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Original Article

Physicochemical Properties of Low and High Amylose Cross-Linked Rice Starches

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ABSTRACT

Background and Objectives: As chemical methods are commonly used to modify the starch properties, in this study, the influence of chemical modification using $POCl_3$ on the rice starch function was evaluated.

Materials and Methods: Rice starch was isolated by alkaline extraction of the proteins and cross-linked rice starches were prepared from reactions between native rice starch (low and high amylose) and varied concentrations of $POCl_3$ (0.1, 0.2 and 0.3 % w/w). The physicochemical, rheological and morphological properties of rice starches (water absorption and solubility, pasting viscosity parameters, light transmittance, freeze-thaw stability, and scanning electron microscopy) were evaluated.

Results: According to SEM micrographs, cross-linking did not affect granule size or shape. The cross-linked starches showed lower solubility than that of the native starches and solubility decreased with increasing the concentration of cross-linking agent. Cross-linking decreased the capability of starches to absorb water at high concentration of cross-linking agent (0.3%). The paste clarity and pasting viscosity parameters of cross-linked samples were lower than their native starches and decreased with $POCl_3$ concentration. The freeze-thaw stability of rice starches improved by cross-linking and increased with an increase in cross-linking agent concentration. Regarding amylose content, low amylose rice starches showed lower water solubility, water absorption and freeze-thaw stability and higher pasting viscosity parameters than those of high amylose ones.

Conclusions: The results of this study suggest that high amylose cross-linked rice starch can be used in food systems in which higher stability, or controlled increase of viscosity is desired during their processing.

Keywords: Rice starch, Low amylose, High amylose, Functional properties

Introduction

Although native starch abundantly is used as a good stabilizer or thickening agent in food formulations (1), but properties such as retrogradation during storage and low shear-thermal resistance have limited the widespread use of native starch in some industrial food applications. Starch modification, which alters the physicochemical characteristics of starch can be used to tailor starch in specific food applications (2). Among different methods of modification, chemical techniques are the most popular ones (3).

Functional groups are added into a starch molecule through chemical modification, changing the physicochemical properties (like stability, solubility, gelatinization temperature and retrogradation behavior) of the starch (4). These changes are occurred through formation of intra- and intermolecular bonds at different parts of the starch chain. Cross-linked starch is an example of chemically modified starches (3), usually produced by $POCl_3$ (Fig. 1) as a cross-linking agent under alkaline pH (~11) during one hour reaction at ambient temperature. Salts (usually NaCl and Na_2SO_4) either may or may not be used during modification (4). The characteristics of the modified starch are greatly influenced by the source of native starch, the kinds and conditions of modification. Therefore, it is very important to choose the proper method of

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modification based on the properties required for the starch (3).

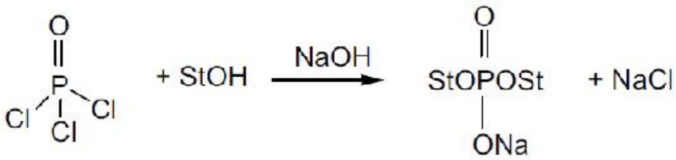

Fig. 1. Chemical reaction of starch cross-linking using POCl3, St = Starch (4).

Rice is considered a good source of starch as it has, on average, 88% of starch (5). Rice starch is a relatively bland ingredient that has the potential to be utilized in lower fat foods (6). As the granule size of rice is comparable to the size of fat globules, it is suited to mimic a full bodied fatty mouthfeel and might provide creamy texture. Therefore, the main objectives of the current research were: 1) To produce high and low amylose cross-linked rice starches and 2) To evaluate the effect of amylose content on the functional properties of produced starches.

Materials and Methods

Materials: Rice grains were obtained from local market in Yasooj, Iran. Other chemical reagents including ethanol, phosphoryl chloride ($POCl_3$), sodium hydroxide (NaOH), sodium sulfate ($NaHSO_4$), ammonium vanadate, ammonium molybdate ($(NH_4)_2MoO_4$), zinc acetate ($Zn(O_2CCH_3)_2$), nitric acid (HNO_3), potassium hydroxide (KOH) and hydrochloric acid (HCl) were purchased from Merck Company (Germany).

Isolation of starch: Five varieties of rice were examined for their amylose content (AACC Method 61-03.01) and then two varieties with high (24.20±0.32) and low (10.03±0.20) amylose content were selected, milled and used for starch isolation. The isolation of rice starch was performed according to Sodhi and Singh (7), through stepwise steeping of milled rice in 0.15% (w/v) sodium hydroxide solution for eight times.

Cross-linking of rice starch: $POCl_3$ was used to cross-link the rice starch chains according to Woo and Seib method (8). In this regard, 0.1, 0.2 and 0.3 g $POCl_3$ was used in the procedure for 100 g (d.w.b.) of rice starch to produce cross-linked starches modified with three concentrations (0.1%, 0.2% and 0.3%) of $POCl_3$.

To calculate the number of cross-linking sites, Seker and Hanna method (9) was used by measuring the absorbance at 460 nm with a UV/VIS spectrophotometer (PG Instruments, T80+, United Kingdom). A calibration curve was used for determination of phosphorus content. Finally, the degree of cross-linking was determined by using equations 1 and 2:

Amount of phosphorous from cross-linkages =phosphorous content of each sample – phosphorous content of native starch (equ.1)

Degree of cross-linking = [(No. of moles of phosphorous in 1g starch) / (No. of moles of glucose units in 1g starch)] × 2 (equ. 2)

Table 1 shows the chemical composition of native and cross-linked rice starches. AACC methods 08–01, 46–13 and 30–25 were used to measure the amounts of ash, protein and fat, respectively (8).

Scanning electron microscopy: A Cambridge scanning electron microscope (SEM) (Model 5526, 20 KV, UK) was used for assessing the microscopic structure of native and cross-linked rice starches. For this purpose, the SEM stub and samples were coated with gold/palladium and photographed.

Water Solubility and absorption: Leach, McCowen (9) was used to measure the water solubility and absorption of the samples. The starch solubility was finally calculated according to Equation 3 after the starch suspensions were heated, centrifuged and the supernatants were then dried.

$$\text{Solubility (\%)} = \frac{\text{Weight of dried supernatant}}{\text{Weight of initial starch}} \times 100 \quad \text{(equ. 3)}$$

To measure water absorption, the sediments, obtained from centrifugation in water solubility measurement, were weighed. Afterwards, the water absorption was calculated using the equation 4.

$$\text{Water absorption(\%)} = \frac{\text{Weight of sediment}}{\text{Weight of initial starch}} \times 100 \quad \text{(equ. 4)}$$

Pasting Properties: Pasting properties were measured according to Bason *et al.* (10) using a rotational rheometer (Brookfield LV-DVIII Ultra, Brookfield Engineering Laboratories, Stoughton, MA, USA). The applied conditions were as follows:

stirring speed (200 rpm), the rate of temperature increase and decrease from 60 to 90 ºC and 90 to 60 ºC (2ºC/min), temperature hold at 90 ºC (30 min).

