The effect of high moisture heat-acid treatment on the structure and digestion property of normal maize starch

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A B S T R A C T
The objective of this study was to analyze the influence of thermal-acid treatment on the formation of resistant starch (RS). The maximum RS content in citric acid–heat treated starches (CAHT) reached 36.55%, which was 7 times higher than that in native starch. According to HPSEC–MALLS–RI analysis, amylopectin was more susceptible to hydrolysis than amylose during citric acid–heat treatment (CAHT). X-ray measurement revealed that even though the starch crystalline pattern was changed from A-type to a more resistant B-type after CAHT, the fraction of crystalline region decreased from 21.16% to 8.37%. The hydroxyls on the starch chains were substituted by the citric acid anhydrides during CAH according to FT-IR analysis, which led to the formation of ester bond cross-linking structures in starch granules, and it could be the main contribution to the increase of RS content in CAHT samples.

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1. Introduction

Starch is one of the most important natural carbohydrates in the human diet. For nutritional purposes, starch in foods can be classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). The foods which are rich in SDS and RS play a positive and important role in preventing human diseases, such as obesity, diabetes and cardiovascular diseases (Fuentes-Zaragoza, Riquelme-Navarrete, & Pérez-Álvarez, 2010; Lehmann & Robin, 2007).

There are several methods to modify the starch in order to increase the content of SDS and RS, including hydrothermal treatment, enzymatic treatment and chemical treatment. Hydrothermal treatment aims to increase the fraction of crystalline region in starch granules, which contributes to the formation of SDS and RS. Li, Ward, and Gao (2011) heated 80 wt.% mung bean starch solution at 120 °C for 12 h and reported that the RS content increased by 34% with the degree of crystallinity increased by 7.5% after heat-moisture treatment. As for chemical modification, chemical reagents are used to prevent the digestion of modified starch by blocking enzyme access and forming atypical linkages. Etherification, esterification and cross-linking are the three typical chemical modifications to produce RS. Carlos-Amaya, Osorio-Diaz, Agama-Acevedo, Yee-Madeira, and Bello-Perez (2011) used dual modifications of cross-linking and esterification in banana starch, and the RS content increased from 21.49% to 29.14%.

Acid modification is one of the chemical treatments to modify the starch used for the production of cationic and amphoteric starches, starch gum candies and paper (Wurzburg, 1986), and it is also a promising way to prepare SDS and RS. Several studies have been reported on the properties of starches which were prepared by acid modification followed by autoclaving and then retrogradation (Aparicio-Saguilán et al., 2005; Köksel, Masatcioglu, Kahraman, Ozturk, & Basman, 2008; Shin, Byun, Park, & Moon, 2004). The acid would first hydrolyze the amorphous parts of the starch granules and then hydrolyze the crystalline region, resulting in the production of shorter chains, which would be disorganized through auto-claving and finally reoriented to form more ordered double helix structure during the retrogradation which would prevent the enzyme hydrolysis (Hoover, 2000). Köksel, Basman, Kahraman, and Ozturk (2007) obtained 13.6–16.7% RS by hydrolyzing normal corn starch with hydrochloric acid and then retrogradation. Ozturk, Köksel, and Ng (2011) obtained 39.5% RS from amylopectin starch samples using the same process. Xie and Liu (2004) used citric acid and dry heated the normal corn starch under 140 °C for 7 h, and finally obtained 68.3% RS after heating at 100 °C in a boiling water bath. During this procedure, the citric acid would esterify with the hydroxyl groups on the starch chains and form the cross-linking structure that led to the high content of RS. In addition, citric acid
2. Materials and methods

2.1. Materials

Normal maize starch was obtained from the Shandong Zhucheng Chemical Industries Co., Ltd. (Zhucheng, China). Type VI-B porcine pancreas α-amylase (EC 3.2.1.1) and guar gum were purchased from Sigma–Aldrich Chemical Co., Ltd (St. Louis, MO, USA) and Sangon Biotech Co., Ltd. (Shanghai, China), respectively. Amyloglucosidase (EC 3.2.1.3) was donated by Wuxi Genencor Bio-products Co., Ltd. (Wuxi, China). Glucose assay kit was purchased from Shanghai Rongsheng Biotech Corporation, Shanghai, China. All other chemicals and reagents were analytical grade and were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preparation of starch samples

