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Full Paper

Resistant starch content, in vitro starch digestibility and physico-chemical properties of flour and starch from Thai bananas

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Abstract: Flour and starch were prepared from six Thai banana cultivars: Kluai Hom, Kluai Khai, Kluai Lebmuenang, Kluai Namwa, Kluai Hakmuk and Kluai Hin, and their resistant starch (RS), in vitro starch digestibility and physico-chemical properties were determined. The RS content of the flour is 52.2-68.1%, with flour from Kluai Hin containing the highest amount of RS, followed by that from Kluai Hakmuk. The starch has a higher RS content (70.1-79.2%), the highest value coming from Kluai Hakmuk starch, followed by Kluai Hom starch. A significant linear relationship between apparent amylose and RS was observed. Interestingly, most of the flour showed a slower rate of in vitro starch digestibility than that of the starch, with Kluai Hin flour exhibiting the slowest rate, followed by Kluai Namwa. Rapid viscosity analysis showed significantly higher peak viscosity of the starch than the flour, the highest final and setback viscosity being obtained from Kluai Hin starch. Differential scanning calorimetry showed an endothermic transition enthalpy over a range of 17.4 J/g for Kluai Lebmuenang starch to 18.6 J/g for Kluai Hin starch. X-ray diffractograms of the starches exhibited a typical B-pattern with Kluai Hin showing the highest degree of relative crystallinity (31.3%) with a sharp peak at 5.5°. The overall results seemed to indicate an effect of the BB genotype on the resistance of banana starch granules to enzymatic digestion due to amylose molecules and the crystallinity of amylopectin.

Keywords: Thai bananas, green bananas, banana flour, banana starch, resistant starch, starch digestibility

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INTRODUCTION

Bananas (*Musa* sp.) are one of the most important tropical fruits consumed worldwide by people of all age groups. The nutritional and functional properties of bananas are known to provide good health. Nutritionally, bananas contain available carbohydrates which provide energy, vitamins B and C, and significant amounts of potassium and magnesium [1]. A substantial percentage of starch in bananas consists of resistant starch (RS), which has the potential to provide significant health benefits akin to those derived from dietary fibre [2]. Due to a high solid content of 40-70% [3], bananas can be processed into flour and starch suitable for making processed health food products.

At present, a healthy choice of functional food products is of increasing interest to consumers. With properties similar to soluble and insoluble dietary fibre in the gastrointestinal tract, RS plays a major role in the health food industry [4-5]. Showing some resistance to human digestive enzymes, the slow release of glucose from RS results in reduced energy intake by the intestinal cells, which is evident from a low glycemic index of the non-digested starch [6]. This can help improve glucose regulation in diabetes and facilitate weight control for the obese [7]. The non-digested starch in the large intestine is fermented by colonic microflora, producing short-chain fatty acids that encourage the growth of beneficial bacteria [1]. This may lead to healthier colon cells and help prevent the development of colon cancer [4]. In addition, a diet high in RS can reduce blood cholesterol and triglyceride levels due to higher excretion rates of cholesterol and bile acids [7]. Overall, increasing the RS content in the diet has the potential to provide several significant health benefits and an added value to food products.

RS is defined as the sum of starch and products of starch degradation not absorbed in the small intestine of a healthy individual [8-9]. There are four types of RS: type I represents physically inaccessible starch which is locked in the plant cell walls of some foodstuffs such as partially milled grains, seeds and legumes. Type II is characterised by native granular starch found in foods containing uncooked starch such as bananas, raw potatoes and beans. The RS content in reference banana flour samples, determined by three laboratories, averages 52.1% (dry matter), while lentil flour has 8.2% RS [10]. A study of Vatanasuchart et al. [11] on eleven banana cultivars grown in Thailand also shows that the RS content observed in the common cultivars ranges between 52.2-61.4% and values for indigenous cultivars are between 50.7-68.1%. Type-III RS is made up of retrograded starch or crystalline non-granular starch such as that found in cooked potatoes, bread crust, cornflakes and retrograded high-amylose maize starch. Type-IV RS refers to specific chemically and thermally modified or repolymerised starch [8-9].

