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Degradation Kinetic Modelling of Ascorbic Acid from Orange Juice †

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Abstract: Vitamin C or ascorbic acid is a basic nutrient, a highly effective antioxidant, widely used as food additive. Therefore, quality control in food industry demands ascorbic acid determination methods. The purpose of this study was to determine vitamin C in natural orange juices by spectrometric and voltammetric methods. Another goal was to determine the kinetic and thermodynamics activation parameters for ascorbic acid degradation in orange juices over time and at different temperatures. It was observed that during storage, ascorbic acid concentrations in orange juices were gradually decreased with time at a rate depending on storage temperature and type of orange juice. The reaction order was determined through integrated graphical analysis where the dependences of ln c_t/c_0 as a function of time reveals the high values for R^2, indicating that the kinetics of the degradation of AA follows first order reaction at both studied temperatures. For studied samples the loss of ascorbic acid was varied between 4.33% and 9.13%. Enthalpy variation (ΔH) and entropy variation (ΔS) of activation process were obtained from the Eyring–Polany model based on transition state theory. The values of activation energy ranged between 7289.24 kJmol^{-1} and 15689.54 kJmol^{-1}.

Keywords: vitamin C; rate constant; activation energy; enthalpy; entropy

1. Introduction

A powerful antioxidant known as ascorbic acid or vitamin C is a hidrosoluble vitamin present in many juices, fruits, biological systems and multivitamin preparation. It is used in treatment of cold, in cardiovascular disease, stroke and cancer [1-3].

The concentration of vitamin C in beverages can be an index of quality during the storage and production process, because ascorbic acid is labile, being sensitive to the light, temperature and heat [4, 5]. Many methods with different detection techniques have been reported: amperometry [3], voltammetry [5-8], spectrometry [9,10], chromatography [11], chemiluminescence [12], capillary zone electrophoresis ultraviolet detection [13]. Electrochemical techniques are the most used because of their sensitivity, selectivity, accuracy, simplicity, miniaturization and low cost. A cadmium oxide nanoparticle modified disposable screen-printed carbon electrode (SPCE) was reported by Gopalakrishnan and co. [14] for non-enzymatic detection of ascorbic acid. Also, an electrocatalytic detection of ascorbic acid using N,N,N′,N′-tetramethyl-para-phenylene-diamine (TMPD) mediated oxidation at unmodified gold electrodes was used by Sabine Kuss and Richard G. Compton [15]. The method offers sensitivity, a good limit of detection (30 μM) for the detection of ascorbic acid in orange juice, without interferences from others reactants. Another voltammetric detection with modified screen-printed electrodes was studied for the determination of ascorbic acid from beverages [6]. The electrode showed a good selectivity and stability. For the ascorbic acid determination in fruit juices,

Rodríguez-Bernaldo de Quirós et al. [2] were developed a rapid and simple HPLC method. They studied the stability of ascorbic acid during the shelf-life of the product and the degradation of about 54% was observed in a tea drink. But the cheapest way to quantify the ascorbic acid remains electrochemical methods. Amanda Silva et al. [8] were developed a square wave voltammetric method using a cobalt phthalocyanine modified carbon paste electrode for the simultaneous determination of ascorbic acid and citric, lactic, malic and tartaric acids in fruit juice without any previous pretreatment.

The purpose of this study was to determine vitamin C in natural orange juices by spectrometric and voltammetric methods. Another goal was to determine the kinetic and thermodynamics activation parameters for ascorbic acid degradation in orange juices over time and at different temperatures.

2. Results

The analyzed samples were natural orange juices: sample 1 - commercial oranges juice-with no sugar added, sample 2 -commercial blood Sicilian oranges juice-with no sugar added, sample 3-commercial oranges juice with sugar and sample 4 - commercial blood oranges juice with sugar.

2.1. AA content in orange juices

In table 1 the content of ascorbic acid in orange juices, determined by the two methods: spectrophotometric and voltammetric are presented. Determination by the voltammetric method is based on the oxidation of ascorbic acid to dehydroascorbic acid. The irreversibility of the oxido-reduction process, which takes place on the surface of the electrodes, was demonstrated using cyclic voltammetry (fig. 1).

2.2. Ascorbic acid degradation during storage

Vitamin C in the orange juices was determined using the voltammetric method at different intervals of time (0, 30, 60, 90, 120 and 150 min) during storage at 4^0C and room temperature at 22^oC. The degradation of ascorbic acid in orange juices was evaluated using zero-order and first-order kinetic models (fig2). The integrated first-order rate law over time is the equation (1). Rate equations and the correlation coefficients R^2 for the zero-order and first-order kinetic models are listed in table 2.

$$\ln C - \ln C_0 = - k_1 t \qquad (1)$$

where: C is the ascorbic acid concentration at time t, C_0 is the ascorbic acid concentration at time 0, k_0 and k are the ascorbic acid degradation rate constant for the zero order and for the first order and t is the storage time.

