

The Proximate and Phytochemical Properties of Red Pitaya (*Hylocereus polyrhizus*) Stem Flour and Its Potential Application as Food Products

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ABSTRACT

Red pitaya fruit and peel have been widely explored for food products due to their functional properties. However, the stem still has limited use. This work was aimed to determine the proximate and phytochemical properties of red pitaya stem by processing it as flour and applied it as food products. The different drying temperature (40, 50, 60°C) on the flouring process was conducted to determine the best drying condition. The best drying temperature was then used to prepare the flour by using whole stem (epidermis and cortex) and peeled stem (cortex only). The result showed that the effective drying temperature on the flouring process was 60°C. The predominant component in the whole and peeled stem flour was fiber (total of hemicelluloses, cellulose and lignin), which contained up to 50.4% and 43.03% respectively. The second largest component was protein which the whole stem flour contained 9.09% of it and the peeled stem flour contained 11.97% of it. Both of the flour contained high vitamin C (3.64–3.76%) and phenolic compounds (43.55–44.54 mg/g). Either whole or peeled stem flour showed antioxidant activity up to 91% of inhibition and antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* as well as *Salmonella typhi*. The resulting flour has been successfully applied as substitute and additional ingredients to make fiber enriched food products.

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INTRODUCTION

Red pitaya or known as red dragon fruit (*Hylocereus polyrhizus*) is a member of the Cactaceae family from the Cactoidea subfamily of the Cactea tribe. This fruit has been popular because of its nutritional composition and antioxidant activity. Many researchers have conducted some studies to explore the potential use of red pitaya fruit and peel as prospective functional food products and sources of natural pigment (Ho & Latif, 2016; Jamilah et al., 2011; Rebecca et al., 2010; Tenore et al., 2012). However, the study on utilization of red pitaya stem for food product has not been conducted yet.

Red pitaya stem is an abundant agricultural waste. In the local pitaya plantations in East Java – Indonesia, as much as ±60 ton per hectare, red pitaya stem can be obtained at every harvest time. During that time, red pitaya stem is only limited to be used for transplant and animal feed. Jafaar et al. (2009) reported that the stem of red pitaya still had a high nutritional value, especially the ascorbic acid content which was found to be higher than the fruit flesh. One of the efforts to exploit red pitaya stem is by making it as flour. The flouring process consists of several stages including removal of thorns and bark, reductions of size, drying, flouring, and sieving. The drying temperature is a critical controlled step to produce pitaya stem flour with high vitamin C, since it is easily destroyed in high temperature.

In the other hand, the research on exploration of agricultural biomass as a source of dietary fiber has increased in the

last decade (Dungani et al., 2016). Pitaya stem can be a new candidate to be utilized as a source of dietary fiber. The consumption of dietary fiber has been correlated with the prevention of many diseases (Dahl & Stewart, 2015). Thus, determination of proximate and phytochemical properties of red pitaya stem flour is important to be done. In this research, red pitaya stem was processed into flour by using the whole stem (epidermis and cortex) and peeled stem (cortex only). The bark stem (epidermis) of red pitaya is green while the cortex is greenish white. The green color of the plant is generally caused by chlorophyll. Chlorophyll and its derivatives have been reported to have anti-mutagenic activity (Ong et al., 1986) and antioxidants (Lanfer-Marquez et al., 2005). Chlorophyll also contributes to the appearance of the final color of product. In addition to the chlorophyll, bark also has high enough fiber content and vitamin C, so the effect of stripping the bark will have an effect on the nutritional composition and appearance of red pitaya stems flour. The obtained red pitaya stem flours then are analyzed to know its proximate and phytochemical properties. By knowing its characteristics, the flour can be applied to create a new fiber enriched food products.

MATERIALS AND METHODS

Preparation and Production of Red Pitaya Stem Flour

The red pitaya stem used in this research was taken from plantation in Banyuwangi, East Java, Indonesia. The thorns were removed

from the stems and then the stems were washed with running water. The whole stem flour was made from both epidermis and cortex of the stem, while peeled stem flour was made only from the cortex of the stem. The whole and peeled stems were thinly sliced (± 0.2 mm) then dried in 50°C using cabinet dryer. The drying process was stopped until it reached a moisture content of $\pm 3\text{-}5\%$. The dried sliced stems were mashed and sieved into 70 mesh size. The obtained flours were then analyzed for proximate and phytochemical analyses and used to make food products hereinafter.

Determination of Drying Temperature for the Production of Red Pitaya Stem Flour

The peeled stem flours dried using three different temperatures (40 , 50 and 60°C) were analyzed for their vitamin C level. The drying time to achieve $\pm 3\text{-}5\%$ of moisture content was also monitored. The vitamin C content and drying time were used to determine the best drying temperature. Statistical analysis was conducted using One Way ANOVA ($P_{\text{value}} < 0.05$) followed by Tukey multiple comparison test. The selection of drying temperature was based on the shortest time while the vitamin C content still could be preserved. The selected temperature was then used to prepare the red pitaya stem flour for further analyses.

Analysis of Red Pitaya Stem Flour

The whole stem and peeled stem of red pitaya stem flour were analyzed for proximate and phytochemical analyses. All

analyses were conducted in triplicate. The difference between samples was determined using T-test ($P_{\text{value}} < 0.05$). The protocol for proximate and phytochemical analyses is described as follows.

