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# Identification and Thermal Degradation Kinetics of Chlorophyll Pigments and Ascorbic Acid from Ditax Nectar (*Detarium senegalense* J.F. Gmel)

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**ABSTRACT:** *Detarium senegalense* J.F. Gmel (ditax) is a forest tree found in Senegal the fruits of which are characterized by an attractive green flesh with a high amount in ascorbic acid. It is generally consumed as a nectar in Senegal. In this study, the main pigments of ditax pulp were identified and quantified by HPLC-DAD. Pheophytin *a* (128 mg/kg), which represents 58% of the total pigments, followed by hydroxypheophytin *a*′ (33 mg/kg), chlorophyll *b* (24 mg/kg), and chlorophyll *a* (20 mg/kg) was the major pigment of ditax pulp. Lutein and $\beta$-carotene were present in lower amounts (4.6 and 3.6 mg/kg, respectively). The thermal degradation kinetics of pheophytin *a*, hydroxypheophytin *a*′, and ascorbic acid were determined at temperatures ranging from 60 to 95 °C in ditax nectar. Pheophytin *a* was the most heat sensitive. Thermal processing induced the formation of degradation products such as pyropheophytin *a* and pyropheophytin *b*. The kinetics parameters have been calculated according to the models of Arrhenius, Eyring, and Ball. Following the Arrhenius relation, activation energies of pheophytin *a*, hydroxypheophytin *a*′, and ascorbic acid were, respectively, 79, 74, and 46 kJ mol$^{-1}$. Losses calculated during isothermal treatments were close to experimental losses in pheophytin *a*, hydroxypheophytin *a*′, and ascorbic acid. The Eyring model can then be used to predict chlorophyll pigments and vitamin C losses during pasteurization of the nectar (<10%).

**KEYWORDS:** *Detarium senegalense*, pheophytin, hydroxypheophytin, vitamin C, fruit juice

## INTRODUCTION

From tropical African regions, *Detarium senegalense* J.F. Gmel is a big forest tree (15−40 m height) belonging to the Ceasalpiniaceae family, and the *Detarium Juss.* species *D. senegalense* is found on the wet dense forest borders, the coastal and septentrional regions, and the Sudan−Guinean zone. In Senegal, *D. senegalense* is one of the most important forest fruit-bearing species in the exploitative economy in Senegal. It grows wildly on the Sine-Saloum islands and in Casamance.[1] The *D. senegalense* fruit, locally called ditax (wolof), is very popular in Senegal due to the very attractive green color of the pulp. From a nutritive point of view, the ascorbic acid content of ditax is very high, between 1.2 and 2.2 g 100 g$^{-1}$ of fresh weight according to geographical origin; saccharose (18−20% dry weight) is the main component of total sugars (23% dry weight), and potassium (1−1.6% dry weight) is the principal mineral.[2] Ditax nectar (fruit pulp with water and sugar) is one of the most popular beverages in Senegal, but the fruit is also directly eaten or used as marmalade or sherbet.

Chlorophyll and carotenoids are very common pigments, which give color to several fruits and vegetables. Chlorophyll degradation during fruit and vegetable processing has been studied, and many reviews are available in these fields.[3−9] Chlorophyll degradation in food may occurr via chemical and biochemical reactions. Chemical reactions involve the formation of pheophytin from chlorophyll by the replacement of Mg from the porphyrin ring via acidic substitution and/or heat treatment or after action of Mg dechelatase. Decarbomethoxylation may occurr during strong heat treatment, leading to the conversion of pheophytin to pyropheophytin.[10,11] Pheophytin formation is accompanied by color modification from green to olive brown.[4,9,12,13]

The kinetics of chlorophyll degradation has been studied extensively. Studies at different temperatures showed the rate of this degradation followed a first-order kinetic model.[14] In green peas, the reaction rate constant ($k$) was between $1.2 \times 10^{-4}$ and $6.2 \times 10^{-4}$ s$^{-1}$ for chlorophyll *a* and between $6.5 \times 10^{-5}$ and $2.6 \times 10^{-4}$ s$^{-1}$ for chlorophyll *b* from 70 to 90 °C.[15] In spinach puree, Schwartz and Von Elbe[11] determined $k$ constants of $0.63 \times 10^{-3}$ s$^{-1}$ for pheophytin *a* and $0.99 \times 10^{-3}$ s$^{-1}$ for pheophytin *b* at 116 °C. Activation energies determined in virgin olive oil were 83.3 kJ/mol for pheophytin *a* and 74.2 kJ/mol for hydroxypheophytin *a* from 60 to 120 °C.[16]

Although an essential parameter of ditax nectar quality, the pigment composition of ditax pulp has never been studied. During pulp extraction, nectar processing, and/or storage, the color changes from green to olive brown. The influence of dissolved oxygen is also an important parameter as highlighted by García-Torres et al.[17] Then, the first objective of our study was to identify the major chlorophyll pigments of ditax pulp and to understand the impact of thermal treatment on these compounds to improve our knowledge of their thermal resistance. To better characterize the decrease of quality of ditax nectar undergoing thermal treatments

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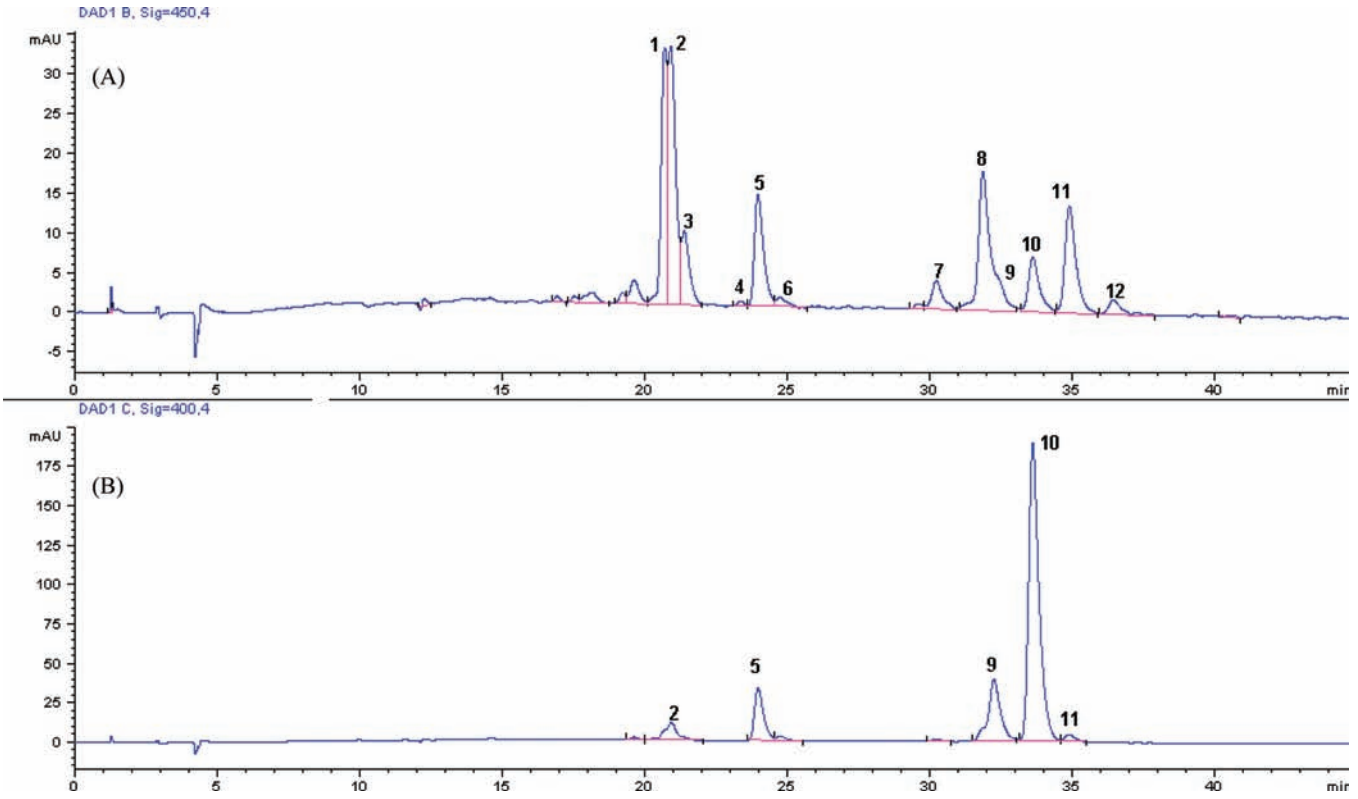


