# Insoluble Dietary Fiber from Breakfast Cereals and Brans: Bile Salt Binding and Water-Holding Capacity in Relation to Particle Size

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#### **ABSTRACT**

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Particle size has been reported to be important in the effect of cereal fiber on colonic function, but few measurements have been made on marketed cereal products. Insoluble fiber was therefore extracted from two oats cereals and from a group of breakfast cereals containing at least 10% neutral detergent fiber (NDF). Mean particle size (MPS), water-holding capacity, and glycocholate and taurocholate binding were measured in pooled NDF from each cereal. Fourteen of the 16 NDF had an MPS of 0.34-0.92 mm. Glycocholate and taurocholate binding varied from 16.2 to 34.2 and from 5.6 to  $12.4 \,\mu \text{mol}/0.2$  g of NDF, respectively. Water-holding capacity varied

from 7.8 to 20.4 g of  $H_2O/g$  NDF. In NDF from wheat (whole or bran) products, positive correlations were found between glycocholate (r = 0.90, P < 0.001) or taurocholate (r = 0.86, P < 0.05) binding and the logarithm of MPS; water-holding capacity was positively correlated (r = 0.85, P < 0.01) with MPS. Similar correlations were found in NDF from unprocessed wheat brans ground at various particle sizes. The water-holding capacity of NDF was also highly correlated with dry bulk. The results indicated that nutritionally important physicochemical characteristics of wheat fiber are adversely affected as particle size is reduced.

Eastwood et al (1973) reported that the physical properties of dietary fiber may be related to its effects on colonic function. Kirwan et al (1974) suggested that the water-holding capacity of wheat bran is a function of particle size and that large particles may be needed to retain water and to prevent the formation of dry, hard feces. However, the fine bran and the coarse bran they used were from two different sources and had different compositions, and their conclusions have not been generally accepted (Anonymous 1975). Heller et al (1980) clearly demonstrated with human subjects that the moisture content of feces following a coarse bran diet is significantly greater than that following a fine bran diet. Their observations correlated with an in vitro assessment of water-holding capacity, indicating that the in vitro characterization of wheat fiber is relevant to physiological effects.

In spite of the importance of the physical properties of dietary fiber, few characterizations of cereal fiber have been reported. Sixteen cereal products were therefore selected from 91 breakfast cereals on the basis of their fiber content, and the neutral detergent fiber (NDF) was examined for particle size, bile salt binding, and water-holding capacity.

Of the various sources of dietary fiber, a special value has been attributed to wheat (Bingham et al 1979). The present results show that bile salt binding and water-holding capacity of wheat fiber are gradually decreased as particle size is reduced.

## **MATERIALS AND METHODS**

Ninety-one cereal products were purchased from local supermarkets, the NDF contents were determined, and those containing at least 10% NDF (14 products) were retained. Two oats products (6 and 7% NDF) were included in the study for comparison.

#### **Analytical Procedures**

NDF was extracted by the method of Goering and Van Soest (1970), modified to include a rapid incubation with  $\alpha$ -amylase from porcine pancreas to remove residual starch.

Amylase solution (5% w/v) was prepared by mixing 5 g of  $\alpha$ -amylase powder (Sigma A6880) for 15 min with 100 ml of buffer solution, pH 7 (61 ml 0.1M Na<sub>2</sub>HPO<sub>4</sub> + 39 ml 0.1M Na<sub>4</sub>PO<sub>4</sub>), centrifuging 10 min at 3,000 rpm and filtering through a coarse sintered glass funnel. After refluxing the cereal sample with neutral detergent, the digest was filtered through a Gooch crucible on the filtering manifold (Goering and Van Soest 1970) and rinsed with hot distilled water. Next, 10 ml of  $\alpha$ -amylase solution and 40 ml of distilled water at 70° C were added to the crucible and yielded a mixture at  $\sim$ 55° C. This was held for 5 min on the filtering

manifold; suction was then applied to the filter, which was then washed with hot distilled water. The bottom of the crucible was stoppered (with a no. 8 rubber stopper), and 10 ml of  $\alpha$ -amylase solution and 40 ml of distilled water at 70° C were again added to the crucible. The crucible was placed in an oven at 55° C. After 60 min of digestion, the crucible was removed from the oven and its contents filtered and washed three times with hot distilled water and twice with acetone. The crucible was dried overnight at 100° C. NDF from each of the samples was pooled from several extractions to obtain enough material for the examination of the physicochemical characteristics.

