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Degradation kinetic modelling of ascorbic acid and colour intensity in pasteurised blood orange juice during storage

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ABSTRACT

The stability of ascorbic acid and colour intensity in pasteurised blood orange juice (*Citrus sinensis* [L.] Osbeck) during one month of storage was investigated at 4–37 °C. The effects of ascorbic acid fortification (at 100, 200 mg L^{-1}) and deaeration, temperature/time storage on the kinetic behaviour were determined. Ascorbic acid was monitored by HPLC–DAD and colour intensity by spectrophotometric measurements. Degradation kinetics were best fitted by first-order reaction models for both ascorbic acid and colour intensity. Three models (Arrhenius, Eyring and Ball) were used to assess the temperature-dependent degradation. Following the Arrhenius model, activation energies were ranged from 51 to 135 kJ mol^{-1} for ascorbic acid and from 49 to 99 kJ mol^{-1} for colour intensity. The effect of storage temperature and deaeration are the most influent factors on kinetics degradation, while the fortification revealed no significant effect on ascorbic acid content and colour intensity.

1. Introduction

Blood oranges, 'Tarocco', 'Sanguinello' and 'Moro', are the commonly cultivated varieties of *Citrus sinensis* (L.) Osbeck in the Mediterranean area. Moro variety is the most colourful of all blood orange varieties (Destani, Cassano, Fazio, Vincken, & Gabriele, 2013; Maccarone, Maccarrone, Perrini, & Rapisarda, 1983; Maccarone, Maccarrone, & Rapisarda, 1985). The red colour (or burgundy colour) of blood orange is primarily associated with anthocyanin pigment (Choi, Kim, & Lee, 2002). Indeed, the characteristic orange colour of carotenoids is masked or partially masked by the water-soluble anthocyanin pigments (Lee, Carter, Barros, Dezman, & Castle, 1990). Another peculiar characteristic of blood oranges is the high concentration of vitamin C, flavanones and hydroxycinnamic acids (Rapisarda, Carollo, Fallico, Tomaselli, & Maccarone, 1998; Rapisarda & Intelisano, 1996). The red colour of the fruit is an important factor affecting consumer appeal and marketability of both fruit and juices (Titta et al., 2009).

In Algeria, blood oranges are consumed mainly as fresh fruit. In addition, many other blood orange products such as juice and juice concentrate are widely consumed. However, obtaining a strong, stable and fresh like colour of fruits and juices, is problematic during processing and storage (Rein, 2005; Yu, Lin, Zhan, He, & Zhu, 2013). Considerable losses were detected in the content of anthocyanins, ascorbic acid (AA) and total phenolics after heat processing (Mazur et al., 2013; Patras, Brunton, Tiwari, & Butler, 2011; Wicklund et al., 2005). Conversely in some cases, heat pasteurisation is reported to have minimal effect on anthocyanin stability including those in blood orange juice (Cisse, Vaillant, Acosta, Dhuique-Mayer, & Dornier, 2009; Kırca & Cemeroğlu, 2003; Torres et al., 2011). In general, storage at high temperatures for a long period of time has negative influence on ascorbic acid (AA) content and anthocyanin pigments (Mazur et al., 2013). Nevertheless, industrialists have to guarantee a minimum food quality loss during this step. Under those circumstances, the storage duration and temperature combinations are important variables to be controlled (Ioannou, Hafsa, Hamdi, Charbonnel, & Ghoul, 2012).

For this, degradation kinetic modelling is an important step to control and predict physico-chemical parameter changes during processing and storage. Furthermore, the empirical approach of the kinetic modelling based on the concept of reaction order (leading to the zero-, half-, first- or second-order reaction modelling) is quite suitable for shelf life modelling (Nicolai & Baerdemaeker, 1998). Cisse, Vaillant, Kane, Ndiaye, and Dornier (2012) have

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improved the ability of Arrhenius, Ball and Eyring model to describe the degradation kinetics of anthocyanin and colour properties of Roselle during storage. For statistical reasons, it is better to estimate kinetic parameters by nonlinear regression method, because such transformation of nonlinear equations to linear forms implicitly alters their error structure and may also violate the error variance and normality assumptions of standard least squares (Chowdhury & Das Saha, 2011).

Few data are available in the literature on the degradation kinetic parameters of colour intensity (CI_{ANC}) and AA content from blood orange juice during storage. Therefore, the aim of this study was to determine the kinetic parameters of both colour intensity (CI_{ANC}) and AA degradation during storage from pasteurised blood orange juice at various temperatures, by using three kinetic models (Arrhenius, Ball and Eyring), at differents ascorbic acid fortification (AA-fortification) and degassing conditions. This approach will give a knowledge about the storage stability of AA content and colour intensity (CI_{ANC}) to provide the best conditions for developing a local blood orange juice.

2. Materials and methods

2.1. Preparation of blood orange juice

Moro blood oranges (*Citrus sinensis* [L.] Osbeck) were harvested at optimal maturity, which is defined by a ratio of soluble solids to titratable acidity of 10, and by burgundy colour of oranges. The harvest from SAOUDI agricultural field (Bejaia, North-east of Algeria) involved a random sampling from 20 trees. Oranges were washed with tap water, cut in half and pressed, using a domestic juicer (Cobra CB-R15-O, Algiers, Algeria). The fresh juice was filtered through a double layer cheesecloth to remove pulp and seeds. The freshly pressed juice was packed in HDPE (High Density Polyethylene) plastic bottles of 1 L capacity and frozen at −20 °C.

Three random bottles of the freshly pressed juice were analysed immediately for moisture content, total soluble solids, total titratable acidity and pH using standard methods (AOAC, 1998). The dissolved oxygen content was measured with an Oxi 730 oxygen electrode, equipped with Cell 325 probe and an Oxicall-SL air calibration beaker (WTW Wissensschaftlich Technishe, Weinheim, Germany). A direct absorbance (A) measurement of the freshly pressed juice at 420, 520, and 620 nm was carried out using UV–visible spectrophotometer (Shimadzu UV-1605 UV–Visible Spectrophotometer, Tokyo, Japan); and the following spectrophotometric attributes were calculated; juice colour intensity (JCI) as the sum of A_{420nm}, A_{520nm} and A_{620nm}; tint as the ratio of A_{420nm} to A_{520nm}; colour proportion of blue (Bl%), yellow (Ye%) and red (Rd%) were calculated by dividing A_{420nm}, A_{520nm} and A_{620nm}, to the juice colour intensity (JCI), respectively (Glories, 1984; Kelebek, Canbas, & Selli, 2008). The total anthocyanins content was assessed by the pH differential method as described by Lee, Durst, and Wrolstad (2005).

