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Effects of aleurone layer on rice cooking: A histological investigation

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ABSTRACT

Understanding how aleurone layer (AL) affects rice cooking behaviour is important for rice processing. Individual effects of AL on rice cooking behaviour were evaluated and histological characters of AL before and after cooking were investigated. AL slightly affected rice cooking quality (optimum cooking time, water absorption, volume expansion ratio and total solids loss) while remarkably affected rice texture (hardness and adhesiveness) and peak viscosity. Histological investigation showed that channels were formed in AL during cooking. The channels facilitated the penetration of water, which could explain why AL exhibited slight effects on rice cooking quality. In addition, thick cell walls and thermally stable aleurone grains were widely distributed in AL. Leached components accumulated on them and formed a reinforced coated film on rice surface during cooking, which may be a possible mechanism accounting for the remarkable effect of AL on rice texture. Histological characters of AL are closely related with rice cooking behaviour.

1. Introduction

Histologically, rice caryopsis is composed of cuticular layer (CL), aleurone layer (AL) and endosperm layer (EL), in centripetal order (Bechtel & Pomeranz, 1977, 1978). CL and AL are known as bran layer and conventionally removed during commercial milling and polishing operations. The resultant EL, namely white rice (WR), is well accepted in markets due to its good cooking behaviour. Now increasing investigations indicated that milling rice to lesser degrees, that is, leaving more CL and AL on rice, can lead to greater grain yield and human health benefits, as well as potential energy savings (Butsat & Siriamornpun, 2010; Roy et al., 2008). However, presence of CL and/or AL may alter rice cooking behaviour. Therefore, it is imperative to figure out how the rice cooking behaviour is influenced by presence of AL and/or CL.

The extent of removal of the bran layer from rice kernels is defined as degree of milling. Several researchers have compared the cooking behaviour of rice with different degree of milling, and showed that presence of bran layer indeed affected rice cooking behaviour, including cooking quality (optimum cooking time, water absorption, volume expansion ratio and elongation ratio) (Mohapatra & Bal, 2006, 2007), texture profile (hardness, adhesiveness, and cohesiveness) (Mohapatra & Bal, 2006; Saleh & Meullenet, 2007), and pasting profile (peak viscosity, final viscosity, breakdown, setback and pasting temperature) (Park, Kim, & Kim, 2001; Perdon, Siebenmorgen, Mauromoustakos, Griffin, & Johnson, 2001; Yoon & Kim, 2004). However, rice caryopsis has an undulating surface, rendering individual removal of CL or AL by mechanical milling a difficult operation (Mohapatra & Bal, 2007; Wood, Siebenmorgen, Williams, Orts, & Glenn, 2012). Therefore, despite the above-mentioned works, the individual effects of CL and AL on rice cooking behaviour have, to the best of our knowledge, never been investigated.

It has been suggested that the CL affected rice cooking behaviour chiefly by acing as an impermeable barrier, preventing the penetration of water into the underlying endosperm (Chen, Chen, & Chang, 2012; Guraya, 2011). However, the mechanism explaining how AL affects rice cooking behaviour is rarely stated. A rice caryopsis can nevertheless be considered as a cellular solid with multiple layers. We assumed therefore that, a histological study could be used to investigate the mechanism of how AL affects rice cooking behaviour.

Using two different rice varieties that are widely cultivated in China, we conducted this study with following objectives: (1) to evaluate the individual effect of AL on rice cooking behaviour

Abbreviations: CL, cuticular layer; AL, aleurone layer; EL, endosperm layer; BR, brown rice; PBR, peeled brown rice; WR, white rice; RVA, Rapid Visco Analyzer; SEM, scanning electron microscopy; FM, fluorescence microscopy.

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and compare the effect with those of CL; (2) to reveal the histological characters of AL *in situ* before and after cooking; and (3) to link the histological characters of AL with the cooking behaviour of rice. Finally, a possible mechanism explaining how AL affects rice cooking behaviour was proposed.

2. Materials and methods

2.1. Materials

Paddies (*Oryza sativa* L.) were obtained from China Oil & Foodstuffs Corporation (Jiangxi, China). A short grain cv. Kongyu 131 (harvested in 2012, Heilongjiang, China) and a long grain cv. Waiyin 7 (harvested in 2012, Jiangxi, China) were selected for our study. Kongyu 131 and Waiyin 7 are widely cultivated in north and south of China respectively. Immediately after the paddy arrived, it was vacuum-packed and refrigerated at 4 °C until used. All experiments were finished within two weeks of the paddy arrival.

2.2. Grain samples, histological staining and stereomicroscopy

Paddies were dehusked with a laboratory husker (Model THU-35A, Satake, Japan). Then the germ of the resultant dehusked rice was manually stripped off to obtain brown rice (BR). CL (pericarp and testa) of BR was carefully removed with a pair of fine forceps and scalpel. The hand-peeled BR was regarded as peeled brown rice (PBR). To prepare white rice (WR), the PBR was further processed with a laboratory polisher (Model TM 05, Satake, Japan) until AL of rice caryopsis were removed.