Freeze- thawed starch gel: The freeze-thaw stability of starch gels was determined according to Van Hung and Morita (11) by freezing the starch gels at -18 ºC for 96 hour, and thereafter, thawing at 30 ºC for 1 hour, for 5 freeze-thaw cycles.

Light transmittance (paste clarity): Bhandari and Singhal (12) method was used to measure the past clarity of samples. For this purpose, the transmittance (%) of pastes was measured at 650 nm.

Statistical analysis: The experiments were conducted according to the completely randomized design. All of the experiments were performed in triplicates. The statistical software SPSS 13 (SPSS Inc., New Jersey, USA) was used to perform the analysis of variance (ANOVA) and also to perform the Duncan's Multiple Ranges test (α<0.05) for grouping of the results.

Results

Study of starch extraction: To achieve a starch with high purity, the absorbance of supernatant obtained from rice flour and NaOH slurries was measured at 280 nm during the extraction stages to ensure that the protein contents of isolated rice starches were 0.5% or less. Fig. 2 shows the absorbance of starch/NaOH slurries at 280 nm which is an indication of protein content during different stages of extraction. Results showed that the protein content of high amylose rice flour was significantly higher than that of low amylose sample (p>0.05) in the first stages of extraction, but by proceeding the extraction and further washing of starches by NaOH, the differences decreased, and the protein content of both kinds of flours reduced to the point, in which very low absorbance (~0.05) was detected at 280 nm. Chemical compositions of rice flours and their corresponding starches are shown in Table 1.

Table 1. Chemical composition of tested starches

	HARF	NHARS	CHARS0.1	CHARS0.2	CHARS0.3	LARF	NLARS	CLARS0.1	CLARS0.2	CLARS0.3
%Lipid	0.52±0.01	0.31±0.02	0.31±0.02	0.41±0.03	0.31±0.03	0.61±0.02	0.44±0.03	0.44±0.01	0.43±0.04	0.42±0.03
%Ash	0.26±0.01	0.25±0.02	0.31±0.02	0.33±0.01	0.41±0.03	0.28±0.02	0.26±0.04	0.32±0.02	0.34±0.03	0.37±0.01
%Protein	7.30±0.23	Lower than 1%	-		-	6.10±0.20	Lower than 1%	-	-	-

* HARF: High amylose rice flour, NHARS: native high amylose rice starch, CHARS: high amylose cross-linked rice starch (using 0.1(CHARS0.1), 0.2 (CHARS0.2) and 0.3% (CHARS0.3) $POCl_3$ in modification process), LARF: Low amylose rice flour, NLARS: native low amylose rice starch, CLARS: low amylose cross-linked rice starch (using 0.1 (CLARS0.1), 0.2 (CLARS0.2) and 0.3% (CLARS0.3) $POCl_3$ in modification process). Each point is the average of three replicates.

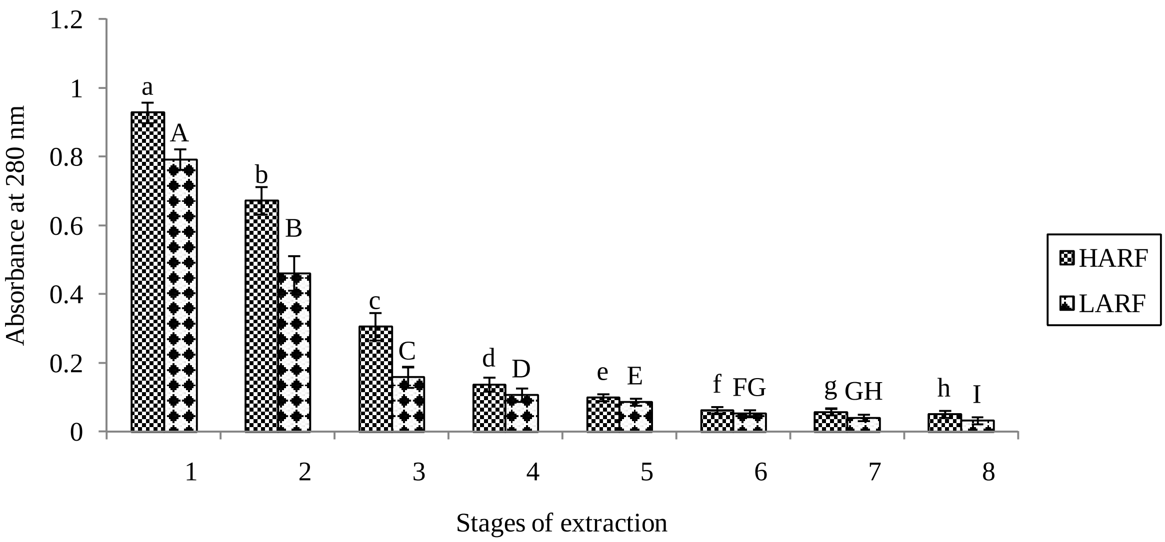

Fig. 2. The absorbance of supernatant achieved from rice flour and NaOH slurries at 280 nm during the extraction stages. HARF: high amylose rice flour, LARF: low amylose rice flour. Each point is the average of three replicates. Different small (for HARF) or capital (for LARF) letters show significant difference between stages of extraction (p<0.05).

Scanning electron microscopy and degree of cross-linking: According to micrographs (Fig. 3), both native and cross-linked rice starches with high or low amylose content, mainly had polyhedral shapes. Also, some oval, irregular, angular, or smooth shapes were detected. The electron micrographs showed that granule sizes were in the range of 2.95 to 4.28 μm.

