International Journal of Agricultural Technology 2020 Vol. 16(2): 517-528
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Effect of harvesting period on physicochemical properties and *in vitro* digestibility of banana flour

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Virulchatapan, P. and Luangsakul, N. (2020). Effect of harvesting periods on physicochemical properties and *in vitro* digestibility of banana flour. International Journal of Agricultural Technology 16(2): 517-528.

Abstract The research finding focused on the effect of unripe bananas with 3 different harvesting times of 60, 70 or 80 days after blooming (DAB) on the physicochemical properties *in vitro* digestibility and yield of banana flour. The results showed that peel color changed from green to light green with increasing DAB and that the diameter and pulp weight of fruit at 80 DAB had significantly ($p \geq 0.05$) larger diameter (35.82 ± 2.74 mm) and higher weight (116.69 ± 1.64 g) than either 60 or 70 DAB. The amount of pulp per finger was not significantly different ($p \geq 0.05$) with values in the range of 57.25 to 69.89%. The yield of banana flour ranged between 19.36-25.12%. The pH of flour from bananas 70 DAB was 5.19 ± 0.09 and 80 DAB was 5.30 ± 0.09 both of which were significantly higher ($p \geq 0.05$) than banana flour from 60 DAB. The total soluble solids of all the samples was not significantly different ($p \geq 0.05$). The swelling power of all samples were not significantly different ($p \geq 0.05$) but solubility of the flour after 80 DAB was the lowest. The time of harvest of all the samples showed no significant effect ($p \geq 0.05$) on gelatinization temperature, which ranged 80.98 to 83.14 ℃, other thermal properties and resistant starch (RS) content ranged between 41.42 to 49.55% but estimated glycemic index of 60 DAB sample (45.77 ± 0.88) with significantly higher ($p \geq 0.05$) than 70 and 80 DAB samples.

Keywords: Harvesting period, Banana flour, Physicochemical property and *In vitro* digestibility

Introduction

Various types of banana (*Musa* triploid and diploid varieties and cultivars) are produced in large quantities in the tropics and are highly nutritious with particularly high levels of fiber, potassium and starch (Jiang *et al.*, 2015; Zhang *et al.*, 2005). Bananas are a major export from several Asian countries including Thailand that exported 1.9 million tonnes in 2017 (FAO, 2018), mainly to Japan and China with a value of more than 90 million dollars per year. Bananas are usually eaten fresh but can be processed into products

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such as flour (Alkarkhi *et al.*, 2011), puree (Yap *et al.*, 2017) and chips (Singthong and Thongkaew, 2009). Banana flour is mainly obtained from unripe bananas because of its starch content and dietary fiber (Campuzano *et al.*, 2018; Singham, 2014) and it has been proposed as an ingredient for noodles (Choo and Aziz, 2010), pasta (Moongngarm *et al.*, 2014; Ovando-Martinez *et al.*, 2009), many other types of bakery products (Agama-Acevedo *et al.*, 2012; Juarez-Garcia *et al.*, 2006), as a thickening agent in some foods such as ice cream (Yangılar, 2015) and as a prebiotic in fermented milk (Batista *et al.*, 2017). Flour from unripe bananas has a low glycemic index that gives it an advantage over many other flours (such as rice and wheat) in that it is considered healthier and especially for those suffering from type II diabetes (Campuzano *et al.*, 2018). In the above research most of the banana flour was obtained from unripe dehydrated bananas (using different stages of ripeness) that were dried by various methods and ground into banana powder. Falade and Oyeyinka (2015) reported that flour obtained from unripe bananas contained a higher proportion of starch and had a brighter color than that obtained from the ripe bananas because starch is converted to sugar, as the fruit ripens, and during processing any sugar will be subjected to the Maillard reaction some of whose products are brown in color. Wang *et al*. (2014) showed that the solubility increased and swelling power decreased of flour with made from increasing ripeness of bananas. Campuzano *et al*. (2018) found that the flour made from unripe banana had a high resistant starch content that results in slow digestibility of starch by an enzyme and low glycaemic index. The resistant starch of unripe banana flour was in the range of 52.2-68.1% that was more than that of ripe banana flour (Vatanasuchart *et al.*, 2012; Wang *et al.*, 2014).

