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Effect of pullulan on the short-term and long-term retrogradation of rice starch☆

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ABSTRACT

The effect of pullulan (PUL) on the retrogradation of rice starch (RS) was investigated by means of rapid visco-analyzer (RVA), rotational rheometer, differential scanning calorimetry (DSC), and X-ray diffraction (XRD). RVA results showed that addition of pullulan significantly decreased the breakdown and setback values, which meant that the short-term retrogradation of RS was inhibited. The dynamic time sweep of samples also proved the retarding effect of pullulan on the retrogradation of RS. DSC curves showed clearly that pullulan significantly reduced the retrogradation enthalpy of amylopectin, and the kinetics of retrogradation was analyzed using the Avrami model. XRD results showed that recrystallinity of RS was reduced from 11.565% to 8.841% with the addition of pullulan and this was in line with the DSC results. It could be concluded that the addition of pullulan apparently influenced not only the short-term retrogradation of amylose, but also the long-term retrogradation of amylopectin.

1. Introduction

Rice is one of the most common crops in the world, the staple food and the major energy source of people in East Asian countries (James & Roy, 2009; Wani et al., 2013). Rice consists mostly of starch, and therefore it is very prone to retrogradation after cooking. The term retrogradation is used to describe the changes that occur in gelatinized starch during storage, especially at low temperature. The components of rice starch, amylose and amylopectin, have unique roles in the retrogradation of starch. Generally, amylose is responsible for the short-term retrogradation, while the long-term retrogradation is ascribed to the reordering of amylopectin which is a much slower process involving mainly the recrystallization of the outer branched-chains of amylopectin (Miles, Morris, Orford, & Ring, 1985; Ring et al., 1987). The retrogradation during production or storage profoundly affects quality, acceptability and shelf-life of starch or starch-containing foods (Karim, Norziah, & Seow, 2000). The firmness of starch gel increases markedly due to the fact that starch molecules in pastes associate together on aging (Seow & Teo, 1996). The phase separation between polymers and water appeared and resulted in the phenomenon of syneresis (Karim et al., 2000). What's more, retrogradation could change the nutritional value of starch with increasing the level of enzyme-resistant starch (Haralampu, 2000).

This tendency of retrogradation limited the large-scale production of rice products in commercial and industrial field, and therefore, effective measures were urgently required to overcome this problem. Blending starch with hydrocolloids, as a safe and effective method, was widely applied in food industry to modify and control the rheological properties (Chaisawang & Suphantharika, 2006; Chen, Tong, Ren, & Zhu, 2014; Pongsawatmanit, Chantaro, & Nishinari, 2013; Wang et al., 2008), retrogradation behavior (Banchathanakij & Suphantharika, 2009; Funami et al., 2005; Tang, Hong, Gu, Zhang, & Cai, 2013), texture (Charoenrein, Tatirat, Rengsutthi, & Thongngam, 2011), syneresis and freeze-thaw stability (Chen et al., 2014; Lee, Baek, Cha, Park, & Lim, 2002; Sae-kang & Suphantharika, 2006). Possible interactions among hydrocolloids, starch molecules, and starch granules, i.e. hydrocolloid molecules interacted with leached starch molecules,

☆ Please note: As pullulan is not a specific chemical, it is a polymer of repeating maltotriose units; it will not have a specific record in PubChem.

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were hypothesized to play an important role in modifying the properties of starch–hydrocolloid combinations (BeMiller, 2011), including retarding the granule swelling and the aggregation of amylose that led to retrogradation (Chen et al., 2014; Fredriksson, Silverio, Andersson, Eliasson, & Åman, 1998).

In the aspects of natural neutral polysaccharide, pullulan is attracting the attention of more and more people, not only because of its preferable film forming ability (Wu & Chen, 2013; Xiao, Tong, & Lim, 2012), but also its unique and multiple benefits on human beings (Singh, Saini, & Kennedy, 2008). Pullulan is a typical linear exocellular polysaccharide produced by *Aureobasidium pullulans* with a starch-like structure of linkage α-D-glucan primarily consisting of maltotriose repeating units interconnected by α-(1 → 6) linkages (Singh et al., 2008). The regular structure confers pullulan the aforementioned distinctive properties compared to other polysaccharides.

