Songklanakarin J. Sci. Technol.
43 (5), 1275-1282, Sep. - Oct. 2021

http://www.sjst.psu.ac.th

Original Article

Effect of pullulanase debranching on the physical and chemical properties of instant Jasmine rice porridges

Jirayus Woraratphoka[1*], Chompoonuch Khongla[1], Sumalee Musika[1], Artit Ausavasukhi[1], Plengpin Pianpumepong[2], Dusadee Teimtes Boonmasongsung[3], and Worawikunya Kiatponglarp[4]

[1] *Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Mueang, Nakhon Ratchasima, 30000 Thailand*

[2] *Faculty of Engineering and Architecture, Rajamangala University of Technology Isan, Mueang, Nakhon Ratchasima, 30000 Thailand*

[3] *Faculty of Business Administration, Rajamangala University of Technology Isan, Mueang, Nakhon Ratchasima, 30000 Thailand*

[4] *Synchrotron Light Research Institute, Mueang, Nakhon Ratchasima, 30000 Thailand*

Received: 24 August 2020; Revised: 16 October 2020; Accepted: 19 October 2020

Abstract

Gelatinized rice starch was debranched with pullulanase at 60°C for 0, 2, 4, 8, 16, and 24 h. Then, freeze/thaw process was applied prior to tray drying, and chemical and physical property evaluations. The starch that was debranched for 16 h showed the highest degrees of hydrolysis and syneresis, namely 18.82 % and 35.40%, respectively. This corresponded to the higher resistant starch (RS) value that slightly increased from 0.32% for 0 h to 0.84% for 16 h. X-ray diffraction and FTIR analyses of 16 h debranched instant rice porridge confirmed the double helix formation and amylose-lipid complexes, which showed increased 12.18% crystallinity and intensity ratios (R) of 1045/1037 cm^{-1} (0.862) and 1014/995 cm^{-1} (1.002), respectively. TGA results revealed that enzymatic treatment slightly altered the decomposition temperature by raising it and gave a faster rate of decomposition. Based on this study, improving rice porridge to be suitable food for health-conscious consumers is possible by pullulanase treatment.

Keywords: pullulanase debranching, rice porridges, resistant starch, amylose-lipid complex

1. Introduction

Instant rice porridges have become more popular as breakfast cereals for a large part of Asia's population, especially in Thailand. Among the many varieties of rice, Kao Dok Mali 105 (*Oryza sativa* L.) or Jasmine rice is the most popular variety for consumption because of its pleasant aroma and tender texture. Consequently, Jasmine rice is widely used as the major component in the production of instant rice porridges. Rice is usually recognized for its fairly high glycemic index (GI), with a recorded range of 54-121, in comparison to other starchy foods (Jenkins *et al*., 1981). However, there have been reports on reduced metabolic responses to and GI values for high-amylose rice variants (Hu, Zhao, Duan, Linlin, & Wu, 2004). The consumption of foods with low glycemic responses is seen as advantageous for the dietary management of metabolic disorders, especially of diabetes and hyperlipidemia (Jenkins *et al*., 1987).

*Corresponding author
Email address: jirayus.wo@rmuti.ac.th

A combination of both physical and chemical factors can mediate the metabolic reaction to starchy food (Björck, Lifebergand, & Ostman, 2000**)**. One of these is modulated by altering the starch structure. Resistant starch (RS) is that starch portion which resists digestion by human enzymes in the upper part of the gastrointestinal tract and reaches the large intestine, where it can be partly or fully fermented with microflora. The major health benefits from RS consumption can be categorized as glycemic response reaction, body weight management, and intestinal wellbeing (Sajilata, Singhal, & Kulkarni, 2006). For resistant starch type 3 (RS3), a long while ago, both *in vitro* and *in vivo* resistance of retrograded amylose to α-amylase digestion were elaborated (Englyst & Cummings, 1985). Upon gelatinization, the released starch molecules continue to re-associate during cooling, creating tightly packed structures with hydrogen bonding and contributing to digestion resistance (Patel *et al*., 2017). The quantity of retrograded amylose, corresponding to RS3, depends on the proportion of amylose and its chain size (Dupuis, Liu, & Yada, 2014). High amylose content and process treatments tend to increase resistant starch proportion (Walter, Silva, & Denardin, 2005). The amylose content of Thai Jasmine rice is usually between 12-17% by weight, which is relatively less in comparison to other rice varieties. The treatment by pullulanase debranching has been used to generate samples with linear, low-molecular weight amylose chains (Guraya, James, & Champagne, 2001; Pongjanta, Utaipatanacheep, Naivikul, & Piyachomkwan, 2008). Pullulanase enzymes rapidly hydrolyze only α-1,6-glycosidic bonds. This releases a mixture of varied chain length amylose molecules, which promote the retrogradation of starch. In addition, retrogradation also increases as starch gels undergo freezing and thawing treatments (Tovar, Carmelo, Eggar, Ana, & Elevina, 2002).

