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Zeta Potential (ζ) and Pasting Properties of Phosphorylated or Crosslinked Rice Starches

Zeta potential (ζ) measurement is not only applied for estimation of colloidal stability but also used for the rapid determination of degree of substitution (DS) of phosphorylated or crosslinked rice starch, obtained by the reaction of rice starch with STPP (sodium tripolyphosphate) or STMP (sodium trimetaphosphate), respectively. The introduction of the phosphate groups into the starch molecules by these reagents enhances the negative charge on the starch particles, which relate to the zeta potential (ζ). The data from ζ and chemical analysis of DS were used in a regression analysis for developing a linear correlation to express the DS and ζ value and some RVA-pasting properties of phosphorylated or crosslinked rice starch based on the ζ value. For the phosphorylated starches, linear relationships among DS, ζ value and peak viscosity (PV) as well as breakdown (BD) with a high correlation value (r^2) were obtained. ζ value decreased with increasing DS, PV and BD, and with the decrease of pasting temperature (PT). For the crosslinked starches, linear relationships with a high correlation value (r^2) among DS, ζ value and PV as well as BD were obtained. ζ value decreased with increasing DS and PT, in contrast with the decreasing of PV and BD. The ζ measurement can be used for the calculation of DS of the phosphorylation or crosslinking reactions. Some RVA-pasting properties of native or modified rice starches can also be predicted from the ζ value.

Keywords: Rice Starch; Zeta potential; Phosphorylation; Crosslinking

1 Introduction

ζ is a consequence of the existence of surface charge, which can give information on electrical interaction forces between the dispersed particles. Zeta potential is defined as the electrokinetic potential at the boundary of the hydrodynamic shear plane of a charged particle adjacent to a solid surface exposed to a liquid. The potential is measured by electrophoresis, in which the particle migrates in an external electric field. Under these conditions, the particle migrates together with the ions in the Stern layer, and it is the potential just outside this boundary layer (also called the shear plane), which determines the speed of particle movement [1]. The speed can be directly related to the magnitude and sign of the surface charge [2]. ζ is lower than the potential at the particle surface.

The distinction between surface potential and ζ should be emphasized. ζ can be measured in a fairly simple manner, whereas the surface potential cannot. The ratio between ζ and the surface potential depends on the thickness of the

double layer. In pure water, a relatively large double layer is usually found, and in this case ζ is a good approximation of the surface potential. The situation is different in salt solutions, where the high level of ions compresses the double layer, and ζ is only a fraction of the surface potential. The zeta potential depends on both the surface charge of the particle and the thickness of the electrical double layer. That is, it describes not only the electrochemical property of a particle surface but also the environmental conditions (e.g., pH and ion concentration). Therefore, ζ should always be stated with medium conditions (e.g., pH, conductivity, temperature, and additives) [3].

According to the electrostatic principles, ζ is calculated by using Smoluchowski's equation [4]:

$$\zeta = 4\pi\eta\mu/\varepsilon$$

where ζ is the zeta potential in mV, η is the viscosity (Poise) of the medium, μ ($\text{cm}^2 \text{s}^{-1} \text{V}^{-1}$) the electrophoretic mobility = $v \cdot L/V$ (v = speed of the particle in cm/s, L = distance between electrodes, V = voltage) and ε is the dielectric constant of the medium.

The concept of ζ has been applied very successfully in the field of colloid science to understand the colloid interactions, and control the stability of a colloid sys-

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tem. The stability of a colloidal system is determined by using DLVO theory, which is to use the sum of the repulsive forces of the electrical double layer and the attractive van der Waals forces to calculate the particle interaction potential. For colloidal stability, the repulsive forces must be dominant [1]. The magnitude of ζ gives an indication of the potential stability of the colloidal system. Generally, when all the particles have a large positive or negative ζ (where the positivity and negativity is greater or lower than +30 mV and –30 mV), they will repel each other and the dispersion is stable. On the other hand, when the particles have low ζ values, there will be no force to prevent the particles from aggregating [5].

In the field of modified starch technology, the ζ concept has not yet been applied. In order to investigate the relationship with the DS and have been measured in this study.

2 Materials and Methods

2.1 Materials

Normal rice starch was supplied by Choheng Rice Vermicelli Co., Ltd. (Nakornpathom, Thailand). All reagents were analytical grades, purchased from Merck (Darmstadt, Germany).

