Antioxidant and stability of dragon fruit peel colour

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KEYWORDS: Dragon fruit; Pitaya; Hylocereus polyrhizus; antioxidant colour; stable colour.

ABSTRACT: Dragon fruit is considered as a functional fruit with a decorative effect. The peel colour was evaluated on activity and stability. More preferred colour was obtained from water extract with a higher antioxidant activity and phenolic content than the ethanolic one. Chlorogenic acid, gallic acid, and quercetin were determined for the first time. Water extract having red-purple colour was stable at higher pH and temperature. Dragon fruit peel is a potential source of red-purple colour with a moderate antioxidant activity for food and cosmetic decorations. Its ecological origin is meeting an economical perspective and the consumers’ preference for green products.

INTRODUCTION

Dragon fruit (Hylocereus polyrhizus) or Pitaya is emerging in health promotion product in addition to its known application in food having nutritional and decorative effects. In the genus Hylocereus, the fruit pulp has a variety of colour, which is white, red, and purple, including red and yellow peel. The red hue prepared from Hylocereus sp, fruit is being particularly requested in the market (1) because of its health benefits and colour. Dragon fruit is gaining much attention recently because of its micronutrient enrichment regulated by the phenolics that possess antioxidant and antiproliferative activities (2) in addition to its attractive colour. The pulp is popularly consumed in fresh fruit, and the juice is an additive to ice-cream colouring application (3). It exhibits anti-inflammatory and antioxidative properties with a suppression effect on cardiovascular disease including cancer prevention potential (4). Dragon fruit juice is therefore industrially manufactured as a functional drink (5) to serve the consumers’ requirement of a natural supplement promoting good health condition. The fruit is considered as a functional food and therefore accounted as a food product with high economic value (6).

Safely concern against synthetic colour has been increased, leading to a high demand for natural colour (4). An ecological product is particularly required by consumers, as well as the application of such raw material into green cosmetics. To prepare the red colour, which is beneficial for health care and used in food and cosmetic products, from the dragon fruit, stability of colour is needed to be clarified in order to vary the practical application of this natural colour in an industry.

Although there were some studies on the colour patterns of the red dragon fruit, the colour was from the pulp (1), which is the edible part of the fruit that alters food security. Therefore, sustainable application of the nonedible part or fruit residue would conserve the ecological consideration. Furthermore, preparation of colour from the fruit peel has rarely been introduced. Also, antioxidant activity, total phenolic and flavonoid contents, and characterization of phenolics in the peel have never been reported. In addition, the stability evaluation of the prepared colour has never been addressed. Thus, the natural colour prepared from the dragon fruit peel is worthy to be extracted using a practical and feasible method. Antioxidant activity by means of ABTS, DPPH, and FRAP assessments was evaluated with an analysis of polyphenols to reveal the other function of the colour. Biological activity and stability of the prepared colour will enable an economic and sustainable utilization of this agricultural product with the limitation of waste discarded. In addition, high profit of the food industry will be attained.

MATERIALS AND METHODS

Sample collection

Dragon fruit (H. polyrhizus), cultivated in Chiang Rai, Thailand, was collected. The fruit was washed with tap water and wiped to dry. The fresh peel was separated from the ripe fruit before cutting into small pieces (2 mm). The sample was further dried at 50°C in a hot air oven and ground into powder.

Colour extraction

The powdered peel was extracted in several conditions in search of the most suitable one. The powder was separately macerated with n-Hexane, EtOH, and deionized water (DI H2O) at various proportions of the sample and solvent, which are 1:10, 1:50, and 1:100. Each maceration was performed for three different durations, 10, 30, and 60 min, and concentrated to dry. The fresh peel was separated from the ripe fruit before drying under ambient temperature for 16 h in a light protection vessel. Before use, the solution was diluted in EIOH (Merck, Germany) to obtain an absorbance of 0.700 ± 0.200 at 750nm with a microplate reader (ASYS, UV/Vis, UK). The stock solution containing 7 mM of ABTS (Fluka, USA) and 2.450 mM potassium persulfate (Fluka, USA) was incubated under ambient temperature for 16 h in a light protection vessel.

