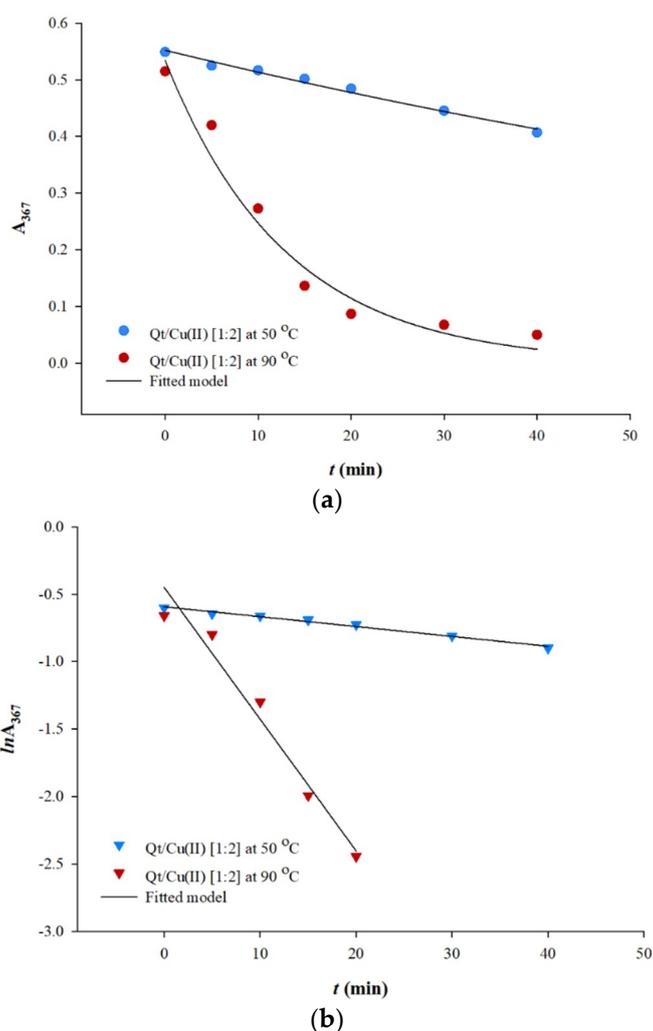


Figure 2. Kinetics of quercetin oxidation (33.3 μM) at pH 7 in the presence of 66.6 μM of Cu^{2+} (a) and verification of pseudo first-order kinetics shown by linear plots (b).

A plot of $\ln A_{367}(t)$ against t is a straight line with the slope equal to $-k$ (Figure 2b). The half-life ($t_{1/2}$) of quercetin could then be calculated:

$$t_{1/2} = \frac{0.693}{k}. \quad (5)$$

The graphical determination of k at 50 and 90 °C gave corresponding values of 0.0073 and 0.0771 min^{-1} . The half-lives determined using the Equation (5) were 94.9 and 9 min, respectively. These findings suggested a significant dependence of quercetin degradation on temperature. To confirm this, the activation energy (E_a) of the reaction was determined as follows [21]:

$$\ln\left(\frac{k_2}{k_1}\right) = \left(-\frac{E_a}{R}\right)\left(\frac{1}{T_2} - \frac{1}{T_1}\right), \quad (6)$$

where k_1 and k_2 are the quercetin first-order decay constants at 50 and 90 °C, respectively, R the universal gas constant ($8.314 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$) and T_1 and T_2 the temperatures 50 and 90 °C, expressed in K. The E_a thus estimated was $59.39 \text{ kJ}\cdot\text{mol}^{-1}$, illustrating a significant dependence on T .

