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Research article

Physicochemical characterization of starch isolated from red *Monascus* rice

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ABSTRACT: Red *Monascus* rice (Ang-kak) is used as a traditional medicine and a natural colorant for food and beverages. Starch isolated from waste Ang-kak may compete economically with other common starch and give new value to this waste. Isolation by using alkaline method gave a product with a starch content of 81%. Impurities in the product are protein, fiber, ash, and secondary metabolites of the *Monascus* rice. Several physicochemical properties of the isolated starch were also investigated. It was found that 88.03% of the isolated starch is amylopectin. Thermogravitation study showed that the isolated starch degraded only slightly at temperature below 230 °C and can be considered as a new source of starch for various applications. © 2013 Curtin University of Technology and John Wiley & Sons, Ltd.

KEYWORDS: *Monascus* pigment; red *Monascus* rice; starch; solubility; thermal analysis

INTRODUCTION

Plant usually synthesizes and stores energy in the form of starch. The diversity of plants causes different structural and functional characteristics of their starch. Starch has been utilized for various applications such as thickeners, colloidal stabilizer, gelling agent, bulking agent, water retention agent, and adhesive. These applications depend on the compatibility, degradability, non-toxicity and abundance of starch.[1]

Wheat, corn, rice, and potato are the common sources of the global starch production with diverse applications.[1,2] Modifications such as hydration, swelling, pasting, digestibility, and other properties can expand its usage.[2] Nowadays, studies to explore new sources of starch have increased significantly.[3–6] Most of these studies focused on finding new and low-cost sources of starch or starch from agricultural waste.

Red *Monascus* rice is commercially known as 'Ang-kak'. It has been known in Chinese culture as a result of traditional fermentation of rice by mold from *Monascus* species. This fermented rice has been used as a natural colorant of food and beverage, which provides several benefits for human body. Traditional Chinese medicine records several functions of red *Monascus* rice, such as improving food digestion and blood circulation and lowering cholesterol level by stopping the action of a key enzyme [e.g. 5-hydroxy-3-methylglutaryl-coenzyme-A (HMG-CoA) reductase] in liver.[7] These benefits come from secondary metabolites of *Monascus* species that include several known-pigments and other compounds. Rubropunctatamine ($C_{21}H_{23}O_4N$, MW = 353), monascorubramine ($C_{23}H_{27}O_4N$, MW = 381), rubropunctatin ($C_{21}H_{22}O_5$, MW = 354), monascrorubin ($C_{23}H_{26}O_5$, MW = 382), monascin ($C_{21}H_{26}O_5$, MW = 358), and ankaflavin ($C_{23}H_{30}O_5$, MW = 386) are the six main pigments reported by Blanc *et al.*[8] Other pigments detected are Xanthomonasin A ($C_{22}H_{26}O_6$, MW = 386) by Martínková *et al.*, monascodilone ($C_{15}H_{12}O_4$, MW = 256), monascopyridine ($C_{21}H_{25}NO_4$, MW = 355), and monascopyridine B ($C_{23}H_{29}NO_4$, MW = 383) by Wild *et al.*[9,10] The other compounds detected as secondary metabolites of *Monascus* are monacolin K, an anti-hypercholesterolemic agent, and γ-aminobutyric acid, a hypotensive agent.[7]

Most studies on red *Monascus* rice were focused on its functional compounds; there is no report on the content and characteristic of its starch. After extracting of its secondary metabolites, starch is left as waste and

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can be utilized for different applications. Ma *et al*. reported that 73% of red *Monascus* rice is starch.[7] This study aimed to investigate the physicochemical properties of starch isolated from red *Monascus* rice.

MATERIALS AND METHODS

Materials

Red *Monascus* rice was purchased from a local store (Pasar Tradisional Gang Baru) in Semarang, Indonesia. This red *Monascus* rice was fermented from mixed rice varieties harvested in East Java. The red *Monascus* rice was ground using mortar and pestle and sieved to obtain 100/120 mesh powder. The powder was kept in a desiccator prior to use.

The rice powder was defatted in a soxhlet extractor at 85 °C for 4 h using 75% aqueous *n*-propanol solution. This process was repeated three times. In the extractions, 25.89% (w/w) propanol-soluble materials in red *Monascus* rice were removed. Lipid and other constituents, such as pigments and polyketides, were mostly removed during the extractions. The defatted rice was subsequently air-dried for 48 h and kept in a desiccator and used as raw material for further studies.

