A Thesis for the Degree of Master of Science

Structural and physicochemical characteristics of granular malic acid-treated heat-stable sweet potato starch

사과산 처리로 열 안정성을 증진시킨 입자상 고구마 전분의 구조 및 이화학적 특성

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농학석사학위논문

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by

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ABSTRACT

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 non-thermal 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 bond 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 53.4% RS fraction which was reduced to 49.9% after cooking, revealing greater heat stability compared with non-thermally 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.

Keywords: Sweet potato starch; Malic acid; Heat stability; Resistant starch; Esterification

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INTRODUCTION

Starch is used in many kinds of foods and served 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) (Englyst, Kingman, & Cummings, 1992). RS is also defined as the sum of starch and degraded starch products that resist digestion in the small intestine of healthy people (Goni, GarciaDiz, Manas, & SauraCalixto, 1996). The RS content of starch is affected by the amylose/amylopectin ratio, physical form, degree of gelatinization, storage, and thermal treatment (Sievert & Pomeranz, 1989; Tovar, 1992). Depending on the cause of resistance, RS can be further divided into five categories (Haralampu, 2000; Nugent, 2005): RS1, physically inaccessible starch due to entrapment; RS2, raw starch granules with crystallinity; RS3, retrograded starch; RS4, chemically modified starch; RS5, amylose-lipid complexes. Chemical modification has been known to not only raise *in vitro* digestion resistance of starches, reducing post-prandial glucose and insulin concentration but also maintain sensory attributes of the final foods (Stewart & Zimmer, 2017). 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 (Nugent, 2005). Upadhyaya, et al. (2016) had reported that consumption of RS4 led to an abundance of *Bifidobacterium adolescentis, Parabacteroides distasonis,* and *Christensenella minuta* in gut. In addition, RS also has health beneficial effects such as prevention of colon cancer, hypoglycemic effects, hypocholesterolemic effects, and inhibition of fat accumulation (Kim & Shin, 2011; Sajilata, Singhal, & Kulkarni, 2006).

Polyfunctional carboxylic acids such as malic, tartaric, citric, and glutaric acid have been used in the synthesis and rheological characterization of hydrogels (Seidel, et al., 2001). Xie and Liu (2004) 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 degree of substitution (DS) of starch by ester bond with the citrate decreased the rate of digestion by pancreatin (Kim & Shin, 2011; Klaushofer, Berghofer, & Steyrer, 1978). Kim et al. (2008) 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 (2007) showed that acid treatment hydrolyzed the branching points

of amylopectin, leading to an increased apparent amylose content. According to Kim and Shin (2011), a structural difference, such as in the number of carboxyl groups can affect the physicochemical properties of starch. However, 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 (Lakso & Kliewer, 1975). It is also naturally produced by many organisms without showing any nutritional harm. 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 (Goldberg, Rokem, & Pines, 2006).

Sweet potato, *Ipomoea batatas*, is a creeping dicotyledonous plant belonging to the Convolvulaceae family. Sweet potato has been a traditional source of starch in the 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 (Shin, Kim, Ha, Lee, & Moon, 2005). 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 (Zhu & Wang, 2014). 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.

MATERIALS AND METHODS

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). GOD-POD assay kit was from Embiel Co. (Gunpo, Korea).

2. Methods

2.1. Preparation of malic acid 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 stainless-steel bowl for 16 h at room temperature. The bowls were then placed in a 50°C air drying oven for

24 h. The dried mixture was ground and placed either in a 130°C air drying oven or at room temperature for 12 h. It was washed thoroughly with distilled water to remove unreacted malic acid, dried in a 50°C air drying oven, and ground. Samples were named according to their processing condition in the concentration-temperature format. Starch sample, which underwent the same procedure with distilled water, was used as the control.

2.2. Optical microscopy of malic acid-treated starch

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 bubble. A digital camera (Nikon, Tokyo, Japan) was used to take the photographs.

