Hydrolysis of native and annealed tapioca and sweet potato starches at sub-gelatinization temperature using a mixture of amylolytic enzymes


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Abstract

This study investigated the effect of annealing treatment (at 50°C for 72 h) on hydrolysis of tapioca and sweet potato starches using a raw starch hydrolyzing enzyme namely STARGEN 001 (a blend from fungal α-amylase and glucoamylase) at sub-gelatinization temperature (35°C) for 24 h. The degree of hydrolysis of the starches was evaluated based on the dextrose equivalent (DE) value. The hydrolyzed starches were then characterized in terms of its morphology, swelling power and solubility, gelatinization and pasting properties, amylose content and x-ray diffraction pattern. After 24 h of hydrolysis, annealed starches were hydrolyzed to a greater degree with higher DE value compared to native starches (40% vs 33% for tapioca; and 29% vs 24% for sweet potato starch). Scanning electron microscopy (SEM) micrographs revealed a more porous granules and rougher surface in annealed starches than their native counterparts. The swelling power and solubility of annealed starches decreased significantly. Annealing was found to affect the pasting properties of the starches appreciably and increase the starch gelatinization temperature. The amylose content in hydrolyzed annealed tapioca and sweet potato starches increased while no significant changes observed in the X-ray diffraction of those starches. This study shows that the annealing treatment can be used as a way to increase the degree of hydrolysis of tapioca and sweet potato starches at sub-gelatinization temperature using a raw starch hydrolyzing enzyme.

Introduction

Hydrothermal modification, including annealing and heat-moisture treatment, is a physical modification of the starch granule that changes the physicochemical properties of starch without destroying the granular structure (Zavareze and Dias, 2011), and involves only heat and water. These physical thermal treatments usually been used to improve starch functionalities for application in the food industry (BeMiller and Huber, 2015). Starch modifications are often performed to enhance starch performance, to alter their susceptibility to enzyme attack (Huang et al., 2016) and to improve particle integrity, solubility, viscosity and textures (Falade and Ayetigbo, 2015). Annealing is defined as incubation of a starch granule in excess water at a temperature above the glass transition temperature but below the gelatinization temperature. It is also associated with the physical re-organization of starch granules when heated in water at a temperature between the glass transition temperature (Tg) and the onset of gelatinisation (To) of the native starch system (Tester, 2000). The annealing treatment provokes a re-organization of starch molecules which modifies the physicochemical properties of starches such as increase in enzymatic susceptibility, decrease in swelling power and solubility, increase in gelatinization temperatures and enthalpy and also narrowing of gelatinization range, stability of paste and crystallinity and decrease in peak viscosity and retrogradation trend (Gomez et al., 2004). Annealing did not influence the amylose and amylpectin ratio or the amylpectin chain-length distributions, but the ordered structures developed a more stable form with a smaller dispersion than those in the original starch (Kohyama and Sasaki, 2006). It has been proposed (Brumovsky and Thompson, 2001) that annealing treatment could be used to enhance the resistant starch level of starch by perfecting its granular or molecular structures. The alteration in granular or molecular structure of starch could further affect the enzymatic resistance of starch.

A new low temperature liquefaction and saccharification enzyme, STARGEN 001 with high granular starch hydrolyzing activity was used in this study to hydrolyze native and annealed tapioca...
and sweet potato starches. This enzyme contains a mixture of α-amylase from *Aspergillus kawachi* and glucoamylase from *Aspergillus niger* and it is produced by GENENCOR international company. STARGEN 001 is widely known as a new granular starch hydrolyzing enzyme that are able to convert starch into dextrin at low temperatures as well as hydrolyze dextrin into fermentable sugars. In normal processing method for production of fermentable sugars, it involves two major steps which are liquefaction and saccharification process. In this process the starch need to be gelatinized at high temperature before being hydrolyzed with thermostable α-amylase. However, with the usage of STARGEN 001 enzyme for hydrolyzing starch in the fermentable sugars production process, the liquefaction and saccharification steps could be eliminated as the enzyme could hydrolyze the starch directly in native granular state at low temperature. This consequently may reduce the production cost for production of fermentable sugars.

