Production of Value Added Flour based on Unripe Papaya from Fruit Processing Industrial Waste

การผลิตแป้งมูลค่าเพิ่มจากมะละกอดิบจากวัสดุที่เหลือทิ้งจากอุตสาหกรรมแปรรูปผลไม้

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ABSTRACT

Papaya fruit (*Carica papaya* L.) belongs to Caricaceae family and is known as a good source of dietary fiber and antioxidants. The objective of this study was to develop the unripe papaya flour (UPF) from waste fruit product. The study was also investigated physicochemical and antioxidant properties of UPF. The small pieces of unripe papaya were heated by hot air oven, then ground by using a high-speed universal grinder and sieved through a 100-mesh sieve. The results showed that particle size of UPF was 140.8±2.1 µm with irregular shape. The water solubility index of UPF was higher than wheat flour, whereas bulk density, the water absorption index and swelling power were not significant differences. Total polyphenol and β-carotene contents of UPF were 85.7±1.6 mg gallic acid equivalent/100 g dry weight and 39±3.3 µg/100 g dry weight, respectively. In addition, UPF had ferric reducing antioxidant power (411.6±38 µmol $FeSO_4$/100 g dry weight) higher than wheat flour. The results suggest that the unripe papaya flour can be regarded as a good ingredient for natural antioxidants for development of healthy products.

บทคัดย่อ

มะละกอ (*Carica papaya* L.) ผลไม้ในตระกูล Caricaceae ที่เป็นแหล่งของใยอาหารและสารต้านอนุมูลอิสระ ซึ่งในงานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาและพัฒนาแป้งมะละกอดิบจากวัสดุเหลือใช้ในอุตสาหกรรมแปรรูปผลไม้และนำมาศึกษาคุณสมบัติทางเคมีกายภาพและคุณสมบัติการต้านอนุมูลอิสระ แป้งมะละกอดิบทำโดยการใช้มะละกอดิบอบแห้งถูกนำมาบดและร่อนผ่านตะแกรง 100 เมซ ซึ่งผลการทดลองพบว่าแป้งมีรูปร่างไม่แน่นอนและมีขนาดอนุภาค 140.8±2.1 ไมครอน ดัชนีการละลายน้ำพบว่าแป้งมะละกอดิบสูงกว่าแป้งสาลีในขณะที่ความหนาแน่นรวม ดัชนีการดูดซึมน้ำและความสามารถในการบวมน้ำไม่แตกต่างกัน และยังพบว่าแป้งมะละกอดิบมีปริมาณสารประกอบฟีโนลิก 85.7±1.6 มิลลิกรัมเทียบเท่ากรดแกลลิคต่อ 100 กรัมแป้งและเบต้าแคโรทีน 39±3.3 ไมโครกรัมต่อ 100 กรัมแป้ง นอกจากนี้แป้งมะละกอดิบยังมีฤทธิ์การต้านอนุมูลอิสระแบบ FRAP (411.6±38 ไมโครโมล $FeSO_4$ต่อ100 กรัมแป้ง) ซึ่งมีค่าสูงกว่าแป้งสาลี ผลจากการศึกษาอาจกล่าวได้ว่าแป้งมะละกอดิบอาจนำไปใช้เป็นส่วนประกอบของผลิตภัณฑ์อาหารที่มีฤทธิ์ต้านอนุมูลอิสระและส่งผลดีต่อสุขภาพได้

Keywords: Unripe papaya, Flour, Antioxidant
คำสำคัญ: มะละกอดิบ แป้ง สารต้านอนุมูลอิสระ

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Introduction

Fruit processing industry is one of many solutions for poor postharvest management practices and growing awareness. Nowadays, waste products of fruit processing have become dominating influence in environmental and economic concern (Sharma et al., 2016). Therefore, the waste utilizes of the fruit processing industries to produce value-added products is one of the main challengeable aspects in the world (Yazid et al., 2018). In nutraceutical products, fruit wastes can be used as a source of polyphenolic and other compounds, proteins, edible oils, dietary fiber and polysaccharides (Wadhwa et al., 2015). In addition to value-added food products, fruit wastes may be used as alternative flour because it is normally derived from natural source, available in abundance and low cost (Li, Zhu, 2017). For example, kiwifruits, lychee and banana may be feasible to develop alternative flour for replacing traditional flours or wheat allergy/intolerance (Li, Zhu, 2017; Queiroz et al., 2015; Segundo et al., 2017). Nowadays, natural food sources are widely used for alternative ingredients as flour replacement due to nutritional components such as dietary fiber and antioxidant properties (Sirichokworrakit et al., 2015). Fruits are commonly known and accepted as a source of phytochemical compounds (Yusufu M.I., 2014). Moreover, it has been studied fruit processing by-products still contained high antioxidants; for example, grape pomace, mango pulp and apple puree (Galanakis, 2012; Saura-Calixto, 1998). The previous study suggested that fiber-rich flour from by-product as an ingredient can improve the nutritional value and antioxidants (Resende et al., 2019). The demand to use novel sources as wheat partial replacement was increased to provide the consumer requirement, enhance nutritional value of food and provide more health benefits.

