

PROXIMATE AND PHYSICO-CHEMICAL ANALYSIS OF PINK GUAVA JUICE FORTIFIED WITH VITAMIN E

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Abstract

This study is focused on determination of proximate and physicochemical properties of pink guava juice (PGJ) fortified with vitamin E. Coffee, tea, or carbonated soft drinks are popular however the customer nowadays is more health conscious and start reducing caffeine-containing beverages from daily intake and replacing it with fruit juices such as PGJ. In addition, beverages with added value such as vitamin E that are highly demanded in the fruit juice market globally. The experimental work of this research are proximate analysis (i.e. protein, carbohydrate, fat, fibre, ash and moisture content) followed by physico-chemical properties (i.e titratable acidity, pH, total soluble solid (TSS), water activity (a_w), viscosity determination and colour analysis (i.e. lightness (L^*), chroma (C^*) and hue angle (H°)). Results showed that pink guava juice (PGJ) contained protein (0.67 ± 0.15^a %), carbohydrate (4.96 ± 0.59^a %), fat (0.27 ± 0.05^a %), total fiber (0.74 ± 0.10^a %), ash (0.64 ± 0.10^a %), moisture (92.72 ± 0.91^a %), acidity (0.41 ± 0.10^a %), pH (3.65 ± 0.12^a), TSS (10.56 ± 0.43^a), a_w (0.99 ± 0.02^a), viscosity (15.33 ± 1.15^a mPa.s), L^* (31.95 ± 0.02^a), C^* (34.65 ± 0.01^a), H° (25.42 ± 0.02^a) and vitamin E content (17.44 ± 1.63^a mg/l). In conclusion, this study will be useful for preliminary information regarding pink guava juice and also Vitamin E-Fortification for fruit juice in the future.

Keywords: Pink Guava Juice, Vitamin E fortification, Vitamin Fortified Fruit Juice, Pink Guava, Fruit Juice.

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Introduction

Guava, (*Psidium guajava* L.) is a member of the large Myrtaceae or Myrtle family. It is native to tropical and warm subtropical areas originated from Central America and was expanded throughout the cool subtropical and tropical regions including Brazil, Mexico, Colombia, Egypt, India, China, Thailand, and Malaysia for the past 400 years (Suwanwong and Boonpangrak, 2021; Nagarajan et al., 2019; Lamo al., 2019; Ninga et al., 2018; Moon et al., 2018; Garbanzo et al., 2017; Aishah et al., 2016a). In Malaysia, Perak state has the largest area for guava plantation and guava is one of the easiest fruits to process whilst providing good characteristics for the food and beverages industry (Aishah et al., 2016a). Figure 1 show the image of pink guava fruit. According to Aishah et al., (2016b), guava does not show problems of physical or biochemical nature in relation to texture, shape or pulp browning during processing. Pink guava has rich tropical aroma, colour, aroma, flavour and functional properties. It has ideal source of carotenoid, flavonoids, triterpenoids, pectin, dietary fibre as well as other phytochemicals like ascorbic acids, anthocyanins and ellagic acids. Guava is consumed in its fresh form but there are varieties of guava processed products such as guava paste, marmalades, jellies, juices, puree, concentrate, syrup, ice cream, canned product, and beverages drinks (Campoli et al., 2019; Lamo et al., 2019; Ninga et al., 2018; Moon et al., 2018; Garbanzo et al., 2017).

