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Modification of tapioca starch granule surfaces on soluble fiber formation

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ABSTRACT

Enzyme hydrolysis, acid hydrolysis and acid hydrolysis combined with ball milling, which was used after acid pretreatment, were applied to modify granular surface of tapioca starch in order to study an efficient method to produce soluble fiber. Microscopic and specific surface area evidence indicated the different mechanisms of each modification method. The enzyme hydrolysis influenced starch granules with various characteristics: medium-size holes, sponge-like erosion, internal cracks and single holes extending into the granule interior. Using acid hydrolysis, starch granules were lost their smoothness with the external corrosion. The small particles and maximum of specific surface area (6.55 m^2/cm^3) were obtained from the combination of acid hydrolysis and ball milling. Soluble fiber formation was achieved by using pyroconversion reaction. The pyroconversion treatment of amyloglucosidase treated starch for 6 h gave the highest total dietary fiber (TDF) content (64.58% w/w db) and high solubility (85.43%). The extensive modification of acid hydrolysis combined with ball milling before pyroconversion provided low value of solubility (23.40%) and TDF content (12.30%). The extensive modification did not improve the solubility and promote the formation of soluble fiber.

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Introduction

Starch is carbohydrate of high natural abundance and the most common constituent of human diet. It is a macromolecular complex of at least two polymeric components, amylose and amylopectin. Amylose is an essentially linear α (1-4) glucan, whereas amylopectin is a highly branched polymer with α (1-4) glucosidic linkage and α (1-6) glucosyl bonds (Bluèon *et al*., 1998). However, native starches have limitations that reduce their functional properties. Therefore, starches were modified to enhance their performance in different applications.

Starches can be modified by enzymatic, chemical and physical methods (Agboola *et al.*, 1991). Porous starch granules are formed by the partial hydrolysis of starch using amylase at temperatures below the gelatinization point. The two common amylases used are α -amylase and glucoamylase. Moreover, mineral acids commonly used are hydrochloric (HCl) and sulfuric (H_2SO_4) acids were also applied to hydrolyze the starches at lower gelatinization temperature. Acid hydrolysis by hydrochloric acid has long been used to modify a starch granule which is called lintnerised starch (Lintner, 1986). Physical modification such as ball milling of starch in conjunction with chemical modification has been used for changing the granular structure and to convert native starch into soluble starch or into small particle of starch (Jane *et al*., 1992).

Traditionally, dietary fiber was defined as the portions of plant foods that were resistant to digestion by human digestive enzyme. It included polysaccharides, lignin and associated plant substances. More recently, the definition has been expanded to include oligosaccharides and resistant starches (AACC, 2001). Dietary fiber can be divided into 2 basic categories: soluble and insoluble. Recent studies have shown that some soluble indigestible saccharides of low molecular weight promote physiologically favorable functions, such as intestinal regularity, moderate postprandial blood glucose levels and lower cholesterol levels (Ohkuma *et al.,* 2000).

The present study was to investigate the different modification methods of tapioca starch granule surface using chemical, enzymatic and physical methods for soluble fiber formation by pyrocoversion reaction.

MATERIALS AND METHODS

Material

Native tapioca starch was obtained from Chaodee Starch Company, Ltd (Nakhon Ratchasima, Thailand)

Preparation of starch surface modification

Acid hydrolyzed starch (AS)

Starches were hydrolyzed with 2.2 M HCl in a shaking water bath at 55°C (30 g dry starch/ 70 mL acid) at 180 rpm for 0-6 h. The granular residue that obtained was washed three times with deionized water. Then, the residues were dried with spray dryer (Niro A/S Gladsaxevej, Denmark) at an inlet temperature of 160°C and an outlet temperature of 70°C according to the method of Atichokudomchai *et al.,* (2000).

Enzyme hydrolyzed starch

Native tapioca starch suspension (30% w/w) in 0.1 M acetate buffer (pH 4.5) with a mixture enzymes (αAAM) and a single enzyme of α-amylase heat stable (αA) (120 U, EC 3.2.1.1 from *Bacillus licheniformis*, Sigma, USA) and amyloglucosidase (AM) (300 U, EC 3.2.1.3 from *Aspergillus niger*, Sigma, USA), with 3% w/w of each enzyme. The suspension was incubated in a shaking water bath at 55°C (30 g dry starch/ 70 mL acid) at 180 rpm for 0-6 h. After that washed and dried in the similar manner to the acid hydrolyzed starch

Ball milled starch (BM)

A suspension of AS hydrolyzed at 6 h in absolute ethanol (10% w/v) was placed in the ceramic container of rotating ball mill. The starch was milled with the ceramic balls (15 mm and 10 mm diameters, 15% w/v of each diameter) at 56 rpm for 3 h. After treatment, the sample was collected and dried at room temperature.

