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Short communication

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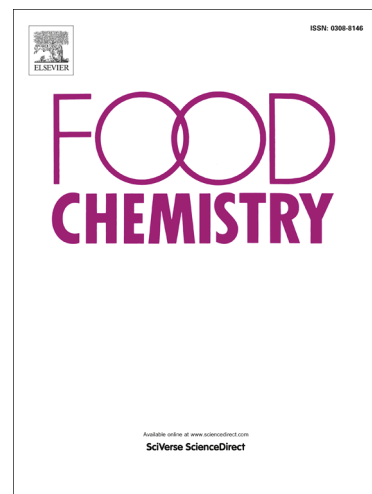
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Washing rice before cooking has no large effect on the texture of cooked rice

Hongyan Li ^a, Muyao Gao ^a, Jingyuan Yang ^a, Jing Wang ^{a*}, Baoguo Sun ^a

^aBeijing Advanced Innovation Center for Food Nutrition and Human Health, School of Food and Chemical Engineering, Beijing Technology and Business University, 11 Fucheng Road, Beijing 100048, China

*Corresponding author:

Jing Wang, e-mail: wangjing@th.btbu.edu.cn, Mobile: +86 13718205789;

E-mail address for other authors:

Hongyan Li: hongyan.li@btbu.edu.cn;

Muyao Gao: maureen1020@sina.com;

Jingyuan Yang: yangjy0801@163.com;

Baoguo Sun: sunbg@btbu.edu.cn;

Abstract

Washing rice before cooking is common to remove dust and any remaining bran produced by the milling process. In this study, the effect of washing procedure on the textural properties of cooked rice, and its relations to starch leaching and the molecular structure of leached starch are investigated. Statistical analysis indicated that the washing procedure does not significantly affect either hardness or stickiness of cooked rice, but the interaction between rice variety and washing times on stickiness is significant. The components of leached materials, chain-length distribution (CLD) and molecular size of leached starch are significantly different from those of the surface materials, but not largely varied between rices with different washing times, which helps explain the molecular mechanism of the causes of these textural results. This indicates for the first time that, the adhering materials on the surface of raw rice grains do not contribute to the texture of cooked rice.

Keywords:

rice washing, hardness, stickiness, leached materials, amylose, amylopectin, chain-length distribution, molecular size

1. Introduction

Washing raw rice with water before cooking is a very common pre-cooking procedure to remove dust and any remaining hull or bran produced during rice milling process (Champagne, Bett-Garber, Fitzgerald, Grimm, Lea, Ohtsubo, et al., 2010). This pre-cooking procedure leads to the drastic loss of essential micronutrients, such as B vitamins, iron, and zinc, thereby causing a lower nutritional quality (Bouis & Welch, 2010). Even though, it is still deemed to be a necessary process before rice cooking. In Pakistan, India or Iran, Basmati rice are washed 3 to 5 times before cooking in excess water, while in Thailand, China, Philippines, Japan and Australia, the low-amylose rice are washed 2 to 3 times before cooking in a rice cooker with the absorption method (Yu, Turner, Fitzgerald, Stokes, & Witt, 2016). It was reported that washing rice for 5 to 10 minutes can remove about 60-80% of lipids adhering on the surface of raw rice (Monsoor & Proctor, 2002), therefore, washing is thought to be a practical way to reduce free fatty acids and off-flavor development in cooked milled rice from lipid oxidation (Yu, Turner, Fitzgerald, Stokes, & Witt, 2016). However, less is known about the effect of washing rice on the cooked rice texture. Yu, Turner, Fitzgerald, Stokes, and Witt (2016) proposed that the washing procedure can remove free starch produced by the milling process which may change rice texture by changing grain-grain adhesion in a similar way to starch/amylose leaching. To verify this hypothesis, the effect of washing rice before cooking on the textural properties (hardness and stickiness) of cooked rice is investigated here, and its molecular mechanism is also uncovered.

