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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

scalpel. For observing the porosity of EL and the thickness of coated film on cooked rice, the uncooked and cooked grain samples were fractured along their cross-sectional axis with a razor blade. During fracturing, efforts were made to produce no physical contact between the razor blade and the fractured surface of the internal endosperm tissues (Kim et al., 2004). With the surface fractured or striped upward, the samples were mounted on an aluminum stub using double-sided stick tape, and sputter coated with a thin film of gold, then examined at various magnifications with an accelerating voltage of 20 kV.

2.8. Fluorescence microscopy (FM)

Endosperm cell and distribution of lipids in rice were observed under FM (DM4000 B LED, Leica, German) equipped with a fluorescence illumination source and a filter combination. Grain samples were cooked to the optimum cooking time and lyophilized. Uncooked and cooked samples were embedded in tissue freezing medium (JUNG, Leica, German) on sample tray and oriented longitudinally. Then samples were allowed to harden in liquid nitrogen. The hardened block was faced on a rotary microtome (CM1900, Leica, German) equipped with cryostat. A representative longitudinal section (ca. 100 μm in thickness) was collected. For observing the endosperm cell, prepared specimens were directly examined and photographed under the FM. To show the distribution of lipids in rice, prepared specimens were immersed in 0.01% aqueous Nile Blue A (Sigma–Aldrich Co., LLC, St. Louis, MO), a lipid-specific probe, for 30 s, then rinsed briefly in water, before fluorescence microscopy (Wood et al., 2012). Fluorescence illumination in the blue region of the spectrum was applied.

2.9. Statistical analysis

The statistical analyses were performed using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL). A comparison of the means was ascertained by Turkey's test, at 5% level of significance using one-way analysis of variance (ANOVA).

3. Results

3.1. Grain samples, histological staining and stereomicroscopy

To investigate the individual effects of AL or CL on rice cooking behaviour, grain samples with varying number of tissue layers were prepared firstly. Stereomicroscopy images of unstained and stained BR, PBR and WR are displayed in Fig. 1. As prepared, BR had three layers namely EL, AL and CL, showing the outmost brown CL (Fig. 1a and g). PBR retained EL and AL, revealing the light-grey AL (Fig. 1b and h). WR merely kept EL, disclosing the innermost white EL (Fig. 1c and i). Chikubu and Shikano (1952) indicated that the CL, AL and EL of rice would be dyed bluish green, purple and pink respectively after staining with May Grunwald's reagent. To verify the grain samples were properly prepared, BR, PBR and WR were stained with this reagent. After staining, BR turned a uniform bluish green (Fig. 1d and j), PBR was a uniform purple (Fig. 1e and k), and WR was a uniform pink (Fig. 1f and l) for both cultivars. Staining results suggested that grain samples with varying number of tissue layers were successfully prepared.

3.2. Presence of CL and/or AL on rice cooking behaviour

3.2.1. Cooking quality analysis

Comparison between BR and WR indicated that presence of bran layer (CL + AL) significantly ($p < 0.05$) increased the optimum cooking time and decreased the water absorption, volume expansion ratio and total solids loss of rice (Table 1). This trend was consistent in both varieties.

To evaluate the individual contribution of CL to the above trend, the cooking quality indicators of BR and PBR were compared. For both varieties, CL remarkably affected rice cooking quality (Table 1). The values of water absorption, volume expansion ratio and total solids loss of BR were significantly lower ($p < 0.05$) than those of PBR. Meanwhile, the optimum cooking time of BR were significantly ($p < 0.05$) higher than that of PBR.

To assess the individual contribution of AL to the effects of bran layer on rice cooking quality, the cooking quality indicators of PBR and WR were compared. AL showed insignificant or marginal influence on rice cooking quality (Table 1). No significant difference ($p > 0.05$) in water absorption was found between PBR and WR for either variety. In addition, the PBR and WR of Kongyu 131 showed insignificant differences ($p > 0.05$) in volume expansion ratio and total solids loss. Although the volume expansion ratio and total solids loss between PBR and WR of Waiyin 7 varied significantly ($p < 0.05$), the differences were much smaller than those between BR and PBR. Take the volume expansion ratio of Waiyin 7 for example, the difference between PBR and WR (0.43) was less than one-third of that between BR and PBR (1.33). The optimum cooking time of PBR differed significantly ($p < 0.05$) from those of WR. However, according to mean value, the differences in optimum cooking time between PBR and WR were much smaller than those between PBR and BR. Using the optimum cooking time of Waiyin 7 as an example, the difference between PBR and WR was 1.00, while the difference between BR and PBR was 16.00.

3.2.2. Texture profile

Comparison between BR and WR indicated that presence of bran layer (CL + AL) significantly ($p < 0.05$) increased hardness and decreased adhesiveness of rice (Table 1). This trend was consistent in both varieties.

