# MATERIALS AND METHODS

## Materials

### 1. Rice samples

Three varieties of low amylose (Pathum Thani 1), medium amylose (RD 7) and high amylose (Leuang 11) Thai milled rices were obtained from Rice Research Institute, Pathum Thani, Thailand.

### 2. Chemical Reagents

- Sulfuric acid (BDH AnalyR®, England)
- Hydrochloric acid (BDH AnalyR®, England)
- Sodium hydroxide (BDH AnalyR®, England)
- Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Germany)
- Ethanol (BDH AnalyR®, England)
- Potassium iodide/Iodine solution
- Enzymatic digestion assay for starch damage (Megazyme International, Ireland)
- Sodium azide (SigmaUltra, Sigma-Aldrich, USA)
- Others

### 3. Equipments and Instruments

- Hammer mill (Ultra-centrifugal mill type ZM1, Retsch, Germany)
- Double-disk stone mill (Locally made, Thailand)
- Basket centrifuge (Type H-130G, Kokusan Ensinki Co., Ltd., Japan)
- Laboratory test sieve 100 and 200 mesh (Endecotts Ltd., England)
- Tecator Kjeltec System (Tecator AB, Sweden)
- Tecator Soxtec System (Tecator AB, Sweden)
- Muffle furnace (FSE-621 Series, Sanyo Gallenkamo, Germany)

- Scanning electron microscopy (SEM) (JEOL, JSM-5600 LV, Japan)
- Differential scanning calorimetry (DSC) (DSC 2920, TA Instruments, USA)
- Rapid Visco Analyser (RVA) (Newport Scientific Pty, Ltd., Australia)
- Size-exclusion chromatography with multi-angle laser light scattering and refractive index detection (SEC-MALLS-RI)
- X-ray diffractometer (JEOL, JDX-3530, Japan)
- Texture Analyzer (TA.XT2) (Stable Micro System, England)
- Hot air oven (Memmert, Germany)
- Spectrophotometer (Spectronic 23, LabMed Inc., USA)
- Viscometer (Brookfield DVIII, Brookfield Engineering Labs Inc, USA)
- Centrifuge (Himac CR 20B2, Hitachi, Germany)

## Methods

### 1. Rice Flour Preparation

Three varieties of low amylose (Pathum Thani 1), medium amylose (RD 7) and high amylose (Leuang 11) Thai rice were used for making rice flour. The rice samples were packed in polyethylene bags and then stored in a cold room at 4$^{o}$C before milling process. Two milling processes, dry-milling and wet-milling, were applied to prepare rice flour samples. For dry-milling process, the polished rice kernels were grounded using hammer mill fitted with a 0.5-mm sieve. For wet-milled process, the rice kernels were steeped in water for 4 hr to soften the kernels and then grounded by double-disk stone mill with water at ratio of water:rice for 2:1 (w/w). The slurry was poured into a thick cloth bag and centrifuged by basket centrifuge for 10 min to remove the excess water. The wet-milled flour was dried in a hot-air oven at 40$^{o}$C for 12 hr to reduce the moisture content to approximately 12%. The dried samples were grounded 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 -18$^{o}$C until used.

## 2. Rice Starch Preparation

Rice starch was isolated from dry- and wet-milled rice flour following the method adapted from Lumdubwong and Seib (2000) as shown in Figure 10. Both dry- and wet-milled rice flour (200 g) were mixed for 3 hr at 25 $^{o}$C with 0.2% (0.05M) NaOH (500 mL) and filtered the slurry through a 200-mesh laboratory test sieve (75 μm opening). The filtrate was centrifuged at 3,000 g for 20 min, the supernatant was discarded, and the sediment was washed twice with water (500 mL) and centrifuged. The residue was suspended in water and adjusted to pH 7 by adding 1 M hydrochloric acid, and the slurry was centrifuged. The supernatant was discarded, and the yellow tailings layer over the starch layer was carefully scraped away and discarded. The starch were carefully scraped away and discarded. The starch was washed three times with water (500 mL). The starch was dried in a hot-air oven at 40 $^{o}$C for 12 hr. The dried starch was grounded using a cyclone mill grinder with a 0.5-mm sieve. Starch samples were passed through a 100-mesh sieve, packed in plastic bags and stored at –18 $^{o}$C before analysis.

