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Water effect on the interaction between amylose and amylopectin during retrogradation

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ABSTRACT

Possible interactions between amylose (AM) and amylopectin (AP) during a temperature cycled retrogradation (4/30 °C 1 day at each temperature, 2 days) in aqueous pastes/gels of corn starch and reconstituted mixtures of AM and AP (1:3 weight ratio) were examined at different water levels (60–90%). With the pastes containing 90% water, no AP crystallization was observed under a differential scanning calorimeter in waxy starch or the starches containing AM. With 80% water, AP crystallization was not observed in waxy starch but it was found in the starches containing AM. With 70% water, AP crystallization was facilitated by the presence of AM. With 60% water, however, AP crystallization was found not to be affected by AM presence. It was evident that the presence of AM (25%) affected AP crystallization during retrogradation but only when the water content in starch pastes/gels was proper (70–80% in this study).

1. Introduction

It has been generally accepted that melting endotherms at a temperature below 100 °C of retrograded starch pastes/gels are attributed solely to amylopectin (AP) crystallization (Liu & Thompson, 1998; Miles, Morris, Orford, & Ring, 1985). However, the exact influence of amylose (AM) on AP crystallization remains unclear. Some studies (Fredriksson, Silverio, Andersson, Eliasson, & Aman, 1998; Gudmundsson & Eliasson, 1990) reported that AM might promote the crystallization of AP if AM was present in a greater amount than AP. However, Klucinec and Thompson (2002) found that AM at a smaller amount (25% or 50%) contributed to the AP crystallization.

In our previous study (Zhou, Baik, Wang, & Lim, 2010), normal corn starch gels (20% or 30% starch solids) retrograded to a larger extent than waxy corn starch gels under isothermal storage and cycled-temperature storage, based on DSC endotherm. When the water content was relatively low (50–60%), however, retrogradation level of normal corn starch was similar to or less than that of waxy corn starch. It was speculated that the AM in normal corn starch had synergistic interactions with AP during retrogradation, which were dependent on water content. The synergistic effect was more apparent when the samples were stored at cycled temperatures than at a single temperature (Zhou et al., 2010). Normal corn starch with 80% water exhibited an obvious peak after a 4/30 °C temperature cycle, i.e. storage at 4 °C for 1 day and then at 30 °C for 1 day, whereas waxy corn starch showed no endothermic peaks under same condition. In the present study, the effect of AM on AP crystallization during retrogradation in corn starches were tested at different water contents (60%, 70%, 80% and 90%) after one 4/30 °C temperature cycle.

2. Experimental

2.1. Materials

Native waxy and normal corn starches (NWX and NNC) were provided by Samyang Genex Company (Seoul, Korea). Purified (defatted) waxy and normal corn starches (PWX and PNC) were obtained by following the procedure of Han and Lim (2004). Isolated AM and AP from normal corn starch (NCAM and NCAP) were obtained by following the procedure of Jane and Chen (1992).

2.2. Preparation of the reconstituted starch

PWX (nongranular) (37.5 mg, dry base) and NCAM (12.5 mg, dry base) were weighed into a capped glass bottle. Consequently, the AM content was 25% of the total starch. The combined material was then dispersed in 90% DMSO, precipitated, and dried using the same method as for the purification of the native starches. As a comparison, granular native waxy corn starch (1.5 mg, dry base) and NCAM (0.5 mg, dry base) were weighed directly in the DSC pan.

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The two kinds of reconstituted starches were termed as WXAM and GWXAM, respectively.

2.3. Molecular weight of NCAM

NCAM (50 mg) was dispersed in 5 ml 90% DMSO (1.0% w/v) in a boiling water-bath for 1 h and magnetic-stirred at room temperature for 12 h. One milliliter of the above starch DMSO solution was mixed with 6 ml ethanol. After centrifugation, the precipitate was redissolved in boiling water (10 mL) and further stirred for 30 min in a boiling water bath. The hot sample solution was filtered using 5 μm nylon membrane and then injected into a HPSEC system (Summit HPLC system, Dionex, USA) using Shodex OHpak SB-804 and SB-802.5 columns (Showa Denko, Tokyo, Japan) for molecular weight determination (Wang, Kim, Kim, Park, & Yoo, 2011). The column temperature was maintained at 50 °C while the temperature of RI detector was set at 30 °C. The elution was performed by using the HPLC grade water at a flow rate of 1.0 ml/min. Glucose, maltotriose, maltopentaose, maltoheptaose, and Shodex pullulan P-82 (Showa Denko, Tokyo, Japan) were employed as standards. AM peak DP was calculated directly from the standard curve. AM average DP_w was calculated as that described by Potocki-Veronese et al. (2005).

