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Measuring Total Dietary Fiber of Resistant-Starch Products Using Different Test Kits

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ABSTRACT

AACC International methods 32-07 (AOAC 991.43) and 32-05 (AOAC 985.29) may be used to measure the total dietary fiber (TDF) in food systems and in food ingredients such as resistant starch (RS). Two enzyme preparation kits for the analysis of TDF by AACC International methods 32-07 and 32-05 were evaluated. A significant difference between the two enzyme preparations was found when used on RS samples. The enzymes offered by one kit produce much lower percent TDF in RS samples than the other enzymes. Based on the enzyme activities and volumes reported, α-amylase from one kit is much more active than the other kit's. While this difference of α-amylase activity does not cause a problem for traditional nonstarch fibers, the use of the more active kit's α-amylase can lead to significant underestimation of the TDF content for RS samples. The buffer system used in AACC International 32-07 and 32-05 seemed to influence the activity of these enzymes differently when used at equal activity levels. The variation in enzyme activity between manufacturers and the potential difference between the buffer systems used in AACC International 32-07 and 32-05 need to be investigated further.

The proposed Codex (2) definition of dietary fiber includes all carbohydrate polymers with a degree of polymerization not lower than 3 that are resistant to digestion and absorption in the small intestine (2,5). Dietary fiber has been shown to have a positive effect on laxation and the attenuation of blood cholesterol or blood glucose levels and/or to be fermentable by colonic microflora (1,3).

AACC International methods 32-07 and 32-05 (4,5) may be used to measure the total dietary fiber (TDF) in food systems and in food ingredients such as resistant starch (RS). Laboratories that use a higher dosage of enzymes than prescribed by the AACC International methods may find that the percent TDF in resistant starch is underestimated. To determine the difference between the enzyme preparations, a high-amylose starch and two RS-containing ingredients were analyzed.

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MATERIALS AND METHODS

Materials

Kit A was an enzyme kit supplied by Megazyme (KTDF-R, Megazyme International, Wicklow, Ireland). The following information for the enzymes in the kit was provided by Megazyme: α-amylase, heat-stable (*B. licheniformis*), 10,000 U/mL on soluble starch at pH 6.0 and 40°C; protease, 50 mg/mL (~350 tyrosine U/mL); amyloglucosidase (AMG) (*A. niger*), 3,200 U/mL on soluble starch at 40°C, pH 4.5.

Kit B was supplied by Sigma (TDF100A, Sigma-Aldrich, St. Louis, MO). The following information was provided by Sigma for the enzymes in the kit: α-amylase, heat stable, A 3306 (lot 064K8806), 30,800 U/mL on soluble starch at pH 6.9 and 20°C; protease, P 3910; AMG, A 9913 (*A. niger*) (lot 064K8807), 7,800 U/mL.

High-amylose starch (TDF Controls Kit [K-TDFC], Megazyme) and RS-containing ingredients (Reference RS and F4-763 RS, Tate and Lyle, Decatur, IL; Hi-maize 260, National Starch and Chemical Co., Bridgewater, NJ) were analyzed.

Principle of the TDF Methods

Dry test samples were analyzed in duplicate. The test samples underwent sequential enzymatic digestion by heat-stable α-amylase, protease, and AMG to remove digestible starch and protein. The digestion residue was treated with alcohol to precipitate soluble fiber before filtering and then was washed with alcohol and acetone, dried, and weighed. The two enzyme kits were used to determine percent TDF by AACC International methods 32-07 and 32-05. A summary of the TDF methods is provided in Table I.

RESULTS AND DISCUSSION

TDF values measured with AACC International method 32-05 were higher than for AACC International method 32-07 for all samples and both enzyme preparations.

Table I. Summary of AACC International methods 32-07 and 32-05

AACC International 32-07	AACC International 32-05
1.000 ± 0.005 g sample	1.000 ± 0.005 g sample
MES-tris[a] buffer at pH 8.2	Phosphate buffer at pH 6.0
50 μL heat-stable α-amylase	50 μL heat-stable α-amylase
35 min at 95–100°C	35 min at 95–100°C
Add 10 mL water	pH adjustment to 7.5 ± 0.1
100 μL protease	100 μL protease
30 min at 60°C	30 min at 60°C
pH adjustment to 4.1–4.8	pH adjustment to 4.5 ± 0.2
300 μL amyloglucosidase	300 μL amyloglucosidase
30 min at 60°C	30 min at 60°C
3 volumes ethanol	3 volumes ethanol
Precipitate (= fiber) recovered and weighed	Precipitate (= fiber) recovered and weighed

[a] 2(*N*-morpholino) ethane sulfonic acid–tris(hydroxymethyl)aminomethane.