2. Materials and methods

Three commercial rice varieties with known amylose content (Am), Pandaroo[®] Glutinous rice (Am~0%), Sunrice[®] Medium grain (Am~21%), Pandaroo[®] Jasmine rice (Am~13%) were purchased from supermarket.

2.1 Rice washing and cooking

As the pre-cooking procedure, a 10-g sample of three commercial rice was subjected to washing procedure by using about 80 ml distilled water in a 100 ml beaker. For each washing procedure, the mixture was firstly stirred for about 20 s with a glass rod, the excess water was immediately drained out of the beaker without any loss of rice grains. The washing procedure was repeated for 0, 3, and 10 times, respectively. To simplify, rices with different washing times were named as W0, W3, and W10, respectively. After the above procedure, distilled water was added to adjust the rice-to-water ratio for 2 different cooking types based on their amylose (Am) content (1:1.3~Am 0 %; 1:1.6~Am 10-25%). The beaker was sealed with aluminium foil, placed on a steaming tray, and steam cooked in a household rice cooker (PESKOE[®], Guangdong, China) for 30 min.

2.2 Texture profile analysis (TPA)

Rice grains (0.5 g) were placed in a single layer on the base plate. Texture analysis was conducted on a Texture analyser (Brookfield Engineering Laboratories, MA, US) with a 35-mm circular probe. The TPA settings are as follows: pre-test speed, test speed, and post-test speed were set at 1 mm/s; compression strain was set at 40%.

Each sample were tested for four times.

2.3 Extraction of the surface materials of raw rice and leached materials of cooked rice

The residual brans and other adhering particles on the surface of raw rice grains, the surface materials, were collected by washing rice and freeze-drying the washing water.

The leached materials of cooked rice were extracted using the same method as reported elsewhere (Li, Fitzgerald, Prakash, Nicholson, & Gilbert, 2017; Li, Wen, Wang, & Sun, 2017). The 10-g of cooked rice was rinsed with 100 ml of boiled distilled water using a glass rod for gently stirring about 10 s before filtering through a 250 μm sieve, the rinsing procedure was repeated with another 50 ml of boiled water, and then the rinsed water was collected, frozen, and freeze-dried for storage and further analysis.

2.4 Composition analysis of the extracted materials

Total solids were measured by weighing the freeze-dried surface materials and leached materials as described above. A Megazyme total starch assay kit (Wicklow, Ireland) was used to measure starch content of the extracted materials, while a BCA protein assay kit (Banxia, Beijing) was used for the determination of protein content.

2.5 Starch isolation and debranching

For the isolation of starch from the surface materials and leached materials, the following method described elsewhere is used (Li, Prakash, Nicholson, Fitzgerald, &

Gilbert, 2016a). All samples were dissolved in a DMSO solution with 0.5% (w/w) LiBr (DMSO/LiBr). A protease (from *Streptomyces griseus* (type XIV), Sigma-Aldrich, China) and sodium bisulfite solution was used to remove protein from the dried powder. The treated sample was dispersed in DMSO/LiBr and the starch then precipitated from the resulting soluble portion by adding 10 mL of ethanol. The extracted starch solution was dissolved again in the DMSO/LiBr solution and stored at room temperature for subsequent analysis by size exclusion chromatography (SEC).

For starch debranching, the above isolated starch (~4 mg) was dissolved in 0.9 mL of deionized water and then mixed with 10 μ L isoamylase (from *Pseudomonas sp.*, Megazyme, Ireland), 0.1 mL acetate buffer solution (0.1 M, pH 3.5), and 5 μ L sodium azide solution (0.04 g mL⁻¹). The mixture was incubated at 37 °C for 3 h. The debranched starch suspension was then heated in a water bath at 80 °C for 2 h, and then freeze-dried. The dried sample was dissolved in DMSO/LiBr solution for SEC analysis.

