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Dissociation of aleurone cell cluster from wheat bran by centrifugal impact milling

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ABSTRACT

Wheat aleurone which contains most of the nutritionally important substances of wheat bran has been widely investigated, and electrostatic separation based on the distinct electrical properties of bran tissues has been proven to be effective for the enrichment of aleurone layer. In order to obtain a suitable starting material containing single-layer bran strips for the electrostatic separation, centrifugal impact milling was utilized to dissociate wheat bran constitutive tissues. During centrifugal impact milling, the outer pericarp was detached when the particle size of the fragments decreased to approximate 460 μm, whereas the aleurone layer consisted of 20–30 intact cells in single-layer was completely dissociated until the particle size decreased to about 200 μm. Moreover, the detached behaviors of bran tissues were well explained by the relationship of their elastoplasticities, the rupture-energy and peeling force generated by the rotating blade tip. After centrifugal impact milling and sieving, the bran fraction with the median particle diameter of 144 μm contained 61% single-layer aleurone cell clusters, indicating that it could be a superior starting material for the electrostatic separation of wheat aleurone.

1. Introduction

Epidemiological studies have indicated that diets high in whole grains may be associated with decreased incidence of chronic diseases (Giacco et al., 2010; Jacobs & Gallaher, 2004; O'Neil, Nicklas, Zanovec, & Cho, 2010). Although the mechanisms underlying of these beneficial effects have not been thoroughly understood, the concept that critical bioactive compounds against the diet-related diseases were concentrated in the bran layers has been generally accepted (Buri, Von Reding, & Gavin, 2004; Surget & Barron, 2005). Lots of studies have shown that the beneficial role of whole-grain consumption may depend on the synergy effects of multiple biochemical compounds in bran, especially in the aleurone layer (Potter, 1996; Slavin, Martini, Jacobs, & Marquart, 1999).

Wheat aleurone layer is composed of thick cells enclosing compounds including dietary fiber (β-glucan and arabinoxylan), antioxidants (feruloyl oligosaccharide and hydroxycinnamic acids) in the cell wall and high amounts of phytate, protein, and micronutrients (minerals, folates, and plant sterols) in the intracellular content (Atwell, 2010; Fincher & Stone, 1986; Rhodes, Sadek, & Stone, 2002; Wilkinson, Laidman, & Galliard, 1984). Due to its nutritional enrichment, wheat aleurone could be separated into purified fractions, and then be used as ingredients for human food or a starting material for extracting bioactive compounds. However, wheat bran which is composed of several adhesive tissues (outer pericarp, intermediate layer and aleurone layer) is usually used as a low-value ingredient in animal feed or in fermentation industry (Fincher & Stone, 1986).

Recently, dry fractionation technologies, which only require physical and mechanical processes, have been used to separate wheat aleurone cell or aleurone cell contents from the wheat bran (Brouns, Hemery, Price, & Anson, 2012; Hemery, Rouau, Lullien-Pellerin, Barron, & Abecassis, 2007). These dry processes have obvious advantages compared with wet processes (chemical or enzyme treatments), because of their higher energy efficiency and requiring no chemical pre-treatment and effluent treatment. Furthermore, dry processes mainly focus on the functionality of phytochemicals rather than on the molecular purity (Schutyser & van der Goot, 2011), so the

Abbreviations: CIM, centrifugal impact milling; D_{50}, median particle diameter; DHD, dehydrodimers of ferulic acid; FAt, 4-O-8′,5′-5″-dehydrotriferulic acid; ARs, alkylresorcinols; p-CA, para-coumaric acid; $F_{(20-40)}$, bran fragments obtained after CIM that passed though the 20-mesh grid, but did not pass though 40-mesh grid; $F_{(40-60)}$, $F_{(60-80)}$, $F_{(80-100)}$, $F_{(100-120)}$, and $F_{(120-150)}$, represent the bran fractions obtained by the corresponding sieving processes.

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processes will be beneficial to the functionality retaining of the copious compounds in wheat bran. As a dry process, debranning is a relatively simple process to produce aleurone-rich fraction (Dexter & Wood, 1996; Harris, Chavan, & Ferguson, 2005). However, debranning needs the wheat kernel as the starting material, and has less yield and product purity (40–60%) (Hemery et al., 2007). Comparing with wheat kernel, wheat bran, which is the main by-product of conventional flour industry and with high nutritional potential, is more suitable for using as the starting material of wheat aleurone separation.

Electrostatic separation based on the movements of charged particles in an electric field is another effective dry fractionation technology for particle mixtures. Due to its sorting fundamental, the particles in the starting materials of electrostatic separation should have distinct dielectric properties or conductivities (Kelly & Spottiswood, 1989). Many studies have shown that the electrostatic properties of bran tissues were different and influenced by the particle size, composition, microstructure, and moisture content. Antoine, Castellon, Toureille, Rouau, and Dissado (2004) observed the dielectric properties of hand-isolated wheat bran tissues using dielectric spectroscopy, and found that the aleurone layer exhibited more capacitive ability than outer pericarp and intermediate layer. As to the bran particles after grinding and tribo-charging, the finer aleurone particles were found obtaining more charge than fine bran particles and coarse aleurone particles (Dascalescu et al., 2010). Hemery, Rouau, et al. (2009) studied the electrostatic properties of aleurone-rich fraction and pericarp-rich fraction with different particle sizes and moisture contents. They found that, when the samples were not dried, the fine aleurone-rich fraction behaved like an insulator, while the pericarp-rich fraction behaved like a conductor. In brief, the previous studies have indicated that the aleurone layer and the other bran tissues exhibited distinct electrostatic properties, which can be used in the process of electrostatic separation. Recently, electrostatic separation has been proven to be effective for the enrichment of aleurone layer from the wheat bran (Bohm & Kratzer, 2005; Hemery, Holopainen, et al., 2011; Stone & Minifle, 1988), but the premise is that the bran strips in the starting material should be dissociated before electrostatic separation.

