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Impact of the Enzymatic Modification of Rice Flours on *In Vitro* Digestibilty and Molecular Properties

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In vitro digestibility was used to analyze the glucose content versus the definite digestion time by simulating the starch metabolic pathway in human. Three types of starch were classified as its *in vitro* digestion rate: rapidly digestible starch, RDS, slowly digestible starch, SDS, and resistant starch, RS. Starch with higher RS content has more dietary fiber, hypoglycaemic effect and reduces cholesterol. High RS and SDS in starch contribute low glycemic index (GI). Enzymatic modification is one of the methods for increasing RS and lowering GI in starch. In this work, four rice flours of one glutinous rice (RD6) and three non glutinous rice flours (Jasmine 105, Pathumthani and Suphanburi rices) were studied with two combinations of enzymatic modification (pullulanase and isoamylase from *Psedomonas sp*. (PII), pullulanase and isoamylase from *Thermus filiformis* (PI2). The *In vitro* digestibility result showed that both enzymatic combinations increased the RS, which caused GI of the modified rice flours to decrease from 76.4-102.2 to 40.4-41.9. They also decreased both RDS and SDS content in the rice flours. Both the enzyme combinations gave a greater number of short chain amylopectin for all studied rice flours. PII resulted in degree of polymerization (DP) of the modified glutinous rice and Suphanburi rice flours higher than that of the other two rice flours modified by PI2. Regarding the x-ray diffraction result, the effect of both enzyme combinations changed the crystalline structure from A+Vh to B+Vh. The morphology of the granules from Scanning Electron Microscope (SEM) showed the rough surface of their granules instead of smooth surface. The thermal properties from Differential Scanning Calorimeter (DSC) indicated that the difference of onset and conclusion temperature (Tc-To) of the modified rice flours was greater than those of their native counterparts. However, both of the two enzyme combinations showed remarkably different results to this value on Suphanburi rice. In conclusion, the enzymatic modification by using two enzyme combinations (pullulanase and isoamylase) affected the molecular properties by shortening molecular chain and rearranging its structure, which resulted in number of RS and lowered GI value. PII showed tendency of greater increase in RS than PI2.

Keywords: *In vitro* digestibility, Enzymatic modification, Rice flour, Molecular properties, Pullulanase, Isoamylase

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Introduction

Presently, healthy foods tend to be the food commodity to meet the need to global consumers. There are many types of healthy foods, such as, low fat, low sodium, trans-fat free or high fiber, high in some certain vitamins and minerals and antioxidants, etc. Regardings to carbohydrate, carbohydrate is a nutrition functioning as providing energy to human from glucose, which is the final metabolite from carbohydrate metabolism. Functional food from carbohydrate can be manipulated by the way of high fiber, low carbohydrate (carb) or low glycemic index (GI) food. Starch is a component of many carbohydrate foods. Starch digestibility performs different rate in different kinds of food depending on the internal structure of starch molecule, other constituents surrounding the starch molecule such as protein, fat and the suscepability towards acid and enzyme hydrolysis of the starch molecule. The slow digestibility type of starch can be utilized to healthy food for diabetics and the person who control the satiety as the weigh management issue.

Starch digestibility can be divided into three types according to the rate of glucose release and its absorption in the gastrointestinal tracts (Englyst *et al*., 1992). Rapid digestibility starch (RDS) is the starch fraction causes a sudden increase in blood glucose level aftyer ingestion. Slow starch digestibility (SDS) is a starch fraction that is digested completely in the small intestine at a lower rate as compared to RDS. Resistant starch (RS) is the starch fraction that cannot be digested in the small intestine, but is fermented in the large intestine to gain the short chain fatty acid. The health benefits of RS have been reported as hypoglycemic effects, substrate for growth of the probiotic microorganisms, prevention of colon cancer, hypochloesterolemic effects, inhibition of fat accumulation (Bjorck *et al*., 1994; Sajilata *et al*., 2006).

