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The Effect of Different Citric Acid Concentrations on Physicochemical and Antioxidant Properties of Red Pitaya Peel Gummy Candies

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ABSTRACT

Red pitaya peels (RPP), often discarded as waste, are rich in betacyanin, making them a potential natural colourant for food products such as gummy candy. However, citric acid may influence the quality and stability of the colourant. This study examined the physicochemical, antioxidant, and sensory properties of RPP gummy candies with different citric acid concentrations. The RPP were dehydrated, ground into powder, and incorporated into gummy formulations. Citric acid significantly reduced water activity ($p<0.05$), lowered pH, and increased titratable acidity. Colour analysis showed a significant rise ($p<0.05$) in a* value with higher citric acid, indicating a redder colour. Samples containing 3% citric acid exhibited a marked reduction ($p<0.05$) in chewiness. Antioxidant properties were also affected, with the 3% citric acid sample showing the highest total phenolic content (0.91 mg GAE/g) and DPPH radical scavenging activity (40.73%). However, total betacyanin content declined significantly ($p<0.05$) in samples with citric acid, ranging from 0.55-0.85 mg/g, compared to 1.79 mg/g with 0% citric acid. Sensory evaluation revealed that the sample F2 with 1% citric acid sample received the highest scores for most attributes, suggesting an optimal balance between flavour and texture. Overall, the study highlights the potential of RPP as a natural colourant and demonstrates how varying citric acid concentrations impact the physical, antioxidant, and sensory qualities of gummy candy. These findings underscore the importance of optimising citric acid levels to maintain both the functional and sensory attributes of RPP-infused gummy candies.

Keywords: Food Waste Utilisation, Functional Food, Natural Additives, Betacyanin, Natural Colourants

Introduction

Dragon fruit, which is also known as Pitaya or Pitahaya (*Hylocereus spp*.), belongs to the family *Cactaceae* and subfamily *Cactoideae*, growing on the cacti plant species of genus *Hylocereus* and *Selenicereus* which is widely cultivated in tropical and subtropical areas.[1] Pitaya fruit is well-known for its unique appearance and nutritional value. It is rich in betacyanins, antioxidants, dietary fibres, vitamins, organic acids, amino acids and sugars.[2] Pitaya peels and seeds have been reported to contain higher concentrations of betacyanins, polyphenols, and amino acids than the pulp, while anthocyanins were mainly detected in the pulp.[3] Pitaya peels are often discarded as waste, causing the by-products of pitaya generated in large quantities during agro-industrial processing.

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In recent years, there has been an increasing interest in finding new uses for fruit peels, from pitaya. Recently, a study conducted on jelly candy made with pectin extracted from the red dragon fruit peel was conducted, resulting in samples with desirable characteristics in terms of texture.[4] In addition, the effect of red dragon fruit (*Hylocereus polyrhizus*) peel on the overall quality of beef sausage was also conducted whereby the incorporation of beef sausages with red dragon fruit peel (RPP) increases the antioxidant properties of the samples and acts as antimicrobial agent.[5] The effect of dragon fruit (*Hylocereus undatus*) peel on the antioxidant properties and dietary fibre of chicken nuggets was also investigated, where the addition of RPP improves the emulsion stability and leads to significant reduction ($p<0.05$) in lipid peroxidation.[6]
Betalains are water-soluble molecules containing nitrogen that can be subdivided into betacyanins with a red-violet hue and the yellow-orange compounds known as betaxanthin.[7] Betacyanin has been reported to exhibit great antioxidant and radical scavenging activities and can be found in plants such as beetroot, roselle, red cactus pears and red pitaya.[8–11] The presence of betacyanin resulted in a deep pink to red colour in pitaya peel and flesh that can potentially be used as natural food colourants. However, several factors in food processing, such as temperature, pH, and the use of additives, may affect its stability in food products.[12–14] It has been reported previously that the excessive use of additives affects the stability of betacyanin under controlled storage conditions of dark and the absence of oxygen, where the addition of ascorbic acid led to the reduction in storage stability as it reacts as prooxidant.[14] However, the stabilising effects of organic acid such as citric acid have also been reported previously, whereby the addition of citric acid had shown to enhance the stability of betanin under thermal

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treatment.[15,16] Thus, further studies regarding the use of natural colourants such as betacyanin should be conducted in order to ensure its feasibility in various food products.

Gummy candy is one of the most significant confectionery products that is produced from a mixture of hydrocolloids (gelling agents including gelatine and pectin), sugar syrup and citric acid. The addition of citric acid in the production of gummy candies leads to the desired elastic texture as it hydrolyses the gelatine.[17] Besides, citric acid also provides tartness flavour, reduces the pH and exhibits antioxidant properties that help to improve its shelf stability.[18] However, the consumption of gummy candies among children has been associated with adverse effects due to their high level of artificial colourants, including tartrazine E102, quinoline yellow WS E104, sunset yellow FCF E110, carmoisine E122, ponceau 4R E124, Allura red AC E129.[19] High consumption of artificial colour through confectionery products has been reported to cause allergic reactions, asthma issues, hyperactivity and behavioural issues in children.[20] This led to an increase in demand for healthy confections with no artificial colourants and encouraged the food industry to be actively in search of suitable natural alternatives to artificial colourants. Red pitaya peel can potentially be used as a natural colourant in food products due to the high amount of betalains that provide an intense red hue colour. The natural betalains from RPP are also safe for human consumption with no allergic reaction and toxicity, as reported through an *in vivo* study of the extract.[21,22] However, the inclusion of natural colourants remains a challenge due to their instability. Specifically, the application of natural colourants from plant extracts remained a challenge in gummy candy production due to the presence of acid as one of its ingredients. To the best of our knowledge, the effects of different citric acids on the production of gummy candy containing natural colourants remain under-explored. Thus, the present study explores the potential use of red pitaya peel in the production of gummy candy, focusing on the effect of citric acid on its stability, whereby the novelty lies in its exploration of RPP as a sustainable, natural colourant source in gummy candy production, while simultaneously investigating the dual impact of citric acid on both the stability of betacyanin pigments and the physicochemical, antioxidant, and sensory qualities of the gummy candy and filling the gap in the understanding of how natural colourants behave in acidified confectionery systems.

