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Thermal Degradation Kinetics of Anthocyanins from Blood Orange, Blackberry, and Roselle Using the Arrhenius, Eyring, and Ball Models

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Anthocyanin stability was assessed over temperatures ranging from 30 to 90 °C for seven products: blood orange juice [*Citrus sinensis* (L.) Osbeck]; two tropical highland blackberry juices (*Rubus adenotrichus* Schlech.), one with high content and the other with low content of suspended insoluble solids (SIS); and four roselle extracts (*Hibiscus sabdariffa* L.). The blackberry juice showed the highest content of anthocyanins with 1.2 g/L (two times less in the roselle extracts and 12 times less in the blood orange juice). The rate constant for anthocyanin degradation and isothermal kinetic parameters were calculated according to three models: Arrhenius, Eyring, and Ball. Anthocyanins in blood orange juice presented the highest rate constant for degradation, followed by the blackberry juices and roselle extracts. Values of activation energies were 66 and 37 kJ/mol, respectively, for blood orange and blackberry and 47−61 kJ/mol for roselle extracts. For the blackberry juices, a high SIS content provided only slight protection for the anthocyanins. The increasing content of dissolved oxygen, from 0.5 to 8.5 g/L, did not significantly increase the rate constant. For both isothermal and nonisothermal treatments, all three models accurately predicted anthocyanin losses from different food matrices.

KEYWORDS: ***Citrus sinensis*; *Rubus adenotrichus*; *Hibiscus sabdariffa*; thermal degradation kinetics; anthocyanins**

INTRODUCTION

Recent toxicological alerts have led to bans on several artificial colorants for foodstuffs. The food industry is now using plant sources to develop various additives that have both coloring and antioxidant properties (*1*). Consequently, the market for natural food colorants is growing by 4−6% per year, as compared with that of artificial colorants, which is growing by only 2−3% per year (*1*).

The main groups of natural food colorants are anthocyanins, betacyanins, carotenoids, curcuminoids, and chlorophylls. The restrictions in the use of certain synthetic colorants greatly reduced the color palette available to the food industry. No color was spared, but that of red is, without doubt, the most affected. The banning of FD&C Red No. 2 (also called Amaranth) in the United States by the U.S. Food and Drug Administration, Orange RN in Britain, and Ecarlate GN and Ponceau GR (both reds) in France has led to an increased use of natural sources for red pigments (*1*). The largest group of water-soluble, natural, red pigments that offers alternatives to synthetic colorants is comprised of the anthocyanins.

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Anthocyanins are flavonoids that are characterized by a flavylium nucleus that exhibits maximum absorbance in the green/blue spectrum at 510 nm. These pigments are widespread throughout the plant kingdom and are responsible for the attractive colors—salmon, pink, scarlet, magenta, violet, purple, and blue—of most fruits, vegetables, flowers, leaves, and roots and other storage organs.

Many edible plants are sources of anthocyanins and flavonoids (*2*−*4*). These include roselle calyx (*Hibiscus sabdariffa*), blackberry (*Rubus* spp.), and blood orange (*Citrus sinensis*), which are particularly targeted by the beverage industry. Roselle calyx contains high amounts of anthocyanins that can be as much as 2.5 g/100 g DW (*5*−*7*). The calyx contains mainly two anthocyanins: delphinidin 3-xylosylglucoside and cyanidin 3-xylosylglucoside. Blackberries (*Rubus* spp.) also present high concentrations of anthocyanins that may be as much as 3.9 g/100 g DW, depending on variety and cultivar (*2*, *8*). Various anthocyanins have been identified in blackberries (*9*), but in the wild tropical highland variety (*Rubus adenotrichus*), found in tropical Latin America studied in this paper, only two anthocyanins predominate, cyanidin 3-glucoside and cyanidin 3-(6′-malonyl) glucoside (*2*). The anthocyanin content in blood orange

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ranges between 1 and 1.5 g/100 g DW. About 10 anthocyanins have been identified in blood orange (*10*), but cyanidin 3-glucoside and an acylated anthocyanin, that is, cyanidin 3-(4′-acetyl)-glucoside, predominate.

Edible sources of anthocyanins are highly appreciated by the food industry for their coloring properties, which can give foods various hues of red and violet. In addition to their potential health effects (*11*), beneficial effects for controlling human diseases have also been reported in the literature (*12*), including the reduction of risks of coronary heart disease, cancer, and stroke (*13*).