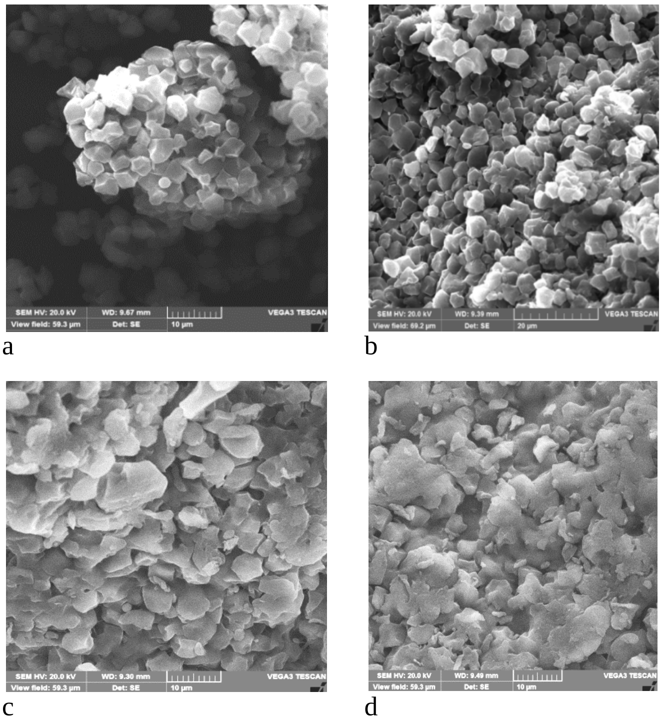

Fig. 3. Scanning electron micrographs of: native (a) and cross-linked (b) low amylose rice starch, and native (c) and cross-linked (d) high amylose rice starch.

Table 2 shows the degree of substitution for cross-linked rice starches. In this study, the degree of cross-linked rice starch substitution varies from 7.67×10^{-5} to 9.98×10^{-5}, depending on $POCl_3$ concentration. Therefore, the degree of substitution increased significantly with increasing the cross-linking reagent and was not affected by amylose content.

Determination of water solubility and absorption indices: Figs.4 and 5 show the water solubility and absorption indices of the native and cross-linked rice starches. Results showed that cross-linked rice starches have lower solubility than those of corresponding native starches and the water solubility decreased significantly with an increase in the concentration of cross-linking reagent. Also, the native high amylose rice starch exhibited higher water solubility than that of the native low amylose sample.

With respect to water absorption, no significant differences were observed between low and high amylose rice starches. In this regard, the native rice starch and the cross-linked ones at lower concentrations of $POCl_3$ (0.1 and 0.2%) did not differ significantly. However, water absorption decreased significantly with an increase in $POCl_3$ concentration from 0.2% to 0.3%.

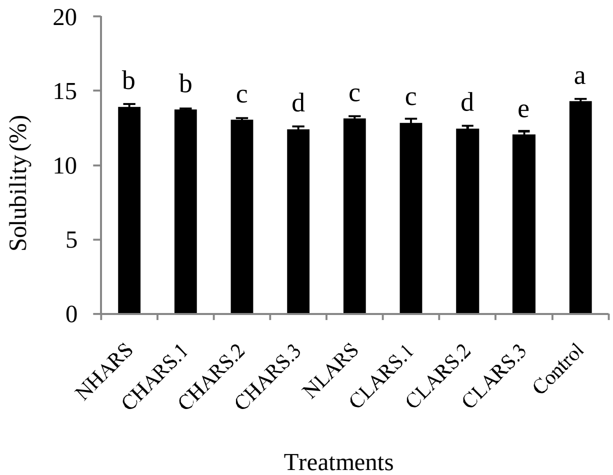

Fig. 5. Water absorption of the high amylose native and cross-linked rice starches. Samples are as follows: NHARS (high amylose native rice starch), CHARS (high amylose cross-linked rice starch [using 0.1(CHARS0.1), 0.2 (CHARS0.2) and 0.3% (CHARS0.3) $POCl_3$ in modification process]), NLARS (low amylose native rice starch), CLARS (low amylose cross-linked rice starch [using 0.1 (CLARS0.1), 0.2 (CLARS0.2) and 0.3% (CLARS0.3) $POCl_3$ in modification process]), Control (Wheat starch). Values with similar letters do not differ significantly ($P<0.05$).

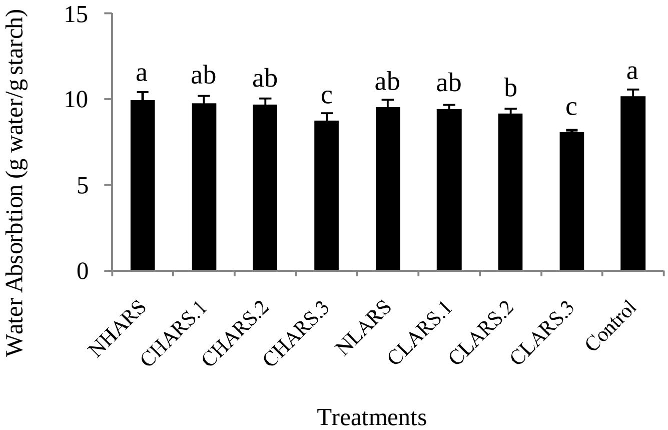

Fig. 4. Water solubility of the low amylose native and cross-linked rice starches. Samples are as follows: NHARS (high amylose native rice starch), CHARS (high amylose cross-linked rice starch [using 0.1(CHARS0.1), 0.2 (CHARS0.2) and 0.3% (CHARS0.3) $POCl_3$ in modification process]), NLARS (low amylose native rice starch), CLARS (low amylose cross-linked rice starch [using 0.1 (CLARS0.1), 0.2 (CLARS0.2) and 0.3% (CLARS0.3) $POCl_3$ in modification process]), Control (Wheat starch). Values with similar letters do not differ significantly ($P<0.05$).

Table 2. Degree of substitution (DS)

Samples	NHARS	CHARS.1	CHARS.2	CHARS.3	NLARS	CLARS.1	CLARS.2	CLARS.3
DS*	7.42×10^{-5}	7.67×10^{-5}	8.06×10^{-5}	9.84×10^{-5}	7.69×10^{-5}	7.95×10^{-5}	8.09×10^{-5}	9.98×10^{-5}

*DS: Degree of substitution

** NHARS: native high amylose rice starch, CHARS: high amylose cross-linked rice starch (using 0.1(CHARS0.1), 0.2 (CHARS0.2) and 0.3% (CHARS0.3) $POCl_3$ in modification process), NLARS: native low amylose rice starch, CLARS: low amylose cross-linked rice starch (using 0.1 (CLARS0.1), 0.2 (CLARS0.2) and 0.3% (CLARS0.3) $POCl_3$ in modification process). Each point is the average of three replicates.

