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2**the Taiwan Institute of Chemical Engineers 42 (2011)** 86–91 **Contents lists available at ScienceDirect Journal of the Taiwan Institute of Chemical**

Engineers journal homepage: www.elsevier.com/locate/jtice Isolation and characterization of

starch from defatted rice bran Cynthia Fabian a, Aning Ayucitra b, Suryadi Ismadji b, Yi-Hsu Ju a,*

5a Department of Chemical Engineering, National Taiwan University of Science and Technology, Taipei 10607, Taiwan b Department of Chemical Engineering, Widya Mandala Surabaya Catholic University, Kalijudan 37, Surabaya 60114, Indonesia

19ARTICLE INFO Article history: Received 19 August 2009 Received in revised form 5 February 2010 Accepted 7 March 2010

Keywords: Gelatinization Starch Rice bran Thermogravimetric analysis ABSTRACT Rice starch is one of the most expensive yet very useful starches due to its unique characteristics. This study aimed to isolate starch from defatted rice bran, an underutilized byproduct of milling and is a relatively inexpensive source of rice starch. Starch was extracted from the bran by first soaking it in water. The mixture was then subjected to blending and washing with water, alcohol and alkali solution. About 83% of the rice bran starch was recovered. Characterization of the rice bran starch showed that its gelatinization and retrogradation properties as well as its granule size are similar to those of starch from rice endosperm. Based on the results of this study, defatted rice bran can be a good source of starch that is suitable for applications in food and pharmaceutical industry and other new applications such as a potential material in the biomedical field. ß 2010

 1. Introduction Starch serves as the major reserve of

polysaccharide in plants that provides the bulk nutrient and energy source in human diet (Galliard, 1987; Shelton and Lee, 2000). It finds wide applications not only in food but also in pharmaceutical and biomedical industries because of its biocompatibility, biodegradability, non- toxicity, and abundant sources (Kaur et al., 2007). One of the most interesting applications of starch is in the biomedical field. Starch has been recognized as a potential material in tissue engineering of bone, bone fixation, carrier for the controlled release of drugs and hormones, and as hydrogels (Chakraborty et al., 2004; Lenaerts et al., 1998; Mano and Reis, 2004; Pal et al., 2006; Pereira et al., 1998; Won et al., 1996). Biodegradable bone cements made from starch are highly advantageous because they can provide for immediate structural support and, as

6they degrade at the site of application, allow the ingrowth of new bone for complete healing of bone fracture

(Domb et al., 1996; Pereira et al., 1998). Nanoparticles, nanospheres, and nanogels from starch have also been used as base materials for nanoscale construction of sensors, tissues, mechanical devices, and drug delivery systems (Chakraborty et al., 2004). From rice starch, a patented process for the production of starch nanoparticles by extrusion method has been described by Giezen et al. (2004). They claimed that the nanoparticles produced can be used as a matrix material like resin materials in coating application, as a thickener, as a fat replacer and as carrier of *

11Corresponding author. Tel.: +886 2 2737 6612; fax: +886 2 2737 6644. E-mail address: yhju@mail .ntust.edu.tw (Y.-H. Ju).

colorants, medicaments, flavors and other compounds such as drugs that require slow-release agent. Rice starch is advantageous for nanotechnology applications and other special applications because among plant starch, it has the smallest and narrowest size range of about 2–10 mm (Dendy and Dobraszczyk, 2001). Its contaminating protein is generally considered to be hypoallergenic since there have been no reports related to the occurrence of allergic reactions after eating rice (Helm and Burks, 1996). However, according to Matsuda et al. (1988), a 16-kDa allergenic protein in rice is present but can be decreased or totally removed by enzymatic decomposition

6as reported by Ito et al. (2005) and Watanabe et al. (1990). Despite the

known unique properties and impressive potential applications of rice starch, it is still not widely used because of its relatively high price compared to other cereal starches and because the co-products of these other grains are currently more valuable than those from rice (Bao and Bergman, 2004; Dendy and Dobraszczyk, 2001). Thus, the possibility of extracting rice starch from cheaper sources would be advantageous. One source of relatively inexpensive rice starch is rice bran, an underutilized byproduct of rice milling. Rice bran is usually not consumed as food because of its high fiber content and possible hull contamination (Luh, 1991). It also has limited food application because of the rapid development of rancidity due to the activation of lipase in bran upon milling that breaks down glycerides into fatty acids (Juliano, 1985). Development of stabilization techniques has led to the use of a small percentage of rice bran as commercial food products. However, most rice bran is either

25**used directly as an ingredient in animal feed**

or as fuel in boiler. Rice bran is an

25**undervalued byproduct of rice milling** and **is rich**

in carbohydrates, protein, lipids, dietary fibers, vitamins and minerals (Saunders, 1990).

