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Physicochemical properties, antioxidant activities and sensory evaluation of butterfly pea (*Clitoria ternatea* L.) ice cream flavoured with citrus juice

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The aim of this study was to investigate the influence of addition of lime and lemon juice on the physicochemical properties, antioxidant activities and sensory properties of butterfly pea ice creams. The addition of citrus juice led to colour changes from bluish to purplish as the pH decreased (from 6 to 4). It also resulted in a reduction of total soluble solids and a slight increment in moisture content. Among the samples, ice cream with lemon was the softest while having the most viscous mixture. Regarding antioxidant activities, all ice creams had similar total phenolic content (0.3 mg gallic acid/100 ml sample). The ice cream samples containing citrus juice had higher 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity and ferric reducing antioxidant power (FRAP) value than the control sample. However, the anthocyanin content of butterfly pea ice creams decreased by the addition of citrus juice. Overall, the addition of lemon juice significantly improved consumer acceptance towards butterfly pea ice cream. Therefore, lemon juice can be incorporated into formulation of butterfly pea ice cream to enhance the amount of natural antioxidants as well the flavour.

Keywords: butterfly pea, *Clitoria ternatea* L., ice cream, physicochemical properties, antioxidant activities, sensory evaluation

INTRODUCTION

Ice cream is a sweet and frozen dairy food created by agitating a pasteurized mixture to incorporate air before homogenizing and freezing to ensure its consistency. The main ingredients in making ice cream are milk fat, milk solids non-fat, sweetener, and stabilizer. In 2020, the global ice cream market was valued at USD 111.58 billion and is expected to grow at a compound annual growth rate (CAGR) of over 5.71% by 2026 (Wood, 2021). One way to break through this competitive market is by introducing nutritious ice creams.

Butterfly pea (*Clitoria ternatea* L.) is a climber which commonly grows in tropical areas including India, Philippines, South and Central America, Caribbean and Madagascar (Jeyaraj et al., 2021). The flower is commonly used in traditional medicines due to its anti-diabetic properties, anti-proliferative properties, antioxidant properties, antimicrobial properties, and anti-compulsive activity (Lakshan et al., 2020). This flower is one of the herbs that commonly be applied as a colourant

in beverages, cuisine, and dessert. The flower can be a substitute for blue synthetic colourant as its blue petals contain anthocyanins. The blue colour would change upon exposure to different pH values; from red to violet, blue, blue-green, green, and yellow when the pH varied from 0.05 to 12.0 (Abdullah et al., 2010). It is hypothesized that the addition of citrus juice (lime and lemon) changes the colour of the ice cream.

According to Vojdani & Vojdani (2014), the amount of synthetic dye used in foods has increased by 500% in the last 50 years and it is reported to cause allergic and immune reactive disorders. The long-term effect of artificial food is that it can cause cancer in humans if consumed above the recommended dosages (Ahmed et al., 2021). As public awareness increases, non-synthetic additives are the current trend in the food sector due to food safety demands and the health advantage of natural nutrients (Neves et al., 2021). In addition, natural colourant such as anthocyanins are known for their possible health benefits as dietary antioxidants (Baskaran et al., 2019; Masaud et al., 2014). Anthocyanins are absorbed as intact molecules in the stomach and have systemic activity, exerting an antineoplastic, anticarcinogenic, antiatherogenic, antiviral, and anti-inflammatory effects, improving memory impairment, as well as decreasing capillary permeability and fragility, inhibiting platelet aggregation and stimulating immune function (Lema, et al., 2022; Limsuwan et al., 2014; Ridzwan et al., 2022).

Consumer demand for nutritious foods has led to the development of healthier ice cream. In this study, the extract of butterfly pea flower was incorporated into ice cream formulations to produce a functional ice cream. Despite its functionality, the extract may provide a vibrant and appealing colour to the ice cream. Citrus juices (i.e. lime and lemon) was added as a flavouring because the aqueous extract of the butterfly pea flower has a bland taste. Citrus juices were added to improve the taste of the ice cream and thus well accepted by the consumer.