2.2.1. Citric acid treated starch (CAT)

Normal maize starch dispersed in citric acid solutions (0.1, 0.5, or 1 M) were placed in sterilized bottles (starch:acid solution = 2:3, w/w) and mixed well. The dispersions were heated at 50 °C in a water bath for different times (1 h, 4 h, or 8 h) and neutralized by 10% (w/v) NaOH solution. After drying, the samples were re-dispersed in water (starch:water = 2:3, w/w) with continuous stirring and stored overnight in a refrigerator (4 °C). Finally, the starch samples were dried at 35 °C in an air-draft oven and ground.

2.2.2. Citric acid-heat treated starch (CAHT)

Normal maize starch dispersed in citric acid solutions (0.1, 0.5, or 1 M) were placed in sterilized bottles (starch:acid solution = 2:3, w/w) and mixed well. The dispersions were heated at 140 °C in an air-draft oven and ground. After that, the samples were re-dispersed in distilled water, neutralized with 10% (w/v) NaOH solution and washed three times with distilled water. Finally, the washed starch was air dried at 35 °C and ground again.

2.3. In vitro digestibility determination

2.3.1. Uncooked samples

The in vitro digestibility of native, CAT, control, and CAHT starches were determined according to the method of Englyst et al. (1992) with a slight modification. 200 mg starch and 2 mL water were added to 50 mL screw-capped polypropylene centrifuge tubes and mixed using a magnetic stirrer for 5 min. Then, 50 mg guar gum, 15 glass beads, and 8 mL sodium acetate buffer (pH 5.2) were added to each tube. After equilibrated at 37 °C for 5 min, 10 mL of porcine pancreatic α-amylase (290 U/mL) and amyloglucosidase (20 U/mL) were added, followed by incubation in a water bath at 37 °C with shaking (160 rpm). After 20 (G20) and 120 (G120) min of incubation, 50 μL of hydrolyzate was removed and 950 μL absolute ethanol was added to stop the enzymatic reaction. The glucose released in each hydrolyzate was determined by using a glucose oxidase peroxidase diagnostic kit (Shanghai Rongsheng Biotech Corporation, Shanghai, China). The RDS, SDS, and RS percentage of each sample were calculated from the values of G20 (glucose released after 20 min), G120 (glucose released after 120 min), FG (free glucose) and TS (total starch) as follows:

\[
RDS = \frac{(G20 - FG) \times 0.9 \times 100}{100}
\]

\[
SDS = \frac{(G120 - G20) \times 0.9 \times 100}{100}
\]

\[
RS = TS - (RDS + SDS)
\]

2.3.2. Cooked samples

Native, CAT, control, and CAHT starch samples (200 mg) were each dispersed in water (2 mL) in 50-mL screw-capped polypropylene centrifuge tubes and mixed well. After mixing, the tubes were placed in a boiling water bath for 20 min. The samples were stirred during heating with magnetic stir bars. After cooking, the tubes were placed in a 37 °C water bath for 10 min until it is cooled. The RDS, SDS, and RS were measured as described for the uncooked samples.

2.4. Degree of substitution

The degree of citric acid esterified to the starch was analyzed by the procedure of Klaushofer, Berghofer, and Pieber (1979), which was based on the reaction of citric acid and Cu²⁺ that formed a stable complex during titration with a solution of copper sulfate. Briefly, the starch sample (450 mg) was dispersed in 2 mL deionized water. The solution was then dissolved in 50 mL of 1 M KOH and boiled in a water bath for 10 min. After the solution was cooled to 25 °C, the pH was adjusted to 8.5 with 5 M acetic acid. The solution was then added to 25 mL sodium borate buffer (pH 8.5) which contained 0.3 g indicator (murexide:sodium sulfate = 1:500, w/w) and dilute to 300 mL with deionized water. The solution was titrated with 0.05 M copper sulfate solution until the red-violet color disappeared.

