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Kinetics of the thermal degradation of phenolic compounds from achiote leaves (*Bixa orellana* L.) and its effect on the antioxidant activity

José Edgar ZAPATA[1]*, Cindy Tatiana SEPÚLVEDA[1], Andrés Camilo ÁLVAREZ[2]

Abstract

This work evaluates the effect of temperature and soluble solids on the degradation rate of phenolic compounds, and antioxidant activity of extracts from *Bixa orellana* L. leaves. The temperatures were studied in the range of typical food processes (70-90 °C) and food storage (−20-37 °C). The results showed that the thermal degradation of the phenolic compounds follows first-order kinetics, in which the degradation rate depends on the temperature, the amount of soluble solids, and the pH. The loss of antioxidant activity also follows first-order kinetics. Under different storage conditions, the half-life times of the total phenols were in the range 40.72-202.47 days, while for the antioxidant activity, the half-times were from 55.87-68.83 days for the ABTS and from 57.85-107.03 days for the FRAP method. The antioxidant activity of the extracts follows the same pattern of thermal degradation as the phenolic compounds. Therefore, we conclude that antioxidant activity is due to its phenolic compounds.

Keywords: radical scavenging capacity; thermal degradation; biological activity; iron reducing capacity; storage stability.

Practical Application: The results from this work deliver useful information for those who are interested in using achiote as an antioxidant ingredient in the food that will be submitted to thermal processes or long periods of storage. This information lets calculate the needed amount of extracts that should be used depending on the process or storage temperature to keep the antioxidant activity to the required levels for a given period.

1 Introduction

Achiote (*Bixa orellana* L.) is a shrub from the intertropical regions of America, belonging to the *Bixaceae* family (Lourido Pérez & Martínez Sánchez, 2010; Viuda-Martos et al., 2012). Its fruits are an ovoid capsule with seeds, which are of great interest as a natural dye in food and cosmetics due to its bixin and norbixin content (Rodrigues et al., 2007; Verbeyst et al., 2010). It has been used in traditional medicine (Bachir Bey et al., 2014) associated with the presence of phenolic compounds (Radhika et al., 2010; Shilpi et al., 2006), which constitutes one of the most important secondary metabolites (Moo-Huchin et al., 2015). Its leaves contain compounds with microbiological (Gómez et al., 2012; Viuda-Martos et al., 2012) and antioxidant activity (Sepúlveda et al., 2016; Viuda-Martos et al., 2012) attributed mainly to its phenolic compounds content (Gómez et al., 2012).

In general, the phenolic compounds have a broad range of biological properties such as antimicrobial properties (Prado et al., 2014; Fleischer et al., 2003; Gutiérrez-Larraínzar et al., 2012; Koolen et al., 2013; Xia et al., 2011), anti-inflammatory (Derringer & Suich, 1980; Rocha Garcia et al., 2012; Tao et al., 2014), anti-allergic (Agati et al., 2012; Medeiros et al., 2008), anti-viral (Sant'Anna et al., 2012; Zhang et al., 2014), anti-cancer (Agcam et al., 2014; Henríquez et al., 2014), antioxidant (Balasundram et al., 2006; Baydar & Baydar, 2013; Bendary et al., 2013; Bonilla et al., 1999; Gutiérrez-Larraínzar et al., 2012; Liu et al., 2012; Zhang et al., 2014), among others (Schulte, 2011; Soobrattee et al., 2005; Williams et al., 2004). However, such compounds can be vulnerable to the light (Arabshahi-D et al., 2007), temperature (Albarici & Pessoa, 2012; Kirca et al., 2007; Kirca & Cemeroğlu, 2003), solids content (Kirca et al., 2007; Williams et al., 2004), pH (Cevallos-Casals & Cisneros-Zevallos, 2004; Fan et al., 2008; He et al., 2015; Reyes & Cisneros-Zevallos, 2007), oxygen (Garzón, 2008; Kirca et al., 2007), and hydrogen peroxide (Ozkan et al., 2002). Also, they can be vulnerable to the kind of solvent in which the extracts are (Padmashree et al., 2012, 2014) being water one of the solvents with lowest thermal stability reported (Padmashree et al., 2014).

