

Preparation, pasting properties and freeze–thaw stability of dual modified crosslink-phosphorylated rice starch

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Abstract

The pasting properties and freeze–thaw stability of one step combined modifications of rice starch through crosslinking and phosphorylation reactions were studied and compared to those modified by crosslinking or by phosphorylation alone. The native rice starch was mixed with a solution of pH 9.5, containing 1% sodium trimetaphosphate (STMP) and 4% sodium tripolyphosphate (STPP). The starch mixture with approximately 27% moisture content was granulated and heated in a hot air oven at 120 °C allowing the gradual evaporation of water to dryness up to 2 h reaction time. The combined modifications provided higher peak, breakdown, final and setback viscosities than that of the phosphorylation alone. However, these pasting properties of both combined modifications and phosphorylation were increased in line with the increase of reaction time. In contrast to that of the crosslinking reaction with 1% STMP which provided the decreasing of those pasting properties with the increase of reaction time. The pasting temperature for both the combined modifications and phosphorylation were decreased in line with the reaction time, while those for the crosslinked starch were increased. The freeze–thaw stability of native and modified rice starch pastes for five cycles were studied. The combined modified rice starch provided the highest freeze–thaw stability, and this type of modified rice starch could be used to prepare food products with good freeze–thaw stability.

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1. Introduction

Wheat and corn starches were phosphorylated in a semi-dry state at 130 °C with 5% sodium tripolyphosphate (STPP) and/or 2% sodium trimetaphosphate (STMP). The best corn starch phosphate, judging from its paste properties, was obtained at an initial pH of 11 with STPP and contained 0.16% P (including 0.02% P from lipid). When corn or wheat starch was heated with a mixture of

5% STPP and 2% STMP, the best product when pasted at 95 °C was obtained at the initial reaction pH of 9.5. Paste clarity of the phosphorylated starches indicated that crosslinking accelerated rapidly above pH 8 with STMP but above pH 10 with STPP (Lim & Seib, 1993). Sago starch was phosphorylated in a semi-dry state with 2% STMP, 5% STPP singly, and in combination at pH levels between 6 and 11. As the reaction pH was increased from 6 to 11, the degree of phosphorylation was observed to decrease, except at pH 9 where the degree of phosphorylation was increased (Muhammad, Hussin, Man, Ghazali & Kennedy, 2000). Different types of starch were phosphorylated to different degrees of substitution using monosodium

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and disodium hydrogen orthophosphate at 160 °C under vacuum. Viscosities of different starch types except corn amylose showed the highest values at the lowest degree of substitution, when the degree of phosphorylation increased the viscosity values decreased. The conditions for starch dry phosphorylation under heat and vacuum were also optimized. Mahmoud, Sitohy, El-Saadany, Labib, & Zagazig, 2000a, 2000b). Recent advances in application of modified starches for bread making were studied. The crosslinked starches made the bread crumb harder because the chemical crosslinking retained the granular structure of starch in the gluten network resulting in increase in the firmness of bread crumb (Miyasaki, Hung, Maeda, & Morita, 2006).

In general, the condition used for crosslinking and phosphorylation are different. Crosslinking occurred at room temperature in chemical solution of phosphorus oxychloride, epichlorohydrin or STMP in alkali solution of sodium hydroxide and sodium carbonate while phosphorylation reaction occurred at high temperature (120–170 °C) in dried state of the mixture of starch and STPP. In order to perform both reactions, crosslinking and phosphorylation should be done consecutively. The starch should be first modified by crosslinking by suspending the starch in alkali solution containing STMP, stirring for a period of time and then neutralizing the reaction mixture with acid. The chemical was removed by washing with distilled water, filtering and drying to obtain the crosslinked starch. Phosphorylation would be followed by mixing STPP solution with the dried powder of the aforementioned crosslinked starch and dry the mixture at low temperature. Then the phosphorylation reaction was started by raising the temperature up to the range of 120–170 °C, the starch mixture was then washed with distilled water to remove the excess STPP, dried, grinding and sieve to obtain dried modified starch powder. It could be seen that two steps modifying by STMP and STPP required many processing steps. The objective of this project is to reduce the processing steps by combining the reaction of rice starch with both STMP and STPP in semi-solid system with low moisture content and heating up the reacting mixture by direct exposing to the hot air to above 120 °C. The product was washed and dried to obtain combined modifying product. It was intended that the crosslinking would occur at the beginning at high moisture content and followed by phosphorylation in dried state. The freeze–thaw stability of singly and combined crosslinking and phosphorylation of rice starch are also studied and compared.

