

## Stability of selected quality attributes of pink guava juice during storage at elevated temperatures

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### Article history

Received: 15 August 2015

Received in revised form:

22 December 2015

Accepted: 6 January 2016

### Abstract

Quality degradation is normally judge by monitoring independently the loss of a certain quality attribute during storage. However, the rate of degradation for each of the quality attributes present in a food product is not the same. This study focus on deterioration of vitamin C, lycopene, total phenolics and antioxidant activity of ready-to-drink pink guava juice (PGJ) during storage at elevated temperatures. Kinetic order, rate constant (k), activation energy (E<sub>a</sub>) and temperature coefficient (Q<sub>10</sub>) of the degradation were derived by applying Arrhenius equation. The results obtained showed that freshly made PGJ contain 39.79±2.18 mg/100 mL of vitamin C, 3.17±0.27 mg/L of lycopene, 28.08±4.11 mgGAE/100 mL of total phenolic content (TPC) and 13.20±1.91 mMTE/100 mL of ferric reducing antioxidant power (FRAP). All quality attributes measured in this study showed zero-order kinetic reaction. The results also showed that FRAP has the highest E<sub>a</sub> of 49.52 KJ/mol and Q<sub>10</sub> of 1.80, followed by vitamin C (E<sub>a</sub>=41.49 KJ/mol; Q<sub>10</sub>=1.64), lycopene (E<sub>a</sub>=31.75 KJ/mol; Q<sub>10</sub>=1.46), and lastly TPC (E<sub>a</sub>=14.11 KJ/mol; Q<sub>10</sub>=1.18). The predicted total depletion of each quality attribute at refrigerated storage (5°C) were 266 days for antioxidant activity, 158 days for vitamin C and lycopene, and 63 days for total phenolics. This study provide useful information on the degradation rate and availability of health beneficial and bioactive compounds present in fruit juice beverage during storage.

### Keywords

Quality degradation

Pink guava juice

Kinetic

Activation energy

Q<sub>10</sub>

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### Introduction

Guava (*Psidium guajava* L.) belongs to the Myrtaceae family, originated in the American tropics and is today distributed throughout the tropical and subtropical areas of the world. Guava can be classified into white and pink guava based on the colour of its flesh. The largest pink guava plantation in the world with over 500 hectares of guava fruit grown is located in Sitiawan, Perak, followed by Johor; both in Malaysia. Both white and pink flesh guava fruits are aromatic, rich in minerals and vitamins. Vitamin C content in guava fruit is known to be significantly higher than most citruses. This vitamin is an important nutrient known for its antioxidant and anticancer properties, and other health promoting effects (Vikram *et al.*, 2005). However, vitamin C is heat sensitive and is easily decomposed when subjected to heat treatment.

In pink flesh guava fruit, its attractive colour is attributed to the presence of the carotenoids pigment, lycopene. The colour intensity of the fruit is directly proportional to the lycopene content in the fruit flesh as well as the colour and lycopene amount in the

juice made from the fruit (Pasupuleti and Kulkarni, 2013). Although lycopene do not have any pro-vitamin A activity but it has been described as one of the most active antioxidants of all the carotenoid pigments (Atasoy, 2012). It has a singlet-oxygen quenching ability twice as high as β-carotene and 10 times higher than α-tocopherol (Atasoy, 2012). Degradation of carotenoids and its health beneficial effects are dependent on various factors. The highly unsaturated carotenoids molecules are prone to oxidative deterioration in which as polyene, lycopene undergoes cis-trans isomerisation induced by light, thermal energy and chemical reactions. Losses of carotenoids specifically of lycopene content during processing and storage have been reported in numerous studies (Sharma and LeMaguer 1996; Anguelova and Warthesen, 2000; Lee and Chen, 2002; Wang and Chen, 2006; Ferreira and Amaya, 2008; Naviglio *et al.*, 2008).

