Dietary fibers: their effects on intestinal digestive enzyme activities

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Three plant foods (cabbage, guava, and mucilaginous isabgol husk) were fed to rats as the source of dietary fiber to study their effect on the activities of small intestinal disaccharidases (sucrase, maltase, and lactase) and alkaline phosphatase. Inclusion of cabbage and guava (at 5 and 10 g/100 g level) and isabgol (1 and 2 g/100 g) in the diets adversely affected the activities of sucrase, maltase, and alkaline phosphatase, which further decreased significantly ($P < 0.05$) with an increase in level of dietary fiber from cabbage and guava. (J. Nutr. Biochem. 5:176–180, 1994.)

Keywords: diet; dietary fiber; disaccharidases; alkaline phosphatase

Introduction

During the process of digestion and absorption, enzymes and substrates interact and the end products of digestion diffuse to the mucosal surface for final hydrolysis and absorption. Dietary fiber, due to its bulk forming effect, could dilute enzymes and absorbable compounds in the gut. Different dietary fiber components have individual and specific effects on jejunal disaccharidase morphology.[1] Addition of mucilaginous substances like pectin and galactomannins in the diet has also been reported to influence disaccharidase levels in the rat jejunum.[2] It can be speculated that because of their gel-forming and absorbent properties these dietary fibers may interfere with bile and pancreatic secretions. Guar gum lowers the lactase and alkaline phosphatase activities significantly,[3] which may be due to a delay in gastric emptying and a direct effect on interaction of digestive enzymes and their substrates in the intestine.[4] Most of the studies on the effect of dietary fiber on enzymes have been conducted on individual components of dietary fiber. But the information on how the whole dietary fiber from different foods affects the different enzyme system is scanty. Therefore, an attempt has been made in the present investigation to study the effect of plant fibers on disaccharidases and alkaline phosphatase in the small intestinal mucosa of rats.

Methods and materials

Fiber sources

Isabgol *(Plantago evata)* and fresh cabbage *(Brassica oleracea)* were procured from a local market and fresh guava fruit *(Psidium guajava)* from the Horticulture Farm of Haryana Agricultural University, Hisar, India.

Diet preparation

All diets were isoproteinous (10%) and prepared according to AIN 76 "Purified Diets for Rats".[5] The dietary fiber content of diets was selected at two levels (5 and 10 g/100 g from cabbage and guava, and 1 and 2 g/100 g of isabgol, a mucilaginous polysaccharide). The fiber-free diet served as a control. Dietary fiber composition and protein content of cabbage and guava (dried and well ground) were estimated before preparing diets. Dietary fiber composition was determined as hemicellulose, cellulose, and lignin by the method of Van Soest and Wine,[6] pectin was determined by gravimetric analysis according to Ranganna.[7] Lignin and non-starch polysaccharide (the sum of hemicellulose, cellulose, and pectin) contents of cabbage and guava were 2.97 and 13.20 g/100 g, and 11.70 and 40.0 g/100 g, respectively. All the diets were analyzed to confirm the specific levels of dietary fiber and protein content *(Table 1)*.

Experimental design

Young white albino Wistar male weanling rats ($n = 42$) weighing 27 to 35 g, were randomly divided into seven groups of six animals and housed in individual cages kept in an air conditioned room ($22 \pm 2°$ C) and fed ad libitum on these diets for 36 days.

On day 37, the animals were lightly anesthetized with diethyl ether. To obtain the small bowel, the abdominal cavity was opened by a mid-line incision, and the intestine was separated by an incision at pyloric sphincter, whereas the ileocaecal junction was located for the lower end of the small intestine. After making these two incisions the intestine was carefully lifted by cutting the mesentries, and all the membranes were cleaned off. A syringe was filled with ice-cold saline (9 g/L) and the entire intestine was flushed with it to remove all debris. Care was taken to keep the intestine from freezing. While being slit open, it was washed and cleaned of all ruminal contents using iced saline. The segment was gently blotted

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dry and the mucosa carefully scraped off with the help of a glass blade, weighed, and homogenized in a glass bottle at low speed for 2 minutes using a Vertis homogenizer (Vertis Company, Gardiener, NY, USA). The mucosa and saline were chilled with crushed ice for at least 5 minutes before and during homogenization. The homogenate was centrifuged at 3000 to 5000 rpm for 10 minutes at 4° C. The supernatant was collected, made up to a volume of 25 mL with ice-cold saline solution and stored under frozen conditions (−5° C) for no longer than 10 days before determining the enzyme activity.