The properties of banana starch and flour are important for their utilisation in industrial food products. Several reports have suggested that banana starch contains a high level of amylose which is associated with high retrogradation [1, 11-12]. From flour of the common and indigenous banana cultivars, Vatanasuchart et al. [10] reported a high content of apparent amylose (24.7-37.1% and 29.9-35.9% respectively). The findings of Nimsung et al. [3], however, indicated a lower amylose content in banana starch isolated from Kluai Khai and Kluai Hom cultivars. A study of Tongdang and Saasagul [13] showed two stages of swelling of starch from Kluai Hin and Kluai Namwa cultivars as a result of a higher proportion of amylose in relation to amylopectin, while the gelatinisation enthalpy of Kluai Hin (20.16 J/g) was higher than Kluai Namwa (17.43 J/g).

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As starchy foods are a main source of energy in the diet of Thai people, a healthier choice of starchy foods that still provide beneficial functions for sustaining good health should be encouraged. Therefore, it is relevant to acquire new knowledge about the health benefits of different banana cultivars grown in Thailand and to hypothesise that both starch and flour from bananas are a good source of RS which is good for health. Our previous study [11] on RS and amylose in banana flour concerned only a chemical aspect, so more data on the functional properties of both banana starch and flour should be of use. In this study, the contents of total starch, digestible starch and RS (non-digestible starch) in six banana cultivars were determined. The rates of in vitro starch digestibility and the physico-chemical properties of banana starch and flour samples were also compared. X-ray diffraction patterns of banana starch samples were examined.

MATERIALS AND METHODS

Sample Preparation

Edible green (unripe) bananas (six cultivars), aged 90-120 days, were collected from Pakchong Research Station, Kasetsart University at Nakhon Ratchasima, and also from local markets. Different species of diploid and triploid genome groups were classified into four common cultivars: Kluai Hom (AAA), Kluai Khai (AA), Kluai Lebmuenang (AA) and Kluai Namwa (ABB), and two indigenous cultivars: Kluai Hakmuk (ABB) and Kluai Hin (BBB) [14] (Figure 1). In processing into flour, the bananas were peeled and sliced into 1-mm-thick pieces, spread evenly on a stainless steel tray, dried in a hot-air oven at 50°C for 8 hr, and then milled and passed through a 100-mesh sieve. Banana starch was prepared according to a water-alkaline extraction process adopted by Zhang et al. [15], as presented in Scheme 1.

Kluai Hom (AAA) Kluai Khai (AA) Kluai Lebmuenang (AA)

Kluai Namwa (ABB) Kluai Hakmuk (ABB) Kluai Hin (BBB)

Figure 1. Different Thai banana cultivars. (AA represents two sets of chromosomes and AAA, ABB or BBB represent three sets of chromosomes inherited from their parents.)

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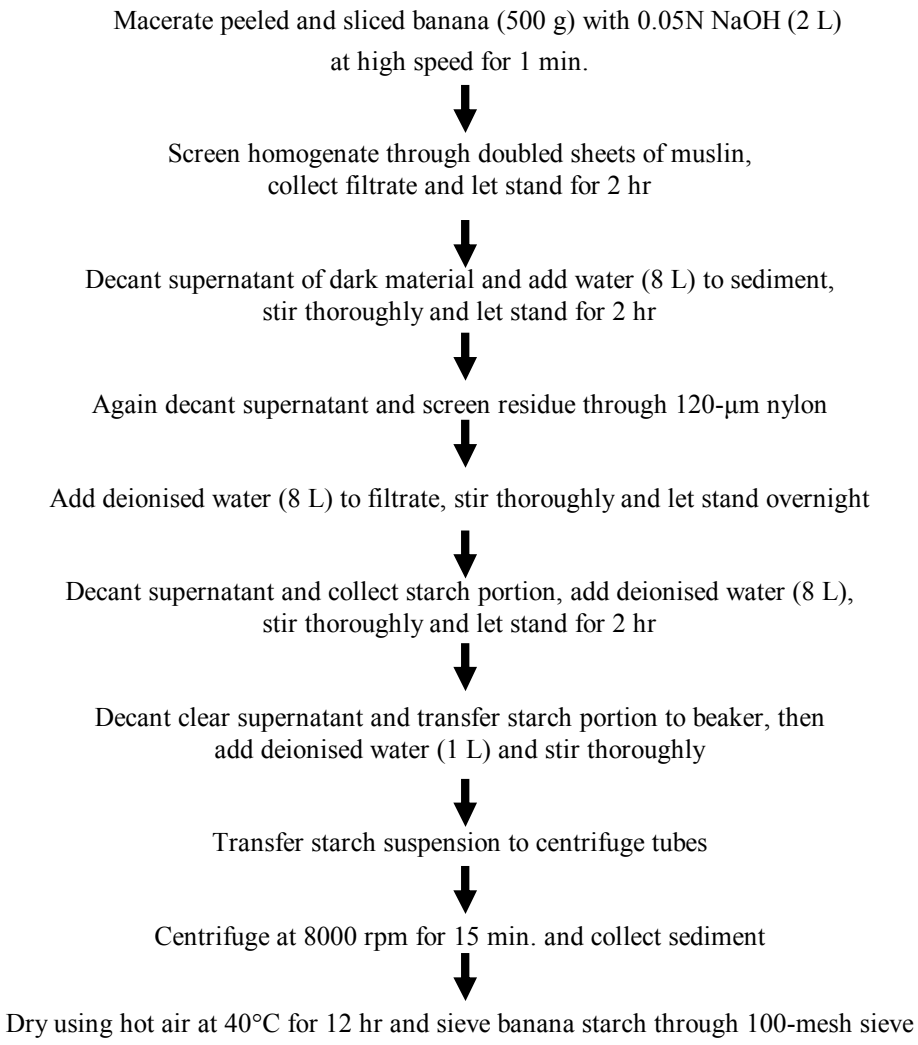

