EXOTHERMIC TRANSITIONS ON COOLING OF GELATINIZED NATIVE RICE STARCH STUDIED BY DIFFERENTIAL SCANNING CALORIMETRY

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Abstract: *Differential Scanning Calorimetric (DSC) experiments were designed to investigate the exothermic events on programed cooling of gelatinized native rice starch-water system. These exothermic transitions, with peak temperatures of 85°C–127°C, were attributed to amylose-lipid complexes recrystallization. Starch concentration and cooling rate showed significant effect on the manifestation of these transitions. The 1:1 starch to water ratio system showed broader and flattens exothermic transitions at higher peak temperatures (120°C–127°C) which were stable over different cooling rates. On the other hand, the 1:2 and 1:3 starch to water ratio systems showed sharpen and narrow exotherms at lower peak temperatures (85°C–100°C) which became smaller with lower cooling rate. These observations suggest the presence of two types of amylose-lipid complexes in native rice starch-water system.*

Keywords: rice starch, thermal analysis, exothermic transitions, amylose-lipid complex

Abstrak: *Eksperimen menggunakan Kalorimeteri Pengimbasan Pembezaan (DSC) telah direkabentuk untuk mengkaji kejadian eksotermik apabila sistem kanji beras natif-air disejukkan. Peralihan eksotermik yang diperhatikan antara suhu puncak 85°C–127°C telah dicadangkan disebabkan oleh penghabluran kompleks amilosa-lipid. Kepekatan kanji dan kadar penyejukan menunjukkan pengaruh yang signifikan terhadap manifestasi peralihan ini. Sistem kanji-air dengan nisbah 1:1 menunjukkan peralihan eksotermik yang lebar dan rata pada suhu yang lebih tinggi (120°C–127°C) dan ianya stabil pada kadar penyejukan yang berbeza. Sebaliknya, sistem kanji-air 1:2 dan 1:3 menunjukkan eksoterma pada suhu yang lebih rendah (85°C–100°C) yang menjadi semakin kecil pada kadar penyejukan yang lebih rendah. Pemerhatian ini mencadangkan kehadiran dua jenis kompleks amilosa-lipid dalam sistem kanji natif-air.*

Kata kunci: kanji beras, analisis terma, peralihan eksotermik, kompleks amilosa-lipid

1. INTRODUCTION

Differential scanning calorimetry (DSC) is a technique commonly employed to probe thermal properties of starch based on the heat flow changes associated with both first-order (melting) and second-order (glass transition) transitions of polymeric materials.[1,2] Normal cereal starches contain lipids (in quantities around 1% on a dry weight basis.[3–5] It has been shown that in rice starches, the internal granular lipids are mainly free fatty acids and lysophospholipids.[6] The lipids exist as amylose-lipid inclusion complexes in native starch granules[5,7] or the amylose-lipid complexes were formed during starch gelatinization.[8] Amylose-lipid complexes have been shown to affect many technologically important properties of starch-containing foods by changing the granule swelling, solubilization, and crystallization of starch polymers.[8] In the case of native rice starch, other than the gelatinization peak (starch crystallite melting), programed heating in a DSC produced melting and crystallization of amylose-lipid complexes within the starch granule. An exotherm was recorded between 110°C and 120°C, which Biliaderis et al.[1] suggested was the result of crystallization of starch-lipid complexes. Generally, amylose-lipid complexes melt (endothermic transition) in the temperature range of 85°C–130°C. The transition is reversible, as an observable exotherm appeared on the DSC cooling curves,[9,10] which is well-defined and more reproducible[2] as compared to those multiple melting thermal profiles on the DSC heating curves.

It is believed that native amylose-lipid complexes formed during cooking or processing do affect the storage stability, for example retrogradation of starch-containing food.[2,11] With the increase in application of rice starch in various food products, (e.g. as a fat replacer in food), the aim of the present work was to investigate the effects of starch concentration, cooling rate and heating-end-temperature (or cooling-start-temperature) on the manifestation of amylose-lipid complexes transition present in native rice starch during cooling in DSC experiment which may have practical significance on food quality and stability.

2. MATERIALS AND METHOD

2.1 Materials

Rice starch was obtained from Sigma Chemical Company, St Paul, MO, USA. Rice starch was defatted by Soxhlet extraction (7 h) with n-propanol/water (3:1, v/v), based on the method of Vasanthan and Hoover.[12] The initial moisture content of the starch was determined from the loss in weight on drying triplicate samples at 105°C to constant weight.

