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Changes in resistant starch from two banana cultivars during postharvest storage

Juan Wang, Xue Juan Tang, Ping Sheng Chen, Hui Hua Huang *

College of Light Industry and Food Science, South China University of Technology, Wushan Road No. 381, Guangzhou 510641, China

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ABSTRACT

Banana resistant starch samples were extracted and isolated from two banana cultivars (*Musa* AAA group, Cavendish subgroup and *Musa* ABB group, Pisang Awak subgroup) at seven ripening stages during postharvest storage. The structures of the resistant starch samples were analysed by light microscopy, polarising microscopy, scanning electron microscopy, X-ray diffraction, and infrared spectroscopy. Physicochemical properties (e.g., water-holding capacity, solubility, swelling power, transparency, starch–iodine absorption spectrum, and Brabender microviscoamylograph profile) were determined. The results revealed significant differences in microstructure and physicochemical characteristics among the banana resistant starch samples during different ripening stages. The results of this study provide valuable information for the potential applications of banana resistant starches.

1. Introduction

Resistant starch (RS) is defined by EURESTA (European FLAIR Concerted Action on Resistant Starch) as "the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals" (Asp, 1992). There are four types of RS: (1) physically entrapped, inaccessible starch within whole or partially milled seeds (RS_1); (2) native granular starch, consisting of nongelatinised granules (RS_2); (3) retrograded starch produced by food processing applications (RS_3); and (4) chemically modified starch (RS_4; Englyst, Kingman, & Cummings, 1992). RS has physiological effects similar to those of prebiotics and dietary fibre: RS stimulates the growth of beneficial bacteria in the gut (e.g., bifidobacteria) and increases the production of short-chain fatty acids associated with gut immune function and microbiota modulation (Fuentes-Zaragoza et al., 2011; Johnson & Gee, 1996). Additionally, RS protects against several diseases, including type II diabetes, colorectal cancer, and other diet-related chronic diseases (Niba, 2002; Topping & Clifton, 2001).

Several studies have focused on the functions of RS. Mutungi, Rost, Onyango, Jaros, and Rohm (2009) studied the crystallinity and the thermal and morphological characteristics of RS_3 from debranched cassava starch. Garcia-Rosas et al. (2009) assessed the changes in maize tortilla RS content and structure during storage. Aparicio-Saguilan et al. (2007) successfully prepared slow-digestible cookies from RS-rich lintnerised banana starch. In addition, Aparicio-Saguilan, Gutierrez-Meraz, Garcia-Suarez, Tovar, and Bello-Perez (2008) investigated the physicochemical and functional properties of cross-linked banana RS. Ble-Castillo et al. (2008) reported that banana RS flour supplementation reduces body weight and insulin resistance in obese individuals with type II diabetes.

Unripe bananas are rich in RS_2 (Johnson & Gee, 1996; Niba, 2002). As tropical and subtropical fruits, bananas are mainly planted in tropical and subtropical zones. RS isolated from different banana cultivars may have different properties. Moreover, with post-harvest storage, numerous enzymes transform the starches in these fruits into different sugars. Consequently, the RS content of bananas at different ripening stages may differ. However, few studies have focused on the changes of banana RS throughout storage. This study assessed the changes in the content, physicochemical and structural properties of RS from two banana cultivars (*Musa* AAA group, Cavendish subgroup and *Musa* ABB group, Pisang Awak subgroup) at different stages of maturity.

2. Materials and methods

2.1. Materials

Two banana cultivars were used in this study: *Musa* AAA group, Cavendish subgroup and *Musa* ABB group, Pisang Awak subgroup. These cultivars were planted and sold in Guangdong province, China. Using the criteria reported by SH Pratt Co. (Luton, United

* Corresponding author. Tel.: +86 2087112851.
E-mail address: fehhuang@scut.edu.cn (H.H. Huang).

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Kingdom; Soltani et al., 2011), the fruits were divided into seven ripening states: 1-entirely green; 2-green with a trace of yellow; 3-more green than yellow; 4-more yellow than green; 5-yellow with a trace of green; 6-entirely yellow; 7-entirely yellow with brown speckles. Bananas at ripening stage 1 were selected and purchased from a local market. Subsets of these bananas were stored at room temperature for different periods of time to attain ripening stages 2–7. However, only bananas at stages 1–5 were used in this study because RS samples from bananas at stages 6 and 7 were colloid-like substances that were difficult to grind. All chemicals used in this study were of analytical grade.

2.2. Isolation and determination of banana resistant starch (BRS) content

Using the method reported by Cheng Yanfeng et al. (2008), bananas were peeled, pulped, and digested with pectinase and amylase to remove pectin, cellulose, protein, and digestible starch. The digested banana pulp was centrifuged at 3 000 rpm for 15 min; the resulting precipitate was dehydrated at 50 °C, ground, and stored at 5 °C. RS content was determined by the method reported by Goni, Garcia-Diz, Manas, & Saura-Calixto (1996). Briefly, the method consisted of the removal of protein and digestible starch, the solubilisation and enzymatic hydrolysis of RS, and the quantification of RS. Human gastric and intestinal conditions (pH and transit time) were simulated.