#### **Bile Salt Binding**

Bile salt solutions were prepared by dissolving sodium taurocholate or glycocholate in Na<sub>2</sub> HPO<sub>4</sub>-NaH<sub>2</sub>PO<sub>4</sub> 0.1 M buffer (pH 7) to obtain a concentration of 12.5  $\mu$ mol of bile salt per ml. Radioactive taurocholic (24-<sup>14</sup>C, NEC-665, New England Nuclear) or glycocholic (Glycine-1-<sup>14</sup>C, NEC-620, New England Nuclear) acid was added to obtain a concentration of 0.0025  $\mu$  Ci per ml.

Eight milliliters of the bile salt solution was added to a 25-ml Erlenmeyer flask containing 0.2 g of dry NDF. The flask was incubated at 37° C for 1 hr in a shaking bath with the speed adjusted to permit continuous mixing. A control flask without NDF was treated in the same manner. After the incubation, approximately 3 ml was centrifuged at  $30,000 \times g$  for 10 min at 10° C. Aliquots (0.8 ml) of the supernatant were pipetted into separate scintillation vials containing 10 ml Aquasol (New England Nuclear). The content was mixed and read on a Beckman LS 9000 scintillation counter.

Bile salt bound to the NDF was determined by the difference in the count between the control and the NDF-treated solutions. This procedure is a modification of the Kritchevsky's method (Kritchevsky and Story 1974).

# Water-Holding Capacity

Water-holding capacity was determined by a method similar to that of McConnell et al (1974). Approximately 20 ml of water was added to a 30-ml centrifuge tube containing 0.8–1.0 g of NDF. This was mixed with a glass rod and shaken for 1 hr in a water bath at  $37^{\circ}$  C, leaving the glass rod in the tube for better mixing. After centrifuging at  $14,000 \times g$  for 1 hr at  $10^{\circ}$  C, the supernatant was discarded and the tube drained for 15 min. The wet NDF was weighed, dried overnight, and weighed again to determine the water content. The water-holding capacity was expressed in grams of water held by 1 g of NDF.

# Mean Particle Size

Approximately 2-3 g of NDF was sifted for 5 min through sieves using an ATM Sonic Sifter at an amplitude that allowed the largest particles to roll on the screen. The mechanical pulsing action was utilized, when needed, to break down cluster particles. Standard

TABLE I
Neutral Detergent Fiber from Breakfast Cereals: Particle Size, Bile Salt Binding and Lignin Content

