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RESEARCH NOTE

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Effects of Raw, Cooked, and Germinated Small Black Soybean Powders on Dietary Fiber Content and Gastrointestinal Functions

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Abstract The effects of raw and processed small black soybean powders on dietary fiber content and gastrointestinal function in rats were investigated. The crude oil, protein, and ash contents of raw small black soybean powder were not significantly different from those of processed small black soybean powders. The germination process increased soluble and insoluble dietary fiber contents significantly, as compared to raw small black soybean powder. The germinated small black soybean powder diet led to a significantly different food intake than the basal diet in both normal and loperamide-induced constipated rats. The body weight gains of the experimental groups, however, were not significantly different from that of the basal diet groups for both the normal and loperamide-induced rats. The gastrointestinal transit times and fecal weights for normal and loperamide-induced rats consuming the processed small black soybean powder diet were significantly different from those on a basal diet. These results suggest that the processes of cooking and germinating the small black soybean might contribute to acceleration of fecal excretion in both experimental normal and constipation model rats.

Keywords: small black soybean powder, dietary fiber, gastrointestinal function, loperamide-induced rats

Introduction

Soybeans are consumed in many countries for their dietary proteins and play an important role in human nutrition. Soybeans are also excellent sources of dietary fiber, minerals, and vitamins (1, 2). In addition, the beneficial effects of soybeans also include reductions in the risks of various cancers, postprandial glycemia, and serum cholesterol, and induction of intestinal microflora (3-5). However, soybeans contain anti-nutritional components as well, such as lectins and enzyme inhibitors, resulting in a limit to their nutritional value. Therefore, various processes such as boiling, germination, and fermentation have been used to inactivate the anti-nutritional enzymes and to remove undesirable flavors and components (6-11).

These processing techniques modify the plant cell wall and alter the chemical, structural, and nutritional properties of the soybeans. It has been reported that dietary fibers, the major components of plant cell walls, are affected by thermal and microbial decomposition during processing through changes in the soluble and insoluble dietary fiber content (12). These dietary fibers are known to have beneficial physiological effects on bowel functions, such as changes in stool output and stool composition (8, 13). In particular, insoluble dietary fibers accelerate colonic transit time to produce large and soft stools through their mechanical action on the gastrointestinal tract, whereas soluble dietary fibers are metabolized by colonic bacteria to improve gastrointestinal functions (14, 15).

Several studies have demonstrated that dietary fiber affects gastrointestinal function and fecal output to normalize the speed of intestinal transit and to increase fecal weight (16, 17). Little information exists, however, on the effect of soybean processing on intestinal functions. In this study, we have used raw and processed (cooked and germinated) small black soybeans to examine the effects of cooked and germinated small black soybeans on dietary fiber content and subsequent intestinal functions in normal and loperamide-induced constipated rats.

Materials and Methods

Preparation of small black soybean powders Small black soybeans were purchased from a local agricultural cooperative market (Imsil, Jeonbuk, Korea). The soybeans were soaked in distilled water (1:4, w/v) at room temperature for 12 hr, frozen, freeze-dried, ground, and passed through a 300-μm sieve to yield the powdered raw product. The cooked small black soybeans were prepared by soaking in distilled water (1:4, w/v) for 12 hr and boiling at 100°C for 30 min. The cooked seeds were frozen, freeze-dried, ground, and then passed through a 300-μm sieve to yield the powdered cooked product. Germination of the soybeans was carried out in an incubator; prior to germination, the small black soybean seeds were soaked in distilled water at room temperature for 12 hr, the water was then drained off and the seeds transferred to the incubator. The seeds were layered over moist filter paper to germinate at 25°C for two days. The germinated soybeans were frozen, freeze-dried, ground, and then passed through a 300-μm sieve to yield the powdered germinated product.

Chemical analysis Crude lipid, protein, and ash contents of the soybean products were determined by the AOAC official methods (18). Dietary fiber content was determined using a dietary fiber assay kit (Sigma, St. Louis, MO, USA) after enzymatic removal of starches and proteins (19). Samples (1 g) were dispersed in phosphate buffer (0.08 M, pH 6.0±0.2) to analyze the soluble dietary fiber

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(SDF) and insoluble dietary fiber (IDF).

