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Porous high amylose rice starch modified by amyloglucosidase and maltogenic α-amylase

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ABSTRACT

Porous starch is attractive by providing high surface area for many applications. In this study amyloglucosidase (AMG) and maltogenic α-amylase (MA) were investigated in direct comparison to elucidate potential effects in producing porous starch using high amylose rice starch as a substrate. Both enzymes generated pores at the surface as illustrated by Scanning Electron Microscopy (SEM). The enzyme-treated granules had higher relative crystallinity as deduced from Wide Angle X-ray Scattering (WAXS). MA treatment increased the number of short amylopectin chains and decreased the molecular weight with extended incubation time. The MA-treated starch had higher solubility whereas swelling capacity, amylose content, peak viscosity, final viscosity, breakdown and setback of both treatments were decreased compared to the control. Enzymatic treatments produced starch with delayed gelatinization temperature and increased the enthalpy. The results demonstrate that porous rice starch can provide different functionalities depending on the enzyme mechanisms, extending the range of applications.

1. Introduction

Rice starch is a small granule starch found in a wide range of amylose/amylopectin ratios. However, the amylose fraction provides heterogeneity and increases the gelatinization temperature and starch retrogradation which may negatively affect the quality of the starch product. Hence, physical, chemical or enzymatic modifications are therefore applied. However, chemical modification is associated with environmental pollution and consumer concerns and enzymatic modification is more selective and clean and can reduce by-product volumes (Le et al., 2009; Oh, Choi, Lee, Kim, & Moon, 2008).

There is a current growing interest to produce porous starches due to their high specific surface area facilitating the access of enzymes and chemical reagents into the starch granules for further modification. Porous starches find applications in various foods as absorbents or carrier agents for volatile compounds, flavorings and protecting the sensitive elements such as vitamin, oil and probiotic (Belingheri, Giussani, Rodriguez-Estrada, Ferrillo, & Vittadini, 2015; Benavent-Gil, Rodrigo, & Rosell, 2018; Li, Ho, Turner, & Dhital, 2016; Xu et al., 2017). Enzyme-assisted modification has been used as an efficient method for obtaining porous starches. For example, a commercial hydrolyzing enzyme (STARGEN™001, a blend of α-amylase (AM) and amyloglucosidase (AMG)) has been used to hydrolyze native and cross-linked corn, tapioca and sweet potato starches in their granular state (Yussof, Utra, & Alias, 2013). The results showed that granule erosion occurred mainly on the surface but pores were found in the interior part of the granules. Native corn starch treated with AM and AMG at sub-gelatinization temperature produced porous starch with microstructure that was dependent on the enzymatic treatment (Dura, Błaszczak, & Rosell, 2014). Moreover, the effect of AMG hydrolysis on granular morphology as evaluated by SEM showed that the pore size radius of hydrolyzed starch granules gradually increased with higher degree of hydrolysis (Chen & Zhang, 2012).

In this study, AMG and maltogenic α-amylase (MA, glucan 1,4-α-maltohydrolase, EC 3.2.1.133) were investigated to produce porous rice starch. It has been identified both AMG and MA having exo-activities (Van Der Maarel, Van Der Veen, Uitdehaag, Leemhuis, & Dijkhuizen, 2002). AMG obligatory and specifically hydrolyzes α-1,4 glucosidic bonds from the non-reducing ends of starch, resulting in the production of glucose. AMG is also capable of hydrolyzing α-1,6 linkages, however at an 80-fold lower rate (Dura et al., 2014; Mertens & Skory, 2007). Catalytically MA is versatile enzyme that in addition to having mainly exo-α-1,4-glucanase activity releasing maltose, also exhibit endo-glucanase activity on accessible intramolecular bonds (Christophersen,

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Otzen, Norman, Christensen, & Schäfer, 1998; Goesaert, Slade, Levine, & Delcour, 2009; Le et al., 2009). Moreover, transglycosylation activity of MA to form various glucosidic linkages such as α,1-3 and α,1-6 linkages from acarbose and gelatinized starch has been reported (Cha et al., 1998; Lee et al., 2002, 2008; Park et al., 2000). MA is used mainly as an anti-staling agent by shortening the outermost chains of amylopectin inhibiting retrogradation of amylopectin as shown for waxy maize starch (Grewal et al., 2015), rice starch and rice meal (Li, Li, Tian, & Park, 2014). The use of MA has not been reported for obtaining porous starch. However, MA has been tested in gelatinized systems aiming at modifying starch to reduce amylolytic digestion by increasing branched side chains (Ao et al., 2007; Miao et al., 2014). Moreover, the combination of MA and α-glucanotransferases such as branching enzyme (BE) has received considerable attention to produce highly branched starch (Le et al., 2009; Lee et al., 2008) and attenuated digestion of maize extruded flours (Martínez, Pico, & Gómez, 2016). We hypothesized that the different catalytic actions of AMG and MA can provide different structural and physicochemical properties of granular rice starch. The aim of this study was to investigate the the different hydrolytic mechanisms of AMG and MA to produce porous starch using high amylose rice starch as a substrate. The morphological, structural and physicochemical properties were characterized.