## 3. Determination of Chemical Properties and Morphological Properties of Rice Flour and Rice Starch

**3.1 Chemical Analysis.** The moisture, crude protein, crude lipid and ash contents for all the rice flour and rice starch were determined following approved methods of AACC 44-15A, 46-11A, 30-10 and 08-01 (AACC, 2000). The conversion factor of N x 5.95 was applied to convert nitrogen to crude protein content. Amylose content was measured using the method of Morrison and Laignelet (1986) as described in Appendix. An enzymatic digestion assay kit (Megazyme International, Ireland) was used to determine starch damage using approved methods of AACC 76-31 (AACC, 2000) as showed in Appendix.

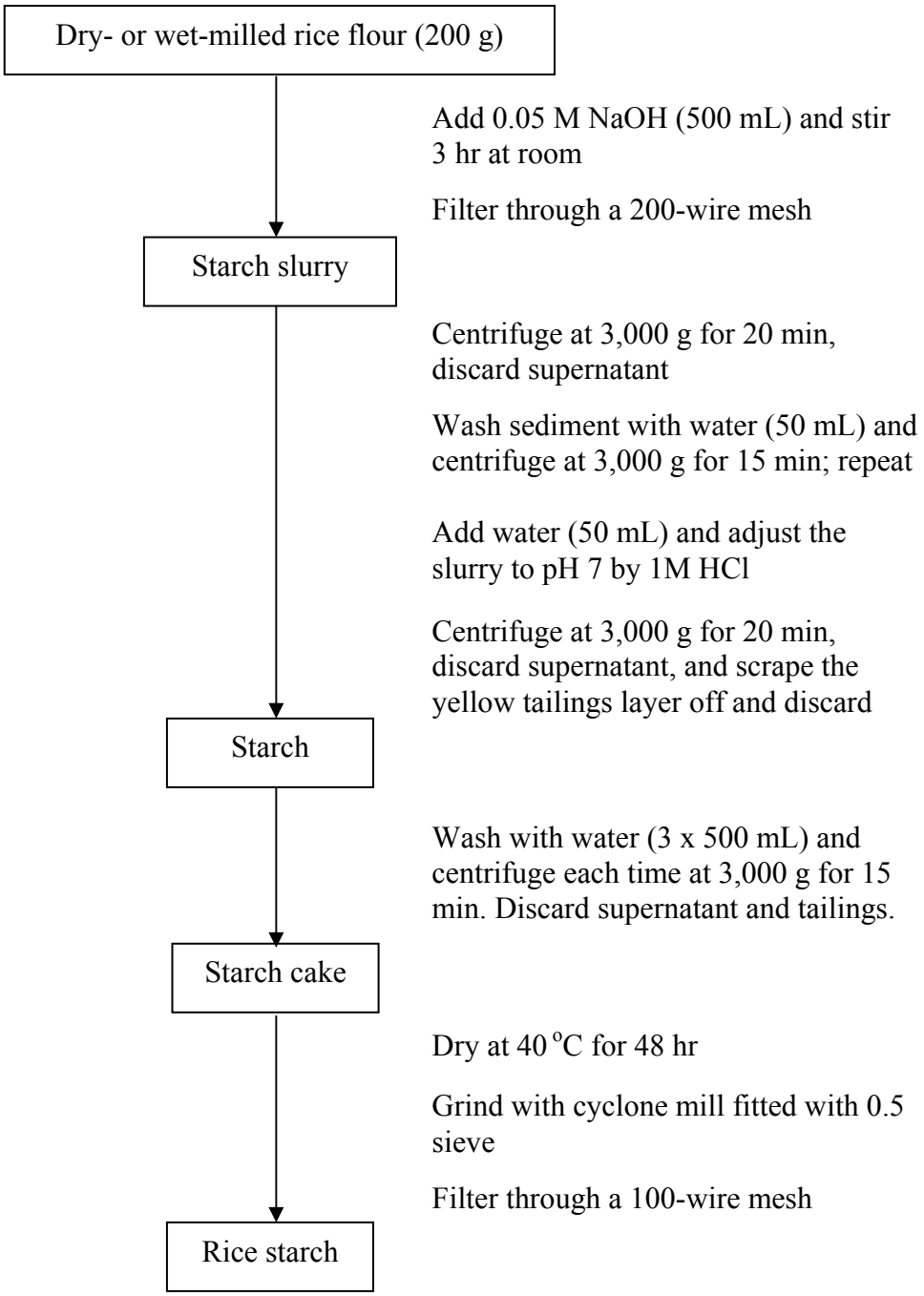


**Figure 10** Isolation of rice starch from dry- and wet-milled rice flour.
Modified from Lumdubwong and Seib (2000)

**3.2 Scanning Electron Microscopy.** Microscopic images of rice starch granules were examined using scanning electron microscopy (SEM) (JEOL, JSM-5600 LV, Japan). Rice flour and rice starch before and after subjected to α-amylase in starch damaged determination were investigated. The samples were mounted on an aluminum stub using double-side tape, sputter-coated with gold and investigated under SEM at an accelerated voltage of 15 kV following the method modified from Vatanasuchart (2004) as showed in Appendix.

## 4. Determination of Physicochemical Properties of Rice Flour and Rice Starch

**4.1 Gelatinization Properties.** Thermal properties of rice flour and rice starch were analyzed by using differential scanning calorimeter (DSC) (DSC 2920, TA Instruments, USA) following the method of Patindol and Wang (2003) as described in Appendix. The instrument was calibrated with indium, and an empty pan was used as reference. 