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Influence of natural colourants annatto and pumpkin powder on the physicochemical properties, antioxidant activities and sensory characteristics of *Carica papaya* L. juice

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Abstract

The processing of papaya juice alters the colour of the juice, resulting in a less visually appealing appearance. To improve the juice's visual appeal, food colouring is required. This study aimed to investigate the effects of annatto and pumpkin powders, as natural colourants, on the physicochemical properties, antioxidant activities, and sensory characteristics of papaya juice. Papaya was blanched in citric acid (0.5% w/v) at 95°C for 2 mins before blending into puree and was treated with pectinase enzyme (20 ppm) at 45°C for 60 mins to enhance juice extraction. The juice (86.5% v/v) was added with 10% w/v sucrose and 2% w/v annatto, and pumpkin powder at different ratios (0:0 (control) (S1), 100:0 (S2), 75:25 (S3), 50:50 (S4), 25:75 (S5), 0:100 (S6)). Then, the papaya juice was analysed for pH, total soluble solids (TSS), turbidity, colour, total phenolic content (TPC), total flavonoid content (TFC), antioxidant activities (DPPH and FRAP assays) and sensory characteristics using check-all-that-applied (CATA) analysis. Higher annatto powder ratios (S2 and S3) appeared more attractive and made the TFC of papaya juice (26.20 and 23.77 mg QE/100 mL) significantly higher compared to other ratios, but resulted in a bitter aftertaste and unpleasant mouthfeel. However, higher ratios of pumpkin powder (S5 and S6) significantly reduce the TPC of papaya juice (26.48 and 28.21 mg GAE/100 mL). Higher ratios of pumpkin powder significantly increased the antioxidant activities (DPPH and FRAP) and overall acceptability scores, but the juice colour was unattractive. A combination of 50:50 ratios of annatto and pumpkin powders provided the optimal balance between attractive appearance, high antioxidant properties, and acceptable sensory characteristics. The findings of this study emphasised the importance of selecting appropriate natural colourants and ratios to improve the quality of processed fruit juices.

1. Introduction

Packaged fruit juices represent one of the most commonly consumed beverages, with the fruit juice market's volume reaching 45.4 billion litres in 2018 and expected to increase to 50.6 billion litres by 2024 (Mordor Intelligence, 2019). However, papaya juice is a less common variation compared to other readily available packaged fruit juices. Papaya's high nutritional value and distinct flavour profile can be processed into a more palatable juice for customers through better processing techniques and formulations of the juice, which may lead to a better sensory perception and consumer preferences, thus increasing its marketability. The quality of packaged fruit juices might be degraded during processing, packaging, and storage, resulting in detrimental biological, chemical, and physical changes (Rai *et al.,* 2022). High levels of pectin, which make up around 30% of the main cell walls of plants and get into fruit juice during processing (Kazemi *et al.,* 2020), have caused the juice to be cloudy and sedimentary, which makes it less appealing (Conidi *et al.,* 2020). To address this issue, Siti Rashima *et al.* (2022) discovered that pectinase enzymes can degrade pectin linkages, facilitate papaya juice extraction, and increase juice yield while also clarifying the juice.

Increasing the enzyme content, on the other hand, might influence the sensory qualities of the juice, resulting in a lighter and less appealing colour by reducing its redness as reported by Siti Rashima *et al.*

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(2022). Colour is an essential attribute of food and beverages, as it serves as an indicative parameter for quality control and influences consumers' purchasing decisions (Dias *et al.,* 2012). Although synthetic colourants are stable, produce attractive colours, and are low-cost, natural food colourants are gradually preferred due to changing consumer lifestyles and concerns about potential adverse health effects (Albuquerque *et al.,* 2020). As a result, after treatment with pectinase enzymes, a combination of natural colourings such as annatto and pumpkin powder can be added to papaya juice to increase its orange-red colour and make the juice more appealing, which could also contribute to its antioxidant benefits and marketability.