2.3. Fourier transform-infrared spectroscopy (FT-IR) analysis

FT-IR spectra (VERTEX80v; Bruker, Billerica, MA, USA) was used to obtain the IR spectra. The spectra were measured ranging from 4000 to 600 cm^{-1}, in transmission mode, at a resolution of 4 cm^{-1}. Samples were diluted with KBr (1:100, v/v) before acquisition.

2.4. Degree of substitution (DS) measurement

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., with some modifications (Xu, Miladinov, & Hanna, 2004). 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 with 0.05 N HCl back to original pH. DS was calculated as follows:

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

where DS is 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 used to back titrate, and V_{HCl} is the volume of HCl used for back titration.

2.5. *In vitro* digestibility of malic acid-treated starches

In vitro digestibility was measured following the method of Englyst et al.

(1992) with slight modifications by Shin et al. (2007). 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 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 used to measure by the GOD-POD kit (Embiel Co., Gunpo, Korea). Amount of glucose after 10 min 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 incubation.

2.6. Analysis of X-ray diffraction patterns and relative crystallinity

X-ray diffraction analysis was performed using an power 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 step time of 0.5 sec. The relative crystallinity was calculated using the software developed by the instrument manufacturer (EVA, 2.0).

2.7. Determination of thermal properties

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

2.8. Determination of apparent amylose content

Apparent amylose contents were measured according to the colorimetric

method outlined by AACC Approved Method 61-03 (AACC, 2000). 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 50% NaOH. The cooled solution (1 mL) was placed in each tube with 9 mL distilled water. Diluted sample solutions were put into a 15-mL tube containing 9 mL 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 colored sample solution was measured at 620 nm. The standard curve used to determine the content of apparent amylose was prepared amylose from potato and amylopectin from maize (Sigma–Aldrich).

2.9. Pasting viscosity of starches

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 starch was added to an RVA canister with 25 mL distilled water. Measurement followed the AACC standard method 2. The starch suspension was equilibrated at 50°C for 1 min, heated from 50 to 95°C for 7 min 30 sec, held at 95°C for 5 min, cooled to 50°C for 7 min 30 sec, and held at 50°C for 2 min. The paddle speed was 960 rpm during the first 10 s, and remained 160 rpm during the

rest of experiment.

