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Kinetics of Ascorbic Acid Degradation and Quality Changes in Guava Juice during Refrigerated Storage

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Abstract In this study, the kinetics of ascorbic acid degradation and quality changes in guava juice during refrigerated storage at various temperatures were investigated The ascorbic acid degradation in all juice samples during refrigerated storage was fitted in first-order reaction with high regression coefficient (r^2≈0.961-0.999). Lowest rate constant (k) was obtained in all juice samples stored at 5°C (3.0-5.8 × 10^{-2} day^{-1}), followed by 10°C (3.8-7.1 × 10^{-2} day^{-1}) and 15°C (5.2-9.1 × 10^{-2} day^{-1}). Accordingly, the half-life ($t_{1/2}$) values of ascorbic acid were longer when juice samples stored at lower temperature. The estimated activation energy (Ea) of ascorbic degradation was in the range of 0.443-0.544 kcal/mol for 30-100% juice samples. Quality attributes of juice samples stored at various refrigerated temperature for 21 days did not showed any significant difference (p>0.05) in pH (3.80-3.83), acidity (0.25-0.29%), and total soluble solids (12.00-12.20 °Brix). Microbial populations were below the detection limit during the whole storage period.

Keywords: *kinetics, ascorbic acid, antioxidant activity*

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1. Introduction

Guava (*Psidium guajava* Linn.) is a tropical fruit and a member of Myrtaceae family. It can be cultivated in tropical/sub-tropical regions and is available throughout the year. There are many cultivars of guava, but the most popular varieties consumed in Thailand are white flesh color such as Kimju, Sithong, Glomsalee and Vietnam. Some guavas of different cultivars have pink flesh color such as Samsi, Beaumount and Kahuakula. Guava is consumed as a fresh or preserved fruit and in the form of juice beverage. The white flesh color is rather used for juice production due to its color, pleasant taste and exotic flavors [1]. From the review literature, several researchers have demonstrated that guavas from different cultivars contain variable vitamin C and antioxidant content, which in turn also depends on the pre- and post-harvest conditions [1,2].

Apart from being the rich source of vitamin C (50-300 mg/100 g edible fruit) and antioxidants, guava also contains vitamin A, vitamin B, folate, dietary fiber and essential minerals; potassium, magnesium, manganese, and iron [2,3]. In general, vitamin C play a vital role in the human body such as synthesis of collagen tissue, related to the nervous system, acts as an antioxidant, increases absorption rate of calcium and folic acid and boosts the immune system. In addition, increased intake of vitamin C is associated with the reduced risk of chronic diseases such as cardiovascular disease and cataracts. Thus, vitamin C could promote health and immunity and prevent DNA mutation induced by oxidative stress [4]. The defensive mechanism of human body is strongly influenced by the hydrophilic antioxidants, particularly the high ascorbic acid and phenolic compounds contained in natural guava fruits [5].

Ascorbic acid is water soluble, heat sensitive and could get oxidized/degraded upon exposure to oxygen, higher temperature and metal ion catalysts. Besides, the pH factor and sugar presence are well related to the ascorbic acid reduction [6,7]. Ascorbic acid degradation in juices during storage is a serious problem of food processing industries [8,9,10], particularly in retaining the high vitamin C content in guava juice for health benefits [11,12]. Due to the heat treatment and pasteurization, a significant amount of vitamin C as well as other essential ingredients is lost, thereby lowering the nutrient quality of processed juices. The retention of ascorbic acid content is usually lowered by bruising, mechanical injuries, and by excessive trimming. Therefore the loss of ascorbic acid could be minimized by retaining the nutrient quality of juices.

From the previous studies, the vitamin C degradation in juice products such as orange, strawberry, pomegranate, and grapefruit were noticed during the pre-harvest and post-harvest periods. Although, there were few studies performed on the ascorbic acid loss in guava juice during processing [13], but there was no information reported on the kinetics of ascorbic acid degradation in guava juice during refrigerated storage. Therefore, the aim of this study was to evaluate the kinetics of ascorbic acid degradation in guava juice prepared at various juice concentrations (30%, 60% and 100% w/w) during refrigerated storage at 5°C, 10°C, and 15°C for 21 days. In

addition, the changes in physical quality and microbiological indexes were also investigated to ensure the products quality and safety for consumption.

2. Materials and Methods

2.1. Chemicals and Culture Media

The chemical reagents such as metaphosphoric acid (HPO_3), sodium hydroxide (NaOH), dithiothreitol (DTT; $C_4H_{10}O_2S_2$), potassium dihydrogen phosphate (KH_2PO_4), and L-ascorbic acid were acquired from Sigma-Aldrich (USA). The culture media namely tetrathionate broth (TTB) with brilliant green and iodine, Rappaport-Vassiliadis (RV), hektoen enteric agar (HEA), xylose lysine deoxycholate agar (XLDA), brain heart infusion broth (BHIB), Baird-Parker medium containing egg yolk tellurite (BP), and Levine's eosin-methylene blue agar (L-EMBA) were obtained from Oxoid (United Kingdom). The bismuth sulfite agar (BSA), trypticase soy broth (TSB), trypticase soy agar (TSA), lauryl sulfate tryptose broth (LSTB) brilliant green lactose 2% bile broth (BGLB), plate count agar (PCA), and dichloran rose-bengal chloramphenicol agar (DRBCA) were purchased from Merk (German).