MATERIALS AND METHODS

Plant Materials

The butterfly pea flowers were collected around Besut District, Terengganu, Malaysia. All ingredients for making ice cream were bought from local supermarkets in Terengganu, Malaysia.

Preparation of Butterfly Pea Extract

The butterfly pea extract was prepared according to method by Saptarini et al. (2015) with modification. 100 grams of butterfly pea flower was added to 100 ml of boiling water for 10 minutes. The extract was filtered using muslin cloth. Then, the extract was kept in a sterile glass bottle that has been wrapped with aluminum foil and stored in a freezer (0ºC) for further use.

Preparation of Butterfly Pea Ice Cream

Butterfly pea ice creams were prepared based on modified formulations of Hapdang et al. (2021), Limsuwan et al. (2014), Singo & Beswa (2019). The list of ingredients used is listed in Table 1. Firstly, egg yolk was whisked before added to milk and whipping cream mixture. Then, the mixture was heated to 40ºC followed by the addition of all other ingredients except butterfly pea extract, vanilla extract and citrus juice. The mixture was heated at 75ºC for 25 seconds before it cooled down using an ice bath. After that, butterfly pea extract, vanilla extract and citrus juice were added into the mixture and homogenized using a hard ice cream machine (model FHICM-45T Euroasia Food Equipment Sdn Bhd, Penang, Malaysia) for 15 minutes. Once ready, the ice cream was put in plastic containers and stored at -18ºC for hardening.

Table 1. Formulations of butterfly pea ice cream.

Ingre-dients (g)	Formulation 1 (control)	Formulation 2 (lime juice)	Formulation 3 (lemon juice)
Milk	1000	1000	1000
Whipping cream	500	500	500
Sugar	300	300	300
Gelatin	2	2	2
Starch	8	8	8
Milk powder	100	100	100
Egg yolk	60	60	60
Vanilla extract	10	10	10
Butterfly pea extract	200	200	200
Citrus juice	-	165	165

Physicochemical Properties of Ice Cream

pH

The pH of melted ice creams was measured using pH meter (Orion 9107BNMD, Thermo Scientific, USA).

Moisture content

The moisture content was determined using the air oven drying method (AOAC Method 925.10) (AOAC, 1990). The sample was dried at 105 °C overnight (24 hours) until a constant weight was reached and cooled in a desiccator for 3 hours. Subsequently, the moisture content was calculated as the weight of water removed during drying divided by the initial weight of the sample.

Colour

The colour of ice cream was measured using a hand-held colorimeter (Chroma meter, CR-400, Konica Minolta, Japan). The measurement was done in triplicate and the L* (represents darkness to lightness), a* (represents greenness to redness) and b* (represents blueness to yellowness) values were compared. The absolute colour difference (ΔE) of ice creams were calculated using:

$$\Delta E = ((L_0 * - L *)^2 + (a_0 * - a *)^2 + (b_0 * - b *)^2)^{\frac{1}{2}}$$

Total soluble solids

The total soluble solid (TSS) content of lime and mandarin juice along melted ice creams were measured by using a digital benchtop refractometer (Easy R40, Mettler Toledo, Switzerland). The TSS values was expressed in degree Brix (°Brix).

Texture analysis

The texture of the ice cream was determined by the method of Januário et al. (2018). The firmness and index of viscosity of the ice cream were measured at room temperature (20°C) using a texture analyzer (TA-XTPlus, Stable Microsystems, Surrey, UK) equipped with a 3.6 cm diameter stainless steel cylindrical probe. Before the test, ice cream samples were tempered to -5°C for 24 hours and cut into 4 cm squares on each side. The conditions for analysis were: penetration distance = 10 mm, force = 1.0 g, probe speed before and during penetration = 1 mm/s, and probe speed after penetration = 10.0 mm/s. The measurement was performed in triplicate for each sample.

First dripping and complete melting

The first dripping and complete melting of butterfly pea ice cream was determined following the method of Akesowan (2008). 25 gram of ice cream at -18ºC was placed on a sieve (2 mm wide, square openings) and the time of the first drop was recorded as the first dripping time. The melted ice cream's volume was measured for every 5 minutes until the time of 40 minutes was reached. The end dripping time was recorded as the complete melting time.