Guava juice is an example of a tropical fruit juice that has gradually become important in recent years due to the overall increase in 'natural fruit' juice consumption as an alternative to the traditional caffeine-containing beverages as coffee, tea, or carbonated soft drinks. By incorporating tropical fruits

into fruit-juice blends, food technologists have been able to exploit their exotic flavours without adding artificial flavours. This is especially true with highly aromatic fruit such as guava that may be able to compete in this market, either as guava juice or as mixtures with other juices (Aishah et al., 2016b). In Malaysia, pink guava juices are marketed with total soluble solid ranged from 9.9°Brix to 10.63°Brix and pH ranged between 3.46 and 3.98 (Silva et al., 2016). Pink guava juice is chosen over ordinary guava juice mainly due to its attractive pink colour originating from lycopene which constitutes more than 80% of its total carotenoids content (Nagarajan et al., 2019; Campoli et al., 2019; Aishah et al., 2016a). The knowledge obtained from this study may provide a new era of knowledge on the proximate and physico-chemical properties in beverages fortified with vitamin E such as pink guava juice. The results obtained may also be used for further determination such as its stability towards gravitational separation. Glover (2019) stated that beverage with added value such as vitamin E are highly demanded in the fruit juice market globally.



Figure 1. Pink guava fruit

Methods

Raw materials

Pink guava puree and guava booster (guava flavour enhancer) were obtained from Golden Hope Food and Beverages Sdn Bhd., Sitiawan, Perak. Tocotrienol palm oil extract (Gold Tri.E 50) was obtained from Sime Darby Biogonic Sdn Bhd., Telok Panglima Garang, Selangor. Food grade citric acid, carmoisine red, sodium benzoate, ascorbic acid (AA), carboxymethylcellulose (CMC), and polysorbate 80 (P80) were purchased from Meilun Food Chemical Sdn Bhd., Klang, Selangor while sucrose was purchased at a local supermarket.

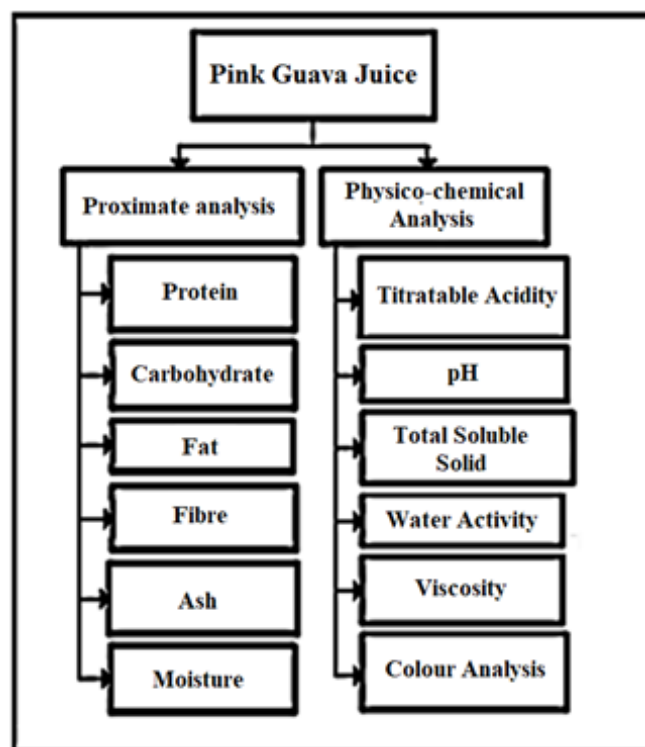


Figure 2. Experimental work of study

Preparation of pink guava juice

Pink guava puree was filtered through 0.045 mm size of orifices sieve and this process was repeated 2 to 3 times to obtain uniform particle size of the guava puree. Additional ingredients such as carmoisine red, sodium benzoate, citric acid and stabilisers were homogenised separately into 100ml solution each using 6003 RCF (20000 rpm) for 5 minutes using laboratory scale homogeniser (T25 Ultra TURRAX®, IKA, Staufen, Germany). Distilled water, sugar and emulsifier were then added and homogenised at 6003 RCF (20000 rpm) for 5 minutes or until all the sugar dissolved. Then, pre-homogenised carmoisine red, sodium benzoate, citric acid and stabilisers were added into sugar solution and homogenised at 6003 RCF (20000 rpm) for 5 minutes. Consequently, the pink guava puree, guava booster and vitamin E were then added and homogenised at 6003 RCF (20000 rpm) for 5 minutes to form a complete full mixture of PGJ. The juice was bottled and processed at 100 °C for 5 minutes, followed by immediate cooling to room temperature. The specific formulation of the single strength pink guava juice is shown in Table 1. In the drink formulation, citric acid was added mainly as an acidulant to reduce the pH of PGJ to between 3.0-3.5 range. Carmoisine red, guava booster and sodium benzoate on the other hand were mainly added as a colour enhancer, flavour enhancer and preservatives, respectively. Amount of vitamin E, stabilisers and emulsifier were added according to Table 1 and experimental work of study is shown in Figure 2.

