Research Article
Degradation Kinetics of Vitamin C in Orange and Orange Juice during Storage

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Abstract: The objective of this study was to determine Vitamin C (Vc) degradation in orange and orange juice during storage at different temperatures. Degradation kinetics of Vc in orange and orange juice was modeled by zero and first order reaction. Vc of orange and orange juices is on the thermal instability during storage, which was found to decrease significantly with increase in storage time and temperature. Compared with zero order, the degradation kinetics was better fitted to the first order reaction model for both orange and orange juice. Following the first order kinetic model, the reaction rate constant (k) of Vc increased from 0.0396 to 0.3573/d with the increase of temperature from 5 to 37°C for orange while the k ranged from 0.0460 to 0.2617/d for orange juice. The results indicated that the temperature had a great deal of influence on the degradation of Vc in orange and orange juice.

Keywords: Degradation kinetics, orange, orange juice, storage, temperature, vitamin C

INTRODUCTION

Oranges are an important crop and the orange product in China is the third in the world (Xuan *et al*., 2006). Orange is known as the most popular fruit in China for the long growing season, the delicate flavor as well as the high nutritional value. Some oranges are eaten in its fresh, whole state and a large portion of oranges is used in the production of orange juice. Orange juice is probably the most popular fruit juice worldwide for its nutritional content, appealing color and refreshing sweet and sour taste and biological active compounds, such as vitamin C (Vc) and so on (Niu *et al*., 2008; Wibowo *et al*., 2015). During storage and transportation, orange or orange juice undergoes a number of deteriorative reactions (ascorbic acid degradation, microbial spoilage, development of off-flavor, changes in color, texture, appearance), resulting in quality degradation (Roig *et al*., 1999; Ayhan *et al*., 2001; Bezman *et al*., 2001; Niu *et al*., 2008; Wibowo *et al*., 2015). Vc is known for its potent antioxidant properties and is lost during handling, processing and storage (Caro *et al*., 2004; Yu *et al*., 2013). Therefore, Vc content is widely considered as an appropriate marker for monitoring quality changes during processing and storage (Polydera *et al*., 2003; Zanoni *et al*., 2005).

Vc is the most sensitive and destructed when a commodity is exposed to adverse handling and storage conditions (Lee and Kader, 2000). Vc can be reversibly oxidized to dehydroascorbic acid (DHAA), without losing Vc functionality. Further degradation of DHAA to e.g., 2, 3-Diketogulonic Acid (DKGA) results in the loss of biological activity. As the most important water-soluble antioxidant nutrient, Vc largely contributes to antioxidant defence against oxidative stress (Plaza *et al*., 2006). This fact is connected with its ability to act as a reducing agent and as an oxygen scavenger (Kitts, 1997). The stability of Vc in fruits and vegetables is related to degree of exposure oxygen and temperature of storage. Vc was measured to have a considerable losses during heat processing (Patras *et al*., 2011; Mazur *et al*., 2014). The high temperature with long storage time had negative impact on Vc (Mazur *et al*., 2014). In this case, it comes to be particularly important to coordinate the storage temperature and storage time (Remini *et al*., 2015). For those reason, degradation kinetic model, as an effective method to predict the physical and chemical parameter changes, are becoming more and more popular for people to control and improve the storage conditions. In recent years, the study of Vc degradation kinetic are mainly concentrate on medicament, juice, vegetables, tea and so on (Phillips *et al*., 2010; Verbeyst *et al*., 2013; Rahman *et al*., 2015). Similar research reported

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that first-order reaction modelling is quite fit with the oxidation kinetics of orange juice (Burdurlu *et al*., 2006). Nevertheless, there is negligible work available in the literature about the degradation kinetic of Vc in orange and orange juice. The objective of this study was to determined Vc changes with the storage temperature and time to construct the degradation kinetic model that are suitable for both orange and orange juice. The research will be available to have a better understanding on the storage stability of Vc content and to offer the best conditions for the storage of orange and orange juice.

MATERIALS AND METHODS

Preparation of orange and orange juice: Oranges were harvested at maturity stage (total soluble solids/titrable acidity = 7) and chosen in the similar size and softness. Some were immediately squeezed using a domestic squeezer (Yoyoung, Guangzhou, China), then filtered through a stainless steel sieve (1 mm) to remove pulp and seed. The juice sample was divided in five copies of equal volume. The juice and the other oranges respectively stored at five different temperature conditions (5, 16, 23, 30 and 37°C) in temperature-controlled storage locker (in the dark). Temperatures were controlled by placing the desiccators in circulation oven set at the desired temperature with the maximum variation of ±1°C. Oranges stored at four thermal conditions (5, 16, 23 and 30°C) were measured once in two d and stored at 37°C should be measured within 2.5 d for the high temperature. Juice stored at two thermal conditions (5, 16°C) was measured every day. The juice stored at 23 and 30°C should be measured within 2.5 d while stored at 37°C should be done within 2 d. Based on preliminary results of the repeated trials, all above processing conditions were selected on account of their effect on the stability of Vc in both orange and orange juice (Remini *et al*., 2015).

