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CHEMICAL, PHYSICAL AND PHYSICOCHEMICAL PROPERTIES OF MODIFIED BANANA STARCH

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ABSTRACT

Unripe banana starch has shown a potential to film formation. However, its hydrophilic nature is caused a high water vapor permeability film. The objective was to determine the effect of modification methods and starch contents on chemical, physical and physicochemical properties of modified banana starch. Sodium trimetaphosphate (STMP) was used to produce cross-linked banana starch (CBS) at different starch contents (4, 6, and 8 g). Another modification method miniemulsion of CBS (MCBS) was obtained from CBS with a mixture of cyclohexane and surfactants. Cross-linked and miniemulsion cross-linked technique did not change in shape and particle size of banana starch granules. CBS and MCBS showed lower swelling power (SP) and solubility (S) values at room temperatures (29 °C) and gelatinization temperature (80 °C) compared to native banana starch (NBS) corresponding to an increase in degree of substitution (DS) of modified starch. Moreover, starch content was directly proportional to DS and inversely proportional to S of modified banana starches. MCBS with 8 g of NBS provided the highest DS and the lowest S values. This might improve film water barrier. Further study on the method of particle size reduction is needed to enhance film properties.

INTRODUCTION

Starch is one of natural carbohydrate polymers that consisting of a large number of glucose units in terms of linear amylose and branched amylopectin packed into semi-crystalline granules. Starch can be found in many kinds of plant such as cereals (rice, wheat and corn), legumes (bean, pea and fabre), tubes or roots (potato and cassava), and green or immature fruits (banana and mango). Banana is easy to grow crop in the tropical and sub-tropical countries, especially in South and Southeast Asia. Raw banana is an interesting new source of carbohydrate especially starch (more than 70% in dry basis) [1]. Moreover, it is also known as a source of resistant starch [2]. Banana flour has been used to form edible film [3].The drawback of banana based film is poor water vapor permeability due to its natural hydrophilicity. There is an effort to incorporate modified banana starch (cross-linked starch) into banana based film formation to overcome this problem [4]. A cross-linked starch which is a chemical modification showed the improvement of the functional properties by increasing granule stability and strengthening with new covalent bonds introduced by functional groups of cross-linker [5]. Sodium trimetaphosphate (STMP), monosodium phosphate (MSOP), sodium tripolyphosphate (STTP), epichlorohydrin (EPI), phosphoryl chloride ($POCl_3$) are the main agents used as cross-linker for food grade starches. STMP is a popular reagent to improve functional properties of starch compared with the others because of its better solubility and uniform distribution [6]. Water-in-oil (w/o) miniemulsion cross-linked technique is another modification method. This technique with mechanical force from high pressure homogenizer was used to produce an anionic starch nanoparticle for drug carrier system [7]. The emulsification of miniemulsion cross-linked technique was become a thermally stable system. Water phase (starch and cross-linker) was dispersed as droplets into oil phase (cyclohexane and surfactant). The cross-linked reaction between starch molecules and cross-linker took place in the droplets. Later, high pressure homogenizer was applied to obtain cross-linked nanoparticle starch. According to the modification methods above, the cross-linked reactions generate intra- and inter chemical bonds which form network structures in starch molecules to stabilize and reinforce the starch molecules [7]. However, there is no work done on the effect of these modification methods on modified banana starch properties.

In this study, the modification methods (cross-linked and w/o miniemulsion cross-linked techniques with mechanical force) and starch contents were studied on the chemical, physical, and physicochemical properties of modified banana starch.

MATERIALS AND METHODS

Materials

Unripe banana (*Musa sapientum* Linn. "Kluai Namwa") was obtained in green stage 112-116 days after petal fall from an orchard of Kasetsart University, Kamphaengsaen Campus, Nakhonpathom, Thailand. Banana flour was prepared by peeling, washing, drying, and grounding with rotary mill. Native banana starch (NBS) was extracted by 0.05 N NaOH solution at ratio 1:5 [2]. Sodium trimethaphosphate (STMP) was obtained from SIGMA Life Science (Shanghai, China). Cyclohexane and tween-80

(HLB 14.9) were purchased from Ajax Fine Chemical (Sydney, Australia). Span-80 (HLB 4.3) was obtained from Merck (Hohenbrunn, Germany).