Scheme 1. Process for isolation of banana starch

Reagents and Chemicals

The hydrolytic enzymes of pancreatic α-amylase (Sigma, no.A3176; 23 IU/mg), pepsin (Merck, no.7190; 2000 FIP-U/g) and amyloglucosidase (Boehringer, no.102857) as well as a glucose oxidase-peroxidase kit (Sigma, no.G3660) were used for RS determination and in vitro starch digestibility test in this study. Amylose and amylopectin used as standards were purchased from Sigma. Other chemical agents were of analytical grade from Merck.

Determination of RS, Total Starch and Amylose

The RS content of banana samples was determined by a direct method of Goni et al. [10]. A ground sample of flour or starch (100 mg) was incubated with a solution containing 20 mg pepsin at 40°C for 60 min. to remove protein. A tris-maleate solution containing 40 mg pancreatic α-amylase was then added and the mixture incubated at 37°C for 16 hr to hydrolyse digestible starch. The hydrolysate was centrifuged and the residue was solubilised with 4M KOH and incubated with amyloglucosidase (80 μL) at 60°C for 45 min. to hydrolyse RS. The glucose content was measured using a glucose oxidase-peroxidase kit. The RS content, comprising fractions of types I and II, was calculated as mg of glucose x 0.9.

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The total starch content was determined according to a modified method of Goni et al. [16]. A 50-mg ground sample of flour or starch was dispersed in 2M KOH (6 mL) and the mixture incubated for 30 min. at room temperature. The solubilised starch was then hydrolysed by adding amyloglucosidase (60 μL) and incubating at 60°C for 45 min. in a shaking water bath. After centrifugation (15 min., 4500*g*), the glucose content in the supernatant was measured using the glucose oxidase-peroxidase kit, and the total starch content was calculated as mg of glucose x 0.9. Digestible starch content was calculated as the difference between total starch and RS or indigestible starch, expressed as per cent of the sample dry weight.

The amylose content of banana flour and starch samples was determined by a colorimetric AACC method [17]. Briefly, a 100-mg sample was gelatinised in the presence of 95% ethanol (1 mL) and 1N NaOH (9 mL) to liberate amylose molecules. Iodine solution (2 mL) was added to form an amylose-iodine complex and absorbance was read at 620 nm. The amylose content was calculated by means of a standard curve and expressed as per cent of sample dry weight.

In vitro Starch Digestibility

Following the method of Goni et al. [16], a ground sample (50 mg) of banana flour or starch was incubated with a solution containing pepsin (20 mg) at 40°C for 60 min. to remove protein, and the volume of the mixture was made up to 25 mL with tris-maleate buffer. Five mL of tris-maleate solution containing α-amylase (3.3 IU) were then added and the mixture incubated at 37°C to hydrolyse digestible starch. An aliquot sample (1 mL) was taken every 30 min. during a 3-hr period and heated at 100°C for 5 min. to inactivate the enzyme. Then amyloglucosidase (60 μL) was added to each of the aliquote samples to hydrolyse the remaining starch at 60°C for 45 min. After centrifugation (15 min., 4500*g*), the glucose content in the supernatant was measured using the glucose oxidase-peroxidase kit, and the digestible starch was calculated as mg of glucose x 0.9. The digestibility was expressed in terms of glucose release per 100 g of sample hydrolyzed at different times (0, 30, 60, 120, 160 and 180 min).

