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Original Research Article

Extraction, biological activities and stability of *Hylocereus polyrhizus* peel extract as a functional food colorant and nutraceutical

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Abstract

Purpose: *To study the biological effects and the stability of H.polyrhizus peel extract as a natural colorant and nutraceutical in foods*
Methods: *Distilled water, 90 % alcohol mixed with citric acid (2 and 4 %) were used for pigment extraction. Betalain content and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity were determined. Cytotoxic effect against human breast adenocarcinoma (MCF-7), human cervical adenocarcinoma (Hela), human colon adenocarcinoma (HT-29), murine leukemia (P388) and human oral cavity carcinoma (KB) were measured with MTT assay. Then, the effectiveness of different carriers for coating the pigment i.e., lactose, starch and maltodextrin were measured. The thermal and pH stabilities of pigments were determined in samples at temperatures of 50, 70 and 90 °C, and at pH values of 2, 7 and 12. Finally, consumer acceptance of cooled jelly containing synthetic dye and betalain pigment was assessed.*
Results: *Distilled water was the best solvent for extraction, producing 59.9 mg/g of betalain, and 42.39 % DPPH radical scavenging activity. The betalain pigment had high antiproliferative effect against P388 cancer, with 77.50 %, cytotoxicity. The best for carriers were 10 % lactose and 10 % matodextrin ($p > 0.05$). The samples were unstable when adjusted pH 2 and 12 at all temperatures. Cooled jelly containing 1 % artificial dye had the highest sensory score, followed by 2.5 % natural colorant.*
Conclusion: *H. polyrhizus pigment has promising potential for application in food products as a functional food colorant and nutraceutical. However, further studies are required the shelf life of pigment.*

Keywords: *Hylocereus polyrhizus, Betalain, Utilized waste, Biological activities, Stability, Natural food colorant, Nutraceutical*

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INTRODUCTION

One of the causes of increasing illness in people is the type of product that they consume. Nowadays, food preservatives and synthetic food additives are popularly used to prolong the shelf life and improve the appearance of food products. However, the use of these additives is

associated with problems concerning their safety and toxicity. Therefore, there is increasing demand for nutraceutical and medicinal products containing natural food antioxidants, based on increasing awareness of the harmful effects of synthetic food additives. In recent years, consumers have focused on processed foods rich in nutritional and functional properties, and free from synthetic food additives.

Dragon fruit (*Hylocereus* spp.) is a good source of nutrients, such as high dietary fibers, vitamins, minerals and its role as an antioxidants. The appearance is unique such as reddish flesh, sweet taste, and crunchy texture. Several studies have shown that dragon fruit contains the betacyanins and betaxanthins act as antioxidants. These substances are potent antioxidants which help in radical scavenging that cause cell damage.[1]. In addition, the antioxidant properties of dragon fruit are helpful in preventing inflammation-based diseases such as gout and other forms of arthritis [2]. Extracts from the fruit of the *H. polyrhizus* exerted anti-proliferative effect on melanoma cells, with the peel extract producing a higher effect than the flesh extract due to higher content of flavonoids and betalains [3]. Nutraceuticals are food substances or parts of food that provide health or medicinal benefits, including the treatment and prevention of diseases. Therefore, food industries endeavor to incorporate nutraceuticals into their food products. In Thailand, the two species of dragon fruit consumed are *H. polyrhizus* and *H. undatus*. The processing of dragon fruits produces numerous waste products from the peel. Typically, the waste from the peel constitutes about 50 % of the weight of the dragon fruit. Betalain is the reddish pigment in *H. polyrhizus* which is a potential source of natural food additive that can be used to fortify foods. Natural pigments are known to be safe, cheap and healthy. However, they are very unstable when subjected to changes in pH, temperature, light, oxygen and storage conditions. Thus, this research focused on the antioxidant and cytotoxic effects, and stability of natural pigments from *H. polyrhizus* peel extract, and also the likelihood of consumer acceptance if it is used as substitute for synthetic colorants and as enriched-antioxidants for industrial food applications.