The main problem of anthocyanins as food colorants is their instability in the presence of endogenous enzymes [e.g., peroxidase, polyphenoloxidase, and β-glucosidase (*1*, *14*)] and heat. Indeed, thermal degradation of anthocyanins is a major problem for the food industry. Degradation kinetics during heating depends mainly on both the specific composition of anthocyanins and the characteristics of the food matrix. For instance, the presence of ascorbic acid (*15*, *16*), phenolic compounds (*17*), micellar systems (*18*), and soluble solids (*19*) may alter degradation kinetics. Hence, thermal stability of anthocyanins has been studied in different food matrices such as sour cherry (*4*), red and black raspberry (*20*), grape (*21*), strawberry (*22*), acerola (*16*), roselle (*23*, *24*), blackberries (*25*), and even juice blends (*26*). Reported results show that rate constants for anthocyanin degradation with respect to temperature can always be assumed to follow a first-order reaction.

Even so, the Arrhenius model (eq 1)—an empirical collision model based on the classic approach used for chemical reactions—is often considered as a reference; other models can also be used to describe the temperature dependence of the degradation reaction rate of anthocyanins (*25*, *27*, *28*). In this study, two other models were investigated. The theoretical Eyring model, also known as the Eyring−Polanyi model, is based on the transition state theory in which the enthalpy of activation (ΔH^*) and entropy of activation (ΔS^*) are the model's parameters (eq 2). The Ball model follows the approach commonly used in food processing for microorganisms destruction. It defines a decimal reduction time (D), which is related to temperature via a z factor (eq 3). The aim of this study was to compare the classical empirical approaches (Arrhenius and Ball models) and an approach based on statistical thermodynamics (Eyring). Then, the ability of these three models to describe the thermal degradation kinetics of anthocyanins was evaluated across different fruit juices and plant extracts, with different contents of dissolved oxygen and insoluble solids.

$$k = k_\infty e^{-\frac{E_a}{RT}} \quad (1)$$

with T expressed in K, k_∞ = pre-exponential factor = value of k at $T \infty$ (1/s), E_a = activation energy (J/mol), and R = gas constant = 8.31 J/mol K.

$$k = \frac{k_B}{h} T \times e^{-\frac{\Delta G^*}{RT}} = \frac{k_B}{h} T \times e^{-\frac{\Delta H^* - T\Delta S^*}{RT}} \quad (2)$$

with T expressed in K, ΔG^* = free activation enthalpy (J/mol), ΔH^* = activation enthalpy (J/mol), ΔS^* = activation entropy (J/mol K), k_B = Boltzmann constant = 1.381×10^{-23} J/K, h = Planck constant = 6.626×10^{-34} J s, and R = gas constant = 8.31 J/mol K.

$$D = D_0 10^{-\frac{T}{z}} \quad (3)$$

with T expressed in °C, D_0 = value of D at T = 0 °C (s), and z factor (°C).

MATERIALS AND METHODS

Plant Materials and Juice Preparation. Fully ripe blackberry fruits (*R. adenotrichus* Schlech.) were harvested in Cartago (Costa Rica) at 1500 m above sea level. A proportion of the collected fruits was pressed, using a discontinuous OTC 25 ton hydraulic press (OTC, Owatonna, MN) to obtain the blackberry reference juice (BRJ). The remaining fruits were sieved with an industrial extractor (sieves 15 and 8 mm) to obtain blackberry pulpy juice (BPJ).

Blood oranges (cv. Sanguinelli, *C. sinensis* L. Osbeck) were purchased from the collection of INRA-CIRAD at the Agronomic Research Station of INRA (Corsica, France). Fruits were hand-squeezed and then filtered through a stainless steel sieve (1 mm). Both blackberry and blood orange juices were placed in amber-sealed vials (125 mL) under nitrogen and kept frozen at −20 °C until analyzed.

Four varieties of roselle (*H. sabdariffa* L.) were used. One came from Guatemala, and the others came from Senegal. The Senegalese varieties were Koor, Vimto, and Thai (*29*) and were harvested in Thiaré Village, Kaolack Region (central Senegal). Varieties Koor and Vimto were of Sudanese origin, whereas Thai, as its name indicates, originated in Thailand. To prepare the roselle extract, the dried calyces were mixed with plain water at a mass ratio of calyx to water of 1:10 at a temperature of 25 °C for 10 h. The extract was then filtered through a stainless steel sieve (1 mm) and placed in amber-sealed vials (125 mL) under nitrogen and kept frozen at −20 °C until analyzed. Commercially, these products are available mainly as a single strength juice for the blood orange and as beverages formulated with water and sucrose for the blackberry and roselle.