The main differences between the two methods are the buffer and pH used for α-amylase hydrolysis (Table I). AACC International 32-07 is more widely used and is said to give more precise results than AACC International 32-05, but both methods can be used to determine TDF.

When both enzyme preparations were used at dosages recommended by the manufacturers, the TDF values were higher for kit A's enzymes for all samples and both assays (AACC International 32-07 and 32-05), as shown in Tables II and III. For AACC International 32-07, the TDF values were ~10–15% lower when the kit B enzymes were used at the recommended dosage levels (Table II). The relative difference between TDF values determined with the two enzymes were even larger for AACC International 32-05 (Table III). For most samples, the relative difference was 9–15%, but for the high-amylose sample the relative difference was >30%. When the kit B α-amylase was used at one-sixth the recommended dosage level, the percent TDF for all samples was higher than those found with kit A enzymes for AACC International 32-07 (Tables II and IV). For AACC International 32-05, the percent TDF values for the lower kit B enzyme dosage were similar to values found with the kit A α-amylase (Tables III and IV).

When the kit A enzymes were used for AACC International 32-07, the results for the RS reference (59.7% TDF) matched the certificate of analysis for this lot (60.7% TDF), which had been determined at a separate laboratory. The results for the Hi-maize 260 (63.3% TDF) closely matched the certificate of analysis provided by the manufacturer (63.5% TDF).

The large differences between percent TDF determined with the enzyme kits suggest the need for a closer look at the enzyme activity, dosage, and buffer systems.

Enzyme activities listed for the AMG preparations were higher than those stated in the official AACC International method (Table IV). AACC International methods 32-07 and 32-05 state the enzyme activities and volumes to be used for the assays. However, both companies recommend a lower volume of their AMG compared to the volume stated in the official methods. After the volume adjustments, the enzyme dosage (activity per gram of sample) was in the range stated in the official method (2,000–3,300 U/mL). The official method uses 300 µL per 1 g of sample, leading to a dosage of 606–1,000 U/g of sample. Kit B recommends 100 µL of AMG at 7,800 U/mL, which equals 780 U/g of sample. Kit A recommends 200 µL of AMG, which equals 640 U/g of sample. The AMG enzyme therefore does not seem to be the problem.

The enzyme activity for kit A's α-amylase (10,000 U/mL) is the same as the activity defined in the official method for this enzyme (10,000 ± 1,000 U/mL) (Table I). The volume recommended by the manufacturer is also the same as prescribed in the official method (50 µL); therefore, the dosage of kit A α-amylase (500 U/g of sample) is the same as required by the official method. The kit B α-amylase (30,000 U/mL) has a much higher activity than prescribed in the official method. The volume recommended by the manufacturer for this enzyme (100 µL) provides a dosage of the α-amylase that is six times higher (3,000 U/g of sample) than the dosage prescribed in the official method (500 U/g of sample).

The activities of both α-amylases were determined on the same substrate (soluble starch) but at slightly different pH levels and different temperatures. The activity of the kit A α-amylase was determined at pH 6.0 and 40°C compared with pH 6.5 and 40°C in the official method. This slight difference in pH would not be expected to make a large difference in the activity of the enzyme. It is very likely that the kit A α-amylase has the same activity as the α-amylase listed in the official method. The activity of the kit B α-amylase was determined at pH 6.9 and 20°C. Again, the pH difference is not likely to cause large activity differences, but the difference in temperature is significant. The activity of a heat-stable α-amylase would be expected to be lower at a lower temperature. It is therefore likely that the kit B α-amylase is more active at 40°C than at 20°C, and the effective enzyme dosage recommended by the manufacturer for the TDF analysis is even more than six times higher than the dosage in the official method.