2.2. Stability during storage

For the storage test, frozen juice sample was thawed to room temperature. After homogenisation, four juices were prepared from the squeezed thawed juice, i.e. non-fortified control juice (NFCJ), ascorbic acid fortified juice with 100 mg L^{-1} (AAFJ-100), ascorbic acid fortified juice with 200 mg L^{-1} (AAFJ-200) and non-fortified deaerated juice (NFDJ) obtained by bubbling argon for 30 min until the dissolved oxygen concentration was less than 1 mg L^{-1}. All juice samples were equally divided (15 mL) into 30 mL glass bottles (to maintain an equal head space of 15 ml) and pasteurised (80 °C/2 min) in a thermostatic oil bath before storage. Samples were rapidly cooled in ice water bath and were stored immediately after processing (time 0) at four different isothermal conditions (4, 20, 30 and 37 °C) in temperature-controlled storage locker (in the dark) for one month; except for NFDJ that was stored at two different temperatures (20 and 30 °C). Samples analysis were carried out for colour intensity (CI_{ANC}) and ascorbic acid content (AA) at different interval of times.

2.3. Determination of ascorbic acid (AA) content

AA content was monitored by the HPLC analytical procedure as outlined by Mertz (2009). Blood orange juice samples of 1 mL were filtered through 0.45 μm membrane filter. Then, 10 μl of the filtered samples were injected onto a DIONEX ULTIMATE 3000 HPLC equipped with diodes array detector (DAD), a quaternary pump and an autosampler. The separation was performed at 30 °C using an ACE 5 C18 column (250 mm × 4.6 mm, 5 microns) (AIT FRANCE, COAL, France). The isocratic solvent system was a 0.01% (pH = 2.6) solution of sulfuric acid, the flow rate was maintained at 0.5 mL min^{-1} for an injection time of 10 min. Eluted AA was monitored by UV detection at 245 nm. Chromatograms were recorded and processed with Chromeleon software. For quantification, a standard curve was generated with the standard solution of AA, within the working range of the samples (six points from 25 to 600 mg L^{-1} with a regression equation $y = 0.878x + 11.929$, $R^2 = 0.998$, a percentage recovery values of 95–104%, a limit of detection (LOD) = 14.58 mg L^{-1} and limit of quantitation (LOQ) = 48.60 mg L^{-1}), and results were expressed as mg of L-ascorbic acid per litre of blood orange juice.

2.4. Measurement of colour intensity (CI_{ANC})

The parameters traditionally used to describe the colour variation of the anthocyanin solutions have mainly been the changes in λ_{max} in the visible part of the spectrum as a measurement of variations in hue, together with changes in absorbance for the variation in colour intensity (CI_{ANC}) (Glories, 1984). UV/Vis absorption spectra (250–600 nm) of blood orange juices were recorded using UV–visible spectrophotometer (Shimadzu UV-1605 UV–Visible Spectrophotometer, Tokyo, Japan). The freshly made juice samples (from the different storage conditions) were filtered through 0.45 μm membrane filter and diluted to 25% (v/v) with warm Milli-Q water. The change in the maximum absorbance (A_{max}) at carrying wavelengths (λ_{max}) presented the change in the colour intensity (CI_{ANC}) related to anthocyanin pigments (Rein, 2005). In our work, wavelength (λ_{max}) was fixed as 515 nm.

2.5. Degradation kinetics modelling during storage

Traditionally, nutrients degradation in foods during their thermal processing and storage has been described in terms of zero, half, first or higher order kinetics (Corradini & Peleg, 2006). The degradation reaction order during storage of both CI_{ANC} and AA from different blood orange juices was predicted using the general rate law in (Eq. (1)).

$$\frac{dX_t}{dt} = -k_X(X_t)^n \quad (1)$$

where k_X is the reaction rate constant ($days^{-1}$), n is the reaction order, X_t could be AA concentration (mg AA/L of juice) or maximum absorbance of CI_{ANC} (A_{515nm}) at any given time (t), and t is the reaction time (days).

The reaction order was determined through linear regression by graphical analysis, where exponent n in Eq. (1) was set to 0, 0.5, 1, and 2 to compare the adjusted coefficients of determination (R^2_{adj}) and root mean square error (RMSE) amongst zero-, half-,

first-, and second-order reactions, respectively (Nayak, Berrios, Powers, & Tang, 2011). The integrated forms of zero-, half-, first-, and second-order models are given in Eqs. (2)–(5).

$$\text{Zero-order}: X_t = X_0 - k_X t \tag{2}$$

$$\text{Half-order}: 2\sqrt{X_t} - \sqrt{X_0} = k_X t \tag{3}$$

$$\text{First-order}: \ln\frac{X_t}{X_0} = -k_X t \tag{4}$$

$$\text{Second-order}: \frac{1}{X_t} - \frac{1}{X_0} = k_X t \tag{5}$$

Using the experimental CI_{ANC} and AA data, the R^2_{adj} and RMSE were observed to be minimum for $n = 1$, predicting a first order reaction.