Vc extraction: 20 mg mash of orange from the temperature-controlled storage locker were weighed and put into mortar with a small amount of 2% oxalic acid and then were grind quickly within five min. The mixture was diluted to 250 mL with 2% oxalic acid then filtered. 20 mL orange juice from the temperature-controlled storage locker was diluted to 250 mL with 2% oxalic acid (Verbeyst *et al*., 2013). All extractions were carried out under reduced light.

Determination of Vc: Vc was determined using a titration method with 2,6-dichloro-indophenol reagents and the procedure described by Kirk *et al*. (1991). Standard indophenol solution was prepared by dissolving 0.05 g 2, 6-dichloro indophenols in distilled water diluted to 250 mL and filtered. To standardize, 0.1 g of Vc was dissolved in 450 mL of 2% meta-phosphoric acid and diluted with water to 500 mL. Ten mL of this solution was pipette into a small conical

flask and 10 mL of 2% metaphosphoric acid (as stabilizing agent) was added. The mixture was titrated with indophenol solution until a faint pink color persists for 15 s. This could calculate solution per milliliter of dye that equaled to the content of Vc (mg/L). 10 mL of extracted juice was pipette into a conical flask with 10 mL of 2% meta-phosphoric acid (as stabilizing agent). It was titrated with the indophenol solution until a faint pink color persists for 15 s. Vc content were expressed as mg/100 g for orange and mg/L for juice (Kenawi *et al*., 1994). All measurements were done in triplicate.

Degradation kinetics model during storage: Generally, most of food nutrients degradation has been explained according to zero, half, first or higher order kinetics (Corradini and Peleg, 2006), which can be described in Eq. (1)-(2) (Teng and Chen, 1999):

Zero-order: $C=C_0-kt$ (1)

First-order: $C = C_0\ exp\ (-kt)$ (2)

where C_0 is Vc concentration (mg/mL of juice or mg/100 g of orange) at the given time $t = 0$, C is a concentration (mg/mL of juice or mg/100 g of orange) at any given time (t), k is the reaction rate constant (/d) and t is the storage time (d). The half-life time ($t_{1/2}$) was calculated from Eq. (3)-(4):

Zero-order: $t_{1/2} = C_0/2k$ (3)

First-order: $t_{1/2} = ln2/k$ (4)

The rate constant k (d^{-1}) varied with the system's absolute temperature, T (K), according to the Arrhenius law from Eq. (5):

$k =A\ exp\ (-E_0/RT)$ (5)

where A is pre-exponential factor, E_0 is activation energy, R is gas constant and T is the absolute temperature (K). For the specified reaction, both A and E_0 are the constants that have no relationship with reaction concentration and reaction temperature.

RESULTS AND DISCUSSION

The V_C degradation kinetics in orange and orange juice: Vc content change in orange and orange juice during storage at various temperatures were represented in Fig. 1 and 2, respectively. In Fig. 1, storage temperature has an important effect on Vc content. Storage times varied between 2 and 6 d, depending on temperatures investigated. Vc content decreased with increasing temperature. In Fig. 2, it was seen that degradation of Vc increased with raising temperature at the first two d of storage. It was also observed that storage time accelerated the degradation of Vc. In Fig. 2, there was an initial high rate of Vc loss at relatively higher storage temperature, followed by a
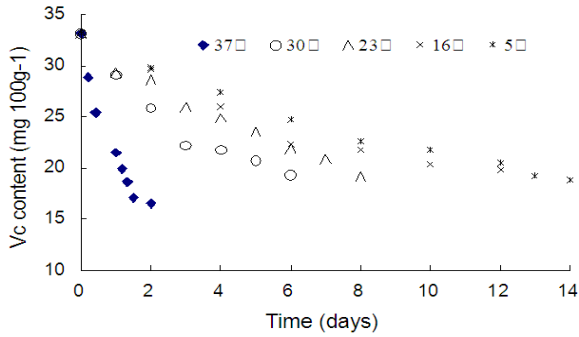
Fig. 1: Degradation kinetics of Vc in orange during storage at 5, 16, 23, 30 and 37 °C

