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Thermal Degradation of Antioxidant Micronutrients in *Citrus* Juice: Kinetics and Newly Formed Compounds

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The thermal degradation kinetics of vitamin C, two carotenoids (β-carotene and β-cryptoxanthin), and hesperidin, as a function of temperature, were determined for *Citrus* juice [*Citrus sinensis* (L.) Osbeck and *Citrus clementina* Hort. ex Tan]. The influence of dissolved oxygen on the rate of ascorbic acid degradation was also assessed. Analysis of kinetic data suggested a first-order reaction for the degradation of vitamin C and carotenoids. The kinetics parameters D_θ, z, and E_a have been calculated. Following the Arrhenius relationship, the activation energy of ascorbic acid was 35.9 kJ mol^{-1} and agreed with the range of literature reported value. The results on vitamin C and carotenoids from citrus juice made it possible to validate the predicting model. Thermal degradation of carotenoids revealed differences in stability among the main provitamin A carotenoids and between these and other carotenoids belonging to the xanthophyll family. The activation energies for the two provitamin A carotenoids were 110 and 156 kJ mol^{-1} for β-carotene and β-cryptoxanthin, respectively. On the other hand, no degradation of hesperidin was observed during thermal treatment. Finally, the vitamin C in citrus juice was not as heat sensitive as expected and the main provitamin A carotenoids present in citrus juice displayed a relative heat stability. The high-performance liquid chromatography−diode array detection−mass spectrometry analysis of degradation products showed that the isomerization of the epoxide function in position 5,6 into a furanoxide function in position 5,8 was a common reaction for several xanthophylls. These findings will help determine optimal processing conditions for minimizing the degradation of important quality factors such as vitamin C and carotenoid in citrus juice.

KEYWORDS: ***Citrus*** **juice; vitamin C; β-carotene; β-cryptoxanthin; HPLC-DAD-MS; thermal degradation kinetics**

INTRODUCTION

The consumption of citrus juices, especially orange juice, was reported to be beneficial for the prevention of degenerative diseases (*1*−*3*). Health benefits of citrus could be attributed to the richness in various antioxidants, vitamin C, polyphenols, and carotenoids, which are able to counteract oxidative stress, a key step in the development of degenerative diseases (*4*). Moreover, citrus juices represent a dietary source of provitamin A carotenoid with β-cryptoxanthin and β-carotene (*5*). However, processing, especially thermal treatments, could cause undesirable reactions such as nonenzymatic browning and nutrient losses (*6*−*8*). Vitamin C (L-ascorbic acid) is a typical heat sensitive micronutrient, and its degradation plays a major role in nonenzymatic browning reactions (*9*). Because HMF is one of the decomposition compounds of ascorbic acid degradation, it is used to evaluate the severity of heating applied to fruit juice during processing (*10*). That is, the oxidation of carotenoids generates losses of color and provitamin A levels as well as the development of off-flavors (*11*). In a food system, the mechanisms involved in the nutrient degradation are complex. The various interactions between the different solutes in citrus juices could modulate nutrient degradation. Likewise, degradation rates depend on environmental conditions such as pH, dissolved oxygen content, metal catalysis, and UV exposure (*12*, *13*).

To predict nutrient damage during thermal treatments, the knowledge of the kinetics behavior of these compounds,

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including the reaction rate as a function of temperature, is required. The degradation kinetics of vitamin C have been extensively studied for food storage at low temperatures and in model systems (*7*, *12*, *14*−*16*). Few data are available for thermal degradation of ascorbic acid in real situations like fruit juices at temperatures as high as 50 °C (*17*−*19*); it seems to depend on the content of dissolved oxygen (*7*).

Recently, the degradation kinetics of total carotenoids were studied by measuring the absorption at 450 nm (*20*). Other studies focused on the degradation of carotenoids in dehydrated or model food systems (*21*−*26*). Kinetic parameters were strongly modulated by the nature of the pigments, the experimental conditions used, and the product's moisture content. In aqueous media, the stability of carotenoids is increased and follows a first-order reaction kinetics, whereas in anhydrous media, decoloring of carotenoids seems to follow zero-order kinetics (*23*, *24*). Nevertheless, the effects of thermal treatment on carotenoids pigments in fruit juices remain unclear because of the lack of data available in the literature, particularly on thermal processing, which induces isomerization and degradation products.

The objective of our study was to understand the impact of thermal treatment on micronutrients and to improve the knowledge of their thermal resistance. To better characterize the decrease of nutritional quality of a citrus juice undergoing conventional thermal treatments such as pasteurization or concentration, kinetic studies were carried out. The influence of temperature (between 50 and 100 °C) on the degradation kinetics of the main antioxidant micronutrient (carotenoids, vitamin C, and hesperidin) of citrus juice was studied to predict the impact of thermal processing on these antioxidants. Experimental data were treated using kinetic models. In addition, several degradation products of carotenoids from heated citrus juice were identified.

