Impact of soybean 7S globulin content on thermal and retrogradation properties of non-waxy maize starch

SHIFENG YU – LIAN-ZHOU JIANG – NARASIMHA KUMAR KOPPARAPU

Summary

In the present study, thermal and retrogradation properties of non-waxy maize starch/soybean 7S globulin mixtures with different water content were evaluated by differential scanning calorimetry. The thermal results showed that soybean 7S globulin can increase onset, peak temperatures and thermal enthalpy of maize starch. The onset and peak temperatures of mixtures are positively related to soybean 7S globulin content. The thermal enthalpy is positively correlated to soybean 7S globulin content when water content is less than 50%, however, it is negatively related to soybean 7S globulin content when water content is higher than 50%. On the other hand, soybean 7S globulin can retard maize starch retrogradation, since the retrogradation enthalpy is negatively correlated to soybean 7S globulin content when water content is higher than 50%, while it is positively related to water content during storage. In addition, maize starch retrogradation enthalpy is the highest when water content of mixture is 50% during the storage period. Therefore, controlling soybean 7S globulin content and water content of mixtures is effective in increasing or decreasing starch gelatinization and retrogradation. These findings may have important application for production of high quality maize starch-based food products.

Keywords

maize starch; 7S globulin; thermal property; retrogradation; soybean

Maize starch and soybean proteins are two major food components in cereal-based food products, and interactions between maize starch and soybean proteins play an important role in properties of starch-based food products [1]. However, interactions between maize starch and soybean proteins are still not clear, which is a serious problem during production of high quality maize starch-soybean protein-based food products. Therefore, it is very important to study these interactions, which can provide useful information for starch food industry and enhance the understanding of functionalities of maize starch and soybean protein in real food systems.

Recently, many researches have reported the effects of proteins on various properties of starch. Proteins can change the structure of starch gel network and can increase gel strength [2], zein and gliadin can increase the viscosity and decrease the peak temperature of amylopectin [3], milk protein can change the pasting behaviour of rice starch [4–5], and soybean protein isolates can increase the onset and peak temperatures of maize starch/soybean protein isolate (SPI) mixtures [1]. On the other hand, low protein contents (1~5%) have no significant effects on reducing the degree of gelatinization [6]. Moreover, protein content has important impact on gel properties of starch/protein mixtures. The degree of elasticity of maize starch/whey protein isolate mixed gel decreased as whey protein isolate content increased [7], soya protein isolate increased the viscosity of starch suspension

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and weakened the structure of starch gel [8–9]. The microstructure of starch-protein gel depended on the proportion of whey protein isolate/cassava starch [10], and interactions between starch and proteins are very complex [11–12]. Other studies focused on the influence of soya protein hydrolysate on maize starch retrogradation [13], and improvement of functional properties of cassava starch/soybean protein concentrate blends by soybean protein concentrate [14]. However, only few reports are available on the impact of soybean 7S globulin on thermal and starch retrogradation of maize starch [1]. The interaction between soybean 7S globulin, thermal and retrogradation properties of maize starch are still not clear. Therefore, the objective of the present study was to investigate the effect of soybean 7S globulin content on thermal and retrogradation properties of non-waxy maize starch, and their interactions in gelatinization and retrogradation during storage.

MATERIALS AND METHODS

Materials

Non-waxy maize starch (amylose content of 30.5%) was purchased from Heilongjiang Jingpo lake agricultural development company (Mudanjiang City, Heilongjiang province, China). Soybean (harvested in Chaoyang City, Liaoning province, China) was purchased from a local supermarket. All the samples were stored at 4 °C in a refrigerator. All other chemicals used in the study were of analytical grade.

Defatted soybean flour

Soybean flour was defatted according to the method of Liu et al. [15] with minor modifications. Soybean seeds were milled using a universal high-speed smashing machine (FW80-1; Tianjin Taisite Instrument, Tianjin City, China), passed through a 60-mesh sieve (250 μm), defatted with n-hexane by Soxhlet extraction (soya flour : hexane = 1 : 5, v/v) for 10 h at room temperature, and then dried at 40 °C for 24 h. The defatted flour was passed through a 100-mesh sieve (150 μm) and stored at 4 °C until experiments.

