Comparison of the Water-Holding Capacity of Wheat Bran Products Prepared by Wet and Dry Smashing Methods *in vitro* and Effect on the Gastrointestinal Retention Time in Rats *in vivo*

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Abstract: Microfibril wheat bran (MFW) prepared by wet smashing of wheat bran using a colloidal mill has the advantages of being more palatable than other wheat bran and easier to apply to various foods. In this study, we investigated water-holding capacity (WHC) and physiological effects of a novel food material, MFW, focusing on shortening of the retention time of the gastrointestinal contents compared to those of dry smashing of wheat bran (DWB) prepared by conventional method, and wheat bran (WB), which is the raw materials.

The mean particle size of MFW was 35μm, and WHC was 5.1 g/g. In contrast, those of DWB were 61 μm and 3.0 g/g, respectively. Those of WB were 420 μm and 5.0 g/g, respectively. The WHC of MFW was 1.7 times greater than that of DWB and comparable to that of WB. The dietary fiber content in MFW, DWB, and WB were 73.5, 66.9 and 70.2%, respectively.

Six-week-old Fisher rats were divided into three groups, and fed for 20 days with AIN-76 chow supplemented with MFW, DWB, or WB to a dietary fiber content of 10%. On days 14–16 of the experimental period, the mean retention time (MRT) of gastrointestinal content and fecal weight were measured using solid phase and liquid phase markers. On day 20 of the experimental period, animals were killed, and the water content, pH,

Abbreviations used: MFW, microfibril wheat bran; WB, wheat bran; DWB, dry smashing of wheat bran; WHC, water-holding capacity; MRT, mean retention time; SCFA, short chain fatty acid; SV, settling volume.

composition of short chain fatty acids (SCFAs) in the cecal content and total amounts of SCFAs in the cecum were investigated. MRT in the MFW group was 15.2 ± 0.8 h in the solid phase, which was significantly shorter than that in the DWB group (18.0 ± 0.9 h) ($p < 0.05$), and comparable to that in the WB (15.5 ± 2.4 h). MRT in the liquid phase was almost the same as that in the solid phase: 14.7 ± 1.0, 18.4 ± 0.8, and 16.0 ± 2.5 h in the MFW, DWB, and WB groups, respectively. The fecal weight, pH, the concentration of SCFA in the cecal content and total amounts of SCFAs in the cecum did not differ among the groups, but the cecal water content was in the order of MFW > WB > DWB, showing a significant difference between each group ($p < 0.05$).

The above finding suggested that MFW is a novel food material with a greater WHC and the ability of shortening the retention time of the gastrointestinal contents compared to DWB.

Key words: Microfibril wheat bran, water-holding capacity, particle size, mean retention time of gastrointestinal contents, rats

Introduction

A potential role of dietary fiber in the prevention of colon cancer has been recognized [1, 2]. Some studies have found that wheat bran is effective in prevention of colorectal cancer in experimental models [3–7]. Shortening of the retention time of the gastrointestinal contents by wheat bran may be one of the mechanisms [8, 9]. However, it is thought that the taste of wheat bran is undesirable, the palatability is poor due to its large particle size, and its application to foods has been limited to cereal foods and cookies. Therefore, in an attempt to apply wheat bran to various foods, we developed microfibril wheat bran (MFW) using wheat bran as the raw material. MFW was prepared by swelling wheat bran with hot water and wet-smashing the swollen wheat bran. The most marked characteristic of MFW is good palatability due to a very small fiber particle size, allowing application to a wide variety of foods [10]. However, fibers with a large particle size were reported to have better physiological effects than fibers with a small particle size [11–14], giving a concern that processing to microfibril may reduce the physiological effects. Therefore, in this study, we investigated the water-holding capacity *in vitro* and the retention time of the gastrointestinal contents in rats *in vivo*. We compared the results with those of the raw material, WB, and dry smashing of wheat bran (DWB) prepared by conventional method to confirm whether MFW preserves the high physiologic effect. The results are described below.