Proximate Analysis

Moisture Content. As much as 2 g of the sample was placed in the crucible which its constant weight has been known. The sample then was placed inside 105°C drying oven (Memmert 600, Germany) for 3-5 h and placed in the desiccators afterwards for allowing cooling. The dried sample was weighed until it achieved its constant weight (weighing difference was less than 0.2 mg). The formula for moisture content calculation described as follows:

$$\text{Moisture content (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Note: W_1 = weight of crucible; W_2 = weight of crucible and sample before drying; and W_3 = weight of crucible and sample after drying

Ash. The ash content measurement was conducted by weighing 2 grams of sample and put it into crucible. The total initial weight of the sample and the crucible was recorded. The sample was then placed in a muffle furnace oven (Daihan Scientific FX-14, Korea) at 550°C for 8 h. The sample was placed in the desiccators for cooling and weighed until it achieved its constant weight. The ash content was calculated as:

$$\text{Ash content (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Note: W1 = weight of crucible; W2 = weight of crucible and sample before ashing; and W3 = weight of crucible and sample after ashing

Reducing Sugar and the Total Sugar. The sample was prepared by dissolving the 5 grams of flour into 100 ml distilled water which stirred for 1 hour. The non-soluble part was separated with the soluble part by

centrifugation $6,708 \times g$ for 10 minutes. The soluble part then taken for further sugar analyses and the added distilled water was calculated as a dilution factor. The concentration of reducing sugar was determined by dinitrosalicylic (DNS) colorimetric assay (Miller, 1959). The total sugar was determined by sulfuric acid phenol method (Dubois et al., 1956). Both of the total and reducing sugar determinations were using glucose as sugar standard, and the formula described as follows:

$$\text{Reducing sugar (\%)} = \frac{\text{Reducing sugar concentration} \times \text{dilution volume}}{\text{Initial weight of sample}} \times 100$$

$$\text{Total sugar (\%)} = \frac{\text{Total sugar concentration} \times \text{dilution volume}}{\text{Initial weight of sample}} \times 100$$

Starch. The concentration of starch was determined by weighing sample of 5 grams and added with 50 ml of distilled water into a 250 ml glass beaker and stirred for 1 hour. The suspension is filtered and washed with distilled water until the volume of filtrate is 250 ml. The filtrate is discarded. The residue on the filter paper was washed 5 times with 10 ml of ether and allowed to evaporate. The residue then washed with 150 ml of 10% alcohol to release dissolved carbohydrates. The residue in the filter paper was then moved to Erlenmeyer and washed with 200 ml of distilled water and

20 ml of 25% HCl. The Erlenmeyer then covered with condenser and then heated in boiling water for 2.5 hours. The solution was allowed to cool then neutralized with 45% NaOH solution and dilution was carried out until the volume was reached 500 ml. The sample then was filtered and the sugar content expressed as glucose was determined from the obtained filtrate by dinitrosalicylic (DNS) colorimetric assay for reducing sugar measurement. Reducing sugar weight was multiplied by 0.9 as the weight of starch. Reducing sugar weight and starch content equations are listed below:

$$\text{Reducing sugar weight} = \text{Reducing sugar concentration} \times \text{dilution volume}$$

$$\text{Starch content (\%)} = \frac{\text{Reducing sugar weight} \times 0.9}{\text{Initial weight of sample}} \times 100$$

Lignocelluloses. The analysis of lignocelluloses (lignin, cellulose, and hemicelluloses) was conducted according to Chesson (1981). As much as one gram of flour sample (a) and 150 ml of distilled water were mixed and heated in a 95°C water bath for 1 hour. The mixture was filtrated and the residue was rinsed with 300 ml of hot water. The residue was dried in an oven to a constant weight (b). The residue was added with 150 ml of H_2SO_4 1N and heated in a 90-100°C water bath for an hour. The mixture was filtered and rinsed with 300 ml of hot water. Then, the residue was dried (c). Dry residue was soaked in 10 ml of 72% H_2SO_4 for 4 hours at room temperature. After that, 150 ml of H_2SO_4 1N was added to the mixture and refluxed in the water bath for 1 hour. The solid was rinsed with 400 ml of distilled water and heated in an oven at 105°C and a constant weight was weighed. The solid was burnt in furnace and the ash was weighed (e). The formula for calculation of percent cellulose, hemicellulose, and lignin described as follows:

$$\text{Hemicellulose (\%)} = \frac{b - c}{a} \times 100$$

$$\text{Cellulose (\%)} = \frac{c - d}{a} \times 100$$

$$\text{Lignin (\%)} = \frac{d - e}{a} \times 100$$

Note: a = weight of sample (gram); b = weight of first residue (gram); c = weight of second residue (gram); d = weight of third residue (gram); e = weight of ash (gram)

Crude Fiber. As much as 2 grams of fat-free sample was added with 50 ml of 1.25% H_2SO_4 and put into 500 ml Erlenmeyer. This mixture was boiled for 30 minutes using condenser, and was added with 3.25% NaOH then boiled again for 30 minutes. The samples then immediately filtered in hot condition using a Buchner funnel with non-gray Whatman 54, 41, or 541 filter paper which had been dried and known for its weight. The residue on the filter paper was washed sequentially with 1.25% hot H_2SO_4 , hot water, and 96% ethanol. The filter paper and its contents were lifted and placed on a crucible (that has known for its weight) and dried at 105°C. The sample was then cooled and guided until a fixed weight was obtained. The weight of the residue represents the weight of crude fiber. The percent of the crude fiber was calculated using the following formula:

$$\text{Crude fiber (\%)} = \frac{\text{Weight of crude fiber}}{\text{Weight of sample}} \times 100$$