Pasting Properties: Table 3 represents the mean values for pasting properties (peak viscosity, breakdown viscosity, final viscosity, setback viscosity, trough viscosity, pasting temperature and peak time) of the native and cross-linked rice starches. The pasting curves of starch samples are also presented in Figs. 6 and 7. According to the results, the pasting properties of the cross-linked starch samples associated with viscosity values decreased significantly ($p<0.05$). Regarding the effect of cross-linking agent concentration, the pasting viscosity parameters including the peak, trough, breakdown and final values decreased with cross-linking agent concentration from 0.1 to 0.3%; however, peak time and pasting temperature values increased. The trend of each starch sample behavior during heating and cooling time is clear in Figs. 6 and 7.

Table 3. Pasting properties of the native and cross-linked rice starches

Pasting properties* / Starch samples	PV(N.m)	TV(N.m)	BV(N.m)	FV(N.m)	SV(N.m)	PeT(min)	PaT(°C)
NHARS	19.28±0.82^{d}	17.90±0.85^{c}	1.38±0.53^{de}	18.46±1.26^{c}	00.56±.02^{a}	53.86±0.80^{c}	70.33±1.28^{e}
CHARS.1	16.26±0.76^{e}	15.00±086^{d}	1.23±0.75^{de}	15.39±1.17^{d}	00.39±.01^{a}	57.66±0.75^{b}	73.33±1.65^{d}
CHARS.2	13.50 ±0.86^{f}	12.66±0.57^{e}	0.83±0.06^{de}	13.09±0.50^{e}	00.43±.04^{a}	60.83±0.95^{b}	89.66 ±1.33^{b}
CHARS.3	12.50±0.43^{f}	12.00±0.50^{e}	˙0.50±0.03^{e}	12.50±0.60^{e}	00.50±.03^{a}	61.00±0.80^{a}	90.93±1.25^{a}
NLANRS	86.66±1.16^{a}	70.33±1.57^{a}	16.33±0.88^{a}	70.46±1.57^{a}	0.13±.02^{b}	48.00±0.95^{e}	68.50±1.01^{f}
CLARS.1	50.33 ±1.07^{b}	39.33±1.75^{b}	11.00±0.26^{b}	39.43±1.16^{b}	00.10±.07^{b}	48.77±0.75^{e}	71.33±1.35^{e}
CLARS.2	23.20±0.96^{c}	18.66 ±1.37^{c}	4.53±0.55^{c}	18.80±0.81^{c}	00.14±.05^{b}	51.33±0.87^{d}	86.60±1.30^{c}
CLARS.3	13.97 ±1.28^{f}	12.00±0.86^{e}	1.97±0.57^{d}	12.16±0.76^{e}	00.16±.04^{b}	51.66±0.97^{d}	85.60±1.43^{c}

* PV, Peak viscosity; TV, trough viscosity; BV, breakdown viscosity; FV, final viscosity; SV, setback viscosity; PeT, peak time; PaT, pasting temperature.

** Torque expressed: T=F×R (N.m).

*** NHARS: native high amylose rice starch, CHARS: high amylose cross-linked rice starch (using 0.1(CHARS0.1), 0.2 (CHARS0.2) and 0.3% (CHARS0.3) $POCl_3$ in modification process), NLARS: native low amylose rice starch, CLARS: low amylose cross-linked rice starch (using 0.1 (CLARS0.1), 0.2 (CLARS0.2) and 0.3% (CLARS0.3) $POCl_3$ in modification process). Each point is the average of three replicates. Mean (±SD, $N.m^{b}$) values followed by the same letter in each column are not significantly different ($P<0.05$).

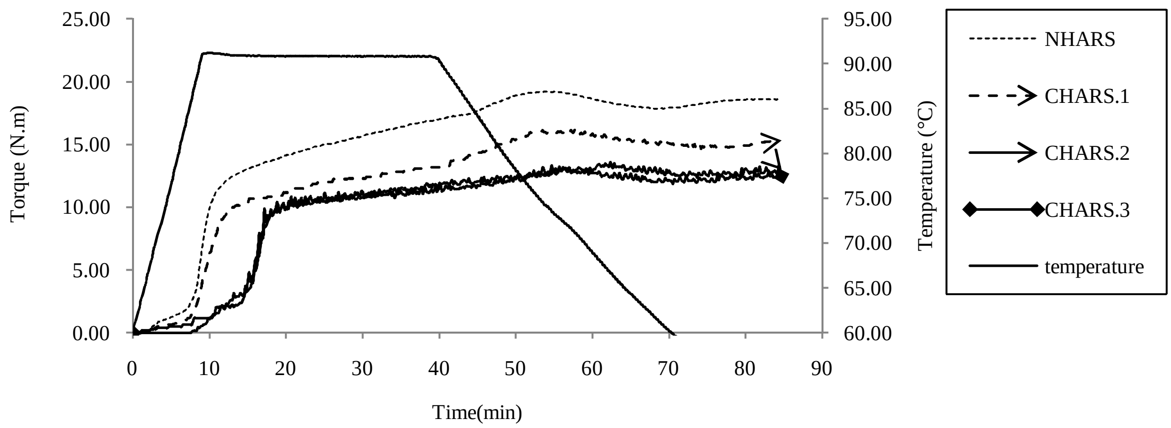

Fig. 6. Pasting curves of the high amylose native and cross-linked rice starches. NHARS (high amylose native rice starch), CHARS (high amylose cross-linked rice starch [using 0.1(CHARS0.1), 0.2 (CHARS0.2) and 0.3% (CHARS0.3) $POCl_3$ in modification process]).

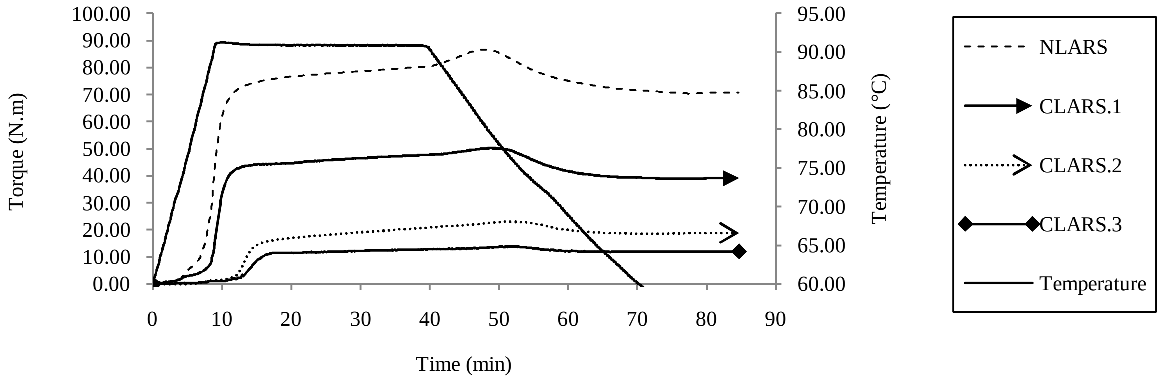

Fig. 7. Pasting curves of the low amylose native and cross-linked rice starches. NLARS (low amylose native rice starch), CLARS (low amylose cross-linked rice starch [using 0.1 (CLARS0.1), 0.2 (CLARS0.2) and 0.3% (CLARS0.3) $POCl_3$ in modification process]).

