OFFICIAL METHODS℠

AOAC Approves Integrated Method for Measurement of Total Dietary Fiber

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A method for measurement of total dietary fiber, including resistant starch (RS) and nondigestible oligosaccharides (NDO; Integrated Total Dietary Fiber method) has been developed and validated. The procedure involves incubation of sample with pancreatic α-amylase plus amyloglucosidase under physiological conditions to simulate digestion of starch in the human small intestine. The high-molecular-weight soluble fiber (HMWSDF) is precipitated with alcohol and recovered with the insoluble dietary fiber (IDF) on a sintered crucible under vacuum, dried and weighed. NDO are not hydrolyzed by the purified enzymes used in the incubation. These are recovered in the aqueous ethanol filtrate and are subsequently measured by high-performance liquid chromatography (HPLC) as low-molecular-weight soluble dietary fiber (LMWSDF). The method was approved by AOAC as First Action Method **2009.01** Total Dietary Fiber (CODEX Definition) in Foods by Enzymatic-Gravimetric-Liquid Chromatographic Method.

The most significant challenge in the development of this method was to obtain values for RS that were in-line with values obtained with ileostomy patients.

Background

Dietary fiber is a multicomponent mixture, thus it is essential that it is clearly defined and that there is a method to allow measurement of the defined components. Interest in dietary fiber is a consequence of the belief that its physiological effects contribute positively to the health/quality of life of the consumer. The term "dietary fiber" was coined by Hipsley in 1953 to cover the nondigestible constituents of plants that make up the plant cell wall. This definition was broadened by Trowell in 1976 to become primarily a physiological definition, based on edibility and resistance to digestion in the human small intestine. The broadened definition includes indigestible polysaccharides such as gums, modified celluloses, mucilages and pectin, and NDO. The definition of dietary fiber that arose from the 27th Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU; ALINORM 06/29/26; Bonn, Germany, November 21–25, 2005), and subsequently modified in 2009, includes RS and leaves the decision on the inclusion of NDO to national authorities.

In developing AOAC *Official Method℠* **985.29** (the Prosky method) and **991.43**, the aim was to measure HMWSDF and IDF. The significance of RS and NDO were not fully appreciated at that time. Subsequently, a survey of scientists (in 1993) showed that 65% of the respondents favored the inclusion of NDO and 80% favored inclusion of RS in the definition of dietary fiber. This led to the development of methods for resistant starch (AOAC Method **2002.02**) and for a number of NDO, including fructo-oligosaccharides (AOAC Methods **997.08** and **999.03**), Polydextrose (AOAC Method **2000.11**), Fibersol 2 (AOAC Method **2001.03**), and galacto-oligosaccharides (AOAC Method **2001.02**). However, one problem remained, which was the issue of "double counting" of a percentage of the various components. AOAC *Official Methods℠* **985.29** and **991.43** measure some of the RS, fructan, etc., thus values specifically determined for RS, fructan, etc. cannot simply be added to values determined for total dietary fiber. A need for an all-encompassing method was evident.

The Challenge of Method Development

The most significant challenge in the development of this method was to obtain values for RS that were in-line with values obtained with ileostomy patients. The method was based on AOAC *Official Method℠* **2002.02** for RS and was designed to give RS values for a number of samples that matched values from in vivo studies. Because the ultimate determination of dietary fiber is gravimetric, the sample size had to be increased 10-fold (matching sample weight used in current AOAC Official TDF methods) and incubation conditions needed to be altered. Purity of enzymes was paramount. The sample is incubated with the enzyme mixture for 16 hours, so even very minor contaminating activities could lead to reduced recoveries of soluble dietary fiber, both

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high and low molecular weight. As much as possible, the gravimetric determinations and HPLC methodology matched that in existing AOAC *Official Methods*℠.

The study was designed and conducted according to AOAC guidelines. A precollaborative ruggedness study was conducted with a number of laboratories to ensure adequate method performance. A call was issued for volunteer laboratories to participate in evaluating the methodology. Collaborating laboratories were sent six samples along with copies of the method, ion exchange resins, and a supply of enzymes. Each laboratory was requested to run each sample in singlet. Laboratories were requested to conduct the analysis, ask questions regarding procedures and write-up, and provide feedback to the study directors on any aspects of the method for which the collaborator might have a concern.

The results of the precollaborative ruggedness study were typical for dietary fiber methods. Repeatability, reproducibility, and the HorRat were within the range of performance characteristics typically found for dietary fiber methods wherein a significant number of manual steps are necessary to carry out the assay. Relevant comments received from the participating laboratories were incorporated as changes to improve the method as appropriate. No procedural changes were found to be necessary, and only minor edits of the text for clarity were incorporated. Therefore, the study directors determined the method was ready for full collaborative study.

Collaborative Study

Eight food samples were selected for the collaborative study. The samples were chosen to be challenging, with emphasis on quantitating products high in RS (legumes, RS ingredient, and whole grain products) and products with typical levels of NDO (all samples). Moist samples were freeze-dried before grinding. All samples were ground to method-specific size and homogenized by thorough mixing before being subdivided into polyethylene bottles and sealed. Samples, copies of the method, report sheets, and sample storage instructions, along with an adequate supply of enzymes and deionizing resins were shipped to collaborating laboratories by express overnight shipment. A total of 16 laboratories reported data for the collaborative study samples.

Applicability

The performance characteristics of the method (accuracy, repeatability, and reproducibility) were acceptable according to AOAC criteria. The method is applicable to determination of total dietary fiber (including RS and NDO) in plant and food products and dietary fiber preparations.

Results

The dietary fiber content of the eight test pairs ranged from 12.13 to 49.35%. Digestion of samples under the conditions of AOAC Method **2002.02** followed by the isolation and gravimetric procedures of AOAC Methods **985.29** and **991.43** results in quantitation of high-molecular-weight dietary fiber (HMWDF). The filtrate from the quantitation of HMWDF is concentrated, deionized, concentrated again, and analyzed by HPLC to determine LMWSDF, i.e., all NDO of DP ≥ 3. Total dietary fiber is calculated as the sum of HMWDF and LMWSDF. Repeatability standard deviations (s_r) ranged from 0.40 to 1.38%, and reproducibility standard deviations (s_R) ranged from 1.20 to 5.52%. This is comparable to other official dietary fiber methods.

Problems Encountered

Many participants were unable to perform the analysis in a timely manner due to other commitments. The method was designed for determination of NDO using the Waters Sugar Pak® HPLC column with D-sorbitol as internal standard. However, some analysts preferred to use the gel-permeation chromatographic arrangement described in AOAC *Official Method*℠ **2001.03**. With this system, D-sorbitol is not a suitable internal standard as it chromatographs with D-glucose. Thus, in these cases, internal standards were not used. To resolve this problem and to make the method more versatile, alternative internal standards have been evaluated, and diethylene glycol (recommended by Okuma, Matsutani Chemical Co.) appears to be the best option.

Conclusion

Interlaboratory evaluation of the method showed that it is suitable for the measurement of total dietary fiber, including RS and NDO, in a wide range of plant and food products. On the basis of the study, it was recommended that the method be granted Official First Action, and it was subsequently adopted as **2009.01**. The method is available online at www.aoac.org (click on "OMA Online") or directly at http://eoma.aoac.org/. The full collaborative study is published in the January/February 2010 issue of *J. AOAC Int.* As part of the evaluation of the method, the second stage of the interlaboratory evaluation will again involve analyzing 16 samples (eight blind duplicates), but in this evaluation, IDF, HMWSDF, and LMWSDF will be measured. This study will be initiated in March/April 2010. ■

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