Although many efforts have been made to investigate the properties and applications of pullulan in many fields such as food and pharmaceutical industry (Singh et al., 2008), there are very few reports focused on the effect of pullulan addition on the properties of starch. Xiong, Song, Yao, and Lu (2013) reported that pullulan reduced the retrogradation enthalpy, the peak viscosity, and final viscosity of high amylose RS by reducing the water mobility and the interactions between starch and water. Our previous study (Chen et al., 2014) investigated the effect of pullulan on the gelatinization and rheological properties of RS, and found that pullulan adsorbed onto the surface of starch granules and coated around these granules during heating when the concentration of pullulan was greater than 0.07%, resulting in the reduction of granule swelling, leached amylose, the subsequent gelatinization and setback values of RS. However, the information about the effects of pullulan on the retrogradation behavior of starch is still insufficient, and relevant investigations and farther quest of deep-seated mechanisms behind these phenomena are still needed.

The objective of this study was to investigate the role and potential usefulness of pullulan in controlling retrogradation of starch-based foods. Since retrogradation was a complex process affected by many factors, it was unlikely that any single method could be able to give a complete picture of the retrogradation properties of starch gels. Therefore, multiple methods including RVA, rheological properties, DSC, and XRD were applied, and the independent evidence derived from those methods might allow us to obtain a comprehensive understanding of the effect of pullulan on both the short-term and long-term retrogradation of rice starch. Furthermore, the Avrami model fitting the DSC data was employed to study the isothermal crystallization kinetics of long-term retrogradation in RS, and the relative crystallinity dada of retrograded samples obtained from the XRD diffraction patterns were also given.

2. Materials and methods

2.1. Materials

Normal rice starch was obtained from Jiangsu Baby Co., Ltd. (Suqian, China). The contents of moisture, ash, protein, lipid and amylose in this rice starch were 11.82%, 0.14%, 0.23%, 0.68%, and 23.30%, respectively. Pullulan was purchased from Hayashibara Biochemical Inc. (Shanghai, China) with 4.50% moisture content and 200,000 MW.

2.2. Pasting properties

RS–PUL mixtures were prepared as follows. RS–PUL ratios were 5/0, 5/0.07, 5/0.10, 5/0.30, and 5/0.50 (w/w), respectively. The calculated amounts of pullulan powder were primarily dissolved thoroughly in distilled water with continuous stirring for 1 h, and then RS was slurried in pullulan solutions at room temperature by magnetic stirring for 30 min to avoid the formation of starch lump. The prepared slurry was transferred into an aluminum canister and stirred using a plastic paddle. The pasting parameters of samples were measured by a rapid visco-analyzer (RVA-RECHMASTER, Newport Scientific Pty. Ltd, Sidney, Australia) in the following manner. The slurry was equilibrated at 50 °C for 1 min, heated to 95 °C within 7.5 min, and then held at 95 °C for 5 min. The hot sample was subsequently cooled to 50 °C within 7.5 min, and maintained at 50 °C for 4 min. Paddle speed was 960 rpm for the beginning 10 s to disperse the sample, and then the speed of paddle was set at 160 rpm during the measurement. Breakdown value representing the differences between peak viscosity and trough viscosity, and setback value representing the differences between trough viscosity and final viscosity were calculated from the RVA curves. The viscosity was expressed in cP units.

2.3. Dynamic viscoelastic measurements

The dynamic time sweep was performed following the method reported by Funami et al. (2005) with a slight modification by using an AR G2 rheometer (TA instrument Inc., USA) isothermally at 4 °C. Parallel plate geometry (60 mm) at gap 500 μm was used for rheological measurements. The slurries, which went through the RVA measurements, were immediately transferred to the rheometer plate. The excess material was wiped off with a spatula. Silicon oil was applied to the exposed surfaces of samples to prevent evaporation during experiments. Prior to measurement, samples were held for 5 min to equilibrate stresses and temperature. G' values were monitored during aging at a constant frequency of 6.28 rad/s and at a constant strain of 3%. The 3% strain was in the linear viscoelastic region according to the strain sweep results (data not shown).

2.4. Differential scanning calorimetry (DSC)

The thermal analysis of RS with different pullulan ratios during storage at 4 °C was performed by using a differential scanning calorimetry (SII NanoTechnology Inc., Japan) under an ultrahigh-purity nitrogen atmosphere. The instrument was calibrated using indium as a standard and an empty aluminium pan was used as the control. The gelatinization and retrogradation properties of samples were determined form the DSC curves. Rice starch containing 0%, 1.4%, 2%, 6%, and 10% pullulan (based on rice starch weight) were prepared, respectively. Three milligrams of prepared samples were mixed with 6 μL of deionized water and hermetically sealed in an aluminium pan. All sealed samples were equilibrated at room temperature for 12 h to hydrate and then heated over a temperature range of 30–100 °C at a constant rate of 10 °C/min to perform the gelatinization process. Subsequently, the gelatinized samples were stored at 4 °C for 1, 3, 5, 7, 14, 21, and 28 days to perform the retrogradation process and then rescanned from 30 to 100 °C to melt the retrograded amylopectin crystallites. Melting enthalpy (ΔH) was calculated from the area of the main endothermic peak and expressed in terms of J/g of dry starch using the equipment software. Measurements were performed in triplicate.

