Kasetsart J. (Nat. Sci.) 43 : 745 - 751 (2009)

Effect of Milling Process on Functional Properties of Legumes

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ABSTRACT

This study demonstrated the effect of milling processing (screw crushing, hammer milling and jet milling) on functional properties, such as antioxidant capacity, total phenolic content and resistant starch content of three legumes (broad beans, black soybeans and green beans). The results showed that broad beans possessed the highest antioxidant capacity and resistant starch content. Black soybeans had the highest total phenolic content. Different milling processes changed the functional properties of samples ($p<0.05$) depending on the particle size of the flour and the volume of the flush air operation. The grinding process, producing a smaller particle size and lower volume of flush air had higher functional properties than the others processes. Therefore, hammer milling was more suitable for grinding to obtain a higher antioxidant capacity, total phenolic and resistant starch content compared to the other process.

Key words: legume, milling, functional properties, antioxidant capacity, resistant starch

INTRODUCTION

For over a decade, interest in developing functional foods has thrived and been driven largely by higher market potential for foods that can improve the health and well-being of consumers. The concept of functional foods includes food or food ingredients that have a beneficial effect on host health and/or reduce the risk of chronic disease beyond basic nutritional functions (Charalampopoulos *et al*., 2002). The food industry is coming up with new products with novel nutrients from natural sources added to meet consumer demand.

Cereals and legumes, in general, play an important role in human nutrition. Recent studies have shown that cereals and beans contain constituents that have demonstrated health benefits for humans, such as antioxidants and anti-disease factors (Ragaee *et al*., 2006; Stratil *et al*., 2007). Antioxidants in beans are related to the presence of phenolic compounds that influence their seed coat color (Beninger and Hosfield, 2003). In this regard, colored dry beans such as red, pinto and black, are expected to possess stronger antioxidant activity than navy beans. Studies have also demonstrated that food with high carbohydrate and dietary fiber, such as resistant starch (Kutos *et al*., 2003; Costa *et al*., 2006) derived from cereals, allowed withdrawal of oral hypoglycemic agents or a reduction in the insulin dose in diabetic subjects (Pathak *et al*., 2000). Additionally, several health claims based on grain dietary components have been approved by the FDA in the USA.

Resistant starch has been recognized as a functional fiber (prebiotic) performing an important role in the digestive physiology of the colon. Like oligosaccharides, especially fructo-

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Received date : 22/01/09 Accepted date : 20/06/09

oligosaccharides, it escapes digestion and provides fermentable carbohydrates for colonic bacteria. Resistant starch has also been shown to provide benefits such as the production of desirable metabolites including short-chain fatty acids in the colon. In addition to its therapeutic effects, resistant starch provides a better appearance, texture and mouth feel than conventional fibers (Martinez *et al*., 1998).

Milling is a process to produce flour involving a number of stages, such as grinding, reduction, shifting and purifying. Several reports have shown the effect of the milling process on antioxidant capacity (Liyana-Pathirana and Shahidi, 2007) and dietary fiber (Glitso and Bach-Knudsen, 1999) of cereals and legumes.

In the present study, the effect of the grinding process on the product quality was assessed. Legumes such as broad beans, black soybeans and green beans were used to determine the change in the functional properties resulting from different milling processes. Further, the products were evaluated for their functional properties with reference to antioxidant activity, total phenolic and resistant starch content.

MATERIALS AND METHODS

Legume grains

Broad bean, black soybean and green bean samples were supplied by Fujicco (Kobe, Japan). All legumes, after harvest, were stored in a cold room before use.

Chemicals

1,1- diphenyl -2- picryl- hydrazyl (DPPH), methanol, 6- hydroxyl- 2,5,7,8 – tetramethyl-chroman- 2- carboxylic acid (Trolox), Folin-Ciocalteau reagent, sodium carbonate, gallic acid monohydrate, ethanol, potassium hydroxide, sodium acetate, acetic acid, hydrochloric acid, sodium hydroxide, food grade calcium carbonate and D(+)-glucose were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). The Resistant Starch Assay Kit (Megazyme, Ireland) was purchased from Biocon Japan Ltd (Nagoya, Japan).

Sample preparation

Legume grains (broad bean, black soybean and green bean) were ground by a hammer mill (model 1018-S-3; Yoshida Seisakusho Co. Ltd., Tokyo, Japan) using a 0.5 mm screen, packed in a plastic bag and stored at 4°C until further use.

Samples (1 g) were extracted with 80% methanol (10 ml) on an ultrasonic machine for 15 min and centrifuged at 2500 g for 10 min. The precipitate was twice rinsed with 80% methanol (10 ml). The supernatant was used for analyses of antioxidant activity and total phenolic content after being diluted to proper concentration with 80% methanol.