Crude Protein. The crude protein content was evaluated using micro-bjeldal method (Bjeldal and McMeekin, 1924). As much as 1 g of the sample was placed inside a bjeldal flask. The sample was then added with 15 g of H_2SO_4 , 1 mg of CuSO_4 catalyst solution, 1 g of catalyst selen, boiling stone and 25 mg of concentrated H_2SO_4 . The mixture then was boiled and the color changed into a clear green. The mixture was then cooled off and diluted using distilled water as needed. 75 ml of 30 % NaOH solution was given

before it was distilled for 5–10 min until the solution reached 150 mL, with 50 mL of 4% H₃BO₃ solution posing as the container. The solution was then titrated using 0.1 N NaCl. The difference in the total value of

the titrated sample and the blank, posed as the total value of nitrogen. The protein content is obtained through the process of multiplying N% with 6.25 convection factor.

$$N (\%) = \frac{(\text{mL of HCl in sample} - \text{mL of HCl in blank}) \times N \text{ HCl} \times 1.4007}{\text{Weight of sample in gram}} \times 100$$

$$\text{Protein content (\%)} = N \% \times 6.25$$

Fat. The fat content was measured by directly extracting the pitaya stem flour with petroleum ether in Soxhlet extractor for 4 h. The residue after solvent removal in round

bottom flask represents the fat content of the sample. The fat content calculation was conducted as follows:

$$\text{Fat content (\%)} = \frac{\text{Weight of the residue}}{\text{Weight of sample}} \times 100$$

Phytochemical Analysis

Vitamin C. Vitamin C was measured by mixing 100 mg of sample with 10 ml of distilled water and then stirred for 2-3 minutes. The solution was added with 5 ml of starch indicator and titrated with 0.1 N

Iodine solution which was standardized with Na₂S₂O₃ solution which has standardized by KI 0.1 N as the primary standard solution. Then, the titration was stopped when the solution titrated with iodine solution was dark blue and lasted for about 1 minute.

$$\text{Vitamin C (\%)} = \frac{\text{Mr of Ascorbic Acid} \times N \text{ iodine} \times \text{iodine (mL)}}{\text{Weight of the sample}} \times 100$$

Antioxidant Activity. Testing of antioxidant activity was carried out using DPPH reagent (2, 2-diphenyl-1-picrylhydrazyl). The test was carried out by adding 0.5 ml 10,000 ppm of the sample with 0.5 ml of DPPH 0.1 mM (in ethanol). The control solutions were made by replacing samples with sample solvents and added with 0.5 ml of DPPH

0.1 mM (in ethanol). Blank solutions were made by using sample solvents, while all tubes were incubated in a dark room for 30 minutes. After that, measurements were taken by reading the absorbance using a spectrophotometer at a wavelength of 517 nm (Marques et al., 2012). The percent of inhibition was calculated using this formula:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - (\text{absorbance of sample} - \text{blank})}{\text{Absorbance of control}} \times 100$$

Total Phenolic Compound. As much as 200 µl of 2,000 ppm flour samples in water were added with 2.5 ml of Folin-Ciocalteu reagent (10% v/v) and 2 ml of Na₂C₂O₃ (7.5% w/v) and homogenized then incubated for 15 minutes at 45 °C. The absorbance of the solution was then measured by a spectrophotometer at a wavelength of 765 nm. The total phenolic compounds expressed as milligrams (mg) gallic acid equivalent per gram of sample (mg g⁻¹ sample). As a standard, gallic acid was used in ethanol at various concentrations (0, 5, 10, 20, 40, 80 and 100 ppm) (Javanmardi et al., 2003).

Antibacterial Activity. A total of 100 mg of red pitaya stem (whole and peeled) flour samples were dissolved into 100 µl distilled water to be tested for antimicrobial activity. Antimicrobial activity was aimed primarily towards pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium*. Each of these pathogenic microbes was grown in nutrient broth to reach OD₆₀₀ = 0.5. Furthermore, antimicrobial testing using the pour plate technique was carried out by adding 100 µl of bacteria to 15 ml of nutrient agar and homogenized. The media was then poured into sterile petri dishes and left until the media was solidified. Sterile cylinder cups were planted partially on the media to contain microbial inoculums and added with 100 µl of positive control,

negative control, and samples. The media then was incubated at 37 °C for 24 hours. Observations were made by determining the diameter of the clear zone formed around the cylinder cup. The positive controls used for *Staphylococcus aureus* and *Escherichia coli* were ampicillin (100 mg ml⁻¹) and chloramphenicol (5 mg ml⁻¹) respectively, while the negative controls used were distilled water as the solvents of the samples.

Toxicity Test. Toxicity test was carried out using the Brine Shrimp Lethality Test (BSLT) method using *Artemia salina*. As much as 10 mg of shrimp eggs *A. salina* was grown in 100 ml of filter-sterilized seawater. The hatching temperature was 25-30 °C and pH 6-7 for 48 h. After the hatching process, the active nauplii were collected and used for the assay. As much as 20 ml of pitaya stem flour in brine solution (250 ppm, 500 ppm, 1000 ppm and 1500 ppm) was put into a petri dish containing 20 nauplii and incubated at room temperature for 24 h and surviving larvae were counted. The experiments were conducted along with control and each treatments were conducted in triplicate.