The stability of the phenolic compounds depends on the source from which they have been extracted (Apak et al., 2013; Padmashree et al., 2014). For that reason, some studies have been carried out to establish the thermal stability of anthocyanins in black carrots (Kirca et al., 2007), *Solanum nigrum* L. leaves (Apak et al., 2013; Padmashree et al., 2014), corn (Cevallos-Casals & Cisneros-Zevallos, 2004), potatoes, carrots, and grapes (Fan et al., 2008; He et al., 2015; Reyes & Cisneros-Zevallos, 2007).

This work aimed to evaluate the effect of temperature (T), soluble solids (SS), and pH on the degradation rate of the phenolic compounds and on the loss of antioxidant activity in *Bixa orellana* L. leaves.

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[1]*Grupo de Investigación "Nutrición y Tecnología de Alimentos", Universidad de Antioquia, Medellín, Colombia*
[2]*Departamento de Ingeniería Química, Facultad de Ingeniería, Universidad de Antioquia, Medellín, Colombia*
*Corresponding author: edgar.zapata@udea.edu.co

2 Materials and methods

2.1 Plant material

Bixa orellana L. leaves were collected from a private farm in the municipality of San Luis, Antioquia, Colombia, located 150 m above sea level. The leaves were identified as *Bixa orellana* L. red variety, from which a specimen is found in the herbarium of the Universidad de Antioquia identified with the number HUA 108450.

2.2 Extraction

The leaves were dried in a conventional oven (Thermo Scientific™, USA) at a temperature of 37 ± 0.2 °C for 48 h. Then, they were submitted to an extraction process with 95% ethanol at 4 ± 0.2 °C for 60 h.

2.3 Total Phenolic Content (TPC)

The total phenolic content was determined using the Folin-Ciocalteu method. Briefly, 500 µL of extract reacts with 250 µL of Folin-Ciocalteu reagent, and 125 µL of 20% Na_2CO_3 were added to the mixture and left at room temperature in the dark for 2 h. Then, the absorbance at 725 nm was measured, and the concentration was calculated from a tannic acid standard curve, expressed as tannic acid equivalents per gram of extract ($mg_{TA} \cdot g^{-1}$) (Singleton & Rossi, 1965).

2.4 Reaction with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS)

ABTS antioxidant activity was determined by the methodology described by Re et al. (1999) with slight modifications. A solution of 7 mM ABTS and 2.45 mM potassium persulfate was prepared and incubated at room temperature in the dark for 16 h to form the radical $ABTS^{\bullet+}$. A 100 µL of the extract was added to 1 mL of the radical solution and incubated at 30 °C in the dark for 30 min. Finally, the absorbance was measured at a wavelength of 730 nm and the results were expressed as equivalent micromoles of Trolox per gram of extract ($\mu mol_{TE} \cdot g^{-1}$).

2.5 Reduction capacity on Fe^{3+} (FRAP)

FRAP was carried out using the methodology proposed by Pulido et al. (2000), in which the FRAP reagent (tripyridyltriazine, iron chloride, and sodium acetate buffer) freshly prepared was heated at 37 °C, mixed with distilled water, and with the extract or with Trolox standard. It was then incubated at 37 °C for 30 min. The absorbance was measured at 595 nm and the results were expressed as equivalent micromoles of Trolox per gram of extract ($mg_{TE} \cdot g^{-1}$).