Pasting Properties, Thermal Properties and X-ray Diffraction Measurement

The pasting properties of 10% starch and flour suspensions in distilled water were determined by using a rapid viscosity analyser (RVA 4D, Newport Scientific, Australia). The determination was started at 50°C; the suspension was then heated to 95°C, maintained for 2.5 min., cooled to 50°C and held for 2 min. The total running time for each sample was 13 min [18].

Thermal properties were determined according to a method adapted from Vatanasuchart et al. [19] using a differential scanning calorimeter (Pyris I, Perkin-Elmer) equipped with a cooling system. Each starch sample (3 mg) was weighed in an aluminum pan and deionised water was added to obtain a 30% starch suspension. The cover was put on and hermetically sealed. Each sample pan was placed in the calorimeter and heated from 0°C to 120°C at 10°C/min. An empty pan was used as a reference and the instrument was calibrated using indium control. Endothermal curves exhibiting onset, peak and end temperatures and melting enthalpy (J/g of the sample weight on dry basis) of duplicate samples were recorded.

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Wide-angle X-ray diffraction patterns of starch samples were examined using a X-ray diffractometer (JDX 3530, JEOL Ltd.) operated at 30 kV and 30 mA and generating monochromatic Cu-K_α radiation of 1.542 Å. Diffractograms were obtained from 4° to 30° (2θ) at a scanning speed of 4°/min. The degree of relative crystallinity was calculated from the ratio of diffraction peak area to total diffraction area [20].

RESULTS AND DISCUSSION

Starch Content

The total, digestible, and resistant starch contents of flour and starch from the six banana cultivars are shown in Table 1. The RS content of the flour ranges between 52.2-68.1% and that of the starch, 70.1-79.2%. Kluai Hin flour has the highest RS content, followed by Kluai Hakmuk. Most of the starch samples have significantly higher RS content than that of the flour. Study by Englyst et al. [5] and Goni et al. [10] on RS in banana flour showed values of 51.3-53.1%. Also, work by Tribess et al. [21] showed a high RS content in banana flour (40.9-58.5%), similar to the findings by Faisant et al. (47.3-57.2%) [2], whereas Rodríguez-Ambriz et al. [22] found a lower RS content (30.4%). However, the overall results from the present study indicate that the indigenous Kluai Hakmuk (ABB) and Kluai Hin (BBB) cultivars in the BB genome group are rich in RS when compared to the common cultivars.

Table 1. Total starch, digestible starch, RS and amylose contents (g/100g dry weight) of different banana cultivars

Sample	Total starch	Digestible starch	RS	Amylose
Flour:				
Kluai Hom (AAA)	91.0 ± 3.1 ^{b}	33.3 ± 1.9 ^{a}	57.7 ± 1.1 ^{de}	25.0 ± 0.3 ^{c}
Kluai Khai (AA)	80.5 ± 0.3 ^{c}	28.2 ± 4.5 ^{ab}	52.2 ± 4.1 ^{e}	24.7 ± 2.5 ^{c}
Kluai Lebmuenang (AA)	72.1 ± 3.4 ^{d}	15.1 ± 3.2 ^{d}	57.0 ± 0.2 ^{de}	31.2 ± 0.8 ^{b}
Kluai Namwa (ABB)	79.7 ± 1.1 ^{c}	23.0 ± 4.8 ^{bc}	56.6 ± 5.8 ^{de}	25.8 ± 2.7 ^{bc}
Kluai Hakmuk (ABB)	72.3 ± 1.8 ^{d}	10.9±0.5 ^{def}	61.4 ± 2.3 ^{d}	27.3 ± 2.8 ^{bc}
Kluai Hin (BBB)	72.7 ± 1.4 ^{d}	4.6 ± 0.6 ^{f}	68.1 ± 2.0 ^{c}	29.9 ± 0.6 ^{bc}
Starch:				
Kluai Hom (AAA)	82.7 ± 1.0 ^{c}	6.6 ± 0.3 ^{ef}	76.1 ± 0.7 ^{ab}	38.6 ± 0.5 ^{a}
Kluai Khai (AA)	96.0 ± 2.1 ^{a}	25.5 ± 3.3 ^{b}	70.5 ± 0.0 ^{bc}	40.9 ± 0.2 ^{a}
Kluai Lebmuenang (AA)	98.0 ± 1.7 ^{a}	22.5 ± 1.4 ^{bc}	75.5 ± 3.2 ^{ab}	42.7 ± 0.5 ^{a}
Kluai Namwa (ABB)	88.9 ± 1.0 ^{b}	13.3 ± 1.6 ^{de}	75.6 ± 0.6 ^{ab}	43.8 ± 1.1 ^{a}
Kluai Hakmuk (ABB)	87.4 ± 1.3 ^{b}	8.1 ± 2.6 ^{ef}	79.2 ± 3.9 ^{a}	39.3 ± 1.6 ^{a}
Kluai Hin (BBB)	87.0 ± 0.4 ^{b}	16.9 ± 6.7 ^{cd}	70.1 ± 4.9 ^{bc}	40.3 ± 7.0 ^{a}

