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Application News

High performance Liquid Chromatograph

Analysis of Dietary Fiber in Functional Beverage Using Automated Dietary Fiber Analyzer Combined with Integrated HPLC

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User Benefits

- The automated dietary fiber analyzer reduces labor and shortens processing time through automated dietary fiber analysis.
- Both insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) can be measured in accordance with AOAC 2022.01.
- Low-molecular-weight soluble dietary fiber (SDFS), which typically shows low recovery rates with the enzymatic-gravimetric method, can be analyzed using HPLC.

■ Introduction

Dietary fiber is an indigestible carbohydrate present in foods such as grains, legumes, fruits, and vegetables[1]. A high intake of dietary fiber has been associated with a reduced risk of mortality from heart disease, stroke, type 2 diabetes, and certain cancers including breast, colorectal, and pancreatic cancer[2]. Adequate dietary fiber intake also helps support healthy weight management and improves the intestinal environment [2].

Dietary fiber can be classified into two types: IDF and SDF. IDF, such as cellulose, hemicellulose, and lignin, is abundant in grains and contributes to water absorption and intestinal function. SDF, including glucomannan, gum arabic, pectin, fucoidan, and β-glucan, is found in foods such as fruits, vegetables, konjac, seaweed, and mushrooms, and is known for its cholesterol-lowering effects [3] .

Traditionally, the enzymatic-gravimetric method (Prosky method) has been widely used for dietary fiber measurement. However, this method often shows low recovery rates for SDFS such as resistant dextrin and polydextrose. AOAC 2022.01[4] analyzes the filtrate from the enzymatic-gravimetric method using HPLC, quantifying fractions of trisaccharides and larger as SDFS.

This article introduces dietary fiber analysis based on the AOAC 2022.01 method, utilizing an automated dietary fiber analyzer (ANKOM Technology)[5] with integrated HPLC.

■ Sample pretreatment of dietary fiber

The dietary fiber pretreatment process involves enzymatic digestion of food, followed by filtration and ethanol precipitation, with independent measurements of IDF and SDF using the enzyme-gravimetric method. SDFS is measured by recovering the filtrate from the enzymatic-gravimetric method, removing proteins and salts from ethanol-soluble components using ion-exchange resin, and then analyzing with HPLC. This article references the latest AOAC 2022.01 method. The AOAC 2022.01 method provides enzyme processing conditions closer to those of the human small intestine by employing a combination of pancreatic α-amylase (PAA) and amyloglucosidase (AMG). The enzymatic digestion time is reduced to 4 hours in AOAC 2022.01, compared to 16 hours in AOAC 2011.25.

The automated dietary fiber analyzer significantly reduces researchers' workload and processing time. Fig. 1 illustrates the dietary fiber pretreatment process using this integrated measurement system. Required steps such as preparing glassware, overnight ashing of glass crucibles, constant-temperature shaking in a water bath, sample transfer, and suction filtration become unnecessary by using disposable filter bags instead of crucibles in the automated system. Computer-controlled operations—including enzyme and solvent dispensing, temperature regulation, stirring, and filtration—eliminate human error and improve precision. Fig. 2 shows the automated dietary fiber analyzer and integrated HPLC.