period of less rapid degradation as the Vc content decreased. The high rate of loss at the beginning of the storage may be attributed to immediate reaction of an amount of ascorbic acid with the dissolved oxygen (Polydera *et al.*, 2003). The retention of Vc is not only dependent on storage temperature but also on storage time. The longer the time and the higher the storage temperature, the greater the degradation of Vc. The trend is consistant with published studies that high storage temperature had a negative effect on Vc retention (Lee and Chen, 1998; Kabasakalis *et al.*, 2000; Elez-Martínez *et al.*, 2006; Cortés *et al.*, 2008).
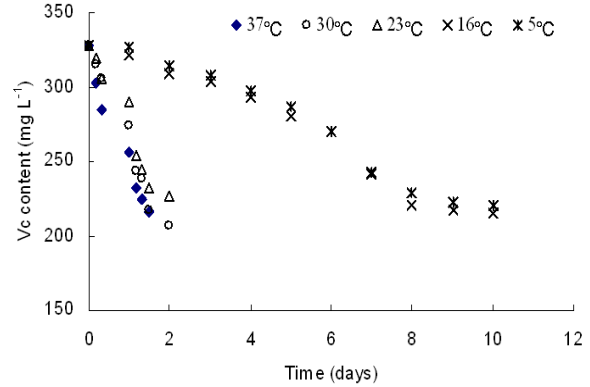

Fig. 2: Degradation kinetics of Vc in orange juice during storage at 5, 16, 23, 30 and 37 °C

V$_C$ degradation rate and reaction order during storage: The loss of Vc was calculated by using the standard equation for a first-order reaction Eq. (2):

$$ln\ C = lnC_0 - kt \tag{6}$$

When Vc retention of orange or orange juice plotted to versus storage time (Fig. 3 and 4), Vc degradation rate k and determination coefficients R^2 of the curves were obtained (Table 1). Under a certain
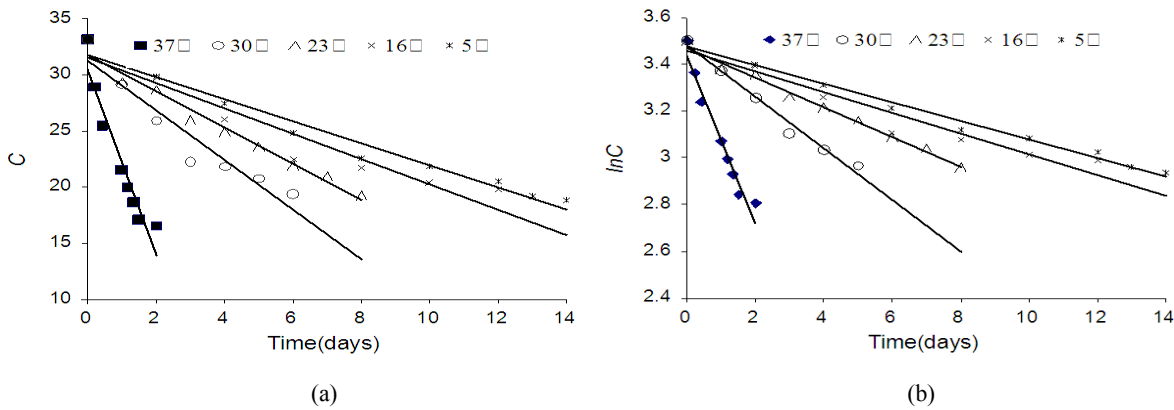

(a)   (b)

Fig. 3: *C-t* reflect the zero order degradation in orange, *lnC-t* reflect the first order degradation in orange
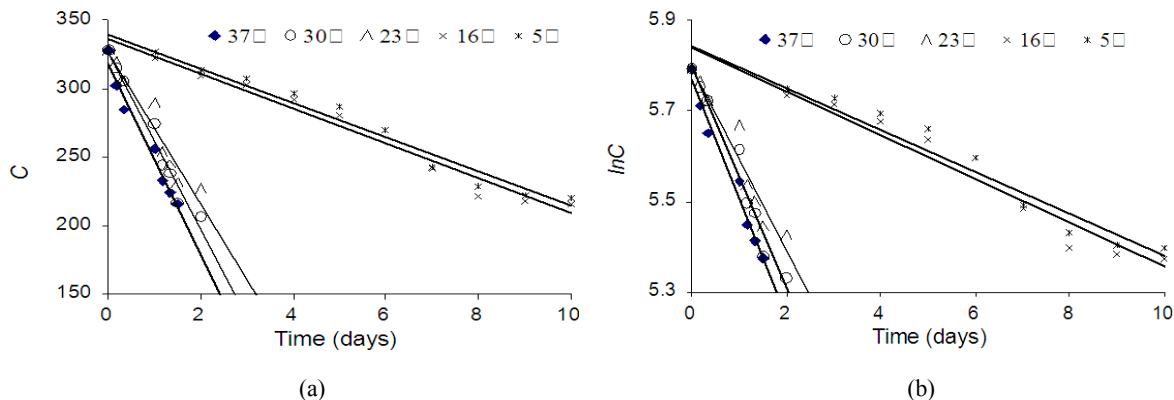

