

Effect of Dry- and Wet-Milling Processes on Chemical, Physicochemical Properties and Starch Molecular Structures of Rice Starches

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ABSTRACT

Rice starches from low amylose (Pathum Thani 1), medium amylose (RD 7) and high amylose (Leuang 11) varieties prepared from dry- and wet-milling processes were investigated. Dry-milled rice starches contained significantly ($p < 0.05$) higher amounts of damaged starch than wet-milled rice starches in all rice varieties. The scanning electron micrographs (SEM) of starch granules confirmed that damaged starches occurred from the dry-milling process more than from the wet-milling process. The enthalpy required to gelatinize dry-milled rice starches (8.11-9.53 J/g) from all rice varieties was significantly ($p < 0.05$) lower than those of wet-milled rice starches (9.24-12.48 J/g). The peak viscosity (257.75-353.80 RVU) and final viscosity (248.64-391.00 RVU) of dry-milled rice starches measured by Rapid Visco Analyser (RVA) were significantly ($p < 0.05$) lower than those of wet-milled rice starches. Rice starch molecular weight distributions obtained by size-exclusion chromatography with RI and multi-angle laser light scattering detection (SEC-RI-MALLS) showed that dry-milled rice starch samples contained lower amounts of the high molecular weight portion of starch (amylopectin) than wet-milled samples. The molecular weight of both amylopectin from dry-milled rice starches ($0.95-1.7 \times 10^7$ Da) was lower than that of wet-milled rice starches ($1.11-1.90 \times 10^7$ Da), suggesting that the dry-milling process caused a higher degree of starch fragmentation than the wet-milling process.

Key words: rice starch, physicochemical properties, dry-milled, wet-milled, starch molecular structure

INTRODUCTION

The application of rice flour includes traditional Asian foods and baked goods, many of which have now spread around the world. Rice flour are used increasingly in breakfast cereals, hypoallergenic foods, infant formula, seasoned multigrain products and reduced calorie items (Yeh, 2004). Thailand is a major exporter of rice flour in the world. In 2005 (January-July), Thailand exported 21,263 tons of non-waxy rice

flour and 37,874 tons of waxy-rice flour, and earned 28.1 million USD (Ministry of Commerce, 2006).

There are three methods (wet-, semidry-, and dry-milling) used to prepare rice flour. Generally, rice flour is made from the wet-milling process. Soaking, adding excess water during grinding, and drying to remove the excess water are the three steps that differentiate wet-milling from dry-milling. The cooling and lubricating effects of water cause a reduced amount of starch

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Received date : 17/07/06

Accepted date : 27/09/06

damaged (Yeh, 2004). However, the wet-milling process consumes a large amount of water, which in turn creates a lot of wastewater. On the other hand, for the dry-milling process, cleaned rice grains can be directly ground to get rice flour without generating any wastewater. However, dry-milled rice flour shows many differences in terms of chemical composition and physicochemical properties compared with the wet-milled rice flour (Chen *et al.*, 1999, Chaing and Yeh, 2002, Yoenyongbuddhagal and Noomhorm, 2002, Suksomboon *et al.*, 2005). More than 90% of rice flour constitutes from starch. Therefore, the functionality of rice flour has been ascribed to starch. However, the data related to the effect of milling process on the properties of starch isolated from dry- and wet-milled rice flour are limited. Thus, the objectives of this research were to investigate the chemical, physicochemical properties, as well as molecular weight distribution of dry- and wet-milled rice starches isolated from dry- and wet-milled rice flours.

MATERIALS AND METHODS

Rice starch preparation

Three varieties Thai milled rice were analyzed: low amylose (Pathum Thani 1), medium amylose (RD 7) and high amylose (Leuang 11). Two milling processes, dry-milling and wet-milling, were used to prepare rice flour. For the dry-milling process, the polished rice kernels were ground by using a hammer mill fitted with a 0.5-mm sieve. For the wet-milling process, the rice kernels were steeped in water for 8 hr to soften the kernels and then ground by a double-disk stone mill using water twice the weight of rice. The slurry was poured into a thick cloth bag and centrifuged by a basket centrifuge for 10 min to remove the excess water. The wet-milled flour was then dried in a hot-air oven at 40°C for 12 hr to reduce the moisture content to approximately 15%. The dried sample was ground using the

hammer mill grinder with a 0.5-mm sieve. Both flour samples were passed through a 100-mesh sieve, packed in plastic bags and stored at room temperature until used. Starch was isolated by the sodium hydroxide (NaOH) method of Lumdubwong and Seib (2000).