**Figure 1.** HPLC elution profile (A) at 450 nm to have a best view of all pigments and (B) at 400 nm to focus on major peaks 9 and 10 of ditax pulp. Peaks: chlorophyll *b*, 1; lutein, 2; chlorophyll *b*′, 3; zeaxanthin, 4; chlorophyll *a*, 5; chlorophyll *a*′, 6; hydroxypheophytin *b*′, 7; pheophytin *b*, 8; hydroxypheophytin *a*′, 9; pheophytin *a*, 10; $\beta$-carotene, 11; and 9-*cis*-$\beta$-carotene, 12.

such as pasteurization, kinetic studies were carried out measuring dissolved oxygen. The influence of temperature (between 60 and 100 °C) on the degradation of the main chlorophyll pigments (hydroxypheophytin and pheophytin) and ascorbic acid of ditax nectar was studied to predict the impact of thermal processing on these compounds. Experimental data were processed using different kinetic models.

## EQUIPMENT AND METHODS

**Chemicals.** Extraction solvents were RPE grade hexane, ethanol, and dichloromethane from Carlo-Erba (Val de Reuil, France). Analytical solvents were HPLC grade methanol (Carlo-Erba) and methyl *tert*-butyl ether (MTBE) from Sigma-Aldrich (Steinheim, Germany). Reagents for analysis were pure grade sodium chloride and sodium sulfate (Carlo-Erba).

**Ditax Pulp and Nectar Formulation.** Fruits were collected in 2008 (pulp 2) from Kabiline 2 village (Ziguinchor, Senegal) and in 2010 (pulp 1) from a Dakar local market. Fruits were characterized by a fresh weight of 49.05 ± 5.98 g and a diameter of 3.83 ± 0.22 cm (the means and standard deviations were obtained for 30 fruits). Percentage of natural pulp extract was 26.19% from fresh fruit. Pulp 2 was extracted immediately, packed in polypropylene bags, and stored at −18 °C before analysis. Pulp 1 was extracted just before analysis without storage. For thermal treatment, nectar at 14% Brix and pH 4.06 was prepared by adding to pulp 1 (15%) water (75%) and sugar (10%). To have drinkable nectar, the pulp ratio in the formulation of nectar had to be ≤15% because of the texture of the pulp (nectar will be very thick and dense). Pulp 2 was used to identify pigment contents of ditax pulp.

**Determination of Ascorbic Acid.** Ascorbic acid was determined by HPLC.[18] Ditax nectar (0.1 mL) was homogenized with 9.9 mL of a 4.5% metaphosphoric acid solution. Extractions were carried out in triplicate. After centrifugation, the supernatant was filtered through a 0.45 $\mu$m membrane and analyzed by HPLC (Spectra SCM 1000 Thermo Scientific, Spectra UV 3000 detector) equipped with an RP 18e Licrospher 100 (250 × 4.6 mm i.d., 5 $\mu$m; Merck KgaA). The isocratic solvent system was a 0.01% solution of $H_2SO_4$, the flow rate was 1 mL $min^{-1}$, and detection was set at 254 nm. Quantification of ascorbic acid was done at 254 nm by an external standard method (calibration curve between 20 and 200 mg $L^{-1}$).

**Determination of Pigment Contents from Ditax Pulp and Nectar.** *Identification of Pigments.* Standards of chlorophyll *a* (from *Anacystis nidulans* algae, Sigma-Aldrich) and chlorophyll *b* (from Spinach, Fluka analytical) were used to prepare standards of epimers, pheophytins, hydroxypheophytins, and pyropheophytins to identify chlorophyll pigments. The epimer of chlorophyll *a* was obtained using a method described by Watanabe et al.[12] Chlorophyll *a* was dissolved in chloroform and settled for 2 h at 4 °C (this treatment promoted epimerization). Pheophytin *a* and pheophytin *b* were prepared from their respective chlorophylls by adding 30 $\mu$L of a solution of 0.1 M HCl in chlorophyll *a* and chlorophyll *b* methanol solutions.[10,12,19−21] Pyropheophytins *a* and *b* were obtained from their respective pheophytin after heating treatment: 200 $\mu$L of chlorophyll in methanol with 30 $\mu$L of 0.1 N HCl was evaporated to dryness and heated in an oil bath for 1 h at 95 °C. The dry extract was diluted with methanol and analyzed by HPLC. Carotenoid standards ($\beta$-carotene and lutein) were purchased from Extrasynthèse (Genay, France). Pigments were identified using retention times, absorption spectra, and co-injection with standards. The UV−visible spectra were compared with those reported in the literature.

*Extraction and HPLC Analysis of Pigments.* Pigments (chlorophyll derivates and carotenoids) were extracted simultaneously according to the method of Dhuique-Mayer et al.[18] adapted to ditax pulp with slight

**Table 1. Spectral Data of Pigments Detected in Ditax Pulp**

| peak | retention time (min) | pigment | $\lambda_{max}$ online (nm) | $\lambda_{max}$ reported[a] (nm) |
|---|---|---|---|---|
| 1 | 20.67 | chlorophyll *b* (470 nm) | **464**, 600, 650 | 466, 600, 650;[a] 462, 600, 648;[b] 465, 602, 650;[c] 457, 597, 646;[f] 460, 647[g] |
| 2 | 20.84 | lutein (450 nm) | 422, **444**, 472 | 444, 472;[a] 424, 447, 474;[f] 425, 447, 476[g] |
| 3 | 21.37 | chlorophyll *b*′ (470 nm) | **462**, 600, 650 | 466, 602, 652;[a] 462, 600, 648[b] |
| 4 | 23.37 | zeaxanthin (450 nm) | 426, **448**, 480 | 425, 450, 479;[e] 430, 450, 476;[d,h] 428, 455, 481[g] |
| 5 | 23.94 | chlorophyll *a* (400 nm) | **432**, 618, 664 | 432, 618, 664;[a] 430, 616, 662;[f] 431, 661[g] |
| 6 | 24.74 | chlorophyll *a*′ (400 nm) | **432**, 618, 664 | 432, 620, 666;[a] 430, 618, 664[b] |
| 7 | 30.21 | hydroxypheophytin *b*′ (450 nm) | **436**, 528, 654 | 436, 528, 600, 652[b] |
| 8 | 31.85 | pheophytin *b* (450 nm) | **436**, 528, 598, 654 | 436, 598, 652;[a] 435, 649[g] |
| 9 | 32.24 | hydroxypheophytin *a*′ (400 nm) | **410**, 506, 536, 610, 666 | 408, 504, 534, 610, 666[b] |
| 10 | 33.60 | pheophytin *a* (400 nm) | **408**, 506, 536, 608, 666 | 408, 506, 536, 608, 666;[b] 411, 666[g] |
| 11 | 34.89 | *β*-carotene (450 nm) | **452**, 476 | 452, 478;[d,h] 454, 476;[f] 455, 481[g] |
| 12 | 36.44 | 9-*cis*-*β*-carotene (450 nm) | 425, **448**, 472 | 425, 449, 475[h] |