2.10. Statistical analysis

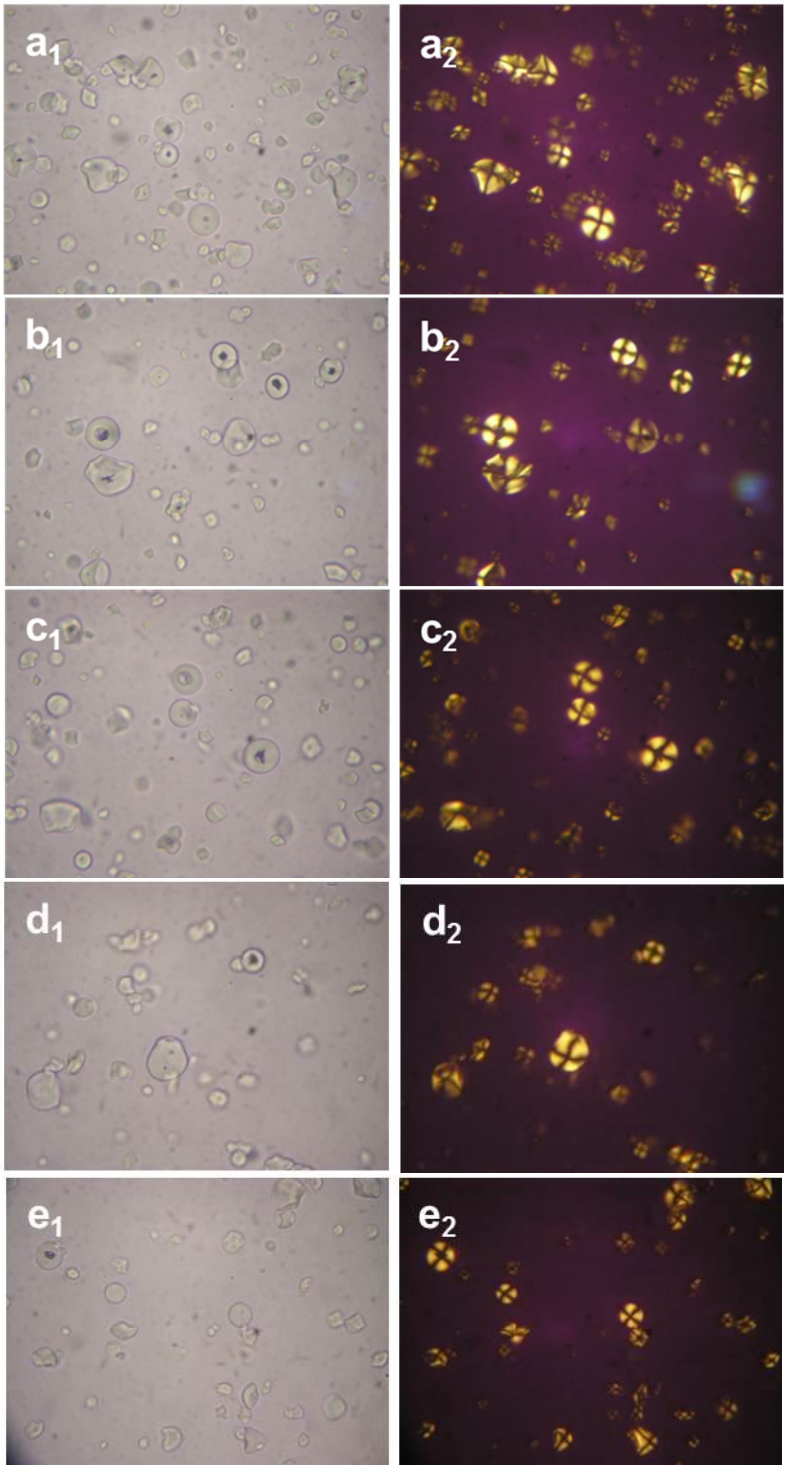
All experiments were triplicated and mean values and standard deviations are reported. Duncan's multiple range test was used to analyze the variance and the mean separations (p <0.05). The statistical analyses were conducted using the SPSS for Windows 22.0 software (IBM, New York, USA).

RESULTS AND DISCUSSION

1. Photomicrographs of starches with different malic acid treatment

The granular shape of the various malic acid-treated starch samples were investigated using a light microscope (Fig. 1). The shape of starches including malic acid-treated samples had round, semi-oval, oval spherical, and round polygonal shape which was consistent to preivious studies (Tian, Rickard, & Blanshard, 1991; Zhu & Wang, 2014). 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. (2004), 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 (Hirashima, et al., 2004). Beyond pH 3.5, the granular shape of the malic acid-treated starches was not ruptured, despite the high concentration of malic acid. Maltese cross was observed using a polarizing plate, confirming the inner ordered semi-crystalline structure and radially ordered alignment of amylose and