2.2. Pasteurized Guava Juice

Guava fruits (*Psidium guajava* Linn.), white flesh color and Kimju cultivar were harvested at the orchard, Sampran, Nakorn Pathom province, Thailand. On the harvested day, the whole fruits were washed with running water, followed by trimming, and then cut into small pieces. The guava pulp was mixed with deionized water at various ratios 30:70, 60:40, and 100:0 w/w to produce the 30%, 60% and 100% guava juices, respectively. Subsequently, the guava-water mixture was coarsely ground using juice extractor (Thai Wasino Electric, Samutprakarn, Thailand) and then the juice was extracted with the aid of hydraulic pressing (Sakaya Automate, Bangkok, Thailand). The extracting juices were further added with granulated sugar (8.0-8.5%), citric acid (0.2-0.25%) and salt (0.16%) to recover the accepted taste. All the mixed juice samples were pasteurized at 85°C for 1 min and then hot filled in plastic bottles (180 ml), and immediately cooled in an ice-water bath.

2.3. Storage Study of Guava Juice

After pasteurization, the finished juice products prepared at various concentrations (30, 60 and 100 %) were stored in refrigerator at different temperatures (5, 10 and 15°C) for 21 days. During the refrigerated storage, there samples were randomly taken at 0^{th}, 3^{rd}, 5^{th}, 7^{th}, 10^{th}, 14^{th}, and 21^{st} days for the following analyses.

2.4. Ascorbic Acid Analysis

The ascorbic acid content was analyzed according to the modified method of María et. al, [14]. Briefly, juice sample (5 g) was placed in volumetric flask, following by addition of 3 ml of 10% metaphosphoric acid (HPO_3) and diluted to 50 ml with distilled water. A volume of 10 ml was pipetted out and adjusted to pH 5-5.2 with 1 M sodium hydroxide (NaOH), then added with 10 mg of dithiothreitol (DTT; $C_4H_{10}O_2S_2$), and was kept in the dark place for 1 h. The samples were diluted to 25 ml with 2% HPO_3 and filtered through 0.45 µm membrane filter before analysis by high performance liquid chromatography (HPLC) with ultraviolet (UV) detector at wavelength 254 nm. The analysis was performed on a Zorbax x 5 µm ODS (octadecylsilane); 25 x 0.46 cm with guard column, flow rate 0.7 ml/min with 10% potassium dihydrogen phosphate (KH_2PO_4) for mobile phase. A standard calibration curve was acquired by using L-ascorbic acid (Merck, Germany). The ascorbic content (mg/100g) was calculated using the CSW 32 software program.

2.5. Degradation Kinetics of Ascorbic Acid

The degradation of ascorbic acid in guava juices upon refrigerated storage was evaluated using the first-order kinetic models as equation 1.

$$C = C_0 \exp(-kt) \quad (1)$$

where C_0 represent the initial concentration of ascorbic acid, C represent the concentration of ascorbic acid at time t, *t* is the storage time and k is the ascorbic acid degradation rate constant ($time^{-1}$).

Half-life ($t_{1/2}$) is the estimated time where the concentration of ascorbic acid decreased by 50% from the initial value ($C = 0.5C_0$). Half-life of each guava juice concentrations at its corresponding storage temperature was determined by using equation 1.

The temperature dependence of the ascorbic acid degradation expressed in terms of the activation energy (E_a) as described by Arrhenius kinetics are as follow:

$$k = k_0 \exp(-E_a / RT) \quad (2)$$

$$\ln k = \ln k_0 - E_0 / RT \quad (3)$$

where, k is the rate constant, k_0 is frequency factor or the Arrhenius constant ($time^{-1}$), E_0 (kcal/mol) is the activation energy, R is the universal gas constant (1.987 kcal/mol K), and T is the absolute temperature in Kelvin.

2.6. Physical Juice Quality

The total soluble solids was measured using model N1 refractometer (Atago, Tokyo, Japan), whilst pH was determined by a Delta 340 pH meter (Mettler Toledo, Columbus, OH, USA) with an InLab 413 probe. The titratable acidity was determined according to the AOAC 942.15. The appearance of juice color was observed by Munsell Book of color under day light fluorescent.