2. Materials and methods

2.1. Chemicals

Rice starch was kindly supplied by Cho Heng Rice Vermicelli Factory Co. Ltd., Nakornpathom, Thailand. Boric acid, hydrochloric acid, iodine, potassium hydroxide, potato amylose, potassium iodide, sodium carbonate,

sodium hydroxide were purchased from Merck, Germany. All chemical reagents are analytical grade. Petroleum ether is analytical grade and was supplied by Labscan Asia, Thailand. Sodium trimetaphosphate and sodium tripolyphosphate are food grade and were supplied by Monsanto, USA.

2.2. Methods

2.2.1. Preparation of modified rice starch

Dissolved 1% STMP (w/w of starch) or 4% STPP (w/w of starch) or the combination of 1% STMP with 4% STPP (dual modifications) based on dried starch weight in distilled water containing solution of 0.5 M NaOH. Proportions of mixture between base solution and distilled water were varied in order to obtain wet powder with $27.58 \pm 0.4\%$ moisture contents and pH 9.5. Then, five hundred gram (dried basis) of rice starch was mixed with the mixture of chemical solution thoroughly in the blender and passed through 14 mesh sifter again to obtain wet agglomerated granules of the rice starch mixture. Next, the sample was transferred to petri-dish and exposed directly without cover in a hot air oven (Model SLM 400–800 Memmert, Universal) at 120 °C for 7.5, 15, 30, 60 and 120 min and then cooled to room temperature. The granules were suspended in distilled water then the solution was adjusted to pH 6.5 by 0.5 M HCl solution. Modified rice starch was washed 3 times (3×1500 ml) with distilled water. The washed water was removed by vacuum-filtration on Buchner Funnel. After that, the modified rice starch was dried at 60 °C (Model SLM 400–800 Memmert, Universal, Germany) overnight. Finally, the modified rice starch was ground with a blender and passed through 80 mesh sifter (Laboratory Test Sieve, Endecotts Ltd., England) to obtain dried powder.

2.2.2. Determination of physicochemical properties of native and modified rice starch

2.2.2.1. Proximate analysis of rice starch. The proximate analysis of native rice starch was performed according to the standard methods described in the AOAC, 1990a, 1990b, 1990c, i.e. moisture, ash, protein, fat, fiber contents. Protein content was estimated from nitrogen content by Kjeldahl method multiplied by 6.25. Carbohydrate content was calculated by subtracting the aforementioned compounds from 100.

2.2.2.2. Amylose content. Amylose content of native rice starch (based on weight free of moisture, protein, fat and ash) was determined by Iodine Affinity method (Knutson, 1986).

2.2.2.3. Determination of phosphorus content and DS (degree of substitution) of starch monosodium phosphate ester and distarch phosphate. Dissolved 2.2275 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in distilled water then made up the solution into 100 ml in volumetric flask, then it was made into

serial dilutions. Add 2 ml of 25% conc. HCl into serial solutions and adjust their volume to 10 ml by adding distilled water, and then mixed 2 ml of vanadate-molybdate reagent, left the solution at room temperature for 45 min. Measure the absorbance of the solutions at 435 nm. Standard curve obtained by plotting absorbance against the phosphorus content.

Phosphorus content was determined by a method modified from standard methods described in the AOAC, 1984. Sodium carbonate (1.5 g) was dissolved in 5 ml of distilled water in crucible. 2.5 g of sample was added and mixed well. The mixture was boiled until dryness and did not provide any fume. Ignite it at 550 °C 12 h in the furnace. The finished product was cooled to room temperature and 2 ml of 25% conc. HCl with 10 ml of distilled water were added. The solution was transferred to beaker contained 20 ml distilled water and made up the volume to 50 ml by adding distilled water. Mix well then filter through glass funnel with filter paper No.1. The solution was again diluted into 250 ml by the addition of distilled water in the volumetric flask. Pipette 10 ml of diluted solution and mixed well with 2 ml of vanadate-molybdate reagent and left the solution at the room temperature for 45 min. Measure the absorbance of the sample at 435 nm with a spectrophotometer model Beckman, DU65. The phosphorus content was obtained from the standard curve. The DS of mono and distarch phosphates are calculated as follows (Wongsagonsup, Shobngob, Oonkhanond, and Varavinit, 2005):

$$\text{DS (starch mono-phosphate)} = 162P/3100 - 97P$$

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

$$\text{DS (distarch phosphate)} = 324P/3100 - 96P$$

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

2.2.2.4. Pasting properties of native and modified rice starch.

The pasting properties of starch was obtained from RVA with 2.5 g dried starch dispersed in 25 ml of distilled water. A heating and cooling cycle was programmed in the following manner; the sample was heated from 50 to 95 °C for 3 min, held at 95 °C for 2 min and cooled to 50 °C within 3 min. The gelatinization parameters such as maximum gelatinization temperature, peak viscosity, holding strength, final viscosity, break down, and set back were determined.

