**Plant pigments obtained from ultrasound−assisted extraction:**

**color properties and antioxidant activities during *in vitro* digestion**

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**Abstract**

Herbal plants are potential sources of natural pigments, providing distinctive colors and exert antioxidant effects that are far more superior than synthetic colorants. The highly efficient extraction   
of natural colorants supports their use as substitutes for synthetic dyes in industrial applications.   
In this study, four major types of plant pigment including anthocyanins from butterfly pea flower (*Clitoria ternatea* L.), betalains from dragon fruit peel (*Hylocereus undatus*), curcuminoids from turmeric rhizome (*Curcuma longa*) and chlorophylls from pandan leaf (*Pandanus amaryllifolius*) were extracted with water and 50% (v/v) aqueous ethanol, compared with 70% (v/v) acidic acetone. Results showed that water was the most effective solvent for extraction of betalains and anthocyanins whereas, 50% (v/v) aqueous ethanolwas suggested for extraction of curcuminoids and chlorophylls since it gave the higher concentration of the required pigment and the most intense color with higher antioxidant properties. In order to enhance extraction efficiency, ultrasound−assisted extraction (UAE, 38.5 kHz) was conducted at 25 and 65°C for 1 h, compared to maceration under agitation at 25°C for 24 h. Among extraction conditions, UAE apparently increased extraction efficiency compared to maceration with agitation. UAE at 25°C gave the highest concentration of the required pigments, total phenol content and associated antioxidant activities based on FRAP and DPPH. However, temperature during UAE at 65oC could also stimulate or inactivate antioxidant capacities of samples. To assess their stability and bioaccessibility, the four pigment solutions obtained from UAE at 25°C were compared. Upon *in vitro* gastrointestinal digestion, all pigment compounds, total phenolics and their antioxidant activities increased on gastric digestion (126.87−260.44% recovery) but, decreased during intestinal digestion (47.44−80.70% recovery). Their respective antioxidant capacities were also recovered 106.66−141.33% during gastric digestion and recovered 54.30−89.77% during intestinal digestion. Results revealed the low stability of pigment compounds and their associated antioxidant activities after gastrointestinal digestion.

**Keywords:** Anthocyanins, Betalains, Curcuminoids, Chlorophylls, Antioxidant, Ultrasound−assisted extraction, *In vitro* digestion

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**1. Introduction**

Color of food products plays a significant role to attract the consumer and also gives information on freshness, safety and sensory characteristics (Ngamwonglumlert *et al.,* 2015). However, natural colorants are now gaining popularity and considerable significance due to consumer awareness since synthetic dyes cause severe health problems (Amchova *et al.,* 2015). Plant colorant is pigment or any other substance obtained from plant sources such as fruits, vegetables, flower, root and seed, providing distinctive color shades. The common pigments that have been extracted and used as additive in foods and beverages are anthocyanins (red−blue color), betalains (pink−red color), carotenoids (orange−yellow color)   
and chlorophylls (green color) (Lauro and Francis, 2018; Delgado-Vargas *et al.,* 2000).   
These natural colors are phytochemicals produced from plant that pose health−related properties such as antioxidant, antiproliferative, cardioprotective and antiinflamatory effects (Sigurdson *et al.,* 2017; Rodriguez-Amaya, 2018).

Conventional solid-liquid procedures, like maceration is commonly used to extract pigments from plant sources (Subhash *et al.,* 2015). Water, as universal solvent, is the most common type of solvent used in the extraction process since it dissolves water soluble pigments, including anthocyanins and betalains (Azeredo, 2009; Boo *et al.,* 2012). However, for the fat soluble pigments such as chlorophylls and carotenoids existing in plastid of plant protoplasm (protoplasts), organic solvent and their aqueous mixtures are recommended since they could disrupt cell walls and dissolve the required pigments efficiently (Delgado−Vargas *et al.,* 2000; Sigurdson *et al.,* 2017). Factors that may have impact on plant extraction include extraction time, temperature, solid:liquid ratio, assistance with microwave and sonication (Boo *et al.,* 2012; Ngamwonglumlert *et al.,* 2015). Conventional maceration is extensively used for plant pigment extraction but it poses some disadvantages such as long exposure time and less yield. To overcome these drawbacks, the assistances of agitation or other mechanical methods have been applied.

Currently, ultrasound assisted extraction (UAE) of the plant pigments is used as it is an effective extraction method, providing high extraction yield with less extraction time (Laqui−Vilca *et al.,* 2018; Maran *et al.,* 2015). UAE is a process that uses acoustic energy transmitted through a solvent medium via pressure waves, inducing vibrational motion of the molecules which can damage the plant cell walls on bursting and make the solvent easily permeate into plant material to extract the desired compounds (Subhash *et al.,* 2015).   
A various sonication studies with varying extraction parameters including exposure time, temperature, power, and solvent to solid ratio were reported. Earlier studies have been reported on the use of ultrasound ranging from 20 to 2,000 kHz with the short extraction time of 15−45 min in increasing extraction efficiency of single plants pigment (Maran *et al.,* 2015; Prakash *et al.,* 2013; Sharmila *et al.,* 2019). However, the comparative studies on effect of UAE focusing on antioxidant capacities among different types of pigmented plants were rare.

*In-vitro* methods simulating human digestion processes, typically including the oral, gastric and small intestinal phases, are widely used to study the gastrointestinal behavior of food or pharmaceuticals (Minekus *et al.,* 2014). However, little is known about the change of pigment compound during digestion process and the effect of physiological conditions occurring *in-vivo* on stability of these compounds.

To date, no comparative data are available on how the plant pigments are extracted with different solvents and extraction methods. The comparative studies on the stability among plant colorants and antioxidant properties during *in−vitro* digestion are relatively few. This study therefore, evaluated the effect of food applicable solvents (water and aqueous ethanol)   
and common solvent (acidic acetone) on four major plant pigment compounds: anthocyanins, betalains, curcuminoids and chlorophylls under conventional process and UAE.   
The appropriate extraction solvents and methods for each plant pigment are suggested.   
The following study is the analysis of pigment compounds and their associated antioxidant capacities that result from physiological condition upon *in−vitro* gastrointestinal digestion.   
The stability and bioaccessibility of the four pigment compounds are assessed.

**2. Materials and Methods**

**2.1 Plant materials and chemicals**

Fresh butterfly pea flower (*Clitoria ternatea*), white dragon fruit (*Hylocereus undatus*), turmeric rhizome (*Curcuma Longa*) and pandan leaf (*Pandanus amaryllifolius*) were obtained from local farm in Chiang Rai Province, Thailand. For white dragon fruits, only peel of fruit was used. All samples were pre−sorted, washed and cut into small pieces before drying in hot air oven (Memmert, D−91107 Schwabch) at 60°C to obtain final moisture content less than 14% w.b. Dried samples were ground into fine powder and kept in sealed plastic bag at 4oC until use. All chemicals were analytical grades and were obtained from Sigma (St. Louis, MO, USA). Enzymes were obtained from Megazyme (Bray, Ireland) and Sigma (St. Louis, MO, USA).

