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Application of red pitaya powder as a natural food colourant in fruit pastille[1]

Aplikasi tepung buah naga merah sebagai pewarna makanan alami pada permen buah

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ABSTRACT

Background: *Confectionary products meet the important consumers' need states of fun and enjoyment, especially among children. Synthetic colourant had been applied as a colouring agent in confectionary products for decades, however various adverse health effects have been reported after consumption. Hence, usage of natural colourant has increased enormously as it confers functional and nutraceutical benefits. Red pitaya, a common and popular fruit cultivated in South-east Asian countries. Its rich betacyanin content that gives the fruit a red-violet colour. Hence, red pitaya is a potential source of natural colorant as an alternative to synthetic colorant.* ***Objective:*** *This research was aimed to produce fruit pastille with red pitaya powder applied as a natural colourant.* ***Method:*** *Production of red pitaya powder was achieved through spray drying process. Fruit pastille was prepared and subjected to antioxidant, stability and sensory analysis.* ***Results:*** *Physicochemical study showed that pastille incorporated with red pitaya powder exhibited significantly (p<0.05) higher antioxidant properties than the blank pastille (control). An eight weeks storage stability study revealed that betacyanin content of pastille incorporated with pitaya powder remained stable for the first four weeks of storage. Besides, no significant change was observed in redness (a*) of pastille throughout the storage study. Sensory study was carried out to assess the consumer preference on pastille incorporated with pitaya powder and synthetic colourant. Colour attribute of pastille incorporated with red pitaya powder has gained significantly (p<0.05) higher liking that the one added with synthetic colour.* ***Conclusion:*** *Red pitaya powder could be a potential natural colourant for gummy confectionary.*

KEY WORDS: *antioxidant; betacyanin; natural colourant; red pitaya; spray drying; stability*

ABSTRAK

Latar Belakang: Produk makanan harapannya dapat memenuhi kebutuhan konsumen dari segi kesenangan dan kenikmatan, terutama di kalangan anak-anak. Pewarna sintetik telah digunakan sebagai zat pewarna pada produk permen selama beberapa dekade. Namun, berbagai efek kesehatan yang merugikan timbul akibat konsumsi permen. Oleh karena itu, penggunaan pewarna alami meningkat sangat besar karena memberikan manfaat fungsional dan nutrasetikal. Buah naga merah merupakan buah umum dan populer dibudidayakan di negara-negara Asia Tenggara, kaya akan kandungan betacyanin yang memberikan warna merah-violet. Buah naga merah merupakan sumber potensial dari pewarna alami sebagai alternatif pewarna sintetik. **Tujuan:** Menghasilkan permen buah dengan tepung buah naga merah sebagai pewarna alami. **Metode:** Produksi tepung buah naga merah diperoleh melalui proses *spray drying*. Permen buah naga tersebut dianalisis kandungan dan stabilitas antioksidan serta uji sensoris. **Hasil:** Hasil penelitian menunjukkan bahwa kandungan antioksidan permen buah dengan tepung buah naga merah lebih tinggi dibandingkan dengan permen buah kontrol (p<0,05). Setelah penyimpanan delapan minggu, diketahui bahwa kandungan betacyanin permen buah dengan tepung buah naga tetap stabil selama empat minggu pertama penyimpanan. Selain itu, tidak ada perubahan signifikan pada warna kemerahan (a*) dari permen buah selama uji stabilitas. Studi sensorik dilakukan untuk menilai preferensi konsumen pada permen dengan tepung buah naga dan pewarna sintetis. Warna permen dengan tepung buah naga merah lebih disukai dibandingkan dengan permen kontrol yang ditambahkan warna sintetis (p<0,05). **Simpulan:** Tepung buah naga merah dapat menjadi pewarna alami yang potensial untuk permen buah.

KATA KUNCI: antioksidan; betacyanin; pewarna alami; buah naga merah; *spray drying*; stabilitas

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INTRODUCTION

Colour plays an important role in enhancing the appearance and improving the quality of food products. Synthetic colour are widely used by manufacturer as economical is always a better choice. However, there is an uncontrolled use of synthetic colour particularly in food products that is mostly consumed by children. Several studies suggested that consumption of high doses of artificial colorants poses negative effect to the consumers such as damage in different organs (1,2). Therefore, the use of synthetic colorants in food faced with controversy, especially in children nutrition when the food is added with the colours at high doses. Researchers and consumers hence switched their attention to natural colourant.

Hylocereus polyrhizus, also known as red flesh red pitaya is a member of the family Cactaceae. Red pitaya is also locally known as dragon fruit or *pitaberry*. This species of red pitaya has an oval appearance an attractive purple-red peel and flesh. The flesh is well-dispersed with small black seeds which is rich in essential fatty acids and it is delicate and juicy (3,4). The fruit is highly cultivated South-East Asia country such as Malaysia, Vietnam and Indonesia. The attractive purple-red colour of *Hylocereus polyrhizus* flesh is contributed by a set of pigments called betalains. Betalains are water soluble nitrogen-containing pigments which can be divided into red-violet pigment called betacyanins and yellow pigment called betaxanthins. According to Ow, Boyce & Somasundram (5), betaxanthins are totally devoid in red pitaya and at least seven identified betacyanins in the *Hylocereus* genus. These betacyanins identified are betanin, isobetanin, phyllocactin, isophyllocactin, betanidin, isobetanidin, and bougainvillein-R-1. Betacyanin pigment has antioxidant properties that exhibit health benefits hence making it a potential source as natural pigment and functional ingredient in food products (6).

Red pitaya can be made into puree, juice or powder to contribute its function as natural colourant in food products. However, puree and juice has lower shelf life compare to powder due to its high moisture content and water activity. Fruit juice powders are produced to maintain and preserve its quality. Among the drying techniques, spray drying is usually applied to produce the fruit juice powder. Spray drying process converts fluid material into dry solid particles which the liquid is being atomized in a hot gas medium to instantaneously obtain the powder (7). Besides, there are various researches done on application of spray dried red pitaya powder in food products such as yoghurt (8). However, there is a limited research on application of spray dried red pitaya powder in solid food product such as sugar confectionary. Soft confectionary such as pastille, gummy bears and jellies are preferred by parents compared to hard candies as it is easier to be consumed by young children. There is also scant in study with regard the stability of red pitaya powder in food model especially solid food. The aim of this research was to produce fruit pastille by incorporating spray dried red pitaya powder as natural colorant. The functional properties and the stability of betacyanin in fruit pastille were also studied.

METHODS

Raw material

All fresh red pitaya fruits were purchased from fruit shop 'MBG' located in Sunway Pyramid, Selangor, Malaysia. All fruits were stored in chiller until further use. Maltodextrin with dextrose equivalent (DE) 10 - 13 % was purchased from V.I.S Foodtech Ingredient Supplies Sdn. Bhd., Kuala Lumpur, Malaysia. Gula Prai brand of granulated sugar and CSR brand of caster sugar were purchased from Cold Storage, Sunway Pyramid, Petaling Jaya, Selangor. Low methoxyl pectin, sodium citrate and citric acid were obtained from Modernist Pantry, USA. Glucose syrup and blackcurrant flavour were obtained from V.I.S Foodtech Ingredient Supplies Sdn. Bhd., Kepong, Kuala Lumpur. AmeriColor electric purple synthetic food colourant was purchased from Bake with Yen Sdn. Bhd., Selangor, Malaysia.

Chemical and reagent

Folin-Ciocalteu's (FC) reagent, sodium carbonate anhydrous, denatured ethanol, 1M sodium hydroxide, di-sodium hydrogen phosphate, potassium dihydrogen phosphate, potassium hexacyanoferrate (III), iron (III) chloride anhydrous, trifluroacetic acid (TFA), methanol, acetonitrile were purchased from Merck, Germany. Sodium nitrite was obtained from VWR, European Community. Aluminium chloride-6-hydrate was obtained from Bendosen Laboratory Chemicals, Norway. gallic acid, catechin, betanin (red beet extract diluted with dextrin), and trichloroacetic acid were obtained from Sigma-Aldrich, USA.

Preparation of Red Pitaya juice

Red pitaya fruit was peeled and cut into smaller pieces. The cut flesh was blended into puree using blender (Model: MX-900M, Panasonic, Malaysia). The puree was filtered three times using muslin cloth to remove seeds and unwanted solids prior to spray drying.

Preparation of Red Pitaya powder

The filtered juice was mixed with distilled water at 1:2 ratio. 15% (w/w) of maltodextrin was added to the red pitaya juice and homogenised using blender (Model: MX-900M, Panasonic, Malaysia The red pitaya juice and maltodextrin mixture was then subjected to laboratory scale spray dryer (Model: SD-06, Lab-Plant, UK) to obtained spray dried red pitaya powder. The spray dryer was equipped with 0.5 mm spray nozzle and 215 mm OD × 500mm long. The inlet temperature of spray dryer was maintained at 140 ˚C, pump speed at 15 (feed flow of 11.58 mL/min), fan setting at 50 (air speed at exhaust of 4.3 m/s) and pressure at 2 bar. The red pitaya powder obtained was collected in Schott bottle wrapped with aluminium foil and stored at room temperature till further use as natural colourant in fruit pastille.

