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Physical Properties and Resistant Starch Content of Rice Flour Residues Hydrolyzed by α-Amylase

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***Abstract*—**Enzymatic modification of rice flour can produce highly functional derivatives use in food industries. This study aimed to evaluate the physical properties and resistant starch content of rice flour residues hydrolyzed by α-amylase. Rice flour hydrolyzed by α-amylase (60 and 300 u/g) for 1, 24 and 48 hours were investigated. Increasing enzyme concentration and hydrolysis time resulted in decreased rice flour residue's lightness (L*) but increased redness (a*) and yellowness (b*) of rice flour residues. The resistant starch content and peak viscosity increased when hydrolysis time increased. Pasting temperature, trough viscosity, breakdown, final viscosity, setback and peak time of the hydrolyzed flours were not significantly different ($p>0.05$). The morphology of native flour was smooth without observable pores and polygonal with sharp angles and edges. However, after hydrolysis, granules with a slightly rough and porous surface were observed and a rough and porous surface was increased with increasing hydrolyzed time. The X-ray diffraction patterns of native flour showed A-type configuration, which hydrolyzed flour showed almost 0% crystallinity indicated that both amorphous and crystalline structures of starch were simultaneously hydrolyzed by α-amylase.

***Keywords*—**α-Amylase, Enzymatic hydrolysis, Pasting properties, Resistant starch

I. Introduction

STARCH modification is the method for developing the physical and chemical properties of the native starch to specific in food applications. Modified starches are obtained by 4 techniques, namely physical, chemical, genetic and enzymatic modifications [1], [2]. The advantages of using the enzymatic modification are fewer by-products, more specific hydrolysis products and high yield, besides better control of the process and end products with particular properties [3].

Amylases are glycoside hydrolases, which act upon the bonds between the glucose unit of the starch polymers. These can be derived from several sources, including plants, animals, and microorganisms. The microbial enzymes have dominated applications in industrial processes [4]. The α-amylase is endo-acting amylases or starch-degrading enzymes which hydrolyse the α-1,4-glycosidic bonds of the starch polymers internally to produce reducing sugars like maltose and glucose. As a result, the viscosity of starch decrease due to the amylose molecular weight reduces [5]-[7]. The amylase treatment is efficient method to reduce the viscosity of oatmeal, increasing its liquidity for spray drying and for use in drinks [8]. Moreover, the method of enzyme hydrolysis was adopted in preparation of resistant starch (RS). RS is defined as starch and products of starch degradation that cannot be absorbed in the small intestine of healthy individuals and, hence, might be fermented in the colon [9]. The method combining α- amylase and pullulanase was used to prepare RS from maize starch. They concluded that the production of RS could be greatly increase by treatment with thermostable α-amylase before debranching with pullulanase [10].

Before designing a successful hydrolysis system, information is required describing phenomena which affect the kinetics of starch hydrolysis. Many researchers have reported the temperature, pH, enzyme concentration, substrate concentration as affected by enzyme hydrolysis [11]-[13]. Changing the enzyme and substrate concentrations affect the rate of reaction of an enzyme-catalysed reaction [14]. The susceptibility of starch to amylase attack depends on the properties of the specific starch, such as e.g. degree of gelatinization, and the characteristics of the specific amylase. Many studies deal with the amylolysis of native starch and focus on the effects of substrate characteristics, such as granule size, shape, structure, and amylose content. In addition, amylases showed widely different activities on various kinds of solubilised starches [15]. High amylose mutant starches exist as well with amylose levels ranging from 40 to 80% [16]-[18]. They are attracting considerable attention because of their potential health benefits and industrial uses. High amylose rice varieties are reported to raise blood glucose less than rice with higher amylopectin content; rice that is high in amylose has a lower GI [19]. In this paper, flour was prepared from white rice cv. Jek Chuey 1 and hydrolyzed by endo α-amylase at varies with the enzyme concentration and hydrolysis time. Therefore, the study aimed to evaluate CIELAB values, resistant starch content, pasting properties, granule morphology and crystalline type of hydrolyzed high amylose rice cv. Jek Chuey 1 flour residues.

II. Materials and Methods

A. Materials and sample preparation

High amylose rice cv. Jek Chuey 1 was obtained from Medifoods (Thailand) Co.,Ltd. The polished rice was milled to flour by using an airflow mill grinder and passed through 100 mesh sieve (0.177 mm sieve). Endo α-amylase preparation derived from *Bacillus amyloliquefaciens* (Amano enzyme INC.)