2.6 Effect of the factors on the degradation rate constant of TPC

We used a factorial design of experiments (3^3) to evaluate the effect of the temperature (70, 80, and 90 °C), the pH (3, 5.5, and 8), and the soluble solids (SS) content (8, 14, and 20 °Brix) on the reaction rate constant (Equation 1) and on the degradation of phenolic compounds (k). For that, in each run, the total phenolic content was determined each 9 h. Additionally, other kinetic parameters such as activation energy (E_a Equation 2), temperature coefficient (Q_{10} Equation 3), and half-life time ($t_{1/2}$ Equation 4) were calculated.

$$\ln c = \ln c_0 - k_1 t \quad (1)$$

$$\ln k = \ln k_0 - \frac{Ea}{RT} \quad (2)$$

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{\frac{10}{T_2 - T_1}} \quad (3)$$

$$-\ln\left(\frac{0.5}{1}\right) = k_1 t_{1/2} \quad (4)$$

2.7 Kinetics of the TPC degradation and loss of antioxidant activity at storage conditions

The degradation of TPC and the loss of antioxidant activity of the ethanolic extract of the *Bixa orellana* L. leaves were studied at a pH of 8 and 11.4 °Brix stored at −20, 8, 23, and 37 °C for 3 months. The kinetic parameters k, E_a, Q_{10}, and $t_{1/2}$ were calculated by Equations 1-4.

2.8 Statistical analysis

The design of experiments was analyzed using Design-Expert 7.1.6 (StatEase® USA). Before performing the analysis of variance (ANOVA) (Montgomery, 2005), it was confirmed a normal distribution and homogeneity of the variances with a 95% confidence interval. It includes the statistical significance of all the values of the adjusted model (p value), the coefficients estimated in each term (β_i), and the coefficient of determination of the model (R^2).

3 Results and discussion

3.1 Effect of temperature, pH, and soluble solids on the degradation rate of total phenolic content

The thermal degradation of the TPC followed a first-order reaction model, which agree with the results found in the degradation of TPC of apple peel (Henríquez et al., 2014), apple juice (De Paepe et al., 2014), orange (Kirca et al., 2007), and pomegranate (Fischer et al., 2013).

Table 1 shows the experimental runs of the factorial design of 3^3 (DOE) in random order. The factors are T, SS, and pH; and the response variable is k calculated with Equation 1. The k values show that the TPC of the *Bixa orellana* L. leaves are more stable to thermal degradation than the TPC of red-, purple-flesh potatoes, carrots, and grapes (Reyes & Cisneros-Zevallos, 2007); and black carrot (Kirca et al., 2007). It could be explained because the k values were one order of magnitude smaller than those reported.

Table 1. Factorial design and kinetic parameters in the degradation of TPC of the extract of *Bixa orellana* L.

Run	T (°C)	pH	SS Brix	k (min^{-1})[a]	$t_{1/2}$ (min)	Ea ($kJ \cdot mol^{-1}$)[a]	Q10	
							70/80	80/90
9	70	3	8	0.00020 (0.07)	12008.46			
26	80	3	8	0.00039 (0.99)	4557.51	53.72 (0.81)	2.63	1.06
21	90	3	8	0.00017 (0.70)	4292.95			
12	70	3	14	0.00017 (0.83)	4140.07			
8	80	3	14	0.00070 (0.93)	1808.18	43.74 (0.77)	2.29	2.10
25	90	3	14	0.00016 (0.87)	1792.89			
33	70	3	20	0.00043 (0.99)	7903.66			
14	80	3	20	0.00042 (0.68)	3974.10	114.37 (0.95)	1.99	4.61
5	90	3	20	0.00036 (0.99)	861.49			
17	70	5.5	8	0.00038 (0.56)	3505.19			
2	80	5.5	8	0.00017 (0.64)	3219.50	26.38 (0.86)	1.09	3.74
32	90	5.5	8	0.00079 (0.97)	2099.32			
27	70	5.5	14	0.00080 (0.83)	1910.64			
3				0.00009 (0.73)				
4				0.00020 (0.73)				
15				0.00043 (0.82)				
19	80	5.5	14	0.00060 (0.86)	1445.00	21.73 (0.97)	1.32	0.87
22				0.00017 (0.70)				
23				0.00077 (0.89)				
30				0.00044 (0.94)				
10	90	5.5	14	0.00015 (0.89)	1257.55			
1	70	5.5	20	0.00006 (0.71)	7894.75			
28	80	5.5	20	0.00033 (0.74)	4043.88	83.79 (0.99)	1.95	2.59
16	90	5.5	20	0.00066 (0.81)	1561.41			
31	70	8	8	0.00039 (0.98)	4304.43			
13	80	8	8	0.00022 (0.99)	4159.52	44.98 (0.77)	1.03	2.32
6	90	8	8	0.00009 (0.84)	1792.15			
7	70	8	14	0.00016 (0.91)	993.48			
29	80	8	14	0.00055 (0.96)	903.84	6.29 (0.91)	1.10	1.03
20	90	8	14	0.00237 (0.87)	880.38			
18	70	8	20	0.00039 (0.66)	3443.29			
11	80	8	20	0.00038 (0.99)	2262.00	39.42 (1.00)	1.52	1.41
24	90	8	20	0.00031 (0.83)	1609.77			