2.2 Determination of Amylose Content

Amylose content of the rice starch was determined using the spectrophotometric method described by Jarvis and Walker.[13] Amylose (Type III from potato, amylopectin free, from Sigma Chemical Company, St Paul, MO, USA) and amylopectin (from potato, Fluka Company, Switzerland) were used as standards.

2.3 DSC Measurement

A modulated DSC (Q100, TA Instruments Inc., New Castle, Del., U.S.A.) was used. The studies were carried out for rice starch : water ratio of 1:1, 1:2 and 1:3 (dry weight basis). Starch was accurately weighed to 0.01 mg in a hermetic aluminium DSC pan, and distilled water was added directly to obtain total weight of 10.00 ± 0.20 mg. The sample pans were hermetically sealed and equilibrated at room temperature for at least one hour. The pan was then placed in the DSC cell, heated from 20°C to 120°C or 140°C at 5°C min^{-1} and cooled at 1°C min^{-1}, 5°C min^{-1} or 10°C min^{-1} with a constant purge of nitrogen gas at 50 ml/min. An empty aluminium pan was used as the reference to balance the heat capacity of the sample pan. All measurements were performed in duplicate. Heat flow and temperature were calibrated using pure indium. Data analysis was carried out using the Thermal Advantage Q series Q100-0021 (TA Instruments Inc., New Castle, Del., U.S.A.). The cooling curves were defined by the complete recrystallization temperatures (T_{cxo}), peak transition temperatures (T_{cx}) and their exothermic enthalpies (ΔH).

2.3 Statistical Analysis

The data was statistically analyzed by one-way ANOVA (for comparing more than two means), using *SPSS Version 12.0 For Windows* (SPSS Inc., Chicago, Illinois). Duncan test was also carried out to perform comparison of means at 95% probability level.

3. RESULTS AND DISCUSSION

3.1 Effects of Defatting on Thermal Transitions

The amylose content of rice starch, determined by amylose-iodine blue complex, was 19.7%. The gelatinization (heating) curves as well as cooling curves of native and defatted rice starches are presented in Figures 1 and 2. Defatting did not appear to alter the main gelatinization temperature of rice starch. Similar observation has been found in other cereal and cassava starches as reported by Vasanthan and Hoover.[12] However, the smaller endotherm peak at about 100°C (curve a), has disappeared after lipid removal (curve b) on DSC heating scan as shown in the heating curves (Fig. 1). After gelatinization, native rice starch-water showed a distinct exothermic phase transition at 80°C to 85°C when it was cooled from 120°C (curve a). However, lipid removal from the starch seems effectively erased the above-mentioned transition (curve b), and no other additional exothermic peak present at lower temperature (<70°C) as depicted in Figure 2. This observation has eliminated the possibility of amylose chain association which gives rise to exothermic transitions at <70°C as reported by Sievert and Würsch.[14] It confirms that the exotherms observed on DSC cooling scan were due to the formation of amylose-lipid complexes present in native rice starch. A similar observation has been reported on whole grain milled rice and milled rice flour.[11]

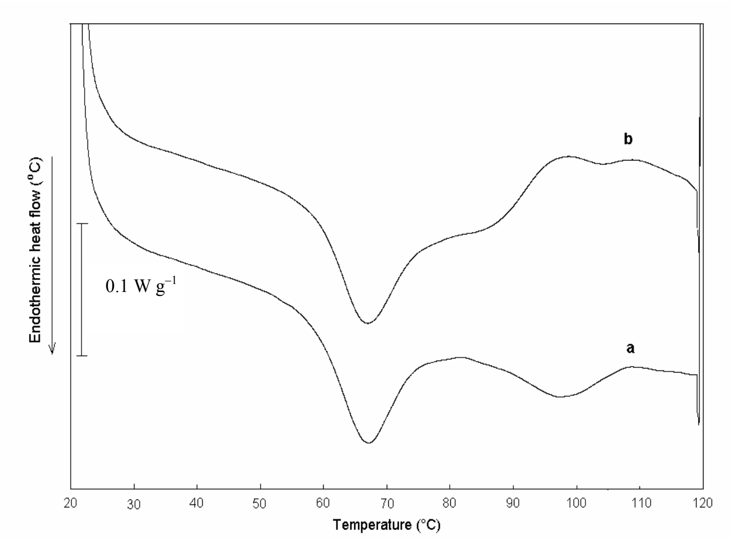

Figure 1: Heating curves of (a) native rice starch-water system and (b) defatted rice starch-water system (starch to water ratio of 1:3).