MATERIALS AND METHODS

Citrus Juices. Four citrus fruits from the Agronomic Research Station selections (San Guiliano, Corsica, France) were used as follows: oranges [*Citrus sinensis* (L.) Osbeck] Valencia SRA 246, cv. Pera SRA 399, Sanguinelli SRA 243, and clementine SRA 85 (*Citrus clementina* Hort.ex Tan) in Tanaka classification. All fruits were harvested between December 2002 and April 2003. Fruits were received at maturity stage (total soluble solids/titrable acidity = 7 for orange and 14 for clementine) and were immediately hand-squeezed, then filtered through a stainless steel sieve (1 mm), and after placed in amber sealed vials (125 mL) under nitrogen and kept frozen (−20 °C) until analyzed. For our study, a juice was chosen for its high contents in provitamin A carotenoids and other xanthophylls. The mix ratio was 1/1/1/1 (v/v/v/v) for Valencia, Pera, Sanguinelli, and Clementine, respectively. Two lots were prepared as follows: Juice 1 was used for studying the degradation kinetics of carotenoids at 55 °C, and juice 2 was used for all of the other trials (**Table 1**). The dissolved oxygen content was 2.5 mg L^{-1} ± 0.03 for all of the treated juices (oxygen meter CellOx325 multilineP3, WTW, Germany).

High-Performance Liquid Chromatography (HPLC) Analysis of Carotenoids from Juice. Extraction, saponification, and analysis were carried out according to Dhuique-Mayer et al. (*5*). First, 10 g of citrus juice was extracted with ethanol/hexane 4/3 v/v. Lycopene was added as the internal standard. Saponification with 10% methanolic KOH was carried out overnight in the dark at room temperature (25 °C). Carotenoids were analyzed by reverse-phase HPLC using an Agilent 1100 system (Massy, France). Carotenoids were separated along a C_{30} column (250 mm × 4.6 mm i.d., 5 μm YMC) (EUROP GMBH, Germany), and the mobile phases were H_2O as eluent A, methanol as eluent B, and MTBE (methyl-*tert*-butyl-ether) as eluent C. The flow rate was fixed at 1 mL min^{-1}, the column temperature was set at 25 °C, and the injection volume was 20 μL. A gradient program was performed as follows: 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, and back to the initial conditions for reequilibration. Absorbance was then assessed at 290, 350, 450, and 470 nm using an Agilent 1100 photodiode array detector. Chromatographic data and UV−visible spectra were treated using the Agilent Chemstation Plus software. Quantification of carotenoids was achieved using calibration curves with β-carotene and β-cryptoxanthin with five concentration levels from 3 to 15 mg L^{-1} (standards from Extrasynthese, Genay, France).

Table 1. Characterization of Citrus Juices Used for Kinetic Experiments

	juice 1	juice 2
TSS (g kg^{-1})[a]	120 ± 2	116 ± 2
TA (g L^{-1})[b]	8 ± 0.1	9 ± 0.2
pH	3.7 ± 0.05	3.6 ± 0.06
	carotenoids (mg L^{-1})	
β-carotene	0.76 ± 0.04	0.75 ± 0.05
β-cryptoxanthin	4.70 ± 0.30	2.43 ± 0.20
zeinoxanthin	0.68 ± 0.05	0.55 ± 0.04
violaxanthin	1.80 ± 0.15	3.70 ± 0.25
zeaxanthin	0.71 ± 0.09	1.12 ± 0.10
lutein	0.76 ± 0.05	0.90 ± 0.09
phytofluene	1.40 ± 0.20	0.71 ± 0.09
phytoene	1.04 ± 0.09	0.57 ± 0.06
ascorbic acid (mg L^{-1})	413 ± 0.5	406 ± 0.6
hesperidin (mg L^{-1})	384 ± 1.2	460 ± 2.1

[a] TSS, titrable soluble solid. [b] TA, titrable acidity expressed as g L^{-1} of citric acid. Values are the means of three determinations ± standard deviations.

Determination of Vitamin C. Ascorbic acid was determined by HPLC (*5*). Orange juice (1 mL) was homogenized with 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 μm membrane and analyzed by HPLC in duplicate using an Agilent model 1100 system equipped with a RP 18e Licrospher 100 (5 μm) column (250 mm × 4.6 mm id) (Merck KgaA, Darmstadt, Germany). 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 245 nm. Quantification of ascorbic acid was carried out by external standard method (calibration curve between 20 and 100 mg L^{-1}).

Determination of Hesperidin. Hesperidin was determined by HPLC according to Dhuique-Mayer et al. (*5*). The HPLC system was Agilent 1100 model using a RP 18e Licrospher 100 (5 μm) column (250 mm × 4.6 mm id) (Merck KgaA). The isocratic solvent system was water/acetonitrile/THF/acetic acid (80:16:3:1;v/v/v/v). Quantification was carried out at 280 nm. The flow rate was fixed at 1 mL min^{-1}. Hesperidin concentrations were determined using an external calibration method. Standards HES was diluted in DMF/water (2:1, v/v) to give 102 mg L^{-1}.

Thermal Treatment. The thermal degradation of citrus juice was studied at temperatures of 75, 80, 90, 95, and 100 °C for carotenoids; 50, 70, 80, 90, and 100 °C for ascorbic acid; and 70 and 90 °C for hesperidin. Juice (15 mL) was heated in sealed pyrex tubes (100 mm length, 16 mm i.d.) to ensure isothermal heating. The 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 juice temperature during the thermal experiments. The time for juice to reach the temperature set up was below 4 min, and the cooling time was about 1 min. Then, the thermal transient could be negligible and the treatment could be considered isothermal. The tubes were taken out of the oil bath (for each time/temperature, two tubes were analyzed for carotenoids and three tubes were analyzed for ascorbic acid) at different times (from 15 to 300 min) and immediately cooled down in an iced water bath. Each juice was stored in amber sealed vials (20 mL) under nitrogen and kept frozen (−20 °C) until analyzed.