Table 1. Formulation of the single strength pink guava juice

Ingredient	Percentage (% w/v)
Pink guava puree	12
Sucrose	9
Citric acid	0.15
Carmoisine red	0.0006
Guava booster	0.046
Sodium benzoate	0.015
Distilled Water	77.5
Carboxymethylcellulose (CMC)	0.2
Tocotrienol palm oil extract (Vitamin E)	0.05
Polysorbate 80 (Food Emulsifier)	1.0

Determination of protein

Protein content of PGJ was measured using protein analyser (Behrotest K90, behr Labor-Technik GmbH, Düsseldorf, Germany) and method used was adopted from the AOAC methods (AOAC, 2016). Three 20 g PGJ samples were placed in the digestion tubes. Then, each of the tube was added with 20 ml concentrated (95 – 98%) sulfuric acid, 0.48 g of mercury oxide tablet as a catalyst and 4.52 g of potassium sulfate. Blanks containing all these reagents were simultaneously processed. The tubes were placed in the preheated digestion block at 420 °C for 2 hours and 30 min. The resulting solutions were cooled at room temperature and diluted by adding 30 ml of water. The tubes were placed in the distillation-titration unit. Then, 20 ml of sodium hydroxide solution were automatically added, and the solutions were distilled for 6 min. About 30 ml ammonia collected in the receiving solution was automatically titrated against the standard 0.25 M hydrochloric acid with colorimetric end point detection.

Determination of carbohydrate

Carbohydrate was measured using the calculation by difference adopted from AOAC methods (AOAC, 2016). Carbohydrate content in PGJ was determined by subtracting the total percentage of other components such as protein, fat, moisture, ash and fibre from 100 using the following equation below;

$$\text{Carbohydrate (\%)} = 100 - (\text{percentage [protein + fat + moisture + ash + fiber]})$$

Determination of fat

Fat determination was carried out using the AOAC methods (AOAC, 2016). Modified Mojonnier method was carried out using three replicates of 10 ml PGJ samples loaded into a Mojonnier fat extraction flask. Two ml ammonium hydroxide, 10 ml of 95% ethanol and 25 ml ethyl ether were added into the flasks and shaken vigorously to neutralize the acid, dissolved protein, prevents gel formation and dissolved lipid in PGJ samples. After that, samples were centrifuged for 30 sec at 121 RCF (600 rpm). Then, clear solvent was decanted from Mojonnier flask into pre-weight Mojonnier fat dish and was dried on a hot plate at 100 °C in a hood. Dish and fat were dried to a constant weight in the oven at 100 °C and finally, the dish was cooled at room temperature and weighed.

Determination of total fiber

Total fiber content of PGJ was measured using fiber apparatus (Fibertec 1023 System E, Foss Analytical AB, Höganäs, Sweden) and method used was adopted from AOAC methods (AOAC, 2016). Ten g PGJ samples was added with 1 ml alpha-amylase. After that, the sample was incubated at 95 °C for 30 min then cooled down to 60 °C. The pH of PGJ samples was adjusted to 7.5 by adding 1 N NaOH and then 100 mg of Neutrase was added and incubated for 30 min at 60 °C. The pH was then adjusted to 4 by using 1 N HCl solution. One mg amyloglucosidase solution was added and incubated at 60°C for 30 min. Finally, the mixture was filtered through Whatman No.4 filter paper, dried in the hot air oven at 50 °C for 12 hours and weighed.