The first order rate constants were calculated from the slope of the straight line and the half-life time, $t_{1/2}$ was calculated from the rate constant as 0.693/k. The results are listed in table 3.

To calculate the activation energy value, determinations were made at different temperatures: 277 and 295 K. To calculate activation energy, the Arrhenius equation which express the dependence of the degradation rate constant on each temperature was used. Enthalpy ($\Delta H^{\#}$) and entropy ($\Delta S^{\#}$) of activation were obtained from the Eyring–Polany model based on transition state theory (2):

$$ln\frac{k}{T} = -\frac{\Delta H\#}{R}\cdot\frac{1}{T} + ln\frac{k_B}{h} + \frac{\Delta S\#}{R} \qquad (2)$$

where: k_B is the Boltzmann's constant (1.381 x 10^{-23} J/K); T is the absolute temperature in Kelvin (K); h is Planck's constant (6.626 x 10^{-34} Js); R is the ideal gas constant (8.314 $J\cdot mol^{-1}K^{-1}$).

Activation enthalpy, $\Delta H^{\#}$ represents the difference in energy between the ground state and the transition state in a chemical reaction. The higher the activation enthalpy, the more energy is required for the products to form. The values for $\Delta H^{\#}$ and $\Delta S^{\#}$ have been determined from kinetic data obtained from $ln\frac{k}{T} = f\left(\frac{1}{T}\right)$. The equation is a straight line with negative slope, $-\frac{\Delta H\#}{R}$ and a y-intercept, $ln\frac{k_B}{h} + \frac{\Delta S\#}{R}$. The Gibbs free energy was obtain using the following thermodynamic equation:

$$\Delta G^{\#} = \Delta H^{\#} - T\Delta S^{\#} \quad (3)$$

The results for activation energy, activation enthalpy, activation entropy and Gibbs free energy of activation are listed in table 4.

2.3. Figures and tables

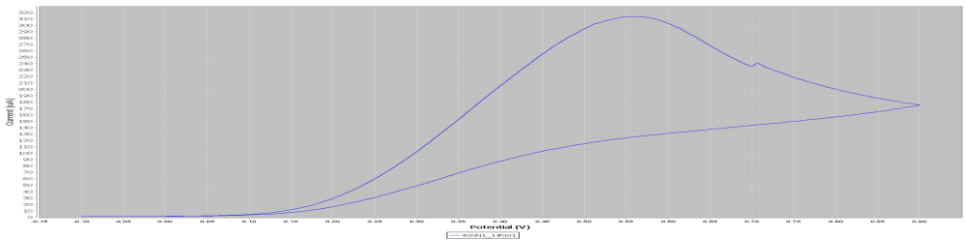


Figure 1. The cyclic voltammogram obtained for the ascorbic acid solution.

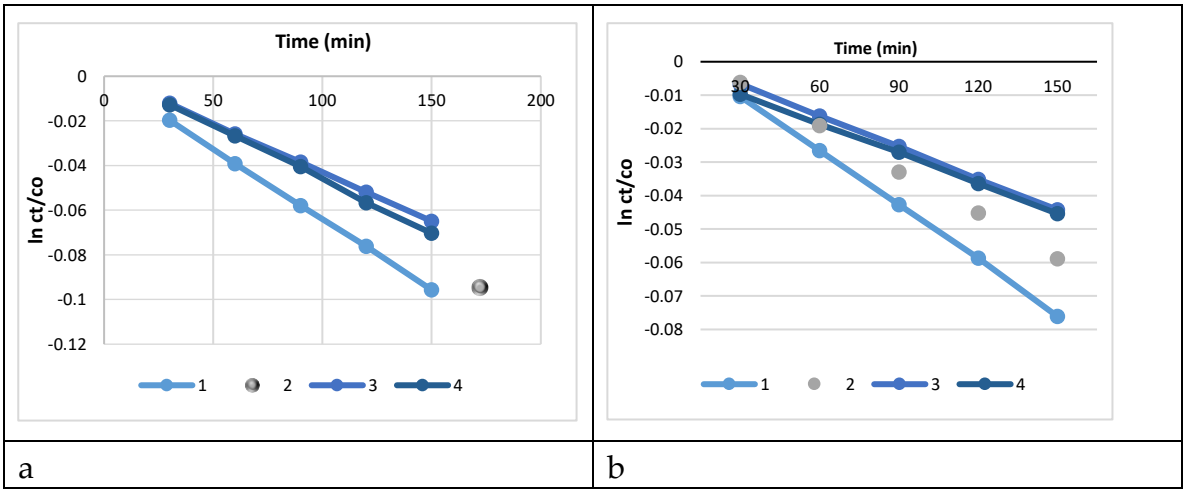
Table 1. The content of AA mg/100 mL in studied samples.