Isolation of soybean 7S globulins

Soybean 7S globulins were obtained according to the method of Nagano et al. [16] with minor modifications. Proteins were extracted by making slurry of defatted soybean flour (pH 8.0) at a flour : water ratio of 1 : 10 with 2 mol·l^{-1} NaOH for 2 h under magnetic stirring at room temperature. The slurry was passed through a 200-mesh sieve (75 μm), and the filtrate was collected and centrifuged (10 400 $\times g$, 15 min, 20 °C). Dry sodium bisulfite (SBS) was added to the supernatant (0.98 g·l^{-1}), then pH was adjusted to 6.4 with 2 mol·l^{-1} HC1, and the mixture was kept in an ice bath overnight. After removing the insoluble fraction by centrifugation (6 500 $\times g$, 20 min, 4 °C), the salt concentration of the supernatant was adjusted to 0.25 mol·l^{-1} by addition of solid NaCl. Then, pH of the supernatant was adjusted to pH 5.0 with 2 mol·l^{-1} HCI. After 1 h, the insoluble fraction was removed by centrifugation (9 000 $\times g$, 30 min, 4 °C), the supernatant was diluted 2-fold with ice-cold water, adjusted to pH 4.8 with 2 mol·l^{-1} HCl, and centrifuged (6 500 $\times g$, 20 min, 4 °C). The obtained precipitate, the 7S globulin fraction, was washed twice with distilled water, adjusted to pH 7.0 with 2 mol·l^{-1} NaOH, and then freeze-dried. The freeze-dried powders were passed through a 100-mesh sieve (150 μm) and stored in a sealed plastic bag at 4 °C. Protein content of soybean 7S globulin powder was 92.5%, as estimated by the Kjeldahl method (N × 6.25).

Formulation of mixtures

Maize starch and soybean 7S globulin were mixed at different ratios (1 : 0, 2 : 1, 1 : 1, 1 : 2, 0 : 1; w/w; dry basis), and the soybean 7S globulin content was 0%, 33%, 50%, 66% and 100%, respectively. Samples were mixed using a mixer (TYXH-II; Shanghai Qiaoyue Electronic, Shanghai City, China).

Differential scanning calorimetry (DSC)

Thermal and retrogradation properties of samples were analysed by a differential scanning calorimeter (DSC, Q20; TA Instruments, New Castle, Delaware, USA) according the method of Yu et al. [17]. Samples of 3.0 mg (dry basis) and distilled water at different ratio (2 : 1, 1 : 1, 1 : 2) were placed in pre-weighed aluminium sample pans (T060601, TA Instruments). The pans were sealed hermetically to prevent moisture loss and incubated overnight. For all DSC runs, a sealed empty aluminium pan was used as a reference. The sample was held isothermally at 20 °C for 1 min before being heated from 20 °C to 130 °C at 10 °C·min^{-1}. The onset temperature (T_o), peak temperature (T_p), and thermal enthalpy (ΔH_g) were determined. The heated sample pans were stored at 4 °C for 14 days, and then were heated again. The onset temperature, peak temperature and retrogradation enthalpy (ΔH_r) associated with the retrograded starch melting peak appearing between 40 °C and 70 °C were calculated.

Statistical analysis

All experiments were performed at least in triplicate, mean values ± standard deviations were used for analyses. One-way analysis of variance (ANOVA) and Duncan multiple-range test were performed by SAS 8.0 (SAS Institute, Cary, North Carolina, USA). The Pearson correlation coefficients were analysed by IBM SPSS Statistics 19.0 (IBM Corporation, New York, New York, USA).

RESULTS AND DISCUSSION

Effect of soybean 7S globulin content on thermal properties of maize starch

A typical DSC curve of maize starch/soybean 7S globulin mixtures is shown in Fig. 1. As shown in Tab. 1, significant differences were observed in T_o and T_p of mixtures with different soybean 7S globulin contents ($p < 0.05$). The lowest T_o and T_p were observed for maize starch, whereas soybean 7S globulin showed the highest T_o and T_p at the same water content. The onset and peak temperatures of maize starch were similar to the earlier reported results [1, 18], but the denaturation temperatures of soybean 7S globulin and SPI were significantly different from maize starch/SPI mixtures [1]. The differences in denaturation temperatures were caused by different kinds of soybean proteins. The Pearson correlation coefficients between thermal properties and soybean 7S globulin content are shown in Tab. 2, T_o and T_p showed a positive correlation with soybean 7S globulin content ($0.988 < r < 0.996$, $p < 0.05$; $0.846 < r < 0.997$, $p < 0.05$, respectively). These results indicate that onset and peak temperatures of maize starch depended on soybean 7S globulin content, higher amounts of protein in the mixtures could increase onset and peak temperatures of maize starch during gelatinization. Similar results were observed for interaction of starch and proteins [1, 19]. Moreover, the higher onset and peak

