

## Changes in structural, physicochemical properties and digestibility of partial hydrolyzed and annealed maize starch



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### ABSTRACT

The objective of this study is to create modified starch with high RS content (especially heat resistant RS) through the combination of two techniques of restricted hydrolysis and annealing. Native maize starch was partially hydrolyzed in HCl solution at room temperature for 4, 14, 23, and 30h. Then, native starch (NC) and partially hydrolyzed starch (PAH) were annealed (ANN) at 50°C for 24h. The structural, physicochemical, and in vitro digestibility properties were measured. The apparent amylose content (AAC) was slightly increased at low hydrolysis level (HL) whilst decreased sharply at high HL. AAC was almost unchanged after annealing. Intrinsic viscosity, molecular weight and degree of polymerization decreased after PAH treatment whilst increased after ANN. The  $\alpha$ -helix/amorphous ratio (AH/AMS) did not change much after PAH although it slightly increased after ANN. Relative crystallinity (DRC) increased slightly at low DH and decreased sharply at too high HL. Furthermore, ANN treatment increased DRC. The crystal pattern (A-type) did not change after PAH and ANN treatment. The gelatinization temperature of starch decreased after double modification. In terms of in vitro digestibility, the content of rapidly digested starch (RDS) increased but resistant starch (RS) significantly decreased after gelatinization pre-treatment. The content of resistant starch (RS) and boiling-stable resistant starch (bRS) increased sharply after PAH and ANN treatment. In particular, the highest bRS reached 24.2% under double treatment. While, the maximum bRS of PAH, ANN, and NC starches were 15.2%, 8.9%, and 4.2%, respectively. Actually, native starch contains poor properties and functions and is not suitable for industrial applications. In fact, the production of chemically modified starch with special technological features or high RS concentration is very interesting. In this study, the combination of the two processing techniques (PAH and ANN) significantly increased RS, especially bRS, which is capable of controlling blood glucose, body weight, and other benefits to human health.

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### 1. Introduction

Starch is one of the main sources of human energy. The digestibility of starch in the human small intestine is divided into three fractions. Slowly digestible starches (SDS) are completely digested in the small intestine but slower than rapidly digestible starch (RDS). SDS has a relatively slow rate of glucose metabolism, which contributes to satiety and good control of diabetes (Lehmann and Robin,

2007). Resistant starch (RS) is not enzymatically digested in the human small intestine (Sajilata et al., 2006). RS has the effect of inhibiting fat accumulation, controls blood cholesterol, and has good effects on diabetes control, obesity reduction, overweight management (Sajilata et al., 2006).

According to Mun and Shin (2006), RS increased 1.5 times, compared with native starch, after hydrolysis with 0.1 M HCl for 30 days. Chung et al. (2009) showed that the content of RS and boiling-stable RS increased significantly under annealing treatment (starch suspension of 30%, w/v; 50°C for 24h). In addition to the formation of RS, some structural and physicochemical properties of modified starch were changed under partial hydrolysis and annealing. After partial hydrolysis, starch had a decrease in average molecular weight. Besides, some other properties also had significant

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changes such as increase of clarity, reduction of viscosity whilst starch gelling ability and gel strength were higher than that of native starch (Huber and BeMiller, 2009).

According to Nakazawa and Wang (2003), annealed starch had an increase in defects and changes in granule size. In addition, other properties of starch also changed after annealing treatment such as increasing the relative crystallinity and gelatinization temperature, formation of double-helix regions (Tester et al., 2000; Waduge et al., 2006; Chung et al., 2009).

