Stability and colour characteristics of encapsulated anthocyanin extract in Pink guava juice during storage

Accepted 17th January, 2018

ABSTRACT

The stability of pink guava juice (PGJ) incorporated with encapsulated anthocyanin from the combination of roselle and senduduk at 70:30 ratio was investigated during storage at 4 and 25°C for 12 weeks, respectively. The degradation in pink guava juice was analysed with spectrophotometer pH differential method while its colour characteristics were evaluated using colour indices such as colour density and CIE L*a*b* parameters. Pink guava juice incorporated with 70:30 ratio of roselle and senduduk in spray dried form stored at 4°C showed good stability as compared to storage at 25°C. PGJ stored at 4°C had a 36% higher total monomeric anthocyanin content as compared to PGJ stored at 25°C. The degradation index (DI) was also found to be two times lower in 4°C storage. A very strong negative correlation (0.930) found between DI and anthocyanin content indicated the decrease in anthocyanin content which resulted to an increase in the DI in pink guava juice and was more severe in room temperature storage. There was an increased colour density which showed an enhancement in anthocyanin content in the juice at 4°C storage. PGJ appeared faded pink colour with a slight yellowish colour at the end of the storage period. The pink guava juice with added colourant was significantly favoured by panellist with the criteria such as taste, sweetness and sourness score the highest compared to other samples. Encapsulated anthocyanin has a potential to be used in fruit juices replacing the artificial colourant.

Keywords: Anthocyanin, encapsulated, stability, colour characteristics, pink guava juice.

INTRODUCTION

Anthocyanins are an important group of water-soluble plant pigments commonly found in various fruits and vegetables. They offer a wide range of attractive colour spectrum from shiny orange, pink, red, purple to blue that has great potential for use as natural food colourants to replace synthetic dyes. However, they lack stability due to processing and storage. The degradation of anthocyanin during storage of food products could affect the colour quality and also the nutritional content. From previous studies, several factors such as species, environmental and agronomic conditions; extraction and processing parameters such as pH, storage temperature, concentration, chemical structure, light, oxygen, proteins, ascorbic acid, sugars, sulfites, enzymes and metallic ions influencing the pigment stability have been investigated (Rein, 2005; Patras et al., 2010; Cavalcanti et al., 2011). In general, anthocyanins are more stable in acidic media at low pH values than in alkaline solutions (Rein, 2005). Therefore, the acidic conditions were mostly studied to consider food with pH in this range such as fruit juices, jams and jellies.

Hibiscus sabdariffa L. also known as roselle is widely grown in Malaysia and other countries such as Indonesia, Africa and America. Its calyxes are brilliant red in colour due to its anthocyanins compound; delphinidin-3-
sambubioside, cyanidin-3-sambubioside, cyanidin-3-glucoside and delphinidin-3-glucoside which are the non-methylated type (Castaneda-Ovando et al., 2009). Although there are many researches conducted on *H. sabdariffa* extract but most are focused on its antioxidant activity (Norhaizan et al., 2010; Tsai and Huang, 2004; Hirunpanich et al., 2006; Tsai et al., 2002) rather than on its anthocyanins pigment stability and application in food product. Study by Chumsri et al. (2008) found that dried roselle calyx powder obtained from vacuum evaporation contained 10% moisture and higher in total anthocyanin content. While Cisse et al. (2011) reported the crossflow of microfiltration roselle extract in 3 months storage at 4 and 20°C was safe for consumption.

*Melastoma malabathricum* L. or *senduduk* is a plant used in traditional Malay folk medicine for treatment of diarrhoea, post-partum treatment, dysentery, toothache, flatulence and haemorrhoids (Sunilson et al., 2008; Susanti et al., 2007). The pulps of *M. malabathricum* fruit are dark red-blue or purple in colour and contained many small white coloured seeds. Two major anthocyanins aglycon in *M. malabathricum* are cyanidin-3-glucoside and cyanidin-3,5-diglucoside (Koay, 2008). Most researches conducted on *M. malabathricum* focused on the identifications and characterization of its anthocyanins structure (Goda et al., 1997; Giusti and Wrolstad, 2003; Terahara et al., 2004; Janna et al., 2006).