2.2.2.5. Determination of thermal properties. Thermal properties of native and modified starch were assessed by DSC (Differential Scanning Calorimeter, Pyris, Perkin Elmer, Belerica, MA, USA). Both native and modified starches (based on weight free from moisture) were dispersed in distilled water to obtain the starch/water ratio of 1:2. Each starch suspension was then transferred to aluminum pan (30 µL) and hermetically sealed. After equilibration at room temperature for 1 h., the sample was heated from

20 to 100 °C at 10 °C/min. An empty pan was used as the reference and the DSC was calibrated with indium. The onset (T_o), peak (T_p), conclusion (T_c) temperatures, and gelatinization enthalpy (ΔH) in J/g of dry starch were recorded.

2.2.2.6. Freeze–thaw stability. Starch paste was prepared by mixing 40 g starch (dried weight) and 460 g of distilled water and then gelatinized in a Brabender Viscoamylograph by heating from 30 °C up to 95 °C at the rate of 1.5 °C/min and kept at this temperature for 15 min before cooling to 50 °C at the same rate. Freeze–thaw stabilities of native rice and some selected modified rice starch pastes were investigated in the following steps .

1. The 25 g accurate weight of the starch paste was added into each of the pre-weighed 50 ml centrifuge tubes, and allowed to cool to 30 °C then these starch paste samples were frozen at –14 °C in a freezer for 22 h.
2. All tubes were removed from the freezer and thawed at 30 °C in water bath for 1 h.
3. Three tubes from each thawing cycle of these samples were centrifuged at 3500 rpm for 15 min. The clear liquid was decanted, and the residue was weighed. The percentage of syneresis was then calculated as the ratio of the weight of the liquid decanted to the total weight of the paste before centrifugation and multiplied by 100.
4. The remaining tubes were then put back to the freezer for further freeze–thaw cycling. Five freeze–thaw cycles were performed for this study.

2.2.3. Statistical analysis

Analytical determinations of individual samples were done in the triplicate and mean values and standard deviations reported. Data were statistically analyzed by independent sample *T*-test and analysis of variance (ANOVA) test procedure and significant different were identified by Tukey's HSD test ($p \leq 0.05$) using SPSS 12.0 for windows (SPSS Inc., IL, USA).

3. Results

3.1. Proximate analysis and amylose content of native rice starch

The proximate analysis and amylose content of native rice starch was found to be 0.5% protein, 0.30% fat, 0.26% ash, 0.16% fiber, 12.30% moisture, 86.48% carbohydrate and 31.02% amylose contents.

3.2. Effect of reaction time on the crosslinking, phosphorylation and dual modifications of rice starches

It was found from Table 1 that the phosphate contents for all of the modifications had the tendency to increase with the increase of reaction times. the modified rice

Table 1

Phosphate content of modified rice starches prepared from various reaction time with $27.58 \pm 0.40\%$ moisture content. Starch was moisten with various moist solution with pH 9.5 and modified temperature at $120^\circ\text{C}^{\text{a}}$

Time (Min)	Phosphate content ^b (%)		
	1% STMP + 4% STPP	1% STMP	4% STPP
0 (native)	$0.007 \pm 0.000^{\text{c}}$	$0.007 \pm 0.000^{\text{cd}}$ (DS = 0.00024)	$0.007 \pm 0.000^{\text{cd}}$ (DS = 0.00012)
7.5	$0.007 \pm 0.000^{\text{c}}$	$0.005 \pm 0.000^{\text{c}}$ (DS = 0.00017)	$0.006 \pm 0.000^{\text{ce}}$ (DS = 0.00010)
15	$0.010 \pm 0.000^{\text{d}}$	$0.006 \pm 0.000^{\text{cd}}$ (DS = 0.00020)	$0.005 \pm 0.000^{\text{ef}}$ (DS = 0.000084)
30	$0.013 \pm 0.000^{\text{e}}$	$0.009 \pm 0.000^{\text{d}}$ (DS = 0.00030)	$0.004 \pm 0.000^{\text{f}}$ (DS = 0.000068)
60	$0.021 \pm 0.000^{\text{f}}$	$0.012 \pm 0.001^{\text{e}}$ (DS = 0.00040)	$0.008 \pm 0.000^{\text{d}}$ (DS = 0.00014)
120	$0.030 \pm 0.001^{\text{g}}$	$0.014 \pm 0.000^{\text{e}}$ (DS = 0.00048)	$0.015 \pm 0.001^{\text{g}}$ (DS = 0.00025)

^a Each value is the mean of two experiments.

^b Means \pm standard deviation.