2.7. Microbiological Indexes

For the detection of *Salmonella* spp. (BAM, 2011), juice sample (50 ml) was diluted with 450 ml of lactose broth, then adjusted to pH 6.8, and was incubated at 35°C for 24 h. The pre-enriched culture of 0.1 ml was transferred to 10 ml tetrathionate troth (TTB) with brilliant green and iodine, and incubated at 35°C for 24 h. As well, the culture of 1 ml also was conveyed to 10 ml Rappaport-Vassiliadis (RV), and incubated at 42°C for 24 h, respectively. A loopful of broths was streaked on Hektoen Enteric Agar (HEA), bismuth sulfite agar (BSA), and

xylose lysine deoxycholate agar (XLDA), and then incubated at 35°C for 24-48 h.

Staphylococcus aureus analysis was performed by direct plate count (BAM, 2001). Juice sample (50 ml) was diluted with 450 ml of Butterfield's phosphate buffered. The serial dilutions of food sample (1 ml) were transferred on Baird-Parker (BP) medium containing egg yolk tellurite in three plates at level 0.3, 0.3 and 0.4 ml per plate, respectively. All plates were inverted and incubated at 35°C for 48 h. The suspected colony were transmitted to brain heart infusion broth (BHIB) and trypticase soy gar (TSA) slant, and incubated at 35°C for 24 h.

The three tubes MPN method (BAM, 2002) was performed to examine the *Coliforms* and *Escherichia coli*. Juice sample (0.1 ml) of three serial dilutions were pipetted into 10 ml lauryl sulfate tryptose broth (LSTB), and incubated at 35°C for 24-48 h. A loopful of positive culture was inoculated in 10 ml brilliant green lactose bile broth 2% (BGLB) and incubated at 35°C, 48 h for *Coliforms*. For E. *coli* detection, a loopful of culture was injected to 10 ml EC broth and incubated at 45.5°C for 24-48 h. The positive cultured broth was streaked on eosin-methylene blue agar (EMBA) and incubated at 35°C for 18-24 h. The E. *coli* colony was streaked on plate count agar (PCA) slant and incubated at 35°C for 18-24 h.

The serial dilution of juice samples were prepared by mixing with 0.1% peptone water. For aerobic plate count (BAM, 2001), the sample (1 ml) was poured on plate count agar (PCA) and incubated at 35°C for 24 h. Yeast and mold (BAM, 2001), the dilution (0.1 ml) was spread on dichloran rose-bengal chloramphenicol agar (DRBCA) plates and incubated at 25°C for 5-7 days.

2.8. Statistical Analysis

Two independent experiments were performed for each study. All the analyses were conducted in triplicate. One-way Analysis of Variance (ANOVA) and Duncan's test for significance were performed using SPSS Statistics version 18.0 (IBM Inc., USA).

3. Results and Discussion

3.1. Quality of Finished Guava Juice

The consumer's preference for taste likeness, the optimum sugar, citric acid, and salt were modified following the formula obtained in this study to improve the sweetness, sourness and saltiness, respectively. The measurement of physical attributes such as pH value, acidity, and total soluble solids were monitored to control the finished juice. All the physical features of 30, 60 and 100% guava juice beverages did not showed any significance difference ($p<0.05$) before and after pasteurization at 85°C for 1 min. All the juice samples had total soluble solids (TSS) of 12 °Brix, pH 3.8, and total acidity 0.28%. As per aroma flavor is concerned, 100 % guava juice beverage had strong flavor compared with 60% and 30% guava juices, respectively. The pathogenic bacteria such as *Salmonella* spp. (cfu/25 ml) and *Staphylococcus aureus* (cfu/ml), including aerobic plate count (cfu/ml) and yeast and mold (cfu/ml) were not detected in finished guava juices, whilst *Escherichia coli* (MPN per 100 ml) and *Coliform* bacteria (MPN per 100 ml) were less than 1.1. Since, the juice production were manufactured at pilot plant, Institute of Nutrition, Mahidol University, the good manufacturing practices (GMP) and hazard critical control points (HACCP) were strictly followed.

After extraction, the ascorbic acid content in 30, 60 and 100% guava juice samples were about 44, 87 and 156 mg/100g, respectively. However, the ascorbic acid content decreased significantly ($p<0.05$) after pasteurization to 33, 67 and 125 mg/100g, thereby resulting about 26, 24 and 20% loss in 30, 60 and 100% juice samples, respectively. The results were in agreement with previous studies of orange juice [8,9,15,16], pomegranate juice [17], and strawberry juice [18] which contained the higher vitamin C; whilst thermal process played an important role for vitamin C degradation by converting the ascorbic acid to dehydroascorbic acid under aerobic condition.