In recent years, there has been an increasing interest in spray drying method due to the cheapest technique to produce encapsulated food material as compared to other methods (Jayasundera et al., 2009). In order to extend the shelf life of the encapsulated food powder, maltodextrin is widely used due to its film-forming capacity and plastic properties as a carrier. Ersus and Yurdagel (2007) reported on the anthocyanin content encapsulated with 20-21 DE maltodextrin in black carrot was 28.45% higher compared to other type of maltodextrin. The half life storage of encapsulated black carrot at 4°C was found to increase thrice than at 25°C storage. Onon et al. (2009) found that red-purple colourant obtained from co-current spray drying, using inlet drying air temperature of 160°C of *Opuntia stricta* fruit juices retained >98% of its colour. The incorporation into food model system also maintained its colour for one month under refrigeration temperature, 4°C.

Currently, tropical fruit juices consumption have increased as an alternative to the traditional caffeine containing beverages (Jagtiani et al., 1998). By incorporating tropical fruits into fruit-juice blends, their exotic strong flavours could contribute to the juice without addition of any artificial flavours. For example, in a highly aromatic fruit such as guava (Floribeth and Lastreto, 1981). Guava or *jambu batu* belongs to the *Psidium guajava* L. of the family Myrtaceae (Morton, 1987). For juice production, a red flesh cultivar is strongly recommended to be developed for the Malaysia growing environment (Kwee and Chong, 1990). Study by Osorio et al. (2011) reported on the characterization of encapsulated pink-fleshed guava fruit extract with different types of carrier agents available. Hobert and Tietze (2001) investigation confirmed that guava fruit has a decreasing effect in cases of high blood pressure and high blood fat readings. The objective of this study was to investigate the degradation and colour characteristics of encapsulated anthocyanin extract incorporated in pink guava juice (PGJ) during storage at 4 and 25°C, respectively.

**MATERIALS AND METHODS**

**Raw materials**

Roselle (*Hibiscus sabdariffa* L.) was obtained from Federal Agriculture Marketing Authority (FAMA) Rengit, Johor. *Senduduk* (*M. malabathricum* L.) was collected from farm wasteland area around Kg. Parit Bulat in Muar, Johor. Pink guava puree for juice making was supplied by Golden Hope Food and Beverages Sdn.Bhd. All places are located in Malaysia.

**Analytical grade chemicals**

Sodium pentaphosphate, sodium acetate, potassium chloride, metallic magnesium and ferric chloride were purchased from Merck (Darmstadt, Germany). Cyanin chloride and delphinidin chloride standard were from Sigma Chemical Co. (Steinheim, Germany). Sodium metabisulphite were purchased from Ajax Finechem (Auckland, New Zealand). Hydrochloric acid concentrated, acetic acid anhydride, sulphuric acid and glacial acetic acid were from R and M Chemicals (Essex, United Kingdom) and ethanol 95% from J. Kollin Chemicals (United Kingdom).

**Food grade chemicals**

Maltodextrin, sodium benzoate and citric acid were purchased from Meilun Chemicals Company (China). Guava booster was provided by Golden Hope Food and Beverages. Sugar was purchased from Giant Hypermarket, Shah Alam, Malaysia.

**Sample preparation**

**Aqueous extraction**

All samples were washed and cleaned prior to further treatments. For *senduduk*, the samples were peeled to remove the skins and unwanted residues. Roselles were washed and the seeds removed. The extraction using water was carried out according to the method of Pin-Der and
Spray drying process

All samples that underwent aqueous extraction were added with food grade citric acid powder to achieve pH 2.3. Initial total soluble solid of the aqueous extract was determined using a handheld refractometer (N2, Atago, Japan). Maltodextrin, the carrier agent was added to obtain 25° Brix. Each preparation was homogenized using a homogeniser (T25, IKA, Germany) at 10000 rpm for 20 min. Thereafter, all combinations were spray dried using a mini spray drier (B-191, Buchi, Switzerland). The inlet and outlet temperatures were controlled at 160 ± 5°C. Powdered samples were kept in an amber bottle at room temperature until further analysis.