METHODS

Cross-linked banana starch (CBS) preparation

CBS was obtained from modified method of Carmona-Garcia *et al.* [6] and Shi *et al.* [7]. In brief, 4, 6, and 8 g of banana starch, 2 g of STMP, 1.5 g of NaCl and 50 mL of distilled water were mixed (designated according to banana starch amount as CBS4, CBS6, and CBS8, respectively). The mixture was adjusted to pH 11.5 with 0.1 M NaOH. The slurry was stirred continuously, warmed up to 45 °C and held at this temperature for 3 h. The slurry was neutralized with 0.1 M of HCl. Then, the CBS was obtained by centrifugation 3000g for 10 min, washed with 150 mL of distilled water, dried in a hot air oven at 60 °C for 12 h.

Miniemulsion cross-linked banana starch (MCBS) preparation

MCBS was produced by w/o emulsification cross-linked technique using STMP as a cross-linker according to Shi *et al.* [7] method. It involves 4 steps to form microsphere preparation. Firstly, the water phase was prepared similar to CBS method with different starch contents. Secondly, the oil phase was obtained by dissolving 9 g of mixture consisting of span-80 and tween-80 at a ratio of 5.04:0.96 (w/w) in 150 mL of cyclohexane and passed through a homogenizer (Polytron PT3100D, Kinematica AG, Lucerne, Switzerland) at 6000 rpm for 20 s. Thirdly, the water phase was mixed into the oil phase with homogenizer at 10,000 rpm for 30 min. Fourthly, acetic acid was added into emulsion system (at a ratio 1:10) to precipitate starch granules, and then separated the sediment from the rest of liquid mixture using a centrifuge (Model 1040 series, Centurion Scientific, UK) at 3000 g for 10 min. Samples were further purified using acetone and finally washed with distilled water. Samples were designated as MCBS4, MCBS6, and MCBS8 according to starch contents, respectively. They were dried in a hot air oven at 60 °C for 12 h. Dried samples were milled, and kept in desiccators until they are tested.

Scanning electron microscope (SEM)

The morphological properties and particle distribution of starch granules were investigated by SEM (S-3400N, Hitachi, Ontario, Canada). The samples were mounted on stub with double-sided adhesive carbon tabs and coated with gold on an ion sputter at 20 mA for 90 s. All samples were examined using an accelerating beam at a voltage 20 kV. Magnifications of 1000X were used. The average particles width was determined by randomly measuring a minimum of 50 particles.

Chemical properties

Chemical compositions such as moisture, protein, ash, fat, and fiber content on dry basis (% db) were determined according to the AOAC method [8]**.** Phosphorus content (% db) and degree of substitution were measured and calculated as described by Kasetkala [9].

Swelling power (SP) and solubility (S)

Swelling power (SP) and solubility (S) in water were measured at 2 temperature levels (room temperature; 29 °C and gelatinization temperature; 80 °C) using a modified method of Carmona-Garcia *et al.* [6] and Schoch [10]. Briefly, a 0.2-0.4 g of sample (A) was mixed with 5 mL of distilled water by a vortex mixer (Fisher Scientific 232, Arizona; USA), put in a controlled temperature water bath (Fisher Scientific Hi-Temp Bath 160 A, Arizona, USA) for 30 min and alternated shaking every 5 min with a vortex mixer. The suspension was then centrifuged at 3000 g for 20 min. The supernatant was decanted out and dried in a hot air oven at 70 °C for 12 h or until constant weight (B) was reached and weighed. The swollen granules remained in a plastic tube was weighed (C) and calculated SP and S as follow;

$$S\ (\%) = \frac{B}{A} \times 100\% \quad (1)$$

$$SP\ (g/g) = \frac{C}{(A \times (100-S)} \times 100 \quad (2)$$

Statistical analysis

A completely randomized experimental design was used to determine the effect of the chemical modification methods and starch contents on the chemical, physical, and physicochemical properties of modified banana starch. Three replicates were used to determine each property. SPSS 16.0 for Windows was used to test analysis of variance (ANOVA) and a Duncan's multiple range tests were used to determine significant differences between treatments at 95 % confidence interval.

