EFFECT OF DEHYDRATION METHODS ON DIGESTED STARCH FRACTIONS OF RETROGRADED DEBRANCHED RICE STARCH

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Received: Jul 19, 2010; Revised: Sept 17, 2010; Accepted: Oct 1, 2010

Abstract

The effect of dehydration methods on the fractions of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) was studied. The rice starch suspension was gelatinized and debranched with pullulanase for 24 h. The debranched rice starch (DBRS) was subjected to aging for 48 h with time temperature cycling treatment with temperatures of 30 and 80°C. After aging, the DBRS was dehydrated using various dehydration conditions and methods: 10 and 24% solid with temperatures of 120 and 140°C for drum drying, 10% solid with inlet temperatures of 150 and 170°C and outlet temperatures of 90 and 110°C for spray drying, and 24% solid with 50°C for 10 h and 80°C for 8 h for hot air oven drying. The lower RS content and the higher RDS and SDS content were observed in all dehydration conditions as compared with the fresh sample before dehydration. The spray drying method showed the highest RS of 43%, the lowest RDS of 15% and SDS of 18%, and the melting temperature of 82-110°C, while the dehydration using the hot air oven and drum drying demonstrated the RS content of 39% and 36%, the SDS content of 20% and 21%, and the RDS content of 16% and 17%. The drum drying method exhibited an A+V type crystalline structure with the minimum relative crystallinity of 19% and the maximum melting temperature of 116-123°C, while that of the other dehydration methods illustrated the B+V type.

Keywords: Dehydration method, rice starch, rapidly digestible starch, slowly digestible starch, resistant starch

Introduction

Starch is divided into 3 types with regard to the rate and extent of starch digestion, including rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst *et al.*, 1992). When carbohydrate foods are consumed into the body, they are digested by enzymes to be glucose and absorbed into the bloodstream as the energy for the body. Cooled

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starchy foods containing a high proportion of RDS result in a high level of glucose and insulin hormone in the bloodstream (Englyst *et al*., 1999), while a high proportion of SDS contributes a medium level of glucose and insulin in the bloodstream (Zhang *et al*., 2008). Since it is hydrolyzed into glucose slowly but completely in the small intestine, it can reduce the risk of chronic diseases such as obesity, diabetes, and cardiovascular disease. In addition, a diet containing a high proportion of RS is healthful because the RS is not digested and absorbed in the small intestine. Then, it passes through the colon and is fermented by microorganisms into short-chain fatty acids (SCFA) which are beneficial to the colon. Therefore, the improvement of food quality with higher SDS and RS is of high interest.

The rearrangement of the crystalline structure of starch into the A and B crystalline pattern could influence the enzyme digestion. Jane *et al*. (1997) believed that the shorter double helix and the crystalline structure of the A-type were easier to hydrolyze by enzymes, resulting in a higher content of RDS and SDS when compared with the B-type crystalline structure which was higher in RS content. The debranching, processing, and aging conditions also affected the content of SDS and RS (Guraya *et al*., 2001; Shin *et al*., 2004). Niba (2003) studied the effects of heat treatment, storage temperature, and time on digestion of corn, rice, cassava, yam, plantain, and cocoyam flour and found that storage at room temperature resulted in the reduction of SDS in all flours except plantain and yam, while the storage at freezing temperature caused a decrease in the SDS content of cocoyam flour.

The dehydration/drying process is one of the important factors for starch production. Chiu *et al*. (1994) reported that the dehydration method influenced the RS content in that, after dehydration of debranched Hylon VII using flash drying, the RS content was the highest as compared with air drying and spray drying. In addition, the dehydration of retrograded debranched corn and potato starch using air drying showed that the RS content was more than 80% (Shi *et al*., 2006). Furthermore, Koksel *et al*. (2008) pointed out that the drying of gelatinized–autoclaved corn starch using oven drying showed a higher RS content than the lyophilization. A similar result was observed by Ozturk *et al*. (2009) in that drying retrograded debranched Hylon V and retrograded-debranched Hylon VII using hot air drying showed a higher RS content than freeze drying.