DS was calculated as follows:

\[
DS = \frac{162W}{100M - (M - 1)W}
\]

In this equation, W (% by weight of substituent) = [bound citrate (g)/sample (g)]-bound citrate (g)/100, and M = molecular weight of the citric acid substituent which was 175.1. Each sample was analyzed in triplicate.

2.5. HPSEC–MALLS–RI system

Starch samples (10 mg) were dispersed in 5 mL of 100 mM NaNO₃/DMSO solution. These suspensions were heated in a boiling water bath for 30 min and then incubated at 50 °C with stirring for
24 h. The starch sample solutions were filtered through a 0.45 µm membrane filter and then 100 µl of the filtrate was injected into the HPSEC system. The HPSEC–MALLS–RI system consisted of a refractive index detector (Waters 2414, Waters Corporation, Milford, MA, USA), a pump (LC-20AB, Shimadzu Corporation, Kyoto, Japan) equipped with a 100 µl sample loop, a multi-angle laser light-scattering detector (DAWN EOS, Wyatt Tech. Corp., Santa Barbara, CA, USA) with a He-Ne laser source (λ = 632.8 nm) and a K-5 flow cell, and a Styraegl HMW 6E and HMW 2 columns (Waters Corporation, Milford, MA, USA). The mobile phase used for HPSEC system was 100 mM NaNO3/DMSO solution that had been filtered through 0.22 µm filters and the flow rate was 0.6 mL/min. Data obtained from MALLS and RI detectors were analyzed using Astra software (Version 5.3.4.20, Wyatt Tech. Corp., Santa Barbara, CA, USA).

From the values of $M_w$ and $R_g$, specific volume for gyration ($SV_g$) could be calculated as follows:

$$SV_g = 4/3\pi (R_g \times 10^{21})^3/(M_w/N) = 2.522R_g^3/M_w.$$  

In this equation, the units for $SV_g$, $M_w$, and $R_g$ were cm$^3$/g, g/mol, and nm, respectively, and $N$ was the Avogadro's number ($6.02 \times 10^{23}$/mol).

2.6. X-ray diffraction

X-ray patterns of starches were analyzed using a X-ray diffractometer (D8 Advance, Bruker AXS, Karlsruhe, Germany) with Cu Kα ($\lambda = 0.15406$ nm) radiation at a voltage of 40 kV and 40 mA. The samples were packed tightly into a circular plastic cell and scanned between 2θ = 3–40° with a scanning speed of 4°/min. The relative crystallinity was calculated using the method of Miao, Jiang, Zhang, Jin, and Mu (2011) as follows:

$$X_c = Ac/(Ac + Ac).$$

In this equation, $X_c$ was the relative crystallinity, $Ac$ was the crystalline area, and $Ac$ was the amorphous area on the X-ray diffractogram.

2.7. Differential scanning calorimetry

The thermal properties of the starches were performed using differential scanning calorimetry (Pyris 1 DSC, Perkin–Elmer, MA, USA). All of the samples were accurately weighed (0.01 mg) on Perkin–Elmer DSC pans. 2 mg of anhydrous starch was mixed with 4 mg deionized water and hermetically sealed in an aluminum pan. The samples were allowed to equilibrate at 25 °C for 15 min before heating in the DSC and then heated from 25 °C to 95 °C at a heating rate of 10 °C/min. An empty pan was used as a reference. The onset temperature ($T_o$), peak temperature ($T_p$), conclusion temperature ($T_c$) and enthalpy of gelatinization ($\Delta H$) were calculated using Pyris software based on the mass of dry solid.

2.8. Fourier transform-infrared spectroscopy (FT-IR)

FT-IR spectra of the starch samples were obtained using a Nicolet Nexus 470 spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with a deuterated triglycine sulfate (DTGS) detector using attenuated total reflectance (ATR) accessory at 4 cm$^{-1}$ resolution for 64 scans. Each spectrum was recorded against an empty cell as background and subtracted from the spectrum of air. The spectra were baseline-corrected and deconvoluted by drawing a straight line at 1100 and 950 cm$^{-1}$ using Omnic software 8.2 (Thermo Fisher Scientific Inc., Waltham, MA, USA). A half-band width of 15 cm$^{-1}$ and a resolution enhancement factor of 1.5 were employed. The absorbance ratio of 1047 cm$^{-1}$/1022 cm$^{-1}$ [$R (1047/1022)$] was obtained from the deconvoluted spectra.