Physicochemical Analysis. All juices or extracts were analyzed for pH, titratable acidity, and density using standard methods (*30*). The total soluble solids (TSS) content was measured with an Abbe refractometer (Atago, Japan), whereas the content of suspended insoluble solids (SIS) was measured as described in ref (*31*). Polyphenols were evaluated as described in ref (*32*). Vitamin C (ascorbic acid and dehydroascorbic acid) was assessed by high-performance liquid chromatography (HPLC) (*33*,*34*) using an Agilent 1100 system (Massy, France), as were sucrose, fructose, and glucose (*35*). The amount of dissolved oxygen was measured with a portable oxygen probe CyberScan DO 300 (Eutech Instruments, Singapore). The total anthocyanin content was assessed by the pH differential method (*36*). All absorbance readings were made against distilled water, which acted as the control. Spectrophotometric measurements were carried out using Shimadzu spectrophotometers (UV-1200 and UV-1605, Kyoto, Japan). Concentrations were expressed as cyanidin 3-glucoside (MW = 449 g/mol) equivalents for blood orange and blackberry and delphinidin 3-xylosylglucoside equivalents for roselle (MW = 577 g/mol). The molar extinction coefficient at pH 1 and 510 nm, used for calculation, was 26900 L/mol cm for cyanidin 3-glucoside (*25*). For delphinidin 3-xylosylglucoside, we used 26000 L/mol cm as determined experimentally using delphinidin chloride as the reference (Extrasynthese, Genay, France). All of the other reagents used were of analytical grade and were purchased from Sigma (L'isle d'Abeau, France).

Thermal Treatment. Juices or aqueous extracts (15 mL) were heated in sealed Pyrex tubes (100 mm long, 16 mm i.d.). The tubes were immersed in a thermostatic bath (AM 3001 K, Fisher Bioblock Scientific, Illkirch, France) that was filled with oil or water. A Heidolph EKT 3001 digital temperature controller (±1 °C), fitted to a filed sealed tube, was used as a control to measure the product temperature during experiments. The time for the solution to reach the temperature setup was between 20 s and 4 min, respectively, for 30 and 100 °C. After the treatment, the tubes were immediately cooled by submersion in an ice bath. The time to reach 25 °C was, at most, 1 min for all trials. Therefore, as compared with the time lag at temperature setup, transient states were deemed to be negligible, and the treatment was assumed to be isothermal. Residual anthocyanin contents were immediately assessed after thermal treatment. All trials and measurements were made in triplicate. For trials with various dissolved oxygen contents, the value was adjusted by bubbling in nitrogen or filtering air through at 0.45 μm. From these experimental data, the parameters of the three kinetic models were identified using linear regressions.

For the nonisothermal treatment, the pasteurization values $F_{70°C}$ were calculated, using 70 °C as the reference temperature and a z factor for microorganisms of 10 °C, according to eq 4.

$$F_{70°C} = \int_0^t 10^{\frac{T-70}{10}} \mathrm{d}t \quad (4)$$

Table 1. Main Characteristics of Juices and Roselle Extracts[a]

		blackberry		extract of roselle calyx[b]			
	blood orange	reference	pulpy	Guatemala	Koor	Vimto	Thai
pH	3.53 (0.05)	2.67 (0.05)	2.68 (0.05)	2.46 a (0.05)	2.23 b (0.05)	2.55 c (0.05)	2.35 b (0.05)
TSS (g/kg)	115 (4)	123 (5)	125 (4)	31 a (2)	32 a (2)	41 b (2)	33 a (2)
SIS (g/kg)	12 (0.5)	12 (0.5)	63 (15)	0	0	0	0
titration acidity (g citric acid/kgTSS)	87 (5)	202 (8)	194 (6)	180 a (6)	199 b (6)	185 a (5)	190 b (4)
glucose (g/kg TSS)	ND	228 (10)	216 (11)	31 a (2)	31 a (2)	32 a (2)	33 a (2)
fructose (g/kg TSS)	ND	228 (10)	224 (12)	40 a (3)	39 ab (2)	35 ab (3)	33 b (3)
sucrose (g/kg TSS)	305 (6)	8 (1)	8 (1)	31 a (2)	32 a (2)	35 a (3)	38 b (3)
ascorbic acid (mg/100 g)	51 (2)	44 (2)	45 (3)	30 a (1)	30 a (1)	34 b (1)	27 c (1)
anthocyanin (mg/L)							
cyanidin 3-glucoside	98(6)	1189 (55)	1233 (49)	315 (23)	250 (18)	718 (54)	306 (25)
delphinidin 3-xylosylglucoside				245 (18)	194 (12)	559 (35)	238 (15)

[a] Numbers in parentheses indicate the standard deviation of three analyses. ND, not determined. [b] Values with similar letters are not significantly different (Tukey, $P > 0.05$).

Experimental Design and Statistics. For the BRJ and the Guatemalan roselle extract, thermal degradation of anthocyanins was studied, following a central composite rotatable design (CCRD) with two variables, temperature (X_1) and dissolved oxygen content (X_2), and five levels for each variable. The temperature and dissolved oxygen content ranged between 35 and 98 °C and 0.4 and 8.5 mg/L, respectively. The rate constant k evaluated experimentally was used as a response and computed, using multiple linear regression (JMP v.5.1; SAS, NC) to fit a second-order polynomial equation (eq 5).

$$k = a_0 + a_1X_1 + a_2X_2 + a_{11}X_1^2 + a_{22}X_2^2 + a_{12}X_1X_2 + \varepsilon \quad (5)$$

where a_n values are constant regression coefficients and ε the error of the model.