Animals and diets Male Sprague-Dawley rats weighing 150-160 g were fed a nonpurified diet for five days, followed by the experimental diets for four weeks. Rats were maintained at 22±2°C and 60±5% relative humidity in a room with a 12:12 hr light: dark cycle and given free access to food and water at all times. Rats were divided into four groups of eight rats each and were fed diets containing a soybean powder-free basal diet or diets supplemented with 10%(w/w) raw, cooked or germinated small black soybean powder for 4 weeks. The diets used in this study consisted of the following ingredients in g/100 g diet: 20 g casein, 0.3 g DL-methionine, 55 g sucrose, 10 g corn starch, 5 g corn oil, 3.5 g AIN-76 mineral (American Institute of Nutrition), 1 g AIN-76 vitamin mix, 0.2 g choline bitartate, 5 g cellulose, and 10 g test substances substituted for corn starch. The test substances were 10% raw, 10% cooked, and 10% germinated small black soybean powders. The experimental constipation animal model was induced by loperamide. Loperamide (1.5 mg/kg, Sigma) was injected subcutaneously as a suspension in 0.9% sodium chloride in rats in the experimental and control groups twice daily, at both 09:00 and 18:00 o'clock, during the experiment after a three-day adaptation period.

Measurement of body weight, food intake, whole gut transit times, and fecal weight Rats were monitored daily for their general health, and body weight and food intake were recorded weekly during the experimental period. The whole gut transit time was determined by the measurement of the initial appearance of dye in feces after injection of 1.5% carmine red dye (2 mL) into the stomach. The fecal weight was measured by collecting feces once a day and allowing the collected feces to air dry for 1 hr before weighing.

Statistical analysis Data were analyzed by ANOVA using the SAS statistical analysis system (SAS Institute Inc., Cary, NC, USA). Differences among samples were analyzed using Duncan's multiple range test ($p<0.05$).

Results and Discussion

Chemical composition The chemical composition of raw, cooked, and germinated small black soybean powders is shown in Table 1. The crude oil, protein, and ash contents of raw small black soybeans were 16.2, 38.5, and 5.2%, respectively, and were not significantly different from those of the processed (cooked or germinated) small black soybean powders. The crude oil contents obtained in the present study were similar, but the crude protein and ash contents were higher for raw and processed products than those previously reported for small black soybeans (20).

The raw small black soybeans contained 1.7% SDF, 18.0% IDF, and 19.7% total dietary fiber (TDF). The germination process, however, resulted in significantly increased SDF and TDF contents as compared with the raw small black soybeans, indicating that structural modifications of the cell wall polysaccharides in the soybean seeds during germination resulted in the production of new dietary fibers (9, 21). Thus, germinated small black soybeans could be a good source of dietary fiber and, as a result, contribute to beneficial physiological effects.

Food intake and weight gain Table 2 presents the results of daily food intake and weight gain of normal and loperamide-induced rats in the experiment that were fed a diet containing 10% raw, cooked, or germinated small black soybean. The food intake of normal and loperamide-induced rats ranged from 20.71 to 23.42 g/day and from 18.2 to 25.03 g/day, respectively. The germinated small black soybean diet led to the highest food intake in both

Table 1. Chemical composition of raw, cooked, and germinated small black soybean powders[1)]

	Crude oil (%)	Crude protein (%)	Crude ash (%)	SDF[3)] (%)	IDF[4)] (%)	TDF[5)] (%)
Raw SBSP[2)]	16.2±0.17	38.5±0.20	5.2±0.04	1.7±0.13	18.0±0.77	19.7±0.90
Cooked SBSP	16.5±0.05	39.3±0.03	5.3±0.02	0.7±0.10	17.7±0.31	18.4±0.20
Germinated SBSP	16.5±0.06	38.1±0.10	5.1±0.02	7.0±0.38	17.0±0.94	24.0±0.56

[1)]Values are mean ± SD.
[2)]SBSP: small black soybean powder.
[3)]SDF: soluble dietary fiber; [4)]IDF: insoluble dietary fiber; [5)]TDF: total dietary fiber.

