J. Appl. Glycosci., **66**, 51–57 (2019)
doi: 10.5458/jag.jag.JAG-2018_0010

Regular Paper

Physicochemical Properties of Starches from Lotus Rhizomes Harvested in Different Months

(Received October 2, 2018; Accepted December 26, 2018)

(J-STAGE Advance Published Date: January 10, 2019)

Yuji Honda,[1,†] Tetsuya Yamazaki,[1] Naoya Katsumi,[1] Naoko Fujita,[2] Kenji Matsumoto,[1] Masanori Okazaki,[1] and Shoji Miwa[3]

[1]*Faculty of Bioresources and Environmental Science, Ishikawa Prefectural University*
(*1–308 Suematsu, Nonoichi, Ishikawa 921–8836, Japan*)
[2]*Department of Biological Production, Faculty of Bioresource Sciences, Akita Prefectural University*
(*241–438 Kaidobata-Nishi, Shimoshinjo-Nakano, Akita 010–0195, Japan*)
[3]*Ishikawa Agriculture and Forestry Research Center*
(*295–1 Saida, Kanazawa, Ishikawa 920–3198, Japan*)

Abstract: We investigated the physicochemical properties of starches extracted from 8 lotus (*Nelumbo nucifera* Gaertn.) rhizomes harvested in different months (September 2012 to May 2013). The physicochemical properties of the lotus starches depended on the harvest date. The peak viscosity (PV) in the Rapid Visco-Analyser analysis, and the viscosity at 65 °C (V_{65}) in the rotational viscometer analysis were significantly lower in SEP starch (extracted from the September-harvested sample) than in the other lotus starches. The Spearman's rank correlation coefficients of potassium ion (K) content vs. V_{65} and of K content vs. PV were 0.905 and 0.714, respectively, indicating that potassium ions are important for expressing the pasting properties of lotus starch. Principal component analysis suggested that the potassium, magnesium, calcium, and phosphorus contents are important for displaying both the pasting and gelatinization properties of the lotus starches. Meanwhile, the cluster analysis revealed that physicochemical properties of the SEP starch were different from those of the starches harvested in other months.

Key words: lotus, *Nelumbo nucifera* Gaertn., physicochemical properties, rhizome, starch

INTRODUCTION

Plants produce starch as a major intracellular storage material. Starch is located in various organs such as cereal seeds (rice, maize, wheat, barley, sorghum, and others), tubers (potato and yam), roots (cassava, sweet potato, and taro), and the seeds of beans and peas.[1)2)] Starch consists of two polysaccharides, amylose and amylopectin. Amylose is a linear polysaccharide of α-1,4 glycosidic bonded D-glucose with a few α-1,6 branch linkages. Amylopectin also comprises linear α-1,4 glycosidic bonded D-glucose, but is highly branched, with α-1,6 glycosidic linkages from the main chain. The compositions and properties of starch are highly variable, and depend on the plant origin and storage region.[3)]

Lotus (*Nelumbo nucifera* Gaertn.) rhizome is cultivated and distributed in China, Japan, Korea, India, and other Southeast Asian countries.[4)] The rhizome is a widely consumed food ingredient in these countries. In Japan, it is served pickled in vinegar, simmered, fried or sautéed. Starch is among the main components of dry lotus rhizome, so its properties largely determine the texture after cooking. Several studies have reported different physicochemical properties in the starches from lotus rhizome and seed.[5)] We also previously investigated the properties of starches from lotus rhizome.[6)7)]

In Japan, the small lotus rhizome is planted in early spring and the rhizome develops with enlarging floating and upright leaves in summer. The enlarged rhizomes are harvested from late summer to the following spring.[8)] The rhizomes are shipped on the harvest day, because they are gradually damaged by exposure to air. The starch in lotus rhizomes accumulates during development, reaching approximately 20 % of the rhizome dry weight in fully matured plants.[9)] Yang *et al*. reported a positive relation between starch synthesis in lotus and enlargement of the rhizome.[9)] Moreover, the physicochemical properties of starches derived from various plants depend on the growing time and/or harvest date of the plants.[10)11)12)13)14)15)16)17)18)] Thus, we hypothesize that the harvest date influenced the

[†]Corresponding author (Tel. +81–76–227–7453, Fax. +81–76–227–7453, E-mail: honda@ishikawa-pu.ac.jp).

Abbreviations: Am, amylose content; DSC, differential scanning calorimeter; FACE, fluorophore-assisted carbohydrate electrophoresis; ICP-AES, inductively coupled plasma-atomic emission spectroscopy; MV, minimum viscosity; PaT, pasting temperature; PCA, principal component analysis; PeT, peak temperature; PV, peak viscosity; R, granule size; RVA, Rapid Visco-Analyser; S, solubility; SO_{65}, solubility at 65 °C; SP, swelling power; SP_{65}, swelling power at 65 °C; T_o, onset temperature; T_p, peak temperature; V_{65}, Viscosity at 65 °C.

Table 1. Harvest dates of lotus rhizomes and temperatures in Kanazawa City.

Harvest date	Sample name	Average Temperature (°C)
Sep.11th, 2012	SEP	23
Oct.30th, 2012	OCT	13
Dec.11th, 2012	DEC	2.5
Jan.08th, 2013	JAN	5.3
Feb.11th, 2013	FEB	5.5
Mar.11th, 2013	MAR	2.2
Apr. 22nd, 2013	APR	7.2
May 21st, 2013	MAY	18
Average		9.5 ± 7.6

physicochemical properties of lotus starches.