The process of pasteurization destroyed the pathogenic microorganisms and inhibited the enzymes, but it resulted in adverse effects on vitamin C content and loss of nutritional value. Therefore, it is recommended that an optimum temperature and time for pasteurization of juice products must be applied. As per previous study, the appropriate pasteurization for guava juice was 85°C for 1 min in which higher vitamin C and good aroma flavor could be retained, and is safe from pathogenic bacteria. Nevertheless, optimum pasteurization for guava juice was different according to the condition utilized by different authors [13,19]. The slight deviation in temperature and time for pasteurization might be due to the preparation process and juice treatments, particularly the pH value of juice. In this study, the pH of juice was 3.8. It must be noted that the pH lower than 4.6 could inhibit the growth of *Clostridium botulinum* and prevent production of botulism toxin.

3.2. Ascorbic Acid Loss in Guava Juice during Refrigerated Storage

During refrigerated storage, it was proved that juice beverages are prone to ascorbic acid degradation and loss of other nutrients essential for health benefits, in particularly during prolonged storage [8,9,18,20]. The results showed that the initial vitamin C content of 30% guava juice was 32 mg/100 g. During storage at 5°C, 10°C and 15°C for 21 days, the vitamin C decreased significantly ($p<0.05$) to 9.6 mg/100 g, 7.0 mg/100 g and 5.2 mg/100 g, respectively (Figure 1a). The maximum percentage of ascorbic acid retained in juices stored at 5°C, 10°C and 15°C for 21 days was about 30, 22 and 16%, respectively. Similar trend was noted in more concentrated juice samples i.e., in 60% and 100% juices, in which vitamin C declined continuously ($p<0.05$) with the increasing storage period at various temperatures. The ascorbic acid content in 60% juice sample was 61 mg/100 g at day 0, and it was reduced to 22.2 mg/100 g, 18.3 mg/100 g and 13.2 mg/100 g at 5°C, 10°C and 15°C, respectively after 21 days of storage period (Figure 1b). The results indicated that about 36, 30 and 22% of ascorbic acid was retained in the 60% juice samples, stored at 5°C, 10°C and 15°C, respectively for 21 days. In case of 100% juice sample, the vitamin C content also decreases considerably from 114 mg/100g to 60.7 mg/100 g, 50 mg/100 g and 36.8 mg/100 g after storage at 5°C,

10°C and 15°C, respectively (Figure 1c). In general, the percentage retention of vitamin C in 100% juice was higher than that of 60% and 30% juice concentrations. It was noted that about 61, 58 and 45% of vitamin C was retained in 100% juice samples stored at 5°C, 10°C and 15°C, respectively for 21 days.