## 2. Materials and methods

### 2.1. Partial acid hydrolysis (PAH)

Partial acid hydrolysis (PAH) was carried out according to the method of Jayakody and Hoover (2002) with slight modifications. Natural corn starch (NC; Roquette Riddhi Siddhi, Uttarakhand, India) was partially hydrolyzed by 2.2 N HCl (12.5g starch per 100 ml acid solution) at room temperature (~30°C) for 4h (H4), 14h (H14), 23h (H23) and 30h (H30). The starch suspension was stirred continuously. The reaction was stopped by adding 1.1 N NaOH until the suspension reached pH 7.0. The sample was then centrifuged (2000×g, 20min). The total sugar content in the supernatant was determined by the phenol sulfuric acid method of Dubois et al. (1956) that was modified by Saha and Brewer (1994). The residue was washed several times with distilled water, dried by a convection dryer at 40°C (until the moisture content is around 11%), ground, and sieved (120 mesh) to a fine powder. The degree of hydrolysis (DH) was calculated according to the method of Robin et al. (1975) with Eq. 1:

$$DH (\%) = \frac{\text{weight of total sugar (g) in the supernatant} \times 0.9}{\text{weight of starch sample (g)}} \times 100 \quad (1)$$

### 2.2. Annealing (ANN)

The ANN was carried out according to the method of Brumovsky and Thompson (2001) and with some modifications. The 30% (w/v) suspensions of NC and PAH samples were incubated at 50°C for 24 h. The suspensions of the samples were stirred continuously in a thermostatic shaker. Then, each sample was centrifuged (2000×g, 20min). The sediment was dried by convection dryer at 40°C (until the moisture content is around 11%), ground, and sieved (120 mesh) to a fine powder. Thus, the NCA, H4A, H14A, H23A, and H30A samples were obtained, respectively

### 2.3. Apparent amylose content (AAC)

The apparent amylose content (AAC) was determined according to the method of Zhu et al. (2008). The result was calculated according to Eq. 2:

$$AAC (\%) = \frac{Abs_{620} - Abs_{510} + 0.0542}{0.3995} \quad (2)$$

where,  $Abs_{620}$  and  $Abs_{510}$  were the absorbances of the sample at 620nm and 510nm, respectively.

### 2.4. Intrinsic viscosity ( $\eta_i$ ) and average molecular weight (Mw)

The intrinsic viscosity ( $\eta_i$ , ml/g) is related to the structure, size, shape, and molecular weight of the starch molecule. Based on the intrinsic viscosity, the average molecular weight is estimated. Starch solutions (from 1.0 to 5.0mg in 1.0ml of 1 M KOH) were prepared (Dokic et al., 2004; Harding, 1997). The starch solutions were determined their kinematic viscosity ( $\eta$ , m<sup>2</sup>/s) by the Ostwald capillary viscometer ( $\emptyset=0.3$ mm, Ref. No 509 03, Germany). The starch solutions were kept at 30°C before analysis. The relative viscosity ( $\eta_{rel}$ ) is  $\eta/\eta_0$  (kinematic viscosity of starch and KOH solution, respectively). The reduced viscosity ( $\eta_{red}$ ) is  $\frac{\eta_{rel}-1}{c}$  ( $c$  is the concentration of starch in KOH solution). From the relative viscosity, subsequent calculations of the reduced viscosity, intrinsic viscosity of solutions, and the average molecular weight of starch samples were conducted according to previous reports (Cowie, 1960; Pimpa et al., 2007).

### 2.5. Fourier-transform infrared spectroscopy (FTIR)

The starch samples were measured by an FTIR-8400S instrument (Shimadzu, Japan) at wavenumbers from 400cm<sup>-1</sup> to 4000cm<sup>-1</sup> (Kizil et al., 2002). The starch sample (2mg) and KBr (200mg) was mixed, finely ground, compressed at 8 bar for 20 min, and put into the FTIR machine.

### 2.6. X-ray diffraction (XRD) and degree of relative crystallinity (DRC)

XRD analysis was performed using an X-ray diffractometer (Model D5005, Bruker, Karlsruhe, Germany) operating at 40 kV and 40 mA (Cu-K $\alpha$  radiation) with a wavelength of 0.15406 nm and scanning angle ( $2\theta$ ) from 3-30°. DRC was determined by Eq. 3:

$$DRC (\%) = \frac{A_c}{A_c + A_a} \times 100 \quad (3)$$

where,  $A_c$  was the area under the crystalline curve,  $A_a$  was the area under the amorphous curve (Nara and Komiya, 1983). XRD graphs and areas were calculated using the Origin 8.5.1 software (OriginLab Corporation, Northampton, Mass, U.S.A.).