In the present study, we investigate the various physicochemical parameters of starches from lotus rhizome harvested from September 2012 to May 2013. The important factors influencing the expression of pasting and the viscous properties of the lotus starch were elucidated by principal component analysis (PCA) and cluster analysis.

MATERIALS AND METHODS

Materials. Lotus rhizomes of the Shinashirobana cultivar were cultivated in April of 2012, and harvested in Konan Town (Kanazawa City, Ishikawa Prefecture, Japan) from September 2012 to May 2013. The harvest dates of the samples and the temperatures in Kanazawa City are summarized in Table 1. Isoamylase from *Pseudomonas amyloderamosa* was purchased from Hayashibara Co., Ltd. (Okayama, Japan). Other reagents were of analytical grade and were obtained commercially.

Preparation of starch from lotus rhizomes. Starches from the lotus rhizomes were purified as described previously.[7] First and second distal rhizomes of each lotus were used for preparation of starch. Total of 112 ± 25 g of starches were obtained from edible parts of 2 lotus rhizomes (1.7 ± 0.2 kg). The starches were named after their month of harvest, i.e., SEP, OCT, DEC, JAN, FEB, MAR, APR, and MAY as shown in Table 1.

Physicochemical analysis of starches from the lotus rhizomes. The physicochemical parameters of starches from the lotus rhizomes were determined by previously reported methods.[7]

The pasting parameters of the lotus starches (6 %, w/w, dry starch basis, 28.0 g total weight) were obtained using a Rapid Visco-Analyser (RVA; model 3DPLUS; Newport Scientific Ltd., Sydney, Australia). The analysis was performed at the Aichi Center for Industry and Science Technology (Nagoya, Aichi, Japan). The viscosity of the lotus starches (6 %, w/w) at 65 °C was determined with a B8R-type rotational viscometer (Toki Sangyo Co., Ltd., Tokyo, Japan). At first, 6 % (w/w) starch suspension was shaken in a shaking water bath (Personal-11; Taitec, Saitama, Japan) for 30 min at 65 °C. The sample was immediately measured at 5-min intervals to 30 min at 100 rpm. The gelatinization parameters of the lotus starches (5 mg in 20 µL of water) were determined by a differential scanning calorimeter (DSC) (DSC 60; Shimadzu Co., Kyoto, Japan). The apparent amylose contents in the lotus starches were determined by the blue values (absorbance at 680 nm) of the starches. The solubilities and swelling powers of the starches (100 mg, dry weight) at 65 °C were determined as previously described,[19] and were calculated as follows:

Solubility (S, %) = [weight of starch in supernatant (mg) / dry weight of starch sample (mg)] × 100

Swelling power (SP) = 100 × [weight of wet precipitate (mg) / dry weight of starch sample (mg) × (100–S)]

Starch granule size was measured using a laser diffraction particle analyzer (SALD-2200; Shimadzu Co.) after sonication in water. The sizes of the starch particles were determined at 25, 50, and 75 % of the frequency distribution data. The branch chain-length distribution of the amylopectin in the lotus starches was analyzed by fluorophore-assisted carbohydrate electrophoresis (FACE).[20][21]

Phosphorus and mineral contents in the lotus starches. Starch (200 mg) was dissolved in nitric acid (specific gravity = 1.38 g/mL, 60 %, 5 mL) in a conical beaker (100 mL) lidded by a watch glass. The sample solution was heated by an electric griddle at 100 °C for 20 min. The sample was recovered after cooling to room temperature. After diluting the sample solution to 100 mL with distilled water, the phosphorus, calcium, magnesium, potassium, and sodium contents in the samples were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES; ICPE-9000; Shimadzu Co.).

Statistical analysis. The parameters in the present study were compared by the Tukey–Kramer multiple comparison procedure using one-way analysis of variance. Spearman's rank correlation coefficients were evaluated in BellCurve for Excel (Social Survey Research Information Co., Ltd., Tokyo, Japan). The PCA and cluster analysis (Ward's method) of the starch property parameters were also performed in BellCurve for Excel.

RESULTS

Pasting properties in RVA analysis.

To elucidate the pasting properties of the 8 starch samples derived from lotus rhizomes, we first determined the RVA parameters. The results are shown in Table 2. The SEP sample exhibited the highest peak temperature and the lowest peak viscosity among the samples. The peak viscosity, which is important for expressing the pasting property of starch, was higher in the OCT, DEC, and JAN, and MAY samples than in the other samples. The breakdown value and peak viscosity were lowest in SEP, and the setback value was higher in DEC and MAY than in the other samples.

Viscosity, swelling power, and solubility of starch suspension at 65 °C.

The viscosity at 65 °C is important for elucidating the pasting property of the lotus starch, because the starches remained viscous at temperatures above 63 °C in the RVA analysis (see Table 2). Moreover, the viscosity at 65 °C clearly varied among the lotus cultivars in our previous

Table 2. RVA parameters of the starches extracted from lotus rhizomes.

