

Effect of Superfine Grinding on Physicochemical Properties, Antioxidant Activity and Phenolic Content of Red Rice (*Oryza sativa* L.)

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Abstract

Red rice gains popularity as a functional crop owing to its high polyphenols content and antioxidant activity. However, active components are discarded in common milling. Superfine ground technology was employed in this paper. To evaluate the influence of superfine ground processing on the physicochemical properties and functional effect of red rice (*Oryza sativa* L.), four powders with the size of 156.74 µm, 69.53 µm, 26.35 µm, and 10.68 µm were prepared by superfine grinding technology in this paper. Results showed that the size was smaller for red rice powders, greater for the bulk density (from 0.624 g/ml to 0.745 g/ml), and smaller for the angle of repose (from 74.67° to 61.41°) and slide (from 38.99° to 26.42°). The values of water solubility index, water holding capacity and enzymatic digestibility by α -amylase significantly increased with the decreasing particle size (P < 0.05). In addition, antioxidant activity and phenolic content were enhanced by superfine ground. These results indicated that superfine ground would improve the physicochemical and functional properties of red rice, which was helpful to promote the overall quality and healthy effect of foods containing red rice.

Keywords

Superfine Ground, Red Rice, Physicochemical Properties, Antioxidant Activity, Phenolic Content

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1. Introduction

In many parts of Asian countries, red rice (*Oryza sativa* L.) was not considered to be a weed but a traditional staple crop [1]. Red rice was also gaining popularity as a functional crop owing to its high polyphenols content [2], anthocyanins [3] and other nutrition components [4] [5]. Previous studies have revealed that the antioxidant activity of this species is due to its biological components [6]-[8]. The growing interest for red rice has resulted in the emergence of various products of colored noodles, cakes, and alcoholic beverages [2]. However, the endosperm of red rice kernels was typically coated with firmly red pericarp that was difficult to remove on common milling processing. Common milling resulted in non-uniform color of red rice powder and poor taste, which reduced the commercial value of red rice products [9]. Furthermore, some active components in red pericarp were discarded. Hence, more effective technology should be adopted to preserve the active components in the maximal degree and obtain more uniform red rice powder, especially for the improvement of texture and overall quality of food containing red rice.

Superfine grinding technology has been applied in biotechnology and food material [10]; however, very limited information is available about the effect of this technology on physiochemical and antioxidant properties of red rice. In the present work, parameters of the physicochemical properties, antioxidant activity and total phenolic content were employed to investigate the application of the superfine grinding technology in red rice.

2. Materials and Methods

2.1. Materials

Red rice was obtained from the Meili Lake Market in Jinan City, Shandong Province, China. Red rice was milled to coarse particles by a disc-mill, then, screened through different sized sieves to separate granulates (d < 1 mm), the superfine powders with the size of 156.74, 69.53, 26.35 and 10.68 μ m were obtained in an BL-32 type micronizer (Beili Powder Machinery Company, Jinan, Shandong, China). The particle size (D₉₀) was tested using a laser particle size instrument (Weina-16, Jinan, Shandong, China). The particle size distributions of the powder were: D₉₀ = 156.74, 59.53, 21.35, and 10.68 μ m. Three measurements were carried out for each red rice powder.

2.2. Bulk Density

The bulk density (g/ml) was the density including pores and interparticle voids. Four types of red rice powders were filled in a 10 ml volumetric flask (W_1) up to the mark and were weighed (W_2) separately. The bulk density of the red rice powders was calculated as follows: $d_0 = (W_2 - W_1)/10$ [11]. Where W_2 was the total weight of the red rice powder and flask, and W_1 was the weight of the flask only. The experiments were repeated five times and the measurement of each sample was repeated three times.

2.3. Angle of Repose and Slide

The angle of repose was measured using the sequence of steps stated here [12]. Firstly, a filler was fixed above some graph paper so that the distance of the paper from the outlet of the filler (*H*) was 3 cm, and the filler was vertical to the paper. Then the red rice powder of different size was separately poured into the filler until the tip of the powder cone touched the outlet of the filler. The diameter (2*R*) of the cone was measured for each type powder. The angle of repose (θ) was calculated as the following formula: $\Theta = \operatorname{arctg}(2R/H)$.

The slide angle was determined according to method of Ileleji and Zhou [13] with some slight modification. The 5.000 g of red rice samples were separately weighed. Then red rice powder was poured on glass plane with a length (*L*) of 130 mm and width of 100 mm. The sliding angle of repose was estimated by gradually lifting the glass plane until the surface of the red rice powder began to be slide. The angle between the inclined glass and horizontal was called the angle of slide. The vertical distance (*H*) between the top of inclined glass plane and the horizontal was measured. The angle of slide (α) was calculated as the following formula: $\alpha = \arcsin(H/L)$.