Degradation Kinetics Modeling. Assuming a first-order reaction for micronutrient degradation, that is, the reaction rate is proportional

Table 2. Degradation Kinetics of Carotenoids from Citrus Juice at 55 °C: Group I, Thermostable Compounds; Group II, Heat Sensitive Compounds

Group I (mg L^{-1})[a]					
time (min)	β-cryptoxanthin	β-carotene	phytoene	phytofluene	zeinoxanthin
0	4.76 a ± 0.26	0.76 a ± 0.02	1.04 a ± 0.03	1.4 a ± 0.04	0.6 a ± 0.03
15	4.75 a ± 0.25	0.74 a ± 0.02	0.93 a ± 0.02	1.23 a ± 0.02	0.57 a ± 0.02
60	4.75 a ± 0.26	0.71 a ± 0.03	0.92 a ± 0.03	1.22 a ± 0.02	0.53 a ± 0.03
120	4.67 a ± 0.23	0.65 a ± 0.02	0.81 ab ± 0.02	1.15 b ± 0.02	0.54 a ± 0.03
240	4.57 ± 0.24	0.68 a ± 0.03	0.79 b ± 0.02	1.12 b ± 0.03	0.55 a ± 0.02
300	4.54 a ± 0.22	0.70 a ± 0.03	0.77 b ± 0.03	1.16 b ± 0.03	0.58 a ± 0.03
Group II (mg L^{-1})[a]					
time (min)	violaxanthin	neoxanthin	zeaxanthin	lutein	
0	1.76 a ± 0.04	0.37 a ± 0.04	0.71 a ± 0.03	0.76 a ± 0.04	
15	0.74 b ± 0.03	0.16 b ± 0.03	0.36 b ± 0.02	0.42 b ± 0.03	
60	0.5 c ± 0.04	0.14 b ± 0.04	0.38 b ± 0.02	0.48 b ± 0.04	
120	0.22 d ± 0.03	ND	0.32 bc ± 0.04	0.43 b ± 0.03	
240	ND	ND	0.32 bc ± 0.04	0.46 b ± 0.03	
300	ND	ND	0.24 c ± 0.03	0.34 c ± 0.03	

[a] Values are the mean of three independent determinations ± standard deviation. Different letters in the same column indicate significant differences for carotenoid content ($\alpha = 0.05\%$). ND, not detected.

to the concentration under isothermal conditions, two models were chosen. The first model is based on the classic approach used for chemical reactions (eq 1), which defines a reaction rate constant (k) that depends on temperature according to an Arrhenius law (eq 2).

$$C = C_0 e^{-kt} \quad (1)$$

$$k = k_\infty e^{-(E_a/RT)} \quad (2)$$

with k_∞ as the pre-exponential factor (s^{-1}), E_a as the activation energy (J mol^{-1}), and R as the gas constant (8.32 J mol^{-1} K^{-1}).

The second model follows the approach commonly used in food processing (eq 3). It defines a decimal reduction time (eq 4), which is related to temperature via a z factor (eq 5).

$$C = C_0 10^{-(t/d)} \quad (3)$$

$$D = \mathrm{Ln}\left(\frac{10}{k}\right) \quad (4)$$

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

with D as the decimal reduction time at temperature T (s), D_0 as the D extrapolated value at 0 °C (s), and z expressed in °C.

The model's parameters were identified, using linear regressions on the logarithmic curves of experimental data. Even if the z value could be estimated from E_a using the relationship $z = \mathrm{Ln}(10)RT^2/E_a$ in a narrow range of temperature (using an average temperature), we have chosen to determine the z value graphically. The micronutrient losses during a thermal treatment were calculated from the general expressions of the micronutrient concentration vs time and temperature (eqs 6 and 7)

$$\text{model 1: } \mathrm{Ln}\left(\frac{C}{C_0}\right) = -k_\infty \int e^{-(Ea/RT)}\,\mathrm{d}t \quad (6)$$

$$\text{model 2: } \log_{10}\left(\frac{C}{C_0}\right) = -\frac{1}{D_0}\int 10^{(T/z)}\,\mathrm{d}t \quad (7)$$

HPLC-Diode Array Detection (DAD)-MS Analysis of Degradation Products from Heated Juice. Citrus juice was heated at 95 °C for 5 h for this experiment. HPLC analyses were carried out with a Hewlett-Packard (HP) model 1050 equipped with a quaternary pump solvent delivery and a DAD switched in line with a Micromass Platform LCZ 4000. The column used was a 250 mm × 4.6 mm i.d., 5 μm, YMC Pack C30 (YMC Inc., Wilmington, NC) equipped with a 20 mm × 4.6 mm, 5 μm precolumn. The column was kept at 27 °C. Absorption spectra were recorded between 220 and 600 nm. Acquisition of the mass data between m/z 100 and 700 was performed in the positive electrospray mode. The program used for data analyses was Masslynx version 3.4. Parameters, and especially cone voltage (15 V), were optimized to avoid fragmentation. The following gradient system was used with H_2O containing 25 mM NH_4OAc (solvent A), methanol containing 25 mM NH_4OAc (solvent B), and MTBE (solvent C): 0−2 min, %A−%B−%C, 40−60−0; 5 min, %A−%B−%C, 20−80−0; 10 min, %A−%B−%C, 4−81−15; 60 min, %A−%B−%C, 4−11−85; 80 min, %A−%B−%C, 4−11−85; 80.01 min, %A−%B−%C, 0−100−0. The flow was 1 mL min^{-1}.

