Rice Starch Isolation from Newly Developed Rice Cultivars by the Improved Alkali Method

(Received April 4, 2002; Accepted July 15, 2002)

Naoko Matsunaga,^{1,*} Setsuko Takahashi¹ and Keiji Kainuma²

¹*Department of Home Economics, Kyoritsu Women's University (2-2-1, Hitotsubashi, Chiyoda-ku, Tokyo 101-8433, Japan)*

²*Bio-oriented Technology Research Advancement Institution (3-18-19, Toranomon, Minato-ku, Tokyo 105-0001, Japan)*

Abstract: A rapid and efficient rice starch isolation method was tested on two varieties of newly developed rice, Hoshinishiki (Kanto181) and Saikaimochi227 (a high-amylose and a high-yield waxy rice), and a reference rice variety, Nipponbare. Rice flour was stirred in 0.05 or 0.075 M aqueous sodium hydroxide for 3 h once or three times, neutralized with 1 M hydrochloric acid and washed with water. Isolated rice starch was compared with the starch isolated by the conventional method in terms of chemical and physical properties. The improved method reduced processing time to two days, while the conventional method required one to three weeks. The improved method lowered damaged starch and protein contents, but slightly lowered starch recovery. Non-waxy rice starches isolated by the two methods differed in physical properties upon testing by photopastography and Rapid Visco Analyser (RVA), whereas no difference was observed in waxy rice. The phosphorus content in Nipponbare starch decreased during the isolation process due to degradation of the lysophospholipid, and the phosphorus content was the lowest in the starch isolated by the conventional method. Sodium content was high in the starch isolated by the improved method, although the influence of residual sodium on the viscosity of the paste was small. To reduce residual sodium in the starch isolated by the improved method, washing with water eight times after neutralization was needed. Thus, we propose the improved procedure for rice starch preparation. Rice flour is washed with 0.05 M aqueous sodium hydroxide three times, neutralized and then washed eight times with water. In the case of high-amylose or waxy rice flours, alkali washing can be reduced to once.

Key words: rice starch, isolation, alkali method

Rice is mostly consumed as milled rice, and the use of rice as starch or flour is rather limited because of the higher price compared to other cereal and tuber starches. However, the virtue of the small particle size and variety of amylose content of rice starch raises the possibility of utilization in wide varieties of industrial materials as well as food ingredients.^{1–3)}

Rice starch consists of up to 90% of milled rice, and the other major component is protein. Since glutelin, which makes up more than 80% of milled rice protein, is denatured and soluble below pH 3 or above pH 10, rice flour has been washed with dilute alkali in order to isolate the starch.^{4,5)} In general, rice starch is isolated from rice flour by washing with alkali or detergent, sonication or enzymatic digestion of the protein.^{2,5)} Detergent washing brings out glycolipids and changes the physical properties of the isolated starch,⁶⁾ and alkali washing causes saponification of the phospholipid.⁷⁾ The protease method could be used for the isolation of rice starch. However, this method requires preliminary testing to determine the optimum condition for the enzymes. The alkali washing method is the most practical in terms of ease for isolating rice starch. Under the alkali washing method, rice flour is washed with dilute alkali one to several times, followed by washing with water to a neutral pH.⁵⁾ The process requires a long time because of sedimentation of the rice starch and the washing out of the alkali with water. Lum-

budwong and Seib²⁾ modified the alkali washing method and isolated rice starch from commercial wet-milled rice flour from Thailand, and compared this with the protease digestion method. They washed rice flour with 0.05 M aqueous sodium hydroxide once, neutralized the starch slurry with acid before washing with water and separated the starch by centrifuge. The starch isolated using this "improved" method was able to have the protein removed successfully.

The objectives of this study were to apply the improved method to different types of rice, and to determine the optimum alkali conditions for rice starch isolation. In this study, two kinds of newly developed rice, Hoshinishiki and Saikaimochi227, were used in addition to a reference rice, Nipponbare. Hoshinishiki, a high-amylose rice, is not preferred as cooked rice;⁸⁾ therefore, its use as rice flour or starch is more promising. Saikaimochi227, a high-yield waxy rice, is preferred as cooked rice,⁸⁾ but could be interesting for utilization as flour or starch.