Materials and Methods

Preparation of small size wheat bran: Wheat bran HF® (Nittou Sefiun Co., Ltd., Tokyo, Japan, designated as WB in this paper) was used as the starting material for MFW and DWB. MFW was prepared by milling WB with a Super-Masscolloider AUTO® (Type MKZA10-15HJ, Masuko Sangyo Co., Ltd., Saitama, Japan) equipped with a non-porous ceramic millstone rotating at 1.450 rpm, after suspending WB in hot water (90°C) for 30 min. The milling procedure was carried out twice and then the sample was lyophilized and passed through a 125 μm sieve (120 mesh). DWB was prepared by milling WB with a jet mill (Type FS-4, Seishin Enterprise, Co., Ltd., Tokyo, Japan). Products were also passed through a 125 μm sieve.

Chemical analysis of the three types of wheat bran: The methods used for measurement of the chemical composition of the wheat bran samples were as follows: Total dietary fiber content was measured by the AOAC method [17]. Acid-detergent fiber (ADF) [18], Acid-detergent fiber-lignin (ADF-lignin) [19] and Neutral-detergent fiber (NDF) [20] were measured. The hemicellulose content was calculated as the difference between NDF and ADF. The cellulose content was calculated as the difference between ADF and ADF-lignin.

Water-holding capacity, settling volume and particle size measurements of the three types of wheat bran: Water-holding capacity (WHC) was measured by the method of Mongeau and Brassard [15]. Briefly 20 ml of water was added to a 50 ml centrifuge tube containing a 1 g sample of the test material. The contents of the tube were mixed with a glass rod and shaken in a water bath at 150 rpm for 1 h at 37°C, then centrifuged at 14.000 × g for 1 h at 10°C. The supernatant was decanted and the tube was drained for 15 min. The wet sample was weighed, dried overnight, and weighed again to determine the water content. The WHC was expressed as grams of water held by 1 g of sample. Settling volume (SV) in water was measured by the method of Takeda and Kiriyama [16] to elucidate water-binding capacity. Briefly, 1 g of bran was

Table I: Composition of experimental diets

Ingredients (g)	Base	WB	MFW	DWB
Corn starch	600.0	477.4	482.6	470.8
Casein	200.0	180.2	181.3	179.7
Corn oil	50.0	50.0	50.0	50.0
Sucrose	100.0	100.0	100.0	100.0
Mineral mixture*	35.0	35.0	35.0	35.0
Vitamin mixture*	10.0	10.0	10.0	10.0
DL-Methionine	3.0	3.0	3.0	3.0
Choline bitartrate	2.0	2.0	2.0	2.0
WB, MFW or DWB**	0.0	142.4	136.1	149.5
Total (g)	1000.0	1000.0	1000.0	1000.0

* The vitamin and mineral mixtures were those of AIN-76 (American Institute of Nutrition, 1977)

** WB, MFW and DWB were expressed wheat bran, microfibril wheat bran and dry smashing of wheat bran respectively.

weighed into a measuring cylinder and 100 ml of water was added while stirring. SVs of each bran were measured after they were left standing for 12 h. The mean particle size of each bran was measured with a particle size analyzer (Type LS 230: Coulter Ltd., FL, USA).

