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Effect of heat processing on thermal stability and antioxidant activity of six flavonoids

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Abstract

The objective of this paper is on the one hand to study the effect of heat processing (30 to 130°C for 2 h) on the stability and antioxidant activity of six flavonoids (rutin, naringin, eriodictyol, mesquitol, luteolin, and luteolin 7-O glucoside), and on the other hand to establish the relation structure–activity–stability of these compounds. The dependency on temperature of the six kinetics was well described by the Arrhenius law and the main parameters provided by this model are determined and compared in this paper. Activation energies found were 107.3 kJ/mol for rutin, 100.6 for naringin, 33.3 for mesquitol, 68.2 for eriodictyol, 51.4 for luteolin, and 120 for luteolin 7-O-glucoside. The data collected showed that glycosylated flavonoids are more resistant than aglycon flavonoids to heat treatment. Moreover, it was also observed that despite the total degradation of some flavonoids, the treated solutions still have an antioxidant activity.

Practical application

Flavonoids are mainly consumed in processed foods. Thus, flavonoids often undergo heat processing. Knowing the thermal stability and the evolution of their antioxidant activity are particularly relevant in the field of food processing. The results of this study allow information to be collected on the relationship between heat stability-flavonoid structure and antioxidant activity. This information will be useful for the formulation of new food products or for the design of new food processes.

KEYWORDS

antioxidant activity, flavonoid, heat processing, modelling, thermal stability

1 | INTRODUCTION

Over the last few decades, epidemiological studies have shown that consuming fruit and vegetables rich in phenolic compounds leads to a general well-being of consumers (Tomás-Barberán, Ferreres, & Gil, 2000). These benefits have been attributed to phenolic compound properties, particularly antioxidant activity which plays a role in this health-promoting capacity (Mellor & Naumovski, 2016). Among phenolic compounds, flavonoids are the most studied class and much interest is focused on them. The antioxidant activity of flavonoids depends on their structure (Heim, Tagliaferro, & Bobilya, 2002; Rice-Evans, Miller, & Paganga, 1996). Modifications of the structure such as alkylation or glycosylation induce a change of their properties; antioxidant activity can be increased or decreased depending on these modifications. Moreover, the human diet is based mainly on the consumption of products having undergone heat treatment, as a transformation step from raw materials to finished products. These processes may have an impact on flavonoid structure and consequently on their antioxidant activities (Bordiga, Travaglia, Mazza, & Arlorio, 2015; Nayak, Liu, & Tang, 2015; Saikia, Mahnot, & Mahanta, 2016).

Flavonoids are classified into nine groups. Compounds belonging to the same group differ from each other in their structure (degree and position of hydroxylation, presence of a substituent, etc.). These differences in structure can affect their stability to heat processing. Most available data concern only two groups—anthocyanins and isoflavones. Studies on anthocyanins have focused on the modeling of flavonoid

degradation (Patras, Brunton, O'Donnell, & Tiwari, 2010) while studies on isoflavones have investigated the thermal stability of several isoflavones according to their structure (Stintzing, Hoffmann, & Carle, 2006; Ungar, Osundahunsi, & Shimoni, 2003). Apart from these two main studies, Buchner, Krumbein, Rhon, and Kroh (2006) carried out preliminary investigations on quercetin and rutin, and suggested the presence of correlations between structure, antioxidant activity, and heat treatment. Few studies have dealt with the identification of new formed products from flavonoid degradation. This is because they are unstable and lead to further degradations (Dall'acqua, Miolo, Innocenti, & Cafieri, 2012; Ramešová, Sokolová, Terábek, & Degano, 2013). Only Buchner et al. (2006) proposed a degradation pathway for quercetin during a thermal process at 100°C under oxygen conditions. Thus, a lot still has to be discovered about the impact of heat treatment on the relationship between structure and antioxidant activity of flavonoids and about the identification of the degradation products. Such findings are essential for the development of a tool to predict the effect of heating on flavonoid structure, antioxidant activity, and biological activities. In this paper, the thermal stability of rutin, mesquitol, naringin, eriodictyol, luteolin, and luteolin 7-O glucoside was investigated. These compounds were heated to different temperatures for 2 hr. The kinetics of their degradations were modeled and the relationship between the various phytochemical structures and their antioxidant activities were studied.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Naringin (purity > 95%), rutin (purity > 95%), 2′-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich Chemical. Eriodictyol, luteolin, luteolin-7-O-glucoside were purchased from Extrasynthese (purity > 98%). Mesquitol (purity > 90%) was extracted from a Kenya tree *Proposis juliflora* (Sirmah, Dumarcay, Masson, & Gerardin, 2009). Trolox and potassium persulfate were purchased from Fluka. Methanol and ethanol were from Carlo Erba and VWR, respectively. All reagents and solvents were of analytical grade.

2.2 | Structural differences between flavonoids

The flavonoids chosen for this study are characterized by a difference in their structure and have a good solubility in water. The flavonoids are subdivided into nine classes. All compounds of the same class have a common structure. Four classes are relevant to study because their common structures differ from each other by one structural element. To study the effect of heat treatment, six molecules belonging to these four classes of flavonoid were selected: flavones (luteolin, luteolin 7-O glucoside) differ from flavonols (rutin) by the absence of the catechol structure. Flavanones (naringin, eriodictyol) differ from flavonols (rutin) by the absence of the enone structure on the C-ring. Flavanols (mesquitol) differ from flavonols (rutin) by the absence of the enone structure and a group carbonyl on the C-ring. In Figure 1, we can see the structure of the six flavonoids. In this study, water was chosen as a food matrix.

2.3 | Model solutions

Aqueous solutions of six flavonoids were prepared according to their solubility limit (naringin 0.29 mM, rutin 0.041 mM, mesquitol 0.93 mM, eriodictyol 0.059 mM, luteolin 0.0087 mM, and luteolin-7-O-glucoside 0.014 mM). Flavonoids were solubilized in the dark at 25°C until maximum solubilization was achieved. The pH value of the different solutions is equal to 6.6 ± 0.1.

2.4 | Thermal treatment of model solutions

Thermal treatments were conducted in an oil-based bath (Hubert, W8518D). The different model solutions were filtered through a syringe filter (0.20 μm) then 10 mL samples of each solution were put into screw-cap pyrex tubes and heated from 70 to 130°C for a total duration of 120 min. Samples were taken every 15 min and cooled in an ice bath to 30°C. Preliminary studies showed that differences in the degradation of flavonoids after 2 hr of heating are not significant. The residual flavonoid content and the antioxidant activity of the solution were measured.