The maturity at which bananas are harvested clearly affects their quality and ripening (Ahmad *et al.*, 2001). There are several ways used to determine the most appropriate time to harvest bananas including shape (called angularity) and thickness (called calliper grade) of the individual fingers. However, the most common method used is based on the number of days after bunch emergence (used throughout the world for fresh bananas for export and called "ribbon tagging"). In Sri Lanka it is 56-63 days for the local market and for export in Ecuador it is up to 84 days maximum and in the Windward Islands up to 91 days maximum (Thompson *et al.*, 2019). However, in some areas where banana cultivation has encountered damage from the natural disasters, such as wind, storm or floods, bunches that are not sufficiently mature may be discarded. Therefore, using unripe banana of different maturities for extraction of flour is a possible solution to reduce wastage from disasters. Also, extraction of flour can be used to add value to bananas when there is an oversupply in the market.

The research findings were investigated the effects of the maturity of bananas harvested 60, 70 or 80 days after blooming (DAB) on physicochemical properties (yield, pH, total soluble solids, swelling power and solubility of flour), pasting properties, thermal properties, resistant starch content and estimated glycemic index of flour made from these fruits.

Materials and Methods

Sample collection

Unripe bananas (*Musa acuminate*, Gros Michel, AAA group 'Kluai Hom Thong') were harvested at different maturities of 60, 70 or 80 days after blooming in November from a farm at Pathumthani province, Thailand with 3 bunches of each harvest maturity.

Physical properties of banana fruit

The color of the peel of each finger was measure using a Minolta CR-400, their diameter using Vernier calipers, their weight and the ratio of pulp (%) between weigh of pulp and whole fruit. Each treatment was replicated three times.

The preparation of banana flour

Each banana fruits was peeled, while still unripe, and immediately dipped in citric acid solution (0.5% w/v) and cut into 5 mm thick slices and immersed again in citric acid solution for 10 minutes then dried in tray dryer (Progress Co., Ltd, Thailand) at 60 ℃ with an air velocity of 1.0 m/s for 16 hours then ground into flour using pin mill (ZM200, Retsch, Germany). The banana flour samples were screened through 200 μm sieve, packed in polyethylene Ziploc plastic pouches and stored in a desiccator at room temperature (about 30^{o}C) and replicated three times.

PH and total soluble solids

The flour samples (8 g) were stirred with 100 mL of distilled water for 5 minutes and allowed to stand for 30 minutes, filtered and the pH (Suntharalingam and Ravindran, 1993) and total soluble solids (TSS) of the filtrate (using hand refractometer, Atago PAL-1, Japan) were measured.

Swelling power and solubility

Swelling power was determined according to method of Leach *et al.* (1959) (Kusumayanti *et al.*, 2015). 0.1 g of banana flour was added to 10 mL of distilled water and heated in a water bath at 60 ℃ for 30 minutes with continuous stirring. The slurry was centrifuged at 1600 rpm for 15 minutes and the sediment was weighted and swelling power was calculated by this equation:

$$\text{Swelling power} = \frac{\text{weight of sediment (g)}}{\text{weight of sample (g)}} \qquad (1)$$

Water solubility was determined using the method of Kainuma and Odat (1967). 0.5 g of banana flour was added to 10 mL of distilled water and heated at 60 ℃ for 30 minutes without stirring. The slurry was centrifuged at 1600 rpm for 10 minutes, then 5 ml of supernatant was separated, dried, weighed and water solubility was calculated using following equation:

$$\text{Solubility (\%)} = \frac{\text{weight of solid after dry (g)}}{\text{weight of sample (g)}} \text{ x 2 x 100} \qquad (2)$$

Pasting properties

The pasting properties of banana flour samples were measured using Approved Method 61-02 (AACC, 2000) with a Rapid Visco-Analyzer (RVA-4 Series, Newport Scientific, Australia). A 3 g sample was dispersed in distilled water (25 ml) and stirred in an RVA container at 160 rpm for 10 s. The temperature profile was started from 50 ℃ for 1 min, then heated to 95 ℃ for 3 min and 42 s, held for 2 min and 30 s and finally cooled to 50 ℃ in 3 min and 48 s at a rate of 6 ℃/min.

Thermal analysis

The gelatinization properties were determined using differential scanning calorimetry (DSC; New Castle, USA). 2g samples were weighed on an aluminum pan, 7 mL of deionized water was added, sealed and kept for 1 day before the analysis. An empty aluminum pan was used as a reference. The heating program was a range of temperature from 10 to 120 ℃ at a heating rate of 10 ℃/min.

