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RESEARCH ARTICLE

In situ Gelatinization of Starch using Hot Stage Microscopy

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Abstract Starch gelatinization is important in food processing and industrial use. Granule swelling and gelatinization temperature of 11 starches from different plants were investigated *in situ* using hot stage microscopy during heating. The amylose content, swelling power, pasting temperature and thermal property of these starches were also measured. The results showed that hot stage microscopy was suitable for measuring granule swelling and the gelatinization temperature of starch during heating. The sectional area swelling percentage of starch granules measured using hot stage microscopy was significantly positively correlated with the swelling power. The gelatinization temperature measured using hot stage microscopy was significantly positively correlated with the pasting temperature and with the thermal property for all 11 starches. For rice starches with the same crystallinity and similar size, the gelatinization temperature was negatively correlated with the amylose content and positively correlated with the swelling power and the sectional area swelling percentage at 95ºC.

Keywords: starch, heating, swelling, gelatinization temperature, hot stage microscopy

Introduction

Starch is stored as discrete semi-crystalline granules in higher plants, and consists of two main components: a mainly linear amylose and a highly branched amylopectin (1). Starches from crops are a major source of nutrition for humans and animals and an important raw material for industry (2). Starches are physically and chemically modified to meet the demands of food and non-food industries. A major step during food processing and industrial use of starch is gelatinization (3,4).

Starch granules are insoluble in cold water. When starch is heated in the presence of excess water, granules absorb water and swell. The absorption of water destabilizes the granule crystalline structure, resulting in a loss of birefringence, which is one definition of gelatinization (5). Swelling and the destruction of the crystalline structure are two important processes of starch gelatinization. The swelling of starch granules can be measured as the swelling power during gelatinization (6). Starch granules are semi-crystalline in structure and exhibit birefringence under cross-polarized light. Generally, starch granules show the characteristic "Maltese Cross" patterns before gelatinization under polarized light. Birefringence indicates that there is a high degree of molecular orientation within starch granules without reference to any crystalline form (7). Loss of birefringence on heating, indicative of disordering processes, is used for determining gelatinization temperatures (8).

There are various methods for determining starch gelatinization and associated properties, including differential scanning calorimetry (DSC), hot stage microscopy, X-ray diffraction, nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy and viscosity measurements (7,9). DSC is the most widely used method to investigate the thermal properties of starch, and can measure the gelatinization temperature range and the energy absorbed during phase transition. However, DSC can not be used to explore the detailed progress of the phase transition, such as the behavior of granule swelling and the loss of crystalline structure (10). Hot stage microscopy is a sensitive and relatively simple method to study the destruction of crystalline structure during starch gelatinization. The advantage of hot stage microscopy is the ability to observe *in situ* the simultaneous behavior of granule swelling and crystallinity disappearance. Yeh and Li (11) determined the sizes and

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distributions of rice starch granules using hot stage microscopy during gelatinization, and discovered that 1) swelling of starch granules reached a maximum 54.7% increase in average sectional area at 75ºC, 2) starch granules proceeded to disrupt and dissolve above 75ºC, and 3) loss of birefringence occurred at a lower temperature than granule rupture. The results of an investigation by Patel and Seetharaman (12) regarding the effect of the heating rate on the morphology and size of wheat starch granules using hot stage microscopy showed that granule morphology differed as a function of the heating rate, and granule swelling was kinetically controlled. Bogracheva *et al.* (13) used hot stage microscopy to view the *in situ* gelatinization process of potato starch, and discovered that melting of the ordered structures in potato starch always began in the hilum area of the granule, followed quickly by swelling of the disrupted part.

In this paper, 11 starches were isolated from different plants. Starch granule swelling and gelatinization temperatures were investigated *in situ* using hot stage microscopy during heating. The physicochemical properties of amylose content, swelling power, pasting property and thermal property were also measured. Correlations between physicochemical properties and the results of hot stage microscopy were analyzed. The objective of this study was to evaluate the feasibility of the hot stage microscopy method for measuring granule swelling and the gelatinization temperature of starch.