Therefore, this study aimed to assess the effects of debranching by using pullulanase enzyme on chemical and physical properties, including resistance to digestion, in instant Jasmine rice porridge.

2. Materials and Methods

2.1 Materials

Jasmine rice (harvested in 2018), broken, from the polishing process, was kindly supplied by Nakhonkong community enterprise, Nakhon Ratchasima, Thailand. Pullulanase enzyme (EC 3.2.1.41) (food grade) iKnowZyme PL was purchased from Reach Biotechnology (Bangkok, Thailand). Resistant Starch kits were purchased from Megazyme (Bray, Co Wicklow, Ireland). All other chemicals and reagents used in the experiments were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Instant rice porridge model

Broken Jasmine rice were boiled in water at the ratio of 1:6 (w/v) for approximately 30 min until completely gelatinized, then cooled down to 60°C. The rice porridges were subjected to pullulanase (EC 3.2.1.41) hydrolysis (15 units per gram porridge). A portion of 500 g were debranched at 60°C for 0, 2, 4, 8, 16, or 24 h in a water bath. The debranched samples were boiled at the end of each time interval for 30 min to deactivate the enzyme. The debranched porridges with different degrees of hydrolysis were stored at 4°C for 24 h. Then one cycle of freeze/thaw process (-20°C/30°C) was applied to the samples to promote syneresis of the retrograded starches. The retrograded rice porridge was dried in a tray drier (Memmert, UF110, Germany) at 60°C for 24 h to reach the moisture content of 6-8%. The dried instant rice porridges were ground and then kept in plastic bags for further analysis.

2.3 Degree of debranching

After each hydrolysis and enzyme deactivation, reducing sugar (Rds) and total sugar (Ts) in the debranched rice porridge samples were analyzed according to DNS method (Miller, 1959) and phenol-sulfuric acid reagent method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) respectively. The level of the debranching of different rice porridge is reported in terms of the degree of debranching (DD):

$$DD\ (\%) = \frac{\text{Rds after hydrolysis}}{\text{TS after hydrolysis}} \times 100 \qquad (1)$$

2.4 Degree of syneresis (DS)

DS of the samples was determined according to Yuan and Thompson (1998) with modifications. Centrifugation was applied at 3,000×g for 10 min to each portion of the debranched starch paste after one cycle of the freeze-thaw process to separate the liquid from the gel. Syneresis water from retrograded gels was collected and weighted. The DS was calculated as follows:

$$DS\ (\%) = \frac{\text{weight of syneresis}}{\text{weight of gel before centrifugation}} \times 100 \qquad (2)$$

2.5 Water absorption index (WAI)

WAI was measured following the method of Anderson, Conway, & Peplinski (2006). A sample of 2.5 g was hydrated by the addition of 30 ml of distilled water. The rice paste was incubated at room temperature for 30 min, while being mixed every 5 min. Then, centrifugation was applied at 2,200×g for 15 min. The supernatant was discarded and then the residue was weighted. The WAI was calculated as follows:

$$WAI = \frac{\text{weight of residue}}{\text{weight of sample}} \qquad (3)$$

2.6 Resistant starch (RS)

The amount of RS was determined using a Megazyme Resistant Starch kit. A 100 mg ground sample was incubated with pancreatic α–amylase (PAA) and amyloglucosidase (AMG) (PAA 0.8kU/ml and AMG 0.34 KU/ml) for 16 h at 37°C in a shaking water bath to hydrolyze digestible starch to glucose. The reaction was terminated with 4 ml 95% ethanol and the RS sediment was recovered by

centrifugation (3,250×g, 10 min). The supernatant was disposed, then the RS sediment was washed twice with 50% ethanol to eliminate the digested starch (DS). The sediment was solubilized in 2 ml of 1.7 M sodium hydroxide in an ice bath, neutralized with 8 ml of 1.2 M sodium acetate buffer, pH 3.8, and the RS was hydrolyzed into glucose with amyloglucosidase (0.1 ml or 0.3 ml, 3300 U/ml, 50°C, 30 min). The absorbance of the released glucose was spectrophotometrically determined at 510 nm using glucose oxidase-peroxidase reagent. RS was calculated as follows, with F = 96.6884:

$$\text{RS (g/100g sample)} = \Delta Ab \times F \times \frac{100}{\text{sample vol.}} \times \frac{1}{1000} \times \frac{100}{\text{Weight}} \times \frac{162}{180} \quad (4)$$

2.7 X-ray diffraction (XRD)

The XRD patterns of instant rice porridges were obtained using an X-ray diffractometer (LabX-XRD-6100, Shimadzu, USA). The diffractometer was operated at 40mA, 40 kV. The scan range of diffraction angle (2θ) was from 5 to 35°θ at 0.05° step size with a count time of 2s. The relative crystallinity of instant rice flours was calculated as the ratio of the crystalline peak area to the total area (crystalline peak and amorphous peak) of a diffractogram.

2.8 Scanning electron microscope (SEM)

Scanning electron micrographs were observed by SEM (FEI, Quanta 250, USA). The rice porridge samples were stuck on an aluminum stub with electrically conductive double-sided adhesive tape. An acceleration potential of 10 kV was used during microscopy.