Light transmittance (paste clarity): Figs. 8 and 9 show the paste clarity indices of the native and cross-linked rice starches. The paste clarity decreased in all starches after the 5th cycle. Also, the cross-linked rice starches had lower clarity than the corresponding native starches and the clarity decreased further with an increase in the concentration of cross-linking agent from 0.1 to 0.2%. No significant differences (P>0.05) was observed between 0.2 and 0.3% $POCl_3$ concentrations.

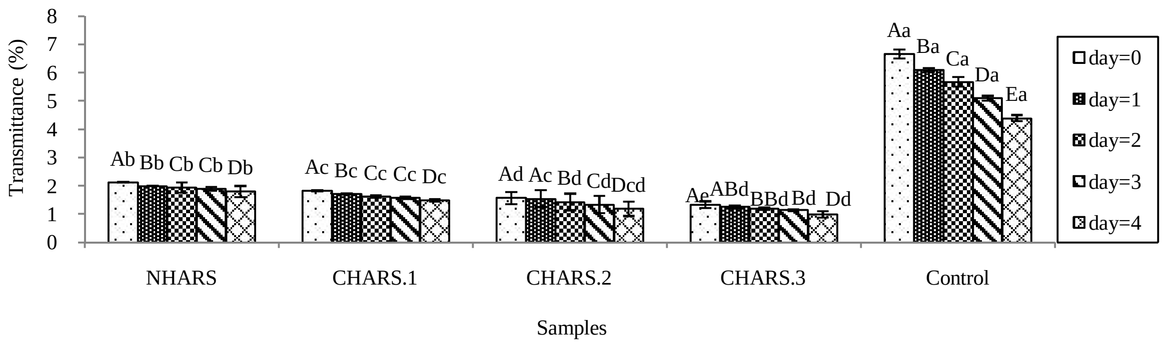

Fig. 8. Effect of cross-linking and storage duration (at 4 °C) on the light transmittance (%) of the high amylose native and cross-linked rice starches. NHARS (high amylose native rice starch), CHARS (high amylose cross-linked rice starch [using 0.1(CHARS0.1), 0.2 (CHARS0.2) and 0.3% (CHARS0.3) $POCl_3$ in modification process]), Control (Wheat starch). For each treatment, different capital letters show differences between the days (5th cycle) (P<0.05). For each day, different small letters show differences between the treatments (P<0.05).

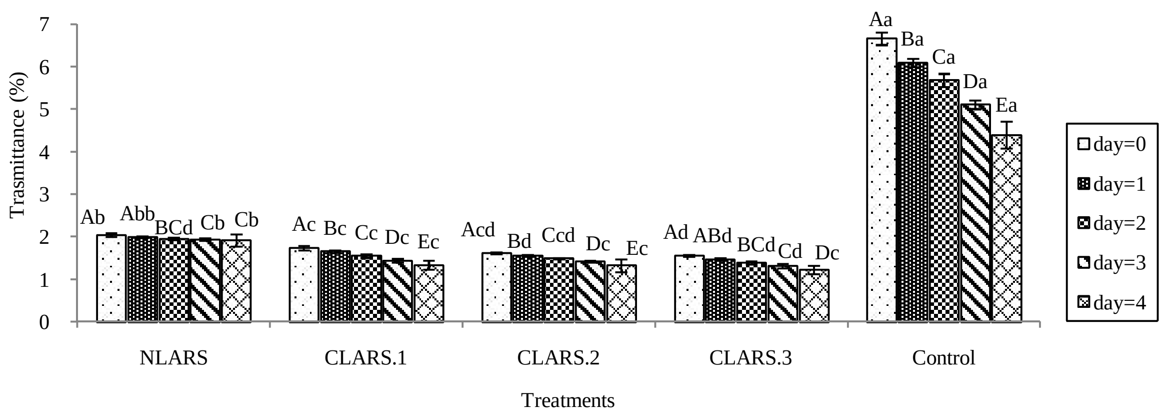

Fig. 9. Effect of cross-linking and storage duration (at 4°C) on the light transmittance (%) of the low amylose native and cross-linked rice starches. NLARS (low amylose native rice starch), CLARS (low amylose cross-linked rice starch [using 0.1 (CLARS0.1), 0.2 (CLARS0.2) and 0.3% (CLARS0.3) $POCl_3$ in modification process]), Control (Wheat starch). For each treatment, different capital letters show differences between the days (5th cycle) (P<0.05). For each day, different small letters show differences between the treatments (P<0.05).

Syneresis of freeze–thawed starch gels: Figs. 10 and 11 show the syneresis data of the pastes (8% solids) prepared from the native and cross-linked rice starches. Syneresis (%) was increased for all samples during storage time. There were significant differences in freeze-thaw stability of pastes prepared from the native and cross-linked starches, whereas the freeze-thaw stability of cross-linked rice starches were more than those of the native starches. $POCl_3$ concentration did not affect the freeze-thaw stability of cross-linked starches ($p>0.05$). Also, freeze-thaw stability of the low amylose rice starches was higher than the high amylose ones.