Materials and Methods

Materials

Red pitaya peels were obtained from a local food business in Kuala Terengganu (5.2961° N, 103.0856° E), while materials such as glucose syrup, sugar, gelatin and citric acid were purchased from a local market located in Gong Badak, Kuala Terengganu. All the chemicals used were analytical grade, which includes distilled water, 80% methanol (Merck, Germany), 0.1M sodium hydroxide (NaOH), phenolphthalein, gallic acid, Folin-Ciocalteu reagent, sodium carbonate, 2,2-Diphenyl-1-picrythydrazyl (DPPH), and 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox).

Red pitaya peel powder preparation

The red pitaya peel powder (RPP) was prepared based on previous method with modifications [4]. Red pitaya peels were obtained from a local food business in Kuala Terengganu whereby the peels were from ripe pitaya fruit. The peels were cleaned and the inner part was then carefully separated from the whole peel and spread on a tray, lined with parchment paper. The peels (inner part) were then dried in a dehydrator at 50°C until the moisture content reach 10%. The dried RPP were then grounded into fine powder by using a blender and sieved through a 60-mesh sieve. The produced RPP powder was then stored at -40°C prior to analysis.

Gummy candies formulation and production

The gummy candy samples were prepared according to previous study with modifications.[23] The samples were prepared with different concentration of citric acid (0%, 1%, 2%, 3%) according to Table 1. Gelatine solution was prepared by dissolving the gelatine with warm water and let to bloom for 10 minutes. Glucose syrup, sugar and citric acid were then added in the remaining water at 70˚C, and the mixture was heated until the temperature of 116˚C was reached. The mixture was then cooled to 60°C and the RPP powder was added and mixed with the concentrated glucose solution. Lastly, the bloomed gelatine was added into the glucose mixture and mixed well forming a gel-like consistency. The air bubbles were removed and the mixture was then poured into moulds. The mixture was then refrigerated at 4˚C for 24 hours. The gummy candies were then demoulded and kept in airtight polypropylene bottles at 25˚C.

Table 1: Formulation of gummy candies with different citric acid concentration

Formulation (%)	**F1**	**F2**	**F3**	**F4**
Water	27	26	25	24
Glucose syrup	30.5	30.5	30.5	30.5
Sugar	32	32	32	32
Gelatine	8	8	8	8
Red pitaya peels	2.5	2.5	2.5	2.5
Citric acid	0	1	2	3
Total	100	100	100	100

Water activity determination

The water activity (a_w) of gummy candies was measured by using a water activity meter (HygroPalm GP23, Rotronic, Switzerland). Approximately 10g of fresh samples were weighed and cut into small pieces with a knife. The samples were then kept in an airtight container and were allowed to equilibrate at room temperature for at least 10 minutes. The samples were then immediately measured in triplicates and results obtained were reported as the mean ± standard deviation.

pH measurement

The pH measurement was conducted with a digital pH meter (EUTECH Instrumentals, Singapore). The pH meter was firstly calibrated with standard buffer solutions of pH 4.0, 7.0 and 10.0. About 1g of red pitaya peel gummy candy dissolved in 9 mL of distilled water and homogenised by a homogenizer (Ultraturrax T-25, IKA Labortechnik, Staufen, Germany). Results of pH values were then recorded as mean ± standard deviation in triplicate measurements.

Total titratable acidity

Total titratable acidity of gummy candies was determined by using the volumetric method.[24,25] Approximately 1 g of the red pitaya peels was homogenized in 9 ml of deionised water. Few drops of phenolphthalein were then added into the sample solution. The homogenized samples were then titrated with 0.1 N of sodium hydroxide (NaOH) until the purple colour change was observed. The samples were analysed in triplicate and the percentage of titratable acidity was calculated using equation 1.

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$$Total\ Titratable\ Acidity, TTA\ (\%) = \frac{Molarity\ of\ NaOH\ x\ mL\ of\ NaOH\ x\ equivalent\ weight\ of\ acid}{Weight\ of\ sample} \times 100 \qquad Eq\ (1)$$

Colour determination

Colour is a vital indicator in terms of quality and acceptability of foods. The colour analysis of the red pitaya peels gummy candies was carried out by using a handheld Hunter colorimeter (Hunter Lab Colorflex EZ (USA)) with CIELAB system. The colour of gummy candies was evaluated by referring to CIE L*a*b* (CIELAB) colour components, where L*= lightness, a*= redness, b*= yellowness. The colorimeter was calibrated with a standard white calibration tile prior to analysis. The measurements were taken on different parts of the sample surfaces in triplicate.

Determination of texture properties

Texture profile analysis of the gummy candies was performed using a texture analyser (Micro TA-TX2, United Kingdom) with the cylinder 45 probe (35 mm in diameter).[26] The analysis was conducted at room temperature with the parameter of pre-test speed of 2 mm/s, test speed of 1 mm/s, post-test speed of 1 mm/s. The distance between the probe and the gummy candy samples was set as 5 mm, trigger force of 5 g and the time between two compression was 5s.

Extraction of gummy candy

The extraction method of RPP gummy candy was performed according to previous study with slight modifications.[26] Approximately 5g of samples were weighed and extracted at room temperature by mixing with 50 mL of 80% of methanol and stirred using a magnetic stirrer for 30 mins. The stirred samples were then centrifuged (Multifuge X1, Thermo Fisher Scientific, USA) at 3,000 rpm for 15 mins at 4˚C. The supernatants were filtered with Whatman No.4 filter paper and the extract was kept at -20˚C prior to analysis.

Total betacyanin content

Total betacyanin content was quantified using the spectrophotometric method by UV-Vis spectrophotometer.[27] Spectrophotometric measurement of the samples was carried out by measuring the absorbance at 538 nm using a UV-Vis spectrophotometer (UV-2600i, Shimadzu, Japan). Total betacyanin content was evaluated and expressed as betanin equivalents (mg/ g of fresh extracts) using the following equation:

$$Total\ Betacyanin\ Content\ (mg/g) = \frac{A \times MW \times V \times DF \times 1000}{\varepsilon LW}$$
Eq (2)

Where A = absorbance at wavelength of 538 nm, MW = molecular weight (550g/mol), V = volume extract (mL), DF = dilution factor, ε = molar extinction coefficient (60,000 L/mol cm) for betanin, L= path length of cuvette (1cm), W = fresh weight of extracting material (g).