Materials and Methods

Plant materials The rhizomes of lotus (*Nelumbo nucifera* Gaertn.) and yam (*Dioscorea opposita* Thunb.), the tuber of potato (*Solanum tuberosum* L.), and the seeds of pea (*Pisum sativum* L.), water caltrop (*Trapa bispinosa* Roxb.) and rice (*Oryza sativa* L.) were used to isolate native starches. Freshly harvested lotus and yam rhizomes, potato tubers and water caltrop seeds, and mature dry pea seeds were obtained from a local natural food market (Yangzhou City, Jiangsu Province, China). Mature seeds of 3 *indica* cultivars of rice, including Guichao 2 (R-G2), Huanghuazhan (R-H) and Nanjing 11 (R-N11), and the 3 *japonica* rice cultivars Guihuahuang (R-G), Wuyunjing 8 (R-W8) and Zhonghua 11 (R-Z11) were harvested from the experimental field of Yangzhou University, Yangzhou, China.

Isolation of native starch Native starch was isolated following a method described by Man *et al*. (14).

Hot stage microscopy Starch suspensions were prepared by suspending approximately 10 mg of starch in 1.0 mL of double distilled water using a vortex mixer. A suspension was transferred onto a slide, covered with a coverslip, and sealed with nail polish to prevent moisture loss during heating. The sealed specimen was then mounted on a hot stage apparatus (MP-10DMFH; Kitazato Co., Ltd., Fuji, Japan) and observed under a long focus M Plan Semi Apochromat objective (20× or 50× magnification) using a polarizing microscope (Olympus BX53; Olympus Optical Co., Ltd., Tokyo, Japan) equipped with cross polarizers. The hot stage was heated from 25ºC to 50ºC at a heating rate of 5ºC/min, and from 50ºC to 95ºC at a heating rate of 1ºC/min.

The swelling of individual starch granule during heating was viewed under normal light and photographed using a CCD camera (Olympus DP72; Olympus Optical Co., Ltd.) from 50ºC to 95ºC at 5ºC intervals. The sectional area of starch granules was determined using the photomicrographs with the JEDA 801D morphological image analysis system (Jiangsu JEDA Science-Technology Development Co., Ltd., Nanjing, China). More than 50 starch granules were measured per sample. Swelling was quantified as the sectional area swelling percentage (ASP) relative to an ungelatinized granule at 50ºC, and calculated using the equation: ASP (%)=At/Ai×100, where Ai and At were the initial sectional area of starch granules at 50ºC and at the specific testing temperature.

The gelatinization temperature was measured according to the method of Konik-Rose *et al.* (15) with some modifications. Starch granules were photographed under polarized light using an Olympus DP72 CCD camera during heating from 50ºC to 95ºC at 1ºC intervals. More than 100 starch granules of each sample were analyzed per experiment. The initial (5%), middle (50%), and end (95%) gelatinization temperatures were recorded at the point when the indicated percentages of starch granules lost their birefringence. Experiments were performed in triplicate.

Swelling power The swelling power of starch was determined by heating starch-water slurries in a water bath at temperatures ranging from 50ºC to 95ºC at 5ºC intervals, according to the procedures of Wei *et al.* (16). The experiments were performed in triplicate.

Amylose content The amylose content of starch was determined following a modified method described by Man *et al.* (14) based on the iodine adsorption method of Konik-Rose *et al.* (17). The experiments were performed in triplicate.

Pasting property The pasting property of starch (8% solids) was evaluated using a Rapid Visco Analyzer (RVA-3D; Newport Scientific, Narrabeen, Australia). A programmed heating and cooling cycle was used where the sample was held at 50ºC for 1 min, heated to 95ºC at a rate of 12ºC/

min, maintained at 95ºC for 2.5 min, cooled to 50ºC at a rate of 12ºC/min, and then held at 50ºC for 1.4 min. The experiments were performed in triplicate.

Thermal property The thermal property of starch was analyzed using a DSC (200-F3; NETZSCH, Selb/Bavaria, Germany) as described previously by Man *et al.* (14). The experiments were performed in triplicate.

Statistical analysis Analysis of variance (ANOVA) using Tukey's test, linear regression, and correlation analysis were evaluated using the SPSS version 16.0 Statistical Software Program. The Pearson correlation coefficient and a two-tailed test of significance were evaluated to compare correlations.

Results and Discussion

Swelling of starch granule during heating The swelling of starch granule was continuously observed *in situ* using a polarized microscope combined with a hot stage. Images of starch granules were viewed and photographed at 5ºC intervals during heating (Fig. 1). Compared to that at 50ºC, the granule size of yam starch did not change at 70ºC. A size increase started above 75ºC. Starch granule swelling was presented as the sectional area swelling percentage from ungelatinized granules at 50ºC. The sectional area swelling percentages for lotus rhizome, pea, potato, water caltrop and yam starch granules at 5ºC intervals from 50°C to 95ºC are shown in Table 1. Different starches showed different swelling processes. Pea and lotus rhizome starches began to swell at 60ºC, while water caltrop starch started to swell at 80ºC. Granule swelling was not measured above 95ºC because of deformation, folding, and disruption of granules. The sectional area swelling percentages of 11 starches at 95ºC are shown in Table 2.