Enzyme assays

The activity of small intestinal alkaline phosphatase was determined by Bedensky's method as described by Hawk and Oser[8] and disaccharidases (sucrase, maltase, and lactase) by the procedure of Dahlquist.[9] Total protein in the mucosa was estimated colorimetrically[10] using bovine serum albumin as standard.

Statistical analysis

The data were subjected to analysis of variance in a completely randomized design using Duncan's Multiple Range Test. Critical difference (CD) was calculated at 5% level of t value using Student's t table.[11]

Results

Intestinal mucosal protein

Total protein of intestinal mucosa varied from 55.08 to 76.75 mg/g mucosal wet weight *(Table 2)*. Feeding dietary fiber from plant sources (cabbage and guava) at 10 g/100 g level resulted in a significant ($P < 0.05$) decrease in intestinal mucosal protein of experimental groups over that of the fiber-free (control) group.

Disaccharidase activity

Sucrase. Wide variations were observed in total activity of sucrase, which ranged from 71.30 (isabgol, 1 g/100 g) to 188.92 units/g mucosa (fiber-free group). The total, as well as the specific activity of sucrase decreased significantly ($P < 0.05$) on feeding different dietary fibers except cabbage and guava at the 5% level. The increase in level of dietary fiber in cabbage and guava significantly lowered the total sucrase activity *(Figure 1)*. Similarly, the specific activity of sucrase was significantly ($P < 0.05$) lower in groups fed with the higher level of cabbage or guava *(Table 2)*. For the animals fed isabgol, total sucrase activity showed an increase at 2 g/100 g inclusion compared with 1 g/100 g; however, the specific activity of this enzyme remained constant (1.20) compared with either guava or cabbage at the 10 g/100 g level (2.09 and 2.08, respectively).

Maltase. The fiber-free group had maximum maltase activity (10.37 units/mg protein) and the guava- (10 g/100 g) fed group had lowest activity (7.10 units/mg protein) *(Table 2)*. Feeding diets containing different levels of dietary fiber resulted in a significant ($P < 0.05$) decrease in intestinal maltase activity. There was a significant ($P < 0.05$) decrease in total maltase activity with an increase in amounts of dietary fiber *(Figure 2)*, whereas the specific activity did not decrease significantly ($P < 0.05$), except in the groups fed cabbage.

Lactase. The fiber-free and isabgol (2 g/100 g) groups had the maximum and minimum lactase activity, respectively. Total, as well as specific activity of intestinal lactase decreased significantly on feeding different dietary fibers *(Figure 3 and Table 2)*. With an increase in levels of dietary fiber from different sources, except guava (10 g/100 g), lactase activity decreased significantly ($P < 0.05$).

Alkaline phosphatase activity. The group fed the 5 g/100 g guava fiber diet showed the highest, while the isabgol-fed group (2 g/100 g) showed the lowest alkaline phosphatase activity. In groups fed isabgol (2 g/100 g) and cabbage (10 g/100 g), it was significantly lower than that of control. With increase in amount of dietary fiber, total activity of alkaline phosphatase decreased significantly ($P < 0.05$) *(Figure 4)*, whereas specific activity increased significantly ($P < 0.05$) in the group fed cabbage (5 g/100 g) and guava (both levels).

Discussion

Feeding of cabbage and guava fibers for 36 days resulted in a significant ($P < 0.05$) decrease in amount of total protein

Table 1 Composition of experimental diets (g/Kg diet)

Components	Cabbage		Guava		Isabgol		
Level of fiber source (g/100 g)	5	10	5	10	1	2	Fiber-free
Egg albumin	99	70	126	123	128	128	128
Sucrose	100	100	100	100	100	100	100
Mineral mixture	35	35	35	35	35	35	35
Vitamin mixture	10	10	10	10	10	10	10
Sunflower oil	100	100	100	100	100	100	100
Choline chloride	2	2	2	2	2	2	2
Cabbage	309	617	—	—	—	—	—
Guava	—	—	96	193	—	—	—
Isabgol	—	—	—	—	10	20	—
Starch	345	66	531	437	615	605	625