To evaluate the individual contribution of CL or AL to the above trend, texture parameters between BR and PBR as well as PBR and WR were compared respectively. Both CL and AL significantly ($p < 0.05$) affected the hardness and adhesiveness of rice (Table 1). Hardness was highest in BR (Kongyu 131, 3392 g; Waiyin 7, 3238 g), intermediate in PBR (Kongyu 131, 2774 g; Waiyin 7, 2916 g), and lowest in WR (Kongyu 131, 2292 g; Waiyin 7, 2321 g). On the contrary, adhesiveness values of BR, PBR and WR ranked in an inverse order. The values of hardness and adhesiveness among BR, PBR and WR differed significantly ($p < 0.05$), suggesting that both CL (BR vs. PBR) and AL (PBR vs. WR) influenced the hardness and the adhesiveness of rice significantly. Notably, according to mean values, the effects of AL exerted on rice hardness and adhesiveness were comparable to those of CL. These results were consistent in both cultivars.

3.2.3. Pasting profile

Comparison between BR and WR suggested that presence of bran layer (CL + AL) significantly ($p < 0.05$) lowered viscosity profiles of rice (Table 1 and Fig. 2). This trend was consistent in both varieties.

To evaluate the individual contribution of CL or AL to the above trend, viscosity parameters between BR and PBR as well as PBR and WR were compared respectively. Both CL and AL significantly ($p < 0.05$) lowered the pasting viscosity of rice (Table 1 and Fig. 2). WR displayed the highest viscosity profiles, followed by PBR, and BR (Fig. 2). Statistical analyses of pasting parameters showed that peak viscosity among BR, PBR and WR differed significantly ($p < 0.05$) and ranked in an order of WR > PBR > BR (Table 1). Similar trends were observed in trough viscosity and final viscosity. The percentage contributions of CL or AL to the declined viscosity by presence of bran layer were calculated according to the data

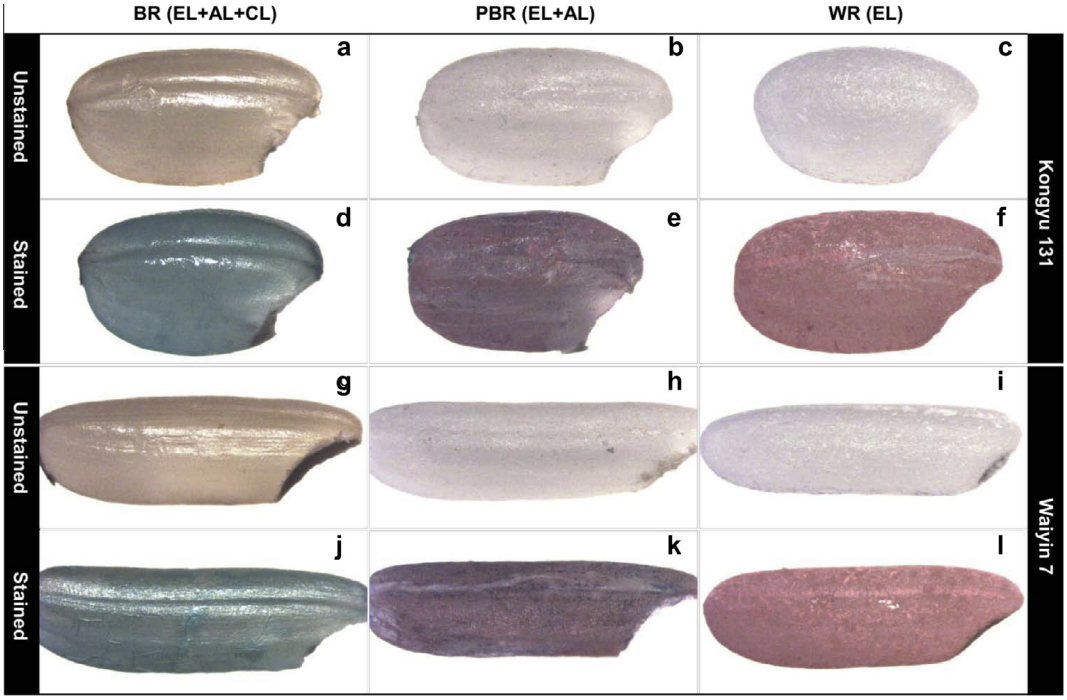

Fig. 1. Stereomicroscope micrographs of unstained (Konyu 131 a–c; Waiyin 7, g–i) and stained (Konyu 131 d–f; Waiyin 7, j–l) kernels of BR, PBR and WR. CL, cuticular layer; AL, aleurone layer; EL, endosperm layer; BR, brown rice; having three tissue layers (EL + AL + CL); PBR, peeled brown rice, retaining two tissue layers (EL + AL); WR; white rice; merely kept EL.