Papaya, one of tropical fruit plants, is widely cultivated and distributed in most tropical countries and islands. In Thailand, papaya is a favorite plant and generally found in many areas. Papaya has culinary use because of its nutritional value and taste acceptability (Annegowda, Bhat, 2016). It found that papaya contains high vitamin C, β-carotene, fiber and antioxidant properties. The study also suggests that unripe papaya contains lower glucose and higher fiber than ripe papaya (Asmah R, 2014). In food industry, papaya can be consumed both fresh fruits of processed food; for example, dried papaya, candy, ice cream, and ingredients of food (Annegowda, Bhat, 2016). However, only middle part of unripe papaya is selected for dried papaya products, but by-products generated during processing including the upper and lower parts of them are discarded as a waste. Numerous environmental problems may be created globally due to large volumes of fruit wastes, innovative technologies and processing of wastes are a major challenge and the aspects to dealt with.

Objectives of the study

The objective of this study was to develop the preparation method of the unripe papaya flour from waste product of fruit industry. Physicochemical and antioxidant properties of the unripe papaya flour were also investigated.

Methodology

1. Unripe papaya flour preparation

The extraction and method preparation of unripe papaya flour (UPF) was modified according to a previously described study (Rajeev Kumar P., 2012; Yusufu M. I. , 2014). The top and bottom parts of unripe papaya waste were soaked with salt for 30 minutes, then rinsed and cut into small pieces. The small pieces of unripe papaya were heated using a hot air oven at 70^{o} C for 8 hours. After heating, dried unripe papaya was ground using a high-speed universal grinder and sieved through a 100-mesh sieve. Unripe papaya flour was kept in aluminum zipper bag and stored at -20^{o} C.

2. Determination of physicochemical properties

2.1 Microstructural property

2.1.1 Scanning electron microscope (SEM)

The morphology and surface appearance were determined using scanning electron microscope (model JSM-IT300, JEOL, Tokyo, Japan)

2.2.2 Particle size distribution

The mean particle size of UPF was determined using a laser diffraction-based Malvern particle size analyzer Mastersizer 3000 (Malvern Instruments Inc., Worcestershire, UK) with a refractive index of 1.33 for water and 1.53 for flour.

2.2 Gel hydration properties

The water absorption index, the solubility index and swelling power were modified according to a previous study (De la Hera et al., 2013). Briefly, flour (50 mg) was dispersed in 1 ml of distilled water, then heated at 90^{o}C for 10 min. After cooling, the sample was centrifuged at 5000 g at 4^{o}C for 15 min. The supernatant was transferred into a microtube and recovered by heating at 105^{o}C until constant weight. Residues (Wr) and dried supernatants (Ws) were weighed and WSI or swelling capacity, solubility index and swelling power (SP) were calculated as follows

$$\text{WAI (g/g)} = (\text{Wr})/\text{Wi}$$

$$\text{WSI (g/100g)} = (\text{Ws})/\text{Wi} \times 100$$

$$\text{SP (g/g)} = (\text{Wr})/(\text{Wi-Ws})$$

2.3 Bulk density

Bulk density was performed following a previous method with minor modifications (Yu et al., 2018). UPF was filled in a graduate cylinder, and then tapped gently until flour sample was tightly packed. The volume of the flour sample after filling was marked. The bulk density was calculated as the weight of flour divided by flour volume (g/ml).

2.4 Color measurement

The colors of flour samples were determined using a CIE Hunter Lab colorimeter. The results were expressed in lightness (*L** value), redness (*a** value) and yellowness (**b* value).

3. Antioxidant activity

3.1 Sample extraction

The method of extraction was done according to a described study by Maisarah et al. (2013). The sample (200 mg) was extracted with 80% methanol then incubated at room temperature with 200 rpm shaker for 2 hours. Thereafter, the mixture was centrifuged at 100 rpm for 15 min. The supernatant was kept and dried with an evaporator.

3.2 Total phenolic compound

Total phenolic content was determined using the Folin Ciocalteu method as described previously method (Maisarah et al., 2013). Briefly, sample in methanol (10 mg/ml) was mixed with 10-fold dilution of Folin Ciocalteu reagent, then incubated for 5 min. Next, 6% sodium bicarbonate solution was added and incubated for 90 min. The absorbance was read using a spectrophotometer at the wavelength of 725 nm. Gallic acid will be used for standard curve. The results were presented as Gallic acid equivalents (GAE) in mg per 100 g DW.

