Fluorescence and Light Microscopic Analysis of Digested Oat Bran

S. H. Yiu

R. Mongeau

Utah State University
MERRILL-CAZIER LIBRARY

FLUORESCENCE AND LIGHT MICROSCOPIC ANALYSIS OF DIGESTED OAT BRAN

[*]S.H. Yiu[a] and R. Mongeau[b]

a. Food Research Centre, Agriculture Canada, Ottawa, Ontario K1A 0C6.
b. Bureau of Nutritional Sciences, Food Directorate, Health
and Welfare Canada, Ottawa, Ontario K1A 0L2.

Abstract

The structural and chemical compositions of digested oat bran were analyzed by fluorescence and other types of light microscopy. The digestion of oat bran was carried out under two conditions - in vitro, by incubation with human saliva, and in vivo, by feeding rats with an oat-bran supplemented diet. Comparison of the digestive breakdown among different structural components of oat bran was conducted by microscopically examining samples obtained from the salivary digestion and from the ileum and contents of the large intestine (LIC) of the rats. Results of the examinations revealed that the (1-3)(1-4)-ß-D-glucan-rich cell wall of the sub-aleurone layer, along with its cellular content, were relatively more digestible than the comparable structures of the aleurone layer. Most of the aleurone structural components, including the cell wall and the phytin globoids, as well as the outer fibrous layers of the bran, remained detectable in the ileum samples, indicating that they were poorly digested by the endogenous gastrointestinal enzymes of the rats. Partial to complete degradations of the aleurone cell wall and the phytin globoids were detected in the digested oat bran tissues found in the LIC of the rats. Microscopic examination of the LIC samples also revealed the presence of the rat intestinal microflora, undigested outer tissues of the bran with its detectable phenolic content, and a large amount of crystalline structures that differed morphologically and chemically from oat phytin globoids. Energy dispersive X-ray microanalysis of the LIC samples revealed their elemental composition to be high in both phosphorus and calcium along with traces of magnesium, potassium and iron.

Introduction

Oat bran is a product separated by mechanical processing of dehulled oat grains (groats). Oat bran is rich in cell wall fibers, particularly (1-3)(1-4)-ß-D-glucan (about 8% - Wood, 1986) which appears to play a major role in lowering serum cholesterol levels in humans and rats (Kirby et al., 1981; Chen et al., 1981; Anderson et al., 1983). Oat bran is also rich in minerals (Peterson et al., 1974), most of which occur as subcellular phytin globoids within the aleurone layer (Pomeranz, 1973). However, interactions between phytate and other dietary components, such as cell wall fibers and various minerals may significantly lower the bioavailability of minerals in animals and humans (Taylor, 1965; Morris, 1986). Hence, the role of oat phytin globoids as a source of dietary minerals and the effect of phytate on mineral availability in humans who ingest oat bran for physiological benefit have to be considered.

Past studies on the nutritional aspects of oat bran mainly regarded the bran as a single entity rather than an organized array of chemically and structurally distinct tissues. Like most plant fibers, oat bran fiber is generally believed to be poorly digested by the human digestive enzymes owing to the lack of specific enzymes such as cellulases in their digestive systems. Hence, degradation of the fiber relies mainly on the microbial fermentation that occurs in the large intestine of the human or the rat (including the cecum) (Nyman and Asp, 1986). However, a recent review on oat bran morphology by Fulcher (1986) describes oat bran components as chemically distinct structures, characterizable by their different cell wall fibers. Studies of plant fiber digestion indicated that some cell walls are more readily digested than the others, depending on their chemical and structural compositions (Akin et al., 1984). Direct comparison between the different oat cell wall components and their digestibilities under comparable conditions is lacking. A clear understanding of the relationship between the oat bran structures and their digestibilities would improve the knowledge of the role of oat bran as a source of dietary fibers, leading to better utilization of the oat fibers for maximum physiological benefits.