[a]The numbers in parentheses correspond to the coefficients of determination of the respective parameter.

The kinetic parameters (Q_{10}, $t_{1/2}$, and Ea) (Table 1) describe the thermal degradation of the TPC at several conditions of T, pH, and SS. The greatest E_a were obtained at pH of 3 and SS of 20 °Brix, while the greatest Q_{10} and $t_{1/2}$ were obtained at pH of 3 and SS of 8 °Brix, which defined the conditions of highest stability of those compounds. On the other hand, the worst conditions (lowest E_a and $t_{1/2}$) were obtained at a pH of 8 and 14, respectively. Those results agree with the reported by Reyes & Cisneros-Zevallos (2007). They found that the lowest degradation rates of anthocyanin from purple- and red-flesh potatoes, carrots, and grapes were at a pH from 1-3. However, they obtained $t_{1/2}$ values lower than those obtained in this work.

The decrease of $t_{1/2}$ as the temperature increased was notable for all the evaluated conditions (Table 1). This result can be attributed to the effect of the temperature on the degradation rate of phenolic compounds (Albarici & Pessoa, 2012; Figueirêdo et al., 2014; Timberlake, 1980; Zhang et al., 2014).

In most of the evaluated conditions, the E_a values are in the same order of magnitude than the reported for açaí drink (49.42 $kJ \cdot mol^{-1}$ at a pH of 5.2) (Albarici & Pessoa, 2012), blackberry juice (75.50 $kJ \cdot mol^{-1}$), and concentrate (65.06 $kJ \cdot mol^{-1}$) (Wang & Xu, 2007); and black carrot concentrate (Kirca et al., 2007), the last four at pH of 4.3.

3.2 Analysis of variance

Table 2 shows the results of the analysis of variance (ANOVA) for the DOE. It shows that both T and pH have a statistically significant effect ($p < 0.05$) on the linear term of degradation of TPC. However, SS is only statistically significant in its quadratic term. Equation 5 represents the mathematical model that expresses the relationship between k and the factors T, pH, and SS obtained in Table 2.

$$k\left(min^{-1}\right) = -2.06 \cdot 10^{-3} + 9.26 \cdot 10^{-6} * T + 4.72 * pH + 2.20 \cdot 10^{-4} * SS - 7.77 \cdot 10^{-6} * SS^2 \quad (5)$$

The plus sign of the factor T in Equation 5 shows a direct relationship between this variable and the degradation rate of TPC. That means, the higher the temperature, the faster the degradation of TPC. This behavior is typical of anthocyanins, which present slow hydrolysis of the glycosidic bond in position 3 and opening of the ring to produce colorless chalcones (Timberlake, 1980). These results agree with the found by Henríquez et al. (2014) and Kirca et al. (2007), who worked with apple peel and black carrot, respectively.

Regarding the difference in signs between the linear and quadratic terms of the SS in Equation 5, they indicate that an extreme point in the response as a function of the factor SS exists. That means that there is a point in which k reaches maximum values that correspond to the highest degradation rate of TPC.