2. Materials and methods

2.1. Materials

Rice starch was a gift from General Food Products Co., Ltd. (Nakhon Ratchasima, Thailand). Amyloglucosidase (EC 3.2.1.3, specific activity 300 U/mL) from *Aspergillus niger* was purchased from Sigma-Aldrich (Steinheim, Germany). Maltogenic α-amylase (EC 3.2.1.133, Maltogenase® L) was kindly provided by Novozymes (Bagsvaerd, Denmark).

2.2. Preparation of porous starch

Rice starch (10 g dry basis, db) was suspended in 20 mL of 20 mM Na maleate and 5 mM $CaCl_2$ buffer at pH 5.5 (MA treatment) or 20 mM Na acetate buffer at pH 4.5 (AMG treatment). The starch suspension was heated in water bath at 60 °C for 0.5 h. Then, AMG or MA (100 U/g starch) was added to the starch suspension. The samples were incubated at 60 °C for 3, 6, 12 and 24 h in a shaking water bath (180 rpm). DI water (40 mL) was added to the suspensions, the starch granules collected on a filter followed by 3 times washing with DI water. The samples were pre-dried at 60 °C for 20 min before drying at 130 °C for 2 h. The dried starch samples were ground and passed through a 100-mesh sieve then stored in a desiccator for further analysis. The starch control samples were prepared with the same procedure without enzyme addition for all treatments.

2.3. Scanning electron microscopy (SEM)

Granular morphology and topography were observed using Field Emission Scanning Electron Microscope (FE-SEM, Carl Zeiss, Oberkochen, Germany). Samples (1 %) was suspended in absolute ethanol. One drop of each sample was placed on a coverslip and dried at 50 °C for 3 h. A thin layer of each sample was placed on aluminum stubs with conductive carbon tape and sputter-coated with gold-palladium. The accelerating voltage was 3.0 kV.

2.4. Specific surface area and total pore volume using nitrogen sorption isotherms through Brumauer-Emmett–Teller (BET) protocol

Firstly, starch samples were dried at 100 °C for 6 h. Then, dried starch samples (200 mg) were degassed at 125 °C for 24 h and immersed in liquid nitrogen (-196 °C). The measurement was carried out by dispensing a specific portion of the nitrogen gas (2 L) and measuring the relative pressure p/po. The pressure change was recorded by BELSORP-mini II. Specific surface area and total pore volume were determined. The monolayer value was calculated using the BET equation (Brunauer, Emmett, & Teller, 1938).

2.5. Relative crystallinity

The relative crystallinity of starch samples was quantified by wide-angle X-ray scattering (WAXS) at beamline 1.3, Synchrotron light research institute, Nakhon Ratchasima, Thailand, described by Boonna and Tongta (2018).

2.6. Determination of amylose content

The amount of apparent amylose was determined by triiodide colorimetry as described by Wickramasinghe, Blennow, and Noda (2009).

2.7. High performance anion exchange chromatography (HPAEC)

The starch samples (5 mg/mL) were gelatinized by boiling and debranched by isoamylase (2 μL, 260 U/mg, Megazyme) and pullulanase (2 μL, 40 U/mg, Megazyme) at 40 °C. Samples of 10 μL were injected into a CarboPac PA-200 column on the HPAEC-PAD system, (Dionex, Sunnyvale, CA, USA) at a flow rate of 0.5 mL/min. Chain fragments were eluted from the column using the following profiles: 0-2 min: 150 mM NaOH isocratic, 5 m in. 150 mM NaOH isocratic, 110 mM NaOAc linear gradient, 130 min: 150 mM NaOH isocratic, 400 mM NaOAc convex gradient. Single peaks were integrated and corrected for the detector response (Viksø-Nielsen, Blennow, Nielsen, & Møller, 1998).

2.8. Molecular weight distribution analysis by size-exclusion chromatography with triple detection array (SEC-TDA)

Molecular composition was determined by size exclusion chromatography (SEC) using a Viscotek System (Malvern, UK) equipped with a GS-520HQ column (Shodex) attached to a TDA302 module (Triple detector array) consisting of a refractive index detector (RI), a four-bridge viscometer detector (VIS) and a light scattering detector (LS). The LS consisted of a right-angle light scattering (RALS) and a low angle light scattering (LALS).

Samples were first prepared in the form of non-granular starch as described by Klucinec and Thompson (1998). Non-granular starch (5 mg) was dissolved in 25 μL of 2 M KOH at 4 °C for 24 h, and re-suspended in 975 μL Milli-Q water mechanically shaken (1200 rpm) at 80 °C for 5 h to a final concentration of 1 mg/mL. The calibration was made using pullulan (50 kDa, polydispersion 1.07, Showa Denko) as standard and samples components were eluted in ammonium formate (10 mM) with a flow rate of 0.5 mL/min. The injection volume was 50 μL and the column temperature was thermostated at 60 °C. Data analysis was performed using the OmniSec Software 4.7 (Malvern Instrument, Ltd.).