2.9 Fourier transform infrared (FTIR)

FTIR spectra were obtained by using a Fourier transform infrared spectrometer equipped with a deuterated triglycine sulphate (DTGS) detector and attenuated total reflectance (ATR) accessory with a diamond crystal (Bruker tensor 27 FT-IR spectrometer, Bruker Optics Ltd, Ettlingen, Germany). Powder samples (moisture content 7-10%) were pressed on the crystal and measured immediately. The spectra were collected at 4 cm^{-1} resolution across 750-4000 cm^{-1} and with 64 scans per recorded spectrum at room temperature. The spectrum baseline correction was done in the region of 1200 - 800 cm^{-1} and then deconvoluted using a deconvoluted factor of 6. The peak intensity ratios (R) of 1014/995 cm^{-1} and 1045/1037 cm^{-1} were calculated to represent the degree of amylose-inclusion (Kumar, Woortman, & Loos, 2013) and degree of short-range molecular order (Van Soest, Tournois, de Wit, & Vliegenthart, 1995) respectively.

2.10 Thermogravimetric analysis (TGA)

TGA determination of lost water was made using thermogravimetry (STA600, Perkinelmer) with an open pan. Samples (7-8 mg) were tested at 10°C/min over the 50-600°C temperature range in nitrogen gas atmosphere.

2.11 Statistical analysis

Data were subjected to the analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test to call significant differences ($p<0.05$). The Statistical Package for Social Science SPSS version 16.0 (SPSS Inc, Ils, U.S.A.) was used.

3. Results and Discussion

3.1 Degree of pullulanase debranching

Degree of debranching obtained at various hydrolysis times, which relates to the number of reducing groups produced by action of pullulanase on rice paste from 0 to 24 h, is depicted in Figure 1A. The degree of debranching significantly increased from 0 to 24 h, reaching its maximum (18.82%) at 16 h and then tending to be unchanged at 24 h of debranching. An increase in the degree of syneresis was consistent with the degree of hydrolysis (Figure 1B), which can be attributed to the hydrolysis by pullulanase enzyme at α-1,6-glycosidic branch points and released linear α-1,4-D-glucan chains (A, B and C linear chains) of amylopectin. These linear fragments could re-associate and aggregate (Trinh, 2015) into indigestible aggregate morphology. Moreover, tightly packed structures were formed in the debranched starch network during the freeze-thaw process, releasing syneresis water from the network and possibly reducing digestibility of the starch (Pongjanta *et al*., 2008).

3.2 Morphological properties of instant rice porridges

SEM micrographs of native broken rice and instant rice porridge are shown in Figure 2. The polygonal shaped starch granules are densely packed in native broken rice as observed in Figure 2 A and D, whereas the rice porridge granules were approximately 1-3 mm in size and had an erosion surface with no starch granularity. During the gelatinization, there was adhesion between rice kernels (Wang *et al*., 2019). Therefore, the disruption of starch granularity meant that the rice porridge became gelatinized during cooking. The gelatinized rice porridge (without enzymatic treatment, 0 h) (Figure 2 B, E) and after pullulanase hydrolysis for 16 h (Figure 2 C, F) showed similar appearances, but the latter had a more dense formation which rendered it more difficult to rehydrate (Deng, Wang, Wang, Zhou, & Xiao, 2012). In addition, it was found that the enzymatic treatment resulted in the porridge having decreased lightness and increased yellowness in color coordinates (data not shown).

3.3 Moisture content and Water absorption index (WAI) of the instant rice porridges

The moisture content, after 24 h drying, of starch tended to increase with hydrolysis time in pullulanase treatment, from 6.88% to 8.86% at 0 and 24 h, respectively (Table 1). The WAI is an indicator of the capacity of instant porridge to absorb water when immersed in excess water, and depends on the capability of hydrophilic groups to bind water and macromolecules to form a gel (González-Soto, Mora-

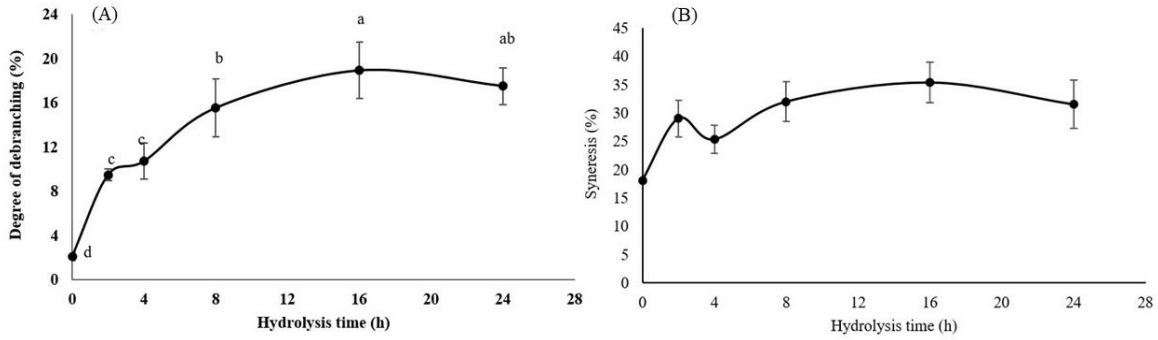

Figure 1. Degree of pulluanase debranching (A) and degree of syneresis (B) of rice porridge, treated by pullulanase (15 unit per gram sample) and incubation at 60°C for 0-24 h. The different letters indicate significant differences ($p \leq 0.05$).