### 2.7. Gelatinization properties

The gelatinization properties of samples were determined according to the method of Hung and Morita (2005) and with some adjustments. The measuring device was a Micro Visco-Amylo-Graph

(803203, Brabender GmbH and Co. KG, Germany). Starch suspensions (8.0%, w/v) were heated from 30 to 93°C at a heating rate of 7.5°C/min. The temperature was kept at 93°C for 15 min and cooled down to 30°C at a cooling rate of 7.5°C/min. Then, the temperature was kept at 30°C for 15 min.

## 2.8. In vitro digestibility

The in vitro digestibility of (non-boiled) corn starch was mainly determined based on the method of [Englyst et al. \(1992\)](#) and described by [Trinh et al. \(2012\)](#). Starch was hydrolyzed with a mixture of pancreatin (P7545, activity 8 USP/g) and amyloglucosidase (AMG 300L, activity 300 U/ml, Novozymes, Bagsvaerd, Denmark). The reaction was stopped after 10 or 240min by boiling (10min). The amount of glucose in the supernatant after centrifugation (5000×g, 10min) was determined using the DNS method ([Miller, 1959](#)). RDS and SDS were calculated by the amount of glucose after 10 and 240min of reaction, respectively. RS constituted the fraction undigested after 240min.

The in vitro (boiled) digestibility was pretreated with 30min-boiling of starch slurry before the measurement mentioned above.

## 2.9. Statistical calculation

All experiments were conducted in triplicate; mean and SD values were reported. The data were calculated differently by one-dimensional Anova calculation ( $p < 0.05$ , Duncan's multiple range test) using SPSS software (released 2008, SPSS Statistics for Windows, Version 17.0, Chicago: SPSS Inc.).

## 3. Results and discussion

### 3.1. Apparent amylose content (AAC)

The degree of hydrolysis (DH) and apparent amylose content (AAC) of corn starch samples were shown in [Table 1](#). In general, AAC decreased at high DH, especially in samples with hydrolysis time >14 h. During the hydrolysis process, the acid agent attacked the starch chains in two stages: (i) the hydrolysis took place rapidly in the amorphous regions, especially at the branch points with  $\alpha$ -1,6 glycosidic bonds; (ii) slower hydrolysis took place in regions of higher crystallinity ([Jayakody and Hoover, 2002](#); [Cui, 2005](#)). Thus, the slight increase in AAC of samples with low DH (H4 and H14 samples) compared with NC was due to the breakdown of  $\alpha$ -1,6 glycosidic bonds in the amorphous regions causing the release of linear segments. However, AAC decreased sharply at high DH (H23 and H30 samples) because the linear chains (containing  $\alpha$ -1,4 glycosidic bonds of amylose and amylopectin molecules) were cleaved into linear segments with smaller molecular weight. During hydrolysis, the color of the starch-iodine complex varies from blue-violet (DP 39-40) to brown (DP 21-24). If the degree of polymerization (DP) is <20, linear molecules cannot bind iodine ([Cui, 2005](#)). In addition, the AACs of the NCA and PAH-ANN starch samples were slightly increased compared with that of the NC and PAH samples, respectively. Practically, these differences were not so much. Similar results were also observed in the study of [Rocha et al. \(2012\)](#).

**Table 1:** Apparent amylose content (AAC), intrinsic viscosity ( $\eta_i$ ), average molecular weight ( $M_w$ ), degree of polymerization (DP) and  $\alpha$ -helix/amorphous ratio (AH/AMS) and degree of hydrolysis (DH)