2.5 | HPLC/DAD analysis

Flavonoid contents were analyzed in an Elite Lachrom HPLC system (VWR HITACHI) which consisted of a quaternary pump (L-2130), an autosampler (L-2200), and a diode-array detector (L-2455). The analyses were carried put on a C18 (150 × 4.6 mm) column (Grace). The different compounds were separated using a water (A) + methanol (B) elution system. Samples were eluted at a flow rate of 1 mL/min with a gradient of 95% water (A) at time 0 up to 100% (B) at 10 min and finally 95% (A) at 20 min before the end of the program. The total duration was 30 min. The injection volume was of 50 μL and the column temperature was set at 50°C. The detection was performed simultaneously at 200, 280, 254, and 350 nm. Flavonoid content was expressed as a percentage compared to the initial content. Then the degradation kinetic was plotted according to heating time. Triplicate measurements were taken for all samples.

2.6 | Identification of the new formed products from flavonoid degradation

A solution of quercetin at 1 mM was prepared in 100 mM KCl and 0.36 mM KOH in order to increase solubility. Aqueous solutions of rutin and eriodictyol were prepared at 0.65 and 0.059 mM, respectively. These three solutions were filtered through a syringe filter (0.20 μm) and heated to 130°C for 2 hr. Qualitative and semiquantitative analyse of new formed products were realized using an HPLC-MS system (ThermoFisher Scientific, San Jose, CA, USA) consisting of a binary solvent delivery pump connected to a photodiode array detector (PDA) and an LTQ mass spectrometer equipped with an atmospheric pressure ionization interface operating in electrospray negative mode (ESI^-). 25

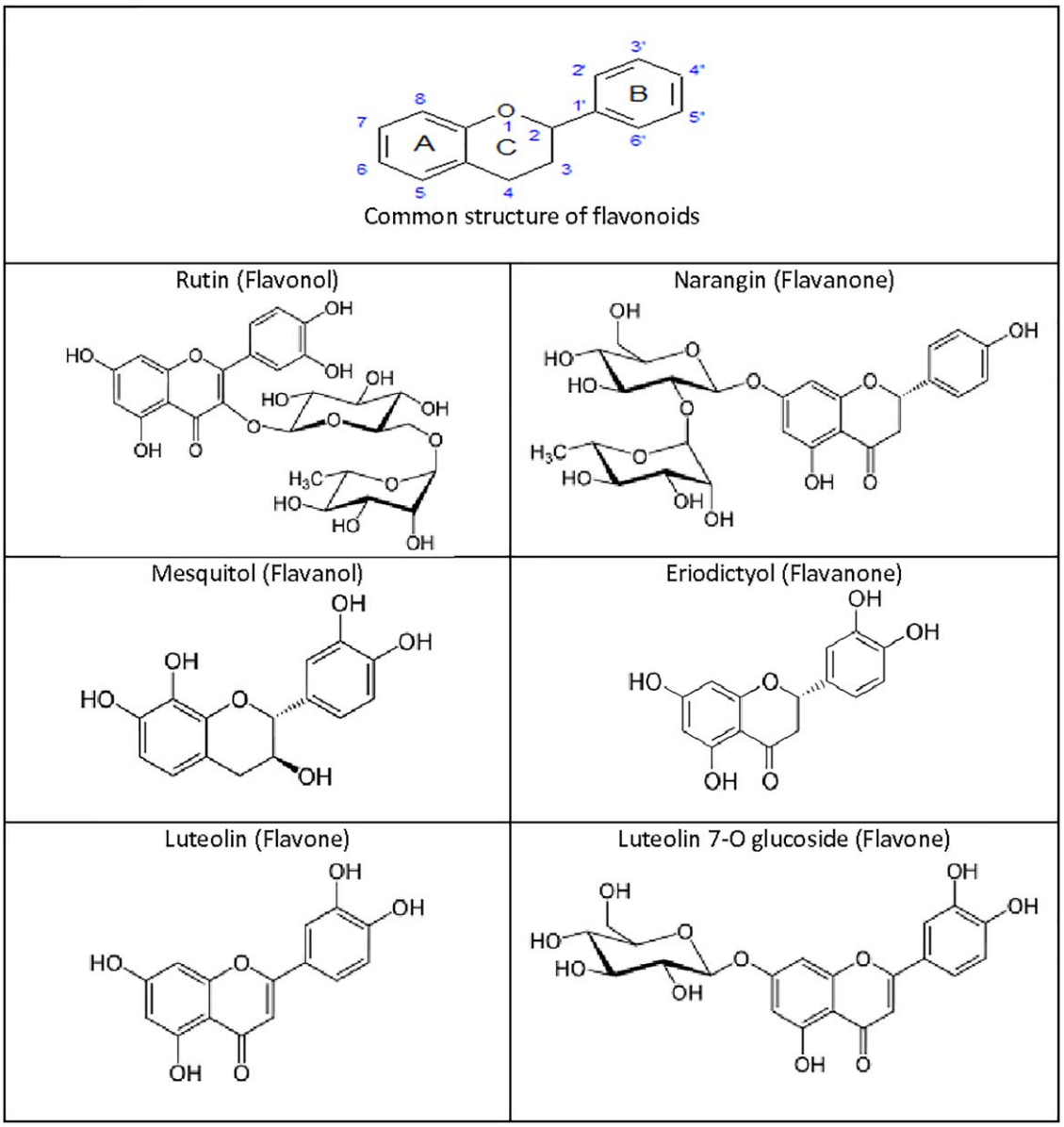

FIGURE 1 Chemical structures of the flavonoid studied

μL of flavonoid degraded solutions was separated on a C18 Alltima (150 mm × 2.1 mm) column (Grace/Alltech). The flow rate was set at 200 μL min^{-1}. The mobile phases for quercetin and rutin were water modified with trifluoroacetic acid (0.1%) for A and acetonitrile modified with trifluoroacetic acid (0.1%) for B. The mobile phase for eriodictyol was water for A and methanol for B.

Degradation products were eluted using a first isocratic step at 15% of B for 3 min and then a linear gradient from 15% to 70% of B for 21 min. Mass spectrometric conditions were as follows for ESI^- mode: spray voltage was set at 5 kV; source gases were set (in arbitrary units min^{-1}) for sheath gas, auxiliary gas, and sweep gas at 40, 10, and 10, respectively; capillary temperature was set at 300°C; capillary voltage at −36 V; tube lens, split lens, and front lens voltages at −80, 44, and 3.25 V, respectively. Ion optic parameters were optimized by automatic tuning using a standard solution of rutin at 0.1 g L^{-1} infused in the mobile phase (A/B: 50/50) at a flow rate of 5 μL min^{-1}. Full scan MS spectra (100 to 2,000 *m*/*z*) and data dependent MS^2 scans for structural investigation were performed using an LTQ (Linear Trap Quadripole). Raw data were processed using the XCALIBUR software program (version 2.1, http://www.thermoscientific.com). MS^2 fragmentation data were compared when possible to available literature in order to identify the nature of degradation products.

2.7 | Antioxidant assay (ABTS)

Antioxidant activity was determined every 15 min during the thermal treatment by the trolox equivalent antioxidant capacity method. ABTS assays were performed using a spectrofluorimeter SAFAS (Xenius) equipped with a 96-well polystyrene plate.