Chemical properties

The moisture, crude protein, crude lipid and ash for all rice starch samples were determined using the Approved Methods 44-15A, 46-11A, 30-10 and 08-01 (AACC, 2000), respectively. The conversion factor (N ¥ 5.95) was applied to convert nitrogen content to the crude protein content. Amylose content was measured using the method of Morrison and Laignelet (1986). An enzymatic digestion assay kit (Megazyme International, Wicklow, Ireland) was used to determine starch damage following the method of Gibson *et al.* (1992).

Microscopic properties

Microscopic images of rice starch granules of dry- and wet-milled rice flours before and after α -amylase treatment were determined by scanning electron microscopy (SEM) following the method of Chen *et al.* (2003). All samples were mounted on aluminum stubs using double-side tape, sputter-coated with gold and investigated using SEM (JEOL, JSM 6301F, Japan) at an accelerated voltage of 20 kV.

Thermal properties

Thermal properties of the rice starch were analyzed by using differential scanning calorimeter (DSC) (TA Instruments, USA) following the method of Patindol and Wang (2003). The sample (4.0 mg, dry basis) was weighed into an aluminum DSC pan and then moistened with 8 mg of deionized water. The pan was hermetically sealed and allowed to stand overnight prior to thermal analysis. Thermal scanning was done from 30°C to 110°C at a heating rate of 10°C/min. The

gelatinization temperature (onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c) and enthalpy change (DH) were determined.

Pasting properties

Pasting properties of rice starch were determined using a Rapid Visco Analyser (RVA) following the method of Bhattacharya *et al.* (1999). Starch (3g, 14% mb) was weighed directly in the aluminum RVA sample canister, and distilled water was added to a total constant sample weight of 28 g. A programmed heating and cooling cycle was used where the samples were held at 50 °C for 1 min, heated to 95 °C in 7.5 min at 6 °C/min, held at 95 °C for 5 min before cooling to 50 °C in 7.5 min, and holding at 50 °C for 1 min. Peak temperature, peak viscosity, breakdown, final viscosity and setback were recorded. All measurements were done in duplicates.

Size exclusion chromatography of starch

Rice starches were extracted according to the method of Jane and Chen (1992). About 100 mg rice starch was placed in 10 mL of 90% (v/v) dimethyl sulfoxide (DMSO) with constant stirring in a boiling water-bath. The solution was stirred for an additional 8 hr at room temperature. Solubilized starch was precipitated by the addition of absolute ethanol (1.5x volume) and centrifuged at 4,000 × g for 10 min. The supernatant was decanted and the precipitate was washed three times with 40 mL absolute ethanol to remove most of the remaining DMSO. Starch precipitate was placed in a boiling water-bath for 3 min to evaporate the ethanol, and allowed to dry at room temperature overnight. Exactly 15 mg of purified dry sample and 5.0 mL of distilled water were placed in a boiling water-bath with constant stirring for 30 min, and then cooled to room temperature. A 1.5 mL starch sample was filtered through a 0.45 mm pore size filter and then injected into SEC-RI-MALLS system consisting of a Varian 9012 HPLC pump (Varian, Inc., Walnut

Creek, CA), a HR 16/50 column (16mm × 50cm, Pharmacia, Sweden) packed with Sephacryl S-500 HR media (exclusion range Mr, 40,000 – 2 × 10⁷ Da, Pharmacia, Uppsala, Sweden), and a Varian 9040 refractive index (RI) detector (Miklus, 1999). A 200 mL sample loop was used and the flow rate was maintained at 1.3 mL/min. The mobile phase consisted of filtered, degassed purified water with 0.02% sodium azide. A multiangle laser light scattering detector at a wavelength of 488 nm (MALLS, Dawn DSP-F, Wyatt Technology, Santa Barbara, CA) was used to determine the molecular mass of starch.