Treatment	Sample	DH (%)	AAC (%)	$\eta_i$ (ml/g)	$M_w$ ( $\times 10^5$ g/mol)	DP	AH/AMS
Acid hydrolysis (PAH)	NC	0.00 <sup>a</sup>	18.07 <sup>d</sup>	100.87 <sup>i</sup>	3.48 <sup>j</sup>	746.44 <sup>i</sup>	0.889 <sup>b</sup>
	H4	1.51 <sup>b</sup>	18.18 <sup>de</sup>	20.51 <sup>g</sup>	0.58 <sup>g</sup>	151.80 <sup>g</sup>	0.891 <sup>c</sup>
	H14	5.78 <sup>c</sup>	18.13 <sup>d</sup>	9.43 <sup>c</sup>	0.24 <sup>c</sup>	69.75 <sup>c</sup>	0.888 <sup>b</sup>
	H23	10.00 <sup>d</sup>	15.99 <sup>bc</sup>	8.29 <sup>b</sup>	0.21 <sup>b</sup>	61.33 <sup>b</sup>	0.894 <sup>d</sup>
	H30	14.99 <sup>e</sup>	15.29 <sup>a</sup>	7.21 <sup>a</sup>	0.18 <sup>a</sup>	53.35 <sup>a</sup>	0.878 <sup>a</sup>
	NCA	-	19.58 <sup>f</sup>	159.05 <sup>j</sup>	5.80 <sup>j</sup>	1176.97 <sup>j</sup>	0.891 <sup>c</sup>
Acid hydrolysis + Annealing (PAH-ANN)	H4A	-	18.40 <sup>e</sup>	30.40 <sup>h</sup>	0.90 <sup>h</sup>	224.92 <sup>h</sup>	0.928 <sup>g</sup>
	H14A	-	18.24 <sup>de</sup>	13.74 <sup>f</sup>	0.37 <sup>f</sup>	101.70 <sup>f</sup>	0.900 <sup>e</sup>
	H23A	-	16.16 <sup>c</sup>	12.19 <sup>e</sup>	0.32 <sup>e</sup>	90.18 <sup>e</sup>	0.902 <sup>f</sup>
	H30A	-	15.86 <sup>b</sup>	11.36 <sup>d</sup>	0.30 <sup>d</sup>	84.06 <sup>d</sup>	0.903 <sup>f</sup>

The results are expressed as mean (n=3). Standard deviations (not shown) were lower than 10% of their means. Numbers in a column with different superscript letters are significantly different ( $p < 0.05$ )

### 3.2. Viscosity, molecular weight ( $M_w$ ), and degree of polymerization (DP)

The reduced viscosity ( $\eta_{red}$ ) of the starch samples was inversely correlated with the degree of hydrolysis ([Fig. 1](#)). The annealed starch had higher  $\eta_{red}$  than that of non-annealed one. In addition, the higher the starch solution concentration was, the higher the  $\eta_{red}$  value was. The intrinsic viscosity ( $\eta_i$ ) of each sample was calculated from the linear trendline ([Fig. 1](#)). In general, both  $\eta_i$ ,  $M_w$ , and DP decreased with increasing DH ([Table 1](#)). In fact, these changes are evidence of the hydrolysis of

starch molecules into smaller ones ([Jayakody and Hoover, 2002](#)). In addition, both  $\eta_i$ ,  $M_w$ , and DP of the NCA and PAH-ANN samples were higher (~1.5 times) than that of the NC and PAH samples, respectively. According to [Tester and Debon \(2000\)](#), a small amount of low-molecular-weight short-chain  $\alpha$ -glucans (products of limited hydrolysis) were dissolved in water during annealing and removed/lost after the later centrifugation. Besides, the formation of  $\alpha$ -helix structure and crystal structure under annealing is also responsible for the increase of  $\eta_i$ ,  $M_w$ , and DP ([Cui, 2005](#); [Huber and BeMiller, 2009](#)).