All the diets contained 10 g sucrose/100 g and 10 g protein/100 g, which also included the amount of protein derived from plant foods. Protein and dietary fiber content of cabbage and guava on a dry matter basis were 7.3 and 1.8 g/100 g and 16.2 and 51.8 g/100 g, respectively. Dietary fiber content of isabgol was 100 g/100 g. Other dietary ingredients were added as per standards for nutritional studies.[5]

Table 2 Effect of different dietary fibers on total protein and specific activities of sucrase, maltase, lactase, and alkaline phosphatase

Source of dietary fiber (g/100 g)		Total protein (mg/g mucosal wet weight)	Sucrase (units/mg protein)	Maltase (units/mg protein)	Lactase (units/g protein)	Alkaline phosphatase (μg Pi liberated/hr/mg protein)
Cabbage	5	76.08 ± 1.7	2.47 ± 0.11	7.98 ± 0.24	402 ± 15.9	387 ± 11.9
	10	55.92 ± 1.4	2.08 ± 0.08	9.28 ± 0.30	371 ± 14.4	224 ± 10.1
Guava	5	76.75 ± 1.4	2.24 ± 0.08	7.90 ± 0.28	424 ± 16.1	367 ± 10.4
	10	55.08 ± 1.6	2.09 ± 0.07	7.10 ± 0.24	516 ± 16.8	290 ± 10.8
Isabgol	1	59.50 ± 1.5	1.20 ± 0.06	10.17 ± 0.29	245 ± 14.4	254 ± 11.5
	2	71.83 ± 2.0	1.20 ± 0.10	7.66 ± 0.25	146 ± 16.9	179 ± 11.1
Fiber-free		67.83 ± 1.4	2.79 ± 0.08	10.37 ± 0.29	525 ± 11.9	279 ± 10.7
SE (M) DF = 35		1.60	0.08	0.27	15.8	11.0
Critical Difference ($P < 0.05$)		4.53	0.12	0.77	44.8	31.2

Values between groups (vertical columns) greater than the Critical Difference are significantly different at the 5% level.
SE (M) derived from analysis of variance table.
One unit of disaccharidase is the activity hydrolyzing 1 μM of substrate per minute.

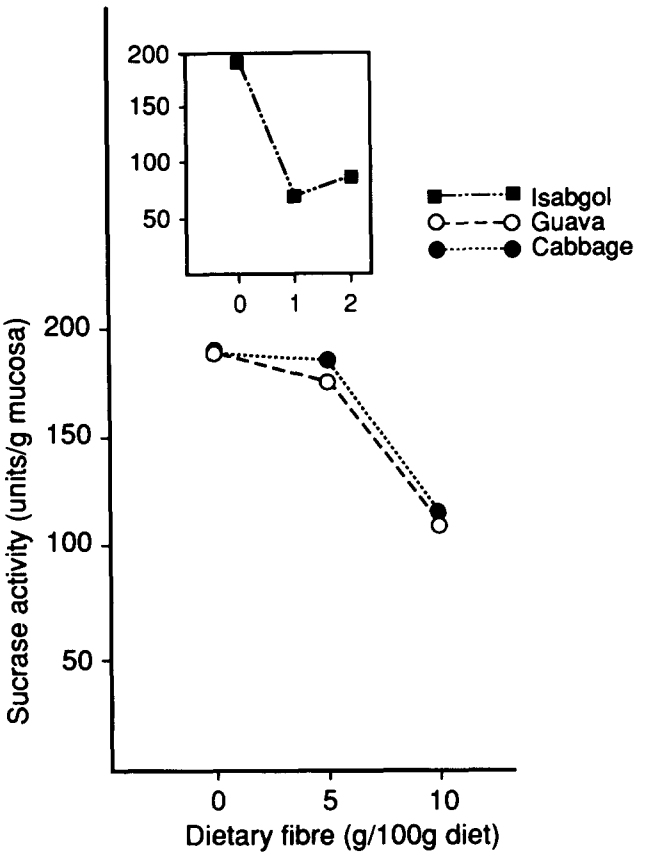

Figure 1 Total sucrase activity in intestinal mucosa of rats fed different dietary fibers.