EXPERIMENTAL

Plant materials and reagents

Fresh *H. polyrhizus* was purchased between October and December 2019, from a local market near Rajabhat University, Chiang Rai Province, Thailand. The samples were kept at 4 ^{o}C before use. The reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Aldrich (USA), Rosewell Park Memorial Institute 1640 medium (RPMI). Fetal bovine serum (FBS), trypsin and MTT reagent 3-(4,5 - dimethylthiazol-2-yl) - 2,5-diphenyltetrazolium bromide were bought from Gibco (USA).

Pigment extraction

Fresh *H. polyrhizus* was washed and peeled, and the peels were cut into small sizes of about 3 mm and dried in a tray drier at 50 ^{o}C for about 24 h. Thereafter, the dried samples were finely ground using blender, and extracted separately with 6 different solvents to obtain pigment extracts. The solvents used were distilled water, 2 % citric acid, 4 % citric acid, 90 % alcohol, 90 % alcohol containing 2 % citric acid, and 90 % alcohol containing 4 % citric acid. The dried samples were extracted with each solvent at the ratio 1:50 (2 g of dried sample and 100 g of solvent). Each mixture was shaken at about 300 rpm for 24 h at room temperature. The supernatant was filtered and the solvent was removed by rotary evaporator at 40 ^{o}C, followed by freeze-drying. A solution of each extract was prepared at a concentration of 2,000 µg/mL and kept at -20 ^{o}C prior to analysis. Betalain content, DPPH radical scavenging and cytotoxicity were measured spectrophotometrically.

Determination of betalain content

A solution of the pigment was prepared. The samples were measured at 525 nm in a visible spectrophotometer. Total betalain content (B) was calculated as shown in Eq 1 [4].

$$B\ (mg/g) = (A \times DF \times MW \times 1000)/\varepsilon L \quad \ldots\ldots\ldots\ldots (1)$$

where A is absorbance of the samples; DF is dilution factor; MW is molecular weight of betalain at 550 g/mol; ε is the molar extinction coefficient (60,000 L/mol) and L is width of cuvette (1 cm).

Antioxidant capacity

The DPPH radical scavenging assay was performed as described by Brand-Williams *et al* [5]. 1 ml of extract was mixed with 3 ml of 0.2 mM DPPH in a tube in the dark place at room temperature for 30 min. The blank used was a mixture of 1 ml of ethanol and 3 ml of DPPH. The absorbance was measured at 517 nm. The percentage inhibition of DPPH radical by each sample was calculated as shown in Eq 2.

DPPH radical scavenging (%) = {(Ac - As)/Ac)100 .....(2)

where Ac, and As are the absorbance of control and treated sample, respectively

Cell culture

Five cell lines were obtained from the National Cancer Institute of Thailand. The cells were grown in RPMI1640 containing 8 % of fetal bovine serum (FBS) and gentamycin (50 µg/mL). The cells were grown at 37 °C under 5 % CO_2.

MTT proliferation assay

Cell viability was determined with MTT assay as described by Mosmann (1983) [6], with a slight modification. Human breast adenocarcinoma (MCF-7), human cervical adenocarcinoma (Hela), human colon adenocarcinoma (HT-29), murine leukemia (P388) and human oral cavity carcinoma (KB) were seeded separately in 96-well plates, each at a density of 1x 10^5 cells/well, and were incubated at 37°C in a 5 % CO_2 incubator for 24 h. The peel extracts were added in each well at final concentration 4000 µg/mL. Cells treated with 1 % DMSO used as a negative control. Untreated cell cultures (control) and blank wells without cells containing 100 µL of medium were incubated for 20 h. Thereafter 10 µL of MTT reagent (5 mg/ml) was added to each well and incubated for 4 h, after which 100 µL of a mixture of 100 % DMSO and 10 % sodium dodecyl sulfate (SDS; volume ratio, 9:1)) was added to the wells, followed by shaking to solubilize the formazan crystal formed. Absorbance was measured at 570 nm. Surviving cell was calculated as a percentage as an index of cytotoxicity. The percentage of cytotoxicity was determined as shown in Eq 3.