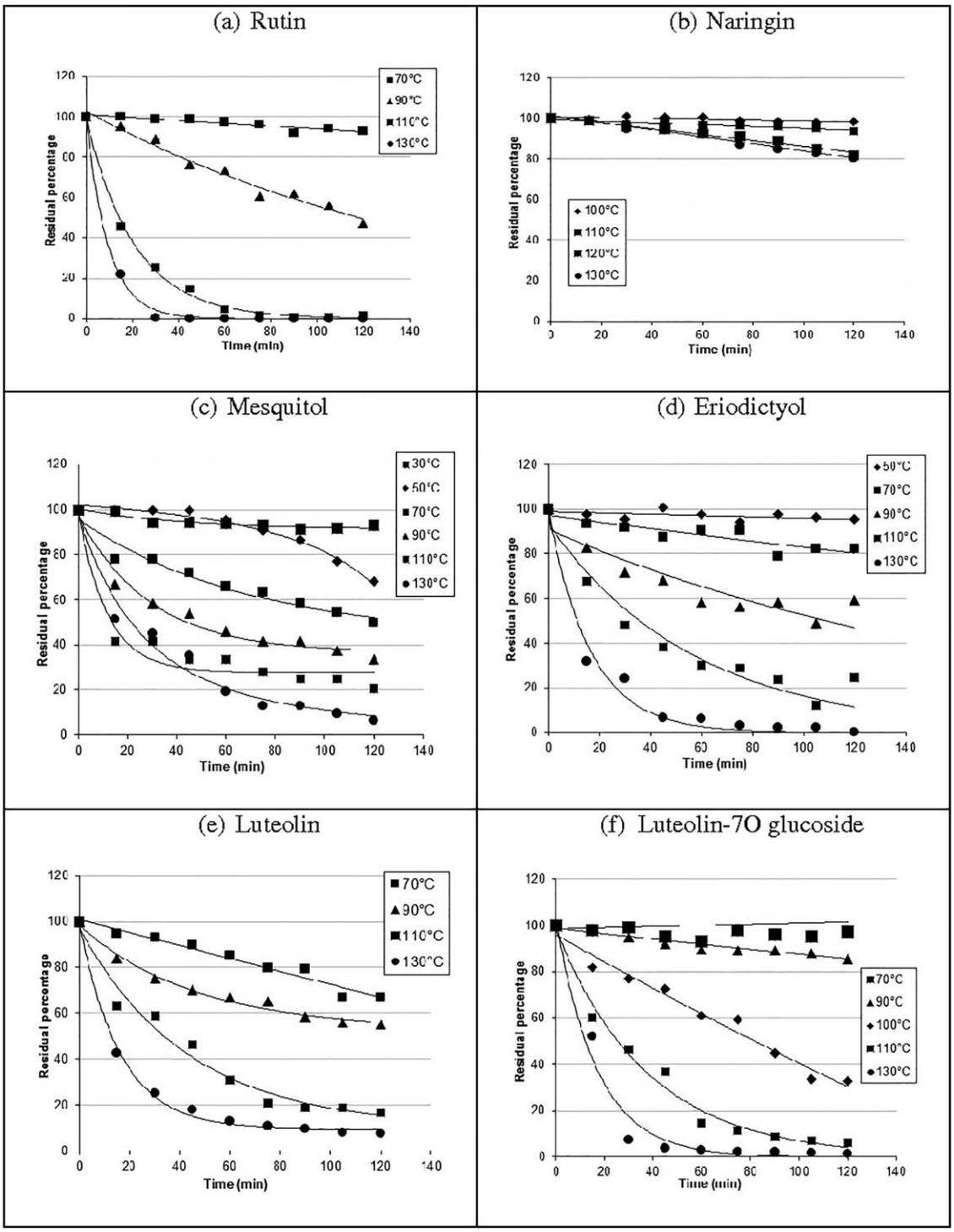

FIGURE 2 Degradation kinetics of flavonoids at different temperatures over 2 hr standard deviations are equal to 2%

Scavenging free radical potentials were tested in an aqueous solution of $ABTS^{\blacksquare+}$ according to the method described by Re, Pellegrini, Proteggente, Yang, and Rice-Evans (1999) with modifications for use in microplates. The percentage of inhibition was calculated by the equation: $(IP\%) = 100 \times (A_C - A_S)/A_C$, where A_C and A_S are the absorbance of the control and test sample, respectively.

Results were expressed as millimoles of TEAC values (Trolox Equivalent Antioxidant Capacity). From this measurement, we determined ΔTEAC. It corresponds to the difference between the total TEAC of the solution and the TEAC due to the residual flavonoid in solution. Triplicate measurements were taken for all samples.

2.8 | Modeling of the degradation kinetics

To estimate the kinetic parameters of the flavonoid degradation during heat treatment, the order of the reaction was determined. The residual

TABLE 1 Kinetic parameters of rutin, naringin, eriodictyol, luteolin, luteolin-7-o-glucoside, and mesquitol during thermal degradation

	Flavonol		Flavanone				Flavone				Flavanol	
	Rutin		Naringin		Eriodictyol		Luteolin		Luteolin glucoside		Mesquitol	
T (°C)	k (min^{-1})	$t_{1/2}$ (h)	k (min^{-1})	$t_{1/2}$ (h)	k (min^{-1})	$t_{1/2}$ (h)	k (min^{-1})	$t_{1/2}$ (h)	k (min^{-1})	$t_{1/2}$ (h)	k (min^{-1})	$t_{1/2}$ (h)
30	ni		ni		nd		ni		ni		0.0009 ± 0.0001	12.83
50	nd		ni		0.0003 ± 0.0001	38.51	nd		nd		0.0020 ± 0.0001	5.78
70	0.0006 ± 0.0001	19.25	ni		0.0017 ± 0.0001	6.8	0.0033 ± 0.0001	3.5	nd		0.0061 ± 0.0002	1.89
90	0.0058 ± 0.0002	1.99	nd		0.0073 ± 0.0002	1.58	0.0050 ± 0.0001	2.31	0.0013 ± 0.0001	8.89	0.0104 ± 0.0002	1.11
100	0.0170 ± 0.0004	0.68	nd		ni		0.0071 ± 0.0001	1.63	0.0086 ± 0.0002	1.34	ni	
110	0.0509 ± 0.0012	0.23	0.0004 ± 0.0001	28.88	0.0187 ± 0.0004	0.62	0.0179 ± 0.0004	0.65	0.0006 ± 0.0077	0.45	0.0140 ± 0.0003	0.83
120	0.0722 ± 0.0017	0.16	0.0016 ± 0.0001	7.22	ni							
130	0.1590 ± 0.0036	0.07	0.0019 ± 0.0001	6.08	0.0469 ± 0.0011	0.25	0.0444 ± 0.0133	0.25	0.0744 ± 0.0223	0.16	0.0236 ± 0.0071	0.49
OH groups	4 (5. 7.3′.4′)		2 (5.4′)		4 (5.7.3′.4′)		4 (5. 7.3′.4′)		3 (5. 3′.4′)		5 (3.7.8.3′.4′)	
Enone motif	Enone motif		Enone motif missing		Enone motif missing		Enone motif		Enone motif		Enone motif missing	
Carbonyl group	Carbonyl group		Carbonyl group		Carbonyl group		Carbonyl group		Carbonyl group		No carbonyl group	
Substituting	Rhamnose-glucose position 3		Rhamnose-glucose position 7		Without substitution		Without substitution		Glucose position 7		Without substitution	
Ea (kJ/mol)	107.3		100.6		68.2		51.4		120.0		33.3	

Note. For a flavonoid given, all constant rates are significantly different.
ni = not investigated; nd = not degraded.