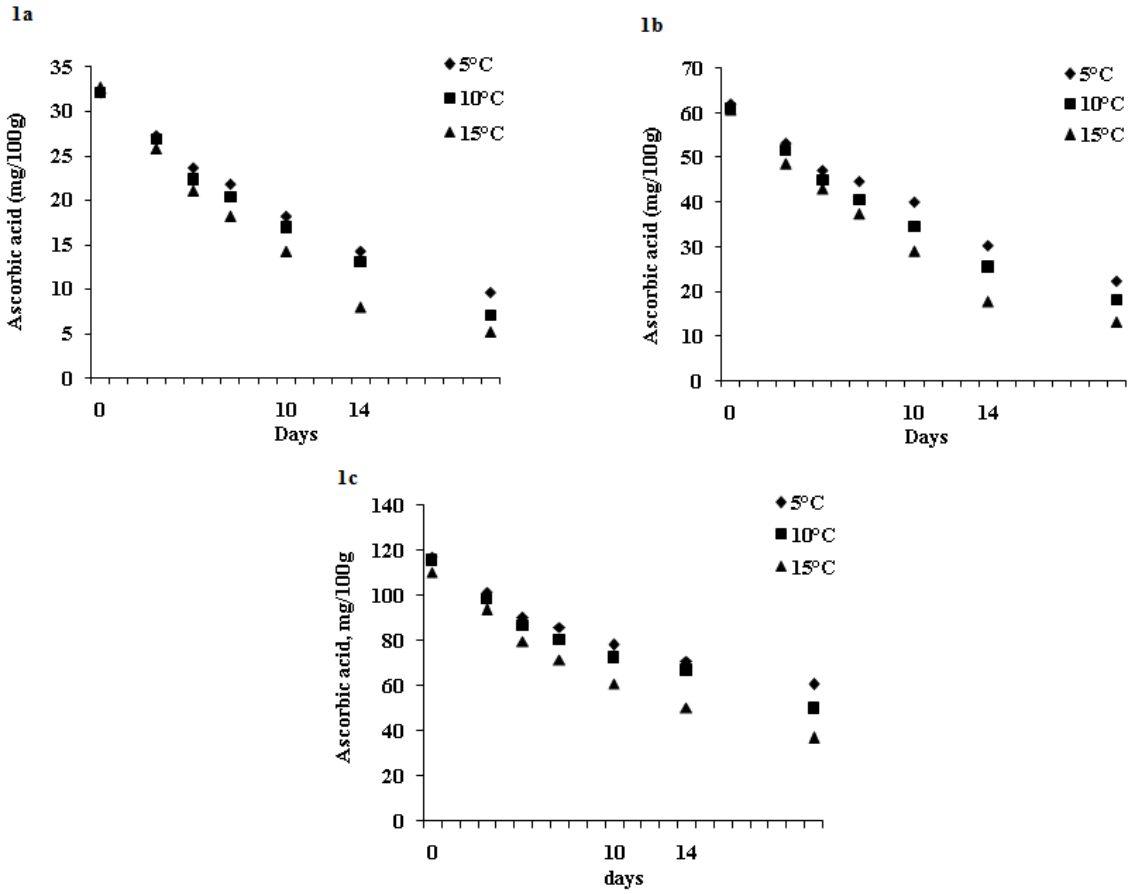

Figure 1. Ascorbic acid degradation in guava juice during storage at 5°C, 10°C and 15°C for 21 days. (1a) 30% guava juice; (1b) 60% guava juice; and (1c) 100% guava juice

The decrease of ascorbic acid with increasing temperature storage was correlated with previous studies [8,18]. It was further noted that increased temperature could promote the reduction-oxidation rates by triggering the reagent substances to move faster and reducing the activation energy of its reaction. Subsequently, the oxidation of ascorbic acid will change its reduced form to become the oxidized form or known as dehydroascorbic acid. The dehydroascorbic acid undergo hydrolysis to 2,3-diketogulonic acid, which then polymerize and form other nutritionally inactive products.

3.3. Kinetics of Ascorbic Acid Degradation in Guava Juice during Refrigerated Storage

The plot of ascorbic acid versus storage time at various temperatures of all juice samples is shown in Figure 1a, Figure 1b and Figure 1c. It was observed that ascorbic acid was dramatically reduced particular between 0-7 days of storage period. This might be due to the effect of dissolved oxygen present in the finished juice which played an important role in oxidization of ascorbic acid at the first step. As the storage time increased, the rate of further oxidation of ascorbic acid could involve the anaerobic reaction [21,22,23].

At the same storage conditions, the highest ascorbic acid content was retained in 100% juice samples followed by 60% and 30% juice samples. It was probably due to the highest juice concentration which could raise the level of ascorbic acid and antioxidants such as polyphenol compounds and flavonoids in the samples [9,24]. Those substances could retard the rate of oxidation reactions. From the review literature, the antioxidant activity in guava fruits is performed by phenolic compounds and vitamin C [5]. For the white flesh guava, the higher antioxidant activity and total phenolic content were retained [2,3].

The ascorbic acid degradation of juices during refrigerated storage is usually described by a first order model [8,15,25]. In this study, the regression coefficient (r^2) of curve plotted between logarithm of ascorbic acid versus time, was very high (0.961-0.999) and rather close to 1 than plot of ascorbic acid against time (0.910-0.972). Hence, the ascorbic acid degradation in guava juice at various concentrations stored at different temperatures was described as a first-order reaction (Equation 1). The temperature dependence of ascorbic acid degradation was determined by using the Arrhenius following Equation 2 and 3. The rate constants, k (day^{-1}) of ascorbic acid degradation in 30% (Figure 2a), 60% (Figure 2b) and 100% juice samples (Figure 2c) were obtained from the plot of logarithm of ascorbic acid retention ratio ($\ln C_t/C_0$) versus time (day) at all storage temperatures. At lower storage temperature (5°C), the rate constant (k, day^{-1}) of ascorbic acid degradation in all juice samples was lowest as compared to the juice samples stored at 10°C and 15°C,

respectively (Table 1). This was consistent with the Arrhenius Theory and in agreement with the previous study, in which the rise in storage temperature led to higher ascorbic acid degradation and retained very less ascorbic acid in the juice samples [8,18,20]. It was further noted that the half-life ($t_{1/2}$) values of ascorbic acid from juice samples stored at higher temperatures were shorter than juice samples stored at lower temperature (Table 1).