Statistical analysis

Completely Randomized Design (CRD) was used as the experimental design. SPSS for Windows program, version 10.0, was employed for analyzing the results obtained from two replications. Analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were used for comparing differences in the mean values at the 0.05 confidence level.

RESULTS AND DISCUSSION

Chemical compositions of dry-milled and wet-milled rice starches

Chemical compositions of dry- and wet-milled rice starches are shown in Table 1. Starches extracted from dry- and wet-milled rice flour contained 10.59 – 11.95 % moisture. Amylose content of dry-milled rice starch was significantly ($p < 0.05$) lower than that of wet-milled rice starch in all rice varieties, whereas, fat content was not significantly ($p > 0.05$) different between dry- and wet-milled starch samples. The ash content of dry-milled rice starch isolated from Pathum Thani 1 (0.30±0.02 %) and RD 7 rice flours (0.34±0.03 %) showed significantly ($p < 0.05$) higher amounts than those of wet-milled samples (0.19 and 0.16%, respectively). However, the ash content of Leuang 11 was not significantly different ($p > 0.05$) between

Table 1 Chemical composition of dry-milled and wet-milled rice starches ^{1/}.

| Sample | Moisture (%) | Amylose (%db) | Protein (%db) | Fat (%db) | Ash (%db) | Damaged Starch (%) |
|----------------|-----------------|----------------|---------------|---------------|---------------|--------------------|
| Pathum Thani 1 | | | | | | |
| dry-milled | 11.50 ± 0.11 bc | 15.28 ± 0.07 a | 1.50 ± 0.06 c | 0.14 ± 0.01 a | 0.30 ± 0.02 b | 5.20 ± 0.24 c |
| wet-milled | 10.59 ± 0.39 a | 19.25 ± 0.42 b | 0.79 ± 0.07 b | 0.11 ± 0.02 a | 0.19 ± 0.01 a | 4.34 ± 0.12 b |
| RD 7 | | | | | | |
| dry-milled | 11.39 ± 0.28 bc | 28.27 ± 0.69 c | 1.64 ± 0.09 d | 0.10 ± 0.00 a | 0.34 ± 0.03 b | 4.42 ± 0.07 b |
| wet-milled | 10.93 ± 0.07 ab | 30.28 ± 0.76 d | 0.55 ± 0.03 a | 0.09 ± 0.01 a | 0.16 ± 0.00 a | 3.74 ± 0.07 a |
| Leuang 11 | | | | | | |
| dry-milled | 11.42 ± 0.37 bc | 37.98 ± 0.83 e | 1.64 ± 0.04 d | 0.09 ± 0.00 a | 0.20 ± 0.00 a | 4.23 ± 0.08 b |
| wet-milled | 11.95 ± 0.01 c | 40.09 ± 0.62 f | 0.85 ± 0.03 c | 0.11 ± 0.01 a | 0.18 ± 0.01 a | 3.53 ± 0.07 a |

^{1/} Values are means of duplicate measurements ± standard deviation. Means for each characteristic followed by the same letter within the same column are not significantly different ($p < 0.05$) by Duncan's multiple range test.

dry- and wet-milled starch samples.

Due to the preparation of wet-milled flour, rice kernels had to be soaked and ground with water. Soluble protein, sugars and non-starch bound lipids were removed, resulting in lower amounts of these components in wet-milled rice flours (Chen *et al.*, 1999, Chaing and Yeh, 2002, Yoenyongbuddhagal and Noomhorm, 2002, Suksomboon *et al.*, 2005). Generally, high purity rice starch contain low amount of protein and lipid. Nevertheless, lipids exist in rice at much lower amount than protein. Therefore, isolation of rice starch mainly involves removing protein and the goal for protein content of isolated rice starch is generally less than 0.5%. Compared with maize and wheat starches, isolation of rice starch from proteinaceous material is very difficult. The starch in rice endosperm is associated with proteins occurring as protein bodies I and II (PB I and PB II). Both proteins are hydrophobic and resist swelling in water at neutral pH. Moreover, PB II is cross-linked with disulphide bonds. Uncharacterized proteinaceous material is also localized in small pockets surrounding both the compound and the individual rice starch granules (Lumdubwong and Seib, 2000). The larger flour particle size of dry-milled rice flours (100-150 mm), compared with particle size of wet-milled