Scanning electron microscopy

The granule morphology of starch samples were observed with field emission scanning electron microscopy (JEOL JSM-7800F, Japan).

Particle size distribution and specific surface area

Particle size distribution and specific surface area of starch samples were determined at room temperature using HORIBA LA-950 (HORIBA Scientific, Japan) laser diffraction as method described by Edwards *et al.* (2008).

Soluble fiber preparation

Preparation of soluble fiber by pyrodextrinization starch was performed with modification of Laurentın *et al.* (2003). The starch surface modification samples of 22 g (moisture content 4-6%) were sprayed with 0.5 mL of 2.2 M HCl, mixed thoroughly, and equilibrated overnight at room temperature. After that, the samples were placed on aluminum tray (10x10 cm) and heated in hot air oven at 150°C for 90 min. Tapioca starch without modified granules surface was prepared for used as a control.

Water solubility

The analysis of water solubility of the soluble fiber was adapted according to Schoch (1964). Samples were dissolved in distilled water to a concentration of 10% w/v. Then, the samples were shaked at room temperature for 30 min. After shaking, the suspensions were centrifuged at 3000g for 15 min, and the amount of dried matter in the supernatant was weighed after drying at 60°C overnight and subsequently in a 130°C oven for 1 h. The solubility calculated from this equation:

$$\% \text{ Solubility} = \frac{\text{weight of dried matter in supernatant} \times 100}{\text{Sample weight as dried matter}}$$

Total dietary fiber

An integrated procedure (AOAC method 2009.01 and 2011.25) was used for measurement of the total dietary fiber, including resistant starch and non-digestible oligosaccharides of DP ≥ 3. Duplicate test portions were incubated with pancreatic α-amylase and amyloglucosidase for 16 h at 37°C in shaking water bath with mixing continuously. The reaction was terminated by pH adjustment to 8.2 and temporary heating. Protein in the sample was denatured and digested with protease. Specific dietary fiber fraction was measured by HPLC.

RESULTS AND DISCUSSION

Starch surface modification

Granular morphology

Structural characteristics observed by scanning electron microscopy of acid, enzyme hydrolyzed starch and acid pretreated starch combined with the ball milling were different. Native starch granules surface appeared to be smooth and had no pore or deep channel (Figure 1 A1 and A2). The granules were round or oval, with a flat surface on one side containing a conical pit. Truncature regions are weak points in the granules structure that led to increased susceptibility (Valetudie *et al.,* 1993).

SEM photographs of acid hydrolyzed starches (AS) are displayed in Figure 1 B and Figure 1 C. The slightly roughened surface due to exo-corrosion was observed for the starch granules hydrolyzed for 1 h (Figure 1 B). At 6 h hydrolysis, severe exocorrosion took place all over the granule surface (Figure 1 C). This was consistent with a study of Atichokudomchai *et al.,* (2000) in that, acid hydrolysis attacked on starch granules as superficial surface erosion showing a rough surface due to exocorrosion. Moreover, at the prolong time of acid hydrolysis (6 h) showed a mass of melted granules involving some intact granules (Figure 1 C 1) and a lot of small granules occurred.

SEM suggested different attack mechanisms of acid and enzyme hydrolysis (Figure 1 B - I). Enzyme molecules influence the starch granule surface in different ways. Five patterns of enzymes attack have been identified: pin-holes, sponge-like erosion, numerous medium-sized holes, distinct loci leading to single hole individual granules, and surface erosion. Generally, enzymes can erode the