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B. Enzymatic modification

Rice flours were hydrolyzed by α-amylase according to the method of Man et al. [20] with some modifications. The flour suspension (35% w/v; dry solid basis) were preheated in the water bath from 65 to 85 °C (1 °C/min) with continuous stirring for gelatinization. Add α-amylase (60 and 300 U/g dry flour) in the gelatinized suspension and incubated in water bath at 85 °C for 1, 24 and 48 h, cooled in cold water at 25 °C. After hydrolysis, undissolved residues were quickly obtained by centrifugation (3000 x g, 10 min) at 4 °C. The residues were subsequently washed three times with double-distilled water to remove residual enzyme. Hydrolysis flour was frozen in refrigerator at -40 °C and then freeze-dried in a freeze dryer at -50 °C and 0.1mPa until constant weight (20 h). The dried flours were ground into powders in a mortar with pestle, and passed through a 100 mesh sieve for further structural analysis.

C. Pasting properties

A rapid visco analyser (RVA-4, Newport Scientific Pvt Ltd., NSW, Australia) was measured pasting properties. The moisture content of the flour samples were determined and used to compensate for differences in moisture content between samples by adjusting the quantities of flour and distilled water used to prepare the RVA sample [21]. RVA tests were performed with around 3 g of flour samples and approximately 25 g of distilled water. After the canister was fitted to the device, the operations were run based on the approved profile. Each sample was held at 50 °C for 1 min, heated to 95 °C at 12 °C/min, held for 2.5 min, cooled down to 50 °C at 12 °C/min, and held at 50 °C for 1.5 min. Total elapsed time was 12.5 min. The RVA measures pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), final viscosity (FV) and peak time (Pt) based on curve. Breakdown viscosity (BD) and setback viscosity (SB) were calculated as the difference between PV and TV, and FV and TV, respectively. PT was defined as the first point at which the viscosity increases by 10 cP or faster within 0.1 min.

D. X-ray diffraction

Native rice flour and rice flour residues were analyzed in an X- ray diffractometer (Model D8 Discover, Bruker AXS Inc., WI, USA) provided with a tube with a copper anode, a detector operated at 40 kV and 40 mA. Diffractograms were obtained from 2θ = 3-60 °C. Crystallinity degree was calculated as the ratio between the absorption peaks area and the total diffractogram area, and expressed as percentage (%), [22].

E. Scanning electron microscopy

Scanning electron micrographs were observed by scanning electron microscope (JSM-6400, JEOL Ltd., Tokyo, Japan). The flour samples were stick on an aluminum specimen stubs, and then coated with gold. An acceleration potential of 10 kV was used during micrography [23].

F. Color measurement

Native rice flour and rice flour residues were measured using color meter (ColorFlex EZ colorimeter, Hunter Associates Laboratory Inc., VA, USA). The CIELAB values were measured as L* value for each scale therefore indicates the level of light (where L* = 100) or dark (where L* = 0), the a* value redness (+a*) or greenness (-a*), and the b* value yellowness (+b*) or blueness (-b*).

G. Resistant starch content

Resistant starch content of the samples were determined following a modified method of Goni et al. [24]. A 100 mg ground sample of flour or starch was dispersed in KCl-HCl, pH 1.5 (10 ml) and incubated with a solution containing 0.2 ml pepsin at 40 ºC for 60 min. to remove protein. A 0.1 M tris-maleate buffer, pH 6.9 (9 ml) and α-amylase solution (1 ml) were then added and the mixture incubated at 37 ºC for 16 h in a shaking water bath.to hydrolyse digestible starch. The hydrolysate was centrifuged at 4600 rpm for 10 min. and the residue was solubilised with 4M KOH (3 ml) and incubated for 30 min. at room temperature. The solubilised starch was then hydrolysed by adding amyloglucosidase (80 µL) and incubating at 60 ºC for 45 min. in a shaking water bath to hydrolyse RS. The glucose content was measured using a glucose oxidase-peroxidase kit. The resistant starch, non-resistant (solubilised) starch and total starch content (resistant starch + non-resistant starch) (%, on a dry weight basis) in test samples were calculated using:

$$RS\,(>10\%),\ Non-RS = \Delta E \times F / W \times 90 \quad (1)$$

$$RS\,(<10\%) = \Delta E \times F / W \times 9.27 \quad (2)$$

where ΔE = absorbance (reaction) read against the reagent blank, F = conversion from absorbance to micrograms (the absorbance obtained for 100 µg of D-glucose in the GOPOD reaction is determined), F = 100 (µg of D-glucose) divided by the GOPOD absorbance for this 100 µg of D-glucose, and W= dry weight of sample analysed = "as is" weight x [(100-moisture content)/100].

