Vietnam Journal of Food Control

A Scientific Journal of National Institute for Food Control

Journal homepage: http://vjfc.vn/

Research Article

Determination of total dietary fiber, soluble dietary fiber, and insoluble dietary fiber in foods using an enzymatic-gravimetric-liquid chromatographic method

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(Received: 02 Jan 2026; Revised: 08 Feb 2026; Accepted: 09 Feb 2026)

Abstract

Dietary fiber has been widely recognized for its beneficial effects on human health. Consequently, fiber is increasingly incorporated into dietary supplements and functional foods to enhance daily fiber intake. However, quality control of these products remains challenging due to the structural diversity of dietary fiber and the need for different analytical approaches for each fiber type. This study aimed to determine soluble dietary fiber (SDF), insoluble dietary fiber (IDF), and total dietary fiber (TDF) in food matrices using a combined enzymatic-gravimetricliquid chromatographic method. The method enables the simultaneous determination of TDF, SDF, and IDF in a single analysis and is particularly suitable for complex food samples with unknown fiber composition. Starch was hydrolyzed using pancreatic α-amylase and amyloglucosidase at 37°C for 4 h, while proteins were digested with protease. IDF was quantified by gravimetric analysis, whereas SDF was determined by liquid chromatography with a refractive index detector. The method was validated in accordance with AOAC guidelines, demonstrating satisfactory specificity, linearity, repeatability (RSD 3.1 - 4.4%), reproducibility (RSD 4.3 - 7.1%), and recovery (92.1 - 105.8%). The validated method was applied to the analysis of 50 randomly collected commercial food samples. Natural food products such as vegetables, fruits, and cereals exhibited high IDF contents (11.3 - 16.7 g/100g), reflecting their characteristic cellulose- and lignin-rich structures. In cereal products, IDF ranged from 5.8 to 9.9 g/100g. In contrast, functional foods and dietary supplements showed significantly lower IDF levels (0.6 - 3.4 g/100g) and predominantly contained soluble dietary fiber, with SDF ranging from 2.1 to 11.8 g/100g.

Keywords: *Dietary fiber, soluble dietary fiber, insoluble dietary fiber, total dietary fiber, enzyme.*

1. INTRODUCTION

The term dietary fiber was first introduced by Hipsley in 1953 to describe the indigestible components forming plant cell walls, including cellulose, hemicellulose, and lignin [1, 2]. With advances in science and technology, the scope of dietary fiber has progressively expanded. In 1976, Trowell *et al.* proposed that dietary fiber should be defined based on its edibility and resistance to digestion in the human small intestine. Accordingly, dietary fiber included indigestible polysaccharides such as gums, modified celluloses, mucilages, and pectins [3]. In 2009, the Codex Alimentarius Commission established a more comprehensive definition of dietary fiber that also encompasses oligosaccharides. According to Codex [4], dietary fiber consists of carbohydrate polymers with three or more monomeric units that are not hydrolyzed by endogenous enzymes in the human small intestine and belong to one of the following categories: Naturally occurring carbohydrate polymers in food products; Carbohydrate polymers obtained from food raw materials by physical, enzymatic, or chemical means; Synthetic carbohydrate polymers.

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https://doi.org/10.47866/2615-9252/vjfc.4631

With increasing recognition of the importance of health and nutrition, dietary fiber is considered an essential component for maintaining gastrointestinal health and preventing chronic diseases such as obesity, diabetes, cardiovascular disease, and colorectal cancer. Dietary fiber comprises both soluble and insoluble fractions, which not only influence nutrient absorption but also play critical roles in metabolism, glycaemic regulation, and modulation of the gut microbiota [5]. Fiber-enriched food products are becoming increasingly prevalent. Therefore, the development of reliable analytical methods for determining dietary fiber content in foods is essential for quality control and consumer protection.

Since the 1970s, analytical methods for determining total dietary fiber as well as specific fiber fractions have been developed and continuously updated, resulting in multiple official methods that remain valid and widely applied [2]. Due to the structural complexity and heterogeneity of dietary fiber, no single analytical method is capable of quantifying all fiber components in food matrices. Comprehensive determination can only be achieved through a combination of analytical approaches.