Preparation of fruit pastille

Fruit pastille was prepared according to formulation of pectin pastille provided by Herbstreith&Fox (9) with some modification. The fruit pastille was demoulded and cut into approximately 1.3 cm × 1 cm cube and coated with caster sugar. Four batches of fruit pastille were prepared. The fruit pastille was collected in Schott bottle covered with aluminium foil and stored in room temperature until further analysis.

Antioxidant analysis

Total phenolic content (TPC)

For sample preparation, 2 g of fruit pastille was dissolved in 20 mL of distilled water (1:10). Standard and sample solutions were pipetted 0.3 mL and mixed with 10 mL of FC reagent (x10 dilution), then stand for 3 minutes. It is then followed by adding 0.8 mL of 7.5% (w/v) sodium carbonate solution into the mixture and vortex. The mixture was incubated in dark for 2 hours and the absorbance was recorded using UV-Vis spectrometer (GENESYS 10UV, Thermo Fisher Scientific, Massachusetts, USA) at wavelength 765 nm. The UV-Vis spectrometer was calibrated using 70% denatured ethanol as blank. Concentration of total phenolic was expressed as milligram of gallic acids equivalents (GAE) per 100 g of fruit pastille. The calibrated standard curve equation for gallic acid was $y = 18.17x + 0.13458$ ($R^2 = 0.993$).

Total flavonoid content (TFC)

For sample preparation, 2 g of pastille was dissolved in 20 mL distilled water (1:10). Standards and sample solutions were being pipetted 0.25 mL and added into test tube, followed by 1.25 mL distilled water and 75 µL of 5% sodium nitrite was added. The mixture was let stand for 6 minutes and 150 µL of 10% aluminium chloride-6-hydrate solution was added into the mixture, then stand for 5 minutes. Then, 0.5 mL of 1M sodium hydroxide and 250 µL of distilled water were added into the mixture. The mixture in test tube was vortexed. The absorbance was measured by using UV-Vis spectrophotometer at wavelength of 510 nm and distilled water was used as blank. Concentration of total flavonoid was expressed as milligram of catechin equivalents (CE) per 100 g of fruit pastille. The calibrated standard curve equation for catechin was $y = 0.0033x + 0.0045$ ($R^2 = 0.9995$).

Ferric reducing antioxidant power (FRAP)

For sample preparation, 2 g of pastille was dissolved in 20 mL distilled water (1:10). Standards and sample solutions were being pipetted 1 mL then mixed with 2.5 mL of 0.2 M phosphate buffer at pH 6.6 and 2.5 mL 1% potassium ferricyanide. The mixture was incubated at 50 ˚C for 20 minutes. Then, 2.5 mL of 10% trichloroacetic acid was added to the incubated mixture and vortexed. The mixture was being pipetted 2.5 mL and transferred into new test tube. Next, 2.5 mL of distilled water and 0.5 mL of 1% iron (III) chloride were mixed into the mixture and incubated for 30 minutes. The absorbance was measured at 700 nm using UV-Vis spectrophotometer and 70% denatured ethanol was used as blank. The antioxidant activity was expressed as milligram of gallic acid equivalents (GAE) per 100 g of fruit pastille. The calibrated standard curve equation was y = 34.105x + 0.12151 (R^2 = 0.99508).

Betacyanin

High performance liquid chromatography (HPLC) (Shimadzu Prominence UFLC, Columbia, USA) was used to identify the betacyanin content in fruit pastille. The HPLC system was equipped with DGU-20A degassing unit, LC-20AT solvent delivery unit, SIL-20A $_{HT}$ auto sampler, SPD-M20A diode array detector and CTO-10AS $_{VP}$ column oven. Betacyanin content of fruit pastille was separated using reversed column Thermo Scientific Hypersil Gold column (5 µm, 150 × 4.6 mm). The mobile phase used for elution is an isocratic solution contains a mixture of 0.5% of 90% trifluoroacetic acid (TFA) and 10% of pure acetonitrile. One liter of mobile phase was prepared by mixing 900 mL of 4.5 mL of TFA with 900 mL of milli Q water and 100 mL of pure acetonitrile. Flow rate of the elution solvent was set at 1.00 mL/min for 15 minutes per injection with injection volume 20 µL. The column condition was set at 25 ˚C and absorbance at 536 nm.

Betanin was used as standard. For sample preparation, 2 g of fruit pastille was dissolved in 20 mL of distilled water (1:10) and the fruit pastille was cut into smaller pieces prior to dissolving it. All standards and sample solution were filtered using Minisart® RC 0.45 µm cellulose acetate syringe filter membrane (Sartorius, Germany) and transferred into 2.0 mL clear vial. The result was reported in mg/100g fruit pastille and the calibrated curve equation was y = 155806.0824x – 2.4312 (R^2 = 0.9962).

Colour measurement

The colour (*L**, *a** and *b** values) of the fruit pastille was measured using HunterLab ColorFlex EZ colorimeter (Hunter Associates Laboratory, Virginia, USA). The *L** value represents the lightness of the sample where a low number (0 – 50) indicates dark and a high number (51 – 100) indicates light. The *a** value represents red vs. green, where a positive number represents red and a negative number represents green. The *b**value represents yellow vs. blue, where a positive number represents yellow and a negative number represents blue as defined by Choudhury (10). The colorimeter was calibrated with standard black tile and white tile prior to sample analysis. Fruit pastille was placed and cover the bottom part of the glass optical cell, then the glass optical cell was placed on the reflectance port to read.

Sensory

Sensory study was conducted to access the consumer preference on fruit pastille incorporated with spray dried red pitaya powder and synthetic colourant. The attributes of the fruit pastille set were colour, aroma, sweetness, sourness, texture, chewiness and overall acceptability. The samples were coded with 3 digits random coding and 9-point hedonic scale was used for the sensory ratings. The sample size of the panellist was 50.

Statistical analysis

Independent t-test was used to determine difference between the sample incorporated with and without red pitaya powder. Repeated measure ANOVA was used to determine significant changes over eight weeks of storage stability study of fruit pastille at significant level $p < 0.05$.

RESULTS

Antioxidant properties

Table 1 shows the TPC, TFC and FRAP of control and fruit pastille added with red pitaya powder. Fruit

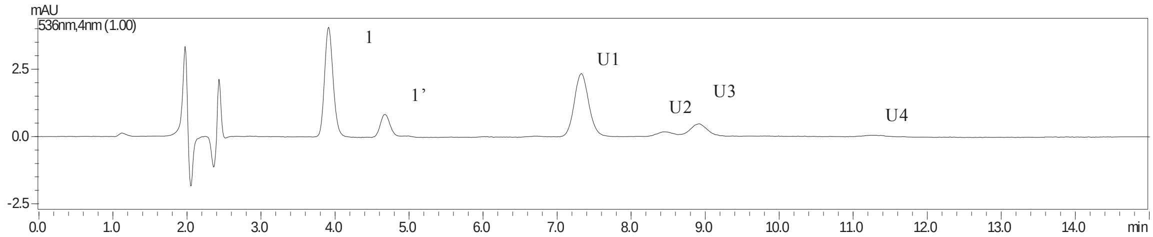

Figure 1. HPLC chromatogram of betacyanins from fruit pastille incorporated with spray dried red pitaya (*H. polyrhizus*) powder.

Table 1. TPC, TFC and FRAP of control and fruit pastille incorporated with red pitaya powder

Antioxidant test	Control	Red pitaya powder
TPC (mg GAE / 100g)	158.00 ± 0.85[a]	249.73 ± 12.9[b]
TFC (µg CE / 100g)	0.62 ± 0.30[a]	4.93 ± 1.61[b]
FRAP (mg GAE / 100g)	244.00 ± 14.66[a]	380.13 ± 35.17[b]

All of the results are presented as mean ± standard deviation of the means. Values are means of triplicates from four separate runs (n = 4). [a,b]Mean values labelled with different subscript letters are significantly different ($p<0.05$)

pastille added with red pitaya powder poses significantly higher TPC, TFC and FRAP as compared to control.

Stability analysis

Betacyanin content

Figure 1 displays the HPLC chromatogram of betacyanin in fruit pastille incorporated with red pitaya powder. Total 6 peaks are shown in **Figure 1**, which retention time are 3.9 min (peak 1), 4.6 min (peak 1'), 7.4 min (peak U1), 8.5 min (peak U2), 9.0 min (peak U4) and 11.4 min (peak U4). From the respective retention time in comparison to commercially obtained betanin standard, peak 1 was identified as betanin while peak 1' was isobetanin. Peak U1, U2, U3 and U4 was unknown due to unavailable of other standards.

According to **Figure 2**, there is a decline trend in concentration of betacyanin in eight weeks of storage. It can be seen that there was a significant decrease in betacyanin content at the fifth week of storage, with betacyanin retention value 77.3%.