Gelatinization temperature of starch during heating Photomicrographs of yam rhizome starch granules are shown in Fig. 2A to illustrate the gelatinization process under polarized light. During heating, the birefringence cross, which is characteristic of crystalline substances, disappeared as the crystalline structure was disrupted (18). Below 70ºC, yam starch granules showed the characteristic "Maltese Cross" pattern. The birefringence intensity of granule faded away with an increase in temperature above 70ºC, and completely disappeared at 86ºC. The loss of birefringence during heating for different starches is shown in Fig. 2B and 2C. The rates of birefringence disappearance for different starches all showed S-curve patterns. The initial gelatinization temperatures varied from 59.8ºC to 79.3ºC, the middle gelatinization temperatures varied from 63.7ºC to 82.7ºC, and the end gelatinization temperatures varied from 67.8ºC to 85.8ºC (Table 2).

Swelling power of starch during heating Swelling power test is a simple analysis that measures the uptake of water during the gelatinization of starch. It can be used to assess the extent of interaction between the starch chains within the amorphous and crystalline parts of the starch granule (19). The swelling powers of lotus rhizome, pea,

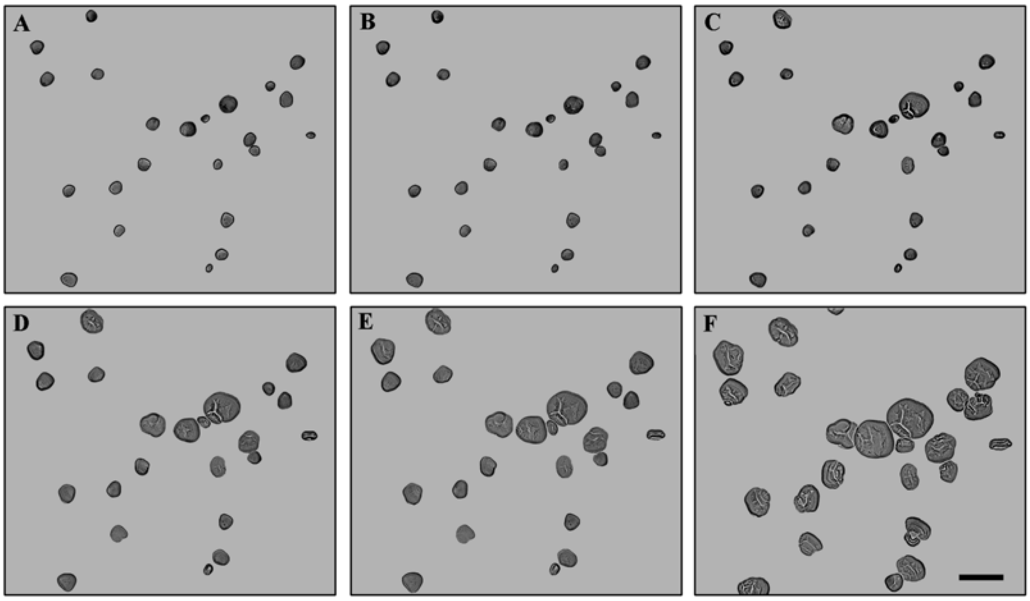

Fig. 1. Photomicrographs of yam starch granules at different temperatures under normal light. Photographs were taken at 50ºC (A), 70ºC (B), 75ºC (C), 80ºC (D), 85ºC (E), and 95ºC (F). Scale bar=50 μm

Table 1. Sectional area swelling percentage (%) of starches measured using hot stage microscopy at different temperatures