Based on the original definition, AOAC Official Method 962.09 determines crude fiber, primarily representing plant cell wall components, using a gravimetric procedure. Subsequently, AOAC Official Method 991.43 became the classical method for determining total dietary fiber. This method separates soluble dietary fiber (SDF), insoluble dietary fiber (IDF), and total dietary fiber (TDF); however, it does not quantify low-molecular-weight dietary fiber (LMWDF) or most types of resistant starch [6, 7]. LMWDF can only be accurately determined using chromatographic techniques. Therefore, beginning with AOAC Official Method 2009.01, liquid chromatography with refractive index detection (LC-RI) was incorporated to quantify this fraction. The chromatographic column conditions have been refined in subsequent AOAC versions to improve the accuracy of LMWDF determination [8, 9].

In the present study, based on AOAC Official Method 2022.01, a combined analytical approach was employed to determine total dietary fiber, soluble dietary fiber, and insoluble dietary fiber in food products, even in the absence of prior information regarding the type of fiber present. The method involves enzymatic digestion under conditions simulating human gastrointestinal digestion, followed by gravimetric determination of insoluble fiber and liquid chromatographic determination of soluble fiber using refractive index detection.

2. MATERIALS AND METHODS

2.1. Research subjects/Materials

Dietary fiber in food products includes the following components: total dietary fiber (TDF), soluble dietary fiber (SDF), insoluble dietary fiber (IDF), high-molecular-weight dietary fiber (HMWDF), non-digestible oligosaccharides (NDO), soluble dietary fiber precipitable in 78% ethanol (SDFP), and soluble dietary fiber remaining soluble in 78% ethanol (SDFS).

The study matrices comprised various food products, including vegetables, fruits, cereals, cereal-based products, dietary supplements, and health supplements collected from supermarkets and pharmacies in the Hanoi market. Fibersol®-2, a resistant starch preparation containing 85.0% dietary fiber, was used as a reference material for method evaluation.

2.2. Chemicals, standards

D-Glucose reference standard was obtained from LGC (Dr. Ehrenstorfer, Germany). Glycerol internal standard solution (100 mg/mL) was purchased from Megazyme. All chemicals used were of analytical grade. Tris base, sodium maleate, and sodium azide were supplied by Sigma-Aldrich (USA). Acetic acid, calcium chloride, and Celite were obtained from Merck KGaA (Germany). The following enzymes and materials were purchased from Megazyme (Ireland): amyloglucosidase (AMG, 3300 U/mL in 50% glycerol), porcine pancreatic α-amylase (50 U/mL) in combination with AMG (3.4 U/mL), protease (50 mg/mL, activity approximately 350 U/mL based on tyrosine release), and ion-exchange resins Amberlite® FPA53 (OH^- form) and Ambersep® 200 (H^+ form). Ethanol, acetone, and certified buffer solutions (pH 4.0, 7.0, and 10.0) were also used in this study.

2.3. Apparatus and equipment

Liquid chromatographic analyses were performed using an LC-40D XR system coupled with a RID-20A refractive index detector (Shimadzu). Chromatographic separations were carried out using a TSK-Gel

G2500PW$_{XL}$ column (7 µm, 7.8 mm i.d. × 30 cm) with a corresponding guard column (Tosoh, Japan). A Micro-Guard De-Ashing refill cartridge (30 × 4.6 mm; Bio-Rad, USA) was used for desalting purposes. A Waters Sugar-Pak column (10 µm, 6.5 mm × 300 mm; Waters, USA) was also employed where appropriate. Drying was conducted using a laboratory oven (Binder, Germany), and ash determination was performed in a muffle furnace (Nabertherm, Germany). Protein analysis was carried out using a Kjeldahl system (VELP, Italy). A rotary vacuum evaporator (Büchi, Switzerland) was used for solvent removal. Ultrasonic extraction was performed using a temperature-controlled ultrasonic bath (Elma, Germany). Enzymatic incubations were conducted in a thermostatically controlled water bath (Memmert, Germany). Filtration was carried out using 50 mL heat-resistant fritted glass crucibles (Pyrex®, USA).

2.4. Research methods

Determination of IDF, SDFP, SDFS, and TDF was carried out sequentially using enzymatic hydrolysis, gravimetric analysis, and liquid chromatographic methods.

