APPENDIX

APPENDIX A

1. Determination of Amylose Contents (Morrison and Laignelet, 1983)

2.1 Apparatus

- Spectrophotometer (Spectronic 23, LabMed Inc., USA)
- Boiling water-bath
- Hot air oven 100 ^{o}C
- Centrifuge

2.2 Reagents

- Amylose Type III from potato (Sigma-Aldrich, No.A-0512, USA)
- Urea-dimethylsulphoxide (UDMSO) solution (mix 9 volumes of DMSO with 1 volumes of 6 M urea)
- I_2-KI solution (2 mg I_2, 20 mg KI/mL)
- Ethanol 95%

2.3 Procedure

2.3.1 Weight rice flour and rice starch (75-85 mg) (correct to 0.1 mg) into a 50 mL screw-cap centrifuge tube.

2.3.2 Add 10 mL of UDMSO, cap the tube and immediately mixed vigorously on a vortex mixer.

2.3.3 Place the tube in the boiling water bath and heat for 5-15 min with intermittent mixing until the solution was almost clear.

2.3.4 Transfer the tube to an oven at 100 ^{o}C to give a total heating time of 60-90 min, then cool and check carefully for the absence of clear gel.

2.3.5 Pipette 1 mL of starch-UDMSO solution and 9 mL ethanol into a 50 mL screw-cap centrifuge tube. The tube was capped and mixed.

2.3.6 Centrifuge at 2000 *g* for 15 min, and decant the supernatant carefully.

2.3.7 Redissolve the starch by addition of 1 mL UDMSO and vortex mixing gently.

2.3.8 Cap the tube and heat at 100 ^{o}C for 15-30 min.

2.3.9 Rinse the starch-UDMSO solution into a 100-mL volumetric flask with approx. 95 mL water.

2.3.10 Add 2 mL of I_2-KI solution, then mix immediately and make to the volume with distilled water.

2.3.11 After 15 min, read the absorbance at 635 nm against UDMSO-I_2-KI solution as blank.

2.3.12 Total amylose content is calculated from the Blue Value, which defined as the absorbance /cm at 635 nm of 10 mg anhydrous starch in 100 mL dilute I_2-KI solution at 20^{o}C as equation (1) and corrected to 20^{o}C using equation (2) with the correction factors shown in Appendix table.

$$\text{Amylose (\%)} = (28.414 \times \text{Blue Value}) - 6.218 \qquad (1)$$

$$(BV_{20} = BV_t + (t - 20) \times \text{correction}) \qquad (2)$$

Appendix Table 1 Correction factors for blue values not measured at 20 ^{o}C

Blue Value (BV)	Correction BV/ ^{o}C
0.22 – 1.80	0.0078
2.00	0.0082
2.20	0.0094
2.60	0.0109
2.80	0.0116
3.00	0.0124
3.20	0.0131
3.40	0.0139
3.60	0.0146
3.74	0.0152
2.0-2.5*	0.010-0.0075

2. Determination of Damaged Starch-Spectrophotometric Method (AACC, 2000)

2.1 Apparatus

- Test tubes, glass, round-bottomed, 12-mL capacity.
- Micropipettes, 100-µL, with tips.
- Pipettes, positive displacement with tips size 5.0, 12.5, and 50.0 mL.
- Bench centrifuge, speed 3,000 rmp.
- Balance, analytical.
- Spectrophotometer.
- Vortex mixer.
- Water bath, thermostatted, set at 40 ^{o}C.
- pH meter.

2.2 Reagents

2.2.1 Sodium acetate buffer (100 mM, pH 5.0) with calcium chloride (5.0 mM). Add glacial acetic acid (5.7 mL, 1.05 g/mL) to 900 mL distilled water. Adjust solution to pH 5.0 by addition of 2M (8 g/100 mL) NaOH solution. Approximately 60 mL is required. Add $CaCl_2$ dihydrate (0.74 g) and dissolve. Adjust volume to 1.0 L, and store buffer at 4 ^{o}C.