The Application of Red Pitaya Stem Flour on Food Products

Red pitaya whole stem and peeled stem flour were applied to enrich several food products, i.e. cake, cookies, noodle, pudding, yoghurt and jelly drink. Descriptive sensory

evaluations were carried out for those products using five selected trained panelist. The selection began with thirty people from both students and staffs in our institution who committed to attend the training and evaluation sessions. The screening procedure to obtain five selected trained panelist was based on Meilgaard et al. (1999). The trained panelists were involved to generate lexicon to evaluate several food products enriched with pitaya stem flour which consist of appearance, aroma, taste, color and texture attributes using a 0–15 scale. The food products composition were added or substituted with pitaya stem flour using following proportions: yoghurt (addition of 1, 2 and 3%), cake (20% substitution of total flour), cookies (25% substitution of total flour), noodle (substitution 5, 10 and 15% of total flour), pudding (addition of 1, 2 and 3%) and jelly drink (addition of 1, 2 and 3%). Analysis of variance (ANOVA) followed with Tukey test were used to determine the differences between samples, excepting cake and cookies data analysis were using one tailed T-test. All statistical analyses were performed using IBM SPSS Statistic 24 (SPSS Inc, USA).

RESULTS AND DISCUSSION

The effect of drying temperature on characteristics of peeled red pitaya stem flour is listed in Table 1. Vitamin C content is an important parameter to be monitored during drying process because vitamin C is thermally unstable. In the other hand, vitamin C is an important nutrient components which has function as antioxidants and prevent various diseases (Chen et al., 2013). The results showed that vitamin C levels of red pitaya stem flour were affected by drying temperatures, where there was a decreasing in vitamin C levels with the increasing of drying temperature. Statistically, there is a significant difference in the level of vitamin C in the treatment between 40 and 50 or 60 °C. However, there is no significant difference between 50 and 60 °C. Another study conducted by El-Ishaq and Birinakem (2015) was in line with this study, where vitamin C levels were lower due to high temperature treatment of fruit juice. Vitamin C is easily oxidized when it is in contact with air or light at high temperatures.

The drying time to achieve $\pm 3\text{-}5\%$ moisture of red pitaya stem flour was monitored. The data is listed in Table 1. The result shows that drying time was affected

Table 1
The effect of drying temperature on characteristics of peeled red pitaya stem flour

Parameter	Temperature (°C)		
	40	50	60
Vitamin C (%)	4.23 ^a \pm 0,24	3.73 ^b \pm 0,10	3.64 ^b \pm 0.13
Moisture (%)	5.13 ^a \pm 1,29	4.88 ^a \pm 1,69	3.61 ^a \pm 0.53
Drying time (h)	48.43 ^a \pm 1,45	37.28 ^b \pm 2.45	19.95 ^c \pm 1.54

Note: Different letter notations behind the mean in each row indicates a significant difference based on Tukey test (P-value \leq 0.05)

by drying temperature, where the higher drying temperature the shorter drying time. Drying temperature of 60°C was selected for further drying process because it took the shortest time but still maintained the vitamin C level which was not significantly different compared to 50°C. The color of peeled red pitaya stem flour under different drying temperatures is shown on Figure 1. The increasing drying temperature made the flour color changed from light green to light yellowish green. The green color was contributed by chlorophyll, a natural pigment present in a plant. Chlorophyll is less stable in high temperature. As reported by Chan-oey et al. (1998), degradation of chlorophyll occurred when it was processed in high temperature.

The selected drying temperature then was applied to proceed for both whole stem and peeled stem of red pitaya to become flour. The obtained flour was analyzed and the results listed in Table 2. The moisture content was maintained at the same level for the two samples. There was a significant difference on protein and starch content between the whole and peeled stem flour. The high protein and starch in peeled stem

flour is due to the cortical cells may contain stored carbohydrates or other substances such as resins, latex, essential oils, and tannins (Limn-acy & aufman, 2012). There was also a significant difference on fat content between whole and peeled stem flour. The high level of fat in whole stem flour is due to the presence of wax in the epidermis of the stem. The stems of plant

Table 2
Proximate analysis of red pitaya stem flour

Parameter	Whole stem flour	Peeled stem flour
Moisture (%)	3.42 ^a ± 0.88	3.61 ^a ± 0.53
Protein (%)	9.09 ^b ± 0.52	11.97 ^a ± 0.87
Fat (%)	0.89 ^a ± 0.26	0.12 ^b ± 0.16
Starch (%)	0.57 ^b ± 0.13	1.91 ^a ± 0.43
emicelluloses (%)	15.40 ^b ± 0.50	27.42 ^a ± 1.60
Cellulose (%)	32.59 ^a ± 0.75	13.14 ^b ± 1.76
Lignin (%)	2.46 ^a ± 0.45	2.47 ^a ± 0.40
Crude fiber (%)	24.48 ^a ± 3.19	14.65 ^b ± 2.42
Ash (%)	1.69 ^a ± 0.56	2.17 ^a ± 0.69
Total Sugar (%)	5.07 ^a ± 0.79	6.56 ^a ± 0.91
Reducing Sugar (%)	1.67 ^a ± 0.63	2.34 ^a ± 0.82

Note: Different letter notations behind the mean in each row indicates a significant difference based on T-test with a P-value of 0.05

