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# Characterization of Vietnamese banana starch and its resistant starch improvement

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**Abstract**

Starch is accumulated in a green stage of banana and banana starch is considered to be a source of resistant starch. The aim of this study is to determine the physicochemical and functional properties of Vietnamese bananas, White Manzano (WM) and Dwarf Cavendish (DC), and investigate amount of resistant starch after debranching and retrogradation of banana starch. The isolated starch granules were smooth, elongated oval with ridges or spherical and approximately 10-50 μm in diameter, depending on banana species as seen by a scanning electron microscope. Amylose contents of WMS were 30.3%, which was higher than that of DCS (26.5%). Both of banana starches exhibited the C-type crystalline structure, low swelling power and high α-amylase hydrolysis resistant capacity. The banana starch after debranching and retrogradation had the B-type structure with significantly improved resistant starch content. The native banana starch analyzed as eaten sample contained 11.2% of resistant starch, whereas the debranched starch had significantly higher amounts of resistant starch (31.8-48.1%). As a result, the debranched banana starch can be used as a functional food with high amount of resistant starch.



## Introduction

Banana (genus *Musa*, AAA group) is one of the well-known fruits widely grown in tropical and sub-tropical areas. Vietnam is one of the ten major banana-producing countries over the world as reported by Food and Agriculture Organization (FAO) in 2003. In Vietnam, banana have been commonly consuming in a ripen stage. Vietnamese banana is also exported to other countries such as China, the United State (US) and the European Union (EU). Banana is rich in carbohydrate and is one of the tender fruits providing more calories. Starch is the principal component of green bananas, which undergoes important changes during ripening. The average starch content drops from 70 to 80% in the pre-climacteric (prior to starch breakdown) period to less than 1% at the end of the climacteric period, while sugars, mainly sucrose, accumulate to more than 10% of the fresh weight of the fruit (Zhang *et al*., 2005). Nowadays, banana starch have been reported to be resistant to α-amylase and glucoamylase hydrolysis, with *in vivo* test showing that 75-84% of the starch granules ingested reached the terminal ileum (Englyst and Cummings, 1986; Faisant *et al*., 1995), which is named resistant starch (RS). RS has been well known to have healthy benefits similar to dietary fiber with being almost totally fermented in the colon to short-chain fatty acids that help to prevent colorectal cancer, to lower the risk of heart disease, and to influence metabolic and inflammatory bowel diseases such as diabetes and diverticulitis. Therefore, banana starch has been classified as RS type 2 ($RS_2$), a native starch that resists to amylase hydrolysis (Englyst *et al*., 1992). However, the consumption of cooked starch in human food is much more common than that of raw starch. RS of the cooked and retrograded starches is classified as RS type 3 ($RS_3$). The relationship between debranching and heat treatments and the formation of RSs and their functional properties from high-amylose starches (Ozturk *et al*., 2009), banana starch (Gonzalez-Soto *et al*., 2007), cassava and potato starches (Hung et al., 2012) or waxy starches (Cai *et al*., 2010; Cai and Shi, 2010) have been recently reported. The amount of RS significantly improved after debranching and retrogradation (Cai *et al*., 2010; Hung *et al*., 2012). There are a number of publications reported in isolation and characterization of banana starch

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as well as formation of resistant banana starch over the world (Bello-Prez *et al*., 1999; Lehmann *et al*., 2002; de la Torre-Gutierrez *et al*., 2007). However, there is not any study on banana starch production in Vietnam. Because Vietnam is one of the top countries growing banana, the banana starch production is really necessary. Therefore, the objective of this study is to determine the physicochemical and functional properties of Vietnamese bananas and investigate amount of RS after debranching and retrogradation.

## Materials and Methods

*Materials*

White Manzano and Dwarf Cavendish bananas were collected when they were three months old from Tra Vinh province, Vietnam. The selected fruits were green and hard, no diseases, moulds and rot. The finger size was about 15 cm in length and 7-8 cm in girth. Ten kilograms of each banana were collected and transported immediately to the laboratory of university, where they were quickly peeled, carefully packaged and stored at 4°C to prevent from change of banana color caused by the oxidation .