When the kinetics was investigated in the presence of L-ascorbic acid (Figure 3a), λ_{max} was recorded at 390 nm and the model best fitted to the kinetic data was:

$$A_{390}(t) = A_{390}(i) + ae^{-kt}. \quad (7)$$

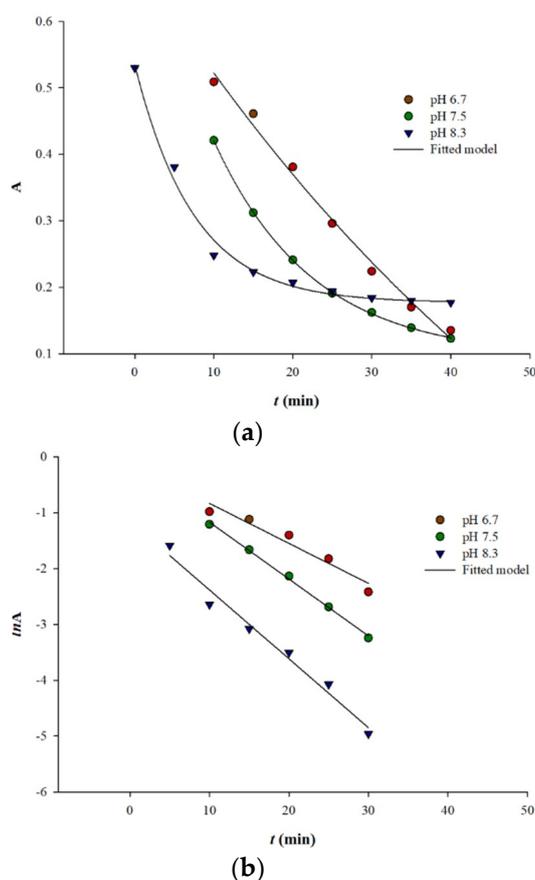


Figure 3. Effect of pH on the kinetics of quercetin oxidation ($33.3 \mu\text{M}$) in the presence of $66.6 \mu\text{M}$ of Cu^{2+} (a) and verification of pseudo first-order kinetics shown by linear plots (b). Reactions were performed in the presence of $40 \mu\text{M}$ L-ascorbic acid and $T = 70 \text{ }^\circ\text{C}$.

In Equation (7), a is a fitting parameter and $A_{390(i)}$ the absorbance at infinite reaction time. For simplicity, $A_{390(i)}$ was considered the absorbance at the end of the assay (40 min). Linearisation of Equation (7) would yield:

$$\ln(A_{390(t)} - A_{390(i)}) = \ln a - kt. \quad (8)$$

A plot of $\ln(A_{390(t)} - A_{390(i)})$ as a function of t would give a straight line with a slope equal to $-k$ (Figure 3b). To ascertain whether pH could affect kinetics, experiments were carried out at pH 6.7, 7.5 and 8.3 at a constant temperature of 70 °C, and in all cases Equation (7) was the model with the highest statistical significance ($R^2 > 0.97$). For pH 6.7, 7.5 and 8.3 the corresponding k determined were 0.0143, 0.0806 and 0.1335 min^{-1} . This fact pointed to the pH dependence of k .

3.3. Response Surface Modelling

The evidence emerged from the preliminary investigation suggested that k may be strongly affected by both pH and T , yet the role of C_{AA} was unclear. To reveal the combined effects of these three variables, a central composite design—uniform precision methodology was employed. This experimental design allowed for the monitoring of the response (k) in relation with simultaneous variation of all three variables and permitted the detection of possible cross effects. The trends recorded were visualised in 3D plots (Figure 4). By using the desirability function, it was possible to estimate the combination of the three independent variables that would yield maximum k (Figure 5), and this also enabled the prediction of conditions under which maximum k could be attained. After omitting all non-significant terms (Figure 5, inset table), the mathematical model was:

$$k \left(\text{min}^{-1} \right) = 0.0288 + 0.0138X_2 + 0.0224X_3 - 0.0225X_2X_3 + 0.0236X_2^2 \left(R^2 = 0.92, p = 0.0003 \right). \quad (9)$$