^{c,d,e,f,g} The same superscript in the same row of each type of reaction is not significant different at $p \leq 0.05$.

starch prepared from the combination of 1% STMP and 4% STPP showed higher phosphate content than those prepared from STMP or STPP individually. However, both modified starch by STMP and STPP provided almost equal value of the phosphate contents at the end of reaction time (120 min) and the summary of those values was almost equal to the value of the combined modification. This demonstrated that the combined modification consisted of half of the phosphate groups from crosslinking and the another half from the phosphorylation reactions. However from the calculation of the DS values, the DS values of the crosslinked starch at 120 min reaction time was 0.00048 which was almost double of the phosphorylated starch (0.00025). The RVA viscograms of modified rice starches prepared from 1% STMP at various reaction times are presented in Fig. 1. As the reaction time was increased from 7.5 min to 120 min, peak viscosity was gradually decreased, while the pasting temperature was increased. The pasting prop-

erties of native and modified rice starch prepared from 4% STPP at various reaction time are shown in Fig. 2. As the reaction time increased from 7.5 min to 120 min, the pasting temperature of modified rice starches was gradually decreased to the lowest pasting temperature at 120 min. The peak viscosity of modified rice starches were lower than that of the native rice starch when the modification time was shorter than 30 min, but the peak viscosity of modified starch was higher than that of the native rice starch when the reaction time was longer i.e., 60 min.

Fig. 3 shows the RVA viscograms of native and modified rice starches prepared from the combined reaction of 1% STMP and 4% STPP at various reaction times. The pasting temperature and peak viscosity of native and modified rice starches followed the same pattern as those of the modified starches prepared from 4% STPP as shown in Fig. 2. However, the peak viscosity of dual modified rice starch obtained from 120 min reaction time was much higher than that of starch modified with 4% STPP.

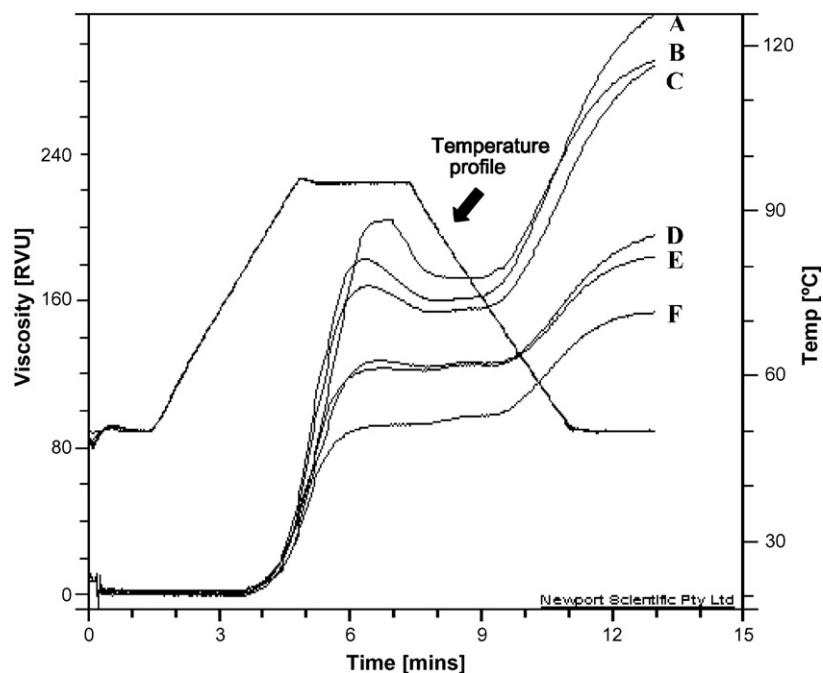


Fig. 1. RVA viscograms of native and modified rice starch prepared by the reaction of native rice starch with 1% STMP of various reaction time with 27% moisture content at pH 9.5, 120°C . (A) native rice starch (B) 7.5 min, (C) 15 min, (D) 30 min, (E) 60 min, (F) 120 min.