TABLE 2 Antioxidant activity and information on the structure of the different flavonoids

	Rutin	Naringin	Eriodictyol	Luteolin	Luteolin glucoside	Mesquitol
Hydroxyl groups (position. number)	4 (5.7.3′.4′)	2 (5.4′)	4 (5.7.3′.4′)	4 (5.7.3′.4′)	3 (5.3′.4′)	5 (3.7.8.3′.4′)
Enone motif	Y	N	N	Y	Y	N
Carbonyl group	1	1	1	1	1	0
Substituting	Rhamnose-glucose position 3	Rhamnose-glucose position 7	No substituting	No substituting	Glucose position 7	No substituting
Antioxidant activity (mmol/l)	$3.15^{a} \pm 0.35$	$0.17^{b} \pm 0.03$	$1.22^{c} \pm 0.06$	$5.56^{d} \pm 1.45$	$4.21^{d} \pm 0.56$	$0.92^{e} \pm 0.01$

Note. Groups found by Tukey analysis are indicated by letters on the antioxidant activity.

concentration was plotted over time according to (1). The constant rate k corresponds to the slope of the straight line obtained.

$$\ln (C_t/C_0) = -kt, \quad (1)$$

where C_0 is the initial flavonoid content (mg/l) and C_t is the flavonoid content (mg/l) after t minutes of heating at a given temperature, k is the reaction rate constant (min^{-1}). Half life time $t_{1/2}$ can be determined by (2). It corresponds to the heating time required to degrade 50% of the flavonoid content (min).

$$t_{1/2} = \ln 2/k. \quad (2)$$

The increase and decrease of temperature during heating were very rapid so we can ignore them and consider that we are in isothermal conditions. Thus, the temperature dependence of the degradation rate constants can be modeled using the Arrhenius law both for temperatures below and above 100°C. Activation energies Ea (kJ/mol) were calculated by the following equation:

$$\ln k = \ln k_O - (Ea/RT), \quad (3)$$

with R is the ideal gas constant (8.314 J K^{-1} mol^{-1}) and T is the heating temperature (K).

2.9 | Statistical analysis

Each experiment was performed using three replicates. The standard deviations were respectively equal to 2% and 2 mmol trolox/L for residual concentration and ΔTEAC. So, the kinetics of the flavonoid degradation and the antioxidant activity determination are repeatable. Moreover, an ANOVA followed by a Tukey test (freeware R© (2.11.1)) was performed to evaluate significant differences on kinetic parameters (k).

3 | RESULTS

3.1 | Degradation kinetics of the flavonoids

The degradation kinetics of rutin, naringin, mesquitol, eriodictyol, luteolin, luteolin-7-O-glucoside in the aqueous system at different temperatures over 2 hr were investigated. The results obtained are summarized in Figure 2. The degradation of rutin (Figure 2a) according to the heating temperature and time showed that, at 70°C, less than 10% of rutin was depleted after 2 hr of heating while only 50% of the flavonoid content was lost at a temperature of 90°C. For temperatures above 100°C, a sharp degradation was observed. Thus, after 45 min at 130°C, rutin was no longer detectable by the HPLC/DAD system. As indicated in Figure 2b, naringin shows a higher stability than rutin when heated to 100, 110, 120, and 130°C. No degradation was observed for temperatures lower than 100°C and less than 2% at 100°C. At a temperature of 130°C, only 20% of naringin content was depleted. Mesquitol was highly sensitive even at low temperatures (Figure 2c). In fact, at 30°C about 10% of reduction was already observed and more than 50% at 70°C. At a higher temperature (130°C) less than 7% of mesquitol content remained in the solution. The degradation kinetic of eriodictyol is presented in Figure 2d. Little degradation—less than 5%—was observed for temperatures below 70°C. For higher temperatures 90 and 110°C, 40% and 25% of reduction were observed respectively, whereas, at 130°C, the residual concentration of eriodictyol was not detectable. Figure 2e shows the luteolin degradation kinetics. It appears that the luteolin content decreased by 30% at a temperature of 70°C. At a temperature of 90°C, the loss of luteolin was more than 45%. Heating to a temperature of 110°C led to a decrease in the luteolin content by more than 80% and more than 90% at 130°C. Luteolin-7-O-glucoside showed a higher stability than luteolin (Figure 2f) when the heat treatment applied was less than 100°C. In fact at 70 and 90°C, the decline in the concentration of luteolin 7-O-glucoside was 10% at 70°C and 15% at 90°C compared with 30% and 45% for luteolin, respectively. At higher temperatures, luteolin-7-O-glucoside became more sensitive than luteolin.

All the flavonoids studied were found to be heat sensitive. Flavonoid degradation increases with heating magnitude and duration. The best curve fit was obtained using an exponential decay. Differences were found between the six flavonoids concerning their thermal stability. These results are in agreement with previous studies which reported a different thermal stability according to the flavonoid structure (Buchner et al., 2006; Stintzing et al., 2006; Ungar et al., 2003). Studies dealing with the modeling of degradation kinetics show either exponential or sigmoidal degradation depending on the kind of flavonoid, the pH, or the temperature (Stintzing et al., 2006; Ungar et al., 2003).