Annatto extracts are natural yellowish-orange colours derived from the outer coatings of the seeds of the tropical plant *Bixa orellana* L. Annatto's primary colouring agents are carotenoids. Annatto's colouring components are the apo-carotenoids bixin (oil-soluble) and norbixin (water-soluble) (Sathiya Mala *et al.,* 2015). Bixin, which is unique to annatto seeds, is widely used as a natural colourant in the food industry (Cardarelli *et al.,* 2008). Norbixin is also extracted from annatto seeds and is particularly useful in water-based food products. In aqueous environments, norbixin's colour can be influenced by factors such as light, pH, and metal ions, and it can be stabilised by natural antioxidants such as ascorbic acid and tocopherols, as well as chelating agents (Moller *et al.,* 2019). Previous research found that spray-dried acid-stable annatto exhibited less precipitation and remained stable in acidic beverages such as lime squash and orange squash during storage (Satyanarayana *et al.,* 2006). Annatto extract has been used to colour a variety of food products, such as dairy, meat, bakery, and extruded products (Zhang *et al.,* 2018). Besides its use as a colourant, annatto extract is known for its antioxidant properties, which are attributed to bioactive compounds such as bixin carotenoids and polyphenols (Quintero Quiroz *et al.,* 2019).

Pumpkin is high in carotenoids, which are responsible for its yellow-orange colour (Luzardo-Ocampo *et al.,* 2021). Pumpkin also contains phenolic components (flavonoids and phenolic acids), carotenoids, and vitamins (Guz *et al.,* 2018). The storage temperature and light exposure affect the degradation kinetics of carotenoids in pumpkin juice concentrate, and the addition of ascorbic acid can protect the carotenoids (Atencio *et al.,* 2022). Pumpkin powder has been added to various foods and beverages, including noodles (Pongjanta *et al.,* 2006), instant drinks (Surya *et al.,* 2021), and muffins (Sathiya Mala *et al.,* 2018), to enhance their nutritive value and overall consumer acceptability. Therefore, the objective of this study was to investigate how different ratios of annatto and pumpkin powders as natural colourants impact the physicochemical properties, antioxidant activities, sensory characteristics, and consumer acceptance of papaya juice.

2. Materials and methods

Fresh papaya (*Carica papaya* L. var. Sekaki) fruits with roughly 70% of the peel displaying a yellow surface (Index 4) were obtained locally from a market in Penang, Malaysia. Pectinase enzyme (PEC-600) from *Aspergillus niger* was obtained from Sunson Industry Group Co., Ltd., China. Natural colouring, annatto, and pumpkin powder were obtained from Baker and Flavorist, Kuala Lumpur, Malaysia. Standard chemicals such as gallic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radicals, and quercetin were purchased from Sigma-Aldrich, Germany. All other chemicals used were of analytical grade.

2.1 Preparation of papaya juice

The papaya juice was prepared according to the method of Siti Rashima *et al.* (2022), with some modifications. Papaya fruits (500 - 600 g) were washed, skinned, and cut into 3 cm^3 cubes. A total of 200 g of papaya cubes were acidified by blanching in citric acid (0.5% w/v) at 95°C for 2 mins. The papaya cubes were drained and placed in chilled water for 1 min before being blended into a puree and placed in bottles. The homogeneous papaya puree was then treated with pectinase enzymes (20 ppm) at 45°C for 60 mins. The bottles were shaken at 200 rpm in an incubator shaker (IKA KS4000, China). After 1 hr, the papaya puree was put in a water bath at 74°C for 2 mins to terminate the enzyme reaction. The puree was filtered, and the juice (86.5% v/v) was added with 10% w/v of sucrose. The mixture was then treated with 2% w/v natural colourants, annatto, and pumpkin powder, in various ratios (100:0 (S2), 75:25 (S3), 50:50 (S4), 25:75 (S5), 0:100 (S6)). The incorporation of 2% of colourants in the papaya juice formulation is justified by the fact that a higher concentration would result in a reddish or yellowish hue, compromising the taste and other sensory characteristics that define the essence of papaya juice. By experimenting with various ratios of annatto and pumpkin powder, the objective was to determine the colour combinations that not only enhance antioxidant activities but also align with consumer acceptance. The control sample (S1) of papaya juice was made by combining papaya juice with 10% *w/v* sucrose without the addition of annatto or pumpkin powder. Then, a total of 1.5% w/v of calamansi juice was added to the mixture, which was pasteurised at 74°C for 7 mins. The pasteurised papaya juice was kept at 4°C prior to analysis.