Statistical tests for evaluating the relevance of the models were the correlation coefficient between the actual and the predicted response (R^2), the probability that tests for the absence of at least one significant regression factor in the model (P), and the probability that tests if the lack of fit of the model is zero (P_{lof}).

RESULTS AND DISCUSSION

Physical and Chemical Characteristics of the Food Matrices. The main physicochemical properties of the blood orange and blackberry juices and roselle calyx extracts are shown in **Table 1**. The blackberry juices presented the highest levels of anthocyanins, followed by roselle extracts and blood orange juice. Contents of the anthocyanin cyanidin 3-glucoside in the blackberry juices are at least two times the content found in the roselle extracts and 12 times that of blood orange juice.

The roselle extracts presented significant differences in anthocyanin contents and titratable acidity according to cultivar. The cultivar richest in anthocyanins is less acid, and in Senegal, to produce a beverage from roselle, the calyces of the more acid cultivars (Koor and Thai) are generally mixed with those of cultivar Vimto to produce an extract with a strong red hue and a more balanced acidity (*29*). Acidity and anthocyanin content appear to be influenced by cultivar and climatic and geographical conditions (*37*, *38*).

Kinetics of Anthocyanin Degradation during Heat Treatment. Examples of the degradation kinetics of total anthocyanins from roselle cv. Thai extract, BRJ juice, and blood orange are shown in **Figure 1**. The decrease of anthocyanins over time fit a first-order equation with a good regression coefficient ($0.93 < R^2 < 0.99$).

The kinetic parameters k and D, respectively, corresponding to Arrhenius and Eyring (eqs 1 and 2) and Ball models (eq 3), were calculated. So, the k and D values ranged between 145 and 1660×10^{-7} 1/s and $13-158 \times 10^3$ s for the blood orange juice, $81-747 \times 10^{-7}$ 1/s and $30-284 \times 10^3$ s for BRJ, $67-671 \times 10^{-7}$ 1/s and $34-341 \times 10^3$ s for BPJ, and $10-751 \times 10^{-7}$ 1/s and $30-2280 \times 10^3$ s for roselle extracts. The reaction rate constant k and D differed significantly among the products tested. Anthocyanins from blackberry juice with low SIS content (BRJ) presented the highest reaction rate for lower temperatures, ranging from 81×10^{-7} 1/s at 30 °C to 582×10^{-7} 1/s at 80 °C for the k parameter. At 90 °C, anthocyanin from blood orange presented the highest reaction rate being 145×10^{-7} 1/s at 90 °C in agreement with previous results (*27*). Differences among reaction rates k appeared to increase at higher temperatures for blood orange juice being only 1.4 times more than the roselle cv. Vimto extract at 60 °C but almost two times more at 90 °C. On the contrary, reaction rate

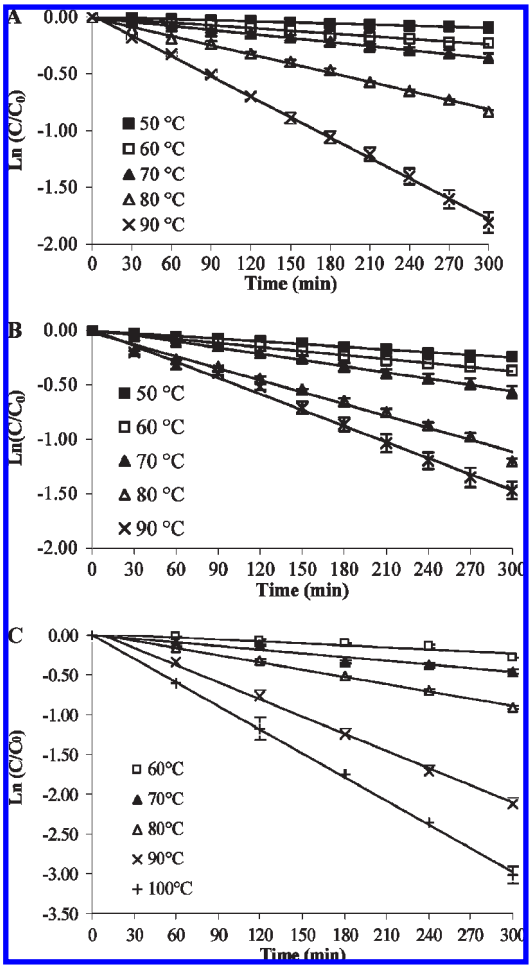

Figure 1. Examples of thermal degradation kinetics of anthocyanins vs temperature in (**A**) roselle cv. Thai extract, (**B**) BRJ, and (**C**) blood orange juice. Standard deviations were evaluated in triplicate.