**2.2 Extraction and preparation of plant pigment powder**

To compare extraction efficiency among solvents, two edible solvents (water and 50% (v/v) aqueous ethanol) was compared with 70% (v/v) acidic acetone. The calculated weight of 2 g dried herbal plants was obtained by deducting the moisture content of each sample which were 13.75, 13.25, 9.63, and 6.57% w.b. for butterfly pea flowers, turmeric rhizome, dragon fruit peels and pandan leaves, respectively. The weighed plant samples were mixed with 200ml of solvent in a flask. The flask was tightly covered with aluminum foil and put in shaker (Incubator shaker, IKA, KS40001, Germany) to perform maceration under agitation (MA) at 25°C for 24 h. In order to enhance extraction efficiency, based on conditions in earlier studies of ultrasound−assisted extraction (UAE), ultrasound at 38.5 kHz was performed for 1 h at 25 and 65°C in sonication bath (Ultrasonic cleaner, Crest, 690 DAE, Malaysia)., the use of ultrasound of 35 kHz ranging from 20 to 2,000 kHz with the short extraction time of 15−45 min in increasing extraction efficiency Each extract was filtered through Whatman No. 4 paper filter and subsequently, the filtrate was evaporated in a rotary evaporator (IKA, RV 10 B, Germany) at 40◦C under vacuum. Finally the remaining extracts were subjected to freeze drying (Christ, Delta 2−24 LSC plus, Germany). The obtained dried pigment was stored at −18°C for further analysis.

**2.3 Color parameters**

The colors of the samples were measured three replicates using a colorimeter that was calibrated with a standard white plate (Hunter Lab, ColorQuest XE). Liquid samples were put in a clear quartz sample holder with 1cm cell path. The quartz provides full transmittance and covers visible spectrum range (400-800nm) used for color measurement. The color was expressed in L\* a\* b\*, where the L\* represents lightness (L\* = 0 yields black and L\* = 100 denotes white), a\* expresses red (+) or green (−), and b\* indicates yellow (+) or blue (−). The hue angle was calculated following Eq (1).

Hue angle (degrees on 360°) = tan^ (−1)〖(b\*)/(a\*)〗 (1)

**2.4 Plant pigment and bioactive compounds**

**2.4.1 Total phenolic content (TPC)**

TPC was determined in all plant pigment powder samples using the Folin−Ciocalteu method. The extract solution (500 µL) was mixed with 2.5 mL of 10% (w/v) Folin−Ciocalteu reagent and 2 mL of 7.5% (w/v) sodium carbonate, mixed well and incubated in for 1 h at 25°C. The absorbance was measured spectrophotometrically at 765 nm and expressed as mg Gallic acid equivalents (GAE) per 100 g of dry samples.

**2.4.2 Total anthocyanins content (TAC)**

TAC in butterfly pea flower pigment powder was measured according to the pH differential absorbance method (Jiang *et al.,* 2019). Briefly, an aliquot (0.3 mL) of anthocyanin sample was mixed with pH 1.0 (potassium chloride buffer, 9.7 mL) and pH 4.5 (sodium acetate buffer, 9.7 mL) solutions, respectively, and equilibrated for 30 min at room temperature in the dark. A microplate spectrophotometer (Thermo Fisher scienfic, Multiskan GO, USA) was used to measure the absorbance at 525 nm and 700 nm, using water as reference.   
The total anthocyanin content was calculated as mg cyanidin−3−glucoside equivalent (mgCyE) per 100 g dry sample.

**2.4.3 Total betalains content (TBC)**

TBC was determined following method of Bucur et al. (2016). Dragon fruit peel pigment powder was extracted with distilled water and subsequently centrifuged at 3000 x g for 10 min. The supernatant was collected and determined spectrophotometrically at 536 nm for betacyanins (BC) and at 486 nm for betaxanthins (BX). The total betalains content was calculated as sum of BC and BX and expressed as mg total betalains content (mg TBC) per 100 g dry sample.

**2.4.4 Total curcuminoids content (TCC)**

TCC was measured referring to method of Martins et al. (2013). Tumeric pigment powder was soaked in 95% (v/v) hexane in ether and subsequently centrifuged at 3000 x g for 10 min. The supernatant was collected and determined spectrophotometrically at 454 nm using a standard curve from analytical−grade curcumin ranging from 1.0 to 8.0 µg/mL. The total curcuminoids content was expressed as mg TCC per 100 g dry sample

**2.4.5 Total chlorophylls content (TCPC)**

Pandan leaf pigment powder was soaked in 80% (v/v) acetone and subsequently filtered through centrifuged at 3000 x g for 10 min. The supernatant was collected and the absorbance was measured at 652 nm by spectrophotometry. The total chlorophylls content was expressed as mg chlorophyll per 100 g dry sample (Witham *et al.,* 1971).

**2.5 Antioxidant activities**

**2.5.1 Ferric reducing antioxidant power assay (FRAP)**

Ferric reducing antioxidant power was assayed using ferric sulfate as standard   
(Benzie & Strain, 1999). The FRAP reagent was prepared freshly by mixing 300 mM acetate buffer (pH 3.6), 40 mM HCl, 10 mM 2,4,6 tripyridyl−s−triazine (TPTZ) and 20 mM FeCl3 at the ratio of 10:1:1 respectively. The extract (400µl) was then mixed with 2.6ml FRAP reagent solution, incubated at 37°C for 30min and absorbance was measured at 595nm against blank   
(Thermo Fisher scienfic, Multiskan GO, USA). FRAP value was calculated as mmole FeSO4 per 100g dry sample.

**2.5.2 DPPH radical scavenging method (DPPH)**

DPPH free radical scavenging activity was determined using Trolox as standard (Molyneux, 2004). Sample extract (50 µL) was mixed with 1950 µL freshly prepared DPPH 60mM solution. The mixture was stored in dark place for 30 min and absorbance was measured at 517 nm against blank. The results were expressed as the equivalent content of Trolox (µmole TE/100 g dry sample).

**2.6 Simulated gastrointestinal digestion**

Simulated GI digestion is performed according to the method described by Tamura   
et al. (2016) with some modifications. This simulated digestion procedure has been primarily used for study the starch digestibility, However, based on the same simulated physiological conditions of human GI tract, the model could be also applied for studying antioxidant substances. Plant pigment−rich solutions of 170 mL were added into a glass reactor connected to circulating water at 37±1◦C (Memmert, D−91126 Schwabach FRG, Germany) and constantly agitated by a stirrer. The glass reactor was fully covered with aluminum foil to protect sample from light and digestion was conducted in darkness. Sample was firstly mixed with α−amylase and pH was maintained around 6.0. The aliquots of 1 mL were collected representing the sample of oral phase (G0) and was rapidly cooled in iced baht for further analysis. To perform subsequent gastric digestion process, pH was adjusted to 2.0 by adding 1M HCl solution. Thereafter, 19 mL pepsin from porcine gastric mucosa was added, and the samples were incubated while continuously stirred for 30 min. The resultant aliquots represented the gastric digested samples (G30). The remaining solution was further submitted to the simulated intestinal digestion process where pH was adjusted to 6.8 with 1N NaOH. Then, 23 mL of intestinal enzyme solution containing pancreatin from porcine pancreas, amyloglucosidase and bile salt were added. The resultant mixture was further incubated for another 2 h and aliquots of the reaction solution were taken after definite time intervals of 0 (I0), 5 (I5), 10 (I10), 15(I15), 30(I30), 60(I60), 90 (I90) and 120 (I120) min, respectively. The obtained digested samples were properly diluted and analyzed for bioactive compounds concentration and antioxidant activity following the methods described earlier. The simulation of the *in vitro* digestion process was performed in triplicate. Furthermore, stability of plant pigments, phenolic compounds and related antioxidant capacities of digested sample at G30 and I120 was calculated based on that of undigested sample at G0.