2.4. Water Solubility Index

The water solubility index (WSI) was determined according to the method of Zhao et al. [10] with some mod-

ifications. The 1.000 g of sample was measured and dispersed in tubers with 50 ml distilled water. The tubers were placed in a reciprocating water bath shaking instrument and shaken for 30 min at different temperatures of 60°C, 80°C, and 100°C, respectively. Then, the tubers were centrifuged at 3000 r/min for 30 min, excess water of the clear supernatant solution was drained off by evaporation. Finally, the samples were dried at 105°C and weight of red rice powder (A) dissolved in water with different temperatures could be obtained. The WSI was caculated as WSI(%) = $A \times 100\%$.

2.5. Water Holding Capacity

This parameter was determined according to the method reported by Wu *et al.* [14] with some modifications. Firstly, the weights of cleaned centrifuge tubes (*M*) and different sized samples (*M*₁) were measured. Then the samples (*M*₁) were dispersed in water (*M*₂) according to $M_1:M_2 = 1:20$ at 20°C and poured into the centrifuge tubes placed in a water bath at 70°C, 80°C, and 90°C, respectively. The tubes were held for 15 min separately and then they were placed in cold water for 30 min, followed by centrifugation for 20 min at 3000 r/min. The supernatant liquid was removed and the centrifuge tubes with the powders (*M*₃) were weighed again. The formula to calculate water holding capacity (*WHC*) was as follows: WHC(g/g) = $(M_3 - M)/M_1$.

2.6. Digestibility with *α*-Amylase

Enzymatic digestibility with α -amylase was performed according to the method described by Liu *et al.* [15]. Approximately 1 g of sample was added to 30 ml of phosphate buffer (0.2 mol/l, pH 6.9) in a test tube and allowed to stand for 30 min in a 95°C water bath. After cooling at 25°C, α -amylase (320 unit, A6380, Sigma-Aldrich) was added and incubated with shaking at 30°C for up to 14 h. After digestion, the undigested sample was removed by centrifugation and analyzed by gravimetric methods.

2.7. Antioxidant Activity

Scavenging activity of the extracts against DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical was measured based on Wu *et al.* [14]. Positive control was prepared by mixing 2 ml of ascorbic acid (0.05 mg/ml) and 3 ml of DPPH (0.04 mg/ml), whereas negative control was prepared by mixing 2 ml of distilled water with 3 ml of DPPH. The 2 ml of the extract (final concentrations were 25, 50, 100, and 150 µg/ml, respectively) prepared from working solution was added to 3 ml of DPPH. The mixture was gently homogenized and left to stand at room temperature for 30 min. Absorbance was read using a spectrophotometer at a wavelength of 517 nm. Activity of scavenging DPPH radicals was calculated using the following equation: Scavenging activity (%) = $[(A_{(-)} - A_{s})/(A_{(-)} - A_{(+)})] \times 100$, where, As is the absorbance of the sample, $A_{(-)}$ and $A_{(+)}$ are the absorbance values of negative and positive controls, respectively.

The reducing power of the extracts from different sized powder was determined by the method of Fu *et al.* [16] with minor modification. Different amounts of working solution (final concentrations were 200, 400, and 600 μ g/ml, respectively) were mixed with 2.5 ml of phosphate buffer (0.2 mol/l, pH 6.6) and 2.5 ml of potassium ferricyanide [0.03 mol/l, K₃Fe(CN)₆]. Aliquots (2.5 ml) of 0.6 mol/l trichloroacetic acid were added to the mixture, which were then centrifuged for 10 min at 1000 × g (Hitachi SCR20BC, Japan). The upper layer of solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.006 mol/l FeCl₃, and the absorbance was measured at a wavelength of 700 nm in a spectrophotometer.

2.8. Total Phenolic Content

Phenolic were extracted by the method of Jeng *et al.* [5]. The content of total phenolic in extract was estimated using the Folin-Ciocalteu assay [17]. The absorption value was determined at a wavelength of 735 nm with gallic acid used as standard.

2.9. Statistical Analysis

The statistical analysis was carried out using SPSS 17.0 (SPSS Inc., Chicago, IL). Results were expressed as mean values \pm standard deviation. Means were compared by univariate variable analysis. A difference was considered statistically significant when P < 0.05.