Notes: 1) Values are means of duplicate analysis. In a column, means not sharing a common letter are significantly different at $P< 0.05$ by ANOVA and DMRT.

2) AAA, AA, etc. are genotypes of banana cultivars.

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In Vitro Starch Digestibility

The experimental values for amylose content of the banana flour and starch were 24.7-31.2% and 38.6-43.8% respectively (Table 1). When the apparent amylose content of both the banana flour and starch was compared to the RS content, a significant linear relationship was observed, with $R^2 = 0.76$ ($p<0.05$) (Figure 3). This indicates that the resistance to enzymatic digestion of banana starch granules comes from amylose molecules. A recent study on four Indian red lentil cultivars [23] also showed a positive correlation between amylose and slow-digestible starch contents, and a negative correlation of amylose with RS contents.

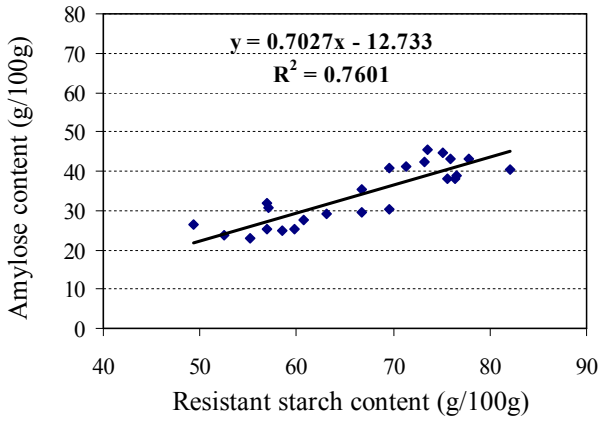

Figure 3. Linear correlation between RS content and apparent amylose content

The in vitro starch digestibility of banana flour and starch from the six cultivars, in comparison to cassava starch, is shown in Figure 4. The flour and starch of Kluai Hin were observed to show lowest digestibility during 30-180 min.of digestion. The present study also shows that the flour is more difficult to digest than the starch and that all banana flour and starch samples exhibit a lower starch digestibility compared to cassava starch.