**2.7 Statistical analysis**

The results are expressed as means ± standard deviation. One way analysis of variance (ANOVA) was used to compare means and Duncan multiple range test (DMRT) were carried out to test any significant differences between the means. Differences between means at 5% level were considered significant. All statistical analysis was performed using SPSS (PASW Statistics18).

**3. Results and Discussion**

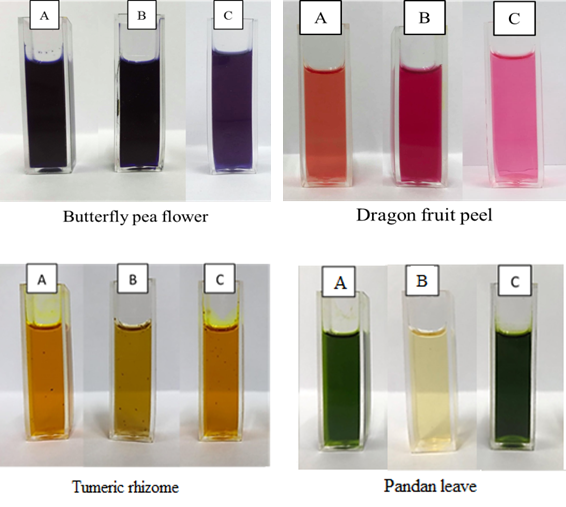
**3.1 Color differences of plant pigments extracted with different solvents**

The plant extracts showed different visual color shades according to the major pigment compound presented in plant materials. Colors of plant pigment extracted with different types of solvent were evaluated. Color appearance and the CIE color space are shown in Fig 1 and Table 1, respectively. For butterfly pea flower extract rich in anthocyanins, samples with the intense blue color were obtained from either acidic 70% acetone (b\*, −6.62), water (b\*, −5.96) or 50% (v/v) ethanol (b\*, −0.40). The hue values of acidic acetone extract (354.80) corresponded to red color tones. Hue values of water extract (264.90) indicated dark blue color whereas, that of aqueous ethanol (102.93) indicated blue−green shades. It was clearly seen that water was much more efficient than aqueous ethanol in extraction of pigment compounds from butterfly pea flower. Anthocyanins are polar molecule which could be extracted by different polar solvents such as acetone, water, and alcohol (Castañeda−Ovando *et al.,* 2009). The extraction under acidic condition was recommended for anthocyanins (Silva *et al.,* 2017). In this study, water extraction showed pH of 5.0±0.5 which help in stabilizing anthocyanins during extraction. In other studies, water extraction of anthocyanins was also applicable for roselle calyx (Aryanti *et al.,* 2019) and butterfly pea flower (Azima *et al.,* 2017).

Dragon fruit peel extracts ranged in color shade from orange red to pink red (Fig 1). Water extract provided more intense redness (a\*, 36.50) than aqueous ethanol extract   
(a\*, 31.53) and acidic acetone extract (a\*, 27.13), respectively. The hue values of water and ethanol extract was 14.93 and 5.63, falling in red shade but that of acidic−acetone was 47.17, indicating the yellow−orange color tone. Betalain is water−soluble pigments which is most stable at pH 5−6 (Azeredo, 2009). Similar to anthocyanins, water extraction at pH about 5.0 was recommended to extract betalians (Prakash *et al.,* 2013). In other studies, water was also used for extraction of betalain in white and red dragon fruit peel (Priatni and Pradita, 2015;   
Ramil *et al.,* 2014).

All solvents could extract yellow pigments from turmeric samples. However, water was the least effective among three solvents. The most intense red-yellow color (a\*, 10.40 and   
b\*, 33.30), corresponding to hue of 67.71 was obtained from ethanol extract followed by that obtained from acetone extract (a\*, 7.26 and b\*, 29.56), corresponding to hue of 61.09. Those of water were a\*, 5.13, b\*, 11.29 and hue, 43.28, indicating the light yellowness. Similarly observations were found in pandan leaf samples. The ethanol extract showed the darkest green color (a\*, −6.50) compared to that from acidic acetone (a\*, −6.01) and water (a\*, −1.63). The similar hue values were obtained from ethanol extract (127.67) and acetone extract (126.40), corresponding to green color shade. Water apparently could not dissolve and release green pigments from samples as hue value was low (86.97). In turmeric, the compounds responsible for the yellow color are the curcumin and curcuminoids whereas, in pandan leaf, the chlorophyll are the major green pigments and alcohol was commonly used for extraction of these fat−soluble compounds (Braga *et al.,* 2003). The uses of water and ethanol in different ratios as cosolvent to improve the extraction efficiency have been reported elsewhere (Surojanametakul *et al.,* 2010; Bagchi, 2012; Putra *et al.,* 2017).

The current results indicated the potential of particular organic solvents in extraction of the required pigment compounds from plant materials. Based on solubility properties of each pigment compounds and the safety applicable for food uses, the appropriate solvent for extraction butterfly pea flower and dragon fruit peel were water and that for turmeric and pandan leaf were aqueous ethanol.



**Fig 1** Visual color of butterfly pea flower, dragon fruit peel, turmeric rhizome

and pandan leaf extracted with different type of solvents:

(A) 70% acidic acetone

(B) water

(C) 50% w/w ethanol

**Table 1** Color parameters of turmeric, dragon fruit peel, butterfly pea flower and pandan leaf

extracted with different type of solvents.

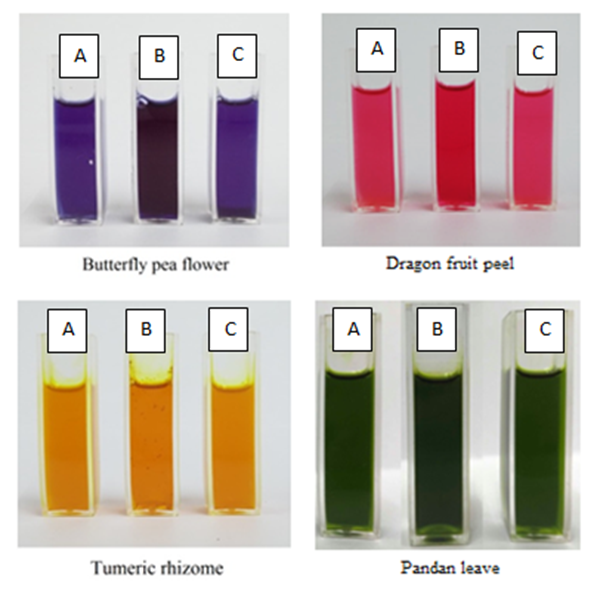
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Samples** | **Type of solvent** | **L\*** | **a\*** | **b\*** | **hue** |
| Butterfly pea flower | 70% acidic acetone | 24.23±0.64b | 2.10±0.30b | -6.62±1.28b | 354.80±3.24a |
| water | 21.27±0.65c | 1.70±0.36b | -5.96±0.78b | 264.90±2.72b |
| 50% w/w ethanol | 26.10±0.95a | 4.9±0.42a | -0.4±1.55a | 252.30±1.11c |
| Dragon fruit peel | 70% acidic acetone | 55.73±2.80a | 27.13±0.67c | 32.27±2.42a | 47.17±0.64a |
| water | 28.27±0.96c | 36.50±2.19a | 7.23±0.78b | 14.93±1.71b |
| 50% w/w ethanol | 33.40±3.67b | 31.53±2.17b | 3.12±2.82c | 5.63±0.64c |
| Turmeric rhizome | 70% acidic acetone | 33.01±1.34b | 7.26±0.52b | 29.56±0.58b | 61.09±1.43b |
| water | 43.71±1.45a | 5.13±0.71b | 11.29±0.69b | 43.28±1.29c |
| 50% w/w ethanol | 31.53±0.76c | 10.40±1.46a | 33.30±2.03a | 67.71±2.01a |
| Pandan leave | 70% acidic acetone | 37.72±0.49b | -6.01±0.06b | 1.57±0.06b | 126.40±0.36a |
| water | 56.70±0.40a | -1.63±0.31a | 31.23±0.29a | 86.97±0.57b |
| 50% w/w ethanol | 36.54±0.29c | -6.50±0.52b | 1.58±0.06b | 127.67±1.12a |

**Note**: Data were expressed as mean ± standard deviation. In a column, values with different superscripts are significantly different (p<0.05).