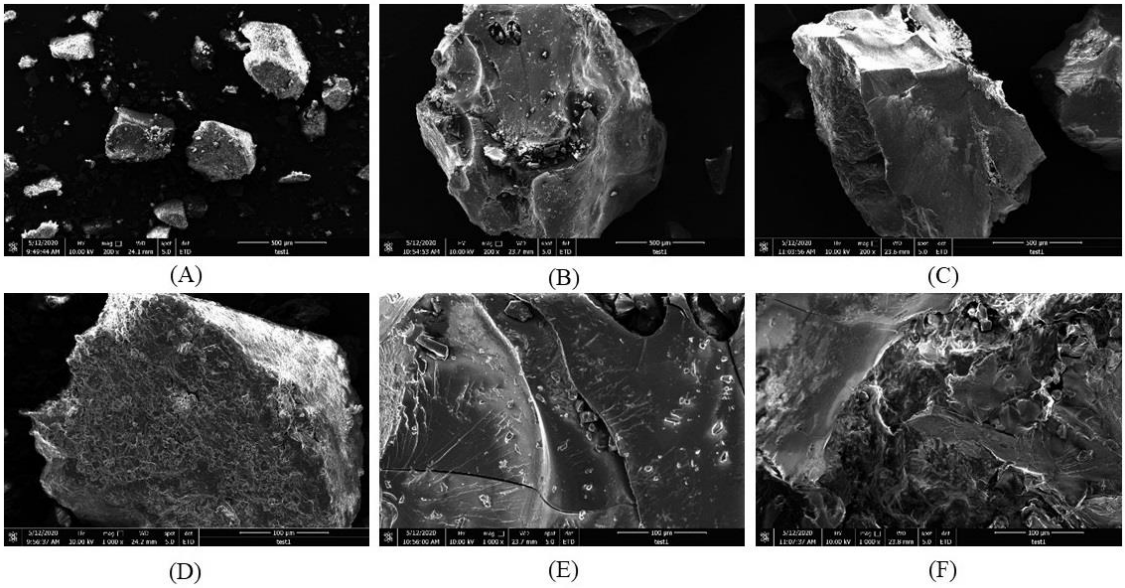

Figure 2. Scanning electron micrographs at 200x and 1,000x of native rice (A, D) and instant rice porridge for 0 h of debranching (B, E) and 16 h of debranching (C, F)

Table 1. Physical and chemical properties of rice porridges debranched for various times

Debranching time (hours)	Moisture content (% d.b.)	Water absorption index, WAI	Resistant starch (% d.b.)
0	6.88 ± 0.04^{b}	7.41 ± 0.66^{a}	0.32 ± 0.01^{c}
2	7.35 ± 0.06^{b}	6.49 ± 0.10^{b}	0.13 ± 0.00^{e}
4	7.44 ± 0.04^{b}	5.98 ± 0.17^{c}	0.24 ± 0.01^{d}
8	8.77 ± 0.03^{a}	5.32 ± 0.09^{d}	0.42 ± 0.01^{b}
16	8.83 ± 0.11^{a}	4.84 ± 0.05^{d}	0.84 ± 0.00^{a}
24	8.86 ± 0.07^{a}	4.93 ± 0.07^{d}	0.78 ± 0.10^{a}

[a-d] Values in the same column with different superscripts are significantly different ($p \leq 0.05$)

Escobedo, Hernández-Sánchez, Sánchez-Rivera, & Bello-Pérez, 2007). Jasmine rice porridge without pullulanase hydrolysis had a WAI of 7.41, which differs from the study of Mayachiew (2014) which showed WAI values of 11.51 and 7.76 for Jasmine and Sangyod rice porridges (drum dried), respectively. This difference might be caused by different cooking and drying processes. When the rice porridge was subjected to pullulanase enzyme, the WAI tended to decrease with debranching time from 7.41 for 0 h to 4.84 for 16 h (Table 1). The low WAI for 16 h treated rice porridge corresponds well to the microscopic structure results. Moreover, Xu *et al.* (2015) reported similar findings on the WAI of extruded rice, namely that α-amylase treatment during extrusion resulted in a substantial decrease in WAI compared to conventional extrusion. The reduction in WAI might be caused by the breakdown of starch particles with an exceptionally high loss of integrity due to the enzymatic treatment.