Preparation of ready-to-drink pink guava juice

Pink guava puree was filtered through two layers of muslin cloth and repeated 2 to 3 times to get a uniform puree pulp size. For the juice making, sugar (9%) was added to water (66%) and thoroughly mixed. The mixture of spray dried anthocyanin extract from roselle and senduduk at the ratio 70:30 was added, 0.015% sodium benzoate and 0.15% citric acid were added and homogenized at 20000 rpm for 5 to 10 min. Pink guava puree (12%) and 0.046% guava booster was then added and thoroughly mixed. The juice was pasteurised at temperature between 90 to 95°C for 2 min and cooled rapidly to 25°C before immediate bottling.

Determination of total monomeric anthocyanin (TMA)

Total anthocyanin analysis was performed using a spectrophotometric differential pH method, with few modifications (Lee et al., 2005). Two lyophilized samples of 500 mg were treated with 10 ml of buffer solution, pH 1.0 (125 ml of 0.2 M KCl and 375 ml of 0.2 M HCl), and 10 ml of buffer solution, pH 4.5 (400 ml of 1 M sodium acetate, 240 ml of 1 M HCl and 360 ml of water, respectively). The mixture was homogenized and centrifuged twice at 4°C at 5000 g for 15 min. The supernatant was collected and its absorbance read by UV-visible spectrophotometer (Heλios α, Thermo Scientific, England) at 524 and 700 nm, respectively. Each sample was analysed in triplicate and the results expressed as the averages of the three measurements.

Total monomeric anthocyanins were calculated as cyanidin-3-glucoside equivalents, using the extinction coefficient of 26,900 L cm⁻¹mg⁻¹, and a molecular weight of 449.2 g/L. Quartz cuvettes of 1 cm pathlength were used and all measurements carried out at room temperature (25°C). Absorbance readings were made against distilled water as a blank. The concentration of anthocyanin (mg/L) was expressed using the following formula:

\[
\text{Total monomeric anthocyanin pigment (mg/L)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l}
\]

Where:

\[
A = (A_{520nm} - A_{700nm})_{\text{pH 1.0}} - (A_{520nm} - A_{700nm})_{\text{pH 4.5}}
\]

DF = Dilution factor;

Pathlength = 1 cm;

\[
\varepsilon = \text{Molar extinction coefficient};
\]

\[
10^3 = \text{Factor for conversion from g to mg}.
\]

Determination of degradation index

The degradation was calculated using absorption at 420, 524 and 700 nm, respectively using the following formula before and after the bisulfite treatment. The bisulfite solution was freshly prepared using 1 g of potassium metabisulfite (K₂S₂O₃) in 5 ml distilled water. The freeze-dried samples of 0.04 g were made into a solution of 20 ml buffer of selected pH. One part of the solution was further diluted with 4 parts of distilled water. 2.8 ml of the diluted solution was added with 0.2 ml of the bisulfite solution. The solution was then vortexed to obtain a homogenous solution before measured using UV-visible spectrophotometer.

\[
\text{Degradation index} = \frac{[(A_{524nm} - A_{700 nm})_{\text{pH 1.0}}]/(A_{524nm} - A_{700 nm})_{\text{pH 4.5}}}{(A_{524nm} - A_{700 nm})_{\text{pH 1.0}} - (A_{524nm} - A_{700 nm})_{\text{pH 4.5}}}
\]

Determination of colour density

Colour density is defined as the sum of absorbances at the maximum wavelength and at 420 nm. (Cevallos-Casals and Cisneros-Zevallos, 2004). The colour density was calculated using the equation at 420, 524 and 700 nm, respectively given as:

\[
\text{Colour density} = (A_{420 nm} - A_{700 nm}) + (A_{524nm} - A_{700 nm})
\]
Determination of colour

The changes in colour were determined using chromameter (CR400, Minolta, Japan). The colour indices were measured using CIE L°C°H° colour space (The International Commission on Illumination, Vienna, Austria) with illuminant of D65 and 2° observer. L° is a measure of lightness ranging from 0 (black) to 100 (white) and colour coordinates, a° which takes positive values for redness and negative values for greenness and b° positive for yellowness colour and negative for blueness. From these coordinates, other colour parameters were calculated; chroma (C°) is the quantitative attribute of colour intensity or saturation. The higher chroma value indicated a more saturated colour was observed. Chroma values were calculated using the equation:

\[
\text{Chroma} = (a°^2 + b°^2)^{\frac{1}{2}}
\]