Table 1
Comparisons in cooking quality, texture and pasting profile of BR, PBR and WR.

Cooking behaviour	Kongyu 131			Waiyin 7		
	BR (EL + AL + CL)	PBR (EL + AL)	WR (EL)	BR (EL + AL + CL)	PBR (EL + AL)	WR (EL)
Cooking quality						
Optimum cooking time (min)	35.11 ± 0.38^{a}	25.00 ± 0.33^{b}	23.00 ± 0.33^{c}	37.33 ± 0.67^{a}	21.33 ± 0.33^{b}	20.33 ± 0.33^{c}
Water absorption (%)	134.96 ± 6.48^{b}	182.89 ± 7.57^{a}	191.17 ± 5.34^{a}	179.94 ± 11.58^{b}	248.95 ± 12.31^{a}	255.06 ± 11.35^{a}
Volume expansion ratio	2.69 ± 0.06^{b}	3.67 ± 0.05^{a}	3.71 ± 0.05^{a}	2.73 ± 0.10^{c}	4.06 ± 0.14^{b}	4.49 ± 0.19^{a}
Total solids loss (mg mL^{-1})	2.50 ± 0.11^{b}	3.85 ± 0.13^{a}	4.02 ± 0.13^{a}	4.71 ± 0.17^{c}	7.71 ± 0.28^{b}	8.24 ± 0.37^{a}
Texture profile						
Hardness (g)	3392 ± 138^{a}	2774 ± 105^{b}	2292 ± 78^{c}	3238 ± 149^{a}	2916 ± 152^{b}	2321 ± 97^{c}
Adhesiveness (g s^{-1})	47.50 ± 8.64^{c}	203.56 ± 34.61^{b}	304.90 ± 57.06^{a}	37.94 ± 7.36^{c}	144.34 ± 29.02^{b}	284.19 ± 50.93^{a}
Pasting profile						
Peak viscosity (cP)	1944 ± 25^{c}	2456 ± 30^{b}	2796 ± 30^{a}	2486 ± 22^{c}	3293 ± 40^{b}	4098 ± 56^{a}
Trough viscosity (cP)	1041 ± 20^{c}	1319 ± 20^{b}	1383 ± 20^{a}	1076 ± 12^{c}	1446 ± 31^{b}	1567 ± 31^{a}
Final viscosity (cP)	2172 ± 15^{c}	2745 ± 19^{b}	2802 ± 23^{a}	2119 ± 14^{c}	2635 ± 20^{b}	2945 ± 8^{a}

Values (means ± SD) with different lowercase letters in a row (within the same variety) are statistically significantly different ($p < 0.05$). BR, brown rice; PBR, peeled brown rice; WR, white rice; CL, cuticular layer, AL, aleurone layer; EL, endosperm layer.

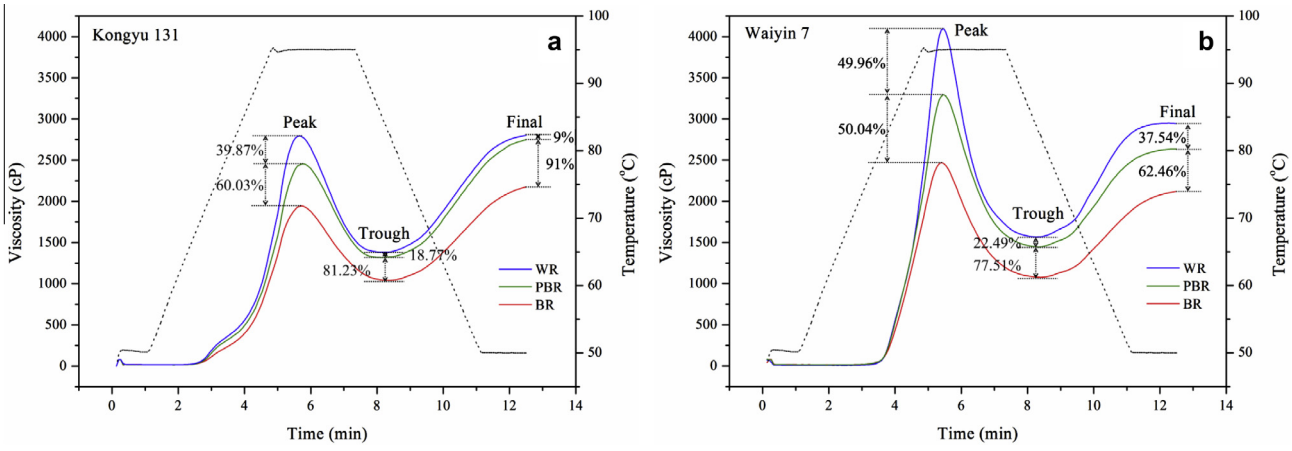

Fig. 2. Pasting profiles of BR, PBR and WR (Kongyu 131, a; Waiyin 7, b). Pasting curves were plotted according average viscosity values ($n = 3$).

in Table 1 (Fig. 2). AL showed 39.87% and 49.96% contribution to the declined peak viscosity of Kongyu 131 and Waiyin 7 respectively, while exhibited much lesser contribution to trough viscosity (18.77% for Kongyu 131 and 22.49% for Waiyin 7).