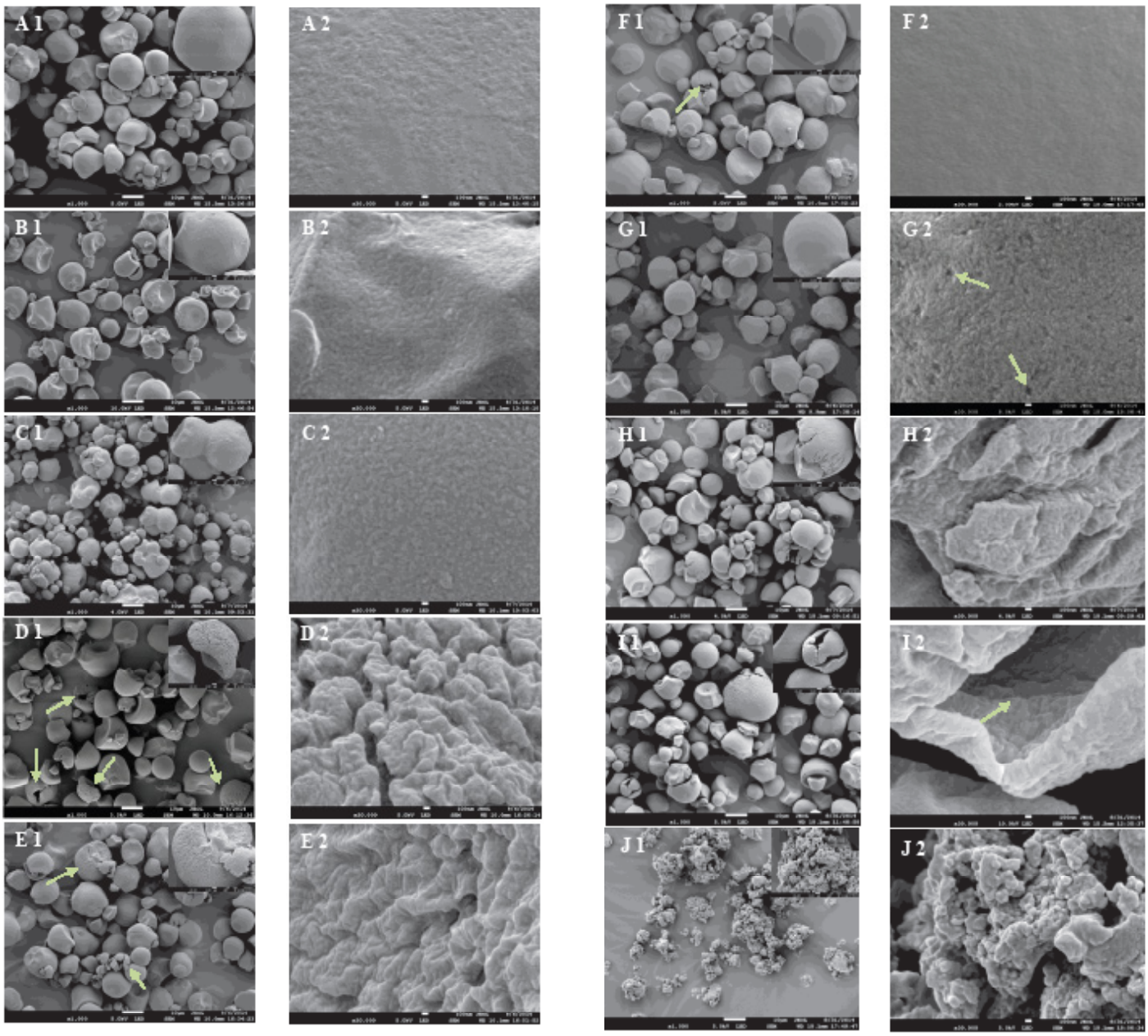

Figure 1 SEM micrographs of (A) native, enzyme and acid hydrolyzed starch. (B) AS 1 h; (C) AS 6 h; (D) AM 1 h; (E) AM 6 h; (F) αA 1 h; (G) αA 6 h; (H) αAAM 1 h; (I) αAAM 6 h and (J) AS 3 h BM 6 h. 1and 2 are the magnification 1000x and 30,000x respectively.

entire granule surface or sections of it (exo-corrosion) or digest channels from selected points on the surface towards the centre of the granule (endo-corrosion). Amyloglucosidase (AM) is an exo-enzyme function by hydrolyzing the terminal or next to terminal linkage starting at the nonreducing end of glucose polymer and producing glucose in this way by hydrolyzing both α (1-4) and α (1-6) linkages (Dura *et al.,* 2014). The hydrolysis at 1 h and 6 h led to destroy the outer surface of starch granules and induced channels with deep internal cracks (Figure 1 D and G at dot line arrows point). Especially for the truncated region of granules, which is the weak point of the granule structure that lead to increased susceptibility of enzyme hydrolysis (Figure 1 D and G at solid line arrows point). The α-Amylase (αA) hydrolyzed starch resulted that some granules have a medium size hole at 1 h of hydrolysis (Figure 1 F1 at the arrow point) but most of granule surface were slightly rough when compared with native starch. At the extensive hydrolysis time of 6 h, some granules exhibited a pin-hole structure (Figure 1 G 2).