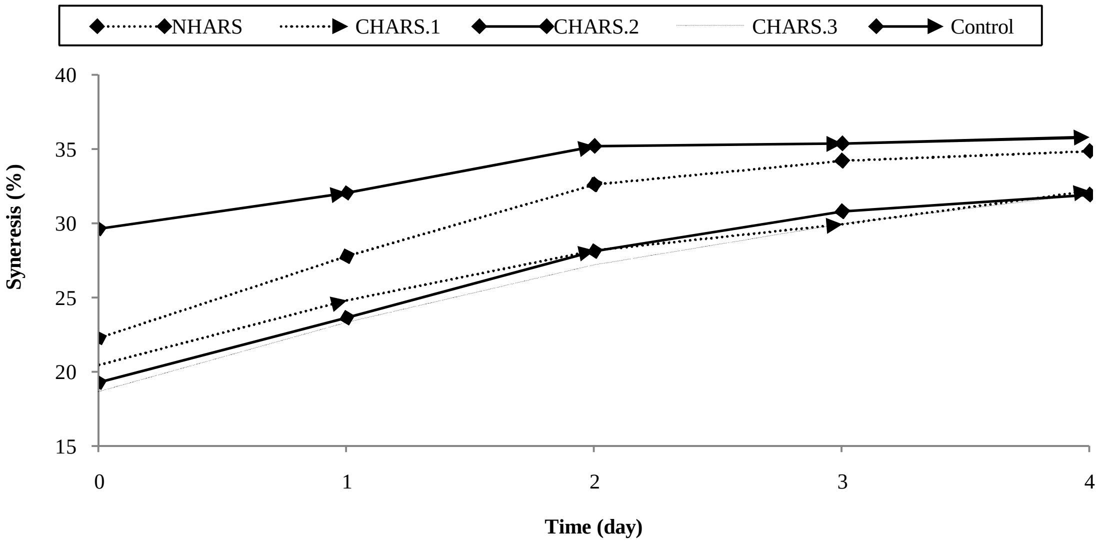

Fig. 10. The Freeze-thaw stability values of the high amylose native and cross-linked rice starches. Samples are as follows: NHARS (high native amylose rice starch), CHARS (high amylose cross-linked rice starch [using 0.1(CHARS0.1), 0.2 (CHARS0.2) and 0.3% (CHARS0.3) $POCl_3$ in modification process]), Control (Wheat starch).

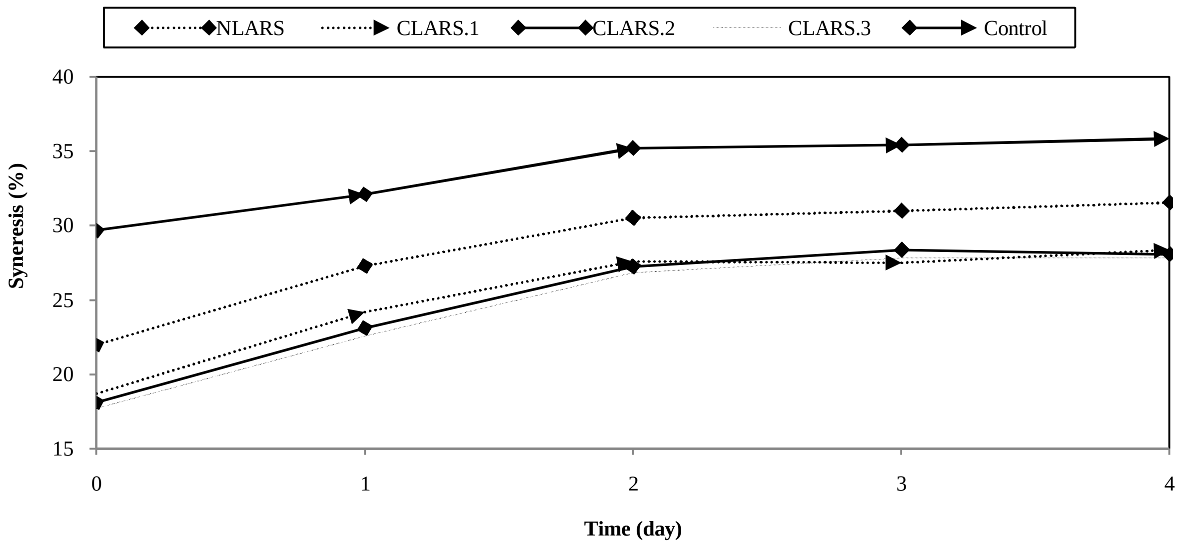

Fig. 11. The Freeze-thaw stability values of the low amylose rice native and cross-linked starches. Samples are as follows: NLARS (low native amylose rice starch), CLARS (low amylose cross-linked rice starch [using 0.1 (CLARS0.1), 0.2 (CLARS0.2) and 0.3% (CLARS0.3) $POCl_3$ in modification process]), Control (Wheat starch).

Discussion

Study of starch extraction: Alkaline steeping method is the usual method of rice starch recovery used in the industry and in researches due to the high solubility of rice proteins in alkaline solutions (13). According to Fig. 2, the protein content of high and low amylose rice starches decreased step by step, by proceeding the extraction, until very low absorbance was detected at 280 nm, meaning the protein was removed remarkably from the starches. It is reported that the protein content of milled rice in a germplasm collection ranged from 4.50-15.90% (14). The isolation of starch through alkaline steeping method (0.1-0.2% NaOH) yielded 73-85% starch (dry basis) and 0.07-0.42% residual protein (15).

Scanning electron microscopy: The granule size of rice starches ranged from 2.95 to 4.28 µm. It is reported that the size of rice starch granules changes from 2 to 7 µm (16). Scanning electron micrographs showed that cross-linking did not affect the size or shapes of granules. No morphological changes were also observed when rice (17) or potato (18) starches were cross-linked by $POCl_3$. Different morphologies with some blister like spots were reported for cross-linked rice starch granules in comparison with native ones (19).

Water solubility and absorption indices: Results indicated that cross-linking as well as $POCl_3$ concentration influenced the intensity of changes in water solubility of the rice starches. The low solubility of cross-linked starch granules even at high temperatures comes from their semi-crystalline structure (20). Subsequently, greater reduction in starch water solubility is supposed to occur with an increase in the concentration of cross-linking agent, which may be due to the higher density of cross-links (21). Reduction of water solubility by cross-linking of different starches and increases in starch granule resistance towards solubility by increasing the concentration of cross-linking agent were reported by other authors (22). Regarding the amylose content, native high amylose rice starch exhibited higher water solubility than that of low amylose sample. It may be attributed to the high water solubility property of amylose, in contrast to amylopectin, the factor which causes amylose to leach out of granules, when starch granules swell (23).

Regarding water absorption, no significant differences were observed between the low and high amylose starches or the native and cross-linked (at 0.1 and 0.2% $POCl_3$) rice starches. Water absorption decreased significantly with the increase in $POCl_3$ concentration from 0.2 to 0.3%. Kaur, Singh (21) reported that cross-linking at lower reagent concentrations did not affect water sorption of potato starch, but the water absorption of cross-linked starch prepared by higher concentration of $POCl_3$ was considerably lower than the native starch (25). It has been demonstrated that starch chains movements may decrease due to the cross-linking, resulting in reduction of water solubility and absorption properties (28). Although important effects on functional properties of starch have been reported due to cross-linking at low concentrations of cross-linking reagent, but contributes little to water sorption properties (24). Majzoobi, Radi (19) declared that the water solubility and absorption indices of cross-linked wheat starch decreased remarkably in comparison with the native starch (23).