Cell cytotoxicity (%) = 100 - (As – Ab)/(Ac -Ab)100 …….. (3)

where As, Ab and Ac are the absorbance of treated sample, blank and control samples, respectively.

Adsorption of concentrated pigments onto carriers

Solutions (0.5 %) of the dried pigments were prepared by dissolving 0.5 g in water and making up the volume to 100. They were adsorbed to solid matrices (lactose, starch, and maltodextrin) at different levels (5 and 10%), and the mixtures were freeze-dried. The water activity was determined using a water activity meter (Dew Point Water Activity Meter 4TE), and the yield (%) was calculated as the ratio of output weight to input weight, expressed as a percentage.

Evaluation of heat and pH stability

The effects of pH and heat on the stability of betalain samples extracted from *H. polyrhizus* were measured. Dried pigment samples were dissolved in phosphate buffer and adjusted to pH values of 2, 7 and 12 using 6M HCl and 1M NaOH. The mixtures were heated to temperatures of 50, 70 and 90 °C. Betalain content and color (CIE) were measured. The CIE color coordinates L*(light/dark), a*(+redness to -greenness) and b*(+yellowness to -blueness) were determined using a colorimeter (Reflection Spectrometer Avantes).

Sensory evaluation

To 100 g of cooled jelly containing 2 % agar, 0.4 % stevia extract and 1 % artificial food additive was added 2.5 or 5 % of natural pigment, followed by organoleptic evaluation. Thirty untrained panelists assessed the acceptability of the jelly samples on a 9-point hedonic scale, with 1 as ‘most unliked’ and 9 as ‘liked most’. The 5 characteristics assessed were color, odor, texture, flavor and overall acceptance.

Statistical analysis

All analyses were done in duplicate, and the results are reported as mean ± SD. A completely randomized experimental design was used. Analysis of variance (ANOVA) was used for statistical analysis with IBM SPSS software, version 24 (IBM Singapore Pte. Ltd., Changi, Singapore). Values of $p < 0.05$ indicated significant differences.

RESULTS

Betalain yield and DPPH radical scavenging activity

The betalain contents and DPPH radical scavenging are presented on Table 1. It was found that solvents containing citric acid decreased betalain content. Increasing citric acid concentration from 2 to 4 % decreased the betalain yield from 11.4 to 6.1 mg/g. In contrast, a sample extracted with distilled water had the highest betalain yield of 59.9 mg/g. Different solvent extracts showed different antioxidant effects. The DPPH radical scavenging effect varied from 6.35 to 44.59 %. The highest DPPH radical scavenging activity was found in extracts from four solvents: 90 % ethanol and 90 % ethanol with 2 and 4 % citric acid, and distilled

Table 1: Betalain contents and DPPH radical scavenging activity of samples with different solvent extraction

Solvent	Betalain (mg/g)	DPPH radical scavenging (%)
Distilled water	59.9 ± 1.16[a]	42.39 ± 11.5[a]
Distilled water + 2 % citric acid	11.4 ± 0.01[b]	18.96 ± 1.18[b]
Distilled water + 4 % citric acid	6.1 ± 0.02[c]	6.35 ± 10.5[c]
90 % ethanol	1.7 ± 0.00[d]	44.59 ± 4.12[a]
90 % ethanol + 2 % citric acid	1.9 ± 0.02[d]	41.85 ± 0.86[a]
90 % ethanol + 4 % citric acid	1.5 ± 0.01[d]	38.81 ± 2.09[a]

Different letters within the same column indicate significant differences ($p \leq 0.05$)

water**.** These results indicated that the reddish pigment in *H. polyrhizus* were soluble in distilled water as well, while other phytochemicals involved in DPPH radical scavenging were soluble in ethanol containing citric acid.