Limited reports on the effects of drying parameters and methods on SDS and RS formation are available. Most studies conducted only the debranching conditions, aging temperature, and aging time on the SDS and RS content. For starch production, dehydration is one of the major steps and should be expected to affect the content of SDS and RS. Therefore, the objectives of this study were to investigate the effect of dehydration conditions and dehydration methods, including drum drying, spray drying, and hot air drying on the starch fractions. Furthermore, the crystalline structure and the thermal properties of the dried sample were also studied.

Materials and Methods

Materials

Rice starch with 30% amylose content was obtained from Cho Heng Rice Vermicelli Factory Co. Ltd. (Nakorn Prathom, Thailand). Commercial pullulanase Promozyme D2 (EC 3.2.1.41, from *Bacillus deramificans,* 1,350 NPUN/g) was purchased from Novozymes A/S (Bagsvaerd, Denmark). Pancreatic α-amylase (EC 3.2.1.1, type VI-B from porcine pancrease, 25 U/mg), amyloglucosidase (EC 3.2.1.3 from *Aspergillus niger,* ≥ 300 U/mL), pancreatin (P1750), PGO enzyme reagent kit (P 7119) and o-dianisidine (D2679) were purchased from Sigma Chemical Co. (St. Louis, Mo., USA). Novelose 330 commercial resistant starch, was a gift from National Starch and Chemical Co. (Bridgewater, NJ, USA). The other chemicals were of analytical grade.

Preparation of Retrograded Debranched Rice Starch

Rice starch suspension (14.5% w/w in 0.1 M acetate buffer, pH 5.0) was gelatinized at 50°C for 10 min and then at 80°C for 20 min with continuous stirring using a 4-bladed propeller overhead stirrer (RW20 digital, IKA Labortechnik, Selangor, Malaysia) and heated at 100°C for 30 min for gelatinization. The paste was cooled to 50°C and pullulanase enyme with a concentration of 60 PUN/g of starch was added. The suspension was incubated with constant stirring at 50°C for 24 h. Then, the activity of the enzyme was stopped by heating at 85°C for 20 min. The debranched rice starch (DBRS) was filtrated to obtain a 24% solid content. The chain length distribution of DBRS was 34.27% of DP 6-12, 44.66% of DP 13-24, 13.22% of DP 25-36 and 7.85% of DP > 37, which was analyzed using a high performance anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD, ICS-2500, Dionex Corp., Sunnyvale. CA, USA). The DBRS was aged with time-temperature cycling (TTC) treatment for 48 h at the temperatures of 30 and 80°C. In the first cycle, the sample was aged at 30°C for 3 h followed by 80°C for 1.5 h. The second cycle was aging at 30°C for 18.5 h followed by 80°C for 1.5 h. In the third cycle, it was aged at 30°C for 18.5 h followed by 80°C for 1.5 h and then cooled down to 30°C for 3.5 h. After TTC aging, the sample was referred to as retrograded debranched rice starch (RDBRS). The RDBRS was subjected to dehydration with 3 different methods including drum drying, spray drying, and hot air oven drying.

Drum Drying

The 24% solid of RDBRS was added with deionized water to prepare a 10% solid content using a blender (National Brand Model MX-T2GN, Matushita Electric (Taiwan) Co., Taipei, Taiwan). The RDBRS with 10% and 24% solid content was passed through a double drum dryer (6 × 8, New Way Manufacturing Co, Ltd., Samutsakorn, Thailand) by setting the distance between the metal drum at 2 mm, the drum speed at 0.5 rpm (47.20 Hz.), and drum temperatures at 120 and 140°C. Then, the samples were ground and sieved (Vibration Sieve Shaker, Fritsch GmbH, Idar-Oberstein, Germany) to obtain a particle size less than 75 microns.

Spray Drying

The solid content of 24% RDBRS was diluted to be 10% using deionized water, then dehydrated using a spray dryer (GEA Niro, Model A/S Gladsaxevej 305 DK 2860, Soeborg, Denmark) with a centrifugal nozzle, and a pressure of 1 bar. The inlet temperatures of 150 and 170°C and outlet temperatures of 90 and 110°C were set. The dried samples were sieved to obtain a particle size less than 75 microns.