Pasting Properties: According to Table 3, the pasting viscosity values of the starch samples decreased significantly ($p<0.05$) with starch cross-linking. Cross-linking may reduce the mobility of starch chains. In addition, the interactions of starch chains with the molecules of water decrease due to the cross-linked bonds. Both of these factors result in lower viscosity of cross-linked starches rather than the native ones at different time-temperature conditions. Also, the resistance of starch granules against temperature increases as a result of cross-linking (3). In other words, the changes occurred in the native starch molecules during heating (like gelatinization), take place to lesser extent for cross-linked starches (19).

In this study, the pasting viscosity parameters including the peak, trough, breakdown and final viscosities decreased; while the peak time and pasting temperature values increased with cross-linking agent concentration. Peak viscosity (PV) is in fact an indication of the viscous load observed in a mixing cooker, which is affected by granule swelling during heating. This means that higher PV is expected from starches showing higher swelling capacity (25). As starches with a low amylose content exhibit higher swelling power, it was expected that low amylose rice starches showed higher PV than those of high amylose ones. Furthermore, the cross-linked rice starches showed lower PV than the corresponding native ones. It may be due to the hard outer crust formed in the cross-linked starches, which inhibits the granules from swelling, and in turn results in lower PV (3). Generally it is believed that with increasing the cross-linkage, a more resistant starch against changes usually occurred during heating, is formed (4).

Holding strength or trough viscosity means the trough at the minimum viscosity of hot paste and it is affected by the rate of granule swelling, amylose exudation, and amylose-lipid complex formation (25). As described before, trough viscosity decreased with cross-linking and cross-linking agent concentration. Yildiz, Yurt (25)explained that the lower water holding capacity of cross-linked starches and subsequently, their lower swelling power result in trough viscosity reduction (31).

Breakdown viscosity (BV) corresponds to the viscosity resulting from the disruption of starch granules as a function of high temperature or shear stress. In such situations, the amylose chains are leached out from granules and oriented (25). As it can be seen from Table 3, in low amylose samples the BV decreased ($P<0.05$) with cross-linking and cross-linking agent concentration, but there was not significant difference between high amylose native and cross-linked rice starches (prepared at different $POCl_3$ concentrations).

Final viscosity (FV) shows the starch ability to form a viscous paste or gel after cooking and cooling. FV is influenced by the retrogradation of soluble amylose during cooling (25). FV values decreased considerably ($P<0.05$) with starch modification and cross-linking agent concentration (Table 3). It is reported that cross-linking postpones the re-association of amylose molecules to form gel after cooling and can delay retrogradation. Similar patterns have been reported for native and cross-linked wheat starches (19).

Set-back viscosity was not affected by cross-linking or cross-linking agent concentration. Moreover, higher setback values were obtained for high amylose starches than low amylose ones. Setback viscosity is the difference between the peak viscosity (PV) and the final viscosity (FV). The final and setback viscosities are particularly important parameters indicating the retrogradation behavior of starch gels. Retrogradation occurs when starch chains and the exuded amylose molecules begin to re-associate during cooling (3). Retrogradation particularly occurs when amylose-containing starches are cooled. Higher setback, indicates a higher retrogradation tendency and more syneresis is likely to take place (4). Subsequently, starches with higher amylose usually show higher setback and more syneresis.

The peak time reflects the temperature at PV (25). The peak time values increased with cross-linking and the concentration of $POCl_3$ ($P<0.05$). Cross-linked starch pastes are more resistant to temperature and shear forces compared to native starch pastes and thus the duration from granule swelling to rupture will increase, resulting in a longer peak time. High amylose rice starches had longer peak time rather than those of low amylose ones. This may be due to amylose leaching from starch granules to the medium during the heating time. Therefore, a harder network with lower swelling power and longer peak time, is formed due to interactions between the leaching amylose and the amylopectin

(26).

Pasting temperature (PT) is the temperature at which the viscosity is increased. This point is supposed to be higher than the point which gelatinization starts. By the use of PT, the minimum temperature needed for cooking a sample, is determined easily (25).

In this study, starch cross-linking results in PT increase, and the PT increased with the intensity of cross-links. As cross-linked starch pastes are more resistant to temperature and swelling, thus, pasting viscosity of cross-linked starches raised as the concentration of cross-linking agent increased.

Light transmittance (paste clarity): Results showed that the paste clarity decreased with cross-linking and $POCl_3$ concentration (from 0.1 to 0.2%). No significant difference ($P < 0.05$) was observed at higher reagent concentrations (0.2 and 0.3%) after the 5th cycle. It is reported that the pastes from high degree cross-linked starches usually show lower clarity or light transmittance compared to the native starches, which is a result of the incomplete gelatinization of these starches and therefore their lower swelling power (3). Kaur, Singh (21) reported that the lower clarity of cross-linked starches may be due to granules resistance to disintegration after gelatinization, while the complete destruction of granules is occurred for native starches. Subsequently, using different $POCl_3$ concentration results in exhibition of different swelling behavior after gelatinization. Denser granules are produced as granules are held together by cross-links at higher cross-linking reagent concentration, resulting in swelling power reduction. Light is more refracted rather than transmitted from the denser granules, resulting in lower past clarity (25).

Syneresis of freeze–thawed starch gels: In freeze-thaw stability tests, it is aimed to cause a temperature fluctuation above Tg by repeating freeze–thaw cycles. By this way, the retrogradation of starch molecules accelerates, resulting in phase separation. During freezing process, the ice crystals are formed in the starch network. During thawing, the ice crystals melt and the produced water separates from the starch matrix which is known as syneresis (27). Results (Figs. 10 and 11) showed that the syneresis (%) increased with storage time. Deetae, Shobsngob (28) declared that by increasing in freeze-thaw cycles, the percentage syneresis of starch pastes would increase. Also, cross-linked rice starches showed higher freeze-thaw stability than those of the native starches. This may be due to the presence of phosphate groups in cross-linked amylopectin which decreases the swelling power and restricts the mobility of cross-linked amylopectin branches (29). Retrogradation is retarded and freeze thaw-stability increases. Similar freeze–thaw patterns have been reported for wheat starch granules (11).