constants for anthocyanins from the blackberry juices differed significantly from roselle extracts at lower temperatures (between 30 and 40 °C), but at higher temperatures (70 and 90 °C), differences became nonsignificant statistically ($P < 0.05$). Roselle extracts presented the lowest reaction rates at low temperatures. However, the roselle cv. Thai extract behaved significantly differently, as compared with the other cultivars, at lower temperatures. For temperatures between 60 and 90 °C, the reaction rate of all roselle cultivars grown in similar conditions in Senegal (Koor, Vimto, and Thai) ranged between 100 and 600×10^{-7} 1/s for k, thus agreeing with previous data obtained by other authors (*24*). The Guatemalan cultivar nevertheless presented a lower reaction rate at 90 °C than did the other cultivars grown in Africa.

The variation between all of these products may be explained by the chemical composition of both products, the content of dissolved oxygen, and the anthocyanin structure. Blood orange juice presented the lowest acidity (i.e., the highest pH) and the highest sucrose and ascorbic acid contents. Previously, low acidity and a pH higher than 3 were shown to considerably reduce anthocyanin stability (*39*). In addition, although the effect of sucrose on anthocyanins is still not clear, reducing sugars, particularly fructose (*40*, *41*), have been shown to indirectly enhance anthocyanin degradation during heating because of the formation of furfural and 5-(hydroxymethyl)furfural, which accelerate the rate of anthocyanin degradation (*42*). The higher content of fructose in the blackberry juices with respect to the roselle extracts may also affect anthocyanin stability. For sucrose, other authors (*43*) have shown that higher content in anthocyanin solutions delays browning, suggesting that sucrose would contribute to anthocyanin stability under heat. With the major pigment in strawberries, pelargonidin 3-glucoside, fructose, arabinose, lactose, and sorbose provided greater pigment degradation than glucose, sucrose, or maltose (*14*). The stability of anthocyanins can also be affected by other components. The presence of higher ascorbic acid content in some food matrices is also believed to affect anthocyanins stability during heating (*3*, *14*, *25*), although how this happens is not yet well understood (*44*, *45*). Anthocyanins are very reactive toward metals. They will form complexes with tin, copper, and iron (*14*). Cyanidin 3-glucoside will form a stable colored complex in the presence of aluminum ions at pH 5.5. Anthocyanins can form weak complexes with flavonols, amino acids, benzoic acids, coumarin, and cinnamic acids.

The anthocyanin degradation rates for the blackberry juices with different SIS contents (**Table 1**) clearly ($P < 0.05$) show that the SIS have a slight protective effect. The two blackberry juices presented similar characteristics, except for the SIS content, which is almost five times higher in the pulpy juice. For the blackberry juice with the high SIS content, rate constant k is considerably smaller for all temperatures tested.

The difference in structure between the main anthocyanins in the products is also a factor to consider. Indeed, the cyanidin-based anthocyanins have quite different stability than delphinidin derivatives. Hydroxyl groups and methoxyl groups on the B ring have an effect on color intensity and stability of anthocyanins (*46*). For roselle extracts, further study is needed using HPLC to differentiate the two anthocyanins that are present into the products.

Effect of Dissolved Oxygen on Kinetic Constants. In addition to intrinsic factors, anthocyanin stability may also be influenced by external factors such as light, copigmentation, metal complexing, and dissolved oxygen. This last factor, often enhanced by extraction procedures, was studied particularly for the BRJ juice and the roselle extract from Guatemala (**Table 2**). For the roselle extract, anthocyanin degradation is only 1.5 times higher at 7.5 mg/L of dissolved oxygen than at 1.5 mg/L at both 45 and 90 °C. For the blackberry juice, it is only 1.1 higher under the same conditions. Meanwhile, in both cases, the k value increased almost 10 times if the temperature increased from 45 to 90 °C. Dissolved oxygen appeared to have a slightly negative effect on anthocyanin stability, at least immediately after thermal treatment. Further impact during storage was not assessed. Other works showed with the juices of blueberry, cherry, current, grape, and strawberry that oxygen and temperature were the most specific accelerating agents (*14*). The effect of oxygen can be explained either by a direct oxidation mechanism or through oxidized compounds that react with anthocyanins to give colorless or brown products. Packaging under nitrogen increased the stability of powdered dyes based on phenolic molecules by slowing the oxidation reactions (*1*).

