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Comprehensive Measurement of Total Nondigestible Carbohydrates in Foods by Enzymatic−Gravimetric Method and Liquid Chromatography

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Total nondigestible carbohydrate (NDC) in foods was determined by combining, not modifications, AOAC Official Methods 991.43, 2001.03, and 2002.02. Total NDC included insoluble dietary fiber (IDF) + high-molecular-weight soluble dietary fiber (HMWSDF), nondigestible oligosaccharides (NDO) not precipitated in ethanol solution, and resistant starch (RS). Eight sources of NDC (cellulose, wheat bran, gum arabic, resistant maltodextrin, polydextrose, fructooligosaccharide, galactooligosaccharides, and RS) were incorporated in different combinations into standard formula bread samples. All of the NDC sources and bread samples were analyzed for their (1) IDF + HMWSDF content with corrections for residual RS amount using AOAC Official Method 991.43, (2) NDO by liquid chromatography (LC) in AOAC Official Method 2001.03, and (3) RS by AOAC Official Method 2002.02. The correlation coefficient (R^2) comparing calculated amounts versus measured amounts of total NDC in 11 bread samples was 0.92. Analysis of commercial food samples was also well matched with the DF + NDO value on their nutritional label. Consequently, we confirmed a single measurement of LC can determine all NDO in foods, and total NDC in foods can be determined by unifying existing AOAC Official Methods.

KEYWORDS: Dietary fiber; soluble dietary fiber; insoluble dietary fiber; nondigestible oligosaccharides; nondigestible carbohydrate; resistant starch; enzymatic−gravimetric method; liquid chromatography

INTRODUCTION

Methods to determine dietary fiber (DF) in foods are an integral part of food labeling requirements, nutrient content claims, health claims, and nutrient databases worldwide. Most counties and international organizations recognize AOAC Official Methods for the analysis of DF. Currently, there are 16 AOAC approved official methods for the measurement of DF in foods including nondigestible carbohydrates (NDC) such as resistant maltodextrin (RMD), fructans, β-glucans, and resistant starch (RS) as referred to in **Table 1**. It is noted that AOAC Official Method 991.43 is the most commonly used modification today (change is use of buffer) of AOAC Official Method 985.29, and both methods are commonly referred to as the "Gold Standard" of total DF analysis. It has been over 20 years since AOAC Official Method 985.29 has been approved, and modifications or advances to this method have not been proposed to include all forms of nondigestible carbohydrates.

It is well-known that AOAC Official Methods 985.29 and 991.43 do not measure soluble nondigestible oligosaccharides (NDO) with degree of polymerization (DP) less than 13, which do not precipitate in ethanol solution (*1*). A second important limitation of these two methods is that they cannot measure all forms of resistant starch (RS) (*2*). Both soluble NDO and RS do not get degraded by human digestive enzymes, and their physiological effects as dietary fiber have been reported by researchers (*3*−*6*). We are still in the midst of a debate on the definition of DF; however, significant international organizations and countries such as AACC International, U.S. Institute of Medicine (IOM), European Union (EU), Food Standards Australia New Zealand (FSANZ), and Japan have included nondigestible oligosaccharides and RS in their DF definition. To correct these omissions and inaccuracies in AOAC Official Methods 985.29 and 991.43, specific methods were developed independently to measure each nondigestible carbohydrate and have been designated as AOAC Final Action approved methods as listed in **Table 1**, such as AOAC 997.08 for fructans by ion-exchange chromatography, AOAC 999.03 for fructans by spectrophotometry, AOAC 2000.11 for polydextrose, AOAC 2001.02 for *trans*-galactooligosaccharides, AOAC 2001.03 for TDF containing resistant maltodextrin, and AOAC 2002.02 for RS (*7*). It appears that a single measurement for all nondigestible oligosaccharides (NDO) is advantageous, and one comprehensive method to measure all nondigestible carbohydrates (NDC) in foods would be helpful internationally in order to

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Table 1. AOAC Official Methods[a] for Analysis of Dietary Fiber and Nondigestible Carbohydrates in Foods