The effects of different physical treatments such as heat-treatment (Shariffa et al., 2009), starch defatting (Uthumporn et al., 2013) and sodium hydroxide (NaOH) treatment (Uthumporn et al., 2012) on the extent of hydrolysis of various starches by STARGEN 001 enzyme at sub-gelatinization temperature has been investigated previously. However, at our best knowledge, the effect of annealing treatment on the efficiency of this enzyme to hydrolyze tapioca and sweet potato starches has not yet been reported. Therefore, this research was designed to study the capabilities of this enzyme to hydrolyze annealed tapioca and sweet potato starches and also to investigate the effect of the treatment on the susceptibility of the starch to enzymatic hydrolysis.

**Materials and Methods**

**Materials**

Tapioca and sweet potato starch were obtained from SIM Company Sdn. Bhd. (Penang, Malaysia).

**Enzyme**

The commercial enzyme, STARGEN 001 enzyme was used to hydrolyze the starches. It is a product of Genencor International (Palo Alto, CA) containing *Aspergillus kawachi* α-amylase expressed in *Trichoderma reesei* and glucoamylase from *Aspergillus niger*. The pH of STARGEN 001 enzyme ranged from 4.0 to 4.5. The specific gravity of STARGEN 001 enzyme is 1.10 – 1.15 g/ml. The recommended temperature for STARGEN 001 enzyme is 20–40°C. The minimum activity of STARGEN 001 enzyme is 456 GSHU/g. GSHU is defined as Granular Starch Hydrolyzing Units.

The enzymes activity was determined by reaction at 37°C with soluble starch (1%) in sodium acetate buffer (pH 4.4). Aliquots were taken after 10 minutes for determining the amount of D-glucose released. The glucose was determined by using dinitrosalicylic acid method. The enzyme activity was 3736 unit/g starch.

**Preparation of annealed starch**

Starch samples were suspended in distilled water (1:5 w/v), then incubated for 72 h in covered beakers in a water bath at 50°C. After incubation, the starch suspensions were filtered and the residues were dried in an air drying oven at 40°C.

**Starch hydrolysis**

The starch was mixed with sodium acetate buffer (25% w/v) with pH 4.4. The enzyme was added (1% w/v) into the starch suspension and the hydrolysis was conducted in an orbital shaker (JEIO Tech, SI-600R, Seoul, Korea) at 35°C with the speed of 150 rpm. After 24 h, the hydrolysis was halted by adding predetermined amount of 2.0 M hydrochloric acid (HCl) until the pH was 1.5-1.6. The pH of starch suspensions was adjusted back to pH 5-6 by washing the starch with distilled water and the starch residues were dried in an air drying oven at 40°C.

**Dextrose equivalent (DE)**

The hydrolysis was performed for a period of up to 24 h and the DE value of the hydrolyzed starch substrate was determined at different time intervals (i.e., 1, 2, 3, 4, 8, 12, 16, and 24 h of hydrolysis time). The reducing sugar value was measured using the dinitrosalicylic acid method (Miller, 1959) to determine the starch’s DE value. A small aliquot was withdrawn from each batch of starch slurry at each time interval. The absorbance was measured at 504 nm using a UV/Visible spectrophotometer (UV-160A, SHIMADZU, Kyoto, Japan). Glucose was used as the standard. Each analysis was performed in duplicate. DE was calculated as follows:

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DE\% = \frac{(g\ reducing\ sugar\ expressed\ as\ glucose)}{(g\ dry\ solid\ weight)} \times 100
\]

**Scanning electron microscopy**

The microstructure of the starch granules after 24 h of hydrolysis were viewed with a field emission scanning electron microscope (FESEM Leo Supra 50VP, Carl-Ziess SMT, Oberkochem, Germany). The
starch granules were stuck onto aluminum specimen stubs with double-sided adhesive tape and sputter-coated with a 20-30 nm layer of gold using a Sputter Coater [Polaron (Fisons) SC515, VG Microtech, Sussex, UK]. The accelerating voltage of the SEM is 5kV.