Temperature (°C)	Lotus rhizome	Pea	Potato	Water caltrop	Yam
50	$100.0\pm0.0^{a1)}$	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
55	103.7 ± 4.1^{a}	105.0 ± 7.9^{a}	101.3 ± 3.4^{a}	100.6 ± 5.8^{a}	101.0 ± 4.3^{a}
60	119.5 ± 29.8^{a}	114.7 ± 12.4^{ab}	102.2 ± 4.3^{a}	101.2 ± 7.5^{a}	100.9 ± 5.3^{a}
65	185.0 ± 83.7^{b}	144.0 ± 34.2^{b}	105.9 ± 8.1^{a}	102.7 ± 7.1^{a}	104.4 ± 5.2^{a}
70	475.7 ± 155.1^{c}	208.0 ± 55.0^{c}	356.6 ± 194.2^{b}	104.4 ± 9.3^{a}	111.8 ± 20.1^{a}
75	779.5 ± 108.7^{d}	335.4 ± 71.4^{d}	602.3 ± 100.3^{c}	103.8 ± 8.5^{a}	147.6 ± 59.5^{b}
80	921.4 ± 125.2^{e}	449.9 ± 95.7^{e}	661.9 ± 101.9^{d}	125.8 ± 45.5^{a}	220.9 ± 91.1^{c}
85	1043.3 ± 141.1^{f}	506.5 ± 108.5^{f}	713.3 ± 110.5^{e}	424.0 ± 185.5^{b}	327.4 ± 117.3^{d}
90	1167.9 ± 157.0^{g}	$530.1\pm121.4f^{g}$	767.7 ± 119.9^{f}	646.7 ± 167.0^{c}	488.3 ± 90.0^{e}
95	1322.1 ± 177.8^{h}	549.9 ± 135.7^{g}	870.1 ± 143.6^{g}	686.3 ± 170.4^{d}	558.2 ± 98.9^{f}

1) Values (mean±SD) with different superscripts within a column are significantly different ($p<0.05$, $n>50$).

Table 2. Sectional area swelling percentage at 95°C and gelatinization temperature of starches measured using hot stage microscopy

Starch	ASP_{95} (%)1)	Gelatinization temperature (°C)2)		
		T_i	T_m	T_e
Lotus rhizome	$1322.1\pm177.8^{f3)}$	61.3 ± 0.1^{c}	65.7 ± 0.3^{b}	69.5 ± 0.5^{b}
Pea	549.9 ± 135.7^{a}	63.5 ± 0.2^{e}	67.1 ± 0.4^{c}	71.9 ± 0.2^{e}
Potato	870.1 ± 143.6^{c}	66.2 ± 0.4^{f}	70.3 ± 0.3^{d}	74.8 ± 0.2^{f}
Water caltrop	686.3 ± 170.4^{b}	79.3 ± 0.1^{h}	82.7 ± 0.0^{f}	85.8 ± 0.4^{i}
Yam	558.2 ± 98.9^{a}	70.3 ± 0.5^{g}	78.8 ± 0.1^{e}	83.8 ± 0.4^{h}
R-G4)	817.4 ± 228.0^{c}	59.8 ± 0.5^{ab}	64.1 ± 0.4^{a}	70.4 ± 0.9^{bc}
R-G24)	702.9 ± 158.7^{b}	59.8 ± 0.6^{a}	63.7 ± 0.4^{a}	67.8 ± 0.1^{a}
R-H4)	988.3 ± 183.5^{d}	61.0 ± 0.4^{c}	66.6 ± 0.2^{bc}	70.3 ± 0.3^{bcd}
R-N114)	1083.5 ± 388.7^{de}	63.3 ± 0.2^{de}	69.5 ± 0.3^{d}	75.8 ± 0.0^{g}
R-W84)	858.6 ± 150.2^{c}	61.0 ± 0.9^{bc}	66.5 ± 0.4^{bc}	70.8 ± 0.3^{cd}
R-Z114)	1126.0 ± 288.8^{e}	61.8 ± 0.7^{cd}	66.8 ± 0.1^{c}	71.4 ± 0.1^{de}

1) Sectional area swelling percentage of starches at 95°C

2) Gelatinization temperature: T_i, initial temperature; T_m, middle temperature; T_e, end temperature

3) Values (mean±SD) with different superscripts within a column are significantly different ($p<0.05$, $n>50$ for ASP_{95} and $n=3$ for gelatinization temperature).

4) Rice cultivars: R-G, Guihuahuang; R-G2, Guichao 2; R-H, Huanghuazhan; R-N11, Nanjing 11; R-W8, Wuyunjing 8; R-Z11, Zhonghua 11

potato, water caltrop, and yam starches were investigated from 50°C to 95°C at 5°C intervals (Table 3). Before gelatinization began, the swelling powers of starches showed a slight variance with an increasing temperature. After gelatinization began, the swelling powers of starches significantly increased as temperatures increased. The swelling powers of pea and lotus rhizome starches began to significantly increase at 60°C, and the swelling power values of potato and water caltrop starches began to significantly increase at 65 and 70°C, respectively, in agreement with the sectional area swelling percentage results for starches during heating (Table 1). The swelling powers of rice starches at 95°C were from 19.6 to 32.1 for the different cultivars (Table 4). Differences in swelling powers among starches can be attributed to interplay of the following factors: 1) the level of lipid-complexed amylose chains (20), 2) the molar proportion of amylopectin unit chains of DP 6-24 (21), 3) the total amylose content (22), and 4) the extent of interaction between starch chains within the amorphous and crystalline domains (23).

