

Comparison of Protease Digestion at Neutral pH with Alkaline Steeping Method for Rice Starch Isolation

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Isolation of rice starch is different from corn, wheat, or potato starch due to the differences in protein properties. Because the majority of rice protein is alkaline soluble, the alkaline steeping method is commonly used in manufacturing rice starch with good recovery and low residual protein content (Yang et al 1984; Resurreccion et al 1993; Ju et al 2000). In general, starch isolated by the alkaline steeping method with 0.1–0.2% sodium hydroxide had 73–85% yield (on a starch dry basis), 0.07–0.42% residual protein, and 0.07–2.6% damaged starch content (Yang et al 1984; Lumdubwong and Seib 2000). However, the alkaline steeping procedure is not preferred in the United States because it generates a large amount of alkali and salt in the process, resulting in a high cost for wastewater treatment. Ultrasonication has also been evaluated, but the resultant rice starch was low in yield and purity (Horiuchi and Tani 1964; Juliano 1984).

Protease digestion has been employed to isolate amaranth, oat, and rice starches (Lim et al 1992; Radosavljevic et al 1998; Lumdubwong and Seib 2000). Amaranth starch was isolated by first steeping amaranth seeds in 0.05% sodium hydroxide solution for 22 hr. The seeds were then washed, ground, and reacted with 0.5–1.0% (v/w on total amount of seeds) alkaline protease at pH 7.5 for 2 hr at 37°C. The starch yield was 80% (based on starch content of amaranth) with a residual protein content of 0.2%. The alkaline protease used in oat starch isolation yielded 78% starch (on a flour dry basis) with 1.1% residual protein at pH 7.5 for 6 hr at 37°C (Lim et al 1992). Lumdubwong and Seib (2000) applied a commercial alkaline protease to isolate rice starch from wet-milled rice flour at pH 10 for 12 hr at 55°C, yielding 95% starch (on a starch dry basis) with 0.52% residual protein content and 2.1% damaged starch content. However, because the protease digestion was conducted at pH 10, alkali was still present in the effluent water, requiring further treatment.

No work has been reported in employing different types of proteases at neutral pH conditions to isolate rice starch. This study was undertaken to investigate the effectiveness of protease digestion in rice starch isolation by using acid-, neutral-, and alkaline-protease at a neutral pH in comparison with the conventional alkaline steeping method.

MATERIALS AND METHODS

Materials

Milled, Mahatma extra long-grain rice was obtained from Riviana Foods Inc. (Abbeville, LA). The rice sample was milled into rice flour with a cyclone sample mill equipped with a 100-mesh screen (3010-018, Udy Corp., Fort Collins, CO). Acid protease (GC 106, activity 1,000 spectrophotometric acid protease units [APU]/g, optimum pH 3.0, and temperature 45–50°C) and alkaline

protease (Protex 6L, activity 580,000 DU/g, optimum pH 9.5, and temperature 60°C) were in liquid form and obtained from Genencor International, Inc (Rochester, NY). Neutral protease (N “Amano” with protease activity ≈150,000–190,000 units/g, optimum pH 7.5, and temperature 55°C) was in dry powder form and obtained from Amano Pharmaceutical Co., Ltd. (Nogoya, Japan). All proteases were food grade and used without further purification.

Starch Isolation, Alkaline Steeping Method and Protease Digestion Method

The alkaline steeping method was modified after the method of Yang et al (1984). Rice flour (100 g, wet basis) was mixed with 300 mL of 0.1% sodium hydroxide (NaOH), stirred for 18 hr at 25°C, filtered through a No. 263 sieve (63 μm), and centrifuged. The top yellowish layer was carefully removed and the bottom starch layer was reslurried with twofold deionized (DI) water and centrifuged. The washed starch was reslurried with DI water and the slurry was adjusted to pH 6.5 with 1*N* hydrochloric acid (HCl) and centrifuged. The starch was washed with twofold volumes of DI water four times and dried in an oven overnight at 45°C. Alkaline-isolated starch was used as the control.