**Swelling and solubility**

The swelling power and solubility of the starches after 24 h of hydrolysis were determined in triplicate as previously described (Schoch, 1964). The hydrolyzed native and annealed starches (100 mg, dry weight) were accurately weighed in a centrifuge tube, to which 10 mL of distilled water were added. The tube was placed in a water bath at 80°C for 30 min until the suspension became translucent. The solution was centrifuged (3500 rpm, 15 min), and then the supernatant was carefully discarded. The swollen starch sediment was then weighed. To determine the amount of soluble starch, an aliquot (5 mL) of the supernatant was dried overnight in an oven at 110°C. Swelling power is defined as the ratio in weight of the wet sediment to the initial weight of dry starch. Solubility is the ratio in weight of the dried supernatant to the initial weight of starch.

**Amylose content**

Amylose content of the starches after 24 h of hydrolysis was determined in triplicate according to procedure that has been described by McGrance et al. (1998) with minor modification. Pure potato amylose and amylopectin (Sigma Chemical Company, Steinheim, Germany) were used as the standards. The results were expressed on a dry basis. Starch (0.1 g, dry weight) was accurately weighed and dissolved by heating in dimethyl sulphoxide (DMSO) for 15 min on a hot plate at 85°C while stirring continuously with a magnetic stirrer bar. After the solution had dissolved, it was diluted to 25 ml with deionized water in a volumetric flask. An aliquot (1 ml) of this solution was diluted with 50 ml of deionized water. Five ml of iodine (0.0025 mol/L) in potassium iodide (0.0065 mol/L) were added with mixing, and the absorbance of this solution in a 1 cm path length glass cell was read at 600 nm using a UV/Visible spectrophotometer (UV-160A, SHIMADZU, Kyoto, Japan). Samples were left for 15 min after the addition of iodine before taking the readings on the spectrophotometer.

**Pasting properties of starch**

The gelatinization temperature of the starches after 24 h of hydrolysis was determined in triplicate using a Rapid ViscoTM Analyzer (Model RVA Series 4, Newport Scientific Pvt. Ltd., Warriewood, Australia). For the determination by RVA, 2 g of a starch sample (corrected to 8% moisture basis) and 25 ml of distilled water were combined and stirred in the aluminum RVA sample canister. The temperature was held at 50°C for 1 min and then raised to 95°C in 3.75 min, held for 2.5 min, cooled to 50°C in 3.75 min, and held for 5 min. The paddle speed was set at 960 rpm for the first 10 s to evenly disperse the starch slurry and then was reduced to 160 rpm throughout the entire experiment. The units of viscosity were expressed as rapid visco units (RVU).

**Differential scanning calorimetry**

The thermal properties of starches after 24 h of hydrolysis were studied using a differential scanning calorimeter (DSC-Q100, TA Instruments, New Castle, DE, USA) equipped with a refrigerated cooling system. A dry sample (2 mg) was loaded into an aluminum pan, and distilled water was added to achieve a starch-water suspension containing 75% water. Samples were heated from 30°C to 130°C at a heating rate of 10°C/min under an oxygen-free, nitrogen gas (N₂) flow rate of 50 ml/min. For calibration, the DSC analyzer was calibrated using indium and an empty aluminium pan (as a reference). Sample pans were heated at a rate of 10°C/min from 10 to 180°C.

**X-ray diffraction**

Crystallinity patterns of the starch granule after 24 h of hydrolysis time were examined by using X-ray diffractometer. The dried starches were conditioned overnight at 100% relative humidity (RH) at room temperature. The starches were scanned by X-ray diffractometer (Diffractometer D5000, SIEMENS, Karlsruhe, Germany). Diffractograms were recorded in the reflection mode in the angular range 4-40°(2θ). The Cu Kα-radiation (λ 1.5406 Å), generated at 40 kV and 30 mA, was made monochromatic using a 15 μm of Ni-foil. Scattered radiation was detected using a proportional detector.