Colour

Figure 3 shows the trend in changes of colour ($L*$ $a*$ $b*$ value) of fruit pastille in eight weeks of storage. This research investigated reading of $b*$ value gradually increased throughout the storage test, indicated that the colour changed from more bluish to more yellowish. Meanwhile, the lightness ($L*$) showed a declined trend however no significance difference between each week throughout the eighth week of storage. The redness ($a*$) was remain stable throughout eight weeks of storage.

Sensory evaluation

The difference between hedonic score of fruit pastille added with synthetic colourant (FPS) and natural colourant (FPN) is displayed in **Figure 4**. There was a significant difference ($p<0.05$) in terms of colour between the samples, with the mean hedonic score 7.28 for FPN compare to 6.24 for FPS. Moreover, the mean hedonic scores for FPN and FPS were, 6.42 and 6.02 for aroma, 5.90 and 6.38 for texture, 6.72 and 6.54 for overall acceptability. However, no significant difference showed in mean hedonic scores between fruit pastille with natural colourant and control under the attributes stated. Moreover, **Figure 5** shows the fruit pastille samples.

DISCUSSION

Antioxidant properties

Red pitaya which is rich in plant source bioactive compound such as phenolic compound, which have been recognized as potential compound to reduce risk of disease such as coronary heart disease, cancer and reduce cholesterol level which mainly contributed by its antioxidant properties (11,12). Therefore, fruit pastille was subjected to various spectrometric methods for the quantification of the antioxidant compound, such as total

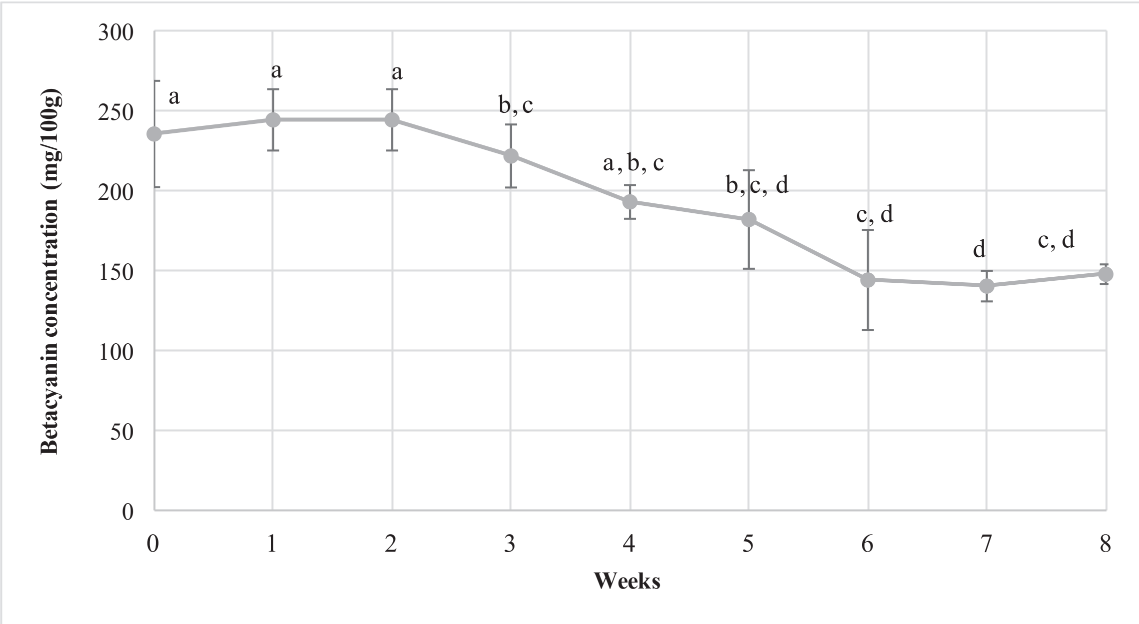

Figure 2. Concentration of betacyanin (mg betanin equivalent / 100g of fruit pastille) in eight weeks of storage

*All of the results are presented as mean ± standard deviation of the means. Values are means of triplicates from four separate runs (n = 4). Mean values labelled with different subscript letters are significantly different (p<0.05)

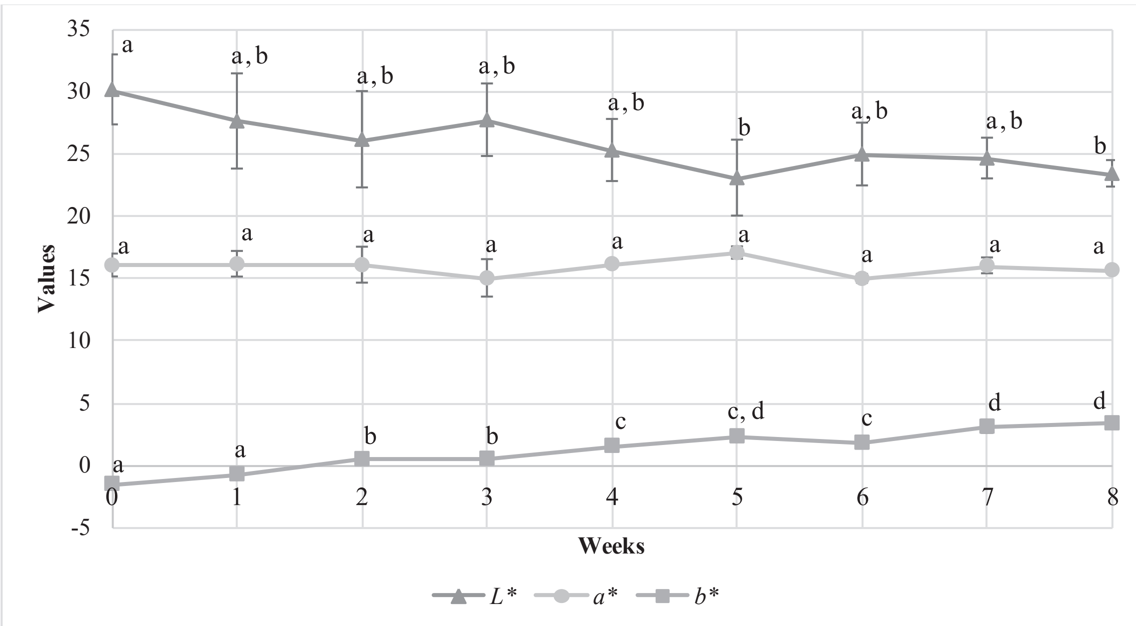

Figure 3. Colour (*L* a* b value) of fruit pastille in eight weeks of storage**

*All of the results are presented as mean ± standard deviation of the means. Values are means of triplicates from four separate runs (n = 12). Points labeled with different subscript letters in the same group are significantly different (p<0.05)

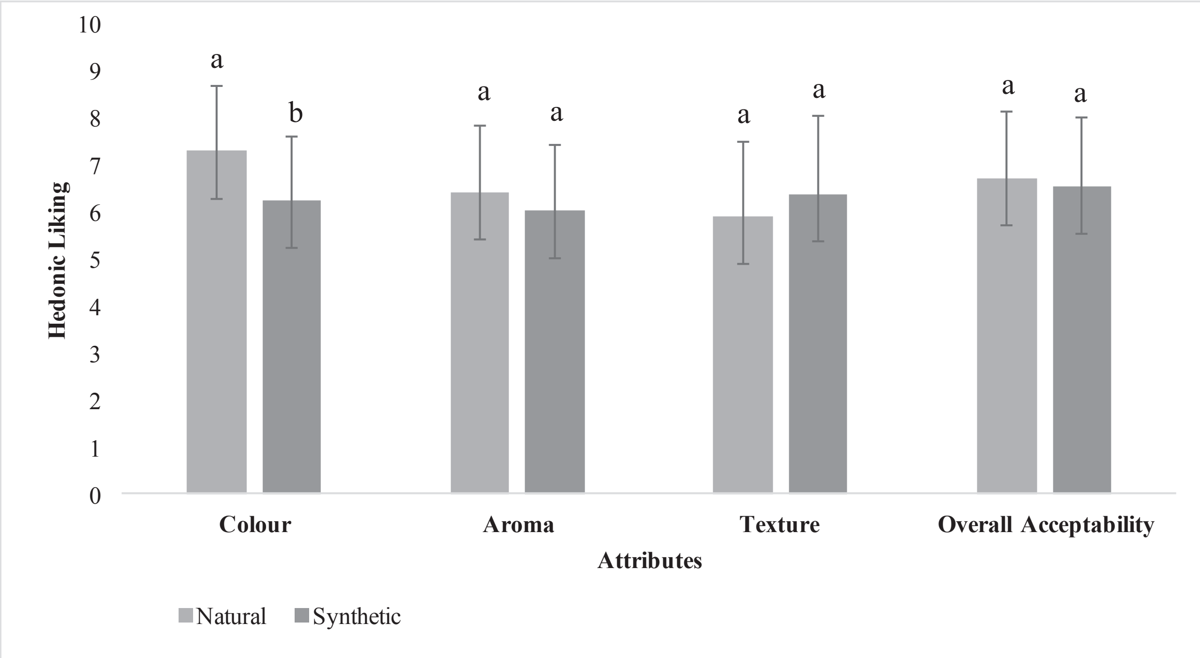

Figure 4. Sensory evaluation on attributes: colour, aroma, texture, overall acceptability of fruit pastille incorporated with natural and synthetic colouring. Bar chart labeled with different subscript letters in the same group are significantly different (p<0.05)

*All of the results are presented as mean ± standard deviation of the mean (n=50). Mean values labelled with different subscript letters are significantly different ($p < 0.05$).