2.6 SEC

SEC is a separating technique which is based on hydrodynamic volume or radius. For branched starch, the molecular size of starch extracted from surface materials and leached materials was characterized in duplicate using an Agilent 1100 Series SEC system with GRAM 30 and 3000 columns (PSS) and a refractive index (RI) detector (RID-10A, Shimadzu Corp., Kyoto, Japan).

For debranched starch, CLDs was measured with another set of columns, GRAM 100 and 1000 columns, and the same RI detector. All the above procedures were performed following the method used in our previous publications (Li, Fitzgerald, Prakash, Nicholson, & Gilbert, 2017; Li, Prakash, Nicholson, Fitzgerald, & Gilbert, 2016a)

2.7 Statistical analysis

Analysis of variance (ANOVA) was carried out to determine rice variety \times washing times effects on the textural properties using the general linear model procedure (Proc glm). For each structural measurement, duplicate analyses were performed. All data were reported as mean \pm standard deviation (SD) using ANOVA with Duncan's pairwise comparisons. ANOVA was carried out using SPSS V. 16.0 software (SPSS Inc., Chicago, IL).

3. Results

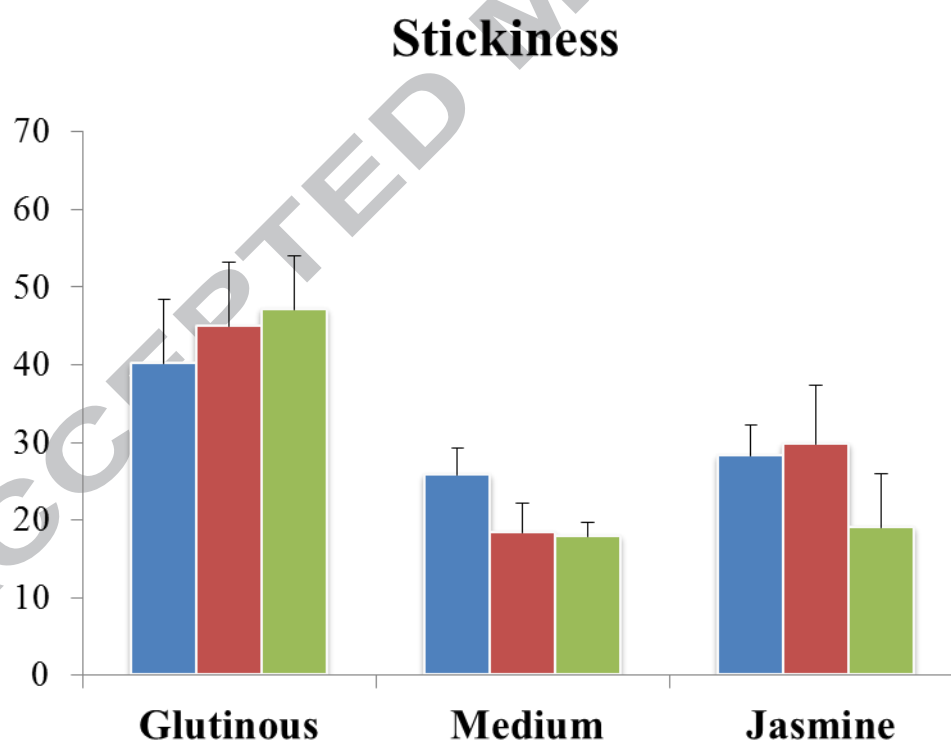
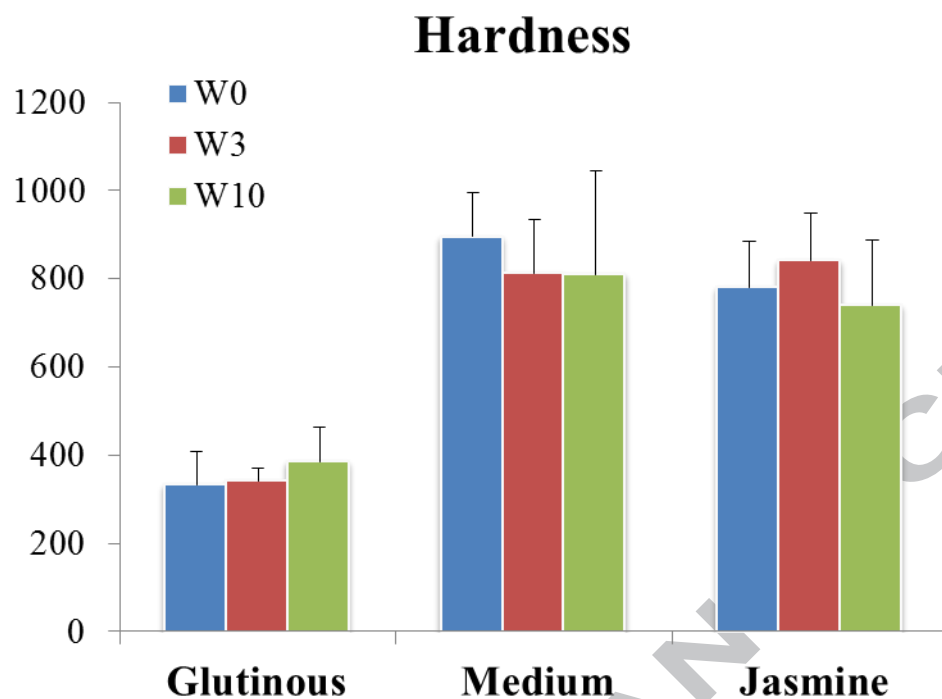