Recently, some methods have been made to modify the starch to increase the RS content or reduce its digestibility by increasing the retrograded starch (RS3). Physical method by hydrothermal treatment has been applied by the method of annealing (ANN) and heat-moisture treatment (HMT) (Chung *et al*., 2009; Lee *et al*., 2012), chemical modification (Han and Bemiller, 2007), enzymatic methods (Pongjanta *et al*., 2009; Man *et al*, 2013a and b; Zeng *et al*., 2014). Among the enzymatic methods, the amylolytic enzymes, namely, α amylase, ß-amylase and amyloglucosidase and the debranching enzyme such as pullulanase, isoamylase were studied to increase RS content or lower digestibility. All the principle of these methods needs the gelatinization step and then followed with the cooling-temperature step to obtain the retrograded starch, which is the expected high RS modified starch. Pullulanases (EC3.2.1.41, pullulan-6-glucanohydrolase) specifically cleave the α-1, 6

glycosidic linkages in pullulan, starch, amylopectin and related oligosaccharides that pullulanases are helpful in RS3 formation as they can catalyze the hydrolysis of α -1, 6 glucosidic bonds in the branching sites of amylopectin, the property of which provides an increased opportunity to molecule aggregation and crystalline structures formation. In recently, pullulanase has been used to increase RS yield from starch. Isoamylase is also direct debranching enzyme which present in plants and bacteria. It can directly hydrolyze α-1, 6-glucosidic bonds of unmodified substrate.

Pullulanases and isoamylases function on the basis of substrate specificity. The major difference between pullulanase and isoamylase that pullulanase hydrolyzes the α-1,6 glucosidic bond in pullulan and amylopectin while it requires each of the two chains linked by an α-1,6- glucosidic bond contains at least two α-1,4-linked glucose units, whereas isoamylase can only hydrolyze the α-1,6 bond in amylopectin and glycogen. Thus, the smallest substrate is suitable for pullulanase than isoamylase. Isoamylase can rapidly depolymerize of and therefore is highly susceptible to retrogradation.

Regardings the debranching enzymes, a little information has been reported for using a combination of these two debranching enzymes to hydrolyze the starch before having it be retrograded. Especially different sources of enzyme, it may function with different potential. Hence, this study is aimed to apply the combination of pullulanase and isoamylase to hydrolyze the different varieties of rice flour to obtain the higher RS content of rice flour. Also, the comparable study of two different source of isoamylase was done. The *in vitro* digestibility and physicochemical and molecular properties of the native and modified rice flours were investigated.

Materials and methods

Material and its preparation

Three types of rice were purchased from the rice mill factory. One lot of rice was purchased and kept in refrigerated room at 4◦C and then was used for whole study. There were glutinous rice (RD6), Jasmine 105, Pathumthani and Suphanburi rices. All the rices have amylose content of 1, 12, 17 and 27%, respectively. The rice flours were prepared by grinding through the sieve with 250 micron. The passed-through rice flours were collected to study as the native rice flours. Then, all native rice flours were modified by using two set combinations of enzymes, pullulanase and isoamylase from *Psedomonas sp.* (PII) and by pullulanase and isoamylase from *Thermus filiformis* (PI2). The modification method was followed according to the method of Zhang and Jin

(2011). First, the 10% concentration (w/w) of rice flours was heated at 80◦C, 20 min. The pullulanase and isoamylase were added with the amount of 4.5 units/ gram and 5 units/gram into the gelatinized rice flour suspension with pH 4.5, respectively. Then, it was incubated at 45◦C, 12 hrs in continuous shaking bath. After that, the incubated flour suspension was transferred to refrigerate at 4◦C, 24 hr. When the time was reached, it was centrifuged to drain the liquid out and washed with ethanol (99.5%). The modified samples were dried at 40◦C in a tray-drier until the moisture to be 11% and finally, grounded to be flour for further analysis.

Analysis Method

All native rice flours and modified rice flours were analyzed as following:

In vitro digestibility (RS, RDS, SDS, hydrolysis index (HI) and Glycemic index (GI))

This was determined according to Megazyme Resistant Starch Assay Kit (AOAC method.2000). Around 100 mg of ground sample material were added with 4.0 ml of pancreatic α-amylase and then incubate tubes at 37℃ with continuous shaking (200 strokes /min). The tubes were removed from the water bath, The samples were centrifuge and the supernatants was analyzed (for RDS and SDS), using the Megazyme D-Glucose (glucose oxidase/ peroxidase). The starch classification based on its digestibility was:

RDS as the starch that was hydrolyzed within 20 min of incubation

SDS as the starch digested during the period between 20 and 120 min.