amylopectin (Blaszczak, Fornal, Valverde, & Garrido, 2005; Kim, et al., 2008). All samples showed 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 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.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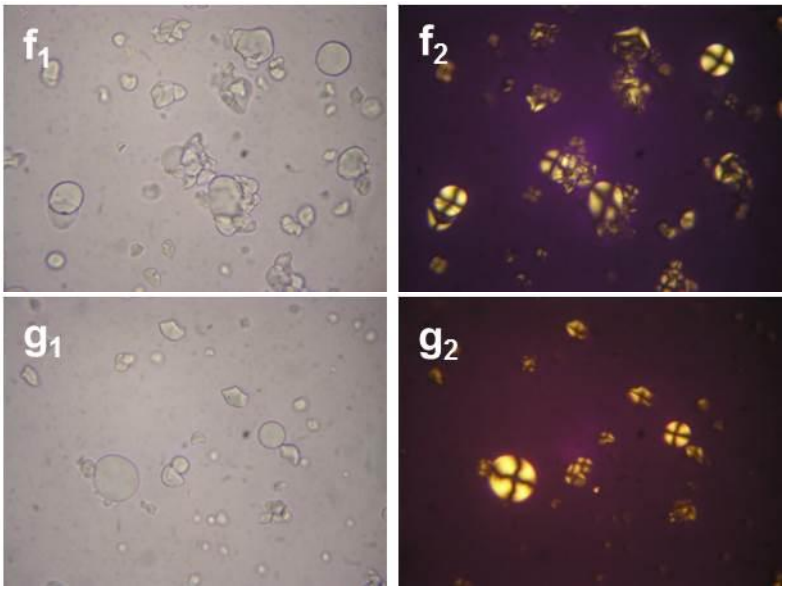

Figure 1. Optical micrographs of raw and malic acid-treated sweet potato starches (400X magnification): (a) raw, (b) 2.0M-25, (c) DW-130, (d) 0.5M-130, (e) 1.0M-130, (f) 1.5M-130, (g) 2.0M-130, 1. under visible light, 2. under polarized light.

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

FT-IR spectroscopy can determine the structural characteristics regarding 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 (Bajpai & Shrivastava, 2005). The peak between 980 and 1200 cm^{-1} can be attributed to C-O stretching vibration (Zarski, Ptak, Siemion, & Kapusniak, 2016). The peak near 1730 cm^{-1} is a carbonyl peak (Kim, et al., 2008; Saikia, Ali, Goswami, & Ghosh, 1995), 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 (Fig. 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 intra-molecular hydrogen bonds by heat, thereby leading to the formation of 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 Fig. 2. This intensity appeared to be highly correlated to both degree 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.