3.4 Resistant starch (RS) of the instant rice porridges

RS is physically inaccessible to digestive enzymes and is a substrate for microflora in the large intestine. The results indicate that an increased pullulanase debranching time resulted in an increase in RS to a maximum of 0.84% at 16 h (Table 1). This is more than in commercial porridge, which does not have any RS content (data not shown). However, the RS contents in these Jasmine rice porridges are considered low when compared with the study of Elmstahl (2002), which showed an RS of 1.2% in rice porridge marketed in Sweden. Normally, the use of pullulanase enzymes to debranch the amylopectin and produce linear amylose chains is an effective way of increasing RS content in various starches. Pongjanta *et al*. (2008) modified 15 % (w/w) high amylose (32.10%) rice starches with different degrees of pullulanase hydrolysis, resulting in the increase of RS content from 4.80 to 12.33% at 0 to 48 h of hydrolysis. Thus, the amount of RS depends on the concentration and chain length distribution of linear chains, incubation temperature, and hydrothermal processing (Guraya *et al*., 2001; Pongjanta *et al*., 2008). The low RS content in this study might be because of medium amylose content in Jasmine rice (14-15%) with a distribution of degrees of polymerization (DP) of amylopectin branch chain lengths of 3-11 (21.38%), 12-24 (64.18%) and 25-40 (11.44%) (Sirivongpaisal, Hill, Pradipasena, & Mitchell, 2005). Moreover, pullulanase could be releasing amylose fragments, but their chain lengths might not be appropriate for the amylose network structure. However, the RS is still very low, and changing to a higher amylose rice variety, or optimizing the conditions of pullulanase debranching, or a combination with isomerase enzyme, may be possible ways to increase the RS content in rice porridge.

3.5 X-ray diffraction (XRD) pattern and relative crystallinity of the instant rice porridges

XRD patterns of native rice starch and instant rice porridges (0-24 h pullulanase debranching and freeze-thaw process) are shown in Figure 3. The diffraction pattern of native rice starch was classified as an A-type pattern as indicated by the typical peaks at 14.9°, 16.9°, 17.9°, 19.9° and 23.0°(2θ). The gelatinized starch exhibited amorphous pattern with a tiny weak peak at 2θ = 19.9° which was attributed to the formation of amylose–lipid complexes (V-type) (Gernat, Radosta, Anger, & Damashun, 1993). Debranching time as well as the recrystallization process influenced the XRD pattern and relative crystallinity of debranched rice porridge, with an increase in crystallinity from 3.87% to 12.18% from 0 to 16 h of debranching time (Table 2). Peak intensities at 13.5° and 19.9°(2θ) were much greater for debranched samples than for the amorphous samples, indicating that the released amylose formed a helical complex with native lipid in rice porridge. Furthermore, with an increase in debranching time, the development of B-type crystals with a peak at 17.3°(2θ) was observed from 16 h to 24 h, which is for retrograded starch. The polymorph of all debranched samples changed from A-type into a combination of B- and V-types, and this pattern of resistant starch is similar to the study of Denchai *et al*. (2019) on gelatinized high amylose rice (Chainat 1 variety) starch. An intense refraction at 2θ = 16.8° and at 17.1°θ representing the B-type was found for retrograded starch (Wang, Li, Copeland, Niu, & Wang, 2015).

3.6 FTIR of the instant rice porridges

The chemical structures of instant rice porridge and conformation changes during the ordered structure formation and amylose-lipid complex formation were investigated by FTIR. The FTIR spectra of instant rice porridge at different debranching times are presented in Figure 4. The typical starch functional group stretching and bending vibrations

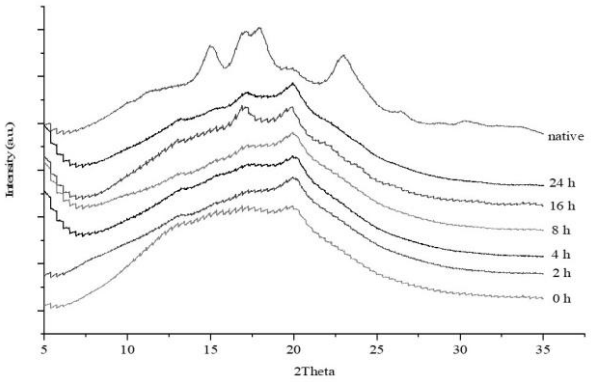

Figure 3. The X-ray diffraction patterns of instant rice porridges debranched for various times

Table 2. Structural characteristics of instant rice porridges with various debranching times as determined by XRD and FTIR

Debranching time (hours)	Relative crystallinity (%)	IR ratio 1045/1037	IR ratio 1014/995
0	3.87	0.853±0.002	0.989±0.007
2	3.80	0.857±0.003	0.990±0.005
4	4.09	0.856±0.003	0.993±0.007
8	4.85	0.858±0.002	1.003±0.007
16	12.18	0.862±0.002	1.002±0.007
24	7.96	0.857±0.002	1.003±0.008