Sample	The Content of Ascorbic Acid mg AA/100 ml	
	Spectrometric method	Voltammetric method
1	29.98±0.24	30.13±0.23
2	32.08±0.31	32.13±0.37
3	42.14±0.43	42.27±0.35
4	38.22±0.22	38.28±0.44

Values are means, n=3.

Table 2. Rate equations and the correlation coefficients.

Sample	Temperature	Zero-order kinetic model		First-order kinetic model	
		Rate equation	R^2	Rate equation	R^2
1	277 K	y = -0.4406x+30.605	0.9979	y = -0.0164x+0.0062	0.9998
2		y = -0.3797x+32.607	0.9919	y = - 0.0132x+0.007	0.9998
3		y = -0.3734x+42.692	0.9979	y = -0.0094x+0.0027	0.9999
4		y= -0.3374x+38.599	0.9995	y= - 0.0089x+0.0007	0.9997
1	295 K	y= -0.553x+30.657	0.9992	y = -0.0006x-0.0011	0.9998
2		y= -0.52x+32.674	0.9994	y = -0.0006x+0.0027	0.9998
3		y= -0.537x+42.815	0.9998	y = -0.0004+0.0009	0.9999
4		y= -0.525x+38.829	0.9989	y= - 0.0005x+0.0021	0.9993



a b

Figure 2. The dependences of ln ct/co as a function of time at 277 K (a) and 295K (b).

Table 3. Values of rate constant and half-life time for both studied temperatures.

Sample	Temperature	Rate constant k (min^{-1})	$t_{1/2}$ (min)	Temperature	Rate constant k (min^{-1})	$t_{1/2}$ (min)
1	277 K	$5x10^{-4}$	1386	295 K	$6x10^{-4}$	1155
2		$4x10^{-4}$	1732.5		$5x10^{-4}$	1386
3		$3x10^{-4}$	2310		$4x10^{-4}$	1732.5
4		$2.97x10^{-4}$	2333		$4.5x10^{-4}$	1540

Table 4. Values for activation energy, activation enthalpy, activation entropy and Gibbs free energy.

Sample	E $kJmol^{-1}$	$\Delta H^{\#}$ $Jmol^{-1}$	$\Delta S^{\#}$ $J{\cdot}mol^{-1}K^{-1}$	$\Delta G^{\#}$ $kJmol^{-1}$	
				277K	295K
1	7289.24	-992.6	-308.48	84456.36	90009
2	8422.80	-1331.9	-310.67	84723.69	90315.75
3	10856.64	-1868.1	-313.60	84999.1	90643.9
4	15689.54	-2930.6	-314.75	84255.15	89920.65

3. Discussions

According to applied pair t-test, no statistically significant differences values of AA content determined by both methods ($t_{calculated} = 0.15 < t_{table} = 2.20$) were found. In the same time, $p = 0.87 > 0,05$ which means that differences are not significant, demonstrating that the two methods: spectrometric and voltammetric can be successfully applied for the determination of ascorbic acid in the analyzed samples.

The reaction order was determined through integrated graphical analysis where the dependences of $\ln c_t/c_0$ as a function of time (fig. 2) reveals the high values for R^2 (table 2), indicating that the kinetics of the degradation of AA follows first order reaction at both studied temperatures. Also, 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 the two temperatures for each type of juice. We selected first order reaction kinetics for which R^2_{adj} ranged between 0.9986 to 0.9996 and RMSE ranged between 0.266 to 0.656. On the contrary, zero-order kinetic model showed a poor performance for fitting the curve for all type of juice. It was observed that the ascorbic acid loss was decreased when the samples were kept at the refrigerator. For sample 1 the loss of ascorbic acid was: 7.33% at 277K and 9.13% at 295K, sample 2: 5.73% at refrigerator at 8.03% at room temperature, sample 3: 4.33% at 277K and 6.29% at 295K and sample 4: 4.44% at refrigerator and 6.79% at room temperature. Also, the ascorbic acid loss was decreased upon sugar presence in studied juices. Oxygen is the most destructive ingredient in juice causing degradation of vitamin C. The sugar presence could decrease the concentration of dissolved oxygen in juice, thus the oxidation process of ascorbic acid was delayed. Grudić et al. [16] found that at room temperature, the ascorbic acid degradation occurs much more slowly: after 6 days the reduction of the concentration is 32% and after 12 days, the percentage is 56%.