3.3. Histological characters of CL, AL and EL

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

The contents of total, hot-water insoluble and hot-water soluble cell wall materials were significantly different ($p < 0.05$) among CL, AL and EL of rice (Table 2). The contents of total and hot-water insoluble cell wall materials ranked in ascending order according to CL, AL and EL. In particular, AL contained a remarkably higher content of hot-water insoluble cell wall materials (Kongyu 131, 15.58 g/100 g; Waiyin 7, 16.25 g/100 g) than EL (Kongyu 131, 2.09 g/100 g; Waiyin 7, 3.16 g/100 g). AL had the highest content of hot-water soluble cell wall materials, followed by CL and EL.

Table 2
Contents of hot-water insoluble, hot-water soluble and total cell wall materials in CL, AL and EL of rice.

Rice fraction	Contents (g/100 g, d.b.)		
	Hot-water insoluble	Hot-water soluble	Total
Kongyu 131			
CL	33.39 ± 1.05[a]	4.07 ± 0.21[b]	37.45 ± 1.24[a]
AL	15.58 ± 1.09[b]	5.20 ± 0.34[a]	20.78 ± 0.84[b]
EL	2.09 ± 0.05[c]	0.95 ± 0.03[c]	3.04 ± 0.07[c]
Waiyin 7			
CL	28.15 ± 1.06[a]	4.06 ± 0.15[b]	32.21 ± 1.10[a]
AL	16.25 ± 0.29[b]	5.96 ± 0.59[a]	22.21 ± 0.36[b]
EL	3.16 ± 0.15[c]	1.13 ± 0.03[c]	4.29 ± 0.18[c]

Values (means ± SD) with different lowercase letters in a column (within same variety) are statistically significantly different ($p < 0.05$). CL, cuticular layer, AL, aleurone layer; EL, endosperm layer.

3.3.2. Microstructural architecture of CL, AL and EL before and after cooking

The microstructural architectures of CL, AL and EL before cooking were clearly revealed *in situ* by SEM and FM (Fig. 3 and Fig. 4a, b, i and j). As shown by SEM, CL, AL and EL were three morphologically distinct tissue layers of rice caryopsis (Fig. 4a and i). In general, the histological characters of the three tissue layers were similar between Kongyu 131 (Fig. 3a–d and Fig. 4a and b) and Waiyin 7 (Fig. 3i–l and Fig. 4i and j). Two closely appressed tissues, pericarp and testa, comprised the CL (Fig. 4a and b and Fig. 4i and j). The outermost pericarp was dense and free from pinhole porosity (Fig. 3a and i). Interior to the pericarp was testa (Fig. 4a and i). The testa was rich in lipids as revealed by FM (the yellow