2.4.1. Enzymatic hydrolysis

Enzymatic hydrolysis was applied to remove digestible starch and protein fractions. Based on previously reported procedures [9, 10], the method was performed as follows: Two test portions were accurately weighed (approximately 1.0 g each) from homogenized samples using an analytical balance. Samples were moistened with ethanol, followed by the addition of maleate buffer (pH 6.0). The mixture was incubated with porcine pancreatic α-amylase and amyloglucosidase (AMG) at 37°C for 4 h. The reaction was terminated by adjusting the pH to 8.2 with Tris base and heating to 95°C to inactivate AMG, thereby preventing further hydrolysis of resistant starch during subsequent processing. After cooling to 60°C, the samples were incubated with protease for 1 h. The pH was then adjusted to 4.5 to inactivate the protease. Hydrolysis time and enzyme concentration are critical parameters in dietary fiber analysis, influencing both hydrolysis efficiency and total analysis time. In this study, the effects of increased enzyme concentration and reduced hydrolysis time were investigated to optimize analytical performance.

2.4.2. Gravimetric method

The gravimetric procedure was used to determine IDF and SDFP as described in the literature [9, 10]. Following enzymatic treatment, the sample solution was filtered through a heat-resistant fritted glass crucible containing Celite as filtration aid. The residue was dried and weighed. Ash and protein contents in the residue were determined separately, and IDF was calculated as the residue weight corrected for ash and protein. The filtrate was retained for SDF determination.

The filtrate was mixed with 78% (v/v) ethanol, thoroughly stirred, and allowed to stand for at least 1 h to facilitate precipitation. The resulting precipitate was recovered by filtration, washed, dried, and weighed. Ash and residual protein were determined and subtracted from the second residue weight to obtain the value of SDFP.

2.4.3. Liquid chromatographic method

Liquid chromatography was employed for the determination of SDFS. Based on previously described procedures [7, 10], the filtrate remaining after 78% ethanol precipitation was concentrated and reconstituted in 8 mL of deionized water. A 5 mL aliquot of the concentrated solution was transferred into a 15 mL centrifuge tube containing mixed ion-exchange resins (1.5 g Amberlite® FPA53, OH^- form, and 1.5 g Ambersep® 200, H^+ form). The mixture was shaken horizontally for 5 min to ensure complete deionization. The deionized solution was filtered through a 0.45 µm membrane filter and analyzed by HPLC equipped with a refractive index detector (HPLC-RI) for quantification of SDFS.

Chromatographic performance may be significantly influenced by sample matrix effects and the type of column employed. Therefore, sample clean-up procedures were evaluated by comparing analyses performed with and without ion-exchange treatment. Chromatographic separation was also investigated using two serially connected TSK-Gel G2500PW$_{XL}$ columns with a guard column and a Micro-Guard De-Ashing refill cartridge (Bio-Rad), as well as a Waters Sugar-Pak column.

2.4.4. Calculation of results

** Determination of IDF and SDFP by the gravimetric method*

The IDF content of the blank was calculated according to Equation:

$$B\ (mg) = \frac{BR1 + BR2}{2} - P_B - A_B \quad (1)$$

Where $BR1$ and $BR2$ are the residue weights of blank 1 and blank 2 (mg), respectively; P_B is the protein mass determined from the first blank residue (mg); and A_B is the ash mass determined from the second blank residue (mg).

The blank was processed in the same manner as the test sample but without addition of sample material.

The IDF content of the test sample, expressed as mg/100 g, was calculated according to Equation:

$$IDF\ (mg/100g) = \frac{[\,(R_1 + R_2)/2 - P - A - B]}{(M_1 + M_2)\,/\,2} \times 100 \quad (2)$$

Where R_1 and R_2 are the first IDF residue weights obtained from test portions 1 and 2 (mg), respectively; M_1 and M_2 are the corresponding sample weights (mg); P is the protein mass determined in the residue of R_1(mg); A is the ash mass determined in the residue R_2 (mg); B is the IDF value of the blank (mg).

The SDFP content was determined in the same manner as IDF, using the residue obtained after precipitation with 78% (v/v) ethanol.

** Determination of SDFS by liquid chromatography*

A standard solution containing glucose and the internal standard glycerol at concentrations of 10 mg/mL each was analyzed. The response factor (Rf) for glucose was calculated according to Equation:

$$Rf = \frac{A_{IS}}{A_{Glu}} \times \frac{C_{glu}}{C_{IS}} \quad (3)$$

Where A_{IS} is the peak area of the internal standard (glycerol); A_{Glu} is the peak area of glucose; C_{glu} is the concentration of glucose (mg/mL); and C_{IS} is the concentration of the glycerol as internal standard (mg/L).