2.2 Methods

2.2.1 Proximate analysis and amylose content of rice starch

Moisture and ash contents of native rice starch were determined according to the standard methods described in the AOAC methods (Official Methods of Analysis, Association of Official Analytical Chemists) [6]. Protein content was calculated from the nitrogen content determined by Kjeldahl method (Model VAPODEST 50 Carousel 250 mL autosample, Gerhardt, Königswinter, Germany and Model Kjeldatherm-Digestion units, with 20 digestion tubes 6100, 250 mL, Gerhardt, Germany) multiplied by 6.25 [7] and fat content of the sample was determined by the method described elsewhere [8]. Carbohydrate content was calculated by subtracting the concentrations of the aforementioned compounds from 100. Amylose content of native rice starch (based on weight that is free of moisture, protein, fat and ash) was determined by the iodine affinity method [9].

2.2.2 Preparation of phosphorylated rice starch [10]

Native rice starch (200 g) was mixed with 180 mL distilled water containing STPP (1, 2, 4 and 6%, w/w, based on the weight of starch). The starch slurry was air dried to 25% moisture content and passed through a 40-mesh sifter to form a granular starch. The starch granules were dried at 40°C overnight. To perform the phosphorylation reaction, the dried starch-STPP mixture was heated in an oven at 120°C for 2 h. Then, the mixture was cooled down to room temperature and dispersed in 1000 mL distilled water. The pH of the starch suspension was adjusted to 6.5–7.0 by adding 0.5 M HCl. Then the starch suspension was washed three times (3×1.6 L) with distilled water. The wash water was removed by centrifugation and the phosphorylated starch dried at 50°C in a hot air oven overnight. The dried starch was ground and sieved through a 200-mesh sifter (Laboratory Test Sieve, Endecotts Ltd., London, UK).

2.2.3 Preparation of crosslinked rice starch [11]

Native rice starch (100 g) was suspended in 120 mL distilled water containing 0.48 g NaOH (0.4%, w/v, NaOH) and 3 g Na_2CO_3 (3%, w/w, based on the weight of starch). The slurry was placed in a water bath at 32°C, while stirring at 1000 rpm. STMP in concentrations of 2, 5 and 10% w/w, based on the weight of starch, was added to the starch slurry and the reaction was performed for 2 h. At the end of the reaction time, 500 mL of distilled water was added to the starch slurry and the pH was adjusted to 6.5–7.0 by adding 0.5 M HCl. The starch suspension was washed three times (3×1.6 L) with distilled water and dried at 50°C in a hot air oven overnight. The dried starch was ground and sieved through 200-mesh sifter (Laboratory Test Sieve, Endecotts Ltd., London, UK).

2.2.4 Determination of phosphorus content

A modification of the standard methods described by the AOAC [12] was used. Sodium carbonate (1.5 g) was dissolved in 5 mL of distilled water in a crucible. A 2.5 g-sample of modified starch was added and mixed well. The mixture was boiled until dryness and provision was made that no fume was produced. It was then combusted at 550°C for 12 h in a furnace (LTD, ECF-12/22A, Leicestershire, UK). The product obtained was then cooled to room temperature. After that, 2 mL of 25% (w/v) HCl in 10 mL distilled water were added. The solution was transferred to a beaker containing 20 mL distilled water. Then, more distilled water was added to the beaker to make up the volume of 50 mL. Next, the well-mixed

ingredients were filtered through a glass funnel with Whatman filter paper no. 1. The solution was, again, diluted to 250 mL by the addition of distilled water. A sample of 10 mL of the diluted solution was mixed with 2 mL vanadate-molybdate reagent and the solution was allowed to stand at room temperature for 45 min. The absorbance of the sample at 435 nm was then measured with a spectrophotometer (Beckman, DU65, Buckinghamshire, UK). Finally, the percentage of phosphorus was calculated from the standard curve.

2.2.4.1 Preparation of standard curve for phosphorus determination

$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ was employed as phosphorus standard in this experiment; $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (2.2275 g) was dissolved in distilled water. The solution was made up to 100 mL, which is equivalent to 0.5% phosphorus content, then diluted into a series. HCl (2 mL of 25%, w/v) was added to the solutions and the volume adjusted to 10 mL with distilled water. Ten milliliters of each diluted solution were pipetted and mixed well with 2 mL of vanadate-molybdate reagent and kept at room temperature for 45 min. The absorbance of the solutions at 435 nm was then measured.

