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Research Article

Structural and Physicochemical Characteristics of Granular Malic Acid-Treated Sweet Potato Starch Containing Heat-Stable Resistant Starch

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This study investigated the structural and physicochemical characteristics of malic acid-treated sweet potato starch. Sweet potato starch mixed with various concentrations of malic acid solution underwent either thermal or nonthermal treatment. Observation of samples under a light microscope ensured the maintenance of granular shape and the Maltese cross. FT-IR spectra displayed a distinct carbonyl peak at 1722 cm^{-1}, and analysis of the degree of substitution (DS) indicated an increase in the extent of ester bonds with increasing concentrations of malic acid. The DS of 2.0M-130 (0.214) was the highest and that of 0.5M-130 was the lowest (0.088) among the reacted starches. In vitro digestion test revealed an increased amount of resistant starch when a high concentration of malic acid was used. In addition, thermally treated samples maintained a higher content of resistant starch (RS) after 30 min of cooking at 100°C. After cooking, 2.0M-130 had an RS fraction of 53.4% which was reduced to 49.9% after cooking, revealing greater heat stability compared with nonthermally treated samples. The structure of malic acid-treated starch was investigated using a differential scanning calorimeter (DSC), an X-ray diffractometer, a rapid visco analyzer (RVA), and analysis of apparent amylose content. The results showed that thermal and malic acid treatment of starch caused not only partial hydrolysis but also rearrangement of the crystalline area and helix structure of starch by esterification. Analysis of malic acid-treated starch, using a rapid visco analyzer showed no pasting properties, due to lack of its swelling caused by the malic acid cross link.

1. Introduction

Starch is used in many kinds of food and serves as a major source of energy for humans. For nutritional purposes, starch can be classified into three categories based on the rate of its enzymatic digestion: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) [1]. RS is also defined as the sum of starch and degraded starch products that resist digestion in the small intestine of healthy people [2]. The RS content of starch is affected by the amylose/amylopectin ratio, physical form, degree of gelatinization, storage, and thermal treatment [3, 4]. Depending on the cause of resistance, RS can be further divided into five categories [5, 6]: RS1, physically inaccessible starch due to entrapment; RS2, raw starch granules with crystallinity; RS3, retrograded starch; RS4, chemically modified starch; and RS5, amylose-lipid complexes. Chemical modification has been known to not only raise in vitro digestion resistance of starches, reducing postprandial glucose and insulin concentration, but also maintain sensory attributes of the final foods [7]. Degradation of RS in the large intestine has physiological

benefits, including the stimulation of intestinal bacterial activity by prebiotic effect leading to the microbial fermentation and production of short-chain fatty acids, which eventually decrease the pH in colon [6]. Upadhyaya, et al. [8] had reported that consumption of RS4 led to an abundance of *Bifidobacterium adolescentis*, *Parabacteroides distasonis*, and *Christensenella minuta* in the gut. In addition, RS also has health beneficial effects such as prevention of colon cancer, hypoglycemic effects, hypocholesterolemic effects, and inhibition of fat accumulation [9, 10].

Polyfunctional carboxylic acids such as malic, tartaric, citric, and glutaric acid have been used in the synthesis and rheological characterization of hydrogels [11]. Xie and Liu [12] used citric acid and high temperature to increase the RS content of corn starch. Compared to inorganic acids, citric acid is nutritionally harmless, and increasing the degree of substitution (DS) of starch by ester bond with the citrate decreased the rate of digestion by pancreatin [9, 13]. Kim et al. [14] reported that glutaric acid treatment at 115°C affected the structural and digestion properties of adley starch but lost the Maltese cross pattern in its granule. Studies on citric acid-rice starch by Shin et al. [15] showed that acid treatment hydrolyzed the branching points of amylopectin, leading to an increased apparent amylose content. According to Kim and Shin [9], a structural difference, such as in the number of carboxyl groups, can affect the physicochemical properties of starch. However, the cross-linking capability of malic acid, in reference to starch, has not yet been reported. Malic acid is a C4 carboxylic acid with two carboxyl groups, comprising 69%–92% of all organic acids in grape berries and leaves [16]. It is also naturally produced by many organisms without showing any nutritional harm. The U.S. Food and Drug Administration classifies malic acid as "generally recognized as safe (GRAS)," and Food Chemicals Codex (FCC) specifications list DL-malic acid as a food-grade organic acid [17]. In industries, Malic acid has been used as food additive in the United States and European Union.

Sweet potato, *Ipomoea batatas*, is a creeping dicotyledonous plant belonging to the Convolvulaceae family. Sweet potato has been a traditional source of starch in Asian countries and is one of the world's most important food crops used as an ingredient in various products such as noodles, breads, and cakes [18]. The potential supply of sweet potato starch is exhaustive and cost-efficient. In addition, its components such as dietary fiber, carotenoids, vitamins, and minerals are health beneficial [19]. Therefore, industrial interest is highly focused on the use of native and modified sweet potato starch. Consequently, extensive research regarding the chemical/physical/enzymatic modifications of sweet potato starch should be performed to deepen the understanding of its functional properties.

The purposes of this study were to produce sweet potato starch with low digestion property and heat stability by malic acid treatment as well as to assess its physicochemical properties, maintaining granule shape.