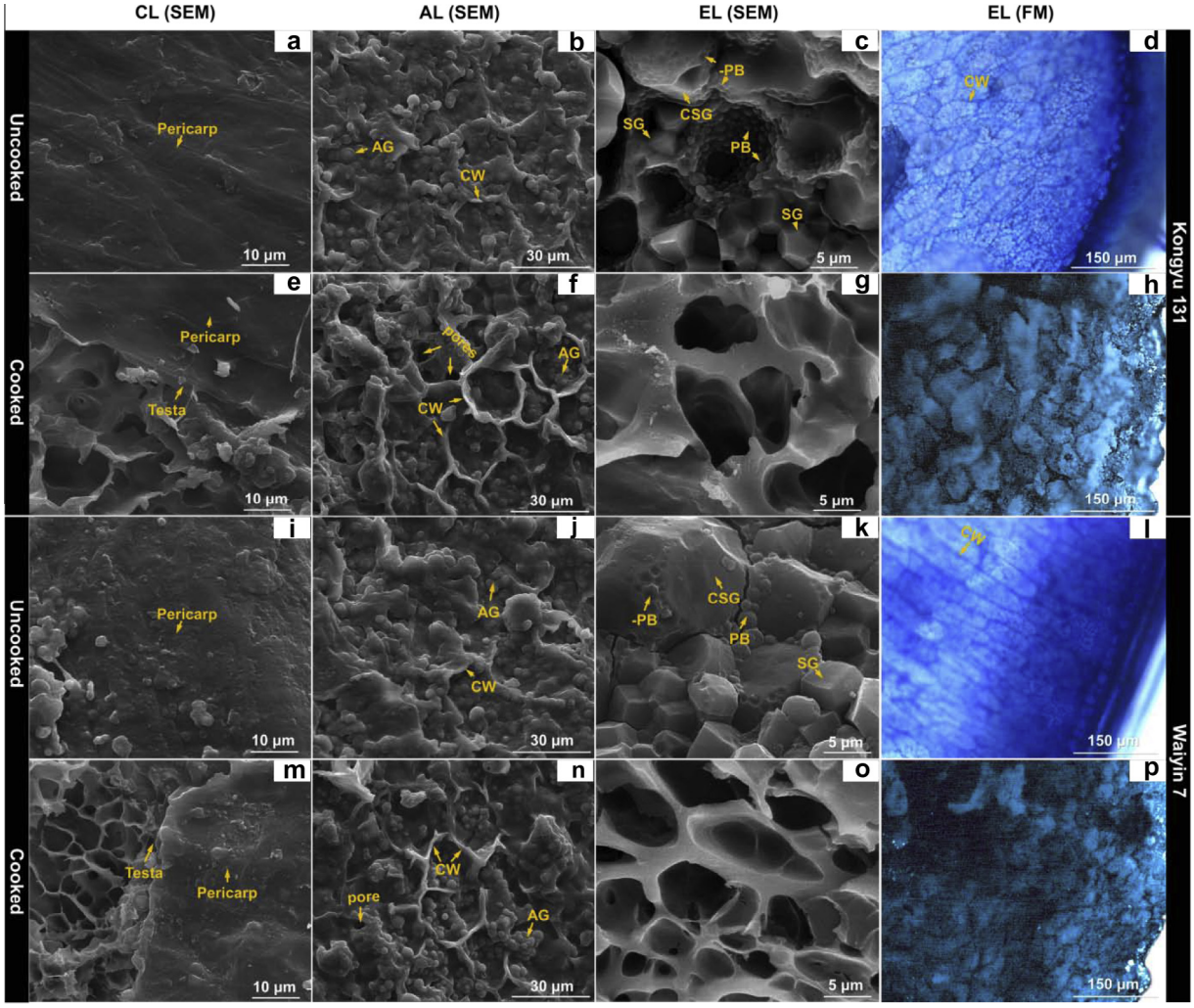

Fig. 3. SEM and FM micrographs of CL, AL and EL of uncooked and cooked rice. SEM shows the microstructural architecture of CL, AL and EL before (Kongyu 131, a–c; Waiyin 7, i–k) and after (Kongyu 131, e–g; Waiyin 7, m–o) cooking, in a vertical view. FM reveals endosperm cell walls before (Kongyu 131, d; Waiyin 7, l) and after (Kongyu 131, h; Waiyin 7, p) cooking, in a section view. CW, cell walls; AG, aleurone grain; SG, starch granule; CSG, compound starch granules; PB, protein body.