Table 2. Food intake and weight gain in normal and loperamide-induced rats fed diets supplemented with raw, cooked, or germinated small black soybean powder for 4 weeks[1)]

Dietary groups	Normal rat		Loperamide-induced rat	
	Food intake (g/day)	Weight gain (g/day)	Food intake (g/day)	Weight gain (g/day)
Basal diet	20.55±0.89b[2)]	10.04±1.01a	18.2±2.76b	4.75±0.22a
10 % raw SBSP[3)] diet	22.30±0.77a	8.82±1.14a	21.53±1.36b	4.31±1.30a
10 % cooked SBSP diet	20.71±1.28b	8.74±1.52a	21.37±1.25b	4.22±2.10a
10 % germinated SBSP diet	23.42±0.86a	7.77±2.57a	25.03±1.66a	4.39±2.04a

[1)]Values are mean±SD.
[2)]Values within the same column followed by different letters are significantly different at $p=0.05$ by Duncan's multiple test.
[3)]SBSP: small black soybean powder.

Table 3. Whole gut transit time and fecal weight in normal and loperamide-induced rats fed diets supplemented with raw, cooked, or germinated small black soybean powder for 4 weeks[1)]

Dietary groups	Normal rat		Loperamide-induced rat	
	Transit time (hr)	Fecal weight (g dry/18 hr)	Transit time (hr)	Fecal weight (g dry/18 hr)
Basal diet	11.50±2.63a[2)]	1.39±0.06c	15.60±1.51a	1.02±0.33c
10 % raw SBSP[3)] diet	7.48±0.61b	1.73±0.05b	8.75±2.18c	2.07±0.24ab
10 % cooked SBSP diet	9.05±1.09b	1.65±0.08b	11.51±1.51b	1.53±0.46bc
10 % germinated SBSP diet	8.13±0.63b	2.01±0.08a	10.61±1.02bc	2.26±0.28a

1)Values are mean±SD.
2)Values within the same column followed by different letters are significantly different at p=0.05 by Duncan's multiple test.
3)SBSP: small black soybean powder.

normal and loperamide-induced rats. The weight gain of normal and loperamide-induced rats ranged from 7.77 to 10.04 g/day and from 4.22 to 4.75 g/day, respectively, which didn't show a significant difference between the groups. Loperamide-induced rats, however, had significantly lower weight gains than the normal rats. The result indicated that a significant improvement in growth performance was not obtained by processes such as cooking and germination.

Whole gut transit times and fecal weights Table 3 presents the results of the measurement for the whole gut transit times and fecal weights in normal and loperamide-induced rats fed various small black soybean powder diets. In normal rats, the whole gut transit times for raw, cooked, and germinated small black soybean powder diets were 7.48, 9.05, and 8.13 hr, respectively, which was significantly less than that of the basal diet (11.50 hr). In loperamide-induced rats, the whole gut transit times for raw, cooked, and germinated small black soybean powder diets were 8.75, 11.51, and 10.61 hr, respectively, which also represented significant decreases compared with that of the basal diet (15.60 hr). The amount of decrease in whole gut transit times for the experimental diet groups were 21.3-35.0% for normal rats and 26.2-43.9% for loperamide-induced rats. Loperamide-induced rats, however, had significantly increased transit times compared with the normal rats. This significant increase observed in loperamide-induced rats might be due to an inhibition of colonic peristalsis by the loperamide (22).

The germinated small black soybean powder diet produced the highest values of fecal weights (2.01 and 2.26 g dry/18 hr) in both normal and loperamide-induced rats, respectively, followed by the raw and cooked small black soybean powder diets, all of which were significantly different from the basal diet. The percentage increase in fecal weights for the experimental diet groups were 19-45% for normal rats and 50-122% for loperamide-induced rats. Several studies have speculated that dietary fibers cause an increase in fecal weight through water retention and partial fermentation of the fiber (23-26). A significant increase in fecal weight following consumption of germinated small black soybean might be also attributed to both adequate fecal water content and the production of short chain fatty acids by microflora in the cecum (27). Indeed, Kanauchi *et al.* (27) also reported that germinated barley foodstuffs are used efficiently by microflora in the cecum during constipation and promote defecation in loperamide-induced rats by adjusting fecal water content to an appropriate level. Consequently, the significant reduction in whole gut transit time and increase in fecal weight for our experimental diet groups suggest that the processed soybean powders might help prevent the retardation of fecal excretion in both the normal and constipated rats by accelerating bowel evacuation.

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