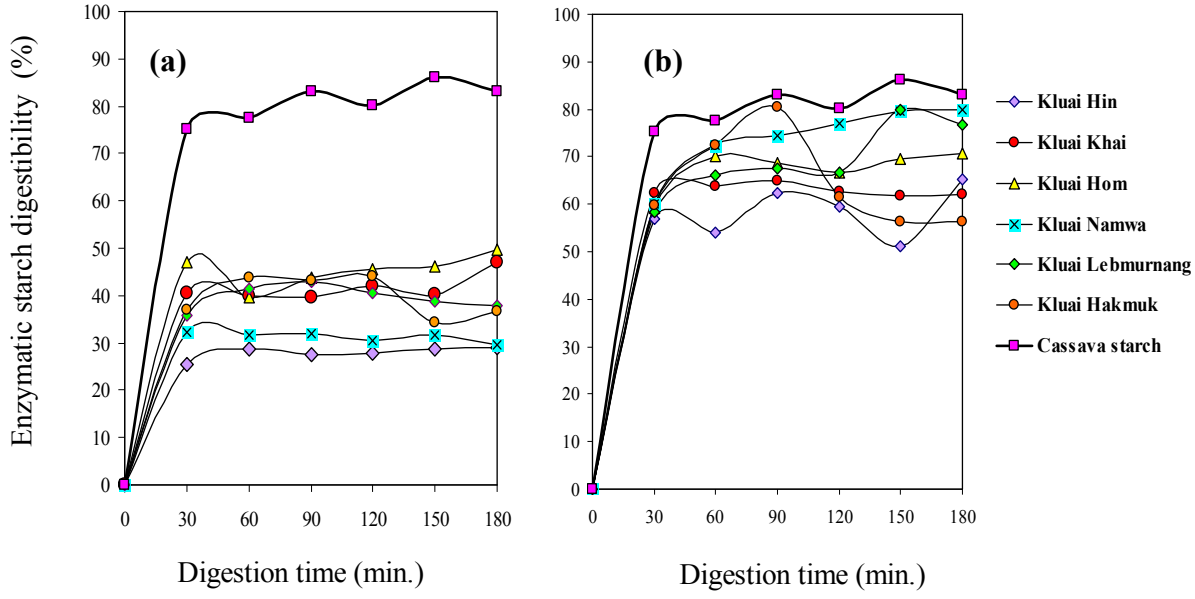

Figure 4. In vitro enzymatic starch digestibility of flour (a) and starch (b) from six banana cultivars as compared with cassava starch

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Several studies have suggested that consumption of unripe bananas confers beneficial effects on human health, a fact often associated with their high content of RS, dietary fibre or non-starch polysaccharides, and nutritive and functional components [1, 2, 24]. In particular, pectin, lignin, cellulose and hemicellulose in banana fruit can promote digestive health [25-26]. The slow digestion rate found among the banana flour might well be explained by the complexation of pectin, cellulose and hemicellulose, which can help resist digestion better than the starch alone.

Pasting Properties

The pasting behaviours of the flour and starch from six banana cultivars are shown in Figure 5. Significantly higher peak viscosities of the starch samples (250.3-304.2 RVU) than those of the flour samples (153.4-213.5 RVU) were observed. Mostly, the banana flour showed a slight change in trough and breakdown viscosities whereas the starch gave greater final viscosities (278.3-356.2 RVU) than the flour (161.0-274.8 RVU), leading to lower setback viscosities of the flour. In particular, the flour from Kluai Hin showed lowest final and setback viscosities while its starch exhibited highest values. The high final viscosities of the starch from Kluai Hin, Kluai Namwa and Kluai Lebmuenang were in accordance with their high amylose content conducive to retrogradation. The pasting of the flour and starch occurred at 79.1-85.2°C and 79.2-83.2°C respectively. These temperatures were higher than for cassava starch (69.5°C), which has a lower amylose content [18]. As previously found, the structural stability of banana starch granules is influenced both by molecules of amylose and the degree of crystallinity in amylopectin [27-28].

Thermal Properties

The thermal characteristics of starch from six banana cultivars (Table 2) as determined by differential scanning calorimetry show that the endothermic gelatinisation enthalpy of Kluai Hin starch is highest and that the starch from Kluai Namwa (ABB) and Kluai Hin (BBB) with the BB genotype has higher thermal enthalpy and higher peak and end temperatures than the starch with AAA and AA genotypes. Also, a broader gelatinisation temperature range of starch from Kluai Namwa, Kluai Hakmuk and Kluai Hin can be observed. Likewise, the findings of Nimsung et al. [3] indicated a higher gelatinisation temperature for Kluai Namwa starch compared to Kluai Hom and Kluai Khai starches. In a similar study [13], the gelatinisation temperature range of Kluai Hin starch was found to be broader (69.8-79.8°C) than that of Kluai Namwa starch (70.9-77.2°C) with the former starch also having a higher gelatinisation enthalpy. The difference in gelatinisation temperature may be attributed to the difference in the amylose content as well as the difference in the size, form and distribution of starch granules [8, 9, 27, 29]. Shamai et al. [20] reported that starch gelatinisation temperature is related to the structural characteristics of crystallinity.