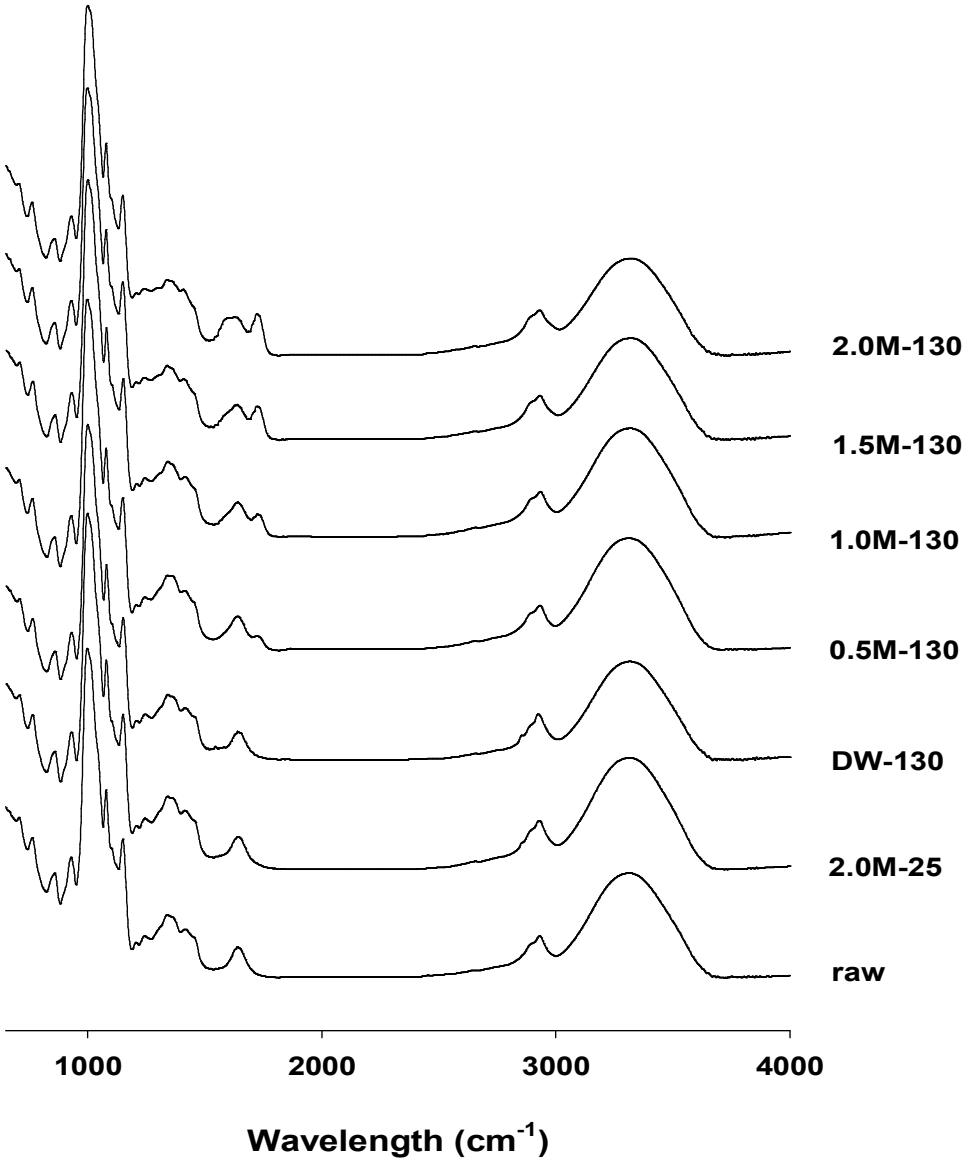

Figure 2. FT-IR spectra of raw and malic acid-treated sweet potato starches.

3. Degree of substitution (DS) of malic acid-treated starches

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 (Shin, et al., 2007). 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 (Xu, et al., 2004). Malic acid, which has two carboxyl groups, can theoretically form two ester bonds, whereas citric acid can make three ester bonds. However, citric acid-treated starch showed a lower DS value, in this study, than that of malic acid-treated starch, which could have resulted from the steric hindrance of carboxyl groups.

4. Determination of starch fractions using the Englyst method

Table 1 presents the proportions 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 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 RS fraction. According to Huber & BeMiller (2000), more the cross-linked starch, the more is 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 (Kim & Shin, 2011; Woo & Seib, 2002). 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 non-reacted 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 DS ($r = 0.983$, $p < 0.01$). The proportion of 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 non-thermally treated samples, whereas the thermally malic acid-treated samples had high content of heat-stable RS in high amount, which indicates the presence of rigid ester bond.

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

Sample	DS[1)]	Non-cooked 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]

1) DS, degree of substitution; RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch; n/d, not detected

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

5. X-ray diffraction of malic acid-treated starches

The X-ray diffraction patterns are shown in Fig. 3. Internal order of a starch granule is demonstrated by X-ray diffraction patterns of A, B, and C types (Cooke & Gidley, 1992). 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 2θ angle. 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 (Hizukuri, Fujii, & Nikuni, 1960), 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. (2011) 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 due to acid hydrolysis and heat treatment during preparation. This result was consistent with the report on

glutarate-treated starch by Kim et al. (2008) and citrate-treated starch by Xie et al. (2006). Hydrogen bonds are known to sustain double helical structure, but when substituted by ester bonds, changes in double helical structure induce rearrangement of crystalline and semi crystalline structure hence lowering relative crystallinity.