RS as the starch not digested during the period for exactly 16 hrs. when the time was reached, the samples were centrifuge and the supernatants were discarded. The residue was analyzed for RS content.

HI was analyzed as this following:

The rate of starch digestion was expressed as a percentage of the total starch hydrolyzed at different times (30, 60, 90, 120, 150, and 180 min). Each treatment was analyzed in triplicate.

A non-linear model established by Goni *et al.* (1997) was applied to describe the kinetics of starch hydrolysis. The first order equation has the form:

$$C = C\infty\,(1 - e^{-kt})$$

where C corresponds to the percentage of starch hydrolyzed at time t,

C_∞ is the equilibrium percentage of starch hydrolyzed after 180 min,

k is the kinetic constant and t is the time (min).

The parameters C_∞ and k were estimated for each treatment based on the data obtained from the *in vitro* hydrolysis procedure.

The area under the hydrolysis curve (AUC) was calculated using the equation:

$$AUC = C_\infty (t_f - t_0) - (C_\infty / k) [1 - Exp[-k[t_f - t_0]]$$

where C_∞ corresponds to the equilibrium percentage of starch hydrolysed after 180 min,

t_f is the final time (180 min), t_0 is the initial time (0 min), k is the kinetic constant.

A hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of each sample by the corresponding area of a reference sample (fresh white bread). Goñi et al. (1997) showed this hydrolysis index to be a good predictor of glycemic response. Expected GI was thus estimated using the model

$$GI = 39.71 + (0.549 \times HI)$$

Average chain length distribution of amylopectin was analyzed by high-performance anion-exchange chromatograph (HPAEC) according to the method of Jane and Cheng (1992)

X-ray diffraction (Model Bruker AXS) was used to analyze starch crystal structure and crystallinity. The native rice flour and the modified rice flours were prepared to the starch from flour. The starch samples were scanned from 4 to 40◦C. The relative crystallinity was calculated as the ratio of the crystalline areas and amorphous regions of x-ray diffractograms.

The native and modified starch samples were observed the granule morphology by using scanning electron microscope (SEM) (JSM 6400, Japan). Dried, ground samples were mounted on an aluminum stub with doubled-size tape and coated with a thin film of gold. The samples were then observed and photographed.

The gelatinization characteristics of both native and modified rice flours were measured and recorded by using differential scanning calorimetry (DSC) (Model Mettler DSC 2, Switzerland). Approximately 5-6 mg of rice flour samples were added to an aluminum DSC pan with adding distilled water to 70% moisture and the sealed, heated from 25-130◦C at 10◦C/min. An empty pan was used as a reference.

Statistical analysis

The experiment was a completely randomized design with three replicates. The data were analyzed by applying analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT) in SPSS.

Results and Discusion

Table 1 Resistant Starch (RS), Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS) content and Glycemic index (GI) of native rice flours and modified rice flours by pullulanase and isoamylase from *Psedomonas sp.* (PII) and by pullulanase and isoamylase from *Thermus filiformis* (PI2).