H. Experimental design and statistical analysis

A 2x3 factorial experiment in a completely randomized design (CRD) with triplicate was conducted for physical properties and RS content. The data were subjected to analysis of varience (ANOVA) using general linear model procedure, SPSS for window version 18.00 (SPSS Inc., USA). Means comparison was performed using Duncan's multiple range test.

III. Results and Discussion

A. Pasting properties

The pasting properties of rice flour residues hydrolyzed by α-amylase are shown in Table I. Result shown a not significantly interaction between enzymatic concentration and hydrolysis time on the all properties parameter of the rice flour

residues. Pt, PT, TV, BD, FV, SB of rice flour residues were not significantly different, while PV of 300 U/g for 48 h was significantly higher than 60 U/g for 24 h. PV, TV, BD, FV and SV of rice flour residues were decreased significantly from the native rice flour, which had PV (2070.33±158.51 cP), TV (1369.67±86.93 cP), BD (700.67±72.39 cP), FV (3203.00±212.49 cP) and SV (1833.33±135.88 cP). While PT and Pt were no significantly different. Hydrolysis of starch is proved to reduce the viscosity of starch suspensions [25]. During enzymatic hydrolysis, complex carbohydrates are hydrolyzed and converted into simpler sugars, either by native enzyme of the grain or through external addition of fungal or bacterial α-amylase [26]. Similarly, the study by Dura et al. [27], PV, TV, FV of corn starch hydrolyzed by α-amylase were decreased significantly from the corn starch and Pt, BD and SV were no significantly different. Due to α-amylase can hydrolyzed the α-1,4-glycosidic bonds of starch in an endo-action. α-1,4-glycosidic bonds within the starch chain were randomly hydrolyzed to rapidly reduce the viscosity of the starch solution during pasting [5]-[7]. However, the change of enzymatic concentration and hydrolysis time were not affected on all pasting parameters of rice flour residues. In this study, aqueous suspension with a flour to water ratio of 1:2. Reactions in the aqueous suspension gave a much high degree of conversion to reducing sugar [28]. This could explain the reaction in the aqueous suspension at 60 U/g dry flour for 1 h gave a much highest degree of conversion to reducing sugar. When increased the enzyme concentration and hydrolysis time, all pasting parameter were not changed.

TABLE I
PASTING PROPERTIES OF RICE FLOUR RESIDUES HYDROLYZED BY α -AMYLASE

Enzyme Concentration (U/g)	Hydrolysis Time (h)	Pt (min)	PT (°C)	PV (RVU)	TV (RVU)	BD (RVU)	FV (RVU)	SB (RVU)
60	1	5.36±1.16	63.50±0.52	87.33±7.23^{ab}	38.00±27.18	49.33±20.21	52.67±21.57	14.67±6.81
60	24	4.70±2.59	62.85±0.57	82.00±5.66^{b}	44.00±24.04	38.00±18.38	57.00±19.80	13.00±4.24
60	48	5.30±0.81	63.30±0.00	96.00±11.31^{ab}	43.50±4.95	52.50±6.36	63.50±2.12	20.00±7.07
300	1	6.03±0.14	63.25±0.99	94.50±6.36^{ab}	48.50±3.54	46.00±9.90	64.00±1.41	15.50±4.95
300	24	5.44±0.33	62.50±0.07	89.50±0.71^{ab}	36.50±9.19	53.00±9.90	48.50±13.44	12.00±4.24
300	48	5.07±1.10	62.75±0.35	104.67±13.28^{a}	38.00±15.87	66.67±12.34	51.00±27.62	13.00±12.78

Means in the same column with different letters are significantly different ($p<0.05$).