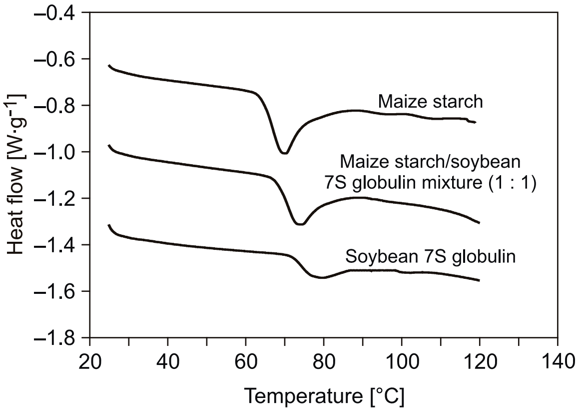

Fig. 1. Typical DSC curve of maize starch, soybean 7S globulin and their mixture.

Water content 66%.

Tab. 1. Thermal properties of maize starch/soybean 7S globulin mixtures with different water content.

Water content [%]	Soybean 7S globulin content [%]	T_o [°C]	T_p [°C]	ΔH_g [J.g^{-1}]
33	0	68.37 ± 0.29^{h}	72.98 ± 0.10^{i}	0.56 ± 0.48^{k}
	33	73.49 ± 0.67^{e}	87.11 ± 0.65^{d}	1.21 ± 0.41^{j}
	50	77.60 ± 0.94^{c}	88.95 ± 0.47^{c}	3.19 ± 0.84^{i}
	66	79.66 ± 0.89^{b}	89.80 ± 0.42^{b}	4.80 ± 0.41^{fg}
	100	83.81 ± 0.21^{a}	90.63 ± 0.16^{a}	3.92 ± 0.22^{hi}
50	0	64.74 ± 0.17^{j}	69.42 ± 0.17^{j}	10.48 ± 0.36^{b}
	33	67.74 ± 0.18^{i}	73.03 ± 0.21^{i}	9.29 ± 0.52^{c}
	50	68.87 ± 0.16^{h}	75.01 ± 0.48^{g}	8.68 ± 0.70^{cd}
	66	70.44 ± 0.50^{g}	78.87 ± 1.90^{f}	5.46 ± 0.19^{f}
	100	75.32 ± 0.16^{d}	81.06 ± 0.66^{e}	3.69 ± 0.17^{i}
66	0	64.51 ± 0.21^{j}	69.69 ± 0.27^{j}	12.04 ± 0.87^{a}
	33	67.60 ± 0.30^{i}	72.78 ± 0.41^{i}	10.36 ± 0.12^{b}
	50	68.38 ± 0.28^{h}	73.82 ± 0.33^{h}	9.24 ± 0.13^{c}
	66	69.68 ± 0.23^{g}	74.88 ± 0.24^{g}	6.25 ± 0.33^{e}
	100	72.10 ± 0.16^{f}	78.09 ± 0.24^{f}	4.93 ± 0.23^{fg}

Means in the same column followed by the same lowercase superscript letters are not different ($p < 0.05$).
T_o – onset temperature, T_p – peak temperature, ΔH_g – thermal enthalpy.

Tab. 2. Pearson correlation coefficients for the relationship between thermal properties, soybean 7S globulin and water content of the mixtures.