2.2.2 Phosphate buffer (0.1M, pH 7.4). Add sodium phosphate monobasic ($NaH_2PO_4 \cdot H_2O$, 13.8 g) to 900 mL distilled water. Adjust pH to 7.4 using 0.1M NaOH (4g/L).

2.2.3 Sulfuric acid (dilute, 0.2% v/v). Carefully add concentrated sulfuric acid (2.0 mL) to 998 mL distilled water.

2.2.4 Fungal α-amylase, from *Aspergillus oryzae*, 1,000 units/mL. Dilute aliquot (1.0 mL) to 20.0 mL with 100 mM sodium acetate buffer (reagent 2.2.1). Store frozen between uses.

2.2.5 Amyloglucosidase, from *Aspergillus niger*, 200 units/mL. Dilute aliquot (1.0 mL) to 10 with 100 mM sodium acetate buffer (reagent 2.2.1). Store frozen between uses.

2.2.6 Glucose oxidase/peroxidase enzyme (GOPOD) system. Final concentration per bottle is at least glucose oxidase, 12,000 units; peroxidase, 650 units; 4-aminoantipyrine, 0.4 m*M*. Dilute contents of 1 bottle to 1.0 L with potassium phosphate buffer/para-hydroxybenzoic acid mixture (reagent 2.2.2).

2.2.7 Reagent blank (in duplicate for each set of determinations). Mix 0.2 mL acetate buffer (reagent 2.2.1) and 4.0 mL glucose oxidase/peroxidase enzymes (reagent 2.2.6).

2.2.8 Glucose reagent (150 µg/0.1 mL). Accurately weigh 150 mg glucose and make up to 100 mL with water.

2.2.9 Glucose standard (in duplicate for each set of determinations). Mix 1 mL acetate buffer (reagent 2.2.1), 0.1 mL glucose reagent (reagent 2.2.8), and 4.0 mL glucose oxidase/peroxidase enzymes (reagent 2.2.6).

2.3 Procedure

2.3.1 Weigh 100 ± 10 mg wheat flour samples accurately into thick-walled glass centrifuge tubes (12-mL capacity).

2.3.2 Before addition of fungal α-amylase solution to tubes, equilibrate both to 40 ^{o}C for 2-5 min.

2.3.3 Add 1.0 mL pre-equilibrate fungal α-amylase (50 units/mL) to each sample tube.

2.3.4 Stir each tube vigorously and immediately on virtex mixer for 5 sec.

2.3.5 Incubate at 40 ^{o}C for exactly 10 min (from time of enzyme addition).

2.3.6 Add 5.0 mL dilute H_2SO_4 (reagent 2.2.3) to terminate reaction.

2.3.7 Centrifuge at 3,000 rpm (1,000 x g) for 5 min.

2.3.8 Transfer aliquots of supernatant solution (0.1 mL) carefully and accurately to bottom of two test tubes.

2.3.9 Add 0.1 mL (2 units) amyloglucosidase solution to each tube.

2.3.10 Incubate tubes at 40^{o}C for 10 min.

2.3.11 Add 4.0 mL GOPOD system (reagent 2.2.6) to each tube (including glucose standards and blank tubes).

2.3.12 Incubate tubes at 40 ^{o}C for 20 min.

2.3.13 Measure absorbance at 510 nm for each sample.

2.4 Calculations

$$\text{Starch damage, \%} = \Delta E \times F \times 60 \times 1/1{,}000 \times 100/W \times 162/180$$
$$= \Delta E \times F/W \times 5.4$$

Where ΔE = absorbance (reaction) read versus GOPOD blank, F = (150 µg glucose/absorbance for 150 µg glucose), 60 = volume correlation (0.1 mL taken to 6.0 mL), 1/1,000 = conversion from µg to mg, 100/W = factor to express starch damage as percent of flour weight, W = weight (mg, as-is basis) of flour analyzed, 162/180 = adjustment of free glucose to anhydrous glucose (as occurs in starch).

3. Scanning Electron Microscopy (Vatanasuchart, 2004)

3.1 Apparatus

- Scanning electron microscopy (SEM, JEOL, JSM-5600 LV, Japan)
- Fine coater
- Aluminum specimen stub
- Double-side adhesive tape

3.2 Procedure

3.2.1 Take a rice flour sample and sprinkle onto double-sided adhesive tape attached to the specimen stub. Remove the excess sample.