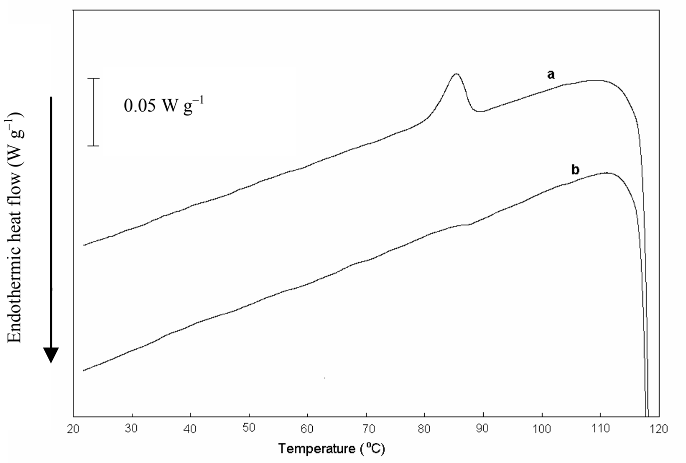

Figure 2: Cooling curves of (a) native rice starch-water system and (b) defatted rice starch- water system (starch to water ratio of 1:3).

3.2 Effects of Starch-water Ratio

Figure 3 shows the exothermic phase transitions during cooling at 5°C min^{-1} on gelatinized native rice starch-water matrices of 1:1, 1:2 and 1:3 (w/w dry starch basis) from 140°C. The exothermic event occurred right after the cooling process was started from 140°C for 1:1 gelatinized starch-water system. Therefore, no observable phase transition was shown for 1:1 gelatinized starch-water system when it was cooled from 120°C (Table 1). The phase transitions were broad and flatten with T_{cxo} of ~119°C for 1:1, sharpen prominently and bigger for 1:2 but became slightly smaller and broader for 1:3, with T_{cxo} of ~89°C and 85°C, respectively. There was an increase in both T_{cxo} and T_{cx} with increased starch concentration, however 1:2 showed the highest enthalpy of crystallization (~ 2.0 J/g). Table 2 gives the T_{cxo}, T_{cx} and ΔH data obtained in which starch to water ratio of 1:1 showed T_{cx} ranged from 120°C to 127°C whereas 1:2 and 1:3 systems showed T_{cx} ranged from 85°C to 100°C.

We suggest that the exothermic peaks showed for 1:1 starch-water systems represent different types of crystalline form from those present in 1:2 and 1:3 starch to water ratio systems. It has been reported that two forms of amylose-lipid complexes exist in many starch-water systems.[15–17] In type I complexes, the helical segments are randomly distributed, it has a lower T_p (melting peak during DSC heat scan) and is assumed to be formed when rapid nucleation occurs, and have little crystallinity which might not be detected using X-ray diffraction.

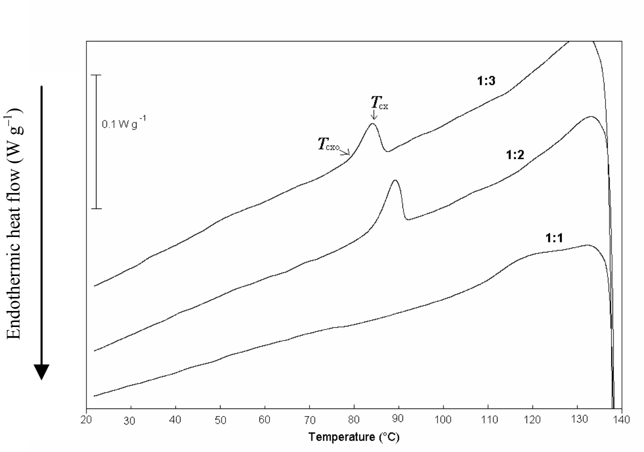

Figure 3: Manifestation of the exothermic event during cooling of 1:1, 1:2 and 1:3 gelatinized native rice strch-water matrix from 140°C at 5°C min^{-1}

Table 1: T_{cxo}, T_{cx} and ΔH of the exotherm obtained on cooling at 1, 5 and 10°C min^{-1} of gelatinized rice starch-water matrix from 120°C.