Amylose content of starch The amylose content is the major factor controlling almost all physicochemical properties of starch, and plays an important role in the internal quality (24). The amylose contents of 11 starches are shown in Table 4. They varied from 10.6% to 44.4%. The lowest value was observed for rice cultivar Nanjing 11 (R-N11) and the highest for pea.

Pasting temperature of starch Pasting temperatures of 11 starches determined using RVA analysis are summarized in Table 4. The pasting temperature is the temperature at which the viscosity begins to increase during the heating process. Significant differences were observed in pasting temperatures among starches from rice cultivars of Guihuahuang, Guichao, Huanghuazhan, Nanjing 11, Wuyunjing 8 and

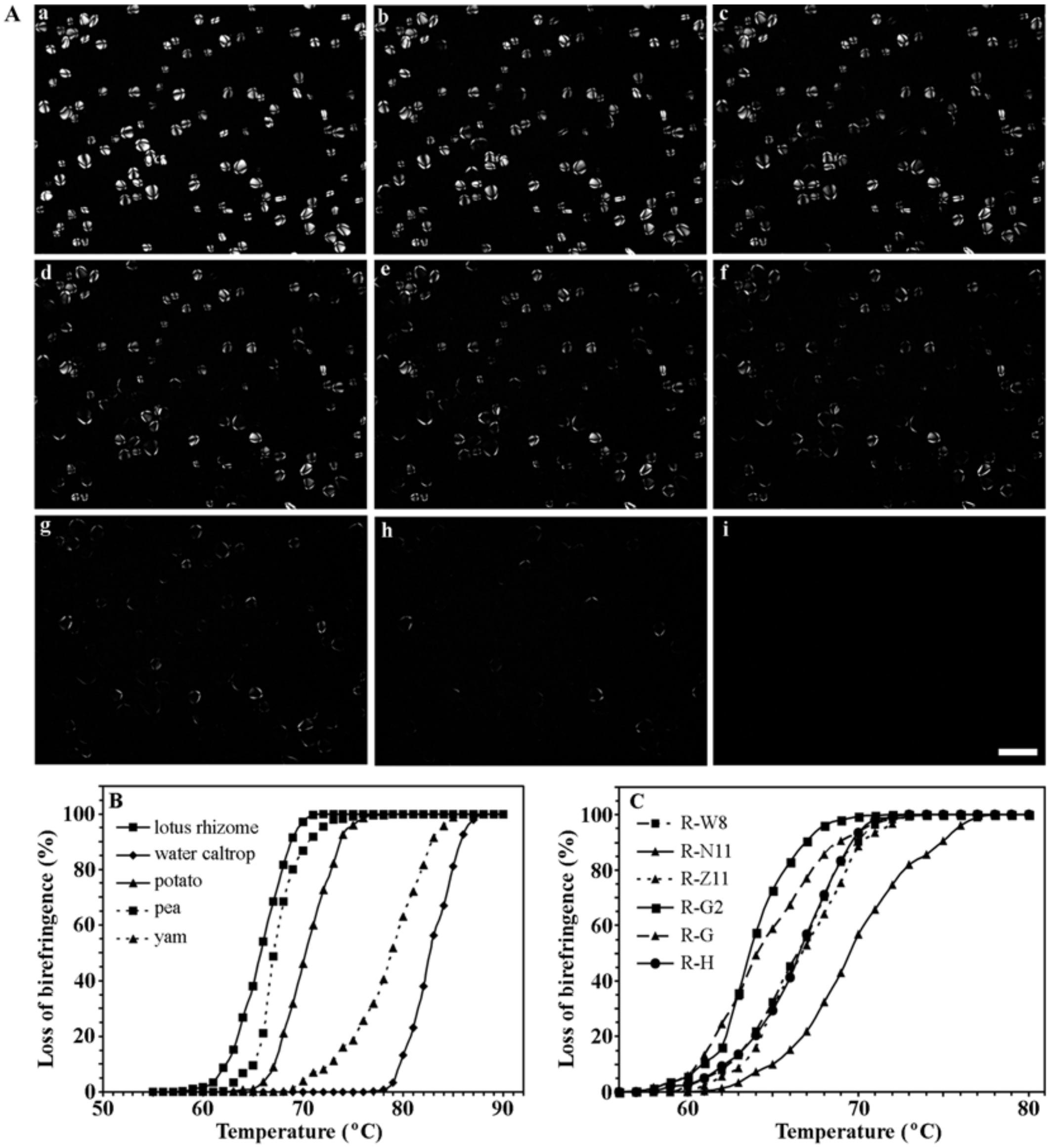

Fig. 2. Loss of birefringence of starch granules. (A) Photomicrographs of yam starch granules under polarized light at 30°C (a), 70°C (b), 75°C (c), 77°C (d), 79°C (e), 80°C (f), 82°C (g), 84°C (h), and 86°C (i). Scale bar=50 μm. (B, C), loss of birefringence of starch granules at different heating temperatures as observed microscopically. Rice cultivars: R-G, Guihuahuang; R-G2, Guichao 2; R-H, Huanghuazhan; R-N11, Nanjing 11; R-W8, Wuyunjing 8; R-Z11, Zhonghua 11

Zhonghua 11. The pasting temperature of the rice cultivar R-W8 starch was the lowest (71.2°C), whereas water caltrop starch had the highest pasting temperature (84.4°C).

Thermal properties of starch DSC analysis measures and records the amount of heat involved in starch gelatinization. Thermal properties of 11 starches were determined using DSC (Table 4). The rice cultivar R-G2 starch showed the lowest onset, peak, and conclusion temperatures, while water caltrop starch showed the highest onset and peak temperatures, in