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Effect of Elevated Temperature Storage on Stability of Pink Guava Juice Added With *Melastoma malabathricum* Fruit

Aishah Bujang[a*], Zati Alyani Othman[b], Nor Farahin Md Ismail[b]

[a]Functional Foods Research Interest Group, CORE of Frontier Materials and Industry Applications, Universiti Teknologi MARA, Shah Alam, 40450 Selangor Darul Ehsan, Malaysia
[b]Food Science and Technology Programme, School of Industrial Technology, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, 40450 Selangor Darul Ehsan, Malaysia

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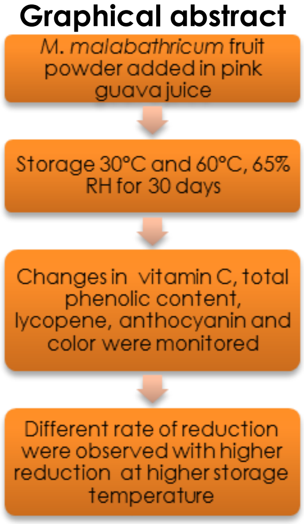
*Corresponding author
aisah012@salam.uitm.edu.my

Graphical abstract



Abstract

This study focus on the stability of natural pigment from *Melastoma malabathricum* fruit in pink guava juice at 30°C and 60°C storage temperature. Analysis on the content of vitamin C, total phenolic content, lycopene, anthocyanin and color were conducted for 30 days. In general, the results showed higher reduction in all quality parameters at higher storage temperature. Freshly made PGJ contains 86.55±5.18 mg/100mL of vitamin C, 5.36±0.27 mg/L of lycopene, 933.50± 60.11 mgGAE/L of total phenolic content (TPC) and 1.36±0.32 mg/L of total monomeric anthocyanin. At day 30 of storage, vitamin C was reduced to 56.4% and 9.4% at 30°C and 60°C respectively. Total monomeric anthocyanins content was reduced to 38.2% and 7.2% at 30°C and 60°C respectively. Total phenolic content remained at day 30 was 12.1% and 7.5% at 30°C and 60°C respectively. While lycopene was found to be least affected by storage temperature compared to other quality attributes analyzed. The total color difference was increased drastically at 60°C between days 0 to 5 while linear increase was observed at 30°C storage. These studies provide useful information on the availability of the health beneficial compounds in fruit juice as well as their stability as natural food colourant during storage.

Keywords: Quality degradation, *Melastoma malabathricum*, pink guava juice, natural pigment

1.0 INTRODUCTION

Consumer demand for natural products has created a strong interest for development of food products using naturally derived colorants. These natural colorants are associated with the image of being healthy and of good quality products while products with synthetic colorants are perceived as harmful, unhealthy and must be avoided. Most plants are a good source of natural pigment that can act as colorant and also contains bioactive compounds that provide health beneficial effects.

"*Senduduk*" (*Melastoma malabathricum*) belongs to the family of Melastomaceae has been reported to grow wild in the Indian Ocean Islands, throughout South and South-East Asia, China, Taiwan, Australia and South Pacific Ocean. Fruits of *M. malabathricum* are technically classified as berries and when they are ripe, they break open irregularly to reveal the soft, dark purple, sweet but rather astringent-tasting pulp and numerous orange seeds [1]. Two major anthocyanins aglycon in *M. malabathricum* are cyanidin-3 glucoside and cyanidin-3, 5-diglucoside [2].

Guava (*Psidium guajava*) is a tropical and semitropical fruit that is known to have about six times higher vitamin C content than other citrus fruits. There are many varieties of guava fruits, which differ in size, shape and taste. However, guava is commonly classified by the colour of its flesh, either white or pink flesh. The pink flesh guava colour comes from the pigment lycopene that contribute to its additional health benefit effects. Guava is a member of Mytacaea family. It is a low evergreen tree or shrub 6 to 25 feet high, with wide spreading branches and square, downy twigs. In Malaysia, the major plantation area of pink guava is in Sitiawan, Perak. The pink guava fruit at this plantation is commercially cultivated and processed into puree [3]. Pink guava contains a lot of phytochemicals and other nutrients including polysaccharides, vitamins, essential oils, lycopene, minerals, enzymes, proteins and dietary fiber [4][5].