B. X-Ray Diffraction Pattern

X-ray diffraction pattern of native rice flour and rice flour residues hydrolyzed by α-amylase are shown in Fig. 1. Native rice flour indicate typical peaks of A-type starch, which has strong diffraction peaks at about 15º and 23º 2θ and a doublet at 17º and 18º 2θ [20], [29]. Rice flour residues hydrolyzed by α-amylase (60 and 300 U/g dry flour) for 1, 24 and 48 h shown disappearance of the separation and broadening of most peaks, indicate that they seemed like mainly composed of amorphous regions (Figs. 1 (a)-(c)). Normally the rice starch was presented the A-type X-ray pattern [30]. The A-type starch granules display a greater susceptibility on enzyme hydrolysis. Due to in A-type starches have branch points scattered both amorphous and crystalline regions. With the scattered branch points, there are lots of short A-chains derived from branch linkages located inside the crystalline regions, which produces an inferior crystalline structure. This inferior crystalline structure containing α-1,6-linked branched points and the short double helices are more susceptible to α-amylase hydrolysis, leading to 'weak points' in the 'A' type starches. These weak points are readily attacked by α-amylase [31]. In the same way, several studies have shown that α-amylases can simultaneously solubilize both amorphous and crystalline regions of starch granules [11], [32], [33]. In reaction in the aqueous suspension at 60 U/g dry flour for 1 h gave a much highest degree of conversion to reducing sugar. Consequently, X-ray pattern of rice flour residues seemed like mainly composed of amorphous regions, for all the crystalline regions were hydrolyzed by α-amylase.

C. Morphological properties

Scanning electron micrographs of rice flour residues hydrolyzed by α-amylase are shown in Fig. 2. The native rice flour granule was polygonal with sharp angles and edged. The surface of the granule was smooth and flat. No pore was observed on the surface (Fig. 2 (a)). Meanwhile, rice flour residues granule were irregular shape. The surface of the granule was porous look like spongy (Figs. 2 (b)-(g)). When flours were hydrolyzed by α-amylase that pitted the starch granule surface first, then penetrated into the interior and hydrolyzed the granule from the inside out [34]. Thus, many pores appeared on the granule surfaces of rice flour residues. This results were agreement with the previous study that the external region of starch granule was easily hydrolyzed to form pores by α-amylase [35].

D. Color

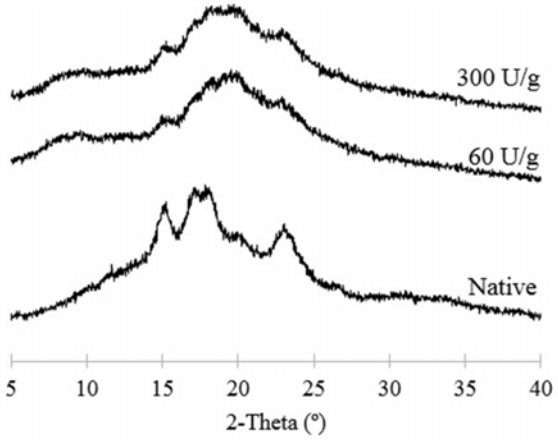
The result of color determination of rice flour residues hydrolyzed by α-amylase are shown in Table II found that the enzyme concentration and hydrolysis time of enzymatic modification of rice flours affected for rice flour residues. Increasing the enzyme concentration and hydrolysis time decreased lightness (L *) but increased redness (a*) and yellowness of rice flour residue. In other word, the rice flour residue had high color intensity. L*, a* and b* values of the rice flour residues were significantly different from the native rice flour. The L* value of all the rice flour residues were lower than the native rice flour (96.31), whereas, the a* and b* values were higher than native rice flour (a*=-0.18, b*=3.24). The changed of color may be caused from the feature of α-

amylase which is dark brown liquid and the maillard reaction between reducing sugar from the hydrolyzed flour, and the amino group in the proteins, during modification. Likewise, the study by Martinez et al. [36], who reported the color of flours after hydrolyzed with α-amylase was darker and more reddish than native flours.