Nutrient loss in fruit juices is of considerable importance since consumers mostly consumed juices to derive their health beneficial effects. This health beneficial effects of fruit juices are also contributed by the phenolic compounds. The polyphenols present

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in guava fruit are ellagic acid and glycosides of myricetin and apigenin (Verma *et al.*, 2015) which contribute to the strong antioxidant activities of the fruit besides vitamin C and lycopene content. The amount of these beneficial compounds in fruit is dependent on the degree of ripeness, variety, climate, soil composition and storage conditions (Haminiuk *et al.*, 2012). The loss of these nutrients has serious consequences on the health-promoting effects of these bioactive compounds hence contributes to the loss of quality.

In order for a product to have a long shelf life, commercially produced products such as fruit juices receives a thermal treatment during processing. The processing step reduces the microbial load and inactivate enzyme present in the juice. It also promotes changes in colour, losses of vitamins and other health promoting compounds. Similarly, the storage conditions of the finished product are also responsible for the product quality deterioration. Knowledge of the nutrient content or other quality indicators kinetic including reaction rate as a function of temperature of storage is useful in predicting their stability. The reaction rate at which selected quality indicator deteriorates as a function of time can be used as a basis to predict the shelf life of a product. Similarly, mathematical models are used in food sciences to describe the reaction rate at elevated temperature. If the temperature-accelerating factor ( $Q_{10}$ ) is known then extrapolation to lower temperatures could be used to predict true product shelf life (Mizrahi, 2000).

The purpose of this study was to determine the degradation rate of vitamin C, lycopene, total phenolic and antioxidant activity during storage of pink guava juice. The degradation of these quality indicators was monitored at elevated temperature of 40°C and 50°C for 2 weeks. Kinetic order and rate constant (k) were derived from the plot of quality loss against storage time and used in calculating the activation energy ( $E_a$ ) and temperature coefficient ( $Q_{10}$ ) value. The information obtained from this study will contribute to the understanding on the mode of deterioration of nutrient and other health beneficial compounds contained in PGJ and to other types of fruit juice beverages available in the market. The elevated storage temperature used in this study will also provide useful data to predict the impact of temperature abuse during processing, transportation and storage as well as predicting the shelf life of a product at lower temperature storage.

## Materials and method

### Raw materials and chemicals

Pink guava puree was obtained from Sime Darby Food and Beverages, Sitiawan, Perak. Sucrose was purchased from TESCO Extra, Section 13, Shah Alam. The food grade chemicals such as citric acid, carmoisine red, sodium benzoate, potassium sorbate, xanthan gum and ascorbic acid were purchased from Meilun Food Chemicals Sdn. Bhd., Klang, Selangor. The analytical grade chemicals were obtained from Merck Sdn. Bhd., Shah Alam, Selangor; which includes potassium chromate, butylated hydroxytoluene (BHT), Folin-Ciocalteu reagent, 2,6-dichlorophenol-indophenol, acetone, ethanol, hexane, sodium carbonate, acetate buffer, 2,4,6-tripyridyl-triazine (TPTZ),  $FeCl_3 \cdot 6H_2O$ , hydrochloric acid and gallic acid.

### Preparation of Sample

Pink guava juice was prepared by adding pink guava puree (12.5%), sucrose (9%), stabiliser (0.20%), citric acid (0.15%), ascorbic acid (0.045%), sodium benzoate (0.015%), potassium sorbate (0.045%) and carmoisine red (0.005%). All the ingredients were homogenised using ultrasonic homogeniser at 20,000 rpm for 5 mins, pasteurised at 90°C for 5 mins and filled in polyethylene bottles. Prepared PGJ samples were stored at 40°C and 50°C in humidity chamber (RH 65%) and were evaluated for 2 weeks of storage at interval of 3 days.

### Determination of Vitamin C

Vitamin C content was determined by spectrophotometric method (Ranganna, 1986) using uv-visible spectrometer (Helios- $\alpha$ , Thermo Electron Corp., USA). Absorbance was read at 518 nm and concentration of vitamin C in samples was calculated based on the standard curve obtained using 0 to 50 mg/100mL range of ascorbic acid concentration.