Antioxidant Activities

Total phenolic content (TPC)

The total phenolic content of ice creams was determined using the method described by Shirazi et al. (2014). 0.1 ml of the methanolic extract was mixed with 0.5 ml distilled water and 0.1 ml of Folin-Ciocalteu's phenol reagent in a test tube covered with aluminum foil before allowing it to stand for 6 minutes. Then, 1 ml of sodium carbonate solution (7% w/v) and 0.5 ml distilled water was added into the mixture. The mixture was kept at room temperature for 90 minutes and the absorbance value was measured at 735 nm by using a UV-VIS spectrophotometer (UVmini-1240, Shimadzu, Japan). Analysis was performed in triplicate and the total phenolic content was expressed as mg gallic acid equivalent per 100 g sample.

DPPH radical scavenging activity

The DPPH radical scavenging activity assay was carried out following the methodology previously reported by Hwang et al. (2009). 0.3 ml of methanolic extract was added to 1.2 ml methanol and 1.5 ml of 0.5 mM DPPH (in methanol). The mixture was kept at room temperature for 90 minutes and the absorbance was measured at 517 nm using UV-VIS spectrophotometer (UVmini-1240, Shimadzu, Japan). 25 mg of Trolox was dissolved in 100 ml methanol to get 1 mg/ml of Trolox standard solution and then the gallic acid was diluted at different concentrations (0.02, 0.04, 0.08 and 1 mg/ml) before used as standard for the calibration curve with methanol as blank. The DPPH test result was calculated according to a calibration curve of gallic acid. Analysis was performed in triplicate and the DPPH was expressed as mg Trolox equivalent per 100 g sample.

Ferric reducing antioxidant power (FRAP)

The FRAP assay was analysed according to Bertoncelj et al. (2007). The stock solutions include 300mM acetate buffer pH 3.6, 10 mM 2,4,6-tripyridly-s-triazine (TPTZ) solution in 40 mM HCL and 20 mM FeCl3•6H2O solution. FRAP reagent was freshly prepared by mixing 50 ml acetate buffer, 5 ml TPTZ solution and 5 ml FeCl3•6H2O solution and then warmed at 37°C before use. 0.2 ml of sample extract was mixed with 1.8 ml of the FRAP reagent. After incubation at 37°C for 10 min, the absorbance of the reaction mixture was measured at 593 nm. A calibration curve was prepared using an aqueous solution of ferrous sulphate, FeSO4.7H2O (50-300 mg/l). The results were expressed as mM Fe2+ per 100 mg sample.

Anthocyanin content

The anthocyanin content was measured according to the method of Hwang et al. (2009). 2 ml of melted ice cream was mixed with 2 ml of 1% HCl in methanol and then centrifuged at 3000 ×g for 10 minutes. 1 ml of the supernatant was added to 9 ml of 1 mol/l HCl and the absorbance was measured at 520 nm using UV-VIS spectrophotometer (UVmini-1240, Shimadzu, Japan). The total anthocyanin content was calculated using formula:

$$Anthocyanin\ content\ \left(\frac{mmol}{ml}\right) = Absorbance_{520} \times 101 \times 18.89$$

Sensory Evaluation

The sensory panel was composed of 40 untrained consumers who were randomly chosen among the students at Faculty of Biosciences, Universiti Sultan Zainal Abidin. The panelists evaluated the three formulations of ice creams in the same session. The sample (18 g) was placed in a lidded transparent cup. Sensory properties of ice cream (colour, aroma, flavour, sweetness, sourness, texture and overall acceptance) were evaluated by panelists based on their preference on a scale between 1 (dislike extremely) and 9 (like extremely). Drinking water at room temperature was provided to clean the mouth before and between tasting the samples. This project was approved by the Universiti Sultan Zainal Abidin Human Research Ethics Committee (UniSZA/UHREC/2021/331).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) software, version 20.0 (SPSS, IBM, Illinois, USA) was used to perform the statistical analysis of all the data. The significant differences among results were determined using one way analysis of variance (ANOVA) and Duncan's Multiple Range Tests ($p<0.05$).