2. Materials and Methods

2.1. Materials. Sweet potato starch was purchased from Seoahn Co. (Buan, Jeollabuk-do, Korea) and DL-malic acid (M1210) was from Sigma Aldrich (St. Louis, MO, USA). The enzymes used in starch digestion were porcine pancreatin (P7545, activity 8 × USP/g, Sigma) and amyloglucosidase (AMG 300L, activity 300 AGU/mL, Novozymes, Bagsvaerd, Denmark).

2.2. Preparation of Malic Acid-Treated Starch. DL-malic acid was dissolved in water to prepare solutions of various concentrations (0.5, 1.0, 1.5, and 2.0 M) with pH adjusted to 3.5 by 10 M NaOH. Sweet potato starch (20 g) and 20 mL of different concentrations of malic acid solution were mixed and kept in a stainless-steel bowl for 16 h at room temperature. The bowls were then placed in an air-drying oven at 50°C for 24 h. The dried mixture was ground and placed either in an air-drying oven at 130°C or at room temperature for 12 h. It was washed thoroughly with distilled water to remove unreacted malic acid, dried in an air drying oven at 50°C, and ground. Samples were named according to their processing condition in the concentration-temperature format. A starch sample, which underwent the same procedure with distilled water (DW-130) and with 2.0 M malic acid solution at 25°C for 16 h (2M-25), was used as the control.

2.3. Optical Microscopy. Malic acid-treated starches were observed under a light microscope (CSB-HP3, Sam Won Scientific, Seoul, Korea) with and without a polarizing plate. Glycerol was used to disperse the sample on a glass slide with minimal air bubbles. A digital camera (Nikon, Tokyo, Japan) was used to take the photographs.

2.4. Fourier Transform-Infrared Spectroscopy (FT-IR). FT-IR spectra (VERTEX80v; Bruker, Billerica, MA, USA) were used to obtain the IR spectra. The spectra were measured ranging from 4000 to 600 cm^{-1}, in the transmission mode, at a resolution of 4 cm^{-1} and normalized using the 1315 cm^{-1} peak of starch CH_2 vibrations. The samples were diluted with KBr (1 : 100, v/v) before acquisition.

2.5. Degree of Substitution (DS). Degree of substitution was determined to estimate the average number of hydroxyl groups substituted with malic acid per anhydroglucose unit in starch. The measurement was performed following the method of by Xu et al. [20], with some modifications. Malic acid-treated starch (0.5 g) was placed in a 250 mL glass beaker with 50 mL of distilled water. The pH was measured after stirring the mix for 1 h at 30°C. To each beaker, 25 mL of 0.5 *N* NaOH was added to release the substituted groups from the malic acid-treated starch, and the solution was stirred for 24 h at 50°C. The excess NaOH was titrated back to original pH with 0.05 *N* HCl. DS was calculated as follows:

$$\mathrm{DS} = \frac{162 \times \left(N_{\mathrm{NaOH}} \times V_{\mathrm{NaOH}} - N_{\mathrm{HCl}} \times V_{\mathrm{HCl}}\right)}{1{,}000 \times W - 116.09 \times \left(N_{\mathrm{NaOH}} \times V_{\mathrm{NaOH}} - N_{\mathrm{HCl}} \times V_{\mathrm{HCl}}\right)}, \quad (1)$$

where DS is the degree of substitution, W is the sample weight (g), N_{NaOH} is the normality of NaOH, V_{NaOH} is the volume of NaOH, N_{HCl} is the normality of HCl, 161 is glucose molecular weight, 116.09 is malic acid molecular weight used to back titrate, and V_{HCl} is the volume of HCl used for back titration.

2.6. In Vitro Digestibility. In vitro digestibility was measured following the method of Englyst et al. [1] with slight modifications by Shin et al. [15]. To prepare enzyme solutions, porcine pancreatin (2 g) was added to 24 mL distilled water in a 50 mL glass beaker and stirred for 10 min. The solution was then centrifuged at 1500 × g, for 10 min at 4°C, to obtain a cloudy supernatant. The supernatant (20 mL) was mixed with 0.4 mL of amyloglucosidase and 3.6 mL of distilled water. To a 2 mL microtube with 30 mg of starch sample, 0.75 mL of sodium acetate buffer (0.1 M, pH 5.2) and a glass bead were added and either cooked for 30 min or not cooked at all. After cooling the tube to 37°C, 0.75 mL of the enzyme solution was added and incubated in a shaking incubator (240 rpm). The tubes were taken out after 10 and 240 min, boiled for 10 min in a heating block to stop the reaction, and cooled to room temperature. The tubes were centrifuged at 5000 × g, for 10 min at 4°C. The amount of glucose in the supernatant was measured by the GOD-POD kit (Embiel Co., Gunpo, Korea). The amount of glucose after 10 min of enzyme reaction at 37°C indicated RDS and that obtained after incubation for 10–240 min was SDS. RS was the starch not hydrolyzed after 240 min of incubation.

2.7. X-Ray Diffraction. X-ray diffraction analysis was performed using an X-ray diffractometer (D8 ADVANCE with DAVINCI, Bruker, Karlsruhe, Germany) operating at 40 kV and 40 mA producing CuK_{α} radiation of 1.5418 Å wavelength, scanning through the 2θ range of 3–30°, and having a step time of 0.5 sec. The relative crystallinity was calculated using the software developed by the instrument manufacturer (EVA, 2.0).