[a] In parentheses, signal detection setting: [a], Kwang Hyun Cha et al.,[25] 2010; [b], Huang et al.,[26] 2008; [c], Giuffrida et al.,[27] 2005; [d], Dhuique-Mayer et al., 2007;[28] [e], Hegazi et al., 1998;[29] [f], Nishiyama et al.,[30] 2005; [g], Montefiori et al.,[31] 2009; [h], Fanciullino et al.,[32] 2006.

**Table 2. Pigment Concentration in Ditax Pulp (Pulp 1, Pigment Analysis of Fresh Pulp; Pulp 2, Pigment Analysis of Pulp after 1 Year of Storage at −20 °C) and Nectar (Native and Heated)[a]**

| | content (mg/kg fresh weight) | | concentration (mg/L) | |
|---|---|---|---|---|
| pigment | pulp 1 | pulp 2 | native nectar | heated nectar |
| chlorophyll *b* | 23.64 ± 1.90 | 2.88 ± 0.16 | | |
| lutein | 4.64 ± 0.20 | 3.47 ± 0.06 | tr | tr |
| chlorophyll *b*′ | 5.19 ± 0.05 | | | |
| chlorophyll *a* | 20.28 ± 6.30 | | | |
| chlorophyll *a*′ | tr | | | |
| hydroxypheophytin *b*′ | 0.48 ± 0.07 | 0.80 ± 0.05 | 0.088 ± 0.003 | 0.057 ± 0.001 |
| pheophytin *b* | 2.69 ± 0.03 | 3.70 ± 0.16 | 0.42 ± 0.02 | 0.26 ± 0.01 |
| hydroxypheophytin *a*′ | 32.95 ± 0.24 | 23.89 ± 1.00 | 2.30 ± 0.05 | 1.23 ± 0.07 |
| pheophytin *a* | 128.02 ± 6.99 | 116.50 ± 5.90 | 11.55 ± 0.57 | 4.30 ± 0.22 |
| *β*-carotene | 3.61 ± 0.3 | 2.69 ± 0.23 | tr | tr |
| pyropheophytin *b* | 0 | 0 | 0 | 0.22 ± 0.004 |
| pyropheophytin *a* | 0 | 0 | 0 | 4.04 ± 0.31 |

[a] Data are means and standard deviations of three determinations. tr, traces.

modifications. Briefly, 1 g of ditax pulp or 3 mL of heated nectar was homogenized by magnetic stirrer with 2 mL of distilled water, 120 mg of $MgCO_3$, and 15 mL of extraction solvent (ethanol/hexane, 4:3 v/v, containing 0.1% BHT as antioxidant) for 10 min. The residue was separated from the liquid phase by filtration with a filter funnel (porosity no. 2) and re-extracted with 15 mL of ethanol/hexane. Ethanol (15 mL) and hexane (15 mL) were successively used to wash the residue. Organic phases were transferred to a separator funnel, and 25 mL of 10% sodium chloride was added followed by 20 mL of distilled water to wash the residue. The aqueous layer was removed. The hexanic phase was dried using anhydrous sodium sulfate and filtered before evaporation to dryness under vacuum at 40 °C. Pigment extracts were dissolved in 500 μL of dichloromethane and 500 μL of an 80:20 (v/v) mixture of MTBE and methanol. This solution was diluted 3.5-fold for the ditax pulp extract and 1.2-fold for the nectar extract in the MTBE/methanol mixture, and samples were placed in amber vials before HPLC analysis. Pigments were analyzed by reverse-phase HPLC using an Agilent 1100 system (Massy, France) according to the method previously used by Dhuique-Mayer et al.[18] Pigments were separated along a $C_{30}$ column YMC (250 × 4.6 mm i.d.; 5 μm; YMC Europe Gmbh, Germany). The mobile phases were $H_2O$ as eluent A, methanol as eluent B, and MTBE as eluent C. Flow rate was fixed at 1 mL $min^{-1}$, column temperature was set at 25 °C, and injection volume was 20 μL. A gradient program was carried out: the initial conditions were 40% A/60% B; 0−5 min, 20% A/80% B; 5−10 min, 4% A/81% B/15% C; 10−60 min, 4% A/11% B/85% C; 60−71 min, 100% B; 71−72 min, back to the initial condition for balance. Absorbance was followed at 290, 350, 400, 450, and 470 nm using an Agilent 1100 photodiode array detector. Chromatographic data and UV−visible spectra were collected, stored, and integrated using Agilent Chemstation plus software.

*Quantification of Pigments.* Concentrations of external standard solutions were determined in appropriate solvent, using absorbance for chlorophyll *a*, chlorophyll *b*,[22] pheophytin *a* [23] and molar extinction coefficient for pheophytin *b*. Results were calculated using linear regression analysis. The calibration curves for the "hydroxy" and "pyro" derivatives were assumed to be identical to their corresponding pheophytin calibration curves.[11] Calculation of the hydroxypheophytin and pyropheophytin concentrations must take into account the difference in molecular weight between pheophytin and hydroxypheophytin and pyropheophytin. The correction factors representing the molar ratios were 1.0182 and 1.0180 for hydroxypheophytins *a* and *b*, respectively; and 0.933 and 0.934 for pyropheophytins *a* and *b*, respectively. Equations