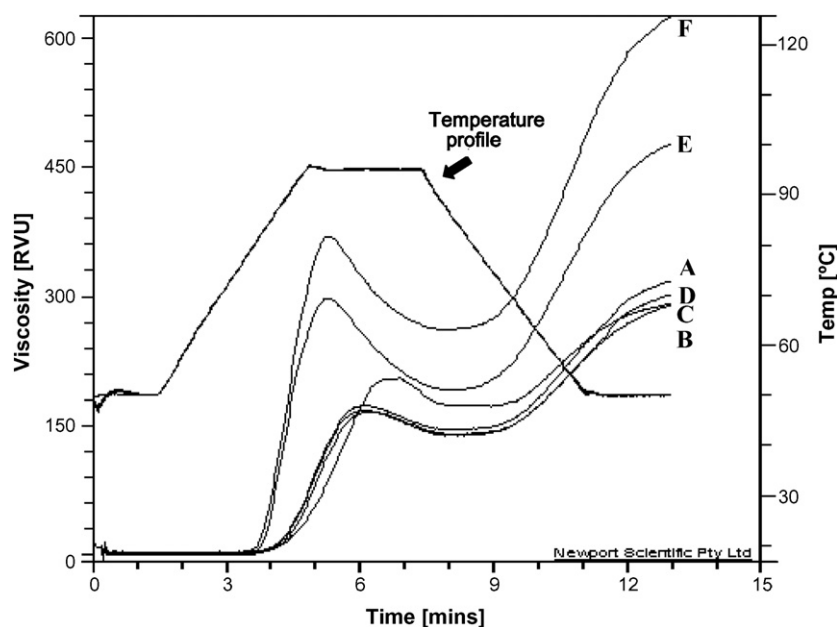


Fig. 2. RVA viscograms of native and modified rice starches prepared by the reaction with 4% STPP by various reaction time with 27% moisture content at pH 9.5, 120 °C. (A) native rice starch, (B) 7.5 min, (C) 15 min, (D) 30 min, (E) 60 min, (F) 120 min.

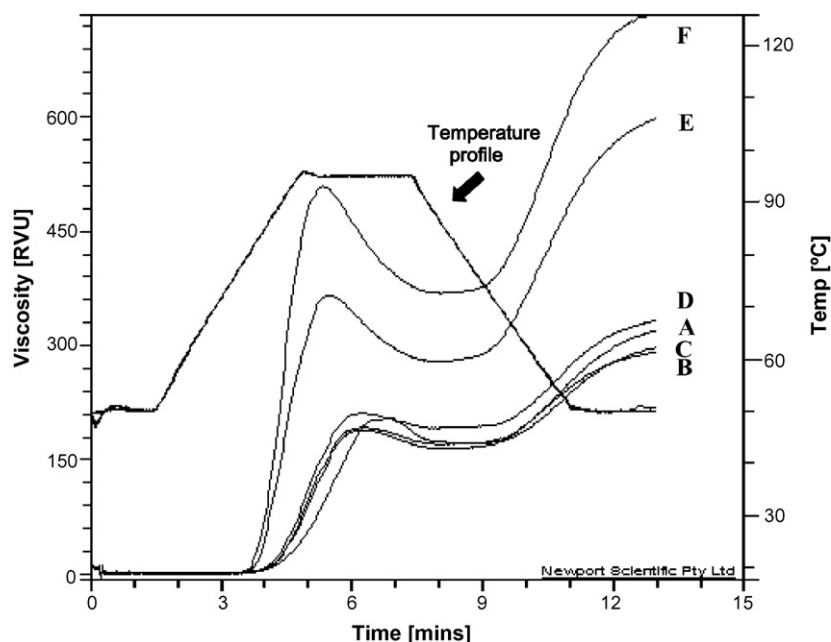


Fig. 3. RVA viscograms of native and modified rice starches prepared by the reaction with combination of 1% STMP and 4% STPP by various reaction time with 27% moisture content at pH 9.5, 120 °C. (A) native rice starch, (B) 7.5 min, (C) 15 min, (D) 30 min, (E) 60 min, (F) 120 min.

3.3. Thermal properties of modified rice starch prepared from various reaction times

Thermal properties of native and modified rice starch determined by DSC: onset (T_o), peak (T_p), conclusion gelatinization temperatures (T_c), and gelatinization enthalpy (ΔH (J/g)) are shown in Table 2–4. It was found that onset temperature of native rice starch was 71.64 °C. The onset temperature of modified rice starch prepared

from the reaction of rice starch with 1% STMP, 4% STPP, or combination of 1% STMP and 4% STPP with various reaction times provided higher value than that of native rice starch during the first period (7.5 min – 30 min) and lower than that of native rice starch during the last period (60 min – 120 min) of reaction time. However, the combined reaction of 1% STMP and 4% STPP provided the lowest onset temperature at the reaction time of 60 min and 120 min.

Table 2
Effect of reaction time on the thermal properties of native and modified rice starch^a

Type of reaction	Time (min)	T_o^b (°C)	T_p^b (°C)	T_c^b (°C)	$T_c - T_o^b$ (°C)	ΔH^b (J/g)
1% STMP \pm 4% STPP	0 (native)	71.64 \pm 0.008 ^c	76.38 \pm 0.001 ^c	83.50 \pm 0.18 ^c	11.86 \pm 0.25 ^c	11.74 \pm 0.12 ^c
	7.50	72.01 \pm 0.10 ^d	76.62 \pm 0.10 ^{cd}	83.48 \pm 0.20 ^c	11.48 \pm 0.22 ^c	11.45 \pm 0.27 ^{cd}
	15.00	72.06 \pm 0.10 ^d	76.90 \pm 0.17 ^d	84.13 \pm 0.37 ^c	12.07 \pm 0.28 ^c	11.39 \pm 0.19 ^{cd}
	30.00	72.00 \pm 0.26 ^d	76.96 \pm 0.27 ^d	84.59 \pm 0.45 ^c	12.59 \pm 0.19 ^c	11.86 \pm 0.007 ^c
	60.00	69.90 \pm 0.03 ^e	75.57 \pm 0.01 ^e	84.18 \pm 0.28 ^c	14.28 \pm 0.27 ^d	10.87 \pm 0.27 ^d
	120.00	67.50 \pm 0.21 ^f	74.34 \pm 0.08 ^f	84.28 \pm 0.58 ^c	16.78 \pm 0.76 ^e	8.99 \pm 0.27 ^e

^a Each value is the mean of two experiments.