For statistical reasons and to better estimate the first order constant rates (Chowdhury & Das Saha, 2011), the experimental CI_{ANC} and AA data were further fitted through nonlinear regression with Eq. (6) and the half-life time ($t_{1/2}$) was calculated from Eq. (7).

$$\frac{X_t}{X_0} = \exp(-k_X t) \tag{6}$$

$$t_{1/2} = \frac{\ln 2}{k_X} \tag{7}$$

The rate constant k_X (days^{-1}) varied with the system's absolute temperature, T (K), according to the Arrhenius law from Eq. (8),

$$\ln k_X = \ln k_{X\,\text{ref}} - \left[\frac{E_{a\,Xi}}{R}\left(\frac{1}{T} - \frac{1}{T_{\text{ref}\,i}}\right)\right] \tag{8}$$

where $k_{X\,\text{ref}}$ is the rate constant (days^{-1}) at reference temperature; $E_{a\,Xi}$ the apparent activation energy (kJ mol^{-1}) for the rate constant of the compound X (AA concentration or maximum absorbance of CI_{ANC}) during i storage temperature range; R is the ideal gas constant (8.314 J mol^{-1} K^{-1}). The reference temperature of storage ($T_{\text{ref}\,i}$) was chosen from the middle of the i studied temperature range, as shown in Eq. (9)

$$T_{\text{ref}\,i} = \frac{1}{n}\sum_{i=1}^{n} T_i \tag{9}$$

Furthermore, enthalpy (ΔH^*_{Xi}) and entropy (ΔS^*_{Xi}) of activation at each studied temperature were obtained from the Eyring–Polany model (i.e., Eq. (10)) based on transition state theory.

$$\ln\frac{k_X}{T} = \frac{-\Delta H^*_{Xi}}{R}\cdot\frac{1}{T} + \ln\frac{k_B}{h} + \frac{\Delta S^*_{Xi}}{R} \tag{10}$$

where k_B is the Boltzmann constant (1.381×10^{-23} J K^{-1}); h is the Planck constant (6.626×10^{-34} J s); T is the absolute storage temperature (K); R is the ideal gas constant (8.314 J mol^{-1} K^{-1}).

The Ball or Bigelow method (i.e., Eqs. (11) and (12)); widely applied in food processing for microorganism destruction; was used to estimate the decimal reduction time (D value), which is related to temperature via the z value.

$$D_X = \frac{\ln 10}{k_X} \tag{11}$$

$$\text{Log}_{10}\left(\frac{D_X}{D_{X\,\text{ref}}}\right) = \frac{-(T - T_{\text{ref}\,i})}{z_{Xi}} \tag{12}$$

where D_X (days) is the storage time required to reduce the compound X by 90%; $D_{X\,\text{ref}}$ is the D_X value at reference storage temperature ($T_{\text{ref}\,i}$) and z_{Xi} (°C) is the storage temperature span necessary for a tenfold decrease in the D_X value. T and $T_{\text{ref}\,i}$ were expressed in °C.

2.6. Statistical analysis and model evaluation

The model's parameters were estimated by least square method through linear regression (for Arrhenius, Ball and Eyring model and reaction order) or non-linear regression for the first order reaction rate constant (using the Levenberg–Marquardt iterative algorithm) using Curve Fitting Toolbox, Matlab ver. 8.0 (The MathWorks Inc., USA, 2012). The goodness of model fitting to the experimental data was evaluated by adjusted coefficients of determination (R^2_{adj}); confidence intervals (*Conf.I*) and root mean square error (RMSE, Eq. (13)). The highest the R^2_{adj} values and the lowest the RMSE values, the better the fitting of the model to the experimental data.

$$\text{RMSE} = \sqrt{\frac{1}{(n-p)}\sum_{i=1}^{n}(X_{t,\text{Mod}} - X_{t,\text{Exp}})^2} \tag{13}$$

where, $X_{t,\text{Mod}}$ is the model predicted value, $X_{t,\text{Exp}}$ is the experimental value, n is the number of the data, and p is the number of parameters.

The analysis of variance (*ANOVA*) was performed using XLSTAT Release 10 (Addinsoft, Paris, France). Tukey's multiple range test (HSD) was used to compare means of the estimated kinetic parameters. Evaluations were based on the $p < 0.05$ significance level.

3. Results and discussion

3.1. Characteristics of the fresh squeezed blood orange juice

The initial physicochemical properties of the fresh blood orange juice are presented in Table 1. Generally, results are consistent with those reported in literature for Moro orange juice (Kelebek et al., 2008), except for tint, anthocyanins, and ascorbic acid that showed a slightly lower concentration. These changes can be attributed to growing, climatic and varietal conditions of the Moro blood orange from Bejaia state.

3.2. Ascorbic acid (AA) degradation during storage

Fig. 1A shows the AA content change in different blood orange juice during storage at 4, 20, 30 and 37 °C.

3.2.1. Reaction order estimation of AA degradation

The order of reaction was estimated graphically by comparing the adjusted coefficients of determination (R^2_{adj}) and root mean square error (RMSE) obtained from plots of AA concentration change as a function of storage time at all temperatures for each

Table 1
Main characteristics of the fresh squeezed blood orange (Moro) juice.

Analysis	Squeezed juice
Juice yield (%)	30.1 ± 3.0
pH	3.29 ± 0.08
Total soluble solids (°Brix)	13.0 ± 0.1
Titratable acidity[a] (g L^{-1})	13.0 ± 0.2
Dissolved oxygen (mg L^{-1})	6.3 ± 0.9
Moisture content (%)	87.3 ± 0.3
Colour intensity	3.74 ± 0.20
Tint	0.94 ± 0.01
% Yellow	41.2 ± 2.1
% Red	20.4 ± 4.1
% Blue	38.7 ± 2.0
Anthocyanin[b] (mg L^{-1})	129.5 ± 7.9
Ascorbic acid (mg L^{-1})	426.0 ± 5.9

Values are the means of three determinations ± standard deviation.
[a] As citric acid.
[b] As cyanidin-3-glucoside.

