Food Chemistry 237 (2017) 936–941

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

The effects of chilling stress after anthesis on the physicochemical properties of rice (*Oryza sativa L*) starch

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ARTICLE INFO

Article history:
Received 8 February 2017
Received in revised form 27 April 2017
Accepted 6 June 2017
Available online 8 June 2017

Keywords:
Chilling stress
Rice starch
Thermal properties
Pasting properties

ABSTRACT

This study investigates the effect of chilling stress, over a period of three days after anthesis, on the physicochemical properties of starches derived from six rice cultivars. Chilling stress significantly affected the grain characteristics and physicochemical properties of rice starches, except for those of two varieties, NJ 9108 and ZD 18. In the other four rice cultivars subjected to chilling stress, the content of medium, and large sized granules showed a decrease, and an increase, respectively. Amylose content increased as a result of chilling stress, thereby resulting in starch with a lower swelling power, water solubility, and higher retrogradation enthalpy and gelatinization temperature. Chilling stress led to deterioration of cooked rice quality as determined by the pasting properties of starch. This study indicated that among the cultivars studied, the two rice varieties most resistant to chilling stress after rice anthesis were NJ 9108 and ZD 18.

1. Introduction

Rice (*Oryza sativa* L.) is one of the world's most important cereal crops, with more than half of the world's population depending on rice and its associated products for most of their calorie and nutritional requirements (Hung, Chau, & Phi, 2016), and is extensively grown by more than half of the world's farmers (Fairhurst & Dobermann, 2002). With improvements in living standards, there has been increased focus on the quality of produced rice (Kong, Zhu, Sui, & Bao, 2015).

Rice yield and quality are associated with the genotype of the rice variety and growing environment (Wu et al., 2013; Falade, Semon, Fadairo, Oladunjoye, & Orou, 2014). The differences in rice quality caused by genotype of the rice variety and growing environment will induce variations in rice processing. Temperature during the grain filling stage is an important environmental factor affecting rice yield and quality, and the optimum temperature for this stage has been shown to be between 21 and 23 °C (Meng & Zhou, 1997). Chilling stress (15–17 °C) after anthesis is an important factor for significant reductions of rice yields in China, leading to the loss of between 5×10^6 and 10×10^6 tons of rice production every year (Yang, Wang, Tu, Zeng, & Li, 2012). Also, chilling stress after anthesis has been found to have a particularly significant effect on rice quality (Song, Sun, Wang, & Liu, 2011). There are multiple studies on the effect of temperature during different growth stages of various grains on the characteristics of starch. Investigations by Lu et al. (2014) suggested that heat stress during grain filling could modify waxy maize (*Zea mays* L) starch. Chun, Lee, Hamaker, and Janaswamy (2015) studied the effect of temperature on the starch of rice grains and concluded that high temperatures during the filling stage caused deterioration in the quality of cooked rice. Starch is an important component of rice grains, comprising more than 80% of its total weight, and is the main factor affecting rice quality. Starch properties depend mainly on physicochemical and thermal characteristics such as granule size, granule size distribution, amylose content, relative crystallinity, swelling power, water solubility, gelatinization, and pasting properties. Among these factors, amylose content is considered one of the most important for determining the quality of rice (Lin, Singh, Chang, & Chang, 2011; Yu, Ma, Menager, & Sun, 2012; Zhu, Liu, Wilson, Gu, & Shi, 2011).

The objective of this study is to obtain an understanding of physicochemical properties of rice starch following chilling stress after anthesis. Since the starch physicochemical properties are the main factors to rice quality. Although there has been some research focussing on the effect of heat stress on starch physicochemical properties, studies on the effect of chilling stress on starch

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http://dx.doi.org/10.1016/j.foodchem.2017.06.039
0308-8146/

physicochemical properties are comparatively few. Further research in this field is of critical importance in efforts to elucidate how starch properties are affected by chilling stress after anthesis. Therefore, as part of these efforts, the physicochemical, thermal and pasting properties of rice starch, obtained from cultivars exposed to chilling stress after anthesis, have been investigated in this study.

2. Material and methods

2.1. Plant materials and experimental design

Experiments were conducted on the Yangzhou University Farm (119°42′ E, 32°39′ N). The soil type is sandy loam, and the preceding crop was wheat. Total N, P and K availability were determined to be 1.4 g·kg^{-1}, 35.1 mg·kg^{-1} and 88.3 mg·kg^{-1}, respectively. Six rice varieties, Wuyunjing 24 (WYJ 24), Nanjing 9108 (NJ 9108), Nanjing 46 (NJ 46), Nanjing 5055 (NJ 5055), Wuyunjing 27 (WYJ 27) and Zhendao 18 (ZD 18), were used in the experiment.