RESULTS AND DISCUSSION

Carotenoids Degradation. To evaluate the degradation behavior of the main carotenoids from citrus juice, a treatment was first performed at 55 °C. Carotenoids could be classified in two groups in terms of their heat sensitivity. The first group (**Table 2**) including provitamin A carotenoids (β-carotene and β-cryptoxanthin), zeinoxanthin (isomer of β-cryptoxanthin), and two colorless precursors (phytoene and phytofluene) revealed a higher heat stability, with losses ranging from 1 to 18% after 15 min of treatment. The second group (**Table 2**) comprised highly oxygenated xanthophylls, which degraded faster, with losses between 30 and 60% after 15 min. Among these xanthophylls, zeaxanthin and lutein were the least damaged by heating. These results are in accordance with previous studies (*27*, *28*) and indicate smaller losses for provitamin A carotenoids than for the other xanthophylls during thermal pasteurization of orange juice. Similar observations were reported by Cortes et al. (*29*) showing that the thermal conservation treatment affects more xanthophylls than provitamin A carotenoids.

To complete kinetic characterization, we selected β-carotene, β-cryptoxanthin, and its isomer zeinoxanthin. The experimental procedure used in this work was not adapted for the second group of carotenoids because of their high rates of degradation at temperatures above 75 °C. As expected, between 75 and 100 °C, degradation kinetics during isothermal treatment fitted the first-order reaction. For the three selected carotenoids, the logarithm of concentration was proportional to treatment time ($0.88 <$ regression coefficient $R^2 < 0.98$). Examples of β-carotene and β-cryptoxanthin are presented in **Figure 1A**,**B**. Kinetic parameters, k and D, can be calculated according to the two models at each temperature (**Table 3**). Similar values were obtained for the three carotenoids. In the temperature range

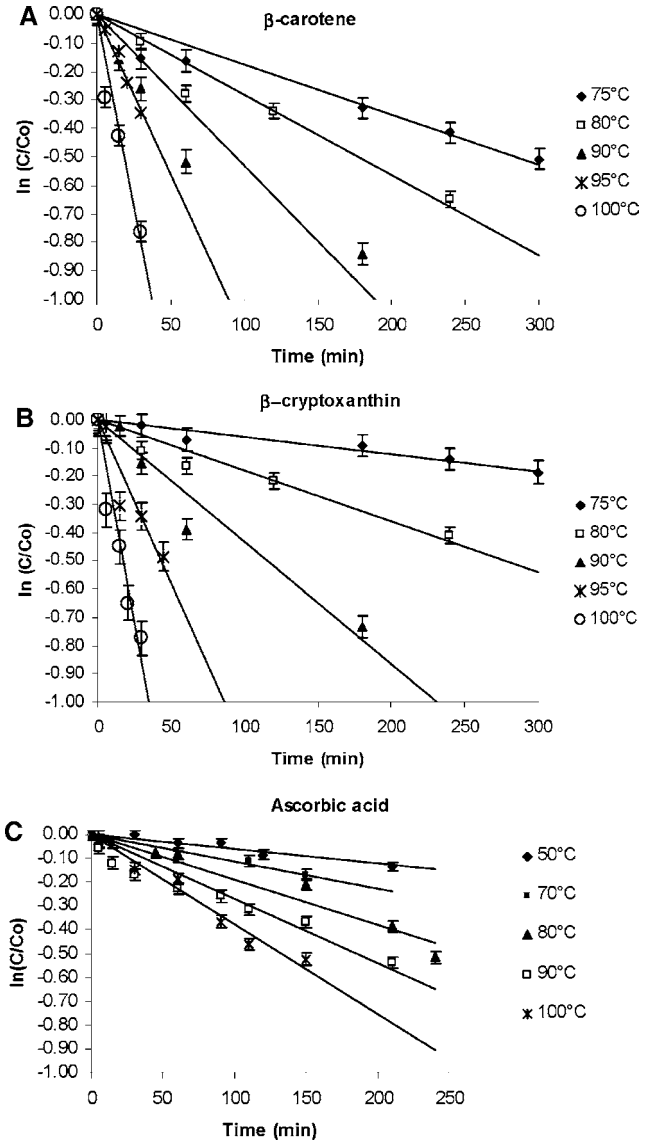

Figure 1. Thermal degradation kinetics of nutrients vs temperature in citrus juice. (**A**) β-Carotene, (**B**) β-cryptoxanthin (mean of two thermal treatments conducted in duplicate $2 \times 2n$), and (**C**) ascorbic acid (mean of tree thermal treatments conducted in triplicate $3 \times 3n$).

studied, the degradation rates were low for citrus juice. During conventional thermal processing of fruit juice, the main provitamin A carotenoids were not significantly affected. These results agree with those previously reported (*27*−*31*), where losses of β-carotene or β-cryptoxanthin were very low during pasteurization or thermal concentration of different citrus juices.