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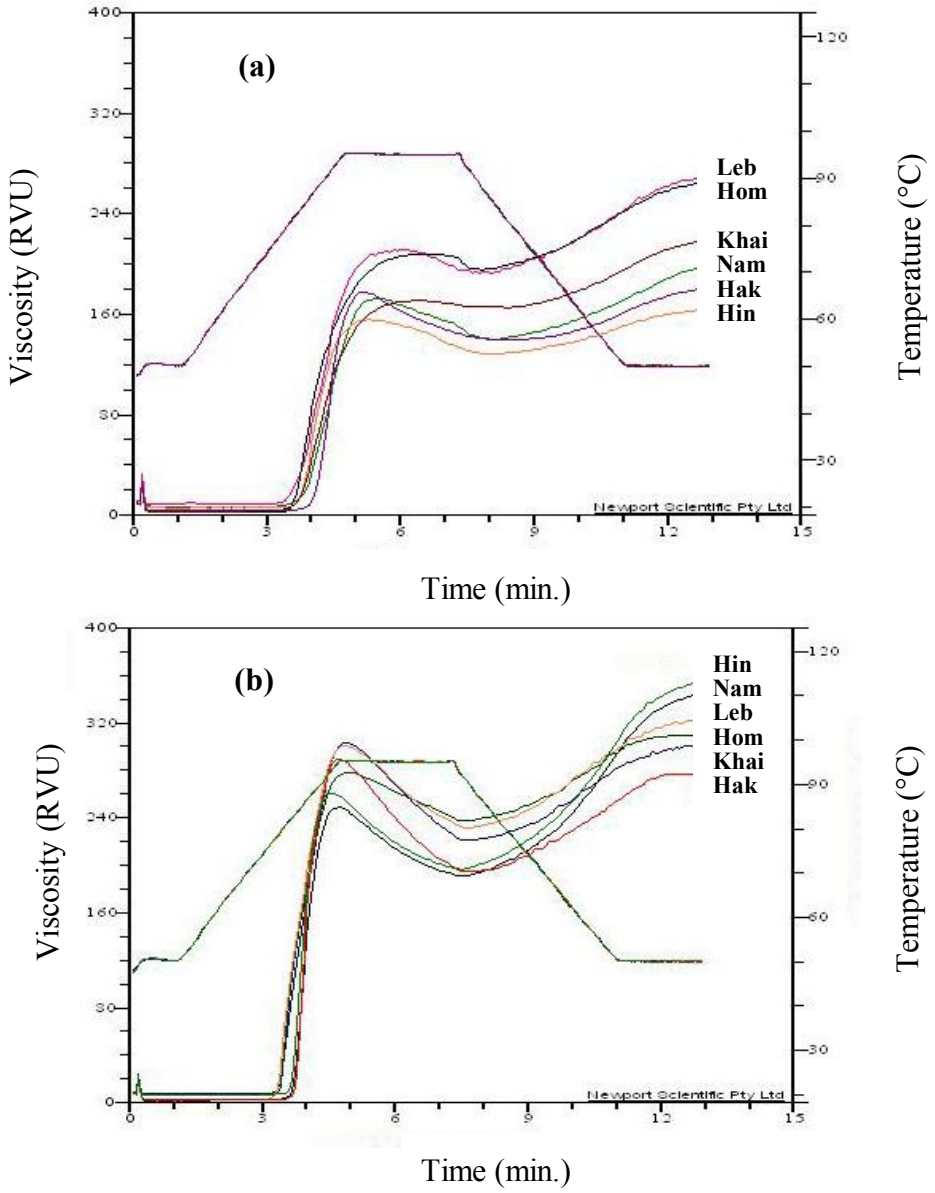

Figure 5. Viscosity curves of flour (a) and starch (b) of six banana cultivars: Kluai Hom (Hom), Kluai Khai (Khai), Kluai Lebmuenang (Leb), Kluai Namwa (Nam), Kluai Hakmuk (Hak) and Kluai Hin (Hin)

Table 2. Thermal properties of banana starch from different cultivars

Starch sample	Enthalpy (J/g)	Gelatinisation temperature (°C)		
		Onset	Peak	End
Kluai Hom (AAA)	18.1 ± 0.1	72.3 ± 0.3	74.7 ± 0.4	77.5 ± 0.5
Kluai Khai (AA)	17.5 ± 0.2	69.0 ± 0.2	72.2 ± 0.1	76.2 ± 0.3
Kluai Lebmuenang (AA)	17.4 ± 0.2	69.1 ± 0.2	72.1 ± 0.1	75.3 ± 0.2
Kluai Namwa (ABB)	18.3 ± 0.1	71.6 ± 0.1	75.8 ± 0.0	79.9 ± 0.1
Kluai Hakmuk (ABB)	17.7 ± 0.2	72.3 ± 0.1	77.1 ± 0.1	83.8 ± 0.1
Kluai Hin (BBB)	18.6 ± 0.1	72.0 ± 0.0	75.9 ± 0.1	79.6 ± 0.1

Note: Values are means of duplicate analysis.

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X-ray Diffraction

X-ray diffractograms of banana starch from the six cultivars showed a typical B-pattern, with peaks at 2θ of about 5.5°, 15°, 17° and 23° (Figure 6). Kluai Hin starch gave the highest degree of relative crystallinity (31.3%) and exhibited a sharper peak at 5.5°. These findings indicate that starch from bananas with BB genotype seem to have a higher degree of relative crystallinity when compared to AA genotype banana starch.