α-Amylase from *Bacillus licheniformis* (>50 units/mL), heat stable α-amylase from *Bacillus licheniformis* for total dietary fiber analyzing, amyloglucosidase from *Aspergillus niger* (≥300 U/mL), protease from *Bacillus sp*. (≥16 U/g), and standard amylose (Type III from potato that is essentially free of amylopectin) were all purchased from Sigma-Aldrich (St. Louis, USA).

Starch isolation

Starch was isolated using the method of Wang and Wang with slight modification.[11] The defatted rice was soaked in 0.1% NaOH solution (1:2 w/v) for 18 h. The mixture was then filtered through a Advantec No.5C quantitative filter paper (pore size < 5 μm). The supernatant was centrifuged (4,500xg, 30 min), and the top layer was carefully separated, while the suspended starch layer was resoaked in 0.1% NaOH solution. The suspended starch was washed twice each with 0.1% NaOH solution and centrifuged (4,500xg, 30 min). The starch was then washed with distilled water and recentrifuged. The starch was next resuspended in water, and the pH of the mixture was adjusted to 6.5 with 1.0 M HCl. This mixture was then centrifuged (4,500xg, 30 min). The precipitated-starch was washed again with distilled water three times to remove excess HCl and dried in an oven (50 °C, 3 h). The dried rice starch was sieved (100/200 mesh) and kept in a desiccator prior to physicochemical analysis.

Starch analyses

Total starch

Total starch analysis was conducted using the enzymatic method of Sáchez-Castillo with slight modification.[12] Dried starch product (0.25 g) was mixed with 40 mL sodium acetate buffer solution (0.2 M, pH 5.0) in a vial. α-Amylase (100 μL) was added to the vial, and the vial was heated in a boiling water bath for 30 min with constant stirring.

After the hydrolysis, the vial was put in a water bath (55 °C). Amyloglucosidase solution (0.5 mL) was added to the sample and kept for 16 h. The mixture was then filtered, and distilled water was added to the filtrate to a final volume of 50 mL.

The hydrolyzed starch (3 mL) was put in a sample tube, and dinitrosalicylic acid solution (1%, 1 mL) was added. The mixture was heated to 90 °C in a water bath until the color of the solution was brownish. Rochelle salt solution (1 mL, 40% w/v) was added to the mixture, and the mixture was cooled to room temperature before it was analyzed using a Jasco UV–VIS spectrophotometer (UV–V 550) at 540 nm to determine its glucose content. The starch content was calculated by multiplying the glucose content by a factor of 0.9.[13]

Dinitrosalicylic acid solution was prepared as follows[14]: dinitrosalicylic acid (1 g), crystalline phenol (200 mg), and sodium sulphite (50 mg) were dissolved and mixed in NaOH (100 mL, 1%). The solution was then stored at 4 °C before use.

Amylose content

Amylose content was determined following the method of Sadasivam and Manickam.[15] Dried starch (100 mg) was mixed with ethanol (1 mL), and NaOH (10 mL, 1 N) and kept for 24 h. The mixture was then transferred to a flask, and distilled water was added to a volume of 100 mL. A small amount (2.5 mL) of the solution was taken and mixed with 20 mL distilled water. Three drops of phenolphthalein were also added to the mixture. HCl solution (0.1 N) was added drop by drop until the pink color of the mixture disappeared. An iodine reagent was prepared by dissolving iodine (0.1 g) and KI (1 g) in distilled water to a total volume of 50 mL. One milliliter of the iodine reagent was put in the sample, and the sample volume was adjusted to 50 mL by distilled water. Its amylose content was determined by a spectrophotometer at 590 nm.

The standard amylose solution was prepared by dissolving amylose (100 mg) in sodium hydroxide (10 mL, 1 N). The volume of the solution was then adjusted to 100 mL using distilled water. Several aliqouts were taken (0.1–1 mL) and mixed with 20 mL distilled water. Three drops of phenolphthalein were added to each solution, before 0.1 N HCl solution was added drop by drop to remove pink color from the solution. Iodine reagent (1 mL) was then added in the solution, and the

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total volume was adjusted to 50 mL by distilled water. All solutions were subjected to spectrophotometer measurement at 590 nm.