The Arrhenius law and the *z* factor fit well the temperature dependence of *k* and *D*, respectively ($0.92 <$ regression coefficient $R^2 < 0.98$) (**Figure 2**). The activation energies ranged between 110 and 156 kJ mol^{-1}, and the *z* factor ranged between 16 and 23 °C (**Table 4**). A higher activation energy of β-cryptoxanthin as compared to β-carotene implies that a smaller temperature change is needed to degrade β-cryptoxanthin more rapidly. For cryptoxanthin isomers, the β form was more affected by a temperature increase than zeinoxanthin. Nevertheless, the E_a and *z* values showed that thermal sensitivity of the three carotenoids was analogous. These results agree with findings by Hallström et al. (*32*), who obtained 140 kJ mol^{-1} and 19 °C for carotenoids in paprika. However, Rios and al. (*22*) reported a E_a value of 154 kJ mol^{-1} for the thermal degradation of bixin in an aqueous model system under

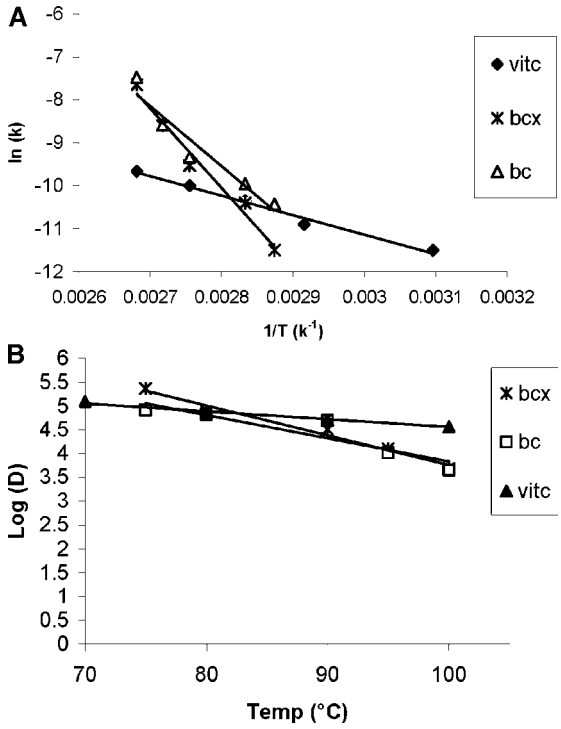

Figure 2. (**A**) Arrhenius plot for the temperature dependence of the rate constant *k* for ascorbic acid (vitC), kβ-carotene (bc), and kβ-cryptoxanthin (bcx) associated with the first order and (**B**) decimal logarithm of *D* value vs temperature for β-cryptoxanthin (bcx), β-carotene (bc), and ascorbic acid (vitC).

Table 3. Isothermal Kinetic Parameters *k* and *D* vs Temperature for the Thermal Degradation of the Nutrients in Citrus Juice

	T (°C)	$k \times 10^{-5}$ (s^{-1})	$D \times 10^4$ (s)
β-carotene	75	2.951	7.802
	80	4.705	4.894
	90	8.837	2.606
	95	18.717	1.230
	100	44.507	0.517
β-cryptoxanthin	75	1.011	22.773
	80	3.007	7.658
	90	7.210	3.194
	95	19.365	1.189
	100	47.950	0.480
Zeinoxanthin[a]	75	2.055	11.203
	80	3.870	5.950
	90	6.491	3.548
	95	20.388	1.129
	100	40.492	0.569
Ascorbic acid[b]	50	1.042 ± 0.02	22.094 ± 0.33
	70	1.924 ± 0.04	11.968 ± 0.28
	80	3.140 ± 0.05	7.334 ± 0.20
	90	4.043 ± 0.08	5.696 ± 0.18
	100	6.319 ± 0.08	3.644 ± 0.15

[a] Structural cryptoxanthin isomer. [b] Kinetic experiments were conducted in triplicate ($3 \times 3n$). For carotenoids, results are the means of two thermal treatments ($2 \times 2n$).

temperatures ranging from 70 to 125 °C. Other studies using color as an indicator for carotenoids concentration reported lower E_a values (around 20 kJ mol^{-1}) as compared to our data (*19*, *20*). This difference may have been a result of the product matrix but also of the indirect measurement of carotenoids, which does not permit ready discrimination between carotenoids and other pigments.

Ascorbic Acid Degradation. As shown by numerous studies on citrus juices (*17*−*19*, *34*, *35*), the thermal degradation of

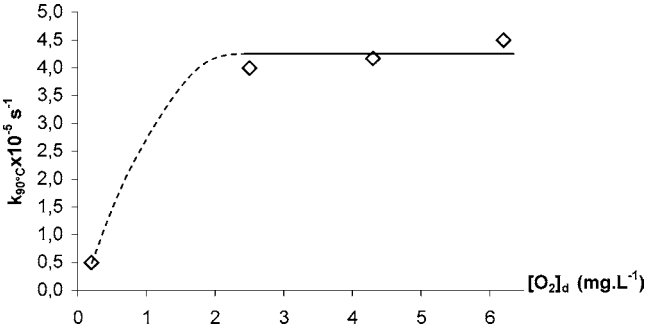

Figure 3. Reaction rate constant of ascorbic acid degradation at 90 °C vs dissolved oxygen concentration in citrus juice.