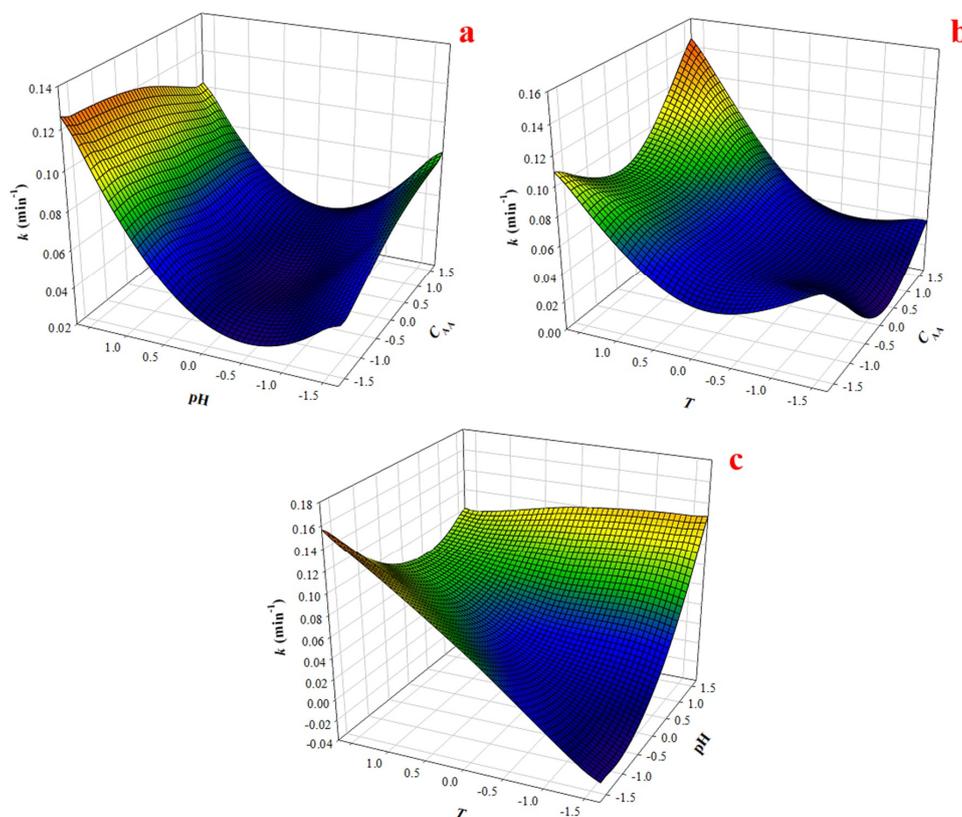


Figure 4. 3D plots illustrating the effect of the simultaneous variation in (a) pH and C_{AA} , (b) T and C_{AA} and (c) pH and T on k .