RESULTS AND DISCUSSION

Physicochemical Properties of Ice Cream

The results of pH, moisture content, colour and total soluble solids of lime and lemon juices and butterfly pea ice creams are presented in Table 2. The addition of citrus juices significantly decreased the pH of ice creams, indicating that the products were more acidic and had a sour taste. This result is in agreement with the finding of Hapdang et al. (2021) who obtained pH value between 5.26 to 5.40 when tangerines were added into the ice cream formulations. This happened because of acidic vitamin C in citrus fruits that naturally reduce the pH level of the final products. Since both citrus juices had similar pH (~2.6), therefore it was expected that the pH of the resulting ice creams was similar too (~4.4). In general, the pH of ice cream is about 6 (Goff & Hartel, 2013), which is similar to the control ice cream without the addition of citrus juice. The moisture content of ice cream is usually around 55 to 76%, which mainly comes from milk (Bajwa et al., 2003; Cakmakci et al., 2016; Murtaza et al., 2004; Rahim & Sarbon, 2019). The moisture content of all butterfly pea ice creams was found to be within this range (approximately 65%).

As shown in Figure 1, the control butterfly pea ice cream had bluish colour (negative value for a* and b*), while the ice creams with citrus juice had purplish colour (positive a* value and negative b* value). The colour of butterfly pea extract was dark blue (L* = 20.29 ± 0.80, a* = 15.19 ± 0.76, b* = -16.43 ± 0.69) at pH 5.6. This is in good agreement with observations of Abdullah et al. (2010) and Escher et al. (2020) who found that the colour of butterfly pea extract pea changed from violet to blue when the pH increased from 5 to 7. Butterfly pea flower contains anthocyanin (a plant pigment) which is responsible for red violet-blue in plant flowers (Saptarini et al., 2015). The colour of this pigment is strongly influenced by the pH of the product; the colour is blue at neutral pH and becomes purple at lower pH (Sutakwa et al., 2021). In terms of lightness, ice cream with lemon juice had the highest value, while the control ice cream was the least. Compared to control ice cream, the absolute colour difference (ΔE) between ice cream flavoured with lime juice and lemon juice was the same due to both juices having similar colour.

The value of total soluble solid depends on the ingredients used such as types of sugar and milk (Rahim & Sarbon, 2019). The total soluble solids of ice creams decreased with the incorporation of citrus juice. Both citrus juices had similar total soluble solids (~7.7°Brix), however ice cream with lemon had higher total soluble solids (29.51°Brix) than the one with lime (27.53°Brix). The total soluble solid and pH influence the taste of ice cream. The sourness of a fruit enhances by its low total soluble solids content, hence affects its sweetness (Ikegaya et al., 2019). Many studies have reported that fruity ice creams exhibited lower total soluble solids and pH values with increasing concentration of fruit in the products, thus imparting the taste of the products (Cakmakci et al., 2016; Gabbi et al., 2017; Singo & Beswa, 2019; Ürkek et al., 2019).

Table 2. Physicochemical properties of lime juice, lemon juice and butterfly pea ice creams.