The Avrami equation, which deals with crystallization at temperatures above the glassy region, has been extensively used by many researchers to provide a convenient empirical means of representing the process of starch retrogradation (Xu et al., 2012).

Therefore, the Avrami's equation, Eq. (1), was used to illustrate the kinetics of retrogradation of starch (especially amylopectin).

$$X(t) = \frac{\Delta H_t - \Delta H_0}{\Delta H_\infty - \Delta H_0} = 1 - \exp\left(-kt^n\right) \quad (1)$$

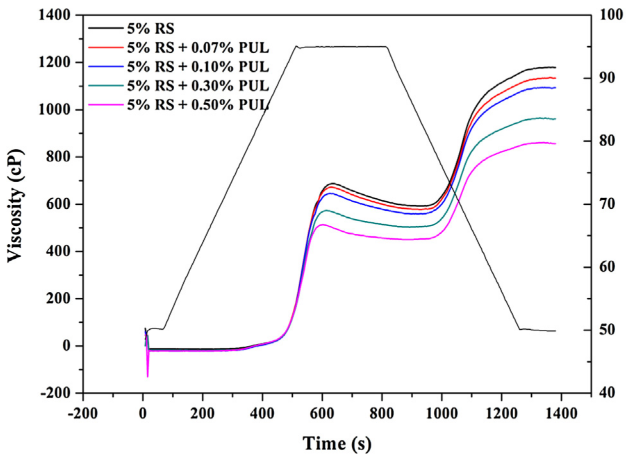

Fig. 1. Pasting curves of RS–PUL mixtures. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

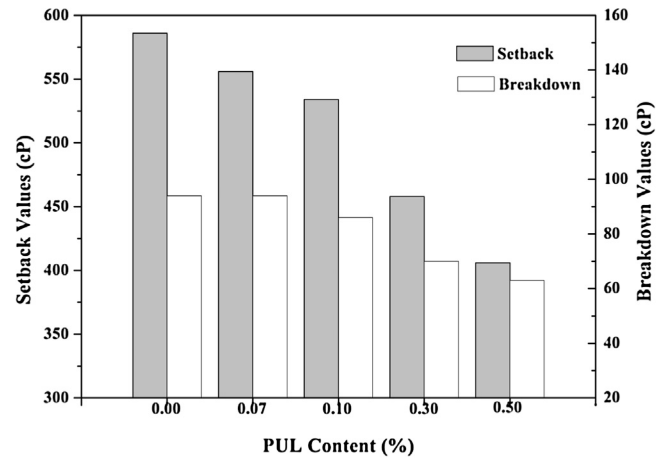

Fig. 2. Variation of setback values and breakdown values with the addition of pullulan. Data are shown as means of triplicate measurements.

where $X(t)$ is the fraction of crystallized starch at time t; ΔH_t is the enthalpy change at time t; $\Delta H\infty$ is limiting enthalpy change (28 days for all samples); k and n are rate constant and the Avrami exponent, respectively.

2.5. X-ray diffraction analysis

The crystalline characteristic of samples was determined by using a Bruker X-ray diffractometer (Bruker AXS Inc., Germany) at 40 kV and 30 mA Cu-*Ka* radiations.

The samples for XRD were prepared by the method of Xu et al. (2013). Briefly, rice starch with 0%, 1.4%, 2%, 6%, and 10% pullulan (based on rice starch weight) was dispersed in distilled water at room temperature by magnetic stirring for 30 min, and then heated at 95 °C in a water bath for 20 min with continuous stirring to obtain a complete gelatinization of the rice starch. Samples were allowed to cool down slowly to room temperature and stored at 4 °C for 7 days. The retrograded samples were dried in a vacuum drying oven at 40 °C for 10 h and milled to pass through a 100-mesh sieve. Then the powders were subjected to moisture equilibrium in a sealed glass desiccator containing saturated sodium chloride (75% relative humidity).

Diffractograms were obtained from 4° to 40° (2θ) at a scan rate of 4°/min. The XRD diffractograms were analyzed using Jade 5.0 software (Materials Data Inc., Livermore, CA, USA) and the relative crystallinity was calculated by dividing the area of peaks by the total area of the diffractogram (Komiya & Nara, 1986).