3.2.2 Place in fine coater for gold coating for 150 seconds.

3.2.3 Place the coated sample in sample chamber in the SEM.

3.2.4 Examine at magnification of 500X, 1,000X and 3,000X with the accelerating voltage of 15 kV.

4. Determination of Gelatinization Properties (Patindol and Wang, 2003)

4.1 Apparatus

- Differential scanning calorimeter (DSC) (DSC 2920, TA Instruments, USA)
- DSC aluminum pan with cover
- Sealing tools

4.2 Procedure

4.2.1 Sample preparation

Weigh 4.0 mg (db) flour sample in the aluminum DSC pan. 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.

4.2.2 DSC Conditions

The sample pas was placed carefully in the DSC and was heated at a heating rate of 10 ^{o}C/min from 30 ^{o}C to 110 ^{o}C. The gelatinization temperature (onset temperature: T_o, peak temperature: T_p and conclusion temperature: T_c) and enthalpy change (ΔH) were recorded. The instrument was calibrated with indium, and an empty pan was used as reference**.**

5. Determination of the Pasting Properties with the Rapid Visco Analyser (AACC, 2000)

5.1 Apparatus

5.1.1 Rapid Visco Analyser (RVA), sample canisters and stirrers (Newport Scientific Pty, Ltd., Australia).

5.1.2 Cyclone mill (fitted with 0.5-mm screen)

5.1.3 Computer loaded with control software (Thermocline for Window)

5.2 Instrument preparation

Switch on instrument and associated computer, and run control software. Switch model 3CR to computer control. Select the test profile for rice that consists of the following time/temperature cycle:

	Temperature (^{o}C)	Time (min:sec)	
	50.0 (Idle temperature)		
1:	50.0	1:00	
2:	95.0	4:45	**Ramp up**
3:	95.0	7:15	**Hold**
4:	50.0	11:06	**Cool down**
End of test		12:30	**Hold**

Enter file name that will be used to record viscosity data of test, and select test run option. Instrument disperses sample by rotating paddle at 960 rpm for first 10 sec of test, after which viscosity is sensed using constant paddle rotation speed of 160 rpm. Heating and cooling is linearly ramped between profile set points. Allow instrument at least 30 min to warm up before use.

5.3 Determination

5.3.1 Weight 3.00 g rice flour or rice starch samples (12% moisture basis) into weighing vessel before transfer into test canister.

5.3.2 Dispense 25.0 mL (±0.1 mL) water (12% moisture basis) into new test canister. Equivalent sample and water mass can be calculated using formular:

$$S = \frac{88 \times 3.0}{100 - M} \text{ for flour and } \qquad W = 25 + (3.0 - S) \text{ for water}$$

Where S = corrected sample weight, W = corrected water weight, and M = actual moisture content of sample (percent as is).

5.3.3 Transfer flour onto water surface in canister. Place paddle into canister and vigorously jog blade through sample up and down 10 times to brake flour or meal lumps on water surface.

5.3.4 Place the paddle into canister, and insert paddle and canister assembly firmly into paddle coupling so that paddle is properly centered. Initiate measurement cycle by depressing motor tower of instrument. (Do not allow flour to stand in the water for more than 1 min before commencing test). Test will proceed and terminate automatically. Discard canister after use.

5.3.5 From recorded viscosity, note peak viscosity, minimum viscosity after peak, and final viscosity at 50 ^{o}C (at 12.5 min). Viscosity is measured in rapid visco units (RVU).

5.3.6 Typically measured pasting properties that characterize rice are as follows:

Pasting temperature—temperature of the initial viscosity increase

Peak Viscosity—maximum viscosity recorded during the heating and holding cycles

Trough—minimum viscosity after peak

Breakdown—the different between peak and trough; an indication of the breakdown in viscosity of the paste during 95 ^{o}C holding period. Breakdown has been related to stability of the starch to heat and shear stress.

Setback (from peak)—the different between final viscosity and peak. Setback is an indication of the starch to retrograde.