MATERIALS AND METHODS

Materials. Reference and high-amylose rice, Nipponbare and Hoshinishiki (Kanto181), respectively, were supplied by the National Agriculture Research Center (Tsukuba, Japan). Waxy rice, Saikaimochi227, was from the Kyushu National Agricultural Experiment Station. The rice was milled and ground to flour by a test mill (Quadrumat-Muhle Nr. 364, Brabender Co.), and passed through an 80-mesh screen.

* Corresponding author (Tel. +81-3-3237-2492, Fax. +81-3-3237-2688, E-mail: unngaun@yahoo.co.jp).

Methods. Moisture content was measured by a moisture balance (MC-30MB, CBC Co., Kyoto, Japan). Nitrogen (N) content was assayed by the micro-Kjeldahl method and protein (%) was calculated as protein = N (%) $\times$ 5.95. The phosphorus, sodium, and chloride contents of the starch were analyzed by the molybdovanadate spectrophotometric method and the atomic absorption spectrophotometric method, respectively, at Japan Food Research Laboratories. Total starch and damaged starch contents were measured by enzyme digestion using analytical kits purchased from Megazyme (Biocon Japan Ltd., Nagoya, Japan).

The gelatinization process and clarity of starch paste were measured by photopastography (ART-3 PPT-1, Hirma Rika Kenkyusho, Kanagawa, Japan). Rice starch (50 mg, dry base) was suspended in water (50 mL). The slurry was poured into a glass cell and stirred at 25°C for 2 min. The transmittance of the starch slurry, upon heating from 25 to 95°C at the rate of 2°C/min, was measured at 372 nm.

The pasting properties of rice starch were measured on a Rapid Visco Analyser (RVA-3D, Foss Japan Ltd., Tokyo, Japan). A slurry of rice starch and water (7.5% starch solid) was heated from 50 to 95°C at the rate of 5.6°C/min. It was held at 95°C for 2 min and then cooled to 50°C at 5.6°C/min.

Isolation of rice starch by the improved method. Rice starch isolation by the improved method was based on the procedure of Lumdubwong and Seib with some modification of sodium hydroxide concentrations and number of alkali washings.²⁾ Rice flour (100 g, dry base) was mixed with 500 mL of 0.05 or 0.075 M aqueous sodium hydroxide (NaOH) and stirred at 25°C for 3 h, after which it was collected by centrifugation (5500 $\times g$, 10 min). The supernatant was discarded, and the yellow-opaque layer of the sediment was removed with a spatula. This alkali washing was done either once or three times. After washing with alkali, the starch residue was again suspended in water (500 mL), filtered through a 100-mesh screen, stirred for 1 h and centrifuged. The starch residue was washed again and then suspended in water for neutralization to pH 7.0 with 1 M hydrochloric acid (HCl). This was followed by stirring for 1 h at room temperature and centrifugation. The starch sediment was washed twice with water as described and then dried at room temperature.

In a separate experiment, the need to wash with water after neutralization for the purpose of removing residual sodium in the isolated starch was examined. Rice flour was washed with 500 mL of 0.075 M NaOH once, washed with water twice and neutralized to pH 7.0 as described above. The starch sediment was then washed with water 2, 4 or 8 times, and the isolated starch was compared with regard to sodium and chloride contents and viscosity of the paste.

The starch recovery was calculated as the percentage of starch obtained in the total starch present in a given quantity of flour.

Isolation of starch by the conventional method. As a conventional method, rice starch was isolated using the procedure of Yamamoto *et al.*³⁾ Rice flour (100 g, dry

base) was suspended in 0.05 M NaOH (500 mL), stirred at 5°C for 3 h and then stored at 5°C until the starch was sedimented, about 24 h. The supernatant was discarded and the starch layer was passed through a 100-mesh screen to remove the yellow-colored material. The alkali wash was repeated twice and the precipitated starch was passed through a 200-mesh screen. The starch residue was washed with water (500 mL) until the pH of the supernatant became 7.0 and then it was dried at room temperature.

All measurements, as well as the isolation of starch, were at least duplicated.

RESULTS AND DISCUSSION

Isolation of rice starch by alkali.