2.6. Statistical analysis

The data were expressed as means of triplicate determinations unless otherwise stated. Statistical significance was assessed with one-way analysis of variance (ANOVA) using SPSS 20.0 (SPSS Inc., Chicago, USA) for windows program. p Value <0.05 was considered to be statistically significant throughout the study.

3. Results and discussion

3.1. Pasting properties

The pasting profiles of RS with or without pullulan were shown in Fig. 1. Important parameters including breakdown and setback values were also analyzed and illustrated in Fig. 2. Pure pullulan solution at a concentration of 0.5% was also run under the same RVA procedure, and the viscosity of pullulan solution was very low and remained almost constant as compared with viscosities of RS and RS–PUL mixtures (data not shown). This indicated that the polysaccharide was throughly dispersed before and during the heating.

As shown in Fig. 1, pullulan apparently affected the gelatinization properties of RS. Addition of pullulan at the concentration tested led to a sharp decrease in peak viscosity. And this might be ascribed to the inhibition effect of pullulan on the swelling and gelatinization of starch granule (Chen et al., 2014). Based on the suppression effect of pullulan on the gelatinization of RS, an increase in pasting temperature was expected. However, the peak temperature were seemly unaffected by the addition of pullulan in the present study. Taking the high pasting temperature (94.2 °C) of RS alone into consideration, the unconspicuous influence of pullulan on the peak temperature might be attributed to the fact that the maximum detectable temperature of the RVA equipment was 95 °C, and therefore any temperature higher than 95 °C would not be detected by this method (Samutsri & Suphantharika, 2012).

Pullulan induced a remarkable reduction of breakdown values and setback values, as shown in Fig. 2. Our previous study (Chen et al., 2014) showed that the effect of pullulan on the gelatinization of RS varied depending on the addition concentration of pullulan. Pullulan could blend well with rice starch and promote the gelatinization of starch granules at low concentration of pullulan, but wrap around the surface of starch granules and suppress the gelatinization of starch at high concentration of pullulan. Similarly, soybean-soluble polysaccharide and gum Arabic were also found to adsorb onto and coat around the starch granules to change the gelatinization and retrogradation properties of starch (Funami et al., 2008). The reduction of breakdown values elucidated that the starch granules would be more compact with the addition of pullulan and this could result in the decrease of leached amylose during heating. Consequently, the short-term retrogradation of starch was inhibited and the setback values decreased with the addition of pullulan. So, as a unique polysaccharide, pullulan might possess the potential capacity to anti-retrogradation of starch.

Many other hydrocolloids, such as xanthan and β-glucans, were incorporated in various kinds of starches. However, unlike the influence of pullulan on the pasting properties of starch, these hydrocolloids significantly increased the peak and final viscosities (Banchathanakij & Suphantharika, 2009; Tang et al., 2013). This difference of pasting properties between starch–pullulan and starch–other hydrocolloids might point towards the different mechanisms of anti-retrogradation. For xanthan gum, etc., the inhibition effect might partially derive from the thickening effect and the water-holding capacity of hydrocolloids (Tang et al., 2013). As

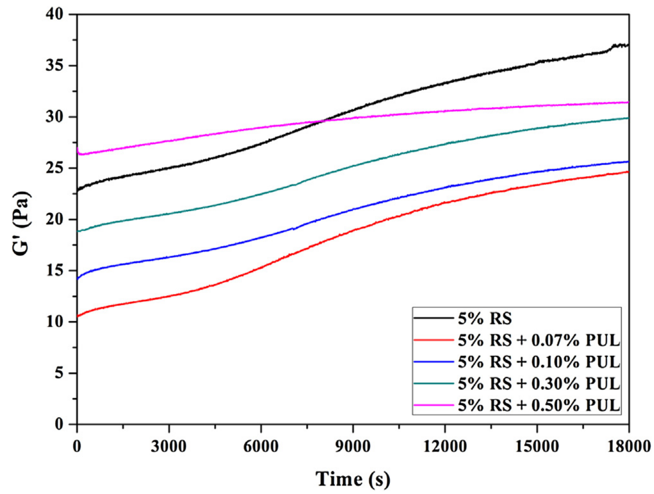

Fig. 3. Changes in G' values during storage at 4 °C for 5 h for RS–PUL mixtures. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