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 $^{o}$C to 110 $^{o}$C at a heating rate of 10 $^{o}$C/min. The gelatinization temperature (onset temperature: $T_o$, peak temperature: $T_p$ and conclusion temperature: $T_c$) and enthalpy change ($\Delta H$) were recorded.

**4.2 Swelling Power and Solubility.** The swelling power and solubility of rice flour and rice starch were obtained following the method of Schoch (1964) with slight modification. The samples (0.5 g) were dispersed in 15 mL of distilled water in 50 mL preweighed centrifuge tubes. The suspensions were heated to 55, 65, 75, 85 and 95 $^{o}$C in a water-bath with periodic mixing over a 30 min period. The cooked paste samples were centrifuged at 2,200 rpm for 15 min. The supernatants were drained and placed in preweighed petridishes and dried at 105 $^{o}$C to constant weight. The weight of swollen matter from cooked paste was determined. The solubility and swelling power were calculated as following equation:

$$\text{Swelling Power (g/g)} = \frac{\text{Weight of swollen matter (g) x 100}}{\text{Weight of sample (g dry basis) x (100 – Solubility)}}$$

$$\text{Solubility (\%)} = \frac{\text{Weight of soluble matter in supernatant (g ) x 100}}{\text{Weight of sample (g dry basis)}}$$

**4.3 Pasting Properties.** Pasting properties of rice flour and rice starch were determined by a Rapid Visco Analyser (RVA) (Newport Scientific Pty, Ltd., Australia) following the approved methods of AACC 61-02 (AACC, 2000) as described in Appendix. The sample (3g, 12% moisture basis) was weighed directly in the aluminum RVA sample canister, and 25 mL distilled water (12% moisture basis) was added. A programmed heating and cooling cycle was used where the samples was held at 50 $^{o}$C for 1 min, heated to 95 $^{o}$C in 3 min 45 sec, held at 95 $^{o}$C for 2 min 30 sec, before cooling to 50 $^{o}$C in 3 min 45 sec. Peak time, peak viscosity, breakdown, final viscosity, and setback were recorded.