Animals and diets: Fifteen 6-week-old male Fisher 344 rats were obtained from CLEA (Tokyo, Japan). Animals were maintained according to our institutional animal care guidelines in a temperature-controlled room (23 to 25°C) with a 12 h light cycle. The rats were housed in wire cages (5 rats per cage) and given free access to food and drinking water. After an acclimatation period of one week, during which the rats were fed a commercial diet (Type MF powder, Oriental Yeast, Tokyo, Japan), rats were housed individually in wire-bottomed metabolism cages and fed a basal diet (Table I) for one week. Rats were then randomly divided into three groups and fed one of the test diets. Compositions of the basal and test diets are given in Table I. MFW, DWB and WB were incorporated at a 10% fiber level in each diet. After 10 days on the starting test diet, fresh fecal pellets were squeezed from each rat and used for the measurement of fecal moisture. During days 14–16, the mean retention time (MRT) of digesta was measured with solid and liquid markers. On day 14, 30 mg of Cr-mordanted cellulose (solid marker) and 20 mg of Co-EDTA (liquid marker) mixed with 2 g of basal diet were given to rats for 1 h after a 17 h starvation period. Following the administration of these markers, rats were resumed on their designated diets. Fecal samples were collected automatically with a feces collector (Type DS-100, Shibata Scientific Technology Co., Ltd., Tokyo, Japan) every hour for 54 h and lyophilized. At day 20, all rats were exposed to pentobarbital (Nembutal, Abbot Laboratories, IL, USA) anesthesia and killed by exsanguination. The cecum of each rat was removed and the pH of the cecal contents was measured. The cecal digesta was weighed, frozen immediately and kept at –20°C for analysis of short chain fatty acids (SCFAs).

Preparation and determination of markers: Cr-mordanted cellulose, prepared as described [21] and Co-EDTA (Tokyo Kasei, Japan) were used to mark the solid and liquid phases of digesta, respectively. The lyophilized fecal samples were subject to wet ashing as described by Luick *et al* [12] and then examined for Cr and Co using an inductively coupled plasma emission spectrometer (ICPS-2000, Shimazu Ltd., Kyoto, Japan).

Analytical methods: Cecal contents and fresh fecal samples squeezed directly from each rat were weighed and then reweighed to determine moisture contents. Concerning measurement of SCFA, details of the sample preparation and analytic conditions were described below. A mixture of the cecal contents (0.2 g) and 1.8 ml of water was homogenized (whereupon the final concentration of organic acids was between 0.2 mM and 30 mM) and the homogenate was centrifuged at 9.000 × g for 10 min at 4°C. A mixture of 0.9 ml of the supernatant and 0.1 ml of 1 mol/liter of perchloric acid was allowed to stand for 2 h at 4°C and then passed through a filter with a pore size of 0.45 µm (Millipore Japan, Tokyo, Japan). The sample was analyzed by HPLC (ICA-3030, Toa Electronics Co., Tokyo, Japan), using two columns (KC-811 Shodex, Showa Denko, Tokyo, Japan) [22].

Calculations: MRT in the whole gut was estimated according to Kikuchi and Yajima [22] by using the following equations:

$$\mathrm{AUC} = \sum_{i=1}^{n} \mathrm{C}i \times \Delta t \qquad [1]$$

$$\mathrm{P}i = \mathrm{C}i \times \Delta t \,/\, \mathrm{AUC} \qquad [2]$$

$$\mathrm{MRT\ (h)} = \sum_{i=1}^{n} ti \times \mathrm{P}i = \sum_{i=1}^{n} ti\,(\mathrm{C}i \times \Delta t\,/\mathrm{AUC}) \qquad [3]$$

where AUC is area under the curve, *ti* is the time elapsed since administration of marker, C*i* is the concentration of marker at *ti*, Δt is a very small interval of time approaching zero duration, P*i* is the probability of the existing marker from *ti* to $ti + \Delta t$. The values of MRT was calculated according to equations [3] with AUC calculated by the trapezoidal method and Δt as the fecal collection interval.

Scanning electron microscopy: Samples of the three types of wheat bran were dehydrated by treating with ethyl alcohol, isoamyl acetate and placing in a critical-point dryer. After coating with osmium tetroxide, specimens were observed with a scanning electron microscope (Type S-570; Hitachi Ltd., Tokyo, Japan).

Statistical Analysis: All data were subjected to a one-way ANOVA. When significant F ratios were found, the individual means were compared by Tukey's test (STATISTICA software, StatSoft, Inc., OK, USA). Statistical significance was accepted at $p < 0.05$.