Starch to water ratio	Cooling rate °C min^{-1}	*Exothermic event on cooling		
		T_{cxo} °C	T_{cx} °C	ΔH J/g
1:2	1	99.2 ± 0.53^a	103.2 ± 0.52^a	0.59 ± 0.06^a
	5	91.7 ± 0.31^b	97.0 ± 0.19^b	0.99 ± 0.02^b
	10	86.0 ± 0.19^c	92.7 ± 0.86^b	1.15 ± 0.10^b
1:3	1	89.7 ± 1.58^a	91.9 ± 1.46^a	0.76 ± 0.08^a
	5	84.7 ± 0.77^b	87.5 ± 0.69^b	1.90 ± 0.14^b
	10	83.1 ± 0.27^c	86.3 ± 0.67^b	1.37 ± 0.15^b

There were no observable transitions for 1:1 gelatinized rice starch-water systems within the temperature range studied.

*Mean ± standard deviation (n = 2). Means within a column (compared within same starch to water ratio) with the same letter are not significantly different at $p < 0.05$.

Table 2: T_{cxo}, T_{cx} and ΔH of the exothermic obtained on cooling at 1, 5 and 10°C min^{-1} of gelatinized rice starch-water matrix from 140°C.

Starch to water ratio	Cooling rate °C min^{-1}	*Exothermic event on cooling		
		T_{cxo} °C	T_{cx} °C	ΔH J/g
1:1	1	119.5 ± 2.06^a	124.6 ± 0.94^{ab}	0.92 ± 0.35^a
	5	118.7 ± 0.15^a	127.1 ± 0.07^a	0.96 ± 0.16^a
	10	108.5 ± 2.12^b	120.2 ± 2.88^b	0.80 ± 0.03^a
1:2	1	94.4 ± 1.87^a	99.3 ± 2.3^a	0.81 ± 0.06^a
	5	89.3 ± 0.47^b	91.9 ± 0.92^b	2.00 ± 0.18^b
	10	88.1 ± 0.09^b	90.6 ± 0.12^b	1.88 ± 0.13^b
1:3	1	89.5 ± 0.20^a	91.6 ± 0.23^a	0.72 ± 0.3^a
	5	85.1 ± 1.48^b	87.9 ± 1.58^b	1.40 ± 0.02^b
	10	83.3 ± 0.42^b	86.7 ± 0.49^b	1.65 ± 0.35^b

*Mean ± standard deviation (n = 2). Means within a column (compared within same starch to water ratio) with the same letter are not significantly different at $p < 0.05$.

Type II complexes are packed in a crystalline register which is believed to have a lamellar-like organization of amylose complexes; i.e., the polysaccharide chains are so folded as to have their chain axes perpendicular to the surface of the lamella and exhibit a V_h-type crystallinity.[18] Tufvesson and Eliasson[16] have reported that in potato starch-monoglyceride-water systems, type I complex started to melt at 88.5°C and type II at 112.9°C during DSC measurements. It is well-documented that the transition of amylose-lipid complex is heat-reversible.[9,10] Therefore, our observations on T_{cxo} ranged from 83°C to 94°C for 1:3 and 1:2 systems, and 109°C–120°C for 1:1 system conformed with the type I and type II complexes behavior. It is anticipated that low moisture contents shift the melting temperatures of inclusion complexes to higher temperatures, crystalline amylose-lipid complexes (type II) are readily formed during cooling of 1:1 starch-water system in which annealing and recrystallization are likely to occur. For 1:2 and 1:3 systems, a decrease of T_{cxo} and T_{cx} with decrease in starch to water ratio is conceivable if we considered the recrystallization as well as the melting of amylose-lipid complex (type I) are solvent-facilitated processes, thus the presence of more water promotes higher mobility of the whole system and made the recrystallization process appeared at lower temperature. The enthalpy of exotherm of 1:2 system was higher compared with the enthalpy of exotherm of 1:3 system, was most probably due to concentration effect, in which the availability of amylose and lipid were higher in 1:2 system.