For all types of processed food products, stability of the product is an important factor to be considered. Over time, product will lose its quality and nutrients due to increased temperature conditions. As the temperature increase, there will be a higher possibility of the product to lose its quality and nutrient content. Therefore, to determine the expiration date of a product, it is important to have knowledge on the extent of deterioration of the product [6].

The purpose of this study was to evaluate the physicochemical properties of pink guava juice (PGJ) added with *M. malabathricum* fruits. In this study, the degradation of the quality attributes of the juice was monitored at the temperature of 30°C and 60°C for 30 days.

2.0 EXPERIMENTAL

2.1 Raw Materials and Chemicals

Pink guava puree was obtained from Sime Darby Food and Beverages, Sitiawan, Perak. The food grade chemicals were purchased from Meilun Food Chemicals Sdn. Bhd., Klang, Selangor and analytical grade chemicals were obtained from Merck Sdn. Bhd., Shah Alam, Selangor.

2.2 Preparation of Sample

M. malabathricum fruits were washed and blended into paste by using the food processor (MK-5080M, Panasonic, Japan). Then, the paste was introduced into freeze dryer at 4.6 Pa, -54°C for 72 hours. It was then ground into fine powder by using the dry food processor (MX-800S, Panasonic, Japan). The powder form extract was stored at the chill temperature of 4°C in amber bottle until further use.

2.3 Preparation of Pink Guava Juice (PGJ)

PGJ was prepared by adding pink guava puree (12.5%), sucrose (9%), stabiliser (0.20%), citric acid (0.15%), ascorbic acid (0.045%), preservative (0.02%), extracts (5000 ppm) and distilled water. All the ingredients were homogenized using ultrasonic homogenizer at 20,000 rpm for 5 mins, pasteurized at 90°C for 5 mins and filled in polyethylene bottles. Prepared PGJ samples were stored at 30°C and 60°C in humidity chamber (RH 65%) and evaluated at every 3 days interval for 30 days of storage.

2.4 Determination of Vitamin C

Vitamin C measured as ascorbic acid content was determined by using spectrometric method (UVA-160921, Helios-α, England) [7].

2.5 Determination of Lycopene

Lycopene content was measured using spectrometric method [8]. Absorbance was read at 503 nm with hexane as blank.

$$\text{Lycopene (mg/L)} = \frac{\text{Absorbance x 31.2}}{\text{Mass of sample (g)}} \qquad (1)$$

2.6 Determination of Total Phenolic Content

Total phenolic content (TPC) was determined by Folin-Ciocalteu method [9] using gallic acid (0-500 mg/L) as standard and result was expressed as gallic acid equivalent (mg GAE/L of juice).

2.7 Determination of Total Monomeric Anthocyanin

Total monomeric anthocyanin content was determined using pH-differential method [10]. Absorbance were read at 524 nm and 700 nm. Anthocyanin content was calculated based on cyanidin-3-glucoside as standard.

$$\text{Anthocyanin (mg/L)} = \frac{(A \times Mw \times DF \times 10^3)}{(\xi \times 1)} \qquad (2)$$

$$A = A_{524\,nm} - A_{700\,nm})_{pH\,1.0} - (A_{524\,nm} - A_{700\,nm})_{pH\,4.5} \qquad (3)$$

Where A is the absorbance of the diluted sample, MW is the molecular weight (49.2) DF is the dilution factor, and ε is the molar absorptivity (26,900).

2.8 Total Colour Difference

The colour of the sample was measured by using chromameter (CR-400 Konica Minolta, Japan). The value L refers to lightness (0: black and 100: white). The +a* value refers to redness and +b* refers to yellowness. The total colour differences (ΔE) was calculated between the initial day and the subsequence day of storage.

$$\Delta E = \sqrt{[(\Delta L)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]} \qquad (4)$$

2.9 Statistical Analysis

All data were expressed as mean values of triplicate analysis and the data obtained were analysed using one-way Analysis of Variance (ANOVA).

