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NONENZYMATIC BROWNING KINETIC REACTION AND ASCORBIC ACID DEGREDATION OF HEAT-TREATED ORANGE JUICE DURING STORAGE

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ABSTRACT

The kinetics of ascorbic acid (AA) degradation and nonenzymatic browning (NEB) of heat-treated single strength orange juice (OJ) over a temperature between 70-95 ^{o}C for 2-10 min. and stored under opaque condition at 4 and 20 ^{o}C for 2 months have been studied. Analysis of kinetic data by measuring absorbance at 420 nm (A_{420}) suggested a zero-order reaction for NEB, while AA degradation followed a first-order reaction. The temperature dependence of NEB and AA degradation were adequately modeled by the Arrhenius equation. Activation energy of NEB and AA degradation as affected by heat-treatment and storage condition followed the same trend. The obtained results indicate that AA degradation can be evaluated by the intensity of brown colour development of OJ.

Keywords: orange juice, ascorbic acid, nonenzymatic browning, reaction kinetic, activation energy.

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INTRODUCTION

Commercial OJ has been traditionally heat-processed to destroy spoiling microorganisms and inactivate enzymes that curb the product quality during storage [Braddock, 1999]. Heat processing often induces undesirable changes in colour, flavour and nutritional value of OJ (Giner *et al*., 2003). Browning of OJ and concentrates during heat processing and storage has been a problem through the history of processing industry. Maintaining the product at low temperature has been the only means to reduce colour and flavour deterioration of processed citrus juices and concentrates in long-term storage (Khalil and Al-Zubaidy, 2010 and Nuray *et al*., 2003).

The abundance of fresh drinks based on fruit juices, especially citrus juices, and minimally processed products which allow consumers to ingest a wide variety of antioxidants in the diet orange juice is an important source of AA, a nutrient that, apart from its vitamin action, is valuable for its antioxidant effect, stimulation of the immune system and other health benefits that are being actively investigated and reported (Zerdin *et al*., 2003 and Miquel *et al*., 2006). In citrus juices, NEB is due to the reactions of sugars with amino acids (Maillard reaction) and AA (degradation) (Johnson *et al*, 1995). NEB significantly influences the commercial value of citrus products, as it is the first visible quality defect to be detected during ambient temperature storage. However, the decomposition of AA is reported to be the major

deteriorative reaction occurring during the storage of OJ (Solomon *et al*., 1995). Lee and Nagy (1988) and Al-Zubaidy and Khalil (2007) also reported a high correlation between the percentage loss of AA and increased browning in grapefruit and OJ. Shinoda *et al*. (2004) have gone beyond of that to say that ascorbic acid contributed most to the browning of citrus juice during storage. AA is thermolabile and highly sensitive to various processing conditions. The mechanism of AA degradation follows aerobic and/or anaerobic pathways and depends upon several processing conditions (Vieira *et al*., 2000).

Kinetic models can be used for objective, fast and economic assessments of food quality. Kinetic modeling may also be employed to predict the influence of processing on critical quality parameters. Regarding the nutrient deterioration, the knowledge of kinetics of AA degradation including the reaction rate as a function of temperature of processing is required. It is necessary to study the effect of different processing temperatures on the retention of AA in the product and kinetic modeling to predict the losses during processing by different heating methods. Attempts have been made to study the kinetics of thermal degradation of fruits during processing (Ahmed *et al.*, 2002). The main objectives of this study were to determine the kinetic parameters of NEB reaction and AA degradation of single strength of OJ as affected by different heat-treatments and storage conditions.

MATERIALS AND METHODS

Sampling preparation: The fresh orange fruits (Chili brand, imported from the country of Chili) used for this investigation purchased from a local fruit market and was kept at 4 ^{o}C until the experiments were carried out (not more than a day). The fruits were washed with running water and pressed manually using a plastic bowl juicer. The produced juice was filtered through muslin cloth where the juice was allowed to flow by gravity.

Thermal treatments: The thermal treatment was carried out for OJ samples at four different temperatures 70, 80, 90 and 95 ^{o}C for 2, 4, 6, 8, and 10 min. by using a thermostatic water bath. The treated samples were immediately brought to room temperature in an ice water bath. 20 mL of each treated juice sample was put in opaque screwed test tubes and stored at 4 and 20 ^{o}C for 2 months.