2.2 Physicochemical analysis

2.2.1 Colour

A sample was placed in a glass sample cell, and the colour (lightness (*L*), redness (+*a*), greenness (-*a*), yellowness (+*b*), and blueness (-*b*)) was measured using a colourimeter (Konica Minolta CM-5). Prior to measurement, the white calibration plate on the target mask and distilled water in a quartz cell were calibrated.

2.2.2 pH and total soluble solids

A pH metre (Mettler Toledo, Germany) and a hand-held refractometer (Milwaukee Instruments MA87, USA) were used to test the pH and total soluble solids (TSS) of papaya juice, respectively.

2.2.3 Turbidity

The turbidity of the juices was determined using a UV-visible spectrophotometer (UV-1650, Shimadzu, Japan) set at 660 nm, and distilled water was used as reference (Lee *et al.,* 2006).

2.3 Total phenolic content analysis

The total phenolic content (TPC) of papaya juice was determined based on the method described by Siti Rashima *et al.* (2022). Papaya juice extract was prepared by mixing the juice (5 g) with 25 mL of 50% ethanol. The solution was mixed well using IKA MS 3 digital shakers, Germany, at 3000 rpm for 1 min. Then, the mixture was centrifuged at 2000×*g* for 10 mins. Then, Folin-Ciocalteu reagent was added to 1 mL of papaya juice extract. After 3 mins of incubation at room temperature, 7.5% Na_2CO_3 was dissolved in the mixture, and the volume was increased to 10 mL with 50% ethanol. After 30 mins, the absorbance of the mixture at 765 nm was measured using a UV-visible spectrophotometer (UV-1650, Shimadzu, Japan). The gallic acid standard solution (0.02-0.10 mg/mL) was used to plot the standard curve. Gallic acid equivalents (mg GAE/100 g) were used to calculate the total phenolic content (TPC) of the juice. All tests were performed in three replicates.

2.4 Total flavonoid content analysis

The total flavonoid (TFC) content of papaya juice was determined by the method described by Siti Rashima *et al.* (2022). A total of 0.6 mL of the papaya juice extract was dissolved in 1.2 mL of 80% methanol and 0.18 mL of 20% $NaNO_2$, before being vortexed. After 6 mins, 0.36 mL of 8% $AlCl_3$ was added to the mixture, which was then incubated for 5 mins. The liquid was then treated with 1.2 mL of 1 mol/L NaOH, which was stirred with a vortex and left at room temperature for 15 mins in a dark room. A UV-visible spectrophotometer was used to measure the absorbance of the mixture at 510 nm (UV1650, Shimadzu, Japan). A calibration curve for the quercetin standard solution (0.005-0.08 mg/ml) was created. The total flavonoid content (TFC) of papaya juice was measured in triplicate and represented as mg QE/100 g.

2.5 2,2-diphenyl-2-picrylhydrazyl radical scavenging assay

The 2,2-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of papaya juice extracts was determined by the method described by Siti Rashima *et al.* (2022). In a test tube, 0.12 mL of papaya juice extract and 4 mL of 0.02 mg/mL methanolic DPPH were mixed, vortexed, and kept at room temperature in a dark room for 45 mins. The mixture's absorbance was then measured at 517 nm using a UV-visible spectrophotometer (UV1650, Shimadzu, Japan). The methanolic DPPH solution served as a control, while methanol was used as a reference. The scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{A}{B}\right) \times 100\%$$

where A = absorbance of sample at 517 nm, B = absorbance of control at 517 nm.

2.6 Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) assay was performed using the method described by Siti Rashima *et al.* (2022). To prepare the FRAP reagent, 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3.6H_20$ were mixed in a 10:1:1 ratio. Then, 4 mL of FRAP reagent and 0.2 mL of papaya juice extract were mixed. After 50 mins, the absorbance of the mixture was measured at 595 nm using a UV-visible spectrophotometer (UV1650, Shimadzu, Japan). Using a calibration curve of $FeSO_4$ standard solution (0.05-0.30 mg/mL), the FRAP test of papaya juice was expressed in $FeSO_4$ equivalents (mg $FeSO_4$/100 g). The analysis was repeated three times.

