# Enzymatic Processing of Wheat Bran: Effects on Nutrient Availability[1]

R. M. SAUNDERS, M. A. CONNOR, R. H. EDWARDS, and G. O. KOHLER, Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, California 94710

**ABSTRACT**

Different commercial cellulolytic enzymes have been used to process bran. Protein availability measured with an *in vitro* system increased by a factor as high as 35% and large amounts of glucose were produced. After wheat bran was processed with one of the enzymes, its nutritive value increased for the rat. Microscopic studies showed that the aleurone cell wall was the primary substrate for these enzymes.

Wheat bran, a by-product of flour milling, is composed of the pericarp and the outermost tissues of the seed, including the aleurone layer. It constitutes almost 10% of the total weight of wheat milled for flour. On a moisture-free basis, bran contains about 17% protein and 70% carbohydrates, about 80% of which is

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cellulose and hemicellulose. Monogastric animals are able to utilize only about 60 to 70% of the protein, and little, if any, of the cellulosic material. Our laboratory is concerned with increasing the nutritive value of wheat bran, and efforts to date have been directed towards increasing protein digestibility. Most of the bran protein and other nutrients are contained in the aleurone cells (1). These cells have heavy cell walls, and the question arises as to whether the protein in raw bran shows relatively low digestibility because of the cell-wall matrix interfering with the digestive process.

Rohrlich and Rasmus (2) showed that increased protein digestibility in aleurone cells depended on physical breakage of the cell wall. Work by our group indicates this is probably true for chicks (3) and is true in *in vitro* studies (4). Olsen and Slinger (5) suggest this may be true for rats. It was considered likely that if the cell wall could be disrupted by cellulolytic enzymes, increases in protein digestibility might occur, and in addition, an increase in metabolizable energy (M.E.) for monogastric animals should be registered by the degradation of the normally nondigestible carbohydrate to digestible carbohydrate.

A recent publication by Neudoerffer and Smith (6) with a similar objective in mind indicates this assumption is correct. After cellulolytic *and* proteolytic hydrolysis of wheat bran, protein digestibility and M.E. increased for rats. Although the authors described the digestible sugars produced by their treatments and noted the protein-digestibility increases, no explanation was offered for the bran moiety or moieties undergoing modification and being responsible for these nutrient increases. Another recent publication by these same authors (7) evaluates different enzymatic strains for solubilizing wheat-bran constituents. This has prompted us to report our experiments herein.

## MATERIALS AND METHODS

The wheat bran was a blend from hard red spring wheat prepared for us through the courtesy of William Johnston, International Multifoods. Its proximate analysis on a dry basis was: protein, 18.2%; fat, 6.0%; ash, 6.9%; and fiber, 12.3%. The cellulolytic enzymes were as follows: Cellulase 36 Concentrate, Miles Laboratories, Elkhart, Ind., source *Aspergillus niger*; "Onozuka" Cellulase, Kanematsu-Gosho, New York, N.Y., source *Trichoderma viride*; and Pectinol 41P Concentrate, Rohm and Haas, Philadelphia, Pa., source unknown.

One gram of bran suspended in 15 ml. of water was immersed in a boiling-water bath for 5 min. After the mixture had cooled to room temperature, 5 ml. of 0.2M sodium acetate buffer, pH 4.0, was added, followed by the cellulolytic enzyme (0, 10, 25, or 50 mg.) and a few drops of toluene. The mixture was gently agitated at 70°F. for 18 hr.; centrifuged; and the supernatant analyzed for sugar, if necessary. The solids were washed once with water (30 ml.), neutralized to pH 7 with a few drops of 10% sodium carbonate solution, then suspended in 10 ml. of 0.2M sodium barbiturate buffer containing 0.15M sodium chloride, pH 7.0. This mixture was then treated with pronase and chick pancreas acetone powder, a method to measure *in vitro* protein digestibility (8).

Sugars were assayed by the phenol-sulfuric acid procedure (9). Paper chromatograms were run on Whatman No. 1 paper in solvent-1-butanol:pyridine:water (6:4:3) (10) and sugars were detected with

alkaline silver nitrate. For column chromatography, 2 g. of bran material was heated under reflux with 50 ml. of 70% ethanol for 1 hr. After centrifugation, one-half of the supernatant was removed, evaporated to dryness, dissolved in a small amount of water, and chromatographed on Dowex 50 ($K^+$) exactly as previously described (11).