Ascorbic acid determination: The quantitative analysis of AA were carried out by using the spectrometric method (Revanasiddappa and Veena, 2008) adopted for control and treated juice samples on day 0 (T_0) and after 2 months of storage at 4 and 20 ^{o}C. Absorbance of treated and control samples were measured at 550 nm against distilled water. The blank was prepared similarly by omitting the AA and its absorbance was measured against distilled water. The difference in absorbance values was used for constructing the calibration curve. However, the degradation rate of AA at T_0 was measured as the ratio of remained (C_t) to its initial value (C_0) in the fresh OJ, while this remaining AA (C_t) was taken as the threshold of AA of heat-treated samples before being stored for 2 months, so degradation rate due to storage condition effect was taken as the ratio of residual AA after the storage period to the remained AA after heat-treatments.

Colour assessment: The brown colour of control and treated juice samples was measured as browning index (BI) according to the method described by Ranganna (1977) using a spectrophotometer (Model 6300, Jenway, U. K,) at 420 nm, on a day 0 and after 2 months of storage at 4 and 20 ^{o}C.

Kinetic calculations: The rate constants for NEB and AA were estimated by a primary model describing the evolution of the concentration of a component, i.e., NEB and AA, with respect to the time and temperature**.** Analysis of kinetic data from BI values of OJ suggested a zero-order reaction (Nuray *et al*., 2003; Valdramidis *et al*., 2007 and Khalil and Al-Zubaidy, 2010). The zero-order reaction employed for this purpose:

$$\mathbf{BI_t = BI_0 - kt} \quad \text{.....(1)}$$

Where:
BI_t = browning index at time t
BI_0 = initial browning index
k = constant rate of zero-order (min^{-1})

Thus, the straight line obtained from plotting BI values against temperatures showed the validity of zero-order reaction kinetics for heat-treated OJ. However, for thermal degradation kinetics of AA in OJ was reported to follow the first-order reaction ((Valdramidis et al., 2007; Al-Zubaidy *et al*., 2009; Al-Zubaidy and Khalil, 2007 and Johnson *et al*., 1995). A general reaction rate expression for degradation kinetics can be written as follows:

$$\mathbf{-d[C]/dt = k[C]} \quad \text{.....(2)}$$

Where:
C = the quantitative value (concentration mg/ml) of the component under consideration (AA in this case)

k = the reaction rate constant
integration of eqn. 2 over any specified heating period t can be written as

$$\mathbf{\ln([C]_0/[C]_t) = kt} \quad \text{.....(3)}$$

where:
$[C]_0$ = the initial concentration of AA at time 0
$[C]_t$ = the value after heating time t (min)

The relationship of reaction rate to temperature was quantified by the Arrhenius equation, where:

$$\mathbf{k = A_0 \exp(-Ea/RT)} \quad \text{.....(4)}$$

where:
Ea = the activation energy of the degradation reaction (kJ/mole)
R = the universal gas constant (8.314 J/mole.K)
T = absolute temperature (K)
A_0 = frequency factor of Arrhenius constant (min−1)

The time required for AA to degrade to 50% of its original value (expressed as half-time, $t_{1/2}$, min.) was calculated from the rate constant as 0.693/rate constant (k) as adapted by Samuel and Jerome (1974).

Statistical analysis: The study was designed as factorial within four temperatures and two storage temperatures with two repetitions. Data were analyzed using Statistical Analysis System (SAS Institute, Cary, NC, USA, 1997) for the analysis of variance (ANOVA). Statistical analysis of the regression and goodness of fit was done using Microsoft Excel 2003 software.

RESULTS AND DISCUSSION

BI values of OJ by using different heat-treatments are shown in Fig. 1. OJ samples at T_0 and stored at 4 ^oC and 20 ^oC showed higher ($p<0.05$) browning colour intensity. A_{420} has been previously used to determine the browning rate in fruit juices and purees (Khalil and Al-Zubaidy, 2010; Khalil, 2009; Shinoda *et al*., 2004; Nuray *et al*., 2003; Johnson *et al*.; 1995; Lee and Nagy, 1988 and Kacem *et al*.; 1987). The BI followed a zero-order reaction (Equ. 1) with respect to treatment times at different temperatures studied and the rate constant (k) increased with temperature increase. Previous kinetic studies on BI reactions based on A_{420} measurement in citrus juices such as orange and lemon juices (Khalil and Al-Zubaidy, 2010), apple juices (Burdurlu and Karadeniz 2003), orange, lemon, grapefruit, and tangerine (Nuray *et al*., 2003), pear puree (Ibarz *et al*. 1999) and pear juice concentrate (Beveridge and Harrison 1984) similarly reported zero-order reaction kinetics. On the other hand, a first-order browning reaction was reported for OJ serum (Johnson *et al*., 1995) and apple juice concentrates (Toribio and Lozano, 1984). The most appropriate model was selected on the basis of determination coefficients (R^2) obtained from regression analysis. Rate constants (k) were determined from best-fit regression equations. The k value, for each replicated study are shown in table 1.