2.7 Sensory analysis

Sensory assessment was carried out in a sensory laboratory in an individual sensory booth, at the School of Industrial Technology, Universiti Sains Malaysia, Penang, Malaysia. Prior to participating in this study, panellists were asked to read and sign a consent form, which was approved by the Human Research Ethics Committee of Universiti Sains Malaysia (USM/JEPeM/22060385).

The check-all-that-apply (CATA) test was used to indicate how the consumers classified the papaya juice by assessing attributes such as colour, odour, aroma, taste, as well as aftertaste and grouping the information for ease of interpretation. The CATA test modified the approach of Siti Rashima *et al.* (2021). Six volunteers, ranging in age from 20 to 24 years old, took part in determining the properties of papaya juice. Before characterising the papaya juice containing varied ratios of annatto and pumpkin powder, they were instructed on the technique for identifying each attribute. Each panellist received six glasses of papaya juice at random, and they were asked to describe the attributes of the juice. All attributes described by the panellists on colour (orange colour, reddish orange colour, reddish colour, attractive and unattractive), odour (sweet odour, sour odour, papaya odour, calamansi odour), aroma (papaya aroma, calamansi aroma) taste (sweet taste, sour taste), aftertaste (bitter aftertaste), and unpleasant mouthfeel were chosen based on the agreement of all panellists. The selected attributes were used for CATA analysis.

The CATA analysis was carried out by 75 panellists who were chosen at random from a group of students and staff ranging in age from 24 to 45 years old. Panellists were served six papaya juices at different ratios of annatto and pumpkin at random. They were asked to mark "tick" at any of a list of attributes that they thought best described the juice. Then, they were asked to mark their acceptability score for the overall acceptability of the papaya juices using 9-point hedonic scales (1 = "dislike extremely"; 9 = "like extremely") (Meilgaard *et al.,* 2016). Participants took approximately 15 to 20 mins to conduct the sensory evaluation.

2.8 Statistical analyses

A statistical package for social science (SPSS 27.0 for Windows, SPSS Inc., USA) was used to run an analysis of variance (ANOVA) on the physicochemical and antioxidant data for six samples of papaya juice. Tukey's range test was used to compare means with a probability value of 0.05. The frequency of each attribute chosen by panellists for CATA analysis was calculated based on the total count. The total counts of attributes determined from CATA analysis for each papaya juice were used in conjunction with physicochemical and antioxidant properties data for Pearson's correlation and correspondence analysis using SPSS software.

3. Results and discussion

3.1 pH, total soluble solids and turbidity properties of papaya juice

Table 1 shows the changes in pH, total soluble solids (TSS), turbidity, and colour of papaya juice with different ratios of annatto and pumpkin powder. The pH of the juice ranged from 4.13 to 4.18. An increased annatto concentration significantly increased the pH of the papaya juice. However, increasing the concentrations of pumpkin powder has no significant effect on the pH of the juice. The addition of annatto and pumpkin powder increased the TSS turbidity of the papaya juice. The content of suspended particles in the juice determined the turbidity of the juice. Increased pumpkin powder ratios significantly increase juice turbidity, as indicated by a higher absorbance value.

Table 1. Effect of different ratios of annatto and pumpkin powders on the pH, total soluble solids (TSS), turbidity, and colour of papaya juice.

Different ratios of annatto and pumpkin powder	pH	TSS (°Brix)	Turbidity (absorbance at 660 nm)
S1 (0:0) (control)	4.12±0.01[c]	14.97±0.06[d]	0.86±0.01[e]
S2 (100:0)	4.18±0.00[a]	15.50±0.00[a]	2.11±0.02[d]
S3 (75:25)	4.15±0.00[b]	15.43±0.06[ab]	2.20±0.01[c]
S4 (50:50)	4.13±0.01[c]	15.20±0.00[c]	2.23±0.03[c]
S5 (25:75)	4.13±0.00[c]	15.33±0.06[b]	2.28±0.03[b]
S6 (0:100)	4.13±0.01[c]	15.40±0.00[ab]	2.33±0.01[a]

Values are presented as mean±SD, n = 3. Values with different superscripts in the same column are statistically significantly different (P<0.05).