Total Phenolic Content

The total phenolic content of the samples was measured by using the Folin-Ciocalteu method based on previous study with slight modifications.[28] The prepared gummy candy extract was kept in a glass vial covered with aluminium foil prior to analysis. The extract was mixed with 0.5 mL of distilled water and 0.1 mL of Folin reagent and the mixture was incubated for 5 minutes in a dark room at room temperature. Then, 1 millilitre of 7% (w/v) of sodium carbonate was added to the mixture, and it was let to stand at room temperature for another 90 minutes. After incubation, the samples absorbance was determined at 765 nm using a UV-Vis spectrophotometer. The total phenolic content of the gummy candy was measured as mg of gallic acid equivalent (mg GAE)/g sample following a gallic acid standard curve produced at 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mg/mL concentrations of standard gallic acid with R^2= 0.97.

DPPH radical scavenging activity

The DPPH radical scavenging activity of the sample extract and standard was measured according to Okafur et al.[29] Briefly, 0.10mM of DPPH was freshly prepared prior to analysis. The stock solution was then further diluted into a number of standard solutions with methanol concentrations ranging from 0 to 300 ug/mL in order to create a calibration curve. Subsequently, 2.0 mL of DPPH solution (0.10 mM) was added into a test tube containing 0.1 mL of sample extract/standard, and the mixture was incubated in darkness for 15 minutes at room temperature The absorbance of the sample/standard was measured at 517 nm following the incubation period. Trolox was used as standard and the DPPH radical scavenging activity (%) was calculated as demonstrated in the following equation:

$$\%\ DPPH\ Radical\ Scavenging\ Activity = \frac{A\ control - A\ sample}{A\ control} \times 100$$
Eq (3)

Where, $A_{control}$ is the absorbance reading of control solution while A_{Sample} is the absorbance reading of the sample/ standard. Meanwhile, the antioxidant activity expressed as Trolox Equivalent (ug/mL) was calculated from the standard curve generated.

Sensory analysis of gummy candy

Acceptance test was performed for gummy candies prepared with different citric acid concentration and the samples were evaluated by 30 untrained panelists (age 18-40 years old, a mixture of local Malay, Indian and Chinese) consisting of staff and students from Universiti Malaysia Terengganu. Each panel was provided with a sample for each formulation that was served in the small and transparent sealed plastic bag labelled with a 3-digit random number. Plain water was provided as palate cleanser and the samples were evaluated in terms of appearance colour, aroma, texture (chewiness), flavour, tartness, and overall acceptability. The attributes were determined by using a 7-point hedonic scale ranges from 1 to 7, where 1= dislike extremely, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately and 7 = like extremely.

Statistical analysis

All the data obtained from experiments were presented as mean ± standard deviation with three replications. A Minitab Statistical Software version 21 was used to perform the statistical analysis of the data. The one-way ANOVA and Tukey's test were used to determine statistical significance at $p < 0.05$ for physicochemical, antioxidant and sensory analysis.

Results and Discussion

Physicochemical properties of gummy candies

Water activity

The water activity of the RPP gummy candies produced with different citric acid (CA) concentrations is shown in Figure 1. Based on the result, the water activity values of the samples were in the range of 0.83 to 0.78. The values obtained were within the range as reported in previous studies of 0.5 to 0.9 and are referred to as intermediate moisture foods.[30,31] The increase in citric acid concentration leads to a reduction in water activity whereby significant reduction ($p<0.05$) was observed for sample F4 (3% CA) with 0.78 ± 0.01 as compared to F1 (0% CA) and sample F2 (1% CA) with 0.83 ± 0.02 and 0.82 ± 0.01 respectively. However, no significant difference ($p>0.05$) was observed between samples F1,F2 and F3.

The result obtained agreed with a study conducted on gummy candy produced with jaban watermelon exocarp powder whereby the water activity of the samples significantly reduced ($p<0.05$) from 0.82 to 0.77 with the increase of CA from 0.75% to 1%.[26] Moreover, a similar trend was also observed from a study conducted on jam produced with young coconut meat and butterfly pea, whereby the addition of 0.8% of CA resulted in a 7% reduction in water activity as compared to the control sample with 0% of CA.[32] The reduction in water activity can be

attributed to the polar nature of CA that can bind naturally with water molecules, which leads to lower availability of free water.[33]
In this study, samples F1, F2 and F3 containing lower amount of CA showed no significant difference indicating that the strength of the acid was inadequate in lowering the water activity. Adequate concentration of CA is required to lower the water activity as CA releases hydrogen ions (H^+) whereby, the higher the concentration of CA, the more hydrogen ions are released and thus, the amount of water trapped by the structure becomes less. Moreover, the presence of sugar in the RPP gummy candy may also affects the water activity due to its ability to bind with water by forming hydrogen bonds between the polyhydroxy group and water ultimately, lowering the water activity of food products. The low water activity inhibits the growth of microorganisms, such as bacteria, yeasts and moulds and improve its shelf life.[34]

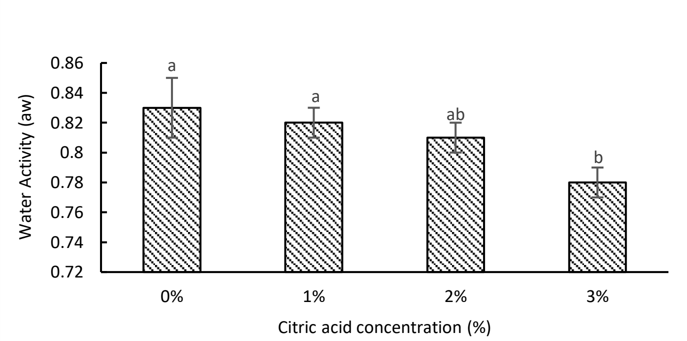

Figure 1: Water Activity of red pitaya peel gummy candies with different citric acid concentration. Bars represent mean values (N=3) where [ab]Mean ± standard deviation values with different letters indicates significant different at $p<0.05$.