### Determination of Lycopene

Lycopene content was measured by spectrophotometric method (Ravelo-Perez *et al.*, 2008). Absorbance was read at 503 nm with hexane as blank. Concentration of lycopene was calculated using molar extinction coefficient of lycopene in hexane ( $17.2 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ ) and molecular weight (536.9 g) substituted in Beer-Lamberts law. The final equation derived and used was:

$$\text{Lycopene (mg/L)} = (\text{Absorbance} \times 31.2) / \text{mass of sample (g)} \quad (1)$$

### Determination of Total Phenolic Content

Total phenolic content (TPC) was determined by the Folin-Ciocalteu method (Katsube *et al.*, 2004) using gallic acid (0 to 500 mg/L) as standard. The results were expressed as gallic acid equivalent (mg GAE/100 mL of juice).

### Ferric reducing antioxidant power (FRAP) assay

The antioxidant activity was measured according to FRAP assay (Thaipong *et al.*, 2006) with the absorbance read at 593 nm. Standard curve was constructed against Trolox concentration (25 to 800  $\mu$ M) and results were expressed in mM TE/100 mL of sample.

### Determination of kinetic order, rate constant (k), activation energy (Ea) and $Q_{10}$ value

The loss of each quality attribute were determined by the decrease in concentration of the nutrient measured over time of storage. The experimental data obtained was transformed into a mathematical model by establishing a kinetic plot of the degradation reaction such that:

$$-d[A]/dt = k[A]^n \quad (2)$$

Where [A] is the measured value of a certain quality degradation attribute, t is the storage time, n is the order of the degradation reaction and k is the rate constant of the degradation reaction. The order of the reaction (n) and the rate constant (k) were determined graphically by plotting the experimental data obtained and analysing the corresponding statistical parameters.

If  $n=0$ , k is the slope of the plot of [A] versus t (Zero-order kinetic).

If  $n=1$ , k is the slope of the plot of  $\ln [A]$  versus t (First-order kinetic).

If  $n \neq 2$ , k is determined from the plot of  $1/[A]$  versus t (Second-order kinetic).

Activation energy (Ea) was calculated based on the Arrhenius equation derived by using two different temperature ( $T_1=40^\circ\text{C}$  and  $T_2=50^\circ\text{C}$ ) and two different reaction rate constant ( $k_1$  and  $k_2$ ) obtained respectively.

$$\ln k_1/k_2 = -Ea/R (1/T_2 - 1/T_1) \quad (3)$$

where R is the gas constant (8.314 J/mol.K).

Temperature coefficient ( $Q_{10}$ ) was determined using equation (4) whereby  $k_2$  and  $k_1$  is the rate

constant and  $T_1$  and  $T_2$  is the temperature at  $40^\circ\text{C}$  and  $50^\circ\text{C}$  respectively.

$$Q_{10} = (k_2/k_1)^{10/(T_2-T_1)} \quad (4)$$

The predicted days of each quality indicator total depletion at refrigerated storage of  $5^\circ\text{C}$  was calculated using the  $Q_{10}$  value obtained.

## Results and Discussion

### Quality parameters of fresh pink guava juice (PGJ)

The freshly prepared ready-to-drink PGJ made was analysed to contain vitamin C of  $39.79 \pm 2.18$  mg/100mL, lycopene content of  $3.17 \pm 0.27$  mg/L, total phenolic content of  $28.08 \pm 4.11$  mg GAE/100mL and ferric reducing antioxidant power of  $13.20 \pm 1.91$  mM TE/100 mL. The suggested recommended daily acceptance (RDA) for vitamin C is within 100-120 mg/day to achieve cellular saturation and optimum risk reduction of heart diseases, stroke and cancer in healthy individuals (Naidu, 2003). The lycopene content obtained in PGJ is comparable to the amount on lycopene in tomatoes (3.1 to 40 mg/100 g) (Nguyen and Schwartz, 1999).