Rice type	(%)	RS	RDS	SDS	GI
RD 6 glutinous rice	Native rice flour	1.6 ± 1.0^{ab}	34.2 ± 5.1^{a}	9.1 ± 1.9^{a}	102.2 ± 0.2^{a}
	Modified rice flours from PII	31.9 ± 0.9^{a}	2.6 ± 0.8^{b}	0.3 ± 0.1^{b}	40.4 ± 0.0^{c}
	Modified rice flours from PI2	8.8 ± 0.2^{b}	2.2 ± 0.1^{b}	1.3 ± 0.0^{b}	41.6 ± 0.0^{b}
Jasmine 105	Native rice flour	0.2 ± 0.1^{ab}	36.0 ± 3.7^{a}	9.0 ± 5.1^{a}	94.8 ± 0.1^{a}
	Modified rice flours from PII	29.2 ± 0.8^{a}	3.6 ± 0.4^{b}	0.4 ± 0.1^{b}	40.6 ± 0.2^{c}
	Modified rice flours from PI2	9.5 ± 1.7^{b}	3.0 ± 0.2^{b}	0.2 ± 0.1^{b}	41.8 ± 0.0^{b}
Pathumthani	Native rice flour	3.0 ± 0.6^{c}	36.0 ± 3.7^{a}	7.5 ± 3.7^{a}	85.2 ± 0.0^{a}
	Modified rice flours from PII	28.4 ± 0.6^{a}	3.6 ± 0.4^{b}	0.1 ± 0.0^{b}	40.6 ± 0.1^{c}
	Modified rice flours from PI2	11.0 ± 0.3^{b}	3.0 ± 0.2^{b}	0.1 ± 0.0^{b}	41.8 ± 0.0^{b}
Suphanburi	Native rice flour	3.2 ± 0.5^{c}	39.0 ± 1.9^{a}	0.9 ± 0.0^{a}	76.4 ± 0.0^{a}
	Modified rice flours from PII	25.3 ± 0.1^{a}	4.1 ± 0.2^{b}	0.1 ± 0.0^{b}	40.7 ± 0.1^{c}
	Modified rice flours from PI2	14.6 ± 0.2^{b}	3.5 ± 0.1^{b}	0.3 ± 0.1^{b}	42.0 ± 0.1^{b}

The superscript a, b, c…. means that the mean values are siginificantly different compared between native rice flour, modified rice flours from PII, modified rice flours from PI2 within the same rice type ($p<0.05$).

As seen in Table1, both modification by two set of enzyme combinations (PII and PI2) remarkably increased RS content of all studied rice flours comparing to their native rice flours, especially the combination of PII enzymes. While RDS content of all rice flours decrease plumetingly, SDS content

decreased significantly on both modified rices. The GI value of all modified rice became the lesser extent with the value about 40, which is named as low GI food (GI≤55). This study indicated that the debranching enzymes of both pullulanase and isoamylase used in this study exhibit the debranching chain, which further rearranged the chain and starch molecule to be more resistant to hydrolysis effect of amylolytic enzyme (Eerlingen *et al*., 1993b; Pongjanta *et al*, 2009). However, the two set of combination of enzymes influenced RS content increasingly with different degree. The modified rice flour from isoamylase from *Psedomonas sp.* (PII) obtained more RS content than that from isoamylase from *Thermus filiformis* (PI2). As for GI value showed in Table 1, all the rice with different amylose content had the close range of GI value (about 40). Eerlingen *et al.* (1993a) reported that the degree of polymerization of RS is independent of the chain length of amylose.

Table 2 Amylopectin distribution (shown as in Degree of Polymerization, DP) of native rice flours and modified rice flours by pullulanase and isoamylase from *Psedomonas sp.* (PII) and by pullulanase and isoamylase from *Thermus filiformis* (PI2).

Rice type		Amylopectin distribution (%)			
		DP 25-35	DP 36-58	DP 59-69	DP>70
RD 6 glutinous rice	Native rice flour	1.7	2.0	0.5	2.6
	Modified rice flours from PII	9.4	58.2	30.8	1.6
	Modified rice flours from PI2	8.2	55.4	32.9	3.5
Jasmine 105	Native rice flour	0.1	1.1	7.1	10.4
	Modified rice flours from PII	1.1	42.8	45.3	10.8
	Modified rice flours from PI2	1.1	45.7	50.4	12.6
Pathumthani	Native rice flour	0.5	4.3	0.5	1.8
	Modified rice flours from PII	1.1	40.6	45.5	12.8
	Modified rice flours from PI2	1.2	56.4	32.2	10.2
Suphanburi	Native rice flour	0.1	2.2	2.3	2.6
	Modified rice flours from PII	1.2	57.4	30.8	10.6
	Modified rice flours from PI2	1.1	30.2	36.3	32.5

From Table 2, the two set of combination of both enzymes increased apparently the long chain amylopectin with DP content of 36-69 whereas the intermediate chain of DP 25-35 increased gradually. This study does not show the result of the short amylopectin chain of DP<12. This result refered that these two combinations of enzymes proceed on debraching amylopectin to obtain more individual chain with still have more glucose polymer in the chain. It was suggested from this study that it may be added some hydrolyzing amylolytic enzyme such as α amylase to obtain the shorter chain in order to gain more crystalline structure for more resistant starch content in the modified starch. From the finding of Shu et al (2007) reported that the increased content of short chains with 8≤DP≤12 and decreased of intermediate and long chains with 24≤DP were clearly associated with the increase of resistant starch in rice, which is a mutant variety high in resistant starch.