AOAC Official Method title	analyte	analytical technique
985.29 Total Dietary Fiber in Foods	fiber/total dietary fiber	enzymatic−gravimetric method
991.42 Insoluble Dietary Fiber in Food and Food Products	fiber/insoluble dietary fiber	enzymatic−gravimetric method, phosphate buffer
991.43 Total, Soluble, and Insoluble Dietary Fiber in Foods	fiber/soluble dietary fiber, fiber/total dietary fiber, fiber/insoluble dietary fiber	enzymatic−gravimetric method, MES−Tris buffer
992.16 Total Dietary Fiber	fiber/total dietary fiber	enzymatic−gravimetric method
992.28 Beta-D-Glucans in Oat and Barley Fractions and Ready-To-Eat Cereals	β-D-glucans	enzymatic−spectrophotometric method
993.19 Soluble Dietary Fiber in Food and Food Products	fiber/soluble dietary fiber	enzymatic−gravimetric method (phosphate buffer)
993.21 Total Dietary Fiber in Foods and Food Products with $\leq$2% Starch	fiber/total dietary fiber	nonenzymatic−gravimetric method
994.13 Total Dietary Fiber (Determined as Neutral Sugar Residues, Uronic Acid Residues, and Klason Lignin)	fiber/total dietary fiber as neutral sugar residue, uronic acid residues, and klason lignin	gas chromatographic−colorimetric−gravimetric method, Uppsala method
995.16 Beta-D-Glucan in Barley and Oats	β-D-glucans	streamlined enzymatic method
997.08 Fructans in Food Products	fructans	ion-exchange chromatographic method
999.03 Measurement of Total Fructan in Foods	fructans/total fructan	enzymatic−spectrophotometric method
2000.11 Polydextrose in Foods	sugars/polydextrose	ion chromotography
2001.02 Determination of *trans*-Galactooligosaccharides (TGOS) in Selected Food Products	sugars/*trans*-galactooligosaccharides	ion-exchange chromatography
2001.03 Dietary Fiber Containing Supplemented Resistant Maltodextrin (RMD)	fiber/total dietary fiber	enzymatic−gravimetric method, liquid chromatography determination
2002.02 Resistant Starch in Starch and Plant Materials	starch/resistant starch	enzymatic digestion
2006.08 Methylcellulose and Hydroxypropyl Methylcellulose Food Gums in Food and Food Products	fiber/soluble dietary fiber, gums	liquid chromatography

[a] See ref *7*.

prevent double counting NDC by applying both the method for total DF (AOAC 985.29 or 991.43) and another method for a specific ingredient (*2*, *8*). Actually, FSANZ is instructing to subtract the double counted nondigestible carbohydrates when using two analytical methods, one for total DF and one for a specific ingredient (*9*).

The objective of this study is to test and verify a single measurement of liquid chromatography (LC) to determine all soluble low-molecular-weight NDO in foods and then the comprehensive method for the determination of all NDC in foods. Since it is considered to be advantageous if all NDC in foods can be determined by combining existing AOAC Official Methods, we have investigated the applicability of these AOAC Official Methods in line: the extension of AOAC 991.43 with RS correction, AOAC 2001.03 to include all NDO by single LC, and independent AOAC 2002.02 for RS.

This method is one of the comprehensive methods preliminarily presented at the group workshop of "Dietary Fiber 2006" held in Helsinki, Finland, on June 11, 2006 (*10*).

MATERIALS AND METHODS

The apparatus and reagents specified in each AOAC Official Method were used. Specialized apparatus and reagents were described here.

Apparatus. The LC system with an oven capable of maintaining a column temperature of 80 °C and LC columns were purchased from Tosoh Corp. (Tokyo, Japan) for LC measurement in AOAC Official Method 2001.03. Column operating conditions were as follows: temperature, 80 °C; mobile phase, distilled water; flow rate, 0.5 mL/ min. LC columns, two TSK-GEL G2500PW$_{XL}$ (7.8 mm i.d. × 30 cm), were connected in series with a guard column, TSK Guard Column PW$_{XL}$ (6.0 mm i.d. × 4 cm). The detector was RI-8020, a refractive index (RI) detector.