Sample	Number of measurements	Pasting Temperature (°C)	Peak Temperature (°C)	Peak Viscosity (RVU)	Minimum Viscosity (RVU)	Final Viscosity (RVU)	Breakdown (RVU)	Setback (RVU)
SEP	3	66.0 ± 0.0	95.3 ± 0.3^{a}	210 ± 3^{a}	116 ± 3^{a}	171 ± 0^{a}	94 ± 1^{a}	54.5 ± 3^{a}
OCT	2	62.9 ± 0.2	76.5 ± 0.0^{b}	280 ± 1^{b}	91 ± 1^{b}	147 ± 0^{b}	190 ± 0^{b}	56.7 ± 1^{ac}
DEC	4	64.7 ± 0.3	79.6 ± 0.4^{c}	310 ± 2^{c}	116 ± 1^{a}	180 ± 1^{d}	195 ± 1^{b}	64.8 ± 2^{b}
JAN	3	64.7 ± 0.0	77.4 ± 0.2^{b}	282 ± 1^{b}	96 ± 0^{c}	154 ± 0^{c}	186 ± 0^{bc}	58.3 ± 0^{ac}
FEB	3	64.3 ± 0.1	79.0 ± 0.4^{c}	253 ± 1^{bd}	94 ± 1^{bc}	148 ± 2^{b}	159 ± 0^{d}	54.1 ± 1^{ac}
MAR	3	63.9 ± 0.0	77.4 ± 0.3^{bde}	217 ± 2^{a}	78 ± 1^{d}	133 ± 1^{e}	139 ± 1^{e}	54.4 ± 1^{ac}
APR	5	63.0 ± 0.1	76.2 ± 0.2^{bdf}	247 ± 5^{bd}	80 ± 1^{bcd}	138 ± 3^{f}	168 ± 4^{f}	58.4 ± 2^{ac}
MAY	3	62.6 ± 0.3	75.1 ± 0.3^{dg}	287 ± 1^{b}	91 ± 1^{bc}	155 ± 1^{c}	197 ± 2^{b}	64.4 ± 1^{b}
Average	3 ± 1	64.0 ± 1.1	79.6 ± 6.5	261 ± 35	95 ± 14	153 ± 16	166 ± 35	58.2 ± 4.3

Results are the means ± standard deviations of independent measurements (n = 2 − 5). Standard deviation was described for evaluation of the variation. Means with different letters within the same column are significantly different ($P \leq 0.05$).

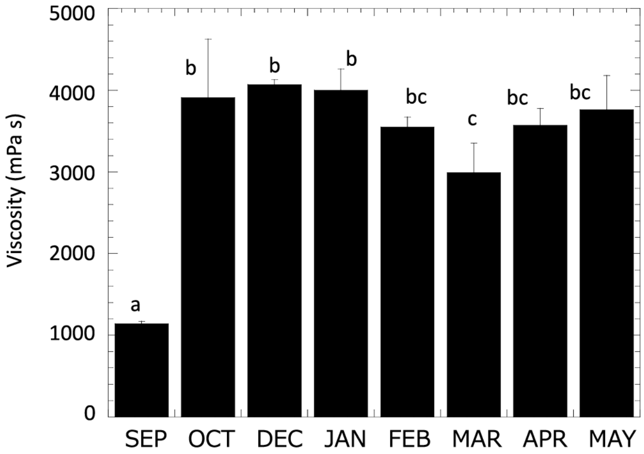

Fig. 1. Viscosity of starches at 65 °C.
The starches were isolated from lotus rhizomes harvested in different months. Different letters indicate significantly different results ($P \leq 0.05$).

study.[7] Figure 1 shows the viscosity of the lotus starches at 65 °C in the present study, determined by the rotary viscometer. The viscosity at 65 °C was over 75 % lower in SEP than in the other starches. The RVA analysis and viscosity at 65 °C support that the pasting property was considerably lower in SEP than in the other samples.

The swelling powers and solubilities of the lotus samples at 65 °C are shown in Table 3, respectively. Several reports have associated the swelling power with the gelatinization and pasting properties of starch.[22][23] In the present study, the swelling power was lowest in SEP and highest in MAY (reaching 60 % in that sample). The solubilities were lower in SEP, DEC, and FEB than in OCT, JAN, MAR, APR, and MAY.

Gelatinization properties.

The gelatinization property is also closely related to the pasting property of starch.[2] Table 4 shows the DSC parameters for determining the gelatinization property of the lotus starches. The peak temperatures were higher in SEP and DEC than in the other samples, but the variation among samples was small (2.5 °C). The enthalpy change ΔH was highest in OCT (17.0, *versus* 10.5–14.5 J/g in the other samples).

Table 3. Swelling power and solubility at 65 °C of the starches extracted from lotus rhizomes.

Sample	Swelling power	Solubility (%)
SEP	14.3±0.2^{a}	3.8±0.4^{a}
OCT	21.0±0.2^{bc}	7.0±0.1^{b}
DEC	18.7±0.3^{bd}	4.5±1.0^{a}
JAN	19.1±0.6^{bc}	6.7±1.8^{b}
FEB	18.6±0.2^{b}	5.6±0.2^{a}
MAR	21.7±0.1^{c}	7.3±0.4^{b}
APR	21.7±0.0^{c}	8.2±0.1^{b}
MAY	24.6±2.0^{e}	8.5±0.7^{b}
Average	20.0±3.0	6.5±1.7

A: Swelling power of starches at 65 °C. The starches were isolated from lotus rhizomes harvested at different dates. **B**: Solubility of the starches at 65 °C. Different letters indicate significantly different results ($P \leq 0.05$).

Table 4. DSC parameters of the starches extracted from lotus rhizomes.