Cereal	MPS <sup>a</sup> (mm)	Bile Salt Binding <sup>b</sup> (μmol/0.2 g of NDF)		Percent	Percent of Ld
		Glycocholate	Taurocholate	NDF°	in NDF
1. Bran Flakes (W+B) <sup>e</sup>	0.34	18.8 ± 1.5	ND	11	7.3
2. Bran Crunchies (W+B)	0.38	$21.6 \pm 0.2$	$7.1 \pm 0.9$	11	8.4
3. Bran Buds (B)	0.44	$25.9 \pm 0.3$	$7.3 \pm 0.8$	25	8.5
4. 100% Bran (B)	0.44	$27.2 \pm 0.6$	$8.2 \pm 0.4$	29	9.4
5. Bran Flakes (W+B)	0.46	$22.4 \pm 1.0$	$5.6 \pm 0.7$	10	9.3
6. All Bran (B)	0.53	$32.6 \pm 0.4$	$9.3 \pm 0.9$	30	8.7
7. Shredded Wheat (W)	0.68	$33.0 \pm 1.0$	$12.4 \pm 0.4$	10	7.8
8. Spoon-Size Shredded Wheat (W)	0.74	$34.2 \pm 1.4$	ND	11	7.6
9. Muffets (W)	0.83	$32.3 \pm 2.0$	ND	11	7.5
10. Unprocessed Wheat Bran (B)	0.92	$33.9 \pm 0.6$	$8.4 \pm 0.7$	43	11.3
11. Wheat Flakes (W)	1.65*	$24.2 \pm 1.7$	ND	13	9.4
12. Wheat Germ (G)	0.73	$18.5 \pm 0.9$	ND	15	9.5
13. Wheat Germ (G)	0.86	$25.4 \pm 0.9$	$6.1 \pm 0.7$	12	6.6
14. Wheat Germ (G)	0.89	$16.2 \pm 1.6$	ND	18	10.1
15. Oatmeal (O)	0.85	$23.1 \pm 2.7$	ND	7	19.2
16. Quick Oats (O)	1.40*	$30.2 \pm 0.6$	$11.0 \pm 0.2$	6	18.2

<sup>&</sup>lt;sup>8</sup>MPS = mean particle size of neutral detergent fiber (NDF). Asterisks indicate MPS estimated with less than 80% of the weight of NDF.

TABLE II Water-Holding Capacity of NDF from Breakfast Cereals

	Gram of H <sub>2</sub> O <sup>a</sup>	Gram of H <sub>2</sub> O <sup>b</sup> Gram of Dry Sample	
Cereal	Gram of NDF		
Bran Flakes (W+B) <sup>c</sup>	$7.8 \pm 0.2$	$0.77 \pm 0.02$	
Bran Crunchies (W+B)	$8.1 \pm 0.4$	$0.89 \pm 0.04$	
Bran Buds (B)	$8.9 \pm 0.4$	$2.31 \pm 0.10$	
100% Bran (B)	$8.2 \pm 0.5$	$2.31 \pm 0.13$	
Bran Flakes (W+B)	$8.1 \pm 0.1$	$0.86 \pm 0.01$	
All Bran (B)	$8.8 \pm 0.2$	$2.58 \pm 0.07$	
Shredded Wheat (W)	$11.1 \pm 0.7$	$1.09 \pm 0.07$	
Spoon-Size Shredded Wheat (W)	$10.8 \pm 0.5$	$1.13 \pm 0.01$	
Muffets (W)	$9.9 \pm 0.4$	$1.08 \pm 0.04$	
Unprocessed Wheat Bran (B)	$10.6 \pm 0.7$	$4.43 \pm 0.30$	
Wheat Germ (G)	$20.4 \pm 1.9$	$2.43 \pm 0.22$	
Ouick Oats (O)	$17.5 \pm 0.6$	$0.86 \pm 0.03$	

<sup>&</sup>lt;sup>a</sup> Mean of three analyses  $\pm$  SEM.

U.S. sieves (12, 16, 20, 30, 40, 50, 60, 70, 100, 140, 200, and 270) were used for particle separation. The amount of material retained on each sieve was weighed, and the mean particle size of NDF from each cereal was determined as described by Ensor et al (1970) using the following formula:

$$MPS = \log^{-1} \left[ \frac{\sum (Wi \log \overline{di})}{\sum W} \right],$$

where MPS = mean particle size, Wi = weight of particles on a sieve,  $\Sigma W = \text{sum of Wi}$ , and  $\overline{di} = [di \times di + 1]^{1/2}$ . (di = size of screen openings of a sieve, and di + 1 = size of screen openings of the upper sieve.)

## Dry Bulk

Eight grams of wheat bran was placed in a 50-ml graduated cylinder, which was tapped with the hand for 2 min before the volume was read.

#### RESULTS

The cereal products selected for this study contained 10-43% NDF, except for two oats products that contained 6 and 7% NDF (Table 1). Cereal products are shown by increasing order of mean particle size (MPS) of their NDF; wheat germ and oats products are not listed among whole wheat and wheat bran products. MPS varied from 0.34 to  $\sim 1.65$  mm, but only two were above 1 mm. MPS values of high fiber products (25-30% NDF) were grouped around 0.5 mm.