2.4. Chain length distribution determined by HPSEC

Purified starch or NCAP (10 mg) was debranched using the method described by Han, BeMiller, Hamaker, and Lim (2003) and analyzed by using the same HPSEC system as described in Section 2.3.

2.5. DSC analysis

DSC thermograms of the starch samples were recorded on DSC6100 (Seiko Instruments Inc., Chiba, Japan). Each of the starch samples (2 mg, dry solids) was weighed into an aluminum DSC pan and distilled water was added. The pan was hermetically sealed after the desired water content of 60%, 70%, 80% or 90% was reached. After heated in a convection oven at 105 °C for 15 min, the sample pans were stored at 4 °C for 1 day and 30 °C for 1 day, and then scanned from 20 to 150 °C at a heating rate of 5 °C/min.

2.6. X-ray diffraction (XRD) analysis

Waxy and normal corn starch suspensions containing 60% and 80% water were made in the conical tube. For gelatinization, these samples were preheated at 70 °C for 10 min with constant stirring (160 rpm) and then in a boiling water bath for 30 min. The resulting starch pastes/gels were retrograded after a 4/30 °C temperature cycle, cut into small pieces and dried in a convection oven at 40 °C for 24 h. Before XRD analysis, the dried retrograded starch pastes/gels were ground to powders and equilibrated in a humidity chamber (85% RH, 25 °C) for 48 h. The final water content of the powders was around 20%. The diffraction patterns were determined by using an X-ray diffractometer (D5005, Bruker, Germany), which was operated at 40 mA and 40 kV, with the diffraction angles of 3–30° (2θ), step size of 0.02° and scan speed of 2°/min.

2.7. Statistical analysis

All numerical results are averages of at least two independent replicates. Data were analyzed by one-way analysis of variance (ANOVA) using ORIGIN 8.0 (OriginLab Inc., USA). The means comparison were determined by Tukey's test ($P < 0.05$).

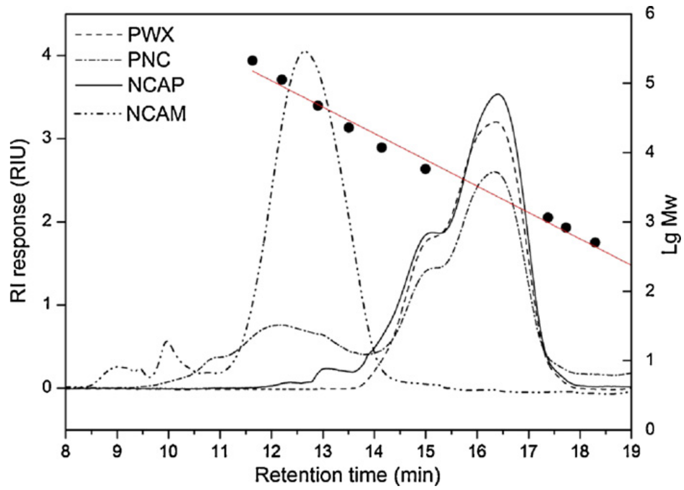

Fig. 1. HPSEC profiles of normal corn amylose (NCAM) and debranched normal corn amylopectin (NCAP), purified normal and waxy corn starch (PNC and PWX).

3. Results and discussion

PNC contained 25.7% amylose based on the HPSEC analysis (Fig. 1). The average DP_w of NCAM was calculated to be 793 which was consistent with that reported by Jane and Chen (1992). The HPSEC profiles of chains released by debranching NCAP and PWX were very similar, showing typical bimodal distributions of long and short chains (Hizukuri, 1985). Table 1 summarized the chain length distribution of the debranched starches analyzed by HPSEC. Compared with PWX, the NCAP contained a slightly higher proportion of long chains (26.8% vs. 22.2%) with a greater average CL_w (28.5 vs. 27.3).

After one temperature cycle at 4/30 °C, no AP crystal endotherm was observed for either waxy starch or the starch containing 25% AM when the water content was 90% (data not shown). It was consistent with the result reported by Longton and Legrys (1981) in which no AP crystallization occurred in wheat starch gels containing more than 90% water. When the water content was reduced to 80%, all the AP samples (NCAP, NWX and PWX) still showed no endotherm (Fig. 2). On the other hand, the starch samples containing AM (25%), i.e. NNC, PNC, WXAM and GWXAM, showed the endotherms with different size responsible for AP recrystallization under the same retrogradation conditions. When the water content was 70%, all the AP samples exhibited smaller enthalpies (expressed on AP basis) than those of AM containing samples (Fig. 2). Klucinec and Thompson (2002) also found that AM (25%) could contribute to AP retrogradation when the water content was 70%. Similarly, Sievert and Wursch (1993) found that AM chain reordering was restricted in the presence of AP on the cooling of AM and AP mixtures containing 78% water.