Effect of milling process on functional properties of broad bean, black soybean and green bean

Different milling processes, such as a screw crusher (Suehiro model ES-100; Suehiro EPM Coporation, Yokkaichi, Japan), a hammer mill (model 1018-S-3; Yoshida Seisakusho Co., Ltd., Tokyo, Japan), and a jet mill (model Co-Jet system α mk III; Seishin Enterprise Co. Ltd., Tokyo, Japan) were used to evaluate the effect of the milling process on the functional properties of broad bean, black soybean and green bean samples. The particle sizes from the three different milling processes were 1000, 250 and 60 μm. Analyses were performed in triplicate.

Antioxidant capacity

DPPH radical-scavenging activity

Antioxidant capacity of the samples was determined with a stable radical, DPPH, as described by Tachibana *et al*. (2001) with some modifications. In brief, antioxidant activity was determined by reacting methanolic extract of a sample (3 ml) with 200 μM DPPH (3 ml). Absorbance of the samples was measured at 515 nm after 40 min incubation at room temperature in darkness. A calibration curve was made using Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), a synthetic, hydrophilic vitamin E analog, as an external standard with a range of concentrations from 0 to 100 μM. Results were expressed as Trolox equivalents (Sensoy *et al.*, 2005).

Phenolic content

Total phenolic content was determined by the Folin-Ciocalteau method, as described by Li *et al.* (2007) with some modifications for initial samples and extruded products. To the methanolic extract of sample (0.2 ml), 1 ml of Folin–Ciocalteu reagent (1:10 diluted) was added. After 4 min, 0.8 ml of saturated Na_2CO_3 solution was added. After 30 min of incubation at room temperature, the sample tube was centrifuged for 10 min at 2500 g. Absorbance of the supernatant was measured at 765 nm. Gallic acid was used for calibration of the standard curve. The results were expressed as milligram gallic acid equivalent (mg GAE)/g dry weight of sample.

Resistant starch content

Resistant starch (RS) content of the sample flours (broad bean, black soybean and green bean) was measured according to the procedure of the Resistant Starch Assay Kit (Megazyme, Ireland). In brief, to each 100 mg of sample, 4 ml of mixture containing pancreatic α-amylase (10 mg/ml) and amyloglucosidase (3 U/ml) was added and incubated in a shaking water bath at 37°C for 16 h. After incubation, 4 ml of ethanol (99%) was added to stop the reaction, the suspension was stirred vigorously and centrifuged at 1500g for 10 min. Supernatant was removed and 8 ml of 50% (v/v) ethanol was added to the residue and stirred. The extraction procedure was repeated three times. The 50% ethanol-washing step was repeated once more. Then, 2 M KOH (2 ml) was added to the residue, with gentle stirring in an ice-water bath to dissolve the residue for 20 min, followed by the addition of 1.2 M sodium acetate buffer (8 ml, pH 3.8) and amyloglucosidase (0.1 ml, 3300 U/ml). Samples were incubated in a water bath at 50°C for 30 min and centrifuged at 1500g for 10 min. To the supernatant (0.1 ml), 3 ml of glucose oxidase-peroxidase-aminoantipyrine (GOPOD, >12 000 U/l glucose oxidase; >650 U/l peroxidase; 0.4 mM 4-aminoantipyrin) was added and the mixture was incubated in a water bath at 50°C for 20 min. Absorbance was measured using a spectrophotometer at 510 nm. A sodium acetate buffer (0.1 M, pH 4.5) and glucose (1 mg/ml in 0.2% benzoic acid) was used as a blank and glucose standard, respectively (Kim *et al.*, 2006). The absorbance was calculated to % resistant starch using an equation from the Kit manual (see Equations 1 and 2). Each analysis was performed in triplicate.

For samples containing > 10% RS,
Resistant starch (g/100 g sample) =

$$\Delta E \times F/W \times 90 \quad (1)$$

For samples containing < 10% RS,
Resistant starch (g/100 g sample) =

$$\Delta E \times F/W \times 9.27 \quad (2)$$

where:

ΔE = absorbance (reaction) read against the reagent blank

F = conversion from absorbance to micrograms

W = dry weight of sample analyzed

Statistical analysis

All experiments were performed in triplicate and results were expressed as means ± standard deviation and analyzed by SAS statistical software, version 9.1. Multiple comparison of the means was carried out by a least significant difference (LSD) test at α = 0.05 level.