3. Results and Discussions

3.1. Bulk Density

Bulk densities of the red rice powders varied with their particle sizes (Table 1). The bulk density of the red rice was in the range of 0.624 g/ml - 0.745 g/ml, and the bulk density increased as the particle size decreased. The results confirmed the significant effect of the particle size on the bulk density (P < 0.05). The reason for this might be that, as particle size decreased, pore spaces between particles decreased resulting in the increase in the bulk density [18].

3.2. The Angle of Repose and Slide

The angle of repose and slide could reflect the variations in the fluidity of the powder. The angle of repose and slide values of red rice particles were showed in **Table 1**, which ranged from 74.67° (156.74 μ m) to 61.41° (10.68 μ m) and 38.99° (156.74 μ m) to 26.42° (10.68 μ m), accordingly. Significant differences (*P* < 0.05) were existed in angle of repose and slide among the red rice particles. Powder of 10.68 μ m had a lower angle of repose and slide than the others followed by 26.35 μ m (65.80° and 30.31°) and 69.53 μ m (70.84° and 35.50°), the highest were 156.74 μ m (74.67° and 38.99°). As the angle of repose and slide increased so did the granular bulk become less flowable [13].

As described above, superfine powders with 26.35 and 10.68 µm had better flow behavior and the surface attachment of the powder would also be higher, which was in agreement with the investigations of Zhao *et al.* [10] and Santomaso *et al.* [19]. The angle of repose and slide of red rice powders usually decreased with powder size reduction, the reason might appear to be the formation of aggregates. The aggregates tended to arrange in a cone the angle of which was much lower than expected for red rice powders. Therefore, the quality of red rice superfine powders would be better, and the mixture in food containing red rice powder would also be uniform and no separable.

3.3. The Water Solubility Index (WSI) and Water Holding Capacity (WHC)

As shown in Figure 1(a), the WSI increased with decreasing size at the same temperature. The WSI increased with increasing temperature, and significant difference was found between the different particles (P < 0.05). The values of WSI of red rice with particle sizes of 156.74 µm - 10.68 varied from 5.42% to 9.33%, 5.93% to 10.15%, 6.04% to 11.47%, 6.16% to 12.61%, for 60°C, 80°C, 100°C, respectively.

Meanwhile, as shown in **Figure 1(b)**, the WHC rised with the size decreasing, and significant difference was found (P < 0.05), however, no significant difference was found when the temperature of samples reached 90°C (P > 0.05). The values of WHC with particle of 156.74 - 10.68 µm varied from 6.52 to 9.62, 8.23 to 11.66, 8.59 to 12.11, 8.70 to 12.34, for 70°C, 80°C, 90°C, respectively. The results indicated that superfine ground enhanced WHC under high temperature of red rice, provided the better hydrophilic and avoided the water lose, which was favor to alleviate the retrogradation of products made by red rice powder.

As demonstrated in **Figure 1(a)** and **Figure 1(b)**, it was noted that the WHC and WSI values of smaller particle size was higher than others during soaking. It might be due to the fact that after superfine grinding, the surface properties, such as surface area and surface energy had been increased. Moreover, the hydrophilic groups in red rice might had been exposed, which resulted in an easy integration with water, and the dispensability and solubility increased, finally, the value of WCH and WSI increased. The results indicated that the size was smaller for the red rice particle and greater for the solubilization and retaining water capacity of red rice powder.

Table 1. Particle size, bulk density, angle of repose angle slide of red rice powder.				
Particle size (µm)	Bulk density (g/ml)	Repose angle (°)	Slide angle (°)	
156.74 ± 12.43	$0.624\pm0.021a$	$74.67 \pm 1.92a$	$38.99 \pm 1.86a$	
69.53 ± 5.78	$0.677\pm0.025b$	$70.84 \pm 1.49b$	$35.50 \pm 1.79b$	
26.35 ± 1.40	$0.716\pm0.014c$	$65.80 \pm 1.35c$	$30.31 \pm 1.33c$	
10.68 ± 0.89	$0.745\pm0.009d$	$61.41 \pm 1.11 \text{d}$	$26.42\pm1.08d$	

Each value represents mean \pm standard deviation of three replicates; different letters in the same column mean significant difference (P < 0.05).



Figure 1. Water solubility index (a), water holding capacity (b), and enzymatic digestibilities by α -amylase (c) of different sized red rice particles.