Amylose content of the sample was calculated by the following equation.

$$\%\text{Amylose} = \frac{\%\text{Amylose (measured for 2.5mLsample)}}{2.5\ \text{mL}} \times 100\ \text{mL} \quad (1)$$

Total dietary fiber (TDF)

Total dietary fiber of the starch was determined based on method of Prosky *et al*.[16] Thermostable α-amylase (0.1 mL) was added to the starch sample (0.25 g) premixed with phosphate buffer (50 mL, pH 6.0). The mixture was incubated in a boiling water bath for 30 min and then cooled to 60 °C. pH of the mixture was adjusted to 7.5 by using NaOH before protease (0.01 g) was added. After 30 min, pH of the mixture was adjusted to 4.5 by using HCl and incubated with amyloglucosidase (300 μL) at 60 °C for another 30 min. Preheated ethanol (250 mL, 95%) was subsequently added to the mixture. The mixture was then filtered (Advantec No. 5C filter paper). The residue was successively washed with 78% ethanol, 95% ethanol, and acetone. The dried residue was weighed, and the total dietary fiber (TDF) content was calculated by using the following equation,

$$\text{TDF} = \frac{\text{Residue Weight} - (\text{Ash Content} + \text{Protein Content})}{\text{Sample Weight}} \quad (2)$$

The ash and protein content was determined using the AOCS Official Method.[17,18]

Swelling and solubility properties

The method of Singh *et al*. was employed to determine the swelling power and solubility of red *Monascus* rice starch.[19] Starch sample (0.5 g) was mixed with distilled water (20 mL) and heated to the desired temperature (30–90 °C) for 30 min. Distilled water was then added to the mixture to a final weight of 25 g, and the mixture was centrifuged (4,500xg, 30 min). The residue (wet) was collected, and its weight was determined.

Ten milliliter supernatant was dried at 105 °C for 3 h. Its dried residue was weighed, and percent solubility was calculated using Eqn (3). The swelling power (SP) was calculated by Eqn (4),[20]

$$\%\ \text{Solubility} = \frac{\text{Dried Residue Weight (g)}}{\text{Dried Sample Weight (g)}} \times 2.5 \times 100 \quad (3)$$

$$\text{SP} = \frac{[\text{Wet Residue Weight (g)} \times 100]}{[\text{Dried Sample Weight (g)} \times (100 - \%\text{Solubility})]} \quad (4)$$

X-ray diffraction (XRD) analysis

Starch sample was well-packed in an aluminum cell prior to XRD measurement. The measurement was performed in a Bruker D2 Phaser X-ray diffractometer operating at 30 kV and 10 mA at ambient temperature. The X-ray generator was run at the Cu K_α wavelength ($\lambda = 1.5406$ Å) with scanning region of diffraction angle (2θ) from 5° to 35°. The specific step size and counting time was set at 0.1° and 2 s, respectively. The obtained diffractogram was used to determine the degree of crystallinity.

To quantify the degree of crystallinity, a method by Nara and Komiya was adapted in this work.[21] The diffractrogram was processed by TOPAS Version 4.2 XRD data analysis program to simulate the amorphous area. Area above the amorphous portion was referred to as the crystalline portion. Both areas then were integrated using this software, and the ratio of crystalline to total diffraction area was taken as the degree of crystallinity.

Thermal decomposition analysis

The stability of starch was studied using a Diamond TG/DTA (Perkin, Elmer, Japan). The temperature was varied from 30 °C to 900 °C. Following the method of Yuliana *et al*.,[6] a starch sample (6 mg) was placed on a platinum pan and heated at 10 °C/min. Nitrogen (3.7 bar) was allowed to pass through the system at 20 mL/min during the process.

Gelatinization and retrogradation analysis

Gelatinization and retrogradation analysis was conducted in a Jade Pelkin Elmer Differential Scanning Calorimetry (DSC) according to the method of Singh *et al*.[5] A 40 μL aluminum pan (TA Instruments, USA) was filled with starch sample (3.5 mg). Distilled water (9 μL) was carefully poured onto the pan before the pan was hermetically covered. The pan was allowed to stay idle for 1 h before heating. An empty aluminum pan was also prepared as the reference.

The sample and reference pan was then heated in the DSC from 20 °C to 100 °C at 10 °C/min. Nitrogen was allowed to pass through the system at 20 mL/min. The gelatinization result was then automatically analyzed and calculated using the Pyris Thermal Data Analyzer. The results obtained were presented as onset temperature (T_o), peak temperature (T_p), and entalphy of gelatinization (ΔH) data.