**Statistical analysis**

All experiments were performed at least twice with three measurements for each analysis. The data was reported in mean ± standard deviation. A one-way analysis of variance (ANOVA) with Duncan’s multiple test range was used to compare significance between sample means at a 5% significance level. The statistically analysis was performed using SPSS 12.0 software (SPSS, Inc., Chicago, IL, USA).
Results and Discussion

Normal tapioca and sweet potato starches were annealed at 50°C for 72 h and then hydrolyzed using STARGEN 001 enzyme for 24 h at 35°C. The degree of hydrolysis of tapioca and sweet potato starches were determined, and then the physicochemical properties of the hydrolyzed starches were analyzed in order to study the action of this enzyme on native and annealed starches. The terms “control”, “native”, “control annealed” and “annealed” used to describe the samples in this research are defined as follows: Control describes the starch that was incubated at 35°C without the presence of the enzyme; native refers to the starch that was hydrolyzed at 35°C using the enzyme; control annealed defines the starch that was annealed and incubated at 35°C without the presence of enzyme; and annealed describes the starch that was annealed and hydrolyzed at 35°C using the enzyme.

Degree of hydrolysis

The enzymatic hydrolysis profile of native and annealed tapioca and sweet potato starches after 24 h of hydrolysis is shown in Figure 1. For the first 12 h of hydrolysis, the rate of hydrolysis of annealed tapioca and sweet potato starches increased insignificantly in comparison to their native counterparts. However, a marked increase in the rate of hydrolysis of annealed tapioca and sweet potato starches was observed after 16 h of hydrolysis time. After 24 h of hydrolysis, annealed starches exhibit higher DE value than native starches for both tapioca and sweet potato starches (40% vs 33% for tapioca; and 29% vs 24% for sweet potato starch). The DE value of the annealed starches was significantly increased due to the higher susceptibility of the annealed starch to α-amylose and glucoamylase during hydrolysis process as compared to native starch. This result is in accordance with previous study (Wang et al., 1997), who studied the annealing effect on the hydrolysis of sago starch granules by a mixture of α-amylose and glucoamylase. It has been reported (Wang et al., 1997) that annealed sago starch has higher susceptibility to enzyme hydrolysis due to two reasons, i.e. disruption of hydrogen bonds between the amorphous and crystalline regions and a slight expansion of the amorphous region after annealing. The swelling of starch granules has been reported to increase the affinity of granular starch hydrolyzing enzyme for starch granules (Li et al., 2014). Certain studies have indicated that annealed wheat, barley and sago starches are more easily hydrolyzed by α-amylose than native starch (Gough and Pybus, 1971; Lorenz and Kulp, 1980). The disruption of the hydrogen bonds in annealed tapioca and sweet potato starches had weakened the granule structure thus allowing the enzyme to penetrate and degrade the α-1,4 and α-1,6 linkages more effectively than native starches. Annealing has also been shown to increase the susceptibility of wheat starch towards fungal α-amylose (Lorenz and Kulp, 1980) and bacterial α-amylose (Lorenz et al., 1980). From the hydrolysis profile, native and annealed tapioca starch exhibit higher DE value as compared to sweet potato starch with or without annealing treatment. This result is in accordance with previous study (Zhang and Oates, 1999), reporting that sweet potato starch is less susceptible than tapioca starch to α-amylose and glucoamylase attack. The presence of truncatures in tapioca starch (Figure 2a, shown by arrows) was the weak points of the granule structure resulting in better susceptibility of tapioca starch to enzymatic attack. The presence of porous structure in tapioca starch is also believed to cause the starch more susceptible to enzymatic hydrolysis. The annealing treatment (performed before the starch being subjected to enzyme hydrolysis) may expand the porous structure thus facilitating the penetration of the enzymes into the granule during hydrolysis. It has been proposed that there are formations of porous structures in annealed starches as a result of annealing leading to more rapid acid hydrolysis of annealed starches relative to their native counterparts (Nakazawa and Wang, 2003). This result suggested that the degree of hydrolysis of starch can be enhanced by performing physical modification such as annealing before the starch being subjected to hydrolysis.