The cloudiness of the juice may be attributed to the colloidal dispersion of electrically charged particles in a complex serum of pectin, sugars, organic acids, and salts as the concentration of pumpkin powder increases. The charged particles are then suspended in the juice, where they create a stable colloidal structure that scatters light and causes the juice to appear cloudy (Li *et al.,* 2021). Furthermore, the pectin molecules in pumpkin powder can form a network in the juice, trapping water and other particles and contributing to the overall turbidity of the papaya juice.

3.2 Colour properties of papaya juice

The papaya juice prepared without the addition of annatto and pumpkin powder is light orange. Blanching papaya fruit, followed by the pectinase enzyme of papaya pulp to facilitate juice extraction, had a substantial influence on the real colour of papaya juice, which turned lighter and reduced the redness and yellowness (Siti Rashima *et al.,* 2022). The processing of papaya juice caused significant colour loss, necessitating the use of colourants to improve the juice's appeal and acceptability. As a result, a natural colouring combination of annatto and pumpkin powder has the potential to be added to the papaya juice formulation to enhance the intensity of the juice's redness and yellowness. The results showed that increasing the concentration of annatto powder significantly ($p < 0.05$)

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reduced the lightness (*L* value) and increased the redness (*a* value) of the papaya juice (Table 2). The pigments bixin and norbixin in annatto, which give a yellow to red colour (Taham *et al.,* 2015), had contributed to the juice's redness. Meanwhile, increasing the ratio of pumpkin powder provided an orange colour due to the presence of pigments, beta-carotene (Lima *et al.,* 2021), which significantly ($p < 0.05$) increased the lightness and yellowness (*b* value) but decreased the redness of the juice. On the other hand, the lightness and colour of the papaya juice with annatto and pumpkin powder are determined by the concentration of the powder used, the pH of the juice, the heating temperature, the storage conditions, and the amount of antioxidant compounds in the juice (Atencio *et al.,* 2022).

Table 2. Effect of different ratios of annatto and pumpkin powders on the colour (lightness (L value), redness (a value) and yellowness (b value)) of papaya juice.

Different ratios of annatto and pumpkin powder	Colour		
	Lightness (*L* value)	Redness (*a* value)	Yellowness (*b* value)
S1 (0:0) (control)	51.68±0.07[a]	36.88±0.08[d]	68.63±0.22[b]
S2 (100:0)	29.28±0.07[f]	41.92±0.45[a]	50.46±0.14[f]
S3 (75:25)	30.51±0.05[e]	41.76±0.03[a]	52.50±0.09[e]
S4 (50:50)	35.91±0.19[d]	40.40±0.05[b]	60.91±0.26[d]
S5 (25:75)	39.16±0.38[c]	38.78±0.04[c]	65.27±0.07[c]
S6 (0:100)	42.99±0.17[b]	35.46±0.06[e]	69.55±0.18[a]

Values are presented as mean±SD, n = 3. Values with different superscripts in the same column are statistically significantly different (P<0.05).

3.3 Antioxidant properties of papaya juice

Table 3 shows the total phenolic and flavonoid content of papaya juice after adding annatto and pumpkin powder in various ratios. The findings demonstrate that the addition of annatto powder to papaya juice results in a significantly higher TPC than pumpkin powder. Annatto powder contains a high level of polyphenols (Quintero-Quiroz *et al.,* 2019), which contributes to the juice's high phenolic content. However, the addition of up to 2% pumpkin powder does not have a significant effect on the TPC of papaya juice. The results showed that papaya juice had a higher TFC (37.45 mg QE/100 mL) than TPC (26.01 mg GAE/100 mL). Previous research discovered that papaya extracts obtained with three different solvents, n-hexane, ethyl acetate, and ethanol, had a higher TFC than TPC (Insanu *et al.,* 2022).