Samples	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
SEP	57.3 ± 0.4^{a}	60.4 ± 0.2^{a}	66.1 ± 0.4	13.3 ± 0.1^{ab}
OCT	54.2 ± 0.4^{b}	58.7 ± 0.2^{b}	66.0 ± 0.4	17.0 ± 2.5^{a}
DEC	57.6 ± 0.3^{a}	60.8 ± 0.2^{a}	65.6 ± 0.3	10.7 ± 0.7^{b}
JAN	57.0 ± 0.3^{c}	60.1 ± 0.4^{ac}	66.4 ± 1.1	10.5 ± 0.8^{b}
FEB	56.8 ± 0.3^{a}	60.0 ± 0.1^{c}	65.9 ± 0.7	12.0 ± 0.9^{b}
MAR	55.7 ± 0.3^{c}	59.5 ± 0.2^{c}	65.3 ± 1.0	14.0 ± 1.0^{ab}
APR	54.4 ± 0.5^{b}	59.0 ± 0.2^{b}	66.1 ± 1.4	14.5 ± 2.0^{ab}
MAY	54.5 ± 0.6^{b}	58.3 ± 0.2^{b}	64.2 ± 0.4	11.8 ± 0.2^{b}
Average	55.9 ± 1.4	59.6 ± 0.9	65.7 ± 0.7	13.0 ± 2.2

Results are the means ± standard deviations of independent measurements (n = 3). Means with different letters in the same column are significantly different ($P \leq 0.05$).

Phosphorus and cation contents.

Several RVA-based studies have reported a positive correlation between the viscosity and phosphorus content of potato starch.[23][24] Lotus starch also contains approximately 50 ppm of phosphorus.[7][25] Divalent cations such as calcium and magnesium can interact with phosphorus groups in the

Table 5. Cation and phosphorus contents in the starches extracted from lotus rhizomes.

Samples	Ca (ppm)	K (ppm)	Mg (ppm)	Na (ppm)	P (ppm)
SEP	24.8 ± 1.0[a]	162 ± 5[a]	3.5 ± 0.2[a]	80 ± 0 [a]	43.9 ± 2.0[a]
OCT	31.8 ± 1.2[b]	262 ± 2[b]	5.6 ± 0.3[b]	135 ± 2[b]	56.3 ± 2.1[b]
DEC	25.2 ± 0.2[a]	289 ± 4[c]	6.3 ± 0.0[c]	61 ± 0[c]	73.6 ± 0.5[c]
JAN	54.8 ± 0.0[c]	436 ± 4[d]	17 ± 0.0[d]	85 ± 1[d]	94.4 ± 1.1[d]
FEB	45.8 ± 0.2[d]	214 ± 3[e]	6.3 ± 0.0[c]	118 ± 2[e]	78.8 ± 0.6[e]
MAR	29.2 ± 0.2[e]	171 ± 4[f]	6.6 ± 0.1[c]	86 ± 1[d]	70.1 ± 1.5[cf]
APR	45.8 ± 0.2[d]	262 ± 2[b]	6.2 ± 0.0[ce]	84 ± 0[d]	69.3 ± 0.6[f]
MAY	20.0 ± 0.1[f]	256 ± 3[b]	2.8 ± 0.1[f]	72 ± 1[f]	50.7 ± 2.0[g]
Average	34.7 ± 12.5	257 ± 85	6.7 ± 4.2	90 ± 24	67.1 ± 16.3

Results are the means ± standard deviations of independent measurements (n = 3). Means with different letters within the same column are significantly different ($P \leq 0.05$).

glucose residues of the amylopectin component of potato starch, reducing the pasting property of the starch.[24] Meanwhile, monovalent cations such as potassium and sodium are abundant in water, which is used in the starch purification process.[26] High concentrations of sodium ions reduce the peak viscosity of starches in RVA analyses.[27] Thus, the contents of phosphorus and divalent/monovalent cations are important for elucidating the pasting properties of lotus starches. Here, we determined the phosphorus, calcium, magnesium, potassium, and sodium contents in the starches by ICP–AES (Table 5). The JAN sample was richer in calcium, potassium, magnesium, and phosphorus than the other samples. The phosphorus and potassium contents were lower in SEP than in the other lotus starches.

Structure of the lotus starches.

The apparent amylose content is indispensable for determining the swelling, gelatinization, and pasting properties of starch, because these properties depend on the amylopectin content.[28][29][30] The apparent amylose contents in the lotus starches are presented in Table S1; see J. Appl. Glycosci. Web site. The contents ranged from 24 to 28 %, being lowest in SEP and slightly higher in JAN, FEB, MAR, APR, and MAY than in the other starches.

The branch-chain distribution of the amylopectin component also affects the gelatinization and pasting properties of starch.[31] In previous studies, increasing the degree of polymerization (DP) of amylopectin from 16 to 25 increased the onset, peak, and conclusion temperatures in the DSC analysis.[32][33][34] RVA analyses have revealed a negative correlation between low proportions of branch chains (DP < 13) and pasting temperatures.[32][33] DSC analysis have also indicated a negative correlation between low proportions of branch chains (DP < 13) and onset temperatures.[34]

Thus, we cleaved the α-1,6 linkages in the amylopectin of the lotus starches and determined the branch-chain distribution in the starches by FACE. The results are presented in Table S2; see J. Appl. Glycosci. Web site. The DP 5–12 values were slightly higher in OCT and JAN than in the other starches, whereas the DP 25–36 was highest in SEP (by a small margin). The DP 13–24 and DP 37–60 values were similar among the studied starches.

Table 6. Spearman's rank correlation coefficients of viscosity at 65 °C, peak viscosity, peak temperature, and swelling power at 65 °C.