Figure 5. Fruit pastille incorporated with synthetic colourant (left) and red pitaya powder (right)

phenolic content (TPC), total flavonoid content (TFC) and ferric reducing agent (FRAP).

TPC assay method measured both bound and unbound phenolic compound. Determination of TPC in this research was through FC method. There were statistically significant differences (*p*<0.05) in antioxidant properties between fruit pastille incorporated with red pitaya powder (sample) and the control, which sample has 1.5 times higher TPC then control. The significant higher value of TPC in sample could be explained by the presence of red pitaya powder as natural colourant. According to Lee (13), red pitaya powder contains a significant amount of total phenolic compound. Betacyanins in red pitaya powder could also contributed to the total phenolic due to a phenol structure in molecule (14). The concentration of betacyanin, expressed as betanin equivalents in fruit pastille was 235.89 mg / 100g of fruit pastille. Besides the phenolic compound, some other organic compounds with reducing ability such as sugar, amino acids, vitamin C and carotenoids may react with FC reagent (13). Therefore, the TPC in control may be contributed by the ingredients which are sugar and glucose syrup.

Red pitaya has also been reported high in flavonoid. Flavonoid content determined in sample was 7 times significantly (*p*<0.05) higher than control. These flavonoids possess a wide range of biological activities such as antioxidant, enzyme inhibiting and antibacterial effects (12) Aluminium chloride ($AlCl_3$) colorimetric method was used to examine the total flavonoid content in fruit pastille. However, only flavones and flavonols were found to complex stably with aluminium chloride hence the method seems to be specific for determination of rutin, luteolin and catechin. (15). Despite of the limitation, Chang et al. (16) stated that this method is sufficient for comparative analysis.

On the other hand, sample has 1.5 times higher ferric reducing antioxidant power compared to control. The reducing capability could be due to the presence of betacyanin, phenolic compound and flavonoid. For control, the antioxidant activity may be contributed by the presence of citric acid that worked as an acid regulator in fruit pastille as well as sugar. According to Prior, Wu & Schaich (17), sugars and citric acid are common interferences with FRAP. Therefore, with all this findings, fruit pastille incorporated with red pitaya powder contains significant antioxidant properties.

Stability analysis

Betacyanin content

Betanin (betanin-*O-ß*-glucoside) was eluted first, followed by isobetanin (isobetanin-*O-ß*-glucoside) in the reversed phase HPLC. This indicates that betanin was a more polar while isobetanin was less polar betacyanin aglycons, which isobetanin created a hydrophobic effect with the mobile phase hence causing the molecule spent more time to be eluted. This can be explained by the molecular structure of both betanin and isobetanin. Kujala et al. (18) explained that branched chain compound elute more rapidly than their corresponding isomers due to the smaller overall surface area. Isobetanin is epimers of betacyanin that have a different configuration at C-15, this allows isobetanin has a greater interaction with the stationary phase and hence greater interaction retention value (19).

Fruit pastille was subjected to storage stability study for eight weeks. It is evident that a decline trend in betacyanin concentration throughout eight weeks of storage. There was a significant (p<0.05) decrease in betacyanin concentration at the fifth week of storage, as through the following week, betacyanin concentration remained stable as no significant decrease observed. The natural degradation of the pigment can be explained by the hydrolysis of betanin into degraded compound, for instance, formation of betalamic acid under storage at 25 ˚C.

Colour

Fruit pastille incorporated with red pitaya powder (sample) was kept in an air-tight glass Schott bottle and stored in the dark at room temperature (25 ˚C) to minimize the degradation rate of betacyanin and maximize the colour stability. The pH of the fruit pastille ranged from 3.4 to 3.6. Moßhammer, Stintzing & Carle (20) confirmed that betalain may be applied over a pH range from 3 to 7, without tonality change.

Based on the observation, colour of fruit pastille gradually changed from red-violet to red colour. The overall colour changes agreed with study conducted by Cai & Croke (21), whom applied *Amaranthus* betacyanin pigment into jellies. They found that with extended storage time, the colour of jellies changed from purplish-red to orange-red then to very light yellow. Based on the colour changes (L^* a^* b^* value) of fruit pastille within eight weeks of storage, for the increased in b^* value, it can be explained by the degradation of betanin that caused formation of yellow degradation product such as betalamic acid which can be formed by cleavage of the cyclo-dopa or neobetacyanins, as well as formation of brown compound, which could be melanin-type polymerization product of cleaved *cyclo*-dopa 5-*O*-*ß*-glucoside Kaimainen et al. (22). Meanwhile, decrease in lightness may be due to the formation of brown pigment. Interestingly, the redness (a^*) was remain stable throughout eight weeks of storage and this may be due to the presence of hylocerenin and phyllocactin that formed red degradation products, thus exhibiting a better colour retention (23). Thus, in the eight weeks of storage, the colour of fruit pastille was fairly stable.

Sensory evaluation

Sensory evaluation is an essential element when a novel food product is developed. In food industries today, prime consideration is given towards the product's palatability and eating quality, followed by quality such as nutritional value and wholesomeness. Therefore, the food product manufacture must ensure that the product is well accepted by consumers. In this study, sensory analysis on the preference and acceptability of fruit pastille was conducted using 9-scale hedonic scale and pastille incorporated with synthetic colour was used as control.

The fruit pastille samples were prepared using the same amount of ingredients. The only variable was the colourant added to it. It is evident that substitution of red pitaya powder with synthetic colourant did not negatively affect the colour hedonic scores, conversely, improvement in the attribute score was observed. From observation, fruit pastille with red pitaya powder (FPN) has a brighter and more appealing red-violet colour, while fruit pastille incorporated with synthetic colorant (FPS) was in heavy and dull red. Hence, this may explained that FPN has higher hedonic score.

In terms of colour and appearance, FPN was described as 'Appealing', 'Attractive', and 'More intense in colour', whereas FPS was described as 'Dull'. Texture wise, although no significant difference between both samples, the hedonic scale of FPS was slightly higher. FPS was described as 'Chewy' and 'Harder' whereas FPN was described as 'Soft'. Most panelists prefer a more chewy texture compare to softer one. However, textural acceptability of fruit pastille ay be dependent on other confounding factors such as culture, experience or habits in consumption. In conclusion, the results showed promising potential of red pitaya powder as natural colourant in fruit pastille due to improvement in colour yet no altering in aroma, texture and overall acceptability.

CONCLUSION

The addition of spray dried red pitaya powder increased the antioxidant properties of the fruit pastille as well as enhanced the appearance of the pastille. The findings showed that there is significant amount of antioxidant such as phenolic and flavonoid presence in the pastille. Based on the results in sensory analysis, fruit pastille incorporated with natural colourant was well accepted by the panellist. In stability study, betacyanin concentration has a significant decrease after 1 month of storage with betacyanin retention value 77.3 %. Colour wise, fruit pastille changed from red-violet to red colour throughout the eight weeks of storage. All the findings suggested that incorporation of red pitaya powder increased the value of fruit pastille in terms of quality and nutritional. Therefore, spray dried red pitaya powder could be a potential natural colourants for gummy confectionary. It is suggested to apply colour retention techniques such as addition of ascorbic acid to prolong the pigment retention in fruit pastille.

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Erratum

Renyoet BS, Martianto D, Iskandar D. Potensi kerugian ekonomi akibat biaya rawat inap dan rawat jalan pada balita yang mengalami obesitas sampai dewasa di Indonesia. Jurnal Gizi Klinik Indonesia 2016;13(2):43-50.

Perbaikan judul dikoreksi oleh penulis menjadi: Potensi kerugian ekonomi akibat biaya rawat inap dan rawat jalan pada balita obesitas yang diprediksi mengalami obesitas saat dewasa di Indonesia.
Terjadi kesalahan pada nama penulis ketiga, yang sebelumnya dilaporkan Dadang Iskandar dikoreksi menjadi Dadang Sukandar.
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Erratum

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The corresponding author was corrected to: Chan Sook Wah, School of Biosciences, Taylor's University No 1, Jalan Taylor's 47500 Subang Jaya, Selangor, Malaysia, *e-mail*: sookwah.chan@taylors.edu.my

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Erratum

Sutiari NK, Rimbawan, Kusharto CM, Ascobat P, Effendi AT. Kromium serum dan asupan mikromineral pada penyandang diabetes tipe 2. Jurnal Gizi Klinik Indonesia 2017;13(4):135-143.