**3.2 Color differences of plant pigment extracts after maceration and UAE**

In this study, attempt has been made to increase efficiency of the solvent extraction   
of plant pigment by UAE. Each sample was extracted with the selected type of solvent and subjected to different extraction conditions; maceration under agitation (MA), UAE at room (25°C) and warm (65°C) temperature. The visual color and color parameters of pigment extract obtained from different extraction conditions are shown in Fig 2 and Table 2, respectively. Overall, UAE could extract the required color from the four plants better than maceration under agitation. Ultrasonic waves produce high pressure cavities in the liquid which can damage the plant cell walls on bursting and make the solvent easily permeate into plant material to extract the desired pigments (Subhash *et al.,* 2015). The effect of UAE in increasing extraction efficiency of the bioactive compounds was extensively reported in various plant materials (Ngamwonglumlert *et al.,* 2015; Braga *et al.,* 2003; Laqui−Vilca *et al.,* 2018; Maran *et al.,* 2015).

According to earlier studies, UAE was more efficient than conventional maceration, resulting in the higher concentration of pigments in the extracts. In this study, the UAE also used much less time (1 h) in extraction plant pigments than conventional macerations (24 h). Based on the visual color, UAE at 25°C provided the more intense color than UAE at 65°C in all samples. Regarding CIE\* color coordinates, UAE at 25°C gave dragon fruit peel extract with the highest redness (+a\*, 15.57), turmeric rhizome extract with the highest yellowness   
(+b\*, 32.47) and pandan leaf extract with a maximum greenness (−a\*, −2.50). The exception was for butterfly pea flower extract in that UAE at 65°C provided the higher blueness   
(−b\*, −7.63) than UAE at 25°C (−b\*, 3.70). It was expected that UAE with warm temperature at 65°C gave the higher extraction efficiency than UAE at ambient. However, high temperature during extraction could either increase or decrease extraction yield. Heat can cause pectin solubilization and depolymerization of cell wall, softening their structure and facilitating the release of bioactive compounds (Hurtado *et. al.,* 2002). However, natural pigments are thermolabile compounds and are subjected to thermal degradation (Laqui−Vilca *et. al,* 2018). Therefore, the appropriate temperature during extraction must be employed. Thermal degradation can cause structural changes of anthocyanins (Jiang *et al.,* 2019). Heat can stimulate oxidation, hydrolysis and decarboxylation of batalains, resulting in the color change to orange−yellow color in purple pitaya juices (Herbach *et al.,* 2004). Thermal decomposition of cucurminoids at higher temperature was also reported in turmeric (Lauro and Francis, 2018) and chlorophylls (Lau *et al.,* 2000), leading to color changes.



**Fig 2** Visual color of butterfly pea flower, dragon fruit peel, turmeric rhizome

and pandan leaf extract obtained from different extraction conditions:

(A) maceration with agitation at 25°C

(B) UAE at 25°C

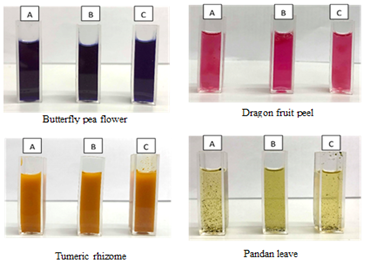
(C) UAE at 65°C

**Table 2** Color parameters of butterfly pea flower, dragon fruit peel, turmeric rhizome and pandan leaf extract obtained from different extraction conditions

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Samples** | **Mechanical extraction** | **L\*** | **a\*** | **b\*** | **hue** |
| Butterfly pea flower | Maceration 25°C | 24.37±0.06b | 0.87±0.06c | -3.00±0.01a | 235.80±0.95b |
| (extracted with water) | UAE 25°C | 24.63±0.49b | 3.70±0.10b | -3.67±0.45a | 265.15±1.08a |
|  | UAE 65°C | 26.47±1.05a | 6.33±0.25a | -7.63±0.49b | 232.13±1.40c |
| Dragon fruit peel | Maceration 25°C | 24.70±0.20b | 5.13±0.06c | 2.57±0.64a | 18.00±1.31a |
| (extracted with water) | UAE 25°C | 25.83±0.42a | 15.57±0.64a | 2.00±0.56a | 15.37±0.91b |
|  | UAE 65°C | 26.00±0.75a | 6.13±0.25b | 1.80±0.44a | 18.30±1.15a |
| Turmeric rhizome | Maceration 25°C | 46.80±1.56a | 9.83±0.92c | 10.17±0.81c | 62.83±2.15b |
| (extracted with 50%  w/w ethanol) | UAE 25°C | 42.67±0.78b | 16.77±0.50a | 32.47±1.92a | 63.20±1.15b |
|  | UAE 65°C | 41.23±0.40b | 14.33±1.46b | 29.27±0.93b | 66.87±0.15a |
| Pandan leave | Maceration 25°C | 30.53±1.30a | -0.70±0.20a | 5.37±0.32ab | 97.13±1.94b |
| (extracted with 50%  w/w ethanol) | UAE 25°C | 27.20±0.53b | -2.50±0.20b | 3.57±0.21c | 104.33±2.60a |
|  | UAE 65°C | 30.23±0.21a | -0.73±0.45a | 6.53±1.70a | 96.33±2.36b |

**Note:** Data were expressed as mean ± standard deviation. In a column, values with different superscripts are significantly different (p < 0.05).

Pigment powders obtained from each plant materials were also re-dissolved in water to evaluate potential use in particular water based food formulation. The visual color of samples shown in Fig 3 differed in terms of solubility and color intensity. Among the extraction methods, samples obtained from UAE at 25°C was better dissolved in water and gave more intense color than other samples. This was clearly observed in particular pandan leaf extract. Therefore, it could be concluded that either water−soluble or fat−soluble dye powders being studied could be applicable for water based food as showing the good dissolvability.



**Fig 3** Visual color of water re-dissolved samples from butterfly pea flower, dragon fruit peel, turmeric rhizome and pandan leaf extracted with different conditions:

(A) maceration with agitation (MA) at 25°C

(B) UAE at 25°C

(C) UAE at 65°C

**3.3 Pigment concentration and antioxidant properties after maceration and UAE**

Major pigment compounds of each sample evaluated were total phenolic content (TPC), total anthocyanin content (TAC) in butterfly pea flower samples, total betalains content (TBC) in dragon fruit peel samples, total curcuminoids content (TCC) in turmeric rhizome samples and total chlorophylls content (TCPC) in pandan leaf samples. In all samples, the highest concentration of pigment compounds was obtained from UAE at 25°C followed by UAE at 65°C and MA at 25°C, respectively. This was corresponded to the color intensity of extracts as shown in Fig 2 and Table 3.