Parameter	Lime juice	Lemon juice	Formulation 1 (control)	Formulation 2 (lime)	Formulation 3 (lemon)
pH	2.65 ± 0.02^{c}	2.62 ± 0.01^{c}	6.41 ± 0.06^{a}	4.35 ± 0.07^{b}	4.48 ± 0.15^{b}
Moisture content (%)	93.71 ± 0.00^{a}	92.08 ± 0.00^{b}	65.00 ± 0.00^{d}	66.33 ± 0.01^{c}	65.44 ± 0.01^{cd}
Colour					
L*	48.57 ± 0.97^{d}	49.16 ± 0.71^{d}	67.33 ± 0.79^{c}	68.78 ± 0.16^{b}	72.11 ± 0.66^{a}
a*	-1.44 ± 0.06^{d}	-1.99 ± 0.05^{d}	-2.11 ± 0.07^{c}	4.71 ± 0.13^{a}	4.04 ± 0.41^{b}
b*	5.86 ± 0.36^{a}	5.65 ± 0.14^{a}	-7.02 ± 0.36^{b}	-9.53 ± 0.13^{c}	-7.38 ± 0.49^{b}
ΔE	-	-	-	7.42	7.82
Total soluble solids (°Brix)	7.80 ± 0.10^{d}	7.63 ± 0.07^{d}	31.65 ± 0.25^{a}	27.53±0.34^{c}	29.51±0.31^{b}
Texture					
Firmness (g)	-	-	1548.00 ± 559.06^{a}	1364.43 ± 128.10^{b}	211.81 ± 37.73^{c}
Viscosity (g.sec)	-	-	40.30 ± 0.70^{c}	64.20 ± 0.30^{b}	65.10 ± 0.30^{a}
Melting properties					
First dripping (min)	-	-	5.21 ± 3.70^{a}	5.01 ± 1.42^{a}	6.54 ± 3.11^{a}
Complete melting (min)	-	-	39.11 ± 0.05^{a}	39.42 ± 0.11^{a}	39.45 ± 0.10^{a}

Different letters in the same row show statistically significant different ($p<0.05$). Values are means ± standard deviation.

Figure 1. The colour and appearance of a) butterfly pea extract, b) lime juice, c) lemon juice, d) butterfly pea ice cream (control), e) butterfly pea ice cream with lime juice and f) butterfly pea ice cream with lemon juice.

The firmness of butterfly pea ice creams was tested as it is an important attribute that relates to 'scoopability'. The control ice cream was the hardest, while ice cream with lime juice was the softest (Table 2). According to Feizi et al. (2021), ice cream with larger air cells is softer and easy to scoop. The firmness of an ice cream also related to its overrun, ice crystal size, ice phase volume and the level of fat destabilization (Muse & Hartel, 2004). Viscosity of ice creams indicates its desirable body and texture (Ürkek et al., 2019). The values of viscosity increased significantly when citrus juices were added. Among the samples, butterfly pea ice cream flavoured with lemon juice had the highest viscosity. This probably happened because juices contained high dietary fibre and thus resulted in more viscous ice cream mixes. A similar observation was reported by Ramadan and Moersel (2009) who found that dietary fibre could cause an increase in viscosity of ice creams.

Another study by Dervisoglu (2006) reported that hazelnut flour that high in fibre enhanced the viscosity of ice cream samples when compared to the control (without hazelnut flour).

The first dripping and complete melting of ice creams are listed in Table 2 and their melted volume over time is presented in Figure 2. All butterfly pea ice creams had similar first dripping and complete melting time. The addition of citrus juices seemed to have no impact on the melting of the ice creams due to its small amount. However, the rate of melting was different between samples. The control ice cream had the highest melting rate (0.21 ml/min), followed by ice cream flavoured with lemon (0.14 ml/min) and the lowest was ice cream with lime (0.08 ml/min). The lemon and lime juice contain fibre which may bind to free water in the ice cream resulting in higher melting point. The melting rate of ice cream can also be affected by numbers of factors including the types and amount of sweetener, additive used, amount of air incorporates (overrun), nature of ice crystals and network of fat globules formed during freezing (Gabbi et al., 2017; Pon et al., 2015; Rahim & Sarbon, 2019). In addition, Sun-Waterhouse et al. (2013) reported that fruit components such vitamin C, polyphenols, pigments and proteolytic enzymes were beneficial in controlling melting time of ice cream.

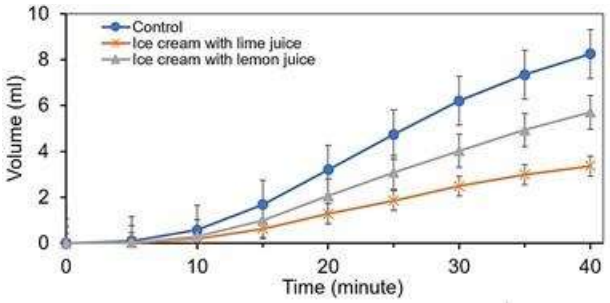

Figure 2. The volume of melted ice creams against time.