^b Mean \pm standard deviation.

^{c,d,e,f} The same superscript in the same row of each type of reaction is not significant different at $p \leq 0.05$.

Table 3
Effect of reaction time on the thermal properties of native and modified rice starch^a

Type of reaction	Time (min)	T_o^b (°C)	T_p^b (°C)	T_c^b (°C)	$T_c - T_o^b$ (°C)	ΔH^b (J/g)
1% STMP	0 (native)	71.64 \pm 0.08 ^{cd}	76.38 \pm 0.0 ^c	83.50 \pm 0.18 ^c	11.86 \pm 0.25 ^c	11.74 \pm 0.12 ^c
	7.50	72.10 \pm 0.11 ^{de}	76.68 \pm 0.10 ^{cd}	83.49 \pm 0.52 ^c	11.38 \pm 0.42 ^c	11.26 \pm 0.67 ^c
	15.00	72.09 \pm 0.01 ^{de}	76.73 \pm 0.09 ^{cd}	83.49 \pm 0.13 ^c	11.40 \pm 0.57 ^c	11.42 \pm 0.33 ^c
	30.00	72.44 \pm 0.28 ^e	77.23 \pm 0.28 ^d	84.30 \pm 0.84 ^c	11.86 \pm 0.57 ^c	10.97 \pm 0.40 ^c
	60.00	71.73 \pm 0.25 ^d	76.68 \pm 0.34 ^{cd}	83.68 \pm 0.56 ^c	11.95 \pm 0.35 ^c	11.12 \pm 0.47 ^c
	120.00	71.17 \pm 0.10 ^c	76.14 \pm 0.09 ^c	83.72 \pm 0.33 ^c	12.55 \pm 0.36 ^c	11.38 \pm 0.21 ^c

^a Each value is the mean of two experiments.

^b Mean \pm standard deviation.

^{c,d,e} The same superscript in the same row of each type of reaction is not significant different at $p \leq 0.05$.

Table 4
Effect time of reaction on the thermal properties of native and modified rice starch^a

Type of reaction	Time (min)	T_o^b (°C)	T_p^b (°C)	T_c^b (°C)	$T_c - T_o^b$ (°C)	ΔH^b (J/g)
4% STPP	0 (native)	71.64 \pm 0.008 ^c	76.38 \pm 0.01 ^{cde}	83.50 \pm 0.18 ^c	11.86 \pm 0.25 ^c	11.74 \pm 0.12 ^c
	7.50	72.29 \pm 0.19 ^d	76.96 \pm 0.25 ^f	84.37 \pm 0.20 ^c	12.08 \pm 0.03 ^e	12.35 \pm 0.38 ^e
	15.00	72.04 \pm 0.08 ^{cd}	76.62 \pm 0.09 ^{ef}	83.25 \pm 0.24 ^c	11.22 \pm 0.17 ^c	9.41 \pm 2.85 ^c
	30.00	72.00 \pm 0.11 ^{cd}	76.50 \pm 0.08 ^{def}	83.33 \pm 0.34 ^c	11.33 \pm 0.34 ^c	11.65 \pm 0.31 ^c
	60.00	70.81 \pm 0.10 ^e	76.08 \pm 0.17 ^{cd}	84.14 \pm 0.70 ^c	13.33 \pm 0.68 ^d	12.38 \pm 0.81 ^c
	120.00	70.28 \pm 0.24 ^f	75.84 \pm 0.25 ^c	84.01 \pm 0.21 ^c	13.72 \pm 0.28 ^d	10.28 \pm 0.13 ^c

^a Each value is the mean of two experiments.

^b Mean \pm standard deviation.

^{c,d,e,f} The same superscript in the same row of each type of reaction is not significant different at $p \leq 0.05$.