Final viscosity—viscosity achieved at the end of the test

7. Determination of the Starch Crystallinity with X-ray Diffractometer (Patindol and Wang, 2003)

7.1 Apparatus

- X-ray diffractometer (JEOL, JDX-3530, Japan).
- Silicon sample cell.

7.2 Sample Preparation

7.2.1 Take a flour or starch sample for about 1 g.

7.2.2 Pack tightly in rectangular silicon cell and spread sample evenly to obtain a smooth surface and place the sample cell in sample holder.

7.2.3 Expose to the X-ray beam.

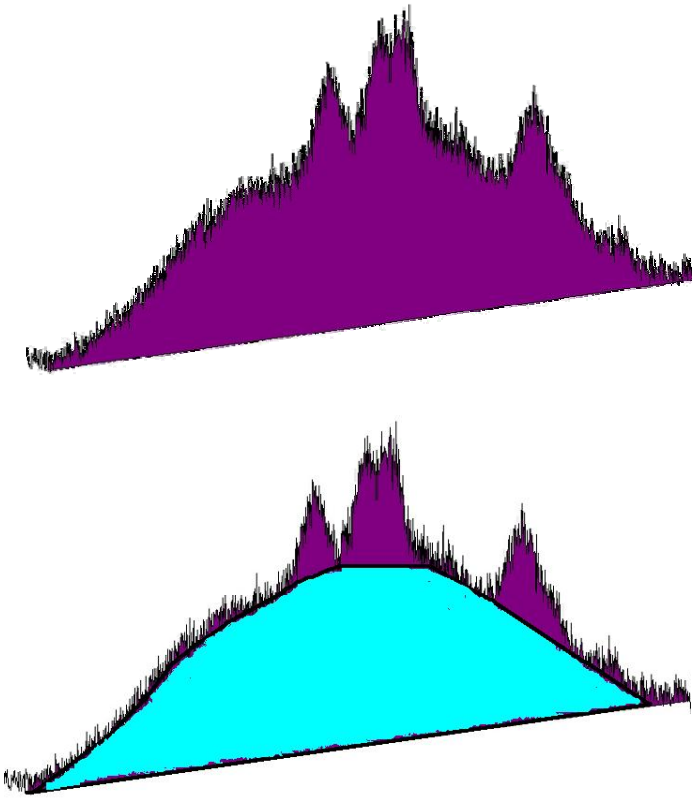
7.3 The X-ray diffractometric Condition

Monochromatic Cu-K_α radiation	1.542 A^o
X-ray generator power	40 mA and 30 kV
Scanning, 2-theta	4^o to 30^o
Step angle	0.02^o
Count time	1 sec
Divergence Slit	1^o
Receiving Slits	0.2 mm
Scattering Slits	1^o
Measurement temperature	Ambient temperature

7.4 Calculation

Amorphous and crystalline sections were examined from the X-ray diffractograms (Appendix Figure 1). Peak baseline (ab) and smooth curve (bold area) were computer-plotted on the diffractogram. The area above the smooth curve was the crystalline portion and the bold area above the peak baseline was the amorphous portion. The degree of relative crystallinity of samples was quantitatively estimated by the ratio of the area above the smooth curve to total diffraction area using the following equation:

$$\% \text{ Relative crystallinity} = \frac{\text{Area above the smooth curve}}{\text{Total diffraction area above the baseline}} \times 100$$



Appendix Figure 1 X-ray diffraction spectra for rice flour showing crystalline (above the smooth curve) and amorphous regions.

8. Determination of Rice Noodle Firmness modified from the method AACC 16-50 (AACC, 2000)

8.1 Apparatus

- Texture Analyser (TA.XT2) (Stable Micro System, England)
- AACC 1mm flat perspex knife blade (A/LKB-F) using 5kg load cell