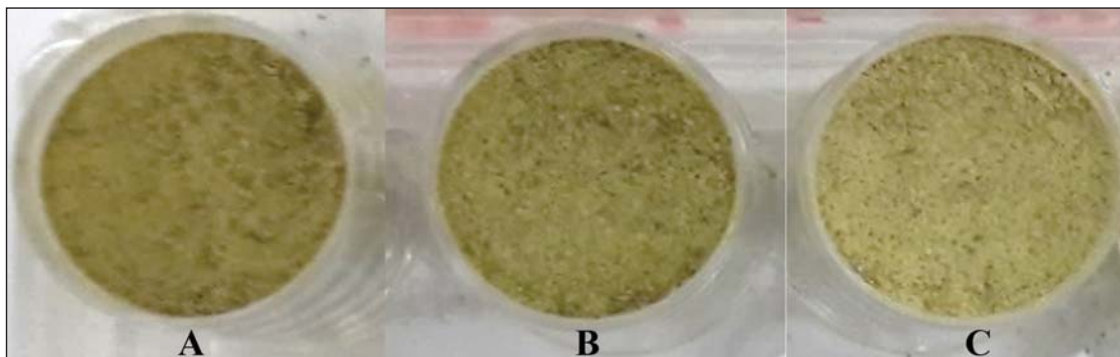


Figure 1. Comparison of peeled red pitaya stem flour's colour on different drying temperature. (A) 40°C; (B) 50°C; (C) 60°C

sometimes covered with smooth layers of wax which give them a whitish surface color and protect them from intense sunlight by acting as a moisture barrier (Raven, et al., 1981). From the data listed in Table 2, it also can be seen that there is a significant difference on crude fiber, hemicellulose and cellulose content between whole and peeled stem flour. Hemicellulose is a polysaccharide matrix found in plant biomass about 20-30% of the plants dry weight. It is stated that hemicellulose is a polysaccharide matrix as a filler of space between cellulose along with lignin in plant cell walls (Bergander & Salmen, 2002). However, there is no significant difference between whole and peeled stem flour on lignin, ash, starch, total sugar and reducing sugar content. Jaafar et al. (2009) suggested that red pitaya stems had a total ash content of 1.5% in young stems and 4.5% in stems that were quite old.

Phytochemical properties of red pitaya stem flour were shown in Table 3. Statistically, there was no significant difference of vitamin C and antioxidant activity for whole stem and peeled stem flour. However, there was a significant difference on the total phenolic compound between whole stem and peeled stem flour.

It indicates that the epidermis contributes to higher phenolic compound than the cortex. The differential accumulation of the total phenols is associated with differential cytological and physiological activities within tissue and organs. The production of these compounds is highly ordered process and regulated by differential expression of genes involved in phenylpropanoid pathway (Chang et al., 2009; Mamti et al., 2006). The phenolic compounds also reported to have antioxidant activity against free radical compounds (Goganayaki et al., 2013). However, in this experiment there was no clear relationship between the levels of phenolic compounds and antioxidant activity. Where the total of phenolic compounds between whole and peeled flour differed significantly, the antioxidant activity was not significantly differed. This can be caused by differences in phenolic components in the epidermis of pitaya stem which can cause different antioxidant activities, depending on the phenolic structure (Nićiforović et al., 2010). This result is also in accordance with other findings reported by Bajpai et al. (2005) and Sengul et al. (2009) which stated no correlation between total phenolic content and antioxidant activities of medicinal plant

Table 3
Phytochemical properties of red pitaya stem flour

Parameter	Whole stem flour	Peeled stem flour
Vitamin C (%)	3.76 ^a ± 0.18	3.64 ^a ± 0.13
Total Phenolic Compound (mg/g)	44.54 ^a ± 0.11	43.55 ^b ± 0.19
Antioxidant Activity (% inhibition)*	90.89 ^a ± 2.77	90.67 ^a ± 0.86

Note: Different letter notations behind the mean in each row indicate a significant difference based on T-test with a P-value of 0.05. Ascorbic acid was used as positive control for antioxidant activity assay. The percent of inhibition of ascorbic acid was 91.46%

extracts. Moreover, the observed antioxidant activity was not only from the phenolic compounds, but also from the presence of other phytochemicals such as pigments and vitamins as well as the synergistic effects among them. On the other hand, the Ciocalteu method used for total phenolic content determination is not an absolute measurement of the amount of phenolic substances (Sengul et al., 2009).

From antibacterial activity test (Table 4), it is now known that both whole and peeled red pitaya stem flour provide antimicrobial activity against *E. coli*, *S. aureus* and *S. typhi* because they showed significant difference in inhibitory size compared to negative controls. Phytochemical compounds such as tannins, flavonoids, alkaloids and several other aromatic compounds which are secondary metabolites of plants, play a role in defense mechanisms to fight predators such as microorganisms, insects, and herbivores (Doughari, 2006).

The toxicity test of pitaya stem flour samples was carried out by the BSLT method using *Artemia salina* larvae. BSLT usually used to determine the

cytotoxicity and effectiveness of traditional medicines derived from plants because this method is very easy, inexpensive, and harmless. The procedure determines IC_{50} of active compounds and extracts in the brine medium. Activities of a broad range of active compounds are manifested as toxicity to the shrimp (Meyer et al., 1982). In this present study, BSLT test was conducted to reveal the toxicity possibility of the pitaya flour. Toxicity test was determined based on the percent of lethality *A. salina* at various concentrations of pitaya flour as shown in Table 5. At the concentrations of 250 and 500 ppm both whole and peeled stem flour give 0% mortality. While at the concentrations of 1,000 and 1,500 ppm whole stem flour, the mortality percentage was 6.67% and 16.67% respectively. At the concentrations of 1,000 and 1,500 ppm of peeled stem flour, the mortality percentage was 3.33% and 13.33% respectively. Based on Friedman test analysis at the concentrations of 1,000 and 1,500 ppm (p-value of 0.519), showed that there was no significant difference in mortality percentage between samples. This indicates that there was no significant