It was observed that during storage, ascorbic acid concentrations in orange juices were gradually decreased with time at a rate depending on storage temperature and type of orange juice (with or without sugar). The rate constants were increased with the increase of storage temperature. The highest rate constant was obtained for sample 1 for both studied temperatures followed by rate constant obtained for sample 2. Samples of blood orange juices reveal smaller values for rate constants and respectively half-life times than the orange juices, samples 1 and 2. The rate degradation of ascorbic acid from green pepper increased with the increase of temperature and the values ranged between: $1x10^{-3}$to $5x10^{-5}min^{-1}$ [16]. According to Polydera et al. [17] a notable difference in acid ascorbic degradation rates was observed when different types of packaging were used for storage of orange juice. Acid ascorbic degradation rates were almost double in case of polypropylene bottles compared to flexible pouches. The highest half-life time was obtained for sample 4 at 277K: 2333 minutes and the smallest half-life time was obtained for sample 1 at 295K: 1155 minutes.

Activation energy ranged between 7289.24 kJmol^{-1} and 15689.54 kJmol^{-1}. The highest values for activation enthalpy, activation entropy were obtained for sample 1 (orange juice with no sugar added). The values of thermodynamics activation parameters could be explained through the type of reaction, that is not spontaneous and it consumes energy, is endothermic. Remini et al. [18] found that the activation enthalpy and entropy for AA degradation vary with the juice type. They found that for blood orange juices values for activation enthalpy and activation entropy were ranged between 49-59 kJ/mol and from 189 to 175 J·mol^{-1}K^{-1}, respective.

4. Materials and Methods

Analytical grade reagents were used. L-Ascorbic acid was purchased from Merck, Darmstadt, Germany. The Fe (III) reagent was prepared by dissolving iron (III) chloride salt in distilled-deionized water and the hexacyanoferrate (III) solutions were prepared by dissolving the potassium salt in water. Metaphosphoric acid (20 g/L) was used for extraction. Solutions of H_2SO_4 0.5M were used for sample preparation and as supporting electrolyte because ascorbic acid oxidation is slower in acidic medium.

For spectrometric analysis, the fruit juices were sonicated, then centrifuged at 5000 rpm for 10 min, using a Hettich Universal 320 centrifuge (Andreas Hettich GmbH & Co. KG). The supernatant was filtered through filter paper (Whatman no. 42). Then, metaphosphoric acid (20 g/L) was used for extraction. Finally, the extract was diluted with metaphosphoric acid. Blank was prepared by the same manner as except for the addition of fruit juice samples. Each of the fruit juice samples was treated separately as described under the general procedure.

Ascorbic acid in orange juices was determinate by UV-VIZ absorption spectrometry and differential pulse voltammetry (DPV) analysis with modified carbon-printed electrodes. The spectrometric method is based on the color reaction between potassium hexacyanoferrate and ascorbic acid in acid medium, when ascorbic acid has a reducing effect. The determinations were done at a wavelength of 700 nm using a UV-VIZ DR 3900 Lange from England spectrometer. The method was optimized in a previous paper [19]. Determination by the voltammetric method is based on the oxidation of ascorbic acid to dehydroascorbic acid. A conventional three-electrode potentiostatic system (Stat400 bi-potentiostat) was used in order to carry out the electrochemical measurements. The three electrodes are integrated in the screen-printed electrodes (SPEs), where working and counter electrodes are based on a carbon ink and the pseudo-reference electrode is made up of silver. A solution of 10^{-2}M ascorbic acid in 0.5M sulfuric acid was prepared and the electrochemical behavior of ascorbic acid on screen-printed electrodes was investigated.

The model's parameters were estimated by least square method. The statistical tools that were used to evaluate the adequacy of fit of the models were adjusted coefficients of determination (R^2_{adj}) and root mean square error (RMSE). Models with highest R^2_{adj} values and lowest RMSE values were used as selection criteria. To determine if the differences between values of AA content obtained by both methods (spectrometric and voltammetric) were significant, a pair t-test was performed. All statistical analyses were carried out at the 95% confidence level and two-tailed hypothesis was applied.

5. Conclusions

According to applied pair t-test, were not found statistically significant differences values of AA content determined by spectrometric and voltammetric methods, demonstrating that the both methods can be successfully applied for the determination of ascorbic acid in the analyzed samples. The study revealed that the loss of ascorbic acid content increases with time and temperature. The acid ascorbic degradation in orange juice can be described by an overall first order reaction. Samples of blood orange juices reveal smaller values for rate constants and respectively half-life times than the orange juices. The highest values for activation enthalpy, activation entropy were obtained for orange juice with no sugar added.

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