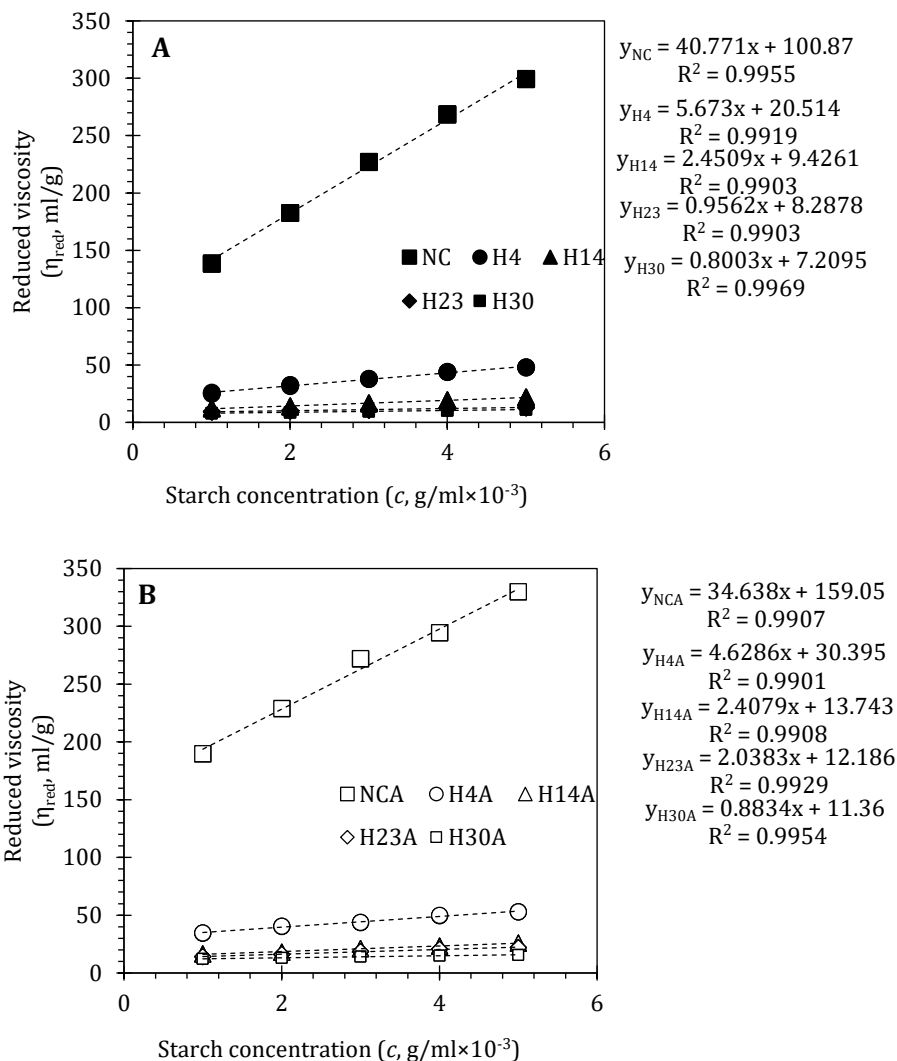


Fig. 1: Reduced viscosity ( $\eta_{red}$ ) of samples at various degrees of hydrolysis

### 3.3. Fourier-transform infrared spectroscopy (FTIR)

FTIR is a useful tool for characterizing chemical transformations in starch (Fig. 2). Overall, there were no significant differences in the FTIR spectra of the samples. The loss or the formation of peaks in the treated starches compared with NC (native starch) was not detected. The FTIR spectra of the PAHs had the presence of two new peaks in the region 2327-2385 $cm^{-1}$  (Fig. 2). However, these were the signal of CO<sub>2</sub> from the atmosphere during the analysis (Abdullah et al., 2018; Kebukawa et al., 2009). Thus, limited hydrolysis treatment and annealing did not form or lose any chemical groups in starch molecules. However, the absorbance of the different peaks reflected the quantification of the chemical structures. The ratio between the peak absorbance of 1047 $cm^{-1}$  ( $\alpha$ -helix) and 1035 $cm^{-1}$  (amorphous) indicated the variation in the degree of crystallinity (Deeyai et al., 2013). The results (Table 1) showed that the NCA and PAH-ANN samples had higher  $\alpha$ -helix/amorphous ratios (AH/AMS) than that of NC and PAH samples, respectively. A similar trend was also reported in the study of Hoove and

Vasanthan (1993). The formation of hydrogen bonds between amylopectin chains and adjacent amylose chains during annealing was also one of the reasons for the increase in the AH/AMS ratio (Huber and BeMiller, 2009).

