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Effect of Dietary Fibers on the Enzymatic Digestion of Casein

P. Howard & R. R. Mahoney*

Department of Food Science and Nutrition, Massachusetts Agricultural Experiment Station, University of Massachusetts, Amherst, Massachusetts 01003, USA

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ABSTRACT

Pectin (P), guar gum (G) and wheat bran (WB) all caused small reductions ($\leq 15\%$) in the digestion of casein by trypsin and chymotrypsin. Lignin (L) and cocoa fiber caused reductions of more than 30% for both enzymes. Lower shaker speeds during digestion further reduced activity of chymotrypsin for P and G but had no effect for WB and L. Casein digestion, as measured by a multi-enzyme assay, was reduced by 5–10% when each fiber was used at a level of 1%; cocoa fiber and lignin had the most effect. The results support the concept that dietary fibers contribute to lower protein utilization in animals by decreasing intestinal proteolysis.

INTRODUCTION

Dietary fiber causes decreased utilization of several nutrients including protein (Southgate & Durnin, 1970; Walker, 1975).

In a study with growing rats, Shah *et al.* (1982) showed that pectin, guar gum and lignin (dietary fiber components) and wheat bran (a complex source of dietary fiber) all decreased protein utilization, decreased true and apparent protein digestibility and increased fecal excretion of both dietary and endogenous nitrogen.

These results could be explained by impaired proteolytic digestion due to changes in either the level or specific activity of digestive proteases. However, a follow-up study using the same fibers (Shah *et al.*, 1985) showed

* To whom correspondence should be addressed.

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that pectin, guar gum and lignin did not reduce the levels of proteases but tended, instead, to increase them. Other studies (Schneeman, 1982) have led to similar results and to the inference that these fibers may affect proteolysis.

The difficulties of measuring proteolytic digestion *in vivo* have led to several in-vitro studies on the interactions of fibers and digestive enzymes (Schneeman, 1978; Harmuth-Hoene & Schwerdtfeger, 1979; Dunaif & Schneeman, 1981; Acton *et al.*, 1982, Isakkson *et al.*, 1982; Gagne & Acton, 1983). However, the results are highly variable, due perhaps to differences in fiber characteristics and also to assay methodology. As a consequence, it is difficult to draw conclusions which may explain the results from animal studies. In this work we have used the same fibers as in the animal studies of Shah *et al.* (1982, 1985) and determined their effect on casein digestion, in-vitro, using both single and multi-enzyme procedures. We have also examined the effect of a food fiber residue (cocoa) on proteolysis to see if the results were consistent with those obtained with the analogous isolated fiber component (lignin).

MATERIALS AND METHODS

Materials

The principal dietary fibers used were pectin, guar gum, lignin and wheat bran; their sources, composition and characteristics have been previously described (Shah *et al.*, 1982). Trypsin (2916 NF units/mg) and chymotrypsin (1170 USP units/mg) used in single enzyme assays were from Calbiochem-Behring (La Jolla, CA). Pepsin, pancreatin (grade VI), amyloglucosidase, 2,4,6-trinitrobenzene sulfonic acid (TNBS), sodium azide and enzymes used in the multi-enzyme assay (trypsin, chymotrypsin, peptidase and protease from *Streptomyces griseus*) were all from Sigma (St Louis, MO). ANRC sodium caseinate was a gift from Dr L. Satterlee (Pennsylvania State University). Cocoa powder (Hershey unsweetened) was purchased from a local store.

Methods

Cocoa powder was defatted in a Soxhlet apparatus using petroleum ether as a solvent and then dried under vacuum at 55°C for 4 days. Soluble and insoluble fiber fractions were prepared from the powder by the enzymatic digestion procedure of Schweizer & Weursch (1979). The fractions were boiled for 30 min to eliminate residual protease activity derived from the isolation method, dried at 55°C under vacuum and then combined.

Single enzyme assays

The control substrate solution was 9 ml of 0·5% sodium caseinate in 0·2M sodium phosphate buffer, pH 8·0. Fibers were added to the caseinate solution at levels of 1–2% and allowed to hydrate overnight at 2°C.

Casein digestion was initiated by adding 0·1 ml enzyme (3 μg trypsin/ml or 3 μg chymotrypsin/ml) to 9 ml substrate solution at 37°C. The digestion mixture was then shaken (60 oscillations per min) for 15 min and the reaction stopped by adding 3 ml 20% trichloroacetic acid. The digests were then diluted with 2 volumes of water (95% ethanol was used for digests containing pectin and guar gum), centrifuged at 10 000 × g for 15 min and the supernatants analyzed for free amino groups by the TNBS method of Fields (1971). Controls in which the enzyme or fiber was omitted were also analyzed. The effect of fiber on digestion was expressed as the percentage change in free amino groups liberated, compared to the fiber-free control.

Multi-enzyme digestions

Casein digestion was measured via the pH drop after multi-enzyme digestion by trypsin, chymotrypsin, peptidase and bacterial protease, as described by Satterlee *et al.* (1979). Fiber components and wheat bran were hydrated overnight at 2°C in 30 ml water containing 0·7% caseinate. Substrate solutions were equilibrated at pH 8·0 and at 37°C prior to digestion with a mixture of proteases. Per cent digestibility of the caseinate was determined from the pH drop via a regression equation (Satterlee *et al.*, 1979). Controls were run in which fiber or enzymes were omitted from the reaction mixture.

