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Physicochemical Properties of Starches from Different Lotus Cultivars in Japan: Shinashirobana Cultivar and Kanasumi-line No. 20

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Abstract: We investigated the physicochemical properties of starches from rhizomes of two lotus (*Nelumbo nucifera* Gaertn.) cultivars, Shinashirobana cultivar and Kanasumi-line No. 20, harvested at the same farm in Japan. A rapid visco analyser analysis indicated that pasting temperatures of the starches from Shinashirobana cultivar and Kanasumi-line No. 20 were 62.1 and 66.5 °C, respectively, and the peak viscosities of the starch of both lotuses were similar 244 and 240 RVU, respectively. The starch from the Kanasumi-line No. 20 at 60 °C had low viscosity, 16.2 mPa·s, but the starch from Shinashirobana cultivar had higher viscosity, 2,720 mPa·s, at 60 °C. The onset and peak temperatures of the differential scanning calorimetry (DSC) curve of the starch from Shinashirobana cultivar were 4–5 °C lower than those of the starch from Kanasumi-line No. 20, although both exhibited similar *ΔH* values (13.4 and 11.6 J/g). The distribution patterns of the branched chain lengths of amylopectin in the two starches indicated that the rate of low-degree of polymerization (DP 5–12) of all branched chains was higher for the starch from Shinashirobana cultivar than that for the starch from Kanasumi-line No. 20. These results suggest that the increased rate of DP 5–12 in the starch from Shinashirobana cultivar is related to the higher viscosity of the starch.

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

INTRODUCTION

Lotus (*Nelumbo nucifera* Gaertn.) rhizome is widely cultivated and distributed in China, Japan, Korea, India, and other Southeast Asian countries.[1)] The rhizomes are mainly consumed as an edible vegetable in these countries. In Japan, lotus rhizome is an important vegetable ingredient of traditional Japanese dishes (Washoku),[2)] such as Tempura (a batter-fried food in Japan), vinegared dishes, sautéed dishes, simmered dishes, and Su-age (naked frying), respectively.

Lotuses cultivated in Japan are categorized into at least 10 types of cultivars on the basis of their morphological characteristics.[3)] Kanasumi-line No. 20 is one of the most popular lotus cultivars in the Ibaraki prefecture, which is a major production area in Japan.[4)] On the other hand, the Shinashirobana cultivar is a major cultivar in the Ishikawa prefecture, where it is called "Kaga lotus." One of the characteristics of the texture of the Shinashirobana cultivar is a greater stickiness than that of lotuses cultivated in other prefectures. Our previous report indicated that the viscosity of the starch obtained from the Kaga lotus (Shinashirobana cultivar) was higher than that of the starch from lotuses cultivated in the Ibaraki prefecture.[5)] According to previous studies on starches from various botanical sources, geography, climate, and growing environment are related to the physicochemical properties of starches.[6)7)8)9)] Therefore, we decided to investigate the differences in starch viscosity between Shinashirobana cultivar and other lotus cultivars cultivated at the same farm to exclude the other factors described above.

In the present study, we investigated the physicochemical properties of starches from lotus rhizomes of Shinashirobana cultivar and Kanasumi-line No. 20 line harvested at the same farm.

MATERIALS AND METHODS

Materials. The lotus cultivars, Shinashirobana cultivar and Kanasumi-line No. 20, were harvested in Konan-town, Kanazawa-city, Ishikawa prefecture in October 2013 after cultivation in April 2013. Isoamylase from *Pseudomonas*

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Abbreviations: DP, degree of polymerization; DSC, differential scanning calorimetry; FACE, fluorophore-assisted carbohydrate electrophoresis; FWHM, full-width at half maximum; RVA, rapid visco analyser; SSIIa, starch synthase IIa; XRD, X-ray diffraction.

amyloderamosa was purchased from Hayashibara Co., Ltd. (Okayama, Japan). Starches derived from corn and potato were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Other reagents were of analytical grade and were obtained commercially.

Preparation of starch from lotuses. Starches from the lotus rhizomes were purified as described previously.[5)10)] Peeled rhizomes were chopped into small pieces and then homogenized in ice-cold water. The homogenates were squeezed in ice-cold water by using double-layered gauze. Next, the extract was left for 3 h at 4 °C. The precipitate was collected after decantation of the slurry supernatant. The precipitate was washed with ice cold water by centrifugation (3,000 × *G* at 4 °C). The starch was dried on a filtration paper, and then dried over P_2O_5 under reduced pressure. A total of 41 g of starch was obtained from 1.8 kg of Shinashirobana cultivar rhizomes. A total of 63 g of starch was obtained from 2.1 kg of Kanasumi-line No. 20 lotus rhizomes. These samples were analyzed without further purification.