(a)   (b)

Fig. 4: *C-t* represents the zero order degradation in orange juice, *lnC-t* represent the first order degradation in orange juice

Table 1: V_C degradation rates k (/days), of reaction order during storage at 37, 30, 23, 16 and 0 °C in the temperature-controlled storage locker

Temperature (°C)	Zero-order				First-order			
	k-value(/days)		R^2		k- value (/days)		R^2	
	Orange	Orange juice	Orange	Orange juice	Orange	Orange juice	Orange	Orange juice
37	8.3182	69.561	0.9235	0.9719	0.3573	0.2617	0.9571	0.9778
30	2.2229	64.722	0.9155	0.9693	0.1102	0.2426	0.9811	0.9719
23	1.6180	55.483	0.9748	0.9341	0.0639	0.2022	0.9892	0.9410
16	1.1259	12.688	0.9134	0.9675	0.0443	0.0479	0.9395	0.9672
5	0.9830	12.467	0.9700	0.9653	0.0396	0.0460	0.9881	0.9549

Table 2: Half-life $t_{1/2}$ (days), activation energy E_0 and pre-exponential factor A in orange and orange juice

Temperature (°C)	$t_{1/2}$ (days)		E_0 (kJ/mol)		A	
	Orange	Orange juice	Orange	Orange juice	Orange	Orange juice
37	2	2	44.068	46.207	1.236×10^7	1.9385×10^7
30	6	2				
23	10	3				
16	15	14				
5	17	15				

temperature, the order of reaction was estimated graphically by comparing determination coefficient (R^2). The higher the linear determination coefficient R^2 is, the better the reaction order is suited. However, the plot of change in logarithm of Vc retentions yielded higher determination coefficients (Table 1). So, the loss of Vc in orange or orange juice at all temperatures was described as a first-order reaction. The reaction rate constant (k) of Vc increased from 0.0396 to 0.3573/d for orange (0.0460 to 0.2617/d for orange juice) with the temperature from 5 to 37°C for orange, which is in agreement with the report of Rahman *et al*. (2015). The degradation of Vc in both orange and orange juice are better fitted with the first-order with R^2 range values of 0.9395-0.9892 for orange (0.9410-0.9778 for orange juice) while those determination coefficients were lower in second-order model with R^2 range values of 0.9235-0.9748 for orange (0.9341-0.9719 for orange juice) (Serpen *et al*., 2007).

The half-life $t_{1/2}$, activation energy E_0 and pre-exponential factor A of V_C degradation reactions in orange: The effect of storage temperature on Vc degradation rate was described adequately by Arrhenius kinetics Eq. (7), as illustrated in Fig. 5. The correlation coefficient r is 0.8890 for orange and 0.9035 for orange juice in this study in the regression analysis. Both of orange and orange juice were observed to have a good fit with first-order kinetics for the significant correlation coefficient ($r_{0.05} < |r| < r_{0.01}$).

$$lnk = ln\, A - E_0/RT \qquad (7)$$

Activation energy valuse and pre-exponential factor A were calculated by using Arrhenius plots of Vc degradation in orange juice were showed in Table 2. Half destruction time of Vc was found higher in orange than orange juice. Vc in orange juice at 37 and 30°C
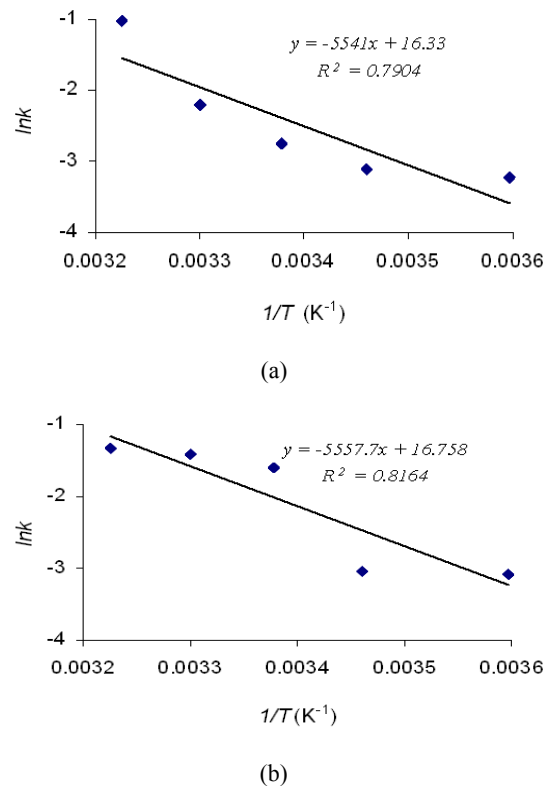