Kalimat simpulan pada bagian *abstract* dikoreksi oleh penulis menjadi: ***Conclusion:*** *The study showed that serum chromium in DMT2 patients is lower than nondiabetic. The magnesium intake among DMT2 is higher as compare to nondiabetic, while zinc and chromium intake are not different in both group* dan abstrak dikoreksi menjadi: **Simpulan:** Kromium serum pada DMT2 lebih rendah daripada nilai kromium serum nondiabetes. Asupan magnesium pada penyandang DMT2 lebih tinggi dibandingkan dengan nondiabetes, sedangkan asupan zink dan kromium pada kedua kelompok tidak berbeda.
Kalimat pada simpulan dan saran dikoreksi menjadi:

Kromium serum DMT2 lebih rendah dibandingkan dengan nondiabetes. Jenis mineral magnesium, zink, dan kromium merupakan jenis mineral yang terkait dengan perbaikan kontrol glikemik dan resistensi insulin. Penyandang DMT2 mempunyai asupan magnesium yang lebih tinggi dibandingkan dengan asupan magnesium nondiabetes dan telah memenuhi kecukupan magnesium yang dianjurkan. Asupan zink dan kromium antara penyandang DMT2 dan nondiabetes tidak berbeda, meskipun sebagian besar penyandang DMT2 (87,5%) mempunyai asupan zink yang cukup. Asupan kromium pada penyandang DMT2 dan nondiabetes tergolong kurang dari nilai kecukupan kromium yang dianjurkan.

Saran untuk penelitian lebih lanjut sebaiknya melakukan pengukuran mineral magnesium, zink, dan kromium pada urin dan serum untuk memberikan gambaran status mineral magnesium, zink, dan kromium pada penyandang DMT2 dan mungkin dapat menggambarkan nilai bioavailabilitas mineral serta melakukan studi eksplorasi pada mikronutrien lain (vitamin).

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Application of red pitaya powder as a natural food colourant in fruit pastille[1]

Aplikasi tepung buah naga merah sebagai pewarna makanan alami pada permen buah

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ABSTRACT

Background: *Confectionary products meet the important consumers' need states of fun and enjoyment, especially among children. Synthetic colourant had been applied as a colouring agent in confectionary products for decades, however various adverse health effects have been reported after consumption. Hence, usage of natural colourant has increased enormously as it confers functional and nutraceutical benefits. Red pitaya, a common and popular fruit cultivated in South-east Asian countries. Its rich betacyanin content that gives the fruit a red-violet colour. Hence, red pitaya is a potential source of natural colorant as an alternative to synthetic colorant.* ***Objective:*** *This research was aimed to produce fruit pastille with red pitaya powder applied as a natural colourant.* ***Method:*** *Production of red pitaya powder was achieved through spray drying process. Fruit pastille was prepared and subjected to antioxidant, stability and sensory analysis.* ***Results:*** *Physicochemical study showed that pastille incorporated with red pitaya powder exhibited significantly ($p<0.05$) higher antioxidant properties than the blank pastille (control). An eight weeks storage stability study revealed that betacyanin content of pastille incorporated with pitaya powder remained stable for the first four weeks of storage. Besides, no significant change was observed in redness (a*) of pastille throughout the storage study. Sensory study was carried out to assess the consumer preference on pastille incorporated with pitaya powder and synthetic colourant. Colour attribute of pastille incorporated with red pitaya powder has gained significantly ($p<0.05$) higher liking that the one added with synthetic colour.* ***Conclusion:*** *Red pitaya powder could be a potential natural colourant for gummy confectionary.*

KEY WORDS: *red pitaya; spray drying; natural colourant; betacyanin; antioxidant; stability*

ABSTRAK

Latar Belakang: Produk makanan harapannya dapat memenuhi kebutuhan konsumen dari segi kesenangan dan kenikmatan, terutama di kalangan anak-anak. Pewarna sintetik telah digunakan sebagai zat pewarna pada produk permen selama beberapa dekade. Namun, berbagai efek kesehatan yang merugikan timbul akibat konsumsi permen. Oleh karena itu, penggunaan pewarna alami meningkat sangat besar karena memberikan manfaat fungsional dan nutrasetikal. Buah naga merah merupakan buah umum dan populer dibudidayakan di negara-negara Asia Tenggara, kaya akan kandungan betacyanin yang memberikan warna merah-violet. Buah naga merah merupakan sumber potensial dari pewarna alami sebagai alternatif pewarna sintetik. **Tujuan:** Menghasilkan permen buah dengan tepung buah naga merah sebagai pewarna alami. **Metode:** Produksi tepung buah naga merah diperoleh melalui proses *spray drying*. Permen buah naga tersebut dianalisis kandungan dan stabilitas antioksidan serta uji sensoris. **Hasil:** Hasil penelitian menunjukkan bahwa kandungan antioksidan permen buah dengan tepung buah naga merah lebih tinggi dibandingkan dengan permen buah kontrol ($p<0,05$). Setelah penyimpanan delapan minggu, diketahui bahwa kandungan betacyanin permen buah dengan tepung buah naga tetap stabil selama empat minggu pertama penyimpanan. Selain itu, tidak ada perubahan signifikan pada warna kemerahan (a*) dari permen buah selama uji stabilitas. Studi sensorik dilakukan untuk menilai preferensi konsumen pada permen dengan tepung buah naga dan pewarna sintetis. Warna permen dengan tepung buah naga merah lebih disukai dibandingkan dengan permen kontrol yang ditambahkan warna sintetis ($p<0,05$). **Simpulan:** Tepung buah naga merah dapat menjadi pewarna alami yang potensial untuk permen buah.

KATA KUNCI: buah naga merah; *spray drying*; pewarna alami; betacyanin; antioksidan; stabilitas

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INTRODUCTION

Colour plays an important role in enhancing the appearance and improving the quality of food products. Synthetic colour are widely used by manufacturer as economical is always a better choice. However, there is an uncontrolled use of synthetic colour particularly in food products that is mostly consumed by children. Several studies suggested that consumption of high doses of artificial colorants poses negative effect to the consumers such as damage in different organs (1,2). Therefore, the use of synthetic colorants in food faced with controversy, especially in children nutrition when the food is added with the colours at high doses. Researchers and consumers hence switched their attention to natural colourant.

Hylocereus polyrhizus, also known as red flesh red pitaya is a member of the family Cactaceae. Red pitaya is also locally known as dragon fruit or *pitaberry*. This species of red pitaya has an oval appearance an attractive purple-red peel and flesh. The flesh is well-dispersed with small black seeds which is rich in essential fatty acids and it is delicate and juicy (3,4). The fruit is highly cultivated South-East Asia country such as Malaysia, Vietnam and Indonesia. The attractive purple-red colour of *Hylocereus polyrhizus* flesh is contributed by a set of pigments called betalains. Betalains are water soluble nitrogen-containing pigments which can be divided into red-violet pigment called betacyanins and yellow pigment called betaxanthins. According to Ow, Boyce & Somasundram (5), betaxanthins are totally devoid in red pitaya and at least seven identified betacyanins in the *Hylocereus* genus. These betacyanins identified are betanin, isobetanin, phyllocactin, isophyllocactin, betanidin, isobetanidin, and bougainvillein-R-1. Betacyanin pigment has antioxidant properties that exhibit health benefits hence making it a potential source as natural pigment and functional ingredient in food products (6).

Red pitaya can be made into puree, juice or powder to contribute its function as natural colourant in food products. However, puree and juice has lower shelf life compare to powder due to its high moisture content and water activity. Fruit juice powders are produced to maintain and preserve its quality. Among the drying techniques, spray drying is usually applied to produce the fruit juice powder. Spray drying process converts fluid material into dry solid particles which the liquid is being atomized in a hot gas medium to instantaneously obtain the powder (7). Besides, there are various researches done on application of spray dried red pitaya powder in food products such as yoghurt (8). However, there is a limited research on application of spray dried red pitaya powder in solid food product such as sugar confectionary. Soft confectionary such as pastille, gummy bears and jellies are preferred by parents compared to hard candies as it is easier to be consumed by young children. There is also scant in study with regard the stability of red pitaya powder in food model especially solid food. The aim of this research was to produce fruit pastille by incorporating spray dried red pitaya powder as natural colorant. The functional properties and the stability of betacyanin in fruit pastille were also studied.

METHODS

Raw material

All fresh red pitaya fruits were purchased from fruit shop 'MBG' located in Sunway Pyramid, Selangor, Malaysia. All fruits were stored in chiller until further use. Maltodextrin with dextrose equivalent (DE) 10 - 13 % was purchased from V.I.S Foodtech Ingredient Supplies Sdn. Bhd., Kuala Lumpur, Malaysia. Gula Prai brand of granulated sugar and CSR brand of caster sugar were purchased from Cold Storage, Sunway Pyramid, Petaling Jaya, Selangor. Low methoxyl pectin, sodium citrate and citric acid were obtained from Modernist Pantry, USA. Glucose syrup and blackcurrant flavour were obtained from V.I.S Foodtech Ingredient Supplies Sdn. Bhd., Kepong, Kuala Lumpur. AmeriColor electric purple synthetic food colourant was purchased from Bake with Yen Sdn. Bhd., Selangor, Malaysia.