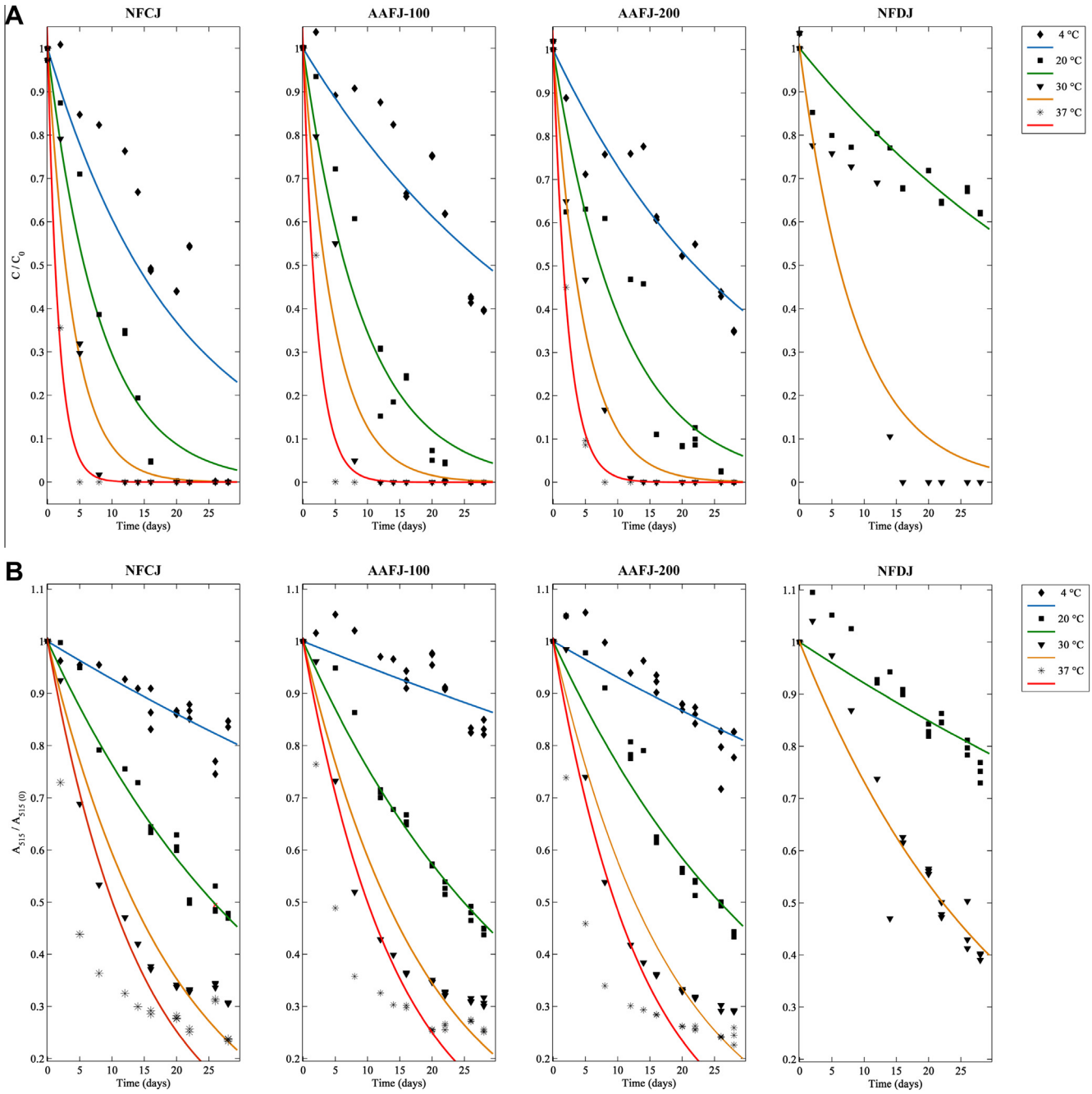

Fig. 1. Degradation kinetics of ascorbic acid (A), colour intensity (B) from different blood orange juices: control (NFCJ), AA-fortified (AAFJ-100 and AAFJ-200) and deaerated (NFDJ); during storage at temperature 4, 20, 30 and 37 °C.

type of juice (Table 2). On the basis of the R^2_{adj} and RMSE range values of the non-linear regression (of each types of juice at different temperatures), the degradation of AA fits better with the first-order model (with R^2_{adj} range values of 0.660–0.907 and RMSE range values of 0.30–0.91), conversely, these coefficients were much lower with second-order model (with R^2_{adj} range values of 0.515–0.662 and RMSE range values of 0.01–0.10), which confirmed that AA degradation in the different studied blood orange juices could commonly be fitted by first-order reaction model (Table 2). On the contrary, zero- and half-order kinetic model showed a poor performance for fitting the curve for all type of juice stored under different temperatures. Moreover, the first-order model was used in most studies to describe the AA degradation in fruit juices during storage (Odriozola-Serrano, Soliva-Fortuny, & Martin-Belloso, 2009; Polydera, Stoforos, & Taoukis, 2003; Torres et al., 2011; Zheng & Lu, 2011).

3.2.2. Effect of storage time on AA degradation

As shown in Fig. 1A, the change of AA content decreased according to the time and temperature storage for all types of juice. The longer the time and the higher the storage temperature, the greater the degradation of AA. Approximately 20% of initial AA content remained after 28 days of storage at 4 °C (in refrigerated conditions) in AA-fortified or non-fortified juice. Whereas, complete degradation was observed for other storage temperatures. Non-fortified deaerated juice (NFDJ) retained more AA during

Table 2
Reaction order estimation of ascorbic acid (AA) degradation and colour intensity (CI_{ANC}) loss based on the adjusted coefficients of determination (R^2_{adj}) and the root mean square error (RMSE) from plots of zero-, half-, first and second-order reactions.

Juice type	Zero-order		Half-order		First-order		Second-order	
	AA	CI_{ANC}	AA	CI_{ANC}	AA	CI_{ANC}	AA	CI_{ANC}
	R^2_{adj}							
NFCJ	0.202	0.474	0.374	NC	0.719	0.698	0.515	0.663
AAFJ-100	0.174	0.570	NC	NC	0.806	0.715	0.566	0.802
AAFJ-200	0.409	0.597	NC	NC	0.907	0.739	0.662	0.829
NFDJ	0.717	0.782	NC	NC	0.660	0.852	0.615	0.705
	RMSE							
NFCJ	206.95	0.33	15.46	NC	0.91	0.14	0.10	0.28
AAFJ-100	227.49	0.17	NC	NC	0.63	0.13	0.07	0.23
AAFJ-200	282.60	0.09	NC	NC	0.41	0.14	0.06	0.23
NFDJ	116.42	0.06	NC	NC	0.30	0.07	0.00	0.18

NC: non-converged points.

storage (7% of AA remained after 28 days of storage at 20 °C). Therefore, AA retention was less improved when the blood orange juice was fortified with AA, but this retention is better when it is degassed even without AA-fortification.