Total titratable acidity and pH
According to Figure 2, a significant reduction ($p<0.05$) in pH was observed with an increase in CA concentration from 0% to 2%. However, no significant difference ($p>0.05$) was observed between F3 (2% CA) and F4 (3% CA). The pH values of the RPP gummy candies range from 2.8 ± 0.05 for samples containing the highest amount of citric acid (F4) to 5.18 ± 0.31 for samples containing 0% of citric acid (F1). The reduction in pH is associated with a significant increase ($p<0.05$) in titratable acidity (TTA) with an increase in citric acid concentration. The reduction of pH was mainly driven by the dissociation of H+ in water, which donates its proton to decrease the overall pH values, which then correlates to the increase in TTA, as observed in this study. Moreover, the pH value of the F1 sample is mainly attributed to the RPP, whereby the pH value of RPP was approximately 4.5 to 5.5, as reported in previous studies.[24,35,36] The result obtained was also in agreement with the previous study conducted on passion fruit jelly incorporated with Dutch eggplant, whereby samples containing 0% CA showed a pH value of 4.89, and the value decreased significantly ($p<0.05$) to pH 3.36 with the addition of 3% of CA due to the high amount of organic acid.[37] The study also reported on the increase in TTA with an increase in citric acid concentrations, whereby samples of passion fruit jelly incorporated with dutch eggplant containing 0% citric acid showed the lowest TTA value of 6.92%, followed by samples containing 3% citric acid with 7.91% of TTA value, 5% citric acid with 8.85% and 7% citric acid with highest TTA value of 10.34%.[37]
Gummy candy is referred to as an intermediate moisture food with water activity values of 0.5 to 0.9.[30,31] The low pH plays a significant role in limiting the growth of bacteria, thus increasing the shelf life of the product.[31] Besides, the addition of acids in fruit-flavoured confectionery such as gummy candy provides tartness in taste and enhances the intensity of fruit flavours and colours in products.[32] In addition, pH plays an important role in ensuring the good gelling properties of products made with hydrocolloids.

Colour
The colour profile of the RPP gummy candy samples is presented in Table 2. In terms of lightness (L^*), the increase in citric acid concentration leads to a significant increase ($p<0.05$) in the lightness of the samples, whereby the values range from 25.62 ± 0.81 for sample with 0% citric acid (F1) to 30.48 ± 1.29 for samples with the highest percentage of citric acid (F4). A similar trend was also observed in terms of redness (a*) and yellowness (b*), whereby the sample containing 0% of citric acid showed values of 13.00 ± 0.68 and 2.53 ± 0.06, respectively. The 3% addition of citric acid (F4) showed significantly higher ($p<0.05$) values of redness (a* = 21.23 ± 2.98) and yellowness (b* = 7.17 ± 1.31) as compared to the F1 sample.
The presence of betacyanin in pitaya peel provides a red-violet colour to the gummy candy samples. The stability of betacyanin in food products is dependent on several factors such as temperature, pH, presence of light and oxygen.[38] Moreover, food additives such as organic acids have also been reported to cause significant effects on betacyanin.[16,39] In terms of pH, the optimal pH range for ensuring the stability of betacyanin is pH 4 to 6, as reported in several studies, whereby the values outside of this range may lead to degradation.[12,24] The pH value of the F1 sample (0% CA) is within the optimal range for betacyanin, which helps maintain the sample's pink-violet colour.
Although the structure of betacyanin and anthocyanins is similar, they were substantially different compounds. In RPP, it was reported to contain anthocyanins with approximately 135.4 ± 9.3 mg/g of dried sample, although it is not the main phytochemicals present in RPP.[40] Anthocyanin has been reported to be much more pH sensitive as compared to betacyanin, where it is more stable under acidic conditions [24] hence, making them a light absorber compound. As such, betacyanin colour was easily degraded by UV light and high temperature as opposed to anthocyanins.[41] The pH sensitivity of anthocyanin is due to its ionic nature whereby at low pH (pH<3), the anthocyanin is predominantly in the form of flavylium cations with a red hue, while at the pH of 5-7, the colour appears to be blue purple.[40] Hence, the increase in the redness (a^*) intensity of the sample with an increase in citric acid can be attributed to the presence of the anthocyanin content that appeared to be red in colour under acidic conditions. Moreover, the increase in yellowness (b^*) with an increase in CA percentage may be due to the increase in the stability of the yellow pigment betaxanthin present in RPP with a reduction in pH. A previous study conducted on yellow pitaya peel showed that highly acidic conditions contributed to the increase in the stability of the yellow pigment for heated samples and during storage, as observed in the present study, whereby the yellow pigment stabilizes with increase in CA although the mixture was heated during the preparation of gummy candy.[42] In addition, the yellow colour may also be attributed to the gelatine, which is slightly yellow in colour.[42]

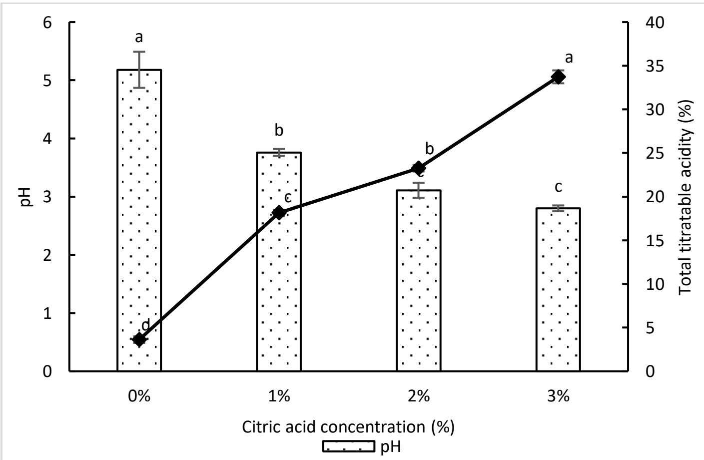

Figure 2: Total titratable acidity (TTA) and pH values of red pitaya peel gummy candies with different citric acid concentration. Bars represent mean values (N=3) where [ab] Mean ± standard deviation values with different letters indicates significant different at $p<0.05$.