Total starch contents. Lotus rhizomes were lyophilized after slicing of the lotus rhizomes. The lyophilized lotus rhizome powder was prepared by grinding with a mortar. Total starch contents of lyophilized lotus rhizomes were determined using a Total Starch assay kit (Megazyme, Wicklow, Ireland) in accordance with the manual.

Rapid visco analyser. Pasting properties were analyzed by using a Rapid Visco-Analyser (RVA; model 3DPLUS; Newport Scientific Ltd., Sydney, Australia). A pasting curve of starch was monitored with starch-water suspensions (6%, w/w, dry starch basis, 28.0 g total weight). The suspension was analyzed as follows: heating from 50 to 90 °C at 6 °C/min (after an equilibration time of 1 min at 50 °C), a holding period at 95 °C for 5 min, cooling from 95 to 50 °C at 6 °C/min and a holding phase at 50 °C for 2 min. The constant rotating speed of the paddle was 160 rpm. The analysis was performed at the Aichi Center for Industry and Science Technology (Nagoya, Aichi, Japan).

Rotational viscometer. Viscosities of the starches were determined using a B8R-type rotational viscometer (Toki Sangyo Co., Ltd., Tokyo, Japan) connected with an HH-type small adaptor (HH-1, φ 16 × 34 mm, and an HH-2, φ 11 × 40 mm; Toki Sangyo Co., Ltd.) at 60 and 65 °C. At first, 6 % (w/w) starch suspension was shaken in a shaking water bath (Personal-11; Taitec, Saitama, Japan) for 30 min at 60 or 65 °C. The sample was immediately injected into the sample adaptor, then a value of the θ value was measured at 5-min intervals to 30 min at 100 rpm. The shear rate in the analysis of starch from Kanasumi-line No. 20 at 60 °C was 99.8 s^{-1} for the HH-1 adaptor. The shear rate in the other analyses of starches was 33.4 s^{-1} with the HH-2 adaptor. Viscosity (η, mPa·s) was calculated by using the following equation according to the manual.

$$\eta = K \times \theta / 100$$

where *K* is a multiplying constant for the instrument; *K* = 10 for the HH-1 and *K* = 50 for the HH-2.

Solubility and swelling power. The solubilities of the starches were determined according to previously reported methods.[11)] The starch (100 mg, dry weight) was suspended in distilled water in a screw-capped plastic tube (15 mL). The suspension was shaken in a shaking water bath for 1 h at 60 or 65 °C. After cooling to room temperature, the sample was centrifuged for 30 min at 20 °C (1,830 × *G*). The supernatant was recovered by decantation, and the sample solution was dried on an aluminum cup for 3 h at 110 °C. The dried sample and wet precipitate were weighed, and solubility and swelling power were determined as described below.

$$\text{Solubility (\%, S)} = [\text{Starch in supernatant (mg)} / \text{Sample of starch (dry weight, mg)}] \times 100$$

$$\text{Swelling power (S.P.)} = 100 \times [\text{Wet precipitate (mg)} / \text{Sample of starch (dry weight, mg)} \times (100\text{-S})]$$

Differential scanning calorimetry. The gelatinization properties of starches were analyzed by using a differential scanning calorimeter (DSC 60; Shimadzu Co., Kyoto, Japan). Starch (5 mg) was loaded onto an aluminum cell, and then 20 μL of water was added. The cell was left for 18 h at 25 °C after sealing the cell. An empty aluminum cell was used as the reference. The scanning temperature range was between 30 °C and 160 °C, and the heating rate was 1 °C/min. Thermodynamic parameters were determined from the DSC curve.

Starch apparent amylose content. The blue value (absorbance at 680 nm) of starch (4 mg) was determined in 0.002 % iodine and 0.02 % potassium iodide solution. The apparent amylose content of lotus starch was calculated as described previously.[12)]