Insufficient understanding of the nutritional quality of oat bran is partly due to the difficulty of extracting individually pure cell wall fractions for enzymatic digestion and to the inadequacy of effective methods capable of analyzing both chemical and structural compositions of the product at the same time. Two different types of microscopic techniques appear suitable for investigating the digestibility of different oat bran components under comparable conditions. Fluorescence microscopy proved to be effective for simultaneously analyzing food structures and their chemical compositions in various food products (Fulcher, 1982, Yiu et al., 1983; Yiu, 1985, 1986). The technique of energy dispersive x-ray microanalysis coupled with scanning electron microscopy enables concomitant analyses of structures and their elemental compositions (Pomeranz, 1973). Using these two techniques as analytical tools, the present study aimed at following structural and chemical changes of oat bran components subjected to _in vitro_ (human salivary) and _in vivo_ (rat intestinal) enzymatic digestion.

Materials and Methods

Oat Bran

Mothers Oat Bran, Creamy High-Fiber Hot Cereal, was obtained from the Quaker Oats Company, Barrington, IL.

In Vitro Digestion of Oat Bran

In vitro digestion of oat bran by enzymes present in the human saliva was conducted according to the method described by Yiu et al. (1987). Oat bran (6g wet weight), either cooked (6 g bran in 60 ml water) for three minutes or soaked in distilled water for the same period of time, was incubated at 37°C with 2 ml human saliva and 5 ml distilled water inside a dialysis tubing (Spectrapor Memb., 32 mm, molecular weight cutoff: 6,000-8,000) and simultaneously dialysed against 200 ml distilled water with constant stirring. After 3 h, the digested contents were removed and immediately prepared for microscopic examination.

In Vivo Digestion of Oat Bran

Five Sprague-Dawley weanling female rats were fed a diet containing 22.4% casein, 0.3% D-L-methionine, 0.2% choline, 3.9% mineral mixture (AIN mineral mixture 76, ICN Nutr. Biochem. Ltd., Cleveland, OH.), 1.1% vitamin mixture (AIN vitamin mixture 76, ICN Nutr. Biochem. Ltd., Cleveland, OH), 11.1% corn oil, 38.3% corn starch and 22.7% oat bran (containing an equivalent content of 4% dietary fiber). The animals were housed in wire-bottom cages. Distilled water was provided ad libitum. After 27 weeks on the diet (to observe the long-term effect of ingesting oat bran in the rat), the animals were anesthetized and later sacrificed with 2% halothane (Ayerst, Montreal, PQ) in oxygen. The whole gastrointestine (GI) was removed and placed in ice-cold Ringer solution. The cecum was separated from the small intestine. The digesta from the distal portion of the ileum (0-6 cm from the cecum) was collected in a plastic scintillation vial, freeze-dried and stored at -70°C until microscopic examination. Pellets, or LIC, from the

distal portion of the large intestine were collected and stored as for the ileum digesta.

Light Microscopy

All samples were first encapsulated in molten 2% agar in a petri dish according to a previously described method by Yiu et al. (1983), and were cut into 1-2 mm blocks after the agar was set firm. They were then fixed in 3% glutaraldehyde (in 0.01M phosphate buffer, pH 7.0) for 24 h according to the method described by Yiu (1986). Fixed samples were dehydrated through methyl cellosolve, ethanol, n-propanol, and r-butanol followed by infiltration with glycol methacrylate monomer for 3-5 days at room temperature prior to polymerisation at 55°C in gelatine capsules. Sections were cut 2 μm thick using an ultramicrotome (Sorvall Inc., Newtown, CT) equipped with a glass knife. All sections were affixed to glass slides for subsequent staining and microscopic examination.