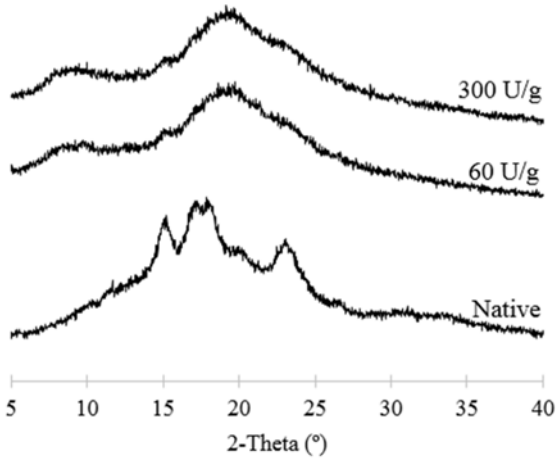
TABLE II
COLOR DETERMINATION OF RICE FLOUR RESIDUES HYDROLYZED BY α - AMYLASE

Enzyme Concentration (U/g)	Hydrolysis Time (h)	L*	a*	b*
60	1	91.89±0.35[a]	0.18±0.03[e]	6.05±0.31[d]
60	24	82.78±0.84[b]	4.10±0.28[d]	17.89±0.42[c]
60	48	77.81±1.58[d]	5.52±0.57[b]	21.39±1.11[a]
300	1	92.33±0.12[a]	0.32±0.02[e]	5.93±0.03[d]
300	24	79.11±0.77[c]	4.96±0.19[c]	19.21±0.34[b]
300	48	73.02±1.45[e]	6.22±0.43[a]	21.72±0.74[a]

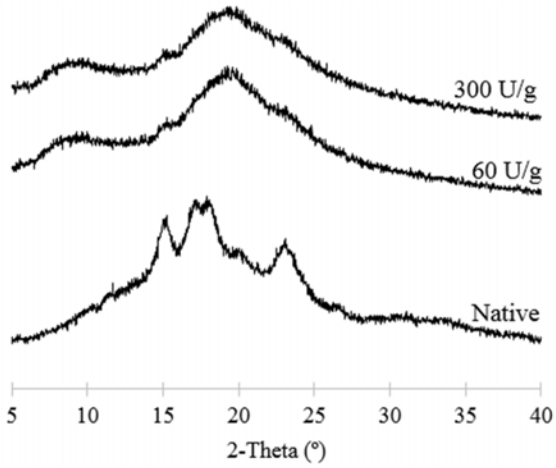
Means in the same column with different letters are significantly different ($p<0.05$).



(a)



(b)



(c)

Fig. 1 X-ray diffraction pattern of rice flour residues hydrolyzed by α-amylase (60 and 300 U/g dry flour) at different hydrolysis time (1 (a), 24 (b) and 48 (c) h).

E. Resistant Starch Content

Resistant starch content is shown in Table III. Native rice flour contained resistant starch as 4.29%, when the hydrolysis time increased the resistant starch increased, whereas the enzymatic concentration had no effects on the resistant starch content. However, no significant change in RS was observed for native rice flour and rice flour residues hydrolyzed by α-amylase (60 and 300 U/g dry flour) for 24 and 48 h, while rice flours were hydrolyzed by α-amylase (60 and 300 U/g dry flour) for 1 h, RS were decreased significantly from the native rice flour. RS3 production can be accomplished by thermal or enzymatic processes, as well as with a combination of both. There are two main steps involved in the production of RS3 by thermal treatments: gelatinization and retrogradation [37]-[41]. A previous study [42] showed that α-amylase treated arrowroot starch had the highest percentage of RS. These results indicate that the amylose content, the molecular mass of starch components, and the amylopectin side chain lengths all strongly influence the formation of RS3.

TABLE III
RESISTANT STARCH CONTENT OF RICE FLOUR RESIDUES HYDROLYZED BY α - AMYLASE

Enzyme Concentration (U/g)	Hydrolysis Time (h)	RS Content (%)
60	1	3.83±0.08[b]
60	24	4.06±0.12[ab]
60	48	3.98±0.06[ab]
300	1	3.83±0.42[b]
300	24	3.97±0.05[ab]
300	48	4.17±0.44[a]