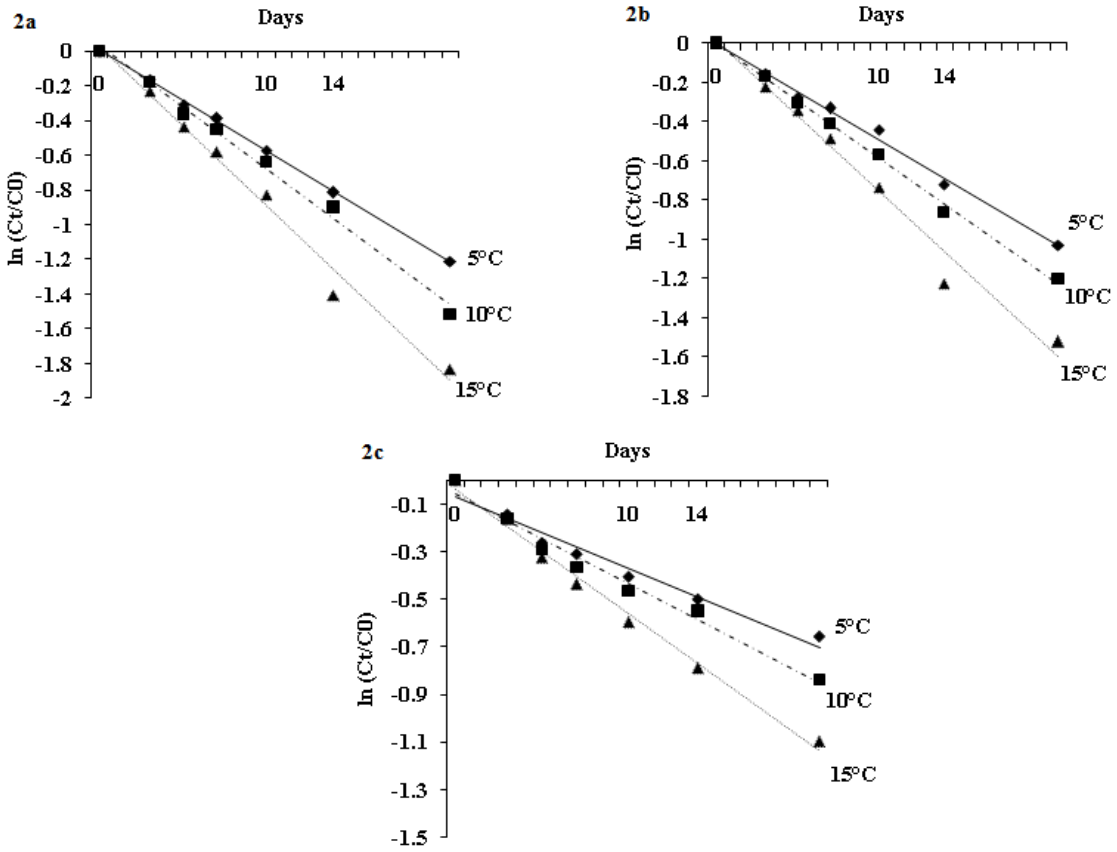

Figure 2. Kinetics of ascorbic acid degradation in guava juice during storage at 5°C, 10°C and 15°C for 21 days. (2a) 30% guava juice; (2b) 60% guava juice; and (2c) 100% guava juice

Table 1. Kinetic and arrhenius parameters for ascorbic acid degradation in guava juice

Juice concentration	Storage temperature	K $\times 10^{-2}$day^{-1}	r^2	$t_{1/2}$ day	Ea Kcal/mol
	5°C	5.79	0.9993	11.97	
30%	10°C	7.07	0.9925	9.80	0.451
	15°C	9.12	0.9876	7.60	
	5°C	4.90	0.9933	14.14	
60%	10°C	5.81	0.9968	11.93	0.443
	15°C	7.65	0.9804	9.06	
	5°C	3.03	0.9612	22.87	
100%	10°C	3.78	0.9777	18.33	0.544
	15°C	5.24	0.9915	13.23	

At same storage condition, the rate constant (k, day^{-1}) of ascorbic acid degradation decreased with the increase in juice concentration. The lowest rate constant was found in 100% juice sample, followed by 60% and 30% juice samples, respectively at all storage temperatures (Table 1). In addition, the higher value of half-life was noticed in ascorbic acid present in highest juice concentration, at same storage temperature (Table 1). This was probably associated with the ascorbic acid and antioxidant levels that obtained in various juice concentrations. Increasing the total antioxidants could protect the ascorbic loss and reduce the oxidation reaction rate. The refrigerated condition of guava juice at 5°C, 10°C and 15°C for 21 days of storage influenced the ascorbic degradation. Furthermore, the addition of sugar and citric acid in juice formula affected the oxidation process by reducing the dissolved oxygen and chelating with metal ions, respectively. It has been reported that sucrose could inhibit the ascorbic acid oxidation in a closed aqueous system [26].

The activation energy (E_a) of vitamin C degradation was calculated by rate constant (lnk) against the reciprocal of the absolute temperature (K) followed Arrhenius Equation 2 and 3 (Figure 3a, Figure 3b and Figure 3c). The estimated values were 0.451 kcal/mol, 0.443 kcal/mol and 0.544 kcal/mol for 30, 60 and 100% juice samples, respectively (Table 1). Therefore samples with higher guava juice concentration showed the retarded rate of ascorbic acid degradation, thus indicating the effectiveness of

high juice concentration for better vitamin C retention in guava juice.