ABTS radical scavenging activity

The activity of the extracts to scavenge 2,2’-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid) (ABTS) radicals or ABTS•+ was determined (9). The stock solution containing 7 mM of ABTS (Fluka, USA) and 2.450 mM potassium persulfate (Fluka, USA) was incubated under ambient temperature for 16 h in a light protection vessel. Before use, the solution was diluted in EIOH (Merck, Germany) to obtain an absorbance of 0.700 ± 0.200 at 750nm with a microplate reader (ASYS, UV/Vis, UK). The samples were mixed with the ABTS solution for 5 min prior to absorbance measurement at 750 nm. The concentration resulting in 50% inhibition (IC50) of ABTS•+ was calculated. The IC50 of a reference compound, ascorbic acid (Fluka,
RESULTS AND DISCUSSION

The colouring application of the dragon fruit comes from the betalain pigments, betacyanins and betaxanthins. Betacyanins include betanins, phyllocactins, and betanidins (6, 12). Betalains in the dragon fruit pulp and peel were reported to be similar (3). Therefore, colour preparation from the peel is theoretically possible for application. In addition, utilization of the nonedible part offering sustainable application of this agricultural product will meet the consumers’ preference on ecological trend. Moreover, higher profit will be feasible for the food industry by transforming the fruit waste into a value-added material. However, none of the peel colour stability has been reported. Different proportion of solvent and extraction time were used to search for an optimized colour extraction condition (7, 8). The sample and solvent ratio of 1:10 was adopted from the pigment recovered in the dragon fruit (4). The most attractive pink colour was obtained from water extraction. The highest yield was obtained at the greatest sample and solvent proportion and at the longest extraction time. Water was found to be the best solvent, followed by ethanol and n-Hexane, respectively (19.31, 2.01, and 0.30%). Antioxidant activity was therefore comparatively assessed in the first and second most preferred colour and yield. ABTS, DPPH, and FRAP assessments were performed in parallel to confirm radical scavenging activity based on a single electron transferring mechanism. The water colour extract was more active than the ethanol one (Table 1), as confirmed by ABTS, DPPH, and FRAP assays. Although its activity was less than that of the positive controls, application of this natural antioxidant is acceptable. The active principle was further measured using the total phenolic content. The better antioxidant activity was consistency with the active content, that is, the water had more phenolics than the ethanol. This finding was in agreement with the previous report claiming dragon fruit peel as a promising source of antioxidant phenolics (2). Thus, the highly preferred pink colour prepared from water was more economically feasible with a better biological activity.

Although betalains in dragon fruit are informatively studied, biologically active phenolics are rarely presented except gallic acid and caffeic acid in dragon fruit seed (13). In addition to caffeic acid and gallic acid, ferulic acid, gallic acid, kojic acid, rosmarinic acid, chlorogenic acid, and quercetin that are widely distributed in plants and known as antioxidants sharing the shikimic biosynthesis pathway were analyzed. This present finding of gallic acid, chlorogenic acid, and quercetin (Table 2) were therefore reported in dragon fruit peel for the first time. However, the content of these phenolics were higher in ethanol extract, which poses a less attractive colour. The preferred red-purple colour of water extract with better antioxidant may be regulated by betalains. Nevertheless, the dragon fruit peel would be applicable as a potential natural source of chlorogenic acid, quercetin, and gallic acid.

Dragon fruit peel water extract, having more attractive colour with better antioxidant activity, was therefore chosen for colour stability evaluation. The colour with a different concentration was prepared in a buffer solution at various pH conditions. A pH higher than 7 was included in this present study because some cosmetic products are prepared at the basic condition. Thus, wider pH stable range would offer several applications of this ecologial colour. The effect of stored temperature was

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**Table 1. Antioxidant activities and total phenolics content of the colour extracts.**

<table>
<thead>
<tr>
<th>Source</th>
<th>ABTS IC50 (μg/mL)</th>
<th>DPPH (μg/mL)</th>
<th>FRAP (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>22.35 ± 1.26</td>
<td>22.35 ± 1.10</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Water</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

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USA), was used for comparison to the IC50 value of the extracts. The measurements were done in triplicate.