Hot Air Oven Drying

The RDBRS with 24% solid content was dried in a hot air oven dryer (Memmert ULE 700 AO, Memmert GmbH, Schwabach, Germany) by setting the temperature of 50°C for 10 h and 80°C for 8 h. Then, the dried sample was ground and sieved to obtain a particle size less than 75 micron.

The Determination of Starch Fractions

The starch fractions (RDS, SDS, and RS) of the samples were measured using a slightly modified method of Englyst *et al.* (1992; 1999). The sample (400 mg) with guar gum (50 mg) was suspended in 20 ml of 0.1 M acetate buffer (pH 5.2) and followed by vortex mixing. Then, the mixture of enzymes of pancreatin and amyloglucosidase (1.6 ml) were added. The samples were incubated at 37°C in a shaking water bath (Tecator 1024, Tecator, Inc., Hoganas, Sweden). After 20 and 120 min of incubation time, 0.4 ml of aliquot was removed into 8 ml of absolute ethanol, mixed well, and centrifuged at 1,500 xg for 3 min. The glucose content in the supernatant was determined using a PGO enzyme reagent kit. The glucose content at 20 and 120 min was referred to as G_{20} and G_{120} respectively.

The RDS is defined as the glucose released after 20 min. The glucose released in the second period (100 min incubation) is defined as SDS. The RS was measured as the starch that remained unhydrolyzed after 120 min of incubation.

Crystalline Structure Property

The crystalline structure was measured using an X-ray powder diffractrometer (Bruker D5005, Bruker GmbH, Karlsruhe, Germany). The RDBRS powder was densely packed into a sample holder and operated at 40 kV and 40mA with CuK_{α} radiation (λ = 1.54Å). Diffractrograms were collected from Bragg's angle (2θ) 4° to 30° with a step size of 0.02°, scan rate of 2.5°/min, divergence slit of 0.5°, anti-scattering of 0.5°, and speed rotation of 30 rpm. The measurement of each sample was performed in triplicate.

The relative crystallinity of the sample was calculated as the ratio of the area of the crystalline peak over the total area using EVA Diffract plus#1 software (Bruker GmbH, Karlsruhe, Germany).

Thermal Property

Thermal properties of the sample were determined using a Pyris Diamond DSC (PerkinElmer Inc., Shelton, CT, USA). The samples (7.5g) were weighed into a 60 µL stainless steel pan and distilled water was added to obtain a dry matter to water ratio of 1:3. The pan was sealed and left overnight at room temperature for equilibration. The samples were heated from 25 to 200°C at a rate of 10°C min^{-1}. Indium was used for the standard and an empty stainless steel pan was used for a reference. The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and enthalpy (ΔH) were calculated automatically using Pyris Dimond sofeware. The measurement of each sample was performed in triplicate.

Statistical Analysis

A completely randomized design (CRD) was performed for the study on the dehydration conditions and dehydration methods on the starch fractions. Analysis of variance (ANOVA) was analyzed using SPSS version 13 (SPSS Inc., IL, USA). The experiment was conducted in duplicate. The differences between mean values were established using Duncan's multiple-range test at 95% significant level. The correlation coefficient was conducted between the relative crystallinity and RS content.

Results and Discussion

Effect of Drum Drying Conditions on the Starch Fractions

The starch fractions of dried RDBRS that were dehydrated by the double drum dryer were demonstrated in Figure 1. The solid content of 10 and 24% and drum temperatures of 120 and 140ºC did not significantly affect the contents of RDS, SDS, and RS ($p > 0.05$). After dehydration, the RDBRS showed the content of RDS, SDS, and RS at 20-22%, 21-26%, and 53-57%, respectively. A decrease in RS content of 27-32% was observed in the dried samples when compared with the fresh RDBRS, whereas the contents of RDS and SDS were increased by 40-46% and 55-78% respectively. The drum drying method contributed directed heat and compression force on the starch samples, resulting in partial destruction of the crystalline structure into the amorphous structure which is more accessible for enzyme digestion. However, the amorphous structure could be partly packed into the dense structure of the SDS which inhibited the enzyme accessibility as noticed from the higher percentage of the increased SDS than that of the increased RDS (Zhang *et al.*, 2006). Due to the fact that the drum drying temperatures of 120 and 140ºC were higher than the meltin g temperature of freeze-dried RDBRS, which was 87-108ºC as measured by DSC, it could result in similar damage of the crystalline structure. Therefore, all the starch fractions were not different.