Depending on the histochemical content of the sample, each section was stained with one of the following reagents according to the methods previously described (Yiu, 1986; Yiu et al., 1987): (1) 0.1% (w/v) aqueous Acridine Orange (BDH Chem. Ltd., Poole, England) for 1-2 min. (2) 0.1% (w/v) aqueous Acriflavine HCl (BDH Chem. Ltd., Poole, England) for 1-2 min, (3) 0.1% (w/v) Cellufluor (Polysciences, Inc., Warrington, PA) in 50% ethanol for 1 min, (4) 0.01% (w/v) aqueous Congo Red (Fisher Scientific Co., Fair Lawn, NJ) for 1-2 min, (5) 0.5% iodine (w/v) in 5% aqueous potassium iodide solution (IKI) for 2 min, or (6) 0.05% (w/v) Toluidine Blue for 1-2 min. All stained sections were rinsed in distilled water, air-dried, mounted in immersion oil, and examined by fluorescence, bright-field, or polarizing optics using a Zeiss Universal Research Photomicroscope (Carl Zeiss Ltd., Montreal, PQ.). The microscope was equipped with both a conventional bright-field illuminating system and a III RS epi-illuminating condenser combined with an HBO 100 W mercury-arc burner for fluorescence analysis. The III RS condenser contained three fluorescence filter systems with a dichromatic beam splitter and an exciter/barrier filter set for maximum transmission at 365 nm/418 nm (FC I), 450-490 nm/>520 nm (FC II) and 546 nm/>590 nm (FC III). Micrographs were recorded on 35 mm Ektachrome 400 Daylight film.

Energy Dispersive X-Ray Microanalysis

Unfixed samples of oat bran, the feed, and contents of the large intestine of two rats were mounted on carbon holders with silver paste. Three specimens were prepared for the examination of each of the above samples. The specimens were coated with carbon using a Speedivac Coating Unit (Model 12E6/1258, Edwards High Vacuum, Oakville, Ont.) and examined on an ISI-DS130 scanning electron microscope (Rayonics Inc. Downsville, Ont.). The microscope was equipped with an energy dispersive X-ray detector and analyzer (TN-5500, Tracor Northern Canada, Rexdale, Ont.), with the detector set at 45° angle to the specimen stage and a working distance of 35 mm. The microscope was operated at an accelerating voltage of 20 keV, no tilt, 5×10^{-9} A probe current and a probe size of 180 nm in diameter. X-ray

spectra were collected for 100 s at each of five selected sites per specimen, and their mineral element contents were qualitatively analyzed.

Quantitative Elemental Analysis

The LIC samples from the two rats were analyzed by Atomic Absorption Spectrophotometry (Model 975, Varian Canada Inc., Ottawa, Ont.). The analysis was conducted at the Land Resources Research Centre, Agriculture Canada, Ottawa, Ont.

Results and Discussion

Structural and Chemical Compositions of Oat Bran

Most of the oat bran structural components and some of their chemical contents can be revealed simultaneously by simple fluorescence microscopic techniques. For example, using Congo Red as a staining reagent for revealing cell wall structures and the FC I filter system for short-wavelength excitation and subsequent fluorescence analysis, it was possible to detect autofluorescence emitted by the outer layers, which include the outermost pericarp, testa and the nucellus, (bluish white fluorescence), the aleurone and part of the sub-aleurone cell walls (blue fluorescence), indicating the presence of phenolic compounds (Fig. 1). Although the identity of these compounds is not fully established, ferulic acid is believed to be one of the major phenolic compounds present on the oat cell walls (Collins, 1986; Fulcher, 1986). The above examination also revealed the concentrated location of $(1-3)(1-4)$-β-D-glucan (β-glucan) (red fluorescence), on the sub-aleurone cell wall (Fig. 1). In addition, the aleurone protein bodies embedded with visible globoids and the sub-aleurone reserves of protein and starch were also revealed in the same section.

Digestion of Oat Bran by Human Salivary Enzymes

Both uncooked and cooked oat bran samples were incubated with human saliva and the effect of cooking on the amylolytic digestion of some of the oat bran components was determined by the following microscopic analysis. Sections from the digested and undigested samples were stained with Cellufluor to detect changes in the cell wall structures. Similar to what was shown previously in rolled oats (Yiu, 1986), cooking induced more cell wall breakdown in the sub-aleurone layer, resulting in a decrease of fluorescence intensity in the cellufluor-bound cell walls. The decrease was probably due to the release of β-glucan from the cell wall into the cooking medium (Yiu et al., 1987). However, the diminished fluorescence intensity was not attenuated after the incubation with saliva (results not shown). The result was not unexpected, since enzymes capable of digesting the β-glucan are absent in human saliva. However, more cell wall breakdown in the sub-aleurone layer would lead to more release of its cellular content, thereby increasing the surface area for other enzymatic digestion, such as starch hydrolysis by amylases which are abundant in human saliva. Hence, the most noticeable difference of the oat bran components after the salivary incubation was the structural changes of the starch granules. After