Many grinding treatments (ball-milling and impact-milling) have been used to decrease the particle size of the wheat bran and corn bran. All these studies focused on the biochemical properties of dietary fibers after grinding, such as redistribution of insoluble fibers to soluble fibers, physiological functions of dietary fiber in the intestine and bio-availability of B vitamins (Stewart & Slavin, 2009; Van Craeyveld et al., 2009; Yu & Kies, 1993; Zhu, Huang, Peng, Qian, & Zhou, 2010). As to ball-milling, the time and energy consumption of cryogenic grinding were reduced for increasing the brittleness of wheat bran, while after ambient grinding, the bran fragments were more dispersed, and could be separated easily by sifting (Hemery, Chaurand, et al., 2011). During ambient ball milling, the hand-isolated aleurone layer was fractured more rapidly than the whole wheat bran (Antoine, Lullien-Pellerin, Abecassis, & Rouau, 2004). Hammer milling, which is a kind of impact milling, was used to break the wheat bran into pericarp–testa particles and aleurone cell particles at the speed of 76–100 m/s (Stone & Minifle, 1988). The study suggested that impact milling might be a better way than ball milling to obtain single-layer bran strips. However, few studies have been done on the dissociation of wheat bran strips using centrifugal impact milling (CIM).

The aim of the present work was to investigate the dissociation status of wheat bran tissues after centrifugal impact milling. We studied the threshold of particle size, at which the aleurone layer was efficiently dissociated from wheat bran as intact cell clusters. Moreover, the proportions of the aleurone layer in the fractions with different particle size were determined to obtain a better starting material for the electrostatic separation of wheat aleurone.

2. Materials and methods

2.1. Materials

Wheat bran (Jimai 22, hard wheat) used in this study was the commercial product supplied by COFCO (China Oil And Foodstuffs Corporation, Beijing, China). The bran was obtained via a conventional roller milling process.

Fluorescent brightener 28, phytic acid, 3,4,5-tri-methoxycinnamic acid, olivetol, p-coumaric acid and pronase were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ferulic acid, agar, ethanol, and glutaraldehyde (AR grades) were purchased from Sinopharm (Beijing, China). Methanol, acetic acid, sodium acetate, and acetonitrile were all high performance liquid chromatography (HPLC) grades, and the ultrapure water was produced by Direct-Q3 UV Water Purification System (Millipore Corporation, Billerica, MA, USA).

2.2. Grinding and sieving processes of wheat bran

Wheat bran was fragmented using a simple blade grinding (Q-500B3, Shanghai, China) until passed through a grid of 0.8 mm. Then, a centrifugal impact milling (DWFJ-5, Jiang Yin Longchang machine manufacturing Co., Ltd, Wuxi, China) was employed to grind the bran fractions (500 g). In the process of CIM (centrifugal impact milling), the flaky bran particles were lined up by the guide blade in the centrifuge rotor, then were impacted and sheared by the high-speed rotating rotor-tooth with a tip speed of ~70 m/s and the gear ring in the crushing chamber. During milling, the temperature was maintained below 15 °C by cold air flow, and the particles were transported out of crushing chamber by the negative pressure air flow, which generated by an air classifier with the speed at 5850 rpm. After milling, the wheat bran powders were separated into seven fractions using six USA standard sieves (sieve openings: 420 μm, 250 μm, 180 μm, 150 μm, 125 μm and 100 μm for 40 mesh, 60 mesh, 80 mesh, 100 mesh, 120 mesh, 150 mesh, respectively). Due to the particles smaller than 100 μm were prone to agglomeration and were difficult to be separated, the six sorts of bran particles larger than 100 μm were chosen and sealed in a desiccator for further measurements.

2.3. Isolation of wheat bran strips from kernel

The outer pericarp, aleurone layer, and the intermediate layer (inner pericarp, testa and hyaline layer), were isolated from wheat kernel (Jimai 22, hard wheat) harvested in 2011 in Shandong province of China. Before isolating, wheat grains were prepared as described by Antoine et al. (2003). The pericarp was peeled firstly from the wheat kernel using a tweezer. Then, a crease incision was made and the endosperm was removed using a scalpel. The intermediate and aleurone layers were separated by inserting a razor blade between them in the last.

The amount of cell walls in hand-isolated aleurone layers was assessed using an adaptation of the method described by Hemery, Lullien-Pellerin, et al. (2009), and Hemery, Rouau, et al. (2009). The hand-isolated aleurone layers were dried, ground and suspended in hexane, then the mixtures were centrifuged (1000 g, 10 min) and the supernatant was discarded. These steps were repeated for twice, and the final sediment was air dried. The dried sample was suspended in water with 1.5% SDS and 5 mmol L^{-1} Na-bisulfite, and a pronase solution was added (0.5 ml, 1 mg pronase/ml water). Then, the mixture was agitated for 1 h at 25 °C, and was centrifuged at 1000 g for 20 min. The pellet was washed extensively by water with intermittent centrifugation, and then was successively suspended in 80% ethanol, absolute ethanol, acetone and ether, with centrifugation between two successive suspensions. The final pellet