2.9. Swelling capacity and solubility of starch

The swelling capacity (SC) and solubility (S) were determined by the method described by Rosell, Yokoyama, and Shoemaker (2011) with a slight modification. Briefly, 100 mg db of the starch was weighed in centrifuge tubes followed by addition of 10 mL of MilliQ water. The samples were heated at 60 °C for 30 min under continuous mixing. Then the dispersion was centrifuged at 4000 *g* for 15 min. The supernatant was transferred to Petri dishes then dried at 110 °C overnight and weighed. The residue obtained after drying of the supernatant

represented the amount of starch solubilized in water. The swelling capacity and solubility were calculated as follows:

$$SC\ (g/g) = \text{sediment weight} / [\text{weight of dried starch} \times (1\text{-}S/100)]$$

$$S\ (\%) = (\text{dried supernatant weight} / \text{weight of dried starch}) \times 100$$

2.10. Pasting properties

The pasting properties were monitored using Rapid Visco Analyser (RVA) (Newport Scientific, Warriewood, Australia). MilliQ water (25 mL) was added to 2.5 g of starch sample (14 % moisture content) in the aluminum RVA canister. The RVA settings were as follows: heating from 50 to 95 °C for 282 s, holding at 95 °C for 150 s and cooling to 50 °C over 300 s. The initial mixing paddle speed was 960 rpm for 10 s followed by160 rpm speed during analysis. Pasting parameters were recorded using Thermocline software (Perten Instruments, Hägersten, Sweden) for Windows.

2.11. Thermal properties

Thermal behavior of the samples was investigated using differential scanning calorimeter DSC 2500 (TA instrument, New Castle, USA), equipped with a thermal analysis data program (Trios, New Castle, USA). Approximately ten mg of the sample was loaded into the aluminum pan and MilliQ water was added to obtain a water-sample ratio of 3:1. The sample pans were hermetically sealed and equilibrated at room temperature for 24 h before analysis. Thermal analysis performed by heating from 25 to 130 °C at a rate of 5 °C/min. Initial temperature (Ti) was defined as the temperature at which the sample started to gelatinize and onset temperature (To), peak temperature (Tp), conclusion temperature (Tc), gelatinization temperature range (Tc-Ti) and enthalpy (ΔH) were determined.

3. Results and discussion

3.1. Granular morphology

The enzymatic modification of rice starch was carried out separately with amyloglucosidase (AMG) or maltogenic α-amylase (MA). To rule out effects of incubation only, the enzyme-action was compared with their controls for each time of incubation, which was performed in the absence of enzyme. SEM micrographs showed that native high amylose rice starch granules displayed polygonal shape and smooth surface with some small holes as evident at high magnification (Fig. 1A). Enzymatic treatment had a clear effect on the granular surface, as seen by several dents and holes which is similar to other previous reports (Benavent-Gil & Rosell, 2017b; Dura et al., 2014; Dura & Rosell, 2016). However, the enzyme had no effect on the general shape of the granules. These results are in accordance with previous reports (Dura et al., 2014; Zhang et al., 2012; Zhao et al., 2018). Specifically, AMG-treated starch displayed big and shallow pores whereas MA-treated starch showed small pores and they seem to be deeper than those of the AMG treatment. It has bee documented that AMG is more active on starch granules having hollow granules (Dura et al., 2014). In addition, the range of pore diameters hydrolysed by AMG was more diverse and wider compared to MA (Fig. 2). As the reaction time increased, the average pore size became wider by AMG and some granules were fragmented after being 24 h modification (Fig. 1B8) that caused the average pore size dropped. Previous studies have demonstrated that corn starch treated with AMG had larger pores and broader pore size distribution compared with α-amylase, showing small pores on the surface (Benavent-Gil & Rosell, 2017a).

The specific surface area, $S_{BET,}$ data confirmed that the specific surface areas increased after enzymatic modifications (Fig. 3), especially for AMG. The highest S_{BET} of AMG and MA treatment was found after 12 h and 24 h modification, respectively. This result could be attributed to AMG and MA absorption on susceptible surface zones and degraded the external part of the granule by exo-corrosion. As the pore size becomes sufficiently wide by exo-erosion activity, endo-erosion can commence, potentially starting at narrow pore structures at the granule surface, progressing towards to the center of the granule (Sujka & Jamroz, 2007). When endo corrosion occurs, the internal part of the granule was corroded through small pores by which enzymes penetrate the granule. Alternatively, it could be due to that MA is also an endo-acting hydrolase enzyme, and hydrolyses α-glucan chains efficiently in the interior of the starch granule. Therefore, the pores of MA-treated starch seem to be narrower but deeper than those of AMG.

3.2. Crystalline structure

To further investigate the effects of enzymatic treatment on the crystalline and amorphous regions, the crystallinity was monitored by wide-angle X-ray scattering. All samples displayed an A-type pattern which strong reflection at 2θ of 15, 17, 18 and 23 (data not shown). The relative crystallinity of the control was typically not different from that of native starch (Table 1). It should however be noted that the control sample for MA, i.e. incubation of the starch at pH 5.5 without MA, resulted in molecular re-arrangement leading to an increase in the relative crystallinity. This increase suggests that annealing took place in a pH dependent manner as suggested (Dura & Rosell, 2016). The relative crystallinity of all enzymatic treatments increased when compared to their buffer controls and native starch, which is consistent with previous report by Zhao et al. (2018). The increase in relative crystallinity of the enzyme treated samples could be an effect of hydrolytic activity mainly in amorphous regions (Dura et al., 2014; Zhao et al., 2018), thereby increasing the proportion of crystalline structure. The relative crystallinity of AMG treatment steadily increased as treatment time was longer; on the other hand, at the initial time of MA hydrolysis (3 h) the crystallinity was increased to 28.09 %. The highest crystallinity was found for the AMG and MA-treated starches, 29.02 % and 33.29 % after incubation for 24 h and 12 h, respectively. This result implies that AMG gradually hydrolyzes in the amorphous region while MA can hydrolyze faster and MA disrupts the crystalline region when the reaction time longer, thereby at 24 h the crystallinity decreased to 27.82 %.