## 5. Determination of Starch Molecular Properties of Rice Flour and Rice starch

**5.1 Starch Crystallinity.** X-ray diffraction patterns were obtained with a diffractometer (JEOL, JDX-3530, Japan) equipped with a copper anode X-ray tube. The diffractometer were operated at 40 kV, 45 mA and the spectra was scanned over a diffraction angle ($2\theta$) range of 4 to 30$^{o}$ at a step size of 0.02$^{o}$ and a count time of 2 sec. Percentage crystillinity was calculated as the percentage peak area to the total diffraction area following the method modified from Patindol and Wang (2003) as described in Appendix.

**5.2 Starch Molecular Properties.** Rice flour and rice starch samples were purified by 10 mL of 90% (v/v) dimethyl sulfoxide (DMSO) according to the method of Jane and Chen (1992) with slightly modification. A 100 mg of rice flour or rice starch was dispersed in 10 mL of 90% dimethyl sulfoxide (DMSO) on the boiling water bath for 1 hr, followed by stirring for 24 hr using a magnetic stirrer at room temperature. The dispersed samples were mixed with four volume of ethanol (40 mL)

to precipitate starch. Ethanol-precipitated starch was separated by centrifugation at 3500 rpm for 10 min. The supernatant was decanted from the starch pellets. Starch pellets were redispersed in 20 mL of ethanol, following with centrifugation and disposal of supernatant. The redispersion was repeated for 2 times. The starch pallets were allowed to dry at room temperature. After the purified starch samples were dried, they were lightly grained to ensure even particle size distribution. Exactly 15 mg of purified dry sample and 5.0 mL of distilled water were placed in 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 5 μm pore size filter and injected into SEC-MALLS-RI system consisting of a Varian 9012 HPLC pump (Varian, Inc., Walnut Creek, CA), a HR 16/50 column (16mm x 50cm, Pharmacia, Sweden) packed with Sephacryl S-500 HR media (exclusion range Mr 40,000 – 2 $x10^7$ Da, Pharmacia, Uppsala, Sweden), and a Varian 9040 refractive index (RI) detector. 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 multi-angle laser light scattering detector at a wavelength of 488 nm (MALLS, Dawn DSP-F, Wyatt Technology, Santa Barbara, CA) was used to determine molecular weight distributions of starch (Miklus, 1999).

Hot water soluble fractions were prepared following method of Mujoo and Ali (1999). The 500 mg of rice flour was placed to a 50 mL centrifuge tube. The 20 mL of distilled water was added. The mixture was heated in a boiling water bath for 30 min with intermittent stirring. Additional 20 mL distilled water was added to the mixture, vortexed briefly and then cooled for 30 min at room temperature. Tubes were centrifuged at 2800 g for 30 min. The supernatant was decanted carefully, loaded in a 3 mL syringe. After filtering through a 5 μm nylon syringe filters, samples was injected into the size exclusion chromatograph.

## 6. Rice Noodle Preparation

Rice noodle was prepared by mix a 40:60 portion of wet-milled rice flour and water following the method of Gallapanayutt (2004). The viscosity of the wet-milled rice flour slurries of all three rice varieties were measured by Viscometer (Brookfield DVIII, Brookfield Engineering Labs Inc, USA). Dry-milled flour slurry from each rice variety was prepared by mixing a portion of dry-milled rice flour and water to obtain the equivalent viscosity with those of the wet-milled rice slurry. Flour slurry was stood at the room temperature with occasionally stirring for 3 hr. The 55 g of rice flour slurry was pour into a 18 x 24 cm stainless steel tray, and then steamed in an electric steamer for 5 min. After 5 min of cooling, the gelatinized sheet was removed from the tray and then put into the plastic bag and kept at 10 $^{o}$C for 6 hr, and then cut to the 3-mm wide noodle. The fresh rice noodle was dried in a hot air oven at 55 $^{o}$C for 2 hr. Dried noodle was packed in the plastic bag before further quality determination.