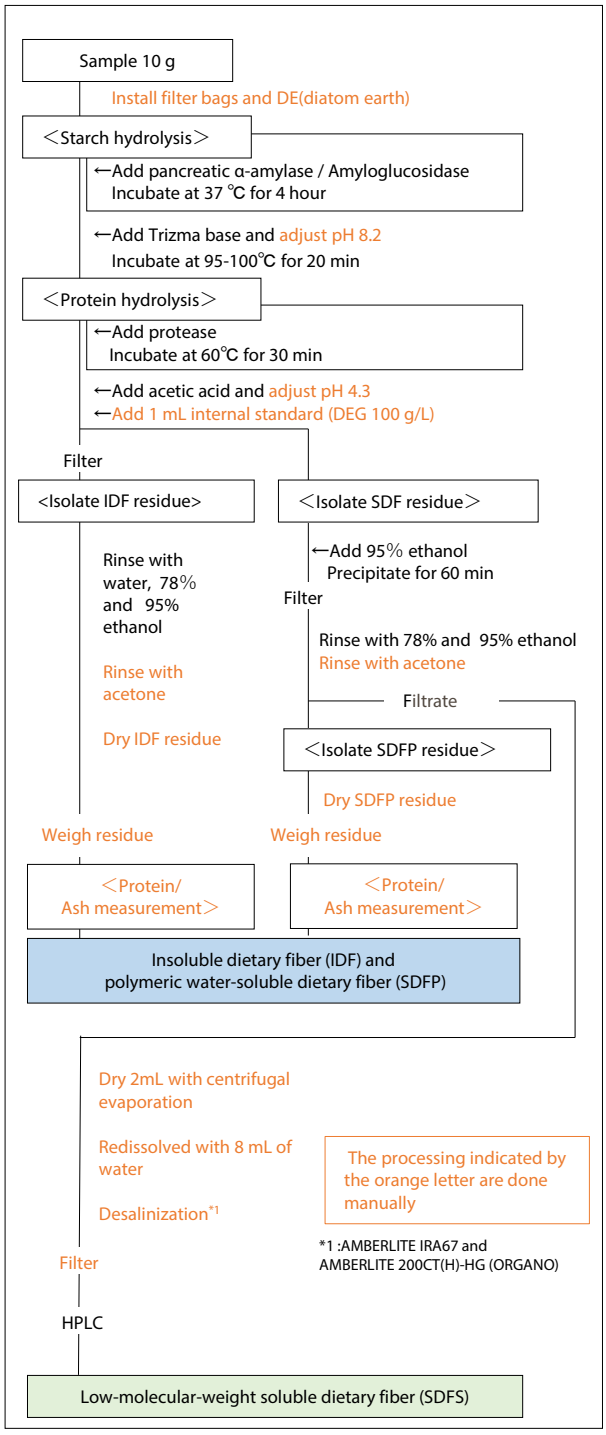

Fig. 1 Sample pretreatment of dietary fiber using automated dietary fiber analyzer

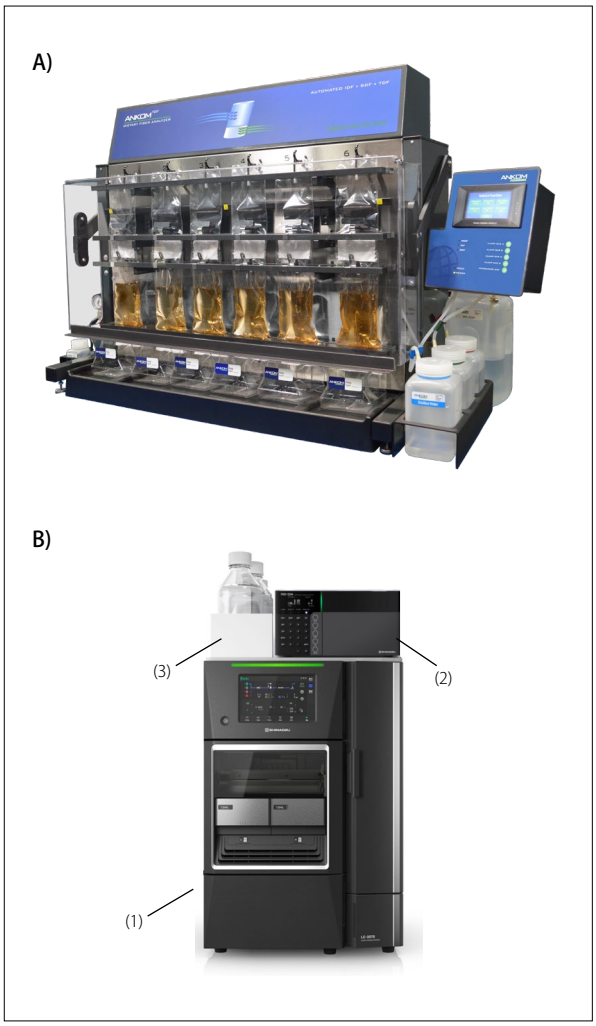

Fig. 2 A) Automated dietary fiber analyzer "Dietary fiber TDF"
B) Integrated HPLC (1)LC-2070 (2)RID-20A (3) Reservoir tray

■ HPLC Analysis of SDFS

Three commercially available functional soft drinks containing resistant dextrin were processed using an automated dietary fiber analyzer. Diethylene glycol (DEG) was used as the internal standard. As these samples consisted mainly of SDFS, HPLC analysis was conducted specifically for this analyte. When determining TDF content in foods that contain other types of dietary fiber in addition to SDFS, the procedure shown in Fig. 1 can also be applied to measure IDF and high-molecular-weight water-soluble dietary fiber (SDFP) by analyzing protein and ash content.

The enzymes used for pretreatment and the reagents for confirming HPLC retention times were supplied in the Rapid Integrated Total Dietary Fiber Assay Kit (reference code: K-RINTDF) from Megazyme. For HPLC analysis, a Bio-Rad desalting cartridge was used as the ion-exchange guard column, Shim-pack™ SUR-Na(G) as the guard column, and two Shim-pack SUR-Na columns connected in series as the analytical columns. Detection was performed using a differential refractive index detector. Fig. 3 shows the chromatogram of the "LC retention time standard" included in the Megazyme K-RINTDF kit. The SDFS fractionation interval was determined based on maltodextrin elution. Chromatograms of the functional beverages are shown in Figs. 4 to 6. HPLC analytical conditions are summarized in Table 1.