The kinetic parameters k_{AA}, $t_{1/2}$, D_{AA} as well as the R^2_{adj}, RMSE of the first-order model through a least square fitting procedure of the AA degradation are given in Table 3. As can be seen, a good fit was obtained by nonlinear regression ($0.737 < R^2_{adj} < 0.998$ and $0.01 < RMSE < 0.17$), except for 4 °C (from AA-fortified or non-fortified juice) and 20 °C (from NFDJ). The degradation rate constants (k_{AA}) increased systematically with temperature for all types of the tested juices (Table 3). Under higher dissolved oxygen conditions (natural juice), the k_{AA} constants in non-fortified control juice (NFCJ) ranged from 49×10^{-3} to 587×10^{-3} days^{-1} for a storage temperature of 4–37 °C (Table 3). These values were greater than the k_{AA} values of AA-fortified juice (k_{AA} ranged from 24×10^{-3} to 463×10^{-3} days^{-1} for a storage temperature of 4–37 °C), which explain the evident stability of ascorbic acid in AA-fortified juice (AAFJ-100 and AAFJ-200) against NFCJ. By contrast, under lower dissolved oxygen conditions, the NFDJ presented the lowest k_{AA} constants compared to other types of juices (k_{AA} varied from 18×10^{-3} to 114×10^{-3} days^{-1} for a storage temperature of 20–30 °C), so degassing contributed to a better stability of AA, even more than AA-fortification. This is probably due to the slow oxygen consumption via oxidative reactions.

For storage temperatures of 4–37 °C, the $t_{1/2}$ varied from 13.9 to 1.2 days in NFCJ and from 28.3 to 1.5 days in AA-fortified juice (AAFJ-100 and AAFJ-200) while a $t_{1/2}$ value of 37.8 days was obtained for NFDJ (Table 3). These results are in accordance with the k_{AA} values of strawberry juice reported by Derossi, De Pilli, and Fiore (2010) which ranged from 121.1×10^{-3} to 198.2×10^{-3} days^{-1} for a storage temperature of 5–25 °C; and those reported by Odriozola-Serrano et al. (2009) which ranged from 67×10^{-3} to 130×10^{-3} days^{-1} for a storage temperature of 5–25 °C.

These observations indicate clearly that the storage temperature is the most influent factor in the AA degradation, while degassing helps to better preserve AA content in juice than the AA-fortification during storage. For example, the degradation of

Table 3
Kinetic parameters of the first-order model for ascorbic acid (AA) degradation and colour intensity (CI_{ANC}) loss in different blood orange juices during storage at temperature 4, 20, 30 and 37 °C.

Juice type	T (°C)	k_X ($\times 10^{-3}$ d^{-1})	*Conf.I* ($\times 10^{-3}$ d^{-1})	$t_{1/2}$ (days)	D_X (days)	R^2_{adj}	*RMSE
AA							
NFCJ	4	49.9	1.2	13.9	46.1	0.737	0.17
	20	122.0	17.8	5.7	18.9	0.943	0.08
	30	249.1	33.7	2.8	9.2	0.974	0.04
	37	587.5	51.6	1.2	3.9	0.982	0.01
AAFJ-100	4	24.5	3.7	28.3	94.1	0.815	0.09
	20	106.3	13.9	6.5	21.7	0.945	0.07
	30	206.4	39.5	3.3	11.2	0.960	0.06
	37	463.5	72.6	1.5	5.0	0.982	0.03
AAFJ-200	4	31.4	2.6	22.0	73.2	0.917	0.05
	20	95.1	15.1	7.3	24.2	0.894	0.10
	30	211.6	24.0	3.3	10.9	0.988	0.03
	37	440.0	20.3	1.6	5.2	0.998	0.01
NFDJ	20	18.3	1.8	37.8	125.6	0.777	0.05
	30	114.4	33.6	6.1	20.1	0.842	0.15
CI_{ANC}							
NFCJ	4	7.5	0.9	92.5	307.2	0.753	0.03
	20	26.9	1.5	25.7	85.4	0.960	0.03
	30	52.1	4.6	13.3	44.2	0.897	0.06
	37	68.8	11.4	10.1	33.4	0.619	0.11
AAFJ-100	4	5.0	1.1	139.0	461.6	0.638	0.04
	20	27.8	1.8	24.9	82.7	0.946	0.04
	30	53.3	4.8	13.0	43.2	0.904	0.06
	37	69.3	10.2	10.0	33.2	0.718	0.10
AAFJ-200	4	7.1	1.2	96.9	322.2	0.752	0.04
	20	26.9	2.2	25.8	85.7	0.919	0.05
	30	54.8	4.6	12.6	42.0	0.921	0.06
	37	72.5	11.2	9.6	31.8	0.699	0.10
NFDJ	20	8.2	1.2	84.7	281.3	0.782	0.04
	30	31.2	2.9	22.2	73.8	0.910	0.06

T: storage temperature (°C).
k_X: rate constant of AA degradation or CI_{ANC} loss at any storage temperature ($\times 10^{-3}$ days^{-1}).
$t_{1/2}$: half-time.
Conf.I: confidence interval were calculated with 95% of probability.
R^2_{adj}: adjusted R^2.
*RMSE: root mean square error between experimental and predicted data.

AA at 20 °C was 6 and 5 times faster in the non-fortified and fortified juice, respectively, compared to the NFDJ.

3.2.3. Effect of storage temperature on AA degradation rates

The temperature dependence of the AA degradation rate constant (k_{AA}) for all types of blood orange juice is shown in Fig. 1A. The kinetic parameters of AA degradation at the reference temperature of 22.7 °C ($T_{ref\ i}$) corresponding to Eqs. (8), (10) and (12) from the Arrhenius, Eyring and Ball models are presented in Table 4. The three models were observed to give a good fit with the temperature dependence of k_{AA} and D_{AA} ($0.933 < R^2_{adj} < 0.991$ and $0.05 < RMSE < 0.26$).

Activation energies ($E_{a\ AA}$) ranged from 51 to 135 kJ mol^{-1} and $k_{AA\ ref}$ varied between 179×10^{-3} and 31×10^{-3} days^{-1} (Table 4), the highest value of $E_{a\ AA}$ and lowest value of $k_{AA\ ref}$ were obtained from NFDJ. The higher activation energy ($E_{a\ AA}$) from NFDJ implies that a smaller temperature change is needed to degrade AA more rapidly than any other type of juice. These values fell within the reported $E_{a\ AA}$ ranges (42–105 kJ mol^{-1}) for AA degradation during storage in various citrus juice over the temperature range of 0–45 °C (Burdurlu, Koca, & Karadeniz, 2006; Polydera, Stoforos, & Taoukis, 2005; Zanoni, Pagliarini, Galli, & Laureati, 2005).