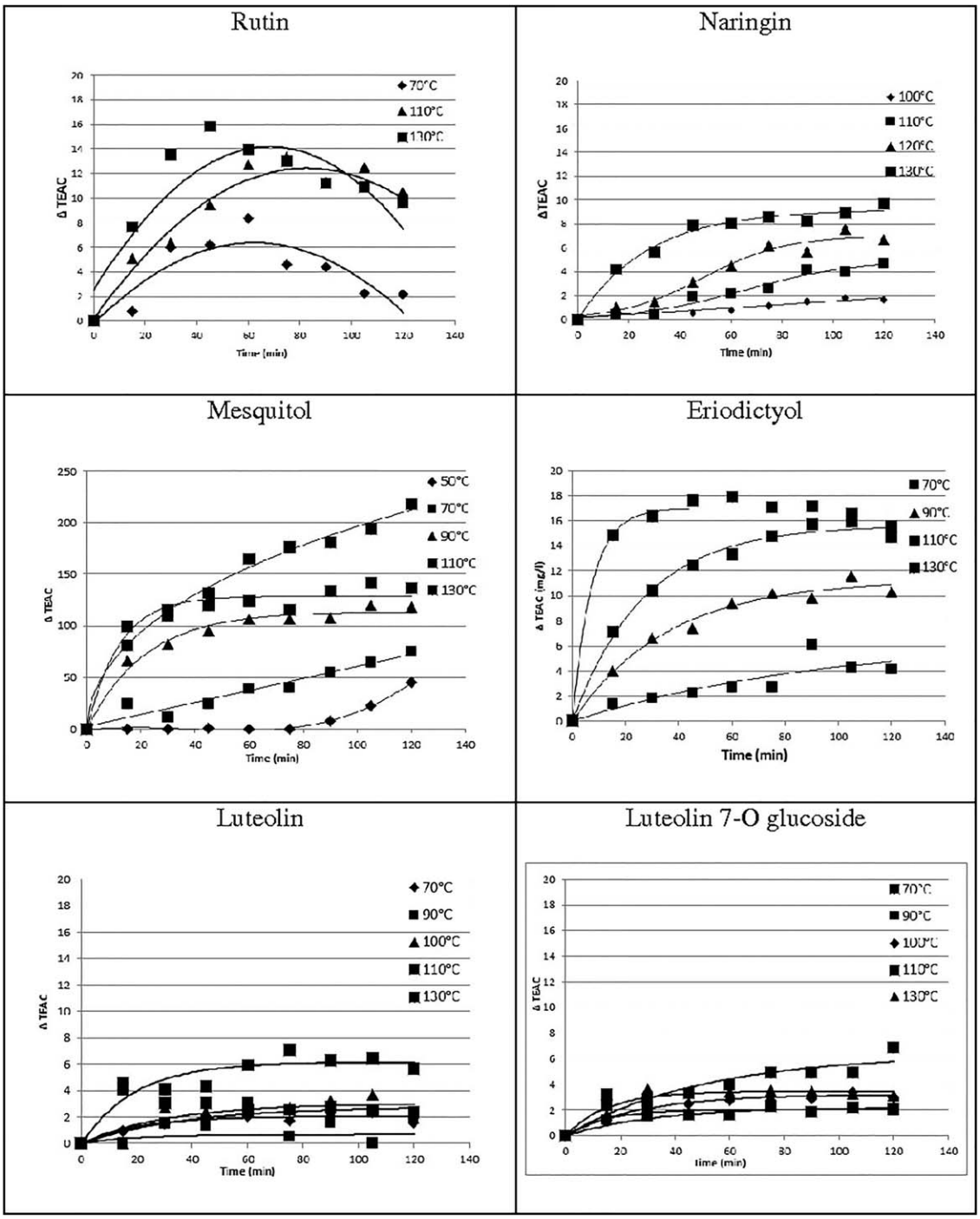

FIGURE 3 Evolution of ΔTEAC (mmol trolox/l) during the thermal treatment standard deviations are equal to 2 mmol trolox/l

3.2 | Kinetic parameters determination

To estimate the kinetic parameters of flavonoid degradation during heat treatment, the order of the reaction was determined. Logarithm (C/Co) was plotted versus heating time. Straight lines were obtained with good determination coefficients (Annex materials). Thus, degradations follow a first-order kinetic. Most studies on the flavonoid degradation have reported that this degradation follows first-order kinetic (Patras et al., 2010). Constant rates and half-life times are given in Table 1. The Arrhenius model was used to calculate temperature dependence. The Arrhenius model is found to be a reliable mathematical method according to the determination coefficient values (Annex materials).

The rate of degradation (k) varies for a given compound according to temperature, and for a given temperature it varies depending on the nature of the compound studied. However, the magnitude of these variations according to temperature is not the same for all studied compounds. As an illustration of this phenomenon, the k values obtained at 130°C were compared. It appears that naringin and mesquitol are the flavonoids that are characterized by the slowest kinetics of degradation

TABLE 3 LC-MS data obtained for the thermal degradation of quercetin, rutin, and eriodictyol

Peak	Retention time (min)	*m/z* values $[M-H]^-$	*m/z* values MS/MS	Suggested compound
Quercetin (1 mM) in 100 mM KCl and 0.36 mM KOH				
P1	9.93	317	191–299–207–163	2-(3′,4′-dihydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2H)-one
P2	11.82	349	330	(3′,4′,5′,6′-tetrahydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2H)-one
P3	12.40	333	ND	(3′,4′,5′-trihydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2H)-one
P4	3.28	181	ND	2-(3,4-dihydroxyphenyl)-2-oxoacetic acid
P5	4.67	544	ND	Unidentified
P6	5.46	169	151	2,4,6-trihydroxybenzoic acid
P7	24.03	297	183	Unidentified
Rutin (0.65 mM) in water				
P1	4.53	278	197	Unidentified
P2	6.52	153	ND	Protocatechuic acid
P3	8.83	1,217	909–601	Dimer
P4	12.12	463	301	Quercetin-glucose
P5	13.41	607	317	Unidentified
P6	17.42	301	179–151	Quercetin
Eriodictyol (0.059 mM) in water				
P1	8.46	197	133	3-(3,4-dihydroxyphenyl)−3-hydroxypropanoic acid
P2	11.87	165	137–121	3-(3,4-dihydroxyphenyl) propanal
P3	13.53	301	286–242–283–257	Unidentified

ND = no data obtained.

with a *k* of 0.0019 and 0.0236 min^{-1}, respectively, followed by eriodictyol and luteolin with, respectively, 0.0469 and 0.0444 min^{-1}. Then, the most rapid kinetics were encountered with rutin and luteolin 7-O glucoside with 0.1590 and 0.0744 min^{-1}, respectively. The analysis of the activation energies found by the Arrhenius law shows differences between the six flavonoids. Mesquitol needs 33.3 kJ/mol to be degraded, 51.4 kJ/mol for luteolin, 68.2 for eriodictyol, 100.6 for naringin, 107.3 for rutin, and 120 for luteolin 7-O glucoside. Activation energy corresponds to the quantity of energy needed to initiate the degradation reactions. The higher the activation energy, the more stable the flavonoid will be at high temperatures. Indeed, the flavonoid which is sensitive to a temperature of 30°C (mesquitol) needs only 33.3 kJ/mol, whereas the flavonoid sensitive to higher temperatures (rutin, luteolin 7-O glucoside) needs more than 100 kJ/mol. The differences observed between the kinetics of the degradation of the six flavonoids cannot be explained solely by their belonging to a given class of flavonoid. The structure of these molecules also seems to be at the origin of these differences. In fact, Buchner et al. (2006) explained that rutin is less sensitive to heat than quercetin because of the presence of a glycosyl moiety. With a view to elucidating the relation between the structure and heat sensitivity of these compounds, the structural characteristics and their kinetic parameters are summarized in Table 1. This table shows a difference between the glycosylated flavonoids and the aglycones. Indeed, activation energy was higher for the glycosylated: rutin (107 kJ/mol), naringin (100 kJ/mol), and luteolin 7-O glucoside (120 kJ/mol). This can be explained by the presence of a substituent, because additional energy is needed to break the osidic bond between the heteroside and the flavonoid (Buchner et al., 2006; Da Costa, Barbosa Filho, Do Nascimento, & Macedo, 2002; Rohn, Buchner, Driemel, Rauser, & Kroh, 2007). However, as rutin is more sensitive to heat than naringin, the difference between the two could be explained by the presence of a double bond in the C-ring C for rutin, and additional energy is needed to break it. For the aglycon flavonoids, mesquitol has low activation energy in comparison to eriodictyol and luteolin. This can be explained by the absence of a carbonyl group for mesquitol, so the energy needed for its degradation will be lower. However, as the change (hydroxylation, glycosylation) in the structure of the six flavonoids takes place in different parts of the molecules, it is rather difficult to attribute the observed behavior to one substitution at a time.