When AP solely exists with a plenty of water in pastes (NWX, PWX and NCAP with 80% and 90% water in this case), the AP side chains are too mobile to associate for crystallization due to the water molecules residing between the chains. However, in the pres-

Table 1
Length and distribution of amylopectin chains in isolated amylopectin (NCAP) and purified waxy corn starch (PWX).[A]

	Average CL_W	Peak DP		Distribution, %	
		Long	Short	Long	Short
NCAP	28.5^a	52.0^a	14.8^a	26.8^a	71.6^b
PWX	27.3^b	52.0^a	14.7^a	22.2^b	77.8^a

[A] Means of triplicates. Values followed by different superscripts in each column are significantly different ($P < 0.05$).

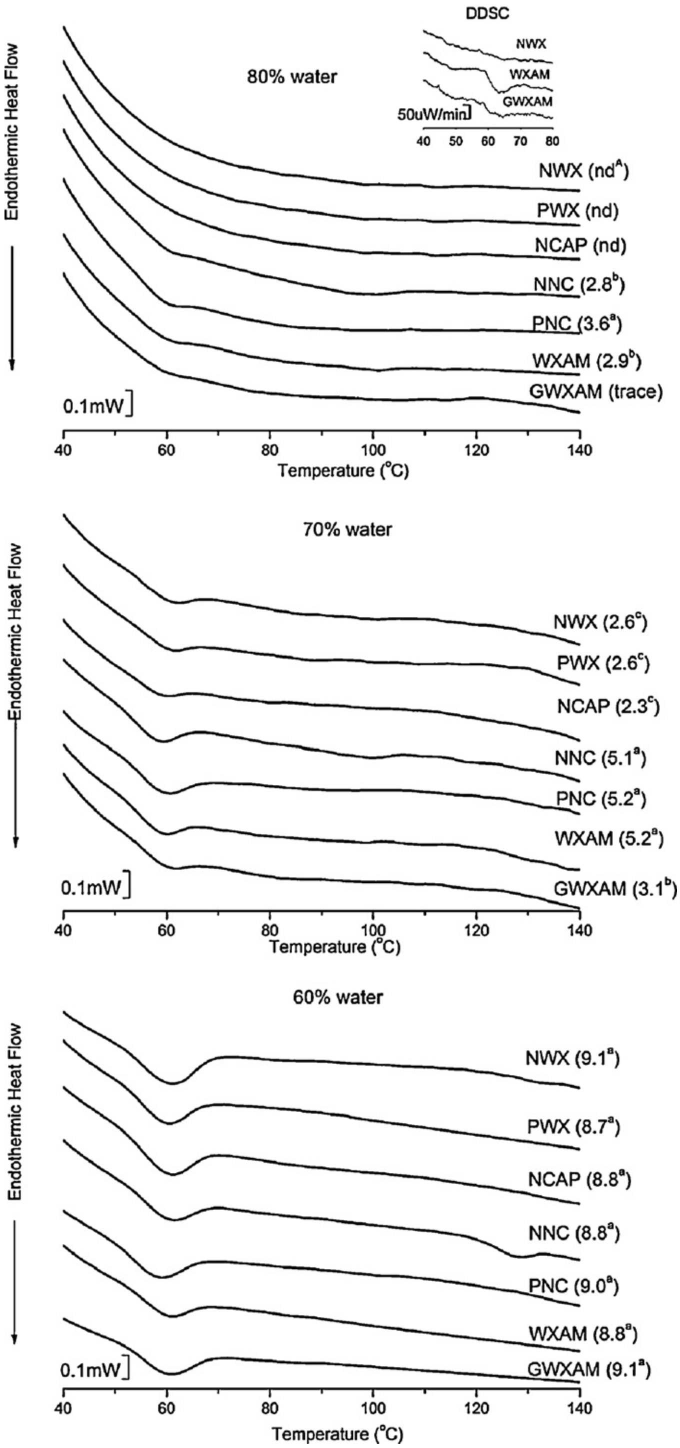

Fig. 2. DSC thermograms of retrograded starch pastes/gels containing 80%, 70% and 60% water: native starch (NWX and NNC), purified waxy and normal corn starches (PWX and PNC), isolated amylopectin (NCAP) and reconstituted starch (WXAM and GWXAM). Melting enthalpies are given in parenthesis (J/g of AP solids). A: not detected.

ence of AM, AP crystallization appeared obvious at the same water content of 80% (Fig. 2). By reducing the water content from 90 to 70%, AM became affecting the AP crystallization. Even though there is a slight difference in molecular structure (chain length and distribution) between the NCAP and PWX, no difference in retrogradation behavior was found in terms of AP crystals.