2.8. Thermal Properties. Thermal properties were determined using a differential scanning calorimeter (Pyris Diamond DSC, Perkin-Elmer, Waltham, MA, USA). Distilled water (40 μL) was added to 10 mg of the sample in a stainless-steel DSC pan and sealed. The pan was kept at room temperature for more than 4 h for equilibration and uniform mixing. The sample pan was heated gradually from 30°C to 130°C at 5°C/min with an empty pan as the reference. To avoid condensation during the scan, dry nitrogen was flushed in the space surrounding the sample chamber. Onset (T_o), peak (T_p), and conclusion (T_c) temperatures, as well as gelatinization enthalpies (ΔH), were measured using the Pyris software.

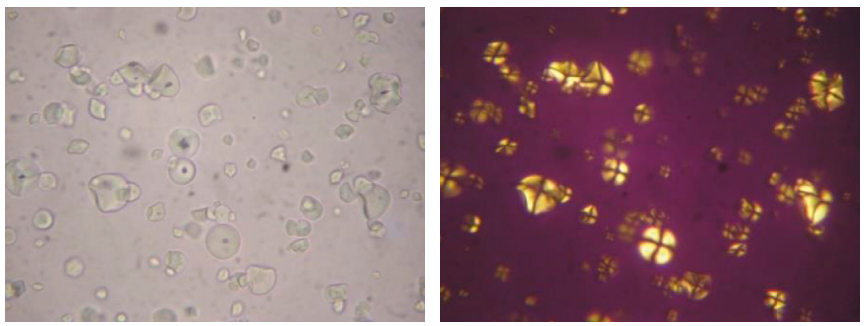
2.9. Apparent Amylose Content. Apparent amylose content was measured according to the colorimetric method outlined by the AACC International Approved Method 61–03 [21]. The samples (20 mg) were precisely weighed in 15 mL tubes and dispersed with 200 μL of absolute ethanol. The tubes were boiled for 10 min after adding 1.8 mL of 50% NaOH. The cooled solution (1 mL) was placed in each tube with 9 mL of distilled water. Diluted sample solutions were put into a 15 mL tube containing 9 mL of distilled water and 100 μL of 1 N acetic acid. Lugol solution (200 μL; 0.2% I_2 + 2.0% KI, Sigma) was added and kept in dark for 20 min. Absorbance of the colored sample solution was measured at 620 nm.

2.10. Pasting Properties. A Rapid Visco Analyzer (RVA-3D, Newport Scientific, Warriewood, Australia) was used to investigate the pasting properties of sweet potato starch and malic acid-treated starch. For each analysis, 2.5 g of starch was added to an RVA canister with 25 mL of distilled water. The measurement followed the AACC standard method 2, which includes a 23 min heating and cooling profile.

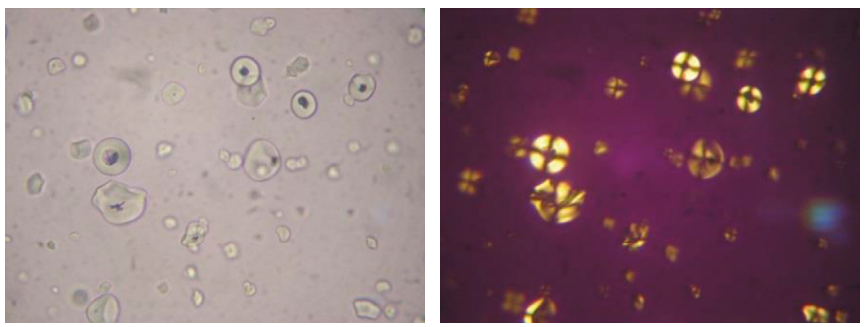
2.11. Statistical Analyses. All experiments were triplicated, and the mean values and standard deviations are reported. Duncan's multiple range test was used to analyze the variance and the mean separations ($p < 0.05$). The statistical analyses were conducted using SPSS for Windows 22.0 software (IBM, Armonk, NY, USA).

3. Results and Discussion

3.1. Photomicrographs. The granular shape of the various malic acid-treated starch samples was investigated using a light microscope (Figure 1). The shape of starches including the malic acid-treated samples had round, semioval, oval spherical, and round polygonal shapes, which was consistent with preivious studies [19, 22]. The malic acid-treated starch granules were not ruptured even at malic acid concentrations as high as 2.0 M. According to Hirashima et al. [23], granules of starch were all broken at pH below 3.0, when more glucose chains were observed, compared to those in higher pH-treated samples. However, in the range of pH 4.0–6.0, the granule shape was retained while at pH above 3.5, less glucose chains leached out, and less fracture of starch granules occurred [23]. Beyond pH 3.5, the granular shape of the malic acid-treated starches was not ruptured, despite the high concentration of malic acid. The Maltese cross was observed using a polarizing plate, confirming the inner ordered semicrystalline structure and radially ordered alignment of amylose and amylopectin [14, 24]. All samples showed the Maltese cross, implying that the regular ordered inner structure of starch was mostly retained even after the thermal treatment in the presence of malic acid. Therefore, it



(a)



