

## EFFECT OF BAGASSE, CORN COBS, GROUNDNUT SHELLS AND THEIR NEUTRAL DETERGENT FIBRE (NDF) FRACTION ON DIGESTIVE ENZYMES

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Bagasse, corn cobs, groundnut shells and their neutral detergent fibre (NDF) fraction were tested for their ability to affect amylase, pepsin, trypsin, chymotrypsin and pancreatin activity.

**Key words:** Dietary fibre, Digestive enzymes, Neutral detergent fibre.

### INTRODUCTION

Though it is an established fact that dietary fibre and lignin are inert substances as far as monogastric digestive tract enzymes are concerned, there are some reports that enzyme activity decreases with increase in fibre concentration [1], probably due to the presence of specific inhibitors in particular fibre source.

No reports are as yet available on the effect of fibres from bagasse, corn cobs, groundnut shells and their NDF fraction on digestive enzymes. Work was, therefore, undertaken to systematically investigate the effect of these fibres on enzymes like  $\alpha$ -amylase, pepsin, trypsin, chymotrypsin and pancreatin, with a view to establishing the suitability of these fibres for use in food systems.

### MATERIALS AND METHODS

**Materials.** Purified bagasse, corn cobs and groundnut shells were procured from local sources,  $\alpha$ -amylase and trypsin (Sigma Biochemicals and organic compounds), pepsin (LOBA) and chymotrypsin and pancreatin (Fluka chemicals and biochemicals) were used.

**Methods.** Purified bagasse was prepared by washing bagasse with cold and hot water followed by drying in tray dryer at 65° for 6 hrs. It was then pulverized in Hammer mill and refluxed twice with water at 100° for 1 hr., filtered through cheese cloth, dried in tray dryer for 2 to 3 hrs at 65° and screened through sieve of 60 mesh. Same procedure was followed for groundnut shells. In case of corn cobs, starch was removed by  $\alpha$ -amylase (*Bacillus subtilis* from Sigma biochemicals and organic compounds) treatment (conc. 0.1%, pH 6.5 to 7.0) at 37° for 24 hrs followed by refluxing with hot water. Enzyme solution to material ratio was 1:20.

NDF fraction were prepared from purified bagasse, corn cobs and groundnut shells by standard method [2,3].

Enzymes were dissolved in their respective buffers as follows: -  $\alpha$ -Amylase-1mg/ml, 0.2M phosphate buffer, pH6.6; Pepsin-1mg/ml, 0.1N HCl, pH 1.80; Trypsin-1mg/ml, 0.2M phosphate buffer, pH 7.6; Chymotrypsin-0.1mg/ml, 0.2M borate buffer, pH 8.0; Pancreatic amylase-1mg/ml, 0.2M phosphate buffer, pH 7.2; Pancreatic lipase-0.4mg/ml, 0.2M Tris -HCl buffer, pH 8.0; Protease-1mg/ml, 0.2M phosphate buffer, pH 7.5.

Suspensions containing 2.5, 5.0 and 7.5 (by weight) of each fibre were made in each of the enzyme solutions. The control for each series contained no fibre. The suspensions were mixed well and incubated for 30 min at 37° for pancreatic  $\alpha$ -amylase, pepsin, trypsin and chymotrypsin and 180 min. at 37° for pancreatic amylase, lipase and protease. After incubation the reaction mixture were filtered and the filtrates were assayed for different enzyme activities using specific substrates as follows:-

Trypsin, 1% Casein; Pepsin, 1% Hemoglobin; Chymotrypsin, 1% Casein; Pancreatic lipase, Olive oil emulsion; Protease, 1% Casein;  $\alpha$ -Amylase, and Pancreatic amylase 2% starch.

Assay for  $\alpha$ -amylase [4,5], Pancreaticamylase [4,5], Lipase [6-8], pepsin [9], protease [9,10], trypsin [11] and chymotrypsin [12] were done by using standard procedure. The activity of the control was taken as 100% and the activity of each sample was expressed as a percentage of the control activity.