		T_o	T_p	ΔH_g
Soybean 7S globulin content	WC 33%	0.993 *	0.846	0.850 **
	WC 50%	0.988 *	0.984 *	–0.947 **
	WC 66%	0.996 *	0.997 *	–0.978 **
Water content	7SC 0%	–0.894 *	–0.845 *	0.929 **
	7SC 33%	–0.871 *	–0.901 *	0.913 *
	7SC 50%	–0.895 *	–0.920 **	0.911 *
	7SC 66%	–0.904 *	–0.967 **	0.984 **
	7SC 100%	–0.970 *	–0.968 **	0.787

* – correlation is significant at 0.05 level (2-tailed); ** – correlation is significant at 0.01 level (2-tailed).
WC – water content, 7SC – soybean 7S globulin content, T_o – onset temperature, T_p – peak temperature, ΔH_g – thermal enthalpy.

temperatures indicated that starch was difficult to gelatinize at higher 7S globulin contents, while it was easy to gelatinize at lower soybean 7S globulin contents. On the contrary, T_o and T_p of soybean 7S globulin decreased with the increasing amount of maize starch. Soybean 7S globulins at higher starch contents were easy to denature, while it was difficult to denature them at lower starch contents. This phenomenon might be caused by the interaction of starch and 7S globulin molecules during gelatinization. When 7S globulin content was higher, more molecular interactions between starch and protein took place, which would retard starch gelatinization with water, and increase the onset and peak temperatures of starch. Similar results were reported for soybean protein isolates/maize starch mixtures [1].

As shown in Tab. 1, the thermal enthalpy increased with soybean 7S globulin content when the water content was 33%, while it decreased with soybean 7S globulin content when the water content was higher than 50%. The thermal enthalpy values showed a negative correlation with soybean 7S globulin content when water content was higher than 50% (Tab. 2). These results indicated that soybean protein played an important role in gelatinization of maize starch/protein mixtures, and higher soybean 7S globulin content could decrease thermal enthalpy and retard complete gelatinization of starch. If soybean 7S globulin content was higher, starch and 7S globulin competed with water molecule, making starch difficult to gelatinize. On the other hand, at lower soybean 7S globulin contents, it was easier to gelatinize probably due to the fact that 7S globulin prevented gelatinization of starch with water molecules. As shown in Tab. 2, the thermal enthalpy values showed a positive correlation with soybean 7S globulin content when water content was 33%, indicating that soybean 7S globulin was difficult to denature at lower water contents, which was caused by starch and 7S globulin competing with water molecule, and the protective effects of starch on proteins against thermal denaturation. In addition, the thermal enthalpy values of mixtures are not the sum of thermal enthalpy of maize starch gelatinization and soybean 7S globulin denaturation together (Tab. 1). These results suggest that starch and soybean 7S globulin have a synergistic or segregative effect on the mixtures, either by combining together or by separating apart. Similar results on starch/protein mixtures were obtained by other researchers [1, 9, 20].

Effect of water content on the thermal properties of maize starch/7S mixtures

As shown in Tab. 2, T_o and T_p of mixtures showed a negative correlation with water content ($0.871 < r < 0.970$, $p < 0.05$; $0.845 < r < 0.968$, $p < 0.01$; respectively). Higher water content in the mixtures could decrease onset and peak temperatures of maize starch or 7S globulin during heating. These results are similar to starch mixtures with different water contents as reported by Mohamed et al. [20] and Li et al. [1]. The change of onset and peak temperatures of mixtures with different moisture content is very similar to results of wheat starch [20] and maize starch/SPI mixtures [1], but the onset and peak temperature values are different. The differences in onset and peak temperature values were caused by the different properties of soybean 7S globulin and SPI. These results indicate that T_o and T_p of mixtures are affected by water content: the mixtures with low water content are hard to gelatinize, but the mixtures with high water content are easy to gelatinize. However, when the water content is higher than 50%, water molecules have little effects on onset and peak temperatures of mixtures. Similar results were observed for wheat starch gelatinization [20]. In addition, the onset and peak temperatures of maize starch/soybean 7S globulin mixtures were higher than those of wheat starch/protein mixtures [9, 20], and T_p was higher than that of 7S of SPI (82.5 °C) reported by SOBRAL et al. [21]. The differences in onset and peak temperatures were probably caused by different water content, and different starch and protein varieties. The influence of water content on starch was confirmed by ELIASSON [22] who found that higher water content of starch resulted in lower onset temperature during gelatinization.