For the mixture enzymes hydrolyzed starch (αAAM), the result showed that some granules were cracked on the surface for 1 h of hydrolysis (Figure 1 H 1). With extensive of hydrolysis (6 h) a single hole at truncated region of some granules extended into the granule interior and lamellar arrangement appeared (Figure 1 I 2). Starch granules are first hydrolyzed at outer surface, with a decrease in granule smoothness or cracking on the surface. Generating a cracking surface permitted the enzyme molecules to penetrate into the granule and disrupted the inside part of the starch granules. At higher degree of hydrolysis, a big hole on granules appeared with hydrolyzed interior. For some granules, the microscopic study was likely to suggest that the enzymes tunneled into the granular interior at the most susceptible part, especially the truncated area (Kimura and Robyt, 1995).

SEM of acid pretreatment combining with ball milling showed granular damage by physical forces (Figure 1 J). After 3 h of milling treatment, the surface of starch granules lost their smoothness and became to be rough and disrupted. Extensive milling also broke the acid hydrolyzed starches into smaller particle sizes and fragments due to generation of more defects or weak point, meanwhile the damaged granules were clumped together either making lumps or adhering to the surface of larger granules. The effect of ball milling treatment may be roughly divided into grinding and mechanical activation. In the ball mill process, the grinding and mechanical activation were dynamic equilibrium processes depending on the granule size of tough-brittle transition (Pourghahramani and Forssberg, 2006).

Table 1 Particle size distribution and specific surface area of native and modified starch granules

Sample name	D10 (μm)	D50 (μm)	D90 (μm)	Specific surface area (m^2/cm^3)
Native tapioca	9.59 ± 0.02 ^{b}	14.71 ± 0.04 ^{b}	21.36 ± 0.08 ^{b}	4.36 ± 0.01 ^{b}
AS 1 h	9.51 ± 0.01 ^{b}	14.57 ± 0.04 ^{b}	21.12 ± 0.11 ^{b}	4.40 ± 0.01 ^{b}
AS 6 h	9.62 ± 0.39 ^{b}	15.93 ± 0.42 ^{c}	27.47 ± 1.49 ^{c}	4.06 ± 0.09 ^{a}
AM 1 h	9.67 ± 0.04 ^{b}	14.68 ± 0.06 ^{b}	21.35 ± 0.14 ^{b}	4.35 ± 0.01 ^{b}
AM 6h	9.71 ± 0.19 ^{b}	14.70 ± 0.20 ^{b}	21.31 ± 0.24 ^{b}	4.34 ± 0.07 ^{b}
αA 1 h	9.76 ± 0.10 ^{b}	14.82 ± 0.15 ^{b}	21.48 ± 0.30 ^{b}	4.31 ± 0.05 ^{b}
αA 6 h	9.72 ± 0.06 ^{b}	14.73 ± 0.09 ^{b}	21.34 ± 0.21 ^{b}	4.34 ± 0.01 ^{b}
αAAM 1 h	9.82 ± 0.24 ^{b}	14.86 ± 0.26 ^{b}	21.54 ± 0.35 ^{b}	4.30 ± 0.09 ^{b}
αAAM 6 h	9.74 ± 0.11 ^{b}	14.72 ± 0.21 ^{b}	21.25 ± 0.58 ^{b}	4.34 ± 0.01 ^{b}
AS 6 h BM 3 h	5.74 ± 0.04 ^{a}	9.96 ± 0.04 ^{a}	17.54 ± 0.60 ^{a}	6.55 ± 0.03 ^{c}

Values followed by the same letter within a column do not differ significantly ($P < 0.05$). D10 and D90 represent the particle diameters that cumulative volume of particles are 10% and 90%, respectively, D50 is median diameter. The specific surface area was calculated from the diameter of the starch granules assuming spherical particles.