## 7. Determination of Rice Noodle Properties

**7.1 Cooking Properties.** Cooking properties were determined according to the method of Yoenyongbuddhagal and Noomhorm (2002b) with slightly modification. Five grams of dried rice noodle was boiled in 150 mL distilled water for 4 min, drained for 5 min and weighed immediately. Cooking water was separated, and dried at 105$^{o}$C to constant weight. Cooking losses during boiling were expressed as percentage of dry matter of the supernatant to dry sample weight. The water absorption index was the percentage of weight increase in the cooked rice noodle sample as compared to the dried starting sample.

$$\text{Water absorption index} = \frac{(\text{Weight of cooked noodle} - \text{Weight of dried noodle})}{\text{Weight of dried noodle}} \times 100$$

$$\text{Cooking loss} = \frac{(\text{Weight of dry matter of supernatant}) \times 100}{\text{Weight of dried noodle}}$$

**7.2 Textural Properties.** Dried noodle was soaked in the water for 10 min, and then boiled in the boiling water for 3 min. Cutting force of rice noodle samples were determine by the Texture Analyzer (TA-XT, Stable Micro Systems, UK) following the method of the approved methods of AACC 66-50 (AACC, 2000) with some modification as described in Appendix. Tensile strength of fresh boiled noodles was also measured following the method of Stable Micro Systems (1995) and Bhattacharya *et al*. (1999). The noodle strand was wound around parallel rollers of a spaghetti/noodle tensile grip analyzer (TA-XT, Stable Micro Systems, UK) to anchor the sample ends and reduce any slippage. The maximum force (N) required breaking the noodle strand (tensile strength), which gave an indication of the sample's resistance to breakdown was recorded.

**7.3 Sensory Evaluation.** Sensory evaluation of six rice noodle samples was determined compared with commercial product. Ten taste panel was performed a quantitative descriptive analysis (QDA) to evaluate cooked noodles. The attributes evaluated for the cooked noodles were turbidity, hardness or firmness, elasticity, and adhesiveness. Perceived intensities were scored on a 15-cm-interval scale. Preference for each perceived intensity and overall preference were also evaluated using a 1 to 9 hedonic scale following the method of Meilgaard *et al*. (1999) and Chen *et al*. (2002) as showed in Appendix.

## 8. Statistical Analysis

8.1 Complete randomize design (CRD) was applied as an experimental design for study the effects of the rice flour and rice starch samples from different rice varieties and milling processes on chemical, physiochemical (except for swelling power and solubility) and rice noodle properties.

8.2 Factorial in complete randomized design (CRD) was used as an experimental design for study the effects of the rice flour and rice starch samples from different rice varieties and milling processes ($n$ =12) and temperatures ($n$ = 5: 55, 65, 75, 85 and 95 $^{o}$C) on the swelling power and solubility of rice flour and rice starch.

8.3 The SPSS for Windows program, version 10.0, was employed for analyzing the statistical results obtained from two replications. Mean with standard deviation for each treatment was calculated. The analysis of variance (ANOVA) and the duncan's multiple range test (DMRT) were used for comparing differences of the mean values at the 0.05 confidence level. Additionally, linear regression analysis was applied for determining 1) a relationship between chemical properties, starch molecular properties and physicochemical properties of rice flour and rice starch and 2) a relationship between chemical properties, starch molecular properties and physicochemical properties of rice flour and rice starch on rice noodle qualities.

## 9. Place and Duration

### 9.1 Places

- Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand
- Whistler Center of Carbohydrate Research, Department of Food Science, Purdue University, IN, USA

### 9.2 Duration

June 2004 –January 2007