Figure 1 The hardness and stickiness of cooked rice for Glutinous, Medium, and Jasmine rice with different washing times before rice cooking (W0, W3, and W10, respectively).

3.1 The effect of washing procedure on the texture of cooked rice

As presented in **Figure 1** and **Table 1**, statistically, the washing procedure has no significant effect on either hardness or stickiness of cooked rice. On the other hand, **Table 1** also shows that variations of rice variety \times washing times for stickiness are significant ($p < 0.01$), indicating a rice variety-specific effect, especially for Medium and Jasmine rice (as also shown in **Figure 1**)

3.2 Component analysis for the surface materials and leached materials

As displayed in **Table 2**, the total solids, starch and protein contents of leached materials for W0, W3, and W10 have no significant differences, indicating that the washing procedure does not affect the components of leached materials. Compared to leached materials, the surface materials of raw rice grains contain significantly different components, e.g. starch accounts up to about 90% in leached materials while the surface materials have significantly higher proportion of protein.

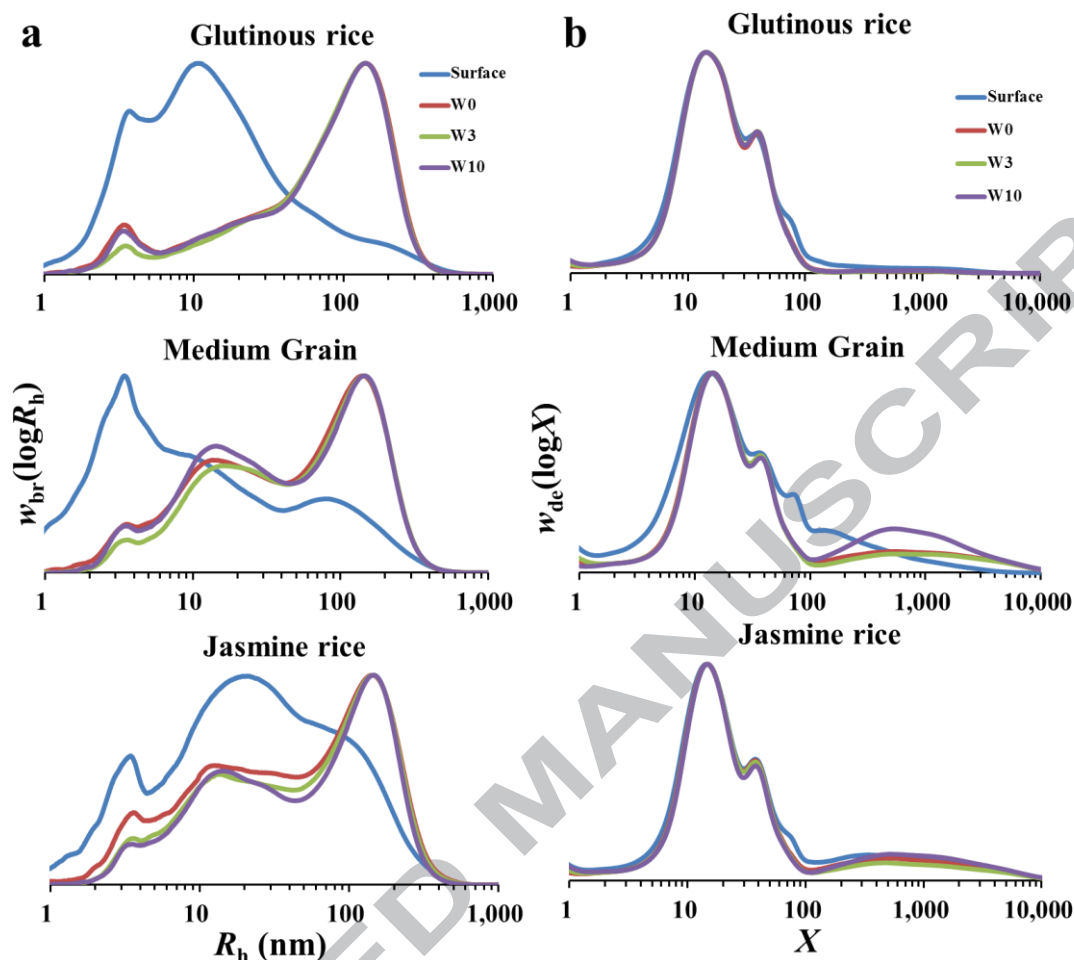


Figure 2 SEC weight distributions of branched and debranched starch molecules. (a) Weight distribution of branched starch, $w_{br}(\log R_h)$. (b) Weight distribution of debranched starch, $w_{de}(\log X)$. All distributions were normalized to the highest peak.

3.3 Starch molecular structures for the surface materials and leached materials

Figure 2a presents typical SEC weight distributions of branched starch molecules.