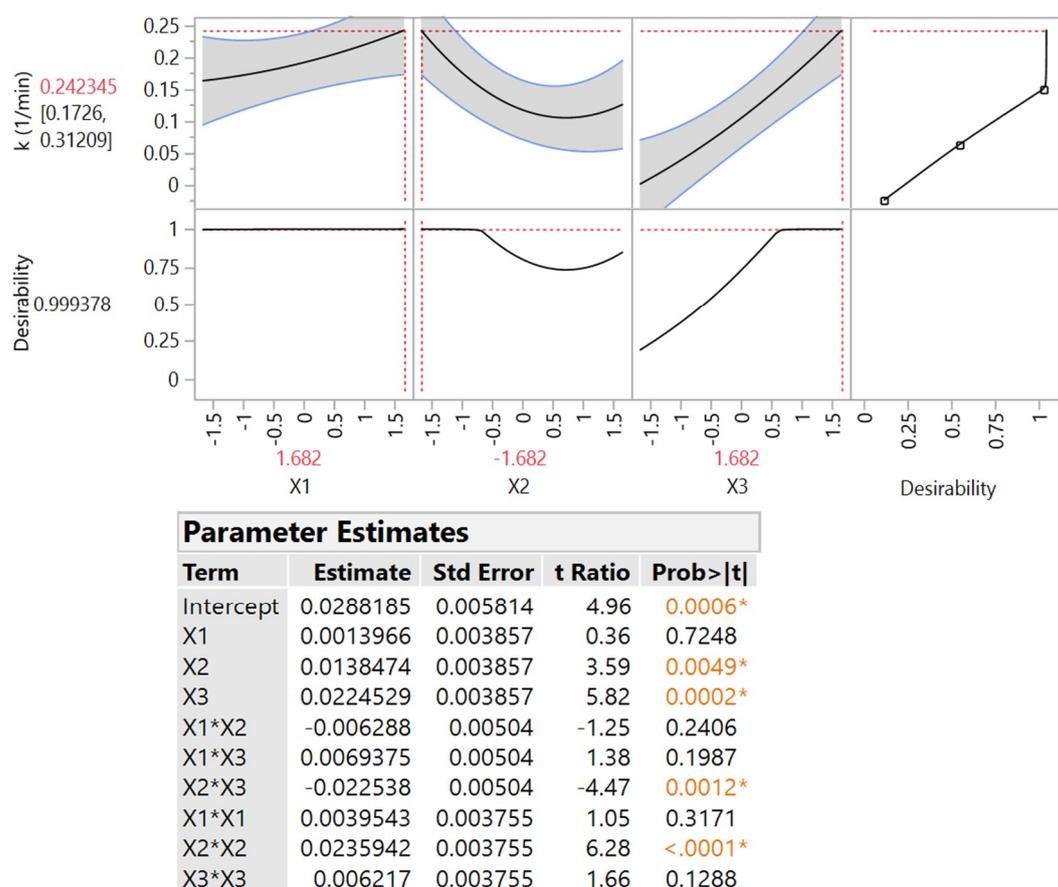


Figure 5. Desirability function and parameter estimates (inset table) determined after implementing a central composite design—uniform precision methodology. Asterisk (*) denotes statistically significant terms.

Out of the three independent variables considered, pH and T were significant and positively affected k , but C_{AA} was not. This outcome showed clearly that any increase in either pH or T up to the limits defined by the model, would accelerate quercetin degradation. The negative effect of the cross term X_2X_3 indicated that acceleration of quercetin due to increased pH could be compensated by lowering T and *vice versa*. Since no cross terms involving X_1 were significant, acceleration of quercetin degradation by switching either pH or T at higher values could not be regulated by modifying C_{AA} (Table 2). Therefore, under the conditions used, the presence of L-ascorbic acid could only contribute towards accelerating or inhibiting quercetin degradation, indirectly. By setting pH and T at their corresponding optimum values 6.7 and 87 °C, the theoretical maximum k was estimated to be $0.2423 \pm 0.0697 \text{ min}^{-1}$ (Figure 5).

The finding that L-ascorbic acid had no obvious effect on quercetin oxidation was rather a paradox, in the light of previous examinations that demonstrated a decline in quercetin degradation rate at pH 8 and 97 °C, more than 7-fold, when L-ascorbic was added at a quercetin/L-ascorbic molar ratio of 2 [5]. Apparently, the relative amounts of all redox species implicated (Cu^{2+} , L-ascorbic acid, quercetin) may play a crucial role in this regard and switching the concentration of any of them could impact the system in a differentiated manner. However, redox potentials and the effect of pH are prominent factors involved in the relevant reactions and the thorough investigation of their involvement might be the key to understanding the behaviour observed.

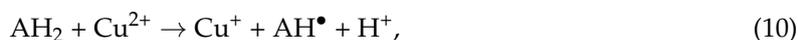
First, it should be noted that quercetin has lower oxidation potential (E_p) than L-ascorbic acid, their corresponding values being 91 and 127 mV [22]. Therefore, it could be supported that quercetin would be preferentially oxidised by Cu^{2+} . In this case, it could be simply argued that this is the reason

for the apparently non-significant effect of L-ascorbic acid on k . Indeed, in a tertiary system containing L-ascorbic acid, Cu^{2+} and quercetin, the flavonoid acted as a protecting agent against L-ascorbic acid oxidation [23–25], a fact attributed to the chelating ability of quercetin. This was because the oxidation of L-ascorbic acid by Cu^{2+} was assumed to be mediated by monohydroascorbate/ Cu^{2+} complex [24], whose formation was presumably inhibited by quercetin.

Table 2. Measured and predicted values of k at all design points used for the response surface methodology.