agreement with the gelatinization temperature results measured using hot stage microscopy (Table 2). A higher gelatinization temperature indicates that more energy is required to initiate starch gelatinization (20). The gelatinization enthalpy values of 11 starches showed significant differences from 9.7 to 18.3 J/g. Variations in gelatinization enthalpy indicate differences in bonding forces between the double helices that form the amylopectin crystallites, which result in different alignment of hydrogen bonds within starch molecules (25).

Correlation analysis between sectional area swelling percentage and swelling power The degree of starch swelling during heating can be quantitatively determined using the sectional area swelling percentage and the swelling power. The sectional area swelling percentages

Table 3. Swelling power (g/g) of starches at different temperatures

Temperature (°C)	Lotus rhizome	Pea	Potato	Water caltrop	Yam
50	2.3 ± 0.3^{a}1)	2.3 ± 0.1^{a}	2.2 ± 0.1^{a}	2.3 ± 0.2^{a}	2.4 ± 0.2^{a}
55	3.4 ± 0.3^{a}	2.6 ± 0.3^{a}	2.3 ± 0.2^{a}	2.3 ± 0.1^{a}	2.6 ± 0.2^{ab}
60	6.3 ± 0.5^{b}	3.3 ± 0.3^{b}	2.6 ± 0.2^{a}	2.4 ± 0.1^{a}	2.6 ± 0.3^{ab}
65	16.5 ± 0.6^{c}	6.0 ± 0.1^{c}	9.0 ± 0.4^{b}	2.5 ± 0.1^{a}	3.0 ± 0.2^{bc}
70	19.4 ± 1.1^{d}	6.9 ± 0.3^{d}	18.7 ± 1.2^{c}	3.1 ± 0.3^{b}	3.3 ± 0.2^{c}
75	20.3 ± 0.2^{d}	9.2 ± 0.4^{e}	18.7 ± 0.7^{c}	3.3 ± 0.4^{b}	5.9 ± 0.2^{d}
80	23.1 ± 0.5^{e}	9.6 ± 0.1^{e}	20.5 ± 1.0^{d}	9.0 ± 0.6^{c}	12.0 ± 0.2^{e}
85	22.8 ± 1.0^{e}	13.1 ± 0.2^{f}	24.6 ± 0.2^{e}	11.4 ± 0.3^{d}	15.7 ± 0.4^{f}
90	28.2 ± 0.9^{f}	14.0 ± 0.2^{g}	24.8 ± 1.2^{e}	14.6 ± 0.2^{e}	17.3 ± 0.2^{g}
95	34.2 ± 0.8^{g}	15.3 ± 0.2^{h}	32.1 ± 1.1^{f}	16.5 ± 0.5^{f}	18.2 ± 0.6^{h}

1)Values (mean±SD) with different superscripts within a column are significantly different ($p<0.05$, $n=3$).