Reagents. The 0.05 M MES−Tris buffer solution was prepared with MES [2-(*N*-morpholino)ethanesulfonic acid], Tris [tris(hydroxymethyl)-aminomethane], and water, adjusting the pH to 8.2 at 24 °C with 6 M NaOH. Enzymes (heat-stable α-amylase, protease, and amyloglucosidase) and a glucose oxidase−peroxidase (GOPOD) reagent for glucose assay were purchased from Megazyme International Ireland Limited (Wicklow, Ireland). The glycerol assay kit was obtained from Roche (Mannheim, Germany). Celite (acid washed) bedded on a crucible was obtained from Sigma (St. Louis, MO). Mixed-bed ion-exchange resin for desalting, purchased from Organo Corp. (Tokyo, Japan), was prepared by mixing 25 g of Amberlite IRA-67 (OH-type) and 25 g of Amberlite 200CT (HG) H (H-type) to fill up a 300 mm × 15 mm i.d. column. Two types of sodium acetate buffer solutions, 1.2 M buffer of pH 3.8 and 0.1 M buffer of pH 4.5, were prepared with acetic acid, 4 M NaOH, and water.

Nondigestible Carbohydrate (NDC) Samples and Their Bread Samples. Eight test ingredients of NDC were cellulose (Avicel FD101; Asahi Kasei Chemical Corp., Tokyo, Japan), wheat bran (Wheat Bran; Con Agra Foods, Inc., Omaha, NE), gum arabic (sample obtained from TIC Gums, Inc., White Marsh, MD), polydextrose (Litesse Ultra; Danisco A/S, Copenhagen, Denmark), resistant maltodextrin (RMD) (Fibersol-2; Matsutani Chemical Industry Co., Ltd., Hyogo, Japan), fructooligosaccharide (FOS) (Ultra-FOS SC; Encore Technologies LLC, Minneapolis, MN), galactooligosaccharide (GOS) (Oligomate 55NP; Yakult Pharmaceutical Industry Co., Ltd., Tokyo, Japan), and resistant starch (RS) (Novelose 260; National Starch & Chemical Co., Bridgewater, NJ). All ingredients were first individually analyzed for their total NDC content and then forwarded to the baking facility of AIB International (Manhattan, KS), where all 11 test breads were baked. These NDC ingredients were incorporated into a standard bread formula with different combinations as shown in **Table 2**. Control bread sample was prepared with no additional NDC.

Commercial Food Samples. Commercial food samples listed in **Table 3** were purchased in several supermarkets in downtown Chicago in September 2005 for total NDC analysis.

Outline of Analytical Procedure. The procedure used in this analysis is the combination of enzymatic−gravimetric AOAC Official Method 991.43 (*7*) for insoluble DF (IDF) and high-molecular-weight soluble DF (HMWSDF), LC measurement in AOAC Official Method 2001.03 (*7*) for one or all low-molecular-weight soluble nondigestible oligosaccharide (NDO), and AOAC Official Method 2002.02 (*7*) for resistant starch (RS). No modifications or changes were made on each AOAC Official Method except for the volume of washings at the alcohol precipitation step, which followed AOAC Official Method 2001.03. The procedure is outlined in **Figure 1**. Briefly, the method started with AOAC Official Method 991.43, the sequential enzymatic digestion by heat-stable α-amylase, protease, and amyloglucosidase to remove starch and protein,

Table 2. Combinations and Ratios of Nondigestible Carbohydrates Added to Test Bread Samples[a]

ingredient	bread 1	bread 2	bread 3	bread 4	bread 5	bread 6	bread 7	bread 8	bread 9	bread 10	control
cellulose	14[b]	14	14	14	14	14	14	14	14	14	–
wheat bran	14	14	14	14	14	–	–	–	–	–	–
gum arabic	7	7	7	7	7	7	7	7	7	7	–
polydextrose	7	–	–	–	7	7	–	–	–	7	–
resistant maltodextrin	–[c]	7	–	–	7	–	7	–	–	7	–
fructooligosaccharide	–	–	7	–	7	–	–	7	–	7	–
galactooligosaccharide	–	–	–	7	7	–	–	–	7	7	–
resistant starch	7	7	7	7	7	–	–	–	–	–	–
bakery flour	700	700	700	700	700	700	700	700	700	700	700

[a] All bread samples were prepared with the same amount of flour, water, wheat gluten, sugar, shortening, salt, and yeast to make up about 1400 g of dough, varying by the amount of nondigestible carbohydrates added and taking out 1048 g to bake two loaves. [b] Amount in grams of ingredient incorporated into bread dough. [c] –, not added.