Inorganic phosphorus content in starch. Starch (200 mg) was dissolved in nitric acid (5 mL) in a conical beaker (100 mL), with a watch glass as a lid. The sample solution was heated at 100 °C for 20 min. After cooling to room temperature, the sample was recovered and neutralized with 1 M NaOH. The sample solution was diluted to 100 mL with distilled water. Phosphorus content was determined by molybdenum blue spectrophotometric analysis.[13)14)15)] The color reagent for the analysis was prepared as follows: Reagent A consisted of hexaammonium heptamolybdate tetrahydrate (2.4 g) and bis[(+)-tartrato]diantimonate(III) dipotassium trihydrate (0.096 g) that were mixed in 120 mL of distilled water. After adding 32 mL of conc. H_2SO_4 carefully, the solution was diluted to 200 mL with distilled water. Reagent B consisted of a L-ascorbic acid (72 g/L) solution. Reagent A (5 volumes) and B (1 volume) were mixed before the experiment. The sample solution (10 mL) and 2 mL of potassium peroxodisulfate solution (40 g/L) were mixed in a phosphorus and nitrogen test bottle (Sansyo Co., Ltd., Tokyo, Japan). The bottle was autoclaved for 30 min at 120 °C. After cooling the sample bottle, 5 mL of the sample solution, and 0.4 mL of the color reagent were mixed and left for 15 min at 30 °C. The absorbance of the sample solution was measured at 880 nm for determination of the phosphate ion concentration by comparison with the absorbance of a standard potassium dihydrogen phosphate solution. Total phosphorus concentration was calculated as

follows:

Total phosphorus concentration = phosphate ion concentration × 0.326.

Optical microscopy. Suspensions of starch were fixed on microscope glass slides and covered with a cover slip. The granule shape and Maltese cross of the lotus starches were observed using a polarized microscope ($\lambda_{530\ nm}$) (MT9300L; Meiji Techno Co., Ltd., Saitama, Japan). For observation of the disappearance of Maltese cross of the starch granules with increasing temperature, a micro heat plate (MP-1000H; Kitazato science Co., Ltd, Shizuoka, Japan) equipped on the microscope was used.[16)17)] The temperature in the experiment was changed as follows: 25→50 °C (5 °C/min), and 50→80 °C (1 °C/min).

X-ray diffraction (XRD) analysis. XRD patterns of starch were analyzed using a Rigaku Miniflex X-ray diffractometer (Rigaku Co., Tokyo, Japan). The starches were placed under an atmosphere of saturated NaCl solution in a desiccator for 18 h at 28 °C before the analysis. The measurement settings were as follows: CuKα tube, 30 kV, 15 mA; scanning speed, 2°/min; diffraction angle (2θ), 3.0–45.0°, step size 0.1°. The relative crystallinity and crystalline indexes of the starches were determined by using previously described methods.[18)19)]

Particle size distribution. Starch granule size was measured using a laser diffraction particle analyzer (SALD-2200; Shimadzu Co., Ltd., Kyoto, Japan) after sonication in water. The size was determined at 25, 50, and 75 % from the frequency distribution data, and 50 % represented the average granule size.

Branch chain-length distribution analysis of starch. Chain-length distributions of lotus starches were determined using a fluorophore-assisted carbohydrate electrophoresis (FACE) method according to a previously reported method.[20)21)] Starch hydrolysates generated by catalysis from reaction with isoamylase were analyzed by capillary electrophoresis performed on a P/ACE MDQ carbohydrate system (AB Sciex, Framingham, USA). The data were independently acquired in triplicate, and the standard error of the values was <5 %.

RESULTS AND DISCUSSION

Morphology of lotus rhizomes from Kanasumi-line No. 20 and Shinashirobana cultivar.

Figure 1A shows rhizomes of Kanasumi-line No. 20 (K) and Shinashirobana cultivar (S) harvested from the same field in Kanazawa-city, Ishikawa prefecture in October 2013. Shinashirobana cultivar has longer rhizome internodes than does the Kanasumi-line No. 20. Additionally, the color of the rhizome surface of the Shinashirobana cultivar was slightly darker than that of the Kanasumi-line No. 20. Figure 1B shows that cross sections of Kanasumi-line No. 20 (K) and Shinashirobana cultivar (S) rhizomes after lyophilization. The same number (n = 7) of larger holes was observed in each cultivar, but there was a greater number of smaller holes in the Shinashirobana cultivar than in the Kanasumi-line No. 20.