3.3 FRAP assay

FRAP assay was performed according to a previous study with minor modifications as describe (Jorjong et al., 2015). In short, Sample was mixed with FRAP reagent and incubated for 30 min. Spectrophotometer was used for analysis at wavelength 565 nm. Iron (II) sulfate solution was used for standard curve. The results were expressed as micromole ferrous ion per gram dry weight (µmol Fe (II)/g DW).

3.4 β-carotene content

3.4.1 Sample extraction

The extraction of UPF was done according to a previous report (Gayosso-García Sancho et al., 2011) with minor modification. Briefly, 1 g flour and 1 g ascorbic acid and distilled water were mixed in a tube. Thereafter, 80% KOH and 0.1% BHT in ethanol was added, then stirred and heated with a water bath shaker at 50^{o} C for 30 minutes. After cooling down, the mixture of hexane and ethyl acetate (8:2) was added and left until the appeal of separation. The upper clear part was kept in amber bottle, then re-extracted the left part until the upper layer become colorless. The total supernatant was dried with nitrogen gas and stored at -20^{o}C for further analysis.

3.4.2 HPLC analysis

The sample was dissolved with 1 ml of hexane and injected into HPLC system with UV-detector. A reverse phase column 5 µm, 150 mm C18 with gradient elution was used. The HPLC conditions were performed according to a previous report (Zhong et al., 2016) with minor modifications. Solvent A [80% acetonitrile, 15% methanol, and 5% dichloromethane (v/v)] and solvent B [30% acetonitrile, 20% methanol, and 50% dichloromethane (v/v)] were used and the condition was follows: 5–70% B (0–18 min), 70–5% B, (18–20 min), and 5% B, (20–25 min) with 0.8 ml/min flow rate and UV detection was performed at 458 nm wavelength. β-carotene was identified by comparing retention time with a reference standard. The concentration of β-carotene was calculated from the standard curve of β-carotene.

4. Statistical analysis

Data are expressed as mean ±SEM, n= 3. Data was analyzed using independent sample t-test. Difference was considered statistically significant at $P < 0.01$.

Results

1. Unripe papaya flour preparation

After all unripe papaya preparation process, unripe papaya flour was produced, and the percent yield was 1.30 ± 0.07%.

2. Physicochemical properties

2.1 Morphology

Particle size distribution was employed by a laser diffraction-based Malvern particle size analyzer. The mean particle size was 140.8±2.1 µm. According to Figure 1 from scanning electron microscope (SEM), the morphology presented irregular shape of flour.

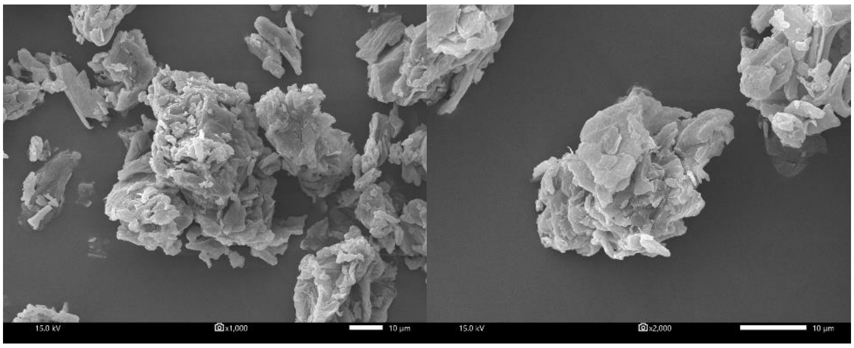

Figure 1 Scanning electron microscopy (SEM) images of UPF (left-1000x, right-2000x)

2.2 Gel hydration properties, bulk density and color measurement

Gel hydration properties were indicated by water absorption index, water solubility index and swelling power. It showed that UPF had significantly higher WSI and SP than wheat flour whereas WAI and bulk density were not difference. The results of color measurement presented in Table 1 Unripe papaya flour exhibited light yellow color (Figure 2).

Table 1 Gel hydration properties, bulk density and color measurement of flours

Sample	WAI (g/g)	WSI (g/100g)	SP (g/g)	Bulk density (g/ml)	Color parameters		
					L*	***a****	***b****
WF	0.81±0.00[a]	3.91±0.58[a]	0.85±0.00[a]	0.53±0.02[a]	34.1±0.0[a]	0.6±0.0[a]	1.8±0.0[a]
UPF	0.77±0.01[a]	15.55±1.37[b]	0.91±0.00[a]	0.58±0.01[a]	30.6±0.0[b]	0.8±0.0[b]	6.0±0.0[b]

WF: wheat flour; UPF: Unripe papaya flour.

