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Reducing phytate content in wheat bran by directly removing the aleurone cell content with teeth roller mill and ultrasonic cleaner

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ABSTRACT

Wheat bran is good for human health due to the abundance of dietary fiber. However, it also contains much phytate which has been well documented as an anti-nutritional factor. This study is the first to explore the effectiveness of reducing phytate content in wheat bran by directly removing the aleurone cell content utilizing different roller mills (smooth roller, coarse smooth roller and teeth roller) and different clean-up methods (brushing and ultrasonic washing). Through analysis of phytate content in wheat bran obtained from different mill systems, the best raw material was found to be obtained from the 5B system. By investigating various roller types and clean-up methods, the optimal technique was found to use a teeth roller mill combined with ultrasonic washing, which could reduce the phytate content by the greatest amount: the reduction of phytate content achieved 62.98% (from 26.80 mg/g to 9.92 mg/g) in wheat bran. The optical microscope observation indicated that ultrasonic washing could wash the aleurone cell content out from broken aleurone cells which were destroyed by the teeth roller mill.

1. Introduction

Wheat bran has attracted wide interest due to its high dietary fiber content, which would help avoid several western diseases, such as hyperlipemia (Brown et al., 1999), hypertension (Whelton et al., 2005), hyperglycemia (Anderson et al., 2004) and diabetes (Montonen et al., 2003). Besides, it could be beneficial to obesity (Tucker and Thomas, 2009), stroke (Steffen et al., 2003) and coronary heart disease (Streppel et al., 2008).

However, many studies have indicated that wheat bran also contains a significant amount of phytate, which is a naturally occurring organic complex and a simple ringed carbohydrate with a phosphate group attached to each carbon (Dost and Tokul, 2006). Although some researches have shown that phytate is health beneficial for its antioxidant and anticancer activities (Shamsuddin, 1995), more studies have proved that phytate is an anti-nutritional factor because of its strong ability of metal-binding or chelation ability. Under acid conditions of gastro, phytate shows a great affinity to essential micronutrients, such as Zn, Ca, Fe, Mg and Mn, then forms insoluble composites that significantly reduce physiological utilization of them under intestinal conditions (Kratzer et al., 1959). Phytate is therefore a significant cause of deficiency in Ca, Zn, and Fe among people whose staple food is wheat-based. Moreover, phytate could impair some enzymatic activity, such as protease, amylase and trypsin, acidic phosphatase and tyrosinase (Urbano et al., 2000), and consequently influences the utilization of protein, starch and fatty acids. Undoubtedly, this anti-nutritional factor is a significant problem in urgent need of a solution for special persons like calcium, iron or zinc deficiency patients, infants and pregnant women.

Different approaches have been proposed for reducing the phytate level in whole grains, for instance, soaking, germination, heating, fermentation and enzymatic hydrolysis. Soaking could reduce phytate content and release P during incubation, particularly with long incubation time, as demonstrated by Esmaeilipour et al. (2013). After germination, the phytate content was reduced by 16%, 30%, 30% and 17% in barley, wheat, rye and oats, respectively (Lee, 1990). Heating is usually less than satisfactory as Porres et al. (2004) just observed a 10% decrease in phytate content by autoclaving on whole lentil seeds at 120 °C for 30 min at an internal

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Nomenclature

Smooth roller	SR
Coarse smooth roller	CSR
Teeth roller	TR
Bran brushing	BB
Ultrasonic washing	UW
[FAt]	Ferulic acid dehydrotrimer concentration
[ARs]	Total alkylresorcinol concentration
[p-CA]	Para-coumaric acid concentration
[phytate]	Phytate concentration
[starch]	Starch concentration
R	Raw material
F	Studied bran fractions
p	Outer pericarp
i	Intermediate layer
aw	Aleurone cell walls
ac	Aleurone cell content
se	Starchy endosperm
1-5 B system	1-5 break mill system

pressure of 1 atm. Significant reductions in phytate of wheat and wheat-containing bread can be brought about by fermentation (Buddrick et al., 2014). Furthermore, both microbial phytase (Blaabjerg and Poulsen, 2010) and endogenous phytase (Esmaeilipour et al., 2012) could degrade phytate effectively. However, these methods mentioned above are very complicated or time-consuming on wheat bran treatment. For instance, soaking wheat bran needed temperature and pH adjusting (Urbano et al., 2007), while it needed 8 h to reduce phytate content in wheat bran from 50.1 to 43.9 mg/g by bakery yeast fermentation at 30 °C (Majzoobi et al., 2014).