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2010.03.013 Rice bran contains high amount of carbohydrates. According to Luh (1991), starch which occurs abundantly only in the endosperm has been identified in the germ and aleurone layers that is part of rice bran. Commercial bran thus contains a fair amount of starch and the value can range from 10 to 55% depending on the type of milling and amounts of endosperm present (Saunders, 1990). Mihara et al. (1974) isolated a starch-rich fraction from full-fat rice bran with similar properties to rice starch. However, the properties and composition of the starch they obtained were not reported. Also, there is no report on starch extraction from defatted rice bran. Thus, in this study, starch is isolated from defatted rice bran. Furthermore, the obtained rice bran starch was characterized to provide information for its possible application. This study is also part of our efforts in developing a method for the total utilization of defatted rice bran. Complete utilization of defatted rice bran would play an important role in lowering the total cost of biodiesel production if rice bran oil is used as the raw material.

15**2. Materials and methods 2.1. Materials Rice bran** fresh from milling **was** purchased from **a local rice mill in** Taoyuan **County, Taiwan.** The **bran**

is not specifically from one variety of rice but is a mixture of rice harvested in northern Taiwan. Bran collected from the mill was stored at 60 8C before use. Defatting of rice bran was done using hexane in a Soxhlet extractor at 60 8C for 4 h. Standards for amylopectin from maize and amylose from potato starch

23**were purchased from Sigma–Aldrich (St. Louis, MO).**

Enzymes for starch analysis such as amylase and glucoamylase were also obtained from Sigma–Aldrich. All other chemicals used were of reagent grade and were also supplied by the said company. 2.2. Preparation of rice bran starch The isolation of starch from rice bran is shown in Fig. 1. About 10 g rice bran was soaked in 50 mL water for 3 h. The mixture was blended in a Philips blender for 5 min. A 60-mesh screen attached ([]GIF$DT)1_.giF Fig. 1. Wet-milling method for the isolation of starch from rice bran. in the blender aided the separation of the extract from the bran when the extract was poured out of the blender. The bran was then blended again with 70% ethanol and 0.1 M NaOH for 5 min for each solvent. The filtrate was

7**centrifuged at** 11 **,000** g **for** 15 **min. The supernatant was** then carefully **separated**

from the solid residue. The residue was reslurried, washed with deionized water and filtered through a 200-mesh screen. The filtrate was filtered again by using a 2.5-mm filter paper (Whatman Grade No. 5) and successively washed with 0.1 M NaOH and deionized water. The residue collected on the filter paper was dried at 55 8C for 48 h. The dried starch was ground with mortar and pestle and stored in a plastic jar at room temperature for later analyses. All extraction experiments and subsequent analyses were carried out at least twice. 2.3. Starch product analysis 2.3.1. Total starch Analysis for total starch extracted was accomplished by using the enzymatic method of Sachez-Castillo et al. (2000) with slight modification. About

0.25 g of the dried starch product was dispersed in 40 mL sodium acetate buffer (0.2 M, pH 5.0) in a 60-mL glass vial.

> 4**One hundred** microliters **of thermostable** a- **amylase (Termamyl**

300L, Trump Chemical Corp., Taiwan)

> 4**was added and the** vial was **heated in a boiling water bath for 30 min with constant stirring. The** tube was then **transferred to a** 55 8C **water bath** and **allowed to equilibrate.**

About 0.5 mL 0

> 4.**2%, w/v, solution of amyloglucosidase** (67.4 **U/mg)**

> 4**was added.** Then, **the tube contents were mixed and incubated for** about **16 h. The**

hydrolyzed sample was filtered, transferred to a volumetric flasks and added with deionized water to a final volume of 50 mL. About 3 mL

> 23**of the** hydrolyzed **sample was** placed in **a 5-mL tube.**

Then, 1% DNS solution

> 17**was added. The mixture was heated**

at 90 8C until its color turned to brownish yellow. After heating, 1 mL of 40%

> 17**Rochelle salt solution was added. The**

> 17**mixture was cooled to room temperature**

before its glucose content was

> 22**analyzed using a** Jasco **UV–vis spectrophotometer (UV-** V 550) **at**

540 nm. The starch content was then calculated by multiplying 0.9 on the glucose content determined (Aman and Hesselman, 1984). 2.3.2. Amylose content The blue value method was used in the determination of amylose content of rice bran starch (Singh et al., 2000). About 0.5 mg starch was placed in a volumetric flask containing 1 mL ethanol and 2.7 mL NaOH (1 M). The mixture was heated at 175 8C for 15 min, cooled and diluted with deionized water to a total volume of 25 mL. About 2.5 mL aliquot of the sample was taken and mixed with 2 mL citric acid (0.15 M), 1 mL