Scanning electron microscopy

Figure 1. Hydrolysis profiles of native and annealed tapioca and sweet potato starches at sub-gelatinization temperature (35°C) for 24 h. The error bar represents ±1 SD (n=3).
The morphology of native and annealed starch after 24 h of hydrolysis can be seen in Figure 2. Generally, there were no changes observed after annealing treatment as control annealed starch (Figure 2c and 2g) shows similar morphology as control native starch (Figure 2a and 2e) for both tapioca and sweet potato starches. This observation is in accordance with previous study (Hoover and Vasanthan, 1994) describing that annealing causes no effect on granule dimensions or shapes. Falade and Ayetigbo (2015) observed insignificant changes in the granule shape of white yam after annealing treatment. However, it has been postulated that annealing could create pores or fissures (Gough and Pybus, 1971; Rocha et al., 2012). In previous study annealing treatment was found to increase the pore size of some barley cultivars slightly (Waduge et al., 2006). After 24 h of hydrolysis, extensively eroded surfaces were observed in hydrolyzed annealed tapioca and sweet potato starches (Figure 2d and 2h) and most of the hydrolyzed annealed tapioca starch show some pits. From this observation, it is believed that the enzyme attacks on annealed starches occurred mainly at the surface of the starch granules and penetrate into the granules through the pores or fissures which have been expanded by the annealing treatment. Although amorphous and crystalline lamellae become more ordered in annealed starch, accessibility to the amorphous regions by enzymes is facilitated by the presence of the pores or fissures (Tester and Debon, 2000). In contrast with their native counterparts, the hydrolyzed native starch showed a single hole on the granule with more extensive hydrolysis of the internal regions of the granule. Enzyme attack occurred at the interior part of the starch granules, leaving a deep round hole on the starch granule surface while some of the granules still have a smooth surface or show only small pits (Figure 2b and 2f). Generally, the α-amylase and glucoamylase attack the annealed tapioca and sweet potato starches uniformly throughout the granule population resulting rough and porous structures in most of the starch granules. In contrasts with native starch, the enzyme action occurred non-uniformly throughout the granule population and it tends to degrade certain granules while some other granules seem to be unaffected. It has been reported that changes to the granule surface on annealing could negate the effect of glucan chain interaction and crystallite perfection on α-amylase hydrolysis and thereby facilitate the entry of α-amylase into the granule interior (Jayakody and Hover, 2008). This could be used to explain the increase in the rate of hydrolysis in some of the annealed starches.

Swelling power and solubility

The results of swelling power and solubility of native and annealed tapioca and sweet potato starches after 24 h of hydrolysis are listed in Table 1. The swelling power of both tapioca and sweet potato starches decreased significantly as the result of annealing treatment. The decrease in granular swelling has been attributed to the interplay of the following factors which are increased crystalline perfection and decreased hydration (Tester, 1997; Waduge et al., 2006), interaction between amylose-amylose and/ or amylopectin-amylopectin (Jacobs et al., 1995), increase in intra-granular binding forces and reinforcement of the granule (Jacobs et al., 1995; Hizukuri, 1996) and V-amylose-lipid complex formation (Jacobs et al., 1995; O’Brien and Wang, 2008).
According to Tester and Sommerville (2001), there is a strong correlation between the swelling of starch and the extent of α-amylase hydrolysis. The porosity of starch granules will be increased as the granules swell, where the semi-crystalline structure is converted into amorphous material which is itself more readily hydrolyzed by α-amylase (Tester, 1997). There are three steps associated with the enzymatic hydrolysis of swollen starch, i.e. diffusion of the enzyme molecule, adsorption of the enzyme onto the solid substrate and hydrolysis. Diffusion is determined largely by the porosity of the solid substrate and the diffusion co-efficient of the enzyme inside the pores. From our observation, even though the swelling power of hydrolyzed annealed tapioca and sweet potato starches decreased significantly, the formation of pores or fissures on the granules as a result of annealing treatment might have facilitated the penetration of α-amylase and glucoamylase into the starch granules and increase the hydrolysis rate.