The branched starch distributions show two populations of α -glucans: amylopectin ($R_h > 50\text{nm}$) and amylose ($R_h \leq 50\text{nm}$). As described elsewhere (Li, Fitzgerald, Prakash, Nicholson, & Gilbert, 2017; Li, Prakash, Nicholson, Fitzgerald, & Gilbert, 2016a), the peak at $R_h \sim 4\text{nm}$ is thought to be residual proteins, due to the incomplete hydrolysis

by protease during the starch extraction procedure. Obviously, the protein peaks of surface materials of all three rice varieties are drastically high, especially for Medium rice displaying the highest peak at $R_h \sim 4\text{nm}$. As shown in **Table 2**, leached amylopectin size of all these rice varieties between different washing times is similar while leached amylose size (Medium and Jasmine) is slightly different between different washing times, indicating washing rice has slight impact on leached amylose size while no influences on leached amylopectin size.

Figure 2b displays typical weight chain-length distributions (CLDs) of debranched starches. As described elsewhere (Li, Prakash, Nicholson, Fitzgerald, & Gilbert, 2016a, 2016b), the components with $X \leq 100$ are defined as amylopectin chains, while those with $X > 100$ are defined as amylose chains. Similar to branched starches, CLDs of leached starch of all three rice varieties are significantly different from starch CLDs of the surface materials. It is noteworthy that, starch of the surface materials for all three rice varieties shows a triple-peak profile which is obviously different from the previous reported dual-peak profile of the typical amylopectin CLDs, and in here another peak at $X \sim 80$ is appeared for amylopectin in the surface materials. As shown in **Figure 2b** and **Table 2**, variations of both leached amylopectin and amylose CLDs of all samples between different washing times are not largely different.