Design Point	Independent Variable Level			Response (k , min^{-1})	
	X_1	X_2	X_3	Measured	Predicted
1	−1	−1	−1	0.0019	0.0030
2	−1	−1	1	0.0871	0.0791
3	−1	1	−1	0.0700	0.0883
4	−1	1	1	0.0769	0.0743
5	1	−1	−1	0.0002	0.0045
6	1	−1	1	0.1250	0.1084
7	1	1	−1	0.0550	0.0647
8	1	1	1	0.0778	0.0784
9	−1.682	0	0	0.0421	0.0377
10	1.682	0	0	0.0403	0.0424
11	0	−1.682	0	0.0600	0.0723
12	0	1.682	0	0.1335	0.1188
13	0	0	−1.682	0.0277	0.0086
14	0	0	1.682	0.0675	0.0842
15	0	0	0	0.0256	0.0288
16	0	0	0	0.0215	0.0288
17	0	0	0	0.0254	0.0288
18	0	0	0	0.0401	0.0288
19	0	0	0	0.0300	0.0288
20	0	0	0	0.0321	0.0288

On the other hand, the progression of the phenomenon as a function of pH was rather obscure. Although at low temperature increased k was recorded for high pH, when the temperature was raised to the maximum value defined by the experimental design (87 °C), maximisation of k occurred at pH 6.7 (Figure 4c). It could be postulated that at 53 °C, quercetin/ Cu^{2+} complex is more stable, representing the main pathway of quercetin oxidation. By contrast, high temperatures might provide the appropriate energy required to destabilise such a complex, giving rise to interactions of Cu^{2+} with L-ascorbic acid. Should this be the case, the increased k observed might be ascribed to oxidising species formed through Cu^{2+} /L-ascorbic acid interactions. Indeed, it has long been known that Cu^{2+} can oxidise L-ascorbic acid as follows [25,26]:



where AH_2 represents L-ascorbic acid. Should the reaction (i) take place, quercetin degradation could be stimulated by the generation of Cu^+ . This is because Cu^+ is more effective than Cu^{2+} in inducing quercetin oxidation [16]. Given that organic radicals may initiate oxygenation of flavonols by dioxygen [27], then reaction of AH^\bullet with quercetin could be another possible cause of the faster quercetin degradation. Furthermore, once Cu^+ is formed, then another reaction would be possible:



The superoxide anion radical ($\text{O}_2^{\bullet-}$) could then react with quercetin in a manner simulating quercetinase (dioxygenase) oxidation [28], giving rise to the depside (DP) and possibly to its decomposition products (a) and (b) [29] (Figure 6).

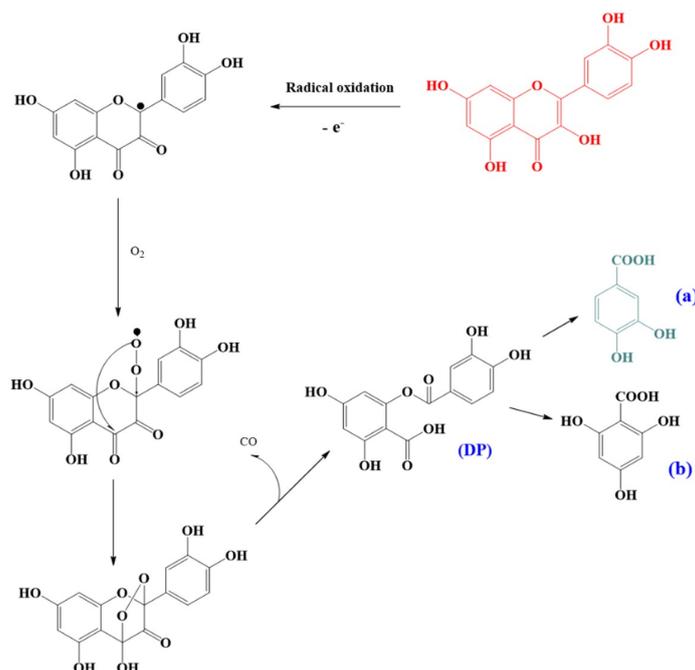


Figure 6. Putative pathway of radical-mediated quercetin oxidation. (DP) depside; (a) protocatechuic acid (3,4-dihydroxybenzoic acid); (b) phloroglucinol carboxylic acid (2,4,6-trihydroxybenzoic acid).