Results

Chemical analysis of the three types of wheat bran: Total dietary fiber content in MFW, DWB and WB were 73.5, 66.9 and 70.2%, respectively (Table II). The cellulose content, hemicellulose content, and lignin contents of MFW were 15.3, 45.1, and 7.2%, those of DWB were 16.4, 41.3, and 6.2%, and those of WB were 16.2, 46.2, and 6.1%, respectively. The chemical composition among MFW, DWB and WB were not different (Table II).

Particle size, WHC and SV: We investigated particle size, WHC and SV used as an index of water-binding capacity of the three types of wheat bran. The mean particle size of MFW, DWB and WB were 35, 61 and 420 µm, respectively. The difference of the particle size was clear using scanning electron microscopy. The particle size was in the order of WB (Fig. 1a) > DWB (Fig. 1c) > MFW (Fig. 1b). The WHC of MFW, DWB and WB were 5.1, 3.0 and 5.0 g/g, respectively. The SV of MFW, DWB and WB were 16.1, 5.4 and 6.9 ml/g, respectively (Table II). Thus, MFW was different from DWB. While it had a smaller mean particle size than DWB, it had greater WHC and SV than DWB.

General observations: At start of this study, the mean initial body weights (150 g) of each group were not different because the rats were stratified by their body weights.

Table II: Chemical composition, water-holding capacity and settling volume of the three types of wheat bran

	Dietary fiber component (%)				WHC (g water/g fiber)	SV (ml/g fiber)	Particle size (µm)
	TDF	Cellulose	Hemicellulose	Lignin			
WB	70.2	16.2	46.2	6.1	5.0	6.9	420
MFW	73.5	15.3	45.1	7.2	5.1	16.1	35
DWB	66.9	16.4	41.3	6.2	3.0	5.4	61

TDF, total dietary fiber. The hemicellulose content was calculated as the difference between NDF and ADF. The cellulose content was calculated as the difference between ADF and ADF-lignin (experimental details see text).