4. Discussions

“Washing rice-hardness” relations. In this study, the washing procedure has no influence on the hardness of cooked rice for all three rice varieties. Since the washing procedure can mainly remove free starch produced by milling process (Yu, Turner,

Fitzgerald, Stokes, & Witt, 2016), and no changes occur to the inner structure of rice grains. As reported in our previous study, the inner structure of rice grains, especially that amylose molecular size and the proportion of amylose branches with DP from 1000 to 2000 are the determinants for the hardness of cooked rice, and how these structural features affect the degree of starch granule swelling during heating, may help explain the mechanism for the hardness of cooked rice (Li, Prakash, Nicholson, Fitzgerald, & Gilbert, 2016a), rather than the adhering materials on the surface of raw rice grains.

“Washing rice-stickiness” relations. Since the washing procedure removes free starch adhering on the surface of raw rice grains, it is proposed that the washing procedure could change the stickiness between cooked rice grains (Yu, Turner, Fitzgerald, Stokes, & Witt, 2016). However, our results deny this proposal. Statistically, the washing procedure has no significant impact on the stickiness of cooked rice (**Table 1**). The first molecular mechanism of stickiness between cooked rice grains was proposed in our recent study (Li, Fitzgerald, Prakash, Nicholson, & Gilbert, 2017), that stickiness increases with increasing total amount of amylopectin, the proportion of short amylopectin chains, and amylopectin molecular size in the leachate. In this study, the components and starch (molecular) structure analysis show that these three rice varieties with and/or without the washing procedure leach similar components, and the leached starch presents similar molecular structure, i.e. similar CLDs and molecular size. Thus, this explains the molecular-based mechanistic reason that the washing procedure has no large effects on the stickiness between cooked rice grains.

On the other hand, it also indicates that the adhering materials on the surface of raw rice grains cannot contribute to the stickiness between cooked rice grains, while the stickiness can mainly be affected by the leaching behavior and molecular structure of leached amylopectin (Li, Fitzgerald, Prakash, Nicholson, & Gilbert, 2017).

5. Conclusions

This study explores the effect of washing rice before cooking on the textural properties of cooked rice. The statistic shows that the washing procedure has no significant impact on either hardness or stickiness of cooked rice. Further investigation shows that the washing procedure does not affect starch leaching behavior and molecular structure of leached starch, i.e. that the components of leached materials, molecular size and CLDs of leached starch for all three cooked rice between different pre-cooking procedures display no large variations. This finding also denies other researchers' proposal that the washing procedure may alter rice texture by removing free starch produced by the milling process. It indicates for the first time that the adhering materials on the surface of raw rice grains do not contribute to the texture of cooked rice. This study provides a new understanding and information for rice industry and consumers to design rice processing steps with desirable palatability.

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Declarations of interest: none.

ACCEPTED MANUSCRIPT

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Table 1 Mean values and Mean square values from ANOVA for rice variety \times washing times effects.

Mean value						Mean Square Values			
Rice variety	Stickiness	Hardness	Washing times	Stickiness	Hardness	Source	df	Stickiness	Hardness
Glutinous	44.1 \pm 8.0 ^a	352.0 \pm 74.9 ^b	W0	31.4 \pm 8.4 ^a	669.8 \pm 264.8 ^a	Rice variety	2	2728.1***	1272277.4***
Medium	20.7 \pm 4.8 ^b	839.8 \pm 158.3 ^a	W3	31.0 \pm 12.9 ^a	665.1 \pm 252.8 ^a	Washing times	2	60.2	5963.8
Jasmine	25.6 \pm 7.7 ^b	779.1 \pm 112.8 ^a	W10	28.0 \pm 14.9 ^a	636 \pm 238.1 ^a	Rice variety \times Washing times	4	169.8**	17888.4

Mean values with different letters in the same column indicate significant difference at the 0.05 level.