### Degradation of vitamin C

Vitamin C is an organic acid with antioxidant properties and one of the major nutritional components in guava fruit. Since vitamin C is easily destroyed during processing, enrichment of the nutrient is commonly practiced by the fruit juice manufacturers to get the desired value of vitamin C content in their product. This water-soluble vitamin is known to be heat sensitive and readily oxidised at elevated temperature. Figure 1 shows the degradation of vitamin C at elevated temperature during storage. As expected, vitamin C content was observed to decrease rapidly at higher temperatures. The amount was found to decrease by 48% at  $40^\circ\text{C}$  and by 79% at  $50^\circ\text{C}$  storage temperature at the end of storage. A study has showed that an increase of temperature by  $10^\circ\text{C}$  caused a distinct decrease in vitamin C (Klimczak *et al.*, 2007). After 6 months of storage at 18, 28 and  $38^\circ\text{C}$ , vitamin C content in orange juice was observed to decrease by 21%, 31% and 81% respectively. Similarly, another study (Kabasakalis *et al.*, 2000) reported a decrease in vitamin C content to 29% in commercial orange juices after 6 months of storage at room temperature. Besides the influence of temperature, greatest decrease in vitamin C during storage is also due to the presence of oxygen, light exposure, moisture content, pH and duration of heat

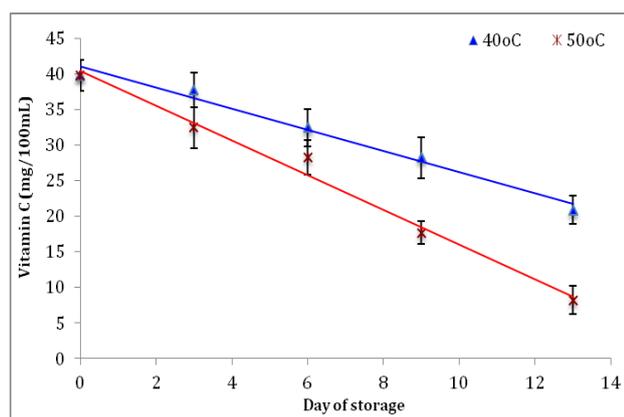


Figure 1. Degradation of vitamin C of pink guava juice during storage

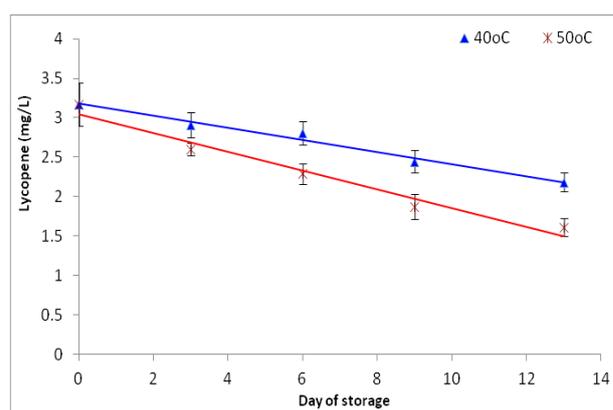


Figure 2. Degradation of lycopene of pink guava juice during storage

treatment (Leskova *et al.*, 2006; Klimczak *et al.*, 2007). The relative instability of vitamin C during processing and storage is well documented by many researchers. However, the prediction of vitamin C losses is complicated due to the lack of information on the mechanism of degradation and the factors that influence its stability (Vikram *et al.*, 2005).

#### Degradation of lycopene

Concentration of lycopene is a significant indicator of PGJ quality since the health promoting effects of consuming PGJ is attributed to the presence of lycopene. To the best of our knowledge, there is no available data on the changes of lycopene content in PGJ during storage. Most studies on lycopene were conducted on tomatoes of different varieties and watermelons (Sharma and LeMaguer, 1996; Henry *et al.*, 1998; Anguelova and Warthesen, 2000; Lee and Chen, 2002; Wang and Chen, 2006; Katherine *et al.*, 2008; Naviglio *et al.*, 2008; Ravelo-Perez *et al.*, 2008). Heat treatment was commonly used to predict the corresponding quality deterioration of lycopene during processing. Figure 2 shows the concentration of lycopene measured against storage time. Lycopene content in PGJ was found to decrease by 32% at 40°C and 49% at 50°C at the end of storage. This observation is in agreement with other researchers (Anguelova and Warthesen, 2000) in which they observed 60% of lycopene in tomato was degraded at 45°C and 30% at lower storage temperature of 6°C after 6 weeks. Another study (Katherine *et al.*, 2008) also reported a decline in lycopene extracted from watermelon with an increase in extraction temperature from 60°C to 75°C. During processing and storage, lycopene undergoes isomerisation and autooxidation causing a decrease in the proportion of all-trans lycopene, colour loss and development of grassy off-flavours (Anguelova and Warthesen, 2000).