Seeds were sown on May 23 and the seedlings were transplanted on June 15. Rice seedlings was transplanted in the pots of soil-based compost (the diameter of pots is 300 mm, the height of pots is 200 mm). One pot had three seedlings, each variety had 30 pots. After anthesis, each variety was transferred into an artificial climate room for three days. The temperature regimens for day and night, over the three days, were 23/15 °C and 17/15 °C, respectively. Natural light was used as the light source; the relative humidity in the artificial climate room was maintained at 65 ± 5%. After three consecutive days of treatment, the pots were transferred outdoors. Protein content of milled rice was determined according to the AACC (1984) procedures. Assays of total starch of milled rice were performed using commercial kits Sigma (HK, China).

2.2. Starch isolation

Starch was isolated according to the method of Wei et al. (2010) with minor modifications. Firstly, in order to remove protein, the rice flour (20 g) was steeped in 0.45% sodium metabisulfite aqueous solution with 10 mg·g^{-1} alkaline protease at 42 °C for 24 h. The starch slurry was sieved (200 mesh), and the residue remaining on the mesh sieve was collected. The residue was mixed with 30 ml deionized water and stirred for 2 min, and the resulting mixture was sieved (200 mesh). The combined starch slurry filtrates were centrifuged at 3000*g* for 10 min. The faintly coloured supernatant liquid was discarded, while the remaining white precipitate was resuspended with 20 ml of deionized water, centrifuged at 3000*g* for 10 min, and the supernatant was again removed. The aforementioned centrifugal steps were repeated five times to ensure thorough removal of impurities. Finally, the starch was then dried at 30 °C at ambient pressure and the dried starch was put through a 200-mesh sieve.

2.3. Granule size analysis

The granule size distribution of starch was studied using a laser diffraction particle size analyzer (Mastersizer 2000, Malvern, England). The starch samples were immersed in absolute ethyl alcohol and stirred at 2000 rpm. The instrument was adjusted to measure starch granule size ranging from 0.1 to 2000 μm. Surface-weighted mean [D (3,2)], and volume-weighted mean [D (4,3)].

2.4. Measurements of iodine absorption spectrum and apparent amylose content (AAC)

Starch was defatted in methanol/water (85:15, v/v) and then dissolved in dimethyl sulphoxide containing urea (UDMSO) solution. The starch-UDMSO solution was treated with I_2-KI solution, according to the method of Man et al. (2012). The iodine absorption spectrum was scanned from 400 to 900 nm using a spectrophotometer (Ultrospec 6300 Pro, Amersham Biosciences, Amersham, UK). Iodine blue value was measured at 680 nm, and the AAC was calculated from the absorbance at 620 nm by reference to a standard curve prepared with amylopectin from corn and amylose from potato.

2.5. X-ray diffraction analysis

X-ray diffractograms were obtained with an X-ray powder diffractometer (XRD) (D8 Advance, Bruker-AXS, Karlsruhe, Germany) operated at 200 mA and 40 kV, over a diffraction angle (2θ) range of 3–40° with a step size of 0.02° and a sampling interval of 0.6 s. Relative crystallinity (%) was calculated with MDI Jade 6 software.

2.6. Determination of swelling power and water solubility

Swelling power and water solubility were determined according to the method of Konik-Rose, Moss, Appels, Stoddard, and McMaster (2001). Starch samples (m_0) were mixed with water (2%, w/v), placed in a 2 ml centrifuge tube (m_1), and heated in a water bath at 95 °C for 30 min. The tubes were gently shaken for one minute. The samples were cooled down to room temperature, centrifuged at 8000*g* for 10 min, and the supernatant was discarded. The colloid remaining in the centrifuge tube was weighed (m_2), and the sediments were dried to constant weight (m_3) at 60 °C. The swelling power and solubility were calculated as follows: swelling power = $(m_2 - m_1)/(m_3 - m_1)$ (g/g); solubility (%) = $100 \times (m_0 + m_1 - m_3)/m_0 \times 100\%$.

2.7. Determination of thermal properties

The thermal properties were studied by using a differential scanning calorimetry (DSC) (Model 200 F3 Maia, Netzsch, Germany) according to Lu and Lu (2012). Five mg starch was mixed with water of two times the weight of starch, and sealed in an aluminum pan at 4 °C overnight. The DSC analyzer was calibrated using a standard pan (empty pan) as reference first and then heated from 20 °C to 100 °C at a rate of 10 °C/min.

2.8. Determination of pasting properties

The starch pasting properties were carried out by using a rapid viscosity analyser (Model 3D, Newport Scientific, Australia) according to Lu and Lu (2012) with minor modifications. Starch sample (2.5 g, dry basis) was mixed with 25 ml of H_2O. The pasting programmed cycle was set in a 13 min. Starch samples started at 50 °C for 1 min and then heated from 50 to 95 °C at 12 °C/min, held at 95 °C for 2.5 min, cooled to 50 °C at 12 °C/min and held 2 min.

2.9. Statistical analysis

The data shown in all the tables represent the mean of the triplicate experiments values. A one-way Analysis of Variance (ANOVA) was used to determine statistically significant differences in means, and an ANOVA and Tukey's test was calculated when statistical differences were observed using the SPSS 16.0 Statistical Software Program. Differences were determined to be statistically significant when $P < 0.05$.