Table 2. Rate Constant k during the Thermal Degradation of Anthocyanins vs Oxygen Concentration and Temperature in Guatemala Roselle Extract, with Model Fittings at $R^2 = 0.98$, $P < 0.0014$, and $P_{lof} = 0.15$, and BRJ, with Model Fittings at $R^2 = 0.98$, $P < 0.006$, and $P_{lof} = 0.75$[a]

		$k \times 10^{-5}$ (1/s)	
O_2 (mg/L)	T (°C)	Guatemala roselle	BRJ
0.45	67.5	2.102 (0.023)	1.770 (0.053)
1.50	45.0	0.553 (0.001)	0.375 (0.004)
	90.0	5.980 (0.051)	3.810 (0.017)
4.50	35.0	0.585 (0.001)	0.286 (0.012)
	67.5	2.750 (0.003)	2.120 (0.005)
	98.5	7.990 (0.256)	7.514 (0.345)
7.50	45.0	0.604 (0.003)	0.557 (0.001)
	90.0	6.752 (0.008)	5.543 (0.002)
8.50	67.5	4.032 (0.002)	3.442 (0.001)

[a] Numbers in parenthesis indicate the standard deviation of three analyses.

Pressed juices show quite different stability from petals extracts. This may be caused by enzymatic activity. In fact, anthocyanins can be degraded by a number of enzymes found in plant tissue such as peroxidases, polyphenoloxidases, and β-glucosidases (*1*, *14*). The glucosidases hydrolyze the pigments to sugars and anthocyanidins. The latter are unstable and degrade to colorless derivatives or perharps become part of the polymeric colored compounds. In our case, the enzymatic degradation of anthocyanins could only take place at temperatures below 60 °C.

Model Validation and Predictions. The isothermal kinetic parameters corresponding to eqs 1−3 for the three models Arrhenius, Eyring, and Ball are presented in **Table 3**. All three models were observed to fit well with the temperature dependence of k and D ($0.916 < R^2 < 0.996$).

Blood orange juice and roselle cv. Thai extract with values of 66 and 61 kJ/mol, respectively, showed the highest activation energies. Increased temperature will therefore degrade anthocyanins more rapidly in these products than in the other juices or extracts. The degradation rate of anthocyanins from the blackberry juices was less affected by temperature increase than those from blood orange juice and roselle extracts, according to the z value and enthalpy of activation ΔH^* (**Table 3**). From this point of view, anthocyanins from blackberry appeared less heat sensitive than the other plant extracts with a stability that was two times higher than that reported previously for a different blackberry variety (*R. fruticosus*) (*25*). The lower acidity and, eventually, the presence of other compounds in tropical highland blackberry may explain this difference. For the roselle cv. Thai extract and blood orange juice, values of activation energy agreed with those found by other authors (*23*, *24*, *27*).

Table 3. Kinetic Parameters for Thermal Degradation of Anthocyanins, Following Different Models[a]

	Arrhenius model			Eyring model			Ball model		
	$k_\infty \times 10^3$ (1/s)	E_a (kJ/mol)	R^2	ΔH^* (kJ/mol)	ΔS^* (J/mol.K)	R^2	$D_0 \times 10^6$ (s)	z (°C)	R^2
blood orange	324 (14.8)	66.04 (1.64)	0.988	63.11 (0.73)	−149 (1.56)	0.987	7.4 (1.05)	36 (1)	0.986
blackberry									
reference	0.015 (0.003)	36.99 (0.40)	0.964	34.24 (0.50)	−232 (1.53)	0.961	1.2 (0.04)	57 (1)	0.981
pulpy	0.013 (0.002)	37.23 (0.32)	0.954	34.48 (0.32)	−233 (1.02)	0.916	1.6 (0.02)	56 (1)	0.948
roselle extract									
Guatemala	1.336 (0.095)	51.12 (1.56)	0.953	48.31 (0.45)	−194 (3.89)	0.976	4.6 (0.89)	43 (1)	0.938
Koor	1.211 (0.810)	49.94 (1.55)	0.990	47.19 (1.55)	−195 (4.47)	0.989	3.7 (0.47)	42 (1)	0.982
Vimto	0.371 (0.078)	47.48 (0.72)	0.980	44.72 (0.71)	−205 (1.83)	0.978	4.3 (0.37)	44 (1)	0.989
Thai	44.20 (0.56)	61.60 (2.64)	0.996	58.78 (2.65)	−165 (1.43)	0.987	14.5 (1.57)	34 (1)	0.994

[a] Numbers in parentheses indicate the standard deviation of three analyses.