Thermal enthalpy of mixtures increased with water content (Tab. 1), and thermal enthalpy of mixture showed a positive correlation with water content (Tab. 2). These results indicated that water molecules play an important role in gelatinization of starch/protein mixtures, and higher water content may increase thermal enthalpy, promote starch gelatinization or protein denaturation, and enhance interactions among starch, protein and water molecules. However, if water content is low, starch and protein compete with water molecules during heating, and it is difficult for starch to react with water molecules, resulting in gelatinization of fewer starch molecules, so the thermal enthalpy is very low. Similar results were observed in the reports of MOHAMED and RAYAS-DUARTE [20] and LI et al. [1]. However, ΔH_g of cowpea flour was lower than maize starch/soybean 7S globulin mixture at a similar moisture content [23]. This difference might have been caused by differences in the structure and properties of cowpea starch and cowpea protein, as compared to maize starch and soybean 7S globulin.

Retrogradation properties

The molecular interactions (hydrogen bonding between starch chains) that occur after cooling of the gelatinized starch gel or paste are known as retrogradation [18, 24, 25]. In general, soybean 7S globulin does not retrograde and recrystallize after denaturation, while retrogradation occurs in starch gel during storage. In this paper, retrogradation of mixtures refers to maize starch retrogradation. The retrogradation properties of gels of maize starch/soybean 7S globulin mixtures stored at 4 °C for 14 days were determined by DSC (typical curve shown in Fig. 2). The values of onset temperature (T_o), peak temperature (T_p), and retrogradation enthalpy (ΔH_r) of retrograded maize starch are shown in Fig. 3, Fig. 4 and Fig. 5.

As shown in Fig. 3A, in starch/7S globulin mixture with 33% water content, the maize starch with soybean 7S globulin content of 66% has the highest onset and peak temperature, however, the maize starch with soybean 7S globulin content of 33% has the lowest onset and peak temperatures during storage. When the water content of mixture was higher than 50%, significant differences in the onset and peak temperatures were observed during 11 days of storage, while no significant differences were seen during the last 3 days of storage (Fig. 3B, 3C. 4B, 4C). Moreover, the onset and peak temperatures of retrograded mixtures showed a negative correlation with soybean 7S globulin content during 11 days of storage, while no obvious correlation was observed in the last 3 days of storage (Fig. 3, Fig. 4). These results indicate that soybean 7S globulin has significant effect on the onset and peak temperatures of retrograded mixtures within 11 days storage, but showed little effects when stored from 11 to 14 days.

On the other hand, higher onset and peak temperatures were determined at lower water contents, with no obvious correlation between onset, peak temperatures and water content when moisture content was higher than 50% (Fig. 3, Fig. 4). These results indicate that water molecule had effects on onset and peak temperature of retrograded starch at lower water content, because starch did not gelatinize completely at low moisture content (it gelatinizes completely when water content is higher than 50%). Similar results of onset and peak temperatures of retrograded maize starch were reported by SANDHU and SINGH [18], which were different from the results for starches [24, 26].

Retrogradation enthalpy of maize starch/soy-

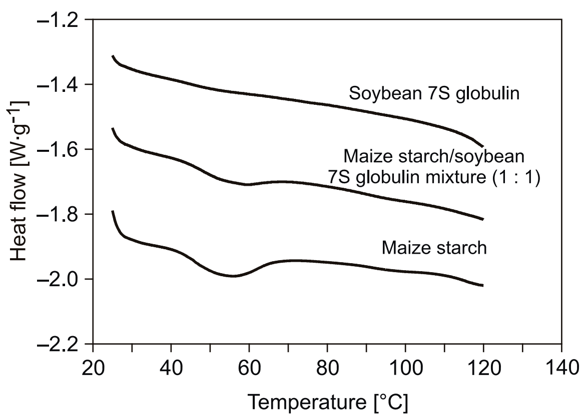

Fig. 2. Typical DSC curve of retrograded maize starch, soybean 7S globulin and their mixture.

Storage for 14 days at 4 °C, water content 66%.