Antioxidant Activities of Ice Cream

The phenolic content, DPPH radical scavenging activity and FRAP value of butterfly pea ice creams are shown in Figure 3, 4 and 5, respectively. All ice creams had similar phenolic content, thus the addition of citrus juices gave no impact on phenolic content of ice cream. However, it resulted in an increment in the DPPH radical scavenging activity and FRAP value. Lime and lemon juice have been reported to contain a considerable amount of antioxidants (Ogundele & Bolade, 2021).

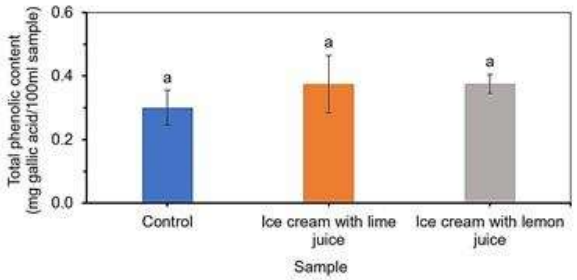

Figure 3. Effect of the addition of citrus juice on the total phenolic content of butterfly pea ice creams. The letters above the bars show statistically significant difference ($p<0.05$).

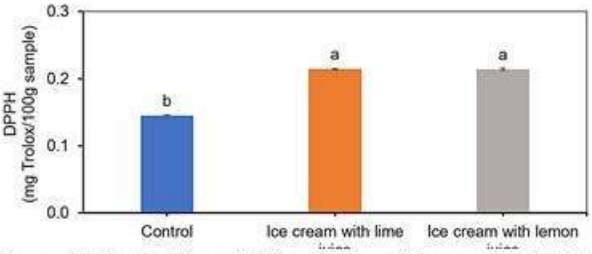

Figure 4. Effect of the addition of citrus juice on the DPPH radical scavenging activity of butterfly pea ice creams. Different letters above the bars show statistically significant difference ($p<0.05$).

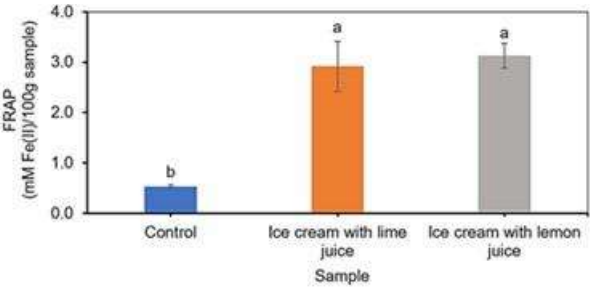

Figure 5. Effect of the addition of citrus juice on the ferric reducing antioxidant power assay of butterfly pea ice creams. Different letters above the bars show statistically significant difference ($p<0.05$).

In fact, the incorporation of citrus fruits had increased the antioxidant activities of food products, such as probiotic yogurt incorporated with orange (Erkaya-Kotan, 2020), functional biscuits with added orange (Rani et al., 2020), bread enriched with lemon pomace (Chang et al., 2015), yanggaeng incorporated with orange (Choi & Lee, 2015) and utilization fruit peels in paneer (Singh & Immanuel, 2014). According to Ogundele & Bolade (2021), the higher biochemical constituents (e.g. ascorbic acid, phenolic compounds. etc) in the citrus juice, the higher the free radical inhibition activities. The citrus juice can reduce Fe (III) ion to its lower valency of Fe (II) ion,

preventing the catalysis of formation of reactive oxygen species which act as an anti-oxidation metallic ion.