3.4. Oxidation Products and Putative Pathways

To substantiate any of the putative pathways proposed, LC-DAD-MS analyses were performed to tentatively identify oxidation/degradation products of quercetin. In Figure 7 it can be seen that two major substances were detected in the sample that exhibited the highest k (sample #12, Table 2). The presence of protocatechuic acid (product (a), Figure 6) was affirmed by its molecular ion at $m/z = 155$. This compound is a typical quercetin degradation product and may be formed under a variety of conditions, including Fe^{2+} and Cu^{2+} -induced thermal degradation [14], electrochemical oxidation [4,30,31], autoxidation [4,32,33], and radical oxidation [4,5].

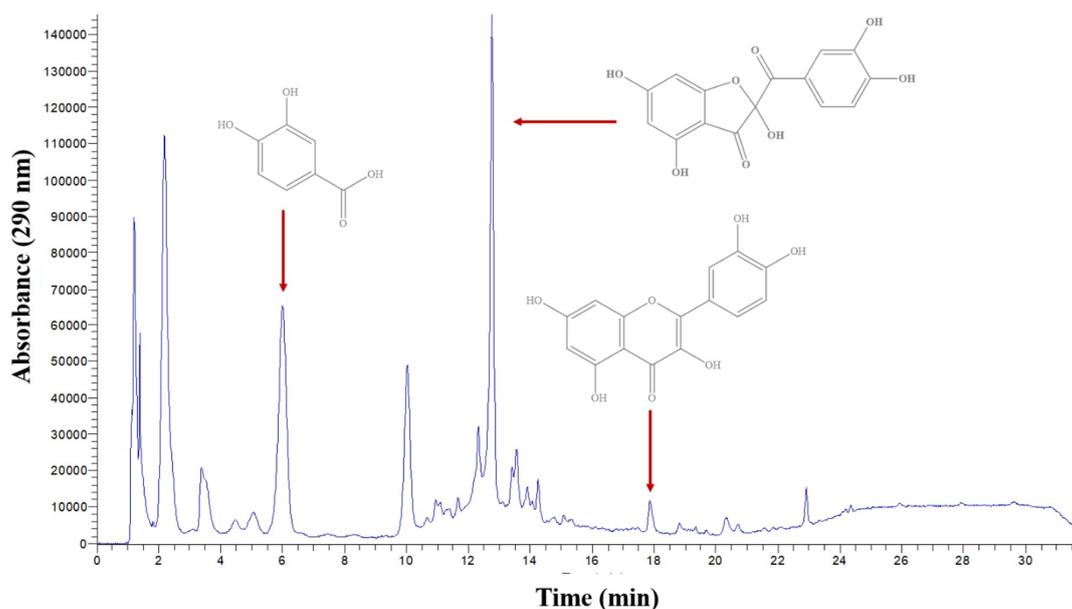


Figure 7. Chromatogram of sample #12 (see Table 2), monitored at 290 nm.

In every case, a mechanism implicating the cascade of reactions shown in Figure 6 was postulated, pointing to a radical-mediated oxidation mechanism. However, it has been demonstrated that Cu^{2+} -mediated quercetin oxidation did not generate protocatechuic acid, which was formed only in the presence of H_2O_2 [5]. Therefore, considering that Reaction (11) took place, the presence of H_2O_2 was rather likely as follows [34]:



On the other hand, the tentative identification of compound (f) (Figure 8), based on its pseudo molecular ion at $m/z = 319$ and a diagnostic Na^+ adduct at $m/z = 341$, strongly suggested the possible involvement of another oxidation pathway for quercetin, since protocatechuic acid has not been reported to derive from the decomposition of (f). In this case, formation of the quercetin/ Cu^{2+} complex (c) happened first, which led to quercetin oxidation through 2H^+ abstraction (Figure 7). Subsequent steps would include the formation of a quinone methide (d) and a hydro-derivative (e) as intermediates, yielding eventually compound (f), which is the 2-(hydroxybenzoyl)-2-hydroxybenzofuran-3(2H)-one (Figure 8).