rice flours (less than 100 mm), caused the difficulty to NaOH to penetrate into the surface of the rice starch granules to dissolve the protein. Thus, result in a higher amount of protein remaining in the dry-milled rice starch samples. In the addition to, the larger flour particle size, the starch granules from dry-milled rice flours contained higher amounts of damaged starch (4.23 – 5.22%) compared with those of wet-milled rice starches (3.53 – 4.35%). The damaged starch can absorb a large amount of water and is mixed in the upper layer of protein after the centrifugation process, resulting in the higher contamination of proteinaceous matter in the dry-milled starch and causing lower starch recovery (57.09 – 66.93% wt of dry flour) compared with the wet-milled starch (67.10- 75.91 % wt of dry flour).

Microscopic properties

The SEM of starch granules from untreated and α -amylase treated dry-milled and wet-milled rice flours are shown in Figure 1. Under microscopy, the dry-milled sample had clumps of starch granules aggregated into particles with the smooth cutting surfaces (Figure 1a); while the starch granules from wet-milled samples were separated as individual granules (Figure 1b). Dry-milled rice flour contained significantly larger

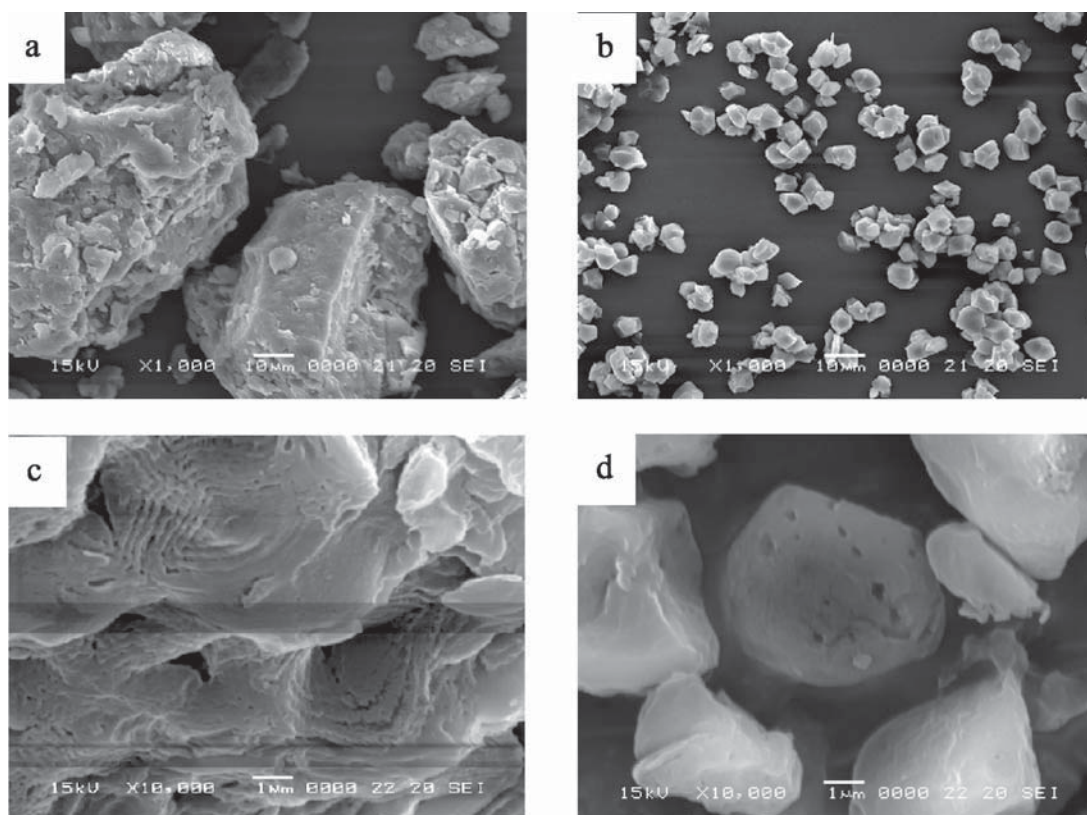


Figure 1 SEMs of starch granules from untreated a) dry-milled and b) wet-milled rice flours; and α -amylase treated c) dry-milled and d) wet-milled rice flours.