Determination of ash

The dry ashing procedure was performed according to AOAC methods (AOAC, 2016). Three PGJ samples with 30 g each were weighed and placed in the 50 ml crucibles and dried in an oven at 105 °C for 72 hours. Then, they were covered, cooled in the desiccators, and weighed. The samples were re-dried for one hour in the oven, cooled, and reweighed. The process was repeated at 1 hour drying intervals until the differences in weight were less than 0.1%. After that, the dried samples in the crucibles were subjected to ashing in an electrical furnace with temperature 550 °C for 16 hours. Finally, they were covered, cooled in the desiccators and the final weight was recorded.

Determination of moisture

Moisture determination was carried out using the AOAC methods (AOAC, 2016). Three 20 g PGJ samples were weighed and filled in 50 ml beaker and then dried in air oven drier at 70 °C for 48 hours. The drying process was continued until three consecutive constant weights (equilibrium) were achieved. Percentage of moisture was calculated using the following equation below;

$$\text{Moisture (\%)} = ((\text{initial weight} - \text{equilibrium weight}) / (\text{initial weight}) \times 100$$

Titrateable acidity

The titrateable acidity method used was adopted from AOAC methods (AOAC, 2016). Approximately 5 ml of pink guava juice was placed in a 250 ml beaker, and 200 ml of distilled water was added. Then, this solution was titrated against standardised 0.1 N NaOH to the phenolphthalein (as an indicator) to the end point (pH 8.2 ± 0.1). The volume of NaOH was converted to grams of citric acid per 100 ml of juice, and the total acidity was calculated using the following equation below;

$$\text{TA (\%)} = ((V \times 0.1\text{N NaOH} \times 0.067 \times 100)) / v$$

Where V is titer volume of NaOH, and v is volume of pink guava juice (ml).

Determination of pH value

The pH values of samples were determined using pH meter (MP320U, Mettler-Toledo, Switzerland). The pH meter was calibrated using buffer solution (pH 7 and 4) before usage. Sample of 100 ml volume was placed in beaker and pH was recorded. Three samples were measured and averaged.

Determination of total soluble solid (TSS)

Hand-held refractometer (Master M, Atago, Tokyo, Japan) was used to measure total soluble solid of samples. One drop of sample was placed onto the surface of the main prism and the °Brix was then observed and recorded. Three samples were measured and averaged.

Determination of water activity (a_w)

Water activity of sample was determined using water activity meter (Aqualab 4TE, Decagon, USA) where 7.5 ml sample was placed into a disposable cup in the instrument. The cup was inserted into the chamber lid and water activity value was obtained and recorded. Three samples were measured and averaged.

Determination of viscosity

Viscosity was measured using a rotatory viscometer (V1-L, Myr, Tarragona, Spain) equipped with spindle protector and temperature sensor (-15 °C to 180 °C). Juice with 900 ml volume was filled in the 1000 ml beaker and viscosity was determined using L1 spindle, with a rotation speed of 30 rpm at room temperature. Three readings were measured and averaged.

Determination of colour

The changes in colour were determined using chromameter (CR400, Minolta, Japan). The colour indices was measured using CIE L*C*H_o colour space (The International Commission on Illumination, Vienna, Austria) with illuminant of D65 and 2o observer. L* is a measure of lightness ranging from 0 (black) to 100 (white) and colour coordinates, a* which takes positive values for redness colour and negative values for greenness and b* positive for yellowness colour and negative for blueness. From these coordinates, other colour parameters are calculated, where 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 as following equation below;

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2}$$

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 following equation below;

$$\text{Hue angle} = (\tan^{-1} a^*/b^*)$$

Determination of vitamin E

Vitamin E was determined following the AOAC methods (AOAC, 1997). 10 μ L sample was injected into high performance liquid chromatography (HPLC) (1200 Series, Agilent, USA). Mobile phase that was used were mixture of acetonitrile, chloroform, and ethyl acetate in 88:4:8 ratio. Flow rate of HPLC was set at 1.3 ml/min and the wavelength at 292 nm then the vitamin E was detected by UV detector and analytical column C18 was used.