The SDFS content was calculated according to Equation:

$$SDFS\ (mg/100g) = \frac{Rf \times m_{IS} \times A_{SDFS}}{A_{IS}} \times \frac{100}{M} \quad (4)$$

Where Rf is the glucose response factor calculated from Equation (3); m_{IS} is the mass of glycerol internal standard added per milliliter of internal standard solution (100 mg); A_{SDFS} is the peak area corresponding to SDFS in the sample chromatogram; A_{IS} is the peak area of the internal standard; and M is the sample weight (g).

** Determination of total dietary fiber (TDF)*

Total dietary fiber was calculated as the sum of the individual fractions: TDF = IDF + SDFP + SDFS

2.4.5. Method validation

The analytical method was validated by evaluating the following performance characteristics: Specificity of the chromatographic method, assessed by analyzing blank samples, standard solutions, and spiked blank samples; linearity, evaluated through construction of calibration curves; Limit of detection (LOD) and limit of quantification (LOQ); Repeatability and reproducibility, expressed as relative standard deviation (RSD%), determined by analyzing six replicates (repeatability) and four replicates (reproducibility) using milk powder as the representative matrix; recovery assessed by spiking Fibersol®-2 dietary fiber material at three concentration levels (low, medium, and high) within the working range. The validation results were evaluated in accordance with the acceptance criteria specified by AOAC and Commission Implementing Regulation (EU) 2021/808 for the corresponding concentration levels [12, 13].

3. RESULTS AND DISCUSSIONS

3.1. Evaluation of the enzymatic hydrolysis process

Starch present in the samples was hydrolyzed using porcine pancreatic α-amylase (PAA) in combination with amyloglucosidase (AMG). This step typically requires a considerable incubation time, thereby prolonging the overall dietary fiber analysis. α-Amylase is inactivated at temperatures above 50°C, which is significantly lower than the gelatinization temperature of starch (approximately 65°C) [10, 11]. Several studies have reported that prolonged hydrolysis with α-amylase may promote starch gelatinization, potentially leading to the

formation of resistant maltodextrins from partially hydrolyzed starch. This phenomenon may result in positive bias in dietary fiber determination [4].

The efficiency of enzymatic hydrolysis was evaluated by reducing hydrolysis time while increasing enzyme concentration. Fibersol®-2 was used as the test material and subjected to two hydrolysis conditions: Procedure 1: PAA (1.8 KU)/AMG (0.136 KU) for 16 h; Procedure 2: PAA (4.0 KU)/AMG (1.7 KU) for 4 h. The results obtained under the two hydrolysis procedures are presented in **Table 1.**

Table 1. *Comparison of hydrolysis results obtained using two enzymatic procedures*

Sample	Declared dietary fiber (%)	Procedure 1			Procedure 2		
		IDF (%)	SDFP (%)	SDFS (%)	IDF (%)	SDFP (%)	SDFS (%)
Fibersol®-2	85.0	0	31.6	51.8	0	31.2	52.1

The results presented in **Table 1** indicate that starch and protein hydrolysis under the two evaluated procedures produced no significant differences in the measured dietary fiber fractions. Procedure 2, which employed a higher concentration of starch-hydrolyzing enzymes, allowed the hydrolysis time to be reduced from 16 h to 4 h while still ensuring complete digestion of starch present in the sample. The comparable values obtained for SDFP and SDFS under both conditions confirm that the shortened hydrolysis time did not compromise analytical accuracy. The reduction in hydrolysis time substantially improves laboratory efficiency, enabling completion of sample preparation within a single working day and facilitating subsequent analytical steps. This modification enhances the practicality of the method for routine application without adversely affecting analytical performance.

3.2. Evaluation of ionic effects

Refractive index detectors (RID) detect analytes based on changes in the refractive index of the mobile phase. The enzymatic sample preparation procedure requires buffered conditions to maintain stable pH for optimal enzyme activity; consequently, the hydrolyzed sample solution contains a high concentration of inorganic ions.

The influence of residual anions and cations on chromatographic analysis was evaluated by comparing filtrates subjected to ion-exchange clean-up with those analyzed without clean-up. Ion removal was performed using mixed ion-exchange resins, Amberlite® FPA53 (OH^- form) and Ambersep® 200 (H^+ form). Briefly, the two resins were directly weighed into a centrifuge tube, followed by addition of the sample solution. The mixture was shaken horizontally to allow complete ion-exchange to occur, then centrifuged. The supernatant was filtered and analyzed by HPLC-RI.