Particle size distribution and specific surface area

Particle size distribution and Specific surface area of the starch samples obtained with different modification mechanisms are shown in Table 1. The results showed that the particle diameter of native and modified starch granules mainly distributed in the range of 9 – 20 μm and present the specific surface area in the range of 4.30 – 4.40 m^2/cm^3 . However, except for the treatment of AS 6 h and AS 6 h BM 3 h, the results were different from other. The AS 6 h had the highest median diameter (15.93 μm) and 90% of cumulative volume particles diameter (27.47 μm) but it had the lowest amount of specific surface area (4.40 m^2/cm^3), because a mass of melted granules occurred and involving some intact granules; therefore, the granules of starch increased the size and decreased the area. For the treatment of AS 6 h BM 3 h, it had the lowest granule distribution in the range of 5 – 17 μm, but it had the highest specific surface area (6.55 m^2/cm^3) because the mechanical force destroyed the starch granules to small particles that lead to the minimum particle size and maximum specific surface area.

Soluble fiber formation

Water solubility and Total dietary fiber

Table 2 shows the characteristics of soluble fiber by pyroconversion reaction. The native tapioca starch, a starting material, had lower total dietary fiber (TDF) with very less solubility in water. For the treatment of Pyro_control. However, it shows the highest solubility after pyroconversion due to the smooth surface of native tapioca starch. It also contained less charcoal when compared with all modified starch surface. However, after pyroconversion treatment,

all of the treated samples can soluble in the water more than 75% ,except the treatment of acid combining with ball mill treatment. The AS 6 h BM 3h after pyroconversion showed lower TDF content (12.30%) and solubility (23.40%) because it lost the characteristic of the granular structure. Therefore, it is difficult to promote the intermolecular bond formation (transglucosidation), leading to the less formation of a typical linkages such as α (1-2) and α (1-3), which are resistant to human enzyme digestion (Lowary and Richards, 1991). For, the pyroconversion treatment of AM treated starch at 1 and 6 h, it showed the highest TDF content of 63.81 and 64.58%, respectively.

From this result, it showed that the extensive modification of the starch surface by the combination of acid hydrolysis and ball milling could not promote the soluble fiber formation. The starch that was not underwent the surface modification (the control) showed the lowest amount of TDF (2.44%). The low amount of surface area and the smooth surface of native starch granules might influence the reaction with the acid in pyrocoversion process. The pyroconversion reaction of the smooth surface granules less occurred than that of the rough surface , resulting in low TDF content of the control after pyroconversion (Pyro_control sample) (45.10%).

All of the treatments of Pyro_modified starch granule surface show lower of solubility than Pyro_control because they were susceptible to the pyroconversion reaction and induced the charcoal occurrence. This leads to future investigate the mild condition of soluble fiber production that promotes the highest solubility and total dietary fiber from modified starch granule surface using pyroconversion reaction.

CONCLUSION

Structural and morphological characteristics, of acid, enzyme hydrolyzed tapioca starch and starch treated with acid combining with the ball milling were different. The combination of acid hydrolyzed starch and ball milling led to the small particles obtained. Specific surface area of modified starch was increased, especially starch treated with acid hydrolyzed starch combined with ball milling. After pyroconversion process, all of the treated samples can be soluble in water more than 75%, except the starch treated with acid combined with the ball milling. The pyroconversion treatment of AM hydrolyzed starch showed the highest total dietary fiber. An extensive modification did not promote the soluble fiber formation.

Table 2 Characteristics of soluble fiber produced by pyroconversion reaction of surface modified tapioca starch

Sample name	% solubility	% TDF
Native tapioca	0.81 ± 0.36^{a}	2.44 ± 0.03a
Pyro_Control	93.39 ± 0.05^{f}	45.10 ± 0.14^{c}
Pyro_AS 1 h	86.22 ± 0.99^{e}	61.76 ± 1.43^{f}
Pyro_AS 6 h	77.85 ± 0.62^{c}	59.01 ± 0.10^{e}
Pyro_AM 1 h	85.13 ± 0.68^{de}	63.81 ± 0.29^{g}
Pyro_AM 6 h	85.43 ± 0.50^{e}	64.58 ± 0.11^{g}
Pyro_αA 1 h	84.92 ± 0.07^{de}	62.13 ± 1.08^{f}
Pyro_αA 6 h	85.62 ± 0.49^{e}	58.99 ± 0.40^{e}
Pyro_αAAM 1 h	83.68 ± 0.74^{d}	56.41 ± 0.18^{d}
Pyro_αAAM 6 h	84.98 ± 0.42^{de}	56.65 ± 0.94^{d}
Pyro_AS 6 h BM 3 h	23.40 ± 0.21^{b}	12.30 ± 1.41^{b}

Difference letters within the same column indicate a significant difference ($p < 0.05$) Pyroconversion of native tapioca starch was used as a control (Pyro_control)

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