the incubation, the cooked oat bran had virtually no detectable starch granule structures, whereas the uncooked samples contained many partially digested starch granules (Fig. 2). This finding, which is in agreement with previous reports (Snow and O'Dea, 1981; Yiu et al., 1987), confirms that uncooked oat starch can be digested by human salivary amylases to a certain extent and that cooking greatly enhances its digestibility. Cooking also induced some release of the aleurone cell contents, but much less than that of the sub-aleurone layer, owing to the relatively sturdy aleurone cell wall. This was demonstrated by a decrease in the amount of phytin globoids, the structure of which can be detected by staining with Acriflavine HCl (Fig. 3) and by viewing under polarized light. However, whether the decrease was due to a loss of the cell contents going into the incubation medium or was caused by enzymatic breakdown remained uncertain. In view of the fact that phytase has never been detected in human saliva, the degradation of oat phytin globoids is expected to be low.

Digestion of Oat Bran by Rat Intestinal Enzymes

Rats fed with an oat-bran supplemented diet were used as experimental models to investigate the digestive breakdown of various oat bran cell wall fibers and the phytin globoids in the gastrointestinal tract.

Digestion of oat bran in the rat ileum

Microscopic examination of the rat ileal contents (the digesta) revealed that most of the sub-aleurone cell wall and its cellular components were partially or completely degraded (Fig. 4) whereas the aleurone cell wall was relatively intact and many phytin globoids remained detectable (Fig. 5). This finding indicates that the former tissue is more susceptible to the environment of the rat digestive system, and it further confirms that the two cell walls are structurally and chemically different from each other. Aside from the host's digestive enzymes, microbial enzymes may also play a role in degrading the oat bran components, even though the number of microflora present in the ileum is relatively small (Borriello, 1986). The manifestation of degradation by the microflora is demonstrated in Fig. 6 which shows penetration of microorganisms through an opening on the aleurone cell wall into the interior of the cell. The opening on the wall was probably caused by mechanical rupture induced during processing or chewing.

Digestion of oat bran in the rat large intestine

Examinations of the contents of the large intestine revealed a different pattern of oat bran digestion. Unlike those present in the salivary-digested samples and in the ileum digesta, most aleurone cell walls were degraded while there was no trace of any detectable sub-aleurone cell wall structure (Fig. 7). Residual bran materials were mostly components of the outer fibrous tissues, including the trichomes (the oat hairs) (Figs. 8a and 8b). The low digestibility of these tissues probably resulted from their chemical composition which includes lignin, cutin and phenolic acids (Fulcher, 1986).

The microbial population present in the large intestine of the rat was undoubtedly one of the major factors for degrading the aleurone and sub-aleurone cell wall fibers. In fact, close contact between the microflora and the cell wall materials was observed in the LIC samples (Figs. 8a and 8b). Although not identified individually, the shapes of these microorganisms, mostly spherical or elongated rods of various sizes and lengths, were revealed by the present microscopic examination (Fig. 9a). Previous studies on the microflora of the large GI tract suggest that they are mostly streptococci, bacteroides, and lactobacilli, all of which can produce enzymes capable of digesting cereals including fibers and phytin (Hill, 1986; Nayini and Markakis, 1986). Furthermore, it appears that the bran materials tended to attract certain types of microorganisms (Fig. 9b) to their vicinity, but more studies would be required in order to confirm this observation.

Many of the phytin globoids remained detectable, after degradation, by staining with Acriflavine HCl (Fig. 10a), or by viewing the same sample under polarized light. The polarizing-microscopic examination also revealed the presence of other crystalline compounds that were structurally different from the phytin globoids and reacted differently to the staining reagent, Toluidine Blue (Fig. 10b). Similar crystalline structures (in much smaller numbers) were detected in the feed sample, and they were presumably the added mineral salts that constituted part of the rat diet. The above speculation was supported by results obtained from energy dispersive X-ray microanalysis of the feed, oat bran and the LIC samples. The major elements that were detected in the feed included sulfur, phosphorus, magnesium and calcium (Fig. 11a) while those detected in oat bran were phosphorus, potassium, sulfur, chlorine and silicon (Fig. 11b). In comparison with the above samples, the LIC contained predominantly phosphorus and calcium, in addition to magnesium, sulfur, potassium, chlorine and a trace of iron (Fig. 11c). These findings were confirmed by data obtained from the atomic absorption analysis which quantitatively estimated the concentrations of some of the above elements in the feed and in the LIC samples of both rats. All four major minerals (Ca, Mg, P, and Fe) were at least ten times more concentrated in the metabolic waste products of the rats than those in the feed (Table 1). Similar findings