2.3. Structural observations of BRS

2.3.1. Light microscopy and polarising microscopy

BRS samples were dissolved in glycerol (50% concentration) and observed under a microscope (Vanox BHS-2, Olympus Corporation, Japan) using both natural and polarised light.

2.3.2. Scanning electron microscopy (SEM)

Particles of BRS powder were scanned, using a S3700N scanning electron microscope (Hitachi, Japan). Samples were fixed on an objective table coated with platinum (10–20 nm thickness).

2.3.3. X-ray diffraction (XRD)

Cu K_α radiation was used to scan BRS samples over the $2\theta = 4$–60° range, with a step interval of 0.04°, a scanning rate of 17.7 s per step, a voltage of 40 kV, and a current of 40 mA. The D8 ADVANCE X-ray diffractometer from Bruker Corporation (Germany) was used for the XRD analyses.

2.3.4. Infrared spectroscopy

BRS samples were pressed in KBr. An infrared spectrometer (VECTOR33, Bruker Corporation, Germany) was used to scan the samples from 4000 cm^{-1} to 400 cm^{-1} of the infrared region.

2.4. Physicochemical properties of BRS

2.4.1. Water-holding capacity (WHC)

WHC was measured by the method reported by Toyokawa, Rubenthaler, Powers, and Schanus (1989). Briefly, 20 ml of starch suspension (5 g/100 ml) were transferred to centrifuge tubes and heated in a water bath for 15 min at 50 °C, 70 °C, or 90 °C. The tubes were centrifuged at 3,000 rpm for 15 min. The supernatant was discarded; tubes containing sediment were placed at a 45° angle for 10 min to allow water drainage and weighed. WHC was calculated by Eq. (1).

$$\text{WHC}\ (\%) = \frac{m_2 - m_1 - m_0}{m_0} \times 100\% \quad (1)$$

where m_0 is the weight of the starch sample, m_1 is the weight of the centrifuge tube, and m_2 is the weight of the starch sample and centrifuge tube following water drainage.

2.4.2. Solubility and swelling power

Solubility (S) and swelling power (SP) were determined, using the method reported by Aparicio-Saguilán et al. (2005). In this experiment, 20 ml of starch suspension (5 g/100 ml) were transferred to centrifuge tubes and heated in a water bath for 30 min at 50 °C, 70 °C, or 90 °C. After the tubes had cooled to room temperature, they were centrifuged at 3000 rpm for 15 min. The sediment and supernatant were separated; the sediment was dried and weighed. S and SP were calculated using Eqs. (2) and (3), respectively.

$$S\ (\%) = \frac{A}{W} \times 100\% \quad (2)$$

$$\text{SP}\ (\%) = \frac{D}{W(1-S)} \times 100\% \quad (3)$$

where A is the weight of dry dissolved solids in the supernatant, W is the weight of the sample, and D is the weight of the sediment.

2.4.3. Transparency

An aqueous starch solution was preparing by mixing 1.0 g of starch with 99.0 g of water. This solution was heated in a boiling water bath for 15 min under continuous stirring and subsequently cooled to room temperature. The transparency of the resulting starch paste was detected at 620 nm (UV-1800 spectrophotometer, Shimadzu Co., Japan). Distilled water was used as a blank control, which was considered to have a transparency of 100%.

2.4.4. Starch–iodine absorption spectra

Spectra of iodine-bound starch samples were determined, using the method reported by Klucinec and Thompson (1998). BRS (50 mg) was dispersed into 10.0 ml of DMSO containing 10% of 6.0 M urea. Subsequently, 2.0 ml of the dispersed solution, 25 ml of distilled water, and 1.0 ml of I_2-KI (2.0 mg I_2/ml and 20.0 mg KI/ml) were pipetted into a 50 ml volumetric flask and mixed. The mixed solution was brought to a volume of 50 ml with distilled water. Control solutions were prepared without BRS. A UV–visible spectrophotometer (UV-1800, Shimadzu Co., Japan) was used to scan each sample from 500 to 800 nm; λ_{max} for each sample was defined as the wavelength that resulted in the highest absorbance value.

2.4.5. Pasting properties

A microviscoamylograph (Visgraph-E, Brabender Instruments, Inc., Germany) was used to determine the viscosity profiles (in Brabender units, BU) of the starch samples. Dispersions of BRS (6%, dry basis) were transferred to the microviscoamylograph and subjected to thorough agitation. The dispersion was brought to an initial temperature of 30 °C and subsequently to 95 °C at a rate of 1.5 °C/min. The temperature of the dispersion was maintained at 95 °C for 30 min; subsequently, the dispersion was cooled to 50 °C at a rate of 1.5 °C/min and maintained at 50 °C for 30 min (Aparicio-Saguilán et al., 2005).

2.5. Statistical analyses

Data were analysed by the SPSS statistical software package, v19.0 (IBM company). Data were expressed as means ± standard deviation. One-way analysis of variance (ANOVA) was used to compare the different BRS samples, Levene's test was used to assess homogeneity of variances, and the Bonferroni test was used for multiple comparisons. Statistical significance was set at