Table 4. Swelling power at 95°C, the amylose content, the pasting temperature, and thermal property of starches

Starch	SP_{95} (g/g)1)	AC (%)2)	P_{temp} (°C)3)	Thermal property4)			
				T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
Lotus rhizome	34.2 ± 0.8^{g}5)	23.9 ± 0.4^{c}	71.4 ± 0.8^{a}	57.3 ± 0.1^{a}	64.7 ± 0.3^{a}	72.8 ± 0.4^{ab}	12.5 ± 0.5^{cde}
Pea	15.3 ± 0.2^{a}	44.4 ± 1.3^{f}	78.9 ± 0.3^{e}	59.5 ± 0.4^{b}	66.4 ± 0.1^{b}	73.8 ± 0.2^{bc}	12.5 ± 0.3^{cde}
Potato	32.1 ± 1.1^{f}	34.7 ± 1.7^{e}	73.4 ± 0.3^{bc}	64.3 ± 0.3^{d}	69.0 ± 0.2^{d}	77.0 ± 0.4^{e}	15.6 ± 0.3^{f}
Water caltrop	16.5 ± 0.5^{ab}	25.7 ± 0.5^{c}	84.4 ± 0.6^{f}	75.3 ± 0.4^{e}	82.4 ± 0.2^{f}	89.7 ± 0.4^{g}	18.3 ± 0.3^{g}
Yam	18.2 ± 0.6^{bc}	34.7 ± 0.9^{e}	83.6 ± 0.5^{f}	64.6 ± 0.4^{d}	76.8 ± 0.1^{e}	89.9 ± 0.3^{g}	13.1 ± 0.4^{de}
R-G	22.8 ± 0.4^{d}	22.8 ± 0.5^{c}	72.0 ± 0.8^{ab}	57.2 ± 0.1^{a}	64.9 ± 0.4^{a}	74.3 ± 0.4^{bcd}	10.5 ± 0.3^{ab}
R-G2	19.6 ± 0.0^{c}	30.0 ± 0.4^{d}	73.8 ± 0.8^{cd}	57.2 ± 0.3^{a}	64.8 ± 0.0^{a}	72.0 ± 0.5^{a}	9.7 ± 0.4^{a}
R-H	26.5 ± 0.8^{e}	19.1 ± 0.7^{b}	72.7 ± 0.5^{abc}	60.8 ± 0.2^{c}	67.1 ± 0.1^{bc}	73.4 ± 0.1^{abc}	11.0 ± 0.7^{abc}
R-N11	32.1 ± 0.5^{f}	10.6 ± 0.6^{a}	79.9 ± 0.3^{e}	59.5 ± 0.4^{b}	69.0 ± 0.4^{d}	83.5 ± 0.5^{f}	13.7 ± 0.6^{e}
R-W8	24.4 ± 0.4^{d}	16.0 ± 1.2^{b}	71.2 ± 0.5^{a}	59.2 ± 0.4^{b}	67.8 ± 0.2^{c}	75.6 ± 0.5^{de}	11.3 ± 0.5^{abc}
R-Z11	27.7 ± 0.2^{e}	17.8 ± 0.9^{b}	75.0 ± 0.3^{d}	60.2 ± 0.4^{bc}	67.1 ± 0.0^{bc}	74.4 ± 0.6^{cd}	11.9 ± 0.4^{bcd}

1)Swelling power of starches at 95°C

2)Amylose content

3)The pasting temperature of starches is measured using RVA.

4)The gelatinization temperature (T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature) and gelatinization enthalpy (ΔH) of starches are measured using DSC.

5)Values (mean±SD) with different superscripts within a column are significantly different ($p<0.05$, $n=3$).

Table 5. Correlation coefficient between the sectional area swelling percentage and swelling power of starches at different temperatures from 50°C to 95°C at 5°C intervals

Starch	Lotus rhizome	Pea	Potato	Water caltrop	Yam rhizome
r	0.930**1)	0.973**	0.959**	0.942**	0.950**

1)** indicates significance at $p<0.01$ level ($n=10$).

and the swelling powers of lotus rhizome, pea, potato, water caltrop, and yam starches were measured from 50°C to 95°C at 5°C intervals (Table 1, 3). Correlations between the sectional area swelling percentage and the swelling power during heating were analyzed (Table 5). Results showed that the sectional area swelling percentage was significantly positively correlated with the swelling power. The correlation coefficients were 0.930, 0.973, 0.959, 0.942, and 0.950 for lotus rhizome, pea, potato, water caltrop, and yam starches, respectively. The sectional area swelling percentage at 95°C (Table 2) was significantly positively correlated with the swelling power at 95°C (Table 4) for all 11 starches. The correlation coefficient was 0.888 ($p<0.01$, $n=11$). The swelling power of starch has been reported to depend upon the water holding capacity of starch molecules due to hydrogen bonding. Hydrogen bonds that stabilize the structure of the double helices in crystallites are broken during gelatinization and are replaced by hydrogen bonds with water, and swelling is regulated by the crystallinity of the starch (6). The sectional area swelling percentage of starch was significantly positively correlated with the swelling power during gelatinization, suggesting that hot stage microscopy can be used to measure the swelling property of starch.