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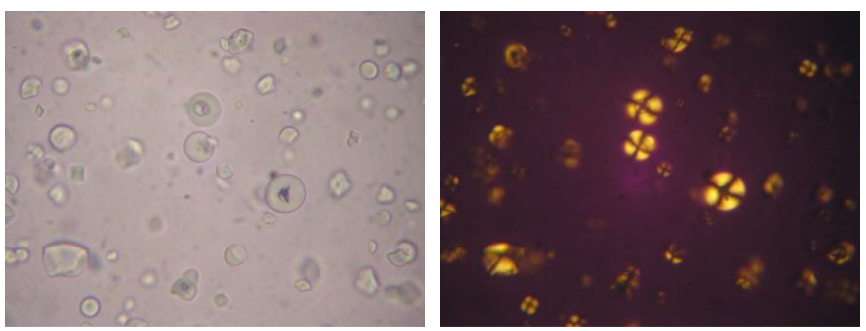

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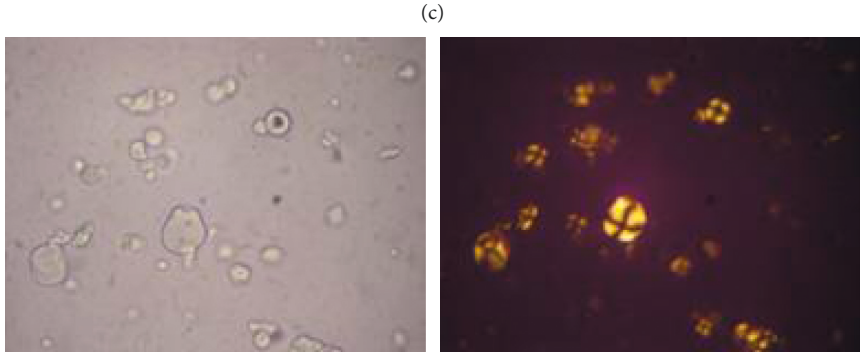

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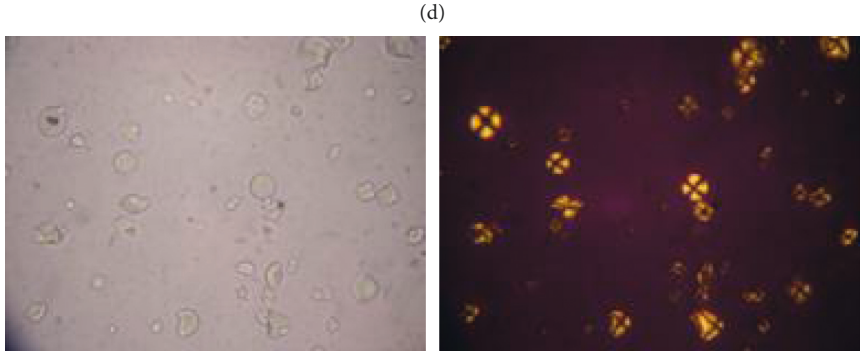

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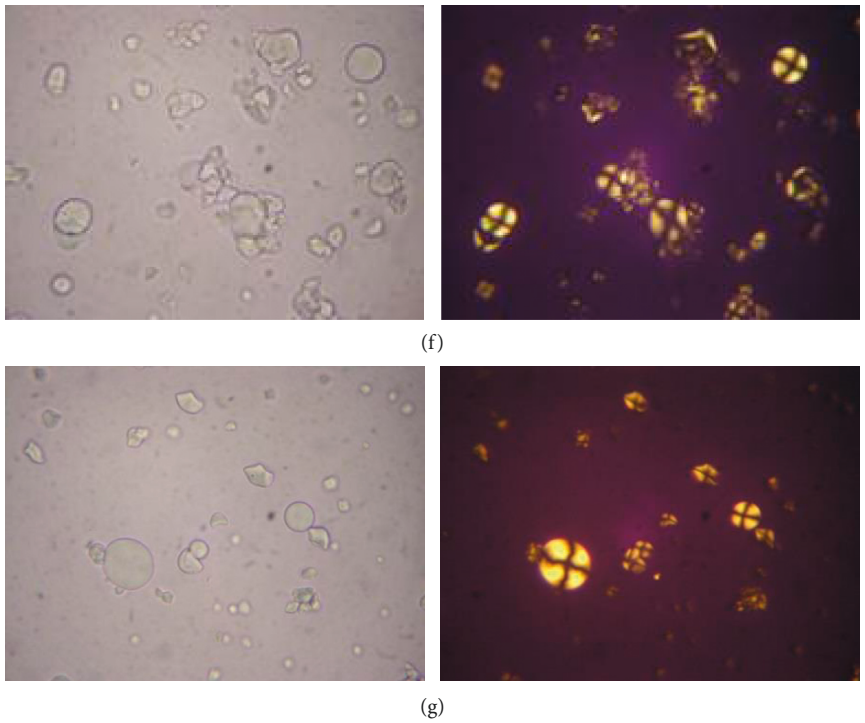

Figure 1: Light micrographs of raw and malic acid-treated sweet potato starches (magnification 400×): (a) raw, (b) 2.0M-25, (c) DW-130, (d) 0.5M-130, (e) 1.0M-130, (f) 1.5M-130, and (g) 2.0M-130; 1-under visible light and 2-under polarized light.

could be assumed that the changes observed were caused not by the change of granular shape but by the effect of malic acid on the inner structure of starch.