**PPH radical scavenging activity**

Antioxidant activity was assayed by means of the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay (9). Samples and standard ascorbic acid were prepared in absolute ETOH [Merck, Germany]. A portion of the test sample was mixed with 6 × 10⁻⁵ M DPPH (Fluka, USA) and reacted for 30 min under ambient conditions in a light protection vessel. The microplate reader was used to monitor the reduction of DPPH⁺ at 517 nm. The ability to scavenge DPPH⁺ (IC₅₀) was compared with the standard. All measurements were done in triplicate.

**Ferric reducing ability of plasma (FRAP)**

The reducing power was examined using a modified FRAP method (10) by preparation of the FRAP reagent in a 10 mM 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) solution (Fluka, USA) in a mixture containing 40 mM HCl, 20 mM FeCl₃, and 0.3 M acetic acid. The sample was incubated at ambient temperature for 1 h. Absorbance was measured using the microplate reader at 750 nm comparing with gallic acid (Fluka, USA). The total phenolic content was compared with the standard curve and expressed as milligrams of gallic acid equivalents per 100 g of crude extract (mg GAE/100 g crude).

**Determination of total phenolic content**

The total phenolic content in the peel extracts was determined as previously described (9). Samples were mixed with Folin-Ciocalteu reagent (Fluka, USA), followed by the addition of 7.5% Na₂CO₃ (Fluka, USA). The solution was incubated at ambient temperature (23°C, 37°C, and 50°C) condition in screw-capped bottles protected from light exposure following 0, 1, 3, 5, and 10 days of storage. Each buffer solution without sample at each pH was prepared in a buffer solution at various pH conditions. A pH 3.6 (Fluka, USA) containing 40 mM HCl, 20 mM FeCl₃, and 0.3 M acetate buffer (pH 3.6 (Fluka, USA)) was used for the calibration curve. The reducing power was determined in triplicate. The reducing power was expressed as an equivalent concentration (EC) similar to that of 1 mM FeSO₄ and ascorbic acid was used as the positive control. The reducing power was determined in triplicate.

**Characterization of phenolics**

Phenolics in dragon fruit peel extracts were characterized using HPLC (Waters 2695, Agilent, USA) with a photodiode array detector (Waters 2996, Agilent, USA) (9). Samples were successively separated on a reversed phase column (Alltech, Prevail C18 5 mm, 250 × 4.6 mm) equipped with a guard column (Alltech, Prevail all-guard cartridge C18 5 mm, 7.5 × 4.6 mm) and eluted with a solvent system consisting of AcCN (A) and 3% acetic acid (B) with the following gradient: 0-3 min 100% B, 3-5 min 85% B, 5-10 min 80% B, 10-15 min 75% B, 15-20 min 70% B, and 20-30 min 50% B at a flow rate of 1 mL/min. All solvents and standards were of HPLC grade. Caffeic acid, ferulic acid, gallic acid, kojic acid, rosmarinic acid, chlorogenic acid, and quercetin that are widely distributed in plants and known as antioxidants sharing the shikimic biosynthesis pathway were analyzed. This present finding of gallic acid, chlorogenic acid, and quercetin (Table 2) were therefore reported in dragon fruit peel for the first time. However, the content of these phenolics were higher in ethanol extract, which poses a less attractive colour. The preferred red-purple colour of water extract with better antioxidant may be regulated by betalains. Nevertheless, the dragon fruit peel would be applicable as a potential natural source of chlorogenic acid, quercetin, and gallic acid.