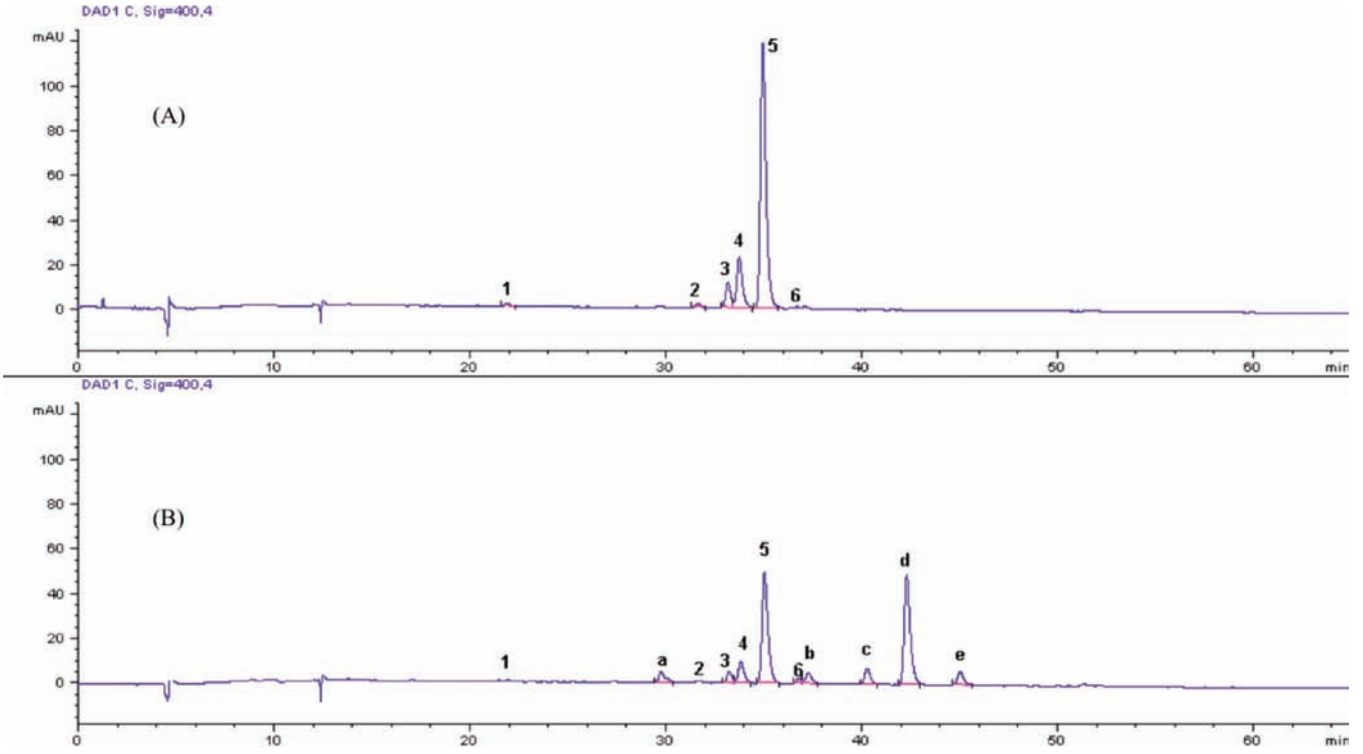


**Figure 2.** Chromatograms at 400 nm of ditax nectar before (A) and after thermal treatment for 2 h at 95 °C (B). Peaks: 1−6, peaks in native nectar; a−e, peaks in heated nectar; lutein, 1; unknown chlorophyll derivate, a; hydroxypheophytin *b*′, 2; pheophytin *b*, 3; hydroxypheophytin *a*′, 4; pheophytin *a*, 5; unknown chlorophyll derivate, *b*; pyropheophytin *b*, c; pyropheophytin *a*, d; unknown chlorophyll derivate, e.

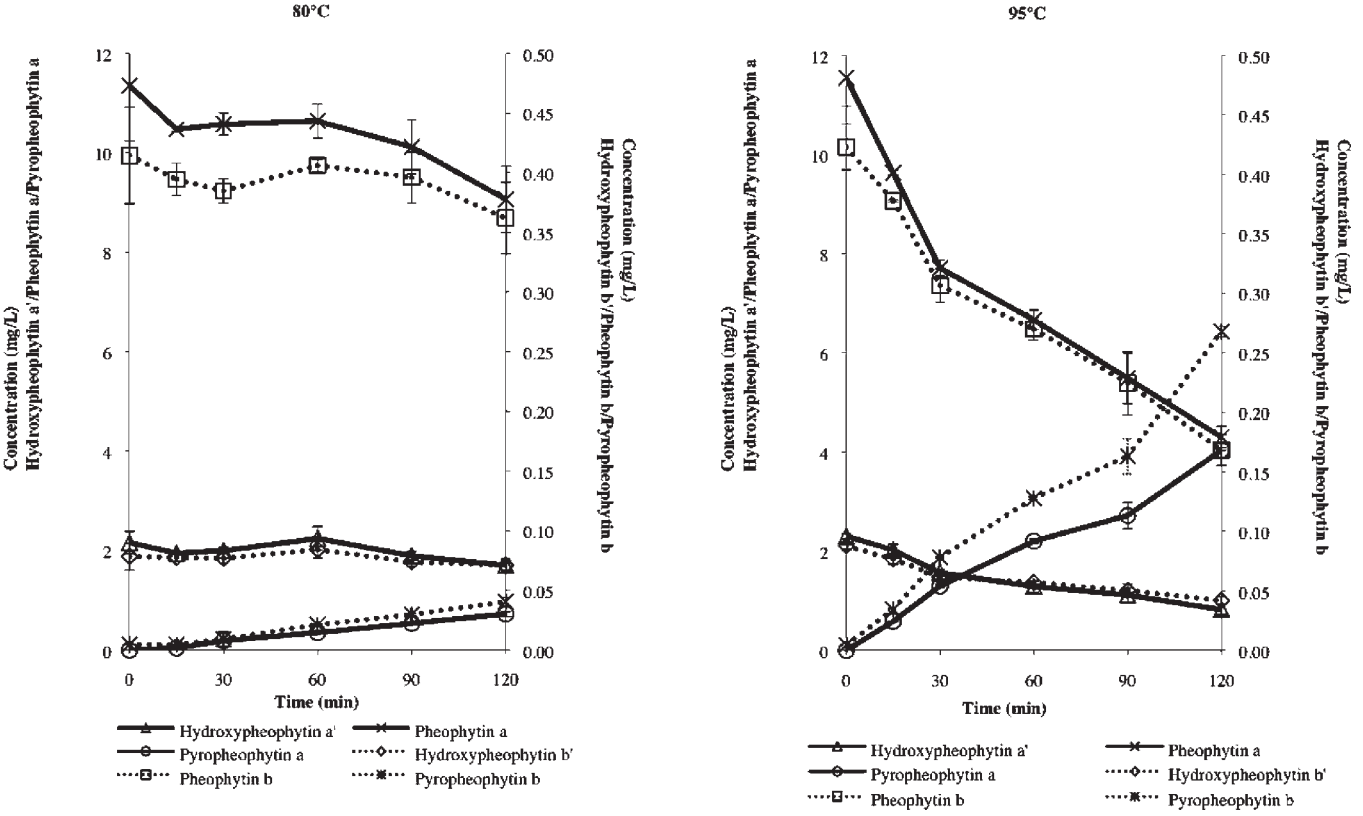


**Figure 3.** Evolution of concentration (mg/L) of main chlorophyll pigments identified in ditax nectar after thermal treatment for 120 min at 80 and 95 °C.


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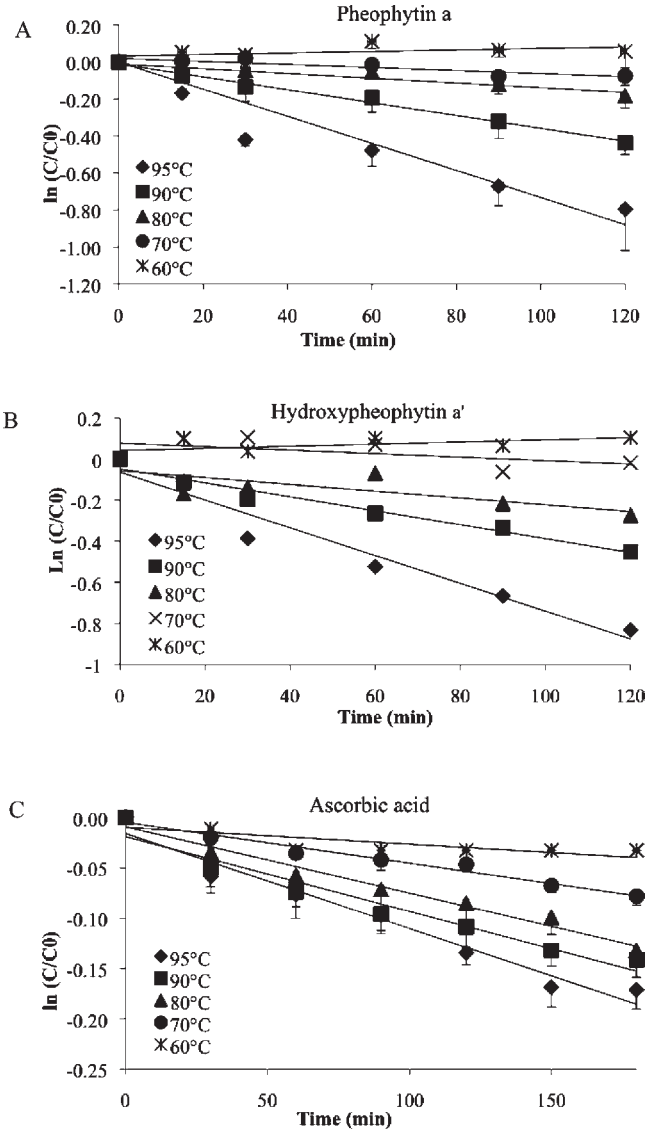