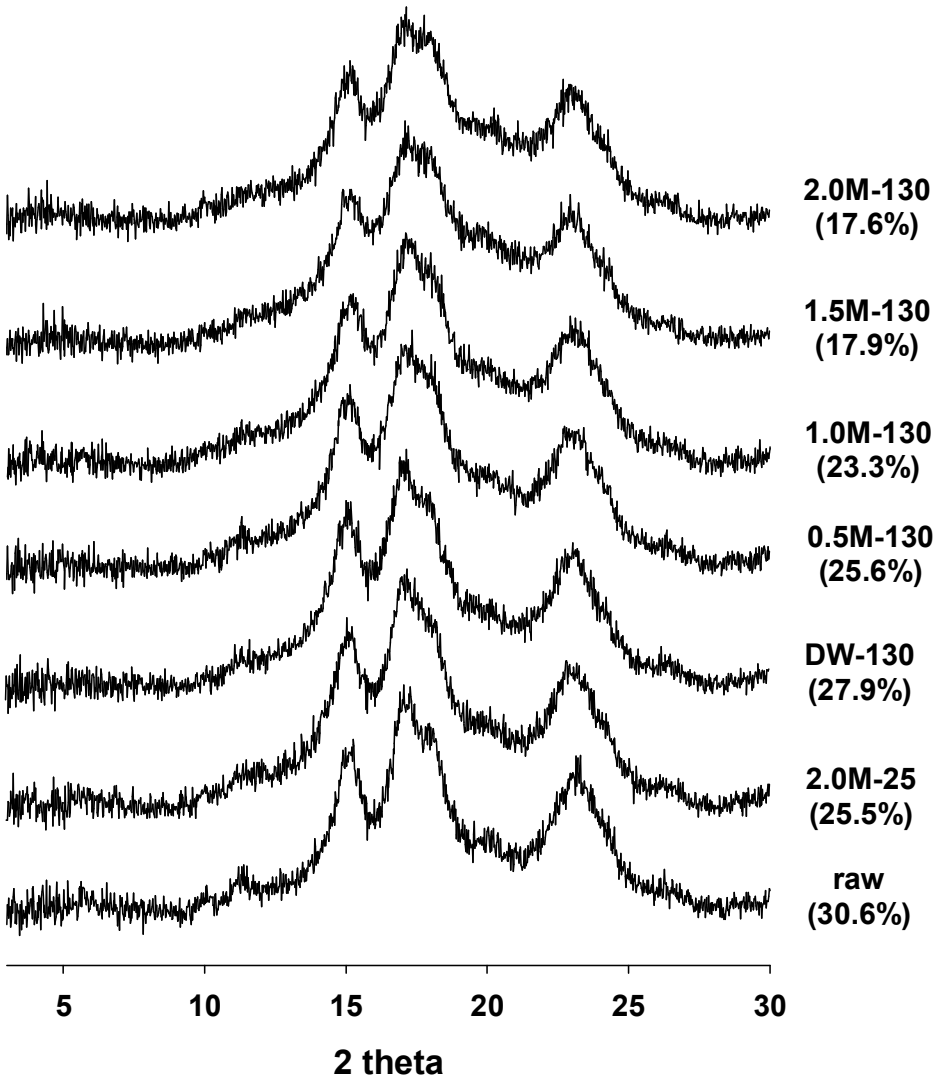

Figure 3. X-ray diffraction patterns and relative crystallinity of raw and malic acid-treated sweet potato starches. Numbers in parentheses indicate the percentage of crystallinity.

6. Thermal properties of malic acid-treated starches

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), T_c-T_o (14.9) and higher ΔH (13.6), which could have resulted from heat-moisture treatment. Increasing 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 (Biliaderis, 1990), also and the quality and quantity of crystalline area by measuring the change in heat energy (Cooke & Gidley, 1992). 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 non-thermally 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 by 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 bond, and is related to increased DS and a higher fraction of heat-stable RS.

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

Sample	T_o [1)] (℃)	T_p (℃)	T_c (℃)	T_c–T_o (℃)	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}

1) T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; T_c-T_o, temperature range of crystal melting; ΔH, enthalpy change

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

7. Measurement of apparent amylose content (AAC)

The apparent amylose contents of various malic acid-treated starches are presented in Table 3. Compared to the raw and non-thermal group, samples with high DS-values showed decreased apparent amylose content. Apparent amylose content 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 (Shin, et al., 2007), but the opposite trend was observed in this study. Mussulman and Wagoner (1968) and Robin et al. (1974) 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 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.