In retrogradation study, the gelatinized-sample was stored at 4 °C for 7 days before it was reheated in the DSC from 20 °C to 100 °C at 10 °C/min. Pyris Thermal Data Analyzer was also used to analysis the data.

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Prior to these measurements, the DSC instrument was calibrated using indium as the reference material.

Statistical analysis

Results reported in this study are the averages of triplicate observations. They are expressed as means ± standard error of mean.

RESULTS AND DISCUSSION

Starch isolation

The purity and yield of crude starch isolated from red *Monascus* rice using the alkaline method is 80.79 ± 1.22% and 68.35%, respectively. Starch granules are susceptible to dissolution by alkaline solution or other hydrogen bond breaking agents.[22] Therefore, starch loss likely occurred and affected the yield of the isolated starch product.

Other components of the isolated starch are the following: fiber (3.14 ± 0.54%), protein (1.07 ± 0.40%), ash (5.66 ± 0.34%), and others (9.34 ± 0.62%). Ash contains magnesium and sodium as metal elements in the rice. Further eliminating of protein is difficult because the remaining protein is strongly associated with starch molecules. Yoon *et al*. reported this protein as starch granule-associated proteins that are known to be specific to starch origin.[23] Red *Monascus* rice starch may have proteins attached to amylose or amylopectin with strong hydrophobic bonds.

Monascus pigments were reported to have water-soluble and water-insoluble constituents, most of which are water-insoluble and can be extracted by organic solvent such as alcohol.[24] Other secondary metabolites of *Monascus* strain, such as monacolin K and γ-aminobutyric acid were identified as polar extractable-compounds.[7] Therefore, other impurities are expected to be the remaining pigments or polyketides from the secondary metabolism of *Monascus* strain that cannot be removed by *n*-propanol extraction.

Because red *Monascus* rice comes from common rice endosperm, its lipid content is similar to that of rice starch. Fujino and Morrison reported that endosperm-rice starch lipid contains internal lipid and minor amount of external lipid.[25,26] The internal lipid, such as free fatty acid, lysophosphatidylcholine, lysophosphatidylethanolamine, monoglycosyl-monoglyceride, and diglycosylmonoglyceride, is a monoacyl lipid that forms complexes with amylose. The external lipid, which is composed of neutral lipid, glycolipids, phospholipids, is associated with protein outside the starch. These minor nonpolar lipids may also exist in starch granules as impurities.

Swelling and solubility properties

Starch from different sources possesses different swelling ability and solubility. Starch granules have amorphous and semi-crystalline growth rings. The amylose part has amorphous conformation, while the amylopectin part contributes to the crystalline structure. Both amylose and amylopectin can be found in amorphous and crystalline lamellae.

The swelling and solubility properties of starch may provide evidence of interaction between amorphous and crystalline domains.[1] This study found that about 88.03% of the starch isolated from red *Monascus* rice is amylopectin, a branched polymer. This high amylopectin content affected the swelling and solubility of the isolated starch, because amylose plays a role in restricting initial swelling.[1,2]

As shown in Fig. 1, both swelling power and solubility of the isolated starch increase with temperature. At 90 °C, solubility of the isolated starch is 11.4%, which is similar to that of white rice.[1]

However, swelling power of the isolated starch increased only slightly, from 5.2 to 8.2 g/g, as temperature was raised from 30 to 90 °C as indicated in Fig. 1. Hence, it can be categorized as low swelling power starch (8.2 g/g at 90 °C). As a comparison, white rice starch with similar amylose content has a swelling power of 23–30 g/g at 95 °C.[1] The big difference in swelling power between *Monascus* rice starch and white rice may be the results of the differences of characteristics of amylose and amylopectin such as molecular weight, distribution, and length of branching.[27] Red *Monascus* rice starch is the product of fermentation. Impurities left in the isolated starch may also interfere with the swelling of starch granules.