V_{65}	PV	T_p	SP_{65}
BD (0.933)	BD (0.923)	PaT (0.958)	SO_{65} (0.976)
K (0.905)	V_{65} (0.905)	T_o (0.927)	$R_{25\%}$ (0.738)
PV (0.904)	K (0.714)	PeT (0.910)	$R_{50\%}$ (0.714)
DP13 (0.738)	SB (0.714)	MV (0.755)	$R_{75\%}$ (0.714)
SB (0.714)	DP37 (–0.762)	SP_{65} (–0.810)	T_o (–0.714)
DP37 (–0.714)		SO_{65} (–0.881)	DP37 (–0.738)
			MV (–0.802)
			T_p (–0.810)
			PaT (–0.850)
			PeT (–0.934)

Abbreviations are defined in Fig. 2. Data with correlation coefficients exceeding ± 0.700 were extracted from Table S4.

Starch properties such as viscosity and gelatinization are known to depend on granule size.[35][36] In a study of lotus starch in China, the granule size positively influenced the swelling power of the starch, and negatively influenced its peak temperature, conclusion temperature and amylose content.[37] Table S3; see J. Appl. Glycosci. Web site, shows the granule sizes in the 25, 50, and 75 % cumulative distributions of the lotus starch particles. The average granule size of the samples ranged from 38.9 to 49.2 µm. Multimodality was not observed in the analysis. The OCT, MAR and MAY particles were approximately 10 µm larger than the other starch particles, and the 25 and 75 % size distributions were also larger in these three samples. In contrast, the 25, 50 and 75 % distributions of the granules were smaller-sized in the SEP starch than in the other lotus starches.

Spearman's rank correlation coefficients.

To elucidate the relationships among the 28 above-determined parameters, we determined the Spearman's rank correlation coefficients of the paired parameters (Table S4; see J. Appl. Glycosci. Web site). The viscosity at 65 °C (V_{65}) in the rotational viscometer analysis and peak viscosity (PV) obtained in the RVA analysis are considered to largely influence the pasting property of the lotus starches, whereas the peak temperature T_p and swelling power at 65 °C (SP_{65}) are indispensable for their gelatinization properties.[38] Therefore, we compared the Spearman's rank correlation coefficients of V_{65}, PV, T_p, and SP_{65} with absolute values exceeding 0.700 (Table 6). The V_{65} and PV were positively correlated with the breakdown (*BD*) values and K contents of the starches, and negatively correlated with DP37. The T_p was strongly correlated with the onset temperature T_o, the pasting temperature PaT, and the peak temperature PeT (with correlation coefficients exceeding 0.900), and negatively correlated with SP_{65} and SO_{65} (with correlation coefficients of –0.810 and –0.881, respectively). The DP37, PaT, PeT, minimum viscosity MV, T_o, and T_p were negatively correlated with SP_{65}, while the SO_{65} and granule sizes ($R_{25\%}$, $R_{50\%}$, and $R_{75\%}$) were positively related to SP.

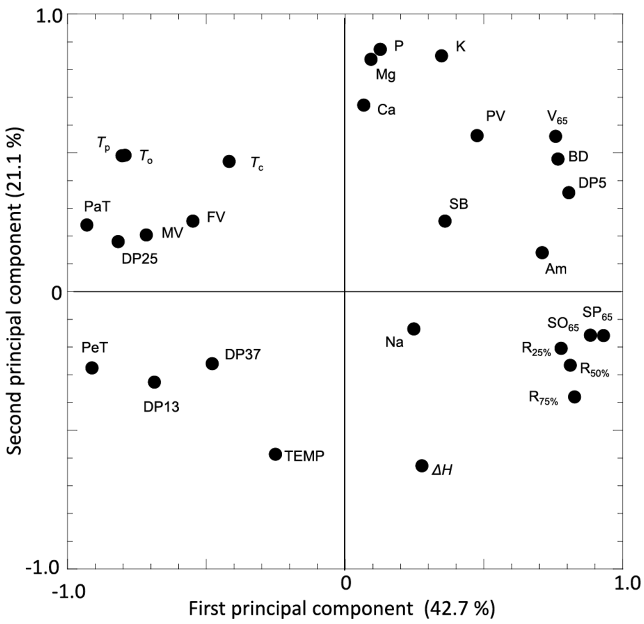

Fig. 2. Principal component analysis: loading plots of PC1 and PC2 showing the variations among the properties of starches in lotus rhizomes harvested in different months.

Variables are defined as follows: TEMP, temperature on the harvest day; PaT, pasting temperature; PeT, peak temperature; PV, peak viscosity; MV, minimum viscosity; FV, final viscosity; BD, breakdown; SB, setback; V_{65} , viscosity at 65 °C; SP_{65}, swelling power at 65 °C; SO_{65}, solubility at 65 °C; T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH, enthalpy change; P, phosphate content; Na, sodium content; Ca, calcium content; K, potassium content; Mg, magnesium content; Am, amylose content, DP5, $DP_{5–12}$; DP13, $DP_{13–24}$; DP25, $DP_{25–36}$; DP37, $DP_{37–60}$; $R_{25\%}$, 25 % granule size; $R_{50\%}$, 50 % granule size (average granule size); $R_{75\%}$, 75 % granule size.

Principle component analysis of the starch properties.