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Table 2: Colour parameters of red pitaya peel gummy candies with different citric acid concentration. L* indicates lightness while a* is the value for redness/blueness and b* indicate yellowness/ greenness. F1- 0% citric acid, F2- 1% citric acid, F3- 2% citric acid, F4- 3% citric acid

Sample	L*	a*	b*
F1	25.62 ± 0.81[c]	13.00 ± 0.68[b]	2.53 ± 0.06[c]
F2	27.40 ± 1.21[bc]	15.13 ± 1.36[b]	4.84 ± 0.54[b]
F3	29.29 ± 0.15[ab]	21.13 ± 1.09[a]	7.03 ± 0.22[a]
F4	30.48 ± 1.29[a]	21.23 ± 2.98[a]	7.17 ± 1.31[a]

[a-c]Mean ± standard deviation. Values within the same column with different letters indicates significant different at $p<0.05$

Texture

Table 3 shows the mean texture of the gummy candies with different citric acid concentrations. In terms of hardness and gumminess, a significant increase ($p<0.05$) was observed with an increase in citric acid from 0% to 2% (F1, F2 and F3), whereby samples containing 2% of citric acid (F2) showed the highest value for both attributes. However, further increase in citric acid led to a significant reduction in hardness and gumminess as compared to samples with a lower acid content of 1% (F2) and 2% (F3), while no significant difference ($p>0.05$) was observed between the control sample (F1) and F4 sample. Moreover, no significant difference ($p>0.05$) was also observed in terms of cohesiveness between all the samples tested. In terms of chewiness, the highest value was observed for sample F3 (2%), in which a significant difference ($p<0.05$) was observed between the sample as compared to sample F4 (3%).

Gelatine is a protein obtained from collagen which forms a three-dimensional network structure to provide chewy properties to gummy candies and has a polyampholytic nature with a mixture of positive and negative charges present on its amino acid structure. The properties of gelatine are, thus, dependent on the pH value whereby a lower pH will result in a positively charged surface of the gelatine due to the protonation of the functional groups, while a higher pH may lead to a negatively charged surface due to the deprotonated functional groups.[43] This creates a repulsion force within the system that may weaken the network. Thus, it is important to achieve a balance in the surface charge to provide greater electrostatic interactions between the negatively charged carboxyl groups and the positively charged amino groups of the gelatine.[43] Moreover, excessive addition of citric acids up to 3% might weaken the gelatine network as it hydrolyses the gelatine as observed in the present study, leading to a reduction of gumminess and chewiness in the RPP gummy candy present in this study.[17]

The hygroscopic nature of sugar presence in the gummy candy mixture minimises the hydrolysis of gelatine by the acids for samples containing 1% to 2% of citric acid as the hydroxyl groups of sugar can create intramolecular hydrogen bonds with water molecules, thus resulting in the formation of stable hydrates and the trapping of water in the gel that lowers the availability of water for hydrolysis. These led to an increase in the chewiness of the sample, while the low hydrolysis of gelatine by the acids led to an increase in hardness and gumminess.[37] The data obtained is also in agreement with the study conducted on confectionery gels made with gelatine, whereby the addition of 0.25% to 1.5% citric acid increased hardness while further increase in citric acid led to a significantly lower value in terms of hardness, which the value was not significantly different ($p>0.05$) as compared to the control sample with 0% citric acid.[44] The increase in the hardness and gumminess of the samples with an increase in citric acid from 0% to 2% may also be due to the increase in molecular mobility of the gelatine under certain degrees of hydrolysis by the acid that led to an increase in the growth of gelatine network within the matrix.[44]

Significant reduction ($p<0.05$) in gumminess, hardness and chewiness for the sample with the highest concentration of citric acid (F4, 3% CA) as compared to the other samples may be attributed to the change in protein interactions under low pH. It has been observed that acidic environments can alter the intra- and intermolecular interactions of gelatine, which in turn affects the elasticity of gel during the initial phases of gelation.[45] The extremely low pH condition can cause the protonation of amino acids in gelatine, preventing the formation of hydrogen bonds and reducing the gel structure.

Table 3: Texture of red pitaya peel gummy candies with different citric acid concentration. F1 - 0% citric acid; F2 – 1% citric acid; F3 – 2% citric acid; F4 - 3% citric acid.

Sample	Hardness	Cohesiveness	Gumminess	Chewiness
F1	1333.66 ± 62.87 [c]	0.86 ± 0.01[a]	1149.05 ± 58.72[c]	1121.43 ± 78.39[ab]
F2	1736.57 ± 147.17[b]	0.89 ± 0.02[a]	1533.70 ± 92.88[b]	1492.95 ± 369.11[ab]
F3	1955.83 ± 183.04 [a]	0.89 ± 0.02[a]	1739.65 ± 140.68[a]	1673.22 ± 128.42[a]
F4	1265.38 ± 195.03[c]	0.89 ± 0.02[a]	1119.03 ± 129.94[c]	1065.67 ± 209.50[b]

[a-c]Mean ± standard deviation. Values within the same column with different letters indicates significant different at $p<0.05$.

Antioxidant Properties

Total Betacyanin Content

According to the results obtained in Figure 3 significant difference ($p<0.05$) was observed in terms of total betacyanin content, while there is no significant ($p>0.05$) difference between samples F3 and F4. Sample A showed the highest total betacyanin content of 1.79 ± 0.05mg/g. followed by sample F3, F4 and F2 with 0.85 ± 0.02 mg/g, 0.75 ± 0.01mg/g and 0.55 ± 0.04 mg/g respectively.