3.0 RESULTS AND DISCUSSION

3.1 Vitamin C Stability

Vitamin C is an essential nutrient needed for maintaining human health and one of the major nutritional components in guava. However, vitamin C is very sensitive to light and heat, hence its content will be degraded when subjected to higher temperature and prolong storage. Figure 1 shows the degradation of vitamin C of PGJ added with *M. malabathricum* during storage.

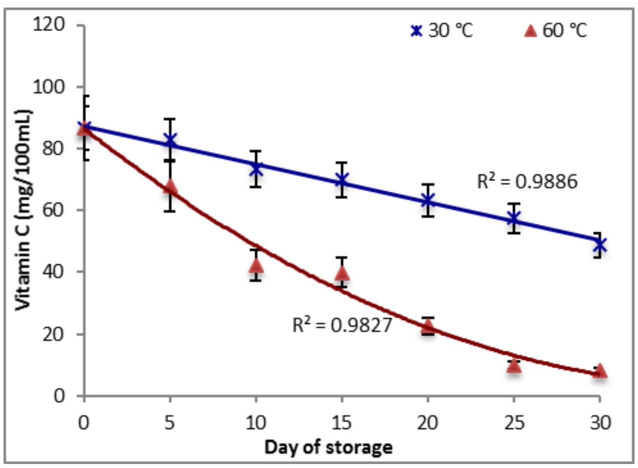


Figure 1 Vitamin C content in pink guava juice added with *M. malabathricum* fruit during storage at different temperature

Vitamin C content in the freshly prepared PGJ added with *M. malabathricum* is 86.55±5.18 mg/100mL. This value is within the range reported by other researcher [11]. According to the study, vitamin C in freshly processed guava and orange juice range from 80.0 to 122.3 mg/100g. The results also showed that the degradation rate of vitamin C increases with the increase of temperature. Similar finding was obtained on vitamin C loss in pomegranate juice that also showed significantly higher rate at higher temperature [12]. Another study reported a decrease in vitamin C in pink guava juice by 48% at 40°C and by 79% at 50°C storage temperature [13].

In this study, linear decrease ($y=-1.200x+86.550$) in vitamin C was observed at 30°C but a polynomial curve ($y=0.057x^2–4.368x+86.550$) was observed at 60°C. At the end of day 30 of storage, vitamin C content remained was 56.4% and 9.4% at 30°C and 60°C respectively.

3.2 Total Phenolic Content (TPC) Stability

Phenolic compounds are secondary metabolites of plants, which contribute to antioxidant activity and also promote health beneficial effects. The stability of phenolic compounds is influenced by many factors such as exposure to light, air or different storage temperatures. Freshly made PGJ added with *M. malabathricum* has TPC of 933.50±60.11 mg GAE/L. Other study [14] obtained TPC value of 383.3 mgGAE/100g in blueberry fruit and 47.13 mgGAE/100g in pineapple juice [15]. In this study, the amount of TPC in freshly prepared PGJ was contributed by TPC in pink guava puree itself and from *M. malabathricum* fruit added in the juice. Figure 2 shows the reduction of TPC in juice added with *M. malabathricum* fruit during storage.

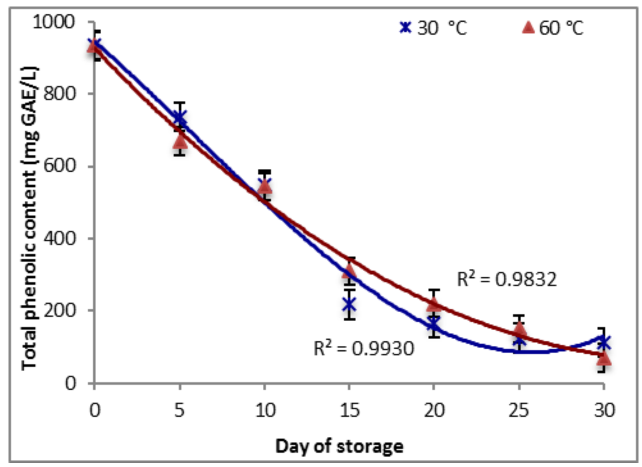


Figure 2 Total phenolic content of pink guava juice added with *M. malabathricum* fruit during storage at different temperature