8.2 Sample preparation

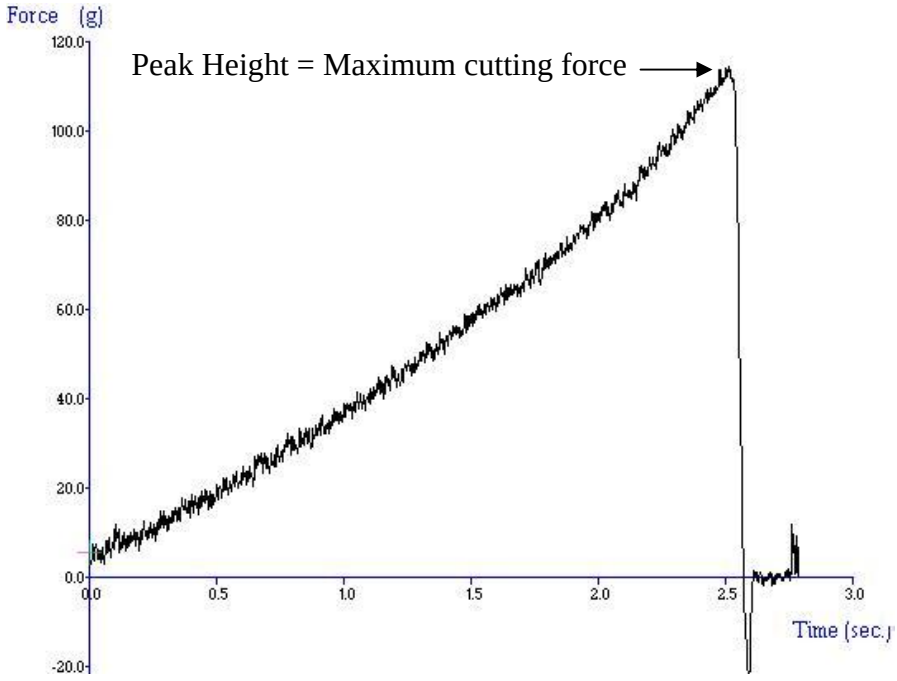
A 25 g of dried rice noodle was soaked in 500 mL of water at room temperature for 10 min. The noodle sample was placed in the 600 mL boiling water for 3 min. After this time it was immediately removed and rinsed with running water for 2 min. After rinsing, the sample was drained in a sieve and left in the sieve for 5 min before textural determination. Testing should be performed immediately following cooking to minimize changes resulting from storage in liquid medium.

8.3 Determination:

The TA-XT2 was set as the condition below

Mode:	Measure force in compression
Option:	Return to start
Pre-Test Speed:	0.5 mm/s
Test Speed:	0.2 mm/s
Post-Test Speed:	10.0 mm/s
Stain:	50%
Trigger Type:	Auto-5g
Data Acquisition Rate:	400pps

Place a strand of rice noodle centrally under the knife blade, with the axis of the product at right angles to the blade. Commence the test and repeat using fresh samples. Firmness of the sample is defined as the maximum force in gram required to cut a piece of rice noodle.



Appendix Figure 2 Force vs. time plot from the measurement of rice noodle firmness.

9. Determination of Rice Noodle Tensile Strength (Stable Micro System,1995)

9.1 Apparatus

- Texture Analyser (TA.XT2) (Stable Micro System, England)
- Spaghetti tensile grips (A/SPR) using 5kg load cell