X-ray diffraction pattern result

The X-ray diffractograms for starch isolated from red *Monascus* rice are presented in Fig. 2. The results were

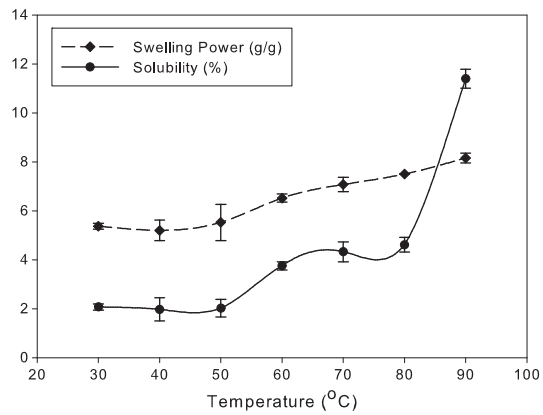

Figure 1. Swelling power and solubility of red *Monascus* rice starch.

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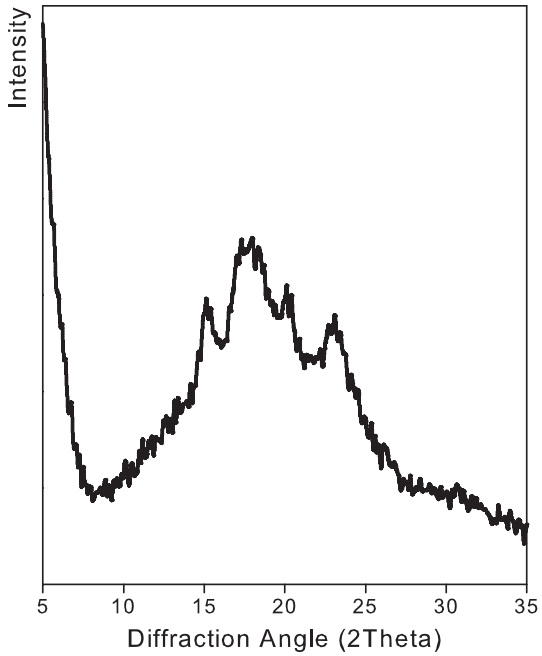

Figure 2. Wide-angle X-ray diffraction spectra of red *Monascus* rice starch.

compared with standard diffraction patterns reported in literatures in order to identify the starch crystalline types.[28–30] Several studies reported different X-ray diffraction patterns for different starches: A-type for cereal starches, B-type for tuber and high amylose starches, C-type for legume, root, and some fruit and stem starches.[29,31–33] Sometimes C-type starch is believed to represent the combination of A- and B-type starches.[29,31–33]

As summarized in Table 1, peaks of the starch isolated from red *Monascus* rice were found at 2θ around 15°, 17°, 18°, 20°, and 23°. The strong reflections found at 15° and 23° and an unresolved doublet at 17°, 18° are the characteristic of A-type starch.[30] However, an additional peak found around 5° is known as the diffraction pattern of B-type starch.[30] Thus, the starch is most likely a C-type starch. Because most peaks typically represented A-type starch, the crystalline type of starch isolated from red *Monascus* rice should be C_A-type starch.

Cereal is usually A-type starch, where its amylopectin has shorter chain length on average than amylopectin of B-type starch such as potato.[34] In this study, the starch isolated from red *Monascus* rice almost reflected the crystallinity type of common rice starch characteristic (A-type starch). By the involvement of *Monascus* mold, starch in red *Monascus* rice possibly underwent slight structural change. The presence of the peak at 5° indicates that crystal of the original starch may have been changed.

Following the method of Nara and Komiya,[21] the degree of crystallinity can be calculated from the ratio of diffraction peak area (crystalline portion) and total diffraction area (crystalline and amorphous portion). Prior to the experiment, the starch sample was also equilibrated to the final moisture content (about 8%). The obtained data were processed by TOPAS Version 4.2 XRD data analysis program. The degree of crystallinity of red *Monascus* rice starch obtained was 38.43%, which is within the range of 15–45% as reported by Zobel for natural starch granules.[29] The degree of crystallinity was also quite similar to the crystallinity of normal rice, which was found to be 38%.[35]

The calculated crystallinity also shows a strong correlation with the amylose content. The amylose content of starch isolated from red *Monascus* rice is low, and it is inversely proportional to the degree of crystallinity.

Thermal properties of starch

Results of the thermal decomposition of the isolated starch are shown in Fig. 3. Heating was started at 45.46 °C. The first peak shows that dehydration of starch sample occurred before 111.21 °C and caused a weight loss of 4.69%. The decomposition was then continued to 230 °C, and the weight of sample decreased to 89.80%. These second peak could be an indication of the initial decomposition of the isolated starch in the absence of water. The derivative weight loss curve in Fig. 3 shows a steep drop at 308 °C. At this temperature, major decomposition occurred, and 46.32% of the sample weight was lost. As heating proceeded, the sample weight kept decreasing until 550 °C.