flour particles in the form of these aggregated starch granules, which were 50 – 150 μ m in diameter compared to the separated starch granules of wet-milled samples which were 5-9 μ m in diameter. Chiang and Yeh (2002) stated that during the soaking process of wet-milling rice flour, protein matrix and other substances were leached out from the surface of starch granules, causing the structure of starchy endosperm to become loosen which resulted in the fine particles and less damaged starch.

After being subjected to α -amylase, damaged starch from both dry- and wet-milled samples was digested. The damaged starch at the smooth cutting surfaces of the aggregated starch granules from dry-milled rice were digested and the growth ring in these damaged starch were

verified. For wet-milled rice samples, damaged starch granules were digested internally and the remnants left appeared to be the shells of granules after treatment with α -amylase. The above evidence suggests that both milling processes can create damaged starches. A severe condition of the dry-milling process created a larger amount of damaged starches located on the surface of aggregated granules, while the wet-milling process created smaller amounts of damaged starches scattered in the samples.

Physicochemical properties of dry-milled and wet-milled rice starches

Thermal properties

The gelatinization characteristics of dry- and wet-milled rice starches measured using DSC

are shown in Table 2. The results show that T_o (64.04 ± 0.04 °C) and T_c (80.24 ± 0.41 °C) of Pathum Thani 1 rice starch from dry-milled samples were significantly ($p < 0.05$) lower than that of wet-milled samples; while, T_o and T_c of RD 7 and Leuang 11 rice starches from dry-milled samples were not significantly ($p > 0.05$) different to wet-milled samples. For all rice varieties, the enthalpy required to gelatinize dry-milled rice starch (8.11 ± 0.16 to 9.53 ± 0.04 J/g) was significantly ($p < 0.05$) lower than that of wet-milled rice starch (9.24 ± 0.04 - 12.48 ± 0.06 J/g). In general, DSC parameters have been reported to reflect the appearance of the crystalline structure influenced by the fine structure of amylose and amylopectin in starch granules. The higher the crystalline structure remaining in the starch granule, the greater the gelatinization enthalpy used during the starch gelatinization process (Chen *et al.*, 1999). Many researchers reported the decrease in DH in flour samples when starch granules were damaged caused by milling method (Chen *et al.*, 1999; Yoenyongbuddhagal and Noomhorm, 2002). Morrison and Tester (1994) found that with gradual ball-milling of wheat and maize starches up to 24 hr, starch granule crystallinity and double helix content decreased while damaged starch increased. They postulated that the primary event caused by mechanical

damage is the conversion of large ordered (crystalline) regions into essentially disordered amorphous material which is freely accessible to external agents such as solvent water and amyolytic enzymes. From the presented results, lower DH and higher damaged starch content measured by an enzymatic method (Table 1) from dry-milled rice starch samples indicate that dry-milling process caused considerably more mechanical damage to the starch granules than wet-milling process.

Pasting properties

The pasting properties of dry- and wet-milled rice starches were determined using RVA. Typical pasting curves and pasting properties obtained from RVA are shown in Figure 2 and Table 3. Dry-milled rice starches provided significantly ($p < 0.05$) lower peak viscosity (257.75 ± 4.24 to 353.80 ± 0.18 RVU) than those of wet-milled rice starches (281.11 ± 5.26 to 370.03 ± 4.82 RVU). The lower peak viscosity was caused by a higher starch damage of dry-milled rice starches, similarly to the result reported by Yoenyongbuddhagal and Noomhorm (2002). The results showed that dry-milled rice starches (134.42 ± 4.24 to 181.55 ± 0.53 RVU) had significantly ($p < 0.05$) lower breakdown than those of wet-milled rice starches (149.00 ± 1.46 to