8.2 Sample preparation

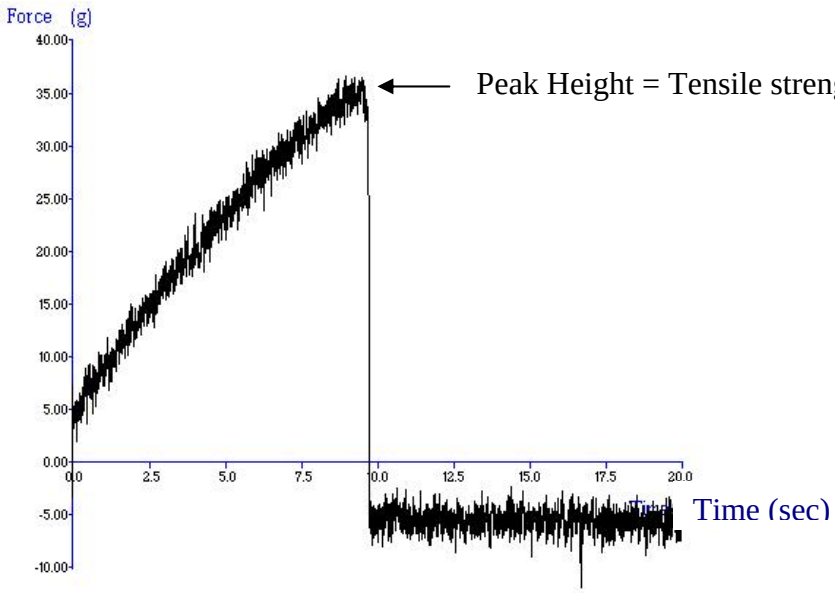
A 25 g of dried rice noodle was soaked in 500 mL of water at room temperature for 10 min. The noodle sample was placed in the 600 mL boiling water for 3 min. After this time it was immediately removed and rinsed with running water for 2 min. After rinsing, the sample was drained in a sieve and left in the sieve for 5 min before textural determination. Testing should be performed immediately following cooking to minimize changes resulting from storage in liquid medium.

9.3 Determination:

The TA-XT2 was set as the condition below

Mode :	Measure Force in Tension
Option:	Return To Start
Pre-Test Speed:	3.0 mm/s
Test Speed:	3.0 mm/s
Post-Test Speed:	5.0 mm/s
Distance:	50mm
Trigger Type:	Auto - 5g
Data Acquisition Rate:	200pps

The noodles are then tested individually by placing one end into the lower rig arm slot and winding the loosened arm sufficiently, in order to anchor the noodle end (away from the slot) by at least two revolutions of the arm. The arm is tightened and the same procedure is performed to anchor the other noodle end to the upper arm. The maximum force (tensile strength) (g) required to break the noodle strand indicated the sample's resistance to breakdown and the distance (mm) to break showed its extensibility.



Appendix Figure 3 Force vs. time plot from the measurement of rice noodle tensile strength.

10. Sensory Evaluations (Meilgaard *et al*., 1999; Chen *et al*., 2002)

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 rice noodle. The panelists were introduced to the reference rice noodle (Top Brand, purchased from Top Supermaket, Kasetsart Branch, Bangkok) and asked for characterizing sensory attributes of the product. After that, they were asked to evaluate the reference rice noodle for the intensity of each attribute (turbidity, hardness or firmness, elasticity, and adhesiveness), when the average intensity of each sensory attribute was marked in the scale. The intensity of each characteristic was evaluated from six dry- and wet-milled rice noodle compared to commercial product, and reference sample was also presented at the time of evaluation. Moreover, the perceived intensities were scored on a 15-cm-interval scale. Acceptance for each perceived intensity and overall acceptance were also evaluated using a 1 to 9 hedonic scale.

Sensory Evaluation Form

Product Sample: Rice Noodle (Sen Lek)

Panelist's Name ---------- Sex ---------- Date ----------

Please describe intensity of sensory characteristics of rice noodle by placing the mark (I) on the scale to locate the intensity of each sensory attribute

1. Turbidity

Clear — Turbid

2. Hardness, Firmness

Fluffy — Firm

3. Elasticity

Slightly — Elastic

4. Adhesiveness (Stick together)

Non-sticky — Sticky

Please determine your acceptance toward each sensory characteristic and evaluate overall acceptance to the product, using 1-9 hedonic scale

Like extremely	Like very much	Like moderately	Like slightly	Neither like nor dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely
9	8	7	6	5	4	3	2	1

Product	_____	_____	_____	_____	_____	_____	_____	
1. Turbidity	_____	_____	_____	_____	_____	_____	_____	
2. Firmness	_____	_____	_____	_____	_____	_____	_____	
3. Elasticity	_____	_____	_____	_____	_____	_____	_____	
4. Adhesiveness	_____	_____	_____	_____	_____	_____	_____	
5. Overall acceptance	_____	_____	_____	_____	_____	_____	_____	

Comments ---------- Thank you ☺

APPENDIX B

Appendix Table 2 Correlation between chemical properties, starch molecular properties and physicochemical properties of rice flour and rice starch.