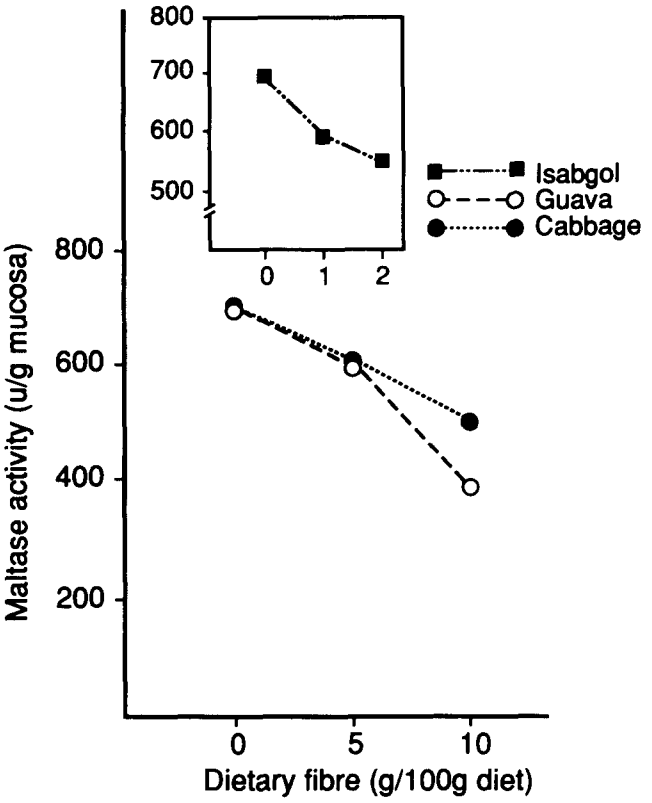

Figure 2 Total maltase activity in intestinal mucosa of rats fed different dietary fibers.

of rat intestinal mucosa. The levels of mucosal protein obtained in the present study are in agreement with those of Thomson et al.,[12] who reported that total intestinal mucosa protein varied from 53.2 to 62.6 mg/g wet mucosa. The lowering effect of dietary fiber on mucosal protein in the present study can be substantiated with that of Kimura et al.,[13] who also observed a decrease in mucosal protein in rats fed okra fiber in the diet. Mucosal protein levels in the proximal small intestine of groups fed different fibers have been found to be significantly less than those of the fiber-free group.[14] On the contrary, no significant difference in mucosal protein concentration between the groups fed different dietary fibers have been observed.[2] Increase or decrease in the intestinal mucosal protein may be because of the abrasive effect of dietary fibers on the intestinal lining, thus resulting in tearing and wearing of tissue affecting turnover rate of protein in the mucosal lining. The altered nutrition in the intestine also causes adaptive responses in the morphology and function of intestinal mucosa.[15]

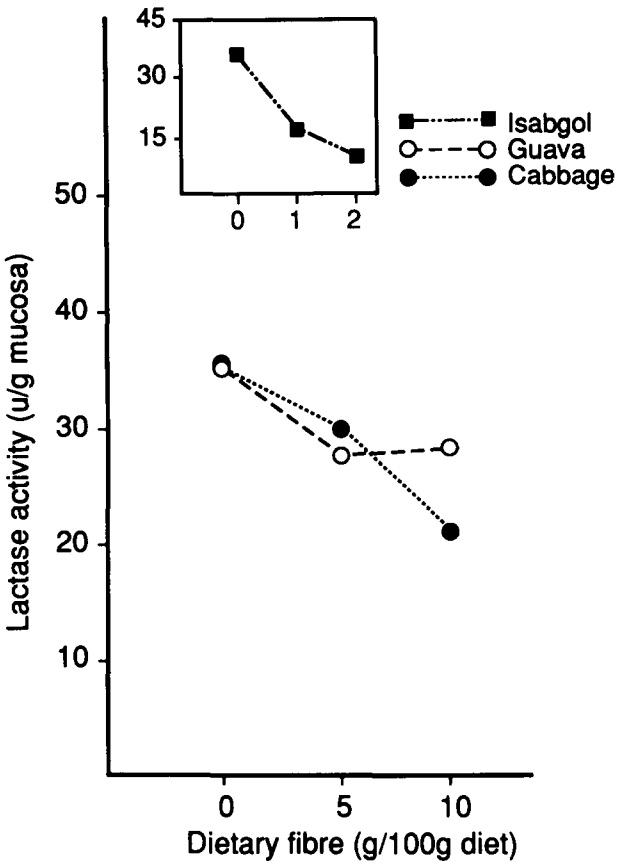

Figure 3 Total lactase activity in intestinal mucosa of rats fed different dietary fibers.