Table 2. ANOVA to evaluate the effect of the factors on the kinetics of the thermal degradation of TPC of the extract from *Bixa orellana* L. leaves.

Source	Sum of squares	Degrees of freedom	Mean square	F value	p value
Model	$1.01x10^{-6}$	4	$2.51x10^{-7}$	27.42	<0.0001
T	$1.44x10^{-7}$	1	$1.44x10^{-7}$	15.67	0.0005
pH	$2.34x10^{-7}$	1	$2.34x10^{-7}$	25.48	<0.0001
SS	$4.18x10^{-9}$	1	$4.18x10^{-9}$	0.46	0.51
SS^2	$5.97x10^{-7}$	1	$5.97x10^{-7}$	65.03	<0.0001
R^2	0.81				

Meanwhile, the plus sign in the coefficient corresponding to the pH in Equation 5 indicates that the higher the pH, the greater the degradation rate of total phenolic, so a pH around 3 affects less this reaction. These results agree with the found by Kirca et al. (2007) and Verbeyst et al. (2010), who reported that increasing the pH, increased the degradation rate of anthocyanins.

Figure 1 shows the response surface graphs in which the effect of the factors T, SS, and pH on k are represented. The plots involving SS show a concave downward shape due to the influence of the quadratic term of the SS factor. In Figure 1, the dome represents the highest values of k, which implies less favorable conditions, which means faster degradation of TPC. In the region that k increases with SS, the results agree with Kirca et al. (2007), who found that the values of k increased with SS in the degradation of black carrot anthocyanins. On the other hand, the region in which the values of k decreased with SS, agree with those reported by Garzón (2008), who found that increases in the medium water activity cause anthocyanin