The solubility of annealed tapioca and sweet potato starches after 24 h of hydrolysis decreased significantly as compared to their native counterparts. In hydrolyzed native starches, the solubility values were less than 1% (Table 1), in contrast with hydrolyzed annealed starches with solubility values of almost 7%. Similar pattern of result was obtained from previous study (Gomez et al., 2004) observing the total lost of solubility in unfermented tapioca starch after 120 h time of annealing treatment and suggesting that there was a clear strengthening of bonds between starch molecules. The amount of soluble components in mung bean starch decreased as the annealing temperature increases (Chung et al., 2000). The decrease in solubility indicated that interactions between amylopectin and/ or amylose and amylopectin helices were increased by annealing time, producing a more stable structure preventing amylase leaching from the granules (Gomez et al., 2004).

### Amylose content

The amylose content of native and annealed tapioca and sweet potato starches after 24 h of hydrolysis are listed in Table 1. From the results, the amylose content of hydrolyzed annealed starches was higher than that of hydrolyzed native starch for both tapioca and sweet potato starch. The increment of amylose content in hydrolyzed annealed starch might be resulted from the degradation of amylopectin which contribute to shorter polymer chains and increased the amylose content value. Due to crystallization of amylose during glucoamylolysis, the amylopectin was preferentially hydrolyzed by glucoamylase which resulting in an increase in amylose ratio in Hylon V and VII starches (O’Brien and Wang, 2008).

### Pasting properties and thermal properties

The pasting properties of hydrolyzed native and annealed tapioca and sweet potato starches are listed in Table 2. The pasting temperature of hydrolyzed annealed tapioca and sweet potato starches increased by almost 7ºC and 4ºC, respectively as compared to hydrolyzed native starch, showing that there was a strengthening of bonds of annealed starch requiring higher temperature to gelatinize the starch granules. Increment in pasting temperature in annealed fermented tapioca starch (Gomez et al., 2004) as the time of annealing was increased indicating that there was a strengthening of the bonds requiring larger temperatures for the gelatinization of the granules. The peak viscosity of hydrolyzed annealed tapioca

<table>
<thead>
<tr>
<th>Sample</th>
<th>Swelling power (g/g)</th>
<th>Solubility (%)</th>
<th>Amylose content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tapioca</td>
<td>Control</td>
<td>15.54 ± 1.08^a</td>
<td>5.38 ± 0.18^a</td>
</tr>
<tr>
<td></td>
<td>Native</td>
<td>15.53 ± 0.63^a</td>
<td>6.65 ± 0.27^a</td>
</tr>
<tr>
<td></td>
<td>Control Annealed</td>
<td>11.76 ± 0.05^a</td>
<td>0.31 ± 0.01^a</td>
</tr>
<tr>
<td></td>
<td>Annealed</td>
<td>10.41 ± 0.10^a</td>
<td>0.28 ± 0.03^a</td>
</tr>
<tr>
<td>Sweet Potato</td>
<td>Control</td>
<td>9.89 ± 0.49^a</td>
<td>4.65 ± 0.28^a</td>
</tr>
<tr>
<td></td>
<td>Native</td>
<td>11.50 ± 0.31^a</td>
<td>6.41 ± 0.40^a</td>
</tr>
<tr>
<td></td>
<td>Control Annealed</td>
<td>8.75 ± 0.11^a</td>
<td>0.04 ± 0.03^a</td>
</tr>
<tr>
<td></td>
<td>Annealed</td>
<td>10.25 ± 0.08^a</td>
<td>0.08 ± 0.01^a</td>
</tr>
</tbody>
</table>

*Values followed by a different letter within the same column are significantly different (P < 0.05)*
starch decreased significantly in relation to its native counterparts. This behavior was influenced by the reorganization of starch molecules, forming a more stable conformation and decreasing the tendency of amylose to leach out at 95°C (Gomez et al., 2004). The reduced viscosity and improved shear stability of tapioca starch on annealing has been attributed to reduced granular swelling and amylose leaching, and increased interaction between starch chains during annealing (Stute, 1992; Hoover and Vasanthan, 1994; Jacobs et al., 1995). In contrast with sweet potato starch, the peak viscosity increased significantly as the result of annealing treatment. It has been postulated that there was an increment in granule rigidity and resistance to shear making it more viscous (Jacobs et al., 1995). The breakdown of hydrolyzed annealed tapioca starch was decreased significantly as compared to its native counterpart. The decrement of breakdown value in hydrolyzed annealed tapioca starch was possibly caused by stabilization of polymer chains, preventing association of amylose molecules after cooling.