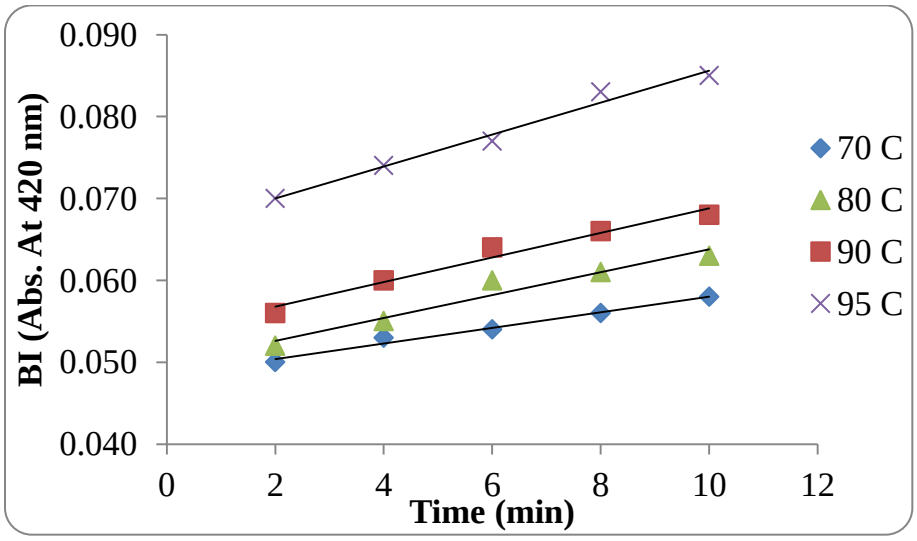

Figure (1): Absorbance values at 420 nm (BI) of orange juice using different heat-treatments at T_0.

Activation energies (Ea, kJ/mole) of browning colour development were calculated as a product of gas constant, R (8.314 J/mole.K), and slope of the graph obtained by plotting lnk versus 1/T (Fig. 2).

Table (1): kinetic parameters for orange juice browning pigment (A_{420})

Storage Temp. °C	Temp.(°C)	K	lnk	Ea(kJ/mol)
Time zero, control (T_0)	70	0.005	-5.300	28.69
	80	0.006	-5.116	
	90	0.008	-4.860	
	95	0.010	-4.580	
4	70	0.003	-5.896	30.76
	80	0.004	-5.655	
	90	0.004	-5.491	
	95	0.006	-5.075	
20	70	0.005	-5.298	14.27
	80	0.005	-5.404	
	90	0.005	-5.234	
	95	0.007	-4.915	

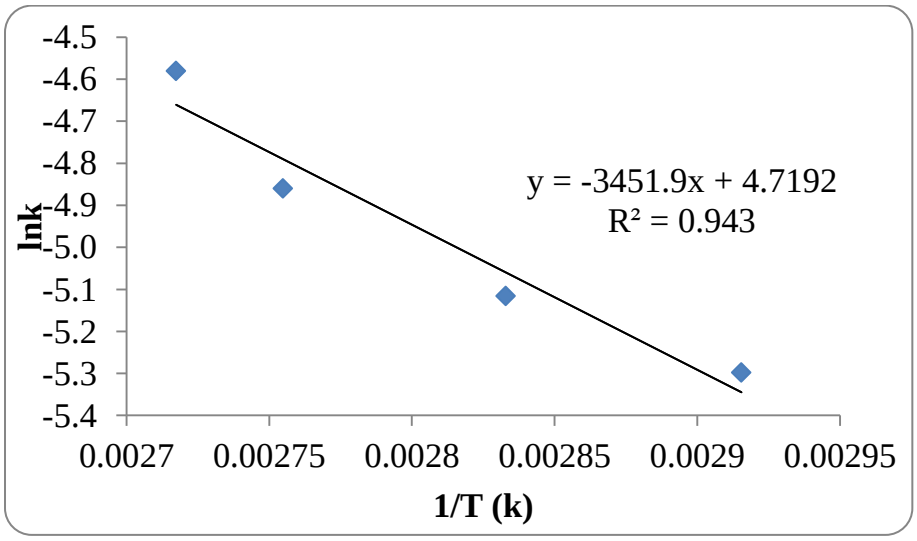

Figure (2): Arrhenius plot for A_{420} of OJ subjected to different heat-treatments.