Effect of Spray Drying Conditions on the Starch Fractions

The results in Figure 2 illustrated that the inlet temperatures of 150 and 170ºC and the outlet temperatures of 90 and 110ºC of spray drying did not show the differences in the RDS, SDS, and RS content of dried RDBRS ($p > 0.05$). It is probably due to the fact that both the inlet and outlet temperatures were not far apart from each other. The dried RDBRS showed the RDS, SDS, and RS content in the range 19-22%, 21-26%, and 53-57%, respectively. As compared with the fresh RDBRS, the RDS and SDS contents in the spray dried samples were higher by 13-22% and 31-60% respectively but the RS content was lower by 13-20%. This tendency is similar to the drum drying result. During spray drying, the heat from hot air may destroy some regions of the crystalline structure into the amorphous structure of the RDS. These regions of the amorphous structure were probably able to be packed into the dense structure of the SDS, similar to the drum drying method.

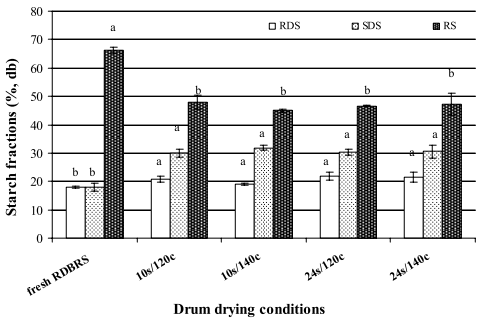

Figure 1. Effect of drum drying conditions on the starch fractions and the fresh retrograded debranched rice starch (RDBRS).[a, b] Means with the different letters above the solid graph are significantly different ($p < 0.05$)

Effect of Hot Air Oven Drying Conditions on the Starch Fractions

The drying with a hot air oven at 50ºC for 10 h and 80ºC for 8 h did not affect the RDS, SDS and RS content ($p > 0.05$) as shown in Figure 3. Although the drying time of 50ºC was longer than that of 80ºC for 2 h, similar partial disorganization of the crystalline structure occurred; consequently, the enzyme digestibility of both samples was not different. The RDS, SDS, and RS contents of hot air oven drying were 20-21%, 26-28%, and 51-54%, respectively. Tribess *et al.* (2009)

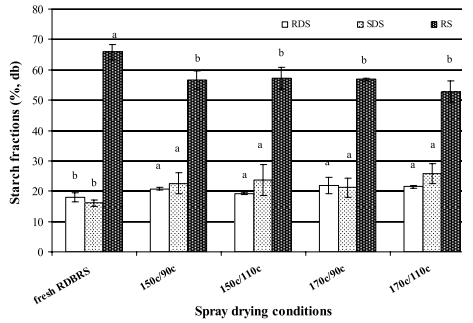

Figure 2. Effect of spray drying conditions on the starch fractions and the fresh retrograded debranched rice starch (RDBRS).[a, b] Means with the different letters above the solid graph are significantly different ($p < 0.05$)

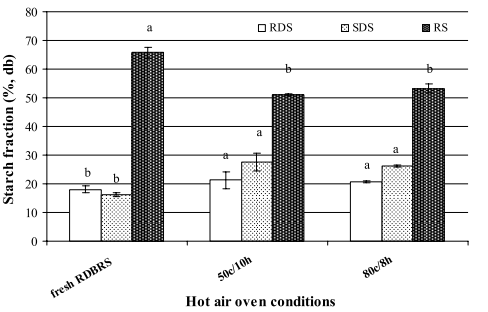

Figure 3. Effect of hot air oven drying conditions on the starch fractions and the fresh retrograded debranched rice starch (RDBRS).[a, b] Means with the different letters above the solid graph are significantly different ($p < 0.05$)