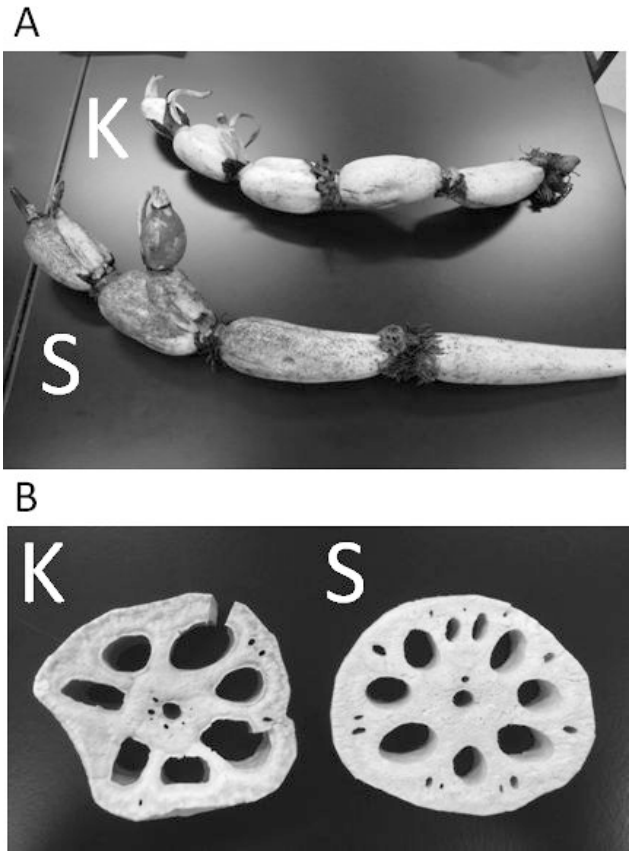

Fig. 1. A: Edible rhizomes of the Kanasumi-line No. 20 (K) and Shinashirobana (S) lotus cultivars harvested in Kanazawa-city, Ishikawa prefecture, Japan. B: Sliced surfaces of lyophilized lotus rhizomes from the Kanasumi-line No. 20 (K) and Shinashirobana (S) lotus cultivar.

Morphology of the starch granules.

Previous studies have indicated that starch granules from lotuses had vertically long oval shapes, and hilum was observed at the end of the starch granules.[5)10)22)23)] Visualization by polarized light microscopy showed that the starch granules exhibited a Maltese cross at the end of the starch granule (Fig. 2 at 50 °C). Previously, we reported that the shape of the starch derived from the Shinashirobana cultivar was slightly different from those of other lotus cultivars by optical microscopy.[5)] Thus, we determined the distribution of the starch granules from the Shinashirobana cultivar and Kanasumi-line No. 20 using a laser diffraction particle analyzer. The analysis showed that the starch granules were normally distributed. Table 1 shows that the radii of the Shinashirobana cultivar starch at 25, 50, and 75 % distribution were slightly smaller than those of the Kanasumi-line No. 20 starch.

Gelatinization properties.

Figure 2 shows the disappearance of Maltese crosses with increasing temperature on a hot stage equipped on the polarized light microscope. Maltese crosses of the Shinashirobana cultivar starch granules began to disappear at 55 °C (arrow) and completely disappeared at 65 °C (Fig. 2B). On the other hand, many of the Kanasumi-line No. 20 starch granules had Maltase crosses that began to disappear at 65 °C and completely disappeared at 77 °C (data not

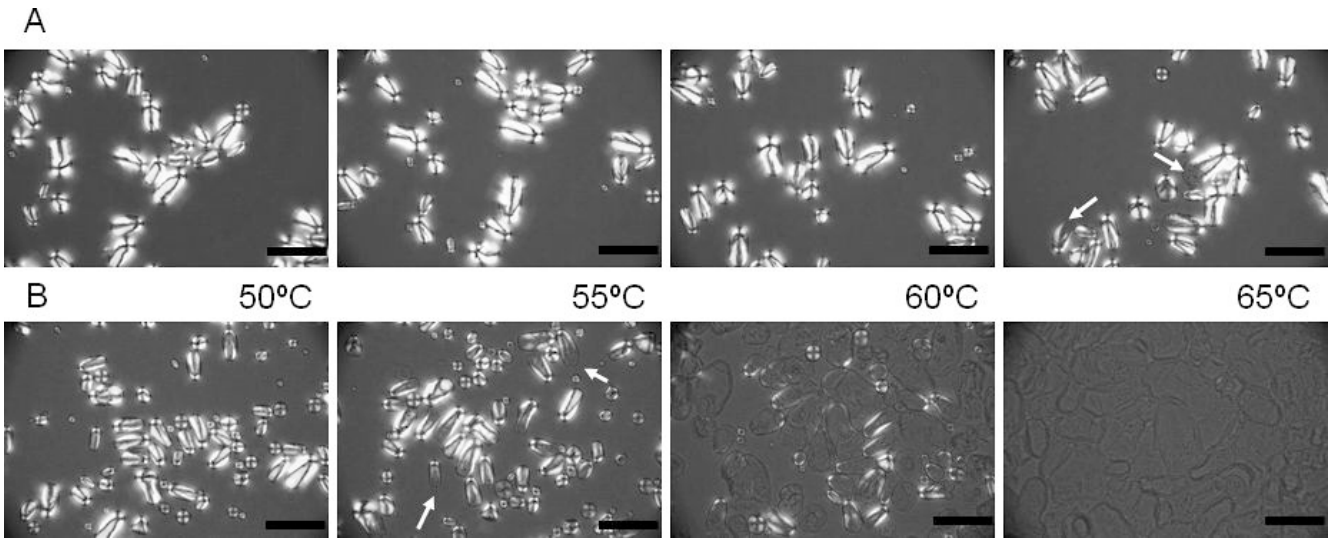

Fig. 2. Temperature dependence of Maltese cross of the starch particles from the Kanasumi-line No. 20 (A) and Shinashirobana cultivars (B).
The temperature in the experiment was changed as follows: 25→50 °C (5 °C/min) and 50→80 °C (1 °C/min). Arrows indicate an early stage of disruption of a starch granule.