For protease digestion, rice flour slurry (25%, w/v, wet basis) was adjusted to pH 6.5 with 0.1*N* NaOH, and the amount of protease addition varied according to the activity of the protease samples. Three levels for each protease were used to identify a suitable usage level for starch isolation. The digestion temperature was 50°C, which was close to the optimum temperature of the proteases according to the manufacturers' product brochures. The levels of acidic protease applied were 0.5, 1.0, and 1.5 mL, neutral protease levels were 5.0, 10, and 15.0 mg, and alkaline protease levels were 0.05, 0.10, and 0.15 mL. The starch-protease mixture was stirred for 18 hr at 50°C, screened through a No. 263 sieve (63 μm), centrifuged, washed with twofold DI water four times, and dried in an oven overnight at 45°C.

Analyses

The moisture, protein, total starch, and damaged starch content of rice flour and isolated starches were determined according to Approved Methods 44-15A, 46-13, 76-13, and 76-31, respectively (AACC 2000). $N \times 5.75$ was used for nitrogen to protein conversion.

The iodine affinity of the rice starch was determined in duplicate with amperometric titration (Schoch 1964) with modifications. Rice starch was defatted with 65% butanol at room temperature overnight. The defatted starch was dispersed in 10 mL of 1*N* KOH, and the mixture was stirred at room temperature for 20 min before measurement.

The pasting properties of rice starches were measured according to Approved Method 61-02 (AACC 2000) with a Rapid Visco Analyser (RVA-4, Newport Scientific Pty, Ltd, Warriewood, NSW, Australia).

A Perkin-Elmer Pyris 1 differential scanning calorimeter (DSC) (Perkin-Elmer Co., Norwalk, CT) equipped with a cooling system was used to determine the gelatinization of rice starch following the method of Wang et al (1992).

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Statistical Analysis

Experimental data were analyzed by using the general linear models procedure (1999 version; SAS Software Institute, Inc., Cary, NC), and least significance differences were computed at $P < 0.05$.

RESULTS AND DISCUSSION

The ground rice flour from the long-grain rice sample used in this study consisted of 90% total starch, 7.67% protein, and 7.6% damaged starch content on a dry basis. The starch yield, residual protein content, damaged starch content, and iodine affinity of alkali- and protease-isolated starches are summarized in Table I. The starch yield by the alkaline steeping method averaged 72.2% and those by protease digestion were 68.2–77.6% on a starch dry basis. The protease digestion method produced a comparable or higher starch yield than the alkaline steeping method, with the exception of neutral protease at lower usage levels (5 and 10 mg). Similarly, the protease-isolated starch had a similar or slightly higher residual protein content (0.07–0.33%) compared with the alkaline-isolated starch (0.15%).

The damaged starch content was significantly lower in the starch isolated by protease digestion. Water binding capacity and solubility of damaged starch granules were much higher than intact starch granules (Evers and Stevens 1985); therefore, it is possible that protease was more effective in liberating damaged starch from the protein-starch matrix into the liquid phase than was the alkali, thus reducing the residual damaged starch content. Furthermore, there was no significant difference in iodine affinity (4.9–5.2) among

starches isolated by the different methods and proteases. These results indicate that protease digestion could efficiently assist in rice starch recovery. Among the proteases and level of protease activity evaluated, alkaline protease at the concentration of 0.1% (v/w on a flour wet basis) produced the highest yield and comparable quality of starch.

The pasting properties of the isolated rice starches are listed in Table II. Starches isolated by acidic protease showed a significantly higher pasting temperature, which agrees with the findings of Lumdubwong and Seib (2000). Protease digestion produced starch with a significantly higher peak viscosity at all usage levels. As the amount of protease increased, the resultant starch generally exhibited a decrease in breakdown and an increase in both setback and final viscosity.