> 13**iodine solution (0.2 g I2, 2 g KI** and 250 **mL water) and 14.5 mL** deionized **water. The** mixture was stored **for 20 min**

before being

> 22**analyzed using a** Jasco **UV–vis spectrophotometer (UV-** V 550) **at**

620 nm. 2.3.3. Total dietary fiber The method of Prosky et al. (1988) was employed for the determination of total dietary fiber (TDF). About 0.25 g rice bran starch was treated with three enzymes. Firstly, the starch was digested with 0.1 mL thermostable a-amylase at pH 6 for 30 min in a boiling water bath. The pH of the mixture was adjusted to 7.5 and 0.01 g of protease (16 U/g) was added. The mixture was incubated for 30

min at 60 8C. Lastly, pH of the mixture was adjusted to 4.5 and amyloglucosidase was added followed by incubation for another 30 min at 60 8C. Then, 250 mL preheated 95% ethanol was added to the mixture and the solution was allowed to precipitate at room temperature for 60 min. The mixture was then filtered using an ashless filter paper (Advantec No. 5C) and the residue was successively washed with 78% ethanol, 95% ethanol and acetone. Also, the ash and protein contents of the residue were analyzed by 88 C. Fabian

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86–91 AOCS standard methods. Then, the TDF content was determined as the weight of residue less its ash and protein content.

3**2.3.4. Swelling and solubility Swelling and solubility of**

rice bran starch were studied using the methods of Singh et al. (2000). About 500 mg starch was added in 20 mL water and the solution was heated at various temperatures (30–90 8C) for 30 min. The mixture was weighed and water was added to make the total weight equal to 25 g. The weighed mixture was then centrifuged and the

10**supernatant was decanted.** The **residue was weighed for** the determination of **swelling power**

by using the following formula (Singh et al., 2000): Swelling power ¼ wet residue weight ðgÞ=500 dried starch weight ðgÞ For starch solubility determination, about 10 mL supernatant obtained from the centrifugation of the starch solution was

10**dried at 105** 8C **for 3 h and** then **weighed.** The **solubility was** calculated using **the following**

formula (Singh et al., 2000): Solubility ¼ dry residue weight ðgÞ 2:5 100=starch weight ðgÞ 2.3.5. Thermal analysis Thermal properties of rice bran starch were analyzed using a DSC Jade (Perkin Elmer) and the methods were based from the study of Singh Sodhi and Singh (2003). About 3.5 mg rice bran starch was weighed and put in a 40-mL aluminum pan (TA Instruments, USA). Distilled water was added

3**to achieve a starch- water suspension containing 70% water.** The sample was **sealed and allowed to stand for 1 h at room temperature before heating in the DSC. The DSC was calibrated using indium and an empty** aluminum **pan was used as** the **reference. Sample pans were heated**

16**from 25 to 100 8C at 10 8C/min.**

9**Onset temperature (To), peak temperature (Tp) and enthalpy of gelatinization** (DHgel) **were calculated** automati- cally using **the**

Pyris thermal data analysis software. After cooling the sample was stored at 4 8C for 7 days. Retrogradation was measured by reheating the sample pan contain- ing rice bran starch

16**from 25 to 100 8C at 10 8C/min.**

The

9**enthalpy of retrogradation** (DHret) **was calculated** automatically and **the** percentage of **retrogradation** (%R) **was calculated as**

follows (White et al., 1989): DHret %R ¼ DHgel A Perkin Elmer Diamond TG/DTA Instrument (Perkin Elmer, Japan) was used for thermal stability studies. Approximately 6 mg rice bran starch was placed on a platinum pan. The sample was heated from 30 to 950 8C at 10 8C/min to determine the temperature at which decomposition occurs. During the entire run, air at atmospheric pressure was allowed to flow through the

system containing the sample at 20 mL/min. 2.3.6. Scanning electron microscopy The scanning electron micrographs of rice bran starch were taken with a Cambridge scanning electron microscope (S-360) at an accelerating voltage of 20 kV. Starch granules were sprinkled onto a double-sided

24**tape attached to a** stub **and coated with gold.**

Table 1 Composition of rice bran starch product. Component % weighta Starch 84.24 Fiber 5.76 Protein 0.66 Ash 9.23 a Dry basis. about 36% was utilized. Using the process in Fig. 1, the starch obtained has a purity of 84% with a corresponding recovery of 83%. The amylose content of rice bran starch is 5.66% which characterizes low-amylose rice. The source of rice bran used in this study may have come from a low-amylose variety of rice in Taiwan. Minor components of the rice bran starch obtained in this study are ash, fiber and protein as shown in Table 1. During starch isolation, it is usually required to remove protein from crude starch obtained. In this study, water and 0.1 M NaOH were used to wash the crude starch product which resulted in reducing the protein content to 0.66%. The protein in the starch product was difficult to remove because it is not only associated with starch granule surface as in the case of wheat starch (Galliard, 1987) but also bound