3.3. Apparent amylose content

The amylose content was determined using iodine complexation, in which its result is summarized in Table 1. Native rice starch contained 30.65 % apparent amylose that is classified in high amylose rice starch (Juliano, 1992). Although the effect of enzymes on the amylose content was minor both AMG and MA significantly decreased the amylose content compared to their control samples. As treatment time increased, the amylose content steadily decreased by AMG whereas the effects of MA on amylose reduction was obvious at 3 h of time incubation as 3.3 % reduction. The maximum decrease in amylose content was found for AMG and MA treatments after incubation for 24 h and 12 h as 5.4 % and 5.2 % reduction in amylose compared to control, respectively. It has been suggested that the rate limiting step for exo-acting enzymes on starch granules is the accessibility of non-reducing ends (Zhang, Dhital, & Gidley, 2013). Both AMG and MA are exo-acting, while the endo-action of MA would in addition cleave the long chain of rice starch (Grewal et al., 2015), thereby MA treatment might cause the reduction of amylose faster. This result is in agreement with the crystallinity data that increased obviously by MA treatment.

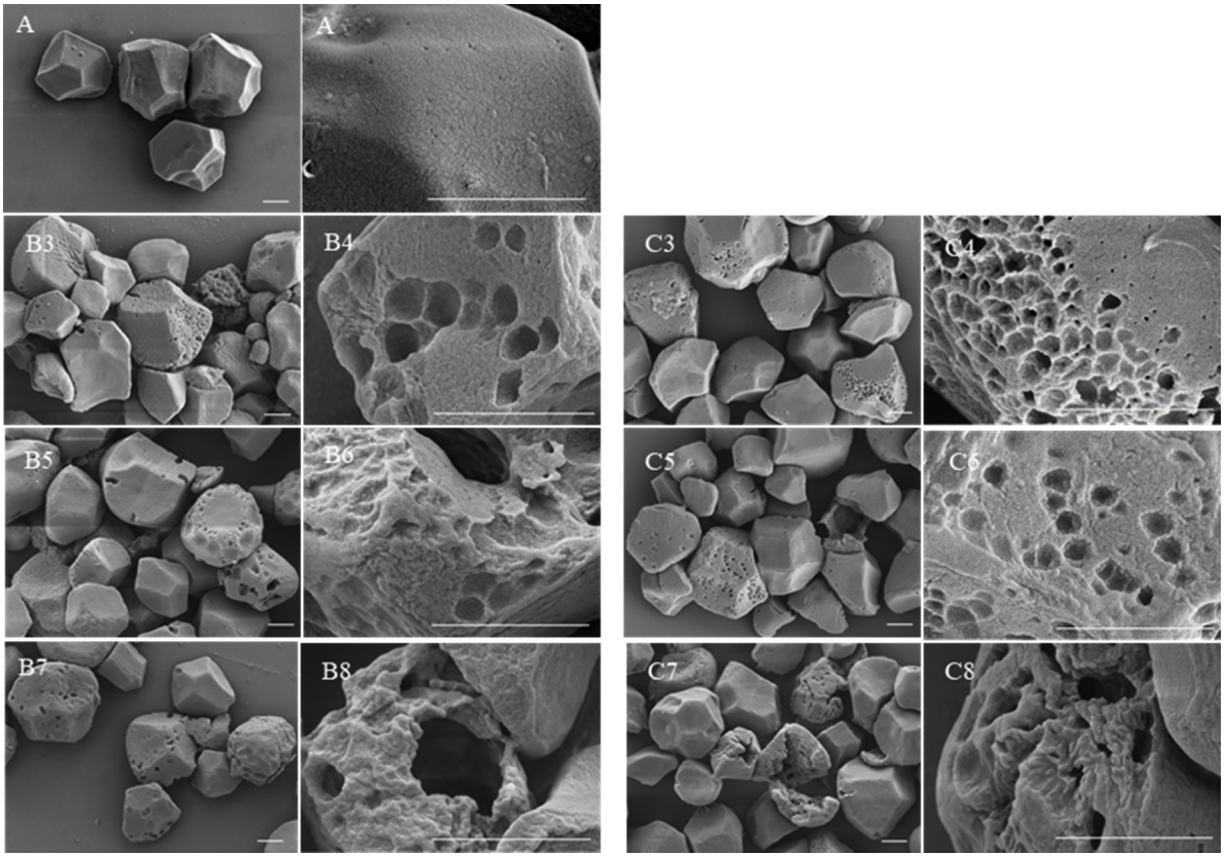

Fig. 1. SEM images of native rice starch (A) and enzymatically treated starch (B; AMG and C; MA) for 3 h (1 and 2), 6 h (3 and 4), 12 h (5 and 6) and 24 h (7 and 8). Scale bar = 2 μm.