3.3 | Antioxidant activity evolution

Before the heat treatment, the antioxidant activity of each flavonoid was characterized. Flavonoids having the highest antioxidant capacity are luteolin, luteolin-7-O-glucoside, and rutin. However, the capacities of the three compounds are different and equal to 5.56 ± 1.45, 4.21 ± 0.56, and 3.15 ± 0.35 mmol/l, respectively (Table 2). This behavior can be explained by the presence in these three compounds of the enone structure (2–3 double bond of the C-ring) conjugated with a carbonyl group in C4, which is missing in the structure of the three other flavonoids. It has been indicated that this structure is responsible for an increase of antioxidant activity (Heim et al., 2002). Among luteolin, luteolin 7-Oglucoside, and rutin, the aglycon flavonoid has a higher TEAC. These results showed that glycosylation or

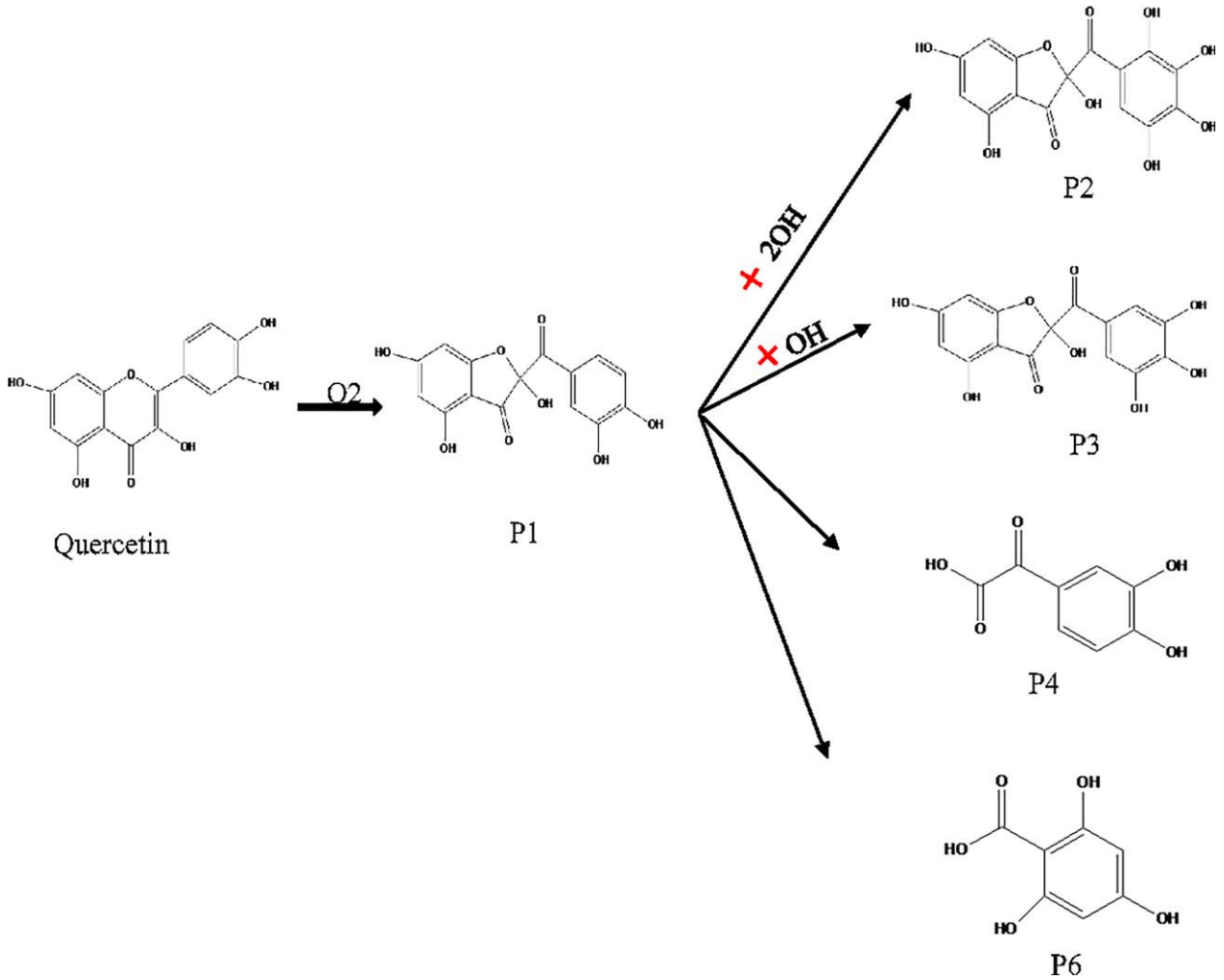

FIGURE 4 Thermal degradation pathway of quercetin

methylation provokes a significant decrease in antioxidant activity and particularly when the substitute is in position 3 (Van Acker et al., 1996). Indeed, the hydroxyl in position 3 confers antioxidant activity to molecules (Heim et al., 2002).

Eriodictyol, mesquitol, and naringin present, respectively, an antioxidant activity of 1.22 ± 0.06, 0.92 ± 0.01, and 0.17 mmol/l ± 0.03. Naringin has a very low antioxidant activity in comparison to eriodictyol and mesquitol because of the glycosylation and the absence of the 3′, 4′-catechol structure. It has been reported that the presence of two hydroxyl groups in position 3′ and 4′ on the B-ring increases flavonoid antioxidant activity (Heim et al., 2002). For eriodictyol and mesquitol, the presence of a hydroxyl group in position 3 does not counterbalance the absence of the enone structure. The number of hydroxyl groups seems to be at the origin of the high antioxidant activity of eriodictyol compared to mesquitol. Antioxidant activity of a flavonoid seems to be linked to some structural elements. All factors that change the flavonoid structure lead to an evolution of antioxidant activity. In Figure 3, the ΔTEAC is given according to heating time at different temperatures. For a given time (t_i), ΔTEAC (mmol trolox/l) is defined as the difference between the measured TEAC of the solution containing both residual flavonoid and product of degradation, and the predicted TEAC, taking into account only the measured residual concentration of the flavonoid studied.