Two explanations for this positive effect of AM on AP crystallization may be possible. The AM chains which randomly distributed in the dilute pastes have much greater specific volumes than the AP chains due to their linearity. More water molecules thus become bound to AM chains than AP chains of the same molecular size. Therefore, the presence of AM may result in reducing the available water for AP to interact, which in turn facilitates the association of AP chains. The other possibility is the cocrystallization of AM and AP chains. As the water content was reduced, AM might be given an increased chance to be entangled with AP. Leloup, Colonna, & Buleon (1991) tested gels of potato amylose and waxy corn starch mixtures as phase-separated systems, however, suggested that AM and AP might cocrystallize when they came in contact with each other at interfaces. When AM is added to the granular waxy corn starch (GWXAM), there is little chance for AM to entangle with AP (Kurakake, Akiyama, Hagiwara, & Komaki, 2009). Water absorbing capacity of AM, which make less water available for AP, might account for the greater degree of retrogradation in GWXAM than in pure AP. In the case of WXAM, AM was homogenously mixed with nongranular waxy corn starch, so that there was more possibility for AM and AP to cocrystallize. Therefore it resulted in a larger endotherm than that of GWXAM. As the endotherm of WXAM was comparable to that of NNC, synergistic interactions of AM with AP might be the main reason for the greater degree of retrogradation in normal corn starch than in waxy corn starch.

When the water content was 60%, no significant difference was observed between AM-containing starches (NNC, PNC, WXAM and GWXAM) and AP starches (NWX, PWX and NCAP) in terms of the melting enthalpy of AP crystals calculated on the basis of AP content (Fig. 2). Zhou et al. (2010) reported that water heterogeneously distributed and possibly limited for the dissolution of some starch molecules when gelatinized with water less than 60%. After gelatinization of starches with insufficient water, the AP chains are readily associate each other during retrogradation resulting in AP crystallization. It was assumed that the contribution of AM to the AP crystallization became less or no obvious as shown in DSC thermograms (Fig. 2) because the chain mobility was highly restricted and the AM and AP chains heterogeneously distributed. Gudmundsson and Eliasson (1990) found synergistic interactions between AM and AP during retrogradation occurred only when AM content was higher than 50%. In their study, the water content of the mixtures was 50% where the AP chains' mobility was more restricted than in our study, so that only large amount of AM (>50%) could affect AP recrystallization.

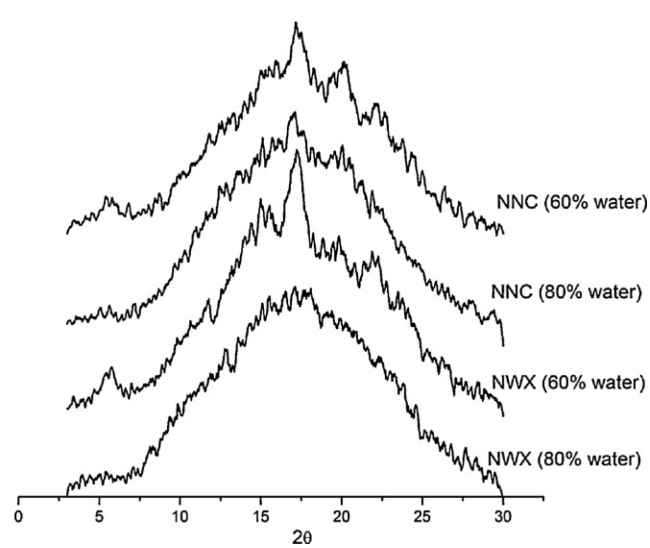

Fig. 3. X-ray diffraction patterns of retrograded native waxy and normal corn starches (NWX and NNC) at different water contents (60% and 80%).

It was also noticeable that the AM-lipid complex endotherm in NNC moved from 100 °C to around 120 °C as the water content was reduced from 70% to 60% (Fig. 2). With the restricted chain mobility, AM might form the complex with lipids more readily with an increased thermal stability.

XRD patterns of the retrograded starches (see Fig. 3) were in agreement with their thermal transition properties. With 80% water, NWX was largely amorphous, whereas NNC exhibited small peaks around 17° and 20° which were responsible for AP recrystallization and formation of AM-lipid complex, respectively. With 60% water, the peak intensity at 17° for NWX was higher than that for NNC due to the larger proportion of AP in NWX than in NNC.

Overall data revealed that there are different characteristics in AP crystallization between normal and waxy corn starches after a short term retrogradation. The AM presence (about 25% in this case) may enhance the AP crystallization during retrogradation of starch pastes/gels, and this effect depends on the residual water content.

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