Hue angle (H°) is the qualitative attribute of the colour expressed as (0°/360°) red, (90°) yellow, (180°) green and (270°) blue and calculated using the equation:

\[
\text{Hue angle} = \tan^{-1}(a°/b°)
\]

Sensory evaluation: Consumer preference test

A sensory evaluation was carried out to determine the acceptability of the pink guava juice added with the spray dried sample; 60 untrained panelists consisting of students and staffs ranging from 18 to 50 years of age evaluated the juice based on the Hedonic scaling method (9-1scoring) with 1=extremely dislike (showing lowest quality), 5= neither like nor dislike (medium quality) and 9=extremely like (highest quality), respectively. Prior to sensory evaluation pink guava juice samples were refrigerated, randomly coded and served (20 ml) at 15°C. All sensory evaluations were carried out in partitioned booths under fluorescence lamp for each panelist to avoid any interference. The panelists were required to rinse their mouth with drinking water between samples. Sensory scores for different attributes such as taste, colour, sweetness, sourness, consistency and overall acceptability were evaluated.

Storage stability study

A storage stability study of ready-to-drink (RTD) pink guava juice incorporated with spray-dried anthocyanin colourant was carried out for 12 weeks. Pink guava juices were stored in glass bottle and wrapped with aluminium foil to eliminate light exposure and to mimic the commercial aseptic carton packaging. Two storage conditions were evaluated at refrigerated temperature, 4°C and room temperature, 25°C. Analyses of total monomeric anthocyanin content, degradation index, colour density and colour analysis were conducted for every week for a period of 12 weeks.

Data analysis

All presented numeric values are means of three or more measurements as stated ± standard deviation (SD). The correlations between methods were determined using analysis of variance (ANOVA) and quantified in terms of the correlation factor, R². One-way ANOVA performed in Statistical Analysis System SPSS version 15.0 (SPSS Inc., Chicago, Illinois) was used to determine whether the differences between measurements are significant. Differences were considered significant at a confidence level superior to 95% (p < 0.05).

RESULTS AND DISCUSSION

Degradation of total monomeric anthocyanin content in pink guava juice during storage

In general, total monomeric anthocyanin content at both storage (4 and 25°C, respectively) showed a similar decreasing trend over storage time. Based on Figure 1, PGJ stored at 4°C had a higher total monomeric anthocyanin content as compared to PGJ stored at 25°C. The total monomeric anthocyanin at 4°C range between the highest of 74.48 mg/L to the lowest of 34.98 mg/L, while total monomeric anthocyanin at 25°C range between 47.40 to 13.54 mg/L during the storage period of 12 weeks, respectively. The total anthocyanin pigments showed gradual decrease throughout the storage period with some fluctuation at certain weeks. These results were similar with report from Zhang et al. (2008), which documented that the degradation of anthocyanins was affected by storage temperature.

The initial total monomeric anthocyanin of spray dried anthocyanin extract added into the PGJ formulation was about 120 mg/L, however, after the completion of PGJ processing, the total monomeric anthocyanin was reduced to about 75 mg/L. Losses of TMA to PGJ processing amount to 38%. The drastic loss of TMA during processing was attributed by the other ingredients mixed to the PGJ formulation and also due to the pasteurisation treatment.

As described by Mercandante and Bobbio (2007), sugar in the pink guava juice system which was 9% of total volume could also have destructive effect on the stabilities of anthocyanin as anthocyanin thermostabilities was reduced when sucrose concentration increased from 0 to 20%. According to Gomez-Plaza et al. (2006), sugar may lead to Maillard type browning reactions and their decomposition product may increase the rate of
anthocyanin degradation. Rosso and Mercandante (2007) reported that the effect of added sugar in the anthocyanin stability depends on its structure, concentration and type of sugar. A low concentration (86 g/L) of sugar would affect its stability.