The addition of pumpkin powder to papaya juice causes a notable decrease in the TPC of papaya juice, as shown in Table 3. Mokhtar *et al.* (2021) reported that pumpkins in various stages of ripeness have relatively low levels of TFC compared to TPC, which could account for the observed decline in TFC in the juice as the concentration of pumpkin powder increases. Another possibility is that the pectin in the pumpkin powder forms a network that traps water and other components, including flavonoid compounds, resulting in cloudy juice (Li *et al.,* 2021). During the filtration step used to extract the juice, larger particles are suspended and removed, along with the flavonoid compounds. Hence, the reduction of TFC in the juice was likely due to the removal of flavonoid compounds and larger particles in the suspension during the extraction process.

The antioxidant properties of papaya juice are attributed to its high content of phenolic and flavonoid compounds, as well as other beneficial phytochemicals such as carotenoids, alkaloids, tannins, tocopherols, and phytosterols, as noted in studies by Gonçalves Rodrigues *et al.* (2019) and Kong *et al.* (2021). The addition of annatto and pumpkin powder to papaya juice significantly increased its DPPH scavenging activities and FRAP value. According to Quintero Quiroz *et al.* (2019), annatto seeds contain polyphenol compounds such as catechin, chlorogenic acid, xanthohumol, hypotaetin, licochalcone, chrysin, and butein that contribute to their high antioxidant activities. Previous studies have found that the use of annatto seeds as a dye for pork patties can enhance their antioxidant properties

Table 3. Effect of different ratios of annatto and pumpkin powders on the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities (DPPH scavenging activity and FRAP activity) of papaya juice.

Different ratios of annatto and pumpkin powder	TPC (mg GAE/100 mL)	TFC (mg QE/100 mL)	DPPH scavenging activity (%)	FRAP activity (mg $FeSO_4$/100 mL)
S1 (0:0) (control)	26.01±1.51[a]	37.45±1.05[a]	29.34 ±0.44[a]	18.36±0.15[a]
S2 (100:0)	36.99±0.94[b]	26.20±1.39[b]	35.62 ±0.27[b]	19.09±0.15[b]
S3 (75:25)	35.74±0.72[b]	23.77±1.05[c]	37.56 ±0.10[c]	19.63±0.05[c]
S4 (50:50)	36.05±2.16[b]	18.60±0.91[de]	37.03 ±0.27[c]	20.04±0.02[d]
S5 (25:75)	26.48±0.72[a]	17.69±0.91[e]	37.15 ±0.35[c]	20.08±0.06[d]
S6 (0:100)	28.21±0.98[a]	19.82±0.53[d]	37.62±0.27[c]	20.07±0.05[d]

Values are presented as mean±SD, n = 3. Values with different superscripts in the same column are statistically significantly different (P<0.05).

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by reducing the levels of thiobarbituric acid reactive substances (TBARS) and peroxide values (POV), as observed by Cuong and Chin (2016). However, increasing the amount of pumpkin powder added did not significantly boost the juice's antioxidant activities, as demonstrated in Table 3. The high antioxidant activities of pumpkin powder may be attributed to the presence of bioactive compounds such as carotenoids (zeaxanthin, lutein, and β-carotene), polyphenols, tocopherols (α-tocopherol and γ-tocopherol), and vitamins (C, B1, folates) (Kulczyński and Gramza-Michałowska, 2019).

3.4 Sensory characteristics and consumer acceptability of papaya juice

The check-all-that-apply (CATA) method has become a popular technique for consumer perception studies due to its simplicity and versatility, and it can be used by both trained and untrained consumers (Lee *et al.,* 2021). CATA questions are used in consumer studies to identify sensory attributes that are associated with a specific product (Piochi *et al.,* 2021). Figure 1 shows the relationship between sensorial profiling of colour (orange colour, reddish orange colour, reddish colour, attractive, and unattractive), odour (sweet, sour, papaya, and calamansi), aroma (papaya and calamansi), taste (sweet, sour, bitter aftertaste, unpleasant mouthfeel), and overall acceptability of papaya juice. Papaya juice with 2% pumpkin powder (S6) and without any colourant (S1) was characterised as having an orange colour, strong papaya odour and aroma, and a strong sweet odour. Papaya juice with 75% annatto and 25% pumpkin powder (S3) had the most attractive colour, but no papaya odour was detected. Juice (S4) with a ratio of 50% annatto and 50% pumpkin powder had a reddish-orange colour. Meanwhile, juice (S5), with a ratio of 75% pumpkin and 25% annatto powder, had a mild sweet odour and taste.