According to the evolution of ΔTEAC with time, three cases can be observed: (i) an increase of ΔTEAC shows that the products formed have an antioxidant activity that is higher than the native flavonoid; (ii) a decrease of ΔTEAC indicates a lower antioxidant activity of the degradation products; and (iii) a ΔTEAC constant means that degradation products have the same antioxidant activity as the native flavonoid.

For luteolin and luteolin 7-O glucoside, standard deviations range between 1 and 2, so there is no increase of ΔTEAC with temperature or heating time. Thus we can conclude that degradation products have an antioxidant activity that is similar to that of native flavonoids.

For the other flavonoids, standard deviations are low in comparison with TEAC values (inferior to 1), so we can state that there is an increase of ΔTEAC during the heat treatment. We notice that this increase is higher when temperature increases. So rutin, naringin, eriodictyol, and mesquitol lead to degradation products with an antioxidant activity. Similar findings were reported when rutin samples were heated to 100°C for 300 min. Despite the small amount of rutin left in the samples after thermal treatment, the solution still possessed antioxidant ability (Buchner et al., 2006).

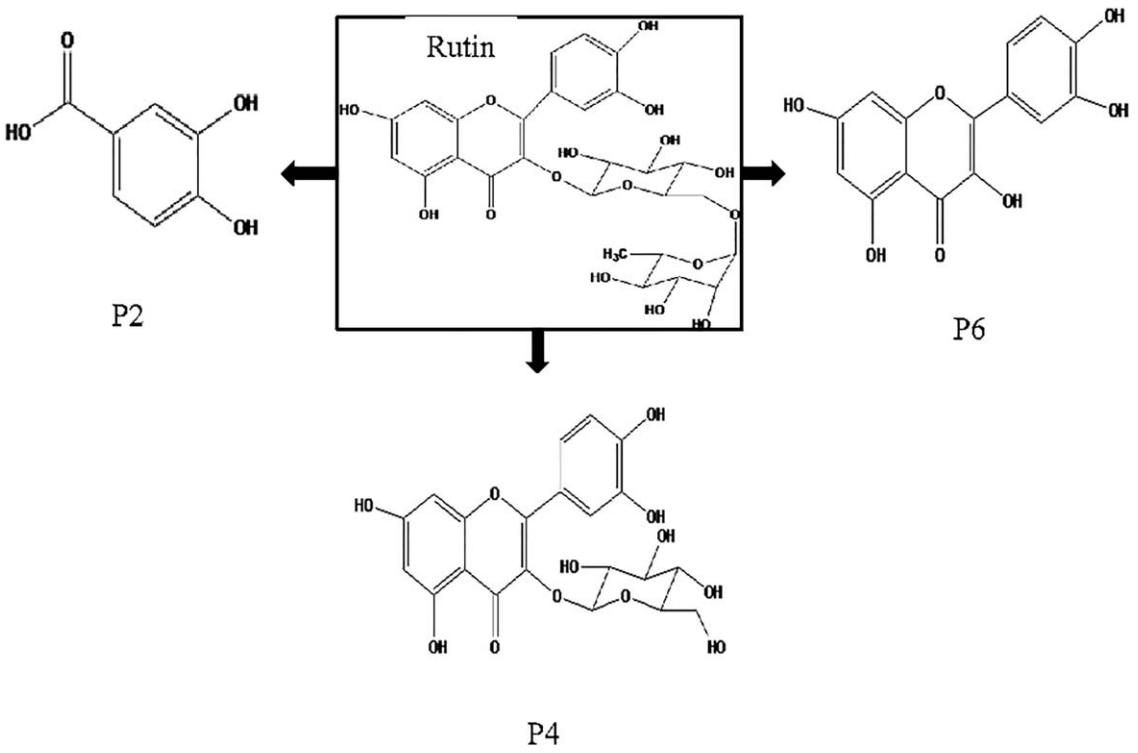

FIGURE 5 Thermal degradation pathway of rutin

Depending on the structure of the molecule and heat treatment behavior, the evolution of the antioxidant activity is different. For mesquitol, an increase of 200 at 130°C after 2 hr was observed, whereas for eriodictyol, the increase was only of 17 and 9 for naringin. Moreover, the antioxidant activity of rutin increased during the first hour of treatment then dropped progressively. This can be attributed to a sequential formation and degradation of the degradation compound during heat treatment.

3.4 | Identification of flavonoid degradation products

As explained before, the evolution of the antioxidant activity of the six flavonoids is different according to the flavonoid structure and the temperature of the thermal treatment. The few data available in the literature on the new formed products after flavonoid degradation indicated the existence of different degradation pathways leading to different new formed products (Dall'acqua et al., 2012; Ramešová et al., 2013). Thus, the new formed products would be different according to the kind of degradation (temperature, oxygen, light) and the structure of the native flavonoid. Results found on thermal degradation of quercetin (aglycon), rutin (glycosylated), and eriodictyol (aglycon without the enone structure) are in agreement with conclusions from the literature. The quercetin is considered as a reference because it has already been studied in the literature and it constitutes the aglycon part of rutin. To identify the new formed products, samples were taken at the end of the thermal treatment and analyzed by LC-MS.

3.4.1 | Identification of new formed products from quercetin degradation

The results obtained showed that quercetin is highly sensitive to temperature. The thermal degradation begins at low temperature during the step of solubilization. Products P1 (2-(3′,4′-dihydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2H)-one), P2 ((3′,4′,5′,6′-tetrahydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2H)-one), P3 ((3′,4′,5′-trihydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2H)-one) were obtained after solubilization at 35°C. During the thermal treatment (130°C, 2 hr), quercetin, P1, and P2 are totally degraded and lead to the formation of P4 (2-(3,4-dihydroxyphenyl)-2-oxoacetic acid), P5 (unidentified product), P6 (2,4,6-trihydroxybenzoic acid), and P7(unidentified product). The molar masses of the new formed products are summarized Table 3 and the quercetin degradation pathway is indicated in Figure 4. The chromatograms obtained by LC-MS are given in Annex materials.

A similar methodology was applied for the other two flavonoids.