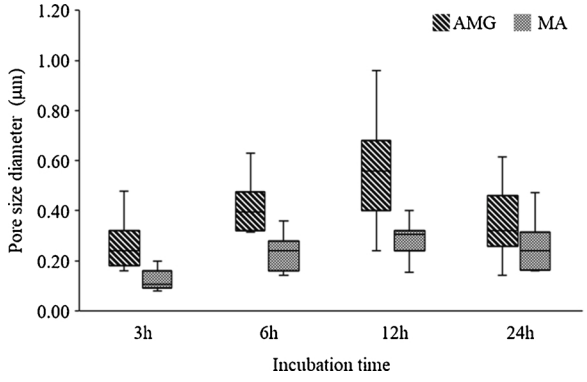

Fig. 2. Pore size analysis of AMG- and MA-treated starches as analyzed from SEM images.

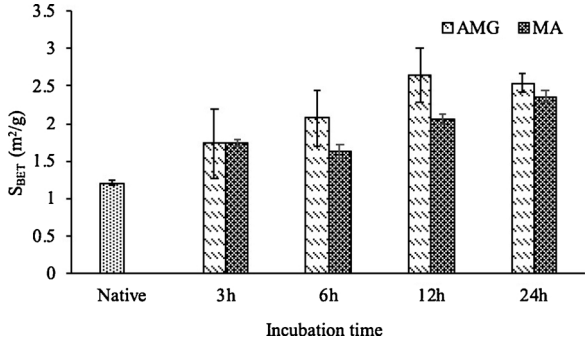

Fig. 3. Specific surface area (S_{BET}) of native, control and enzyme-treated starches at 3, 6, 12 and 24 h.

3.4. Chain-length distribution

As expected, the changes in the amylopectin side chain distribution after enzymatic treatments was a minor due to the complexity of starch granular structure that resists to enzyme attack (Božić, Lončar, Slavić, & Vujčić, 2017). However, a clear trend in the chain length distribution was observed (Fig. 4). At 3 h of incubation, the short amylopectin chains with a degree of polymerization (DP) of 6–12 were decreased whereas DP 13–22 were increased for AMG treatment (Fig. 4A1). When the incubation time was longer, B-long chains (DP21-30) were hydrolyzed, causing an increase in short so called B-chains (DP13-20) (Fig. 4A2). The reduction of short amylopectin chains could be due to that the exo-action of AMG mainly attacks the non-reducing ends of short chains mostly from the external layer of starch granules (Bertoft, 2017; Pérez, Baldwin, & Gallant, 2009). On the contrary, MA treatment increased the proportion of DP2-16 and decreased the proportion of DP17-40 (Fig. 4B1). This phenomenon was more obvious with longer incubation time (24 h). Miao et al. (2014) demonstrated that MA hydrolyzed gelatinized starch thereby increasing the amount of short amylopectin chains. This is likely attributed to the endo-action of MA that acts mainly on long chains, producing short chains (Grewal et al., 2015).

3.5. Starch molecular weight fraction

The molecular weight of native, control and the enzymatically treated starch samples was analyzed by SEC. Two starch fractions, I and II, which refer to high and low molecular weight, respectively, were generally detected for all samples (Fig. 5). For the untreated samples, these corresponded to amylopectin and amylose, respectively. The result showed molecular weight of fraction I and II of native rice starch

Table 1
Relative crystallinity, amylose content, swelling capacity and solubility of native, control and enzyme-treated starches.

Samples	Relative crystallinity (%)	Amylose content (%)	Swelling capacity (g/g)	Solubility (%)
Native	23.77 ± 0.18 a	30.65 ± 0.11 ij	2.89 ± 0.11 bcd	0.40 ± 0.10 b
AMG-3 control	23.69 ± 0.08 a	30.92 ± 0.03 k	3.26 ± 0.27 ef	1.18 ± 0.19 d
AMG-3	25.57 ± 0.69 cd	30.65 ± 0.09 ij	2.80 ± 0.33 bc	0.40 ± 0.26 b
AMG-6 control	23.16 ± 0.19 a	30.17 ± 0.12 h	3.14 ± 0.11 de	1.08 ± 0.10 d
AMG-6	25.80 ± 0.60 cd	29.56 ± 0.17 f	2.64 ± 0.08 ab	0.39 ± 0.18 b
AMG-12 control	24.27 ± 0.08 abc	29.46 ± 0.04 ef	3.49 ± 0.31 fg	1.17 ± 0.32 d
AMG-12	28.82 ± 0.78 e	28.26 ± 0.03 a	3.01 ± 0.18 cde	0.12 ± 0.01 a
AMG-24 control	22.72 ± 1.58 a	30.57 ± 0.05 i	3.83 ± 0.31 h	1.86 ± 0.27 g
AMG-24	29.02 ± 0.16 e	28.94 ± 0.07 b	2.87 ± 0.31 bcd	0.10 ± 0.00 a
MA-3 control	26.04 ± 1.20 d	30.97 ± 0.01 k	3.01 ± 0.18 cde	0.46 ± 0.12 b
MA-3	28.09 ± 0.55 e	29.94 ± 0.10 g	2.74 ± 0.14 abc	1.45 ± 0.21 ef
MA-6 control	25.69 ± 0.79 cd	30.43 ± 0.06 i	2.93 ± 0.23 bcd	0.80 ± 0.17 c
MA-6	27.73 ± 0.74 e	29.16 ± 0.09 cd	2.66 ± 0.20 ab	1.67 ± 0.24 fg
MA-12 control	25.35 ± 0.39 bcd	30.60 ± 0.06 i	3.11 ± 0.27 de	1.05 ± 0.31 d
MA-12	33.29 ± 0.59 f	29.00 ± 0.03 bc	2.48 ± 0.21 a	1.82 ± 0.08 g
MA-24 control	23.94 ± 0.16 ab	30.83 ± 0.23 jk	3.55 ± 0.14 g	1.29 ± 0.22 de
MA-24	27.82 ± 0.04 e	29.30 ± 0.11 de	2.76 ± 0.07 abc	2.56 ± 0.22 h

Values followed by different letters within a column are significantly different ($p < 0.05$).