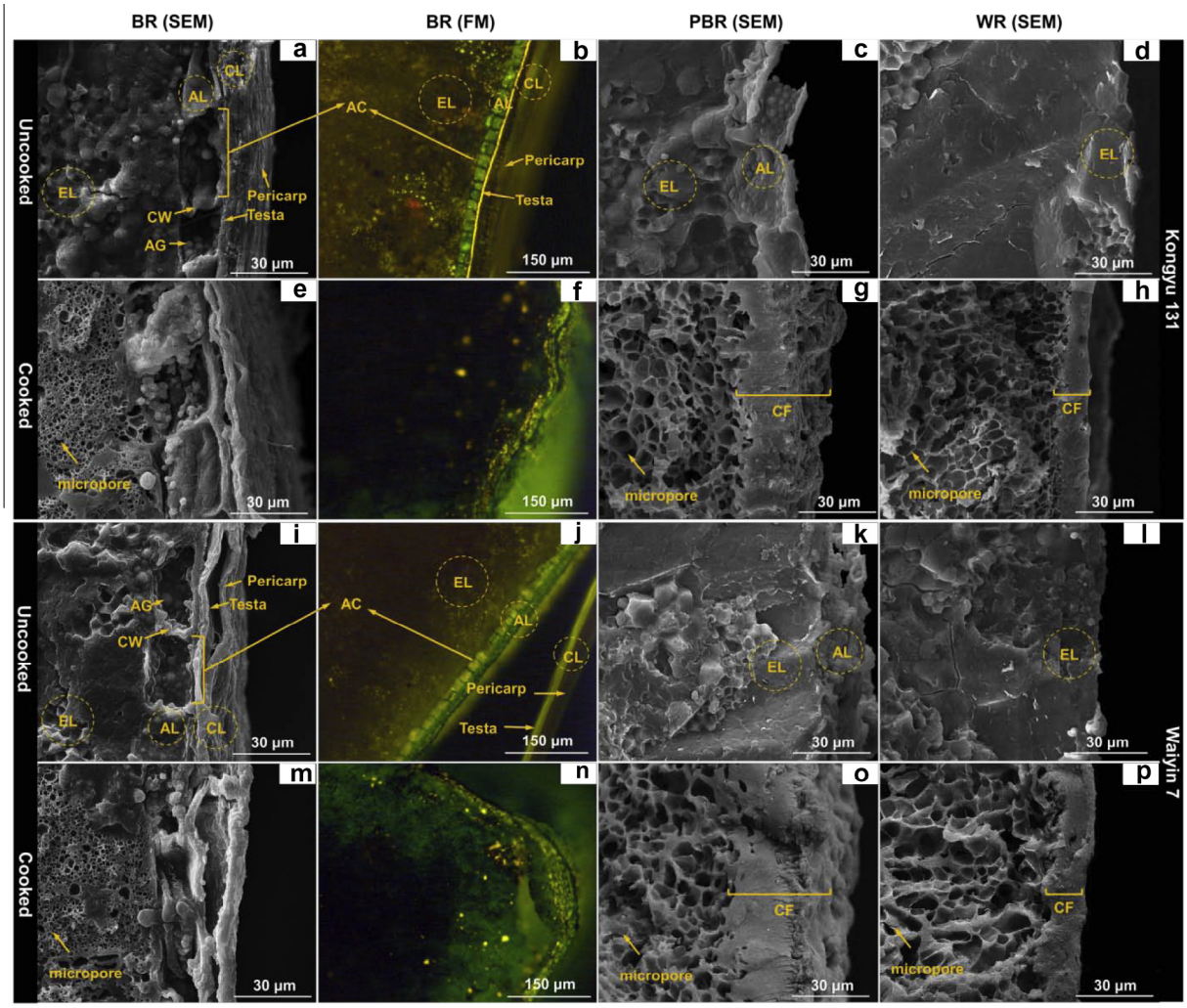

Fig. 4. SEM and FM micrographs of uncooked and cooked BR, PBR and WR. SEM disclosed the microstructural architecture of CL, AL and EL before and after cooking (Kongyu 131, a, e; Waiyin 7, i, m), in a section view. SEM also reveals the formation of coated film and micropore of BR, PBR and WR during cooking (Kongyu 131, a, c, d, e, g, h; Waiyin 7, i, k, l, m, o, p). FM illustrates the distribution of lipids in rice before and after cooking (Kongyu 131, b, f; Waiyin 7, j, n). The yellow material stained with Nile Blue A is neutral lipid enriched in aleurone cell and testa. CL, AL and EL were marked by dashed circles. AG, aleurone grain; AC, aleurone cell; CW, cell wall; CF, coated film. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

material stained by Nile blue A) (Fig. 4b and j). Immediately beneath the testa was the AL, a single layer of cuboidal aleurone cells (Fig. 4a, b and i, j). The thick cell walls of the aleurone cell were clearly visible in transverse sections of Kongyu 131 (Fig. 4a) and Waiyin 7 (Fig. 4i). This very thick cell wall is consistent with the high contents of cell wall materials of AL (Table 1). However, the profile of the cell walls of aleurone cell was barely discernible in the vertical view of AL (Fig. 3b and j), which probably was due to the covering of cytoplasmic components. The aleurone cells were loosely packed with scattered aleurone grains, lipid bodies and intracellular voids (Fig. 3b, j and Fig. 4a, i). The lipid bodies in aleurone cell can be detected by direct visualisation using fluorescence probe Nile Blue A and FM (Fig. 4b and j). Unlike AL, the inner EL was compact without intracellular voids (Fig. 4a and i). The EL was highly organised by polyhedral starch granules and ellipsoidal protein bodies (Fig. 3c and k). The starch granules were tightly packed and clustered into compound starch granules, and these compound starch granules were surrounded by protein bodies (Fig. 3c and k). The cell walls of the endosperm cells were too thin to see clearly by SEM. This low thickness agreed with the low content of cell wall materials of EL (Table 1). Nevertheless, the cell wall of endosperm cell could still be probed with FM by taking advantage of the autofluorescence characteristics of phenolic compounds in plant cell wall (Fig. 3d and l). These findings are consistent with those of Vidal et al. (2007), who found the thickness of endosperm cell wall was difficult to determine with SEM but could be estimated using a confocal laser scanning microscope at 1 μm.