Table 2 Gelatinization characteristics of dry-milled and wet-milled rice starches ^{1/}.

| Sample | T_o (°C) | T_p (°C) | T_c (°C) | Enthalpy (J/g) |
|----------------|--------------------|--------------------|--------------------|--------------------|
| Pathum Thani 1 | | | | |
| dry-milled | 64.04 ± 0.04 a | 71.60 ± 0.05 a | 80.24 ± 0.41 a | 8.11 ± 0.16 b |
| wet-milled | 65.94 ± 0.08 b | 71.98 ± 0.15 a | 81.08 ± 0.33 b | 12.48 ± 0.06 e |
| RD 7 | | | | |
| dry-milled | 72.80 ± 0.15 d | 77.62 ± 0.18 d | 84.77 ± 0.05 d | 9.53 ± 0.04 c |
| wet-milled | 73.02 ± 0.64 d | 78.16 ± 0.28 e | 85.15 ± 0.06 d | 10.66 ± 0.32 d |
| Leuang 11 | | | | |
| dry-milled | 69.73 ± 0.10 c | 74.58 ± 0.18 b | 83.10 ± 0.16 c | 7.08 ± 0.06 a |
| wet-milled | 70.12 ± 0.15 c | 75.86 ± 0.03 c | 83.20 ± 0.49 c | 9.24 ± 0.14 c |

^{1/} Values are means of duplicate measurements \pm standard deviation. Means for each characteristic followed by the same letters within the same column are not significantly different ($p > 0.05$) by Duncan's multiple range test.

199.58±0.47 RVU) due to lower number of swollen starch granules (lower peak viscosity). After cooling to 50 °C, both dry- and wet-milled rice starches showed increasing viscosity. Final viscosity was significantly ($p<0.05$) lower for dry-

milled rice starches (248.64±0.39 to 391.00±2.47 RVU) than those of wet-milled rice starches (260.04±4.30 to 411.88±6.57 RVU). Dry-milled starches showed lower setback than those of wet-milled rice starches, except for Leuang 11 rice