Glycocholate binding was measured on four aliquots of each fiber extract, and mean values varied from 16.2 to  $34.2 \,\mu \text{mol}/0.2 \,\text{g}$  of NDF (Table I). NDF from wheat germ breakfast cereals bound 18.5, 25.4, and 16.2  $\mu$ mol; NDF from oats products bound 23.1 and 30.2  $\mu$ mol. The relation between particle size and bile salt binding was studied in the wheat products (excluding wheat germ) with MPS in the range of 0.34–0.92 mm (Table I, 1–10). Sample 11 was not retained because its MPS, in addition to being relatively imprecise, was clearly outside the range of other samples. A significant correlation was observed (r = 0.90, P < 0.001) between glycocholate binding and the logarithm of the fiber MPS (Fig. 1).

The measurement of taurocholate binding was restricted to the most representative samples of Table I: one Bran Flakes (5), the Bran Crunchies (2), the three high-fiber cereals (3, 4, and 6), one Shredded Wheat (7), one unprocessed wheat bran (10), one wheat germ (13), and one oats product (16). Taurocholate binding was measured in four aliquots of the different fiber extracts, and values varied from 5.6 to 12.4  $\mu$ mol/0.2 g of NDF (Table I). Although the values from six wheat products were significantly correlated (r = 0.86, P < 0.05, Fig. 2) with the logarithm of MPS in the range corresponding to 0.38–0.68 mm, the value from the unprocessed wheat bran (10, MPS = 0.92 mm) appeared to disagree with the correlation.

Water-holding capacity was measured on three aliquots of each NDF (Table I, 1–10, 14, and 16). Values varied from 7.8 to 11.1 g of  $\rm H_2O/g~NDF$ , except in oats and wheat germ, where values were 17.5 (MPS = 1.4 mm) and 20.4 (MPS = 0.86 mm), respectively (Table II), indicating that the type of cereal or the part of the kernel greatly influenced the water-holding capacity. The right column of Table II shows the results of water-holding capacity per gram of the whole product (calculated using NDF values from Table I). Figure 3 shows the positive correlation (r = 0.85, P < 0.01) between water-holding capacity and MPS of fiber in wheat products.

<sup>&</sup>lt;sup>b</sup>Mean of four analyses ± SEM. ND = not determined.

 $<sup>^</sup>c$ NDF = neutral detergent fiber after digestion with  $\alpha$ -amylase from porcine pancreas (Sigma No. 6880). Mean of two samplings at six-month interval; each with duplicate analyses.

<sup>&</sup>lt;sup>d</sup>L = permanganate lignin (Mongeau and Brassard 1982).

<sup>&</sup>lt;sup>e</sup>Letters in parenthesis indicate the composition of products. W = whole wheat, B = wheat bran, G = wheat germ, O = oats.

<sup>&</sup>lt;sup>b</sup>Calculated using neutral detergent fiber values from Table I. See Table I for mean particle size.

<sup>&</sup>lt;sup>c</sup>Letters in parenthesis indicate the composition of products. W = whole wheat, B = wheat bran, G = wheat germ, O = oats.

Factors other than particle size may have been involved in the relationships (Figs. 1-3) between particle size and bile salt binding or water-holding by NDF of wheat products, because the products were made from different sources of wheat and were processed in various ways. The same physicochemical characteristics were therefore studied in four unprocessed wheat brans. Figure 4 shows glycocholate binding by NDF from AACC bran after milling through different sizes (20-, 40-, 60-, and 80-mesh screen) with an intermediate Wiley mill; the correlation (r = 0.84) was not significant. The same was true with the NDF from three other unprocessed brans (data not shown). However, when NDF was first extracted and then milled through different screen sizes, the linearity was improved (r = 0.996, P < 0.01), although this was not the manner in which the fiber from processed cereals was studied.