RESULTS AND DISCUSSIONS

Chemical compositions

Certain CBS (CBS6 and CBS8) had lower moisture content than NBS as shown in Table 1 ($p \leq 0.05$). The reduction of the moisture content of CBS6 and CBS8 might be related to the new covalent bonds created from the reaction between OH^- groups in starch molecule and polyphosphate group of cross-linker in the amylopectin molecule during chemical modification process, which resulted in decreasing possibility of reaction between starch chains and the water molecules [5] and [6]. However, all MCBSs showed slight higher moisture content than CBS and NBS (Table 1). This might be resulted from starch molecules of MCBS reacted with water molecule and they were trapped in droplets that covered with surfactant which only small amount of water could pass through during drying process [7]. Ash content of modified banana starch (CBS and MCBS) increased due to the sub-products of reaction containing Na and P, which might be retained in the modified starch [6]. The protein contents in CBS and MCBS decreased compared to NBS (Table 1), except in MCBS4. This effect was due to partial solubilization of proteins with the reagents used in the cross-linked modification [6]. CBS had higher fat content than NBS and MCBS ($p \leq 0.05$) (Table 1). This finding was opposite to the work of Carmona-Garcia *et al.* [6] that showed a lower fat content in cross-linked starch than native starch. It might be resulted from a different technique of starch purification [6]. Increase in starch content showed higher phosphorus content and DS significantly ($p \leq 0.05$) in CBS and MCBS (Table 2). MCBS8 showed the highest phosphorus content and DS (Table 2). Increase in phosphorus content and DS might be due to the modification process corresponding to the increase in ash content as mentioned earlier.

Table 1 Chemical compositions of native (NBS), cross-linked (CBS), and miniemulsion cross-linked starch (MCBS) with different starch contents (4, 6 and 8 g).

Starch	Moisture (% db)	Ash (% db)	Protein (% db)	Fat (% db)
NBS	$8.70^{a}\pm0.02$	$0.22^{g}\pm0.01$	$1.64^{a}\pm0.33$	$0.23^{b}\pm0.07$
CBS4	$8.85^{a}\pm.0.01$	$1.00^{b}\pm0.00$	$0.13^{c}\pm0.05$	$2.04^{a}\pm0.26$
CBS6	$6.67^{b}\pm0.56$	$0.88^{d}\pm0.04$	$0.71^{b}\pm0.22$	$1.28^{a}\pm0.25$
CBS8	$6.01^{b}\pm0.10$	$0.79^{e}\pm0.02$	$1.43^{a}\pm0.29$	$1.43^{a}\pm0.35$
MCBS4	$11.51^{a}\pm2.90$	$1.03^{a}\pm0.02$	$1.83^{a}\pm0.18$	$0.26^{b}\pm0.13$
MCBS6	$11.04^{a}\pm3.15$	$0.49^{f}\pm0.04$	$1.29^{a}\pm0.10$	$0.25^{b}\pm0.12$
MCBS8	$11.33^{a}\pm0.17$	$0.92^{c}\pm0.01$	$0.83^{b}\pm0.01$	$0.56^{b}\pm0.17$

Values represent the means±SD; n = 3. Values in a column followed by different letters as superscripts were significantly different from each other according to Duncan's multiple range tests ($p\leq0.05$)

Table 2 Phosphorus content and degree of substitution (DS) of native (NBS), cross-linked (CBS), and miniemulsion cross-linked starch (MCBS) with different starch contents (4, 6 and 8 g).