PCA reveals the overall similarities and differences among the lotus-starch samples. Figure 2 is a loading plot of the first and second principal components (PC1 and PC2, respectively) of the 8 starches. The PC1 and PC2 explained 42.7 and 21.1 % of the overall variation, respectively. Moreover, the SP_{65}, SO_{65}, $R_{75\%}$, $R_{50\%}$, DP5, $R_{25\%}$, BD, V_{65}, and amylose content (Am) exceeded 0.5 in PC1, suggesting that these parameters contribute significantly to the pasting properties and gelatinization of the lotus starches. In contrast, PaT, PeT, DP37, T_p, T_o, MV, DP25, and FV locate at negative values of PC1, indicating that these variables negatively influence the pasting properties and gelatinization. The significant variables in PC2, with positive values above 0.5, were P, K, Mg, Ca, PV, and V_{65}. These results suggest that the mineral contents in the lotus starches positively influence the pasting and gelatinization properties of the starches. Figure 3 is a score plot of the lotus starches. The OCT, JAN, MAR, and MAY samples scored positively along PC1, whereas the SEP, DEC, and FEB scored negatively along this component. In the cluster analysis, the SEP sample was significantly separated from the other lotus starches (Fig. S1; see J. Appl. Glycosci. Web site), suggesting that the physicochemical properties differed between SEP and the other starches.

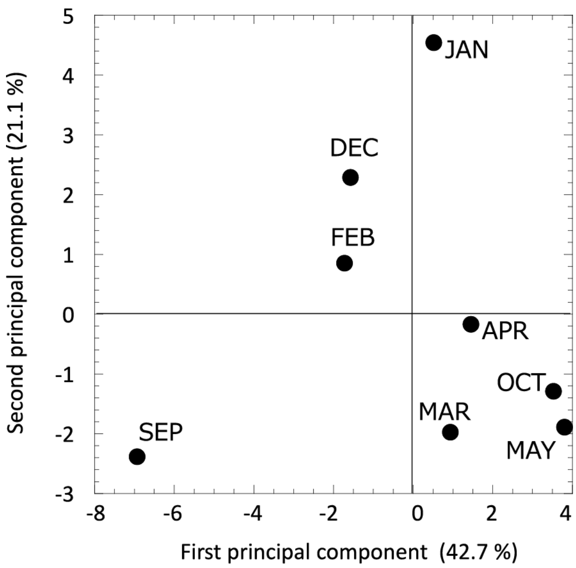

Fig. 3. Principal component analysis: score plot of PC1 and PC2 showing the variations among the properties of the starches in lotus rhizomes harvested at different dates.

DISCUSSION

Herein, we determined the physicochemical properties of lotus starches harvested from September 2012 to May 2013. The physicochemical properties were found to depend on the harvest date. The peak viscosity in the RVA analysis and viscosity at 65 °C in the rotational viscometer analysis were much lower in SEP than in the other lotus starches. Also, SEP yielded the highest peak temperature in the RVA analysis. These results indicate a much lower pasting property in the SEP starch than in the other lotus starches. In various previous studies, the pasting properties of starches from potatoes, yams, and water caltrops significantly depended on growth time.[13)14)15)16)17)18)] In an RVA analysis of potato starch, the peak viscosity gradually increased with increasing growth time of the potatoes.[14)] The authors reported that the phosphorus and apparent amylose contents are important factors of viscosity expression. In the present study, the Spearman's rank correlation coefficients for the peak viscosity of lotus starch indicated a greater influence of the K content than the amylose and phosphorus contents (Table 6 and Table S4; see J. Appl. Glycosci. Web site). The correlation coefficient between V_{65} and K was 0.905, suggesting that K is a dominant factor for expressing the pasting properties of lotus starches.

Jane investigated the swelling power and solubility of starch under the effects of various ions, dividing the monatomic ions into two classes based on the Hofmeister series.[39)] The first class included salting-out (structure making) ions such as K^+ and F^-, the second comprised salting-in (structure breaking) ions such as Na^+ and Li^+.[40)] The swelling power and solubility of potato starch containing 0.1 M cationic solution decreased in the order $Li^+ > Na^+ > K^+$, consistent with the Hofmeister series.[40)] The thermal parameters for gelatinization (T_p and ΔH) also increased along the Hofmeister series. In the present study, the K was well correlated with V_{65} (0.905) and PV (0.714), and lowly correlated with T_p, ΔH, SP_{65}, and SO_{65}. As described above, K classifies as a structure-making ion along the Hofmeister series. In the present study, the K contents of the lotus starches ranged from 162 to 436 ppm (see Table 5), lower than reported in previous studies. However, we consider that the K^+ play an important role in the physicochemical properties of lotus starch. We speculated that K prevents water molecules in the solvent from penetrating the internal starch, lowering its swelling power and solubility. The JAN lotus starch, with the highest K content, expressed higher values of SP_{65}, SO_{65}, V_{65}, and peak viscosity than the other starches. The structure-making effect of K^+ was not reflected in the pasting and thermal properties of the lotus starches, indicating that the Hofmeister series was inapplicable to our results. On the other hand, the values of P, K, Mg, and Ca exceeded 0.6 in PC2 (see Fig. 2), suggesting that these cations exert a considerable collective effect on the expressed starch properties. The relationship between K^+ and the properties of lotus starch should be elucidated in further analysis.