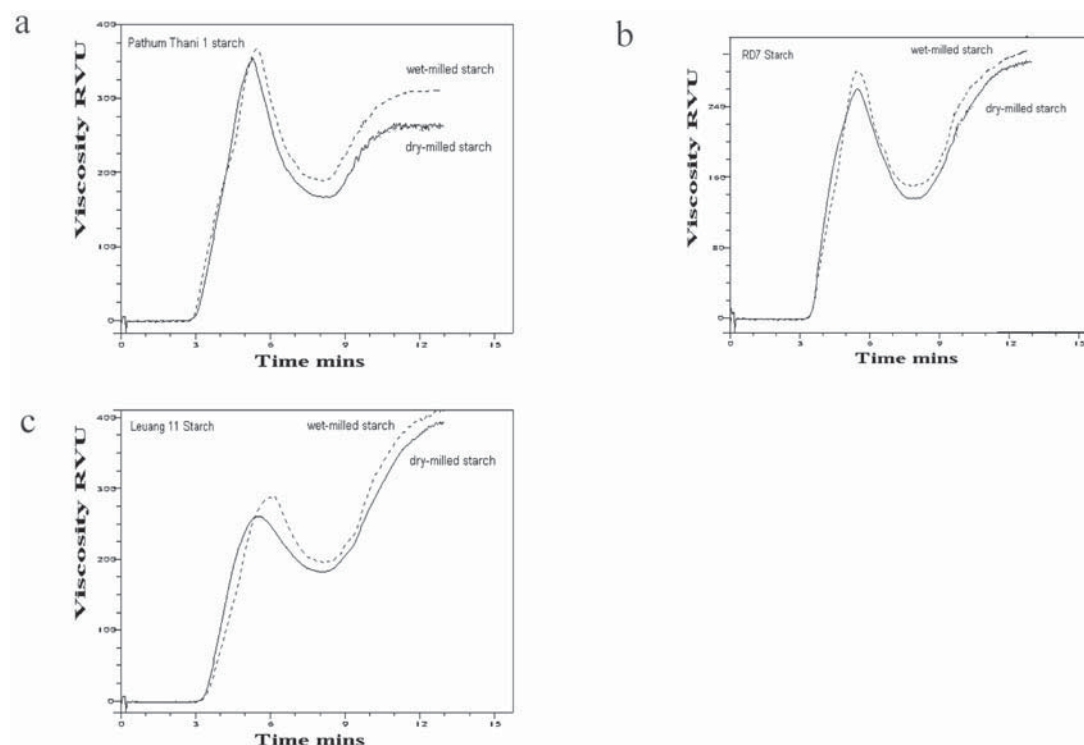


Figure 2 Pasting properties of (—dry-milled, - - - wet-milled) rice starches from three rice varieties: a) Pathum Thani 1 b) RD 7 and c) Leuang 11.

Table 3 Pasting properties of dry- and wet- milled rice starches ^{1/}.

| Sample | Pasting Temp. (°C) | Viscosity (RVU) | | | |
|----------------|-----------------------|-----------------|----------------|----------------|----------------|
| | | Peak Visc. | BreakDown | Final Visc. | SetBack |
| Pathum Thani 1 | | | | | |
| dry-milled | 71.60 ± 0.05a | 353.80 ± 0.18d | 166.63 ± 0.29c | 248.64 ± 0.39a | 57.66 ± 6.43a |
| wet-milled | 72.43 ± 0.53a | 370.03 ± 4.82e | 190.97 ± 6.08e | 309.20 ± 3.00c | 91.58 ± 4.72b |
| RD 7 | | | | | |
| dry-milled | 77.78 ± 0.21c | 262.50 ± 3.07a | 134.42 ± 2.12a | 253.22 ± 7.60a | 104.22 ± 7.11b |
| wet-milled | 78.18 ± 0.32c | 281.11 ± 5.26b | 149.00 ± 1.46b | 260.04 ± 4.30b | 112.62 ± 5.01c |
| Leuang 11 | | | | | |
| dry-milled | 76.55 ± 0.28b | 257.75 ± 4.24a | 181.55 ± 0.53d | 391.00 ± 2.47d | 209.46 ± 1.94d |
| wet-milled | 76.70 ± 0.99b | 294.71 ± 1.71c | 199.58 ± 0.47f | 411.88 ± 6.57e | 214.46 ± 3.00d |

^{1/} Values are means of duplicate measurements ± standard deviation. Means for each characteristic followed by the same letters within the same column are not significantly different ($p\Delta 0.05$) by Duncan's multiple range test.

starches. This result implies that dry-milled samples had a lower degree of recrystallization of gelatinized starch during cooling. From all of the results above, it can be concluded that the milling process influences pasting viscosity properties by the mechanical damage of starch granules.

Size exclusion chromatography of starch

Molecular distributions of starches from dry- and wet-milled starches resolved by SEC-RI-MALLS are shown in Figure 3. The chromatograms illustrate that high molecular weight fractions (amylopectin) eluted at the void volume at 30-45 mL, followed by a broad fraction of amylose at 45-95 mL. The RI profiles show that starches from dry-milled samples contained

smaller amounts of high molecular weight fractions (amylopectin) but greater amounts of low molecular weight fractions (amylose) than those of wet-milled starch samples. The MALLS data show that the average molecular weight of amylopectin from dry-milled rice starches ($4.45\text{--}5.20 \times 10^7$ Da) was smaller than that of wet-milled rice starches ($4.64\text{--}5.42 \times 10^7$ Da). Morrison and Tester (1994) found similar results as soluble extracts separated on SEC from ball-milled wheat starch provided a breakdown of amylopectin to low molecular weight fragmented amylopectin that co-eluted with amylose. The presented results indicate that during dry-milling process, starch was susceptible to fragment to lower molecular weight fractions more than wet-milling process. In