Chemical and reagent

Folin-Ciocalteu's (FC) reagent, sodium carbonate anhydrous, denatured ethanol, 1M sodium hydroxide, di-sodium hydrogen phosphate, potassium dihydrogen phosphate, potassium hexacyanoferrate (III), iron (III) chloride anhydrous, trifluroacetic acid (TFA), methanol, acetonitrile were purchased from Merck, Germany. Sodium nitrite was obtained from VWR, European Community. Aluminium chloride-6-hydrate was obtained from Bendosen Laboratory Chemicals, Norway. gallic acid, catechin, betanin (red beet extract diluted with dextrin), and trichloroacetic acid were obtained from Sigma-Aldrich, USA.

Preparation of Red Pitaya juice

Red pitaya fruit was peeled and cut into smaller pieces. The cut flesh was blended into puree using blender (Model: MX-900M, Panasonic, Malaysia). The puree was filtered three times using muslin cloth to remove seeds and unwanted solids prior to spray drying.

Preparation of Red Pitaya powder

The filtered juice was mixed with distilled water at 1:2 ratio. 15% (w/w) of maltodextrin was added to the red pitaya juice and homogenised using blender (Model: MX-900M, Panasonic, Malaysia The red pitaya juice and maltodextrin mixture was then subjected to laboratory scale spray dryer (Model: SD-06, Lab-Plant, UK) to obtained spray dried red pitaya powder. The spray dryer was equipped with 0.5 mm spray nozzle and 215 mm OD × 500mm long. The inlet temperature of spray dryer was maintained at 140 ˚C, pump speed at 15 (feed flow of 11.58 mL/min), fan setting at 50 (air speed at exhaust of 4.3 m/s) and pressure at 2 bar. The red pitaya powder obtained was collected in Schott bottle wrapped with aluminium foil and stored at room temperature till further use as natural colourant in fruit pastille.

Preparation of fruit pastille

Fruit pastille was prepared according to formulation of pectin pastille provided by Herbstreith&Fox (9) with some modification. The fruit pastille was demoulded and cut into approximately 1.3 cm × 1 cm cube and coated with caster sugar. Four batches of fruit pastille were prepared. The fruit pastille was collected in Schott bottle covered with aluminium foil and stored in room temperature until further analysis.

Antioxidant analysis

Total phenolic content (TPC)

For sample preparation, 2 g of fruit pastille was dissolved in 20 mL of distilled water (1:10). Standard and sample solutions were pipetted 0.3 mL and mixed with 10 mL of FC reagent (x10 dilution), then stand for 3 minutes. It is then followed by adding 0.8 mL of 7.5% (w/v) sodium carbonate solution into the mixture and vortex. The mixture was incubated in dark for 2 hours and the absorbance was recorded using UV-Vis spectrometer (GENESYS 10UV, Thermo Fisher Scientific, Massachusetts, USA) at wavelength 765 nm. The UV-Vis spectrometer was calibrated using 70% denatured ethanol as blank. Concentration of total phenolic was expressed as milligram of gallic acids equivalents (GAE) per 100 g of fruit pastille. The calibrated standard curve equation for gallic acid was $y = 18.17x + 0.13458$ ($R^2 = 0.993$).

Total flavonoid content (TFC)

For sample preparation, 2 g of pastille was dissolved in 20 mL distilled water (1:10). Standards and sample solutions were being pipetted 0.25 mL and added into test tube, followed by 1.25 mL distilled water and 75 µL of 5% sodium nitrite was added. The mixture was let stand for 6 minutes and 150 µL of 10% aluminium chloride-6-hydrate solution was added into the mixture, then stand for 5 minutes. Then, 0.5 mL of 1M sodium hydroxide and 250 µL of distilled water were added into the mixture. The mixture in test tube was vortexed. The absorbance was measured by using UV-Vis spectrophotometer at wavelength of 510 nm and distilled water was used as blank. Concentration of total flavonoid was expressed as milligram of catechin equivalents (CE) per 100 g of fruit pastille. The calibrated standard curve equation for catechin was $y = 0.0033x + 0.0045$ ($R^2 = 0.9995$).

Ferric reducing antioxidant power (FRAP)

For sample preparation, 2 g of pastille was dissolved in 20 mL distilled water (1:10). Standards and sample solutions were being pipetted 1 mL then mixed with 2.5 mL of 0.2 M phosphate buffer at pH 6.6 and 2.5 mL 1% potassium ferricyanide. The mixture was incubated at 50 ˚C for 20 minutes. Then, 2.5 mL of 10% trichloroacetic acid was added to the incubated mixture and vortexed. The mixture was being pipetted 2.5 mL and transferred into new test tube. Next, 2.5 mL of distilled water and 0.5 mL of 1% iron (III) chloride were mixed into the mixture and incubated for 30 minutes. The absorbance was measured at 700 nm using UV-Vis spectrophotometer and 70% denatured ethanol was used as blank. The antioxidant activity was expressed as milligram of gallic acid equivalents (GAE) per 100 g of fruit pastille. The calibrated standard curve equation was y = 34.105x + 0.12151 (R^2 = 0.99508).

Betacyanin

High performance liquid chromatography (HPLC) (Shimadzu Prominence UFLC, Columbia, USA) was used to identify the betacyanin content in fruit pastille. The HPLC system was equipped with DGU-20A degassing unit, LC-20AT solvent delivery unit, SIL-20A $_{HT}$ auto sampler, SPD-M20A diode array detector and CTO-10AS $_{VP}$ column oven. Betacyanin content of fruit pastille was separated using reversed column Thermo Scientific Hypersil Gold column (5 µm, 150 × 4.6 mm). The mobile phase used for elution is an isocratic solution contains a mixture of 0.5% of 90% trifluoroacetic acid (TFA) and 10% of pure acetonitrile. One liter of mobile phase was prepared by mixing 900 mL of 4.5 mL of TFA with 900 mL of milli Q water and 100 mL of pure acetonitrile. Flow rate of the elution solvent was set at 1.00 mL/min for 15 minutes per injection with injection volume 20 µL. The column condition was set at 25 ˚C and absorbance at 536 nm.

Betanin was used as standard. For sample preparation, 2 g of fruit pastille was dissolved in 20 mL of distilled water (1:10) and the fruit pastille was cut into smaller pieces prior to dissolving it. All standards and sample solution were filtered using Minisart® RC 0.45 µm cellulose acetate syringe filter membrane (Sartorius, Germany) and transferred into 2.0 mL clear vial. The result was reported in mg/100g fruit pastille and the calibrated curve equation was y = 155806.0824x – 2.4312 (R^2 = 0.9962).

Colour measurement

The colour (L^*, a^* and b^* values) of the fruit pastille was measured using HunterLab ColorFlex EZ colorimeter (Hunter Associates Laboratory, Virginia, USA). The L^* value represents the lightness of the sample where a low number (0 – 50) indicates dark and a high number (51 – 100) indicates light. The a^* value represents red vs. green, where a positive number represents red and a negative number represents green. The b^*value represents yellow vs. blue, where a positive number represents yellow and a negative number represents blue as defined by Choudhury (10). The colorimeter was calibrated with standard black tile and white tile prior to sample analysis. Fruit pastille was placed and cover the bottom part of the glass optical cell, then the glass optical cell was placed on the reflectance port to read.

Sensory

Sensory study was conducted to access the consumer preference on fruit pastille incorporated with spray dried red pitaya powder and synthetic colourant. The attributes of the fruit pastille set were colour, aroma, sweetness, sourness, texture, chewiness and overall acceptability. The samples were coded with 3 digits random coding and 9-point hedonic scale was used for the sensory ratings. The sample size of the panellist was 50.

Statistical analysis

Independent t-test was used to determine difference between the sample incorporated with and without red pitaya powder. Repeated measure ANOVA was used to determine significant changes over eight weeks of storage stability study of fruit pastille at significant level $p < 0.05$.

RESULTS

Antioxidant properties

Table 1 shows the TPC, TFC and FRAP of control and fruit pastille added with red pitaya powder. Fruit

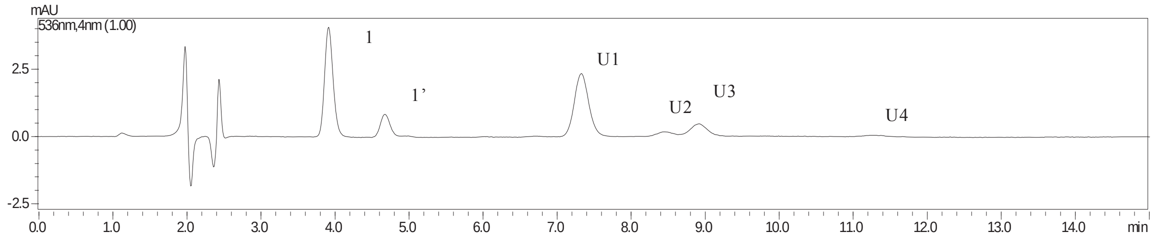

Figure 1. HPLC chromatogram of betacyanins from fruit pastille incorporated with spray dried red pitaya (*H. polyrhizus*) powder.

