NOTE

Beta-Glucanase as an Aid in Measuring Neutral Detergent Fiber in Barley Kernels[1]

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The neutral detergent fiber (NDF) technique as developed by Goering and Van Soest (1970) is a preferred method for estimating plant cell wall constituents. The cell walls consist of cellulose, hemicellulose, lignin, silica, cutin, insoluble minerals, lignified nitrogen compounds, lignocellulose, gums, and pectins (Van Soest 1978). However, gums and pectins are removed in the process of determining cell wall constituents by NDF (Bailey and Ulyatt 1970); therefore, Van Soest (1978) proposed that a suitable method be developed for measuring these soluble substances in a procedure separate from that for insoluble fiber. Gums are not considered a part of dietary fiber, which is currently defined as cellulose, hemicellulose, lignin, and pectin.[2]

The determination of NDF in barley with the α-amylase enzyme modification for concentrate feedstuffs developed by Robertson and Van Soest (1977) has involved filtration difficulties in our laboratory. Barley in aqueous solutions has a high viscosity (Göhl 1977), which was suspected to be the cause of the problem. Viscous barley aqueous solutions are generally attributed to a water-dispersible polysaccharide commonly called β-glucan (Greenberg 1974, Preece and MacKenzie 1952). According to Wood et al (1977), β-glucan has been broadly established as a linear molecule composed of β-1,3- and β-1,4- linked D-glucopyranosyl units. The enzyme β-glucan endohydrolase from *Bacillus subtillis* specifically hydrolyzes 1,4- linkages in β-glucans containing both 1,3- and 1,4-linkages such as occur in barley and oat β-glucans. It has no action on either β-glucans having only 1,4- linkages (cellulose) or β-1,3-glucans of the callose type, which are known to be associated with β-1,3:1,4- glucans in barley endosperm and other plant tissues (Anderson et al 1978). Barley varies considerably in β-glucan content (Göhl 1977), and these complex carbohydrates have also been reported in oats although in lesser amounts than in barley (Anderson et al 1978).

The aim of the present investigation was to determine whether the addition of β-glucanase would speed the filtration in the determination of the NDF of barley and other grains without dissolving the fiber.

MATERIALS AND METHODS

Grains

Samples of barley, oats, wheat, and corn were included in the comparisons. The five barleys were four isogenic types of Glacier (CI 9676), two covered and two hull-less cultivars grown in 1971, and a hull-less isogene of Betzes (CI 6398) grown in 1978. The three samples of oats were grown in 1977, 1978, and 1979, and the wheat and corn were samples grown in 1979. All grains were ground through a Cyclotec sample mill with a 0.5-mm screen.

Alphacel

The alphacel was finely ground wood cellulose available from ICN Nutritional Biochemicals, Cleveland, OH.

Neutral Detergent Solution

The neutral detergent solution was prepared by the procedure of Goering and Van Soest (1970).

α-Amylase

The α-amylase was Type III-A, a crude bacterial preparation from *Bacillus subtillis* obtained from Sigma Chemical Company, St. Louis, MO. The product had an α-amylase activity of 50-100 units per milligram of solid. One gram of enzyme preparation was dissolved in 45 ml of water, filtered, and combined with 5 ml of ethoxyethanol. The solution was stored at 5° C and used within seven days.

β-Glucanase

The β-glucanase was obtained as Cereflo 200L from Novo Laboratories Inc., Wilton, CT. The product had a standardized activity of 200 β-glucanase units per gram at 30° C at pH 7.5 and an α-amylase activity not below 100 kilo Novo units per gram determined at 37° C and pH 5.7. The glucanase activity of Cereflo peaks at approximately 50° C and decreases linearly to zero at approximately 75° C. Cereflo is a purified bacterial β-glucanase preparation produced by submerged fermentation of a selected strain of *Bacillus subtillis*. With storage of 5° C for six months, no loss of activity was detected.