2.9. Scanning electron microscopy

The surface structure of starch granules was observed using a scanning electron microscopy (Quanta-200, FEI Company, Eindhoven, Netherlands). The starch samples were sprayed on an aluminum plate using double-sided adhesive and coated with a thin film of gold. The samples were examined at 5 kV accelerating voltage. Each CAHT sample was taken at magnifications of 600× and 1200×, respectively.

2.10. Statistical analysis

The statistical analysis of the results was conducted by the analysis of variance (SAS Statistic Package, SAS Institute Inc., Cary, NC, USA). Significant differences were defined as $P \leq 0.05$.

3. Results and discussion

3.1. The influence of thermal–acid treatment conditions on the digestion properties of starch samples

In order to study the impact of acid concentration level, thermal intensity and their combination on the digestion properties of normal starch, the resistant starch (RS) and slowly digestible starch (SDS) contents in starch granules after citric acid hydrolysis combined with heat treatment were studied. The digestion properties of both cooked and uncooked starch were also compared in Table 1.

As expected, the RDS content in cooked native starch increased as compared with the raw samples to 88.00%, while SDS and RS contents decreased to 6.98% and 5.02%, respectively. The increased digestibility in cooked native starch was due to the disruption of double helix which forms the starch crystallites at the granule surface and/or to the crystallite reorientation during the cooking process (Chung, Liu, & Hoover, 2009).

With acid treatment alone, the RDS content in uncooked CAT samples increased dramatically while the SDS and RS content decreased as compared with raw starch. The results seemed that the acid treatments have negative effects on the SDS and RS formation, but in fact there are two factors contributed to the final digestion properties of resulted starches. Acid hydrolysis would increase SDS and RS contents due to the formation of short chains and their reorganization during retrogradation under low moisture content condition (Köksel et al., 2007). However, boiling of starch suspension under high moisture content would disrupt the crystallite and starch granules, resulting in higher RDS content (Lee, Shin, Kim, Choi, & Moon, 2011). It was obvious that in the uncooked CAT samples, the boiling effect was the dominate factor, but in cooked CAT samples, the increased SDS and RS content as compared to the cooked native starch did reflect the effect of acid hydrolysis. However, the level of acid concentration and time did not affect the RDS, SDS or RS contents both in cooked and uncooked samples. Due to the low acid hydrolysis temperature, no significant degree of DS was observed for any of the CAT samples.

Among the uncooked CAHT samples, the digestibility of starch produced with the low citric acid concentration (0.1 M) was similar to that of the control, which suggested that the effect of the acid hydrolysis at low acid concentration would be compensated by the heat moisture treatment. At higher citric acid concentrations, there was a decreasing trend in RDS and SDS contents and an increasing trend in RS contents, suggesting the increasing indigestibility of CAHT, which was also indicated by the increasing DS from 0.01 to 0.10 among the uncooked CAHT samples. For cooked CAHT samples treated at different acid concentrations, similar trends were found in RDS, SDS and RS contents as compared to the correspond-
For amylose, with molecular weights of 1.07 × 10^6 for amylopectin and peak 2 for amylose, the starch exhibited two peaks: peak 1 for amylopectin and peak 2 for amylose. The molecular weights of peak 1 and peak 2 were 1.07 × 10^6 and 7.50 × 10^5, respectively. The results were close to that reported by Mirzaei, Pourjafar, and Homayouni (2012), with the MW of amylopectin and amylose of native maize starch being 59.61 ± 1.08 g mol⁻¹ and 3.84 ± 0.73 g mol⁻¹, respectively. However, a much higher amylose molecular weight was reported by Mun and Shin (2006), they suggested the Mw of amylose may not be hydrolyzed at low acid concentration (0.1 M) during CAHT, and as the acid concentration increased further, the amylose would also be hydrolyzed.