Chromatograms of samples analyzed with and without ion removal are presented in **Figure 1.**

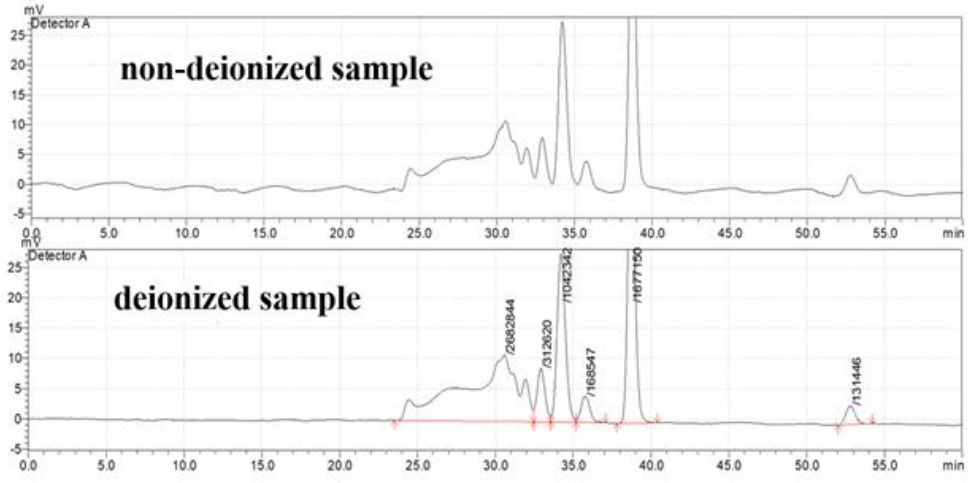

Figure 1. *Chromatograms comparing the effectiveness of the ion-removal process*

Deionization significantly improved the cleanliness of the hydrolyzed solution, resulting in a more stable chromatographic baseline for the ion-exchange-treated sample compared with the sample without ion removal. The deionization step not only reduced analytical error but also contributed to prolonging the lifetime of the analytical column. Therefore, ion removal prior to chromatographic analysis is considered essential.

3.3. Evaluation of chromatographic separation columns

Liquid chromatography was employed for the determination of SDFS, which comprises low-molecular-weight dietary fibers such as fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS). The chromatographic separation was evaluated using two columns specifically designed for saccharide analysis: the Waters Sugar-Pak column and the TSK-Gel G2500PW$_{XL}$ column. The results are presented in **Figure 2.**

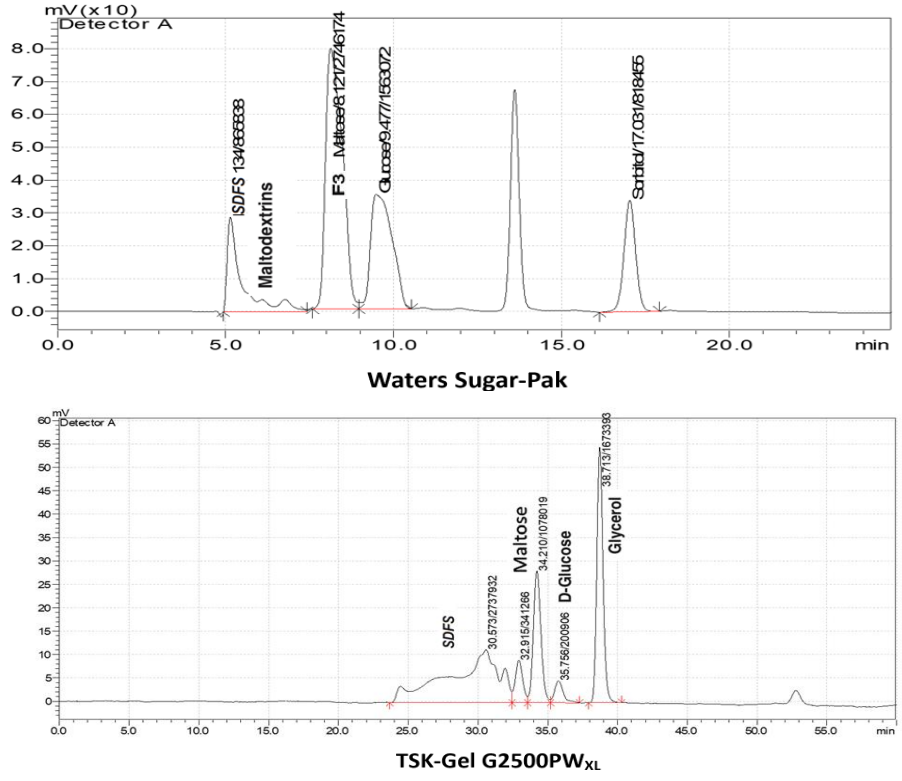

Figure 2. *Chromatograms comparing retention time standards obtained using the Waters Sugar-Pak column and the TSK-Gel G2500PW$_{XL}$ column*