#### Degradation of total phenolic content (TPC)

TPC is usually measured due to its strong correlation and contributions towards antioxidant activity. Figure 3 shows the degradation of phenolic compounds in PGJ during storage at elevated temperature. The results obtained show greater reduction occurred at 50°C in which TPC was reduced by 45% compared to reduction by 38% at 40°C. Total phenolic compounds measured by Folin-Ciocalteu reagent reacted not only with phenols but also with other reducing compounds such as carotenoids, amino acids, sugars and ascorbic acid (Vinson *et al.*, 2001). At the end of storage, the juice showed less degradation in TPC than expected. It is possible that during storage of juice, some compounds are formed that react with Folin-Ciocalteu reagents and significantly enhance TPC (Vinson *et al.*, 2001).

Other study on TPC in orange juice has also showed that time and temperature of storage has significant effects (Klimczak *et al.*, 2007) when stored at 18, 28 and 38°C for 4 months. The content of polar phenolic compounds in camelina oil stored at 50°C and 65°C was reduced to 72% and 21% respectively of its initial value after 15 days of storage (Abramovic *et al.*, 2007). Another study also reported that TPC of packaged drink decreased significantly starting from the second month onwards (Siah *et al.*, 2011). Their result showed that at the end of the second month of storage, about 50% of TPC was lost from all the Centella drinks packed using different packaging materials.

#### Degradation of antioxidant activity

The defensive effects of natural antioxidants in fruits are related to three major groups: vitamins, phenolic and carotenoids. Ascorbic acid and phenolics are known as hydrophilic antioxidants while carotenoids are known as lipophilic antioxidants (Thaipong *et al.*, 2006). In this study, antioxidant activity determined as ferric reducing antioxidant power which measured both the hydrophilic and

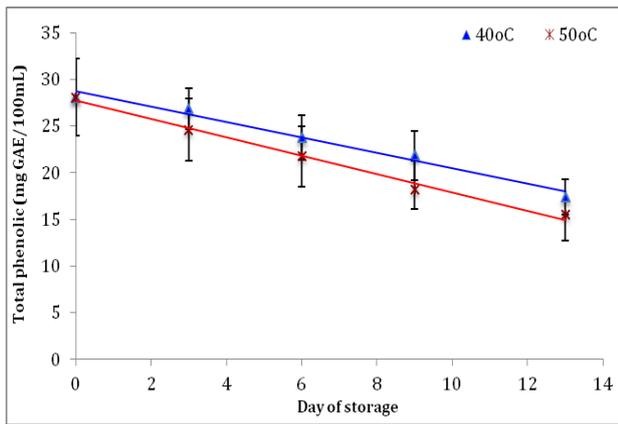


Figure 3. Degradation of total phenolic of pink guava juice during storage

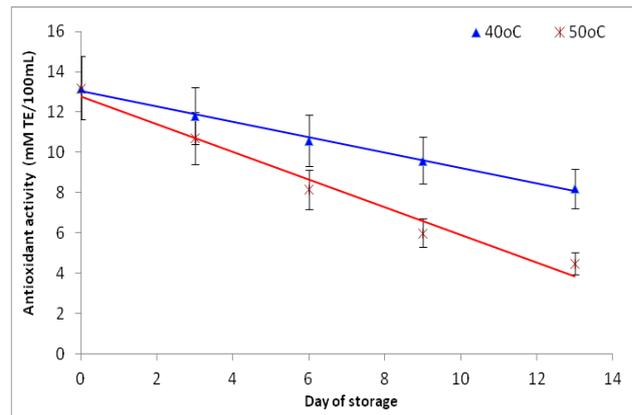


Figure 4. Degradation of ferric reducing antioxidant power of pink guava juice during storage