Microstructural architecture of CL, AL and EL after cooking is clearly revealed *in situ* by SEM and FM (Kongyu 131, Fig. 3e–h; Waiyin 7, Fig. 3m–p). The pericarp and testa of CL were still intact even though the inner endosperm had been gelatinized completely (Fig. 3e and m). Comparing with CL, changes of AL upon cooking were more noteworthy. The thick cell walls of aleurone cells were disclosed in the vertical view of AL (Fig. 3f and n), probably due to the dissolution of cytoplasmic components that initially filled in AL. Meanwhile, channels were formed in AL (Fig. 3f and n), and many aleurone grains were still morphologically visible after cooking. Instead, the structural entity of EL was totally ruptured into a honeycomb-like matrix (Fig. 3g and o), indicating that starch granules and protein bodies were thermally unstable. Cell walls of endosperm were completely disrupted (Fig. 3h and p) upon cooking owing to their low thickness and weak mechanical strength.

3.3.3. Differences in porosity and coated film among cooked BR, PBR and WR

Porosity and coated film of cooked BR, PBR and WR are presented in Fig. 4. No obvious difference in porosity between PBR (Fig. 4g and o) and WR (Fig. 4h and p) could be discerned. However, the porosity of BR (Fig. 4e and m) was obviously smaller than that of PBR and WR. Most interestingly, a coated film was much easier to form on AL than on CL and EL. The coated film of cooked PBR was presumably 30–50 μm in thickness (Fig. 4g and o), while it was only about 5–15 μm for WR (Fig. 4h and p). In contrast, the CL and AL of BR remained morphologically identifiable, indicating that the coated film hardly formed on the glossy and dense CL of BR.

4. Discussion

Presence of bran layer (CL + AL) affects rice cooking behaviour. Evaluating the individual contribution of AL and CL to the effect would provide a rational basis for a reasonable milling of rice. To that end, grain samples with varying number of tissue layers, namely BR (EL + AL + CL), PBR (EL + AL) and WR (EL), were prepared (Fig. 1), and their cooking behaviours including cooking quality, texture profile and pasting profile were evaluated (Table 1 and Fig. 2). Comparative study of BR, PBR and WR suggested that: (1) AL exhibited an insignificant or marginal influence on rice cooking quality, including optimum cooking time, water absorption, volume expansion ratio and total solids loss. It was CL that should take the primary responsibility for the significant increase of optimum cooking time and decrease of water absorption, volume expansion ratio and total solids loss of rice. This agrees with the results of Desikachar, Raghavendra Rao, and Ananthachar (1965) and Roy et al. (2008), in which partial removal of CL by milling can greatly increase the water absorption of rice. Chen et al. (2012) also reported that partial removal of CL by plasma treatment could greatly decrease the optimum cooking time of rice. SEM and FM in this study showed that the dense pericarp and testa were enwrapping rice while cooking was ongoing (Fig. 4e, f, m and n). The testa was fatty (Fig. 4b and j) forming an impermeable barrier for water. Therefore, it was difficult for outer water to enter the grain, while the soluble solids inside the grain were hard to leach out. Moreover, the rice kernel could not expand freely and get fully gelatinized, unless a sufficient amount of water was absorbed. These could explain how the CL affected rice cooking quality; (2) AL showed comparable effects with CL on rice texture. Both AL and CL significantly ($p < 0.05$) increased the hardness and decreased the adhesiveness of rice. This result is consistent with those of Mohapatra and Bal (2006) and Saleh and Meullenet (2007), who found that continuous removal of bran layer by milling could decrease the hardness and increase the adhesiveness of rice; (3) AL showed comparable effects with CL on rice peak viscosity. Both of them significantly ($p < 0.05$) decreased the peak viscosity. Peak viscosity of rice was generally found to increase with increasing removal of bran layer by milling (Park et al., 2001; Perdon et al., 2001; Yoon & Kim, 2004), which agrees with our results. The significant effect of AL and CL on rice peak viscosity may be attributed to the enrichment of lipids and amylase in them. As shown by FM, rice lipids are predominantly located in the testa and aleurone cells (Fig. 4b and j). Batey (2007) pointed out that lipids usually lower the peak viscosity of RVA, presumably due to the lipids inhibiting the swelling of starch granules by reducing the rate of water imbibition, while amylase will digest the starch during RVA test. This can be substantiated by the fact that the viscosity of rice with bran was significantly increased by the addition of amylase inhibitor (Perdon et al., 2001). Therefore, presences of AL and/or CL would decrease the peak viscosity of rice.