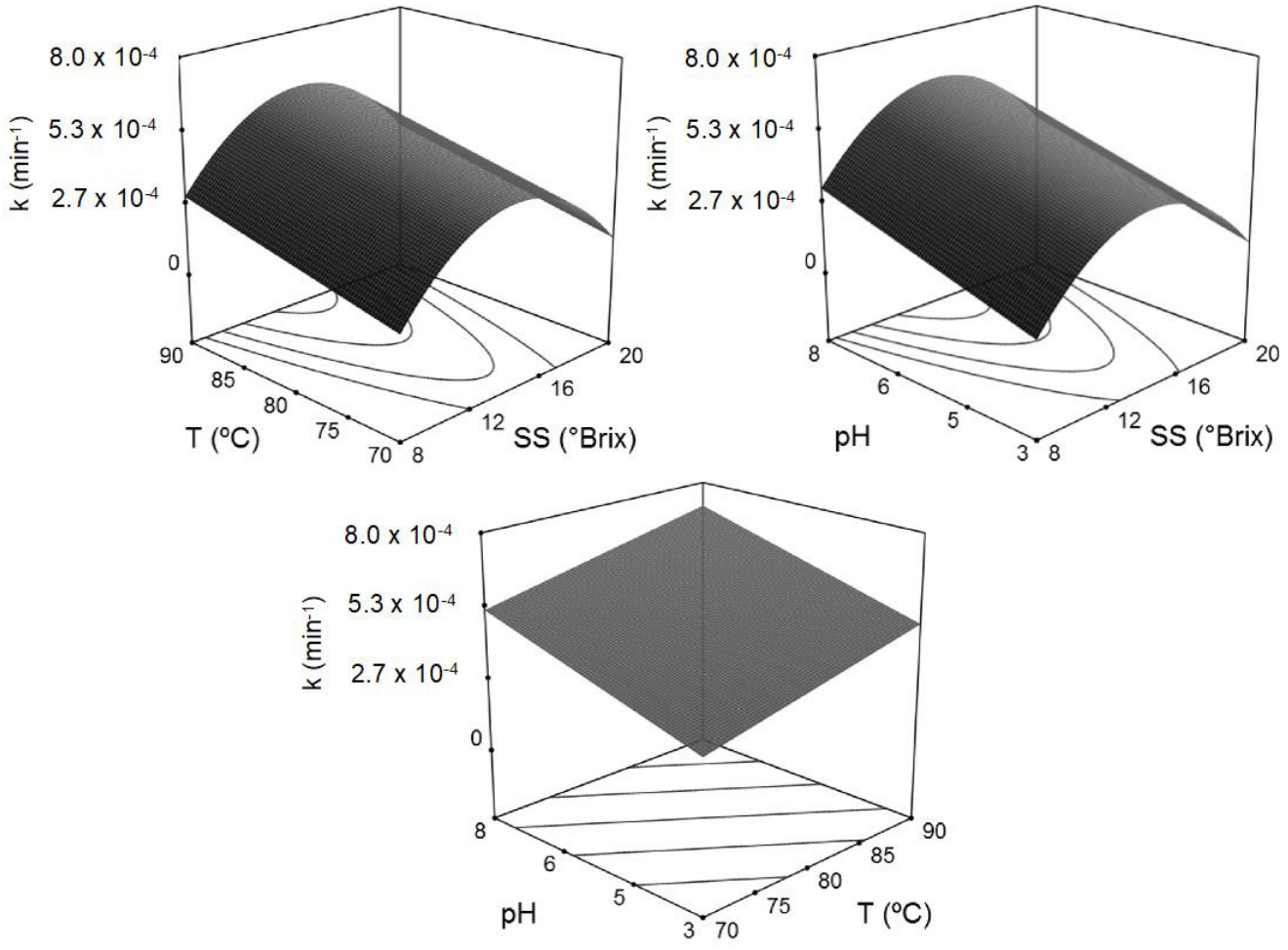

Figure 1. Response surface plots of k as a function of SS, T, and pH in the thermal degradation of TPC from the ethanolic extract of *Bixa orellana* L. leaves.

degradation probably due to higher interaction between the water and the flavylium cation to form the pseudo base unstable (Olaya et al., 2009). This result is relevant taking into account that decreases in the SS cause increments in the water activity (Zapata & Montoya, 2012).

The plot that represents the factors of pH and T presents a planar shape due to the absence of quadratic terms of both factors. It shows that the lower levels of the factors are those with the lowest k.

Figure 2 depicts the decrease in total phenolic concentration in extracts of *Bixa orellana* L. leaves as a function of time at the temperatures −20, 8, 23, and 37 °C. The thermal degradation of TPC was adjusted to a first-order reaction model; as found in anthocyanins of black carrot (Kirca et al., 2007), potatoes (Reyes & Cisneros-Zevallos, 2007), açaí concentrate (Albarici & Pessoa, 2012), and *Bixa orellana* L. seeds (Sepúlveda & Zapata, 2019). The degradation rate of total phenolic increased as the storage temperature increased, while the $t_{1/2}$ decreased. The same occurs at processing conditions (70-90 °C). The temperature factor (Q_{10}) showed that the changes in degradation rates also increased with increases in temperature, presenting a smaller increment in the degradation rate in temperatures in the from −20-8 °C. The positives effects of T on the degradation rate of the TPC have been reported in olive oil (Owen et al., 2000), carrots (Kirca et al., 2007), red-, purple-flesh potatoes, carrots, and grapes (Reyes & Cisneros-Zevallos, 2007), *Bixa orellana* L. seeds (Figueirêdo et al., 2014; Sepúlveda & Zapata, 2019), açaí concentrate (Albarici & Pessoa, 2012), and *Origanum vulgare* (Zhang et al., 2014).

It is essential to highlight the difference between the parameters of the thermal degradation that Table 1 and Table 3 show. Because the processing temperatures (70, 90 °C) are higher than the storage temperatures (−20, 37 °C), it indicates the high temperatures deteriorates the structure of the phenolic compounds (Timberlake, 1980).