Pullulanase from *B. acidopullulyticus* (≥ 400 U/ml), α-amylase from *A. oryzae* (~ 30 U/mg) and amyloglucosidase from *A. niger* (≥ 300 U/ml) used in this study were purchased from Sigma-Aldrich Co. (St. Louis, MO, US). Pectinase from *A. aculeatus* (Pectinex Ultra SPL) was obtained from Novozymes Corp. (Denmark). Cellulase (40,000 U $g^{-1}$) was obtained from Amano Pharmaceutical Co., Ltd (Nagoya, Japan). Other chemicals were purchased from Merck Co. (Darmstadt, Germany).

*Starch isolation*

Unripe banana (200 g) were peeled and cut into small pieces (1-2 $cm^3$). Then they were macerated with 200 ml $NaHSO_3$ 0.01M, pH=4.5 at low speed for 10 min. Cellulase (4 U/g banana pulp) and pectinase (6 U/g banana pulp) were added and then the mixture was incubated at 40°C for 2 h. The suspension was sieved through 140-mesh sieve for 3 times and finally washed with distilled water for several times. The slurry was stood for 15 min for starch sedimentation and then centrifuged at 2,500 × g for 15 min. The white starch was collected and dried in the oven at 40°C for 36 h. Finally, the dried starch was ground into powder and stored at room temperature in a plastic bag. The same procedure done without adding enzyme was used for cassava starch as a control.

*General analysis*

Amylose content of starch was determined according to the method previously descried by Hung and Morita (2005). Protein content was determined using a Kjeldahl digestion system (KI 26, Gerhardt, Germany) based on the Association of Official Analytical Chemists (AOAC) method (AOAC, 1995). Lipid contents were determined by extraction with hexane for 6 h using a Soxhlet apparatus. Ash content was determined by burning in a muffle furnace at 550°C for 3 h according to the AACC Approved Methods 08-01 (AACC International, 2000). Total starch was calculated as follows: total starch (%, db) = 100% - protein content (%, db) – lipid content (%, db) – ash (%, db).

*Physicochemical properties of banana starch*

Morphology of banana starch granules were determined using a scanning electron microscope (SEM) as previously described by Hung and Morita (2005). The crystalline structure of the native and debranched banana starches was observed using an X-ray diffractometer (Rigaku Co., Ltd., Rint-2000 type, Tokyo, Japan) according to the operation described by Hung and Morita (2005). The X-ray diffraction system was operated at 40 kV and 80 mA and diffractograms of the starches were recorded from 2° 2θ to 35° 2θ with a scanning speed of 8°/min and scanning step of 0.02°. The crystallinity and amorphous areas on the diffractograms were automatically recorded by X-ray diffractometer. The ratio of the crystallinity area to the total diffraction area was calculated as the degree of crystallinity (%). Swelling power was determined by the difference in weights of cooked starch and native starch according to the method of Sasaki and Matsuki (1998).

*Degree of enzymatic hydrolysis*

Native starch (100 mg) was mixed well with 10 ml of distilled water and then added with 2 ml of α-amylase solution (240 U/ml, Sigma-Aldrich Co.) and gently mixed. The tube was incubated for 0, 3, 6, 12, 24 and 48 h at 37°C. After each interval time, one milliliter of aliquots was taken, added with 2 ml of ethanol and then filled up by distilled water to make up 10 ml of solution. The mixture was centrifuged at 2,500 × *g* for 10 min. An aliquot of the supernatant were analyzed for soluble carbohydrate according to the phenol-sulfuric acid colorimetric method of Dubois *et al*. (1956). The degree of hydrolysis was expressed as the amount of maltose which is released per 100 mg of dry starch.

*Resistant starch production*

For producing RS, the WMS were debranched by pullulanase, autoclaved and re-crystallized at different storage temperature based on the method of Gonzalez-Soto *et al*. (2007) with slight modification

as follows. The starch suspension (10% w/w, db) was cooked in a boiling water bath for 10 min with continuously stirring, autoclaved at 121°C for 30 min and then incubated with pullulanase (20 U/g starch, Sigma-Aldrich Co.) with constant stirring for 24 h at 50°C. After 24 h, the debranched starch gel was autoclaved at 121°C for 30 min, cooled down and stored at 25°C, 4°C or -18°C for another 24 h. The debranched starches were then dried at 50°C in an oven overnight and stored in closed glass containers until further use.