Statistical analysis

The results obtained were subjected to analysis of variance (ANOVA) using IBM SPSS Statistics version 15.0 (IBM, Armonk, New York). The Duncan's post hoc multiple comparisons was used to obtain statistical comparisons among sample means and differences were only considered significant at a confidence level superior to 95% ($p < 0.05$)

Result and Discussion

Proximate analysis of PGJ

The results of proximate analysis of PGJ after processing was carried out to obtain the percentage of moisture content, ash, protein, total fiber, fat and carbohydrate in PGJ. Table 2 shows the composition of protein, carbohydrate, and fat of PGJ were 0.67 ± 0.15^a %, 4.96 ± 0.59^a % and 0.27 ± 0.05^a %, respectively. Studies done on several fruit juices such as guava, grape, apple, pineapple, lemon lime and orange found that the composition for protein, fat and carbohydrate were observed to be in range of 0.05 to 1.5%, 0.1 to 0.3% and 4.0 to 23.05%, respectively (Okokon and Okokon, 2019; Chuku and Akani, 2015 and Muramatsu et al., 2010). It means that the result of this study is an agreement with the finding by Okokon and Okokon, (2019), Chuku and Akani, (2015) and Muramatsu et al. (2010).

Table 2. Proximate composition of PGJ after processing

Composition	(%)
Protein	0.67 ± 0.15^a
Carbohydrate	4.96 ± 0.59^a
Fat	0.27 ± 0.05^a
Total fiber	0.74 ± 0.10^a
Ash	0.64 ± 0.10^a
Moisture content	92.72 ± 0.91^a

Data are mean \pm S.D. (n=3)

^a Different letter in the same column indicates significant difference at $p < 0.05$

In addition, result also showed that the moisture content, ash and total fibre composition of PGJ were 92.72 ± 0.91^a %, 0.64 ± 0.10^a % and 0.74 ± 0.10^a %, respectively. Researches done by Okokon and Okokon, (2019), Chuku and Akani, (2015) and Muramatsu et al. (2010) found that the moisture content, ash and total fibre percentages were in a range of 87% to 93%, 0.3 to 0.8% and 0.1 to 2.5%, respectively for fruit juices such as guava, grape, apple, pineapple, lemon, lime, orange, cherry and apricot juice.

Physicochemical properties of PGJ

The results of physicochemical properties of PGJ after processing are as shown in Table 3 for titratable acidity, pH, total soluble solid, water activity, viscosity and colour value lightness, chroma and hue angle. Results show that titratable acidity value is at 0.41 ± 0.10^a %. Lamo et al. (2019), Okokon and Okokon, (2019) and Silva et al. (2016) found that the titratable acidity values for guava juice were in range of 0.46% to 0.67%. Low titratable acidity values were also inherent in other fruit juices such as apple juice, banana juice, grape juice, papaya juice and star fruit juice.

Table 3. Physico-chemical Properties of PGJ after Processing

Physico-chemical properties	PGJ After processing
Titrateable acidity (% citric acid)	0.41 ± 0.10 ^a
pH	3.65 ± 0.12 ^a
Total soluble solid value (°Brix)	10.56 ± 0.43 ^a
Water activity (a _w)	0.99 ± 0.02 ^a
Viscosity (mPa.s)	15.33 ± 1.15 ^a
Lightness (L*)	31.95 ± 0.02 ^a
Chroma (C*)	34.65 ± 0.01 ^a
Hue angle (H°)	25.42 ± 0.02 ^a
Vitamin E (mg/l)	17.44 ± 1.63 ^a