lipophilic antioxidants present in PGJ. Figure 4 represents the FRAP of PGJ at different storage temperatures. At storage of 50°C, FRAP values decreased more progressively by 76% of its initial value after 2 weeks. However, when stored at 40°C, FRAP values were decreased less progressively by 38% after 2 weeks. This observation is in agreement with the study of the effect of storage temperature on antioxidant capacity and phenolic compounds in strawberries (Jin *et al.*, 2011) and in *Centella asiatica* (Siah *et al.*, 2011). The reducing value of antioxidant activity in juices was due to the transformation of existing structure and interaction of phenolic antioxidant with other food components (Nicoli *et al.*, 1999). Previous study (Gardner *et al.*, 2000) observed that both vitamin C and total polyphenols in fruit juices strongly correlated with the antioxidant capacity determined by FRAP assay. Klimczak *et al.* (2007) stated that antioxidant activity of orange juice evaluated by FRAP method is significantly related to the vitamin C concentration in the juice.

Based on the percentage of degradation of other quality indicators measured, the decrease in antioxidant activity at 40°C seems to be contributed mostly by the deterioration of vitamin C followed by phenolic compounds and finally by the deterioration of lycopene in the juice. At 50°C, greater decrease in antioxidant activity was seen to be affected mainly by the higher deterioration of vitamin C followed by lycopene and finally by phenolic compounds deterioration. Study by Bahorun *et al.* (2004) showed existence of a positive relationship between TPC and its antioxidant capacity in several Mauritian vegetables. Another study by Igual *et al.* (2010) also reported correlation between antioxidant capacity with total phenols. They stated that total phenols played a major role in the antioxidant capacity of grapefruit juices compared to vitamin C, malic acid and citric acid.

*Kinetic order, rate constant (k) and activation energy (Ea) and temperature coefficient (Q<sub>10</sub>)*

Many studies have been carried out to quantify the rate of destruction of nutrients during storage. However, large differences in kinetic parameters, activation energy and reaction rate values have been observed between researchers. The variation observed are attributed to the fact that nutrient destruction is a complex function of many variables such as pH, oxygen, salt, sugar, presence of enzymes, amino acids and metal catalysis. In this study, the degradation of all quality attributes followed zero-order kinetic as indicated in Figure 1-4 except for lycopene at 50°C which indicates a first-order kinetic. The rate constant (k), linearity of curve (R<sup>2</sup>), Arrhenius activation energy (Ea) and Q<sub>10</sub> value are indicated in Table 1. Higher k values at 50°C than at 40°C indicates that faster loss of quality indicators in PGJ occur at higher storage temperature. Ferreira and Amaya (2008) reported that the Ea values for different heating methods were within the range of 7.54 to 125.6 kJ/mol. Higher Ea implies that a smaller temperature change is needed to degrade nutrients more rapidly.

Degradation of vitamin C usually follows first-order kinetics but at higher storage temperatures, there can be departure from first-order reaction. This departure could be attributed to acceleration effects from many breakdown products of vitamin C degradation at higher storage temperatures (Jawaheer *et al.*, 2003). First-order kinetic reaction for vitamin C degradation in citrus juice investigated for eight weeks of storage was observed by Burdurlu *et al.* (2006). While zero-order kinetic of vitamin deterioration was reported by previous studies (Laing *et al.*, 1978; Davies *et al.*, 1991; Lee and Coates, 1999) which similar with the result of this study. The Ea value obtained in this study was 41.49 KJ/mol

Table 1. Degradation kinetic of quality attributes of pink guava juice during storage