shown). These results suggested that the starting temperature of the crystal disruption of Shinashirobana cultivar starch was approximately 10 °C lower than that of Kanasumi-line No. 20 starch.

Next, we determined the thermodynamic parameters associated with gelatinization of both of the starches by DSC analysis (Table 2). All of the temperatures related to gelatinization of the Shinashirobana cultivar starch were approximately 5 °C lower than those of the Kanasumi-line No. 20 starch. Table 2 shows that the ΔH of gelatinization of the Shinashirobana cultivar starch is slightly higher than that of the Kanasumi-line No. 20 starch, but the ΔH values of both starches are most likely similar judging from the standard deviation of the values. These results suggested that gelatinization of the Shinashirobana cultivar starch began at an approximately 5 °C lower temperature than that of the Kanasumi-line No. 20 starch.

By comparing with gelatinization temperatures between the Shinashirobana cultivar starch and the Kanasumi-line No. 20 starch, the difference of the gelatinization temperature observed with the microscopy was 2 times larger than that with DSC method. At present, we considered the cause of this difference as follows. Firstly, the gelatinization of each starch granule in the suspension was independently observed in the case of the microscopic method while the DSC method mainly detected gelatinization of amylopectin in the starch. Secondly, the thermal conductivity of the hot stage equipped on microscopy would be lower than that of the inside of the DSC cell. Thus, the observation of the disruption of starch granules would be delayed in comparison with DSC method, resulting in large difference in gelatinization temperature between the DSC and the microscopy method.

Pasting properties.

Table 3 shows the RVA parameters of the lyophilized rhizome powders of the edible parts and rhizome starches from Shinashirobana cultivar and the Kanasumi-line No. 20. In the case of the lyophilized rhizome powder, peak viscosity and breakdown of the Shinashirobana cultivar were approximately two times higher than those of the Kanasumi-line No. 20. In contrast, both starches had similar values for peak viscosity and break down. The pasting temperature of the lyophilized rhizome powder containing starch from the Shinashirobana cultivar was approximately 5 °C lower than that of the Kanasumi-line No. 20. These results indicated that viscosity of Shinashirobana cultivar starch was expressed at a 5 °C lower temperature in comparison with Kanasumi-line No.20 starch, whereas the peak viscosities of starch were similar between the two cultivars in the RVA analysis. In general, root and tuber crops contain 16−24% starch in the edible parts. The properties of starch vary depending on the plant origin and storage region.[24)25)] We also determined the total starch contents in the lyophilized rhizome powders of both of the lotuses, which were 60.1 ± 1.0 % (Shinashirobana cultivar) and 42.6 ± 0.1 % (Kanasumi-line No. 20). Therefore, we considered that the difference in peak viscosity in the RVA analysis for the lyophilized rhizome powders was related to the starch content of the rhizomes.

Table 1. Granule size distribution of starches from both lotus cultivars.

		Granule size (μm)
Kanasumi-line No. 20 starch	25 %	29.0 ± 0.02
	50 %	38.2 ± 0.08
	75 %	53.2 ± 0.26
Shinashirobana cultivar starch	25 %	27.3 ± 0.04
	50 %	35.9 ± 0.01
	75 %	50.5 ± 0.20

Results are the mean ± standard deviation (n = 3).

Viscosity, solubility, and swelling power of starches.

As described in Pasting properties, the temperature range of the starches for the Shinashirobana cultivar starch in the

Table 2. DSC parameters of starches from both lotus cultivars.

	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
Kanasumi-line No. 20 starch	58.4 ± 0.09	61.8 ± 0.14	67.6 ± 0.63	11.6 ± 2.2
Shinashirobana cultivar starch	53.6 ± 0.30	57.3 ± 0.40	64.5 ± 0.92	13.4 ± 0.6

T_o, T_p, T_c, and ΔH indicate onset, peak, conclusion temperature, and enthalpy change. Results are the mean ± standard deviation (n=3).

Table 3. RVA parameters of lyophilized rhizome powders and starches from both lotus cultivars.