Because the starch used in this work was isolated from rice fermented by *Monascus* mold, structural change may have occurred and resulted in physical property changes such as thermal stability. Singh *et al*. reported that the decomposition temperature of common rice starch and

Table 1. X-ray diffraction data of red *Monascus* rice starch.

5°	15°	17°	18°	20°	23°	Degree of Crystallinity	Crystall Pattern
~5[a]*	15.08[a] (5.87 Å)[b]	17.34[a] (5.11 Å)[b]	17.91[a] (4.95 Å)[b]	20.09[a] (4.42 Å)[b]	23.05[a] (3.86 Å)[b]	38.43%	C_A

*A strong intensity is detected on 5° 2θ, but its highest point probably occurs at or less than 5°.
[a]Intensity.
[b]The value in parentheses represent the d-spacings.

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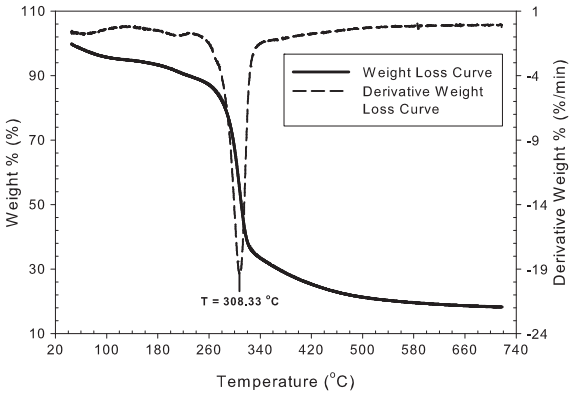

Figure 3. Thermogravimetric curve of red *Monascus* rice starch.

waxy-rice starch is 273–288 °C and 268–275 °C, respectively.[19] Starch isolated from red *Monascus* rice has better thermal stability because major decomposition occurred at 308 °C. For application of red *Monascus* rice starch, temperature higher than 230 °C should be avoided to minimize the degradation of starch granules.

Differential scanning calorimetry was used to study gelatinization and retrogradation of the isolated starch, and the results are shown in Fig. 4. Gelatinization occurs due to the presence of water. Water in starch acts as a dispersant to increase starch swelling by creating bonds with the exposed hydroxyl groups of amylose and amylopectin.[1,36] French reported that water in hydrated granules is primarily associated with the amorphous region,[33] although Wu and Sarko suggested that crystal structure may also contain added-water, especially for B-type starch, since B-type starch has more open-packed structure.[32]

Gelatinization of starch isolated from red *Monascus* rice started at about 71 °C. Near that point, the starch

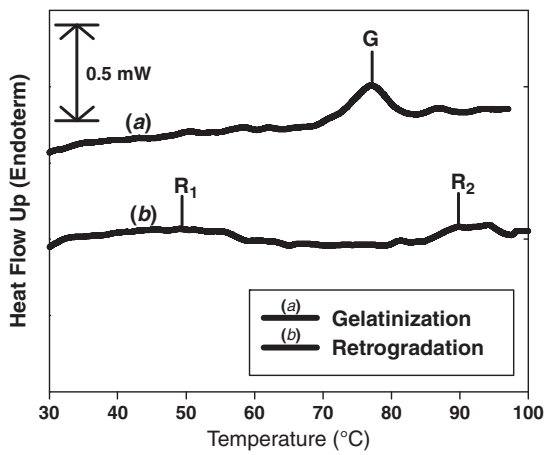

Figure 4. Differential scanning calorimetry (DSC) curves of red *Monascus* rice starch; G = peak of gelatinization; R_1 = 1st peak of retrogradation; R_2 = 2nd peak of retrogradation.