The freeze-thaw stability of the low amylose rice starches was higher than those of high amylose samples which could be attributed to higher amylopectin content of LARS and lower tendency of amylopectin to retrograde. Greater syneresis was also reported for high amylose corn and potato starches (18). The linear structure of amylose causes the molecules re-associate easily through formation of hydrogen bonds, in contrast to the “tumbleweed-like” structure of amylopectin, resulting in formation of a crystalline structure and retrogradation (4). Good freeze-thaw stability was reported previously, for rice starch (20).

Conclusion

Cross-linking of rice starch with $POCl_3$ yielded a modified starch with no changes in the morphology, but significant effects were observed on the physico-chemical properties of the starch as a result of molecular changes. In general, gelatinization peak time and freeze-thaw stability increased by cross-linking, whereas paste clarity, solubility and water absorption decreased. Cross-linking and the levels of cross-linking reagent had a strong influence on the properties of rice starch as the paste clarity, pasting viscosity parameters, water solubility and absorption decreased with cross-linking and cross-linking reagent concentrations. High amylose rice starches exhibited higher water solubility, water absorption and freeze-thaw stability and lower pasting viscosity parameters than those of low amylose samples. Therefore, the use of high amylose cross-linked starch in food production systems, where higher stability and controlled increase of viscosity are desired during their production, may be recommended.

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References

1. Considine DM. Foods and food production encyclopedia: Springer Science & Business Media; 2012.

2. Hermansson A-M, Svegmark K. Developments in the understanding of starch functionality. Trends in Food Science & Technology. 1996;7(11):345-53.

3. Singh J, Kaur L, McCarthy O. Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications—A review. Food Hydrocolloids. 2007;21(1):1-22.

4. Thomas DJ, Atwell WA. Starches: Eagan press St. Paul, MN; 1999.

5. Limberger VM, Brum FB, Patias LD, Daniel AP, Comarela CG, Emanuelli T, et al. Modified broken rice starch as fat substitute in sausages. Food Science and Technology (Campinas). 2011;31(3):789-92.

6. Cody T, Olabi A, Pettingell A, Tong P, Walker J. Evaluation of rice flour for use in vanilla ice cream. Journal of Dairy Science. 2007;90(10):4575-85.
7. Sodhi NS, Singh N. Morphological, thermal and rheological properties of starches separated from rice cultivars grown in India. Food Chemistry. 2003;80(1):99-108.
8. Committee AACC. Approved methods of the American association of cereal chemists: Amer Assn of Cereal Chemists; 2000.
9. Leach HW, McCowen L, Schoch TJ. Structure of the starch granule. I. Swelling and solubility patterns of various starches. Cereal Chemistry. 1959;36(6):534-44.
10. Bason M, Booth R, Hazelton J. RVA: new development and standardization. Applications of the Rapid Visco Analyzer. 1996.
11. Van Hung P, Morita N. Physicochemical properties of hydroxypropylated and cross-linked starches from A-type and B-type wheat starch granules. Carbohydrate Polymers. 2005;59(2):239-46.
12. Bhandari PN, Singhal RS. Effect of succinylation on the corn and amaranth starch pastes. Carbohydrate Polymers. 2002;48(3):233-40.
13. Lumdubwong N, Seib P. Rice starch isolation by alkaline protease digestion of wet-milled rice flour. Journal of Cereal Science. 2000;31(1):63-74.
14. Kennedy G, Burlingame B. Analysis of food composition data on rice from a plant genetic resources perspective. Food Chemistry. 2003;80(4):589-96.
15. Bao ZJ, Bergman CJ. The functionality of rice starch. Starch in Food: Structure, Function and Applications. 2004;2:258.
16. Wani AA, Singh P, Shah MA, Schweiggert□Weisz U, Gul K, Wani IA. Rice starch diversity: Effects on structural, morphological, thermal, and physicochemical properties—A review. Comprehensive Reviews in Food Science and Food Safety. 2012;11(5):417-36.
17. Yeh A-I, Yeh S-L. Some characteristics of hydroxypropylated and cross-linked rice starch. Cereal Chemistry. 1993;70:596-601.
18. Singh N, Chawla D, Singh J. Influence of acetic anhydride on physicochemical, morphological and thermal properties of corn and potato starch. Food Chemistry. 2004;86(4):601-8.
19. Majzoobi M, Radi M, Farahnaky A, Jamalian J, Tongdang T. Physico-chemical properties of phosphoryl chloride cross-linked wheat starch. Iranian Polymer Journal. 2009;18:491-9.
20. Ferrini LM, Rocha TS, Demiate IM, Franco CM. Effect of Acid-Methanol Treatment on the Physicochemical and Structural Characteristics of Cassava and Maize Starches. Starch□Stärke. 2008;60(8):417-25.
21. Kaur L, Singh J, Singh N. Effect of cross□linking on some properties of potato (*Solanum tuberosum* L.) starches. Journal of the Science of Food and Agriculture. 2006;86(12):1945-54.
22. Neelam K, Vijay S, Lalit S. Various techniques for the modification of starch and the applications of its derivatives. International Research Journal of Pharmaceutics. 2012;3(5):25-31.
23. Lii C-Y, Tsai M-L, Tseng K-H. Effect of amylose content on the rheological property of rice starch. Cereal Chemistry. 1996;73(4):415-20.
24. Chilton W, Collison R. Hydration and gelation of modified potato starches. International Journal of Food Science & Technology. 1974;9(1):87-93.
25. Yildiz Ö, Yurt B, Baştürk A, Toker ÖS, Yilmaz MT, Karaman S, et al. Pasting properties, texture profile and stress–relaxation behavior of wheat starch/dietary fiber systems. Food Research International. 2013;53(1):278-90.
26. Tester RF, Morrison WR. Swelling and gelatinization of cereal starches. II. Waxy rice starches. Cereal Chemistry. 1990;67(6):558-63.
27. Yuan R, Thompson D. Freeze-thaw stability of three waxy maize starch pastes measured by centrifugation and calorimetry. Cereal Chemistry. 1998;75(4):571-3.
28. Deetae P, Shobsngob S, Varanyanond W, Chinachoti P, Naivikul O, Varavinit S. Preparation, pasting properties and freeze–thaw stability of dual modified crosslink-phosphorylated rice starch. Carbohydrate Polymers. 2008;73(2):351-8.
29. Chung H-J, Woo K-S, Lim S-T. Glass transition and enthalpy relaxation of cross-linked corn starches. Carbohydrate Polymers. 2004;55(1):9-15.