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Application News

No.L507

High Performance Liquid Chromatography

Analysis of Low Molecular Weight Soluble Dietary Fiber by Prominence-i

Research into the physiological effects of dietary fiber has been ongoing since the results of epidemiological research showing dietary fiber reduces incidence of diseases that include digestive system disorders, circulatory system disorders, and diabetes. This research has revealed a variety of effects of dietary fiber, including the reduced absorption of lipids like cholesterol from the intestines, and assistance with adsorption and excretion of harmful substances. This functionality has garnered interest in recent years, with chemically synthesized dietary fiber being added to an increasing number of processed foods. Some of nutritional labeling of foods show dietary fiber content, and such labeling is a regulatory obligation in some countries.

Many substances can be classified as dietary fiber, with definitions varying by country and policy. Dietary fiber mainly refers to: cellulose, hemicellulose, and lignin that make up cell walls; animal polysaccharides like chitin and chitosan (insoluble dietary fiber), polysaccharides like pectin (high molecular weight soluble dietary fiber), and chemically synthesized polysaccharides like polydextrose and indigestible dextrin (low molecular weight soluble dietary fiber).

Dietary fiber is normally measured using an enzymatic-gravimetric method (prosky method). This involves subjecting a sample to an enzymatic reaction, filtering the sample, and measuring the weight of residue as the amount of dietary fiber. A problem with this method is its low rate of recovery for low molecular weight soluble dietary fiber, such as polydextrose and indigestible dextrin. Total dietary fiber is obtained by adding the quantitative result obtained by the enzymatic-gravimetric method to the quantitative result obtained by HPLC analysis of filtrate obtained from the enzymatic-gravimetric method. Japan's food labeling standards (Cabinet Office Ordinance No. 10, dated March 20, 2015) specify dietary fiber to be measured by a prosky method or HPLC method.

This application news describes an analysis of the amount of low molecular weight soluble dietary fiber by an enzymatic-HPLC method, based on the Food Labeling Standards — Appendix: Methods of analysis of nutritional composition[1].

The instrument used was a Prominence-i integrated HPLC system installed with an RID-20A differential refractive index detector.

Pretreatment of Low Molecular Weight Soluble Dietary Fiber in a Beverage

Two types of cold beverages containing indigestible dextrin were prepared according to the analytical method[1]. The pretreatment procedure is shown in Fig. 1. After treatment with three different enzymes (after the step "*" in Fig. 1), samples would normally require ethanol precipitation and filtration steps. However, these steps can be omitted when analyzing samples that clearly contain only low molecular weight soluble dietary fiber. The steps were omitted for both the beverages analyzed, since they were presumed to contain only low molecular weight soluble dietary fiber. Samples were then passed through an ion-exchange column before being used as the HPLC sample solution. Please refer to the analytical method[1] for further information.

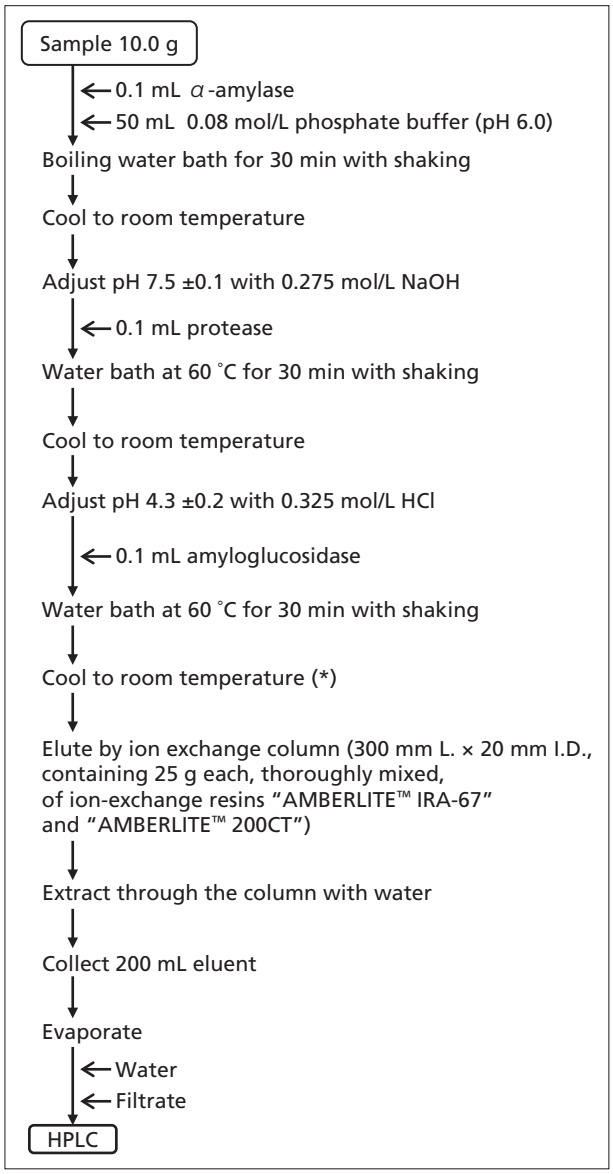

Fig. 1 Pretreatment Procedure

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HPLC Analysis of Low Molecular Weight Soluble Dietary Fiber