Figure 5 shows the effect of grinding on taurocholate binding by NDF from four different unprocessed brans. Taurocholate binding was significantly correlated (r = 0.98, P < 0.02) with the particle size of NDF from AACC bran, but the correlation was not significant in the three other brans.

Figure 6 shows a significant correlation between water-holding capacity and mean particle size of NDF from the four unprocessed wheat brans. The relation appeared to be true even when the MPS was as high as 1.2 mm in bran D, in which a high linearity (r = 0.996, P < 0.01) was observed.

Figure 7 shows a linear relationship between the dry bulk of wheat bran and the MPS of its fiber. The correlation was particularly high (r = 0.998, P < 0.01) with AACC bran and with

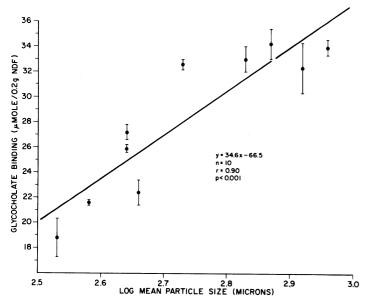


Fig. 1. Glycocholate binding and particle size of neutral detergent fiber (NDF) from wheat breakfast cereals. Mean of four analyses ± SEM.

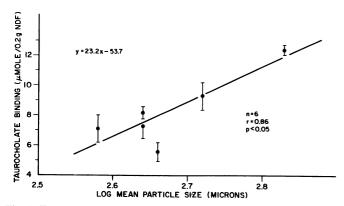


Fig. 2. Taurocholate binding and particle size of neutral detergent fiber (NDF) from wheat breakfast cereals. The mean of four analyses  $\pm$  SEM.

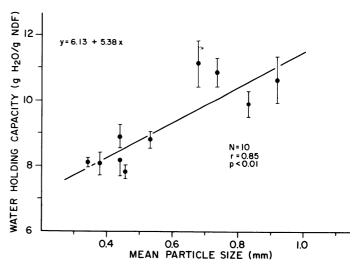


Fig. 3. Water-holding capacity and particle size of neutral detergent fiber (NDF) from wheat breakfast cereals. Mean of three analyses  $\pm$  SEM.

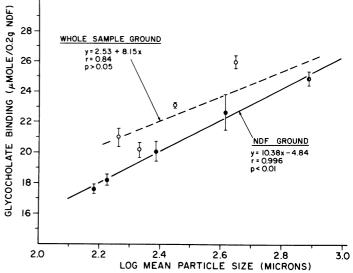


Fig. 4. o = binding with neutral detergent fiber (NDF) extracted after grinding the entire sample.  $\bullet = binding$  with NDF extracted prior to its grinding. o = binding with unground NDF from original sample.

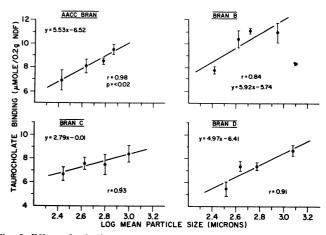


Fig. 5. Effect of grinding on taurocholate binding by neutral detergent fiber (NDF) from unprocessed brans. Mean of four analyses ± SEM. ● to far right of each curve represents NDF extracted from unground (ie, aspurchased) bran. The other circles (from right to left) represent the brans milled through the 2.0-, 1.0-, or 0.5-mm sieve, using an EDS Wiley Mill.

bran D. Water-holding capacity was also highly correlated with the dry bulk (Fig. 8) of bran C (r = 0.994, P < 0.01) and of bran D (r = 0.998, P < 0.01). The correlation was significant in AACC bran (r = 0.997, P < 0.05) but not in bran B (r = 0.90).