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}

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

8. Pasting properties of malic acid-treated starches

The pasting properties of raw and malic acid-treated starches are shown in Fig. 5. 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 (Agboola, Akingbala, & Oguntimein, 1991). Starch samples, which only conditioned in malic acid solution (2.0M-25), showed similar RVA curve with raw starch. Raw starch and 2.0M-25 had peak viscosity at 88.25°C and also had similar setback viscosity. As DW-130 indicated changes in structure which seemed to be the effect of slight 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 lower and linear RVA profiles (1.0M-130, 1.5M-130, 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 non-swelling property (Xie & Liu, 2004). Higher the concentration of malic acid solution used, lesser viscosity was observed. Shukri and Shi (2017) 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 (Agboola, et al., 1991), 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.

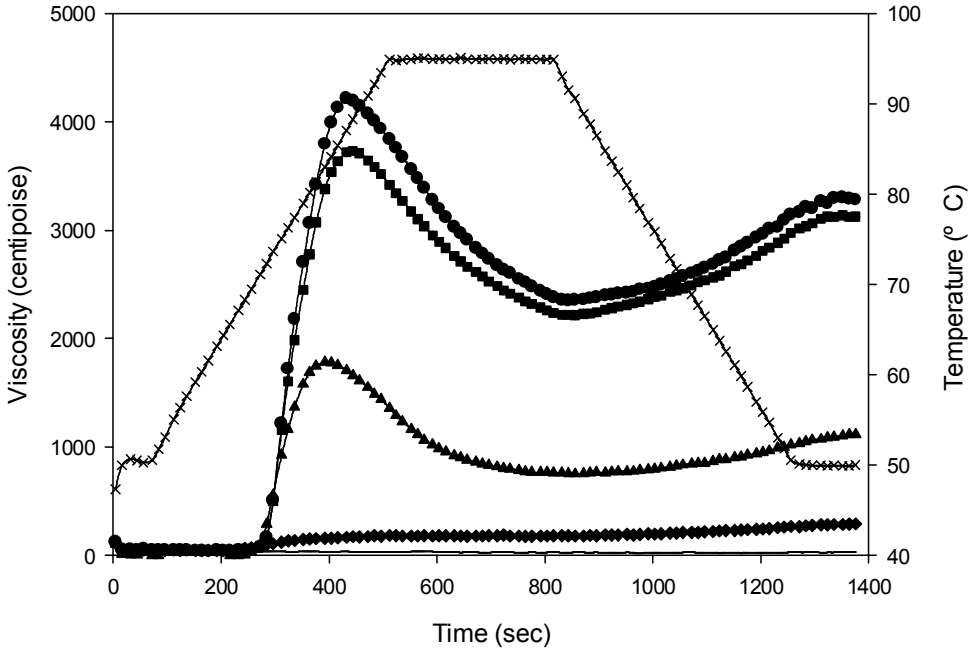

Figure 4. Rapid visco analyzer: raw and malic acid-treated sweet potato starches: ●, raw starch; ■, 2.0M-25; ▲, DW-130; ♦, 0.5M-130; ─, 1.0M-130, 1.5M130, 2.0M130; X, temperature.

CONCLUSION

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. 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 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 non-swell properties caused by 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.

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국문 초록

전분은 Englyst 등에 의해 소화되는 시간을 기준으로 하여 속소화성 전분 (rapidly digestible starch, RDS), 지소화성 전분 (slowly digestible starch, SDS), 저항전분 (resistant starch, RS)으로 분류되었다. 가교결합방법은 다양한 전분에서 저항전분의 함량을 높이기 위해서 사용되고 있다. 하지만 generally regarded as safe (GRAS) 물질로 설정된 사과산은 아직까지 전분의 가교결합에 사용한 연구가 많이 없다. 또한 고구마는 척박한 토양에서 잘 자라고 전분 함량이 높아서 고구마 전분에 대한 집중적인 연구가 진행되고 있다. 본 연구에서는 사과산을 여러 농도로 처리하여 제조한 고구마 전분의 구조와 이화학적 특성을 알아보았다.