3.3 Effects of Cooling Rate

Figure 4 gives a comparison of the DSC thermograms obtained using cooling rates of 1, 5 and 10°C min^{-1} on the 1:2 system. Generally, the transitions occurred at lower temperatures and were larger when a faster cooling rate was employed. Slower cooling rate of 1°C min^{-1} produced much broader and smaller crystallization peak as compared to cooling rates of 5°C min^{-1} and 10°C min^{-1}. Similar observations were obtained for 1:3 systems (Table 2). However, there is no specific trend observed for 1:1 systems in which we supposed it was due to the crystalline formed (type II) were more stable and not affected by cooling rate significantly. In contrast, the exothermic peak at ~85°C–100°C shown for the 1:2 and 1:3 samples cooled at 10°C min^{-1} were the largest, became smaller when it was cooled at 5°C min^{-1}, and nearly diminished when it was cooled at 1°C min^{-1}. This observation strengthen our assumption that the complexes formed at 1:2 and 1:3 systems are mainly type I crystalline which would not be stable during prolonged heat treatment[17] (cooling rate as low as 1°C min^{-1} from 140°C, in this case, similar to prolonged heat treatment). Tufvesson and Eliasson[16] have stated that it was possible to transform amylose-lipid complex from type I into type II when it was heat-treated, as type I was then partially or totally melted. In addition, there was an increase in T_{cxo} (from 88°C to 94°C for 1:2 system, 83°C to 90°C for 1:3 systems) and T_{cx} (from 91°C to 99°C for 1:2 systems, 87°C to 92°C for 1:3 systems) with decreasing cooling rate, again, this point to the fact that low cooling rate induced annealing which has allowed reorganization of basic structure segment and results in higher peak transition temperature.

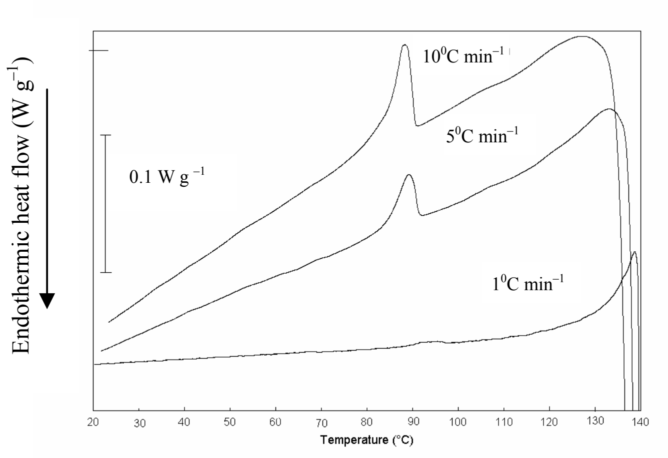

Figure 4: Manifestation of the exothermic event during cooling of 1:2 gelatinized native rice starch-water matrix from 140°C at 1, 5 and 10°C min^{-1}.

3.4 Effects of Heating-end-temperature or Cooling-start-temperature

Table 1 shows the T_{cxo}, T_{cx} and ΔH data obtained at heating-end-temperature of 120°C. There is no significant influence of heating-end-temperature (140°C of Table 1) observed for 1:3 gelatinized native rice starch-water systems for all the phase transition parameters. However, the T_{cxo} and T_{cx} for the 1:2 system became higher at heating-end-temperature of 120°C but the ΔH showed lower value as compared to heating-end-temperature of 140°C. This may be attributed to the amount of amylose-lipid complex present at higher starch to water ratio (1:2) was not completely melted at 120°C as compared to those of 1:3 system, therefore the recrystallization enthalpy showed lower value which indicates less recrystallization (insufficient melting) if the system was heated until 120°C, instead of 140°C. However, the system that has not been completely melted provides less 'raw material' for reorganization during the cooling process, thus higher thermal stability (shown by peak transition temperature) was achieved.

4. CONCLUSION

The DSC studies of native rice starch revealed the exothermic event during cooling was due to amylose-lipid complexes recrystallization. Starch to water ratio of 1:1 showed vastly different exothermic peak (in appearance and temperature location in thermograms) as compared to starch to water ratio of 1:2 and 1:3. In addition, the exothermic peak of 1:1 system was not affected by cooling rate as compared with those of 1:2 and 1:3 systems in which, higher cooling rate produced larger exothermic peak. It is believed that the differences in responses exhibited by the native rice starch system to these factors are due to the presence of two types of amylose-lipid complexes.

5. ACKNOWLEDGEMENT

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