WAI: water absorption index; WSI: water solubility index; SP: swelling powder.

Data were expressed as mean ± SEM, n = 3.

Values followed by different letters within each column indicate significant differences (P<0.01).

Figure 2 Wheat flour (left) and Unripe papaya flour (right)

3. Antioxidant properties

The effects of unripe papaya flour on different antioxidant activity are shown in Table 2 The total polyphenol content of UPF was 2-fold higher than wheat flour. The content of β-carotene in Unripe papaya flour was 39±3.3 µg/100 g flour. Moreover, the FRAP value of UPF had significantly higher than wheat flour.

Table 2 The total phenolic content and antioxidant activity of wheat flour and unripe papaya flour

Sample (per 100 g DW)	**TPC (mg GAE)**	**FRAP (µmol Fe (II))**	**β-carotene content (µg)**
WF	36.09±1.27[a]	7.19±0.08[a]	ND
UPF	85.67±1.62[b]	411.58±38 [b]	39±3.3

WF: wheat flour; UPF: Unripe papaya flour; ND: not determined.

Total phenolic content (TPC) was expressed as milligram gallic acid equivalents (GAE)/100 g DW. FRAP was expressed as micromole ferrous/100 g DW.

Data were expressed as mean ± SEM, n = 3. Values followed by different letters within each column indicate significant differences ($P<0.01$).

Discussion and conclusion

In the current study, unripe papaya flour was made from the top and bottom part of waste unripe papaya from fruit processing industry using a hot air oven and a high-speed universal grinder, then sieved through 100-mesh for particle size. It has been shown that the particle size of flour could affect quality of food product, especially bakery product (Sakhare et al., 2014). For wheat flour has average particle size around 200 µm (Codex alimentarius, 1995). In the current study, the average particle size of papaya flour (<150 µm) was observed which are similar range of particle size of other flours from waste products including pineapple stem (9.96 µm), unripe banana (80-156 µm) and passion fruits (<400 µm) (López-Vargas et al., 2013; Nakthong et al., 2017; Segundo et al., 2017). The different particle size of flour made from fruit waste depend on species and the method of preparation (Yu et al., 2018). The different particle size can be affected to the specialty and quality of end products (Sakhare et al., 2014). It suggested that flour representing particle size > 150 µm could decrease quality and sensory attributes of food product (Segundo et al., 2017). Meanwhile, small size of flour can increase hydration and improve quality of bakery products (Sakhare et al., 2014; Segundo et al., 2017). According to the observed particle size, it suggests that UPF may be applied as wheat partial replacement in bakery products. The morphology of unripe papaya flour showed irregular shape which may be due to the milling method. However, it still restores substances of granule including dietary fiber indicating by the compact and aggregate structure. From previous study suggested morphological properties of flour were correlated with amylose content, swelling power and water–binding capacity (Pelissari et al., 2012; Singh et al., 2003; Yu et al., 2018). The findings indicate that water solubility index and swelling power of UPF exhibited higher than wheat flour, suggesting that dietary fiber content in papaya might play a role in these properties (Oloyede, 2005). In a previous study, WSI contributes to final quality of food product in relation to the ability to dissolve component in aqueous solution, resulting in increase in stickiness of food products. The current findings suggest that UPF could increase stickiness in food product which might be applied in a food product concerning in viscosity such as pancake and cereal drink (Kraithong et al., 2018). The color of flour could be one of the important factors for the consumer's selection (Franklin et al., 2017; Klunklin, Savage, 2018). The results indicate that lightness (*L** value) of UF had lower than WF, whereas redness (*a** value) and yellowness (**b* value) of UPF had higher than WF. It suggests that high light-yellow color of unripe papaya might be partly attributed to the color of β-carotene. Furthermore, the total polyphenol and FRAP of UPF were significantly higher than that of wheat. It has been shown that flour made from papaya contains phytochemical components such as β-carotene, vitamin C, procyanidin, gallic acid, catechin, ρ-coumaric acid, epicatechin and quercetin (Oboh et al., 2015). These components demonstrated antioxidant activity by acting as a reductant in a redox-linked reaction, wherein Fe^{3+} is reduced to Fe^{2+}at low pH (Pulido et al., 2000). In addition, β-carotene has the ability to quench singlet oxygen and scavenge free radicals against lipid peroxidation (Krinsky, Johnson, 2005). It is suggested that phytochemical compounds are partly responsible for the antioxidant activity of UPF.

In conclusion, the particle size of unripe papaya by-products flour (UPF) prepared by heating, high-speed grinding and sieving was<150 µm with yellow color and an irregular shape. UPF also had high water solubility index and β-carotene content with antioxidant activity. The results suggest that the unripe papaya flour can be regarded as a good source of natural antioxidants for development of healthy products.

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