Raw starch granules are semi-crystallites comprising amylose and amylopectin polymers. The degree and type of crystallinity present is dependent mainly on the structural characteristics of amylopectin and three types (A, B and C) of crystalline structure of starch granules have been distinguished [29]. According to several reports, banana starches can have A-type, B-type or a mixture of the two, depending on varietal source, growing conditions and other factors. However, B-type diffraction patterns for banana starch have often been reported [2,13,15].

In the present study, a B-type crystalline structure of banana starch granules was found, with a distinctive peak at 2θ of about 5.5°, which is considered to be a fingerprint for the B-type structure and is in agreement with the report by Shamai et al. [20]. Also, according to another study by Williamson et al. [30] on the enzymatic resistance of different polymorphs, the B-type structure is digested more slowly than the A-type by α-amylase. Thus, the B-type crystalline structure of banana starch observed in this study should be related to a high level of RS, which would consequently result in a high gelatinisation enthalpy, particularly for BB genotype bananas such as Kluai Hin and Kluai Namwa.

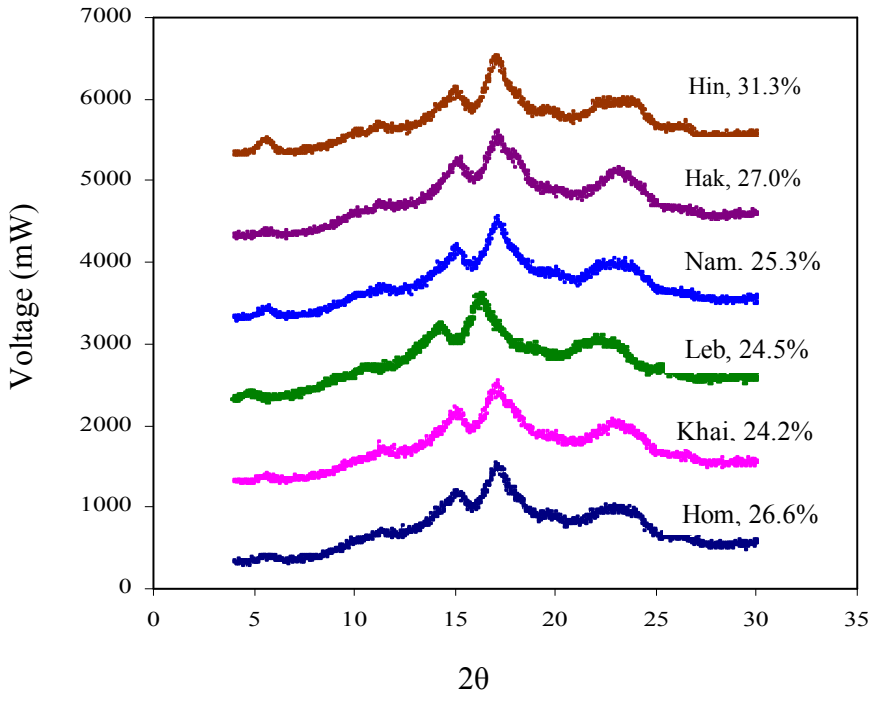

Figure 6. X-ray diffraction patterns of banana starch from different cultivars: Kluai Hom (Hom), Kluai Khai (Khai), Kluai Lebmuenang (Leb), Kluai Namwa (Nam), Kluai Hakmuk (Hak) and Kluai Hin (Hin), shown with degree of relative crystallinity in percentage.

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CONCLUSIONS

The high content of RS found in both starch and flour samples of all six cultivars of Thai bananas indicates that they are a healthy choice for consumption. Their genotypes seem to influence the RS content and in vitro starch digestibility as well as physical and structural properties. The highest RS content was found in starch from Kluai Hakmuk (ABB genotype) and in flour from Kluai Hin (BBB genotype) (79.2% and 68.1% respectively). A significant linear relationship between apparent amylose and RS contents of the banana flour and starch samples was observed ($R^2 = 0.76$), indicating that the starch stability comes from amylose molecules. More importantly, Kluai Hin starch showed the slowest in vitro digestibility rate and highest thermal enthalpy. According to the X-ray diffraction patterns, this should result from a more confined structure of amylopectin crystallinity of Kluai Hin starch compared to other starches.

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