Table 4. Values of the Parameters of the Models Used to Represent the Degradation Kinetics of the Nutrients in Citrus Juice

	T range (°C)	Ln k_∞ (k_∞ s^{-1})	E_a (kJ mol^{-1})	log D_0 (D_0 s)	*z* (°C)
β-carotene	75–100	27.415	110.0	8.267	22.5
β-cryptoxanthin	75–100	42.410	156.0	10.028	15.9
zeinoxanthin[a]	75–100	31.519	122.8	8.737	20.2
ascorbic acid[b]	50–100	1.81 ± 0.04	35.9 ± 0.5	6.14 ± 0.2	64.0 ± 0.6

[a] Structural cryptoxanthin isomer. [b] Kinetic experiments were conducted in triplicate (3 × 3*n*). For carotenoids, results are the means of two thermal treatments (2 × 2*n*).

ascorbic acid followed a first-order reaction (**Figure 1C**). Regression coefficients obtained on logarithmic curves were above 0.925. The *k* and *D* values are presented in **Table 3**. Evaluated in triplicate, the reproducibility of the procedure was good with a standard deviation around 2%. For all of the temperatures tested, the degradation rates were surprisingly very low. At these temperatures, we noticed that the ascorbic acid in the citrus juice studied was not as sensitive as expected. As for carotenoids, the effect of temperature on reaction rate can be accurately represented using the models (**Figure 2**). The E_a and *z* factor were 36 kJ mol^{-1} and 64 °C. These results fall within the ranges usually reported in the literature for ascorbic acid degradation in various citrus juices at similar temperatures (20−96 °C): 21−53 kJ mol^{-1} for E_a and 36−118 °C for *z* (*19*, *34*, *35*). Factors that could contribute to the wide distribution of these kinetic parameters include of course intrinsic characteristics of the product such as variety and maturity, pH, and probably dissolved oxygen level.

The concentration of dissolved oxygen in citrus juice decreased after all thermal treatments, dropping from 2.5 to 1.6 mg L^{-1}. This decrease reveals oxygen consumption via oxidative reactions. Because of the highly reducing property of ascorbic acid, the degradation reaction in citrus juice may depend on oxygen level (*36*, *19*). Alwazeer et al. (*37*) demonstrated that gassing a juice with N_2 after thermal treatment increased the ascorbic acid stability. To evaluate the hypothesis, degradation kinetics were studied at 90 °C, varying the initial dissolved O_2 concentration in the juice (by N_2 stripping or aerating the standard juice at 2.5 mg L^{-1}). The reaction rate was strongly affected by O_2 concentration (**Figure 3**). Degradation of ascorbic acid is around 10 times slower at 0.2 mg L^{-1} of dissolved oxygen than at 2.5 mg L^{-1}. Above 2.5 mg L^{-1}, the reaction rate *k* reached a limit. These results prove that the thermal degradation of ascorbic acid in the juice goes through an oxidative mechanism.

Hesperidin Degradation. For this compound, no significant decrease was noticed during thermal treatments (losses <2 % at 90 °C after 240 min). In the temperature range studied, the thermal stability of this phenolic compound appeared very high. These results were in agreement with those reported by Sanchez-Moreno et al. (*38*), showing that pasteurization did not modify hesperetin content. This behavior is completely different from anthocyanins that are strongly damaged by heating: 69% losses reported after 120 min at 90 °C in blood orange juice (*39*). The difference between the chemical structures of these molecules belonging to the subclasses of flavonoids was based on the c-ring and particularly with the presence of positive charge.

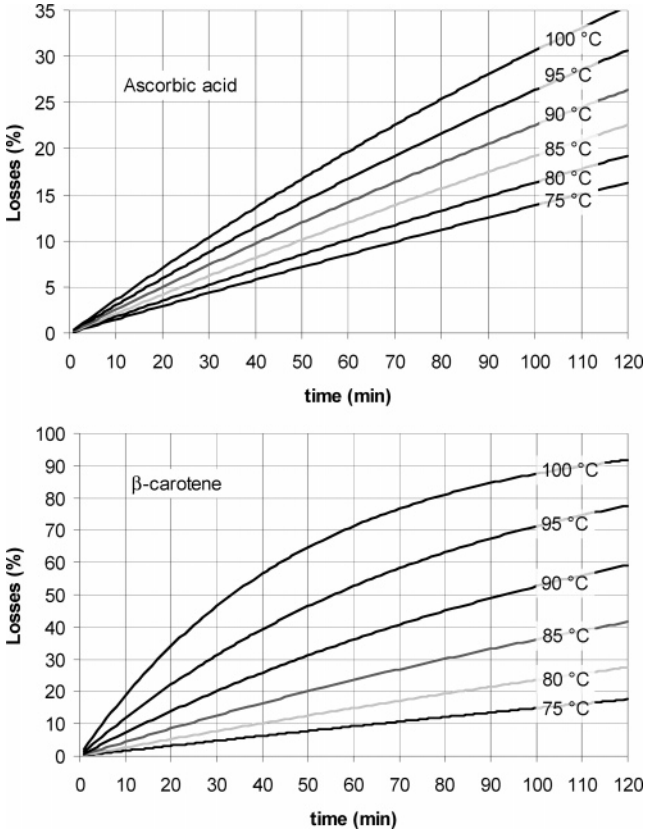

Figure 4. Estimated losses for β-carotene and ascorbic acid during isothermal treatments at different temperatures (*D*/*z* model).