Resistant starch content (RS) and estimated glycemic index (eGI)

The resistant starch content and estimate glycemic index were analyzed using the method of AACC method 32-40.01 (AACC, 2000) with slight modification. Each sample (100±5 mg) was incubated with 4 mL of pancreatic α-amylase (10 mg/mL) in 4 mL of 0.1 M sodium maleate buffer (pH 6.0) and shaken in a water bath at 37 ℃. For the eGI analysis, it was incubated for 30, 60, 90, 120, 150 or 180 min and resistant starch was incubated for 16 hr. After incubation, 8 ml of ethanol (99%v/v) was added and centrifuged at 1500 g for 10 min. The supernatant was decanted into a 100 mL of volumetric flask and the sediment washed with 8 mL of ethanol (50%v/v) and centrifuged again for 10 min. The supernatant was combined, volume adjusted to 100 mL with sodium acetate buffer (pH 4.5) and glucose content was measured using a glucose oxidase-peroxidase kit. RS content was analyzed using a Resistant Starch Assay Kit (Megazyme, Ireland) according to the manufacturer's instructions.

The kinetics of starch hydrolysis of the banana flour was calculated using the following equation (Goñi *et al.*, 1997):

$$C = C\infty \, (1 - e^{-kt}) \qquad (3)$$

Where C, C∞, and k were the percentage of starch hydrolyzed at time t (min), the equilibrium percentage of starch hydrolyzed after 180 min and the kinetic constants, respectively.

The hydrolysis curve area (AUC) was calculated using the following equation:

$$AUC = C\infty \, (t_f - t_0) - (C\infty / k)(1 - \exp^{(-k(tf - t0))}) \qquad (4)$$

Where t_f and t_0 were the final time (180 min) and the initial time (0 min), respectively. The hydrolysis index (HI) was calculated by dividing the area under the hydrolysis curve (0-180 min) of the sample by the area of a reference sample (white bread).

The eGI was calculated using the Goñi *et al*. method (1997) with the following equation:

$$eGI = 39.71 + 0.549HI \qquad (5)$$

Statistical analyses

The data of experiments were analyzed using one-way analysis of variance (ANOVA). The means were compared using Duncan's Multiple Range Test comparison ($p < 0.05$) by SPSS V.24.0 statistical software (SPSS Institute Inc., Chicago, IL, USA).

Results

Physical properties of banana fruit

Physical properties of ‘Kluai Hom Thong’ bananas which were harvested at 60, 70 or 80 days DAB as are shown in Table 1. It was observed that the peel color changed when increasing DAB. The brightness (L^*) and yellowness (b^*) values for the 80 DAB fruit were the highest. The results of the diameter of all bananas were significantly different ($p < 0.05$). The diameter of the fruit was significantly different ($p < 0.05$) with the 80 the largest and 60 DAB the smallest. The same effect was observed on the weight pulp per fruit. The was no significantly differences ($p \geq 0.05$) in the pulp:peel ration with values in the range of 57.25 to 69.89.

Physicochemical properties of banana flour

The physicochemical properties of banana flour from unripe bananas with different harvesting periods were presented in Table 2. The results showed that the percentage of flour yield was in the range of 19.36 to 25.12 which was not significantly different ($p \geq 0.05$) between harvest maturities. The pH of the samples for 70 and 80 DAB had pH levels of 5.19 ± 0.09 and 5.30 ± 0.09, respectively, which was significantly higher those from 60 DAB. TSS of the samples were not significantly different ($p \geq 0.05$), with values in the range of 0.9 to 1.0.

The result of swelling power and water solubility are presented in Table 2. The samples from 70 and 80 DAB had swelling powers of 3.31 ± 0.28 and 3.48 ± 0.33 respectively, which were not significantly different ($p \geq 0.05$) to 60 DAB. The water solubility values of the 80 DAB samples were significantly lower ($p \geq 0.05$) then the other two at 5.56 ± 0.46 %.

Pasting properties

There were no significant differences ($p \geq 0.05$) between the three harvest maturities on pasting temperature (PT), but the bananas harvested 60 DAB had significantly lower ($p \geq 0.05$) pasting viscosity (PV) than the other two and breakdown (BD) of the 70 DAB was significantly higher ($p \geq 0.05$) than the other two. The final viscosity (FV) was highest for 60 DBA followed by 80 and 70 DAB and setback (SB) was higher for 60 DBA with no significant differences ($p \geq 0.05$) between 80 and 70 DAB.