2.2.5 Determination of DS

The degrees of substitution (DS) by phosphate monoester and phosphate diester groups were calculated according to:

$$\text{DS of starch monoester} = \frac{162P}{3100 - 97P}$$

where P = % phosphorus (dry basis) of the phosphorylated starch and

$$\text{DS of starch diester} = \frac{324P}{3100 - 96P}$$

where P = % phosphorus (dry basis) of the crosslinked starch.

2.2.6 Pasting properties of native and modified rice flour

To determine the pasting properties of native or modified rice starches from Rapid Visco Analyser measurements (Model RVA 3D+, Newport Scientific, Warriewood, Australia), 2.5 g of dried flour was dispersed in 25 mL distilled water. A heating and cooling cycle was programmed in the following manner: The sample was heated from 50 to 95°C during 3 min, held at 95°C for 2 min and cooled to 50°C during 3 min. The pasting parameters such as pasting temperature (PT), peak viscosity (PV), holding strength

(HS), final viscosity (FV), breakdown (BD), and setback (SB) were determined according to the following definitions:

- Peak viscosity is the highest viscosity during the heating step.
- Holding strength is the lowest viscosity at the end of the 95°C heating stage.
- Final viscosity is the viscosity at the end of 50°C cooling stage.
- Breakdown is the ratio of changing in viscosity from peak viscosity to holding strength.
- Setback is the changing in viscosity from holding strength to final viscosity.

2.2.7 ζ measurement

Eight milligrams of fine, modified rice starch (pass 200 mesh sifter) were suspended in 40 mL de-ionized water (DI-water). The suspension was well mixed and allowed to sediment for 5 min. The supernatant with the fine particles was injected directly into the capillary cell of a Malvern Zetasizer 3000 (Malvern Instrument Inc., London, UK). The measurements were carried out in triplicate for the time interval of 30 s for one injection.

3 Results and Discussion

The native rice starch employed in this investigation contained 0.5% protein, 0.08% fat, 0.26% ash, 12.5% moisture and 86.66% carbohydrate (31.02% amylose).

The native rice starch was phosphorylated by STPP (1, 2, 4, and 6%, w/w, based on the weight of starch) as well as crosslinked by STMP (2, 5, 10%, w/w, based on the weight of starch) to obtain phosphorylated and crosslinked rice starches, respectively. Both phosphorylated and crosslinked rice starches contained phosphate groups providing negative surface charges, which was measured by ζ .

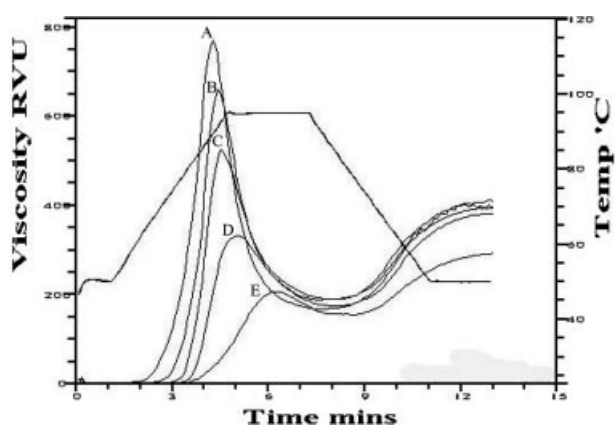
The results of the pasting properties as measured by the RVA are presented in Fig. 1A and Tab. 1 for the phosphorylated and Fig. 1B and Tab. 2 for the crosslinked rice starches. When the concentration of STPP used for phosphorylation increased, PV and BD were increased, whereas PT and pasting time decreased. This is characteristic of phosphorylated starch [13]. For the crosslinked starch, the PV and BD decreased with the increase in STMP concentration, which is also a characteristic of crosslinked starches [10]. Both types of starches were

Tab. 1. Pasting properties of phosphorylated rice starch obtained from the reaction of rice starch with various STPP concentrations.