Betacyanin is a natural colour pigment that is easily degraded, heat-labile, and less stable than synthetic colourants.[27] Several studies have reported on the optimal pH for ensuring the stability of betacyanin that ranges between pH 4 to 6, whereby values beyond this range may lead to degradation.[46–48] This resulted in high betacyanin content for samples with 0% citric acid, as the pH was recorded at 5.18 ± 0.31, while a significant reduction in pH for samples with the addition of citric acid led to a significant decrease ($p<0.0.5$) in TBC. However, in the present study, an increasing trend in TBC was recorded for samples with the addition of citric acid from 1% to 3%. Although previous studies reported on the reduction of betacyanins with a reduction in pH, it may depend on the type of acids used. The reduction of TBC was observed with the addition of ascorbic acid, which acts as a prooxidant [14] meanwhile, the addition of citric acid has been reported to increase the stability of betacyanin.[15,39] In this study, it was postulated that the increased value in TBC might be due to the citric acid acting as an acidifying agent that plays a vital role during the extraction process which may result in a higher yield of TBC as compared to those with lower concentrations of CA.[49] Additionally, CA is commonly used because it acts as a neutralising agent for the electrophilic centre of betalains, which improves its stability.[50]

Previous studies have reported on the improved stability of betacyanin in food with the addition of citric acids, which agrees with the result obtained in the present study for samples containing CA, whereby the increase in CA concentration from 1% to 3% led to a significant increase in TBC ($p<0.05$). A study conducted on the fermented red dragon fruit juice showed an increase in the stability of betacyanin during four weeks of storage with an increase in CA concentration from 0% to 0.2%.[39] Moreover, a study conducted on the thermally treated purple pitaya juice also reported an increase in betacyanin retention by 55% with the addition of 1% of citric acid,[15] while a previously published study on the effects of organic acids on the retention of betanin showed 1.5 times increase in the half-life value of betanin with the addition of 10 000 ppm (1% w/v) of citric acid.[51] The stabilisation effect of betacyanin in samples containing CA may be attributed to its several protective mechanisms, as reported in previous studies, whereby CA may act as a reliable pH regulator and buffering agent, which prevents the substantial change in pH that can cause betacyanin degradation in food products.[18,52] In addition, the presence of CA may also act as a metal cation chelating agent, where it prevents the degradation of betacyanin by binding with metal cations that are naturally present in foods.[39] Besides that, it has also been reported that CA may act as a sequestrant through partial neutralisation of the electrophilic centre of betacyanin, hence reducing the degradation effect of water molecules.[51,53]

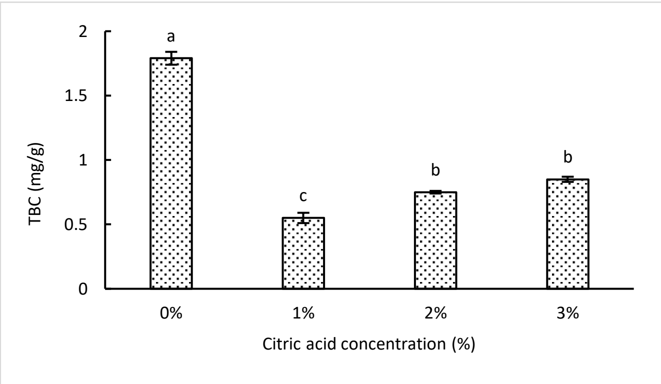

Figure 3: Total Betacyanin Content (TBC) of red pitaya peel gummy candies with different citric acid concentration. Bars represent mean values (N=3) where [a-c]Mean ± standard deviation values with different letters indicates significant different at $p<0.05$.

Total Phenolic Content

The total phenolic content (TPC) of samples with different citric acid concentrations is presented in Figure 4. From the result obtained, a significant increase ($p<0.05$) in TPC was observed, with an increase in citric acid concentration from 0% to 3%. Sample containing 3% CA (F4) showed the highest TPC of 0.91 mg GAE/g, followed by samples F3, F2 and F1 with 0.76 mg GAE/g, 0.62 mg GAE/g and 0.55 mg GAE/g, respectively.

A previous study reported on the high amount of TPC in the methanolic extract of red pitaya peel with 7.63 mg/g GAE as compared to white pitaya peel (5.74 mg/g of GAE) and papaya peel (3.45 mg/g of GAE).[54] It has also been reported previously that the red pitaya peel contained a higher amount of TPC with 28.16 mg/100g as compared to 19.72 mg/100 g in the pulp.[55] Several phenolic compounds have been reported in the red pitaya peel, such as chlorogenic acid, gallic acid, caffeic acid, ferulic acid, *p*-coumaric acid and quercetin.[56,57] The addition of RPP in foods such as biscuits and cakes have also been reported to increase the TPC of these products.[58,59] However, phenolic compounds may be partly loss during heat processing such as cooking.[60] In the present study, the RPP was added to the mixture when the temperature reached 60℃. Although the temperature was considered moderate, some polyphenols appeared to be more heat-sensitive than others. For instance, due to heat processing, anthocyanins can undergo a multitude of mechanisms such as glycosylation, nucleophilic attack of water, cleavage and polymerisation that will cause the loss of this pigment and their degradation.[61] Therefore, with increasing temperature, the degradation of these compounds occurs, and as such, both the colour intensity determined by monomeric anthocyanins and their amount decrease depending on time and temperature.[62] It has been proven that in all food pigments, including anthocyanins, stability decreases with increasing temperature.

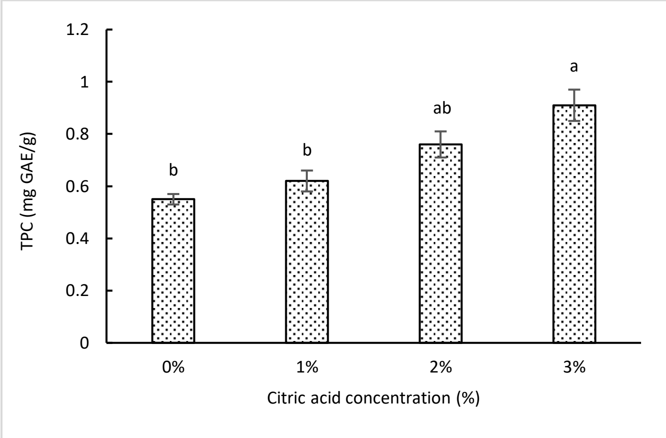

Figure 4: Total Phenolic Content (TPC) of red pitaya peel gummy candies with different citric acid concentration. Bars represent mean values (N=3) where [ab]Mean ± standard deviation values with different letters indicates significant different at $p<0.05$.