3.2. FT-IR. FT-IR spectroscopy can determine the structural characteristics, in terms of the functional groups in malic acid-treated starches. The peak in the range of 3000 to 3500 cm^{-1} indicates the presence of hydroxyl groups in starch, and the one at 2930 cm^{-1} indicates C-H bond stretching [25]. The peak between 980 and 1200 cm^{-1} can be attributed to C-O stretching vibration [26]. The peak near 1730 cm^{-1} is a carbonyl peak [14, 27], and the starches that reacted with malic acid at 130°C commonly had a remarkable carbonyl peak at 1722 cm^{-1} regardless of malic acid concentration (Figure 2). However, the starches kept at room temperature for 12 h, or at 130°C without malic acid, had no carbonyl peak. These results could be explained by the destruction of some parts of inter- and intramolecular hydrogen bonds by heat, thereby leading to the formation of an ester bond between starch and malic acid. The peak intensity of thermally treated samples with a higher concentration of malic acid was higher compared with those with low concentration of malic acid as shown in Figure 2. This intensity appeared to be highly correlated to both degrees of substitution and RS content of malic acid-treated starch. As the peak intensity of samples increased, the content of RS also gradually increased ($p < 0.05$). The RS content of DW-130 and 2.0M-130 were 27.0% and 53.4%, respectively. This result was consistent with the report on glutarate-treated starch by Kim et al. [14].

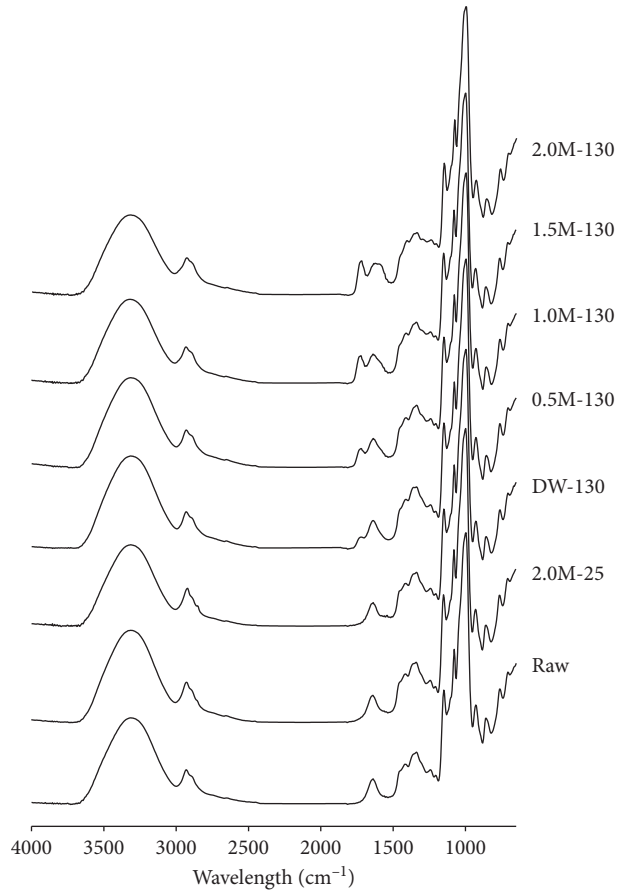

Figure 2: FT-IR spectra of raw and malic acid-treated sweet potato starches.

Table 1: Degree of substitution and in vitro digestibility of raw and malic acid-treated sweet potato starches.

Sample	DS	Noncooked starch			Cooked starch		
		RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)
Raw	n/d	14.5 ± 1.20^{a}	52.9 ± 4.20^{d}	32.6 ± 3.07^{bc}	59.6 ± 5.90^{c}	19.8 ± 2.45^{d}	20.6 ± 3.52^{a}
2.0M-25	n/d	15.5 ± 0.25^{a}	55.2 ± 2.41^{d}	29.3 ± 2.24^{ab}	67.9 ± 3.38^{de}	13.7 ± 4.04^{c}	18.4 ± 0.80^{a}
DW-130	n/d	21.1 ± 0.73^{b}	51.8 ± 1.76^{d}	27.0 ± 1.79^{a}	71.8 ± 3.71^{e}	8.67 ± 3.65^{b}	19.5 ± 1.16^{a}
0.5M-130	0.088 ± 0.0068^{a}	36.4 ± 1.53^{c}	28.7 ± 1.19^{c}	34.9 ± 1.51^{c}	69.9 ± 1.79^{e}	3.57 ± 2.65^{ab}	26.6 ± 4.27^{b}
1.0M-130	0.127 ± 0.0094^{b}	48.5 ± 2.73^{e}	12.8 ± 0.87^{b}	38.7 ± 1.86^{d}	64.0 ± 0.31^{cd}	1.83 ± 0.69^{a}	34.2 ± 0.96^{c}
1.5M-130	0.184 ± 0.0058^{c}	46.9 ± 1.16^{e}	6.63 ± 1.86^{a}	46.5 ± 2.83^{e}	52.8 ± 1.55^{b}	3.87 ± 2.61^{ab}	43.3 ± 1.93^{d}
2.0M-130	0.214 ± 0.0029^{d}	41.9 ± 0.74^{d}	4.72 ± 0.70^{a}	53.4 ± 0.69^{f}	45.2 ± 0.84^{a}	4.86 ± 2.39^{ab}	49.9 ± 2.57^{e}

DS, degree of substitution; RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch; n/d, not detected. The values with different superscripts in each column are significantly different ($p < 0.05$) by Duncan's multiple range test.