As described above, the peak viscosity was lower in the SEP sample than in other lotus starches, lowering the pasting properties of this sample. We consider that the decrease derives from the shorter growing time of lotus rhizomes in September than in the other months. Several RVA analyses have reported a gradual increase in peak viscosity with growing time [14)15)16)17)18)]. The starch properties of lotus rhizomes are probably changed by regulation of the enzymes involved in starch synthesis (sucrose synthase, glucose-1-phosphate uridylyltransferase, adenosine diphosphate-glucose pyrophosphorylase, granule-bound starch synthase, soluble starch synthase, starch branching enzyme, and starch debranching enzyme). Lotus internodes develop through four stages: the stolon stage (elongation in a single direction), initial swelling (cessation of longitudinal growth and initiation of girth increase), middle swelling (continued swelling with gradual starch accumulation), and later swelling (cessation of enlargement with rapid starch accumulation).[9)] Indeed, starch accumulation has been confirmed in lotus rhizome cultivated in China, indicating that the starch content increases in each growth stage.[9)] Moreover, transcriptomic analysis has revealed changes in the gene expression patterns as the lotus rhizomes develop.[9)] In the present study, the developmental stage of the SEP lotus was midway between the middle swelling and later swelling stages. Thus, we speculated that starch synthesis was incomplete in the SEP lotus rhizome, and complete in the other lotus starches. In immature lotus rhizomes that are still synthesizing starch, the pasting properties should be lower than in mature rhizomes. By genetically analyzing the starch-synthesis enzymes in lotus, we could better understand the different pasting properties of starches from lotus rhizomes.

We newly report the physicochemical properties of starches from lotus rhizomes harvested on different dates. The harvest date influenced the physicochemical properties of the lotus starches. Especially, the pasting properties were lower in the September-harvested sample than in the samples harvested in other months, while the other parameters were not significantly changed. The statistical analysis revealed that phosphorus, calcium, magnesium, and potassium are important for expressing the pasting property. Further analysis of ionic effects on lotus starch would elucidate how ions influence the properties of lotus starch.

CONFLICTS OF INTEREST

The authors declare no conflict of interests.

ACKNOWLEDGMENTS

We are grateful to Mr. Tohru Miyano for providing the lotus samples and Ms. Fumi Kawashima for her technical assistance during the course of this study.

REFERENCES

1) S.C. Zeeman, J. Kossmann, and A.M. Smith: Starch: its metabolism, evolution, and biotechnological modification in plants. *Annu. Rev. Plant Biol.*, **61**, 209–234 (2010).
2) Y. Ai and J.l. Jane: Gelatinization and rheological properties of starch. *Starch–Stärke*, **67**, 213–224 (2015).
3) N. Singh, J. Singh, L. Kaur, N.S. Sodhi, and B.S. Gill: Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chem.*, **81**, 219–231 (2003).
4) Y. Zhang, X. Lu, S. Zeng, X. Huang, Z. Guo, Y. Zheng, Y. Tian, and B. Zheng: Nutritional composition, physiological functions and processing of lotus (*Nelumbo nucifera* Gaertn.) seeds: a review. *Phytochem. Rev.*, 1–14 (2015).
5) F. Zhu: Structures, properties, and applications of lotus starches. *Food Hydrocoll.*, **63**, 332–348 (2017).
6) Y. Honda, T. Mishima, H. Koga, K. Matsumoto, Y. Maeda, F. Kawashima, M. Okazaki, and S. Miwa: Physicochemical properties of starch from Kaga lotus rhizome. *J. Appl. Glycosci.*, **61**, 27–29 (2014).
7) T. Yamazaki, N. Katsumi, N. Fujita, K. Matsumoto, M. Okazaki, S. Miwa, and Y. Honda: Physicochemical properties of starches from different lotus cultivars in Japan: Shinashirobana cultivar and Kanasumi-line No. 20. *J. Appl. Glycosci.*, **63**, 61–68 (2016).
8) J.-I. Masuda, T. Urakawa, Y. Ozaki, and H. Okubo: Short photoperiod induces dormancy in lotus (*Nelumbo nucifera*). *Ann. Bot.*, **97**, 39–45 (2006).
9) M. Yang, L. Zhu, C. Pan, L. Xu, Y. Liu, W. Ke, and P. Yang: Transcriptomic analysis of the regulation of rhizome formation in temperate and tropical lotus (*Nelumbo nucifera*). *Sci. Rep.*, **5**, 13059 (2015).
10) M. Asaoka, J. Blanshard, and J. Rickard: Effects of cultivar and growth season on the gelatinisation properties of cassava (*Manihot esculenta*) starch. *J. Sci. Food Agric.*, **59**, 53–58 (1992).
11) T. Noda, Y. Takahata, and T. Nagata: Developmental changes in properties of sweet potato starches. *Starch-Stärke*, **44**, 405–409 (1992).
12) T. Noda, Y. Takahata, T. Sato, H. Ikoma, and H. Mochida: Combined effects of planting and harvesting dates on starch properties of sweet potato roots. *Carbohydr. Polym.*,

33, 169–176 (1997).