Papaya juice with a high content of annatto powder was found to have a strong sour odour and a lower papaya odour. The addition of 2% annatto powder (S2) decreased the papaya aroma and sweetness, but increased the calamansi aroma, and produced a bitter aftertaste and an unpleasant mouthfeel (Figure 1). Consumer evaluations of papaya juice with and without annatto and pumpkin powder revealed that consumers greatly preferred the papaya juice without any colourant (S1), while they moderately and slightly preferred the papaya juice with annatto and pumpkin at ratios of 75:25 (S5) and 50:50 (S4), respectively. Studies on the addition of annatto to various food products, such as soy-wheat cake (Dzah *et al.,* 2016) and spreadable liver pates (Martín-Sánchez *et al.,* 2017), have reported higher acceptability scores. The reddish colour of annatto has been shown to improve the appearance of foods and beverages, making them more attractive and improving consumer acceptability.

The correspondence analysis performed on the physical properties, antioxidant activities, and sensory characteristics of papaya juice is shown in Figure 2. Papaya juice with higher ratios of annatto, specifically 100:0 (S2) and 75:25 (S3), exhibited similar characteristics, such as reddish colour, bitter aftertaste, unpleasant mouthfeel, high total phenolic content (TPC), high redness (*a* value), and attractive appearance. The analysis revealed a strong relationship between the reddish colour and the phenolic acid content of the juice. Conversely, the opposite region at the upper right, representing papaya juice with a high ratio of pumpkin

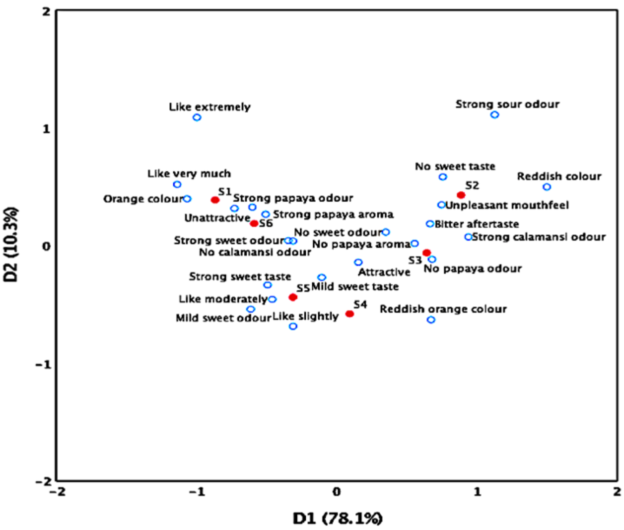

Figure 1. Correspondence analysis (CA) plot of the sensory properties using check-all-that-apply (CATA) questions of papaya juice without (S1) and with the addition of annatto and pumpkin powder at different ratios: S2 =100:0, S3 = 75:25, S4 = 50:50, S5 = 25:75 and S6 = 0:100.

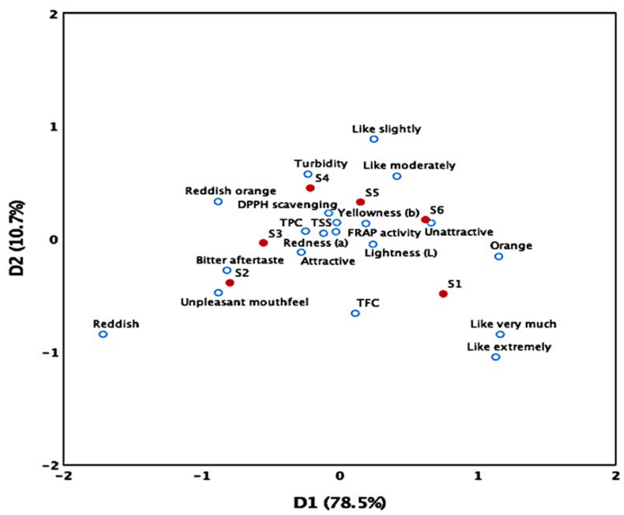

Figure 2. Correspondence analysis (CA) plot of the relationship between physicochemical, antioxidant and sensory properties of papaya juice without (S1) and with the addition of annatto and pumpkin powder at different ratios: S2 =100:0, S3 = 75:25, S4 = 50:50, S5 = 25:75 and S6 = 0:100.