It appears that the α-amylases for kits A and B have different activities in the two different buffer systems used for AACC International 32-07 and 32-05. Kit A's α-amylase gave significantly different percent TDF values with the two assays (Table II and III). The kit B α-amylase

Table II. Total dietary fiber (TDF) values for AACC International method 32-07 using enzymes A and B on starch and resistant starch (RS)

Sample	Enzyme A TDF (%)[a]	Enzyme B TDF (%)[a]
Reference RS	55.4	49.7
Hi-maize 260	63.3	53.8
F4-763 RS	59.7	52.1
High-amylose starch	26.5	23.5

[a] Values represent the mean of three independent analyses performed on different days. Each analysis was done on duplicate samples.

Table III. Total dietary fiber (TDF) values for AACC International method 32-05 using enzymes A and B on starch and resistant starch (RS)

Sample	Enzyme A TDF (%)[a]	Enzyme B TDF (%)[a]
Reference RS	65.7	57.6
Hi-maize 260	69.4	59.2
F4-763 RS	66.8	60.5
High-amylose starch	37.8	24.4

[a] Values represent the mean of three independent analyses performed on different days. Each analysis was done on duplicate samples.

Table IV. Total dietary fiber (TDF) values for AACC International methods 32-07 and 32-05 using kit B's enzymes at one-sixth dosage level

Sample	AACC International 32-07 TDF (%)[a]	AACC International 32-05 TDF (%)[a]
Reference RS	64.1	68.3
Hi-maize 260	65.5	68.6
High-amylose starch	41.9	41.0

[a] Values represent the mean of two independent analyses performed on different days. Each analysis was done on duplicate samples.

Table V. Effective enzyme dosages with recommended volumes

	α-Amylase			Amyloglucosidase		
Method	Activity (U/mL)	Volume (µL)	Dosage (U/g of sample)	Activity (U/mL)	Volume (µL)	Dosage (U/g of sample)
AACC International 32-07 and 32-05	10,000 ± 1,000	50	500	2,000–3,300	300	606–1,000
Kit A	10,000 ± 1,000	50	500	3,200	200	640
Kit B	30,000	100	3,000	7,800	100	780

gave different values only when the recommended dosage was used. When one-sixth of the recommended dosage was used (500 U/g of sample), the percent TDF values for AACC International 32-07 and 32-05 were very similar for the RS samples tested (Table V). While the kit A α-amylase appeared to have a lower activity in the buffer system of AACC International 32-07, at reduced levels, the kit B α-amylase seemed to have very similar activity in the different buffer systems (Table V).

The differences between the enzyme preparations have not been noted in the literature. The control samples in the official method are designed to test the contamination of the enzyme preparations used. Wheat starch and corn starch controls are mentioned to monitor the α-amylase activity in the assay. Both of these starches are negative controls, and the expected TDF values for them are 0–1%. These controls are useful to confirm that the α-amylase used is active enough, but they will not reveal an α-amylase that is too active. There also seems to be a difference in enzyme activity related to the different buffer systems used in AACC International 32-05 and 32-07. Since both methods are approved for the measurement of TDF in foods, this difference should be further explored.

When the AACC International TDF methods were developed, they were designed to ensure complete hydrolysis of all starch in the sample, and only a negative control was recommended. Since the tests were developed, RS-containing ingredients have been developed and measured by AACC International TDF assays. The activity of α-amylase does not impact the measurement of more-traditional fibers such as oat fiber, cellulose, or other non-starch fibers. Only when RS samples are measured does a high α-amylase activity become problematic and lead to underestimation of the true fiber content of the samples.

CONCLUSIONS AND RECOMMENDATIONS

The buffer systems used in AACC International 32-07 and 32-05 seemed to influence the activity of the two α-amylases differently when both enzymes were used at 10,000 U/mL. The activity of the kit A α-amylase seemed higher in the 2(*N*-morpholino) ethane sulfonic acid (MES)-tris buffer system used in AACC International 32-07, while kit B's α-amylase had similar activity in both buffer systems.

When measuring TDF in RS, it is critical that the enzyme dosage and activity prescribed in the AACC International methods be followed. Since the AACC International 32-07 method is used to determine the fiber content of foods around the world, it is important that these fiber values are accurately measured by all laboratories. Reliable tests are important for research and improve the ability of food companies to offer consumers products containing beneficial fibers. The variation in enzyme activity between manufacturers and the potential difference between the buffer systems used in AACC International 32-07 and 32-05 need to be further investigated.

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