Cytotoxic effects

The *H. polyrhizus* peel extract exhibited different antiproliferative effects against the 5 cancer cell lines, as revealed in MTT assay (Table 2). The peel extract showed highest cytotoxicity against P388, followed by Hela, KB, MCF-7 and HT-29, with percentage cytotoxicity values of 77.50, 41.52, 21.94, 21.20 and 19.24 %, respectively. After treatment of the cells with the extracts, the toxicity produced resulted in smaller amount of formazan crystals when compared with control (Figure 1). These results indicate that the peel extract was able to inhibit cell proliferation at concentration of 4000 µg/mL.

Table 2: Cytotoxic effect of distilled water extract of *H. polyrhizus* peel (4,000 µg/mL) on cancer cell lines

Cell lines	Cytotoxic activity (%)
MCF-7	21.20 ± 3.50[c]
Hela	41.52 ± 3.18 [b]
KB	21.94 ± 4.51[c]
HT-29	19.24 ± 4.12[c]
P388	77.50 ± 2.02 [a]

Different letters within the same column indicate significant differences ($p \leq 0.05$)

Adsorption of concentrated pigments on carriers

This study was carried out to select a suitable carrier for coating the pigment prior to use. The data obtained in studies on the adsorption of betalain extracted from *H. polyrhizus* peel as a natural food are presented on Table 3. These results indicate that lactose and maltodextrin gave higher yield than starch, and the lower free water. At 10 % adsorbent level, the highest betalain concentrations were 9.30±0.35 and 8.83 %. The control (without carrier) had the lowest yield, whereas all carriers improved the bulk density of the extract, leading to a higher yield of pigment. Moreover, the increased amounts of pigment resulted in lower free water.

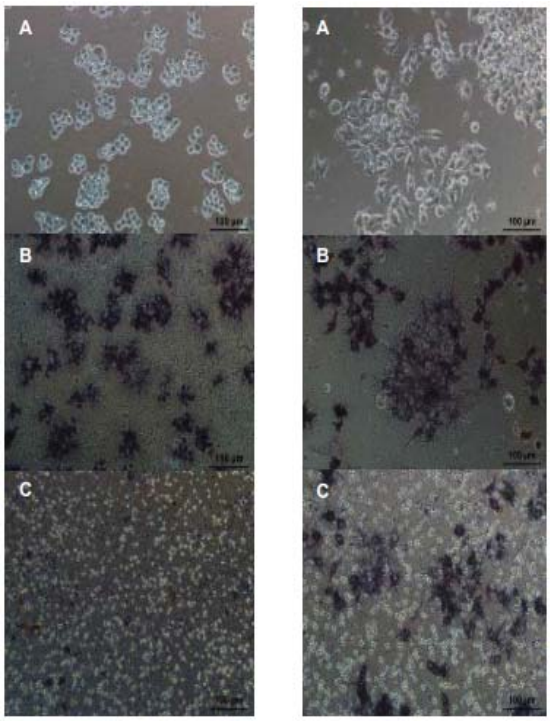

Figure 1: Photomicrographs (x200) of *H. polyrhizus* peel extract-treated P388 (left) and Hela cells (right). Cells before addition of MTT reagent (A); cells without treatment (B), and cells treated with extract at a dose of 4,000 µg/mL (C)

A comparison showed that 10 % maltodextrin yielded 8.83±0.39 % betalain, while starch carrier yielded 8.07 % betalain. In addition, samples encapsulated with lactose had more homogeneous texture than those encapsulated with maltodextrin. Lactose gave a color closest to the control sample (pigment without carrier) which had dark pink color (Figure 2).