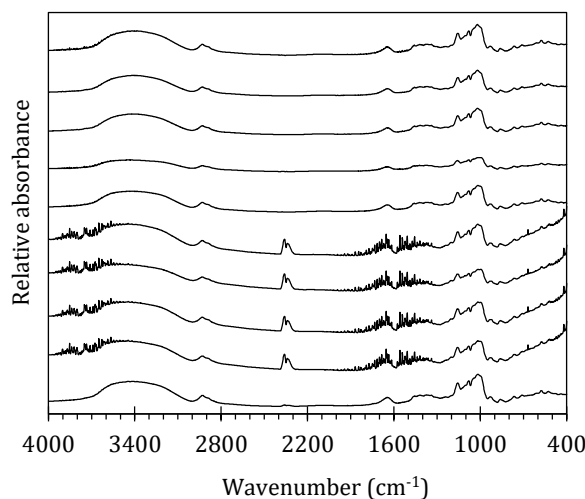


Fig. 2: FTIR spectra of samples (from top to bottom: H30A, H23A, H14A, H4A, NCA, H30, H23, H14, H4, NC)

### 3.4. X-ray diffraction pattern (XRD) and degree of relative crystallinity (DRC)

Fig. 3 showed that all corn starch samples had A-type crystals because they contain characteristic peaks at positions 15°, 17°, 18°, 20°, 23° ( $2\theta$ ) (Gomes et al. 2010). The degree of relative crystallinity (DRC) of all starch samples was calculated based on the XRD spectrum (Fig. 3). The results showed that increasing DH caused a slight increase in DRC. However, if the DH is too high, the DRC will decrease sharply and lower than that of the native starch (NC). Similar trends were also noted in the study of Jayakody and Hoover (2002) and Nagahata et al. (2013). Accordingly, the linear chains formed from the depolymerization process will participate in the structure of new crystals in the early stages of hydrolysis, thereby increasing DRC. However, at a

higher degree of hydrolysis, the depolymerization is strong and acts directly on the crystalline regions thereby reducing DRC.

The results showed that the DRC of annealed starches (NCA, PAH-ANN) was higher than that of corresponding samples (NC, PAH). Thus, the annealing process rearranged the  $\alpha$ -helix structure thereby increasing the DRC of starch. Similar results were also observed in the study of Waduge et al. (2006). According to Waduge et al. (2006), DRC depends on the following factors: Amylopectin content, crystal form, and the amount of  $\alpha$ -helix structures in the crystal region. XRD spectra (Fig. 3) of all samples had the same crystal pattern. Thus, the important reason for the increase in DRC was the increase in AH/AMS ratio under annealing treatment.