The activation enthalpy (ΔH^*_{AA}) and entropy (ΔS^*_{AA}) for AA degradation were found to vary with the juice type (Table 4). For AA-fortified or non-fortified juice, the values of ΔH^*_{AA} and ΔS^*_{AA} were in narrow range, between 49–59 kJ mol^{-1} and from −189 to −175 J mol^{-1} K^{-1}, respectively (Table 4). Conversely, the NFDJ presented significantly the highest values of ΔH^*_{AA} and ΔS^*_{AA} (ΔH^*_{AA} = 133 kJ mol^{-1} and ΔS^*_{AA} = 80 J mol^{-1} K^{-1}). The former data indicate that k_{AA} from NFDJ was more affected by temperature, over the range of 20–30 °C, than the other types of juice (Table 3). The value of ΔH^*_{AA} and ΔS^*_{AA} from NFCJ (Table 4) were closer to that reported by Alzubaidy and Khalil (2007) obtained for AA degradation in single-strength lemon juice during storage at 25 °C (ΔH^*_{AA} = 55 kJ mol^{-1} and ΔS^*_{AA} = −81 J mol^{-1} K^{-1}). The positive values of ΔS^*_{AA} for NFDJ (Table 4) suggest an increase in entropy variation upon achieving the transition state, which often indicates a dissociative mechanism (Espenson, 1981).

The thermal resistance approach of AA degradation in terms of z_{AA} value varied between 32 and 13 °C, where $D_{AA\ ref}$ varied between 13 and 76 days for all types of juice (Table 4). Alvarado and Viteri (1989) reported a z values of 35.8–46.5 °C for AA in various citrus fruits under thermal treatment over the temperature range of 20–92 °C; although no available data exists on the Ball-Bigelow model parameters (z, D_{ref}) of AA degradation in food during storage.

For the three tested models, Tukey's test showed significant differences ($p < 0.05$) in kinetic parameters of AA degradation from NFDJ compared to the other juice types (Table 4). Furthermore, Fig. 2A shows similar slopes of temperature dependence of k_{AA} from AA-fortified or non-fortified juice than NFDJ. This means firstly that k_{AA} from AA-fortified or non-fortified juice have a similar temperature dependence and secondly that k_{AA} from NFDJ are more sensitive to temperature change than the other juice types. In other words, the temperature dependence of the k_{AA} from NFDJ is smaller than that of both AA-fortified or non-fortified juice.

3.3. Colour intensity (CI_{ANC}) loss during storage

Loss in colour intensity (CI_{ANC}) of different blood orange juice upon the application of the different storage temperatures is shown in Fig. 1B. As can be seen, the CI_{ANC} of all types of juice during storage underwent a substantial depletion throughout the storage time irrespective of the storage temperature.

Table 4
Kinetic parameters of ascorbic acid (AA) degradation and colour intensity (CI_{ANC}) loss from different blood orange juices during storage temperature 4, 20, 30 and 37 °C following Arrhenius, Ball and Eyring model.

Juice type	Arrhenius model				Ball model				Eyring model			
	$E_{a\ AA}$ (kJ mol^{-1})	k_{AAref} ($\times 10^{-3}$ d^{-1})	R^2_{adj}	RMSE*	z_{AA} (°C)	$D_{AA\ ref}$ (days)	R^2_{adj}	RMSE*	ΔH^*_{AA} (kJ mol^{-1})	ΔS^*_{AA} (J mol^{-1} K^{-1})	R^2_{adj}	RMSE*
AA												
NFCJ	51 ± 32 [b]	179 ± 100 [a]	0.939	0.26	32 ± 17 [a]	13 ± 5 [b]	0.956	0.10	49 ± 32 [b]	−189 ± 108 [b]	0.933	0.26
AAFJ-100	62 ± 16 [b]	131 ± 37 [a]	0.989	0.13	26 ± 6 [a]	18 ± 4 [b]	0.991	0.05	59 ± 16 [b]	−156 ± 55 [b]	0.988	0.13
AAFJ-200	56 ± 20 [b]	134 ± 47 [a]	0.979	0.16	29 ± 8 [a]	18 ± 4 [b]	0.989	0.05	53 ± 20 [b]	−175 ± 68 [b]	0.977	0.16
NFDJ	135 ± nd [a]	31 ± nd [a]	nd	nd	13 ± nd [a]	76 ± nd [a]	nd	nd	133 ± nd [a]	80 ± nd [a]	nd	nd
	Arrhenius model				Ball model				Eyring model			
Juice type	$E_{aCI_{ANC}}$ (kJ mol^{-1})	$k_{CI_{ANC}ref}$ ($\times 10^{-3}$ d^{-1})	R^2_{adj}	RMSE*	$z_{CI_{ANC}}$ (°C)	$D_{CI_{ANC}ref}$ (days)	R^2_{adj}	RMSE*	$\Delta H^*_{CI_{ANC}}$ (kJ mol^{-1})	$\Delta S^*_{CI_{ANC}}$ (J mol^{-1} K^{-1})	R^2_{adj}	RMSE*
CI_{ANC}												
NFCJ	49 ± 12 [a]	30 ± 7 [a]	0.989	0.10	34 ± 12 [a]	79 ± 20 [b]	0.980	0.06	46 ± 13 [b]	−211 ± 43 [b]	0.988	0.10
AAFJ-100	58 ± 28 [a,b]	28 ± 13 [a]	0.964	0.22	28 ± 16 [a]	86 ± 38 [b]	0.948	0.12	56 ± 28 [ab]	−180 ± 94 [ab]	0.961	0.22
AAFJ-200	51 ± 13 [b]	31 ± 7 [a]	0.989	0.10	32 ± 11 [a]	78 ± 21 [b]	0.981	0.06	49 ± 13 [b]	−203 ± 45 [b]	0.988	0.11
NFDJ	99 ± nd [a]	12 ± nd [a]	nd	nd	17 ± nd [a]	194 ± nd [a]	nd	nd	96 ± nd [a]	− 50 ± nd [a]	nd	nd

Values are mean ± 95% confidence interval.
Values with different letters (a-b) were significantly different (Tukey, $p < 0.05$) for the four types of juice.
R^2_{adj}: adjusted R^2.
*RMSE: root mean square error between experimental and predicted data.
nd: not determined.