TABLE 1

Concentration of Selected Elements in
Rat Feed and Contents of the Large Intestine

Sample	% Ca	% Mg	% P	% K	μg/g Fe
A	6.39	0.53	6.39	0.66	906
B	6.31	0.58	6.88	0.59	752
C	0.51	0.06	0.80	0.52	60

A = LIC from rat 1
B = LIC from rat 2
C = feed for both rats
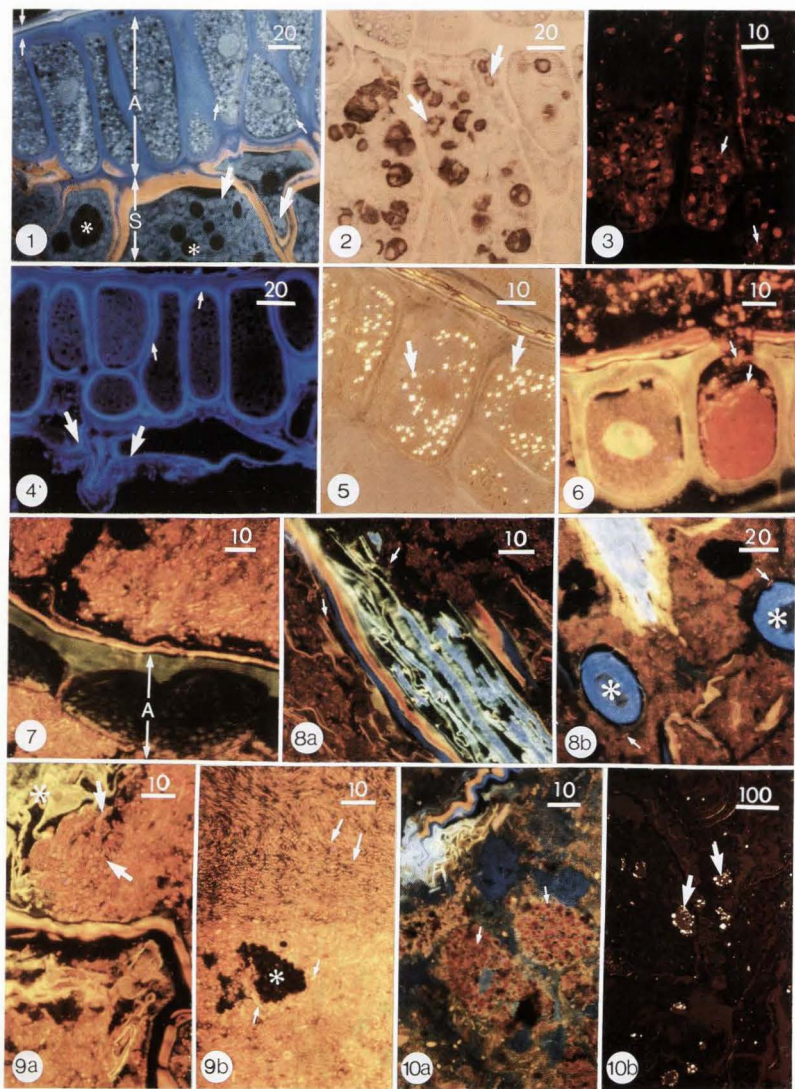
were reported in an unrelated study which showed that the levels of several mineral elements, including Ca and Fe, were higher in the fecal matter than in the diet enriched with corn bran (Dintzis et al., 1985). The present result indicates that mineral elements present in the diet were not completely absorbed by the rats. Although it is known that cereal phytate can chelate dietary calcium to form insoluble calcium phosphate complexes (Taylor, 1965), there is no direct evidence in the present study to suggest that the detected crystalline structures may contain the above complexes. However, more investigations are currently being conducted in order to determine the chemical composition of these structures and the effect of low and high levels of dietary cereal fiber on mineral absorption in rats.
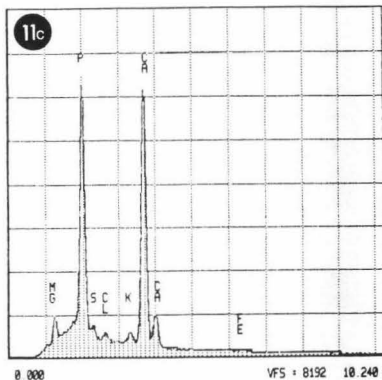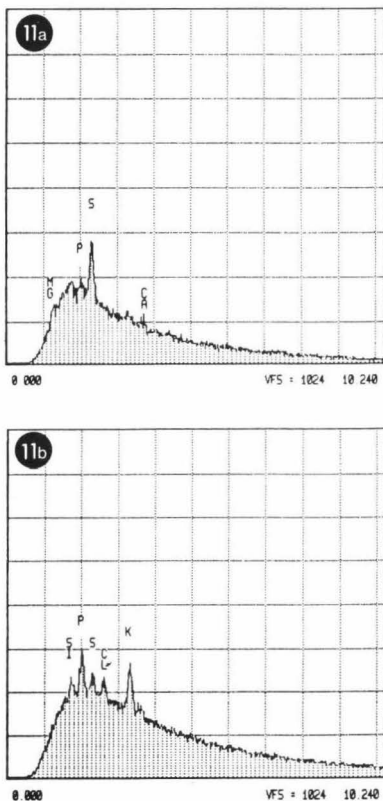
Fig. 11. Energy dispersive X-ray analysis spectra from (a) the feed, (b) oat bran, and (c) contents of the large intestine of one rat.