% STPP	Pasting properties						
	Peak viscosity [RVU]	Trough [RVU]	Breakdown [RVU]	Final viscosity [RVU]	Setback [RVU]	Pasting time [min]	Pasting temp. [°C]
0	207.42	155.42	52.00	293.25	137.83	6.27	80.70
1	332.50	175.08	157.42	381.75	206.67	5.00	75.90
2	526.33	191.08	335.25	395.92	204.83	4.53	72.75
4	660.17	188.92	471.25	408.17	219.25	4.47	67.85
6	770.67	168.08	602.58	392.83	224.75	4.27	61.05

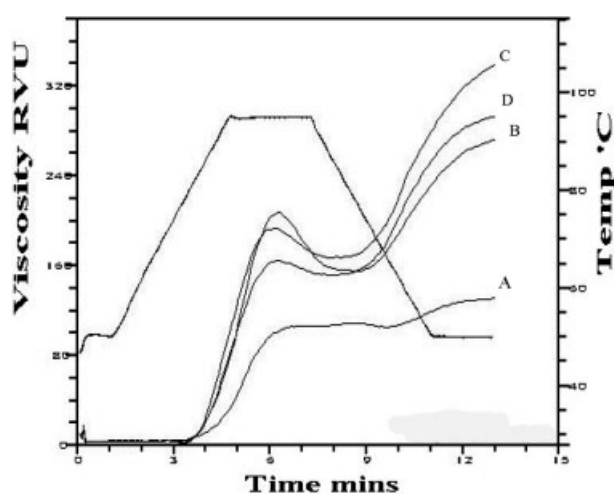
Tab. 2. Pasting properties of crosslinked rice starch obtained from the reaction of rice starch with various STMP concentrations.

% STMP	Pasting properties						
	Peak viscosity [RVU]	Trough [RVU]	Breakdown [RVU]	Final viscosity [RVU]	Setback [RVU]	Pasting time [min]	Pasting temp. [°C]
0	207.42	155.42	52.00	293.25	137.83	6.27	80.70
2	193.17	167.17	26.00	338.67	171.50	6.13	80.70
5	164.50	151.75	12.75	272.00	120.25	6.27	81.50
10	106.75	98.67	8.08	130.83	32.17	6.93	88.65

**Fig. 1A.** Pasting profiles of phosphorylated rice starch with various STPP concentrations; A: 6% STPP, B: 4% STPP, C: 2% STPP, D: 1% STPP, and E: native rice starch.

analyzed for phosphorus content, which was calculated to determine DS, and subjected to ζ analysis in DI water. The results are shown in Tabs. 3 and 4.

For the phosphorylated starches, a linear relationship with a high correlation value (r^2) of DS as a function of ζ value and PV as well as BD and PT was found (Figs. 2, 3, and 4, respectively). When the ζ value decreased the DS, PV and

**Fig. 1B.** Pasting profiles of crosslinked rice starch with various STMP concentrations; A: 10% STMP, B: 5% STMP, C: 2% STMP, and D: native rice starch.

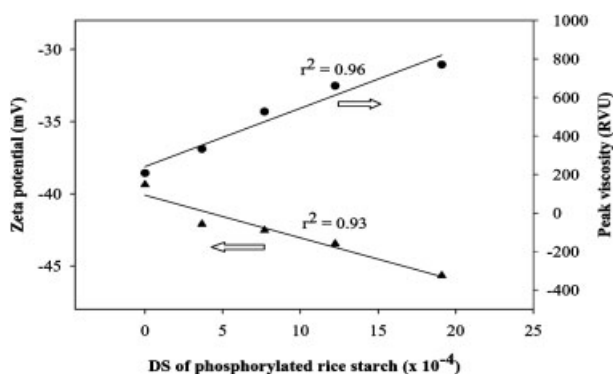
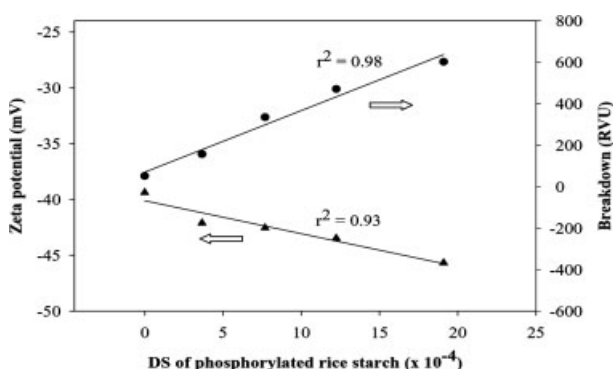
BD increased, in contrast with the decreasing of PT in the phosphorylation reaction. In general, the phosphate group provided a negative charge, which was the higher the more phosphate groups were present per starch particle. The phosphate group also

Tab. 3. Phosphorus content, degree of substitution (DS) and ζ values in DI water of phosphorylated rice starch obtained from the reaction of rice starch with various STPP concentrations.