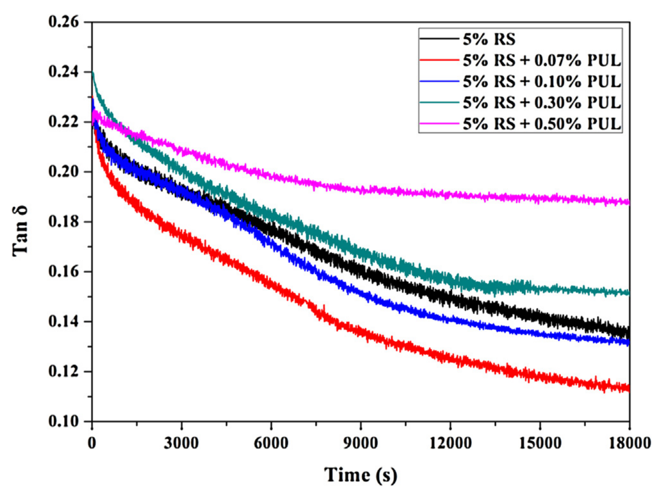

Fig. 4. Changes in tan δ values during storage at 4 °C for 5 h for RS–PUL mixtures. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

for pullulan, alternative explanations are needed to be proposed, which will be discussed in the following sections.

3.2. Dynamic viscoelastic properties

In order to further investigate the retarding effect of pullulan on the short-term retrogradation of starch, time sweep of samples was performed. Taking the rapid process of short-term retrogradation of amylose into consideration, 5 h was selected as the time needed to perform the time sweep experiment. The storage modulus (G') and loss tangent (tan δ) as a function of time for 5 h at 4 °C were shown in Figs. 3 and 4.

In the rheology analysis of gelatinized starch, the G' value is more closely related to the aggregation of amylose at the early aging stage, because the initial gel network structure development is governed by amylose gelation (Karim et al., 2000). Upon cooling, the water can be easily expressed from the gel duo to the aggregation of starch molecules and then the phenomenon of sample dehydration occurred in the process of time. Although dehydration might also lead to the increase in G', this increment in G' was essentially derived from the aggregation of starch molecules. For the above reason, the changes of G' values during aging were selected as an indicator which monitoring the gelation or retrogradation behavior of rice starch, especially the short-term retrogradation of amylose.

The G' values of RS increased during the experiment and continued to rise steadily without a plateau even after 5 h (Fig. 3). This non-plateau behavior of RS was also observed by Kim and Yoo (2006) who studied the rheological properties of rice starch–xanthan gum mixtures, and by Tang et al. (2013) who evaluated the effect of xanthan on the retrogradation of RS. These results indicated that the rapid aggregation of amylose occurred at the early stage and consequently formed a three-dimensional gel network, which was well known as short-term retrogradation resulted from the recrystallization of amylose (Miles et al., 1985).

Compared with RS alone, the minimum addition concentration of pullulan, i.e. 0.07%, resulted in the greatest reduction of G'. Furthermore, the continuous increase of pullulan gave rise to a significant increase in G' values of RS–PUL mixtures. This interesting impact of pullulan on the G' might be attributed to the combined effects of multifactor. On the one hand, pullulan could wrap up the surface of starch granules during gelatinization (Chen et al., 2014), and restrict the amylose from leaching out, resulting in the inhibition of amylose gelation and the reduction of G'. On the other hand, however, the possible molecular interactions between amylose and polysaccharides (BeMiller, 2011) in the continuous phase might promote the formation of tri-dimension gel structure and then increase the G' values. Apparently, the higher the pullulan added, the higher the G' increments. This explained why G' values of RS–PUL mixtures increased with pullulan. The enhancement effect and weaken effect of pullulan on the tri-dimension network gel structure of RS worked together and eventually led to the changes in G' values, as shown in Fig. 3.

In order to further investigate the effect of pullulan on the gelation of amylose, the changes of dynamic mechanical loss tangent (tan δ) during storage were illustrated in Fig. 4. The tan δ values increased with the increase in pullulan concentration, but decreased with the extending storage time. This indicated that the retrogradation occurred in all the samples. The mixtures that contained 0.7% and 1.0% pullulan had lower tan δ values than RS did, while the mixtures with 3% and 5% pullulan had higher tan δ values than starch alone did. This suggested that the gelation behavior of RS was indeed affected by pullulan.