The protein and damaged starch content of the isolated starches affected the starch pasting properties. The protein content was slightly correlated with the breakdown viscosity of starch ($r = 0.58$, $P < 0.05$), whereas the peak viscosity was negatively correlated with the damaged starch content ($r = -0.78$, $P < 0.05$). According to Lim et al (1999), protein content of isolated rice starches was negatively correlated with peak viscosity and positively correlated with pasting temperature. In the studies of Sanchez et al (1986) and Sabularse et al (1992), it was reported that damaged starch was negatively correlated with peak viscosity. The present results also suggests that damaged starch content might play a more important role than protein content in determining peak viscosity of starch.

The onset and peak gelatinization temperatures of isolated rice starches were 74.0–75.9°C and from 78.9–80.6°C, respectively. The enthalpy values of the starches were 12.1–14.6 J/g. There were no significant differences in thermal properties among the isolated rice starches, which is different from the results by Lumdubwong and Seib (2000), possibly because of a higher alkaline concentration in alkali digestion and the alkaline condition in protease digestion employed in their study. Although alkali might modify the pasting properties of starch, in the present study, it did not change the ordered structure within the starch granule. Therefore, all starches showed similar gelatinization temperatures and enthalpies.

CONCLUSIONS

Food-grade protease, acid-, neutral-, or alkaline-type, was capable of recovering 68–75% of the available rice starch from a ground long-grain milled rice flour at pH 6.5 after 18 hr of digestion at 50°C, which was as effective as the alkaline steeping method. The protease digestion method produced starch with higher or comparable yield, reduced damaged starch content, and exhibited properties comparable with the alkali method. Protein waste could also be recovered as a value-added by-product. The results demonstrate that a protease-assisted isolation procedure could be developed to

TABLE I
Starch Yield, Protein Content, Damaged Starch, and Iodine Affinity of Isolated Rice Starches^a

Method, Protease Level	Starch Yield (% starch db)	Protein (% db)	Damaged Starch (% db)	Iodine Affinity (% db)
Alkaline steeping	72.2b	0.15c	2.3a	5.2a
Acidic protease				
0.5 mL	75.5b	0.23b	1.9b	5.0a
1.0 mL	73.4b	0.16c	1.9b	5.0a
1.5 mL	74.1b	0.07d	1.8b	5.2a
Neutral protease				
5.0 mg	69.4c	0.33a	2.0b	5.2a
10.0 mg	68.2c	0.17c	1.6c	5.0a
15.0 mg	75.4b	0.17c	1.5c	5.2a
Alkaline protease				
0.05 mL	70.2bc	0.16c	1.8b	5.0a
0.10 mL	77.6a	0.19bc	1.6c	5.0a
0.15 mL	74.8b	0.17c	1.6c	4.9a

^a Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

TABLE II
Pasting Properties of Isolated Rice Starches^a

Method, Protease Level	Pasting Temp (°C)	Peak Time (min)	Rapid Visco Analyser Viscosity (RVA units)			
			Peak	Breakdown	Setback	Final
Alkaline steeping	82.4b	5.9a	234d	110d	107b	232c
Acidic protease						
0.5 mL	85.5a	6.0a	258c	126c	82c	215c
1.0 mL	86.0a	6.1a	271b	125c	125a	221c
1.5 mL	84.7a	6.1a	272b	111d	117ab	278a
Neutral protease						
5.0 mg	82.8b	5.9a	267bc	143a	97c	223c
10.0 mg	82.8b	6.1a	273b	139a	90c	225c
15.0 mg	83.2b	6.1a	289a	144a	102bc	247b
Alkaline protease						
0.05 mL	83.6b	6.2a	291a	132b	94c	252b
0.10 mL	83.6b	6.2a	275b	125c	95c	245b
0.15 mL	83.6b	6.2a	270b	114d	122a	278a

^a Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

produce rice starch with good yield and quality without the generation of alkali and salt in the process. Because of the potential of microbial spoilage under the conditions employed in this study, further study will be conducted to optimize the protease-assisted isolation procedure in order to shorten the reactions time or to incorporate preservatives to prevent starch slurry from spoilage.

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