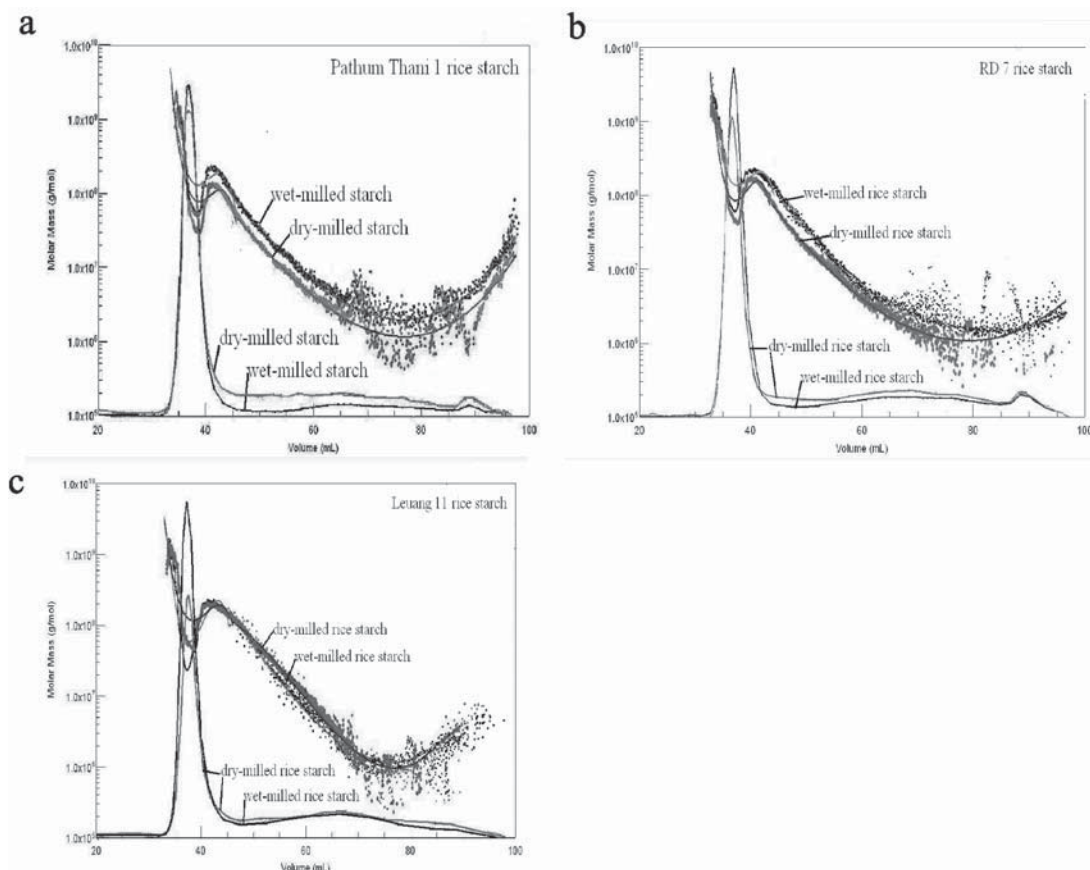


Figure 3 Molar Mass vs. Volume Chromatogram of (—dry-milled, —wet-milled) rice starch from three rice varieties: a) Pathum Thani 1 b) RD 7 and c) Leuang 11

addition, the starch fragmented products might influence the physicochemical properties of rice flours as discussed earlier.

CONCLUSIONS

Rice starch isolated from dry- and wet-milled rice flours had differences in chemical composition and physicochemical properties. Dry-milled rice starches contained greater amounts of protein and damaged starch than those of wet-milled rice starches. The molecular size distributions indicate that during dry-milling process, starch especially amylopectin, was susceptible to fragment to lower molecular weight fractions more than in the wet-milling process. In conclusion, a higher amount of damaged starch and greater degree of starch fragmentation during dry-milling process caused lower gelatinization enthalpy and lower RVA viscosity profiles than in wet-milled rice starches.

ACKNOWLEDGEMENTS

The authors wish to thank The Commission on Higher Education, Ministry of Education, THAILAND for financial support. We also appreciate the technical support for SEC-RIMALLS provided by Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, IN, USA.

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