Sample	Pasting temperature (°C)	Peak temperature (°C)	Peak viscosity (RVU)	Minimum viscosity (RVU)	Final viscosity (RVU)	Breakdown (RVU)	Setback (RVU)
Kanasumi-line No. 20 lyophilized rhizome powder	71.9 ± 0.7	80.0 ± 0.5	35.4 ± 0.1	13.9 ± 0.2	17.2 ± 0.1	21.6 ± 0.2	3.3 ± 0.1
Shinashirobana cultivar lyophilized rhizome powder	65.2 ± 0.3	76.4 ± 0.4	70.0 ± 0.4	28.3 ± 0.6	35.6 ± 0.7	41.7 ± 0.4	7.4 ± 0.1
Kanasumi-line No. 20 starch	66.5 ± 0.0	79.0 ± 0.3	240 ± 3	86.4 ± 1.4	150 ± 1	154 ± 2	63.6 ± 0.3
Shinashirobana cultivar starch	62.1 ± 0.3	77.4 ± 0.5	244 ± 1	84.0 ± 1.0	147 ± 1	160 ± 0	62.9 ± 0.6

Each parameter was analyzed in duplicate and is shown as the mean ± standard deviation.

Table 4. Viscosity of starches from both lotus cultivars at 60 and 65 °C.

		Viscosity (mPa·s)
Kanasumi-line No. 20	60 °C	16.2 ± 2.8
	65 °C	2030 ± 40
Shinashirobana cultivar	60 °C	2720 ± 20
	65 °C	3410 ± 90

Viscosity of the starches were determined by using a B8R-type rotational viscometer (Toki Sangyo Co., Ltd., Tokyo, Japan) connected with an HH-type small adaptor (HH-1, φ 16 × 34 mm, and an HH-2, φ 11 × 40 mm, Toki Sangyo Co., Ltd.) at 60 and 65 °C. The shear rate of the Kanasumi-line No.20 starch was 99.8 s^{-1} for the HH-1 adaptor (*). The shear rate of the other starches was 33.4 s^{-1} for the HH-2 adaptor. Results are the mean ± standard deviation (n = 3). Other conditions are described in the text.

viscous state started at 62.1 °C, whereas that of the Kanasumi-line No. 20 starch started at 66.5 °C. Also, the microscopic observation with increase of temperature indicated that swelling of Shinashirobana cultivar starch were observed at 60 °C while the Kanasumi-line No. 20 starch kept Maltese crosses. Thus, we considered that the lower temperature onset of the Shinashirobana viscous starch relative to that of the Kanasumi-line No. 20 viscous starch was a characteristic of the Shinashirobana cultivar starch. To investigate the differences in the viscosities between the Shinashirobana cultivar starch and Kanasumi-line No. 20 starches, we determined the viscosity of the starch gel by using a rotational viscometer at 60 and 65 °C (Table 4). There was a major difference in the viscosities at 60 °C between the Shinashirobana cultivar and Kanasumi-line No. 20 starches. The viscosity of the Shinashirobana cultivar starch was 168 times higher than that of the Kanasumi-line No. 20 starch at 60 °C. On the other hand, the viscosity of the Shinashirobana cultivar starch was 1.7 times higher than that of the Kanasumi-line No. 20 starch at 65 °C.

The solubilities and swelling powers of both starches were also determined at 60 and 65 °C (Table 5). The solubility of the Shinashirobana cultivar starch was five times higher than that of the Kanasumi-line No. 20 starch at 60 °C, although both starches had similar solubilities at 65 °C. The swelling power of the Shinashirobana cultivar starch was 1.8 times higher than that of the Kanasumi-line No. 20 starch at 60 °C, but the swelling powers of both starches were similar at 65 °C. These results indicated that the expression of viscosity and the swelling power of the starch gel were related to DSC parameters (T_o and T_p) of the starches from the Shinashirobana cultivar and the Kanasumi-line No. 20.

X-ray diffraction.

Figure 3 shows the XRD patterns of starches from corn, potato, and rhizomes of the Kanasumi-line No. 20 and Shinashirobana cultivars. Peak numbers are labeled in a manner described in a previous report.[26] Sharp peaks were observed at 2θ = 5.7° (1), 15.0°(3b), and 17.0°(4a), and broad peaks were also detected at around 24.0° (6a and 6b) in both of the starches from the lotuses. Each of the corresponding XRD peaks were found in starches from corn (A type) and potato (B type) as shown in Fig. 3. Therefore, we determined that the Shinashirobana cultivar and Kanasumi-line No. 20 starches were both C-type starches that contained A- and B-type starches.