*Determination of RS content*

Resistant starch contents (%RS) of the native and debranched starches were determined according to the method of Englyst *et al*. (1992) with a slight modification. One gram of starch (db) was mixed with 25 ml of acetate buffer (pH 6.0) and then boiled for 30 min in a water bath. The suspension was treated with amylase (7,000 U/g starch) at 37°C for 2 h and then with amyloglucosidase (50 U/g starch) at 60°C for 30 min. The mixture was centrifuged (1500 × *g*, 15 min) and the sediment was washed with distilled water for three times and then dried at 50°C for 48h. The $\%RS_3$ was calculated as weight of remained residue (db) compared to that of the initial sample. A blank with no starch was used to minus the contamination of the enzymes.

*Statistical analysis*

One-way ANOVA was applied to analyze the data ($p<0.05$). All tests were carried out at least in duplicate and the data were expressed as means ± standard deviation.

## Results and Discussion

*Chemical composition of banana starches*

Chemical compositions of Vietnamese banana starches are shown in Table 1. Using cellulase and pectinase for hydrolysis of cellulose and pectin in the banana pulp, most of contaminations were removed from starch granules. The total starch contents of white manzano starch (WMS) and dwarf cavendish starch (DCS) were 99.6% and 99.5%, respectively. The values of ash content was 0.09% in DCS and 0.08% in WMS. DCS contained higher amounts of lipid and protein (0.09% and 0.34%, respectively) as compared with that of WMS (0.06% and 0.22%, respectively). Other studies reported that the total of contaminations such as protein, lipid and ash in their extraction method was less than 5% (Bello-Prez *et al*., 1999; Lehmann *et al*., 2002; de la Torre-Gutierrez *et al*., 2007), which indicate that the isolated starches in this study were high purity. Thus, the enzymatic

Table 1. Chemical composition of banana starches[1,2]

| Sample | Protein (%) | Ash (%) | Lipid (%) | Total starch (%) | Amylose content (%) |
|---|---|---|---|---|---|
| WMS | 0.22 ± 0.01 | 0.08 ± 0.01 | 0.06 ± 0.00 | 99.6 ± 0.1 | 30.3 ± 1.5 |
| DCS | 0.34 ± 0.02 | 0.09 ± 0.01 | 0.09 ± 0.01 | 99.5 ± 0.1 | 26.5 ± 3.5 |

[1]WMS, White Manzano starch; DCS, Dwarf Cavendish starch.
[2]Data are mean values of three measurements

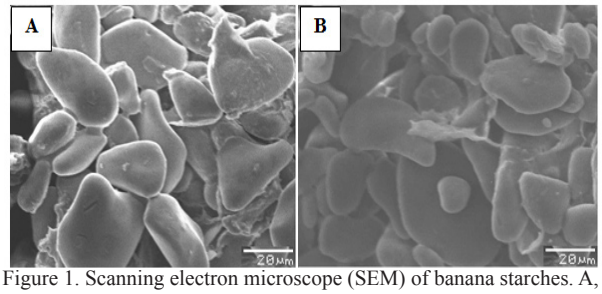

Figure 1. Scanning electron microscope (SEM) of banana starches. A, White manzano starch; B, Dwarf Cavendish starch

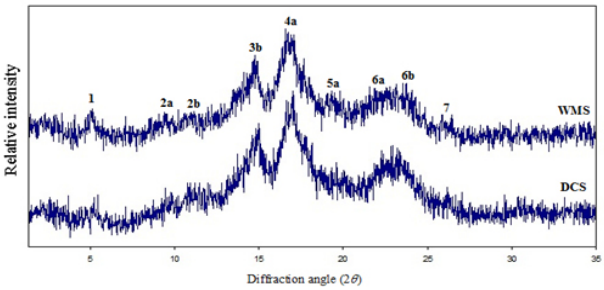

Figure 2. X-ray diffraction pattern of native banana starches. WMS, White manzano starch; DCS, Dwarf Cavendish starch

treatments method in this study are possibly used to isolate banana starch with high purification.

Amylose content of WMS (30.3%) was significantly higher than that of DCS (26.5%). The higher amylose contents of banana starches have been reported for *Musa* var. *valery* (40.7%) (Waliszewski *et al*., 2003) and criollo (87%) (Bello-Perez *et al*., 1999). However, the lower amylose contents of banana starches have also been reported for cavendish banana starch (19.5%) (Ling *et al*., 1982) and macho (18%) banana starches (Bello-Perez *et al*., 1999). Therefore, the difference in varieties and growing location affected the amylose content of banana starch resulting in different physicochemical and functional properties of the isolated starches.