3.4. Freeze–thaw stability of native and selected modified rice starch paste

Freeze–thaw stability of 8% dried basis of native and selected modified rice starch pastes subjected to five freeze–thaw cycles at thawing conditions of 30 °C for 1 h is illustrated in Table 5. It could be seen that native rice

starch and modified rice starches showed similar patterns with highest % syneresis in the first cycle and then % syneresis gradually decreased to the fifth cycle. Native rice starch showed the decreasing of % syneresis from cycle 1 to cycle 5 of 28.86%, 22.26%, 10.61%, 1.25% and 1.31%, respectively. The % syneresis of modified rice starch with 4% STPP was decreased from cycle 1 to cycle 5 of

Table 5
Percent syneresis of 8% native and selected modified rice starch pastes frozen at -14 °C for 22 h and thawed at 30 °C for 5 freeze–thaw cycles^a

Starch modified with (reaction time 120 min)	(%) Syneresis ^b				
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Native rice starch	28.86 \pm 2.16 ^c	22.26 \pm 2.40 ^d	10.61 \pm 2.76 ^e	1.25 \pm 1.36 ^f	1.31 \pm 0.64 ^f
1% STMP and 4% STPP (combined)	5.91 \pm 5.36 ^c	3.71 \pm 2.10 ^{cd}	4.08 \pm 2.14 ^c	4.06 \pm 1.17 ^c	22.22 \pm 0.99 ^{cd}
1% STMP	33.47 \pm 3.04 ^c	23.01 \pm 3.65 ^d	13.84 \pm 3.24 ^e	5.04 \pm 1.83 ^f	2.72 \pm 0.10 ^g
4% STPP	16.86 \pm 1.21 ^c	16.26 \pm 1.09 ^c	11.46 \pm 0.32 ^d	10.01 \pm 2.42 ^d	7.31 \pm 3.66 ^d

^a Each value is the mean of three experiments.

^b Mean \pm standard deviation.

^{c,d,e,f,g} The same superscript in the same row of each type of reaction is not significant different at $p \leq 0.05$.

16.86%, 16.26%, 11.46%, 10.01% and 7.31%, respectively. Percent syneresis of the combination of 1% STMP and 4% STPP was decreased in the first cycle to 5.91% and 2.22% for the last cycle. In contrast with, the % syneresis of the first cycle of modified rice starch with 1% STMP was increased up to 33.47% and 2.72% for the first and last cycle, respectively.

4. Discussion

In general, the condition used for crosslinking and phosphorylation are different. Crosslinking reaction occurred at room temperature in alkaline condition of STMP (Puchongkavarin H., 1999) while phosphorylation reaction proceeded at high temperature (120–170 °C) (Solarek, 1986) in dried state from the mixture of starch and STPP at neutral condition (Lim and Seib, 1993; Sitohy, Labib, El-Saadany, & Ramandan, 2000). In order to perform both reactions, crosslinking and phosphorylation reactions should be conducted consecutively, and therefore too many steps must be required. In order to reduce too many processing steps the combined reaction mixture of rice starch, STMP and STPP with low moisture content (27%) was performed by directly exposed to hot air in an oven allowing the gradual evaporation of water to dryness for 2 h reaction time. In this study, the crosslinking reaction occurred when the mixture was wet and phosphorylation reaction proceeded when it was dried. The pH 9.5 was selected by the use of 1% STMP and 4% STPP. However, various concentration of STMP combined with 4% STPP was also studied. Currently, there was no phosphorylated starch produced with lower concentration than 4% STPP. The suitable 27% moisture content of the reacting mixture of the combined modification reaction in the semi-dry system was introduced. Higher quantity of water content of the starch mixtures would caused in gelatinization of starch during heating over 120 °C for the phosphorylation reaction (Hoover and Vasanathan, 1994). The temperature of the reaction at 120 °C was selected while it was the lowest temperature used for the phosphorylation reaction. By increasing the pH of the crosslinking reaction of rice starch and STMP from 9–10 provided the increasing of phosphate content of the modified rice starch in contrast with the phosphorylation reaction with STPP provided the decreasing of phosphate content (data not shown). This demonstrated that STMP preferred higher pH to produce crosslinked starch while that of STPP preferred neutral pH to produce phosphorylated starch. For the investigation of various pH ranging from 9–10 of reaction of the combined 1% STMP and 4% STPP, it was found that pH 9.5, 27% moisture content of the reacting mixture and reaction temperature of 120 °C with the reaction time 120 min provided both characteristics of crosslinking and phosphorylation reactions which could be observed from the pasting properties. Lower pH than 9.5 dominated the phosphorylation reaction while higher pH dominated the crosslinking reaction (Lim & Seib, 1993). Because of the same method of all

types of the modification were used, DS values of the individual crosslinked starch (1% STMP) and phosphorylated starch (4% STPP) should be equal to that of the DS values of the combined modification (1% STMP and 4% STPP). Then the DS values of the combined modification could be estimated from the values of the individual modification of crosslinking and phosphorylation, which were 0.00048 for the crosslinking and 0.00025 for the phosphorylation reactions, respectively.