Table 3. Result of Analysis for Total Nondigestible Carbohydrates (NDC) in Commercial Foods and Comparison with Value on Nutritional Label

		total NDC (% to sample weight, dry basis)	
commercial food samples[a]	nondigestible ingredients shown on ingredient panel	analytical value[b]	labeled value[c]
yogurt	resistant maltodextrin	21.66	20.3
orange juice	orange pulp, resistant maltodextrin	11.57	9.8
nutrition beverage	oat fiber, soy fiber, fructooligosaccharide, cellulose, carrageenan	7.87	6.8
breakfast cereal	oat, resistant maltodextrin, brown rice, rye, wheat bran	24.41	22.4
biscuit cereal	wheat bran, resistant maltodextrin	19.62	17.7

[a] These commercial food samples were purchased in supermarkets in downtown Chicago in September 2005. [b] Total NDC = (IDF + HMWSDF + part of RS) + NDO − RS correction (third crucible) + RS. Average of duplicate analyses. [c] Calculated from the nutrition panel on the label as the sum of dietary fiber and separately stated NDO contents.

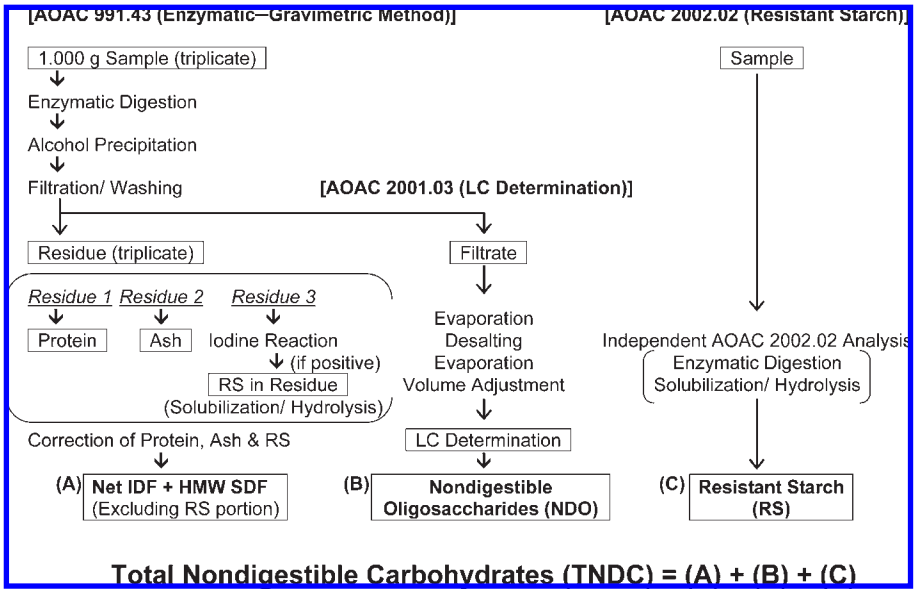

Figure 1. Outlined procedure of comprehensive analytical method for total nondigestible carbohydrates (NDC).

then alcohol precipitation, and filtration. The sum of net IDF and high-molecular-weight SDF was corrected after alcohol precipitation for protein, ash, and the RS portion remaining as IDF. The filtrate of alcohol precipitation was desalted, and all soluble low-molecular-weight NDO was determined by LC in AOAC Official Method 2001.03. RS was determined independently by AOAC Official Method 2002.02. In this study, we measured TDF described in AOAC Official Method 991.43 (the sum of IDF and HMWSDF); however, the separation of these two values is possible, if required, by following the procedure for IDF and SDF as described in AOAC Official Method 991.43.

Sample Preparation. The dried, ground, sieved with a 1.70 mm opening mesh, and defatted (if containing >10% fat) sample weighing accurately 1.000 ± 0.005 g was transferred into a 500 mL tall-form beaker, whose weight was recorded beforehand. Along with triplicate test samples three blanks were also assayed to see any contribution from reagents to the residue. To the sample dispersed and hydrated completely (e.g., with sonication) in 40 mL of MES−Tris buffer solution was added 50 μL of heat-stable α-amylase solution. The sample in a beaker covered with aluminum foil was shaken at 95 °C for 30 min in a shaker water bath and then cooled to 60 °C to add 100 μL of protease solution. After being held at 60 °C for 30 min with continuous agitation, 5 mL of 0.561 M HCl was added, followed with additional 1 M NaOH or 1 M HCl to adjust the pH to 4.1−4.8 at 60 °C. In the same manner 200 μL of amyloglucosidase was added and held at 60 °C for 30 min.