*Scanning electron microscopy (SEM) of Vietnamese banana starches*

Morphology of Vietnamese banana starches observed by SEM is given in Figure 1. All of starch granules had a smooth surface but varied in shapes and sizes. WMS granules showed an irregular shape in which some of granules appeared microscopically as rectangular-shape and the others were elongated ovals with ridges, whereas the DCS granules seemed to be more regular in shape with spheroid form in almost granules. Moreover, the estimated sizes of

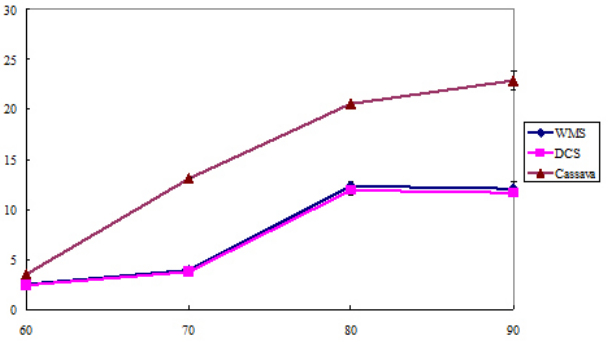


Figure 3. Swelling power of native banana starches. WMS, White manzano starch; DCS, Dwarf Cavendish starch

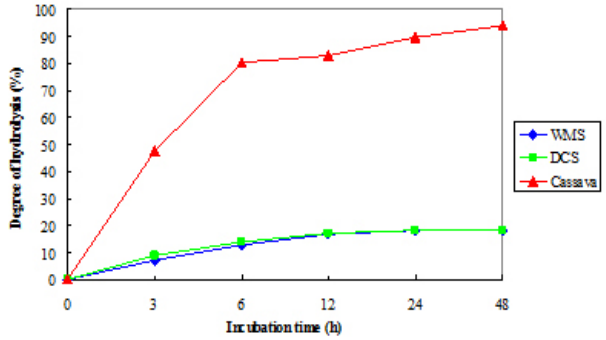


Figure 4. Enzymatic hydrolysis of native banana starches. WMS, White manzano starch; DCS, Dwarf Cavendish starch

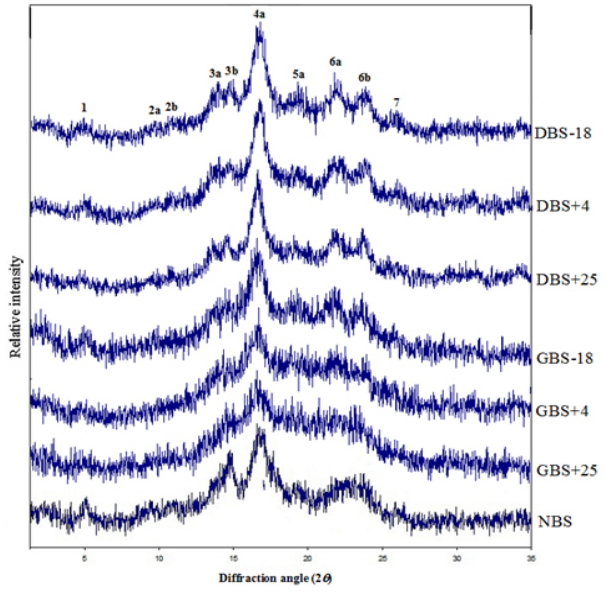


Figure 5. X-ray diffraction patterns of native and debranched banana starches at different storage conditions. NBS, native banana starch; GBS+25, Gelatinized banana starch at 25°C; GBS+4, Gelatinized banana starch at 4°C; GBS-18, Gelatinized banana starch at -18°C; DBS+25, Debranched banana starch at 25°C; DBS+4, Debranched banana starch at 4°C; DBS-18, Debranched banana starch at -18°C

WMS and DCS were about 20-33 µm and 18-50 µm, respectively. The sizes of DCS granules were considered to be more irregular than WMS. This observation was in agreement with other studies (Eggleston *et al*., 1992; Jane *et al*., 1994).