Rice starch was isolated using the improved method with one or three washes of 0.05 or 0.075 M NaOH, shown as 0.05M-1, 0.075M-1, 0.05M-3 or 0.075M-3, respectively. These results were compared with the starch obtained by a conventional method. The conventional method required one to three weeks because the sedimentation of small particles of rice starch took about 24 h, and washing out the alkali to a neutral pH of 7 required more than 15 washes with water. Thus, the improved method could reduce the processing time to two days, whereas the conventional method required one to three weeks.

When rice flour was mixed with five times the amount of 0.05 M NaOH at 25°C, the pH of the slurry was approximately 12.0. Upon suspension in water after the alkali washes, the pH of the starch slurry was high, around 11.9. The long steeping time of rice flour or its starch residue in an alkali condition may cause damage to the rice starch granules. To avoid damaging the starch, neutralization of the alkali condition is desirable. In the improved method, the rice flour slurry was neutralized by 1 M HCl after suspension in alkali long enough to denature the protein.

The starch recovery and components of the isolated starch are shown in Table 1. Starch recovery of Nipponbare, a reference and non-waxy rice, was 65.3–76.3% by the improved method and 79.6% by the conventional method. Starch recovery by the improved method decreased with higher concentrations of aqueous NaOH or more washes with aqueous NaOH. Damaged starch content was lower in the starch isolated by the improved method (5.2–5.5%) than the conventional method (9.0%). Protein content of the starch isolated by the improved method was lower than the conventional method, except for the sample isolated with 0.05M-1. Thus, the improved method resulted in lower damaged starch and protein contents. It is assumed that some of the non-damaged starch was removed during the step where damaged starch was scraped off, resulting in slightly lower starch recovery using the improved method. Considering the recovery and purity of the isolated starch, 0.05M-3 was the optimum condition for the isolation of Nipponbare starch.

Using the improved method, 0.05M-1 and 0.05M-3 were applied for the isolation of Hoshinishiki and Saikaimochi227 starches (Table 1). Hoshinishiki (a high-

Table 1. Starch recovery and components of rice starches isolated by two methods.

	Total starch (%)	Starch recovery (%)	Damaged starch (%)	Protein content (%)	Phosphorus* ($\times 10^{-3}$ mol)	Sodium* ($\times 10^{-3}$ mol)
Nipponbare						
Flour	90.4			7.41	4.7	0.04
Starch						
Conventional		79.6	9.0	0.127	0.13	0.33
0.05M-1		76.3	5.2	0.227	0.93	0.91
0.05M-3		75.2	5.5	0.104	0.24	0.87
0.075M-1		70.8	5.2	0.069	0.67	0.75
0.075M-3		65.3	5.5	0.068	0.35	0.95
Hoshinishiki						
Flour	89.7			4.74	3.9	n.d.**
Starch						
Conventional		74.7	5.9	0.270	0.68	n.d.
0.05M-1		76.9	4.0	0.265	0.80	n.d.
0.05M-3		64.7	n.d.	0.267	n.d.	n.d.
Saikaimochi227						
Flour	89.0			5.26	3.8	n.d.
Starch						
Conventional		69.6	4.3	0.067	0.12	0.07
0.05M-1		76.4	2.9	0.069	0.15	0.22
0.05M-3		62.9	n.d.	0.067	n.d.	n.d.

*The amount of phosphorus and sodium in 100 g (dry weight) of flour or starch. **Not determined.

amylose rice with *Wxa* as its predominant gene)⁹ showed a high content of residual protein in the starch. It has been reported that residual protein in non-waxy Indica rice starch (predominant gene *Wxa*) is three times higher than that in non-waxy Japonica rice starch with a similar amylose content.¹⁰ Hoshinishiki and Saikaimochi227 starches isolated with 0.05M-1 were slightly higher in starch recovery and lower in damaged starch and protein content compared to those starches isolated by the conventional method. Increasing alkali washes to three times, 0.05M-3, did not increase protein removal but lowered the yield compared to 0.05M-1. Hence, for the procedure using 0.05 M NaOH, alkali washing once, 0.05M-1, is recommended for the isolation of Hoshinishiki and Saikaimochi227 starches.