The role of UAE in maintaining and improving antioxidant activity of plant extract was well reported (Ngamwonglumlert *et al.,* 2015; Braga *et al.,* 2003; Laqui−Vilca *et al.,* 2018; Maran *et al.,* 2015). In addition, temperature was also main factor affecting stability of phytochemicals, including plant pigments.

Thermal degradation caused the decline bioactive compounds and their related antioxidant activities. In butterfly pea flower, total anthocyanins content from UAE at 25°C was 204.22 mg/100g associated with the highest total phenolic content of 59.99 mgGAE/100g. These agreed to reference data with some differences that may be due to genetic variability, growing conditions and extracting solvent. UAE at 25°C increased TAC to about 5.8 folds of MA whereas UAE at 65°C increased 0.28 folds of MA. The raised temperature of 60°C was also reported to decrease anthocyanin content, phenolics and antioxidant capacities in anthocyanin-rich fruit (Erick *et al.,* 2017). In the sample obtained from UAE at 25°C, the related antioxidant activities by FRAP was also the highest among extractions (684.76 mmol FeSO4/100g). However, the highest DPPH was obtained from UAE at 65°C (1082.48 umol Trolox/100g). Structural changes of polyphenol compounds including anthocyanins as a result from heat can influence their antioxidant activities (Arancibia−Avila, 2012).

Dragon fruit peel after MA at 25°C showed total betalains content of 13.49 mg/100g which conformed to the range reported by other studied of 0.4-20 mg/100 g (Priatni and Pradita, 2015; and Ramli *et al.,* 2014). TPC of sample after MA was found in lower amount of 7.96 mg/100g. It was expected for the lower TBC than TPC in the sample studied. However, the higher TBC than TPC was also found in pittaya fruit in other study (García−Cruz *et al.,* 2017). Considering MA as reference, UAE at 25°C raised TBC in dragon fruit peel samples to the greater level of 19.85 mg/100g, compared to UAE at 65°C (16.22 mg/100g). The reduction of TBC in red pittaya during extraction and processing at temperature between 50−85°C was also reported (Gengatharan *et al.,* 2015). Another study by Herbach et al. (2004) showed degradation of betalains in red beet after heat treatments at 60-86°C. However, the slight increase in TPC during warm temperature of 65°C during UAE was also observed. This was contributed to the disrupted plant cell wall in which phenolic compounds were easily released (Hurtado *et. al.,* 2002). The high bioactive compounds in UAE samples contributed to the increase in antioxidant activities based on FRAP (136.77−150.72 mmole FeSO4/100g) and DPPH (95.22−98.21 umol TE/100g).

In turmeric rhizome, total curcuminoids content in ethanol extraction was reported in range 10−50 mg/100g (Surojanametakul *et al.,* 2010; Bagchi, 2012). Result of this study also showed the similar range of 42.49 mg/100g after ethanol extraction using MA at 25°C. However, UAE at 25°C apparently increased about 4 folds amount of curcuminoids in MA sample. This was, corresponding to the greatest FRAP and DPPH which increased to 1.65 and 2.07 folds, respectively. Comparing to UAE at 25°C, the lesser amount of TCC, TPC and associated antioxidant activities in sample after UAE at 65°C were observed. Temperature between 60−80°C decreased phenolic content in turmeric rhizome extracts (Prathapan *et al.,* 2009).

Ethanol extract of pandan leaf under MA showed chlorophylls content of 1.38 mg/100g. The previous research reported a wide range of chlorophylls in ethanol extract between 3−60 mg/100g (Putra *et al.,* 2017; Areesrisom *et al.,* 2018). To this study, the greater chlorophyll content after UAE at 25 °C apparently increased to 1.43 folds of MA sample, contributing to the increase to 1.35 folds in FRAP but no change in DPPH of chlorophylls rich extracts.   
In earlier report, chlorophyll in broccoli juices was found apparently decreased after thermal processing at 50−120°C (Van Loey *et al.,* 1998). The temperature during UAE in extraction of plant pigment should be optimized to minimize effect of heat in destroying plant pigment structure and reducing antioxidant activities.

**Table 3** Pigment content and antioxidant activities of butterfly pea flower dragon fruit peel, turmeric rhizome, and pandan leaf extracted at various conditions.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Samples** | **Extracting condition** | **Pigment compound concentration** | **Total phenolic content (mg GAE/100g)** | **FRAP (mmol FeSO4/100 g)** | **DPPH (µmol Trolox/100g)** |
| Butterfly pea flower | Maceration 25°C | 34.87±21.67 c | 39.50±0.14c | 443.33±17.00c | 629.53±18.39 c |
| UAE 25°C | 204.22±29.69a | 59.99±1.18 a | 684.76±1.21a | 776.77±32.57 b |
| UAE 65°C | 56.89±10.16b | 42.32±2.83b | 497.53±28.73b | 1082.48±33.15a |
| Dragon fruit peel | Maceration 25°C | 13.49±0.21c | 7.96±0.14c | 103.78±0.40c | 77.48±1.20b |
| UAE 25°C | 19.85±0.09a | 8.77±0.73b | 136.77±5.25b | 95.22±2.03 a |
| UAE 65°C | 16.23±1.54b | 9.26±0.59a | 150.72±11.70a | 98.21±3.11 a |
| Turmeric rhizome | Maceration 25°C | 42.49±9.53c | 111.37±2.80c | 1004.10±46.19b | 3868.28±123.80c |
| UAE 25°C | 177.57±136.71b | 211.89±7.11a | 1652.18±176.60a | 7992.68±505.29a |
| UAE 65°C | 192.44±14.31a | 155.82±19.18b | 613.12±57.00c | 4733.14±262.92b |
| Pandan leave | Maceration 25°C | 1.38±0.27b | 31.89±0.44c | 427.17±13.97b | 656.94±10.63 a |
| UAE 25°C | 1.97±0.19a | 49.25±1.68a | 575.46±47.44a | 654.94±338.65 a |
| UAE 65°C | 0.46±0.30c | 46.89±0.95b | 262.43±12.31c | 675.53±77.01a |

**Note:** Data were expressed as mean ± standard deviation. In a column, values with different superscripts are significantly different (p<0.05).

Pigment compounds were total anthocyanins content (TAC, mgCyE /100g dry sample) in butterfly pea flower, total betalains content (TBC, mg TBC/100g dry sample) in dragon fruit peel, total curcuminoids content (TCC, mg TCC/100g dry sample) in turmeric rhizome and total chlorophylls content (TCPC, mg TCPC/100g dry sample) in pandan leaf.

**3.4 Digestive recovery of plant pigment compounds and antioxidant properties during *in−vitro* gastrointestinal digestion**

In order to investigate the changes upon *in-vitro* gastrointestinal digestion, four pigment extracts obtained from UAE at 25°C was compared. Samples were firstly subjected to amylase at pH 6.0 in the oral phase (G0) and subsequently incubated with pepsin at pH 2.0 for the gastric digestion (G30) and with pancreatin at pH 6.8 to simulate small intestine digestion (I120), respectively. After each step of the *in−vitro* digestion, samples were analyzed in terms of their pigment compounds content and antioxidant capacities. In sense of comparing, concentration of pigment compound and TPC of each plant extracts were converted into percentage in dry weight basis. Fig 4 illustrated the changes in pigment compounds, TPC and antioxidant capacities following *in−vitro* gastrointestinal digestion.