The phosphate group presented in the starch molecule affected the pasting properties measured by RVA-viscoamylograph. When the heat was applied to the phosphorylated starch with H-bonding between molecules would be destroyed and let the absorption of water into the starch granules to form high swelled starch granules which resulted in the increasing of peak viscosity. However, the swelling of phosphorylated starch was limited since it could be broken down after high absorption of water. In contrast with the crosslinking reaction which inhibit the swelling without the breakage of starch granules therefore it resulted in the lowering of peak viscosity and breakdown. Combined modification of crosslinking and phosphorylation provided the enhance of peak viscosity. Because of the resistance to breakage of starch granules by crosslinking reaction and promotion for high water absorption ability of starch granules by the phosphorylation reaction would enhance the water absorption ability with the slow breakage of starch granules. However, the pasting properties of modified starches prepared from various concentration of STMP (0.25, 0.5, and 1%, data not shown) combined with 4% STPP were also investigated, but the combination of 1% STMP and 4% STPP provided the highest peak viscosity. Therefore any degree of crosslinking by STMP was affected by the phosphorylation reaction with STPP for the promotion of the dramatic increase of the peak viscosity.

From the thermal property of crosslinked rice starch, gelatinization temperature (onset temperature) should be increased in line with the degree of crosslinking (Varavinit, Hanwasdi, & Shobsngob, 1999). In contrast with the phosphorylated starch providing lower gelatinization temperature (Solarek, 1986). For the reaction of native rice starch with the combined of 1% STMP and 4% STPP provided the increase of the gelatinization temperature at the first period of reaction time (7.5–30 min). It could be the occurring of crosslinking reaction, which provided higher gelatinization temperature. However at higher reaction time (60–120 min), it provided much lowering of the gelatinization temperature than the reaction of native rice starch with 1% STMP or 4% STPP. This confirmed the enhance of the phosphorylation reaction of the combined reaction of 1% STMP and 4% STPP much more than the others at final state. For the reaction of native rice starch with 1% STMP or 4% STPP provided higher gelatinization temperature on the first period of reaction time (7.5–30 min) which confirmed the occurring of crosslinking reaction in wet condition and on the last period of reaction time (60–120 min) provided lower gelatinization temperature

which confirmed the occurring of phosphorylation reaction.

The freeze–thaw stability of native and modified rice starches were compared. The freezing and thawing was performed for 5 cycles. Native and modified rice starches showed the decreasing of % syneresis from cycle 1 to cycle 5, which were not the normal freeze–thaw stability property. The water separation or % syneresis of any starch pastes should be increased with the increase of freeze–thaw cycle (Varavinit, Anuntavuttikul, & Shobsngob, 2000). In the case of native rice starch with rather high amylose showed a rather high % syneresis for the first freeze–thaw cycle, but a surprisingly low syneresis after the second cycle, where it appeared to have good freeze–thaw stability (Varavinit, Shobsngob, Varayanond, Chinachoti, & Nai-vikul, 2002). However, it was found that during these cycles the amylose rice starch gel had changed from a smooth gel to a rough-textured porous gel (rough surface) with a sponge-like structure that allowed it to reabsorb the separated water. Thus syneresis was reduced unless this rough-textured gel was pressed to squeeze out the absorbed water. This retrogradation phenomenon occurred when the rice starch gel was frozen and ice crystals spread within the gel. Upon subsequent thawing at a lower temperature (30 °C), the ice crystals melted and rough-textured with relatively high porosity was obtained. In the first freezing cycle, only a small quantity of the rough porous gel was formed and it was not enough to form a spongy structure and a high syneresis value was observed. From the second to the fifth cycle, the quantity of rough-textured gel accumulated sufficiently to form the sponge-like structure.

It was found that the combined modified rice starch of 1% STMP and 4% STPP provided the highest freeze–thaw stability among the modified rice starches with 1% STMP or 4% STPP because of the branching of the phosphate groups and the effects of the dramatic high peak and final viscosities of the starch paste. However, the formation of sponge-like structure was not occurred during the freeze–thaw cycles. This type of modified rice starch could be selected for preparing food products with good freeze–thaw stability.

5. Conclusion

Dual modifications of rice starch by crosslinking and phosphorylation reactions with 1% STMP and 4% STPP was conducted with 27% moisture content of the mixture at initial pH 9.5. The reaction mixture was directly exposed to hot air in the oven at 120 °C allowing the gradually evaporation of wet to dryness after 2 h reaction time. The crosslinking reaction was occurred when the reaction mixture was wet and phosphorylation reaction when it was dried. DS of the dual modifications was found to be 0.00048 and 0.00025 for the crosslinking and phosphorylation reactions, respectively. It was found that the peak viscosity of the dual modified rice starch increased dramatically in comparison with the native rice starch.

Freeze–thaw stability is very good in comparison with the conventional phosphorylated rice starch. The process is very convenience to produce a dual modifications of rice starch in a larger scale.

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