The phosphorus content is shown in Table 1. Since the phosphorus in non-waxy rice starch is mostly lysophospholipid (LPL), and only 2–12% of the total phosphorus stays as glucose 6-phosphate ester,^{7,11} a trace of phosphorus is an indication of degradation of LPL. It has been reported that the steeping of rice flour in aqueous NaOH causes some degradation of LPL to phosphoglycerol and free fatty acid (FFA).⁷ Other had shown that the saponified phosphoglycerol part of LPL in wheat starch is released in water, whereas the FFA part stays in the starch.¹² The phosphorus content (Table 1) was lower in the starch isolated by the conventional method (0.004% or 0.13 mmol/100 g starch, dry base) than the improved method (0.007–0.028% or 0.24–0.93 mmol/100 g starch, dry base). Apparently, the conventional method degraded LPL in starch due to the steeping in alkali for a long time.

Photopastograms of rice starch isolated by the improved and conventional methods.

The physical properties of the rice starches were tested by photopastography. Photopastograms of Nipponbare starches obtained under various conditions showed similar curves (Fig. 1). The temperature of the onset of rising

Fig. 1. Photopastograms of 0.1% Nipponbare starch paste isolated under various alkali conditions.

—, conventional; ○, 0.05M-1; ●, 0.05M-3; △, 0.075M-1; ▲, 0.075M-3.

transmittance was slightly higher in 0.05M-3 and 0.075M-3, and transmittance of the conventional method at 70 to 90°C was slightly lower than that of the starches obtained using the improved method. The same tendency was shown in Hoshinishiki, and the starch isolated by the conventional method was slightly lower in transmittance at 70 to 95°C. On the other hand, Saikaimochi227 starch did not show any differences between isolation methods in the photopastograms (data not shown).

Pasting properties of isolated rice starch by the improved and conventional methods.

The pasting properties of the rice starches are provided in Table 2. Non-waxy rice starches isolated by the improved method were larger in breakdown and higher in final viscosity. In contrast, there was no difference in the viscosity of waxy rice (Saikaimochi227) starches isolated by the two methods, as was shown in the photopastograms. Set-back, calculated by subtraction of peak viscosity from final viscosity, differed among the three rice

Table 2. Pasting properties of rice starches isolated by two methods.

	Pasting temperature (°C)	Peak viscosity (RVU)	Minimum viscosity (RVU)	Breakdown (RVU)	Final viscosity (RVU)	Set-back* (RVU)
Nipponbare						
Conventional	66.3	93	60	33	99	6
0.05M-1	66.0	136	80	50	165	29
0.05M-3	66.6	129	84	44	138	9
Hoshinishiki						
Conventional	73.4	84	74	11	108	24
0.05M-1	69.0	79	52	27	137	58
0.05M-3	68.8	88	64	31	122	34
Saikaimochi227						
Conventional	62.1	147	71	76	84	-63
0.05M-1	61.7	147	70	77	83	-64
0.05M-3	61.6	146	67	79	81	-65

*Set-back was calculated by subtraction of peak viscosity from final viscosity.

starch varieties, where the units were Saikaimochi227 < Nipponbare < Hoshinishiki. The result indicates that the retrogradation tendency is the lowest in waxy rice starch and the highest in high-amylose rice starch. The set-back of non-waxy starch tended to be higher in the starch high in residual phosphorus. Presumably, the phospholipid was responsible for the elevation of paste viscosity of non-waxy starch upon cooling. On the other hand, no increase in paste viscosity was shown in Saikaimochi227 starch upon cooling.

The pasting curves of Nipponbare rice starches isolated by the improved method were high in peak and final viscosities, and large in breakdown (Fig. 2). The starches isolated by 0.05M-3 and 0.075M-3, as well as that isolated by the conventional method, were low in set-back, indicating that those starches were lower in the retrogradation tendency. The starch isolated by the conventional method was about 70% in peak viscosity and 60–80% in final viscosity compared with the improved method. It is assumed that some damage occurred in the starch isolated by the conventional method because of the long-term steeping in alkali.

Nipponbare starch isolated by 0.075M-3 showed a rapid increase in viscosity after passing the pasting temperature. Yamamoto *et al.*⁵⁾ had shown that the rice starch isolated with many alkali washes increases viscosity rapidly, and is higher in alkali number and lower in iodine affinity.

Fig. 2. Pasting curves of Nipponbare starch slurry at 7.5% starch solid and the heating and cooling rate of 5.6°C/min.

—, conventional; ○, 0.05M-1; ●, 0.05M-3; △, 0.075M-1; ▲, 0.075M-3; ○, 0.075M-3 with 8 washes; ···, temperature.