Bran was processed for a feeding experiment as follows. One kg. of bran suspended in 6 liters of water was heated to 95°C., then cooled. One liter of 0.2M sodium acetate buffer (pH 3.8) and 50 g. of Pectinol 41P were added, and the mixture was stirred at 70°F. for 16 hr. The mixture was heated to 95°C., cooled, frozen, and lyophilized. Water was sprayed onto the product to achieve a final moisture level of about 12%, then the product was Wiley-milled (20 mesh). In the "Pectinol control" bran, the same procedure was followed except that the Pectinol 41P was added *after* the 16-hr. stirring, immediately before heating and lyophilization. The "untreated bran" underwent no processing except for the Wiley milling.

The rat feeding experiment was carried out as follows. Young male Sprague-Dawley rats were placed in individual cages and equilibrated for several days on a daily intake of 5 g. of a semi-purified nutritionally adequate diet as described by Rice et al. (12). For a period of 7 days, groups of five rats were each fed daily 2 g. of the various bran samples (moisture-free basis) plus 5 g. basal diet. Thus, a total of 70 g. of each bran sample was fed to five rats. A control group continued to receive the basal diet only (5 g. per rat per day). After completion of the 7-day feeding period, all rats were then continued for a 2-day period on the basal diet only (5 g. per rat per day), in order to eliminate undigested intestinal residues from the body-weight gains. Feces were collected quantitatively from each rat over the entire period of 9 days, and the moisture-free fecal weights were recorded.

## RESULTS AND DISCUSSION

*In vitro* protein-digestibility measurement of the untreated bran used was 69 ±1%. After Wiley milling (20 mesh), this figure increased to 72.0%, and the corresponding *in vivo* figure was 73.0%. When raw wheat-bran was separately pretreated with the three cellulolytic enzymes, this figure increased by only a few percent. However, when the bran was preheated in water to about 95°C. for a few minutes, the cellulolytic treatments caused marked increases in *in vitro* protein digestibility. Presumably the cellulose or hemicellulose (or both) is structurally modified during this wet heating. Table I lists the *in vitro* protein digestibility as a function of cellulolytic-enzyme concentration (Pectinol 41P and Cellulase 36 Concentrate) and time of treatment. The relatively low level of 2.5% added enzyme caused increases in protein digestibility from 69 ±1% to about 87%. More intensive treatment raised the figure to a high of 93.1%, a net improvement of 34.9%. The value of 87% is the same as that obtained *in vivo* by Neudoerffer and Smith (6) on their cellulase-proteinase processed wheat bran. In their case, this represented a net improvement of 45% since their original bran had a protein digestibility of 60%.

Examination of the carbohydrates solubilized by the cellulolytic enzymes showed large additional amounts of glucose and some additional xylose and arabinose present in the hydrolysates after treatment with Cellulase 36 or

TABLE I. IN VITRO PROTEIN DIGESTIBILITY OF WHEAT BRAN AFTER TREATMENT WITH ENZYMES

| Enzyme Pretreatment | Protein Digestibility After[a]: 1 day at 70°F. | 2 days at 70°F. | 1 day at 95°F. | 2 days at 95°F. | 1 day at 104°F. | 2 days at 104°F. |
|---|---|---|---|---|---|---|
| 1% Pectinol 41-P concentrate | 80.6 | 84.9 | 84.0 | ... | 85.5 | 86.7 |
| 2.5% Pectinol 41-P concentrate | 86.2 | 87.1 | 85.7 | ... | 88.1 | 89.3 |
| 5% Pectinol 41-P concentrate | 86.7 | 88.9 | 87.1 | ... | 89.3 | 90.7 |
| 1% Cellulase 36 concentrate | 78.9 | 80.7 | 78.1 | 79.7 | 79.6 | 84.2 |
| 2.5% Cellulase 36 concentrate | 81.9 | 84.0 | 82.8 | 85.2 | 88.1 | 89.9 |
| 5% Cellulase 36 concentrate | 87.1 | 86.4 | 86.9 | 88.2 | 90.7 | 93.1 |

[a]Protein digestibility in untreated bran, 69.0%.