## **DISCUSSION**

Dietary fiber consists of a sponge matrix with specific physicochemical properties dependent on the structure and composition of its components (Eastwood and Kay 1979). Several articles have indicated that particle size can influence other physicochemical characteristics of dietary fiber (Eastwood et al 1973, Heller et al 1980, Kirwan et al 1974). In the present article, the effects of particle size were studied on one active property (bile salt binding) and one passive property (water-holding capacity) of NDF from cereal products. Most of the products were prepared from wheat, which is a relatively weak bile salt binder. Nevertheless, Figs. 1, 2, 4, and 5 indicate that wheat fiber (excluding that of wheat germ products) binds substantially less bile salt when its MPS is decreased from about 0.8 mm to 0.3 mm, a range which corresponds to the particle size in most breakfast cereals. The decreased binding reflects the alteration in the integrity of the plant cell wall during grinding. Figure 4 also indicates that grinding may affect the fiber particles in different ways, depending whether whole bran or its NDF is ground; grinding NDF leads to smaller MPS. On the other hand, particle size affects the yield of the neutral detergent method, so that NDF residues obtained

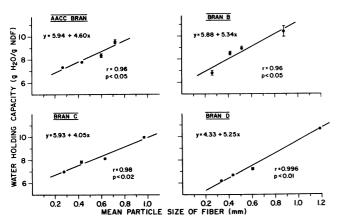


Fig. 6. Effect of grinding on water-holding capacity of neutral detergent fiber (NDF) from unprocessed brans. Mean of three analyses  $\pm$  SEM.

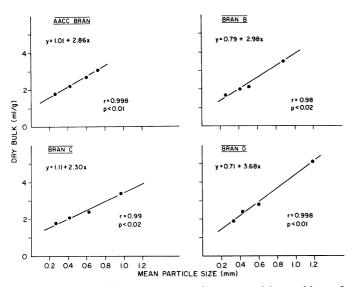


Fig. 7. Effect of grinding on dry bulk of unprocessed brans. Mean of duplicate analyses.

before or after grinding may contain slightly different components. This may explain why different binding capacities were obtained with NDF having similar particle size (ie, why the dashed line was higher than the solid line in Fig. 4). All other grindings (Figs. 5–8) were performed on whole bran.

Eastwood and Kay (1979) reported that bile acid adsorption by fiber was partly attributable to an effect of lignin. The lignin content of each cereal product was measured and expressed as percent lignin in the NDF (Table I). No significant correlation existed between the lignin content of fiber and its bile salt binding. Thus, the variations in binding cannot be attributed to variations in the lignin content. On the other hand, oats fiber contained a larger proportion of lignin (Table I, 15 and 16), but the two values do not suggest that oats fiber binds more glycocholate than wheat fiber in vitro.

Different experimental conditions may lead to different binding values, and comparisons of binding must be made on values measured under the same experimental conditions. Table I provides data on glycocholate binding for all breakfast cereals that contained 10% NDF or more and for two oats products. However, because the preparation of relatively large amounts of NDF from each breakfast cereal involved repetitive, time-consuming extractions, further studies were restricted to the most representative samples.

Figure 2 suggests that taurocholate binding correlates with the logarithm of MPS, but the relatively low value obtained with unprocessed bran (Table I, 10) is not in agreement with such a correlation. The discrepancy may be due to the different sources of wheat used; indeed, Fig. 5 shows that this unprocessed bran (bran C) bound 6.5–8.5  $\mu$ mol of taurocholate/0.2 g of NDF, whereas bran B bound 8–11  $\mu$ mol/0.2 g of NDF. The correlations in Fig. 5, although significant only in AACC bran, are in agreement with Fig. 2 and suggest that reducing the particle size of wheat bran reduces taurocholate binding to the fiber. This may hold over a wide range of particle sizes, because bran D had an MPS of 1.2 mm.

Table III shows that the results on water-holding capacity of AACC bran in relation with particle size are in agreement with those of Heller et al (1980). In spite of differences in the absolute values, the same decrease was observed upon reduction of particle size. Heller et al (1980) obtained a 31% decrease in water-holding capacity when MPS was reduced by 0.54 mm, whereas the present study shows a 24-27% decrease when MPS is reduced by 0.44 mm. The water-holding capacity of AACC bran or of wheat fiber in general (Figs. 3 and 6) appears to decrease gradually with a reduction of its particle size.