When the gelatinized dispersion of starch was cooled, a loose paste or gel would form depending on concentration of starch (Appelqvist & Debet, 1997). In the present study, a three-dimensional network gel was established through the rearrangement of starch molecules with the phenomena that storage modulus (G') predominated over the loss modulus (G'') and the two moduli did not intersect together. The short-term development of the gel structure was considered to be ascribed to the reordering and crystallization of amylose that leached out from the starch granules. Obviously, the gel characteristics of RS were changed by the incorporation of pullulan. As we all known, tan δ is an important indicator of the relative contributions of the viscous and elastic components to the viscoelastic properties of samples (Karim et al., 2000). According to Fig. 4, the tan δ values of RS–pullulan mixtures were higher than that of RS alone when the concentration of pullulan was higher than 0.3%, indicating that there were relatively more viscous components and less elastic components in RS–pullulan mixtures than in RS. In other words, the aggregation or short-term retrogradation of amylose chains was retarded. It is noteworthy that the effective inhibition concentration of pullulan on the short-term retrogradation of amylose is higher than 0.3%.

From what have been discussed above, we could draw a conclusion that the short-term retrogradation of RS, especially amylose, during storage was apparently affected by the addition of pullulan and the effect of pullulan was greatly dependent on the pullulan

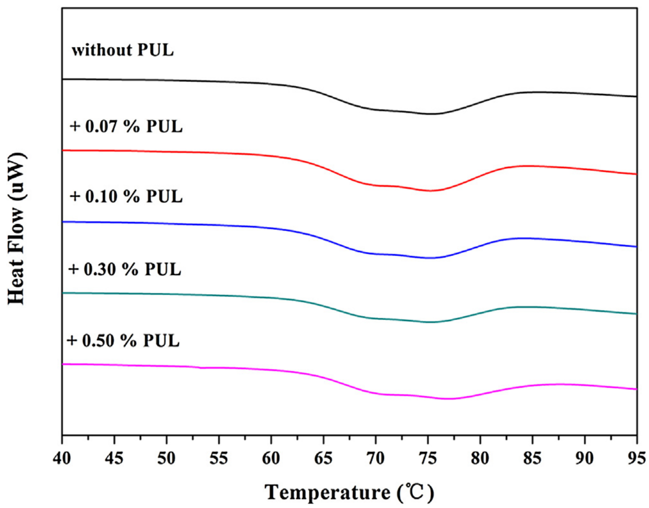

Fig. 5. Gelatinization thermograms of RS–PUL mixtures.

concentration. Pullulan could retard the aggregation of amylose especially when the concentration of pullulan was high enough.

3.3. Thermal analysis

The gelatinization properties of RS–PUL mixtures determined by DSC were summarized in Fig. 5 and Table 1. The gelatinization temperature (onset temperature, T_o; peak temperature, T_p; and conclusion temperature, T_c) seemed to be unaffected by the addition of pullulan at the concentrations tested in present study as compared to those of the control. The weak influence of polysaccharides on the temperature parameters of DSC curves was also observed by Banchathanakij and Suphantharika (2009) who studied the effect of different β-glucans on the gelatinization and retrogradation of rice starch, and this phenomenon could be interpreted by the adequate hydration theory according to Khanna and Tester (2006). The gelatinization enthalpy of RS was 13.12 J/g, which was not in good agreement with previous results reported by Wu, Chen, Li, and Li, (2009) and Wani et al. (2013), suggesting that diversity in rice cultivars apparently affected properties of rice starch (Wani et al., 2012). Compared with the control, the RS–PUL mixtures exhibited a slight decrease in enthalpy. It might be due to the fact that the hydrophilic characteristic of pullulan was well and thus resulted in the lower heat transfer rates and the decreased mobility of water in the system (Krüger, Ferrero, & Zaritzky, 2003).

The peak retrogradation temperature was approximately 23 °C lower than that of the gelatinization. And the temperature ranges of the retrogradation samples were broader than the counterpart of the gelatinization process (data not shown). These results indicated that the storage of gelatinized starch at 4 °C formed less perfect and more heterogeneous crystallites than those existed in native starches (Karim et al., 2000). This tendency was in good agreement with previous results (Banchathanakij & Suphantharika, 2009; Wu et al., 2009). The enthalpy values of the retrogradation peak below 60 °C reflected the melting of retrograded amylopectin rather than amylose, because the melting temperature of retrograded amylose or that of amylose-lipid complex could be much higher than that of amylopectin (Creek, Ziegler, & Runt, 2006; Shamai, Bianco-Peled, & Shimoni, 2003).