,* indicate significance at 0.01, and 0.001 levels, respectively.

Table 2 Components and starch molecular parameters for the surface and leached materials.

Sample		Components analysis			Amylopectin						
		Total solids ^a	Starch content	Protein content	$\bar{R}_{h,Ap}$	$6 < X \leq 12$	$12 < X \leq 24$	$24 < X \leq 36$	$36 < X \leq 100$	\bar{X}_w	\bar{X}_N
Glutinous	Surface	$14.1 \pm 2.1a$	$85.1 \pm 1.5\%a$	$12.4 \pm 0.9\%a$	$104.4 \pm 1.0b$	$25.1 \pm 0.4\%a$	$38.3 \pm 0.2\%a$	$15.1 \pm 0.1\%a,b$	$21.5 \pm 0.7\%a$	17a	25a
	W0	$69.9 \pm 3.0b$	$92.1 \pm 1.8\%b$	$4.1 \pm 0.6\%b$	$117.8 \pm 2.4a$	$24.7 \pm 0.0\%a,b$	$39.8 \pm 0.2\%b$	$14.9 \pm 0.0\%b$	$20.6 \pm 0.2\%a$	17a	25a
	W3	$72.2 \pm 1.7b$	$92.2 \pm 0.8\%b$	$3.7 \pm 0.1\%b$	$116.7 \pm 0.7a$	$24.2 \pm 0.4\%b$	$40.0 \pm 0.1\%b$	$15.3 \pm 0.1\%a$	$20.5 \pm 0.1\%a$	17a	25a
	W10	$67.3 \pm 2.5b$	$92.9 \pm 0.5\%b$	$4.5 \pm 0.1\%b$	$117.6 \pm 0.9a$	$24.1 \pm 0.1\%b$	$40.0 \pm 0.2\%b$	$14.9 \pm 0.1\%b$	$21.0 \pm 0.4\%a$	17a	25a
Medium	Surface	$10.6 \pm 2.9a$	$54.7 \pm 2.2\%a$	$36.1 \pm 5.2\%a$	$104.9 \pm 4.8b$	$27.4 \pm 0.2\%a$	$35.2 \pm 0.5\%b$	$13.7 \pm 0.2\%b$	$23.7 \pm 0.4\%a$	17a	25a
	W0	$28.6 \pm 1.8b$	$91.3 \pm 0.0\%b$	$6.2 \pm 0.3\%b$	$118.5 \pm 0.7a$	$25.7 \pm 0.2\%b$	$40.9 \pm 0.1\%a$	$14.8 \pm 0.0\%a$	$18.6 \pm 0.3\%b$	17a	25a
	W3	$23.2 \pm 2.9b$	$92.1 \pm 1.4\%b$	$4.5 \pm 0.0\%b$	$120.0 \pm 1.3a$	$25.1 \pm 0.3\%b,c$	$41.4 \pm 0.1\%a$	$15.1 \pm 0.1\%a$	$18.4 \pm 0.3\%b$	17a	25a
	W10	$28.7 \pm 2.2b$	$93.2 \pm 0.8\%b$	$4.5 \pm 1.6\%b$	$118.5 \pm 2.9a$	$25.0 \pm 0.2\%c$	$41.5 \pm 0.0\%a$	$14.8 \pm 0.3\%a$	$18.8 \pm 0.0\%b$	17a	25a
Jasmine	Surface	$17.4 \pm 0.8a$	$60.6 \pm 0.7\%a$	$27.6 \pm 0.1\%a$	$99.4 \pm 0.7b$	$24.3 \pm 0.1\%a$	$40.9 \pm 0.2\%b$	$14.3 \pm 0.1\%b$	$20.5 \pm 0.3\%a$	17a	25a
	W0	$27.4 \pm 1.8b$	$90.2 \pm 0.9\%b$	$5.9 \pm 0.6\%b$	$119.3 \pm 1.3a$	$23.6 \pm 0.2\%a,b$	$42.8 \pm 0.0\%a$	$14.8 \pm 0.0\%a$	$18.9 \pm 0.3\%a,b$	17a	25a
	W3	$26.3 \pm 2.7b$	$90.5 \pm 0.6\%b$	$6.6 \pm 0.9\%b$	$119.8 \pm 0.9a$	$23.2 \pm 0.2\%b$	$43.4 \pm 0.5\%a$	$14.9 \pm 0.3\%a$	$18.5 \pm 0.4\%b$	17a	25a
	W10	$29.2 \pm 1.2b$	$92.8 \pm 0.6\%b$	$5.1 \pm 0.5\%b$	$120.4 \pm 0.2a$	$23.0 \pm 0.6\%b$	$43.5 \pm 0.7\%a$	$14.5 \pm 0.0\%a,b$	$19.1 \pm 1.3\%a,b$	17a	25a