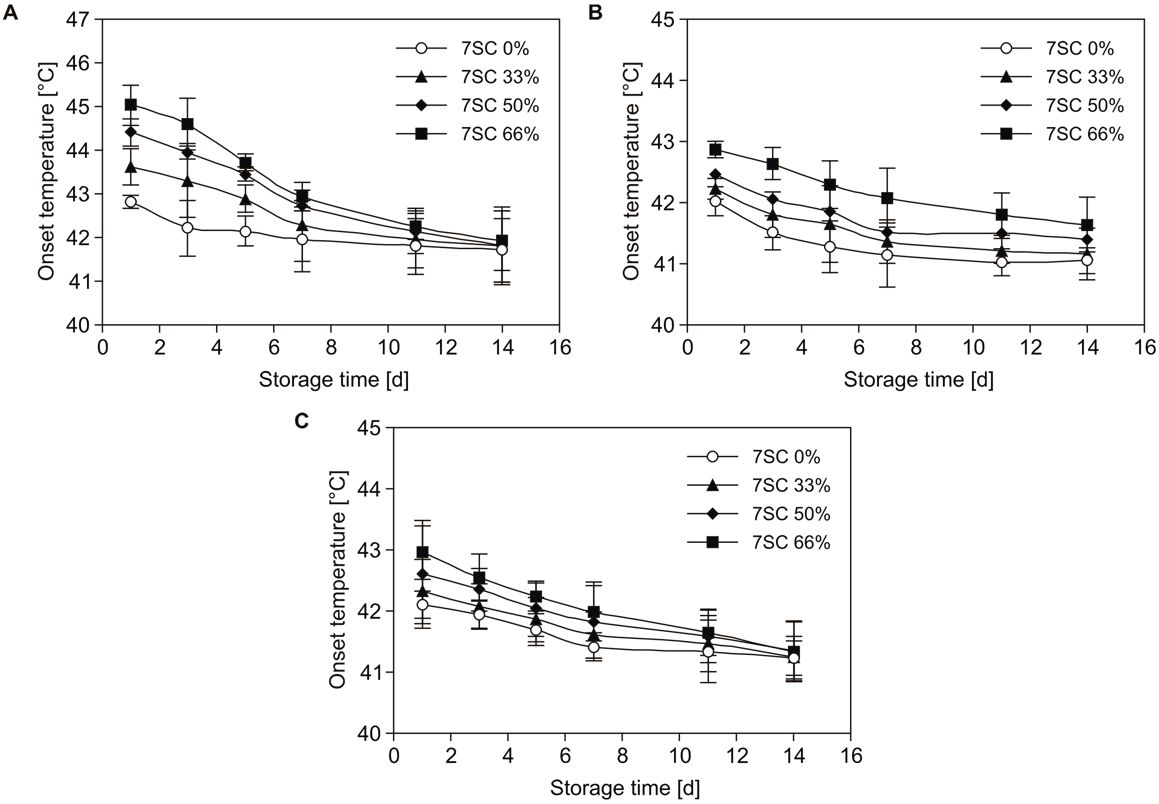

Fig. 3. Onset temperature of retrograded maize starch/soybean 7S globulin mixtures.

A – water content 33%, B – water content 50%, C – water content 66%. 7SC – soybean 7S globulin content.