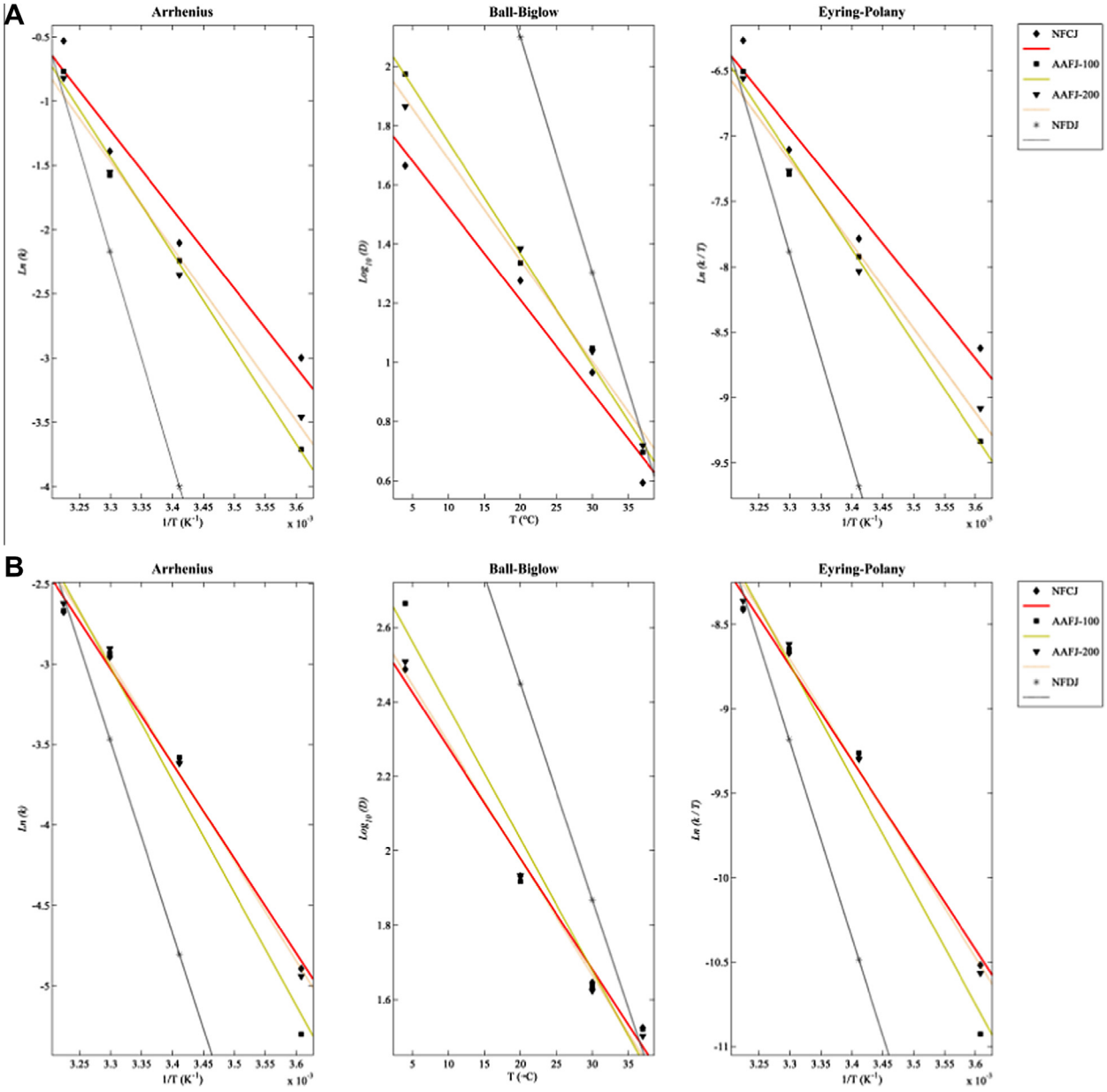

Fig. 2. Effect of the storage temperature on ascorbic acid degradation (A), colour intensity loss (B) following Arrhenius, Ball–Bigelow and Eyring–Polany models (lines) from different blood orange juices during storage at temperatures 4, 20, 30 and 37 °C.

3.3.1. Reaction order estimation of the CI_{ANC} loss

The estimated RMSE and R^2_{adj} amongst zero-, half-, first-, and second-order reactions used to describe variation in CI_{ANC} for each juice type as function of storage time at different temperatures are displayed in Table 2. Similar range of R^2_{adj} values (for each juice type at different temperatures) were obtained for both first- (R^2_{adj} ranged from 0.698 to 0.853), and second-order (R^2_{adj} ranged from 0.663 to 0.829) kinetic model (Table 2). However, first-order model seems to be most appropriate for the four types of juice, because of the RMSE range values obtained (RMSE ranged from 0.07 to 0.14) were lower compared to those of second-order model (RMSE ranged from 0.18 to 0.28). In contrast to first-order model, zero- and half-order kinetic model showed a weak performance for fitting the curve under all storage temperatures for all type of juice. Furthermore, a simple first-order model is employed by numerous studies to describe anthocyanin pigment loss frequently related to CI_{ANC} loss in fruit juices and concentrates during storage at different temperatures (Kırca & Cemeroğlu, 2003; Odriozola-Serrano et al. 2009; Wang & Xu, 2007).

3.3.2. Effect of storage time on the loss of CI_{ANC}

The effect of storage time and temperature on the loss of CI_{ANC} from different blood orange juices to the first-order kinetic model is shown Fig. 1B. In accordance with AA degradation (Fig. 1A), CI_{ANC} of different blood orange juices consistently decreased as the storage temperature increases. Consequently, the longer the time and the higher the storage temperature, the more substantial loss of CI_{ANC} (Fig. 1B). However, CI_{ANC} loss occurred much lower than AA degradation (Fig. 1). As expected, non-fortified deaerated juice (NFDJ) retained more CI_{ANC} during storage (about 75% and 40% of CI_{ANC} remaining after 28 days of the storage at 20 and 30 °C, respectively) with respect to the other juice types (about 45% and 30% of CI_{ANC} remaining at the end of the storage at 20 and 30 °C, respec-

tively). Thereby, retention of CI_{ANC} from blood orange juice was improved by the deaeration rather than by the AA-fortification.