The curve plots showed in Figure 2 indicates that TPC was degraded at almost similar rate with $y=0.0381x^3-0.660x^2-41.648x+944.66$ and $y=0.707x^2-49.448x+925.55$ at 30°C and 60°C respectively. At day 30 of storage, TPC remained was 12.1% at 30°C and 7.5% at 60°C. This result was in contrast with the reported result of TPC in carrot puree [16]. According to the study, after 20 minutes of treatment, TPC was reduced from 102.8 mgGAE/100g to 93.03 mgGAE/100g (9.2% loss) at 70°C and slight reduction to 102.3 mgGAE/100g (0.5% loss) at 22°C.

3.3 Total Monomeric Anthocyanin Stability

Anthocyanins are flavonoid compounds found in fruits and vegetables having red to purple pigment colour that also contribute to the antioxidant properties. Figure 3 shows the stability of total monomeric anthocyanin at storage temperature of 30°C and 60°C.

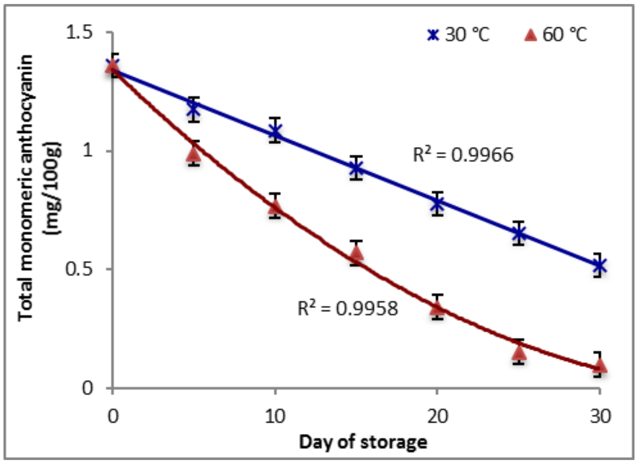


Figure 3 Total monomeric anthocyanin content of pink guava juice added with *M. malabathricum* fruit during storage at different temperature

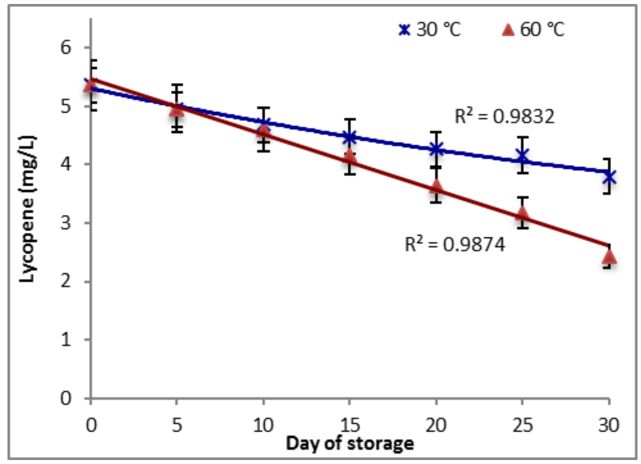


Figure 4 Lycopene content of pink guava juice added with *M. malabathricum* fruit during storage at different temperature

The total monomeric anthocyanin content in the freshly prepared PGJ added with *M. malabathricum* fruit is 1.36±0.32 mg/L. Linear reduction in monomeric anthocyanin was obtained with y=-0.028x+1.342 for 30°C storage and polynomial curve with $y=0.001x^2$–0.066x+1.34 for 60°C storage. At day 30 of storage, the total monomeric anthocyanins at 30°C was reduced to 38.2%, while at the storage of 60°C, it was reduced to 7.2%.