"Onozuka" Cellulase, whereas only additional glucose was found after treatment with Pectinol 41P. Figure 1 shows a quantitative column fractionation of the sugars extracted from an unprocessed control bran and from bran treated with 5% Pectinol 41P. Little change obviously occurred in the disaccharides, trisaccharides, and larger oligosaccharides, but a large increase in glucose is apparent in the processed material. Figure 2 shows the sugars solubilized from bran after a 16-hr. period at 70°F. after addition of 5% "Onozuka" Cellulase or 5% Cellulase 36 Concentrate. By comparison with the control bran (Fig. 1), it is obvious that glucose, xylose, arabinose, fructose, and galactose have been produced during enzymatic hydrolyses. Neudoerffer and Smith (6), in their work, apparently found no pentoses but did note an increase in M. E. ascribed to the hydrolyzed cellulose products.

Microscopic examination of the bran after cellulolytic hydrolysis but before addition of the protease demonstrated the specific substrate under attack. Figure 3 shows the regular aleurone layer in the control experiment. The thick cell-wall honeycomb matrix is clearly visible and intact except where smashed cells are

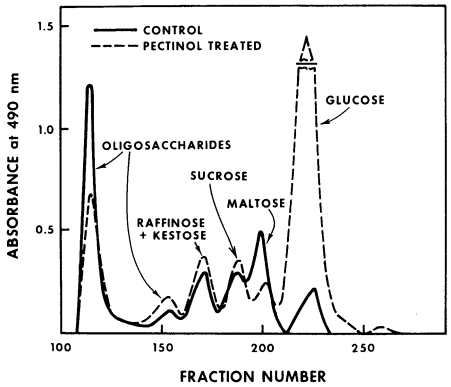



Fig. 1 (left). Sugars in wheat bran before and after processing with Pectinol 41P.

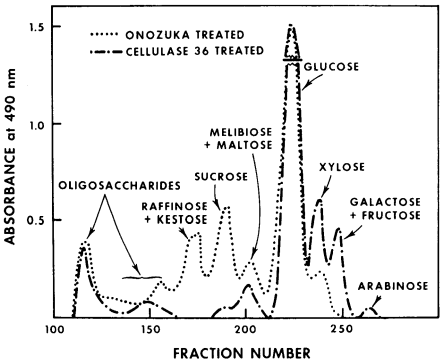
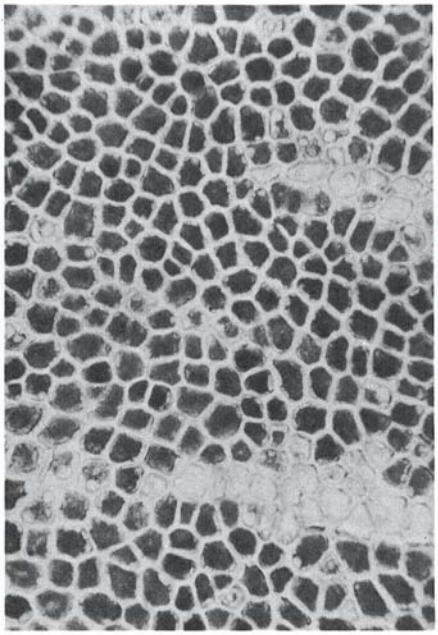
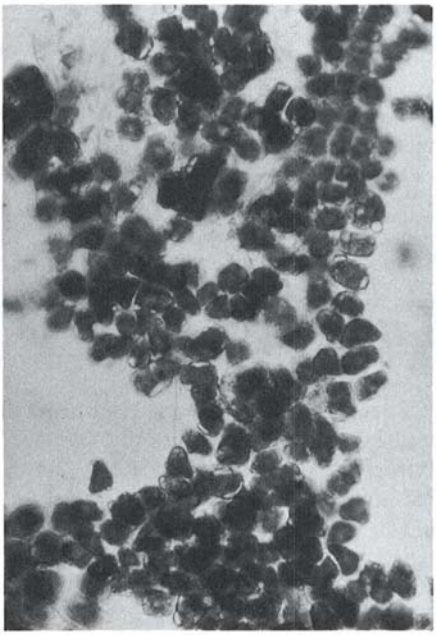



Fig. 2 (right). Sugars in wheat bran after treatment with Cellulase 36 Concentrate or "Onozuka" Cellulase.