Table 2 showed the retrogradation enthalpy changes of the samples during storage at 4 °C. As the samples were stored for increasing time, the amylopectin retrogradation enthalpy became more pronounced, which meant that more energy was required for melting a large amount of amylopectin crystallites formed during storage. The enthalpy of RS increased significantly from 0 to 6.88 J/g as the storage time increased from 0 to 28 days. This trend was also found in other cereal starch (Xu et al., 2012). The enthalpy values decreased with the addition of pullulan compared with control, and the more the pullulan added, the lower the enthalpy values became. The enthalpies of RS–PUL decreased from 6.10 to 4.55 J/g with the concentration of pullulan increased from 0.07 to 0.5% when stored for 28 days. These results suggested that pullulan could inhibit the retrogradation of rice starch especially the crystallization of amylopectin. Some β-glucans, i.e. curdlan, oat, barley and yeast β-glucans, were also found to have the ability to decrease the retrogradation enthalpy of rice starch (Banchathanakij & Suphantharika, 2009).

The phenomena above might be attributed to the interactions between RS and pullulan. When RS was heated in pullulan solution, the swelling and gelatinization of starch granules were suppressed at relatively high pullulan concentration duo to the competition of soluble polysaccharides against starch granules for available water (Brennan & Cleary, 2007), leading to insufficient gelatinization of rice starch. As a result, these partial gelatinized starch, which have higher proportion of ordered or denser structure (Fu, Wang, Li, Zhou, & Adhikari, 2013), might decrease the mobility of starch macromolecular chains and therefore inhibited the crystallization progress of starch molecules. Furthermore, suppression effect of pullulan on swelling of starch granules also reduced the quantity of leached amylose (Chen et al., 2014). According to existing research (Russell, 1987), the amylose leached out from granules during gelatinization had been suggested to have synergetic effects on the retrogradation of amylopectin, so in view of the reduction of leached amylose, we could also expect the inhibition of amylopectin retrogradation.

According to previous studies, the retrogradation process of gelatinized starch would be analyzed using the theoretical concepts of the polymer crystallization kinetics, and therefore the Avrami model was a useful tool for studying the kinetics of starch retrogradation (Tian et al., 2009; Xu et al., 2012). Table 2 showed the Avrami exponent (n) and rate constant (k) of Avrami model. Parameter k depended on the crystals growth and crystals nucleation constants, while the parameter n was depended on the nature of crystal nucleation and the crystal dimension (Del Nobile, Martoriello, Mocci, & La Notte, 2003). As could be seen in Table 2, experimental data were fitted well with Avrami model with high determination coefficient (0.9663–0.9833). Compared with the RS alone, RS–PUL had a slower recrystallization rate (k), whereas Avrami exponent (n) was dependent on the concentration of pullulan. The n values of RS–PUL were higher than that of RS except for the samples contained 0.07% and 0.10% pullulan. There results suggested that the retrogradation process of RS was slowed down by the incorporation of pullulan, especially when the addition level of pullulan was high er than 0.10%.

It could be concluded that addition of pullulan might absorb water and decrease the mobility of the starch chains and the quantity of leached amylose, and in turn retard the retrogradation of the amylopectin.

3.4. XRD pattern analysis

XRD analysis was applied to further prove the inhibition effect of pullulan on the long-term retrogradation of rice starch. The XRD patterns and relative crystallinity of samples were illustrated in Fig. 6. Native RS showed a typical A-type XRD pattern with strong peaks at 2θ close to 15, 17.5, 18.7 and 23.3°, which was in good agreement with precious reports (Wu et al., 2009; Zhang et al., 2014). However, the typical B-type XRD pattern formed when RS was subjected to gelatinization and retrogradation, and a well-defined peak at 2θ close to 17° was observed in all samples. The transformations of crystallinity from A-type to B-type

Table 1
Gelatinization temperatures and enthalpy of RS–PUL mixtures at various ratios[a].

PUL conc (%)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_g (J/g dry starch)
0.00	64.20 ± 0.98a	75.30 ± 0.11a	79.56 ± 0.23ab	13.12 ± 0.27b
0.07	64.50 ± 0.36a	75.23 ± 0.06a	79.33 ± 0.25ab	13.08 ± 0.24b
0.10	63.90 ± 0.44a	75.20 ± 0.10a	79.30 ± 0.26a	13.03 ± 0.31b
0.30	64.77 ± 0.71a	75.33 ± 0.15ab	79.57 ± 0.38ab	12.87 ± 0.38ab
0.50	65.50 ± 1.41a	75.77 ± 0.50b	79.97 ± 0.45b	12.43 ± 0.23a

T_o: onset temperature; T_p: peak temperature; T_c: conclusion temperature.
[a] Tests were performed in triplicate. Mean ± standard deviation values in the same column for each sample followed by different letters are significantly different ($p < 0.05$) by Duncan's test.