The changes in pH and the concentration of acids in foods may also affect the stability of phenolic compounds. The increase in TPC with an increase in CA concentration, as observed in the present study, is correlated with several studies where the stability of phenolic compounds improves under acidic conditions.[63–66] A study conducted on the effect of pH on the total phenolic content of mustard seeds showed a significant increase ($p<0.05$) in TPC with lower pH.[65] In addition, a study conducted on the effect of citric acid on the phenolic stability of sliced button mushrooms reported that the inclusion of 3% w/v citric acid resulted in higher TPC.[63] Similar findings were also obtained from sliced *P.embelica* juice, whereby higher stability of phenolic compound was observed when the pH was adjusted to a lower value of 2.5 with citric acid as compared to pH 3.5.[67] However, the result obtained is contrary to the result obtained previously on roselle beverages, whereby different concentrations of CA do not significantly affect ($p>0.05$) TPC. This may be due to the lower amount of citric acid added in the previous study (0.02 to 0.03% w/v) as compared to the present study with 1 to 3% w/v. The stability of phenolic compounds has been reported to be highly influenced by pH, where its sensitivity is highly related to the structure of phenols. Phenolic compounds such as gallic acid, caffeic acid and chlorogenic acid that are found in red pitaya peel have been reported to be more stable under low pH as opposed to epigallocatechin and ferulic acid, which are stable under high pH conditions. [60,66] Thus, the increase in citric acid resulted in low pH that helps to improve the stability of phenolic compounds in RPP.

DPPH Radical Scavenging Activity

Figure 5 shows the effect of different citric acid concentrations on the antioxidant activity of RPP gummy candies as opposed to Trolox. Results showed an increasing trend whereby sample A with 0% citric acid had equivalent to 57.53 ug TEAC/g, sample B with 1% citric acid had 85.3 ug TEAC/g, sample C with 2% citric acid had 168.7 ug TEAC/g and sample D with 3% citric acid has 171.18 ug TEAC/g. Meanwhile, Figure 6 shows the radical scavenging activity of RPP gummy candies. The ability of antioxidants to reduce the DPPH radical concentration in the reaction medium is assessed spectrophotometrically by monitoring the drop in absorbance at a characteristic wavelength. Results reported there was a significant difference ($p<0.05$) between samples F1 (0% CA) and F2 (1% CA) with samples F3 (2% CA) and F4 (3% CA), while there was no significant difference between samples F1 (15.46%) and F2 (21.63%), and between samples F3 (40.21%) and F4 (40.73%). The DPPH radical scavenging activity (%) showed an increasing trend with the increasing of citric acid concentration. This result agreed with the previous study,[32] where the increase in citric acid concentration increased the antioxidant activity in young coconut meat added with butterfly pea sheet jam, with the highest concentration of citric acid (0.8%) giving 30.75% DPPH scavenging activity. A recent study supported this data where citric acid has been recently associated as an antioxidant and anti-inflammatory agent in cells, which helps to improve the overall immune system.[68] Moreover, a study on methanolic extracts of aerial parts of *B. madagascariensis* and *B. purpurea* trees showed IC_{50} of 18.24 ± 0.82 µg/ml and 50.54 ± 2.18 µg/ml, respectively, which highly corresponded to the present of citric acid dominantly present as secondary metabolite in these extracts, potentially act as an oxygen scavenger and retards unsaturated lipid/free fatty acid oxidation by inhibition of essential agents like metals-based oxygen scavengers.[69]

It is also worth noting that this result also corresponds to the TPC data obtained in the current study. Previously, the TPC of 0.91 mg GAE/g was obtained in F4 (3% CA), and the trend declined with the decreased concentration of CA. This was supported by the findings from Abdl Aziz et al.,[64] where the authors observed an increase in DPPH scavenging activity that correlates with the amount of TPC. Specifically, TPC measures phenolic compounds, including organic acids such as citric acid, where some organic acids can exhibit antioxidant activity. Moreover, citric acid with the presence of chitosan had shown strong colour-retaining properties in litchi pericarp, suggesting that citric acid can prevent discolouration and inactivation of post-harvest enzymes such as polyphenol oxidase and anthocyanins that were responsible for browning of fruits pericarp.[70]

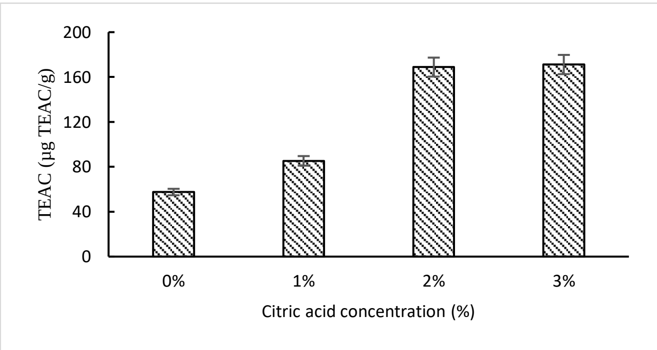

Figure 5: Trolox Equivalent Antioxidant Capacity (TEAC) of red pitaya peel gummy candies with different citric acid concentration. Bars represent Mean ± standard deviation (N=3).

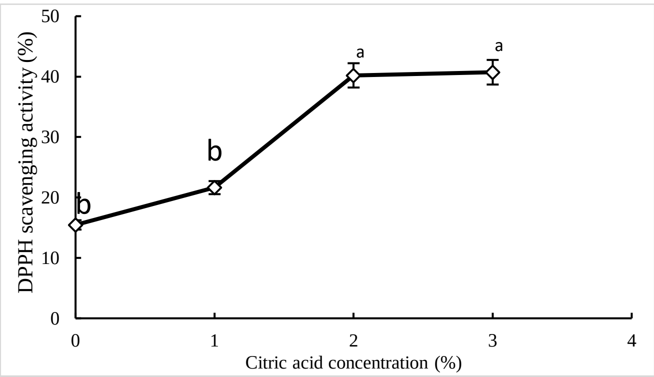

Figure 6: DPPH Radical Scavenging Activity of red pitaya peel gummy candies with different citric acid concentration. Bars represent mean values (N=3) where ^{ab}Mean ± standard with different letters are significantly different at $p<0.05$.