also founded that different air temperatures (52-58ºC) at the same air velocity of drying process did not affect on the RS type II content of banana flour. When compared with the fresh RDBRS, the RS content of the dried sample was decreased by 19-22% whereas the RDS and SDS contents were increased by 14-18% and 62-71% respectively. This tendency was similar to the drum and spray drying results. It suggested that after drying, a partial ordered structure of the RS was destroyed to become the RDS and SDS. The structure of SDS contains amorphous components and double helical components with partially ordered structure (Guraya *et al*., 2001).

Comparative Methods of Dehydration on the Starch Fractions

The different drying methods were used to dehydrate the RDBRS, including drum drying (10% solid and the drum temperature of 120ºC), spray drying (10% solid, the inlet temperature of 150ºC and the outlet temperature of 110ºC), and hot air oven drying (24% solid and the temperature of 50ºC for 10 h). The RS fraction of dried samples was different depending on the dehydration method ($p < 0.01$) but the RDS and SDS fractions were not different ($p > 0.05$). All dried samples showed the highest RS fraction of 48-56%, followed by the SDS fraction of 24-30% and the RDS fraction of 20-22% as illustrated in Table 1. The spray dried sample showed higher RS (55.93%) than the hot air (51.07%) and drum (48.06%) drying methods. When compared with the fresh RDBRS, it also indicated a minimum decrease in RS of 15% while the drum drying method resulted in a maximum decrease in RS of 27%. Chiu *et al*. (1994) found that the dehydration of the debranched Hylon VII starch using filtration followed by flash drying yielded 30.60% RS which was higher than that of air drying at room temperature (26% RS) and spray drying (25% RS). Shi *et al*. (2006) also reported that the dehydration of the retrograded debranched waxy maize starch and the retrograded debranched low amylose potato starch using filtration followed by air drying resulted in the RS content of more than 80%. In our study, the drum drying caused a greater extent of damage to the RS structure. It was probably due to the fact that the RDBRS directly contacted the hot surface of the metal rollers combined with the compression force of the rollers for 60 sec. Incontrast, the dehydration with the spray dryer and hot air oven involved

Table 1. The starch fractions of dried retrograded debranched rice starch (RDBRS) with different dehydration methods

Dehydration methods	Starch fractions (%, db)		
	RDS	SDS	RS
Drum drying [1]	22.11 ± 0.99 [b]	29.83 ± 1.45 [c]	48.06 ± 2.44 [c]
Spray drying [2]	20.42 ± 0.26 [b]	23.64 ± 5.17 [c]	55.93 ± 3.22 [b]
Hot air oven drying [3]	21.31 ± 3.94 [b]	27.62 ± 4.19 [c]	51.07 ± 0.25 [c]
Novelose 330	13.26 ± 0.14 [a]	21.27 ± 0.69 [b]	65.47 ± 0.56 [a]
Fresh RDBRS	18.04 ± 1.50 [b]	16.14 ± 1.02 [a]	65.81 ± 2.51 [a]

[1] 10% solid, drum temperature of 120ºC

[2] 10% solid, inlet temperature of 150ºC and outlet temperature of 110ºC

[3] 24% solid, temperature of 50ºC for 10 h

[a, b, c] Means with the different letters in the column are significantly different ($p < 0.01$).

only hot air with a lesser processing time and a much lower temperature respectively. It could destroy less of the crystalline structure. Therefore, the RS fraction of spray drying was higher than that of the other methods but lower than the commercial RS (Novelose 330), which was 65.46%.

As aforementioned, the RS content reduced as a result of all thermal dehydration methods. When compared with the fresh RDBRS, the RS content of all thermal dehydration methods was decreased by 13-27% whereas the RDS and SDS contents were increased by 6-23% and 46-85% respectively. It indicated that the destruction of the crystalline structure was not to an extent as great as the percentage of the increased SDS fraction that appeared in all dehydration methods. Therefore, the thermal dehydration perhaps destroyed only the less perfectly ordered structure into the partially ordered and amorphous structure of the SDS to a greater extent than the amorphous structure of RDS.