**Figure 4.** Thermal degradation kinetics of (A) pheophytin *a*, (B) hydroxypheophytin *a*′ (means of two treatments in duplicate 2 × 2*n*), and (C) ascorbic acid (means of three treatments in duplicate 3 × 2*n*).

can be used for concentration calculation of *a*′ and *b*′ chlorophyll derivatives. Quantification of carotenoids was achieved using calibration curves with $\beta$-carotene and lutein at five concentration levels.

**Heat Treatment.** The thermal degradation of ditax nectar was studied at 60, 70, 80, 90, and 95 °C for pigments and ascorbic acid. For pigments, the nectar (15 mL) was heated in sealed Pyrex tubes (100 mm length, 16 mm i.d., 2 mm thick) to ensure isothermal heating. Tubes were immersed in an oil bath with a temperature control (AM 3001K, Fisher Bioblock Scientific, Illkirch, France). A digital temperature probe (Heidolph EKT 3001 ± 1 °C) fitted to a sealed Pyrex tube was used to measure the nectar temperature during heating. For ascorbic acid, nectar (50 mL) was heated in amber flasks (60 mL), which were immersed in a water bath (Memmert). The time for nectar to reach the temperature set and the cooling time were under 4 min. Then the thermal transient could be negligible, and the thermal treatment was assumed to be isothermal. Tubes or flasks were taken out of the oil or water bath (for each time/temperature, two tubes were analyzed for pigments and three flasks for ascorbic acid) at different times (from 15 to 120 min for pigments and from 30 to 180 min for ascorbic acid) and immediately cooled in an ice bath. Each nectar was stored in amber sealed flasks and kept frozen (−20 °C) until analyzed. The dissolved oxygen content was measured with an oxygen meter (Multi 350i with a sensor for dissolved oxygen ConOx; WTW, Germany).

**Table 3. Isothermal Kinetic Parameters *k* and *D* versus Temperature for the Thermal Degradation of Pheophytin *a*, Hydroxypheophytin *a*′, and Ascorbic Acid in Ditax Nectar**

| compound[a] | $T$ (°C) | $k \times 10^{-6}$ ($s^{-1}$) | $D \times 10^{5}$ (s) |
|---|---|---|---|
| pheophytin *a* | 60 | 7 | 3.29 |
| | 70 | 10 | 2.30 |
| | 80 | 20 | 1.15 |
| | 90 | 60 | 0.38 |
| | 95 | 100 | 0.23 |
| hydroxypheophytin *a*′ | 60 | 9 | 2.56 |
| | 70 | 10 | 2.30 |
| | 80 | 30 | 0.77 |
| | 90 | 60 | 0.38 |
| | 95 | 100 | 0.23 |
| ascorbic acid | 60 | 3 | 7.68 |
| | 70 | 7 | 3.29 |
| | 80 | 10 | 2.30 |
| | 90 | 10 | 2.30 |
| | 95 | 20 | 1.15 |

[a] Pheophytin *a* and hydroxypheophytin *a*′ results are the means of two thermal treatments (2 × 2*n*). For ascorbic acid, experiments were conducted in triplicate (2 × 3*n*).

For nonisothermal treatment, the pasteurization values $F_{70\,°C}$ and the cooking values $F_{100\,°C}$ were calculated, using 70 °C as the reference temperature and a *z* factor for microorganisms of 10 °C, according to eq 1 and 100 °C as reference temperature and a calculated *z* factor for related compounds for $F_{100\,°C}$ according to eq 2.

$$F_{70°C} = \int_0^t 10^{(T-70)/10}\,dt \quad (1)$$

$$F_{100°C} = \int_0^t 10^{(T-100)/z}\,dt \quad (2)$$

**Kinetic Modeling of Degradation.** Assuming a first-order reaction for pigments and ascorbic acid degradation, that is, the reaction rate is proportional to the concentration under isothermal conditions, three models were chosen.[24] The first model is based on the classical approach used for chemical reactions, which defines a reaction rate constant ($k$) that depends on temperature according to an Arrhenius law (eq 3), with $T$ expressed in K, $k_\infty$ = pre-exponential factor = value of $k$ at $T_\infty$ ($s^{-1}$), $E_a$ = activation energy (J $mol^{-1}$), and $R$ = gas constant = 8.31 J $mol^{-1}$ $K^{-1}$.

$$k = k_\infty \exp\left(-\frac{E_a}{RT}\right) \quad (3)$$

The second model follows the theorical Eyring−Polanyi approach based on the transition state theory in which the enthalpy of activation ($\Delta H^*$) and the entropy of activation ($\Delta S^*$) are the model's parameters (eq 4), with $T$ expressed in K, $\Delta G^*$ = free activation enthalpy (J $mol^{-1}$), $\Delta H^*$ = activation enthalpy (J $mol^{-1}$), $\Delta S^*$ = activation entropy (J $mol^{-1}$ $K^{-1}$), $k_B/h$ the Boltzmann and Planck constants ratio = 2.084 × $10^{-11}$ ($K^{-1}$ $s^{-1}$), and $R$ = gas constant = 8.31 J $mol^{-1}$ $K^{-1}$.

$$k = \frac{k_b}{h} T \exp\left(-\frac{\Delta G^*}{RT}\right) = \frac{k_b}{h} T \exp\left(-\frac{\Delta H^* - T\Delta S^*}{RT}\right) \quad (4)$$

The third model called the Ball or Bigelow model generalizes the approach commonly used in food processing for microorganism destruction. It defines a decimal reduction time, which is related to temperature via a $z$ factor (eq 5), with $T$ expressed in °C, $D_0$ = value of $D$ at $T$ = 0 °C (s), and $z$ factor (°C).

$$D = D_0 10^{-T/z} \quad (5)$$

Pigments and ascorbic acid losses during a thermal treatment were calculated from the general expression of the pigments and ascorbic acid concentration versus time and temperature from the Eyring model (eq 6)

$$\frac{C}{C_0} = \exp\left(-\frac{k_B}{h}\exp\left(\frac{\Delta S^*}{R}\right)\int_0^t T\exp\left(\frac{-\Delta H^*}{RT}\right)\mathrm{d}t\right) \quad (6)$$