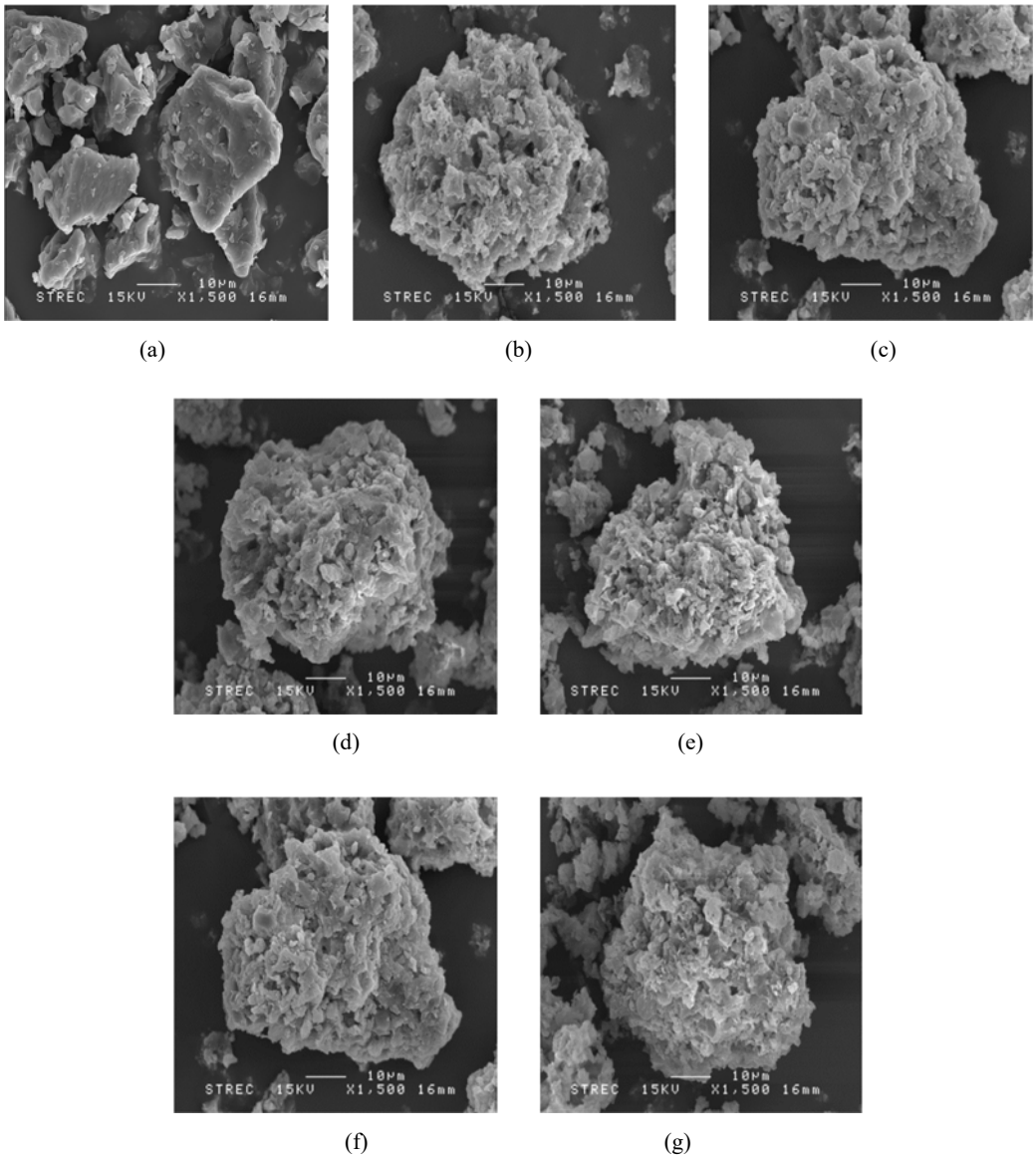
Means in the same column with different letters are significantly different ($p<0.05$).

The linear fractions released after fungal α-amylase action followed by debranching in the arrowroot starch were more appropriate in size for reassociation. This difference makes their pairing and recrystallization easier and hinders the access of digestive enzymes [42]. In contrast, RS content in this study

decreased slightly, so α-amylase was used in this study may not be appropriate to increased RS content.

IV. Conclusion

Rice flour residues hydrolyzed by 300 u/g α-amylase for 48 h had highest resistant starch content. Pasting temperature and viscosity of rice flour residues were lower than native flour. Increasing the hydrolysis time, resistant starch content and physical properties were changed significantly. The color of rice flour residues was the yellow brown, which have related to the concentration of enzyme. Due to the color of α-amylase was dark and reddish. The x-ray diffraction pattern seemed like mainly composed of amorphous regions. While granule morphology was an irregular shape and the surface was porous look like spongy.



(a) (b) (c)

(d) (e)

(f) (g)

Fig. 2 Scanning electron micrograph of native rice flour (a) and rice flour residues hydrolyzed by α-amylase 60 U/g at 1 h (b), 24 h (c), 48 h (d) and 300 U/g at 1 h (e), 24 h (f), 48 h (g)

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