Table 6. Correlation coefficient between the gelatinization temperature and physicochemical property of starches

Gelatinization temperature (°C)	AC (%)	ASP_{95} (%)	SP_{95} (g/g)	P_{temp} (°C)	Thermal property			
					T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
T_i	0.249	0.416	0.405	0.813**1)	0.967**	0.967**	0.856**	0.884**
T_m	0.188	0.407	0.372	0.852**	0.920**	0.987**	0.937**	0.817**
T_e	0.167	0.422	0.358	0.881**	0.872**	0.968**	0.971**	0.796**

1) ** indicates significance at $p<0.01$ level ($n=11$).

Correlations between the gelatinization temperature and the physicochemical properties The initial, middle, and end gelatinization temperatures of 11 starches were measured using hot stage microscopy. Correlation coefficients were determined to study the relationships between the gelatinization temperature measured using hot stage microscopy and the physicochemical properties of amylose content, sectional area swelling percentage, swelling power, pasting temperature, and thermal property (Table 6). Gelatinization temperatures (initial, middle, and end temperatures) measured using hot stage microscopy were significantly positively correlated with the pasting temperature and thermal property. The gelatinization temperatures determined using hot stage microscopy were similar to results of DSC measurement and in agreement with the result of Yeh and Li (11). A significant positive correlation between gelatinization and pasting temperatures has also been reported by Wickramasinghe *et al.* (26). The gelatinization temperatures obtained using hot stage microscopy, RVA, and DSC were significantly positively correlated, indicating that hot stage microscopy could be used to determine the gelatinization temperature of starch.

The gelatinization temperature of starch is related to a variety of factors, including the size, proportion, and kind of crystalline organization, and the ultrastructure of starch granules (27). Previous research showed that lotus rhizome, pea, potato, water caltrop, and yam starches have significantly different sizes and morphologies (28). Potato starch has B-type crystallinity, lotus rhizome, yam and pea starches all have C-type crystallinity, and water caltrop starch has A-type crystallinity (28). Starches from rice cultivars have A-type crystallinity, and show similar granule sizes (29). The present results showed that gelatinization temperatures measured using hot stage microscopy had no correlation with the amylose content, the sectional area swelling percentage, or the swelling power for all 11 starches with different sizes and crystalline types (Table 6). In order to exclude the effect of granule size and crystalline type on the physicochemical properties, correlation coefficients were compared between the gelatinization temperature measured using hot stage microscopy and the amylose content, the sectional area swelling percentage, and the swelling power of starches from 6 rice cultivars having the same crystalline type and similar granule sizes (Table 7). Results showed that initial, middle and end temperatures were significantly negatively correlated with the amylose content and positively correlated with the sectional area swelling percentage and the swelling power. A similar negative correlation between the gelatinization temperature and the amylose content has also been reported by Fredriksson *et al.* (30). Differences between correlation coefficients in Table 6 and Table 7 indicated that the granule size and the crystalline type had significant effects on the physicochemical properties of starch.

Table 7. Correlation coefficient between the gelatinization temperature and the amylose content, the sectional area swelling percentage, and the swelling power of starches from 6 rice cultivars

Gelatinization temperature (°C)	AC (%)	ASP_{95} (%)	SP_{95} (g/g)
T_i	-0.884*1)	0.846*	0.959**
T_m	-0.938**	0.828*	0.962**
T_e	-0.907*	0.737	0.932**

1)* and ** indicate significance at $p<0.05$ and $p<0.01$ level, respectively ($n=6$).

In conclusion, starch granule swelling and gelatinization temperatures could be measured using hot stage microscopy. The sectional area swelling percentage of starch was significantly positively correlated with the swelling power. The gelatinization temperature was significantly positively correlated with the pasting temperature and the thermal properties. For rice starches with the same crystalline type and similar granule sizes, the gelatinization temperature was negatively correlated with the amylose content and positively correlated with the swelling power and sectional area swelling percentage.

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