Loss of anthocyanin pigments are also probably due to oxidation as well as, condensation of anthocyanin pigments with ascorbic acid (Choi et al., 2002), added in the juice. Ascorbic acid can act as a molecular oxygen activator that produces free radicals but in the presence of anthocyanins, part of it can be remained due to the antioxidant properties attributed by the anthocyanins (Mercandante and Bobbio, 2007). According to Wrolstad et al. (2005), the presence of ascorbic acid in anthocyanin solution will accelerate the destruction process by condensation of anthocyanin with other phenolic compound to form coloured polymeric pigment.

The high temperature led to a faster anthocyanin degradation, which was expected, since these pigments are highly thermo sensitive (Tonon et al., 2010). This negative influence of temperature on anthocyanin stability was observed by Pacheco-Palencia et al. (2007), who verified the anthocyanin stability in the whole, semi-clarified and clarified acai pulp, had a degradation rate 3.5 times higher when samples were stored at 20°C as compared to 4°C. Study by Laleh et al. (2006) on the stability of anthocyanin in red orange juice at 15, 25 and 35°C during a 15 days period showed that the increase in temperature accelerates the destruction of anthocyanins, respectively. The percentage of anthocyanin destruction reached over 60 to 80% in just 15 days at 25°C, suggesting that the speedy destruction of anthocyanin at higher temperatures could be due to hydrolyzation of 3-glycoside structure.

Degradation index of pink guava juice during storage

Figure 2 shows a degradation index of PGJ stored at 4 and 25°C for 12 weeks of storage, respectively. The PGJ stored at 25°C had a significant increase (p<0.05) in degradation index at the end of storage period compared to 4°C storage. The PGJ stored at 4°C for 12 weeks was about two times lower in its degradation index than after 12 weeks storage at 25°C. The DI drastically increased throughout the 3 months of storage at 25°C. These results are in line with the report of Giusti and Wrolstad (2001), who reported that storage temperature had a clear effect on the pigment degradation kinetics of coloured model juices. Refrigerated temperatures drastically decreased the rate of anthocyanin degradation with estimated half-life of over a year. In addition, there was a very strong negative correlation (0.930) between DI and anthocyanin content (Figure 3). These indicated that the decrease in anthocyanin content caused an increase in the DI in pink guava juice and was more severe in room temperature storage.

Colour density and chromaticity

It is well known that processing and storage conditions have significant influence on the PGJ colour. As reported by Alighourchi and Barzegar (2009), it was necessary to objectively measure colour as well as, pigment concentration to investigate colour quality in a systematic manner. It was indicated that for some fruit products colour deterioration cannot be characterized by changes in total anthocyanins alone. Therefore, the colour density and CIE \(L^*C^*H^*\) value were measured to evaluate the changes in the
colour quality of the juice.

According to Figure 4, the colour density trend of the PGJ in 12 weeks of storage at 4°C showed an increased value to 37.95 ± 0.63 by the end of the storage from 33.99 ± 1.17 depicting 11.7% increase. At 25°C storage, the value of colour density ranged from 0.53 to 0.40 showing 25% colour degradation during storage. The increase in colour density showed an enhancement in anthocyanin content in the juice. This result was in accordance with the result of Khandare et al. (2010), who reported a 36% increase in colour density of black carrot (Daucus carota ssp. sativus) as a result of processing, however, contrary with the report of Zhang et al. (2008), who observed a colour density degradation in cyanidin-3-glucoside during storage. The result for colour density has always been associated with chroma value as both parameters would have a linear relationship. Both parameters showed an intensity in colour of the juice. An increase in colour density would result in an amplified reading in chroma value.

As for the chromaticity parameter, the lightness value fluctuate at 4°C significantly throughout 3 months of storage (Figure 5) in which the highest value reached up to 37.77 ± 0.19 and the lowest value was 35.18 ± 0.07. At 25°C storage, there was also a fluctuation from the first to sixth
week and the value was almost constant from week 7 to the end of the storage period.