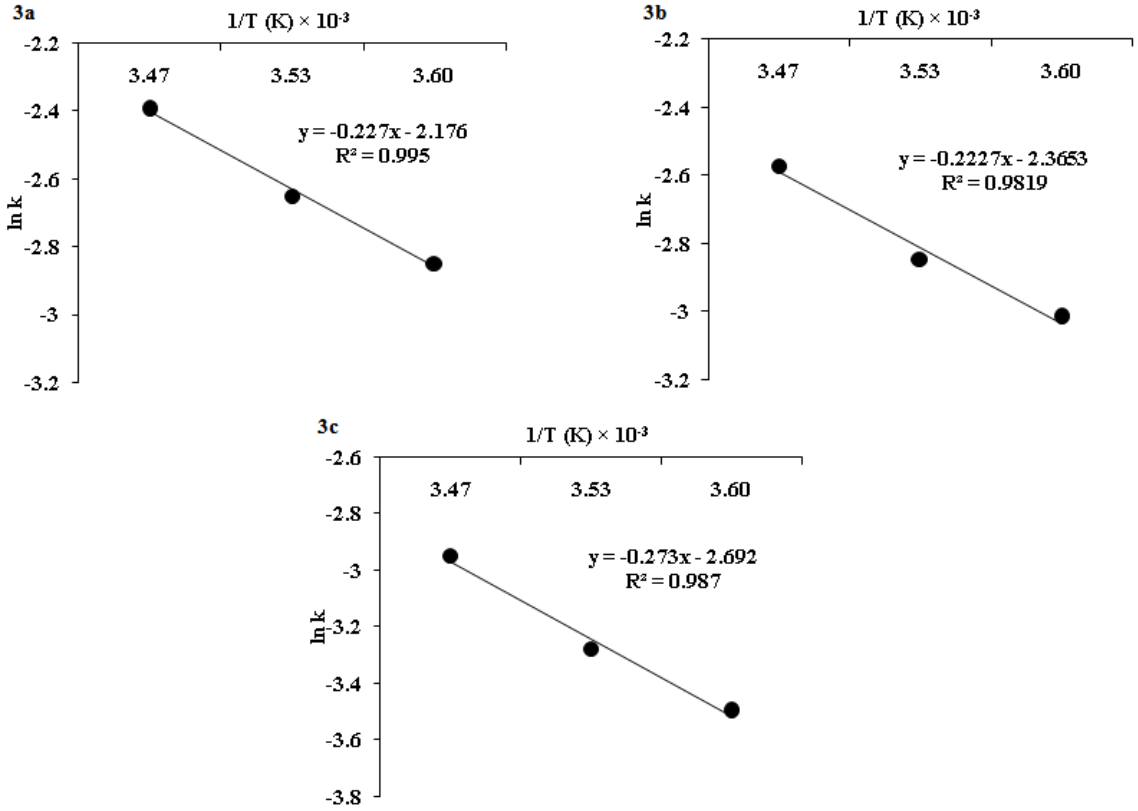

Figure 3. Arrhenius plot of ascorbic acid degradation in guava juice. (3a) 30% guava juice; (3b) 60% guava juice; and (3c) 100% guava juice

Table 2. Change in pH, acidity, and total soluble solids of guava juice during refrigerated storage

Juice	Parameters	Temp	Storage Time (days)						
%		°C	0	3	5	7	10	14	21
		5	3.82	3.83	3.81	3.83	3.82	3.81	3.82
	pH	10	3.83	3.81	3.80	3.81	3.82	3.81	3.82
		15	3.82	3.81	3.83	3.82	3.82	3.80	3.81
		5	0.28	0.28	0.27	0.28	0.26	0.28	0.28
30	**Acidity, %**	10	0.27	0.29	0.27	0.26	0.27	0.25	0.25
		15	0.28	0.28	0.28	0.27	0.26	0.26	0.29
		5	12.10	12.05	12.05	12.20	12.00	12.20	12.10
	Brix, %	10	12.00	12.05	12.20	12.05	12.15	12.10	12.20
		15	12.05	12.10	12.15	12.10	12.05	12.00	12.00
		5	3.82	3.81	3.83	3.82	3.82	3.83	3.82
	pH	10	3.81	3.83	3.80	3.82	3.81	3.81	3.80
		15	3.82	3.81	3.81	3.80	3.82	3.81	3.82
		5	0.28	0.27	0.27	0.26	0.27	0.27	0.26
60	**Acidity, %**	10	0.28	0.26	0.26	0.28	0.27	0.28	0.27
		15	0.27	0.28	0.27	0.27	0.28	0.27	0.27
		5	12.10	12.05	12.15	12.00	12.15	12.15	12.10
	Brix, %	10	12.00	12.10	12.20	12.10	12.20	12.20	12.05
		15	12.10	12.00	12.10	12.10	12.00	12.15	12.15
		5	3.82	3.81	3.81	3.83	3.82	3.82	3.82
	pH	10	3.82	3.82	3.82	3.81	3.81	3.81	3.81
		15	3.81	3.82	3.82	3.82	3.82	3.80	3.82
		5	0.28	0.28	0.26	0.26	0.25	0.26	0.26
100	**Acidity, %**	10	0.28	0.28	0.28	0.27	0.28	0.26	0.29
		15	0.28	0.27	0.27	0.28	0.26	0.28	0.28
		5	12.05	12.05	12.00	12.00	12.20	12.02	12.00
	Brix, %	10	12.05	12.10	12.00	12.10	12.15	12.10	12.20
		15	12.00	12.15	12.10	12.00	12.20	12.15	12.10