Data are mean ± S.D. (n=3)

^a Different letter in the same column indicates significant difference at p<0.05

As shown in Table 3, the pH of PGJ is 3.65 ± 0.12^a. This result is an agreement with the research done by Lamo et al. (2019), Okokon and Okokon, (2019) and Silva et al. (2016), found that pH value of guava juice was in a range of 3.2 to 4.29. Citric acid and sodium benzoate were used to reduce the pH and acted as suitable preservatives added in PGJ formulation to control microbial growth. According to Parker and Pace (2017), acidulant were used to reduce the pH of fruit products such as fruit juices to a value below 4.5 primarily to promote the sour taste, control microbial growth, delay enzymatic browning during storage and provide suitable pH for benzoic acid functionality.

The result showed that the °Brix of PGJ was 10.56 ± 0.43^a. Lamo et al. (2019) and Silva et al. (2016) reported that the total soluble solid values for guava juice were observed to be in a range of 8.2 to 12.5. Water activity value was 0.99 ± 0.02 and similar results were also reported by Shamsudin et al. (2005), who found that for fruits and vegetables products, the water activity values typically range from 0.97 to 1 due to high moisture content (92.9%) in the fruit juice. In this study, the existence of high water activity values were due to the addition of 78-79% of water in PGJ formulation.

The results in Table 3 show that the viscosity of PGJ measured at room temperature was 15.33 ± 1.15^a mPa.s. Surajbhan et al. (2012) and Shamsudin et al. (2005) reported that the viscosity of guava juice measured at room temperature was observed to be in a range of 15.68 to 35.00 mPa.s. According to Surajbhan et al. (2012) there was no fixed range of viscosity value in guava juice because viscosity value was prone to changes depending on temperature, concentration, and total soluble solids.

Result on colour properties showed that the L* value, C* value and H° value for PGJ were 31.95 ± 0.02^a, 34.65 ± 0.01^a and 25.42 ± 0.02^a, respectively. On the other hand, Osorio et al. (2011) reported that the colour properties such as L* value, C* value and H° value for guava aqueous extract were 49.50 ± 0.09, 24.06 ± 0.08 and 62.75 ± 0.06, respectively. Aishah et al. (2016a) stated that PGJ stored at higher temperature produce faster colour change compare to lower temperature. The most affected quality attributes were anthocyanins and total phenolic contents, followed by vitamin C and the least affected was lycopene.

Result on vitamin E stability upon processing showed that processing of PGJ reduced to 96.5% of vitamin E initial content from 500mg/l to 17.44 ± 1.63^a mg/l. Vitamin E was observed to be less stable to heat after undergoing processing treatment (at 100°C). Aishah et al. (2016b) and Nattawan et al. (2014) stated that vitamin E were relatively unstable and start to deteriorate when heating at 30°C and above.

Conclusion

Proximate and physico-chemical properties in PGJ was fortified with vitamin E was determined and this result can be used as a primary research data for further future PGJ related studies. Results showed that PGJ contained protein (0.67 ± 0.15^a %), carbohydrate (4.96 ± 0.59^a %), fat (0.27 ± 0.05^a %), total fiber (0.74 ± 0.10^a %), ash (0.64 ± 0.10^a %), moisture (92.72 ± 0.91^a %), acidity (0.41 ± 0.10^a %), pH (3.65 ± 0.12^a), TSS (10.56 ± 0.43^a), a_w (0.99 ± 0.02^a), viscosity (15.33 ± 1.15^a mPa.s), L^* (31.95 ± 0.02^a), C^* (34.65 ± 0.01^a), H^o (25.42 ± 0.02^a) and vitamin E content (17.44 ± 1.63^a mg/l). Further study can be performed involving PGJ such as determination of the best stabiliser, emulsifier, emulsification performance, vitamin E stability and sensory evaluation.

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