The two beverages were prepared according to the procedure shown in Fig. 1, and analyzed by HPLC. The results of HPLC analysis are shown in Fig. 2 and 3 alongside the results of HPLC analysis of maltotriose. Analytical conditions are shown in Table 1. The saccharides detected were divided into monosaccharides, disaccharides, and trisaccharides or larger on the chromatograms, with trisaccharides or larger being regarded as the dietary fiber fraction. The published analytical method[1)] states to analyze the trisaccharide maltotriose under the same conditions as the sample, then to include all peaks that elute equal to or earlier than maltotriose in the dietary fiber fraction. The published analytical method[1)] states to use either a ligand exchange column or gel filtration column for HPLC. Analysis was performed using a Shim-pack SPR-Na ligand exchange column. Ligand exchange columns are mainly used in carbohydrate analysis, and are excellent at separating carbohydrates. Ligand exchange columns can easily separate glucose from other carbohydrates, and are therefore suited to analyses using glucose as an internal reference standard as shown below.

The actual quantification analysis was performed using glucose produced by hydrolysis of starch as an internal reference standard. The amount of glucose in each sample was calculated by a separate pyranose oxidase method, and the amount of low molecular weight soluble dietary fiber was obtained by multiplying the peak area ratio of the dietary fiber fraction to the glucose peak by the amount of glucose. Depending on the sample and HPLC column type, glucose may not separate from accompanying components. If glucose does not separate from other components, quantification can be performed by adding a known concentration of glycerin or another internal reference standard after the enzyme pretreatment steps, then calculating amounts based on the peak area ratio of the dietary fiber fraction to the internal reference standard. Analytical sensitivity will differ when a compound other than glucose is used as the internal reference standard, and results will need to be corrected accordingly by calculating a coefficient relative to analytical sensitivity for glucose.

The amount of dietary fiber is shown by adding the amount of low molecular weight soluble dietary fiber calculated by HPLC analysis to the result obtained by a separate prosky method. This method of calculation includes oligosaccharides in the dietary fiber fraction, so to show the amount of dietary fiber in the sample minus indigestible oligosaccharides, the amount of indigestible oligosaccharides must be deleted from the results. Please refer to the analytical method[1)] for further information.

Table 1 HPLC Analytical Conditions

System	: Prominence-i, RID-20A
Column	: Shim-pack SPR-Na (250 mm L. × 7.8 mm I.D., 8 µm) × 2
Guard Column	: Shim-pack SPR-Na (50 mm L. × 7.8 mm I.D., 8 µm)
Mobile Phase	: Water
Flowrate	: 0.50 mL/min
Column Temp.	: 80 ˚C
Injection Volume	: 20 µL
Detection	: RID-20A

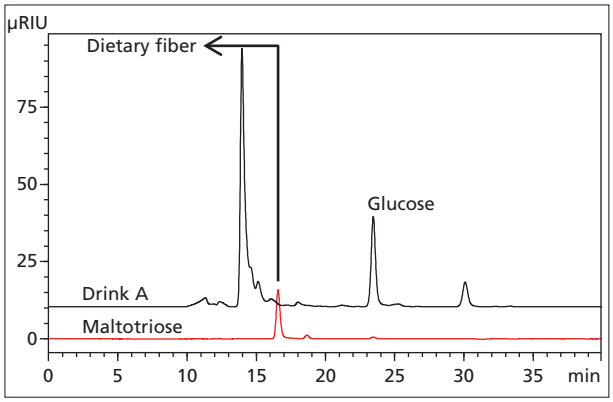

Fig. 2 Analysis of Low Molecular Weight Soluble Dietary Fiber in Beverage A

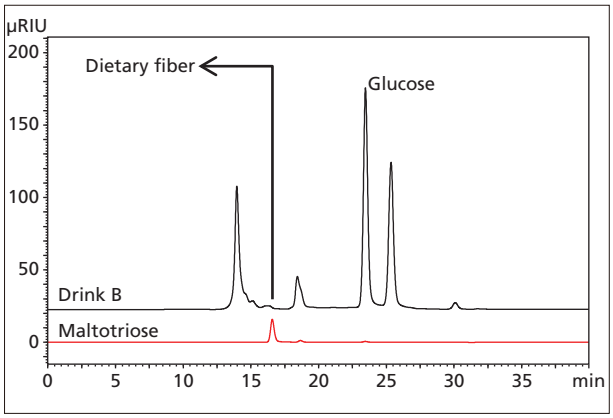

Fig. 3 Analysis of Low Molecular Weight Soluble Dietary Fiber in Beverage B

1) Food Labeling Standards
— Appendix: Methods of analysis of nutritional composition (Notification No. 139 from the Deputy Secretary General of the Consumer Affairs Agency, dated March 30, 2015) [In Japanese]

First Edition: Oct. 2016

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