Table 4. Experimental and Calculated Losses of Anthocyanins as Compared to Different Isothermal and Nonisothermal Treatments of Roselle Cv. Vimto Extract

Isothermal					
thermal treatment		losses (%)			
T (°C)	t (min)	experimental[a]	Arrhenius model	Eyring model	Ball model
35	60	1.0 (0.3)	1.2	1,2	1.2
75	30	4.3 (0.2)	4.9	4.8	4.6
85	30	6.6 (0.5)	7.7	7.6	7.6
85	120	28.8 (0.5)	27.3	27.0	27.2
85	240	49.9 (0.4)	47.2	46.7	47.0
90	90	24.4 (0.6)	25.8	25.5	26.5

Nonisothermal (**Figure 2**)						
T_{setup} (°C)	t_{setup} (min)	$F_{70°C}$ (min)	experimental[a]	Arrhenius model	Eyring model	Ball model
thermal treatment			losses (%)			
70	30	42	6.4 (0.5)	5.4	5.4	5.3
85	30	1309	13.4 (0.1)	16.3	16.2	16.8
90	5	1070	2.0 (0.5)	3.0	3.0	3.2

[a] Numbers in parentheses indicate the standard deviation of three analyses.

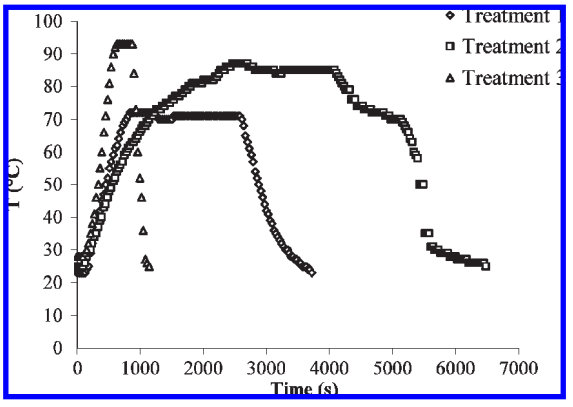

Figure 2. Time and temperature profile used to validate the models using roselle cv. Vimto extract. Treatment 1, stage at 70 °C at 30 min, $F_{70°C}$ value = 42 min; treatment 2, stage at 85 °C at 30 min, $F_{70°C}$ value = 1309 min; and treatment 3, stage at 90 °C at 5 min, $F_{70°C}$ value = 1070 min.

To compare the accuracy of the three models in predicting experimental results, additional trials were performed at different isotherms for roselle cv. Vimto extract. Experimental losses were then compared with calculated values (**Table 4**). In all cases, the three models gave similar results, with estimates being close to the experimental values. Model accuracy was thus verified, and the approach followed in this study was validated for isothermal treatments.

The accuracy of the models was also assessed for nonisothermal treatments, simulating real heating processes. Hence, three

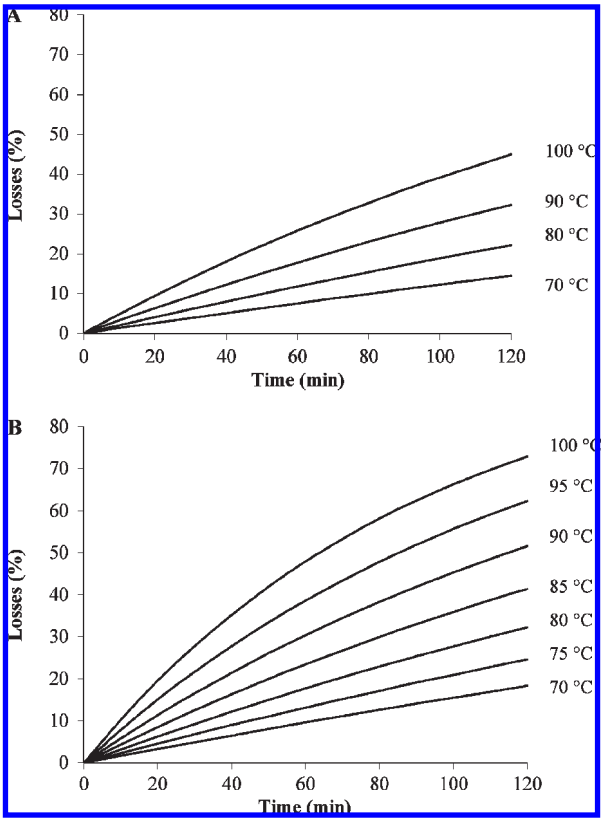

Figure 3. Examples of estimated anthocyanin losses during isothermal treatments at different temperatures using the Eyring model. (**A**) Roselle cv. Vimto extract. and (**B**) blood orange juice.

treatments with different time and temperature profiles were tested (**Figure 2**). Treatment 1 (30 min at 70 °C) simulated classic low-temperature pasteurization; treatment 2 (30 min at 85 °C) simulated pasteurization at a higher temperature and longer residence time; and treatment 3 (5 min at 90 °C) simulated a flash pasteurization at a high temperature. Different $F_{70°C}$ values were calculated, and experimental losses were analyzed and compared with predicted values (**Table 4**). As with the isothermal treatment, all models gave similar results, and estimates were close to experimental values.