According to studies of Hemery et al. (2009) and Chen et al. (2013), phytate was mainly present in the aleurone layer and not found in the intermediate layer and outer pericarp. Removing the aleurone content from wheat bran and then adding the treated wheat bran to the flour in proportion could therefore be considered as a simple and timesaving way to reduce the phytate content of whole wheat flour. However, to the best of our knowledge, this approach of directly removing the aleurone cell content in whole wheat flour has not been the subject of much research.

The aim of this study is thus to examine the effect of reducing phytate content in wheat bran by removing aleurone cell content directly with a roller mill (such as a smooth roller (SR) mill, a coarse roller (CSR) mill, or a teeth roller (TR) mill) and a clean-up method (bran brushing (BB), or ultrasonic washing (UW)), and provide a novel method of phytate reduction in wheat bran.

2. Materials and methods

2.1. Materials and reagents

Wheat bran (Zhengmai 9023, hard wheat; Zhengmai 366, hard wheat; Xinong 979, hard wheat; and a spot of soft wheat. All of those wheats grown at Zhengzhou in 2013) used in this study was the commercial product supplied by Jinyuan Flour Factory (Zhengzhou, China).

Phytic acid (>95%) standard, p-CA (para coumaric acid) standard, o-CA (ortho coumaric acid) standard and olivetol (5-pentylresorcinol) standard were obtained from Sigma-Aladdin (St. Louis, MO, USA). Other chemicals were of analytical grade unless stated otherwise.

2.2. Milling

Wheat bran samples were milled by a roller mill (MDDK × 2, Wuxi Bühler Machinery Co., Ltd., Wuxi, China) with TR, SR or CSR and then the treated samples were sieved by an Analytical Sieve Shaker (Retsch AS200 digit Analytical Sieve Shakers, Germany) at amplitude 70 and interval for 3 min with 250 μm and 125 μm sieves (ISO 3310-1, Retsch, Germany).

2.3. Bran brushing

Wheat bran samples were brushed by a bran brusher (FFR545, Anyang Shuangshi grain & oil machinery limited liability company, Anyang, China), and then sieved by an Analytical Sieve Shaker (amplitude 70, interval off, 3 min) with a 250 μm sieve.

2.4. Ultrasonic washing

Wheat bran samples were washed three times with distilled water (1 g samples: 10 mL distilled water) under an ultrasonic field by an ultrasonic cleaner (KH-250DB, Kunshan Hechuang Ultrasonic Co., Ltd., Kunshan, China) with ultrasonic frequency 25 KHz for 15 min, and then filtered with multilayer fine gauzes and dried in a blast oven at 45 °C for 3 h. The samples were then sealed in a desiccator for further measurements.

2.5. Optical microscope observation

Cross-sectional observation of the wheat grain kernels and wheat bran samples required paraffin-sectioning, and the method used for this was as follows. Samples were embedded in 2% agar and fixed in 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0), dehydrated in a graded ethanol series via tissue dehydration (Leica, ASP 200S, Germany), and then were embedded in paraffin. Polymerized samples were then sectioned (in 60 μm sections, less than 60 μm may cause the loss of the aleurone cell content) in a rotary microtome (Leica, PM2245, Germany) using a steel knife. Prior to staining, the sections were transferred onto a microscope slide, then heated to be dewaxed in an oven at 50 °C, with the agar section containing the wheat bran sample being fixed on the slide. The agar sections containing the samples were then stained with hematoxylin-eosin staining solution and iodine solution. After each staining, the sections were rinsed with distilled water and then dried. The samples were then observed with an optical microscope (Nikon, T1-SAM, Japan). The surface observation of wheat bran samples did not require paraffin-sectioning, and their staining and observation were carried out as above. Since wheat bran flour samples of particle size less than 125 μm contained much starch, which could easily agglomerate during staining, they could be directly observed under an optical microscope observation without staining.

2.6. Starch assay

The content of starch was established by AACC method 76.13 (AACC, 2000).

2.7. Phytate assay

Phytate was measured according to the colorimetric method reported by Buddrick et al. (2014) and determined by a microplate reader. Samples (1 g each) of wheat bran were extracted with