Among 1% (w/v) pigment rich solution, the highest pigment concentration was curcuminoids (390.46 mgTCC/100g d.b.) in turmeric rhizome followed by anthocyanins   
(11.31 mgCyE/100g d.b.) in butterfly pea flower, chlorophylls (2.69 mgTCPC/100g d.b.)   
in pandan leaf and betalains (0.55 mgTBC/100g d.b.) from dragon fruit peel, respectively.   
For total phenolic content, the concentration were in line with pigment compounds since turmeric rhizome showed the highest amount of 150.23 mgGAE/100g d.b. whereas, dragon fruit peel showed the lowest concentration of 14.96 mgGAE/100g d.b. However, TPC in butterfly pea flower and pandan leaf were in similar range of 39.77 and 45.58 mgGAE/100g d.b., respectively.

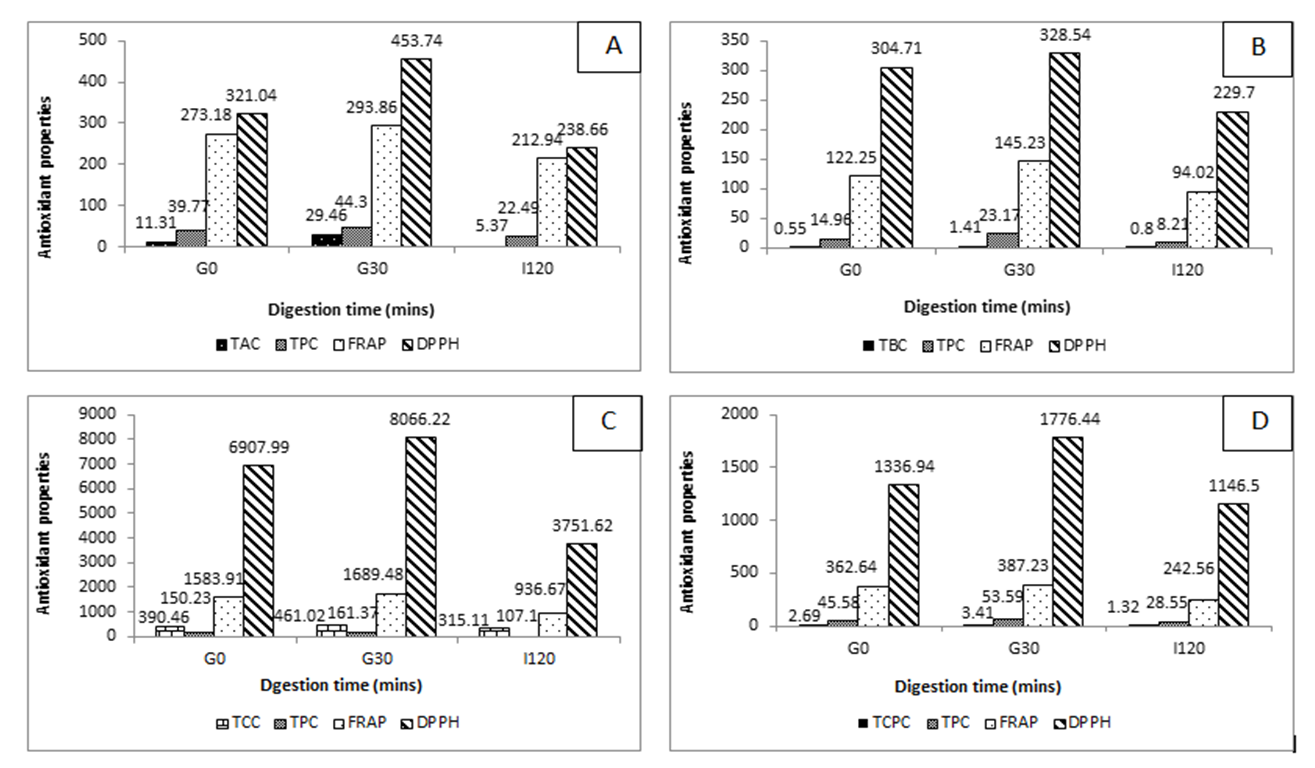
During *in vitro* digestion, overall trend was the increase in all values upon gastric digestion (G30) and gradually declined along intestinal digestion (I0−I120), regardless the type of pigment in sample (Fig 4). In common, all plant pigments studied, anthocyanins, betalains, curcuminoids and chlorophyll are not stable compounds. Many factors can affect the stability of these antioxidant compounds such as pH, oxygen, additives, and the presence of enzymes (David *et al.,* 2019). Their passage through the gastrointestinal tract and subjecting to acidic−neutral−alkaline conditions and digestive enzymes significantly influence their stability and bioaccessibility.

The recovery rate of each pigment compound was also measured by the content of pigment compounds in the digesta (G30 and I120) with respect to the initial pigment content of the undigested pigment solutions (G0). After subjecting samples to simulated gastric digestion, an apparent increase of 260.44% total anthocyanins, 354.49% betalains, 118.07% total curcuminoids and 126.875% total chlorophylls to the corresponding undigested sample were obtained after stomach digestion. These findings suggest that plant pigment compounds were stimulated when exposed to acidic condition in stomach. Acidic conditions caused transformation of pigment compound structure into a more stable form. The low pH value in stomach mainly contributes to the high stability of anthocyanins, which at pH between 1.5−2.0 presented in a stable flavylium cation (Castañeda−Ovando *et al.,* 2009; David *et al.,* 2019). The increase of betalains under acidic condition was also well reported by Azeredo (2009) as well as in curcuminoids by Papillo *et al.,* (2019). However, Chen and Roca (2018) reported that the oxidation of chlorophylls occurred at low pH, resulting in the molecule transformation during digestion. To the present study, the high stability of chlorophylls during gastric digestion was observed. Overall, the increase in antioxidant activities by FRAP (106.66−118.79% recovery) and DPPH (107.82−141.33% recovery) were in accordance to the change in concentration of pigment compounds and TPC as they increased upon gastric digestion.

After duodenal digestion at pH 6.8, a decrease of pigment compounds concentration was observed and the corresponding recovery was in range of 47.44−80.70%, regarding to the undigested sample. The corresponding antioxidant activities also decreased with the lower recovery rate of FRAP about 59.13−89.77% and DPPH about 54.30−85.75%. The lower recovery of plant pigments after intestinal digestion can be explained by the low stability of these compounds under neutral or alkaline conditions (Liu *et al.,* 2014). For anthocyanins, the structural change of the red to orange flavylium cation to a colorless, less stable chalcone was reported (Castañeda−Ovando *et al.,* 2009). Other studies also found that simulated intestinal digestion had a dramatic impact on the anthocyanins from different fruits. Pomegranate juice anthocyanins decreased by 97% during this stage of digestion (Pérez−Vicente *et al.,* 2002), while blueberry anthocyanins decreased more than 80% after intestinal digestion (Correa−Betanzo *et al.,* 2014).

In other studies, the apparent reduction of curcuminoids, TPC and antioxidant activities after *in vitro* digestion were also reported in turmeric (Papillo *et al.,* 2019). The increased stability of curcumin at acidic pH may be contributed by the conjugated diene structure. However when the pH is adjusted to neutral-basic condition, proton removed from the phenolic group leads to the destruction of this structure (Kumavat *et al.,* 2013). For betalains, the study in red beet showed the decrease in concentration of betalains during intestinal digestion (Sawicki *et al.,* 2019). Acidification was found to induce re-condensation of two major species of betalains; betaxanthins and betacyanins, while alkaline conditions brought about chemical bond hydrolysis (Herbach *et al.,* 2006). The lower chlorophylls content after the intestinal digestion was also reported in seaweed (Chen and Roca, 2018) and broccoli chlorophylls (Scrob *et al.,* 2019). The gastric environment causes chlorophyll transformation into Mg2+−free derivatives such as pheophytins and pyropheophytins, following an oxidized reaction during digestion (Scrob *et al.,* 2019). To this study, however, compared to the initial content of the undigested pigment solutions, a significant decrease in recovery of chlorophylls and their related antioxidant capacities was observed after the pancreatic digestion.