Table 3 Crystallinity and crystal structure from X-ray diffraction of native rice starch and modified rice starch by pullulanase and isoamylase from *Psedomonas sp.* (PII) and by pullulanase and isoamylase from *Thermus filiformis* (PI2).

Rice type		Native rice starch	Modified rice starch from PII	Modified rice starch from PI2
RD 6 glutinous rice	crystallinity (%)	38.91	44.65	39.75
	crystal structure	A + V_h	B + V_h	B + V_h
Jasmine 105	crystallinity (%)	33.99	40.38	39.70
	crystal structure	A + V_h	B + V_h	B + V_h
Pathumthani	crystallinity (%)	30.76	39.89	39.12
	crystal structure	A + V_h	B + V_h	B + V_h
Suphanburi	crystallinity (%)	29.84	34.81	38.16
	crystal structure	A + V_h	B + V_h	B + V_h

Table 3 shows the crystallinity and crystal structure of the native and modified rice starch. All native rice starch shows the double peak 2θ at 17 º- 18 º and single peak 2θ at 15 ºand 23 ºfrom x-ray diagram (figure not shown), which indicated the crystal starch of A structure of cereal starches. In addition, the small single peak 2θ at 20 º show the crystal structure of V_h of amylose-lipid complex occurred in all studied rice starches (figure not shown) (Cheetham and Tao, 1998; Derycke *et al*., 2005). After the rice modified from PII and PI2, all the crystal structure changed to B type, which is normally found in the tube and root starches. The B type of crystal structure showed the double

peak 2θ at 22 ºand 24 ºand single peak 2θ at 5.6 ºand 17 º(Buleon *et al*., 1998). All the modified starch also showed apparently the small peak of V_h of amylose-lipid complex. This result concurred with the study of Shi and Goa (2011), who reported the B + V_h of crystal structure found in the modified waxy rice starch by pullulanase and Zhou *et al*. (2014) also reported the B + V_h of crystal structure of indica rice starch found in the heat-moisture treatment modified starch. Shu *et al*. (2006) reported that the B + V_h of crystal structure is the structure that more resistant to hydrolysis of α amylase.

Regardings to the crystallinity, rice starches modified from PII show the higher crystallinity content than rice starches modified from PI2 and the native counterparts, repectively. This is exceptional for Suphanburi rice starch, which the modified from P11 had less crystallinity than the modified from P12. However, all the modified rice starches from both set of enzymes show the crystallinity content higher than their native counterparts. This indicated the studied enzymes functioned as debranching the amylopectin chain to obtain the shorter starch molecule, which easily rearranged their chains to be the crystalline structure. Pullulanase and isoamylase from *Psedomonas sp.* (PII) tended to have the debranching power in lower amylose rice than pullulanase and isoamylase from *Thermus filiformis* (PI2). Gunaratne and Hoover (2002) reported that the average chain length of amylopectin and the short chain amylopecin of DP 10-13 affected the crystallinity of flour. Song and Jane (2000) have been previously reported that the degree of crystallinity can be attributed to crystal size, amount of crystallinity orientation of the double helices and extent of interaction between the double helices.