NDF Modification with α-Amylase

The α-amylase-modified NDF was determined by the method described by Robertson and Van Soest (1977). Five-tenths gram of the ground grain sample was weighed into a 600-ml beaker. Then 50 ml of the cold neutral detergent solution was added to the sample, heated to boiling, and refluxed for 30 min. At that time, an additional 50 ml of the cold neutral detergent solution and 2 ml of the α-amylase solution were added. The beaker was returned to the hot plate and refluxed for 1 hr after the initial onset of boiling, after which the solution was filtered through a weighed sintered-glass crucible. The residue was washed four times with boiling water and twice with acetone. If filtering problems occurred, the residue was rinsed at least once with boiling water, and then about 30 ml of hot (80° C) water and 2 ml of the α-amylase enzyme solution were added. The crucible was let stand for about 10 min, filtered, and washed twice with boiling water and twice with acetone. Then the crucible and contents were dried overnight at 105° C and weighed.

NDF Modification with α-Amylase and β-Glucanase

One milliliter of the β-glucanase and 1 ml of water were added to 0.5 g of the ground grain sample in a 600-ml beaker. These were mixed thoroughly and let stand for 10 min before the addition of 50 ml of the cold neutral detergent solution. The determination of the NDF then continued as described under the NDF modification with α-amylase. Four concentrations of the β-glucanase, 0.3, 0.5, 0.8, and 1.0 ml, were compared to determine the level required for optimum total time of filtration, ie, about 5 min. Nubet, a hull-less barley cultivar having a relatively high viscosity in solution (cP

TABLE I
Filtration Times for Nubet Barley with Different Amounts of β-Glucanase

β-Glucanase (ml)	Time for Filtration (min)[a]	NDF[b] (%)[a]
0.3	...[c]	...
0.5	8	6.0
0.8	5	5.9
1.0	5	5.9

[a] Average of duplicate determinations.
[b] Neutral detergent fiber.
[c] Would not filter.

[1] Published with the approval of the director of the Montana Agricultural Experiment Station, Journal Series 1086.
[2] Leon Prosky, personal communication.

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TABLE II
Neutral Detergent Fiber (%)[a] Determined with α-Amylase and with α-Amylase and β-Glucanase

Material	α-Amylase	α-Amylase and β-Glucanase
Barleys		
Glacier-1, covered, long awn	12.9	12.2
Glacier-2, covered, short awn	14.3	14.5
Glacier-3, hull-less, long awn	5.6	5.6
Glacier-4, hull-less, short awn	7.0	6.9
Oats		
1977	28.9	28.8
1978	32.2	33.0
1979	23.9	22.8
Wheat	8.9	9.1
Corn	9.6	9.7
Alphacel	94.7	94.1

[a]Average of duplicate determinations.

3.95[3]) was used for these comparisons.

The NDF-modified procedure with β-glucanase was attempted with barley without the addition of α-amylase because the β-glucanase solution contained a supplementary amount of α-amylase.

Percentage recoveries of NDF using the modified procedure with α-amylase and β-glucanase or α-amylase alone as described above were compared for samples of barley, oats, wheat, corn, and alphacel.

RESULTS AND DISCUSSION

The NDF determined with different amounts of added β-glucanase with the hull-less barley Nubet is shown in Table I. Amounts of 0.8 and 1.0 ml of β-glucanase provided optimum filtration rates. To avoid problems with barleys, which can contain larger quantities of β-glucans, the larger quantity of β-glucanase is suggested and was used in the remainder of our comparisons.

The addition of β-glucanase without α-amylase after 30 min of refluxing with the Nubet barley was unsuccessful because the solution would not filter. Therefore, the starch in barley must be solubilized by heating before the addition of α-amylase, as in the NDF-modification with α-amylase.

The percentage of NDF recovered using the modified procedure with α-amylase and β-glucanase or α-amylase alone is shown in Table II. Duplicate analyses exhibited very small variation, and the values obtained from both procedures were comparable. The barleys in particular gave very close agreement, even though the NDF values determined with the α-amylase only were very difficult to filter. Therefore, we concluded that Cereflo 200L β-glucanase did not solubilize an appreciable quantity of the NDF.

None of the oat samples tested in this study gave any problem with filtration, so the proposed modified procedure using β-glucanase is of no value for oats unless filtration difficulties arise.

The addition of Cereflo 200L to ground barley before the α-amylase modified procedure for NDF gave a rapid filtering time with no appreciable difference in the amount of fiber as determined by the standard procedure. This method could be applied to other grains that contain sufficient quantity of β-glucans to cause filtering problems.

[3]C. W. Newman, unpublished data.

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