Starch	Phosphorus (% db)	DS
NBS	$0.009^{g}\pm0.000$	-
CBS4	$0.014^{f}\pm0.000$	$0.001^{c}\pm0.000$
CBS6	$0.030^{c}\pm0.000$	$0.002^{b}\pm0.000$
CBS8	$0.029^{d}\pm0.000$	$0.002^{b}\pm0.000$
MCBS4	$0.025^{e}\pm0.001$	$0.001^{c}\pm0.000$
MCBS6	$0.045^{b}\pm0.000$	$0.002^{b}\pm0.000$
MCBS8	$0.062^{a}\pm0.000$	$0.003^{a}\pm0.000$

Values represent the means±SD; n = 3. Values in a column followed by different letters as superscripts were significantly different from each other according to Duncan's multiple range tests ($p\leq0.05$)

Morphological properties

Appearances of native starch (NBS) and modified starches (CBS and MCBS) under SEM were shown (Fig 1) similar morphology as seen in Carmona-Garcia *et al.* [6]. NBS, CBS and MCBS showed compacted granules, irregularly shape with ellipsoidal and triangular elongation similar to the finding of Nimsung *et al.* [2] and Carmona-Garcia *et al.* [6]. However, Waliszewski *et al.* [1] found that starch granule was in elongate and spherical forms. There was no significant difference in particle size distribution on the effect of two modification methods and starch contents ($p>0.05$).The size of NBS was in a range of 11.42 to 21.50 µm in width, which is narrow range compared to Waliszewski *et al.* [1] (14 to 88 µm in width) and Nimsung *et al.* [2] (18-30 µm in width). The particle size of modified starch (CBS and MCBS) granules was in a range of 16.50 to 17.84 µm in width. Size distributions of native and modified starch were shown in Fig. 2. It was found that most of the majority of size was in between 15.01-20.00 µm in width.

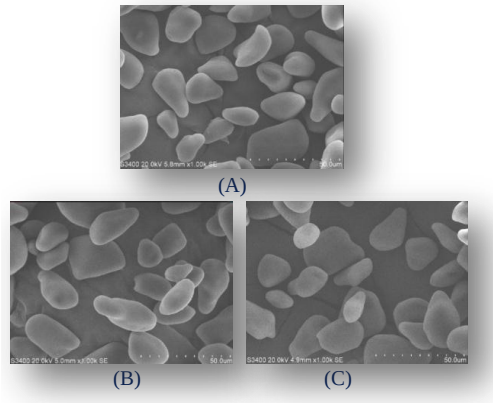


Fig 1 Scanning electron micrographs of native banana starch (NBS) (A), cross-linked banana (CBS) (B) and miniemulsion cross-linked banana starch (MCBS) (C) granules with magnification 1000X.

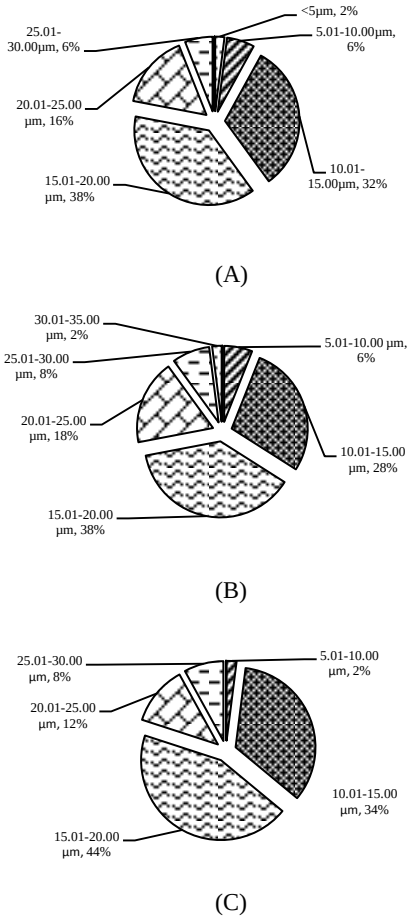
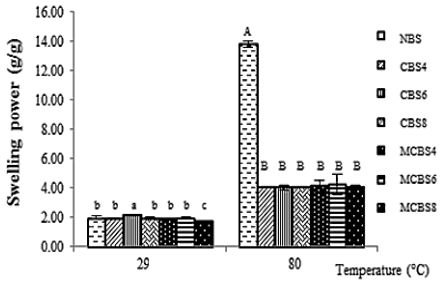