### RESULTS AND DISCUSSION

The effect of bagasse, corn cobs, groundnut shells and their neutral detergent fibre fractions on  $\alpha$ -amylase, pepsin, trypsin and chymotrypsin are recorded in Table 1. Incubation of purified bagasse fibre and its neutral detergent fibre fractions with all these enzymes showed that the fibre sources did not have much effect on all these enzymes even though their concentrations were increased from 2.5

Table 1. Effects of bagasse, corn cobs, groundnut shells and their NDF fraction on some digestive enzymes.

Sources	Concentration (%)	% Enzyme activity retained after contact with enzyme for 30 minutes			
		Alpha amylase	Trypsin	Pepsin	Chymotrypsin
1. Bagasse	2.5	97.25 ± 4.24	99.87 ± 1.11	98.91 ± 1.21	97.99 ± 2.01
	5.0	98.98 ± 0.97	98.92 ± 0.91	99.72 ± 0.83	99.69 ± 1.11
	7.5	98.41 ± 1.43	99.99 ± 0.91	99.90 ± 1.49	98.51 ± 3.11
2. B. NDF	2.5	99.05 ± 0.92	99.71 ± 0.91	99.09 ± 1.11	99.83 ± 1.21
	5.0	98.52 ± 2.28	99.69 ± 0.91	99.19 ± 1.21	99.61 ± 1.39
	7.5	99.04 ± 0.95	99.51 ± 0.69	99.32 ± 0.91	98.99 ± 1.91
3. Corn Cobs	2.5	99.09 ± 1.09	97.89 ± 3.11	95.13 ± 3.99	97.92 ± 2.99
	5.0	98.18 ± 2.33	98.19 ± 2.17	98.13 ± 3.11	98.05 ± 2.19
	10.0	99.27 ± 1.00	99.21 ± 1.31	97.11 ± 2.91	99.39 ± 0.91
4. C. NDF	2.5	97.62 ± 3.01	99.18 ± 0.89	99.00 ± 1.11	99.91 ± 0.91
	5.0	98.99 ± 2.00	99.39 ± 0.91	98.89 ± 2.71	99.00 ± 1.00
	10.0	99.92 ± 1.00	99.01 ± 1.07	97.99 ± 3.91	99.39 ± 1.71
5. Groundnut shells	2.5	98.70 ± 2.19	98.91 ± 1.71	97.71 ± 2.11	98.13 ± 2.07
	5.0	99.17 ± 1.09	98.41 ± 2.07	96.71 ± 3.91	98.92 ± 1.91
	10.0	99.19 ± 0.91	98.39 ± 2.73	96.71 ± 4.11	98.92 ± 2.11
6. G. NDF	2.5	98.97 ± 1.69	99.09 ± 1.09	99.31 ± 1.09	97.52 ± 2.91
	5.0	99.13 ± 0.91	98.39 ± 2.11	99.69 ± 0.91	98.19 ± 2.09
	10.0	98.70 ± 1.19	98.11 ± 2.19	97.99 ± 2.17	97.92 ± 3.07

Average of three reading ± S.D.; B. NDF, C. NDF, G. NDF = Neutral detergent fibre fraction of bagasse, corn cobs groundnut shells respectively.

to 7.5%. Retention of  $\alpha$ -amylase with bagasse and its fibre constituents was observed to be as much as 97% while in case of alfalfa, oat bran and lignin retention of  $\alpha$ -amylase activity to 87, 72 and 100% respectively has been reported [13,14]. In case of pepsin with fibre concentration 2.5 to 7.5% and incubation period upto 30 mins., the inhibition of enzyme was not more than 4% while % inhibition of pepsin has been reported 2% for pectin [15].