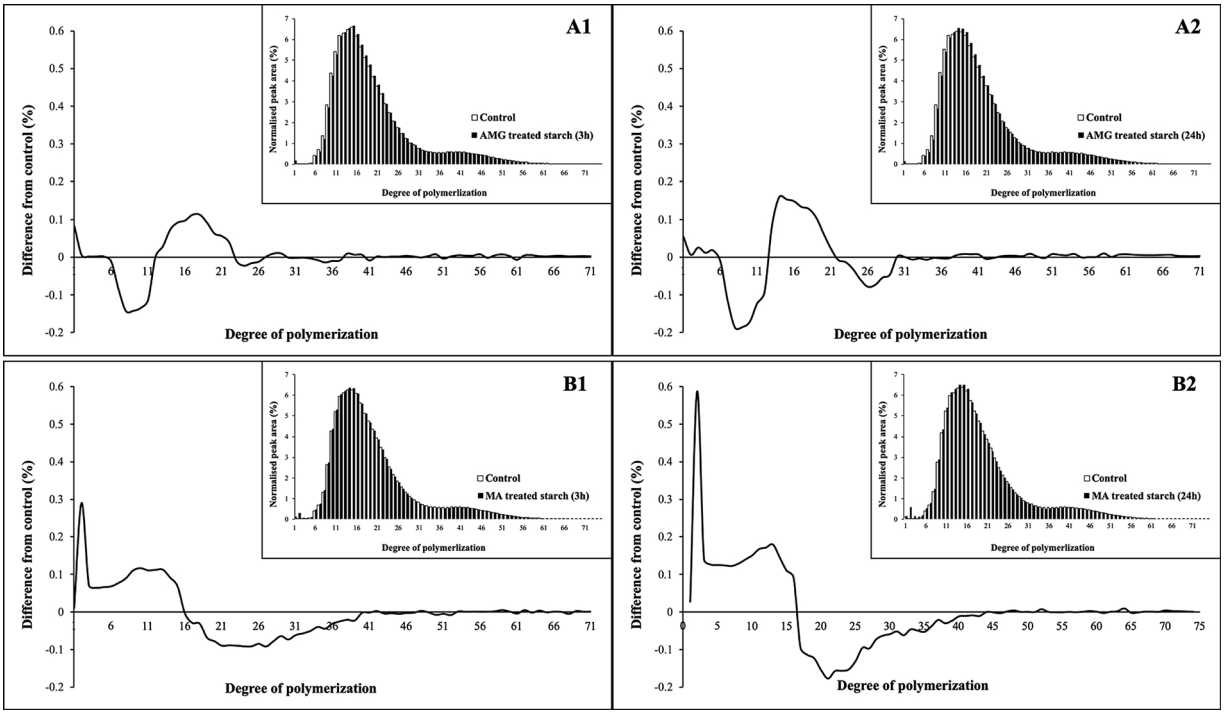

Fig. 4. Chain length distribution analysis (bar graph) and difference plots (line graph) of enzyme-treated starch in relative with the control. (A1 and A2) AMG-treated starch, (B1 and B2) MA-treated starch after 3 h and 24 h modification, respectively.

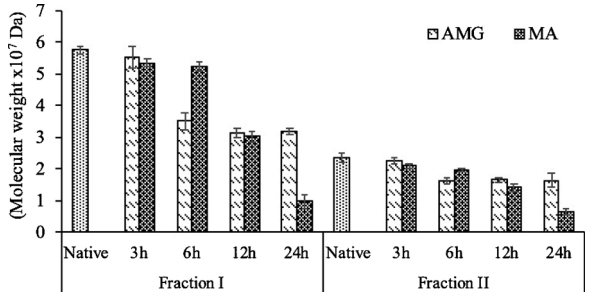

Fig. 5. Molecular weight of fractions of native, control and enzyme-treated starches.

were 5.8×10^7 and 2.4×10^7 Da, respectively. After modification with AMG for 6 h, fraction I and II decreased moderately. MA treatment caused a more extensive decrease in fraction I and II as incubation time extended, decreasing from 5.75×10^7 to 0.98×10^7 and from 2.36×10^7 to 0.65×10^7 Da respectively, after incubation for 24 h. Such effects has been reported elsewhere (Ao et al., 2007; Christophersen et al., 1998; Grewal et al., 2015; Miao et al., 2014). Interestingly, at 6 h there was a break point in which the molecular weight fractions of MA-treated starch were significantly higher than AMG treated starch but after 24 h the MA-treated starch decreased significantly in molecular weight fractions as compared to AMG-treated starch. This could be explained by the AMG has a slower catalytic rate towards α-1,6 glucosidic bonds as compared to the faster hydrolysis of

α-1,4 bonds (Mertens & Skory, 2007). On the other hand, the endo acting MA can continue hydrolysis that caused dramatically decreased in the molecular weight of starch sample.