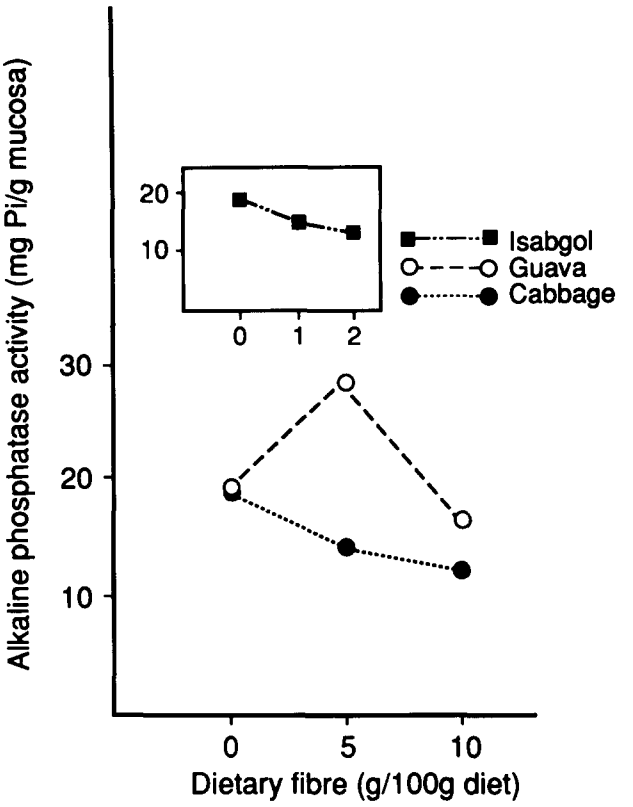

Figure 4 Total alkaline phosphatase activity in intestinal mucosa of rats fed different dietary fibers.

Jejunal mucosal lactase, sucrase, and maltase levels have been reported to be lower in the fiber-supplemented groups of rats than in a fiber-free group.[2] Because there was no significant difference in mucosal protein concentration between the groups, any alteration in activities of the intestinal disaccharidases brought about as a result of feeding different types and levels of dietary fiber may be explained by the effect of dietary fiber components on metabolic activity of bacterial flora.[16] The flora may effect the disaccharidases activity either by affecting substrate availability, enzyme inactivation, or the metabolism. It has also been reported[16] that sucrase activity of the fiber-fed group was similar to that of the basal diet, whereas alkaline phosphatase activity decreased. Inclusion of 10% gobo dietary fiber and okra resulted in decreased specific as well as total activity of alkaline phosphatase as compared with the fiber-free group.[13] On the other hand, specific activity of alkaline phosphatase has been reported to be variably elevated in the proximal intestine of animals given the fiber supplement (alfalfa, guar gum, and metamucil) as compared with the fiber-free control.[14]

The difference in the total and specific activity observed in this study may be due to differences in protein content, which is an agreement with the findings of Calvert et al.,[14] who suggested that the apparent elevation in enzyme specific activity was not as a result of changes in total enzyme activity but due to the lower levels of mucosal protein in the fiber-fed group. However, studies on the effect of dietary fiber on subsequent levels of digestive enzyme activities are not consistent, even within the same laboratory. Thus, pectin-containing diets have been reported to decrease, to have no effect, or to increase the activities of mucosal alkaline phosphatase, lactase, and invertase.[12,17,18] Changes in specific activity of an enzyme may be due to the effect of dietary fiber interfering with normal digestion and absorption of organic nutrients via intraluminal effects rather than decreasing the absolute enzyme activities of the villus surface.[4,19]

The findings of the present study show that supplementation of rat diets with guava, cabbage, or isabgol fiber leads to a decrease in specific activities of sucrase, maltase, and lactase in the small intestinal mucosa of Wistar rats even though the diets contained constant levels of disaccharide (10 g sucrose/100 g). Only groups fed isabgol-supplemented diets showed a similar trend for alkaline phosphatase.

While inclusion of 5 g/100 g cabbage or guava led to an increase in total protein as compared with the fiber-free control, higher levels of supplementation resulted in a significant decrease. This trend was reversed for isabgol. One explanation for the differences observed between the effects of guava and cabbage fiber on the one hand, and of isabgol on the other may be the much higher content of soluble fiber in the latter. It may be concluded that there is some direct interaction of fiber with substrates (disaccharides) present in intestinal mucosa that become unavailable, resulting in their low inducible effect.

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