13) K. Sriroth, V. Santisopasri, C. Petchalanuwat, K. Kurotjanawong, K. Piyachomkwan, and C.G. Oates: Cassava starch granule structure-function properties: influence of time and conditions at harvest on four cultivars of cassava starch. *Carbohydr. Polym.*, **38**, 161–170 (1999).
14) Q. Liu, E. Weber, V. Currie, and R. Yada: Physicochemical properties of starches during potato growth. *Carbohydr. Polym.*, **51**, 213–221 (2003).
15) T. Noda, S. Tsuda, M. Mori, S. Takigawa, C. Matsuura-Endo, K. Saito, W.H.A. Mangalika, A. Hanaoka, Y. Suzuki, and H. Yamauchi: The effect of harvest dates on the starch properties of various potato cultivars. *Food Chem.*, **86**, 119–125 (2004).
16) C.-C. Huang, M.-C. Lin, and C.-C. Wang: Changes in morphological, thermal and pasting properties of yam (*Dioscorea alata*) starch during growth. *Carbohydr. Polym.*, **64**, 524–531 (2006).
17) P.-Y. Chiang, P.-H. Li, C.-C. Huang, and C.-C. Wang: Changes in functional characteristics of starch during water caltrop (*Trapa Quadrispinosa* Roxb.) growth. *Carbohydr. Polym.*, **104**, 376–382 (2007).
18) C.-C. Wang, P.-Y. Chiang, P.-H. Li, and C.-C. Huang: Physicochemical properties of water caltrop (*Trapa taiwanensis* Nakai) starch during growth period. *Carbohydr. Polym.*, **71**, 310–315 (2008).
19) K. Kainuma, T. Oda, and S. Suzuki: Study of starch phosphate monoester. *J. Technol. Soc. Starch*, **14**, 24–28 (1967). (in Japanese).
20) M.G. O'Shea and M.K. Morell: High resolution slab gel electrophoresis of 8-amino-1,3,6-pyrenetrisulfonic acid (APTS) tagged oligosaccharides using a DNA sequencer. *Electrophoresis*, **17**, 681–686 (1996).
21) N. Fujita, H. Hasegawa, and T. Taira: The isolation and characterization of a waxy mutant of diploid wheat (*Triticum monococcum* L.). *Plant Sci.*, **160**, 595–602 (2001).
22) S. Akuzawa and A. Kawabata: Relationship among starches from different origins classified according to their physicochemical properties. *J. Appl. Glycosci.*, **50**, 121–126 (2003).
23) S. Srichuwong, T.C. Sunarti, T. Mishima, N. Isono, and M. Hisamatsu: Starches from different botanical sources II: Contribution of starch structure to swelling and pasting properties. *Carbohydr. Polym.*, **62**, 25–34 (2005).
24) D.P. Wiesenborn, P.H. Orr, H.H. Casper, and B.K. Tacke: Potato starch paste behavior as related to some physical/chemical properties. *J. Food Sci.*, **59**, 644–648 (1994).
25) A. Suzuki, M. Kaneyama, K. Shibanuma, Y. Takeda, J. Abe, and S. Hizukuri: Characterization of lotus starch. *Cereal Chem.*, **69**, 309–315 (1992).
26) I. Zaidul, N. Norulaini, A. Omar, H. Yamauchi, and T. Noda: Correlations of the composition, minerals, and RVA pasting properties of various potato starches. *Starch-Stärke*, **59**, 269–276 (2007).
27) L. Day, C. Fayet, and S. Homer: Effect of NaCl on the thermal behaviour of wheat starch in excess and limited water. *Carbohydr. Polym.*, **94**, 31–37 (2013).
28) T. Sasaki and J. Matsuki: Effect of wheat starch structure on swelling power. *Cereal Chem.*, **75**, 525–529 (1998).
29) T. Sasaki, T. Yasui, and J. Matsuki: Effect of amylose content on gelatinization, retrogradation, and pasting properties of starches from waxy and nonwaxy wheat and their F1 seeds. *Cereal Chem.*, **77**, 58–63 (2000).
30) R. Juhász and A. Salgó: Pasting behavior of amylose, amylopectin and their mixtures as determined by RVA curves and first derivatives. *Starch-Stärke*, **60**, 70–78 (2008).
31) J. Jane, Y.Y. Chen, L.F. Lee, A.E. McPherson, K.S. Wong, M. Radosavljevic, and T. Kasemsuwan: Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chem.*, **76**, 629–637 (1999).
32) Y. Nakamura, A. Sakurai, Y. Inaba, K. Kimura, N. Iwasawa, and T. Nagamine: The fine structure of amylopectin in endosperm from Asian cultivated rice can be largely classified into two classes. *Starch-Stärke*, **54**, 117–131 (2002).
33) T. Noda, Y. Nishiba, T. Sato, and I. Suda: Properties of starches from several low-amylose rice cultivars. *Cereal Chem*, **80**, 193–197 (2003).
34) S. Srichuwong, T.C. Sunarti, T. Mishima, N. Isono, and M. Hisamatsu: Starches from different botanical sources I: Contribution of amylopectin fine structure to thermal properties and enzyme digestibility. *Carbohydr. Polym.*, **60**, 529–538 (2005).
35) E. Chiotelli and L.M. Martine: Effect of small and large wheat starch granules on thermomechanical behavior of starch. *Cereal Chem.*, **79**, 286–293 (2002).
36) S. Dhital, A.K. Shrestha, J. Hasjim, and M.J. Gidley: Physicochemical and structural properties of maize and potato starches as a function of granule size. *J. Agric. Food Chem.*, **59**, 10151–10161 (2011).
37) L. Lin, J. Huang, L. Zhao, J. Wang, Z. Wang, and C. Wei: Effect of granule size on the properties of lotus rhizome C-type starch. *Carbohydr. Polym.*, **134**, 448–457 (2015).
38) P. Steeneken: Rheological properties of aqueous suspensions of swollen starch granules. *Carbohydr. Polym.*, **11**, 23–42 (1989).
39) J.L. Jane: Mechanism of starch gelatinization in neutral salt solutions. *Starch-Stärke*, **45**, 161–166 (1993).
40) H. Zhou, C. Wang, L. Shi, T. Chang, H. Yang, and M. Cui: Effects of salts on physicochemical, microstructural and thermal properties of potato starch. *Food Chem.*, **156**, 137–143 (2014).