Commercial fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) contain trisaccharides, including fructosyl-β-(2→1)-fructosyl-β-(2→1)-fructose (F3) and glucosyl-maltotriose (GM3), at levels of up to 15% (w/w). When analyzed using the Waters Sugar-Pak column, these compounds co-eluted with maltose, making their determination not feasible. In contrast, the TSK-Gel G2500PW$_{XL}$ column was able to resolve the trisaccharide fraction from maltose [9, 10], demonstrating superior separation performance. This column is therefore more suitable for the analysis of food samples supplemented with these soluble dietary fiber components.

3.4. Evaluation of the method performance compared with the traditional method

AOAC 991.43, developed by AOAC International, combines enzymatic and gravimetric techniques for the analysis of dietary fiber in foods. At the time of its adoption, this method was considered the “gold standard” for determining dietary fiber content; it remains valid and is still widely applied in Vietnam. The performance of the enzymatic-gravimetric-liquid chromatography method was evaluated by comparing dietary fiber composition results with those obtained using the traditional AOAC 991.43 method on food ingredient samples, including Raftilose P95 (soluble fiber FOS), polydextrose, and Fibersol®-2. The analytical results are presented in **Table 2**.

Table 2. *Comparison of dietary fiber analysis results obtained by AOAC 991.43 and the proposed method*

Sample	Declared dietary fiber (%)	AOAC 991.43		Research methods		
		IDF (%)	SDF (%)	IDF (%)	SDFP (%)	SDFS (%)
Raftilose P95 (FOS)	90.2	0	0	0	0	84.5
Polydextrose	90.0	1.0	0	0	1.1	85.4
Fibersol®-2	85.0	0	32.0	0	31.2	52.1

The results in **Table 2** show that AOAC 991.43 cannot quantify dietary fiber in products supplemented with low-molecular-weight soluble fibers such as FOS, GOS, and polydextrose. Applying AOAC 991.43 to these samples would therefore lead to negative bias (underestimation). The proposed method provides a comprehensive determination of dietary fiber fractions, yielding results of 84.5% and 85.4% for Raftilose P95 and polydextrose, respectively. Fibersol®-2 showed a total dietary fiber content of 83.3% (31.2% SDFP and 52.1% SDFS), which is comparable to the declared value of 85.0%.

3.5. Method validation

Validation of the analytical method for dietary fiber determination using the enzyme-gravimetric-liquid chromatography procedure was carried out according to the guidelines of AOAC and EC 2021/808 [12, 13]. The validation parameters included specificity, calibration curve, LOQ, LOD, repeatability, within-laboratory reproducibility, recovery, and measurement uncertainty.

3.5.1. Specificity

Specificity was evaluated by analyzing a blank sample, a standard solution, and a spiked sample using HPLC-RID. The spiked sample was prepared with glucose as the analyte standard, while glycerol was used as the internal standard. The blank sample showed no glucose signal, whereas the standard and spiked samples exhibited glucose peaks at the same retention time. The analytical results are presented in **Figure 3.**