This indicates some damage to the starch. Even though a damaged starch content of 0.075M-3 was the same as the other starches obtained using the improved method (Table 1), repeated alkali washing of rice flour could result in some physical changes in the starch.

Residual sodium content in isolated rice starches.

To see the influence of residual sodium on the pasting properties of rice starch, Nipponbare flour was washed with 0.075 M NaOH once, neutralized and washed with water 2, 4 or 8 times. The residual sodium content was 0.017, 0.013 or 0.006% (0.75, 0.58 or 0.27 mmol/100 g starch), respectively, where the starch obtained by the conventional method was 0.007% (0.33 mmol/100 g starch). The washing of rice starch with water decreased the residual sodium content, but the chloride content was similar among the samples (0.005–0.008%, or 0.19–0.23 mmol/100 g starch, dry base).

The pasting curves of the starches isolated with 0.075M-1 and various numbers of washes with water are shown in Fig. 3. Although slight differences were observed in the pasting curves among the 0.075M-1 starches, the differences were rather small compared to the differences in the pasting curves between the starch isolated by the improved and conventional methods. The starch high in residual sodium was slightly high in peak viscosity, had a

Fig. 3. Pasting curves of Nipponbare starch prepared using 0.075 M aqueous sodium hydroxide and different washing times with water after neutralization.

Starch slurry (7.5% starch solid) was heated and cooled at the rate of 5.6°C/min. —, conventional; △, 2 times; ▲, 4 times; ○, 8 times; ···, temperature.

large breakdown and was low in final viscosity. It is assumed that negatively charged ions, which were balanced with sodium ions, repelled each other upon heating and caused more swelling and rupturing of the starch granules.

To reduce the processing time and avoid damaging the starch, the improved method is recommended for rice starch preparation. Comparing the starch recoveries and protein contents of the isolated starches, the improved method of 0.05M-3 was the best for any type of rice.

This study was performed as a part of the project, "Studies of New Rice Production Technology for the Next Millennium," undertaken by the Japanese Ministry of Agriculture, Forestry, and Fisheries, Japan. We thank the National Agriculture Research Center and Kyushu National Agricultural Experiment Station for providing rice samples. We are grateful to Dr. P.A. Seib for advice and discussion, and Ms. T. Sekiguchi for the preparation of samples and technical assistance.

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新形質米における改良したアルカリ法による

米澱粉の調製

松永直子¹, 高橋節子¹, 貝沼圭二²

¹ 共立女子大学家政学部

(101-8433 東京都千代田区一ツ橋 2-2-1)

² 生物系特定産業技術研究推進機構

(105-0001 東京都港区虎ノ門 3-18-19)

短時間に米澱粉を調製する方法について、アルカリによる洗浄条件を検討した。試料は二種の新形質米、ホシニシキ（高アミロース）ならびに西海糯 227 号（多収糯）とし、基準米として日本晴を用いた。米澱粉は、米粉を 5 倍量の 0.05 または 0.075 M 水酸化ナトリウム水溶液に 1 または 3 回懸濁し、1 M 塩酸で中和・水で洗浄後、遠心分離により抽出し、得られた米澱粉の化学・物理的性質を従来法と比較した。澱粉の調製に要する時間は、従来法の 1-3 週間に対して本方法は 2 日間と短縮され、本方法により抽出された米澱粉はタンパク質含量と損傷澱粉量が少なかったが、収量は従来法に比べて低くなった。粳種の米澱粉はフォトペーストグラフィーならびにラピッドビスコアナライザーにおいて調製法による差が認められたのに対し、糯種においては差が認められなかった。粳米澱粉中にリン脂質として存在するリンの含量は従来法による米澱粉が最も少なく、調製時における脂質の分解により流出したと考えられた。本方法で調製された澱粉のナトリウム含量は従来法よりも高く、ナトリウムを減らすためには中和後の水での洗浄は 8 回を要した。以上の結果より、米澱粉の抽出方法として、本方法の 0.05 M 水酸化ナトリウム水溶液洗浄 3 回・中和後の水での洗浄 8 回が従来法に変わる方法として用いられることが明らかになった。ただし、高アミロース米ならびに糯米については 0.05 M 水酸化ナトリウムによる洗浄を 1 回に減じることが可能であった。