After annealing at 50°C for 72 h, the To (onset temperature), Tp (peak temperature) and Tc (conclusion temperature) of annealed starches increased significantly than the counterpart starch before annealing (Table 2). This infers that annealing treatment has re-ordered the starch chains resulting in more ordered structure. The increase in gelatinization temperature has been shown to be most pronounced for To and least for Tc (Jayakody and Hoover, 2008). The formation of enhanced ordered structures allowed significant increase in the more porous structures in starch which might promote more rapid hydrolysis of crystalline structures by the enzymes. The increase in Tp indicates hydrolysis of the amorphous structure by α-amylase and glucoamylase because the amorphous regions facilitate the melting of crystalline structure (O’Brien and Wang, 2008).

X-ray diffraction

The X-ray diffraction patterns of native and annealed tapioca and sweet potato starches after 24 h of hydrolysis are presented in Figure 3. Both hydrolyzed annealed and hydrolyzed native tapioca and sweet potato starches exhibited typical A-type patterns with strong peaks at 2θ about 15 °, 17 °, 18 ° and 23 °. Annealing treatment at 50°C may modify the B-type crystalline structure (Kohyama and Sasaki, 2006). However, in this study, no significant changes

### Table 2. Pasting and thermal properties of native and annealed tapioca and sweet potato starches after 24 hours of hydrolysis at 35°C.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pasting properties</th>
<th>Thermal properties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pasting temperature (°C)</td>
<td>Peak viscosity (RVU)</td>
</tr>
<tr>
<td>Tapioca</td>
<td>70.42 ± 0.23 a</td>
<td>29.20 ± 0.51 a</td>
</tr>
<tr>
<td>Native annealed</td>
<td>68.53 ± 0.05 b</td>
<td>43.19 ± 0.13 b</td>
</tr>
<tr>
<td>Control annealed</td>
<td>76.91 ± 0.63 c</td>
<td>39.11 ± 0.44 c</td>
</tr>
<tr>
<td>Tapioca annealed</td>
<td>76.38 ± 0.23 c</td>
<td>33.42 ± 0.36 c</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>74.70 ± 0.26 a</td>
<td>3.45 ± 0.21 a</td>
</tr>
<tr>
<td>Native annealed</td>
<td>75.13 ± 0.14 b</td>
<td>4.22 ± 0.25 b</td>
</tr>
<tr>
<td>Control annealed</td>
<td>78.65 ± 0.36 c</td>
<td>9.61 ± 0.05 c</td>
</tr>
<tr>
<td>Tapioca annealed</td>
<td>78.70 ± 0.22 c</td>
<td>10.47 ± 0.69 c</td>
</tr>
</tbody>
</table>

Values followed by a different letter within the same column are significantly different (P < 0.05)
were observed in the X-ray diffraction pattern of hydrolyzed native and hydrolyzed annealed tapioca and sweet potato starches. This observation is in accordance with previous study (Gough and Pybus, 1971; Stute, 1992; Zavareze and Dias, 2011; Wang et al., 2014), suggesting that annealing does not result in changes in the wide angle X-ray diffraction patterns. It has been claimed that annealing of tapioca starch close to the onset temperature of gelatinization combined with the addition of α-amylase were found to increase the relative crystallinity of starch by removal of the amorphous region without changing the X-ray pattern (Tukomane et al., 2007).

Conclusion

This STARGEN 001 enzyme has demonstrated a good ability to hydrolyze starch at native granular state without a need for starch gelatinization process. In this study, it has been proven that the annealing treatment may increase the susceptibility of tapioca and sweet potato starches towards hydrolysis at sub-gelatinization temperature (35°C) for 24 h.

Figure 3. X-ray diffraction (XRD) pattern of (a) native tapioca, (b) annealed tapioca, (c) native sweet potato, and (d) annealed sweet potato starches after hydrolysis at sub-gelatinization temperature (35°C) for 24 h.

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