Conclusion

The present study demonstrates that the digestive breakdown of various oat bran components differs, depending on the structural and chemical compositions of the component and its location in the gastrointestinal system. The sub-aleurone layer, which had high polysaccharide concentrations mainly in the forms of starch and β-glucan, was most susceptible to the host's digestive enzymes in the saliva (for the starch hydrolysis) and in the proximal intestinal tract (for the β-glucan-rich cell wall degradation). Degradations of most of the aleurone cell wall and some of the phytin globoids occurred chiefly in the lower intestine where the microflora played a significant role in degrading the above materials. In contrast with the aleurone and sub-aleurone layers, the outer bran tissues of oats (including the trichomes and the pericarp) were mostly undigested by the animals. In addition, the present study also revealed that contents of the large intestine of the rats contained numerous insoluble crystalline structures and had high mineral concentrations, particularly phosphorus and calcium. The findings suggest that the mineral supplement that was incorporated in the diet was not completely absorbed by the rats.

Acknowledgements

the staffs, particularly Mr. Ernie Bond, of the Electron Microscopy Unit, Plant Research Centre, Agriculture Canada, for assisting the EDX-microanalysis. This publication is contribution 731 from the Food Research Centre, Agriculture Canada, and contribution 255 from the Nutrition Research Division, Health and Welfare Canada.

References

Akin DE, Rigsby LL, Brown RH. (1984). Ultrastructure of cell wall degradation in Panicum species differing in digestibility. Crop Sci. 24:156-163.

Anderson JW, Chen WJL, Story L, Story J. (1983). Hypocholesterolemic effects of soluble-fiber rich foods for hypercholesterolemic men. Clin. Res. 31:613A.

Borriello SP. (1986). Microbial flora of the gastrointestinal tract. In: Microbial Metabolism In The Digestive Tract. Hill MJ (ed.), CRC Press, Inc., Boca Raton, FL. P.2-20.

Chen WJL, Anderson JW, Gould MR. (1981). Effects of oat bran, oat gum and pectin on lipid metabolism of cholesterol-fed rats. Nutr. Rep. Int. 24:1093-1098.

Collins FW. (1986). Oat Phenolics: structure, occurrence, and function. In: Oats: Chemistry and Technology. Webster, FH (ed.), American Association of Cereal Chemists, Inc., St. Paul, MN. P.227-296.