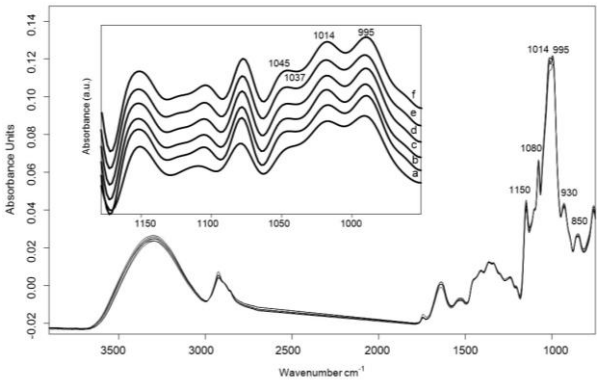

Figure 4. ATR-FTIR spectra of instant rice porridges debranched for various times: (a) 0 h, (b) 2 h, (c) 4 h, (d) 8 h, (e) 16 h, and (f) 24 h (Insert shows the deconvoluted ATR-FTIR spectra).

appeared in the respective ranges 900–1200 and 2800–3400 cm^{-1}. The characteristic peaks of amylose (for C–C and C–O bonds) were located at approximately 995, 1080, and 1150 cm^{-1}, respectively. The vibration of the glycosidic bond (C–O–C) and stretching of bond (O–H) appeared at 850 and 3250 cm^{-1}, respectively (Nikonenko, Buslov, Sushko, & Zhbankov, 2000). The IR absorbance band at 1047 cm^{-1} was sensitive to the amount of ordered starch or crystallites, which were more organized than those of the amorphous region. Thus, the intensity ratio (R) of 1045/1037 cm^{-1} is useful to quantify the degree of short-range order (double helix formation) in starch (Van Soest *et al*., 1995). The R 1045/1037cm^{-1} value increased with prolonged debranching time, to a maximum for 16 h (0.862) (Table 2), which corresponded to the results on the maximum degree of branching (Figure 1A), on resistant starch (Table 1), and on crystallinity percentages (Table 2). These results confirm that debranching by pullulanase enzyme resulted in the release of short-chain amylose molecules, which assembled into a double helix form of RS after the freeze/thaw process.

The band at 995 cm^{-1} represents hydrated crystalline domains related to hydrogen bonding (Van Soest *et al*., 1995). Moreover, the inclusion complex peak appeared at 1014 cm^{-1} and this peak was obviously dominant with an increase in the concentration of guest molecules (Trinh, 2015). Therefore, in this experiment, the R of 1014/995 cm^{-1} represented molecular order of amylose-lipid complexes, with R ratio range 0.989-1.003 (Table 2). These correlated to the formation of V-type crystals with the constant crystalline peak at 19.9°(2θ) of XRD (Figure 3).

3.7 Thermal stability of the instant rice porridges

The thermal stability of the instant rice porridges was measured by TGA and also the derivative curves are shown in Figure 5. TGA curve revealed that thermal degradation of instant rice porridge showed three steps of weight loss. The first step initiated at around 49-240°C, corresponding to the elimination of residual water in the sample, which ranged from 5.85 – 9.36%. The second stage at around 241.71-352.76°C was due to the pyrolysis of starch. The third stage observed at the temperature range of 360-480°C may be ascribed to the decomposition of the other components, such as cellulose, hemicellulose, and lignin present in the starch sample (Khawas & Deka, 2017). The second stage is the main degradation stage where the pyrolysis of starch is demonstrated by a sharp peak in the derivative curve. The instant rice porridge with enzymatic treatment (4-16 h pullulanase debranching) showed endothermic peak starting at around 256.99-258.92°C, which is slightly higher than that of 0 h debranched baseline sample (241.71°C). Moreover, the enzyme-treated instant rice porridge showed a faster rate of decomposition at the second stage with higher temperatures than that of non-enzyme-treated (0 h) case (Table 3) with the range of 51.06-52.54% and 58.06%, respectively. It is evident that the sharp interval of weight loss indicated significant quantities of homopolysaccharide (Di-Medeiros *et al*., 2014). The faster rate of decomposition might be because of the crystal formation during starch modification with enzymatic treatment. However, the presence of weight loss at about 457.03°C indicated amylose-amylose, amylose-amylopectin, and amylose-lipid interactions (Shin, Woo, & Seib, 2003) not observed in all the instant rice porridges.