Table 2
Change in retrogradation enthalpy and Avrami recrystallization kinetic parameters of RS–PUL mixtures stored at 4 °C[a].

PUL conc (%)	Enthalpy change (J/g)							Avrami parameters		
	1 Day	3 Day	5 Day	7 Day	14 Day	21 Day	28 Day	n	k (h^{-n})	r^2
0.00	2.06 ± 0.14c	3.18 ± 0.12d	3.96 ± 0.19d	4.75 ± 0.23d	6.07 ± 0.22e	6.57 ± 0.31d	6.88 ± 0.28d	0.738	0.288	0.9833
0.07	1.83 ± 0.17bc	2.67 ± 0.21c	3.33 ± 0.15c	3.98 ± 0.25c	5.28 ± 0.27d	5.60 ± 0.26c	6.10 ± 0.31c	0.721	0.275	0.9751
0.10	1.68 ± 0.17b	2.43 ± 0.22c	2.68 ± 0.17b	3.67 ± 0.23bc	4.86 ± 0.16c	5.16 ± 0.23bc	5.61 ± 0.22b	0.734	0.263	0.9672
0.30	1.32 ± 0.23a	1.97 ± 0.21b	2.45 ± 0.22b	3.26 ± 0.27b	4.30 ± 0.22b	4.68 ± 0.32b	5.20 ± 0.23b	0.797	0.207	0.9736
0.50	1.11 ± 0.13a	1.62 ± 0.18a	1.97 ± 0.21a	2.75 ± 0.18a	3.66 ± 0.21a	4.00 ± 0.28a	4.55 ± 0.18a	0.814	0.186	0.9663

[a] Tests were performed in triplicate. Mean ± standard deviation values in the same column for each sample followed by different letters are significantly different ($p < 0.05$) by Duncan's test.

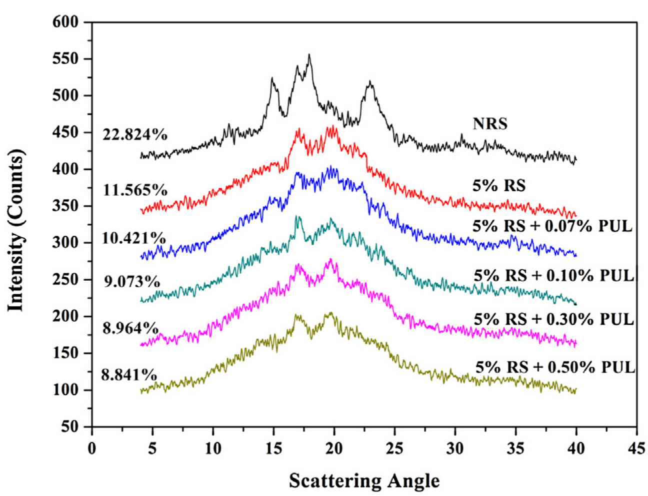

Fig. 6. X-ray diffraction patterns of native rice starch and gelatinized RS pasta containing various ratios of pullulan after storage at 4 °C for 7 days.

after gelatinization and retrogradation reflected the different state of molecular aggregation between native starch and pasted starch. The peak at 20° was assigned to a well-formed V-type XRD pattern, which composed of amylose and naturally occurring fatty acids and phospholipids in the granules (Lalush, Bar, Zakaria, Eichler, & Shimoni, 2005).

Apparently, it was noted that the relative crystallinity decreased from 11.565% to 8.841% with the concentration of pullulan increased from 0 to 0.5%, indicating that there was indeed a retarding effect of pullulan on the recrystallization of amylopectin, just proved the conclusion attained from the DSC experiment above.

4. Conclusions

In summary, this study demonstrated that the addition of pullulan to RS not only could inhibit the short-term retrogradation of amylose, but also could retard and decelerate the long-term retrogradation of amylopectin. The lower retrogradation of starch was ascribed to the inadequate gelatinization and lower leached amylose in the presence of pullulan. Therefore, pullulan could be used as a new aging inhibitor to develop starch-based food with longer shelf lives and better mouthfeel.

The effect of pullulan on the retrogradation of RS was dependant on the addition quantity of pullulan. However, from a business perspective, the application amount could not be very high, because the price of pullulan was much higher than that of rice starch. So the reasonable addition concentration of pullulan should be considered between the anti-retrogradation effect and the cost of the products. Furthermore, the deep-seated mechanism on the molecular level involved the molecular characteristics of both pullulan and starch should be investigated further to uncover the essence behind the phenomenon.

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