The Crystalline Structures of the Dried RDBRS

The crystalline structures of dried RDBRS are presented in Figure 4. There are 2 types of crystalline pattern. The dehydration of RDBRS using spray drying and hot air oven drying showed a similar XRD pattern as the commercial RS (Novelose 330), with a singlet peak at 2θ of 17° and a doublet peak at 22° and 24° which is a characteristic of B type starch (Buleon *et al.*, 1998). In addition, a singlet peak at the 2θ of 19.7° also appeared, indicating a characteristic of the V type crystalline pattern (Derycke *et al.*, 2005). Thus, the spray and hot air dried RDBRS were classified to be a B+V type similar to the Novelose 330. In contrast, the drum dried RDBRS showed a different diffraction pattern with the singlet peaks at 2θ of 15° and 23° and a doublet peak at 17° and 18° which was a characteristic of the A type starch that observed in native rice starch (Figure 4).

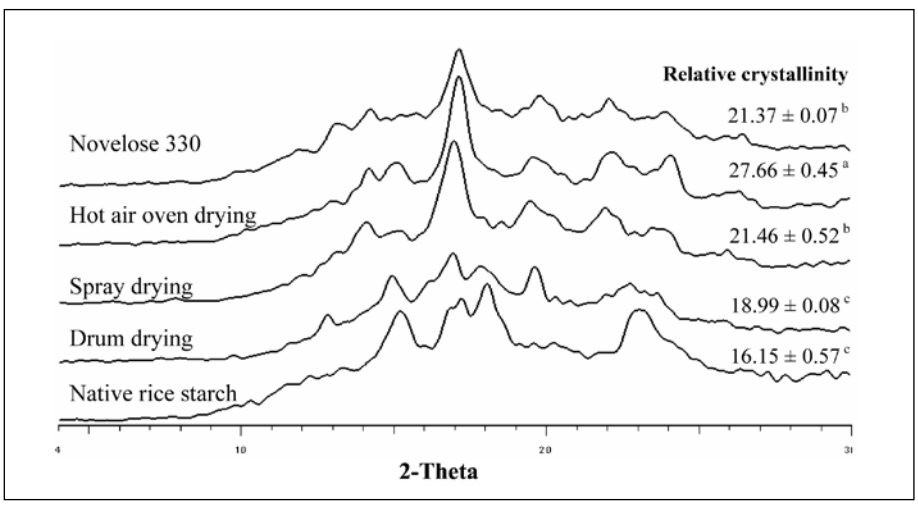

Figure 4. XRD patterns of the dried retrograded debranched rice starch (RDBRS) with different dehydration methods as compared with the native rice starch and the commercial RS (Novelose 330). Drum drying with 10% solid, drum temperature of 120°C; Spray drying with 10% solid, inlet temperature of 150°C and outlet temperature of 110°C; Hot air oven drying with 24% solid, temperature of 50°C for 10 h. [a, b, c] Means with the different letters in the graph are significantly different ($p < 0.01$)

Furthermore, it also showed a singlet peak at 2θ of 19.7°, a characteristic of the V type cryst alline pattern. Therefore, the drum dried RDBRS had a crystalline structure of A+V type. The result suggested that spray drying and hot air oven drying did not change the crystalline structure of RDBRS which showed the B type (data not shown), while the drum drying caused a transformation of RDBRS crystalline structure from B type to A type which may be a result of direct contact with the hot metal rollers and their compression force. Similar results were reported by Ao *et al.* (2007), who found that a slow digestion property of maize starch that was prepared by debranching and dehydration using a spray dryer showed the crystalline structure of B+V type.

The relative crystallinity in Figure 4 demonstrated that the dehydration methods influenced the crystallinity of the dried RDBRS ($p < 0.01$). The maximum relative crystallinity of 27.66% was observed in the hot air dried sample, followed by 21.46% in the spray dried sample which was not different from that of Novelose 330 (21.37%) and the drum dried sample (18.99%). Regarding the crystalline property, the RS content of dried RDBRS had no significant correlation with the relative crystallinity ($p > 0.05$). It indicated that the RS content did not depend on the crystallinity. The differences in the crystallinity of dried RDBRS could be due to the crystalline size, amount of crystalline regions, orientation of the double helices within the crystalline structure, and extent of interaction between double helices (Song and Jane, 2000).