Overall, the digestive recovery of four pigment compounds and their associated antioxidant capacities after a whole process of digestion were assessed and these revealed the low bioaccessibility of plant pigment compounds upon digestion.

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**Fig 4** Antioxidant properties of pigment extracts during *in vitro* gastrointestinal digestion:

(A) butterfly pea flower

(B) dragon fruit peel

(C) turmeric rhizome

(D) pandan leaf

**Note:** Antioxidant properties were total anthocyanins content (TAC, mgCyE/100g d.b.) in butterfly pea flower, total betalains content (TBC, mgTBC/100g d.b.) in dragon fruit peel, total curcuminoids content (TCC, mg TCC/100g d.b.) in turmeric rhizome and total chlorophylls content (TCPC, mg TCPC/100g d.b.) in pandan leaf, Total phenol content (TPC) in mgGAE/100g d.b.), FRAP in mmole FeSO4/100g d.b. and DPPH in umol TE/100g d.b.

**4. Conclusion**

Regarding types of solvent and extraction condition studied, the four plant pigment extracts showed varying concentration of pigment compound, resulting in different shade of visual color, color coordinates and the associated antioxidant activities. The appropriate solvent suggested for butterfly pea flower anthocyanins and dragon fruit peel betalains was water whereas, that for turmeric curcuminoids and pandan leaf chlorophylls was aqueous ethanol. UAE has been proved to significantly improve extraction efficiency. However, the appropriate temperature during UAE should be considered. Results showed the low digestive recovery of the studied plant pigments upon *in−vitro* digestion which indicated the poor bioaccessibility.

**References**

Amchova, P., Kotolova, H., and Kucerova, RJ. 2015. Health safety issues of synthetic food colorants. Regulatory Toxicology and Pharmacology. 73: 914−922.

Arancibia−Avila, P., Namiesnik, J., Toledo, F., Werner, E., Martinez-Ayala, L., A., Rocha−Guzmán, E., N., Gallegos−Infante, A., J., and Gorinstein S. 2012.   
The influence of different time durations of thermal processing on berries quality.   
Food Control. 26: 587−593.

Areesrisom, P., Toakaenchan, N., Kawaree, R., Thonnalak, T., and Areesrisom, K. 2018. Effects of shading during cultivation on chlorophyll and 2−Acetyl−1−Pyrroline contents of *Pandanus amaryllifolius roxb* Leaf. Journal of Agriculture. 1: 353-362.

Aryanti, N., Nafiunisa, A., and Wardhani, D., H. 2019. Conventional and ultrasound−assisted extraction of anthocyanin from red and purple roselle (*Hibiscus sabdariffa L.*) calyces and characterisation of its anthocyanin powder. International Food Research Journal. 26(2): 529−535.

Azeredo, H., M., C. 2009. Betalains: properties, sources, applications, and stability – a review. International Journal of Food Science and Technology. 44(12): 2365−2376. doi:10.1111/j.1365−2621.2007.01668.x

Azima, S., A., M., Noriham, A., and Manshoor, N. 2017. Phenolics, antioxidant and color properties of aqueous pigmented plant extacts: *Ardisia colorata var. elliptica*, *Clitoria ternatea*, *Garcinia mangostana* and *Syzygium cumini*. Functional foods. 1: 232−241.

Benzie, F., F., I., and Strain, J., J. 1999. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology. 299: 15−27.

Braga, M., E., M., Leal, P., F., Carvalho, J., E., and Meireles, M., A., A. 2003. Comparison of yield, composition, and antioxidant activity of turmeric (*Curcuma longa L.*) extracts obtained using various techniques. Journal of Agricultural and Food Chemistry.   
51(22): 6604−6611. doi:10.1021/jf0345550

Boo, H., O., Hwang, S., J., Bae, C., S., Park, S., H., Heo, B., G., and Gorinstein, S. 2012. Extraction and characterization of some natural plant pigments. Industrial Crops and Products. 40: 129−135. doi:https://doi.org/10.1016/j.indcrop.2012.02.042

Bucur, L., Taralunga, G., and Schroder, V. 2016. The betalains content and antioxidant capacity of red beet (*Beta vulgaris L. subsp. vulgaris*) root. Farmacia. 64: 2.

Castañeda−Ovando, A., Pacheco−Hernández, M., d., L., Páez-Hernández, M., E., Rodríguez, J., A., and Galán−Vidal, C., A. 2009. Chemical studies of anthocyanins: A review. Food Chemistry. 113(4): 859−871. doi:https://doi.org/10.1016/j.foodchem.2008.09.001

Chen, K., and Roca, M. 2018. In vitro digestion of chlorophyll pigments from edible seaweeds. Journal of Functional Foods. 40: 400−407. doi:https://doi.org/10.1016/j.jff.2017.11.030

Correa−Betanzo, J., Allen−Vercoe, E., McDonald, J., Schroeter, K., Corredig, M., and Paliyath, G. 2014. Stability and biological activity of wild blueberry (*Vaccinium angustifolium*) polyphenols during simulated in vitro gastrointestinal digestion. Food Chemistry. 165: 522-531. doi:https://doi.org/10.1016/j.foodchem.2014.05.135

David, L., Danciu, V., Moldovan, B., and Filip, A. 2019. Effects of In vitro gastrointestinal digestion on the antioxidant capacity and anthocyanin content of Cornelian cherry fruit extract. Antioxidants. 8(5). doi:10.3390/antiox8050114

Delgado−Vargas, F., Jiménez−Aparicio, A., and Paredes−Lopez, O. 2000. Natural pigments: carotenoids, anthocyanins, and betalains−characteristics, biosynthesis, processing, and stability. 40.

Erick, C., López−Vidaña, Isaac Pilatowsky Figueroa, Farid, B., Cortés, Benjamín A., Rojano, and Arturo Navarro Ocaña. 2017. Effect of temperature on antioxidant capacity during drying process of mortiño (*Vaccinium meridionale Swartz*), International Journal of Food Properties. 20(2): 294−305. DOI: 10.1080/10942912.2016.1155601

García−Cruz, L., Dueñas, M., Santos-Buelgas, C., Valle−Guadarrama, S., and Salinas−Moreno, Y. 2017. Betalains and phenolic compounds profiling and antioxidant capacity of pitaya (*Stenocereus spp*.) fruit from two species (*S.Pruinosus and S.stellatus*).   
Food Chemistry. 234: 111−118. doi:https://doi.org/10.1016/j.foodchem.2017.04.174

Gengatharan, A., A., Dykes, G., and Choo, W., S. 2015. Stability of betacyanin from red pitahaya (*Hylocereus polyrhizus*) and its potential application as a natural colourant in milk. 51.

Herbach, K., M., Stintzing, F., C., and Carle, R. 2004. Impact of thermal treatment on color and pigment pattern of red beet (*Beta vulgaris* L.) preparations. food science. nd.

Hurtado, C., Greve, L., Labavitch, J. 2002. Changes in cell wall pectins accompanying tomato (*Lycopersicon esculentum Mill*) paste manufacture. J Agric Food Chem. 50: 273−8.