3.3. Degree of Substitution (DS). The DS of thermally treated samples is shown in Table 1. The value increased with the concentration of malic acid. While 2.0M-130 had the highest DS value of 0.214, 0.5M-130 had the lowest DS (0.088) among the malic acid-treated starches. This substitution level was much higher than that in citric acid-substituted starches (0.027), reacted at 128.4°C for 13.8 h [15]. DS is generally related to the number of carboxyl groups in organic acids and the steric hindrance between them, which can interrupt the ester bond formation. For example, acetic acid, which has only one carboxylic group and smaller molecular size compared to other organic acids, has the ability to reach a high substitution level [20]. Malic acid, which has two carboxyl groups, can theoretically form two ester bonds, whereas citric acid can make three ester bonds.

3.4. In Vitro Digestion of Samples. Table 1 presents the proportion of RDS, SDS, and RS fractions. The concentration gradient of malic acid without thermal treatment showed no significant difference in RS content (data not shown). Regarding the digestibility of DW-130, raw, and 2.0M-25, there were no significant differences between RS and SDS content, but a difference was observed in RDS, which could have been due to the high heat treatment. On the other hand, thermal treatment along with malic acid increased the content of RS. Thermally and malic acid-treated starch showed an increased RS content from 27.0% (DW-130) to 53.4% (2.0M-130). The DS of substituted starches and RS fractions were highly correlated ($r = 0.967$, $p < 0.01$). In addition, malic acid and heat treatment decreased SDS from 51.8% (DW-130) to 4.72% (2.0M-130) depending on the concentration of the malic acid solution. However, the content of RDS was the highest in 1.0M-130 (48.5%) and decreased with increasing concentrations of malic acid. This result suggested that some parts of the amylose and amylopectin structure were destroyed under acidic heating conditions so the RDS fraction increased until 1.0M-130 but the other part forming ester bonds with malic acid became the RS fraction. According to Huber & BeMiller [28], the more the cross-linked starch, the more the entry of α-amylase molecules through the starch porous channel inhibited, and hence, more resistance to digestion. Therefore, as the DS of malic acid-treated starch increased, intrusion of α-amylase was inhibited by the ester bond between malic acid and starch chain. However, if the cross link fully blocked α-amylase intrusion, there should be no increase of RDS. The interference of cross links in the complexation of α-amylase and starch might be another reason for the increased RS [9, 29]. Despite the diffusion of α-amylase after granule intrusion, the cross-linked part of starch chain resists digestion.

Since the starch used in food industry usually undergoes a cooking step, high heat treatment could possibly destroy the ester bond, thereby decreasing RS. Digestion fractions of cooked samples were also investigated. Apart from the nonreacted starches that showed considerable decrease in RS content, 2.0M-130 had 49.9% of RS fraction, which reduced from 53.4%. Other malic acid-treated starches also had decreased RS content but they were still highly correlated with the DS ($r = 0.983$, $p < 0.01$). The proportion of the RS fraction of DW-130 samples decreased from 27.0% to 19.5% upon cooking. The cooking procedure changed the RS proportions drastically from that of the nonthermally treated samples, whereas the thermally malic acid-treated samples had high content of heat-stable RS in high amount, which indicates the presence of a rigid ester bond.

3.5. X-Ray Diffraction. The X-ray diffraction patterns are shown in Figure 3. The internal order of a starch granule is demonstrated by X-ray diffraction patterns of *A*, *B*, and *C* types [30]. Native sweet potato starch showed the *C*-type pattern with diffraction intensities at 5.6°, 11.5°, 15.3°, 17.4°, 23.2°, and 26.3° at the angle 2θ. *C*-type starches have been subclassified into C_a, C_b, and C_c on the basis of their resemblance to either A-type, B-type, or that between A-type and B-type, respectively [31], and the sweet potato starch used in this study belonged to the C_b-type. Malic acid-treated starches maintained the same X-ray diffractograms, but with differences in crystallinity and peak intensity. As the concentration of malic acid solution increased, the peak intensity and crystallinity decreased (DW-130; 27.9%, 2M-130; 17.6%), indicating the influence of heat-moisture treatment and the possibility of acid hydrolysis. The decreased crystallinity of DW-130 was seemed to be caused by the heat-moisture treatment. Lee et al. [32] reported that hydrothermal treatment decreased the major peaks' intensities and their crystallinity. The crystallinity of malic acid-treated samples was lower than that of raw starch