In case of trypsin and chymotrypsin percentage retention of enzyme activity was more than 97%, indicating that, there was insignificant inhibition of enzyme activity upto 30 min. incubation. However, 100 and 104% retention of trypsin and 99 and 104% retention of chymotrypsin activity have been reported in case of cellulose acetate and pectin respectively [16].

As shown in Table 1 more than 97% retention of  $\alpha$ -amylase activity was observed in case of corn cobs, which is in close agreement with that of for the other fibres, like pectin, where retention of  $\alpha$ -amylase activity is more than 100% [13]. In case of pepsin the percentage inhibition of enzyme activity was not more than 4%, which is also in close agreement with other fibres like pectin, where percentage inhibition is only 2% [15]. The percentage enzyme activity retained in case of chymotrypsin and trypsin when incubated with purified corn cobs and its NDF fraction was more than 97%. No comparative data are available in this respect. However, in case of wheat bran and agar agar, more than 96% retention of trypsin

activity [15] and more than 98% retention of chymotrypsin activity has been reported with cellulose acetate [1].

The retention of  $\alpha$ -amylase activity was more than 97% for groundnut shells as shown in Table 1, which is in close agreement with other fibres like pectin where retention of  $\alpha$ -amylase activity is more than 100% [13]. In case of pepsin at all concentration of fibre, the percentage inhibition of enzyme was not more than 4%, where as only 2% inhibition has been reported for pectin [15]. In case of trypsin and chymotrypsin more than 98% activity was retained, while in case of wheat bran and agar agar, percentage enzyme activity retention for trypsin was more than 96% [1,15] and percentage retention of chymotrypsin activity in case of cellulose acetate is more than 98% [1].

The effects of purified bagasse, corn cobs, groundnut shells and their NDF fraction on pancreatic enzyme like amylase, lipase and protease are recorded in Table 2. Even in this case percentage activity of pancreatic amylase retained was more than 97%. These results are in close

Table 2. Effects of bagasse corn cobs, groundnut shells and their NDF fraction on pancreatic amylase, protease and lipase.

Sources	Concentration (%)	% Enzyme activity retained after contact with enzyme for 180 minutes		
		Pancreatic amylase-II	Protease	Pancreatic Lipase II
1. Bagasse	2.5	99.30 ± 1.21	98.97 ± 1.11	98.93 ± 2.09
	5.0	99.23 ± 2.01	99.00 ± 1.00	97.92 ± 1.91
	7.5	99.39 ± 2.09	98.10 ± 2.00	98.88 ± 3.01
2. B. NDF	2.5	98.71 ± 2.11	99.21 ± 0.67	98.71 ± 2.11
	5.0	98.39 ± 1.79	98.85 ± 0.90	99.88 ± 1.09
	7.5	98.49 ± 1.29	99.96 ± 0.81	98.88 ± 2.00
3. Corn cobs	2.5	97.37 ± 2.11	98.07 ± 2.19	97.11 ± 3.11
	5.0	97.41 ± 1.91	97.69 ± 2.11	98.09 ± 2.91
	10.0	97.86 ± 2.11	98.11 ± 1.11	97.19 ± 2.11
4. C. NDF	2.5	98.89 ± 1.81	98.57 ± 1.75	97.81 ± 3.01
	5.0	98.37 ± 1.09	98.39 ± 1.37	97.13 ± 2.19
	10.0	98.41 ± 2.11	98.71 ± 2.11	97.41 ± 2.17
5. Groundnut shells	2.5	97.13 ± 3.91	97.91 ± 2.11	97.61 ± 3.69
	5.0	97.07 ± 3.11	97.79 ± 3.01	96.57 ± 3.08
	10.0	97.10 ± 3.18	96.81 ± 4.11	96.61 ± 3.99
6. G. NDF	2.5	98.19 ± 1.71	97.31 ± 1.71	97.07 ± 3.11
	5.0	98.89 ± 1.31	98.11 ± 2.19	98.09 ± 1.92
	10.0	99.00 ± 1.00	98.09 ± 2.18	97.11 ± 2.99