Sensory Analysis

Table 4 shows the sensory acceptability of RPP gummy candies with different CA concentrations. From the result obtained, sample F2 with 2% CA showed significantly higher acceptance scores ($p<0.05$) for all of the attributes except for tartness as compared to other samples, which indicated that sample F2 is the best sample in terms of sensory acceptance terms of appearance and texture, sample F4 with 3% CA showed a significantly lower score ($p<0.05$) with 4.57 ± 1.52 and 4.07 ± 1.48 respectively (neither like nor dislike) as compared to sample F1, F2 and F3 whereby no significant difference ($p>0.05$) were observed between these samples. The reduction in appearance and texture for the sample with the highest CA concentration may be attributed to the high lightness (Table 2) and soft texture due to the low value of hardness (Table 3) that is unfavourable by the panels. Sample F2 with 2% citric acid showed the highest score for appearance and texture as the presence of citric acid up to 2% concentration resulted in a mild acidic condition that increased the growth of gelatine network within the matrix, producing gummy candies with desirable chewiness and appearance.[44] Nevertheless, excessive citric acid in gummy candies may weaken the gelatine network, which leads to soft texture and reduction in gumminess that lowers the acceptance scores, as observed for sample F4 with 3% CA.[71] The result obtained was also in agreement with the previous study conducted on citrus jelly, whereby the acceptance score for texture increases with the addition of 0.2% to 0.6% of citric acid due to the mild acidic condition.[72]

Moreover, no significant difference ($p>0.05$) was observed in terms of colour and aroma between all of the samples, which may indicate that there is no perceivable difference in terms of colour and aroma with the addition of citric acid. In terms of taste, sample F2 showed a significantly higher acceptance score ($p<0.05$) with 5.80 ± 1.38, followed by F3, F4 and F1 with 5.57 ± 1.14, 4.70 ± 1.51, 3.43 ± 1.48 respectively. Sample F1 with no citric acid showed a significantly low score ($p<0.05$) for taste (3 = dislike slightly) as compared to the other samples. The higher acceptance score for F2 may be attributed to the balance in taste between sourness and sweetness for the sample as compared to sample F1 whereby the absence of citric acid led to high sweetness that is least accepted by the panels. The least preferred sample was F4, with no significant difference noted between F1 and F3 might show the balance between the preferred taste between the panellists, whereby some panellists did not prefer the sharp and sour taste of the RPP gummy candy. Meanwhile, some panellists could not associate the taste of sweet sugar (F1). The addition of citric acid also led to a significant increase ($p>0.05$) in tartness, with the highest score shown by sample F3 (2% CA) with 5.70 ± 1.06. In this study, the F4 showed significant tartness, including a sharp and sour taste, which was correlated with the percentage of citric acid present. However, a further increase in citric acid to 3% led to a reduction in the acceptance score as the sample was deemed too sour and undesirable to the panels. The result obtained corroborated the study conducted on red guava gummy candies with the addition of pineapple peel extract, whereby the gummy candy with the addition of 14g of pineapple peel extract was highly accepted by the panel in terms of taste due to the balance in sweet in sour taste.[73]

The overall acceptance of the samples increased with the presence of citric acid, whereby sample F1 without citric acid showed the lowest score of 4.30 ± 1.39, indicating neither like nor dislike, while samples containing 1% and 2% of CA showed a higher score of 5.83 ± 1.23 and 5.70 ± 1.15 respectively indicating like slightly. However, the excessive addition of citric acid to 3% (F4) led to a significant reduction in the overall acceptance with 4.40 ± 1.34, where the score was not significantly different ($p>0.05$) as compared to sample F1. Therefore, based on the results, the addition of 1% to 2% of citric acid is recommended in RPP gummy candies as the panels highly accepted them, while the excessive addition of citric acid up to 3% resulted in a significant reduction in sensorial acceptance.

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Table 4: Mean score of sensory attributes of red pitaya peel gummy candies with different citric acid concentration. F1 - 0% citric acid; F2 – 1% citric acid; F3– 2% citric acid; F4 - 3% citric acid

Sample attributes	F1	F2	F3	F4
Appearance	5.90 ± 1.03[a]	6.43 ± 0.90[a]	5.67 ± 1.42[a]	4.57 ± 1.52[b]
Colour	6.10 ± 1.03[a]	6.37 ± 1.00[a]	5.93 ± 1.23[a]	5.63 ± 1.22[a]
Aroma	4.93 ±1.62[a]	5.13 ±1.33[a]	5.13 ±1.50[a]	4.47 ±1.48[a]
Texture (Chewiness)	5.13 ± 1.59[a]	5.83 ± 1.46[a]	5.50 ± 1.20[a]	4.07 ± 1.48[b]
Taste	3.43 ± 1.48[c]	5.80 ± 1.38[a]	5.57 ± 1.14[ab]	4.70 ± 1.51[bc]
Tartness	3.20 ± 1.81[b]	5.10 ± 1.69[a]	5.70 ± 1.06[a]	5.37 ± 1.67[a]
Overall acceptability	4.30 ± 1.39[b]	5.83 ± 1.23[a]	5.70 ± 1.15[a]	4.40 ± 1.34[b]

[a-c]Mean ± standard deviation values within the same row with different letters indicates significant different at $p<0.05$.

Conclusion

In conclusion, the development of gummy candy using red pitaya peel with varying citric acid concentrations (0%, 1%, 2%, and 3%) was successful. The study revealed that citric acid concentrations significantly affected the physicochemical, antioxidant, and sensory properties of the candy. As citric acid concentration increased, the total titratable acidity rose, pH decreased, and water activity decreased. The betacyanin pigment was most stable at a pH of 5.1, preserving the appealing pink-violet colour. The gummy candy with 2% citric acid exhibited the best texture and overall palatability. Additionally, higher citric acid concentrations led to increased total phenolic content and enhanced DPPH radical scavenging activity. To the best of our knowledge, this research on RPP could potentially make promise in food product development, such as in confectionaries like jellies and marshmallows, and at the same, providing a healthier alternative to synthetic dyes. Thus, the use of RPP, which is typically regarded as waste, supports the clean label trend by promoting natural ingredients with minimal additives whilst promoting sustainability in food processing. Despite its potential, the research presents some limitations, such as the instability of betacyanin in the presence of citric acid, which may compromise the colour stability of products over time associated with the large-scale processing and drying of RPP.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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