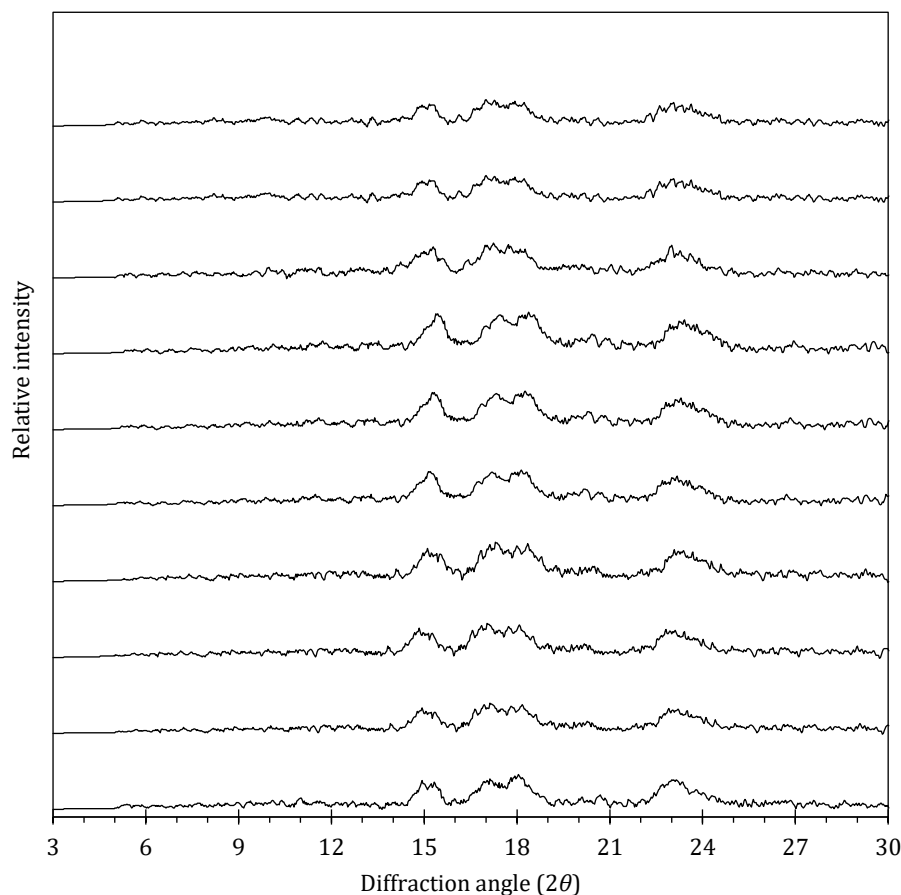


Fig. 3: XRD and DRC of samples (from top to bottom: H30A, H23A, H14A, H4A, NCA, H30, H23, H14, H4, NC)

### 3.5. Gelatinization properties

The gelatinization properties of starch were shown in Fig. 4. Gelatinization occurred strongly for the non-hydrolyzed samples (NC and NCA). However, the hydrolyzed samples (PAH and PAH-ANN) showed no signs of gelatinization under the condition of measurement. Thus, the gelatinization temperature of the starch samples increased after PAH treatment. Similar results were also observed in the study of Brumovsky and Thompson (2001). The results also showed that the gelatinization

properties of the samples without/with annealing treatment did not differ much.

### 3.6. In vitro digestibility

The digestibility fractions of the non-boiled and boiled samples were shown in Table 2. In both treatment stages, the native starch (NC) had the lowest RS (18.02%) and bRS (4.15%) content compared with the others. In contrast, the H23A sample had the highest concentrations of RS (38.64%) and bRS (24.23%). Clearly, after being

boiled, RDS increased and RS decreased significantly. After hydrolysis, the RS and bRS content of starch increased as the degree of hydrolysis (DH) increased.

However, RS and bRS were decreased when DH was too high.

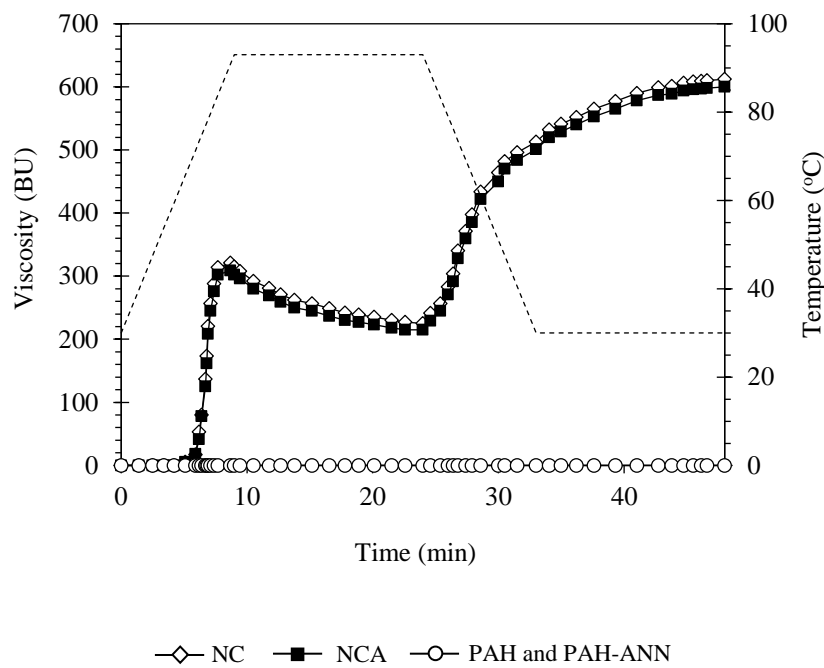


Fig. 4: Amylograph of samples

PAH-ANN samples also tended to be similar. According to Cui (2005), the higher the degree of crystallinity (DRC) starch was, the better its resistance to enzymatic hydrolysis was. During the initial period of hydrolysis, the amorphous region was rapidly degraded. Then, crystals rearrangement took place and DRC was increased (Fig. 2) leading to the increase of RS and bRS. At the latter stages, the hydrolysis gradually attacked the crystalline region causing the decrease of DRC (Fig. 2). This change led