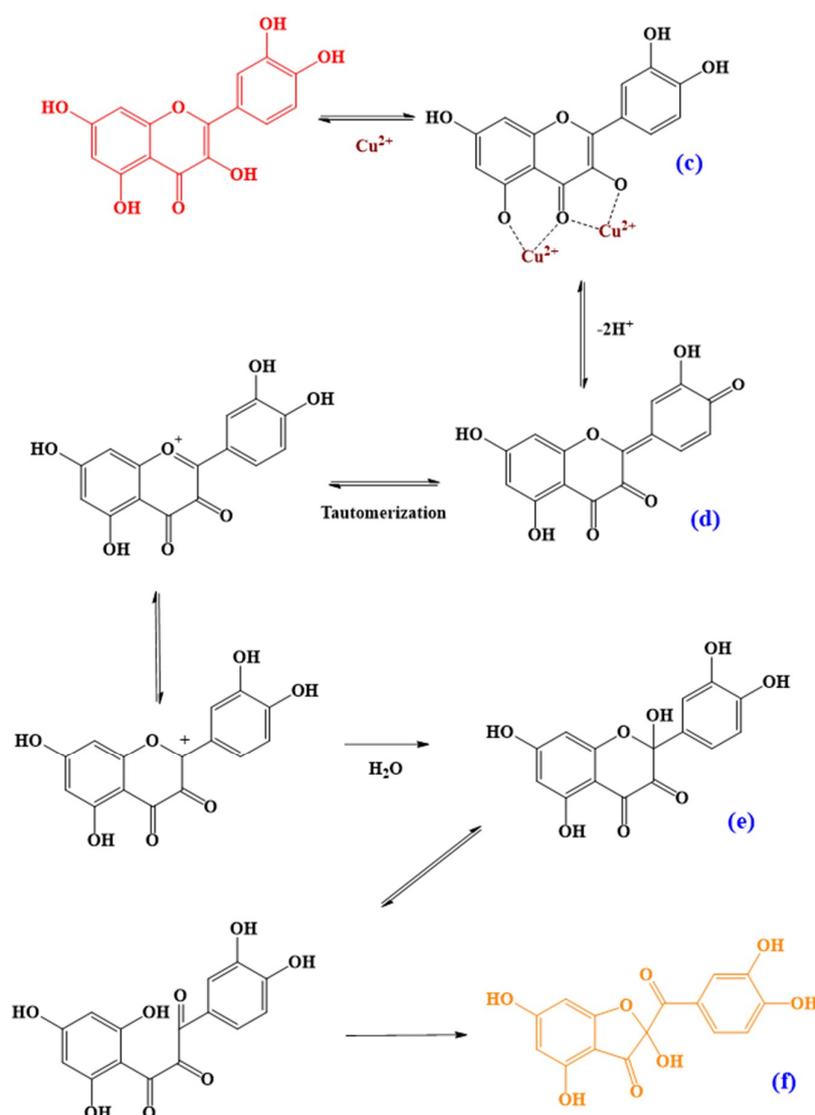


Figure 8. Putative pathway leading to the formation of the 2-(hydroxybenzoyl)-2-hydroxybenzofuran-3(2H)-one.

4. Conclusions

The kinetic investigation of Cu^{2+} -induced quercetin oxidative degradation showed that it obeyed pseudo first-order kinetics irrespective of the presence of L-ascorbic acid and that the reaction was significantly affected by T . Modelling of the effect of L-ascorbic acid by deploying response surface methodology revealed that L-ascorbic acid did not have direct involvement, but its role in quercetin oxidation was implied by the putative pathways proposed. Therefore, an empirical pseudo first-order model was proposed, as a function of T and pH. The tentative identification of protocatechuic acid and 2-(hydroxybenzoyl)-2-hydroxybenzofuran-3(2H)-one suggested that, apart from a mechanism of 2H^+ abstraction as a result of complexation with Cu^{2+} , quercetin may be oxidised through the involvement of L-ascorbyl or other radicals (superoxide anion). Evidence also emerged indicating that the relevant reactions were rather pH-dependent.

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Conflicts of Interest: The authors declare no conflict of interest.

Nomenclature

k	first-order quercetin decay constant (min^{-1})
C_{AA}	L-ascorbic acid concentration (μM)
E_{a}	activation energy ($\text{kJ}\cdot\text{mol}^{-1}$)
R	universal gas constant ($\text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$)
T	temperature ($^{\circ}\text{C}$ or K)
t	time (min)
$t_{\frac{1}{2}}$	half-life of quercetin (min)

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