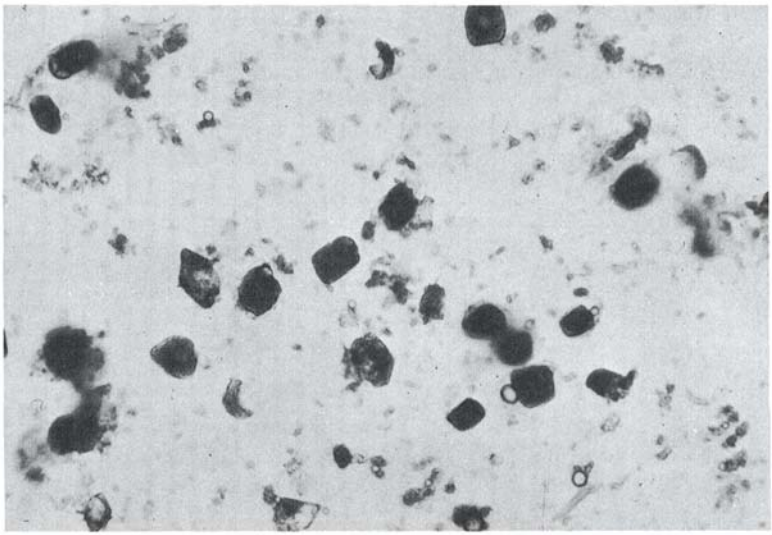


**Fig. 3 (top left). Section of aleurone layer of wheat bran in control experiment.**

**Fig. 4 (top right). Section of aleurone layer of wheat bran after treatment with 5% Pectinol 41P.**

**Fig. 5 (bottom). Single wall-less aleurone cells present in wheat bran after treatment with 5% Pectinol 41P.**

prevalent, certainly as a result of the milling process. Figure 4 shows the aleurone layer after treatment with 5% Pectinol 41P. The cell-wall matrix has undergone severe alteration and has virtually disappeared. Figure 5 shows a different field after the same treatment. Here, the cell wall has disappeared and the aleurone cell "contents" are now freely floating around. Cellulase 36 and "Onozuka" Cellulase showed similar wall disruption, although few single wall-less cells were visible. If a massive amount (50%) of enzyme was used to process the bran, the aleurone cell-wall matrix disappeared entirely but the other cellular layers, the cross-cells and epidermal cells, appeared to have undergone little modification. It has been observed that after bran was fed to calves, the aleurone layer disappeared, but similar branny residues with the appearance of cross-cells or epidermal cells persisted in the feces[2]. The presence of free-floating aleurone-cell "contents" may be due to the fact that enzymes high in polygalacturonase activity, like Pectinol 41P, cause loss of tissue coherence (maceration) and separate cells from each other (13). A large proportion of the cells have retained their characteristic shape, whereas others show varying degrees of damage. Thus, within the aleurone cell wall it is possible that the cell contents are confined in another distinctly contoured membrane.

Bran treated with Pectinol 41P was fed to rats, and compared with untreated bran. The results are shown in Table II. An increase of about 25% has been recorded both in body-weight gain and in bran dry-matter disappearance. Undoubtedly this increase is due to increased protein utilization and utilization of glucose which has previously been unavailable.

It is obvious that such enzymatic treatment of bran can cause nutritive increases for monogastric animals. It is hoped that the apparent increases in protein digestibility and digestible carbohydrates will be large enough to make these processes economically possible.

TABLE II. WEIGHT GAIN BY RATS FED BRAN WITH AND WITHOUT ENZYMATIC PROCESSING, AND DRY-MATTER DIGESTIBILITY OF THE BRANS

| Supplement | Total Weight Gain (5 rats/7 days) g. | Total Weight Change on Basal (5 rats/2 days) g. | Total Corrected Net Weight Gain (5 rats) g. | Net Moisture-Free Fecal Weight (5 rats) g. | Digestibility[a] % |
|---|---|---|---|---|---|
| Basal 5 g./rat/day | −18 (0) | +4 (0) | ... | 3.98 | ... |
| Untreated bran | +46 (+64) | −11 (−15) | +49 | 48.07–3.98 | 37 |
| Pectinol control | +46 (+64) | −7 (−11) | +53 | 44.71–3.98 | 42 |
| Pectinol-treated | +60 (+78) | −9 (−13) | +65 | 37.64–3.98 | 52 |

[a] % Digestibility $= \frac{\text{Feed intake} - \text{fecal weight}}{\text{Feed intake}} \times 100$

[2] Unpublished work.

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