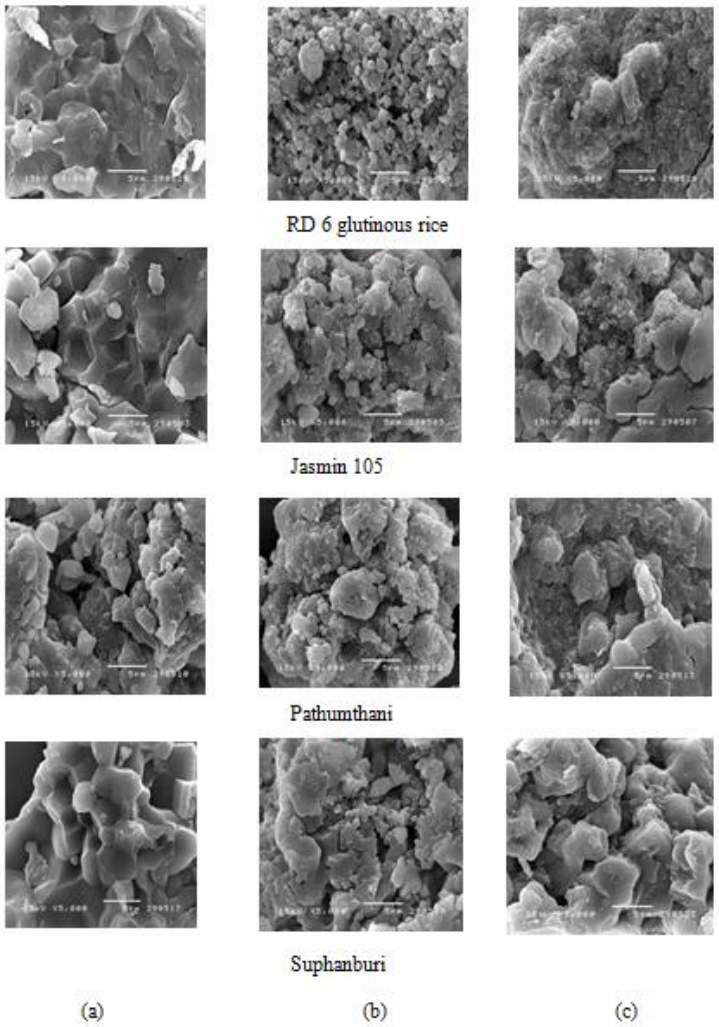

Fig. 1 The starch granule morphology from Scanning Electron Microscope (SEM) of native rice flours (a) and modified rice flours by pullulanase and isoamylase from *Psedomonas sp.* (PII) (b) and by pullulanase and isoamylase from *Thermus filiformis* (PI2) (c).

Fig 1 shows the rice starch granule morphology. All native rice starch granule exhibited the irregular polygonal shape with smmoth surface. After treated with both enzyme combinations (PII and PI2), the modified starch granule of all rice varities remained the same shape with more rough and porous surface. This is the cause from the hydrolysis of enzyme used in this study. Both the modified starch granule looked denser than than the native ones. This structure is caused from the recrystallization of the gelatinized starch. The

debranched and shorter amylose and amylopectin chain moved closer to restructure or recrystallize again. This increase the crystallinity of starch molecule, that shows in Table 3. The same results were reported by Shi and Gao (2011); Zhang and Jin (2011); Zhou *et al* (2014).

Table 4 Thermal properties from Differential Scanning Calorimeter (DSC) of native rice flours and modified rice flours by pullulanase and isoamylase from *Psedomonas sp.* (PII) and by pullulanase and isoamylase from *Thermus filiformis* (PI2).