*X-ray diffraction patterns of Vietnamese banana starches*

Crystallinities of Vietnamese banana starches, WMS and DCS, are shown in Figure 2. Both of the banana starches showed the C-type crystalline structures with the major peaks which are representative for the B-type structure at 15.8 Å (line 1), a strong peak at 5.2 Å (line 4a) and a medium intensity double peaks at 4.0 and 3.7 Å (lines 6a, 6b), while a single peak at about 5.9 Å (line 3b), representative for the A-type crystalline structure, appeared instead of the double peaks (line 3a, 3b) as classified by Zobel (1988). Thus, there is no difference in the type of crystallinity of two Vietnamese banana varieties. Several previous studies also reported that the banana starches exhibited the C-type crystalline structure (Chang *et al*., 1991; Waliszewski *et al*., 2003), whereas the banana starches were also found to have the B-type structure (Lii *et al*., 1982; Faisant *et al*., 1995; Teixeira *et al*., 1998). These results indicate that the type of crystallinity of banana starch is dependant on both the varieties and growing condition of banana. The B- or C(B)-type crystalline structure of banana starches have been reported to be highly resistant to hydrolysis by enzymes (Englyst and Cummings, 1986; Cummings and Englyst, 1991; Eggleston *et al*., 1992; Englyst *et al*., 1996; Faisant *et al*., 1995).

*Swelling power of Vietnamese banana starches*

Swelling powers of WMS and DCS are given in Figure 3. The results indicate that the swelling powers of two kinds of banana starches were not significantly different when cooking at different temperatures. However, the swelling powers of the banana starches were significantly lower than those of the cassava starches. Kayisu *et al*. (1981) also reported that the valery banana starch had the swelling power and solubility close to those of sorghum starch, but significantly lower than those of tapioca and potato starches. The interaction between starch chains within both the amorphous and crystalline domains is responsible for the swelling power and solubility of starch (Zhang *et al*., 2005). The different amylose content, molecular weight distribution, degree of branching, length of branches and conformation of the molecules affected the swelling power and solubility of starch (Ratnayake *et al*., 2002). In addition, the amylose-lipid complexes or protein within granules is also influenced in these properties (Hoover and Hadziyev, 1981; Han and Hamaker, 2002).

*Degree of enzymatic hydrolysis*

Native banana starches showed highly impressive

Table 2. Degree of crystallinity of native and debranched banana starches[1,2]

| Sample | Type of Crystallinity | Degree of crystallinity (%) |
|---|---|---|
| NBS | C | 32.71a |
| GBS+25 | C | 32.13a |
| GBS+4 | C | 36.78c |
| GBS-18 | C | 34.94b |
| DBS+25 | B | 34.45b |
| DBS+4 | B | 36.74c |
| DBS-18 | B | 34.50b |

[1]NBS, native banana starch; GBS+25, Gelatinized banana starch at 25°C; GBS+4, Gelatinized banana starch at 4oC; GBS-18, Gelatinized banana starch at -18°C; DBS+25, Debranched banana starch at 25°C; DBS+4, Debranched banana starch at 4°C; DBS-18, Debranched banana starch at -18°C. [2]The data followed by the different letters are significantly different ($p<0.05$)

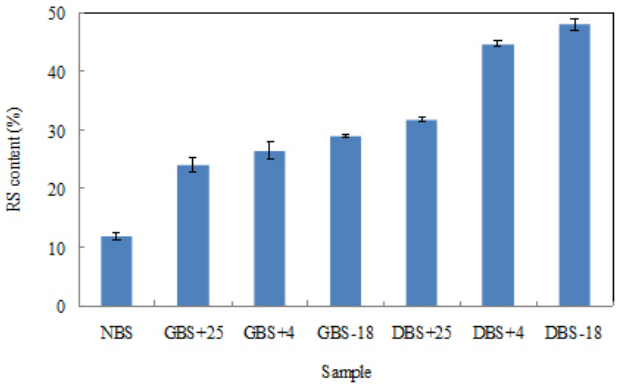


Figure 6. Resistant starch concentrations of native and debranched banana starches at different storage conditions. NBS, native banana starch; GBS+25, Gelatinized banana starch at 25°C; GBS+4, Gelatinized banana starch at 4°C; GBS-18, Gelatinized banana starch at -18°C; DBS+25, Debranched banana starch at 25°C; DBS+4, Debranched banana starch at 4°C; DBS-18, Debranched banana starch at -18°C