Table 1. Physical properties of unripe bananas at 60, 70 or 80 days after blooming

Harvesting period (day)	Color of peel			Diameter of fruit (mm.)	Amount of pulp ^{ns} (%)	Weight of pulp (g/fruit)
	L*$^{/1}$	a*$^{/2\ ns}$	b*$^{/3}$			
60	54.98 ± 3.12^{b}	-16.39 ± 0.85	26.30 ± 2.27^{b}	31.79 ± 4.46^{c}	59.44 ± 2.17	85.24 ± 2.13^{b}
70	56.41 ± 4.25^{b}	-16.60 ± 0.76	26.83 ± 5.33^{b}	33.87 ± 3.27^{b}	62.42 ± 8.64	99.80 ± 9.45^{ab}
80	59.88 ± 5.34^{a}	-16.52 ± 0.89	29.15 ± 1.72^{a}	35.82 ± 2.74^{a}	63.21 ± 6.68	116.69 ± 1.64^{a}

Data are means ± standard deviation (n=3). Values within the same column with different letters were significantly different at $p<0.05$.

^{ns}/ Not significant for t-test at 95% level confidence.

[1] / Lightness

[2] / Greenness

[3] / Yellowness

Table 2. Physicochemical properties of banana flours produced from unripe bananas at 60-, 70-, and 80-days of the harvesting periods

Harvesting period (days)	yield^{ns} (%)	pH	TSS$^{/1ns}$ (°Brix)	Swelling power^{ns}	Solubility (%)
60	22.68 ± 3.32	4.72 ± 0.02^{b}	0.95 ± 0.00	3.50 ± 0.35	7.59 ± 0.71^{a}
70	22.92 ± 1.03	5.19 ± 0.09^{a}	0.90 ± 0.05	3.31 ± 0.28	7.04 ± 0.38^{a}
80	23.20 ± 1.90	5.30 ± 0.09^{a}	0.90 ± 0.00	3.48 ± 0.33	5.56 ± 0.46^{b}

Data is mean ± standard deviation (n=3). Values within the same column with a different letter were significantly different at $p<0.05$.

^{ns}/ Not significant for t-test at 95% level confidence.

[1] / Total soluble solids

Table 3. Pasting properties of banana flour produced from unripe bananas at 60, 70 or 80 days after blooming

Days after blossoming	PT[/1ns] (℃)	PV[/2] (cP)	FV[/3] (cP)	BD[/4] (cP)	SB[/5] (cP)
60	81.72 ± 0.74	6374.50 ± 45.96^{b}	5643.50 ± 12.02^{a}	1688.00 ± 41.01^{c}	957.00 ± 16.97^{a}
70	82.01 ± 0.37	7251.50 ± 54.45^{a}	5026.00 ± 33.94^{c}	2704.50 ± 91.22^{a}	479.00 ± 2.83^{b}
80	82.68 ± 0.46	7325.00 ± 24.04^{a}	5346.00 ± 24.04^{b}	2389.50 ± 37.48^{b}	410.50 ± 37.48^{b}

Data are means ± standard deviation (n=3). Values within the same column with a different letter were significantly different at $p<0.05$.

[ns]/ Not significant for t-test at 95% confidence level.

[1]/ Pasting temperature

[2]/ Peak viscosity

[3]/ Breakdown (peak viscosity- holding strength)

[4]/ Final viscosity

[5]/ Setback from trough (final viscosity - holding strength).

Thermal properties

There were no significant differences ($p \geq 0.05$) between the three harvest maturities for onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy gelatinization (ΔH) for the banana flour. T_o ranged from 74.23 to 74.48 ℃, T_p ranged from 76.56 to 77.41 ℃, T_c ranged from 80.61 to 81.01 ℃ and ΔH ranged from 3.06 to 3.75 ℃, respectively.

Table 4. Thermal properties of banana flours produced from unripe bananas at 60, 70, and 80 days after blooming

Days after blossoming	To[/1ns] (℃)	Tp[/2ns] (℃)	Tc[/3ns] (℃)	ΔH [/4ns] (J/g)
60	74.23 ± 0.22	76.95 ± 0.15	80.63 ± 0.40	3.75 ± 0.28
70	74.48 ± 0.26	77.41 ± 0.49	81.01 ± 0.69	3.19 ± 0.37
80	74.45 ± 0.33	76.53 ± 1.56	80.61 ± 0.55	3.06 ± 0.41

Data is mean ± standard deviation (n=3).

[ns]/ Not significant for t-test at 95% level confidence.

[1]/ Onset temperature

[2]/ Peak temperature

[3]/ Conclusion temperature

[4]/ Enthalpy gelatinization.

Table 5. The resistant starch (RS, %) and estimated glycemic index (eGI) of banana flours produced from unripe bananas at 60, 70, and 80 days after blossoming

Days after blossoming	**RS** $^{/1ns}$ **(%)**	**eGI** $^{/2}$
60	41.74±0.32	45.77 ±0.88^{a}
70	46.35±3.2	43.86 ±0.18^{b}
80	43.26±0.03	43.43 ±0.33^{b}

Data is mean ± standard deviation (n=3). Values within the same column with a different letter are significantly different at $p<0.05$.