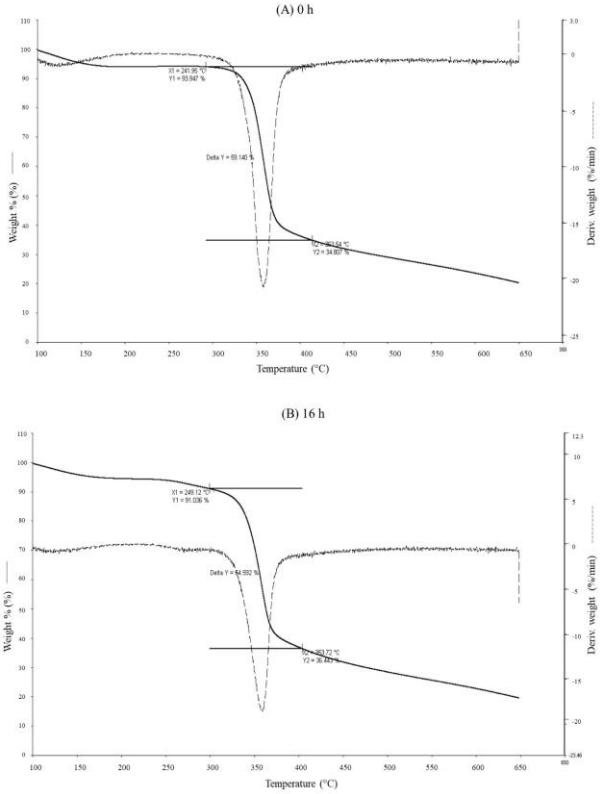

Figure 5. TGA curves of instant rice porridges debranched for various times; (A) 0 h, and (B) 16 h.

Table 3. TGA parameters of instant rice porridges debranched for various times.

Debranching time (hours)	First stage (%)	Second stage (%)	Third stage (%)	Residue (%)	Onset temp. of 2nd stage (°C)
0 h	5.85	58.06	15.27	20.62	241.71
2 h	6.86	54.16	15.63	22.53	249.33
4 h	8.74	51.71	18.43	20.89	258.92
8 h	9.06	51.06	20.03	19.68	258.83
16 h	9.36	52.54	18.33	19.59	256.99
24 h			ND		

ND = not determined

4. Conclusions

The results of this study revealed that a higher degree of pullulanase debranching by prolonged treatment was closely related to elevated retrogradation (syneresis) and resistant starch (RS) content. The formation of amylose-lipid complexes, referred to as RS type 5, was typically found in broken rice porridge after freeze/thaw processing, whereas pullulanase debranching with the recrystallization process induced an increase in double helix formation for retrograded resistant starch type 3. The information obtained in this study can be useful as a guideline for improving rice porridge to be more attractive to health-conscious people.

Acknowledgements

Thank you to School of Applied Biology, Faculty of Sciences and Liberal Arts, and the Research and Development Institute, Rajamangala University of Technology Isan which provided valuable laboratory support and research equipment. This research was supported by Rajamangala University of Technology Isan Research Fund and The Thailand Research Fund (RDG61A0025-02). We would also like to thank Ms. Martha Maloi Eromine for the support in editing English language in this manuscript.

References

Anderson, R. A., Conway, H. F., & Peplinski, A. J. (2006). Gelatinization of corn grits by roll cooking, extrusion cooking and steaming. *Starch-Stärke, 22(4)*,130-135.

Björck, I., Lifebergand, H., & Ostman, E. **(**2000**)**. Low glycemic index of foods. *British Journal of Nutrition, 83(1)*, S149-155.

Denchai, N., Suwannaporn, P., Lin, J., Soontaranon, S., Kiatponglarp, W., & Huang, T.-C. (2019). Retrogradation and digestibility of rice starch gels: the joint effect of degree of gelatinization and storage. *Journal of Food Science, 84(6)*, 1400-1410.

Di-Medeiros, M. C., Pascoal, A. M., Batista, K. A., Bassinello, P. Z., Lião, L. M., Leles, M. I. G., & Fernandes, K. F. (2014). Rheological and biochemical properties of *Solanum lycocarpum* starch. *Carbohydrate Polymers, 104*, 66-72.

Deng, H., Wang, S., Wang, Z, Zhou, Z., & Xiao, H. (2012). Effects of enzyme treatments and drying methods on gelatinization and retrogradation of instant rice porridge. *Food Science and Technology Research, 18(3)*, 341-349.

Dubois, M., Gilles, K., Hamilton, J., Rebers, P., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28(3)*, 350-356.

Dupuis, J. H., Liu, Q., & Yada, R. Y. (2014). Methodologies for increasing the resistant starch content of food starches: A review. *Comprehensive Reviews in Food Science and Food Safety, 13*, 1219-1234.

Elmstahl, H. L. (2002). Resistant starch content in a selection of starchy foods on the Swedish market. *European Journal of Clinical Nutrition, 56(6)*, 500-505.

Englyst, H. N., & Cummings, J. H. (1985). Digestion of the polysaccharides of some cereal foods in the human small intestine. *The American Journal of Clinical Nutrition, 42*, 778-787.

Gernat, C., Radosta, S., Anger, H., & Damashun, G. (1993). Crystalline parts of three different conformations detected in native and enzymatically degraded starches. *Starch-Stärke, 45*, 309-314.

González-Soto, R. A., Mora-Escobedo, R., Hernández-Sánchez, H., Sánchez-Rivera, M., & Bello-Pérez, L. A. (2007). Extrusion of banana starch: Characterization of the extrudates. *Journal of the Science of Food and Agriculture, 87*, 348-356.

Guraya, H. S., James, C., & Champagne, T. (2001). Effect of cooling and freezing on the digestibility of debranched non-waxy and waxy starch and physical properties of the resulting material. *Starch-Stärke, 53*, 64-74.