To differentiate the CL, AL and EL of rice, a complex dye, May Grunwald's reagent (Chikubu & Shikano, 1952) was used. This dye consists of equivalent amount (1.25 g/L) of eosin Y (Aladdin, shanghai, China) and methylene blue (Aladdin, shanghai, China) dissolved in methyl alcohol. Rice kernels of BR, PBR, and WR were directly immersed in dye solution for 2 min. Thereafter, stained samples were rinsed twice with distilled water and then eluted once with ethanol.

Stained and unstained kernels of BR, PBR, and WR were examined and photographed under a stereomicroscope (SMZ800, Nikon, Japan).

2.3. Cooking quality analysis

2.3.1. Optimum cooking time

The optimum cooking time was determined following the standard Ranghino test (Juliano & Bechtel, 1985). Rice kernels in boiling water (98 ± 1 °C) were removed at a specific time interval during cooking and pressed between two glass plates until no opaque core or uncooked centre was left. This cooking period was recorded as the optimum cooking time and is required to ensure that the starch in all rice samples has been gelatinized to the same degree. Other cooking parameters, including water absorption, volume expansion ratio and total solids loss are based on the optimum cooking time.

2.3.2. Water absorption

Cooked grain samples were blotted with filter paper to remove surface water and then weighed. An increase in its weight was calculated. Water absorption was reported as percentage of water adsorbed by one gram of rice in dry basis (Juliano & Bechtel, 1985).

2.3.3. Volume expansion ratio

The volumes of both uncooked and cooked grain samples were measured using the volume displacement method. The volume expansion ratio was expressed as the ratio of the volume of cooked grain sample to that of their uncooked counterparts (Juliano & Bechtel, 1985).

2.3.4. Total solids loss

Total solids loss was determined by drying an aliquot of cooking water (2 mL) at 105 °C for 12 h. The resultant dry matter was weighed accurately and expressed in milligrams. The dry matter content in per milliliter of cooking water was regarded as total solids loss (Zhou, Robards, Helliwell, & Blanchard, 2007).

2.4. Texture profile

Textural analysis of cooked rice was performed on a texture analyser (TA. XT. plus, Texture Technologies Corp., UK) according to the method of Mohapatra and Bal (2006) with minimal modification. In brief, three cooked rice grains (cooked at the optimum cooking time) were arrayed on the platform and tested when the samples were still warm. A two-cycle compression program (TPA) was employed with pre-test, test and post-test speed of 0.5, 0.5 and 5 mm/s, respectively. Rice kernels were compressed to 90% deformation by a cylindrical probe P/36R having a diameter of 26 mm. This experiment was repeated 10 times for each sample. Textural parameters of TPA curves, including hardness and adhesiveness were calculated by the software program called Texture Expert Excede Version 1.0 (Stable Micro Systems Software).

2.5. Pasting profile

BR, PBR and WR were ground by an electric blender (Panasonic, model MX-795N, Japan) and sieved through a 100-mesh screen. The resultant flours were used to determine pasting profiles by Rapid Visco Analyzer (RVA-TecMaster, Newport Scientific Pt. Ltd., Australia) based on AACC (2000) method 61-02. Rice flour (3 g, 12% moisture basis) was added to 25 ml deionized water in a RVA aluminum test canister, following procedural stirring, heating (50–95 °C) and cooling. The total run time was 12.5 min. Analyses were conducted in triplicate. A plot of paste viscosity in cP units versus time was used to determine peak viscosity, trough viscosity and final viscosity. Average curves were also drawn.

2.6. Contents of cell wall materials in CL, AL and EL

Cell wall materials could be regarded as dietary fibre or non-starch polysaccharides in rice (Lai, Lu, He, & Chen, 2007), and prepared according to the modified enzymatic-gravimetric method using MES-TRIS buffer (AACC method 32-07, 2000). One gram of defatted and dried flour that obtained from CL, AL and EL was briefly subjected to sequential enzymatic digestion by heat-stable a-amylase, protease, and amyloglucosidase. The total content of cell wall materials was equivalent to total dietary fibre content. The hot-water insoluble and hot-water soluble fractions of the cell wall materials were separated by boiling original cell wall materials in hot water (98 ± 1 °C) for 1 h as previously reported (Lai et al., 2007). The contents of total, hot-water insoluble and hot-water soluble cell wall materials were expressed as g/100 g in dry basis. Assays were conducted in triplicate.

2.7. Scanning electron microscopy (SEM)

Histological characters of CL, AL and EL before and after cooking were observed using SEM (Quanta-200, FEI Company, Netherlands). Cooked BR (at the optimum cooking time) and uncooked BR were lyophilized. To show microstructural changes of CL, AL and EL of rice during cooking, the kernels of uncooked and cooked BR were totally or partially striped with a pair of fine forceps and

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