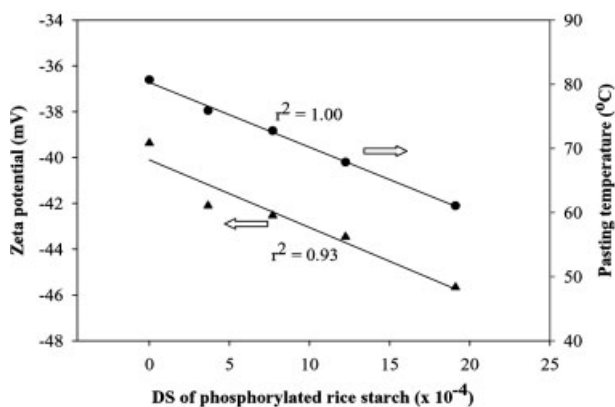
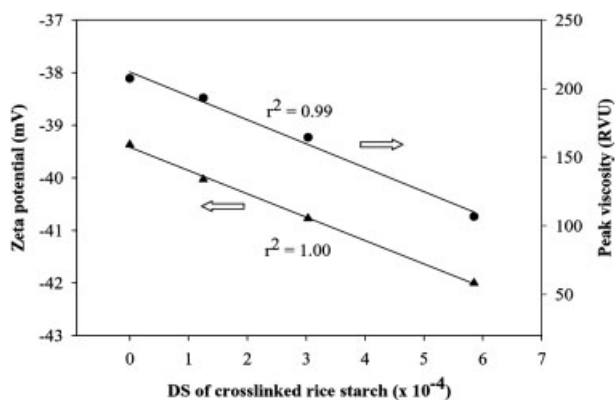
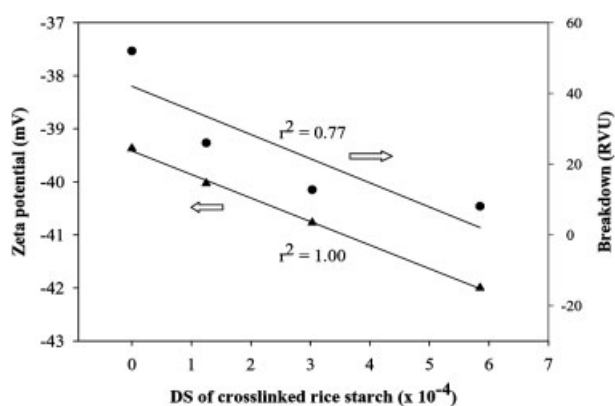
% STPP	% Phosphorus	DS	ζ in DI water [mV]
0	0	0	−39.37(0.64) ^a
1	0.0070	3.66×10^{-4}	−42.10(1.54) ^{ab}
2	0.0147	7.69×10^{-4}	−42.53(0.85) ^b
4	0.0234	12.24×10^{-4}	−43.47(2.08) ^{bc}
6	0.0365	19.10×10^{-4}	−45.67(1.86) ^d

Tab. 4. Phosphorus content, degree of substitution (DS) and ζ in DI water of crosslinked rice starch obtained from the reaction of rice starch with various STMP concentrations.

% STMP	% Phosphorus	DS	ζ in DI water [mV]
0	0	0	−39.37(0.64) ^a
2	0.0012	1.25×10^{-4}	−40.03(0.83) ^{ab}
5	0.0029	3.03×10^{-4}	−40.77(1.89) ^{ab}
10	0.0056	5.85×10^{-4}	−42.00(1.40) ^b

**Fig. 2.** DS as the function of ζ value and peak viscosity of phosphorylated rice starch.**Fig. 3.** DS as the function of ζ value and breakdown of phosphorylated rice starch.

provided a higher negative ζ value. For further understanding, the crosslinking reaction of rice starch with STMP, the results of the linear relationship with a high correlation factor (r^2) of DS as the function of ζ value, as well as the pasting properties of PV, BD and PT are shown in Figs. 5, 6, and 7, respectively. The ζ value could determine the DS value and the pasting properties of PV, BD

**Fig. 4.** DS as the function of ζ value and pasting temperature of phosphorylated rice starch.**Fig. 5.** DS as the function of ζ value and peak viscosity of crosslinked rice starch.**Fig. 6.** DS as the function of ζ value and breakdown of crosslinked rice starch.