Hu, P., Zhao, H., Duan, Z., Linlin, Z., & Wu, D. **(**2004**)**. Starch digestibility and the estimated glycemic score of different types of rice differing in amylose contents. *Journal of Cereal Science, 40*, 231-237.

Jenkins, D. J., Wolever, T. M., Taylor, R. H., Barker, H., Fielden, H., Baldwin, J. M., Bowling, A. C., Newman, H. C., Jenkins, A. L., & Goff, D. V. **(**1980**)**. Glycemic index of foods**:** a physiological basis for carbohydrate exchange. *The American Journal of Clinical Nutrition, 34(3)*, 362-366.

Jenkins, D. J., Wolever, T. M., Collier, G. R., Ocana, A., Rao, A. V., Buckley, G., Lam, Y., Mayer, A., & Thompson, L. U. (1987). Metabolic effects of a low-glycemic-index diet. *The American Journal of Clinical Nutrition, 46(6)*, 968-75.

Khawas, P., & Deka, S. C. (2017). Effect of modified resistant starch of culinary banana on physicochemical, functional, morphological, diffraction, and thermal properties. *International Journal of Food Properties, 20(1)*, 133-150.

Kumar, K., Woortman, A. J., & Loos, K. (2013). Synthesis of amylose–polystyrene inclusion complexes by a facile preparation route. *Biomacromolecules, 14(6)*, 1955-1960.

Mayachiew, P. (2014). *Development of healthy instant legume-rice porridges* (Research reports in Thai, Thaksin University, Songkhla, Thailand). Retrieved from http://kb.tsu.ac.th/jspui/handle/123456789/3274

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry, 31*, 426-428.

Nikonenko, N. A., Buslov, D. K., Sushko, N. I., & Zhbankov, R. G. (2000). Investigation of stretching vibrations of glycosidic linkages in disaccharides and polysaccharides with use of IR spectra deconvolution. *Biopolymers, 57*, 257-262.

Patel, H., Royall, P. G., Gaisford, S., Williams, G. R., Edwards, C. H., Warren, F. J., . . . Butterworth, P. J. (2017). Structural and enzyme kinetic studies of retrograded starch: Inhibition of α- amylase and consequences for intestinal digestion of starch. *Carbohydrate Polymers, 164*, 154-161.

Pongjanta, J., Utaipatanacheep, A., Naivikul, O., & Piyachomkwan, K. (2008). Enzymes-resistant starch (RS III) from pullulanase-debranched high amylose rice starch. *Kasetsart Journal (Natural Science), 42(5)*, 198-205.

Sajilata, M. G., Singhal, R. S., & Kulkarni, P. R. (2006). Resistant starch–A review. *Comprehensive Reviews in Food Science and Food Safety, 5*, 1-17.

Shin, M., Woo, K., & Seib, P. A. (2003). Hot-water solubilities and water sorptions of resistant starches at 25°C. *Cereal Chemistry, 80(5)*, 564-566.

Sirivongpaisal, P., Hill, S. E., Pradipasena, P., & Mitchell, J. R. (2005). Amylose content and amylopectin fine structure of Thai rice starches. *Proceedings of Starch Update 2005: BioThailand the 3rd Conference on Starch Technology*, 221-226.

Tovar, J., Carmelo, K., Eggar, H., Ana, R., & Elevina, P. (2002). Resistant starch formation does not parallel syneresis tendency in different starch gels. *Food Chemistry, 76*, 455-59.

Trinh, K. S. (2015). Recrystallization of starches by hydrothermal treatment: digestibility, structural, and physicochemical properties. *Journal of Food Science and Technology, 52(12)*, 7640-7654.

Van Soest, J. J., Tournois, H., de Wit, D., & Vliegenthart, J. F. (1995). Short-range structure in (partially) crystalline potato starch determined with attenuated total reflectance Fourier-transform IR spectroscopy. *Carbohydrate Research, 279*, 201-214.

Walter, M., Silva, L. P., & Denardin, C. C. (2005). Rice and resistant starch: Different content depending on chosen methodology. *Journal of Food Composition and Analysis, 18*, 279-285.

Wang, S., Li, C., Copeland, L., Niu, Q., & Wang, S. (2015). Starch retrogradation: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety, 14(5)*, 568–585.

Wang, L., Wang, M., Lv, R., Guo, M., Ye, X., Ding, T., & Liu, D. (2019). Modelling the physical properties change of canned glutinous rice porridge during cooking. *RSC Advances, 9*, 5521-5529.

Xu, E., Wu, Z., Wang, F., Li, H., Xu, X., Jin, Z., & Jiao, A. (2015). Impact of high-shear extrusion combined with enzymatic hydrolysis on rice properties and Chinese rice wine fermentation. *Food Bioprocess Technology, 8*, 589-604.

Yuan, R. C., & Thompson, D. B. (1998). Free-thaw stability of three waxy maize starch pastes measured by centrifugation and calorimetry. *Cereal Chemistry, 75(4)*, 571-573.