Recently, Katsumi *et al*. proposed a "crystalline index" for starches from various sources, which is an index of the regular structure and definite direction of crystalline materials.[19] A decrease in the crystalline index of starches indicated a loss of a regular structure or of holding voids of starches, which suggested that the crystallinity of starch was decreased. The 3b (15.0°) and 4a (17.0°) peaks were useful for calculation of the crystalline indexes of A- and B- type starches (Fig. 3), and to compare the crystalline indexes between the two starches, we selected these peaks for calculation of the index. The XRD peaks of 3b and 4a were normalized after subtraction of background peaks. Height and full-width at half maximum (FWHM) of the peaks were used for calculation of the crystalline indexes of the starches. Finally, crystalline indexes of the starches were

Table 5. Solubility and swelling power of starches from both lotus cultivars at 60 and 65 °C.

	60 °C	65 °C
Solubility (%)		
Kanasumi-line No. 20 starch	2.3 ± 0.2	6.0 ± 0.2
Shinashirobana cultivar starch	10.2 ± 1.7	7.0 ± 0.4
Swelling power		
Kanasumi-line No. 20 starch	9.6 ± 1.0	19.8 ± 0.2
Shinashirobana cultivar starch	17.3 ± 0.5	23.6 ± 0.5

Results are the mean ± standard deviation (n = 3).

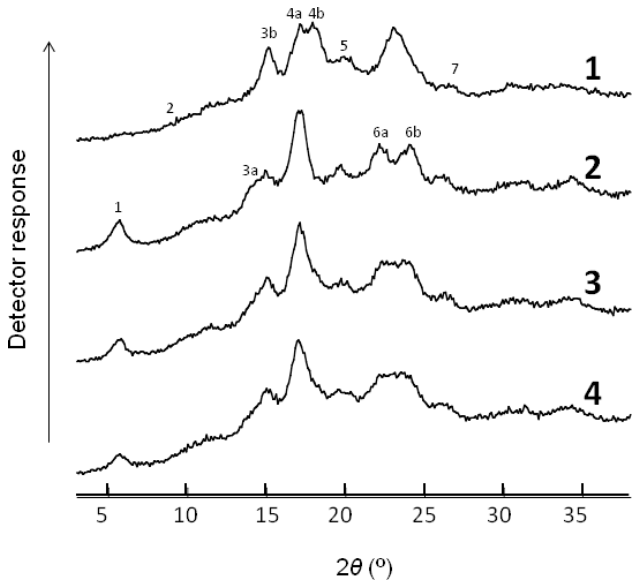

Fig. 3. X-ray diffraction spectra of starches from corn (**1**, A type), potato (**2**, B type), and Kanasumi-line No. 20 lotus (**3**), and Shinashirobana lotus cultivars (**4**), respectively.

Peak numbers are labeled in the manner described in a previous report.[26)]

calculated according to the following equation:

$$\text{Crystalline index} = \text{Peak height 3b} / \text{FWMH 3b} + \text{Peak height 4a} / \text{FWHM 4a}$$

The crystalline indexes of the starches were 690 (Shinashirobana cultivar starch) and 789 (Kanasumi-line No. 20 starch). The indexes indicated that the crystallinity of the Shinashirobana cultivar starch was slightly lower than that of the Kanasumi-line No. 20 starch. We also obtained relative crystallinities of the starches according to a previously reported method.[18)] The relative crystallinities of the Shinashirobana cultivar and Kanasumi-line No. 20 starches were 22.0 and 24.0 %, respectively, which indicated similar crystallinity.

Phosphorus and apparent amylose content.

The phosphorus content of starch from potato is a very important factor because the content determines its viscosity.[27)28)] The phosphorus contents of the Shinashirobana cultivar and Kanasumi-line No. 20 starches were estimated to be 52.4 ± 0.1 and 58.0 ± 5.2 ppm, respectively, which indicated that the phosphorus contents were similar. The apparent amylose contents (%) of the Shinashirobana cultivar and Kanasumi-line No. 20 starches were also similar (Shinashirobana cultivar, 28.2 ± 0.4 %; Kanasumi-line No. 20, 29.5 ± 0.4 %). These results suggested that the phosphorus and apparent amylose contents of the starches were unrelated to the difference in viscosity between the Shinashirobana cultivar starch and Kanasumi-line No. 20 starch.

Chain-length distribution.