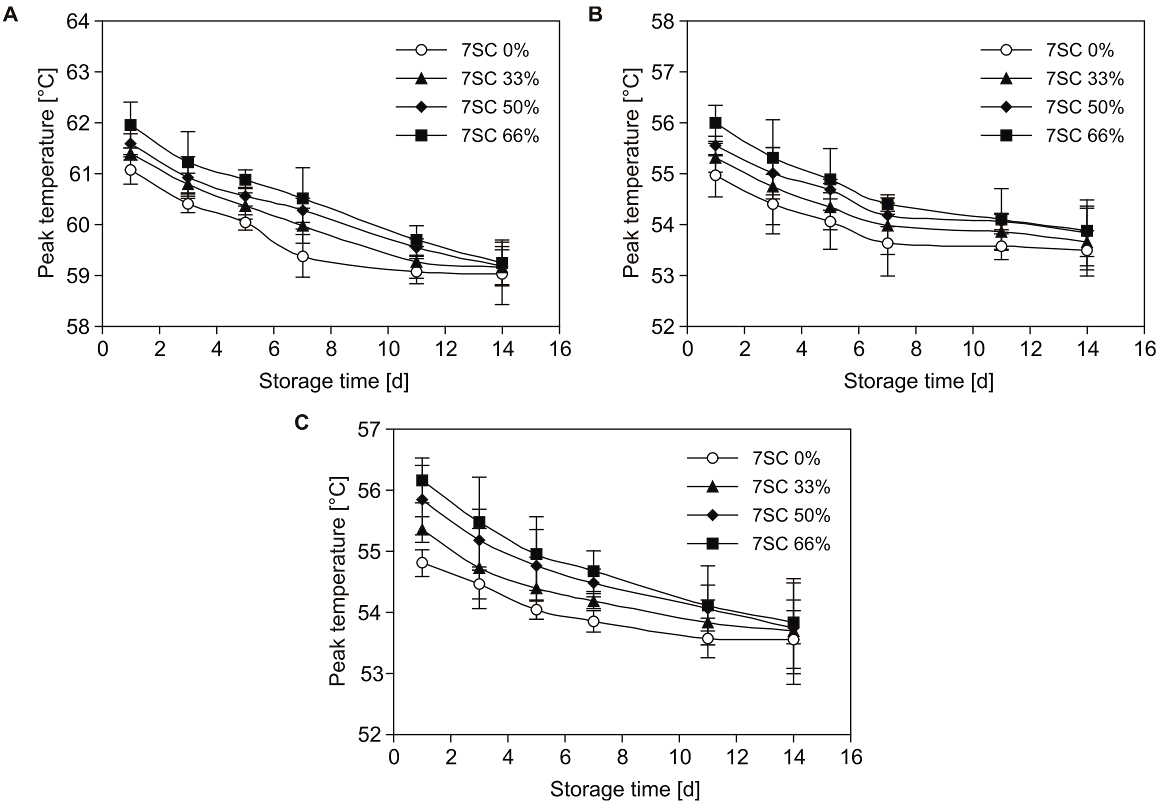

Fig. 4. Peak temperature of retrograded maize starch/soybean 7S globulin mixtures.

A – water content 33%, B – water content 50%, C – water content 66%. 7SC – soybean 7S globulin content.