고구마전분을 다양한 농도(0.5 M - 2.0 M)의 사과산 용액과 혼합한 뒤 16시간 동안 상온에 보관하였다. 그 후 시료들을 건조하고 12시간 동안 130°C 의 드라이 오븐에서 건열 처리하여 사과산과 전분을 반응시킨 시료와 12시간 동안 열처리 없이 상온에서 보관한 시료를 만들었다. 그 후 반응하지 않은 사과산은 모두 증류수와 에탄올 세척으로 제거해주었다.

현미경 관찰 결과, 모든 전분에서 동그란 형태의 전분입자가 보존되었고, 전분 내부의 규칙적인 구조에 의해 나타나는 Maltese cross 도 발견되었다. 푸리에변환 적외분광 분석의 결과에 의하면 사과산과 열을 처리 전분에서만 1722cm^{-1} 에서 에스터 결합을 나타내는 카보닐 피크가 관찰되었다. 또한 사과산 용액의 농도가 증가함에 따라서 치환도가 증가하는 것을 확인하였으며 2.0M-130시료에서 0.214로 가장 높은 치환도를 가졌고 0.5M-130시료가 0.088로 사과산 처리 시료 중 가장 낮은 치환도를 나타내었다. *In vitro* 소화율을 확인한 결과, 치환도와 저항전분 함량이 양의 상관관계를 가진다는 것을 통계적으로 확인하였다 ($r=0.967$, $p<0.01$). 열과 사과산을 처리한 전분에서 저항전분 함량은 100°C 에서 30분간 끓이는 가열과정을 거쳐도 그 감소량이 다른 전분들 보다 낮았다. 특히 2.0M-130시료는 53.4%의 저항전분 중 93.4%가 보존되었으며 여전히 치환도와 높은 상관관계를 나타내었다 ($r=0.983$, $p<0.01$). 즉, 사과산과 열을 처리하여 열에 큰 안정성을 가지는 저항전분을 제조할 수 있었다.

사과산 처리에 의해 저항전분이 증가하게 된 구조적 이유를 X 선 회절분석, 시차주사열량분석, 겉보기 아밀로오스 함량분석, 신속점도측정의 결과를 통해 밝혀내었다. X 선 회절에서 고구마 전분이 일반적으로 나타내는 C 형의 X 선 회절결과를 유지하여

입자내부의 결정구조가 파괴된 것이 아니라는 것을 확인하였다. 또한, 전분의 나선구조에 존재하던 수소결합이 에스터 결합으로 치환된 것과 그에 따른 구조적 변화는 시차주사열량분석과 겉보기 아밀로오스 함량의 결과로 뒷받침 되었다. 시차주사열량분석의 결과 사과산 처리전분에서 T_o, T_p, T_c, ΔH 값이 모두 감소하였는데 이는 전분의 나선구조에 존재하던 수소결합이 에스터 결합으로 치환되었기 때문이다. 겉보기 아밀로오스 함량 또한 감소하였는데 이는 전분사슬의 부분적인 분해뿐만 아니라 사과산과 전분의 에스터 결합에 의하여 나선구조의 변형이 일어났기 때문이다. 신속점도측정 결과에서 사과산과 전분이 가교결합을 형성한 전분은 점도를 나타내지 않아 호화가 일어나지 않았으며 노화도 일어나지 않았다.

결과적으로 사과산처리 전분은 높은 저항전분함량과 열 안정성을 가져 저소화성 소재로 사용될 수 있을 뿐만 아니라 가교결합에 의해 점도를 나타내지 않고 호화, 노화가 일어나지 않아 제품의 점도 및 노화조절제로서 이용될 수 있을 것이다.

주요어: 고구마전분, 사과산, 열 안정성, 저항전분, 에스터 결합

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