Table 3: Yield and water activity of samples with different carriers

Carrier	Yield (%)	Water activity (a_w)
Control (without carrier)	1.06± 0.04^{e}	0.62 ± 0.03 ^{a}
5 % Lactose	5.02 ± 0.02^{c}	0.24 ± 0.01^{b}
5 % Starch	4.09 ± 0.32^{d}	0.58 ± 0.04^{a}
5 % Maltodextrin	4.73 ± 0.19^{cd}	0.17 ± 0.01^{c}
10 % Lactose	9.30± 0.35^{a}	0.06 ± 0.00^{d}
10 % Starch	8.07 ± 0.34^{b}	0.17 ± 0.03^{c}
10 % Maltodextrin	8.83 ± 0.39^{a}	0.06± 0.01^{d}

Different letters within the same column indicate significant differences ($p \leq 0.05$)

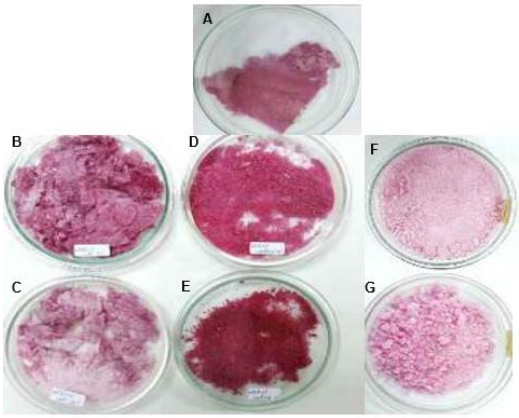

Figure 2: Betalain samples extracted from *H. polyrhizus* peel with different carriers after freeze drying. A (without carrier); B, C (5 and 10% starch); D, E (5 and 10% lactose); F, G (5 and 10 % maltodextrin), respectively

Heat and pH stability

The stability values of betalain extracted from *H. polyrhizus* peel at pH values of 2, 7, 12 and 5.1 (control pH) when heated to temperatures of 50, 70 and 90 ºC are shown in Figure 3A. The results showed that increases in pH increased the stability of betalain (64.02 %) up to pH 7.0. The stability of betalain was increased from 72.42 mg/g at pH 2.0 to 113.10 mg/g at pH 7.0, whereas it was decreased to 23.81 mg/g at pH 12. These results indicate that betalain is not suitable for use in high alkaline foods, since the high alkalinity may degrade its structure.

The color of extracted betalain varied with pH and temperature, as shown in Figure 3B,C,D). (The color stability of betalain pigment at a given pH depends on the structure of betalain. Under neutral or slightly acidic condition, the betalain pigment exists predominantly in the reddish color, while at highly alkaline pH, the pigment is unstable and colorless. The results showed that pH had a great effect on the stability of betalain pigment. The lightness (L*) was increased at pH 12, whereas pH 2 and 7 had no effect. Redness (a*) value increased at pH 2, while at pH 12, the color of the extract was changed from red to green. Yellowness (b*) value of the extract increased at highly acidic and highly alkaline pH. However, temperature had very little effect on color change.

Temperature was a major factor that destabilized the betalain structure: an increase in temperature resulted in greater yellowness in betalain pigment. Except at pH 7, temperature did not affect betalain structure. The results demonstrated that the pigment was more stable at neutral pH (7), while it had low stability at highly alkaline conditions (pH 12). From these results, it is very clear that the use of betalain extract as natural food colorant in neutral and light acidic products such as ice cream, bakery, drink, milk and jelly, is feasible.

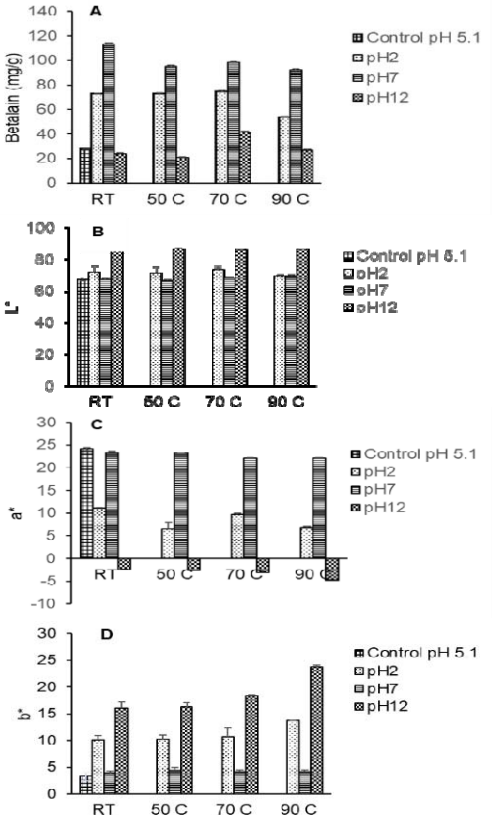

Figure 3: Effect of pH and temperature on betalain contents (A), and color stability L* (B), a*(C) and b* (D) respectively