RESULTS AND DISCUSSION

The effect of fiber components, wheat bran and cocoa fiber on digestion of casein by trypsin and chymotrypsin is shown in Table 1. Wheat bran, pectin and guar gum caused only small decreases ($\leq$15%) in protease activities. However, lignin caused a decrease of 73% and 48% in the activity of trypsin and chymotrypsin, respectively. Cocoa fiber, which contains 64% lignin (Southgate *et al.*, 1976) also caused a substantial decrease in activity of both enzymes. There has always been some concern that purified, isolated lignin might not behave like food lignin because of the vigorous treatment used in its preparation. The fact that cocoa fiber had a similar effect to purified lignin dispels some of this concern and supports the use of isolated lignin in fiber studies.

TABLE 1
Effect of Fibers on the Activity of Trypsin and Chymotrypsin on Casein

Fiber (level)	*Decrease in activity (%)*[a]	
	Trypsin	*Chymotrypsin*
Pectin (1%)	8·0	15
Guar gum (1%)	9·0	13
Lignin (2%)	48	73
Wheat bran (2%)	11	8
Cocoa fiber (1%)	33	30

[a] Compared to a control which contained no fiber; average of three determinations.

The results for pectin, guar gum and wheat bran differ somewhat from earlier studies which indicate either a larger reduction in trypsin activity, e.g. 24% for guar gum (Harmuth-Hoene & Schwerdtfeger, 1979); 40–85% for pectin, guar gum, and lignin (Isaksson *et al.*, 1982) or essentially no reduction (Dunaif & Schneeman, 1981). This may reflect differences in the experimental conditions and design, e.g. different fiber concentrations or the use of synthetic substrates to measure activity.

We observed that small changes in the speed at which digests were shaken (60 oscillations/min) had no effect on casein digestion in the presence of wheat bran and lignin; however, large differences were observed when guar gum and pectin were present. When the digests were shaken at only 30 oscillations per minute, the decrease in activity of chymotrypsin on casein was 37% for pectin and 34% for guar gum, i.e., the decrease in activity more than doubled. These findings support the concept that soluble fibers such as pectin and guar gum inhibit proteolysis by forming viscous solutions which limit enzyme and/or substrate diffusion (Arnal-Peyrot & Adrian, 1974). Diffusion in the gut due to peristaltic movement could well be lower than that which occurred *in vitro*. It is therefore possible that the results reported here for pectin and guar gum underestimate the in-vivo effect. Diffusional limitations could also explain why we have observed lower digestibility with casein than studies which used much smaller synthetic substrates (Dunaif & Schneeman, 1981).

In contrast to the above, the absence of a shaking effect with lignin digests implies that the decrease in proteolysis with this fiber is more likely to be due to chemical interaction with enzyme or substrate than due to some physical restriction.

The effect of fibers on casein digestion using the multi-enzyme technique is

TABLE 2
Reduction in In-vitro Digestibility of Casein in the Presence of Fibers[a]

Fiber	*Fiber level*		
	0·5%	*1·0%*	*2·0%*
Pectin	4·8	6·5	10·5
Guar gum	2·2	7·7	nd
Lignin	6·1	9·8	12·8
Wheat bran	3·5	5·3	7·5
Cocoa fiber	4·8	8·4	14·0

[a] Values listed are averages of three determinations.
nd, not determined due to high viscosity.

shown in Table 2. All the fibers tested caused some discernible reduction in digestion. The greatest effects were again observed in the presence of lignin and cocoa fiber. However, it was not greatly different from that of the other fibers.

The values for decrease in digestibility of caseinate in the presence of pectin, lignin and wheat bran are generally somewhat higher than values of 2–6% previously reported (Acton *et al.*, 1982; Gagne & Acton, 1983). This may reflect the higher fiber: protein ratios used in this study, ranging from 0·71 (0·5% fiber) to 2·8 (2·0% fiber).

The results for pectin, guar gum and wheat bran are generally consistent with those in the single enzyme assays. However, the decrease in digestibility with lignin and cocoa fiber is lower than would be expected given the large decreases in trypsin and chymotrypsin activity. The reasons for this are not clear but may reflect the additional action of peptidase and bacterial protease in this system.

Taken together the results of this study indicate that the dietary fiber components and wheat bran used in in-vivo studies do cause a reduction in casein digestion. The reduction observed *in vitro* is not large but may underestimate the in-vivo effect because of the difficulties in mimicking the conditions in the intestine, especially the effect of peristaltic motion. The reduction in true nitrogen digestibility determined with these fibers (Shah *et al.*, 1982) is similar to, or even lower than that found in the multi-enzyme assay. However, this may simply illustrate the fact that *in vivo* the animal can secrete more enzyme to compensate for reduced activity (Shah *et al.*, 1985, 1987) and thereby reduce the net observed effect. The means by which fibers reduce enzyme activity is still not clear. For viscous polysaccharides it may be by diffusional limitations. However, for lignin some direct chemical effect seems more likely.

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