The specific volume for gyration (SVg), calculated by assuming that the gyration occurs in a sphere pattern, provides the gyration volume on a basis of unit mass that gives the mass-based information on the density and degree of branching, which was inversely related to the degree of molecular compactness (You & Lim, 2000). Amylopectin has more compact shape than amylose and thus had smaller SVg value in the native maize starch sample. The results showed that amylose may not be hydrolyzed at low acid concentration (0.1 M) during CAHT, and as the acid concentration increased further, the amylose would also be hydrolyzed.

The logarithmic slope between Mw and RS data (Table 1), the decreased SVg values suggested that acid hydrolyzation and cross-linking of amylopectin occurred at the same time leading to the compactness of chain conformation increased. The SVg values in peak 2 also showed a decreased trend as the acid concentration increased, which could be attributed to that the hydrolyzed amylopectin chains that mixed into peak 2 would twined with amylose chains and thus increased the compactness of the chain conformation.

The logarithmic slope between Rg and Mw reveals three different dimensional chain conformations (sphere, random coil, and rod shapes) and can be estimated based on the equation: Rg = kMw^n. For a spherical conformation, the relationship between average molecular weight (Mw) and radius of gyration (Rg) yields a line with a slope of about 0.33. In the case of rod shaped molecules, while for random coil in a good solvent, the slope should be between 0.5 and 0.6 (Lee, Han, & Lim, 2006). As shown in Table 2, amylopectin and amylose in native starch showed a sphere and a random coil shape, respectively. However, the conformational structure variation of starch chains in the native maize starch exhibited two peaks: peak 1 for amylopectin and peak 2 for amylose, with molecular weights of 1.07 × 10^6 and 7.50 × 10^5, respectively. The results were close to that reported by Mirzaei, Pourjafar, and Homayouni (2012), with the Mw of amylopectin and amylose of native maize starch being 59.61 ± 1.08 g mol⁻¹ and 3.84 ± 0.73 g mol⁻¹, respectively. However, a much higher amylose molecular weight was reported by Mun and Shin (2006), they suggested the Mw of amylose may not be hydrolyzed at low acid concentration (0.1 M) during CAHT, and as the acid concentration increased further, the amylose would also be hydrolyzed.

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after citric acid-heat treatment, the slope values of both peaks in CAHT samples decreased as the acid concentration increased without showing either sphere or linear shape. As previously discussed with the SVg data and DS data (Table 1), the decreased slope values revealed that both fractions were cross-linked and the intensity of the cross-linking structure increased with the acid concentration increased.

The broadness of the molecular weight distribution in starch can be defined by polydispersity index (PDI) based on the equation: \( \text{PDI} = \frac{\text{M}_w}{\text{M}_n} \). PDI of amylose was larger than that of amylopectin in native maize starch which can be attributed to the fact that the amylose chains, with a random coil shape, are distributed in a broader molecular weight range than that of amylopectin chains. After citric acid-heat treatment, the PDI of peak 1 remained constant. During citric acid-heat treatment, the starch would gelatinize in the aqueous solution and exposure amylopectin homogeneously into the acid solution, which led to a homogeneous acid hydrolyzation and cross-linking of the amylopectin and thus presented a uniform molecular weight distribution in peak 1 at each acid concentration. The PDI of peak 2, however, increased first and then decreased as compared with the native starch. The results suggested that the hydrolyzed amylopectin fraction which was composed of short linear chains would twine with the unhydrolyzed amylose and thus increased the broadness of the molecular weight distribution of peak 2 in 0.1 M CAHT sample. As the acid concentration increased further, the amylose would also be hydrolyzed and generated shorter linear chains, which led to the decrease of the PDI values in 0.5 M and 1 M CAHT samples.