3.6. Swelling capacity and solubility index

The swelling capacity and solubility of the starch samples (Table 1) demonstrates that the swelling capacity of the enzyme-treated starches was significantly lower than their corresponding buffer controls, showing that less amount of water was absorbed in the enzyme-treated starch. The decreased swelling capacity of the hydrolyzed starch samples was supposedly directly attributed to the formation of porous structure after enzymatic modification as confirmed by SEM, which weakened the general granular structure of the starch. Alternatively, more hydrophobic surfaces were created at the internal walls of the pinholes thereby restricting binding of water (Dura & Rosell, 2016). Another explanation can be that the decrease of the swelling capacity of the enzyme-treated starch might be an effect of internal molecular rearrangement in the starch granules forming more ordered double helical segments of the amylopectin side-chains (Zavareze & Dias, 2011).

The solubility index was significantly decreased by AMG treatment (Table 1). This was in contrast with the increase found for the MA treated starch granules. This phenomenon was more pronounced with longer incubation time. The solubility index indicates the amount of solid compounds released from the starch (Dura et al., 2014). A decrease in solubility of the AMG-treated starch could be explained that AMG attacking amylopectin and releasing glucose occurs at the exterior parts of the molecules. Such trimming can induce the restructuring of starch chains that might be caused more complex structure which decreased the compounds releasing from starch granules (Xie, Li, Chen, & Zhang, 2019). For MA, enhanced solubility can likely be an effect of its endo-activity producing small starch fragments from the starch granules which adheres to the starch granules. These results were consistent with chain length distribution results that showed that the short chains were increased by MA treatment whereas they were decreased by AMG treatment. Moreover, it was observed that the swelling capacity and solubility index of all the control samples were higher than native starch. This may be explained by the fact that during incubation, water penetrates into the granules, disrupting amorphous region and allowing the granules to swell freely (Zhao et al., 2018) and releasing of soluble compounds from the granule.

3.7. Pasting properties

Pasting properties provide information about gel formation dynamics during shear, shear resistance and retrogradation behavior. The pasting parameters of native, control and enzymatically treated starches (Table 2) shows that the enzymatic treatments affected the pasting properties of starch by delaying the pasting formation. At all incubation times, pasting temperature was significantly increased by AMG while MA caused the higher pasting temperature during incubation for 12 h and 24 h. These data agree with the swelling capacity since starch swelling is caused by water absorption before gelatinization process (Dura et al., 2014), resulting in that AMG- and MA-treated starches both had lower swelling capacity as compared to their control samples. Enzymatic treatment decreased the peak viscosity, breakdown, final viscosity and setback values compared to their controls, which agrees with a previous report (Dura et al., 2014). Peak viscosity of AMG-treated starch decreased by up to 15 % whereas that of MA-treated starch decreased by 31 % after 24 h of incubation. The reduction in peak viscosity of the enzyme-treated starches is possibly a direct effect of disintegration of the granular surface and fragmentation of granules at longer treatment times. The breakdown parameter represents granule stability, indicating the disrupted granules better resisted the shear force during heating (Karim et al., 2008). After being modified by AMG and MA for 24 h, the breakdown value decreased by 27 % and 51 %, respectively. The reduction could be attributed to the weakened structure after enzymatic treatments. Importantly, the enzyme treatments significantly decreased setback value compared to their control. This effect was pronounced with extended incubation time, showing 50 % and 40 % decrease after 24 h modification with AMG and MA, respectively, suggesting that low amount of amylose chains disabled the capacity for gel formation (Dura & Rosell, 2016).

3.8. Thermal properties

The thermal properties as deduced by DSC (Table 3, DSC curve indicated in Supplementary data) demonstrated that the initial temperature (Ti), which was the temperature where the starch showed a minor gelatinization, and onset temperature (To) were higher for the enzyme-treated starches than for the the non-treated control samples. This suggests that the gelatinization process was delayed, which agrees with the results of the pasting properties (Table 2). A significant increase in Ti was observed for the AMG-treated samples but not for the MA-treated starches. Higher gelatinization regimes of enzyme-treated starches have been implied to the structural stability of starch granules due to higher degree of order and crystallinity (Kaur, Singh, Ezekiel, & Sodhi, 2009). Alternatively, the increase of Ti and To found for the enzyme treated samples could be related to the reduction of swelling capacity previously mentioned. Moreover, a significant narrowing in

Table 2
Pasting properties of native rice starch, control and enzyme-treated starches.