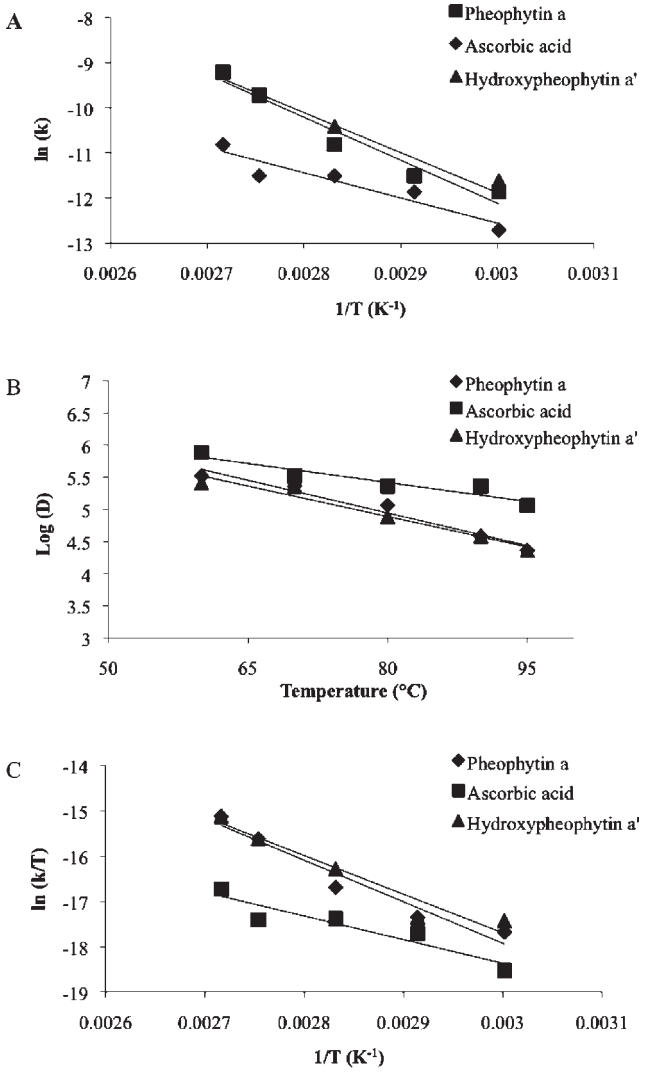


**Figure 5.** (A) Arrhenius plot for the temperature dependence of the rate constant $k$ for pheophytin $a$, hydroxypheophytin $a'$, and ascorbic acid. (B) Decimal logarithm of $D$ value versus temperature for pheophytin $a$, hydroxypheophytin $a'$, and ascorbic acid. (C) Eyring plot for pheophytin $a$, hydroxypheophytin $a'$, and ascorbic acid.

## RESULTS AND DISCUSSION

**Pigment Identification and Quantification.** This paper presents the first qualitative−quantitative investigation of the pigment composition in ditax green pulp. A typical chromatogram of pigment extract is shown in Figure 1A. Twelve peaks corresponding to eight chlorophyll and four carotenoid pigments were identified in ditax pulp (Table 1). Among chlorophyll pigments pheophytin $a$ (Figure 1B) was the major component of ditax fresh pulp (128 mg/kg; 59% total pigments) followed by hydroxypheophytin $a'$ (32.9 mg/kg; 15% total pigments), chlorophyll $b$ (23.6 mg/kg; 11% total pigments), and chlorophyll $a$ (13 mg/kg; 9% total pigments). Lutein and $\beta$-carotene were the main carotenoid pigments, but zeaxanthin and 9-*cis*-$\beta$-carotene were also present in trace amounts (Table 2).

The presence of chlorophyll isomers such as chlorophyll $a'$ and chlorophyll $b'$ in ditax pulp were probably formed during pulp extraction and storage. It is well documented that chlorophylls and their derivatives are susceptible to epimerization at C-13[2] during cooking or storage of green vegetables.[8,26,33] In addition, the presence of hydroxypheophytin $a'$ could be attributed to pheophytin oxidation. Differences in pigment contents between pulp 1 (fresh) and pulp 2 (stored) could be attributed to storage effect (Table 2). The main difference between the two pulps was the absence of chlorophyll $a$ and epimers of chlorophylls $a$ and $b$ in stored ditax pulp (pulp 2). Besides the lower amount of chlorophylls and carotenoids in stored ditax pulp, chlorophyll $b$ decreased drastically from 23.6 to 2.9 mg/kg. Theoretically, both chlorophyll $a$ and chlorophyll $b$ should be present in larger amounts in green plants, but in our study pheophytin $a$ and hydroxypheophytin $a'$ were substantially higher than the other chlorophyll pigments, which may be accounted for by partial conversion or degradation of chlorophylls in ditax during all steps from harvest to transport of fruit and pulp extraction in laboratory. Pheophytin $a$ is also the major component of olive oil (9.12 mg/kg,[20] 19.36−25.04 mg/kg[27]) but in lower quantity than in ditax. The natural acidity of ditax pulp (pH 4.06) may contribute to the high amount of pheophytin. In kiwi fruit (Hayward genotype), only chlorophyll $a$ (11.2 mg/kg) and chlorophyll $b$ (0.53 mg/kg) were found.[30] Several studies have also demonstrated the presence of hydroxyl-containing derivatives in *Gynostemma pentaphyllum* (traditional Chinese herb), mandarin fruit, broccoli, olive, and banana, with the amount increased following a rise in maturity.[20,26,34−36] Furthermore, the anomalous ratio between chlorophyll $a$ and chlorophyll $b$ could

**Table 4. Kinetics Parameters for Thermal Degradation of Pheophytin $a$, Hydroxypheophytin $a'$, and Ascorbic Acid following Different Models**

| | Arrhenius | | | Eyring | | | Ball | | |
|---|---|---|---|---|---|---|---|---|---|
| | $k_\infty$ ($s^{-1}$) | $E_a$ (kJ $mol^{-1}$) | $R^2$ | $\Delta H^*$ (kJ $mol^{-1}$) | $\Delta S^*$ (J $mol^{-1}$ $K^{-1}$) | $R^2$ | $D_0 \times 10^7$ (s) | $z$ (°C) | $R^2$ |
| pheophytin $a$ | $1.43 \times 10^7$ | 79.19 | 0.95 | 76.28 | −117.56 | 0.95 | 4.53 | 29 | 0.96 |
| hydroxypheophytin $a'$ | $2.62 \times 10^6$ | 73.86 | 0.94 | 70.95 | −131.65 | 0.94 | 2.61 | 32 | 0.95 |
| ascorbic acid | $6.63 \times 10^1$ | 46.41 | 0.89 | 43.5 | −219.6 | 0.88 | 0.98 | 51 | 0.96 |