Rice type		Native rice flour	Modified rice flours from PII	Modified rice flours from PI2
RD 6 glutinous rice	T_o (℃)	63.57 ± 0.60^{a}	56.92 ± 0.12^{b}	53.11 ± 0.14^{c}
	T_p (℃)	70.57 ± 0.02^{c}	82.64 ± 0.11^{b}	92.67 ± 0.47^{a}
	T_C (℃)	77.54 ± 0.37^{c}	98.38 ± 0.46^{b}	104.53 ± 0.53^{a}
	$T_c - T_o$ (℃)	14.56 ± 0.60^{c}	41.51 ± 0.41^{b}	50.79 ± 0.21^{a}
	ΔH (J/g)	2.95 ± 0.05^{b}	3.93 ± 0.03^{a}	1.97 ± 0.01^{c}
Jasmine 105	T_o (℃)	63.12 ± 0.17^{a}	61.45 ± 0.02^{b}	53.74 ± 0.37^{c}
	T_p (℃)	69.75 ± 0.08^{c}	85.59 ± 0.59^{a}	77.69 ± 0.45^{b}
	T_C (℃)	76.13 ± 0.25^{c}	94.07 ± 0.18^{a}	88.68 ± 0.46^{b}
	$T_c - T_o$ (℃)	12.61 ± 0.15^{c}	32.91 ± 0.56^{b}	34.94 ± 0.09^{b}
	ΔH (J/g)	3.05 ± 0.05^{a}	1.57 ± 0.04^{c}	2.48 ± 0.04^{b}
Pathumthani	T_o (℃)	66.74 ± 0.37^{c}	73.95 ± 0.07^{b}	76.63 ± 0.53^{a}
	T_p (℃)	72.79 ± 0.45^{c}	82.80 ± 0.14^{a}	77.84 ± 0.23^{b}
	T_C (℃)	81.12 ± 0.16^{c}	87.96 ± 0.06^{b}	89.76 ± 0.34^{a}
	$T_c - T_o$ (℃)	14.63 ± 0.18^{ab}	14.41 ± 0.56^{ab}	13.44 ± 0.23^{bc}
	ΔH (J/g)	2.44 ± 0.37^{a}	0.23 ± 0.10^{b}	0.60 ± 0.04^{b}
Suphanburi	T_o (℃)	61.47 ± 0.09^{b}	54.79 ± 0.35^{c}	88.96 ± 0.06^{a}
	T_p (℃)	67.59 ± 0.40^{c}	92.57 ± 0.33^{b}	99.77 ± 0.33^{a}
	T_C (℃)	73.79 ± 0.12^{c}	104.23 ± 0.11^{b}	110.19 ± 0.13^{a}
	$T_c - T_o$ (℃)	12.17 ± 0.24^{c}	49.76 ± 0.21^{a}	21.44 ± 0.09^{b}
	ΔH (J/g)	2.71 ± 0.15^{a}	1.98 ± 0.03^{b}	0.23 ± 0.02^{c}

The superscript a, b, c…. means that the mean values are siginificantly different compared between native rice flour, modified rice flours from PII, modified rice flours from PI2 within the same rice type ($p<0.05$).

Table 4 shows the thermal properties from Differential Scanning Calorimeter (DSC). It was clearly seen that the combination of enzymes of both studied enzyme sets (PII, PI2) decreased the onset temperature (T_0) of all studied rice except Pathumthani rice, however, increased the peak (T_p) and conclusion temperature (T_c) significantly. As mentioned by Man et al (2013b), the onset temperature involved the gelatinization temperature that uncoiling and melting of the external chains of amylopectin that are packed together as double helix in clusters. Gelatinization temperature is influenced by the molecular structure of crystalline region, which corresponds to the distribution of amylopetin short chains and not by the proportion of the crystalline regions, which corresponds to the amylose/ amylopectin ratio. The lower content of T_0 of both modified rice flour comparing to the native rice flour refered to the rearranged molecule architecture of the modified starch after debranching by enzymes had the rearranged weak crystalline region (Sievert and Lausanne, 1993). However, T_p and T_c of the modified rice flours were higher than that of the native rice flour. This may be the reason of more crystalline region (Table 3) of the modified rice flours.

Conclusion

The combination of the two debranching enzymes (pullulanase and isoamylase) could increase resistant starch (RS) content, decrease rapid digestibility starch (RDS) and slow starch digestibility (SDS) contents. Different sources of isoamylase affected the different increasing degree in RS content. These two enzymes function as debranching α -1, 6 glucosidic bonds in the branching sites of amylopectin to gain more intermediate and long chain with DP 36-69. The modified starch molecule changed the crystal structure to the B + V_h, which is the structure that more resistant to hydrolysis of α amylase. Higher crystallinity of the modified rice starch may contribute to have more crystalline region that affected to peak and conclusion temperature from DSC shifted to higher values. It was suggested from this study that adding more hydrolyzing amylolytic enzyme such as α amylase may increase more shorter chain, which could be formed by aggregation of these shorter chains to be amylose helices in a crystalline B-type structure.

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