Sensory properties

The sensory properties of jelly containing 1 % synthetic and natural pigment (2.5 and 5 %) were evaluated by 30 panelists. The results are shown in Table 4. The cooled jelly containing synthetic

Table 4: Sensory scores of cooled jelly samples containing synthetic or natural pigments

Sample	Color	Odor	Texture	Flavor	Overall liking
1 % Synthetic color	7.10 ±1.93[a]	7.17±1.70[a]	7.33±1.39[a]	6.93±2.05[a]	7.60±1.24[a]
2.5 % Betalain pigment	6.97±2.12[b]	6.80±1.62[b]	6.87±1.75[b]	5.57±1.94[b]	6.30±1.61[b]
5 % Betalain pigment	7.47±1.54[a]	5.10±2.07[c]	5.00±2.22[c]	4.77±2.11[c]	5.37±2.09[b]

colorant had bright pink color, while jelly containing 2.5 and 5 % natural pigment showed the deep pink and dull red colors, respectively (Figure 4.). The highest overall liked color was found in the jelly containing 1 % synthetic dye, whereas there was significant difference between jelly containing 2.5 and 5 % natural pigment ($p > 0.05$). The results showed slight and insignificant color scores of jelly samples containing 1 % synthetic and natural pigment (2.5 and 5 %), with values of 7.10, 6.97 and 7.47, respectively.

Most panelists preferred the color of betalain pigment, but it decreased the odor, texture, and flavor of the jelly. However, cooled jelly with 2.5 % betalain pigment gave higher scores in odor, texture and flavor than cooled jelly containing 5 % betalain pigment. Most of the panelists reported that too much coloring addition gave rise to a soft texture and a little smell. Therefore, 2.5 % betalain pigment was suitable and could be used as a substitute for synthetic color.

Figure 4: Cooled jelly incorporated with synthetic colorant (left) and 0.25 % red dragon peel extract (middle) or 0.5 % red dragon peel (right)

DISCUSSION

Betalain pigment can be solubilized in distilled water and alcoholic solvent due to its structure which is similar to that of anthocyanin, both of which are hydrophilic natural coloring substances [7]. The present study found that distilled water extracted the betalains pigment better than alcoholic solvent. On the other hand, alcoholic solvent gave an extract with higher DPPH radical scavenging effect than water. This may be due to the fact that water extracts only polar substances, while alcohol extracts both polar and non-polar bioactive substances, resulting in an extract with a higher inhibition of DPPH radical.

Dragon fruit is also rich in flavonoids. Several studies have been reported organic solvents such as methanol and ethanol are more effectively for their extraction than polar solvents due to the polarity of flavonoids. [8]. Therefore, flavonoids contained in *H. polyrhizus* may help to increase the inhibition of free radicals. Increasing the efficiency of extraction can be also achieved by changing the solvent pH and ionic strength, both of which affect the solubility of the bioactive compounds and their interactions with the plant matrix.

Mai *et al* [9] studied investigation of the effect of solvent pH on the reconstruction of flavonoids, and found that reconstructions increased in acidic solvent at pH 2.5 - 3.5 and decreased at alkaline pH. It was similar results with Motikar *et al* [10] studied influence of pH on polyphenols. It was found that pomegranate peel extraction was affected by solvent pH, with the best results showed in acidic pH [10]. In addition, at a lower pH, it supports cleavage of phenolics bonds between proteins and carbohydrate structure, leading to the readily penetrate the micelles. [11,12]. This is at variance with the results obtained in the present study which showed that adjusting pH with citric acid had no effect on betalain extraction. Moreover, increasing the amount of citric acid actually decreased the betalain content and decreased the DPPH free radical scavenging potential of the extract. It may be possible that the amount of citric added was not enough to produce acidic pH in the solvent.