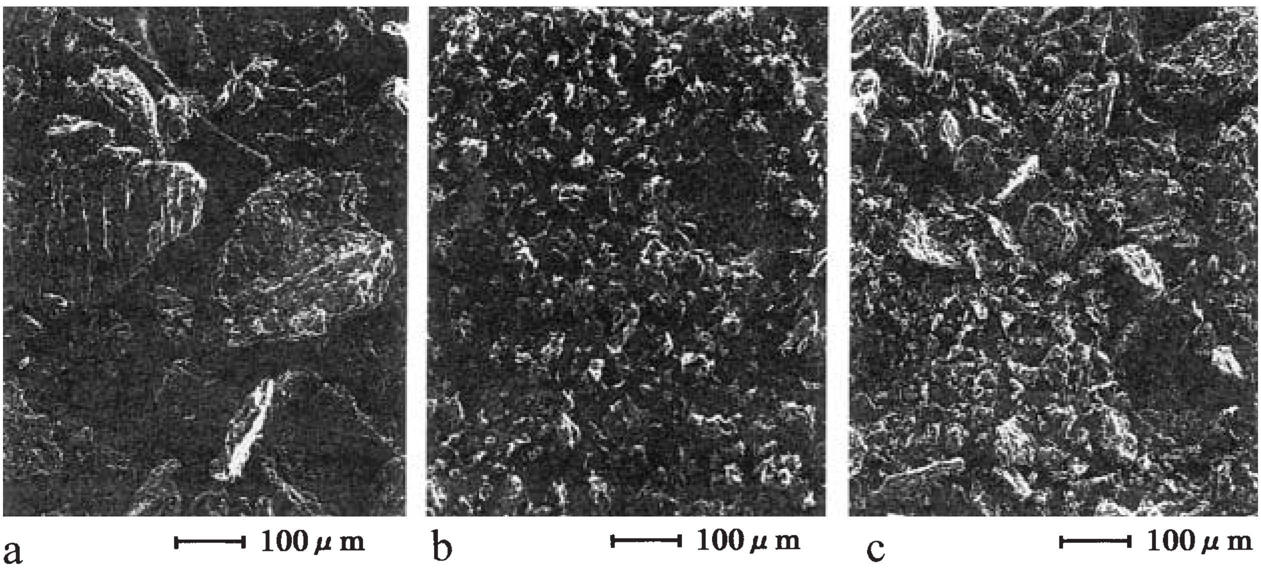

Figure 1: The particle size of three types of wheat bran as viewed under an electron microscope. The particle size of MFW (b) is smaller than those of WB (a), DWB (c). Magnification × 100.

Body weights and food intakes were recorded weekly throughout the study. No effects of diets on food intakes or body weights were seen. The body weights of rats in the WB, MFW and DWB diet groups at the end of the experiment were 215.2 ± 14.6, 221.0 ± 17.4 and 222.3 ± 10.2 g, respectively.

Gastrointestinal MRT of diegesta: Focusing on the solid phase, the MRT of the MFW group showed a significantly ($p < 0.05$) lower value compared to that of the DWB group. The MRT did not differ from that of the WB group, even though the particle size of MFW was 8.3% that of WB. Similar relationships were observed for MRT of the liquid phase (Table III).

Cecal and fecal analysis: While no significant difference was seen in the cecal weight among the three groups, the moistures of cecal contents were significantly different; MFW > WB > DWB (Table IV). No significant differences of fecal weights were observed among the three groups, however, the numbers of fecal pellets of WB and MFW groups were significantly ($p < 0.05$) higher as compared to that of DWB group (Table IV). Nor were significant differences in the fecal moisture seen among the three groups (Table IV).

Concentration of SCFA, total amounts of SCFA and pH in cecum: No significant differences were observed in cecal SCFA concentrations, total amounts of SCFA and pHs for the three groups (Table V).

Table III: Effect of three types of wheat bran on gastrointestinal mean retention time of rats

	Solid phase (h)	Liquid phase (h)
WB	$15.5 \pm 2.4^{*, a, b}$	$16.0 \pm 2.5^{a, b}$
MFW	15.2 ± 0.8^{a}	14.7 ± 1.0^{a}
DWB	18.0 ± 0.9^{b}	18.4 ± 0.8^{b}

* Values are means and standard deviations for five rats. Means in a column not sharing a superscript letter were significantly different, $p < 0.05$.

Discussion

In this study, we compared dietary fibre content, WHC, SV and physiological effects of MFW with those of DWB and WB, focusing on the effect of shortening the retention time of the gastrointestinal contents and evaluated MFW.

Van Soest reported that grinding of wheat bran gradually decreases WHC [23]. The WHC of MFW was approximately 1.7-times greater than that of DWB and was comparable to that of WB. To investigate why MFW preserved greater WHC than DWB *in vitro*, the dietary fiber content of each wheat bran, and cellulose content, hemicellulose content and lignin content, which are constituents of wheat bran, were measured, and these were almost unchanged (Table II). Mongeau and Brassard noted that the variation found in WHC did not seem to be due to compositional changes but to structural changes of the fiber matrix during grinding of wheat bran [15]. Cadden also reported that the matrix structure of wheat bran was collapsed by grinding and the difference of physical structure was related to WHC [24]. So we investigated the differences in the particle structures using scanning electron microscope, but the obvious structural differences were not observed in this study. At this point, more study should be needed.