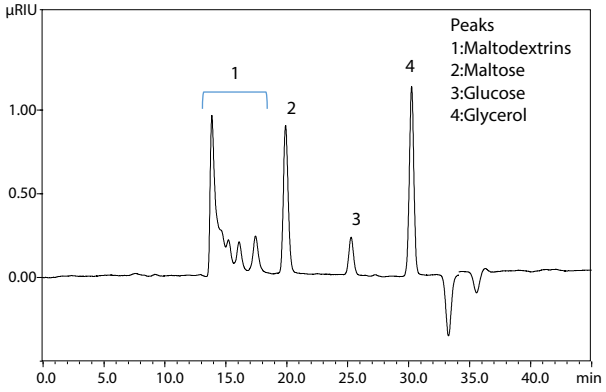

Fig. 3 Chromatogram of LC retention time standard

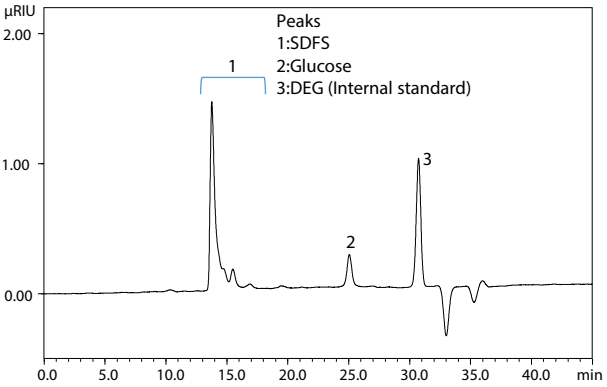

Fig. 4 Chromatogram of SDFS in functional beverage A

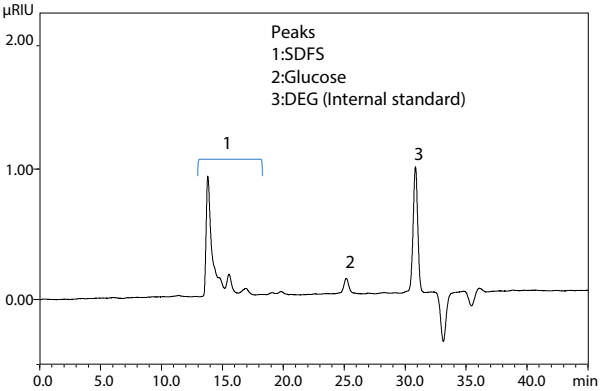

Fig. 5 Chromatogram of SDFS in functional beverage B

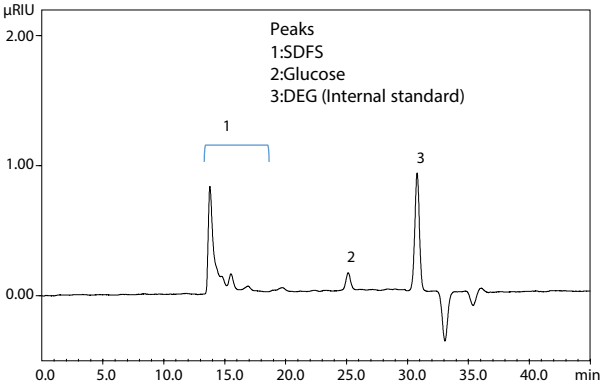

Fig. 6 Chromatogram of SDFS in functional beverage C

Table 1 Analytical conditions

System	: LC-2070
Cation and anion deionization cartridge	: Bio-Rad Micro-Guard De-Ashing Refill Cartridges *1
Guard Column	: Shim-pack SUR-Na(G) *2 (50 mm × 7.8 mm I.D., 8 µm)
Column	: Shim-pack SUR-Na *3 × 2 (250 mm × 7.8 mm I.D., 8 µm)
Mobile phase	: Water
Column temperature	: 80 ℃
Flow rate	: 0.5 mL/min
Injection volume	: 50 µL
Vial	: Shim-vial™-H glass *4
Detection	: Refractive index detector (RID-20A) Polarity +, Cell temp. 40 ℃, Response 1.5 sec

*1 Cat No. 125-0118, 125-0139(Holder) , *2 P/N：228-59529-02
*3 P/N：228-59529-01, *4 P/N：227-34500-01

The SDFS content per 100 g of the functional beverage was calculated based on the HPLC results. First, 10 mg/mL standard solutions of D-glucose and DEG (internal standard) were analyzed by HPLC. The response factor (RF) was calculated from the peak area ratio and mass ratio according to Formula 1. The RF obtained for this analysis was 0.75.