Average of three readings ± S.D.; B. NDF, C. NDF, and G. NDF = Neutral detergent fibre fraction of bagasse, corn cobs and groundnut shells respectively.

agreement with reported values for cellulose, pectin and guar gum, where percentage enzyme activity retention is more than 100% [16]. No effect was observed in case of protease activity. In case of pancreatic lipase percentage inhibition was not more than 3%, which is similar to observations with pectin, where percentage inhibition was nil [1,13].

From the forgoing discussion, it is clear that effect of

fibres prepared from bagasse, corn cobs and groundnut shells on digestive enzymes are seems to be insignificant.

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Table 2. Effect of bagasse, corn cobs, groundnut shells and their NDF fraction on pancreatic amylase, protease and lipase.

Substrate	Control (%)	Enzyme activity retained after contact with enzyme for 180 minutes	
		Protease	Amylase
1. Bagasse	100	98.75 ± 2.11	98.33 ± 1.89
	2.5	99.23 ± 1.91	97.95 ± 1.91
	5.0	99.79 ± 1.99	98.88 ± 1.81
2. Corn cobs	100	98.71 ± 2.11	98.31 ± 1.81
	2.5	98.59 ± 1.79	98.81 ± 1.99
	5.0	98.48 ± 1.79	98.88 ± 1.99
3. Groundnut shells	100	98.75 ± 2.11	98.33 ± 1.81
	2.5	97.75 ± 2.19	97.11 ± 2.11
	5.0	97.49 ± 2.01	96.99 ± 2.01
4. C-NDF	100	97.98 ± 2.11	97.19 ± 2.11
	2.5	98.94 ± 1.81	98.57 ± 1.71
	5.0	98.79 ± 1.71	97.13 ± 1.71
5. D-NDF	100	98.41 ± 2.11	97.81 ± 2.11
	2.5	97.71 ± 2.11	97.81 ± 2.11
	5.0	97.75 ± 2.11	97.81 ± 2.11
6. D-NDF	100	97.75 ± 2.11	97.81 ± 2.11
	2.5	97.75 ± 2.11	97.81 ± 2.11
	5.0	97.75 ± 2.11	97.81 ± 2.11

Average of three readings ± S.E. D.NDF, C.NDF and D-NDF = Neutral detergent fibre fraction of bagasse, corn cobs and groundnut shells respectively.

From the foregoing discussion, it is clear that effect of bagasse, corn cobs and groundnut shells on digestive enzymes is in close agreement with reported values for cellulose, pectin and guar gum, where percentage enzyme activity retention is more than 100% [16]. No effect was observed in case of protease activity. In case of pancreatic lipase percentage inhibition was not more than 3%, which is similar to observations with pectin, where percentage inhibition was all [1,12].

Retention of amylase with bagasse and its fibre component was observed to be as much as 97% while in case of alfalfa, oat bran and lignin retention of amylase activity to 87, 72 and 100% respectively has been reported [1,3,14]. In case of pepsin with fibre concentration 2.5 to 7.5% and incubation period upto 30 min, the inhibition of enzyme was not more than 4% while 7% inhibition of pepsin has been reported 2% for pectin [15].

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As shown in Table 1 more than 97% retention of α-amylase activity was observed in case of corn cobs, which is in close agreement with that of for the other fibres like pectin, where retention of α-amylase activity is more than 100% [13]. In case of pepsin the percentage inhibition of enzyme activity was not more than 4%, which is also in close agreement with other fibres like pectin, where percentage inhibition is only 2% [15]. The percentage enzyme activity retained in case of chymotrypsin and trypsin when incubated with bagged corn cobs and guar gum was more than 97%. No comparative data are available in this respect. However, in case of wheat bran and guar gum, more than 96% retention of trypsin