The slight degradation of the lightness may be due to the polymerisation of anthocyanins at high temperature during juice pasteurisation indicating a loss of red colour in pink guava juice. Study by Alighourchi and Barzegar (2009) verified that the L*, a*, b*, and chroma values of stored reconstituted pomengranate juice (RPJ) at 4°C were higher than those stored at 20 and 37°C, respectively indicating that temperature significantly affects the colour parameters.

The chroma value (C*) reflects colour saturation with higher values corresponding to higher colour brilliance. Differences of C* values were observed at both storage temperature until the end of the storage (Figure 6).

The highest value (12.19 ± 0.09) on first week and the lowest (9.97 ± 0.45) during sixth week were observed at 4°C storage. A higher chroma was observed at lower temperatures during storage period. At 25°C storage, the highest value reported was 12.45 ± 0.01 and the lowest value was 9.02 ± 0.04. These values fluctuate significantly throughout the storage. The value increased during third and ninth week with a subsequent decrease. Stintzing et al. (2006) verified an increase of C* in anthocyanin samples after 22 days of storage. After 2 h of heating, C* value exceeded the initial values. This can be attributed to an increase in colour purity through thermal treatment (Sadilova et al., 2009). An increasing trend of the chroma in pomegranate juice colour was previously observed (Marti et al., 2002), which was in accordance with the present study.

Following processing and storage, PGJ showed a
significant increase in hue angle (H°) indicating a shift to a yellowish tint in chromaticity diagram. The H° value varied within the range of 50° to 70° for both storage temperatures (Figure 7). PGJ stored at room temperature showed a significantly higher (p < 0.05) value as compared to PGJ stored at 4°C. At the end of the storage, PGJ appeared faded pink colour with a slight yellowish colour. The result was in accordance with the report of Sadilova et al. (2009), as subsequent heat treatment, both strawberry and elderberry juice samples showed a significant increase of H° values which indicates a shift to yellowish tints. The differences in H° value shifts demonstrated that the effects of individual saccharides on hue angle values in the course of heating cannot be generalised, since they strongly depend on the genuine plant matrix.

Sensory evaluation: Comparison between anthocyanin incorporated ready-to-drink pink guava juice with commercial pink guava juice in the market

Figure 8 shows the results for the sensory evaluation. The total of 60 panelists evaluated the PGJ sample produced together with two other commercial PGJ in the market (Brands 1 and 2). Based on the results, the colour of the anthocyanin incorporated PGJ was significantly preferred by panelists with the highest score (7.18 ± 1.44) as compared to the other two juices. For the taste attributes, PGJ added with spray dried colourant obtained the highest score (6.66 ± 1.85) which is significantly different to the other PGJ brands. The highest score in taste could be contributed by the formulation used that successfully blends the sweetness.
and the soursness together that made it more preferred by the panelists. The mean score for sweetness and soursness attributes was also the highest with 6.47 ± 1.86 and 6.44 ± 1.92, respectively. However, the consistency in all samples showed no significant difference in the attribute. All PGJ have the same body and gave similar mouth feel to most of the panelists.

For the overall acceptability of the sample attribute, most panelists ranked the PGJ added with natural colourant as the most acceptable and preferred, followed by PGJ of the Brand 1 and finally the PGJ of Brand 2. The PGJ with added colourant was significantly different from the other two brands. This indicates that the juice added with spray dried colourant of roselle and senduduk at 70:30 ratio have a potential to be used in juice replacing the artificial colourant and was well received by most of the panelists.

**Conclusion**

Results from this study showed that total monomeric anthocyanin content significantly decreased (p<0.05) with temperature and time as well as, the degradation index. As the storage time increased, a significant increase of colour density was obtained at all storage temperature. Pink guava juice incorporated with 70:30 ratio of roselle and senduduk in spray dried form stored at 4°C showed good stability as compared to storage at 25°C. Chill temperature was the best condition to maintain anthocyanin properties. The pink guava juice with added colourant was significantly favoured by the panelist indicating that the juice added with spray dried colourant of roselle and senduduk at 70:30 ratio have a potential to be used in juice replacing the artificial colourant.

**ACKNOWLEDGEMENT**

This study was funded by Ministry of Higher Education, Malaysia (FRGS grant: 600-IRDC/ST/FRGS 5/3/1335).

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