CL had remarkable effects on both rice cooking quality and texture profile. Interestingly, AL showed insignificant or marginal effects on rice cooking quality, but exhibited remarkable effects on rice texture profile, suggesting that the mechanism about how AL affects rice texture and cooking quality is different from that of CL. We assumed the histological characters of AL were related to this unanticipated cooking behaviour. To test this assumption, contents of cell wall materials and microstructural architecture of AL were studied (Table 2, Figs. 3 and 4). As shown by SEM and FM, the thick cell walls, aleurone grains and lipids were the major components in uncooked AL (Fig. 4a, b, i and j). This is in accordance with several investigations where transmission electron microscope was applied (Bechtel & Pomeranz, 1977; Kang, Hwang, Kim, & Choi, 2006; Kim et al., 2004). In contrast to transmission electron microscopy, the SEM specimens of this study were prepared either by careful hand-peeling or merely fracturing, involving no procedure like fixing and ultrathin sectioning. Therefore, the SEM used in this paper could also disclose the spatial and architectural information of AL. Before cooking, many intracellular voids existed in the cuboidal aleurone cells which loosely packed with aleurone grains (Fig. 4a and i). After cooking, channels that presumably stemmed from the intracellular voids formed in AL (Fig. 3f and n). In addition, thick cell walls and a part of the aleurone grains maintained their physical structures under the cooking procedure (Fig. 3f and n). These thermally stable cell walls may widely distribute in AL because AL possessed high contents of hot-water insoluble cell wall materials (Kongyu 131, 15.58 g/100 g; Waiyin 7, 16.25 g/100 g) (Table 2).

Unlike AL, the structural entity of EL, including starch granule, protein bodies and cell walls, was totally ruptured into a honeycomb-like matrix after cooking (Fig. 3g, h, o and p). The micropores in the honeycomb-like matrix of cooked grains resulted from the sublimation of absorbed water during freeze-drying (Fig. 4e, g, h, m, o and p). Thus, the porosity accounted for the water absorption of grain samples. Water absorption is the major determinant for controlling the quality of cooked rice because the cooking of rice is a hydrothermal process. Saleh and Meullenet (2007) proposed that water absorption by the kernel during cooking dictated the hardness of cooked rice. Mohapatra and Bal (2006) further confirmed that water absorption was negatively correlated with hardness and positively associated with adhesiveness of cooked rice. Both the low porosity (Fig. 4e and m) and low water absorption (Table 1) of BR indicated that CL exhibited a great influence on water absorption of rice. Therefore, CL affected hardness and adhesiveness of cooked rice chiefly by retarding water to diffuse into the underlying EL during cooking. Although AL was rich in lipids as FM showed (Fig. 4b and j), no significant difference in water absorption was found between PBR and WR (Table 1), suggesting that AL did not affect water absorption of rice. The similar porosity of cooked PBR and WR reconfirmed this conclusion. This result was probably due to the channels formed in AL, which facilitated the flow of water into the EL during cooking. Therefore, AL did not affect rice texture by reducing water absorption as CL did. Ogawa, Glenn, Orts, and Wood (2003) suggested that the coated film on rice surface contributed to the texture and eating quality of rice. When grain samples were cooking in excess water, various cellular components leached into the cooking water. As cooking progressed, the water evaporated and the leached components precipitated, creating a coated film on the surface of the rice grains (Ogawa et al., 2003). Tamura and Ogawa (2012) reported that the thickness of the coated film on the dorsal side tended to be different from that on the ventral side of rice grains. Our study showed that the coated film was much easier to accumulate on AL than on CL and EL (Fig. 4e, g, h, m, o and p). As shown in Fig. 3, the thick cell walls and partial aleurone grains maintained their physical structures under the cooking procedure (Fig. 3f and n). These "structure-maintaining components" formed a skeleton frame in AL. Leached components would easily accumulate on this skeleton

frame. The thermally stable cell wall materials and aleurone grains immerged in the coated film like a reinforced concrete structure, resulting in higher hardness and lower adhesiveness of rice.

5. Conclusions

AL exhibited insignificant or marginal influence on rice cooking quality (optimum cooking time, water absorption, volume expansion ratio and total solids loss). It was the CL that should have taken the primary responsibility for the inferior cooking quality of rice covered with bran. Therefore, merely removing CL of rice during commercial milling operations is enough to achieve similar cooking quality with WR. However, merely removing CL of rice could not achieve similar texture profile (harness and adhesiveness) and peak viscosity with WR, because AL also imparted some extent effects on these properties. Histological investigation showed that thick cell walls were widely distributed in AL, and acted as a skeleton frame for the accumulation of leached components during cooking. The accumulated components formed a reinforced coated film on rice surface, which could explain why AL remarkably affected the texture profile of rice. This possible mechanism suggested that degrading the physical structure cell walls in AL (e.g. enzymatic treatment) may be a feasible way to eliminate the effects of AL on the texture profile of rice.

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