### Table 2
Structural characteristics of native and acid-treated starches.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mw (g/mol)</th>
<th>Rz (nm)</th>
<th>Mass fraction (%)</th>
<th>SVg (cm³/g)</th>
<th>PDI</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peak 1</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>(A) structural characteristics of peak 1</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Native</td>
<td>1.07 ± 0.02 × 10⁶ a</td>
<td>331.3 ± 24.7 a</td>
<td>66.4 ± 1.4 a</td>
<td>0.85 ± 0.08 a</td>
<td>1.30 ± 0.03 a</td>
<td>0.33 ± 0.02 a</td>
</tr>
<tr>
<td>Control</td>
<td>0.96 ± 0.09 × 10⁶ a</td>
<td>305.4 ± 25.4 a</td>
<td>65.3 ± 0.9 a</td>
<td>0.75 ± 0.07 a</td>
<td>1.26 ± 0.05 a</td>
<td>0.32 ± 0.01 a</td>
</tr>
<tr>
<td>0.1 M CAHT</td>
<td>3.30 ± 0.22 × 10⁶ b</td>
<td>195.0 ± 15.6 b</td>
<td>50.5 ± 2.3 b</td>
<td>0.57 ± 0.04 b</td>
<td>1.23 ± 0.10 a</td>
<td>0.26 ± 0.02 b</td>
</tr>
<tr>
<td>0.5 M CAHT</td>
<td>1.51 ± 0.09 × 10⁶ c</td>
<td>95.2 ± 4.2 c</td>
<td>24.2 ± 2.1 c</td>
<td>0.14 ± 0.00 c</td>
<td>1.32 ± 0.09 a</td>
<td>0.19 ± 0.01 c</td>
</tr>
<tr>
<td>1 M CAHT</td>
<td>8.28 ± 0.06 × 10⁶ d</td>
<td>52.0 ± 4.5 d</td>
<td>15.0 ± 3.3 d</td>
<td>0.04 ± 0.01 d</td>
<td>1.35 ± 0.11 a</td>
<td>0.09 ± 0.01 d</td>
</tr>
<tr>
<td><strong>Peak 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B) structural characteristics of peak 2</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native</td>
<td>7.50 ± 0.28 × 10⁶ a</td>
<td>187.0 ± 1.9 a</td>
<td>33.6 ± 1.3 d</td>
<td>2.20 ± 0.03 a</td>
<td>2.80 ± 0.05 b</td>
<td>0.55 ± 0.03 a</td>
</tr>
<tr>
<td>Control</td>
<td>6.17 ± 0.14 × 10⁶ b</td>
<td>177.3 ± 1.8 ab</td>
<td>34.7 ± 0.9 d</td>
<td>2.28 ± 0.03 a</td>
<td>2.88 ± 0.04 b</td>
<td>0.59 ± 0.05 a</td>
</tr>
<tr>
<td>0.1 M CAHT</td>
<td>6.60 ± 0.32 × 10⁶ ab</td>
<td>166.0 ± 5.3 b</td>
<td>49.5 ± 1.2 c</td>
<td>1.75 ± 0.04 b</td>
<td>3.68 ± 0.11 a</td>
<td>0.43 ± 0.01 b</td>
</tr>
<tr>
<td>0.5 M CAHT</td>
<td>2.44 ± 0.05 × 10⁶ c</td>
<td>63.1 ± 3.1 c</td>
<td>76.8 ± 2.2 b</td>
<td>0.26 ± 0.00 c</td>
<td>2.12 ± 0.02 c</td>
<td>0.23 ± 0.01 c</td>
</tr>
<tr>
<td>1 M CAHT</td>
<td>1.60 ± 0.12 × 10⁶ d</td>
<td>43.2 ± 3.3 d</td>
<td>85.0 ± 3.3 a</td>
<td>0.13 ± 0.00 d</td>
<td>1.29 ± 0.04 d</td>
<td>0.14 ± 0.00 d</td>
</tr>
</tbody>
</table>

CAHT: citric acid-heat treated starch; SVg: specific volume for gyration; PDI: polydispersity index; slope: logarithmic slope between \( R_g \) and \( M_w \).

Average of triplicate measurements, \( n = 3 \), ± means standard deviation.

Values in the same column with different letters are significantly different (\( P < 0.05 \)).

**Fig. 1.** X-ray diffraction patterns of native and modified starches.
and remained unchanged. The modified starch samples displayed decreased $T_o$, $T_p$, $T_c$, and $\Delta H$, which could be attributed to the high moisture hydrothermal treatment. In that condition, the starch was almost completely converted from the ordered to the disordered form and starch granules were disrupted to form a more compacted crystalline matrix in starch consists with higher resistant digestion property. However, in our study, the CAHT starches with lower relative crystallinity obtained higher resistant digestion property, which suggested that the increasing RS content may be attributed to the formation of cross-linked structure which limited starch chain mobility, rather than the crystalline structure. Furthermore, as the acid concentration increased, the intensities of diffraction peaks at 5.6° decreased as compared with the control sample, which might be due to the formation of more compacted chain structure during the reaction.