Samples	Pasting tempe (°C)	Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)
Native	79.2 ab	2621 c	550 bcd	2920 de	840 e
AMG-3 control	79.2 ab	3117 f	718 efg	3010 e	611 bcde
AMG-3	80.8 cd	2722 cd	561 cd	2578 c	417 ab
AMG-6 control	78.8 a	2992 ef	717 efg	2802 d	526 abcd
AMG-6	80.8 cd	2599 c	604 cdef	2450 bc	455 ab
AMG-12 control	79.5 abc	2966 ef	631 cdefg	2776 d	441 ab
AMG-12	81.2 d	2759 cd	589 cde	2552 c	382 ab
AMG-24 control	79.6 abc	3126 f	766 g	2968 de	608 bcde
AMG-24	85.6 e	2658 c	559 cd	2403 bc	304 a
MA-3 control	78.8 a	3031 ef	764 g	3028 e	761 de
MA-3	79.2 ab	2204 ab	523 bc	2297 ab	617 bcde
MA-6 control	79.2 ab	2752 cd	697 defg	2788 d	733 de
MA-6	80.0 abcd	2215 b	410 ab	2274 ab	469 abc
MA-12 control	78.8 a	3001 ef	754 fg	3039 e	793 e
MA-12	80.4 bcd	2200 ab	408 ab	2308 ab	517 abcd
MA-24 control	78.8 a	2914 de	691 defg	2941 de	718 cde
MA-24	80.8 cd	2021 a	336 a	2119 a	433 ab

Values followed by different letters within a column are significantly different ($p < 0.05$).

Table 3
Thermal properties of native rice starch, control and enzyme-treated starches as determined by DSC.

Samples	Ti (°C)	To (°C)	Tp (°C)	Tc (°C)	Tc-Ti (°C)	ΔH (J/g °C)
Native	60.1 a	69.5 cd	74.9 def	80.7 h	20.6 h	14.0 f
AMG-3 control	63.4 b	69.0 a	74.3 ab	79.8 bcd	16.5 g	10.9 ab
AMG-3	66.4 defgh	69.9 e	74.7 cd	80.2 cdefg	13.9 bcdef	13.7 ef
AMG-6 control	65.0 c	69.0 a	74.0 a	79.2 a	14.2 cdef	10.9 ab
AMG-6	66.3 defg	69.3 bc	74.0 a	79.6 ab	13.3 abcd	14.7 g
AMG-12 control	65.2 cde	69.9 e	74.9 de	79.8 bc	14.6 ef	10.8 ab
AMG-12	67.8 i	70.5 gh	75.0 def	80.2 cdefg	12.5 a	13.7 ef
AMG-24 control	65.2 cde	70.5 gh	75.5 h	80.2 cdefg	15.0 f	10.9 ab
AMG-24	66.7 hi	70.9 ij	75.1 efg	80.4 fgh	12.7 ab	13.0 d
MA-3 control	64.6 bc	69.4 c	74.3 ab	79.5 ab	15.0 f	13.0 d
MA-3	65.5 cdef	69.8 de	74.5 bc	79.9 bcdef	14.5 def	14.0 f
MA-6 control	66.1 defg	70.0 e	74.5 bc	79.9 bcde	14.2 cdef	12.4 c
MA-6	66.4 defghi	70.4 fg	74.9 def	80.3 efgh	13.5 abcde	14.1 f
MA-12 control	66.6 efghi	70.7 hgij	75.1 efg	80.3 defgh	13.8 bcdef	11.4 b
MA-12	67.5 ghi	71.0 i	75.4 gh	80.5 gh	13.1 abc	13.9 f
MA-24 control	66.7 fghi	70.1 ef	75.2 fgh	80.2 cdefg	13.5 abcde	10.5 a
MA-24	67.1 ghi	70.9 ij	75.5 h	80.3 cdefg	13.2 abcd	13.1 de

Ti = initial temperature, To = onset temperature, Tp = peak temperature, Tc = conclusion temperature, ΔH = enthalpy change.
Different small caused letters within a column denote significant differences ($p < 0.05$).

the gelatinization temperature range was observed for the AMG samples, indicating that the starch granules were more homogeneous and cooperative melting of crystallites took place (Jacobs & Delcour, 1998). In addition, enzyme-treated starches exhibited significantly higher enthalpy (ΔH) than the control, indicating the amounts of molecular order structure was induced (Zhao et al., 2018). However, the ΔH of control samples is lower than that of native starch. These might be due to the effect of annealing which rearrange the double helix to be more perfect as shown on higher Ti. These rearrangements could result in the lower amount of molecular order structure, double helices (Tester & Debon, 2000). Overall, the higher of gelatinization temperature and the enthalpy of enzymatically treated starches made the granules more crystalline which was consistent by WAXS data.

4. Conclusions

Porous high amylose rice starch was produced by using AMG and, to our best knowledge, for the first time using MA. The hydrolytic activity affected the granular structure in both the amorphous and crystalline parts. MA specifically increased the short amylopectin chains and increased solubilization. The peak viscosity, final viscosity, breakdown and setback values of pasting properties were reduced by both enzymatic treatments whereas the gelatinization temperature increased. The enzymatic treatments led to an increased relative crystallinity, producing starch granules more homogeneous with respect to thermal properties. Our data provide information on different actions of the two enzymes on granular rice starch with many of the effects generating beneficial physicochemical properties as compared to gelatinized starch.

Author contributions

Thewika Keeratiburana: Conceived and designed the analysis; Collected the data; Performed the analysis; Performed the statistical analysis; Wrote the paper.

Aleksander Riise Hansen: Contributed data and analysis tools.

Siriwat Soontaranon: Collected the data WAXS data collection.

Andreas Blennow: Conceived and designed the analysis; Other contribution; Manuscript correction.

Sunanta Tongta: Conceived and designed the analysis; Other contribution; Manuscript correction.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.carbpol.2019.115611.

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