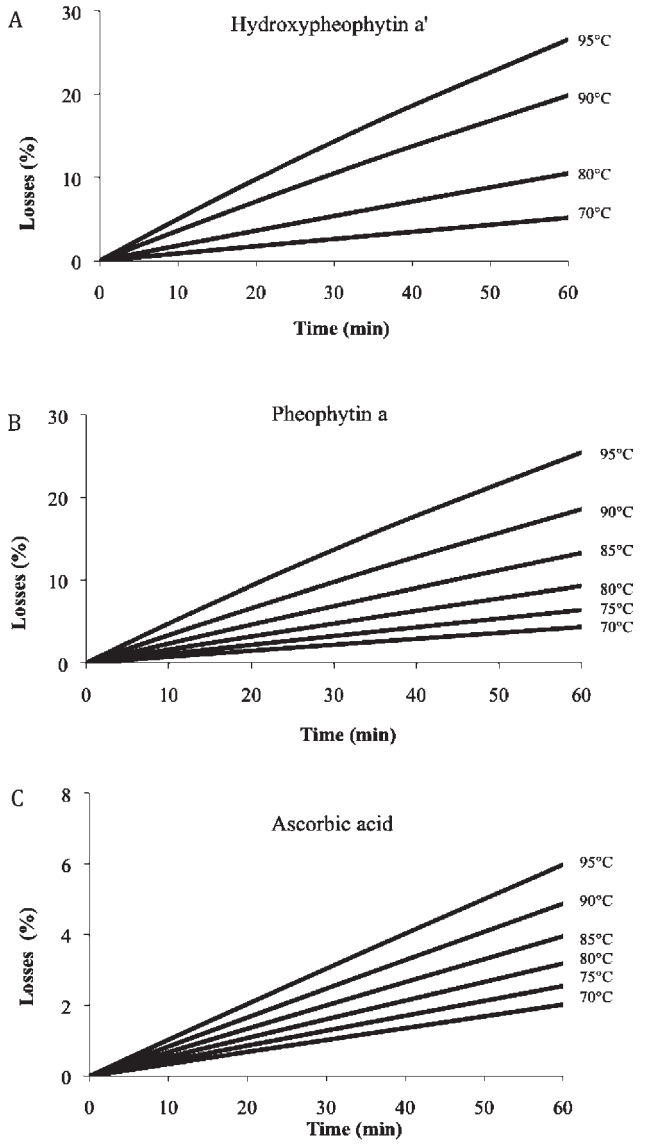


**Figure 6.** Examples of estimated (A) pheophytin *a*, (B) hydroxypheophytin *a*′, and (C) ascorbic acid losses at different temperatures using the Eyring model.

be explained by the alteration rate of chlorophyll *a*, which is faster than chlorophyll *b* degradation (4−10 times in spinach puree during storage).[37]

**Thermal Degradation Kinetics in Ditax Nectar.** *Attempt To Identify and Quantify Degradation Products from Heated Ditax Nectar.* HPLC chromatograms of a ditax nectar pigment extract at initial time (A) and after 2 h of heating at 95 °C are illustrated in Figure 2. As in ditax pulp, pheophytin *a* (peak 5) was the main chlorophyll present in native ditax nectar. After thermal treatment, even if five peaks of chlorophyll derivatives were detected (a−e), the main change was the decrease of pheophytin *a* (peak 5) and the appearance of pyropheophytin *a* (peak d). Compared to ditax pulp, the absence of chlorophylls *a*, *a*′, *b*, and *b*′ in ditax nectar could be explained by the alteration of these compounds during nectar processing (dilution, straining, stirring). Pyropheophytin *a* (peak d) was not detected at 60 °C and appeared after 60 min of heating at 70 °C, 30 min at 80 °C, and 15 min at 90 and 95 °C. The content of these degradation products increased

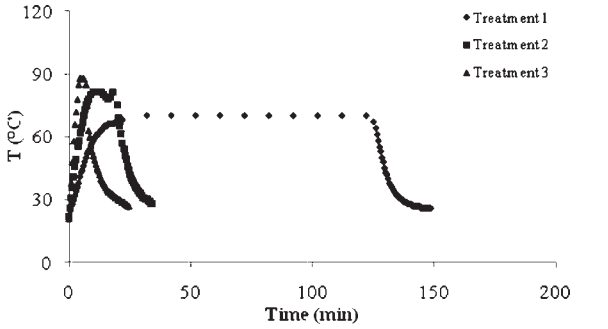


**Figure 7.** Nonisothermal treatments of ditax nectar: treatment 1, 70 °C/100 min, $F_{70\ °C}$ = 105.1 min; treatment 2, 80 °C/10 min, $F_{70\ °C}$ = 126.9 min; treatment 3, 85 °C/3 min, $F_{70\ °C}$ = 187.4 min.

gradually from the initial time of their appearance until the final phase of heat treatment (Figure 3). Identification of pyropheophytin *a* was supported by the diminution of pheophytin *a* in heated nectar, which was converted into pyropheophytin *a*. Several studies have reported the formation of pyropheophytin from pheophytin during heat treatment of green fruits and vegetables by decarbomethoxylation of pheophytins at C-13[2,11,16,33,37,38].

Native nectar was found to contain 11.55 mg/L pheophytin *a* (80% of total chlorophyll pigments) and 2.3 mg/L hydroxypheophytin *a*′ (16% of total chlorophyll pigments). After 2 h of heating at 95 °C, pheophytin *a* represents only 42% of total chlorophyll pigments and hydroxypheophytin *a*′ only 12% of total chlorophyll pigments. Pheophytin *b* represents 2.9% of total chlorophyll pigments in native nectar and 2.6% in heated nectar (Table 2). As pheophytin *a* and hydroxypheophytin *a*′ were the major components of ditax nectar, we selected them to complete kinetic characterization of ditax nectar. The ascorbic acid degradation kinetic was also studied because of the richness of ditax nectar in this micronutrient.

*Pheophytin a and Hydroxypheophytin a*′ *Degradation.* As expected, between 60 and 95 °C, degradation kinetics during isothermal treatment of these chlorophyll derivatives fitted the first-order reaction. The logarithm of concentration against treatment time is presented in Figure 4A,B. The kinetic parameters *k* and *D* were calculated according to the Arrhenius and Ball models at each temperature (Table 3). Similar values were obtained for both pheophytin *a* and hydroxypheophytin *a*′. The degradation rate of pheophytin *a* increased noticeably with temperature, *k* values at 60 and 70 °C were smaller by factors of approximately 2−3, 6−9, and 10−14 than those at 80, 90, and 95 °C, respectively. The marked effect of temperature on the degradation of chlorophyll pigments has been demonstrated in virgin olive oil,[16] in pureed coriander leaves,[39] in broccoli juice,[40] in frozen green peas,[15] and in spinach puree,[38] where in general the reaction rates doubles or triples for each 10 °C increase in temperature. The Arrhenius and Eyring laws and the *z* factor match well the temperature dependence (Figure 5). The kinetic parameters corresponding to eqs 3−5 for the three Arrhenius, Eyring, and Ball models are presented in Table 4. The activation energies were 79 and 73 kJ mol$^{-1}$, respectively, for pheophytin *a* and hydroxypheophytin *a*′ and *z* factors were 29 °C for pheophytin *a* and 32 °C for hydroxypheophytin *a*. The enthalpy of activation ($\Delta H^*$) was 76.3 kJ mol$^{-1}$ for pheophytin *a* and 70.9 kJ mol$^{-1}$ for hydroxypheophytin *a*′, whereas the entropies of activation ($\Delta S^*$) were −117.6 and −131.6 (J mol$^{-1}$ K$^{-1}$).