Dintzis FR, Watson PR, Sandstead HH. (1985). Mineral contents of brans passed through the human GI tract. Am. J. Clin. Nutr. 41:901-908.

Fulcher RG. (1982). Fluorescence microscopy of cereals. Food Microstruc. 1:167-175.

Fulcher RG. (1986). Morphological and chemical organization of the oat kernel. In: Oats: Chemistry and Technology. Webster FH (ed.), American Association of Cereal Chemists, Inc., St. Paul, MN. P.47-74.

Hill MJ. (1986). Metabolism of carbohydrates and glycosides. In: Microbial Metabolism in the Digestive Tract. Hill MJ (ed.), CRC Press, Boca Raton, FL. P.31-42.

Kirby RW, Anderson JW, Sieling B, Rees DE, Chen WJL, Miller RE, Kay RM. (1981). Oat bran intake selectively lowers serum low-density lipoprotein cholesterol concentrations of hypercholesterolemic men. Amer. J. Clin. Nutr. 34:824-829.

Morris ER. (1986). Phytate and dietary mineral bioavailability. In: Phytic Acid: Chemistry & Applications. Graf E (ed.), Pilatus Press, Minneapolis, MN. P.57-76.

Nayini NR, Markakis P. (1986). Phytases. In: Phytic Acid: Chemistry & Applications. Graf E (ed.), Pilatus Press, Minneapolis, MN. P.101-118.

Nyman M, Asp N-G. (1986). Fermentation of dietary fibre in the intestinal tract: comparison between man and rat. British J. Nutr. 55:487-496.

Peterson DM, Schrader LE, Youngs VL. (1974). Elemental composition of developing oat plants. Crop Sci. 14:735-739.

Pomeranz Y. (1973). Structure and mineral composition of cereal aleurone cells as shown by scanning electron microscopy. Cereal Chem. 50:504-511.

Snow P, O'Dea K. (1981). Factors affecting the rate of hydrolysis of starch in food. Am. J. Clin. Nutr. 34:2721-2727.

Taylor TG. (1965). The availability of the calcium and phosphorus of plant materials for animals. Proc. Nutr. Soc. 24:105-112.

Wood PJ. (1986). Oat β-glucan: structure, location, and properties. In: Oats: Chemistry and Technology. Webster FH (ed.), American Association of Cereal Chemistry, Inc., St. Paul, MN. P.121-152.

Yiu SH, Altosaar I, Fulcher RG. (1983). The effects of commercial processing on the structure and microchemical organization of rapeseed. Food Microstruc. 2:165-173.

Yiu SH. (1985). A fluorescence microscopic study of cheese. Food Microstruc. 4:99-106.

Yiu SH.(1986). Effects of processing and cooking on the structural and microchemical composition of oats. Food Microstruc. 5:219-225.

Yiu SH, Wood PJ, Weisz J. (1987). Effects of cooking on starch and β-glucan of rolled oats. Cereal Chem. In press.

Discussion with Reviewers

F.R. Dintzis: It would be helpful for the authors to comment about the 22.7 % oat bran content in the diet, especially since I, and probably most readers, do not know if this is a high dose of bran. Was the transit time for the rats about normal once they adjusted to the diet? I ask this because the fermentation of oat bran in the gut should be dependent on transit times and the reader should know if physiological status of the rats was about normal.

Authors: Oat bran contains about 17% dietary fiber (Mongeau R, Brassard R. 1986. J. Food Sci. 51:1333-1336.), and its presence in the feed (22.7%) amounts to a total concentration of approximately 4% fiber. This is not a high dose of bran. The intestinal transit time for the animals averaged about 13-15 h, not significantly different from that of rats fed with a diet containing rat chow.

F.R. Dintzis: Could the authors give the reader some estimate of the weight percent of 'oat bran' recovered from LIC?

Authors: About 20.9% ±1.7% of the ingested oat fiber was recovered from LIC of the five rats.

F.R. Dintzis: The peaks in Figs. 11a and 11b are relatively convincing. The detectability of Mg and Ca in Fig. 11c seems questionable. I wonder if it would be appropriate to include a blank for a control, i.e., just the EDX spectra taken from a silver paste coated carbon stub?