Thermal Properties of the Dried RDBRS

The thermal transition parameters were summarized in Table 2. The endothermic transition temperature of the RDBRS was 115.8-122.7°C after subjecting to the drum drying. The spray dried and hot air dried RDBRS showed similar transition temperatures of 81.5-109.7°C and 83.3-106.9°C respectively. However, the endothermic transition temperature of Novelose 330 was 109.6-127.4°C. The result of thermal transition temperatures was associated with that of the crystalline pattern. The crystalline structure of drum dried

Table 2. Thermal transition parameters of dried retrograded debranched rice starch (RDBRS) with different dehydration methods as compared with the commercial RS (Novelose 330)

Dehydration methods	T_O (°C)	T_P (°C)	T_C (°C)	ΔH (J / g)
Native rice starch	72.7 ± 0.1 [d]	77.7 ± 0.1 [c]	82.0 ± 0.1 [d]	14.7 ± 1.8 [a]
Drum drying [1]	115.8 ± 0.3 [a]	117.0 ± 0.1 [a]	122.7 ± 0.3 [a]	2.6 ± 0.0 [c]
Spray drying [2]	81.5 ± 0.2 [c]	99.4 ± 0.1 [b]	109.7 ± 2.4 [b]	12.3 ± 2.7 [a]
Hot air oven drying [3]	83.3 ± 0.5 [c]	99.5 ± 1.3 [b]	106.9 ± 0.1 [c]	6.2 ± 0.5 [b]
Novelose 330	09.6 ± 0.7 [b]	118.8 ± 0.2 [a]	127.4 ± 0.4 [a]	13.4 ± 1.6 [a]

T_o, T_p, T_c = onset, peak, and conclusion temperature respectively; ΔH = enthalpy

[1] 10% solid, drum temperature of 120°C

[2] 10% solid, inlet temperature of 150°C and outlet temperature of 110°C

[3] 24% solid, temperature of 50°C for 10 h

[a, b, c, d] Means with the different letters in the column are significantly different ($p < 0.01$).

RDBRS was the A+V type which had higher stability than the B type (Gidley, 1987). It was possible that the drum drying process destroyed the ordered structure and induced the rearrangement of the ordered structure into the perfect crystalline structure, consequently elevating the melting temperature.

During aging, the starch molecules re-associated as double helices and could form the ordered structure of RS stabilized by hydrogen bonding (Eerlingen and Delcour, 1995). After dehydration process, the partial parts of the double helices and the order structure were destroyed, resulting in the lower levels of hydrogen bonds.

The results in Table 2 showed that the enthalpy of spray dried RDBRS was highest at 12.3 J/g, followed by hot air dried RDBRS at 6.2 J/g and drum dried RDBRS at 2.6 J/g. The RS content of dried RDBRS (Table 1) had a good positive correlation with the enthalpy ($r = 0.99$, $p < 0.05$). Cooke and Gidley (1992) also inferred that the enthalpy value was referred to the loss of double helix structure rather than the loss of crystallinity.

Conclusions

The conditions of each dehydration method had no effect on the fractions of RDS, SDS, and RS. The starch fractions of all dried RDBRS showed a similar trend with the highest content of RS and a similar content of the RDS and SDS. Each condition of the dehydration methods showed the reduction of the RS content but an increase in the RDS and SDS as compared with the fresh RDBRS due to the destruction of the crystalline structure after the dehydration process. The spray drying method produced the dried RDBRS with the highest RS content and enthalpy. The spray dried and hot air dried RDBRS showed a B+V type crystalline pattern and a similar melting temperature of 82-110°C. The drum drying method transformed the crystalline structure of dried RDBRS from a B+V type to an A+V type, resulting in the higher melting temperature.

Acknowledgement

This work was financially supported by National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Thailand.

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