Figure 2 also shows the behavior of the antioxidant activity measured with two methods (ABTS, and FRAP) as a function of time for the extract during 91 days of storage at four temperatures (−20, 8, 23, and 37 °C). It shows that the antioxidant activity is inversely proportional to the temperature on both ABTS and FRAP methods. In both cases, the loss of activity follows first-order kinetics like in the degradation of phenolic compounds from other reports (De Paepe et al., 2014; Fischer et al., 2013; Henríquez et al., 2014; Kirca et al., 2007).

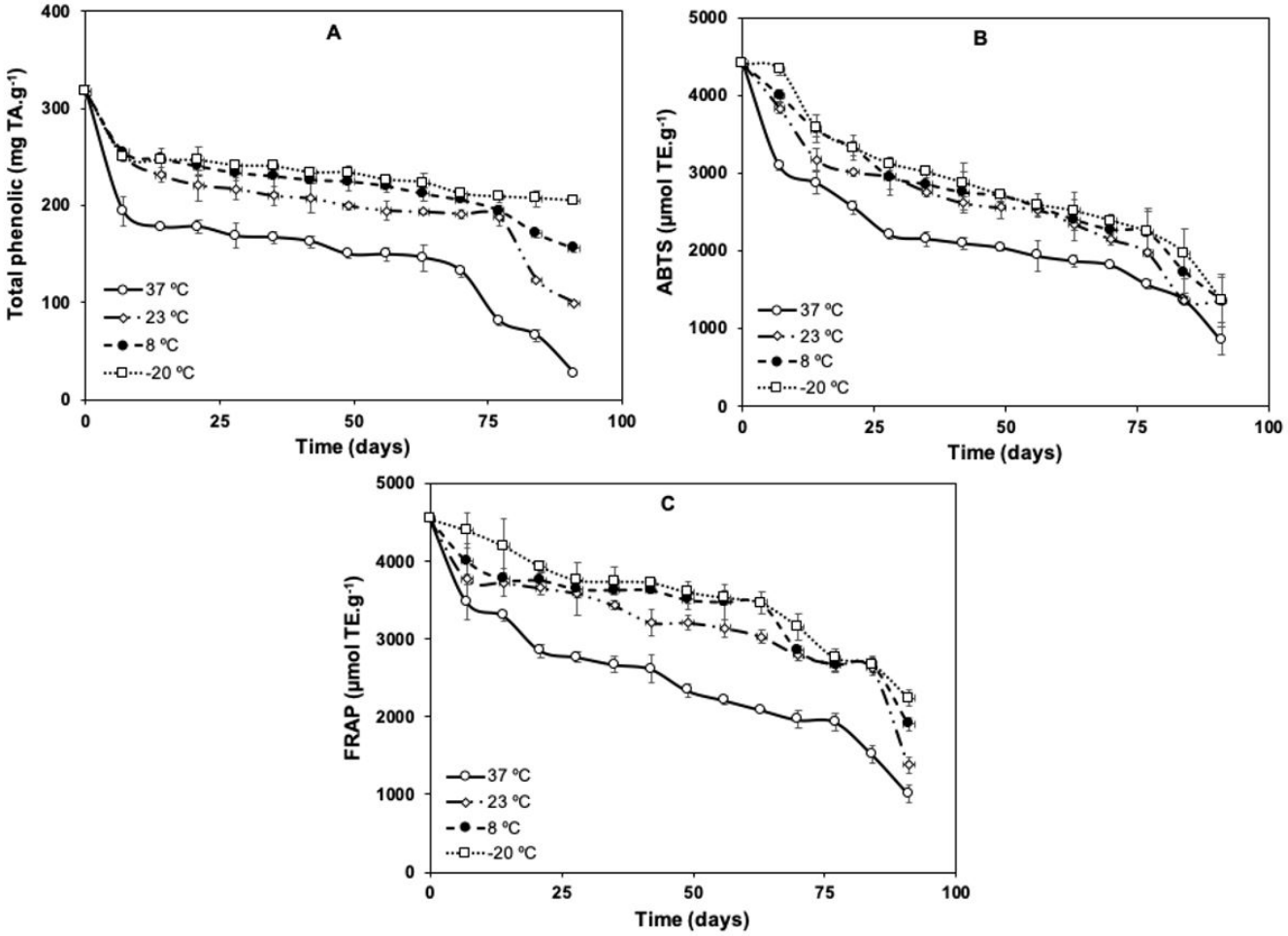

Figure 2. Stability of the leaves extract as a function of time: (A) total phenolic compounds; (B) antioxidant activity (ABTS); and (C) antioxidant activity (FRAP).

Table 3. Kinetic parameters of the degradation of TPC and antioxidant activity during storage of the extracts of *Bixa orellana* L. leaves.

T (°C)	FT		ABTS		FRAP	
	k ($days^{-1}$)[a]	$t_{1/2}$ (days)	k ($days^{-1}$)[a]	$t_{1/2}$ (days)	k ($days^{-1}$)[a]	$t_{1/2}$ (days)
−20	0.0034 (0.71)	202.47	0.010 (0.87)	68.83	0.0065 (0.90)	107.03
8	0.0055 (0.75)	125.72	0.010 (0.91)	67.48	0.0067 (0.71)	104.13
23	0.0083 (0.87)	83.08	0.011 (0.92)	63.51	0.0080 (0.79)	86.91
37	0.017 (0.80)	40.72	0.012 (0.91)	55.87	0.0120 (0.90)	57.85
E_a ($kJ \cdot mol^{-1}$)	17.10 (0.90)[a]		2.11 (0.71)[a]		6.12 (0.66)[a]	
Q_{10} (8/−20)	1.19		1.01		1.01	
Q_{10} (23/8)	1.32		1.04		1.13	
Q_{10} (37/23)	1.66		1.10		1.34	

[a]The numbers in parentheses correspond to the coefficients of determination of the respective parameter.

Table 3 also shows the thermal parameters of the loss of antioxidant activity of extracts of *Bixa orellana* L. leaves. It shows that k increase and the $t_{1/2}$ decrease as the temperature increase in the same way and with similar values than the degradation of TPC. This result confirms than the