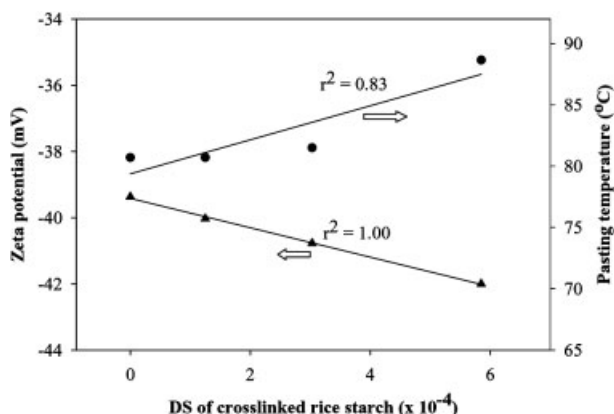


Fig. 7. DS as the function of ζ value and pasting temperature of crosslinked rice starch.

and PT for the crosslinking reaction. When the ζ value decreased, the DS value and the PT were increased in contrast with the decreasing of PV and BD.

Starch has never been studied by ζ measurement. However, a ζ value lower than -30 mV of native or modified starch particles indicates that the particles could suspend very well in DI water. On the contrary, a ζ value close to 0 mV is accounted for the agglomeration of the particles. The chemical modification of rice starch by phosphorylation or crosslinking through the reaction of rice starch with STPP or STMP introduced phosphate groups into the starch molecules, which provided a stronger negative charge on the rice starch granules and a higher colloidal stability in DI water. Rice starch granules are normally as small as $5\text{ }\mu\text{m}$ in diameter [14] which can be suspended in DI water.

4 Conclusion

The ζ measurement is not only applied for determination of colloid stability, but can also be employed for the rapid estimation of low DS values (maximum 9.10×10^{-4} and 5.85×10^{-4}) and pasting properties of phosphorylated or crosslinked rice starch.

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References

- [1] D. B. Genovese, J. E. Lozano: The effect of hydrocolloids on the stability and viscosity of cloudy apple juices. *Food Hydrocoll.* **2001**, 15, 1–7.
- [2] R. J. Hunter: *Zeta Potential in Colloid Science, Principle and Applications*, Academic Press, London, **1988**, p. 4.
- [3] D. Lin, P. J. Brixius, J. J. Hubbuch, J. Thommes, M. Kula: Biomass/adsorbent electrostatic interactions in expanded bed adsorption: A zeta potential study. *Biotechnol. Bioeng.* **2003**, 83 (2), 149–157.
- [4] D. L. Shaw: *Introduction to Colloid and Surface Chemistry*, Butterworth, London, **1980**, p. 56.
- [5] P. Sherman: Rheology of dispersed systems, in *Industrial Rheology*, Academic Press, London, **1970**, p. 97–183.
- [6] AOAC, Official Method of Analysis, Ash and Moisture Content, 15th ed., Association of Official Analytical Chemistry, Arlington, USA, **1990**, p. 777.
- [7] AOAC, Official Method of Analysis, Protein, 15th ed., Association of Official Analytical Chemistry, Arlington, USA, **1990**, p. 781.
- [8] AOAC, Official Method of Analysis, Fat, 15th ed., Association of Official Analytical Chemistry, Arlington, USA, **1990**, p. 780.
- [9] C. A. Knutson: A simplified colorimetric procedure for determination of amylose in maize starches. *Cereal Chem.* **1986**, 63(2), 89–92.
- [10] D. B. Solarek: Phosphorylated starches and miscellaneous inorganic esters. Chapter 7, in *Modified Starches: Properties and Uses* (Ed. O. B. Wurzburg) CRC Press, Boca Raton, FL, **1986**, p. 103.
- [11] H. Puchongkavarin: *Modification of sago and tapioca starch of partially substitution wheat flour in alkaline noodle production* [A thesis submitted in partial fulfillment of the requirement for the degree of Master of Science (Biotechnology)]. Faculty of Science, Mahidol University, Bangkok, **1999**, p. 13.
- [12] AOAC, Official Methods of Analysis: Phosphorus in Flour, Association of Official Analytical Chemistry, Arlington, USA, **1984**, p. 251.
- [13] S. Lim, P. A. Seib: Preparation and pasting properties of wheat and corn starch phosphates. *Cereal Chem.* **1993**, 70, 137–144.
- [14] H. Puchongkavarin, W. Berghaller, S. Shobsngob, S. Varavinit: Characterization and utilization of acid-modified rice starches for use in pharmaceutical tablet compression. *Starch/Stärke* **2003**, 55, 464–475.

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