Authors: Both Mg and Ca peaks consistently appeared in the X-ray spectra of the LIC samples, but were absent from the spectra taken from a carbon coated specimen stub. The X-ray spectra of the background are not shown because there was no evidence of any spectral interferences.

D.J. Gallant: The peak/background ratio (Fig. 11c) seems very high, comparing to Figs. 11a and 11b, and is more comparable to a bran (or aleurone layer) spectrum than a spectrum from organic-rich material. Generally, the spectrum of bran is about ten times richer in K and P (and in Mg) than the other elements, but it does not appear so in Fig. 11b. Could you explain that? What was the surface topography of your sample during the analysis?

Authors: X-ray spectra taken from protein bodies of the aleurone layer of freeze-fractured oat grains contained the 3 major peaks (at a ratio of P:K:Mg/4:3:1, results are not presented in this paper), and all of them had high peak/background ratios, similar to what you suggested. However, similar spectral profiles were not recorded on samples taken from the feed or processed oat bran due to the presence of starch granules which greatly masked details of the bran structures. No attempt was made to extract the bran from the samples for the sake of preserving as much chemical and structural contents as possible. The background counts shown in Figs. 11a, 11b and 11c are similar but are presented at different scales. The low peak/background ratios shown in Figs. 11a and 11b were probably due to the presence of elements at low concentrations.

F.R. Dintzis: I find it most interesting that the phytin globoids are still detectable after passage through the small intestine where most mineral absorption is thought to occur. I do hope the authors are obtaining some measure of recoverable globoid content in digesta retrieved from the ileum and perhaps from the colon.

Authors: We did not try extracting undigested globoids from the digesta but we did measure the phytate content in LIC using the AOAC method. About 60% of the ingested phytate was recovered in the LIC of the rats.

L.U. Thompson: What happened to the phytic acid which no longer is detectable in the bran?

Authors: Phytic acid may form soluble or insoluble complexes with minerals. Alternatively, it may be degraded by enzymes present in the intestine.

L.U. Thompson: The presence of crystalline salts in the LIC suggests that the minerals which were added to the diet were not completely solubilized in the gastrointestinal tract. To confirm this, did you try testing in vitro the solubility of the mineral mix under the pH conditions of the GI tract?

Authors: No, we did not test the solubility of the mineral mix under the pH conditions (around pH 6.8-7) of the GI tract. Since its presence was detected in samples which had been fixed in glutaraldehyde at pH 6.8-7, we assumed that its solubility was low.

L.U. Thompson: What was the [phytic acid] [calcium] / [zinc] molar ratio in the diet? Would you expect the results to be different if the calcium content was more or less?

Authors: The molar ratio was [5.97] [150] / [0.84] = 1066. The high ratio may affect the zinc absorption. The apparent absorption of Zn in the five rats was -5.1%. Different results could be expected if the calcium content was changed; the higher the value of the ratio, the lower the absorption of Zn.

L.U. Thompson: Zinc, which is known to tightly bind to phytic acid, was not detected in the LIC or ileum contents. Does it mean than zinc was not preferentially bound by phytic acid in oat bran, or was the zinc concentration just below the sensitivity of the methods used for measurement?

Authors: Atomic absorption spectroscopy revealed that the zinc content present in the fecal matters of the five rats was 879 ±56 μg/g fecal dry weight. It is possible to detect very low levels (<0.1%) of zinc by x-ray microanalysis (Chandler et al. 1977. Histochem. J. 9:103) provided that ideal operating conditions, such as higher accelerating voltage (>20 keV), thin specimen, and longer time of analysis (>100 s), are met. The present study focussed on detecting the presence of lighter elements, such as P, K, and Ca. No attempt was made to detect Zn either microscopically or via X-ray microanalysis.

L.U. Thompson: Can you conclude from your data that phytic acid does not affect the fiber breakdown in the GI tract?

Authors: About 60% of the ingested phytate and <20% of the original oat fiber were recovered from the LIC samples (measured by the AOAC methods). Neither the above result nor data presented in this study can conclude the relationship between phytic acid and fiber degradation.