Table 1. TPC, TFC and FRAP of control and fruit pastille incorporated with red pitaya powder

Antioxidant test	Control	Red pitaya powder
TPC (mg GAE / 100g)	158.00 ± 0.85[a]	249.73 ± 12.9[b]
TFC (µg CE / 100g)	0.62 ± 0.30[a]	4.93 ± 1.61[b]
FRAP (mg GAE / 100g)	244.00 ± 14.66[a]	380.13 ± 35.17[b]

All of the results are presented as mean ± standard deviation of the means. Values are means of triplicates from four separate runs (n = 4). [a,b]Mean values labelled with different subscript letters are significantly different (p<0.05)

pastille added with red pitaya powder poses significantly higher TPC, TFC and FRAP as compared to control.

Stability analysis

Betacyanin content

Figure 1 displays the HPLC chromatogram of betacyanin in fruit pastille incorporated with red pitaya powder. Total 6 peaks are shown in **Figure 1**, which retention time are 3.9 min (peak 1), 4.6 min (peak 1'), 7.4 min (peak U1), 8.5 min (peak U2), 9.0 min (peak U4) and 11.4 min (peak U4). From the respective retention time in comparison to commercially obtained betanin standard, peak 1 was identified as betanin while peak 1' was isobetanin. Peak U1, U2, U3 and U4 was unknown due to unavailable of other standards.

According to **Figure 2**, there is a decline trend in concentration of betacyanin in eight weeks of storage. It can be seen that there was a significant decrease in betacyanin content at the fifth week of storage, with betacyanin retention value 77.3%.

Colour

Figure 3 shows the trend in changes of colour ($L*$ $a*$ $b*$ value) of fruit pastille in eight weeks of storage. This research investigated reading of $b*$ value gradually increased throughout the storage test, indicated that the colour changed from more bluish to more yellowish. Meanwhile, the lightness ($L*$) showed a declined trend however no significance difference between each week throughout the eighth week of storage. The redness ($a*$) was remain stable throughout eight weeks of storage.

Sensory evaluation

The difference between hedonic score of fruit pastille added with synthetic colourant (FPS) and natural colourant (FPN) is displayed in **Figure 4**. There was a significant difference (p<0.05) in terms of colour between the samples, with the mean hedonic score 7.28 for FPN compare to 6.24 for FPS. Moreover, the mean hedonic scores for FPN and FPS were, 6.42 and 6.02 for aroma, 5.90 and 6.38 for texture, 6.72 and 6.54 for overall acceptability. However, no significant difference showed in mean hedonic scores between fruit pastille with natural colourant and control under the attributes stated. Moreover, **Figure 5** shows the fruit pastille samples.

DISCUSSION

Antioxidant properties

Red pitaya which is rich in plant source bioactive compound such as phenolic compound, which have been recognized as potential compound to reduce risk of disease such as coronary heart disease, cancer and reduce cholesterol level which mainly contributed by its antioxidant properties (11,12). Therefore, fruit pastille was subjected to various spectrometric methods for the quantification of the antioxidant compound, such as total

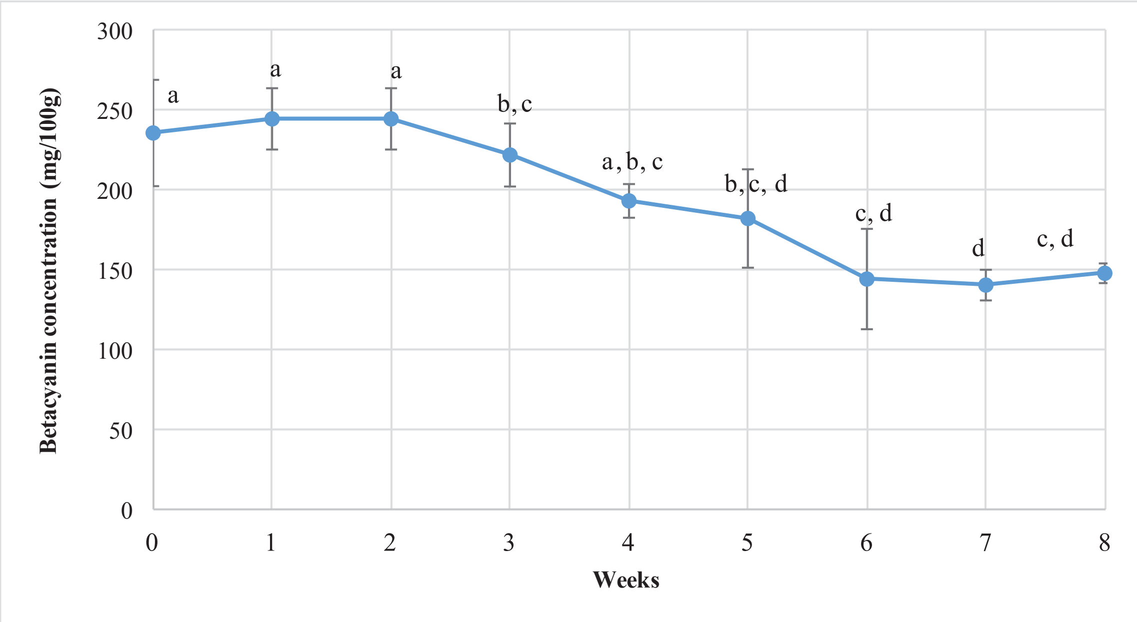

Figure 2. Concentration of betacyanin (mg betanin equivalent / 100g of fruit pastille) in eight weeks of storage

*All of the results are presented as mean ± standard deviation of the means. Values are means of triplicates from four separate runs (n = 4). Mean values labelled with different subscript letters are significantly different (p<0.05)

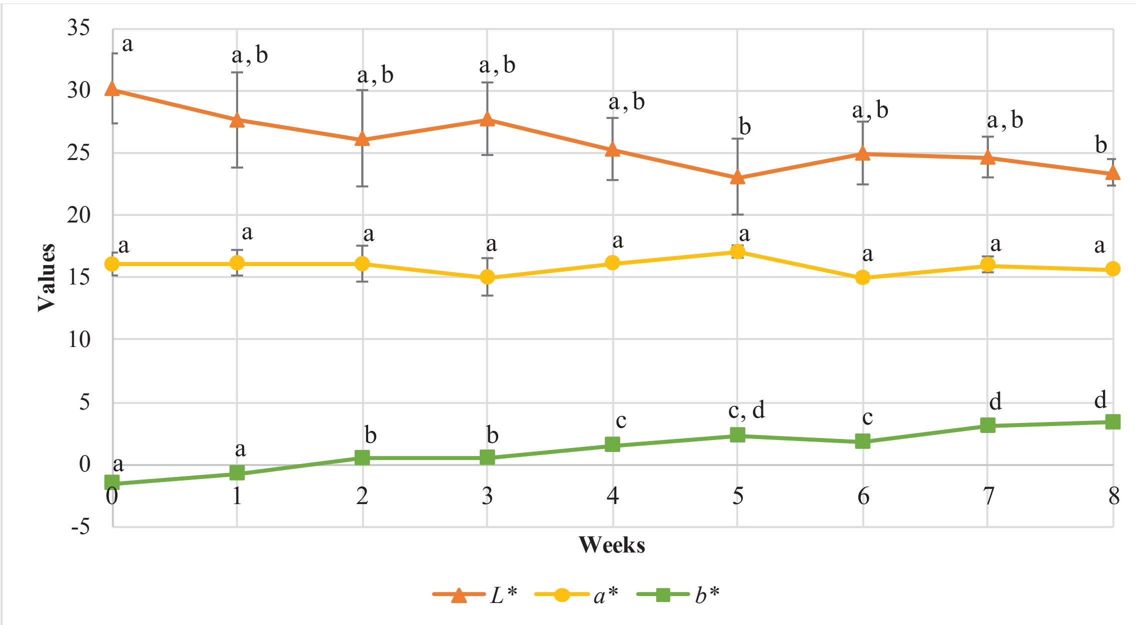

Figure 3. Colour (*L* a* b value) of fruit pastille in eight weeks of storage**

*All of the results are presented as mean ± standard deviation of the means. Values are means of triplicates from four separate runs (n = 12). Points labeled with different subscript letters in the same group are significantly different (p<0.05)