Jiang, T., Mao, Y., Sui, L., Yang, N., Li, S., Zhu, Z., and He, Y. 2019. Degradation of anthocyanins and polymeric color formation during heat treatment of purple sweet potato extract at different pH. Food Chemistry. 274: 460−470.

Kumavat, S., D, Chaudhari, S., Y., Borole, P., Mishra, P., Shenghani, K., and Duvvuri, P. 2013. Degradation studies of curcumin. Pharmacy review and research. 3(2): 50−55.

Lauro, G., and Francis, F., J. 2018. Naural food colorants. Tayloy and Francis. 1: 1−226.

Lau, M., H., Tanga, B,. G., and Swanson. 2000. Kinetics of textural and color changes in green asparagus during thermal treatments. Journal of Food Engineering. 45(4): 231−236.

Laqui−Vilca, C., Aguilar−Tuesta, S., Mamani−Navarro, W., Montaño−Bustamante, J., & Condezo−Hoyos, L. 2018. Ultrasound−assisted optimal extraction and thermal stability of betalains from colored quinoa (Chenopodium quinoa Willd) hulls. Industrial Crops and Products, 111, 606−614. doi:https://doi.org/10.1016/j.indcrop.2017.11.034

Liu, Y., Zhang, D., Wu, Y., Wang, D., Wei, Y., Wu, J., and Ji, B. 2014. Stability and absorption of anthocyanins from blueberries subjected to a simulated digestion process.   
Int J Food Sci Nutr. 65(4): 440−448.

Maran, J., P., Priya, B., and Nivetha, C., V. 2015. Optimization of ultrasound-assisted extraction of natural pigments from *Bougainvillea glabra* flowers. Industrial Crops and Products. 63: 182−189. doi:https://doi.org/10.1016/j.indcrop.2014.09.059

Martins, R., M., Pereira, S., V., Siqueira, S., Salomão, W., F., and Freitas, L., A., P. 2013. Curcuminoid content and antioxidant activity in spray dried microparticles containing turmeric extract. Food Research International. 50(2): 657−663. doi:https://doi.org/10.1016/j.foodres.2011.06.030.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., and Brodkorb, A. 2014. A standardised static in vitro digestion method suitable for food−an international consensus. Food and Function. 5(6): 1113−1124. doi:10.1039/C3FO60702J

Molyneux, P. 2004. The use of the stable free radical diphenylpicryl-hydrazyl. Original article. 26(2): 211−219.

Ngamwonglumlert, L., Devahastin, S., and Chiewchan, C. 2015. Natural colorants: Pigment stability and extraction yield enhancement via utilization of appropriate pretreatment and extraction methods. Food Science and Nutrition. nd.

Papillo, V., A., Arlorio, M., Locatelli, M., Fuso, L., Pellegrini, N., and Fogliano, V. 2019. In vitro evaluation of gastro−intestinal digestion and colonic biotransformation of curcuminoids considering different formulations and food matrices. Journal of Functional Foods.   
59: 156−163. doi:https://doi.org/10.1016/j.jff.2019.05.031

Pérez−Vicente, A., Gil−Izquierdo, A., and García−Viguera, C. 2002. *In vitro* gastrointestinal digestion study of pomegranate juice phenolic compounds, anthocyanins, and vitamin C. Journal of Agricultural and Food Chemistry. 50(8): 2308−2312. doi:10.1021/jf0113833

Prakash, M., J., Manikandan, S., and Mekala, V. 2013. Modeling and optimization of betalain extraction from *Opuntia ficus−indica* using Box–Behnken design with desirability function. Industrial Crops and Products. 49: 304−311.

Prathapan, A., Lukhman, M., Arumughan, C., Sundaresan, and Raghu, K., G. 2009. Effect of heat treatment on curcuminoid, colour value and total polyphenols of fresh turmeric rhizome. Food Science and Technology 44: 1438−1444.

Priatni, S., and Pradita, A. 2015. Stability study of betacyanin extract from red dragon fruits (*Hylocereus polyrhizus*) peels. Procedia chemistry. 1: 438−444.

Putra, M., D., Daramawan, A., Wahdini, I., and Abasaeed, A., E. 2017. Extraction of chlorophyll from pandan leaves using ethanol and mass transfer study. J. Serb. Chem. 82 (7−8): 921−931.

Ramli, N., S., Ismail, P., and Rahmat, A. 2014. Influence of conventional and ultrasonic-assisted extraction on phenolic contents, betacyanin contents, and antioxidant capacity of red dragon fruit (*Hylocereus polyrhizus*). The Scientific World Journal.   
1: 1−8.

Rodriguez−Amaya, D., B. 2018. Update on natural food pigments − A mini−review on carotenoids, anthocyanins, and betalains. Food Research International. nd.

Sawicki, T., Martinez−Villaluenga, C., Frias, J., Wiczkowski, W., Peñas, E., Bczek, N., and Zieliski, H. 2019. The effect of processing and in vitro digestion on the betalain profile and ACE inhibition activity of red beetroot products. Journal of Functional Foods.   
55: 229−237. doi:https://doi.org/10.1016/j.jff.2019.01.053

Scrob, T., Hosu, A., and Cimpoiu, C. 2019. The influence of in vitro gastrointestinal digestion of *Brassica oleracea* florets on the antioxidant activity and chlorophyll, carotenoid and phenolic content. Antioxidants. 8(212): 1−11.

Sharmila, G., Muthukumaran, C., Suriya, E., Muppidathi Keerthana, R., Kamatchi, M., Kumar, N. M., and Jeyanthi, J. 2019. Ultrasound aided extraction of yellow pigment from *Tecoma castanifolia* floral petals: Optimization by response surface method and evaluation of the antioxidant activity. Industrial Crops and Products.   
130: 467−477. doi:https://doi.org/10.1016/j.indcrop.2019.01.008

Sigurdson, G., T., Tang, P., and Giusti, M., M. 2017. Natural colorants: food colorants from natural sources. Annual Review of Food Science and Technology. 8(1): 261−280. doi:10.1146/annurev−food−030216−025923

Silva, S., Costa, E., Calhau, C., Morais, R., and Pintado, M. 2017. Anthocyanin extraction from plant tissues: A review. Critical Reviews in Food Science and Nutrition.

57(14): 3072−3083 DOI: 10.1080/10408398.2015.1087963

Subhash, C., Vivekananda., and Das, A., K. 2015. Classification of extraction methods. Essentials of botanical extraction. 1: 83−136.

Surojanametakul, V., Satmalee, P., Saengprakai, J., Siliwan, D., and Wattanasiritham, L. 2010. Preparation of curcuminoid powder from turmeric root (*Curcuma longa Linn*) for food ingredient use. Kasetsart J. (Nat. Sci.). 44: 123−130.

Tamura, M., Singh, J., Kaur, L., and Ogawa, Y. 2016. Impact of structural characteristics on starch digestibility of cooked rice. Food Chemistry. 191: 91−97. https://doi.org/https://doi.org/10.1016/j.foodchem.2015.04.019.

Van Loey, A., Ooms, V., Weemaes, C., Van den Broeck, I., Ludikhuyze, L., Indrawati, Denys S., and Hendrickx, M. 1998. Thermal and pressure-temperature degradation of chlorophyll in broccoli (*Brassica oleracea L. italica*) juice: A Kinetic Study. J. Agric. Food Chem. 46: 5289−5294.

Witham, F., H., Blaydes, D., F., and Devlin, R., M. 1971. Experiments in plant physiology,   
Van Nostrand, New York.