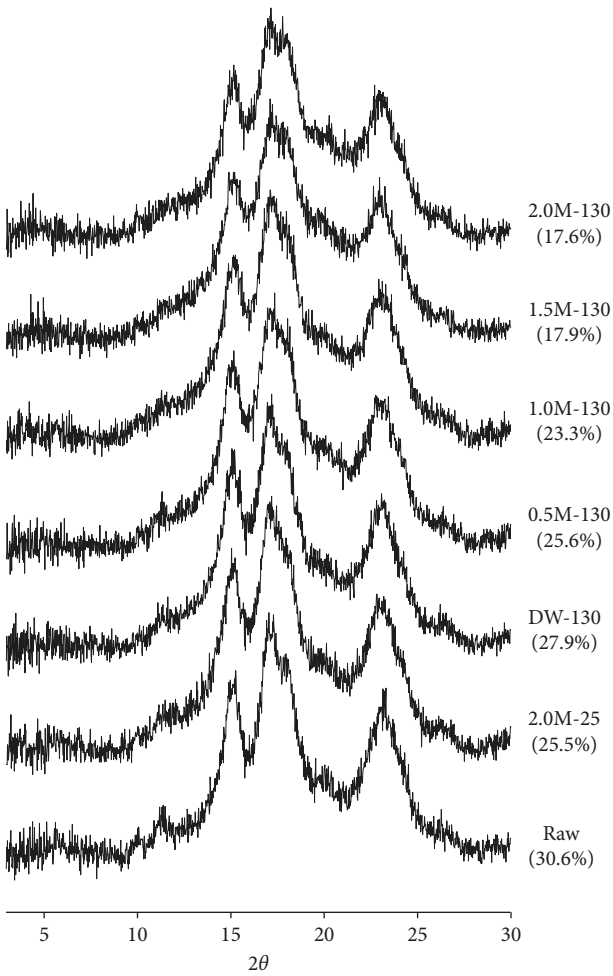

Figure 3: X-ray diffraction patterns and relative crystallinity of raw and malic acid-treated sweet potato starches. Numbers in parentheses indicate the percentage of crystallinity.

due to acid hydrolysis and heat treatment during preparation. This result was consistent with the report on glutarate-treated starch by Kim et al. [14] and citrate-treated starch by Xie et al. [33]. Hydrogen bonds are known to sustain a double helical structure, but when substituted by ester bonds, changes in double helical structure induce rearrangement of the crystalline and semicrystalline structure, hence lowering relative crystallinity.

3.6. Gelatinization Parameters. The gelatinization parameters of various malic acid-treated starches are shown in Table 2. Raw and 2.0M-25 samples had no significant difference in the T_o, T_p, T_c, ΔH, and even $T_c - T_o$. However, DW-130 had lower T_o (57.7), T_p (66.6), T_c (71.3), and $T_c - T_o$ (14.9), and higher ΔH (13.6), which could have resulted from the heat-moisture treatment. Increasing the concentration of malic acid decreased T_o, T_p, T_c, and ΔH, but increased $T_c - T_o$. Consequently, 2.0M-130 had lower T_o (44.0), T_p (50.8), T_c (62.0), and ΔH (2.89) and higher $T_c - T_o$ (18.1) compared with other samples. DSC measures the primary hydrogen bonds that stabilize the double helices within the granules [34] and the quality and quantity of crystalline area by measuring the change in heat energy [30]. The decreased T_o, T_p, T_c, ΔH, and increased $T_c - T_o$, in this study, suggested that the internal crystalline structure and helical structure of the malic acid-treated starch could have been disrupted and become a heterogeneous structure with rearrangement compared to the unmodified starches. If most of the crystalline area was destroyed, no peak would be expected in the X-ray diffractogram; however, the X-ray diffraction patterns of malic acid-treated starch were almost the same as those of raw starch and nonthermally treated starches. Therefore, malic acid, which penetrated into starch granules, may have not only partially hydrolyzed the starch chain into shorter chains, but also rearranged the crystalline structure of the granules by substituting the hydrogen bonds with ester bonds. With increased short chain, melting temperature decreased, which could be highly related to the decrease of SDS and increase of RDS. Decrease of ΔH corresponds to a reduced amount of hydrogen bonds and is related to increased DS and a higher fraction of heat-stable RS.

3.7. Apparent Amylose Content. The apparent amylose contents of various malic acid-treated starches are presented in Table 3. Compared to the raw and nonthermal group, samples with high DS values showed decreased apparent amylose content. Apparent amylose contents of 2.0M-25 and DW-130 showed no significant difference with raw starch ($p > 0.05$). However, the increasing concentration of malic acid with heating decreased the apparent amylose content of the samples and that of 2.0M-130 was the lowest (20.7%). The citric acid-treated starch had increased amount of apparent amylose content with the same treatment because of the hydrolysis, which mainly occurred at the branching point [15], but the opposite trend was observed in this study. Mussulman and Wagoner [35] and Robin et al. [36] suggested that acid hydrolysis mainly occurred during the conditioning step. However, structural analyses showed that most of the starch chain breakdown occurred during the heating step under the influence of both acid and heat. Consequently, the decline of apparent amylose content was due to the rearrangement of the starch helix by the ester bond upon malic acid treatment, and there could be increased amount of short chains due to hydrolysis, which are too short to form the iodine complex.

3.8. Pasting Properties. The pasting properties of raw and malic acid-treated starches are shown in Figure 4. The use of thermal treatment with malic acid can lead to lower peak viscosity because of less swollen granules. Cross-linked starches reacted under low pH conditions are reported to have reduced swelling power [37]. Starch samples, which were only conditioned in malic acid solution (2.0M-25), showed a similar RVA curve with raw starch. Raw starch and 2.0M-25 had peak viscosity at 88.3°C and also had similar setback viscosity. As DW-130 indicated changes in structure which seemed to be the effect of slight the heat-moisture treatment, lower peak viscosity, and setback viscosity were detected by RVA, which could also be due to the effect of heat-moisture treatment. Malic acid-treated starches had

Table 2: Gelatinization parameters of raw, control, and malic acid-treated starches.