3.4. Changes in Physical Quality of Guava Juice during Refrigerated Storage

Over prolonged storage at 5°C, 10°C and 15°C for 21 days, all guava juice samples did not showed any significant difference ($p>0.05$) in terms of pH (3.80-3.83), acidity (0.25-0.29%) and total soluble solids (12.00-12.20 °Brix) as shown in Table 2. It could be implied that the juices were stable and safe for consumption throughout the storage period. These results were supported by the microbiological indexes, in which aerobic bacterial counts (cfu/ml), yeast and mold (cfu/ml), *Salmonella* (cfu/25ml) and *Staphylococcus aureus* (cfu/ml) were not detected. *E. coli* and *Coliforms* were present in less than 1.1 MPN/100 ml at the end of storage period (Table 3). These facts hinted that the appropriate processing and pasteurizing conditions were applied for the production of guava juice beverage to conserve juice quality and retain the vitamin C content. Nevertheless, after storage for over 14 days, the slightly sedimentation were observed at the bottom in all the juice samples. The highest sedimentation was found in 100% juice, followed by 60% and 30% juice samples (Table 3). In fact, for the higher juice concentration samples, the bottom sedimentation was clearly visible due to the increased fruit pulps. The difference in the density of pulp particles and juice medium or continuous phase accelerate the sedimentation in bottle juice. The slight change in juice color was noticed during refrigerated storage at various temperatures in all the samples. The juice color was changed from light green (2.5GY 9/4) at beginning to a little bit brown green (5GY 9/4) at the end of storage period at all storage temperatures. This might be due to the effect of ascorbic acid oxidation. These have been reported previously that decomposition reactive products are formed by the degradation of vitamin C. These compounds might be combined with amino acids, thus results in formation of brown pigments, hydroxymethylfurfural (HMF) [8,23].

Table 3. Physical characteristics and microbiological indexes in guava juice during refrigerated storage

Juice (%)	Storage days	Physical characteristics (5, 10 and 15 °C)	Microorganism Indices (5, 10 and 15 °C)
30	0, 3, 5, 7 and 14	slightly cloudy, retained fresh guava aroma and slightly pale green color	Not detected* < 1.1**
	21	a little bit sedimentation, less fresh guava flavor and slightly brown green color	
60	0, 3, 5, 7 and 14	cloudy juice, decent flavor aroma of fresh guava and slightly green color	Not detected* <1.1**
	21	a little sedimentation, less flavor aroma and slightly pale brown green color	
100	0, 3, 5, 7 and 14	more cloudy, strong flavor aroma of fresh guava and slightly green color	Not detected* <1.1**
	21	a few sedimentation, less aroma and slightly pale brown green color	

*Aerobic bacteria count (cfu/ml), Yeast and mold (cfu/ml), *Salmonella* (cfu/25ml), and *Staphylococcus aureus* (cfu/ml).
***Escherichia coli* (MPN/100ml) *and Coliform* (MPN/100ml).

Furthermore, the amount of vitamin C required for adults is approximately 80 mg/day based on the Recommended Dietary Allowances (RDAs). The rate of ascorbic acid degradation could be prevented by storing juices at lower temperatures. It was noted that all juice samples stored at ≤10°C for 7 days resulted about 30% vitamin C losses. However, prolonged storage time resulted in rapid vitamin C degradation of about 40 %, 51 %, and 65 % at day 10, day 14, and day 21, respectively.

4. Conclusion

The ascorbic acid degradation in guava juice was significantly ($p<0.05$) reduced by refrigerated storage temperature and time and the juice concentration. The lowest rate constant (k, day^{-1}) of ascorbic degradation was found in guava juice samples stored at 5°C, followed by 10°C and 15°C, respectively. Moreover, the half-life ($t_{1/2}$) values of ascorbic acid in juice samples were longer when stored at lower temperature. The activation energy (Ea, kcal/mol) of ascorbic degradation decreased with increasing guava juice concentrations. The juice quality and microbiological safety in all guava juice during refrigerated storage for 21 days did not showed significant difference ($p>0.05$), thus was safe for consumption. However, the refrigerated storage of guava juice at 5°C for 7 days could provide more health benefits.

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