3.4.2 | Identification of new formed products from rutin degradation

As for quercetin, the thermal degradation of rutin was assessed by LC-MS (chromatograms presented in Annex materials). It appears that after 2 hr of heating, six products were observed. Four molecules were identified: P2 (protocatechuic acid), P3 (dimer of rutin), P4 (quercetin-glucose), and P6 (quercetin). A degradation pathway is presented in Figure 5. For the dimer of rutin, different structures can be found (Anthoni, Chebil, Lionneton, Magdalou, Humeau, & Ghoul, 2011), they are proposed in Figure 6. These results indicated that the thermal degradation

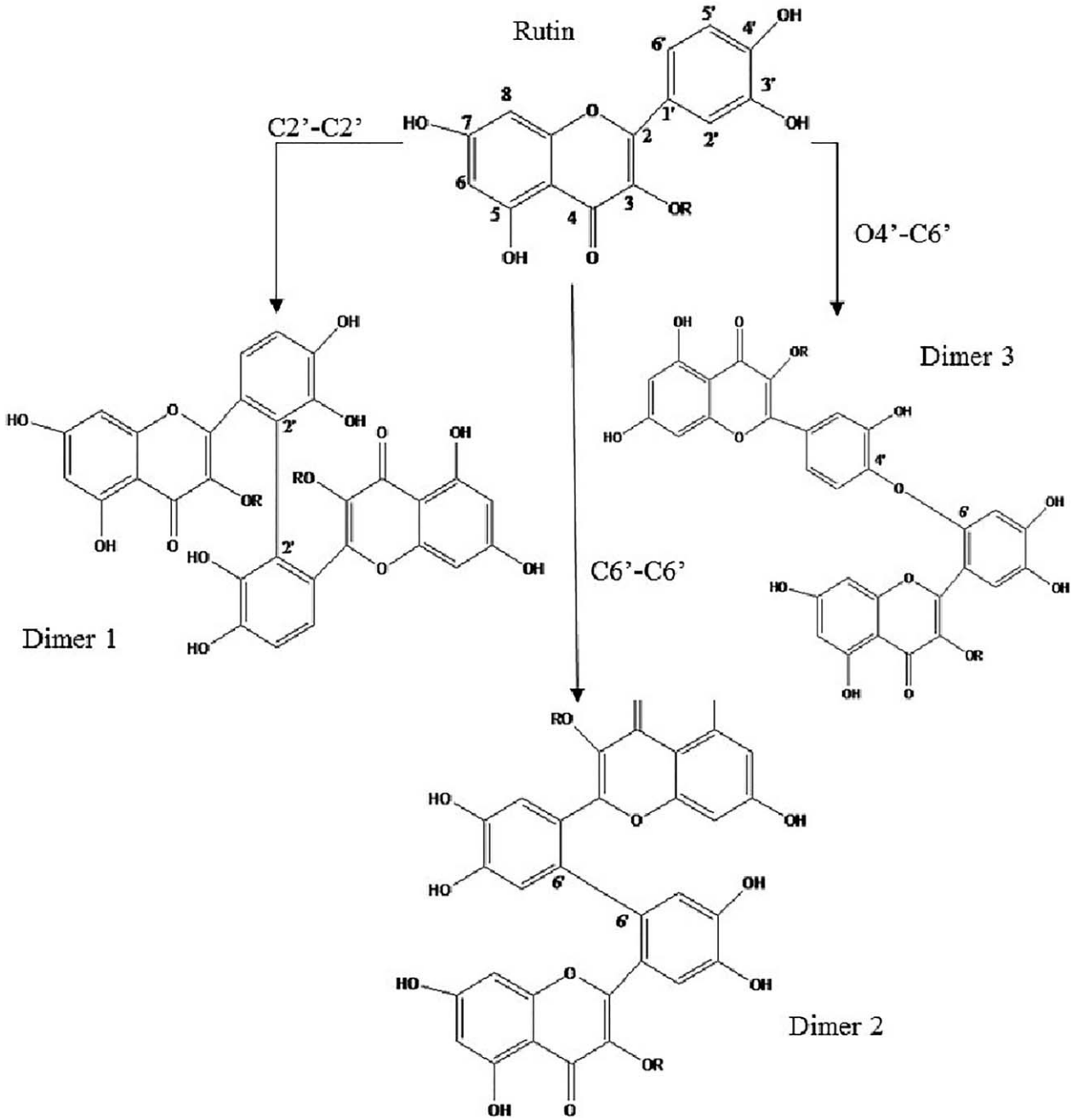

FIGURE 6 Structures of rutin dimer (Anthoni et al., 2011)

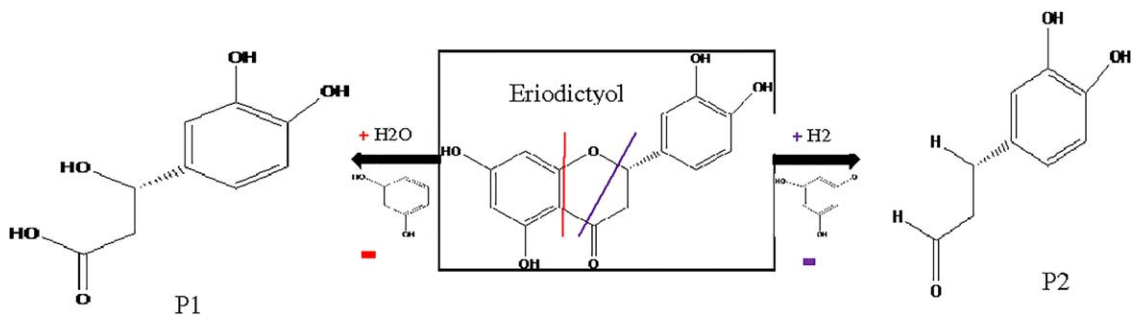

FIGURE 7 Thermal degradation pathway of eriodictyol

pathway of quercetin and rutin are not similar despite the presence of genin in the two structures.

3.4.3 | Identification of new formed products from eriodictyol degradation

The chromatograms of the thermal degradation of eriodictyol are indicated in Annex materials. The molar masses of the different by-products are summarized in Table 3. After 2 hr of thermal treatment, three products P1 (3-(3,4-dihydroxyphenyl)−3-hydroxypropanoic acid), P2 (3-(3,4-dihydroxyphenyl) propanal), and P3(unidentified product) were observed. A thermal degradation pathway is shown in Figure 7.

4 | CONCLUSION

Temperature has an effect on the stability of flavonoids and their biological activity. According to their structure, flavonoids are more or less sensitive to heat treatment. Glycosylated flavonoids are more resistant to heat treatment than aglycon flavonoids. The degradation depends on structural solidity. Therefore, a double bond needs more energy in order to be degraded. Modifications of structure lead to changes in antioxidant activity. According to the degradation products synthesized: (i) antioxidant activity can decrease, which means that degradation products have a lower antioxidant activity, (ii) antioxidant activity remains constant, indicating that degradation products have the same antioxidant activity as native flavonoids, and (iii) antioxidant activity can increase, so the degradation products have a higher antioxidant activity. Degradation products of three flavonoids were identified and we concluded that the thermal degradation pathways are different according to the structure of the flavonoid. The new formed products exhibit residual antioxidant activity sometimes superior to that of native molecules.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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