Fig 2 Particle size distribution of native banana starch (NBS) (A), cross-linked banana starch (CBS) (B) and miniemulsion cross-linked banana starch (MCBS) (C).

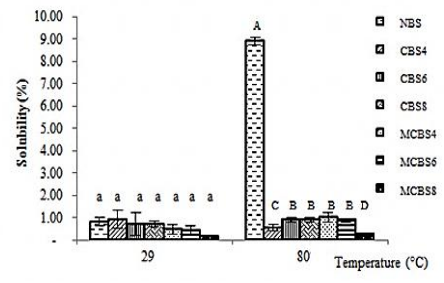
Swelling power and solubility

The swelling power (SP) and solubility (S) behaviors of NBS and modified banana starches at room temperature 29 ˚C and gelatinization temperature 80 ℃ were shown in Fig 3. The SP of CBS and MCBS were shown that they had fairy restricted swelling power [1]. At 29 ˚C, CBS6 showed the highest SP; while MCBS8 showed comparatively lower

SP corresponding to the highest DS. That might be due to the significant difference in chemical compositions that resulted from chemical modification reaction [12]. At gelatinization temperature 80 ˚C (Fig 3, A), CBS has shown lower SP value than Carmona-Garcia *et al.* [6]. MCBS showed no significant difference in SP with CBS. Shi *et al.* [7] has mentioned that the increase of particle size of MCBS was due to the network formed by chemical bonding such as hydrogen bonds and covalent bonds when temperature of medium was increased. However, modified banana starches (CBS and MCBS) were comparatively lower SP values than NBS (Fig 3, A). However, it was found that SP of NBS in this study was lower than the result of Nunez-Santiago *et al.* [13] but slightly higher than Carmona-Garcia *et al.* [6]. These might be due to the differences of cultivars of banana and the modification process.



(A)



(B)

Fig 3 Swelling power (A) and solubility (B) of native banana starch (NBS) and modified starches (CBS and MCBS) at 29 and 80 °C.
[a, A] Different letters were significantly different at the same temperature ($p \leq 0.05$)

Starch solubility (S) of NBS, CBS and MCBS were shown in Fig 3, B. NBS and modified starches did not show significant difference in S values at 29 ˚C. However, NBS showed the highest than the others at 80 ˚C. The increase in starch content of CBS was directly proportional to the increasing of S; but the result of MCBS was inversely proportion to S. However, at both temperatures revealed that MCBS8 exhibited higher resistance to water soluble than other modified starches ($p \leq 0.05$) as seen in Fig 3, B.

CONCLUSION

Chemical modification methods (CBS and MCBS) were affected to the chemical compositions and physicochemical properties of modified banana starch. However, they did not exhibit difference in morphological properties and particles size distribution from native banana starch (NBS). The chemical reaction that caused chemical bonds between hydroxyl groups of starch molecule and phosphate groups of STMP had the potential to enhance the strength and stability of modified starch. Starch content was directly proportional to DS of modified starches, while it was inversely proportional to S of modified starches at 80 ˚C. Higher starch content of MCBS exhibited the higher DS and good resistance to water at 80°C. Further study in modification method to obtain nanoparticle size preparation is needed to explore. The nanoparticle will be benefit as a bio filler to enhance water barrier in biopolymer packaging material.

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