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powder (27: 75 (S5) and 0:100 (S6)), showed a positive relation to yellowness (*b* value), higher FRAP activity, lighter colour, and more orange colour, which were related to an unattractive appearance. The results suggest that colour was the most significant parameter for the changes in papaya juice appearance with and without the addition of annatto and pumpkin powder. Although the orange colour was considered unattractive, it was positively related to a high overall acceptability score given by consumers.

Pearson's correlation coefficients were used to verify the relationships between colour (*L*, *a*, and *b* values) and other physicochemical properties, antioxidant activities, and sensory characteristics of papaya juice with different ratios of annatto and pumpkin powder (Table 4). The redness (*a* value) of the juice showed a strong positive correlation ($p<0.05$) with the TPC. Changes in the redness or yellowness of the juice after the addition of annatto and pumpkin powder did not significantly ($P>0.05$) influence the DPPH scavenging activity or FRAP activity. The high redness (*a* value) of the juice was positively correlated with the reddish and orange-reddish colour perceived by consumers. Papaya juice (S6) with a higher lightness (*L* value) showed a strong positive correlation ($r = 0.959$) at $p<0.01$ with papaya aroma. The yellowness (*b* value) of the papaya juice containing only pumpkin powder showed a significantly negative correlation with a bitter aftertaste and unpleasant mouthfeel.

4. Conclusion

Increasing the ratio of annatto powder resulted in a significant improvement in the redness and attractiveness of the colour, as well as higher levels of total phenolic content (TPC) and total flavonoid content (TFC) in the papaya juice. Increasing the pumpkin powder ratio up to 50% resulted in an increase in DPPH scavenging activity and FRAP activity. Consumers gave higher ratings for the overall acceptability of the papaya juice containing higher ratios of pumpkin. To summarise, a desirable colour and high antioxidant properties, along with acceptable sensory characteristics, can be achieved by adding annatto and pumpkin powder at 50:50 ratios. The results of this study provide valuable insights into the impact of using natural colouring in various ratio combinations on the physicochemical properties,

Table 4. Pearson correlation coefficient of colour (*L*, *a* and *b*) with physicochemical properties, antioxidant activities, and sensory characteristics of papaya juice containing different ratios of annatto and pumpkin powder.

Variables	Colour properties		
	Lightness (*L* value)	Redness (*a* value)	Yellowness (*b* value)
Physicochemical properties			
TSS (°Brix)	-0.746	0.445	-0.602
Turbidity	-0.703	0.319	-0.329
TPC	-0.771	0.850*	-0.877*
TFC	0.540	-0.144	0.064
Antioxidant activities			
DPPH scavenging	-0.629	0.309	-0.314
FRAP	-0.382	0.015	0.062
Sensory characteristics			
Orange colour	0.860*	-0.970**	0.933**
Reddish-orange colour	-0.875	0.973**	-0.882*
Reddish colour	-0.767	0.875*	-0.978**
Sweet odour	0.290	-0.69	0.667
Sour odour	-0.626	0.707	-0.886*
Papaya odour	0.781	-0.694	0.743
Calamansi odour	-0.476	0.863*	-0.912*
Papaya aroma	0.959**	-0.824*	0.761
Sweet taste	0.786	-0.718	0.885*
Sour taste	-0.361	-0.361	0.139
Bitter aftertaste	-0.898*	0.782	-0.878*
Unpleasant mouthfeel	-0.743	0.811	-0.954**
Attractive	-0.401	0.840*	-0.717
Unattractive	-0.530	-0.914*	0.758

*significantly different at P<0.05, **significantly different at P<0.01.

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antioxidant activities, and sensory characteristics of fruit juice to enhance its appearance.

Conflict of interest

All authors have no conflicts of interest to disclose.

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