antioxidant properties of the extracts of *Bixa orellana* L. leaves are due to the phenolic compounds (Makwana et al., 2015). It also has been reported in rudge gourd (Padmashree et al., 2012) *Solanum nigrum* L. (Padmashree et al., 2014) and *Bixa orellana* L. seeds (Sepúlveda & Zapata, 2019).

The antioxidant activity determined by the ABTS method presented higher k and lower E_a and $t_{1/2}$ values than those obtained from the FRAP method. It suggests that the antioxidant activity measured by the ABTS method degrades more quickly than the one measured by the FRAP method since the activity decreases more rapidly (higher k and lower $t_{1/2}$) and requires less energy (lower Ea) to start the degradation reaction. On the other hand, the temperature factor (Q_{10}) shows that the activity degradation rates measured with both methods increase with temperature.

Evaluating the stability of antioxidant activity as a function of time is important because the antioxidant activity is one of the most vital activities of phenolic compounds (Balasundram et al., 2006; Baydar & Baydar, 2013; Bendary et al., 2013; Bonilla et al., 1999; Gutiérrez-Larraínzar et al., 2012; Liu et al., 2012; Zhang et al., 2014).

4 Conclusions

The results from this study show that the thermal degradation of phenolic compounds present in extracts of *Bixa orellana* L. leaves depends on the T, pH, and SS. The thermal degradation of the phenolic compounds and the antioxidant capacity of the extracts of *Bixa orellana* L leaves follows first-order kinetics. On the other hand, the antioxidant activity of these extracts decreased as a function of time and temperature with the same pattern as those of the phenolic compounds, so the antioxidant activity of these extracts can be mainly attributed to the phenolic compounds.

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