Figure 6 exhibits the anthocyanin content in butterfly pea ice creams. In contradiction to data DPPH radical scavenging activity and FRAP, the anthocyanin content in the control sample was the highest (288.54 mmol/ml). The difference is mainly due to irreversible degradation of anthocyanin pigments by ascorbic acid in the juices (Enaru et al., 2021), as proven by pH reduction in the ice cream with lime and lemon (Table 2). Anthocyanin has low stability towards relative humidity, light, pH, temperature, sugars, oxygen levels, sulfur dioxide or sulfites, enzymes, co-pigments and metal ions. Moreover, the presence of oxygen in the environment accelerates the degradation of anthocyanin by ascorbic acid, resulting in the formation of polymeric pigment that whitens the anthocyanin pigment (Enaru et al., 2021).

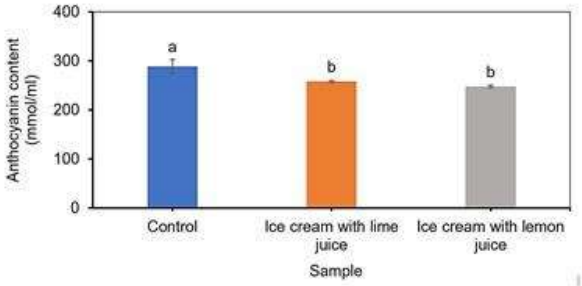

Figure 6. Effect of the addition of citrus juice on the anthocyanin content of butterfly pea ice creams. Different letters above the bars show statistically significant difference ($p<0.05$).

Acceptance of Ice Cream

The results of the consumer's acceptance of butterfly pea ice creams are shown in Table 3. Overall, consumers liked ice cream flavoured with lemon juice the best as it obtained the highest rating for all attributes (colour, aroma, flavour, sweetness, sourness, texture and overall acceptance). All samples received ratings higher than 6 for all attributes, implying that the consumers liked at least slightly the products. The high acceptability of the products by consumers was an interesting result considering that this type of product is not currently available in the market.

For aroma, there was no significant difference between control and ice creams with citrus juices. This means that the untrained panelists preferred both ice creams with or without citrusy aroma. Meanwhile, in terms of flavour, consumers liked ice cream with lemon rather than lime due to its stronger tangy aroma.

Table 3. Mean of hedonic ratings for consumers' acceptance of butterfly pea ice creams.

Attribute	Formula 1 (control)	Formula 2 (lime juice)	Formula 3 (lemon juice)
Colour	6.73±1.66^{b}	7.50±1.34^{a}	7.28±1.38^{ab}
Aroma	7.10±1.69^{a}	6.88±1.36^{a}	7.15±1.33^{a}
Flavour	7.15±1.59^{ab}	6.58±1.71^{b}	7.60±1.48^{a}
Sweetness	7.03±1.73^{ab}	6.78±1.76^{b}	7.60±1.10^{a}
Sourness	6.05±1.84^{b}	6.33±1.85^{b}	7.58±1.26^{a}
Texture	6.38±1.88^{b}	6.93±1.56^{b}	7.70±1.16^{a}
Overall acceptance	6.85±1.56^{b}	6.68±1.53^{b}	7.80±1.14^{a}

Values represent means ± standard deviation; n = 40

All ice creams received the same ratings for sweetness. Both flavored ice cream had the same pH, however, consumers favored ice cream with lemon as it had lesser sourness than ice cream with lime.

CONCLUSION

In conclusion, enrichment of citrus juices (lime and lemon) affected the physicochemical properties, as well as improved the antioxidant activities and sensorial properties of butterfly pea ice creams. A significant increment of DPPH radical scavenging activities and FRAP assay showed that citrus and butterfly pea have synergistic effects as natural antioxidants that could be beneficial for new food development. The purplish colour of ice creams due to low pH of citrus juice made them favoured by consumers. The data of instrumental texture properties supported the sensorial scores, therefore making butterfly pea and citrus juice were good complementary in producing healthy and flavourful ice creams. Manipulating food ingredients, especially those with high in antioxidants is vital in developing nutritious and high quality products that could cater individuals with different nutrition needs and preferences.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors participated in the design of the experiment, performed the experiments, analyzed

the experimental results, reviewed and edited the manuscript. All authors have read and agree to publish the manuscript.

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