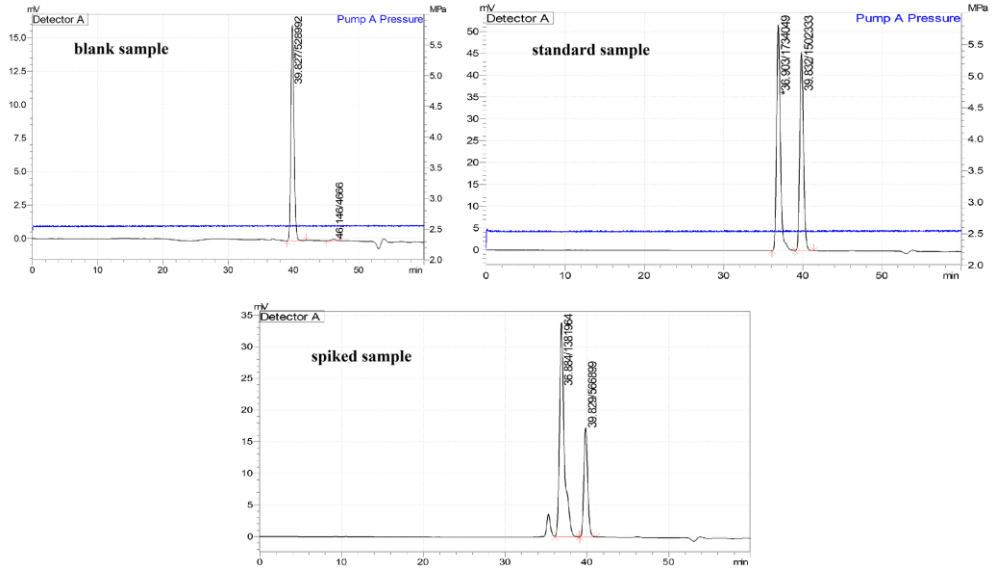

Figure 3. *Chromatograms for specificity evaluation*

3.5.2. Calibration curve

The internal standard calibration curve was established over the concentration range of 50 mg/L to 900 mg/L. The relationship between the response ratio of the glucose standard to the glycerol internal standard and the corresponding glucose concentration was determined. The calibration results are presented in **Figure 4.**

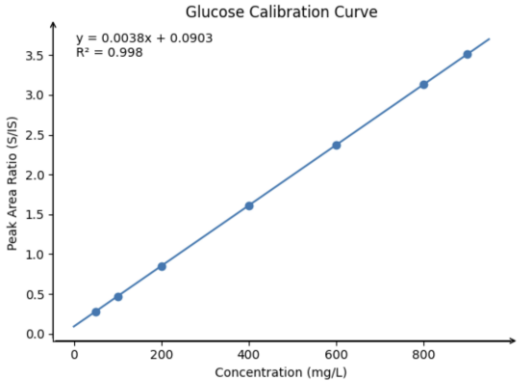

Figure 4. *Calibration curve equation using glycerol as the internal standard*

The calibration results in **Figure 4** show that the coefficient of determination was $R^2 = 0.998$, and the deviation at all concentration levels did not exceed 15%. This indicates that the calibration curve constructed over the glucose concentration range of 50 mg/L to 900 mg/L meets the AOAC requirements.

3.5.3. Limit of detection and limit of quantification

A low concentration sample was analyzed in 10 replicates. The results for the LOD and LOQ are presented in **Table 3.**

Table 3. *Limit of detection and limit of quantification of the method*

	SDFS	**SDFP**	**IDF**
LOD (g/100g)	0.03	0.1	0.1
LOQ (g/100g)	0.1	0.3	0.3

According to Circular 29/2023/TT-BYT issued by the Ministry of Health on the declaration of ingredients and nutritional values on food labels, macronutrients (sugars, protein, carbohydrates, and fat) at levels ≤ 0.5 g/100 g are considered insignificant and are not required to be declared on food labels. The method has limits of quantification of 0.1 g/100 g for SDFS and 0.3 g/100 g for both SDFP and IDF; therefore, it meets the sensitivity requirements and is suitable for the analysis of dietary fiber in food testing and nutritional assessment.

3.5.4. Repeatability and reproducibility

The powdered milk sample was analyzed in six replicates to evaluate repeatability. Within-laboratory reproducibility was assessed by performing the analyses on different days, by different analysts, with four replicate determinations. The results are presented in **Table 4.**

Table 4. *Results of repeatability and reproducibility analysis*

Parameter	**SDFS (%)**	**SDFP (%)**	**IDF (%)**	**TDF (%)**
Mean value (repeatability, n = 6)	3.23	0.52	0.49	4.23
Mean value (reproducibility, n = 4)	3.36	0.56	0.54	4.47
Repeatability RSD_r	3.89	4.39	4.28	3.08
Reproducibility RSD_R	4.66	4.59	7.09	4.26

The repeatability of the method for each fiber fraction ranged from 3.08% to 4.39%, while the reproducibility ranged from 4.26% to 7.09%. All results met the AOAC acceptance criteria [12], indicating that the method is suitable for the analysis of dietary fiber content in foods.