$$Response\ factor(RF) = \frac{PA_{IS}}{PA_{Glu}} \times \frac{Wt_{IS}}{Wt_{Glu}} \quad \text{—Formula (1)}$$

PA_{Glu} : Peak area of D-glucose
PA_{IS} : Peak area of DEG internal standard
Wt_{Glu} : Mass of D-glucose in standard 1 mL
Wt_{IS} : Mass of DEG in standard 1 mL

Then, the amount of SDFS per 100 g of each sample was calculated according to Formula 2.

$$\mathrm{SDFS}(mg/100g) = \frac{RF \times \mathrm{Wt}_{IS} \times PA_{SDFS}}{PA_{IS}} \times \frac{100}{M} \quad \text{—Formula (2)}$$

RF： Response factor calculated from formula (1)
Wt_{IS} ： Mass in mg of internal standard contained in 1 mL of internal standard solution (100 mg/mL)
PA_{SDFS} ：Peak area of SDFS
PA_{IS} ：Peak area of internal standard DEG
M：Sample amount (g)

Table 2 summarizes the measurement results of SDFS in functional beverages. The labeled content of resistant dextrin—a type of SDFS indicated on product packaging—per 100 g of beverage was compared with the HPLC measurement results. The calculated values ranged from 102% to 110% of the labeled content, indicating good agreement.

Table 2 Comparison between labeled and measured low-molecular-weight dietary fiber contents in functional beverages

Sample	Functional beverage A	Functional beverage B	Functional beverage C
Measured content (g/100g) Average (n = 2)	1.46	1.10	1.10
Labeled content (g/100g) (Conversion as 1mL = 1 g)	1.43	1.03	1.00
Measured /Labeled (%)	102	107	110

Samples of functional beverages containing dietary fiber powder (resistant dextrin) were processed according to Fig. 1, followed by a spike-and-recovery test. Table 3 presents the results of the spike-and-recovery test. The recovery rates obtained ranged from 92% to 106%, indicating favorable accuracy.

Table 3. Results of the spike-and-recovery test (Average, n = 2)

Sample	Functional beverage A	Functional beverage B	Functional beverage C
Spike amount (g/100g)	1.39	1.02	1.00
Content of spiked sample (g/100g) Average (n = 2)	2.88	2.18	2.02
Recovery rate (%)	102	106	92

■ Conclusion

High-performance liquid chromatography (HPLC) analysis was performed to quantify low-molecular-weight soluble dietary fiber (SDFS) in functional beverages containing resistant dextrin. Sample pretreatment was conducted using a fully automated dietary fiber analyzer (ANKOM Technology), which markedly reduced manual labor and processing time by automating the pretreatment workflow. The adoption of disposable filter bags further minimized processing time, thereby decreasing operator workload and conserving valuable working hours.

Quantification of SDFS was achieved using integrated HPLC. The Shim-pack SUR-Na column, utilized for this analysis, provides high-resolution separation of target saccharides by combining size-exclusion and sodium-type ligand-exchange chromatographic modes. This configuration is particularly suitable for the analysis, as SDFS is operationally defined as trisaccharides and larger oligosaccharides.

A comparative assessment between the labeled resistant dextrin content on product packaging and the measured SDFS values in the functional beverages demonstrated strong concordance. Additionally, spike-and-recovery experiments confirmed the favorable accuracy of the analytical method.

■ Acknowledgement

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<References>

1) D Mudgil, S. Barak, Int J Biol Macromol, 61 (2013), pp. 1-6
2) Weiwen Hong et al., Nutrition Volume 111, July 2023, 112035
3) D Mudgil et al., J Anim Sci, 98 (2020), p. Skaa303
4) AOAC Official Method 2022.01 Insoluble, Soluble, and Total Dietary Fiber in Foods and Food Ingredients: Rapid Integrated Enzymatic-Gravimetric-Liquid Chromatography
5) ANKOM TDF Dietary Fiber Analyzer | ANKOM Technology

<Abbreviations>

TDF = IDF + SDF = IDF + SDFP + SDFS
Total dietary fiber (TDF)
Insoluble dietary fiber (IDF)
Soluble dietary fiber (SDF)
Polymeric water-soluble dietary fiber (SDFP)
Low-molecular-weight soluble dietary fiber (SDFS)

Enzymes and Reagents
Pancreatic α-amylase (PAA)
Amyloglucosidase (AMG)
Diethylene glycol (DEG)

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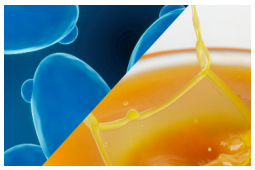

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