Table 4
Antibacterial activity of red pitaya stem flour

Sample	Inhibitory size (mm)		
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella typhi</i>
Positive Control	21,47 ± 0,35	21,29 ± 0,03	31,82 ± 0,77
Peeled Stem Flour	8,50 ^a ± 0,22	8,54 ^a ± 0,19	8,94 ^a ± 0,15
Whole Stem Flour	8,44 ^a ± 0,11	8,86 ^a ± 0,27	9,03 ^b ± 0,11
Negative Control	7,95 ^b ± 0,23	7,53 ^b ± 0,16	7,56 ^c ± 0,04

Note: Positive control for *Escherichia coli* and *Staphylococcus aureus* is Ampicillin (100 mg/ml), while positive control for *Salmonella typhi* is Chloramphenicol (5 mg/ml), negative control is distilled water. The letters behind the numbers in one column show a significant difference of treatments and negative control based on Tukey test with a P-value of 0.05

Table 5
 Toxicity test based on mortality percentage of *Artemia salina* L.

Flour concentration (ppm)	Mortality percentage of <i>Artemia salina</i> L. (%)	
	Peeled stem flour	Whole stem flour
0	0	0
250	0	0
500	0	0
1,000	3.33 ^a ± 5.77	6.67 ^a ± 5.77
1,500	13.33 ^a ± 5.77	16.67 ^a ± 5.77

Note: The same letter notations behind the mean indicate no significant difference based on Nonparametric Test with a P-value of 0.05

difference of possible toxic effect in both flours in those concentrations. Compared to 250-500 ppm, the higher concentration of the flour increased the percentage of nauplii's mortality. Pitaya flour addition has increased the viscosity of the medium due to its high fiber content, causing an increase of osmotic pressure, disrupting nauplii movement and driving the nauplii to death. The addition of the flour higher than 1,500 ppm to growth medium for BSLT assay was not possible to be done due to viscous effect caused. However, the determination of C_{50} could not be conducted yet. On the other hand, it is possible that phytochemical compounds contains in the flour may cause the death of *Artemia salina* larvae. From this data, it still cannot be concluded yet whether the toxicity is caused by viscosity or the presence of phytochemical compounds. It is suggested that further BSLT assay using phytochemical extract of the flour instead of using the whole flour should be conducted. Compared to the other medicinal plants which mostly showed C_{50} value at concentration below 1,000 ppm (Krishnaraju, et. al., 2005; Madjos

Luceño, 2019), this flour was assumed to be less toxic.

The addition of pitaya stem flour on several food products and their descriptive sensory evaluation are listed in Table 6. In yoghurt, pudding and jelly drink, the trained panelists were revealed ten specific attributes. Most of those attributes were found to be significantly different ($p < 0.05$) among of yoghurt, pudding and jelly drink samples, except for smoothness and sourness in yoghurt. The higher concentrations of pitaya stem flour in those three products, the consistency of the products become more viscous. The increase of viscosity of the product may be due to the presence of soluble fiber in pitaya stem flour. Soluble fibers thicken when mixed with fluids and have been reported to have beneficial physiological effects in human, animal, and in vitro models (Dikeman & Dahey Jr., 2006). The bitterness in those three food products were also found to be significantly different ($p < 0.05$), whereas bitterness might be caused by phytochemical compounds presence in pitaya stem flour. The addition of pitaya stem flour also affected the colour

Table 6
Descriptive sensory analysis of food products enriched with pitaya stem flour

Attributes		Yoghurt			
		Pitaya flour addition (%)			
		0	1	2	3
Appearance	Smoothness	8.12 ^a	7.63 ^b	6.91 ^b	6.32 ^b
	Greenish colour	1.61 ^d	3.83 ^c	6.34 ^b	8.1 ^a
Texture in mouth	Thickness	5.61 ^a	5.72 ^a	5.64 ^a	5.9 ^a
	Graininess	1.1 ^b	2.4 ^a	2.78 ^a	3.01 ^a
Taste	Sourness	3.03 ^a	3.21 ^a	3.44 ^a	3.61 ^a
	Astringent	1.82 ^b	2.23 ^b	3.41 ^a	4.43 ^a
	Bitterness	2.21 ^d	3.62 ^c	5.42 ^b	7.31 ^a
	Green tea like	2.01 ^d	5.63 ^c	8.22 ^b	12.4 ^a
Attributes		Pudding			
		Pitaya flour addition (%)			
		0	1	2	3
Appearance	Syneresis	8.12 ^a	6.56 ^b	5.02 ^c	3.87 ^d
	Smoothness	12.87 ^a	11.54 ^b	9.67 ^c	8.03 ^c
	Greenish colour	1.56 ^c	5.6 ^c	9.94 ^b	13.9 ^a
Texture in mouth	Thickness	8.06 ^b	9.65 ^b	11.01 ^a	11.98 ^a
	Smoothness	12.1 ^a	11.98 ^a	11.87 ^a	10.56 ^b
Taste	Sweetness	13.03 ^a	11.21 ^b	10.44 ^b	9.61 ^c
	Astringent	1.82 ^c	2.21 ^c	3.47 ^b	4.49 ^a
	Bitterness	2.21 ^d	5.62 ^c	7.42 ^b	10.31 ^a
	Green tea like	2.01 ^d	5.63 ^c	8.78 ^b	12.48 ^a
Attributes		Jelly drink			
		Pitaya flour addition (%)			
		0	1	2	3
Appearance	Smoothness	8.12 ^a	8.02 ^a	7.72 ^b	7.01 ^b
	Greenish colour	2.01 ^d	4.56 ^c	8.04 ^b	12.34 ^a
Texture in mouth	Thickness	4.06 ^c	5.65 ^b	6.01 ^b	7.98 ^a
Taste	Sweetness	13.83 ^a	11.61 ^b	10.74 ^b	9.81 ^b
	Astringent	2.02 ^d	6.21 ^c	8.47 ^b	10.49 ^a
	Bitterness	2.21 ^d	6.62 ^c	8.42 ^b	10.31 ^a
	Green tea like	2.01 ^d	6.63 ^c	8.78 ^b	12.48 ^a
Attributes		Cake			
		Pitaya flour substitution			
		0	20		
Appearance	Puffiness	12.4 ^a	10.04 ^b		
	Uniformity	14.1 ^a	11.3 ^b		
	Greenish colour	3.8 ^b	10.87 ^a		
Texture	Puffiness	12.4 ^a	8.7 ^b		
	Crumbliness	3.53 ^a	2.83 ^a		