RESULTS AND DISCUSSION

Functional properties of broad beans, black soybeans and green beans

Radical-scavenging activity employing DPPH has been extensively used in the field of food processing for screening the antioxidant capacity of agricultural produce (Sanchez-Moreno, 2002). In this study, DPPH was expressed as mmol Trolox/ g. The antioxidant capacity of the raw materials of cereals and legumes is shown in Table 1. The methanolic extract from samples exhibited obviously different antioxidant capacity. These values ranged from 0.03 to 0.28 mmol Trolox/ g. Among the samples analyzed, broad beans showed the highest antioxidant capacity, with 0.28 mmol Trolox/ g, followed by black soybeans, with 0.13 mmol Trolox/ g. The lowest antioxidant capacity was obtained from green beans with 0.03 mmol Trolox/ g.

The content of total phenolic compounds has been used for determining antioxidant capacity. In this study, phenolic content was expressed as mg GAE/g. The phenolic content of samples is presented in Table 1. The methanolic extracts of samples showed obviously different amount of total phenolics, with a range from 0.32 to 4.05 mg GAE/g. As was the case with antioxidant activity, black soybeans showed the highest content of phenolics, with 4.05 mg GAE/g, followed by broad beans, with 1.80 mg GAE/g. The lowest phenolic content was obtained from green beans with 0.32 mg GAE/g.

In this study, resistant starch was measured according to the procedure described by in the Resistant Starch Assay Kit and expressed as % (w/w). The resistant starch content of legumes is shown in Table 1, with values ranging from 0.63 to 1.40% (w/w). Among the materials screened, broad beans contained the highest content of resistant starch with 1.40% (w/w), followed by green beans, with 0.63% (w/w). The least content of resistant starch was obtained from black soybeans with 0.06% (w/w).

Effect of milling process on functional properties of broad beans, black soybeans and green beans

Milling is an important step to reduce the particle size of starch/flour during processing. A screw crusher produces coarse particles (Sander and Schonert, 1999), whereas a hammer mill produces fine particles (Sowbhagya *et al*., 2007). A jet mill produces much finer particles than a hammer mill (Picot and Lacroix, 2003). The effects of the milling process on antioxidant capacity and the phenolic and resistant starch content of materials used in this study are shown in Figures 1, 2 and 3. Milling with a hammer mill showed the highest efficiency for all functional properties: antioxidant capacity (0.28, 0.13 and 0.03 mmol Trolox/g); phenolic content (1.80, 4.05 and 0.32 mg GAE/g); and resistant starch content (1.40, 0.06 and 0.63% (w/w)) for the broad bean, black soybean and green bean samples, respectively. Since particle size is an important parameter in choosing an extraction procedure (Franco *et al*., 2007), and higher performance is associated with a smaller particle size, it was considered that the hammer mill was more suitable than the screw

Table 1 Antioxidant capacity, total phenolic and resistant starch content of legumes evaluated by DPPH radical-scavenging activity, the Folin-Cioculteau method and the Resistant Starch Assay Kit.

Sample	Antioxidant capacity (mmol Trolox/g sample)†	Total phenolic content (mg GAE/ g sample) †	Resistant starch content (% w/w) †
Broad bean flour	0.28 ± 0.01^{a}	1.80 ± 0.06^{b}	1.40 ± 0.05^{a}
Black soybean flour	0.13 ± 0.01^{b}	4.05 ± 0.04^{a}	0.06 ± 0.01^{c}
Green bean flour	0.03 ± 0.00^{c}	0.32 ± 0.04^{c}	0.63 ± 0.09^{b}

† Mean ± SD of triplicate analysis. Values with different letters in the same column are significantly different at $p<0.05$.

crusher. The jet mill had a slightly different efficiency with regard to the functional properties compared with the hammer mill, because the jet mill process flushed a large quantity of air (nozzle pressure = 7.0 kg/cm^3; air volume = 2.6 m^3/min) that produced higher oxidation during the grinding process (Wachiraphansakul and Devahastin, 2007). This reduced the antioxidant capacity and phenolic content. Results from this study also showed that different milling processes had a slight effect on the resistant starch content of the raw materials. The three milling processes of screw crushing, hammer milling and jet milling respectively produced resistant starch content values for broad beans of 1.27, 1.40, 1.32% w/w; for black soybeans of 0.03, 0.06,0.08% w/w; and