Under isothermal conditions, anthocyanin losses for roselle cv. Vimto extract and blood orange juice estimated for instance by the Eyring model are plotted in **Figure 3**. Classic pasteurization does not significantly damage anthocyanins, with estimated losses being less than 5% for 5 min of pasteurization at 85 or 90 °C. Also, the prediction of anthocyanin losses for a given $F_{70°C}$ value at different setup temperatures can be easily calculated by the

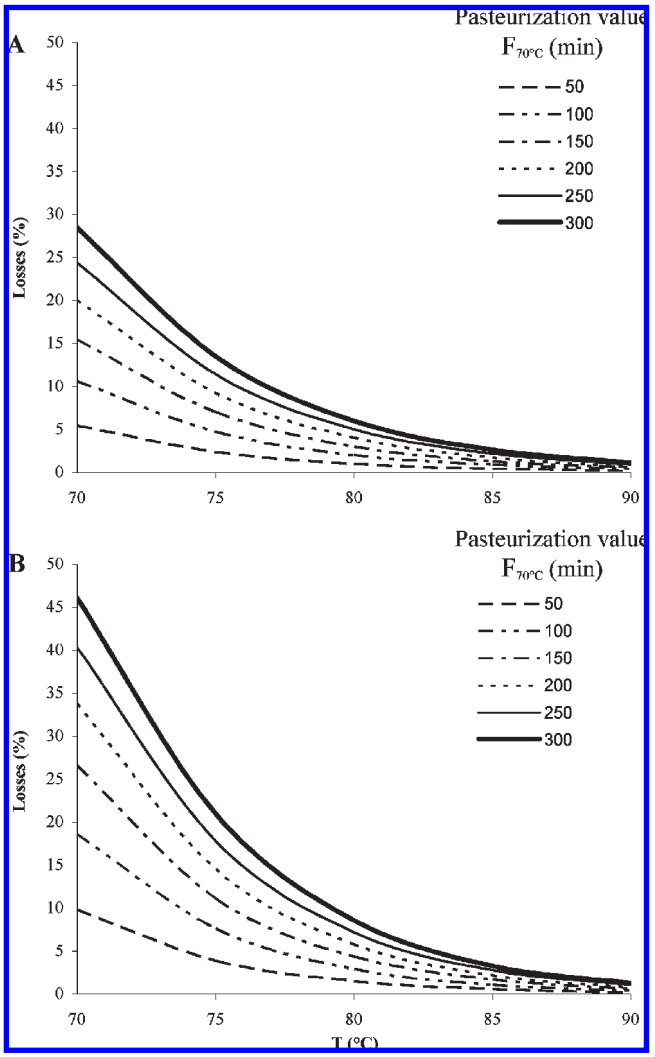

Figure 4. Estimated anthocyanin losses during isothermal treatments with varied setup temperatures and different $F_{70°C}$ values using the Eyring model. (**A**) BRJ and (**B**) roselle cv. Thai extract.

models, as shown in **Figure 4**. For example, for $F_{70°C}$ values between 100 and 200 min, typically used for thermal treatment of fruit beverages, temperatures above 80 °C maintained the losses of anthocyanins at the end of the process below 10% for both the BRJ and the roselle cv. Thai extract.

Our study evaluated the impact of temperature (30−90 °C) on anthocyanins degradation in blood orange juice, two tropical highland blackberry juices, and four roselle extracts. The data show that the thermal degradation of the anthocyanins can be described using first-order reaction kinetics. Variation of degradation rate constants with temperature and time can be described using as well the Arrhenius, Eyring, or Ball equations. Anthocyanins in blood orange juice had the highest rate constant for degradation, followed by the blackberry juices and roselle extracts. For the blackberry juices, a high SIS content provided only slight protection against anthocyanin degradation. The effect of the temperature seems to be predominant on the deterioration of anthocyanins than the effect of dissolved oxygen. Using kinetic parameters, Arrhenius, Ball, and Eyring models were developed to predict the losses of anthocyanins during heat treatment. Model accuracy in isothermal and anisothermal treatment conditions was verified. These models could easily be used as tools to predict losses during different heat treatments. The sensitivity of anthocyanins in the various products may be explained by the chemical composition, dissolved oxygen content, and anthocyanin structure. However, this study on real products was not able to separate the effects of the structure and the effect of the food matrix, which will require further studies on model solutions.

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Supporting Information Available: Table of isothermal kinetic parameters k and D vs temperature for the thermal degradation of anthocyanins in blackberry and blood orange juices and roselle extracts and figure of rate constant k (1/s) during the thermal degradation of anthocyanins vs oxygen concentration and temperature. This material is available free of charge via the Internet at http://pubs.acs.org.

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