^{ns}/ Not significant for t-test at 95% level confidence.

^{1}/ Resistant starch content

^{2}/ Estimated glycemic index.

The resistant starch (RS) and estimated glycemic index (eGI)

The RS content and eGI of the samples are presented in Table 5. The banana flour made from 60, 70 and 80 DAB had the RS contents of 41.74±0.32%, 46.35±3.2% and 43.26±0.03% respectively. The eGI of flour from 60 DAB was 45.77±0.88 which corresponded negatively to its RS content.

Discussion

Physical properties of banana fruit

The degradation of chlorophyll during banana ripening and the unmasking of carotenoids is well established in bananas and would explain the effect on peel color (Moongngarm *et al.*, 2014). Also, the progressive increase in diameter of bananas during growth and maturation and changes in their pulp:peel ratio is well established Kheng *et al.* (2012) and Moongngarm *et al.* (2014).

Physicochemical properties of banana flours

The yield of banana flour increased slightly but not significantly during maturation, which supports the findings of Falade and Oyeyinka (2015) who reported that the yields of banana flour depended on the increase of the moisture content of banana pulp with increasing maturity of banana.

The tendency of the pH of the banana flour samples to slightly increase was compatible with the results of Mustaffa *et al.* (1998), who reported that the pH of bananas started to increase from week 3 until week 12 of development on the plant. The pH changes might also be dependent on malic acid content which was found to be low at the first stages of ripening but increased during ripening (Wills *et al.*, 1984). The fact that the TSS of the banana flour was not affected by harvest maturity might be explained by the fact that the fruit from all three maturities were still unripe and had similar low sugar content (data not shown). This result was similar to the research of Alkarkhi *et al.* (2011). They reported that pH and TSS of banana flour increased with the increasing of ripeness.

The swelling power of all the banana flour samples were not significantly different but their solubility decreased, which might be the reason for the lower sugar content found in the unripe banana flour with the increasing harvest maturity (data not shown) and changing of the strength structure from the less solubility of starch molecule.

Pasting properties of banana flours

Pasting temperature values of the samples are related to the temperature where the initial viscosity changes. The PV value had the highest viscosity while gelatinization is related with hydrogen bond and amylose content. The PV of the banana flours at the 70 and 80 DAB was higher than that of 60 DAB which might be related with the higher swelling power of those samples (Moongngarm *et al.*, 2014). In addition, the FV and SB of banana flours at the 60 DAB had higher values than those of the banana flours from the other two harvest times. The SB value can represent the repacking of amylose in starch granules. This refers to the reorganization of the starch molecules of the banana flour at the 60 DAB proved to have the tighter and more have more stable starch molecules that those of banana flours from the other two of later harvest periods.

Thermal properties of banana flours

The result of T_o corresponded with the previous result of pasting temperature but the flour samples from all three harvesting times did not significantly ($p \geq 0.05$) affect $T_{o,}$ T_p, T_c and ΔH value. This result might be explained by all the samples from the three different harvesting periods were all unripe bananas which had a similar starch molecular structure.

Resistant starch and estimated glycemic index of banana flours

RS of banana flour samples relates to the starch digestion which corresponds to the degree of resistance to the hydrolysis of enzymes in the human digestive system. The banana flour samples of the banana flours from the 70 and 80 DAB had higher RS content than those from 60 DAB. According to Moongngarm *et al.* (2014) and Inchuen (2002), banana flour obtained from unripe banana harvested between 75 to 105 days had higher RS content with increasing the harvesting time because bananas accumulate more starch as the fruit size increased, which was evidenced by the increase in diameter and weight of fruit. The amount of resistant starch was negatively related to the estimated glycemic index (eGI). The increase in resistant starch could lead to the lower of eGI (Ratnaningsih *et al.*, 2017) as was shown by the 70 and 80 DAB, which were lower than that of the 60 DAB.

In conclusion, this study indicated that the time between blossoming and harvest of bananas affected their physical properties, including the color of peel, size, and weight of fruit, pH, solubility, eGI and some characters of pasting properties. The properties of banana flour harvested 70 or 80 DAB were similar. However, the bananas harvested at 80 DAB were recommended for utilization in production of the unripe banana flour as they gave the highest yield of flour.

Acknowledgement

The authors would like to offer gratefully thank Professor Keith Thompson for suggestion, help and language corrections of this paper.

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(Received: 6 August 2019, accepted: 18 February 2020)