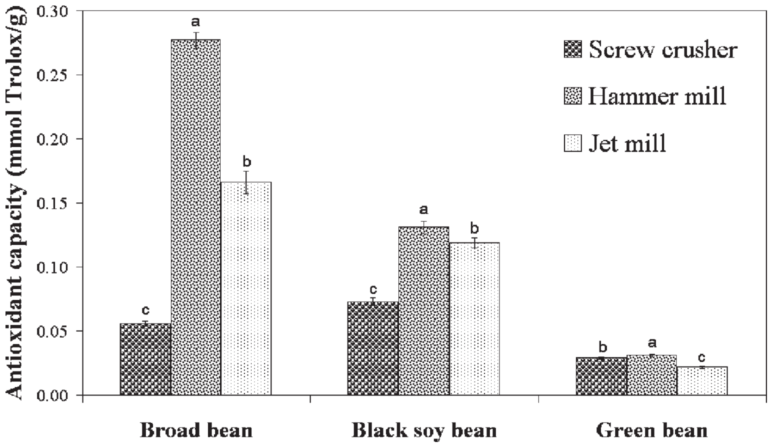

Figure 1 Effect of milling process (screw crusher, hammer mill, and jet mill) on the antioxidant capacity of broad beans, black soybeans and green beans. Each value is presented as the means ± standard error of triplicate analysis. The vertical bars indicate standard errors. Values not having similar superscripts are significantly ($p < 0.05$) different.

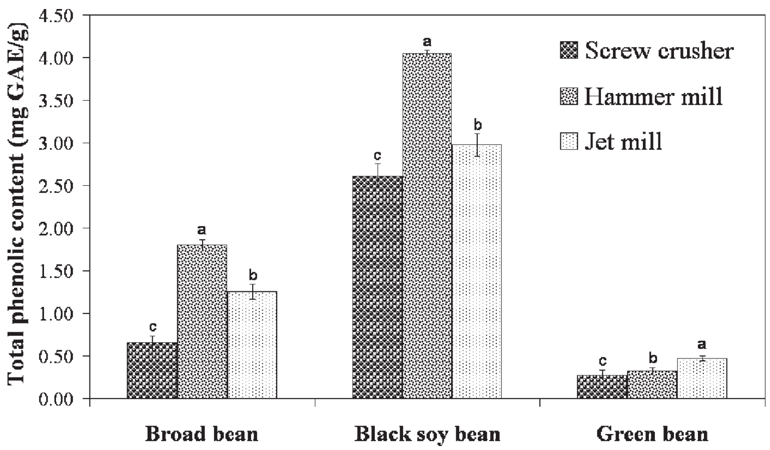

Figure 2 Effect of milling process (screw crusher, hammer mill, and jet mill) on the phenolic content of broad beans, black soybeans and green beans. Each value is presented as the mean ± standard error of triplicate analysis. The vertical bars indicate standard errors. Values not having similar superscripts are significantly ($p < 0.05$) different.

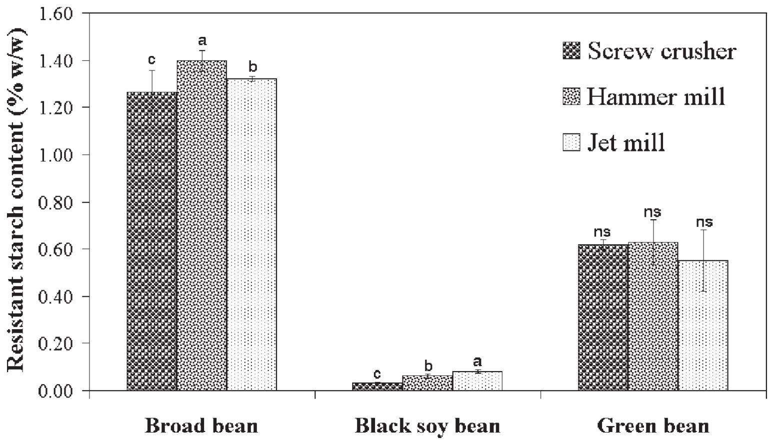

Figure 3 Effect of milling process (screw crusher, hammer mill, and jet mill) on the resistant starch content of broad beans, black soybeans and green beans. Each value is presented as the means ± standard error of triplicate analysis. The vertical bars indicate standard errors. Values not having similar superscripts are significantly ($p < 0.05$) different.

for green beans of 0.62, 0.63, 0.55% w/w, because the physical operation had no influence on changing the structure of starch.

CONCLUSION

Broad beans possessed the highest antioxidant capacity and resistant starch content (0.28 mmol Trolox/ g, and 1.40% w/w, respectively). Black soybeans had the highest phenolic content (4.05 mg GAE/g). Different milling processes affected the functional properties of broad beans, black soybeans and green beans. Hammer milling was more suitable for grinding compared to screw crushing and jet milling. Further studies are in progress to evaluate the effect of an extrusion process on the physical, chemical and functional properties of the cereal and legume products to optimize the conditions for the selection of suitable extruded products for sensory evaluation.

ACKNOWLEDGEMENTS

This research work was supported by Kirin Holdings Co., Ltd (Japan) during a UNU-Kirin Fellowship at the National Food Research Institute, Tsukuba (Japan) in 2007-08.

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