Sample		Amylose						
		$\bar{R}_{h,Am}$	Am content	$100 < X \leq 1000$	$1000 < X \leq 2000$	$2000 < X \leq 20000$	\bar{X}_w	\bar{X}_N
Glutinous	Surface	-	$5.8 \pm 0.0\%$ a	-	-	-	-	-
	W0	-	$2.1 \pm 0.6\%$ b	-	-	-	-	-
	W3	-	$1.8 \pm 0.9\%$ b	-	-	-	-	-
	W10	-	$2.3 \pm 0.5\%$ b	-	-	-	-	-
Medium Grain	Surface	11.5 ± 0.0 c	$16.9 \pm 2.9\%$	$14.9 \pm 2.2\%$ a	$1.4 \pm 0.5\%$ c	$0.6 \pm 0.2\%$ c	274 ± 23 c	1003 ± 153 b
	W0	14.7 ± 0.2 b	$18.7 \pm 0.4\%$ a	$10.6 \pm 0.3\%$ b	$3.4 \pm 0.0\%$ b	$4.7 \pm 0.1\%$ b	690 ± 13 b	1405 ± 4 a
	W3	15.6 ± 0.0 a	$17.1 \pm 0.1\%$ a	$9.1 \pm 0.2\%$ b	$3.3 \pm 0.0\%$ b	$4.7 \pm 0.1\%$ b	765 ± 17 a	1386 ± 4 a
	W10	14.5 ± 0.5 b	$20.9 \pm 4.6\%$ a	$9.9 \pm 0.3\%$ b	$5.6 \pm 1.0\%$ a	$5.5 \pm 0.0\%$ a	659 ± 43 b	1434 ± 24 a
Jasmine rice	Surface	15.0 ± 0.3 a	$20.9 \pm 0.8\%$ a	$12.7 \pm 0.3\%$ a	$3.6 \pm 0.2\%$ a,b	$4.6 \pm 0.4\%$ b	594 ± 11 a	1425 ± 8 a
	W0	14.6 ± 0.1 b	$18.6 \pm 1.3\%$ a	$11.1 \pm 1.1\%$ a,b	$3.3 \pm 0.2\%$ a,b	$4.2 \pm 0.1\%$ b	649 ± 29 a	1409 ± 8 a
	W3	14.8 ± 0.0 a,b	$15.8 \pm 0.5\%$ b	$9.1 \pm 0.3\%$ b	$2.8 \pm 0.1\%$ b	$3.9 \pm 0.0\%$ b	685 ± 0 a	1395 ± 0 a
	W10	14.8 ± 0.1 a,b	$20.8 \pm 0.9\%$ a	$11.4 \pm 0.8\%$ a	$3.8 \pm 0.5\%$ a	$5.6 \pm 0.4\%$ a	660 ± 100 a	1434 ± 50 a

^a the unit of total solids is mg/ g rice kernels.

Highlights

- Statistic shows that washing times have no effects on hardness and stickiness.
- The washing procedure does not affect components and structure of leached material.
- This denies the hypothesis proposed by other researchers in a recent review paper.