The *H. polyrhizus* peel extract showed the highest antiproliferative activity against P388 followed by Hela cancer cells. Although the extract ratio is high (1:50) but the extract still had a good effect in inhibiting cancer cells. The principle of MTT assay for checking viability of cell lines is that viable cells transform the yellow colored MTT to a purple-colored formazan crystals in the mitochondria.

As a result, cells treated with extract showed smaller formazan crystal than control. These phenomena may be due to phytochemicals contained in *H. polyrhizus* peel extract, which were polyphenols, flavonoids and betalains. At high concentration of the extract (4,000 µg/mL), these compounds altered structures of the cell lines, resulting in cell death.

Putthawan *et al* [13] reported that as the concentration of extract increased to 4,000 µg/mL, loss of cell adhesion, reduced cell density, and membrane blebbing occurred. The results of this study demonstrate that the peel of *H. polyrhizus* is a source of antioxidants that prevent disease and have potential applications in the pharmaceutical and food industries.

Carriers serve as shells that encapsulate the extract for increased yield, reduced free water content, and prevention of degradation. Lactose and maltodextrin at 10 % adsorbent levels had the highest betalain concentrations, followed by starch. A similar result was obtained by Shukla *et al* [14] who found that lactose was the best of several tested carriers, followed by starch. It was noticed that the smaller the size of carrier, the better the penetration of the pigment. Considering a_w, an increase in the amount of carrier decreased the moisture content. Maltodextrin and lactose at 10 % had the highest capacity to reduce the amount of free water, while starch had the lowest capacity. Due to the fact that starch had a larger particle size than any other carrier, as well as the lowest solubility, it had low extract penetration. The carries help to reduce the free water by replacing the porosity due to evaporated water.

Results from the effect of pH suggest that betalain is not suitable for use in highly alkaline foods because high alkalinity may degrade its structure, while slight acidic and neutral pH sustain its color. Betacyanin pigment refers to the extraction product of betalains [15]. The red pigment is stable in acidic condition within the range of pH from 4 to 7 at which the pigment retains its red color**.** According to Shukla *et al* [16], pH value affected the stability of carotenoids extracted from sour orange and grape fruit peels: increases in pH resulted in decreases in carotenoid pigment, while neutral pH (7) led to the highest retention of carotenoids. Jackman and Smith [17] reported that variations in pH from 3.5 to 7 did not induce changes in betalain color.

The sensory scores of cooled jelly revealed that the one most preferred by consumers was cooled jelly containing the natural pigment. However, the synthetic dye had the most score for overall acceptance because most consumers liked its flavor and texture more than those of the natural pigment. Most synthetic food additives do not affect the smell and taste of food. In contrast, the texture and smell of cooled jelly were affected by the added betalain pigment due to the acidic effect of betalain on food components, which made the texture too soft. The smell might have resulted from the aroma of the raw natural plant.

However, blanching may help reduce the smell of plants, but too prolonged blanching may affect the structural stability of phytochemicals. Manihuruk *et al* [18] found that adding natural dye reduced the texture score of beef sausage, which is similar to the results of this study. Peel extract at high concentration up to 40 % was ineffective in increasing the reddish intensity of beef sausages, but rather the color was changed to yellow, indicating that excessive addition of natural pigment affects the texture and color of food. Therefore, the use of natural betalain pigment should be controlled.

CONCLUSION

Red dragon peel extract provides phytochemicals with a powerful antioxidant potential capable of preventing cancer, especially murine leukemia cancer. Therefore, it can potentially be used as a source of food colorant and betalain-enriched extracts in functional foods and pharmaceuticals. However, the amount used is limited by pH, thermal processing and other ingredients of foods. Future studies are necessary to prolong the stability of the pigment and its shelf life.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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