to a decrease in RS (Nagahata et al., 2013). Both NCA and PAH-ANN samples had higher RS and bRS than NC and PAH samples, respectively. According to Chung et al. (2009), an increase in RS content was associated with increased chain interactions in starch molecules (amylose-amylose and/or amylose-amylopectin). In addition, increased DRC content after annealing resulted in increased RS and bRS (Waduge et al., 2006).

Table 2: Digestion fractions (%) of starch samples

Sample	Non-boiled			Boiled		
	RDS	SDS	RS	RDS	SDS	RS
NC	12.04 <sup>c</sup>	69.94 <sup>f</sup>	18.02 <sup>a</sup>	69.62 <sup>g</sup>	26.23 <sup>f</sup>	4.15 <sup>a</sup>
H4	15.08 <sup>f</sup>	59.3 <sup>f</sup>	25.60 <sup>e</sup>	65.02 <sup>e</sup>	24.00 <sup>d</sup>	10.98 <sup>d</sup>
H14	9.89 <sup>b</sup>	62.08 <sup>g</sup>	28.03 <sup>f</sup>	56.77 <sup>b</sup>	28.07 <sup>g</sup>	15.16 <sup>f</sup>
H23	25.60 <sup>i</sup>	50.60 <sup>c</sup>	23.80 <sup>d</sup>	66.38 <sup>f</sup>	25.40 <sup>e</sup>	8.22 <sup>c</sup>
H30	37.59 <sup>i</sup>	41.86 <sup>a</sup>	20.55 <sup>b</sup>	72.57 <sup>h</sup>	21.11 <sup>c</sup>	6.32 <sup>b</sup>
NCA	12.72 <sup>d</sup>	65.69 <sup>h</sup>	21.59 <sup>c</sup>	64.38 <sup>e</sup>	26.73 <sup>f</sup>	8.89 <sup>c</sup>
H4A	14.00 <sup>e</sup>	56.03 <sup>d</sup>	29.97 <sup>g</sup>	60.74 <sup>c</sup>	24.97 <sup>e</sup>	14.28 <sup>e</sup>
H14A	8.39 <sup>a</sup>	57.84 <sup>e</sup>	33.77 <sup>h</sup>	52.72 <sup>a</sup>	27.32 <sup>f</sup>	19.96 <sup>g</sup>
H23A	17.51 <sup>g</sup>	43.85 <sup>b</sup>	38.64 <sup>j</sup>	62.96 <sup>d</sup>	12.82 <sup>b</sup>	24.23 <sup>i</sup>
H30A	19.97 <sup>h</sup>	43.38 <sup>b</sup>	36.64 <sup>i</sup>	69.20 <sup>g</sup>	9.39 <sup>a</sup>	21.41 <sup>h</sup>

The results were expressed as mean (n=3). Standard deviation (not shown) was lower than 10% of their mean. Numbers in a column, with different superscript letters, were significantly different (p < 0.05)

#### 4. Conclusion

Treatment of PAH and ANN altered the structural properties associated with changes in the digestibility and gelatinization properties of corn starch. DRC was closely related to changes in digestive fractions. Samples treated with only partial hydrolysis showed higher RS and bRS content than that of NC samples. PAH-ANN treatment significantly increased RS content. Especially, PAH-ANN treatment produced starch with bRS (24.23%) much

higher than NC crude starch (4.15%). This type of starch has potential applications in the production of boiling-stable resistant starch, capable of controlling blood glucose, body weight, and other benefits to human health.

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## Compliance with ethical standards

## Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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