The distribution of the branched chain lengths of the Shinashirobana cultivar and Kanasumi-line No. 20 starches were analyzed by FACE. The peak in the branched chain length in both of the starches was degree of polymerization (DP)12 (data not shown), a finding that was comparable to that (DP 13) in previous reports.[10)29)] Figure 4 indicates the difference in branched chain distribution between the Shinashirobana cultivar and Kanasumi-line No. 20 starches. The data were calculated by subtracting the molar amount of isoamylase-hydrolyzate of the Kanasumi-line No. 20 starch from that of the Shinashirobana cultivar starch. The chain content between the DP 5 and 12 of the Shinashirobana cultivar starch was higher than that of the Kanasumi-line No. 20 starch. It has been reported that a high proportion of DP 6−12 unit chains in amylopectin was related to high viscosity of starches.[29)30)] The proportion of DP 5−12 unit chains in the Shinashirobana cultivar and Kanasumi-line No. 20 starches were 30.2 ± 0.1 and 26.9 ± 0.4 %, respectively (data not shown), which indicated that the value of the Shinashirobana cultivar starch was slightly higher than that of the Kanasumi-line No. 20 starch.

Gelatinization of the Shinashirobana cultivar starch began 5 °C lower than that of the Kanasumi-line No. 20 starch, as shown in the DSC analysis (Table 2). The gelatinization temperatures of starches are regulated by enzymes related to starch synthesis in plants. The distribution of chain length of isoamylase-hydrolyzate of starches provides properties of enzymes which related to synthesize starch molecules.[31)] The distributions of starches from various plants, such as rice, wheat, maize, and barley, have been investigated by FACE and high-performance anion exchange chromatography using pulsed amperometric detection.[31)32)33)34)] SSIIa is one of the key enzymes for determination of the gelatinization properties of starches; thus, low enzyme activity decreases the gelatinization starting temperature of a starch.[35)] The difference in the distribution between the Shinashirobana cultivar and Kanasumi-line No. 20 starches (Fig. 4) was similar to that of starches from SSIIa mutants of rice (*Indica vs. Japonica* having inactive SSIIa) and maize (wild type *vs. su2-Ref*).[33)35)] SSIIa has a role in elongation of short A-chains to long A-chains and short B-chains with DP up to approximately 24 in the case of amylopectin from rice.[36)] Low enzyme activity led to a decrease in the middle chain lengths (DP 13−24) in amylopectin, which resulted in an increase of the short chain lengths (DP 5−12). The increase of the short chain length was related to a lower gelatinization temperature than those of intact starches.[35)] Thus, we speculated that SSIIa could be a key enzyme related to the lower gelatinization temperature and higher viscosity at 60 °C of the Shinashirobana cultivar starch relative to those of the Kanasumi-line No. 20

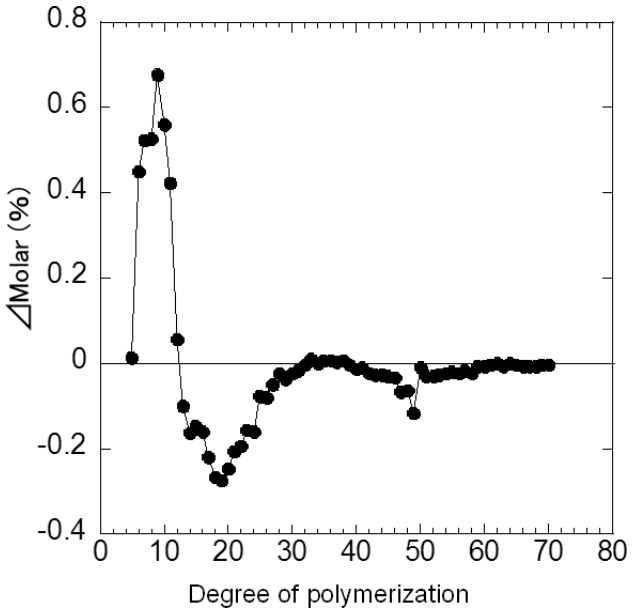

Fig. 4. The difference in the relative molar amounts of the branched chain length distributions between the starches from the Shinashirobana cultivar and Kanasumi-line No. 20.

The chain-length distribution of lotus starch was determined by using a FACE method according to a previously reported method.[20)21)] The data was calculated by subtracting the molar amount of isoamylase-hydrolysate of the Kanasumi-line No. 20 from that of the Shinashirobana cultivar. The data were independently acquired in triplicate, and the standard error of the values was <5%.

starch.

In conclusion, we think that the chain length distribution of amylopectin of a starch is a key factor related to the 5 °C lower gelatinization temperature of the Shinashirobana cultivar starch relative to that of the Kanasumi-line No. 20 starch. Further investigation of the activity and expression level of starch-synthesizing enzymes in lotus cultivars is necessary to elucidate the relationship between gelatinization temperature and enzymes in lotuses.

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