3.4. Enzymatic Digestibility

The digestibility of different sized red rice powder by α -amylase was shown in **Figure 1(c)**. The values of starch digestibility by α -amylase of red rice with particle sizes of 156.74 µm, 69.53 µm, 26.35 µm and 10.68 µm was 37.10%, 46.25%, 53.52% and 60.58%, respectively. Significant differences were found between the four particles (P < 0.05) and the enzymatic hydrolysis increased with the decreased parameters of red rice powder. After superfine ground of red rice, most of the crystalline state converted to a non-crystalline state with reduced mean granule particle size and increased granule specific surface area [20], and structure of cellulose and hemicelluloses in red pericarp of red rice kernels was broken [21], which made powder susceptible to enzymatic hydrolysis.

3.5. Antioxidant Activity and Total Phenolic Content

The scavenging activity on DPPH radical was related to the concentration of extracts, the activity increased as a result of increasing concentration for each stage (**Table 2**). The scavenging effect of extracts from four stages of FLJ flowers on the DPPH radical ranked as 10.68 μ m > 36.25 μ m > 69.53 μ m > 156.74 μ m, and was 92.62%, 88.41%, 80.03% and 74.28% at the concentration of 200 μ g/ml, respectively. A significant difference of the scavenging effect on DPPH was found between particles of 69.53 μ m and stage particles of 36.25 μ m (P < 0.05), while no significant difference was found between particles of 36.25 μ m and particles of 10.68 μ m (P > 0.05). The order of reducing capacity in different sized particles at the amount of 600 μ g/ml, comparing with the positive controls, was BHA > 10.68 μ m > 36.25 μ m > 69.53 μ m > 156.74 μ m (**Table 2**). Hu *et al.* [22] found superfine grinding enhanced antioxidant activity by increased extraction of green tea, although the differences were

Particle size (µm)	DPPH radical scavenging activity (%)	Reducing power	Total phenolic contents (mg GAE/g)		
156.74 ± 12.43	$74.28 \pm 4.02a$	$0.196\pm0.011a$	$6.36 \pm 0.24a$		
69.53 ± 5.78	$80.03\pm3.65a$	$0.231\pm0.017a$	$10.12\pm0.33b$		
26.35 ± 1.40	$88.41 \pm 3.78b$	$0.309\pm0.021b$	$12.64 \pm 0.56c$		
10.68 ± 0.89	$92.62 \pm 3.98b$	$0.338\pm0.018b$	$12.98\pm0.48c$		

Table 2. DPPH radical scavenging activity, reducing power and total phenolic contents (mg GAE/g) of different sized red rice particles.

The concentration of red rice extract was 200 μ g/ml for DPPH radical scavenging activity determination; the concentration of red rice extract was 600 μ g/ml for reducing power determination. Each value represents mean \pm standard deviation of three replicates; different letters in the same column mean significant difference (P < 0.05).

not statistically significant (P > 0.05). Report in fiber from wine grape pomace showed that superfine grinding strengthened the ABTS radical scavenging activity and ferric reducing antioxidant power [23]. Observation in wheat bran dietary fiber, chelating activity and reducing power increased after superfine grinding, however, DPPH radical scavenging activity decreased [24]. These results above indicated superfine grinding treatment influenced the antioxidant activity depending on the methods employed for antioxidant activity determination and food materials.

Phenolic compounds including phenolic acids and flavonoid were a group of aromatic secondary metabolites ubiquitously distributed in natural plants, crops, fruits, and vegetables. These compounds were of great interest in the food industry due to their beneficial health effects [25] [26]. In the present study, we evaluated total phenolic contents in extracts of four sized red rice powder (**Table 2**). The most abundant total phenolic content occurred in rice of 10.68 μ m, followed by rice with 36.25 μ m, 69.53 μ m, and 156.74 μ m, and their contents were 12.98 GAE/g, 12.64 GAE/g, 10.12 GAE/g, and 6.36 mg GAE/g in extracts, respectively. The soluble phenolic compounds increased with the smaller particle, therefore, superfine ground enhanced the antioxidant activity and related compounds of red rice, which was in agreement with the observation in tea powder [23] and in dietary fiber of wheat bran [24] and wine grape pomace [23].

4. Conclusion

The size was smaller for red rice powders, greater for the bulk density and smaller for the angle of repose and slide. Water solubility index, water holding capacity and enzymatic digestibility by α -amylase significantly increased with the decreasing size (P < 0.05). Furthermore, superfine ground enhanced the antioxidant activity and phenolic content. Overall, superfine ground would improve the physicochemical and functional properties and it appeared to be a promising strategy for red rice processing.

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