3.5.5. Recovery

Fibersol®-2 (containing 31.2% SDFP and 52.1% SDFS) was used as the spiking material at three concentration levels-low (1.0%), medium (5.0%), and high (10.0%) in a milk powder matrix in which no dietary fiber was detected. The recovery results are presented in **Table 5.**

Table 5. *Recovery analysis results*

SDFS			SDFP			TDF		
Spiking level (g/100g)	**Content (g/100g)**	**Recovery (g/100g)**	**Spiking level (g/100g)**	**Content (g/100g)**	**Recovery (g/100g)**	**Spiking level (g/100g)**	**Content (g/100g)**	**Recovery (g/100g)**
0.31	0.32	102.6	0.52	0.48	92.1	0.83	0.80	96.0
	0.33	105.8		0.50	96.0		0.83	99.6
	0.30	96.2		0.51	97.9		0.81	97.2
1.56	1.53	98.1	2.61	2.52	96.7	4.17	4.05	97.2
	1.55	99.4		2.50	96.0		4.05	97.2
	1.51	96.8		2.55	97.9		4.06	97.5
3.12	3.07	98.4	5.21	5.32	102.1	8.33	8.39	100.7
	3.01	96.5		5.14	98.7		8.15	97.8
	3.14	100.6		5.31	101.9		8.45	101.4

The recovery of the method for each fiber fraction ranged from 96.2% to 105.8% for SDFS, 92.1% to 102.1% for SDFP, and 96.0% to 101.4% for TDF. All results met the AOAC acceptance criteria [12], indicating that the method is suitable for the analysis of dietary fiber content in foods.

3.6. Application of the method to sample analysis

The developed method was applied to determine dietary fiber content in 50 samples of foods and dietary supplements collected from the Hanoi market. The analytical results are presented in **Figure 5.**

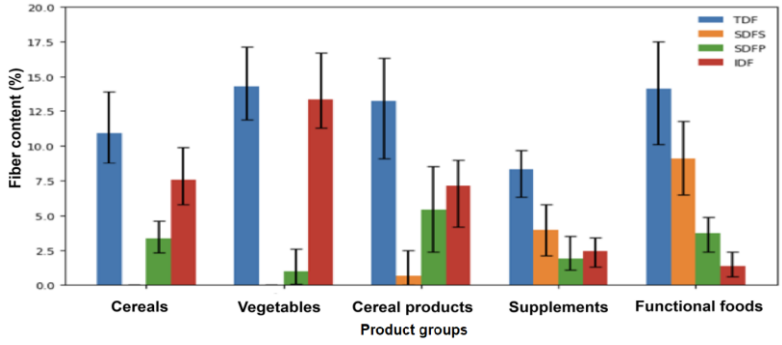

Figure 5. *Results of dietary fiber analysis by product group*

In vegetables and cereals, the predominant fiber fraction is IDF (11.3 - 16.7 g/100 g), reflecting the characteristic cellulose and lignin structure. In cereals, IDF ranges from 5.8 to 9.9 g/100 g. Functional foods and dietary supplements contain significantly lower levels of IDF (0.6 - 3.4 g/100 g). Soluble dietary fiber is mainly present in functional foods and supplements, ranging from 2.1 to 11.8 g/100 g.

Natural foods such as vegetables and cereals are the primary sources of insoluble fiber, which plays an important role in supporting the mechanical aspects of digestion. In contrast, dietary supplements and functional foods containing added fiber ingredients mainly provide soluble fiber. SDFS does not precipitate in ethanol and can only be determined when the appropriate enzyme-chromatographic procedure is applied. If only the currently adopted standard method in Vietnam (TCVN 9050:2012, equivalent to AOAC 991.43) is used, the results will be underestimated because AOAC 991.43 does not quantify SDFS and therefore does not accurately reflect the total dietary fiber content of the samples. The proposed method enables detailed separation and quantification of fiber fractions, particularly SDFS, which can only be detected using HPLC-RID analysis.

4. CONCLUSION

The developed method is suitable for determining dietary fiber content in samples with unknown composition. The procedure simulates food hydrolysis conditions closely resembling digestion in the small intestine and enables the determination of all dietary fiber components as defined by Codex. The method was applied to analyze 50 food samples randomly collected from the market. The results showed that agricultural products predominantly contain fiber in the form of insoluble dietary fiber. In contrast, supplemented products contain high levels of soluble fiber. Some functional foods declare only a single type of soluble fiber (e.g., inulin, GOS, FOS, polydextrose); however, the analytical results still detected other fiber components, possibly originating from excipients used in the formulation of these products.

ACKNOWLEDGEMENTS

This research was funded by the National Institute for Food Control (NIFC), under project code NIFC.DTCS.23.04.

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