likely started to lose its structure stability due to exposure to heat, and it rapidly swelled in the presence of water. As heating proceeded, the amylopectin region that is responsible for the crystallinity region began to swell, paste, lose its optical birefringence, uncoil, and dissociate of its double helices.[37,38] Stable crystals mostly gelatinized at 76.96 °C. This observed temperature was slightly higher than that of normal rice starch. Sodhi and Singh reported an onset and peak temperature of 66.0–67.26 °C and 69.74–71.94 °C, respectively, for rice starch.[39] Hence, the onset and peak temperatures of starch isolated in this study are 7–8% higher than those of other starches. X-ray diffraction analysis obtained in this study also indicates that the starch of red *Monascus* rice is C-type (a combination of A-type and B-type starch). However, because the diffraction pattern was dominated by A-type, its crystal manner may follow that of A-type starch. It was reported that A-type polymorph usually has close-packing structure, which means that the crystalline structure is relatively compact with low water content.[32] Thus, the relative high transition temperature during gelatinization may also imply that crystal swelling of A-type starch occurred after other water-rich region gelatinized. Moreover, the characteristic of starch gelatinization may also be influenced by its botanical origin.[27] The properties of starch obtained in this study may also be influenced by *Monascus* mold during production of red *Monascus* rice.

After subjected to gelatinization, storage at 4 °C offered chances for the gelatinized starch granules to reassociate, which resulted in the change of the rheological properties (firmness), crystallinity, and water-holding ability (syneresis) of starch.[40] It was reported that amylopectin recrystallization process has maximum rate of nucleation and propagation at 4–6 °C.[41] Therefore, reheating the sample in DSC gave different profile with different endothermic peak (Fig. 4).

The first step of retrogradation process actually occurred before any retrogradation peak can be detected by DSC. This step is the gelation of amylose solubilized during gelatinization that occurred rapidly and irreversibly. The following step then proceeds much slower and is thermally reversible where recrystallization of amylopectin within the gelatinized granule occurred.[42] This slow process was the one observed in the retrogradation profiles (Fig. 4) and is referred to as first retrogradation.

The first retrogradation of starch isolated from red *Monascus* rice was observed to have quite broader range of temperatures (T_o–T_c = 21.49 °C) with its peak (T_p) at 49.10 °C. This temperature is two times larger than that of gelatinization (10.12 °C), while its peak is only 64% of that of gelatinization. The big difference is likely due to the formation of less perfect crystallites during the storage of gelatined starch at 4 °C for 7 days.[42] The wide

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Table 2. Thermal properties of red *Monascus* rice starch.

Category	T_o (°C)	T_p (°C)	T_c (°C)	T_o–T_c (°C)	ΔH^* (J/g)
Gelatinization	71.40	76.96	81.52	10.12	6.04
Retrogradation	36.95[a]	49.10[a]	58.44[a]	21.49[a]	4.20[a]
	85.43[b]	89.77[b]	96.69[b]	11.26[b]	2.72[b]

T_o = onset temperature, T_p = peak temperature, T_c = final temperature, ΔH_{gel} = enthalpy of gelatinization.
*The values of enthalpy are based on dry starch weight.
[a]Parameter for first peak of retrogradation.
[b]Parameter for second peak of retrogradation.

melting range and low endothermic peak may imply the existence of large amount of amylopectin crystal with varying stability and heterogeneity, while narrow range of melting temperatures and high transition peak indicate more homogeneous crystal quality and stability.[43]

At higher temperature, other endothermic transition belonging to the second retrogradation was unexpectedly observed. This second transition temperature started at 85.43 °C and peaked at about 90 °C. This peak may be related to the melting of the rest most stable crystal formed during cooling and storing of gelatined starch at 4 °C. Amylopectin in starch gel that can reassociate and reorganize during DSC scan may have taken part in this second endothermic process. Other possibility is the impurities in the isolated starch. During cooling and storing at low temperature, the impurities may have rearranged or formed new complexes with gelatined starch, which may contribute to the occurence of the second retrogradation process.

Overall, gelatinization enthalpy (6.04 J/g) was found to be higher than the enthalpy observed in first retrogradation (4.20 J/g) or second retrogradation (2.72 J/g). The ratio of the enthalpy needed to retrograde most of starch (first retrogradation) to the gelatinization enthalpy is about 7 : 10. Thermal properties of red *Monascus* rice starch are summarized in Table 2.

CONCLUSIONS

In this work, starch with a purity of 81% was isolated from commercial red *Monascus* rice using alkaline method. The impurities contains protein, fiber, ash, and others components, which is expected from the left secondary metabolites of *Monascus* strain, such as pigments or polyketides. The remaining pigments give special properties of starch isolated from red *Monascus* rice. Therefore, this starch can be used as additive in various processed food products. Its solubility in water was found to be quite low, which makes it eligible as fat substitute in food. Heating of the isolated starch to temperature higher than 230 °C should be avoided to minimize degradation of starch granules.

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