24**to the amylose and amylopectin of the starch**

forming a carbohydrate–protein complex (Chrastil, 1990). 3.2. Thermal properties The thermal decomposition profile of rice bran starch shows three events (Fig. 2). The first thermal decomposition occurred in the temperature interval of 28–131 8C, which corresponds to the dehydration of the starch sample. The second and third events were consecutive and correspond to decomposition in the temperature interval of 210–540 8C. At 302 8C, rice bran starch had a major decomposition that resulted in a mass loss of 45%. Rice bran starch

21**in any application should avoid being subjected to such high temperature to ensure no** significant **thermal degradation.** As shown in **the**

gelatinization thermogram in Fig. 3, rice bran starch gelatinization is characterized by a broad endothermic peak at 67–78 8C. At this temperature range, irreversible granule swelling, loss of birefringence, and loss of crystallinity occurred after the regions of amorphous starch first melted or undergone glass transition (Bao and Bergman, 2004; Slade and Levine, 1988). The onset and peak gelatinization temperatures for rice bran starch were found at 67 8C and 73 8C, respectively. The gelatinization e[]GIF$DT)2_.giF(nthalpy is about 9.56 J/g. This heat energy is the amount required 3. Results and discussion 3.1. Starch yield and purity The wet-milling process for the isolation of rice bran starch shown in Fig. 1 yielded four fractions, viz. starch, protein, course Fig. 2. Thermogravimetric curves of rice bran starch. Weight loss curve (—). fiber and fine fiber. In this study, rice bran with a starch content of Derivative of weight loss (—). ([]GIF$DT)3_.giF ([]GIF$DT)4_.giF Fig. 3. DSC curve of gelatinization of rice bran starch. Fig. 4. DSC curve of retrogradation of rice bran starch. to completely gelatinize starch in rice which is critical to the rice processor, who must optimize heat input, cooking time, and temperature and, at the same time, minimize the cost of the entire process (Bao and Bergman, 2004). Both the transition temperature and enthalpy observed for rice bran starch in the present study were found to agree, within experimental error, with those earlier reports on rice starch (Lii et al., 1995; Russell and Juliano, 1983; Singh Sodhi and Singh, 2003). The endothermic peaks of starch, after storing the gelatinized rice starch at 4 8C for 7 days, appeared between 39 and 60 8C as can be observed in Fig. 4. Retrogradation occurred at about 52 8C which is lower than the gelatinization temperature of 73 8C. According to Ward et al. (1994),

20**recrystallization of amylopectin branch chains has been reported to occur in a less ordered manner in** a **stored**

Table 2 Thermal properties of rice bran starch. Thermal property Rice bran Rice starch (Singh starch Sodhi and Singh, 2003) Gelatinization Onset temperature, To (8C) Peak temperature, Tp (8C) Enthalpy, DHgel (J/g) 63.97 72.62 9.555 Retrogradation Peak temperature (8C) Enthalpy, DHret (J/g) Retrogradation (%) 51.85 2.929 30.65 Decomposition temperature (8C) 302.73 67.26 71.94 11.88 – – 31.23 – starch gel

18**than in native** starch. **This explains the occurrence of amylopectin retrogradation** endotherm **at a temperature range below that for gelatinization.**

The enthalpy of retrogradation

14**provides a quantitative measure of energy transformation that occurs during the melting of re-** crystallized rice bran **starch. The** enthalpy needed for **the** break down **of** retrograded **starch** is 31% of **the**

enthalpy needed to gelatinize the native starch because of the less ordered structure of the re-crystallized starch granules. The thermal properties of rice bran starch obtained in this study and rice starch from previous study are summarized in Table 2. ([]GIF$DT)5_.giF Fig. 5. SEM images of rice flour (a) and rice bran starch (b) (c). 9[]GIF$DT)6_.giF(0 C. Fabian

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86–91 4. Conclusions Rice bran starch was isolated from defatted rice bran using a wet-milling process. About 83% of rice bran starch was recovered. The starch product contains 0.66% protein. The size of starch (2– 8 mm), gelatinization (73 8C) and retrogradation (31%) properties are within the range reported for rice starch. Thus, rice bran starch maybe used as functional ingredient in food and pharmaceutical industry like starch from rice endosperm as well as find new applications such as a potential material for biomedical applica- tions since it is from a cheap raw material and can compete economically with other cereal starches. References Aman, P. and K. Hesselman, ‘‘Analysis of Starch and Other Main Constituents of Cereal Grains,’’ Swed. J. Agric. Res., 14, 135 (1984). Bao, J. and C. J. Bergman, ‘‘The Functionality of Rice Starch,’’ Starch in Food: Structure, Fig. 6. Swelling power and solubility of rice bran starch. Function and Application, (2004). Chakraborty, S., B. 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