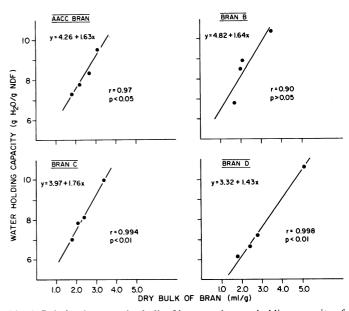


Fig. 8. Relation between dry bulk of brans and water-holding capacity of their neutral detergent fiber. Mean of four analyses  $\pm$  SEM.

TABLE III
Water-Holding Capacity of AACC Bran

	Gram of H <sub>2</sub> (	Gram of H <sub>2</sub> O Retained per Gram of Bran				
Bran	Calculated <sup>a</sup>	Measured on	Values from			
	from Fig. 6	Whole Bran	Heller et al <sup>b</sup>			
Coarse <sup>c</sup>	4.03	3.99	5.25			
Fine <sup>d</sup>	3.07	2.92	3.60			

<sup>&</sup>lt;sup>a</sup> Measured on neutral detergent fiber and calculated assuming 40% neutral detergent fiber.

Table III shows that, in AACC bran, the presence of starch and other digestible components did not substantially change the water-holding capacity, as comparable values were obtained with NDF and with the whole sample. Water-holding values in Table II were measured on NDF residues because it better corresponds to conditions in the colon, after digestible material has been removed. Schaller (1978) reported that starch and other digestible material may contribute to the water-holding capacity as measured in vitro. Although such a contribution was not observed in AACC bran, it may be important in low-fiber products.

According to Rasper (1979), the water retention of cereal fiber correlates positively with insoluble noncellulosic polysaccharides but negatively with cellulose. All the wheat products used for correlation studies had constant proportions of insoluble hemicellulose (around 67% of total NDF) and cellulose (around 24% of total NDF). These results are reported elsewhere (Mongeau and Brassard 1982). The variations found in water-holding capacity did not seem to be due to compositional changes but to structural changes of the fiber matrix during grinding.

Table II suggests that NDF from wheat germ breakfast cereals holds more water than NDF from other wheat products. Mongeau and Brassard (1982) showed that the hemicellulose, cellulose, and lignin contents of wheat germ NDF are comparable to those of other wheat products; these results also show that the proportion of pentose sugars in the hemicellulose fraction from wheat germ is comparable to that in other wheat products. The present results do not provide information on the relationship between the particle size of NDF from wheat germ products and water-holding capacity.

Figure 8 shows a significant correlation between water-holding and dry bulk in three unprocessed brans. This is in agreement with the results of Van Soest (1981), who reported that grinding of wheat bran gradually decreases bulk volume as well as water-holding capacity. Aleurone cells in bran are like holes in a sponge, absorbing water. When particle size is reduced, fewer pores and voids remain to hold water, the water-holding capacity becomes lower, and the physiological usefulness is lessened (Schaller 1978).

Several studies on humans have indicated that coarse wheat bran

has better effects on the colon than fine bran (Brodribb and Groves 1978, Frexinos and Louis 1978, Heller et al 1980, Kirwan et al 1974). A correlation between in vitro assessment of water-holding capacity and colonic function (Heller et al 1980) may exist only for wheat fiber, as nonwheat fibers tend to be extensively digested by colonic bacteria (Stephen and Cummings 1979).

Insoluble dietary fiber from wheat binds less taurocholate and glycocholate and holds less water in vitro when its MPS is decreased, suggesting a negative alteration of physicochemical characteristics of the fiber matrix during grinding.

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<sup>&</sup>lt;sup>b</sup>Heller et al 1980.

<sup>&</sup>lt;sup>c</sup> Mean particle size was around 0.72 mm.

<sup>&</sup>lt;sup>d</sup>Mean particle size was around 0.27 mm, except in Heller's fine bran, in which it was 0.18 mm.