(a)

(b)

Fig. 5: V_C first order degradation *lnk-1/T* in orange (A) and orange juice (B)

were found almost similar. The activation energy values were 44.068±0.67 and 46.207±0.27 kJ/mol for orange and orange juice respectively. There was reported that the chemical reaction activation energy values E_0 were obtained in the range of 51 to 135 kJ/mol for Vc degradation during storage in various orange and orange juice in the temperature range of 0-45°C (Polydera *et al*., 2005; Zanoni *et al*., 2005). The activation energy values of Vc in the experiment were 44.068 and 46.207 kJ/mol for orange and orange juice respectively (Table 2) suggesting that the V_C was prone

Table 3: V_C content of prediction and test in orange and orange juice

Temperature (ºC)	Predicted value		Measured value		Error (%)	
	Orange (mg/ 100g)	Orange juice (mg/L)	Orang (mg/ 100g)	Orange juice (mg/L)	Orange	Orange juice
37	17.92	246.09	16.55	255.86	7.64	-3.97
30	30.36	278.67	28.94	274.48	4.68	1.50
23	31.36	294.46	28.68	289.96	8.54	1.53
16	32.02	312.11	29.72	322.05	7.18	-3.18
5	32.64	320.12	29.91	326.85	8.36	-2.10

to degradation reaction during storage for both of orange and orange juice. Vc is usually degraded by oxidative processes such as light, oxygen, heat, peroxides and enzymes (García *et al*., 2001; Bull *et al*., 2004; Torregrosa *et al*., 2006). But in our study, the first-order kinetic mode of V_C degradation for both orange and orange juices was constructed without taking photosynthesis and oxygen action into consideration. Therefore, further research dealing with the impact of light and oxygen treatments on V_C degradation in orange and orange juice should be addressed to make sure the model more accurate.

Degradation dynamics model of V_C: V_C degradation kinetics model of first order Eq. (8) was acquired from Eq. (2) and Eq. (5). With data E_0, A and R substituted into Eq. (8), Eq. (9) and Eq. (10) were obtained to be applied to calculate original bulk of V_C in orange and orange juice, storage period and residual quantity of V_C during the storage:

$$t = (lnC/C_0)/[A \, exp(-E_0/RT)] \tag{8}$$

$$t = (lnC/C_0)/[1.236 \times 10^7 exp(-5.541 \times 10^3/T)] \tag{9}$$

$$t = (lnC/C_0)/[1.9385 \times 10^7 exp(-5.5577 \times 10^3/T)] \tag{10}$$

The predicted values and measured values of vitamin C content on different temperature treatment was obtained by Eq. (9) in orange with 2 days storage and by Eq. (10) in orange juice with 1 d storage. The result (Table 3) revealed that the prediction error is within 9%. Figure 5 showed the linear correlation analysis on the predicted value and measured value of Vc content, which helps to calculate the determination coefficient $R^2 = 0.9932$ in orange and $R^2 = 0.9423$ in orange juice. As a result, the correlation coefficient $r = 0.9966$ is significant at the 0.01 ($r_{0.01} = 0.959$), proving the Vc degradation reaction models corresponding to the first order are effective in both of orange and orange juice.

CONCLUSION

Vc degradation in both orange and orange juices increased with increasing temperature during storage at 5-37°C. The increased temperature and storage time

resulted in a great of decrease on Vc. Vc degradation in orange and orange juices at all storage temperatures was better suited by a first-order reaction model through nonlinear regression methods. Following the Arrhenius model, the reaction rate constant (*k*) of Vitamin C increased from 0.0396 to 0.3573/d with the increase of temperature from 5°C to 37°C for orange while the *k* ranged from 0.0460 to 0.2617/d for orange juice. The results indicated that the temperature made a great contribute to Vitamin C degradation during storage.

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