Table 5. Comparison between Experimental and Calculated Losses of Nutrients during Isothermal Treatments

		losses (%)		
nutrient	heat treatment *T* (°C)/*t* (min)	experimental[a]	classical model	*D*/*z* model
β-carotene	85/120	36.5 ± 2	42.0	41.3
β-cryptoxanthin	85/120	35.0 ± 3	29.6	28.7
ascorbic acid	75/ 30	4.1 ± 0.07	4.5	4.4
	90/90	21.4 ±0.7	20.6	20.5

[a] Experimental data are the means of three determinations ± standard deviations.

Model Validation and Predictions. To check the ability of the models to foresee results, new kinetics were measured during isothermal treatments not tested for identifying parameters. Experimental losses were then compared with calculated values (**Table 5**). In all of the cases, we noticed that both models gave similar results. Estimations were close to the experimental values especially for ascorbic acid. Model accuracy was then verified, and the approach followed for this study was validated for isothermal treatments. To conclude definitively, the models accuracy should also be evaluated for no-isothermal treatments that are closer to real heating processes. The developed models

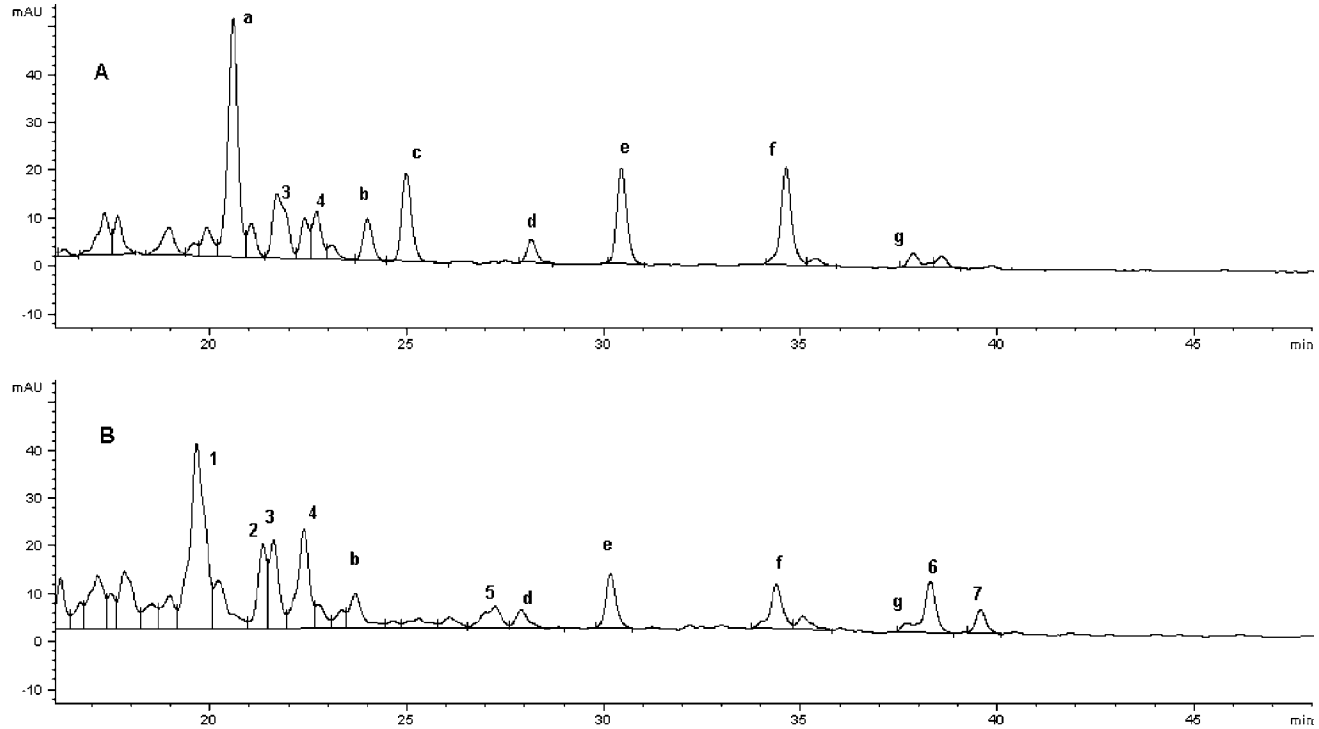

Figure 5. Chromatograms at 400 nm of citrus juice before (**A**) and after thermal treatment for 5 h at 95 °C (**B**). Key: 1–7, peaks in heated juice; a–g, peaks in native juice.

Table 6. Chromatographic and Spectral Data Obtained by HPLC-DAD-MS Detection of the Carotenoids in Heated Juice and in Native Juice

retention time (min)	carotenoid[a]	*m*/*z*	spectral maxima (nm)	oxygen atoms (*n*)	%III/II	juice native	(±)[b] heated
19.65	auroxanthin (*cis* or *trans*)	602	380, 402, 426	4	>100	–	+ (1)
20.61	violaxanthin (*cis*)	602	328, 416, 436, 464	4	87	+ (a)	–
21.33	auroxanthin (*cis* or *trans*)	602	380, 402, 426	4	>100	–	+ (2)
21.60	mutatoxanthin (*cis* or *trans*)	585	408, 428, 452	3	50	–	+ (3)
22.38	mutatoxanthin (*cis*)	585	408, 428, 452	3	66	+	+ (4)
24.01	zeaxanthin	569	430, 450, 476	2	19	+ (b)	+
25.06	antheraxanthin (*cis*)	585	421, 442, 468	3	53	+ (c)	–
27.23	zeinoxanthin (*cis*)	–	338, 424, 444, 468	1	–	–	+ (5)
28.18	zeinoxanthin	553	422, 446, 474	1	43	+ (d)	+
30.47	β-cryptoxanthin	553	427, 450, 477	1	20	+ (e)	+
34.65	ζ-carotene (*cis*)	541	296, 78, 400, 424	–	70	+ (f)	+
37.87	β-carotene		452, 478	–	12	+ (g)	+
38.28	ζ-carotene (*cis* or *trans*)	545	380, 402, 426	–	>100	–	+ (6)
39.55	ζ-carotene (*cis* or *trans*)	545	380, 402, 426	–	>100	–	+ (7)