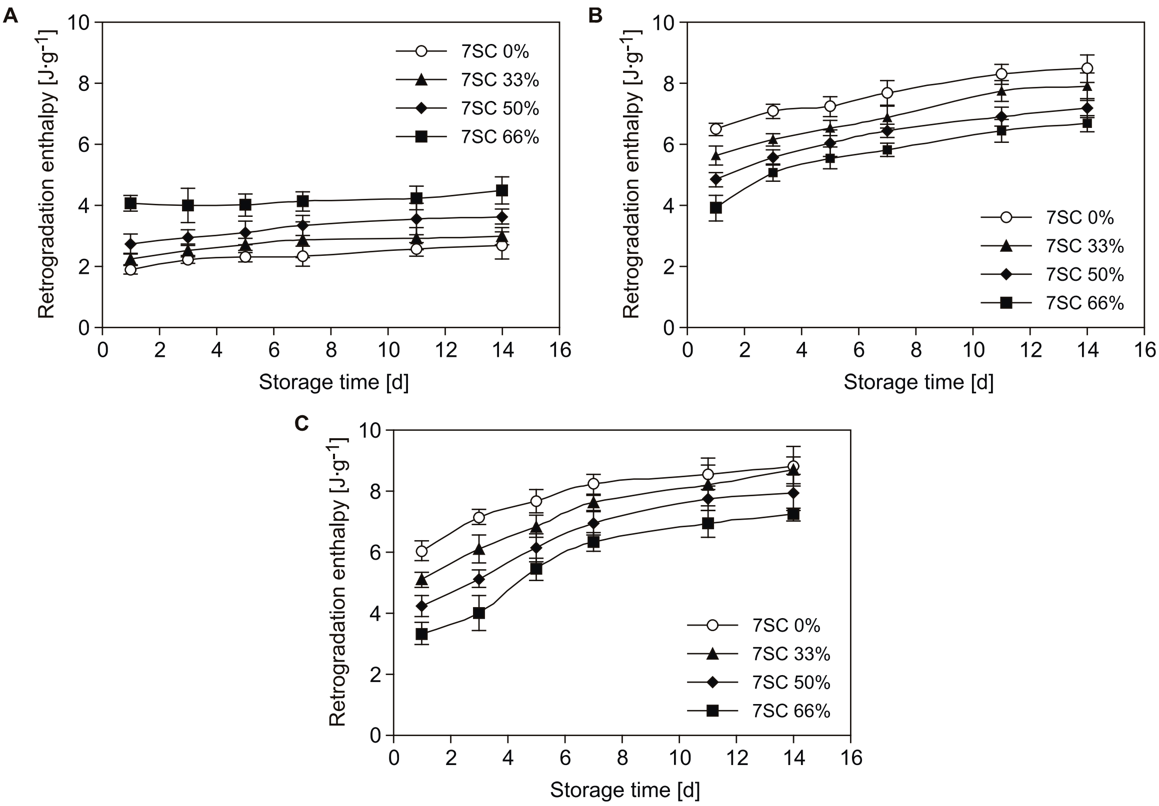

Fig. 5. Retrogradation enthalpy of retrograded maize starch/soybean 7S globulin mixtures.

A – water content 33%, B – water content 50%, C – water content 66%. 7SC – soybean 7S globulin content.

bean 7S globulin mixtures is shown in Fig. 5. As shown, retrogradation enthalpy increased within 7 days of storage, and then increased slowly up to the maximum values when stored for 11 days. No significant changes were observed from 11th to 14th day of storage. The retrogradation enthalpy of mixtures without soybean 7S globulin were the highest, while mixtures with soybean 7S globulin of 66% had the lowest retrogradation enthalpy. These results indicated that soybean 7S globulin significantly affected the retrogradation enthalpy of the mixture during storage. Soybean 7S globulin content was positively related to retrogradation enthalpy of retrograded mixtures when the water content was 33%, while a negative correlation were observed when the water content was higher than 50% (Tab. 3). Similar results were observed for retrogradation of waxy maize starches by Liu

Tab. 3. Pearson correlation coefficients for the relationship between retrogradation enthalpy, soybean 7S globulin and water content of the mixtures during storage.

		ΔH_r (day 1)	ΔH_r (day 3)	ΔH_r (day 5)	ΔH_r (day 7)	ΔH_r (day 11)	ΔH_r (day 14)
Soybean 7S globulin content	WC 33%	0.885	0.898	0.813	0.980 *	0.935	0.913
	WC 50%	–0.980 *	–0.999 **	–0.996 *	–0.994 *	–0.976 *	–0.983 *
	WC 66%	–0.985 *	–0.980 *	–0.990 *	–0.981 *	–0.932	–0.906
Water content	7SC 0%	0.823	0.881	0.907 *	0.912 *	0.892 *	0.898 *
	7SC 33%	0.792	0.865 *	0.896 *	0.932 **	0.910 *	0.927 **
	7SC 50%	0.735	0.792	0.893 *	0.925 *	0.947 **	0.943 **
	7SC 66%	0.689	0.773	0.901 *	0.953 *	0.942 **	0.945 **

* – correlation is significant at 0.05 level (2-tailed); ** – correlation is significant at 0.01 level (2-tailed).
WC – water content, 7SC – soybean 7S globulin content, ΔH_r – retrogradation enthalpy.

and Thompson [24]. In addition, retrogradation enthalpy was positively related to water content during storage (Tab. 3). However, retrogradation enthalpy of mixtures was the highest when water content was 50%, but it was the lowest when water content was 33% (Fig. 5). These results indicated that water content had significant influence on starch retrogradation, as it was easy to retrograde at water content of 50%, and it retarded retrogradation when water content was higher than 50% or less than 33%. Similar results were observed by Liu and Thompson [24] and Zhou et al. [26] for waxy maize starch retrogradation.

CONCLUSIONS

Soybean 7S globulin and water had significant influence on the thermal and retrogradation properties of non-waxy maize starch. Soybean 7S globulin can increase onset temperature, peak temperature and thermal enthalpy of maize starch. The onset and peak temperatures of the mixture were positively related to soybean 7S globulin content, and thermal enthalpy was positively related to soybean 7S globulin content when water content was 33%. However, thermal enthalpy was negatively related to soybean 7S globulin content when water content was 50% and 66%. Soybean 7S globulin could retard maize starch gel retrogradation when water content was higher than 50%. Retrogradation enthalpy was negatively related to soybean 7S globulin content, but positively related when water content was 33%, during storage. Therefore, soybean 7S globulin was effective in controlling gelatinization and starch retrogradation in starch/protein mixtures. These findings may be very useful for food industry for processing of high quality starch-based foods.

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