**Colour stability evaluation**

Stability of colour was evaluated using the CIELAB system (7,8,11). Color parameters, which are L*, a*, and b* including ΔE, were measured using the colorimetric spectrophotometer [UltraScan Vis, HunterLab, USA] at different colour extract concentrations (0.1, 0.4, and 1.0 mg/mL) in Sorensen’s phosphate buffer, pH 3, 5, 7, and 9, stored under various temperature (23°C, 37°C, and 50°C) condition in screw-capped bottles protected from light exposure following 0, 1, 3, 5, and 10 days of storage. Each buffer solution without sample at each temperature condition was used as a reference in the spectrophotometers.
monitored at 23°C, 37°C, and 50°C (7,8). The CIE system was applied in this research because $L^*$, $a^*$, and $b^*$ colour spaces are more convenient than the tristimulus values (11). Lightness in terms of strong, moderate, and pale along a grey scale is recorded by $L^*$ ranging from white (100) to black (0). The colours green (-) and red (+) are shown by $a^*$ coordinate. The $b^*$ is the hue of blue (-) and yellow (+). Changing of colour is determined in terms of a colour difference ($\Delta E$), where $\Delta E = (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2/2$ (14). Colour stability was therefore monitored by $\Delta E$. Those with less than 5 were focused because colour difference will be distinguished at $\Delta E = 5$ (15). In relation to this postulate, the dragon fruit peel colour at all concentration and temperature in buffer pH 9 was stable (Table 3). In addition, the extract concentrated at 0.1 mg/mL remained stable following 50°C storage in buffer, pH 3 and 7, similar to the colour at 37°C and pH 5 that was not changed at the same concentration. This was consistent with the stability of betalains over pH 3–7 (16). However, according to Stark (14) and Gonne!, changing of colour is perceivable by the human eye at $\Delta E$ of 1.5 and 1, respectively. Therefore, the most stable condition of the dragon fruit peel colour would be in buffer pH 9 stored at 50°C and concentrated at 0.4 mg/mL with the minimum $\Delta E$ of 0.09, followed by 1.0 mg/mL ($\Delta E = 0.13$). At this buffer condition, 37°C and 23°C maintained the colour quality at 0.4 mg/mL ($\Delta E = 0.12$) and 1.0 mg/mL ($\Delta E = 0.63$), respectively. In terms of changing the colour that is regulated by three coordinate parameters ($L^*$, $a^*$, and $b^*$), minimal shift of these values will minimize $\Delta E$. These parameters were subjectively evaluated. $\Delta L^*$ was rarely changed at pH 9 especially at 50°C, followed by 37°C and 23°C. Red and purple betalains of dragon fruit colour are mainly from betacyanins, which are 3,4-dihydroxyphenylalanine mainly in glycosides and acylglycosides (18).

Considering the betacyanin structure having basic moieties in the molecules (3), they should be more stable in alkaline media bases on acyl migration effect. The migration is pH dependent under acidic conditions, resulting in unstable betalains (12). Greater stability of betacyanins at higher pH condition is different from betaxanthins that impart the yellow-orange shade of the dragon fruit. Moreover, increasing of betacyanin ratios was exhibited by a reduction of $L^*$ (18). This parameter was decreased in all condition but least changed in buffer pH 9, conferring higher betacyanins exhibiting the red shade and confirming colour stability of the dragon fruit peel consequently. In addition, the red shade ($a^*$) that hardly shifted (Table 3) was prepared in red-purple colour stability. Stability of colour at the best storage condition was further examined. Parameters relevant to betacyanins were analyzed. Colour differences were all less than 1 all throughout the examination period (Table 4), similar to $L^*$ and $a^*$. Interestingly, red-purple colour was stable at high temperature (50°C). High temperature largely degraded the yellow pigments, betaxanthins, as betacyanins were reported stable after heating at 85°C for 1 h (19).

**CONCLUSION**

Dragon fruit peel is a potential source of red-purple colour for food and cosmetic decorations. Its ecological origin is meeting an economical perspective and the consumers’ preference for green products as well. In addition, the moderate antioxidant activity based on phenolics indicates multifunction of this fruit waste application. Furthermore, dragon fruit peel is applicable as an alternative source of natural chlorogenic acid, quercetin, and gallic acid for other health-care application.

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