Table 3 displays the estimated kinetic constants ($k_{CI_{ANC}}$, $t_{1/2}$ and $D_{CI_{ANC}}$) along with the R^2_{adj}, RMSE of the first-order model fitted through nonlinear regression method to describe the loss of CI_{ANC}. As can be observed (Table 3), the fitting of the first order-kinetic model to the experimental data were good irrespective of the storage temperature and juice formulation ($0.619 < R^2_{adj} < 0.960$), except for 4 and 20 °C (from AA-fortified or non-fortified juice), and 20 °C (from NFDJ). Moreover, the good fit was confirmed by the low root mean square error ($0.03 < RMSE < 0.11$). The CI_{ANC} loss rate constant ($k_{CI_{ANC}}$) exhibited a similar trend from AA-fortified or non-fortified juice, as rate constant increased from 5.0×10^{-3} to 72.5×10^{-3} days^{-1} from 4 to 37 ° C (Table 3). Whereas, NFDJ presented not only the lowest $k_{CI_{ANC}}$ constants compared to the other juice types (Table 3), but also the higher temperature-dependence of CI_{ANC} loss. Indeed, the $k_{CI_{ANC}}$ constant from NFDJ at 30 °C (31.2×10^{-3} days^{-1}) was 4-fold higher than that at 20 °C (8.2×10^{-3} days^{-1}). Whereas, AA-fortified or non-fortified juice showed a $k_{CI_{ANC}}$ increase of only 2-fold from 20 to 30 °C (Table 3).

For storage temperatures of 20–30 °C, the $t_{1/2}$ of CI_{ANC} loss ranged between 25.7–12.6 days from AA-fortified and non-fortified juice and ranged from 84.7–22.2 days from NFDJ (Table 3). These results are consistent with loss rate of anthocyanins pigments (which is related to CI_{ANC} loss rate) obtained by Kırca & Cemeroğlu (2003) from blood orange juice and concentrate during storage; ranged from 2.0×10^{-3} to 3.3×10^{-3} days^{-1} at 5–37 °C.

In accordance with AA degradation, the present findings indicate that CI_{ANC} loss during storage is mainly related to the storage temperature impact in addition to the degassing effect. However, AA-fortification (for the tested concentrations) have no effect on the rate constants of CI_{ANC} loss compared to NFCJ.

3.3.3. Effect of storage temperature on the CI_{ANC} loss rates

As for AA degradation; Arrhenius, Eyring and Ball models (corresponding to Eqs. (8), (10) and (12), respectively) fit well the temperature-dependence rate constant ($0.948 < R^2_{adj} < 0.989$ and $0.06 < RMSE < 0.22$) of CI_{ANC} loss from different blood orange juice (Fig. 2B). The corresponding kinetic parameters of CI_{ANC} loss at the reference temperature of 22.7 °C ($T_{ref\ i}$) from the three models are presented in Table 4.

Activation energies ($E_{aCI_{ANC}}$) and $k_{CI_{ANC}ref}$ from Arrhenius model were 49–99 kJ mol^{-1} and 31×10^{-3}–12×10^{-3} days^{-1}, respectively (Table 4). The highest value of $E_{aCI_{ANC}}$ and lowest value of $k_{CI_{ANC}ref}$ were obtained by NFDJ (Table 4). Consequently, the rate of CI_{ANC} loss from NFDJ seemed to be more temperature-dependent than the other studied juices. Except for NFDJ, similar values of $E_{aCI_{ANC}}$ (22.3–30.6 kJ mol^{-1}) were found from pasteurised roselle extracts during storage (Cisse et al., 2012).

The activation enthalpy ($\Delta H^*_{CI_{ANC}}$) values was between 46 and 96 kJ mol^{-1} and z values varied between 34 and 17 °C for all juices (Table 4). The NFDJ obtained the highest value of $\Delta H^*_{CI_{ANC}}$ and lowest value of $\Delta H^*_{CI_{ANC}}$ (Table 4). Indeed, k_{CI} from NFDJ was more affected by temperature, over the range of 20–30 °C, than the other types of juice (Table 3). In other words, the CI_{ANC} loss from NFDJ was more sensitive to temperature change with respect to the juice tested. Cisse et al. (2009) have reported similar findings to those obtained in the present work while processing blood orange juice.

In general, ΔH^*_{AA} and ΔS^*_{AA} had a broader range than that found in CI_{ANC} loss (Table 4), which indicate the strong influence of storage temperature on AA degradation. The ΔH^*_{Xi} values were closer to $E_{a\ Xi}$ values in both AA degradation and CI_{ANC} loss during storage and also similar trends were observed regarding the effect of storage time and temperature. Therefore, CI_{ANC} loss were strongly correlated with AA degradation.

4. Conclusion

This present study evaluated the effect of ascorbic acid fortification (at 100, 200 mg L^{-1}) and deaeration, temperature/time storage on the kinetic behaviour of ascorbic acid degradation and colour intensity loss from Moro blood orange juice. Ascorbic acid degradation and colour intensity loss during storage were best explained by first-order kinetic model through nonlinear regression methods. The storage temperature-dependence of the degradation rate constant was well described by Arrhenius, Ball and Eyring model. Similar trends were observed for both ascorbic acid degradation and colour intensity loss during storage at 4–37 °C. The impact of storage temperature and degassing are the most influent factors in the degradation of ascorbic acid and colour intensity, while ascorbic acid fortification have no significant effect on the degradation kinetic during storage. For this, deaeration can be considered by industrial producers of juice as an alternative processing solution for blood orange juice storage to avoid loss of quality. One of the main advantages of this processing solution is to promote the production of pure natural juice where nothing is added, nothing is removed. Therefore, further studies on the characterisation of the degradation products during storage might be needed to understand the degradation mechanisms involved during storage.

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