Ea in the present study were 28.68, 30.76 , and 14.27 kJ/mole for T_0 and storage at 4 and 20 °C, respectively. Storage at 4 °C had recorded higher Ea than stored orange samples at 20 °C and even at T_0 using different heat treatments. The different Ea values of OJ samples, either treated with different heat-treatments or stored at different storage temperatures, are temperature dependent. Since Ea recorded the highest value with OJ samples stored at 4 °C, it emphasizes that maintaining OJ samples at low storage temperature will keep brown colour at lower level. Khalil and Al-Zubaidy (2010), Nuray *et al*. (2003), and Handwerk and Coleman (1988) have reported that keeping citrus juices at low temperature had decreased the intensity of their brown

colour. However, giving Ea of 28.68 kJ/mole at T_0 of OJ sample was due to the direct effect of heat-treatments used in the experiments.

The degradation of AA had followed the first-order reaction (Equ. 2) using different heat-treatments and storage conditions (Fig. 3). It can be noticed that higher temperature led to higher degradation ($p<0.05$) of AA and higher increase in browning was accompanied by higher degradation of AA of OJ. The later notice has been emphasized by some authors (Al-Zubaidy and Khalil, 2007; Shinoda *et al*., 2004 and Lee and Nagy, 1988) who stated that there is a strong relationship between degradation of AA and the development of brown colour of citrus juices. Using linear regression, degradation data were analyzed using standard integrated rate equation to determine the overall order and rate constant for the degradation reaction.

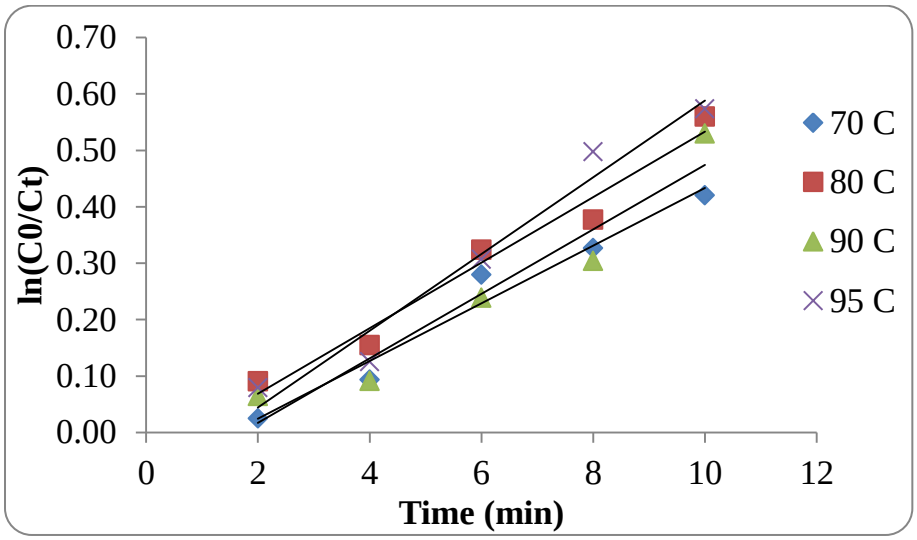

Figure (3): First-order plot of ascorbic acid degradation in orange juice treated with different temperatures and times.

Table (2) indicates rate constants, Ea, and half-life ($t_{1/2}$, min.) for AA in OJ samples at T_0 and after 2 months of storage. It seems that kinetics of either AA degradation or browning development had followed the same trend where rate constant of AA degradation increased with temperature increase as well. Similarly, it can be noticed that Ea recorded less values at T_0 and storage at 20 ^{o}C than with storage at 4 ^{o}C. On the other hand, either kinetic models of AA degradation or brown colour development can be used to predict the deterioration level of OJ. In addition, the determination coefficients (R^2) recorded to be > 0.9 from regression analysis were obtained for both Arrhenius plots of AA degradation (Fig. 4) and BI (Fig. 2) of OJ samples. The rate constants for AA degradation in OJ increased from 0.037 min^{-1} for 70 ^{o}C to 0.073 min^{-1} for 95 ^{o}C, and the $t_{1/2}$ decreased from 18.24 min to 9.49 min as the temperature increased from 70 to 95 ^{o}C. Previously, it has been shown that the degradation of AA in citrus juices is a temperature dependent (Al-Zubaidy *et al*., 2009; Al-Zubaidy and Khalil, 2007; Vieira *et al*., 2000; Johnson *et al*., 1995; Roos and Himberg, 1994).