Table 6 (continue)

		Cake			
Attributes		Pitaya flour substitution			
		0			20
Texture	Softness	13.5 ^a			12.3 ^b
Taste	Sweetness	11.3 ^a			10.2 ^b
	□reen tea like	2.1 ^b			12.2 ^a
Aroma	Bitterness	1.8 ^b			4.23 ^a
	□reen tea like	2.1 ^b			12.2 ^a
	Baked	14.52 ^a			14.1 ^a
	□rassy like	2.2 ^b			11.4 ^a
		Noodle			
Attributes		Pitaya flour substitution (%)			
		0	5	10	15
Appearance	Smoothness	11.3 ^a	11.43 ^a	11.01 ^a	10.97 ^a
	□irmness	12.41 ^d	11.8 ^c	11.01 ^b	8.1 ^a
	□reenish colour	2.03 ^d	3.03 ^c	5.21 ^b	10.03 ^a
Texture	Elasticity	13.01 ^a	12.4 ^a	11.8 ^a	9.6 ^b
	Softness	11.1 ^a	11.4 ^a	10.08 ^a	9.7 ^a
Taste	Starchy like	3.03 ^a	3.21 ^a	3.44 ^a	3.61 ^a
	□rassy like	1.82 ^b	2.23 ^b	3.41 ^a	4.43 ^a
	□reen tea like	2.21 ^d	3.62 ^c	5.44 ^b	7.31 ^a
Aroma	Bitterness	1.1 ^b	1.5 ^b	1.7 ^b	1.86 ^a
	□rassy like	1.3 ^a	1.7 ^a	1.9 ^a	2.01 ^a
	□reen tea like	1.5 ^c	2.4 ^b	3.14 ^b	4.51 ^a
		Cookies			
Attributes		Pitaya flour substation (%)			
		0			25
Appearance	Baked colour	12.1 ^a			10.5 ^b
	Uniformity	13.1 ^a			13.1 ^a
	□reenish colour	2.6 ^b			4.67 ^a
Texture	□ardness	6.63 ^a			6.63 ^a
	Crunchiness	10.03 ^a			9.1 ^a
	Softness	9.1 ^a			9.1 ^a
Taste	Sweetness	12.01 ^a			12.01 ^a
	□reen tea like	2.6 ^b			9.2 ^a
	Bitterness	3.3 ^b			6.23 ^a
Aroma	□reen tea like	3.3 ^b			8.65 ^a
	Baked	12.4 ^a			10.3 ^b
	□rassy like	2.1 ^b			5.3 ^a

Note: The scale ranging from 0 – 15 (low to high). Different letter notations behind the mean in each row indicate a significant difference based on Tukey test (P-value <0.05) and T-test (P-value <0.05) (only for cake and cookies data analysis)

of the three food products. The more pitaya stem flour added, the colour of the yoghurt, pudding and jelly drink became more greenish (Figures 2D, 2E and 2F).

The attributes assessment in cake, cookies, and noodle were listed in twelve specific attributes. The attributes of texture (puffiness and softness) in cake among the tested samples had significantly different ($p < 0.05$). Substitution of wheat flour in

the cake making also made the total gluten content in the dough reduced. Gluten is a protein in wheat flour and plays an important role in cake baking performance, which contributes to the rise ability of the dough and maintain the cake's shape when it is baked (Chhatkar et al., 1995). The high fiber content in pitaya stem flour causes an increase in the water binding capacity that makes texture of the cake becomes less puff