A study on storage stability of black carrot anthocyanins in various types of juices stored at different temperature for 180 days showed that at 37°C storage, degradation of anthocyanins occurs at a much faster rate than at 4°C storage [17]. Other natural pigment also showed similar effects at elevated temperature. Other study showed that at 85°C, degradation of betalain pigment was significantly higher compared at 25°C and 4°C [18]. It is generally known that natural colorants are easily degraded, heat liable and low in stability due to their natural structures compared to synthetic colorant.

3.4 Lycopene Stability

Lycopene is a major pigment present in pink guava flesh that gives the significant pink colour of the fruit. It is known as the most abundant carotenoid found in pink guava, which made up more than 80% of total carotenoids. Figure 4 shows the content of lycopene in PGJ added with *M. malabathricum* fruit powder during storage at different temperature. The lycopene content in freshly prepared PGJ added with *M. malabathricum* is 5.36±0.27 mg/L. Based on other study [19], content of lycopene in pink guava range from 1.35 to 3.55 mg/100g of fresh weight.

Higher rate of lycopene loss was observed at elevated temperature of 60°C with linear reduction curve of y=0.951x+5.469. For storage at 30°C, the reduction curve was non-linear with $y=0.001x^2$-0.062x+5.299. At day 30 of storage, concentration of lycopene left at 30°C is 3.80 mg/L (70.9%) and at 60°C is 2.43 mg/L (45.3%).

According to a study [20], the stability of lycopene and its cis-isomers changes during heating when there was a significant change of all trans-lycopene at the first 12 hours, however after 12 hours the content of lycopene began to decline. They also stated that the levels of all the mono-cis forms of lycopene were found to decrease with increasing heating time.

3.5 Stability of Color

Color is one of the indirect methods to measure the quality of the food or beverage products. The degradation of colour is due partly by the polyphenol oxidation and it can occur at any stage of processing and storage [21]. Figure 5 shows the total color difference of PGJ during storage at different temperature.

In this study, the color of PGJ is contributed by both lycopene and anthocyanins from PGJ and *M. malabathricum* respectively. The results obtained showed that the increase in total color difference was faster at the initial days of storage. At 60°C, rapid increase in color difference observed between day 0 to day 5 followed by a constant curve and later decrease in ΔE between days 20 to 30. The sharp dropped in ΔE at the end of storage period for 60°C curve may be due to the transformation of pigments and other reactions that occur during the storage period. While at 30°C, an almost linear increase was observed. This indicates that the initial pinkish color of PGJ becomes darker and brown in color towards the end of storage.

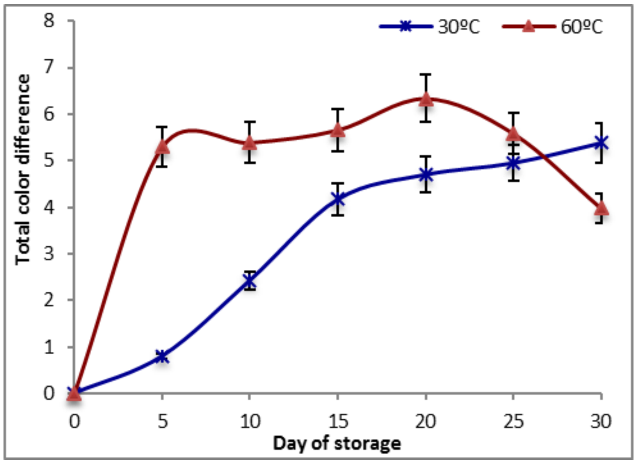


Figure 5 Total color difference of pink guava juice added with *M. malabathricum* fruit during storage at different temperature

4.0 CONCLUSION

On overall, the results from this study showed that vitamin C, total phenolics, total monomeric anthocyanins and lycopene content were decreased with the increase of storage temperature and duration of storage. For total color difference, faster rate of color change was observed at 60°C compared to at 30°C. The most affected quality attributes were anthocyanins and total phenolic contents followed by vitamin C and the least affected was lycopene.

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