Table (2): Rate constant (k), half-life (min), and Ea of AA degradation in OJ samples.

Storage Temp. °C	Temp. (°C)	k	$t_{1\2}$ (min)	lnk	Ea
time zero, control (T_0)	70	0.037	18.24	-3.297	25.95
	80	0.058	11.95	-2.847	
	90	0.061	11.36	-2.797	
	95	0.073	9.49	-2.617	
4	70	0.017		-4.075	42.18
	80	0.022		-3.817	
	90	0.032		-3.442	
	95	0.049		-3.016	
20	70	0.051		-2.976	10.05
	80	0.057		-2.865	
	90	0.058		-2.847	
	95	0.068		-2.688	

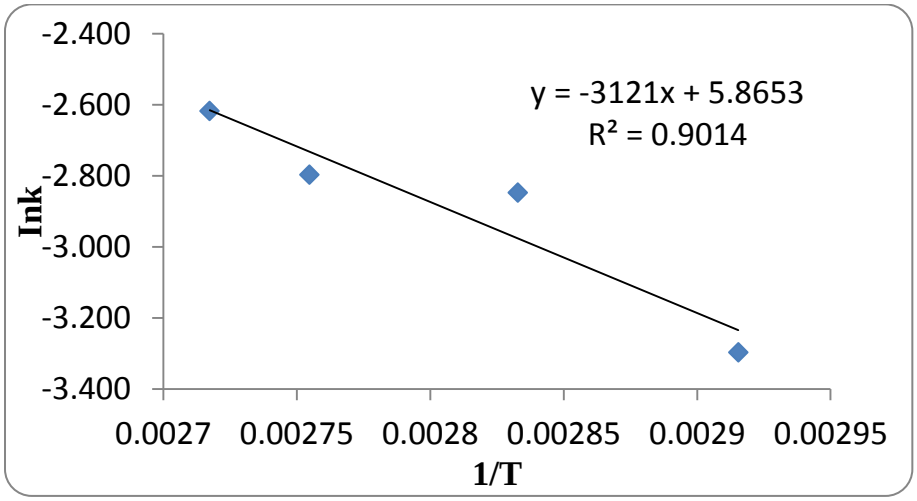

Figure (4): Arrhenius plot of AA degradation of orange juice subjected to different heat-treatments.

The implemented modeling approaches could be further developed by incorporating prior knowledge of kinetic process during the parameter estimation as this approach was previously suggested and applied (Geeraerd *et al.* 2004 and Valdramidis *et al.* 2007). This would require the collection of additional biochemical information on the browning and AA dynamics of different juice products under different heat-treatments and storage conditions.

حركيات تفاعلات الاسمرار غير الأنزيمية لعصير البرتقال المعامل حرارياً أثناء الخزن

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الخلاصة

تمت دراسة حركيات تحطم حامض الاسكوربك والتفاعل البني غير الأنزيمي لعصير البرتقال المعامل حراريا بمدى درجة حرارة 70-95 ^{5}م لمدة 2-10 دقيقة وخزنه في ظروف مظلمة عند درجة حرارة 4 و 20 ^{5}م لمدة شهرين. اقترح تفاعل المرتبة صفر للتفاعل البني غير الأنزيمي عند تحليل البيانات الحركية بقياس الامتصاص الضوئي عند طول موجي 420 نانومتر، بينما اتبع تحطم حامض الاسكوربك تفاعل المرتبة الأولى. وجد انه بالإمكان التعبير عن اعتماد التفاعل البني غير الأنزيمي وتحطم حامض الاسكوربك على درجة الحرارة بشكل كافي بواسطة معادلة ارهينيس. اتبع تأثير درجة الحرارة وظروف الخزن على طاقة التنشيط للتفاعل البني غير الأنزيمي وتحطم حامض الاسكوربك نفس الاتجاه. تشير النتائج الى انه يمكن تقييم تحطم حامض الاسكوربك من خلال شدة تطور اللون البني لعصير البرتقال.

الكلمات الدالة: عصير البرتقال، حامض الاسكوربيك، الاسمرار غير الانزيمي، مركبات التفاعل، طاقة التنشيط.

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