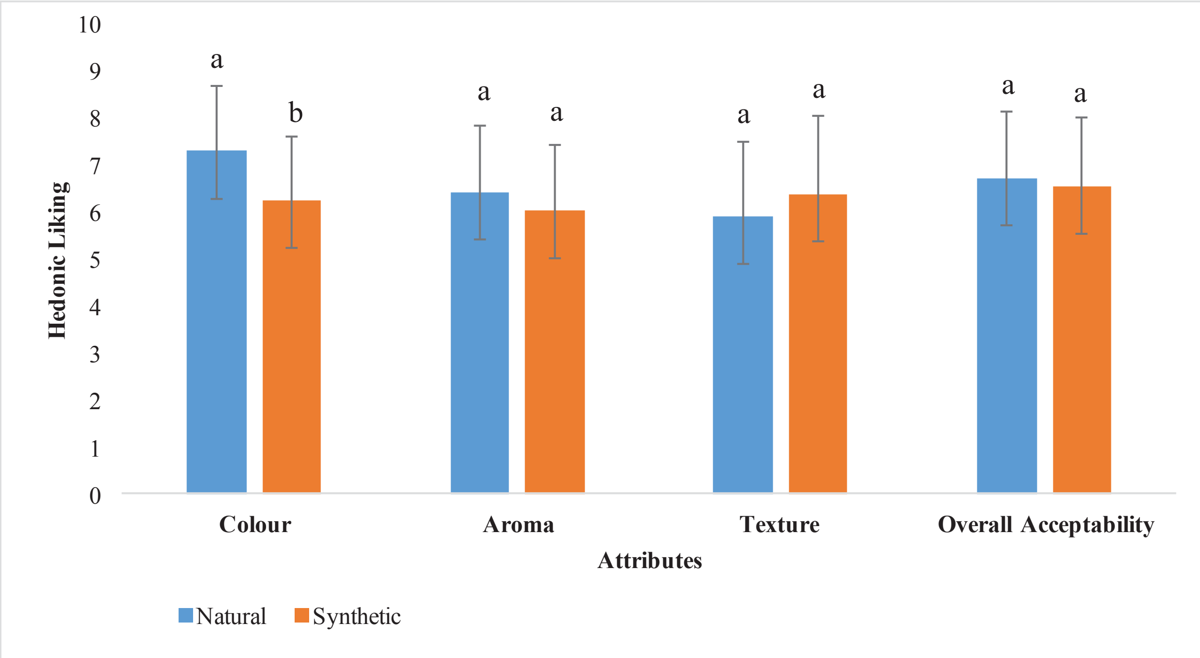

Figure 4. Sensory evaluation on attributes: colour, aroma, texture, overall acceptability of fruit pastille incorporated with natural and synthetic colouring. Bar chart labeled with different subscript letters in the same group are significantly different (p<0.05)

*All of the results are presented as mean ± standard deviation of the mean (n=50). Mean values labelled with different subscript letters are significantly different (p < 0.05).

Figure 5. Fruit pastille incorporated with synthetic colourant (left) and red pitaya powder (right)

phenolic content (TPC), total flavonoid content (TFC) and ferric reducing agent (FRAP).

TPC assay method measured both bound and unbound phenolic compound. Determination of TPC in this research was through FC method. There were statistically significant differences (*p*<0.05) in antioxidant properties between fruit pastille incorporated with red pitaya powder (sample) and the control, which sample has 1.5 times higher TPC then control. The significant higher value of TPC in sample could be explained by the presence of red pitaya powder as natural colourant. According to Lee (13), red pitaya powder contains a significant amount of total phenolic compound. Betacyanins in red pitaya powder could also contributed to the total phenolic due to a phenol structure in molecule (14). The concentration of betacyanin, expressed as betanin equivalents in fruit pastille was 235.89 mg / 100g of fruit pastille. Besides the phenolic compound, some other organic compounds with reducing ability such as sugar, amino acids, vitamin C and carotenoids may react with FC reagent (13). Therefore, the TPC in control may be contributed by the ingredients which are sugar and glucose syrup.

Red pitaya has also been reported high in flavonoid. Flavonoid content determined in sample was 7 times significantly (*p*<0.05) higher than control. These flavonoids possess a wide range of biological activities such as antioxidant, enzyme inhibiting and antibacterial effects (12) Aluminium chloride ($AlCl_3$) colorimetric method was used to examine the total flavonoid content in fruit pastille. However, only flavones and flavonols were found to complex stably with aluminium chloride hence the method seems to be specific for determination of rutin, luteolin and catechin. (15). Despite of the limitation, Chang et al. (16) stated that this method is sufficient for comparative analysis.

On the other hand, sample has 1.5 times higher ferric reducing antioxidant power compared to control. The reducing capability could be due to the presence of betacyanin, phenolic compound and flavonoid. For control, the antioxidant activity may be contributed by the presence of citric acid that worked as an acid regulator in fruit pastille as well as sugar. According to Prior, Wu & Schaich (17), sugars and citric acid are common interferences with FRAP. Therefore, with all this findings, fruit pastille incorporated with red pitaya powder contains significant antioxidant properties.

Stability analysis

Betacyanin content

Betanin (betanin-*O-ß*-glucoside) was eluted first, followed by isobetanin (isobetanin-*O-ß*-glucoside) in the reversed phase HPLC. This indicates that betanin was a more polar while isobetanin was less polar betacyanin aglycons, which isobetanin created a hydrophobic effect with the mobile phase hence causing the molecule spent more time to be eluted. This can be explained by the molecular structure of both betanin and isobetanin. Kujala et al. (18) explained that branched chain compound elute more rapidly than their corresponding isomers due to the smaller overall surface area. Isobetanin is epimers of betacyanin that have a different configuration at C-15, this allows isobetanin has a greater interaction with the stationary phase and hence greater interaction retention value (19).

Fruit pastille was subjected to storage stability study for eight weeks. It is evident that a decline trend in betacyanin concentration throughout eight weeks of storage. There was a significant (p<0.05) decrease in betacyanin concentration at the fifth week of storage, as through the following week, betacyanin concentration remained stable as no significant decrease observed. The natural degradation of the pigment can be explained by the hydrolysis of betanin into degraded compound, for instance, formation of betalamic acid under storage at 25 ˚C.

Colour

Fruit pastille incorporated with red pitaya powder (sample) was kept in an air-tight glass Schott bottle and stored in the dark at room temperature (25 ˚C) to minimize the degradation rate of betacyanin and maximize the colour stability. The pH of the fruit pastille ranged from 3.4 to 3.6. Moßhammer, Stintzing & Carle (20) confirmed that betalain may be applied over a pH range from 3 to 7, without tonality change.

Based on the observation, colour of fruit pastille gradually changed from red-violet to red colour. The overall colour changes agreed with study conducted by Cai & Croke (21), whom applied *Amaranthus* betacyanin pigment into jellies. They found that with extended storage time, the colour of jellies changed from purplish-red to orange-red then to very light yellow. Based on the colour changes (L^* a^* b^* value) of fruit pastille within eight weeks of storage, for the increased in b^* value, it can be explained by the degradation of betanin that caused formation of yellow degradation product such as betalamic acid which can be formed by cleavage of the cyclo-dopa or neobetacyanins, as well as formation of brown compound, which could be melanin-type polymerization product of cleaved *cyclo*-dopa 5-*O*-*ß*-glucoside Kaimainen et al. (22). Meanwhile, decrease in lightness may be due to the formation of brown pigment. Interestingly, the redness (a^*) was remain stable throughout eight weeks of storage and this may be due to the presence of hylocerenin and phyllocactin that formed red degradation products, thus exhibiting a better colour retention (23). Thus, in the eight weeks of storage, the colour of fruit pastille was fairly stable.

Sensory evaluation

Sensory evaluation is an essential element when a novel food product is developed. In food industries today, prime consideration is given towards the product's palatability and eating quality, followed by quality such as nutritional value and wholesomeness. Therefore, the food product manufacture must ensure that the product is well accepted by consumers. In this study, sensory analysis on the preference and acceptability of fruit pastille was conducted using 9-scale hedonic scale and pastille incorporated with synthetic colour was used as control.

The fruit pastille samples were prepared using the same amount of ingredients. The only variable was the colourant added to it. It is evident that substitution of red pitaya powder with synthetic colourant did not negatively affect the colour hedonic scores, conversely, improvement in the attribute score was observed. From observation, fruit pastille with red pitaya powder (FPN) has a brighter and more appealing red-violet colour, while fruit pastille incorporated with synthetic colorant (FPS) was in heavy and dull red. Hence, this may explained that FPN has higher hedonic score.

In terms of colour and appearance, FPN was described as 'Appealing', 'Attractive', and 'More intense in colour', whereas FPS was described as 'Dull'. Texture wise, although no significant difference between both samples, the hedonic scale of FPS was slightly higher. FPS was described as 'Chewy' and 'Harder' whereas FPN was described as 'Soft'. Most panelists prefer a more chewy texture compare to softer one. However, textural acceptability of fruit pastille ay be dependent on other confounding factors such as culture, experience or habits in consumption. In conclusion, the results showed promising potential of red pitaya powder as natural colourant in fruit pastille due to improvement in colour yet no altering in aroma, texture and overall acceptability.

CONCLUSION

The addition of spray dried red pitaya powder increased the antioxidant properties of the fruit pastille as well as enhanced the appearance of the pastille. The findings showed that there is significant amount of antioxidant such as phenolic and flavonoid presence in the pastille. Based on the results in sensory analysis, fruit pastille incorporated with natural colourant was well accepted by the panellist. In stability study, betacyanin concentration has a significant decrease after 1 month of storage with betacyanin retention value 77.3 %. Colour wise, fruit pastille changed from red-violet to red colour throughout the eight weeks of storage. All the findings suggested that incorporation of red pitaya powder increased the value of fruit pastille in terms of quality and nutritional. Therefore, spray dried red pitaya powder could be a potential natural colourants for gummy confectionary. It is suggested to apply colour retention techniques such as addition of ascorbic acid to prolong the pigment retention in fruit pastille.

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