**Table 5. Experimental and Calculated Losses of Pheophytin *a*, Hydroxypheophytin *a′*, and Ascorbic during Different Nonisothermal Treatments of Ditax Nectar**

| | | | pheophytin *a* | | | hydroxypheophytin *a′* | | | ascorbic acid | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $T$ (°C) | $t$ (min) | $F_{70\ °C}$ (min) | $F_{100\ °C}$ (min) $z$ = 29 °C | exptl | calcd | $F_{100\ °C}$ (min) $z$= 32 °C | exptl | calcd | $F_{100\ °C}$ (min) $z$= 51 °C | exptl | calcd |
| 70 | 100 | 105.1 | 10.5 | 8.9 | 8 | 13.3 | 0.3 | 9.6 | 31.2 | 9.7 | 4.3 |
| 80 | 10 | 126.9 | 3.1 | 3.4 | 2.4 | 3.7 | 13.7 | 2.8 | 7.3 | 7.1 | 1 |
| 85 | 3 | 187.4 | 1.7 | 1.3 | 1.3 | 1.97 | 5.2 | 1.5 | 3.8 | 6 | 0.5 |

A higher activation energy of pheophytin *a* as compared to hydroxypheophytin *a′* implies that a smaller temperature change is needed to degrade pheophytin *a* more rapidly. Nevertheless, the $E_a$ and $z$ values showed that the thermal sensitivities of the two chlorophyll derivatives were analogous. These results agreed with findings by Aparicio-Ruiz et al.[16] and Schwartz and Von Elbe,[11] who obtained for pheophytin *a* activation energies of 83.26 and 86.65 kJ mol$^{-1}$, respectively, in virgin olive oil and spinach puree. Weemaes et al.[40] found in broccoli juice an activation energy of 105.49 kJ mol$^{-1}$ for pheophytin *a*. For hydroxypheophytin *a*, Aparicio-Ruiz et al.[16] found an activation energy of 74.2 kJ mol$^{-1}$. In fact, there is no data about $z$ factors for pheophytin *a* and hydroxypheophytin *a*. The $z$ factor was calculated from bibliographic data according the following equation $z = \mathrm{Ln}\,(10)RT^2/E_a$. In virgin olive oil,[16] we estimated $z$ respectively at 30.3 and 34.02 °C for pheophytin *a* and hydroxypheophytin *a*; these data are close to our results. In broccoli[40] and spinach puree,[11] we found $z$ factors of, respectively, 24.58 and 33.44 °C for pheophytin *a*. Gupte et al.[41] found a $z$ value of 33.3 °C for chlorophyll *a* in spinach puree. Aparicio-Ruiz et al.[16] obtained an enthalpy of activation of 81 kJ mol$^{-1}$ for pheophytin *a* and of 76.3 kJ mol$^{-1}$ for hydroxypheophytin *a*, and entropies of activation of −119.5 and −150.2 J mol$^{-1}$ K$^{-1}$, respectively, for pheophytin *a* and hydroxypheophytin *a* in virgin olive oil. The enthalpy and entropy values for thermal degradation of chlorophyll have been reported in coriander (87.9 kJ mol$^{-1}$ and −0.5 J mol$^{-1}$ K$^{-1}$), mint (20.4 kJ mol$^{-1}$ and 0.7 J mol$^{-1}$ K$^{-1}$), and spinach puree (59.4 kJ mol$^{-1}$ and −138 J mol$^{-1}$ K$^{-1}$).[16,38,42]

*Ascorbic Acid Degradation.* As shown by numerous studies on fruit juices, the thermal degradation of ascorbic acid followed a first-order reaction (Figure 4C). Regression coefficients obtained on logarithm curves were around 0.94. The $k$ and $D$ values are presented in Table 3. The degradation rates were very low for all of the temperatures tested. Ascorbic acid in ditax nectar was not sensitive, as expected. Degradation rates in ditax nectar were less than those found by Dhuique-Mayer et al.[28] in citrus juice ($k$ range from $1.042 \times 10^{-5}$ to $6.319 \times 10^{-5}$ s$^{-1}$ and $D$ value range between $3.644 \times 10^4$ and $22.094 \times 10^4$ s). As for pheophytin and hydroxypheophytin, the effect of temperature on the reaction rate can be accurately represented using the three models (Figure 5). The $E_a$ and $z$ factor were 46.4 kJ mol$^{-1}$ and 51 °C, respectively. These results fell within the ranges usually reported in the literature for ascorbic acid degradation in various fruit juices, especially in citrus juices at similar temperature between 20 and 100 °C: 21−53 kJ mol$^{-1}$ for $E_a$ and 36−118 °C for $z$ factor.[27,43−45] As compared to pheophytin and hydroxypheophytin, ascorbic acid was less sensitive to thermal treatment in ditax nectar regarding thermodynamic parameters. $\Delta H^*$ was 43.5 kJ mol$^{-1}$, whereas $\Delta S^*$ was −219.6 J mol$^{-1}$ K$^{-1}$.

As mentioned by García-Torres et al.,[17] ascorbic acid is one of the most important food components in juice that is affected by dissolved oxygen. The concentration of dissolved oxygen in ditax nectar decreased after all treatments from 2.5 to 2.1 to 1.3−0.7 mg/L whatever the heat treatment between 60 and 95 °C and 15−120 min. Even if this decrease was not marked, it could reveal slow oxygen consumption via oxidative reactions. Initial dissolved oxygen in ditax nectar is nearer than in citrus juice[28] and lower than in single-strength orange juice (6.5 mg L$^{-1}$ in normal plant operation conditions).[17] However, the final concentration of dissolved oxygen after 120 min of treatment is higher than in orange juice packaged in PET bottles after 180 days of storage (0.4 mg L$^{-1}$) as found by Ros-Chumillas et al.[46]

*Model Validation and Predictions.* The Eyring model was used to compare experimental losses in pheophytin *a*, hydroxypheophytin *a′*, and ascorbic acid with calculated values during isothermal treatments. Assessed losses were close to the experimental values in all cases. Model accuracy was verified, and the approach followed for this study was validated in that case. Figure 6 shows pheophytin *a*, hydroxypheophytin *a′*, and ascorbic acid losses for ditax nectar estimated by the Eyring model for isothermal processing.

*Effect of Classical Pasteurization on Pheophytin a, Hydroxypheophytin a′, and Ascorbic Acid Degradation in Ditax Nectar.* As a final conclusion, the accuracy of the Eyring model was verified for nonisothermal treatments, simulating real heating processes. Hence, three treatments with different time and temperature profiles were tested (Figure 7). Treatment 1 (100 min at 70 °C) simulated a low-temperature pasteurization; treatment 2 (10 min at 80 °C) simulated a standard pasteurization, and treatment 3 (3 min at 85 °C) simulated pasteurization at a higher temperature. Different $F_{70\ °C}$ and $F_{100\ °C}$ values were calculated, and experimental losses were analyzed and compared with predicted values (Table 5). Under nonisothermal treatments, the accuracy of the Eyring model was validated only for pheophytin *a* (estimated losses were close to experimental losses). For ascorbic acid, estimated losses were under experimental losses for the three treatments. For hydroxypheophytin *a′*, estimated losses were higher than experimental losses for treatments 1 and 2. Differences may be provided during extraction and dosage of pigments and ascorbic acid.

For pheophytin *a* and hydroxypheophytin *a′* calculated losses were less than 3% at 80 and 85 °C and 1% for ascorbic acid even if the $F_{100\ °C}$ values for the three coumpounds were less than treatment time at each treatment. Therefore, pasteurization does not significantly damage these components.

Our study presents the pigment composition from ditax pulp and thermal degradation kinetics of the main pigments and ascorbic acid from ditax nectar. Results show that pheophytin *a* was the main chlorophyll pigment responsible for the green color

of the pulp. Thermal processing of ditax nectar induced the formation of degradation products such as pyropheophytin *a* and pyropheophytin *b*. Pheophytin *a* was the most heat sensitive; nevertheless, classical pasteurization does not significantly affect the three compounds studied.

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