[a] Tentative identification. [b] +, presence in juice; –, absence in juice. Key: 1–7, peaks in heated juice; a–g, peaks in native juice.

could be easily used as tools to predict losses during whatever heat treatment, for example, isothermal (**Figure 4**). Calculations confirmed that classical pasteurization treatments do not significantly damage the nutrients studied. Losses were below 2% for 5 min of pasteurization at 85 °C.

Tentative Identification of Degradation Products from Heated Citrus Juice. The spectral, chromatographic, and mass data obtained by HPLC-DAD-MS detection are summarized in **Table 6** and illustrated by **Figure 5**. Peak 1 was tentatively identified as auroxanthin *Z* (*cis*) or *E* (*trans*). A molecular ion at *m*/*z* 602 was observed, which is consistent with the formula $C_{40}H_{56}O_4$. The UV−vis spectra showed absorption maxima at 380, 402, and 426 nm, which are characteristic of a chromophore containing seven conjugated carbon−carbon double bonds. Spectral data were in agreement with the presence of two furanoxide functions in 5,8- and 5′8′-positions This identification is supported by the disapperance of violaxanthin in heated juice, which could have been converted into auroxanthin through the isomerization of its epoxide functions in 5,6- and 5′6′-positions into furanoxide functions, respectively, in 5,8- and 5′8′-positions. These results are in agreement with those reported by Philip et al. (*40*) who suggested that acidic conditions and heating promoted furanoid formation through epoxide isomerization. Moreover, auroxanthin was identified in previous studies particularly in processed citrus juice (*41*, *42*). Note that auroxanthin was already present after 15 min at 95 °C, but after 5 h, the content was three times less (data not shown). Peak 2 displays identical spectral data than those of peak 1 but with a lower intensity, suggesting that it could be an isomer of auroxanthin, either *Z* or *E*. Two other peaks 3 and 4 with similar spectral characteristics were attributed to mutatoxanthin isomers. These peaks were already present in native juice but at a lower level. These compounds could derive from *Z*-antheraxanthin recently identified in citrus juices (*43*) coming from the isomerization of the 5,6-epoxide group into the 5,8-furanoxide group as previously described for auroxanthin. This agrees with the disappearance of *Z*-antheraxanthin in heated juice. Peak b identified as zeaxanthin in native juice was recovered in heated

juice. UV−vis spectra corresponding to peak 5 showed a marked "*cis* peak" at 338 nm and was tentatively identified as *Z*-zeinoxanthin. Zeinoxanthin was recently identified (*44*, *45*) as an isomer of β-cryptoxanthin in orange juice. The authors suggest that α-cryptoxanthin and zeinoxanthin were often confused in the literature, with zeinoxanthin designed as α-cryptoxanthin. Like Shlatterer and Breithaupt (*45*), we did not observe the characteristic fragment of α-cryptoxanthin at *m*/*z* 535 but only the molecular ion at *m*/*z* 553. Peaks 6 and 7 from heated juice were identified as isomers of ζ-carotene (*Z* or *E*) with *m*/*z* 545 and a UV−vis spectra characteristic of a conjugated system containing seven carbon−carbon double bonds. In native juice, ζ-carotene was identified with a "*cis* peak" at 296 nm (peak f). It was also present in heated juice together with other new isomers (peaks 6 and 7). Under our conditions, thermal processing seemed to increase ζ-carotene isomerization in citrus juice.

Our study evaluated the impact of thermal factor on tree types of antioxidant micronutrients—vitamin C, carotenoids, and polyphenol (hesperidin)—in a real product (citrus juice). We conclude that thermal treatments in reference to time/temperature of classical pasteurization conditions do not damage provitamin A carotenoids, polyphenol as hesperidin, and, more surprisingly, vitamin C in citrus juice. However, the model developed in our work could predict the optimal processing conditions that minimize the degradation of vitamin C and carotenoid in citrus juice. Furthermore, xanthophyll carotenoids were shown to be more heat sensitive than carotene and more likely to generate degradation products. Thermal processing of citrus juice induces the formation of carotenoid degradation products such as furanoids and *cis* isomers. Violaxanthin was the most heat sensitive, and its complete conversion to auroxanthin results in a visually colorless juice. Further experiments are therefore needed to predict degradation kinetics of xanthophylls at temperatures higher than 75 °C. Although some authors reported the lowest contents of vitamin C and carotenoid in thermally processed citrus (*46*), the storage conditions and not the thermal factor could be a cause of micronutrient losses in fruit juice processing, and further studies must be conducted on the role of other factors such as oxygen and light on the juice's oxidative degradation.

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