Sample	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	ΔH (J/g)
Raw	60.5 ± 0.28^{e}	68.2 ± 0.07^{f}	72.4 ± 1.59^{d}	11.9 ± 1.83^{a}	16.1 ± 0.89^{d}
2.0M-25	60.7 ± 0.21^{e}	68.2 ± 0.16^{f}	72.6 ± 0.37^{d}	11.8 ± 0.31^{a}	16.6 ± 0.46^{d}
DW-130	57.7 ± 0.13^{d}	66.6 ± 0.31^{e}	71.3 ± 0.64^{d}	13.6 ± 0.77^{ab}	14.9 ± 0.66^{d}
0.5M-130	52.4 ± 0.19^{c}	63.9 ± 0.25^{d}	68.4 ± 0.26^{c}	16.0 ± 0.33^{bc}	12.1 ± 1.63^{c}
1.0M-130	46.0 ± 0.10^{b}	60.1 ± 0.48^{c}	65.3 ± 0.36^{b}	19.4 ± 0.26^{c}	10.5 ± 1.54^{c}
1.5M-130	45.4 ± 0.73^{ab}	53.0 ± 0.47^{b}	63.5 ± 0.19^{ab}	18.2 ± 0.73^{c}	7.50 ± 1.07^{b}
2.0M-130	44.0 ± 2.04^{a}	50.8 ± 1.02^{a}	62.0 ± 2.46^{a}	18.1 ± 4.34^{c}	2.90 ± 0.72^{a}

T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; $T_c - T_o$, temperature range of crystal melting; ΔH, enthalpy change. The values with different superscripts in each column are significantly different ($p < 0.05$) by Duncan's multiple range test.

Table 3: Apparent amylose content of raw and malic acid-treated sweet potato starches.

Sample	Apparent amylose content (%)
Raw	26.9 ± 0.75^{d}
2.0M-25	26.4 ± 0.59^{cd}
DW-130	26.4 ± 0.13^{cd}
0.5M-130	25.4 ± 0.63^{c}
1.0M-130	23.0 ± 0.68^{b}
1.5M-130	23.6 ± 0.65^{b}
2.0M-130	20.7 ± 0.42^{a}

The values with different superscripts in each column are significantly different ($p < 0.05$) by Duncan's multiple range test.

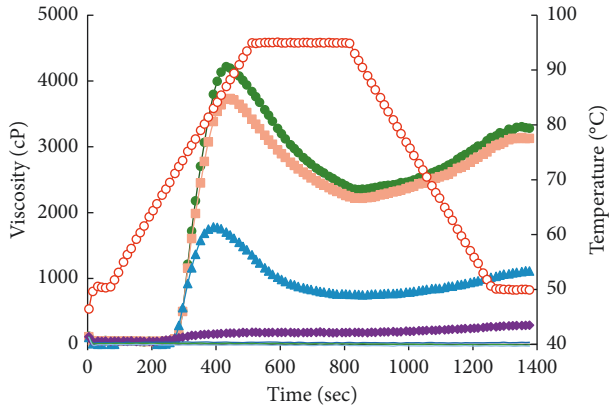

Figure 4: Rapid visco analyzer: raw and malic acid-treated sweet potato starches: ●, raw starch; ■, 2.0M-25; ▲, DW-130; ♦, 0.5M-130; —, 1.0M-130, 1.5M-130, 2.0M-130; ○, temperature.

lower and linear RVA profiles (1.0M-130, 1.5M-130, and 2.0M-130). This result was similar to the dramatically decreased pasting viscosity and linear RVA curve of citric acid-treated starch due to its nonswelling property [12]. The higher the concentration of malic acid solution used, the lesser the viscosity observed. Shukri and Shi [38] had reported that high level of cross-linking inhibits the swelling of starch granules because of less hydrogen bonding between the helical structures in starch. Similar to the previous studies on citrate and acetate starches that reported increased stability of cooked starch as compared to native starch [37], malic acid-treated starch with high DS also showed heat-stability not only toward gelatinization but also toward retrogradation due to the malic acid cross link.

4. Conclusions

Heat treatment with a high concentration of malic acid solution on sweet potato starch caused considerable changes in the internal structure of starch maintaining its granular shape. Moreover, DS values and FT-IR spectra showed ester bond formation between malic acid and the starch. The RS fraction of sweet potato starch drastically increased with the ester bond formation, and these RS fractions had remarkable heat-stable characteristics. Structural analyses by light microscopy, XRD, DSC, RVA, and AAC obviously demonstrated a low degree of hydrolysis in starch chains (even at pH 3.5 of malic acid solution) upon thermal treatment with malic acid and rearrangment of the crystalline area and alterations of the double helix by the substitution of the hydrogen bond by an ester bond. Rapid visco analyzer (RVA) showed no pasting property of malic acid-treated starches due to its nonswell properties caused by the malic acid cross-link. This information about the structural characteristics and heat-stable properties of RS in digestion can be used to develop a low-digestible food ingredient and lead to further application of the study.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

This article is based on the thesis submitted by Chinwoo Kwon as part of the requirements for his master's degree at Seoul National University [39].

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this article.

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