	AM	DS	Protein	AP_T	AM_T	$MwAP_T$	$MwAM_T$	T_o	T_p	T_c	ΔH	SP55	S 55	SP95	S 95	PV	BD	FV
DS	-0.38																	
Protein	-0.14	0.67**																
AP_T	-0.75**	-0.12	-0.16															
AM_T	0.76**	0.13	0.16	-0.97**														
$MwAP_T$	-0.76**	-0.07	-0.17	0.77**	-0.75**													
$MwAM_T$	-0.84**	0.09	-0.11	0.63**	-0.60**	0.78**												
T_o	0.61*	0.07	0.21	-0.32	0.32	-0.62**	-0.87*											
T_p	0.54*	0.14	0.28	-0.28	0.28	-0.60*	-0.84*	0.98**										
T_c	0.41*	0.36	0.53**	-0.28	0.27	-0.54*	-0.74*	0.91**	0.93**									
ΔH	-0.10	-0.57*	-0.64**	0.51*	-0.51*	0.47*	0.25	-0.11	-0.18	-0.29								
SP55	-0.46*	0.92**	0.78**	-0.01	0.02	0.03	0.24	-0.06	0.01	0.27	-0.60**							
S 55	-0.45*	0.81**	0.71**	0.04	-0.02	0.04	0.19	-0.02	0.08	0.22	-0.60**	0.91**						
SP95	-0.25	-0.49*	-0.82**	0.49*	-0.51*	0.38	0.32	-0.30	-0.33	-0.53**	0.52**	-0.54**	-0.47*					
S 95	0.38	0.03	-0.51*	-0.39	0.40	-0.42*	-0.42*	0.33	0.27	0.11	-0.02	-0.26	-0.27	0.33				
PV	-0.73**	-0.11	-0.16	0.64**	-0.63*	0.81**	0.92**	-0.89**	-0.85*	-0.78**	0.38	-0.07	0.07	0.31	-0.53**			
BD	-0.83**	-0.14	-0.26	0.92**	-0.92**	0.83**	0.80**	-0.59**	-0.55**	-0.56**	0.47*	-0.04	0.02	0.56**	-0.37	0.81**		
FV	0.58**	0.35	0.41*	-0.86*	0.86**	-0.66**	-0.49*	0.23	0.23	0.30	-0.66*	0.33	0.27	-0.66**	0.16	-0.47*	-0.85**	
SB	0.64**	0.39	0.42*	-0.83*	0.83**	-0.80**	-0.69*	0.51*	0.51*	0.55*	-0.71**	0.30	0.28	-0.61**	0.32	-0.73**	-0.89**	0.92**

** Correlation is significant at $p < 0.01$, * Correlation is significant at $p < 0.05$; AM=Amylose content, DS=Starch damage content, Protein=Protein content, AP_T=Proportion of amylopectin from total starch fraction, AM_T= Proportion of amylose from total starch fraction, $MwAP_t$=Molecular weight of amylopectin from total starch fraction, $MwAM_t$=Molecular weight of amylose from total starch fraction, To, Tp, Tc=onset, peak, conclusion gelatinization temperature, ΔH=gelatinization enthalpy, SP 55=Swelling power at 55°C, S 55=Solubility at 55°C, SP 95=Swelling power at 95°C, S 95=Solubility at 95°C, PV=Peak viscosity, BD=Breakdown, FV=Final viscosity, SB=Setback.

CIRRICULUM VITAE

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BIRTH PLACE : Umper Muang, Nakhon Ratchasima

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PRESENTATIONS : Poster presentation “Effect of rice varieties and milling processes on rice noodle properties” AACC/TIA joint meeting, September 19-22, 2004, San Diego, CA, USA

: Poster presentation “Comparison of chemical, physicochemical properties and starch molecular structures in dry- and wet-milled rice flours” Starch Update 2005: The 3 rd International Conference on Starch Technology, November 4-5, 2005, Bangkok, Thailand

: Oral presentation “Effect of dry- and wet-milling processes on rice flour and rice noodle properties” The 32nd Congress on Science and Technology of Thailand (STT. 32), October 10-12, 2006, Bangkok, Thailand