resistance to the digestion of α-amylase as compared to cassava starch (Figure 4). During the first 6 h of treatment, the degree of hydrolysis of the cassava starch rapidly increased and reached 80.2%, whereas that of the banana starches slowly increased and only 12.8% of WMS and 14.1% of DCS hydrolyzed. During 48 h of *in vitro* hydrolysis, the degree of hydrolysis was 18.1% for WMS and 18.2% for DCS, which were significant lower than cassava starch (94.1%). Thus, it can be assumed that WMS and DCS had the similar ability to resist α -amylase hydrolysis, which was about 5 times higher as compared to the cassava starch. The high resistance to hydrolysis by enzymes of the native banana starches has been widely reported (Englyst and Cummings, 1986; Cummings and Englyst, 1991; Eggleston *et al*., 1992; Faisant *et al*., 1995; Englyst *et al*., 1996), which could be due to the fact that banana starch without pores and channels, such as potato, yam, and lily starches, was digested through surface erosion of the granule (Gallant *et al*., 1992; Jane *et al*., 1997). In addition, the degree of hydrolysis are also affected by granule size, amylose:amylopectin ratio, length of amylose chain, presence of amylose-lipid complexes, degree of starch crystallinity and surface structure of starch granules (Cummings and Englyst, 1991). Thus, the starches isolated from Vietnamese unripe bananas had large capacity to resist the hydrolysis of α-amylase, which can be used for functional food processing.

*Effects of debranching and crystallization on RS3 formation*

The debranching followed by retrogradation of starch has been reported to produce high amount of $RS_3$ (Berry, 1986; Gonzalez-Soto *et al*., 2007; Cai *et al*., 2010; Hung *et al*., 2012), which is due to re-crystallinity of the short linear chains produced after debranching. The effects of debranching and retrogradation condition on the re-crystallinity of the banana starch are shown in Figure 5. The banana starches, which were gelatinized and retrograded by autoclaving treatment and storing at the different temperatures without debranching, had the C-type crystalline structures similar to the native starch. However, the crystallinities of the debranched banana starches changed to the typical B-type crystalline structure for all samples at different retrogradation conditions. These results are due to the re-organization of the short linear chain molecules during the retrogradation process, which was also reported by Hung *et al*. (2012) for cassava and potato starches and Cai and Shi (2010) for waxy maize, waxy wheat and waxy potato starches. The degrees of crystallinity of the gelatinized starches stored at 4°C and -18°C were significantly higher than that of the native starch or the gelatinized starch stored at 25°C, whereas the degrees of crystallinity of the debranched starch at different storage temperatures were significantly higher than that of the native starch (Table 2). The results also indicate that the gelatinized starch stored at 4°C had no significant difference in degree of crystallinity to the debranched starches at the same condition, which were the highest as compared to those stored at 25°C or -18°C. Thus, the double helices formed by the linear chains had more dense crystalline structure at 4°C resulting in the highest degree of crystallinity of the gelatinized and retrogranded starches.

RS concentrations of the native, gelatinized and debranched starches are given in Figure 6. The native banana starch analyzed as eaten sample had a high amount of RS (11.2%), which was higher than did cassava and potato (Hung *et al*., 2012). The gelatinized and retrograded banana starches at different storage condition improved the amount of

RS in a range of 24.2 - 29.0%. After debranching, the debranched banana starch had significantly higher amounts of RS (31.8 - 48.1%) than did the native or the gelatinized and retrograded banana starches. In addition, the results show that the amount of RS at lower storage temperature was higher than that at higher one. The starch after debranching and storing at -18°C had the highest amount of RS. The effect of storage temperature on RS formation was also reported by Gonzalez-Soto *et al*. (2007), who stated that when starchy material was stored at high temperatures (i.e., 60°C), the material had low RS content. Thus, the re-crystallinity of the short-chain molecules at low temperature caused their structure more dense structure resulting in more resistance to enzyme digestion.

## Conclusion

Two kinds of Vietnamese banana starches are successfully isolated and characterized by enzymatic isolation method in this study. The highly purified starches were obtained with different amylose content. The banana starches had the smooth surface and irregular shape, C-type crystalline structure, low swelling power and high RS content. The amount of RS was improved by debranching and retrogradation at different storage condition. The banana starch after debranching and storing at -18°C had the highest RS content, which can be used as a functional food with high health benefits.

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