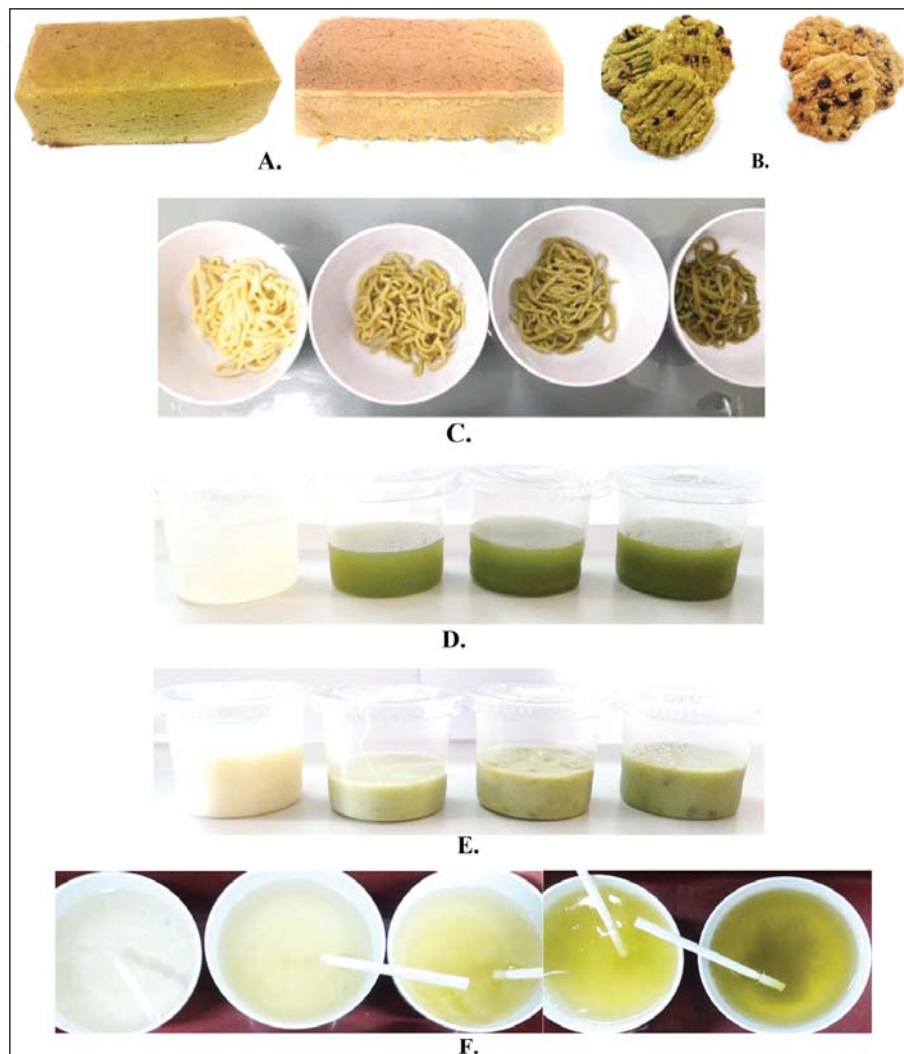


Figure 2. Various food products enriched with pitaya stem flour. A. Cake (Left: 20 % substitution, Right: control); B. Cookies (Left: 25 % substitution, Right: control); C. Noodle (left to right: 0, 5, 10, 15 % substitution); D. Pudding (left to right: 0, 1, 2, 3 % addition); E. Yoghurt (left to right: 0, 1, 2, 3 % addition); F. Jelly drink (left to right: 0, 1, 2, 3, 4 % addition)

and soft (Yamazaki et al., 2005). Therefore, it is recommended to apply pitaya stem flour on cake products lower than 20%. In addition, substitution of wheat flour with pitaya stem flour made the colour of the cake more greenish (Figure 2A). In pitaya flour addition ranging from 5-15% were found to have no significantly different ($p>0.05$) among most of attributes in noodle samples, however elasticity, firmness, and greenish color given significantly different ($p<0.05$). The higher pitaya stem flour addition makes the color become more greenish (Figure 2C) and the texture less elastic. The elasticity is caused by gluten contained in wheat flour (Shewry et al., 1995). Substitution of wheat flour with pitaya stem flour reduced the gluten in the noodle so that the texture becomes less elastic. The appearance (baked and greenish color), taste (bitterness), and aroma attributes of cookies were found to be significantly different ($p<0.05$). The color of pitaya stem cookies represented in Figure 2B. However, the texture assessment in the cookies were found to have no significantly different ($p>0.05$) between the samples.

CONCLUSION

The red pitaya stem flour can be prepared using 60°C drying temperature. This temperature can reduce the moisture content efficiently while the vitamin C content still preserved. The predominant component in whole stem flour was cellulose, followed by hemicellulose and protein. While the predominant component in peeled stem flour was hemicelluloses, followed by cellulose and protein. Both whole and peeled pitaya

stem flour can be used as functional food because it contains high vitamin C and phenolic compounds. Moreover it also shows antioxidant activity and antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* as well as *Salmonella typhi*. The resulting flour has been successfully applied as substitute and additional ingredients to make fiber enriched food products.

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2. The CEE sends the article-identifying information having been removed, to three reviewers who are specialists in the subject matter represented by the article. The CEE requests them to complete the review in three weeks.

Comments to authors are about the appropriateness and adequacy of the theoretical or conceptual framework, literature review, method, results and discussion, and conclusions. Reviewers often include suggestions for strengthening of the manuscript. Comments to the editor are in the nature of the significance of the work and its potential contribution to the field.

3. The CEE, in consultation with the Editor-in-Chief (EiC), examines the reviews and decides whether to reject the manuscript, invites the author(s) to revise and resubmit the manuscript. The CEE may seek additional reviews. Final acceptance or rejection rests with the CEE and EiC, who reserve the right to refuse any material for publication. In rare instances, the manuscript is accepted with almost no revision. Almost without exception, reviewers' comments (to the author) are forwarded to the author. If a revision is indicated, the editor provides guidelines for attending to the reviewers' suggestions and perhaps additional advice about revising the manuscript.
4. The authors decide whether and how to address the reviewers' comments and criticisms and the editor's concerns. The authors return a revised version of the paper to the chief executive editor along with specific information describing how they have answered the concerns of the reviewers and the editor, usually in a tabular form. The author(s) may also submit a rebuttal if there is a need especially when the author disagrees with certain comments provided by reviewer(s).
5. The CEE sends the revised paper out for re-review. Typically, at least one of the original reviewers will be asked to examine the article.
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