On measurement of MRT of gastrointestinal content, MRT in both solid and liquid phases in the MFW group were significantly shorter than those in the DWB group, and comparable to those in the WB group (Table III). Considering that the mean particle size of MFW was approximately $\frac{1}{12}$ of that of WB and approximately ½ of that of DWB, these results of MRT are very interesting, because wheat bran with a large particle size is thought to increase the volume of gastrointestinal content by holding much water inside and outside the fiber particles and facilitate movement of gastrointestinal content [25]. However, Kikuchi and Yajima noted increased WHC rather than fiber particle size as a factor affecting shortening of the retention time of the gastrointestinal contents in cellulose [22], suggesting that MFW also preserved a high MRT-short-

Table IV: Effect of three types of wheat bran on the weight and moisture of cecal contents, and stool output in groups of WB, MFW and DWB

	Cecum		Feces		
	Digesta weight (g)	Moisture (%)	Dry weight (g/day)	Number of pellets (n/day)	Moisture (%)
WB	$2.54 \pm 0.49^{*, NS}$	$75.4 \pm 0.9^{**, b}$	1.83 ± 0.19^{NS}	33 ± 4^{a}	52.4 ± 3.6^{NS}
MFW	2.82 ± 0.36	79.1 ± 2.1^{a}	1.80 ± 0.19	34 ± 2^{a}	54.1 ± 1.9
DWB	3.07 ± 0.58	71.7 ± 1.2^{c}	1.80 ± 0.08	28 ± 2^{b}	52.0 ± 1.0

* Values are means and standard deviations for five rats.
** Means in a column not sharing a superscript letter were significantly different, $p < 0.05$.
NS, not significant.

Table V: Effect of the three types of wheat bran on the cecal concentration of SCFAs, amount of SCFAs and pH

	Acetate		Propionate		Butyrate		Total SCFA		pH
	(µmol/g wet wt)	(µmol/cecum)	(µmol/g wet wt)	(µmol/cecum)	(µmol/g wet wt)	(µmol/cecum)	(µmol/g wet wt)	(µmol/cecum)	
WB	$27.6 \pm 7.0^{*,\mathrm{NS}}$	$70.1 \pm 23.4^{**,\mathrm{NS}}$	$7.4 \pm 1.2^{*,\mathrm{NS}}$	$18.8 \pm 5.3^{**,\mathrm{NS}}$	$17.4 \pm 6.1^{*,\mathrm{NS}}$	$44.4 \pm 19.6^{**,\mathrm{NS}}$	$52.3 \pm 11.5^{*,\mathrm{NS}}$	$133.3 \pm 42.1^{**,\mathrm{NS}}$	$6.57 \pm 0.25^{\mathrm{NS}}$
MFW	26.7 ± 7.4	76.6 ± 27.8	6.6 ± 1.4	18.6 ± 4.9	12.7 ± 3.8	36.7 ± 14.8	45.9 ± 11.9	131.8 ± 46.1	6.94 ± 0.22
DWB	25.1 ± 6.9	74.2 ± 10.9	7.0 ± 1.2	21.0 ± 1.4	13.7 ± 4.7	40.3 ± 8.3	45.8 ± 12.6	135.4 ± 19.5	6.74 ± 0.18

* Values are means and standard deviations for five rats expressed as µmol/g wet wt cecal contents.
** Values are means and standard deviations for five rats expressed as µmol/cecum.
NS, not significant.

ening effect due to the "bulky" content resulting from increased WHC.

Cummings *et al* reported that decrease in MRT is dependent on increase in the amount of fecal excretion [26]. It is also reported that SCFAs produced in the intestine stimulate gastrointestinal movement and secretion of intestinal juice [27, 28], and affect MRT [29]. In this study, the amounts of fecal excretion or the SCFA concentration did not differ among the three groups (Tables IV and V). However, the number of pellets of feces excreted in a day was significantly decreased in the DWB group compared to that in the WB and MFW groups (Table IV). This may be because the "bulking effect" of DWB in the DWB diet was weaker than that in the case of the other two diets, resulting in slow movement of the content. The water content in the cecal content was significantly increased in the order of MFW > WB > DWB, but the fecal water content did not differ among the three groups, which may have been caused by normalizing action on fecal water content by water reabsorption from the distal colon and rectum.

In conclusion, it was shown that MFW shortens retention time of the gastrointestinal contents by the preserved high WHC compared to DWB.

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