### 3.4. Thermal properties of native and modified starch samples

The gelatinization parameters [onset ($T_o$), peak ($T_p$), and conclusion ($T_c$) temperatures] and gelatinization enthalpy ($\Delta H$) of the native and CAHT starches were measured to determine the thermal properties of the CAHT starches (Table 3). All the modified starch samples displayed decreased $T_o$, $T_p$, $T_c$, and $\Delta H$, which could be attributed to the high moisture hydrothermal treatment. In that condition, the starch was almost completely converted from the ordered to the disordered form and starch granules were disrupted by the high moisture content during the hydrothermal treatment (C. J. Lee et al., 2011). When compared with the control, $\Delta H$ in CAHT samples decreased with the increasing reaction intensity, whereas the $T_o$ and $T_c$ remained unchanged. The $\Delta H$ value represents the number of double helices which constitutes the crystalline region that unravel and melt during gelatinization (Cooke & Gidley, 1992). The decrease in $\Delta H$ was due to the citrate substitution that altered the chain packing and generated more amorphous region which can be proved by the decreased values of $R(1047/1022)$.

### Table 3

<table>
<thead>
<tr>
<th>Samples</th>
<th>$T_o$ ($^\circ$C)</th>
<th>$T_p$ ($^\circ$C)</th>
<th>$T_c$ ($^\circ$C)</th>
<th>$\Delta H$ ($kJ/g$)</th>
<th>$R(1047/1022)$</th>
<th>$X_c$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>63.91 ± 0.16 a</td>
<td>68.30 ± 0.01 a</td>
<td>71.58 ± 0.39 a</td>
<td>8.70 ± 0.56 a</td>
<td>0.86</td>
<td>21.16</td>
</tr>
<tr>
<td>Control</td>
<td>49.24 ± 0.37 c</td>
<td>56.24 ± 0.59 b</td>
<td>62.71 ± 0.43 b</td>
<td>4.30 ± 0.12 b</td>
<td>0.83</td>
<td>13.33</td>
</tr>
<tr>
<td>0.1 M CAHT</td>
<td>51.00 ± 0.01 c</td>
<td>56.54 ± 0.13 b</td>
<td>62.01 ± 0.08 b</td>
<td>5.32 ± 0.05 c</td>
<td>0.83</td>
<td>13.29</td>
</tr>
<tr>
<td>0.5 M CAHT</td>
<td>51.98 ± 0.26 c</td>
<td>57.13 ± 0.00 b</td>
<td>63.45 ± 0.05 b</td>
<td>3.52 ± 0.05 c</td>
<td>0.82</td>
<td>9.92</td>
</tr>
<tr>
<td>1 M CAHT</td>
<td>53.82 ± 0.33 b</td>
<td>57.64 ± 0.71 b</td>
<td>63.23 ± 0.04 b</td>
<td>0.48 ± 0.02 e</td>
<td>0.80</td>
<td>8.37</td>
</tr>
</tbody>
</table>

CAHT: citric acid-heat treated starch; $T_o$: onset temperature; $T_p$: peak temperature; $T_c$: conclusion temperature; $\Delta H$: enthalpy; $R$: ratio of 1047 cm$^{-1}$/1022 cm$^{-1}$; $X_c$: relative crystallinity.

Average of triplicate measurements, $n$ = 3, $t$ means standard deviation. Values in the same column with different letters are significantly different ($P < 0.05$).
and $X_c$ in Table 3. $T_o$ represents the melting temperature of the weakest crystallites (Larsson & Eliasson, 1991; Nakazawa & Wang, 2003). The increased $T_o$ in 1 M CAHT sample, which corresponded with the increased acid concentration compared with the 0.1 M and 0.5 M CAHT samples, may be attributed to the fact that the new formed double helices were affected by the more compacted chain structure which altered the chain rearrangement in amorphous phase. Gunaratne and Hoover (2002) also reported that the melting temperature of starch was controlled indirectly by the surrounding amorphous region, and the reduction in granular swelling during heat treatment would enhance the stabilization effect of the amorphous region on crystallite melting.