Quality attribute	Rate, k (M/day)		Linearity, R <sup>2</sup>		Ea (KJ/mol)	Q <sub>10</sub>	Predicted total loss at 5°C (days)
	40°C	50°C	40°C	50°C			
	Vitamin C	1.4889	2.4392	0.9831	0.9877	41.49	1.64
Lycopene	0.0763	0.0523	0.9813	0.9903	31.75	1.46	158
Total phenolics	0.8303	0.9820	0.9802	0.9910	14.11	1.18	63
Antioxidant activity	0.3802	0.6853	0.9952	0.9762	49.52	1.80	266

which is comparable with the reported values. Study conducted on vitamin C degradation during storage at 28, 37 and 45°C on orange, lemon, grapefruit and tangerine juice gave Ea values of 25.16, 12.77 and 18.94 kcal/mol respectively (Burdurlu *et al.*, 2006). Another study (Vikram *et al.*, 2005) found that Ea values of vitamin C varied with method of heating. They obtained Ea of 64.78 KJ/mol for microwave, 47.27 KJ/mol for ohmic, 39.84 KJ/mol for conventional heating and 37.12 KJ/mol for infrared heating. Higher Ea values were obtained for vitamin C loss at frozen temperature, Ea for frozen green peas, spinach, green beans and okra ranged from 98 to 112 KJ/mol (Giannakourou and Taoukis, 2003).

Thermal degradation reaction of lycopene in PGJ shows different kinetic order at different storage temperature with higher k value at lower temperature. Zero-order kinetic with rate constant, k of 0.0763 M/day was obtained at 40°C, while a first-order kinetic was obtained at 50°C with k of 0.0523 M/day. Zero-order reaction kinetics was also reported for the oxidation of  $\beta$ -carotene when subjected to oxidation by Henry *et al.* (1998) and in degradation of other health-related compounds in fruits and juices (Ozhan *et al.*, 2004; Koca *et al.*, 2007). Similar observation was obtained in the study of lycopene stability during heating in a model system (Lee and Chen, 2002). Their result showed that degradation of lycopene during heating at 50°C, 100°C and 150°C fit a first-order kinetic. Other researcher (Sharma and LeMaguer, 1996) reported that kinetics of lycopene degradation during heating of tomato pulp at 50°C, 75°C and 100°C followed a pseudo first-order reaction. In this study the Ea of lycopene in PGJ is 31.75 KJ/mol and Q<sub>10</sub> of 1.46. To the best of our knowledge, no study was conducted to determine the Ea and Q<sub>10</sub> values of lycopene in fruit juices.

For total phenolics and antioxidant activity, degradation curves plotted showed zero-order kinetic for both storage temperature. Similar with lycopene, no study was conducted to determine the kinetic order,

reaction rate, Ea and Q<sub>10</sub> values of total phenolic and antioxidant activity deterioration in fruit juices. The results in this study showed that the Ea value of TPC and antioxidant activity in PGJ are 14.11 KJ/mol and 49.52 KJ/mol respectively, while the Q<sub>10</sub> values are 1.18 and 1.80 respectively.

Based on the Q<sub>10</sub> value obtained, total depletion of the quality indicators were predicted at lower temperature storage. TPC in PGJ will be totally depleted at 63 days, vitamin C and lycopene will be totally depleted at 158 days and finally antioxidant activity at 266 days of storage at refrigerated temperature of 5°C.

## Conclusion

This study showed that the stability of vitamin C, lycopene, TPC and FRAP content in PGJ were affected by storage temperature and duration of storage. Different rate constant, Ea and Q<sub>10</sub> values were obtained indicating that the rate of degradation for each quality indicators are not the same. Therefore, it is concluded that health beneficial properties availability and amount in the juice varies during storage. Further investigation should be carried out to find ways to stabilize these bioactive compounds during storage in order for the consumers to obtain the maximum health promoting effects provided by fruit juice consumption throughout the shelf life of the product.

## Acknowledgements

The authors would like to thank Sime Darby Foods and Beverages Sdn. Bhd. for the supply of pink guava puree. This study was funded by Institute of Research Management and Innovation (IRMI), Universiti Teknologi MARA (600-RMI/DANA 5/3 PSF 20/2014).

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