3.5. FT-IR spectroscopy of native and modified starch samples

The FT-IR spectrum of starch has been shown to be sensitive to changes in structure on a molecular level (short-range order), such as starch chain conformation, crystallinity and retrogradation (van Soest, Tournois, de Wit, & Vliegenthart, 1995). The IR absorbance bands at 1047 cm$^{-1}$ and 1022 cm$^{-1}$ were the characteristic bands of crystalline and amorphous structure in starch, respectively. The ratio of intensity of 1047 cm$^{-1}$/1022 cm$^{-1}$ can express the degree of order in starch (Capron, Robert, Colonna, Brogly, & Planchot, 2007; van Soest et al., 1995). The ratios of the absorbance between the bands at 1047 cm$^{-1}$ and 1022 cm$^{-1}$ of different starch samples were presented in Table 3. With an increase in acid concentration, the ratio of 1047 cm$^{-1}$/1022 cm$^{-1}$ decreased while the degree of substitution increased (Table 1), indicating that the citrate substitution altered chain packing and generated more amorphous regions. Similar results obtained from the X-ray diffraction data (Fig. 1), in which the CAHT exhibited greater amorphous content as the reaction intensity increased. Xie, Liu, and Cui (2006) also reported that the ratio of 1047 cm$^{-1}$/1022 cm$^{-1}$ of citric starch decreased with an increase of reaction time in a low moisture content condition.

A new peak at 1724 cm$^{-1}$ appeared in all CAHT samples, but this was not observed in their controls (Fig. 2). The band at 1724 cm$^{-1}$ was associated with the stretching vibration of the C=O bond from the acetyl group. The peak signal was very weak in the 0.1 M CAHT sample, however, with an increase of acid concentration, the intensity became stronger, which was consistent with the degree of substitution and the digestibility of the CAHT samples (Table 1). The results indicated that the ester was bonded with the starch chains and formed the cross-linking structure.

3.6. Microscopic observations of native and modified starch samples

The granular structures of native and CAHT starches exhibited remarkable differences in their shape when observed under the SEM (Fig. 3). The granules of native maize starch were smooth, irregular shaped and had a few pores on the surface (Fig. 3a). However, the control and CAHT samples showed bigger, more irregular and disrupted structure compared to the native maize starch (Fig. 3b-h), which may be due to the destruction of granular structure of the native starch during heating and recrystallization during storage at 4°C for 24 h. Lee et al. (2011) also reported that the potato starch exhibited the aggregation of granules and a distorted granular shape after 40% moisture heat treatment.

With the citric acid concentration increased, the surface of the starch samples became more irregular, rougher and appeared to contain more small hollows. As previously discussed in HPSEC analysis, after citric acid-heat treated, the CAHT samples consisted of two fractions, one for the hydrolyzed amylopectin and one for the combination of hydrolyzed amylopectin and amylose, which were both highly compacted. After being dried and stored at 4°C, these two fractions would combine with each other and generate larger particles compared with the native starch (Fig. 3). As the acid concentration reached 1 M, lots of small globules formed on the surface of the starch granule, which could be attribute to the fact that some small fragments which consisted by the highly cross-linked hydrolyzed chains (components of the peak 1 and peak 2 in HPSEC analysis) would not combined with each other and remained outside.
4. Conclusion

Heat treatment combined with the citric acid hydrolysis increased the RS content (up to 35.62% after cooked) of normal maize starch. Amylose showed a better stability during citric acid–heat treatment as compared with that of amylepectin at low acid concentration. The citrate substitute could bond with the starch chains and form cross-linking structure in CAHT samples, which was confirmed by FT-IR analysis and DS parameters. With the reduction of relative crystallinity in CAHT samples, the resistant digestion property was enhanced, indicating that the increasing RS content in CAHT samples might be ascribed to the formation of cross-linked structure, rather than the crystalline structure. Therefore, the citric acid–heat treatment is a potential way to increase RS content.

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References