Determination of Insoluble Dietary Fiber and High-Molecular-Weight Soluble Dietary Fiber (AOAC Official Method 991.43). To each of the three digested test solutions and the three blank digestions was added 4 volumes of 95% ethanol (heated to 60 °C in advance). After 1 h rest at room temperature to form precipitate, the sample solution was filtrated by suction using a water aspirator or vacuum pump through ca. 1.0 g of Celite layered on a Pyrex glass crucible filter that previously has been dried to constant weight. The 500 mL tall-form beaker and residue

were washed two times with 20 mL of 78% ethanol using a glass rod to scrape precipitate clinging to the wall of the beaker, two times with 10 mL of 95% ethanol, and two times with 10 mL of acetone.

The filtrate and washings were quantitatively transferred to a 1 L round-bottom flask and reserved for determination of low-molecular-weight NDO by LC. The crucible containing the DF residue was dried in an air oven at 105 °C overnight and then cooled in a desiccator. The crucible carrying the residue and Celite was weighed to the nearest 0.1 mg to calculate residue weight by subtracting the weight of the dry crucible with Celite.

The weight of residue after subtracting protein, ash, RS remaining as IDF, and the blank residue weight represents weight of "net IDF and HMWSDF" in this method. If necessary, IDF and HMWSDF are independently obtainable by following the procedure for IDF and "soluble dietary fiber" described in AOAC Official Method 991.43. The triplicate crucibles served to determine protein, ash, and RS contents: one triplicate from each test sample to determine protein by AOAC Official Method 960.52 (*7*) using $N \times 6.25$ as conversion factor; another triplicate for ash analysis, incinerated at 525 °C for 5 h and weighed to the nearest 0.1 mg after cooling in a desiccator to calculate ash weight by subtracting the weight of the dry crucible with Celite; the third triplicate for RS correction.

Correction of Resistant Starch by the Third Crucible (AOAC Official Method 2002.02). The analysis for RS correction with the third triplicate was conducted only when the residual RS was detected. Iodine−starch reaction was used to check whether undegraded starch (RS) remains on the crucible or not. If no residual starch was detected, the following procedure for RS correction was omitted. The procedure followed the AOAC Official Method 2002.02, starting from solubilization of RS with KOH.

The residue with Celite on the third crucible was scraped, weighed, transferred to a 200 mL beaker, and suspended with 20 mL of 2 M KOH by stirring with a magnetic stirrer bar. RS was carefully dissolved by stirring for ca. 20 min in an ice−water bath over a magnetic stirrer, ensuring that the residue was being vigorously and immediately stirred while adding KOH solution in order not to form a coagulation of starch material. After adding 80 mL of 1.2 M sodium acetate buffer (pH 3.8) while stirring, 10 mL was taken from the 100 mL solution and transferred to a test tube. Soon after adding 0.1 mL of amyloglucosidase solution, the test tube was capped, mixed with a Vortex stirrer, and incubated in a water bath at 50 °C for 30 min with intermittent mixing. The tube was centrifuged at 3000 rpm for 10 min to obtain supernatant, 0.1 mL aliquots (in duplicate) of which (either diluted or undiluted) were transferred into glass test tubes (16 × 100 mm) to react with 3.0 mL of GOPOD reagent. Reagent blank solutions and glucose standards (in quadruplicate) were prepared by mixing 3.0 mL of GOPOD reagent with 0.1 mL of 0.1 M sodium acetate buffer (pH 4.5) or 0.1 mL of glucose (1 mg/mL), respectively. Samples and standards were incubated at 50 °C for 20 min to measure the absorbance at 510 nm against the reagent blank by spectrophotometry. AOAC Official Method 2002.02 was referred to for details of operation and calculation.

Determination of Nondigestible Oligosaccharide by LC (AOAC Official Method 2001.03). The filtrate and washings collected in a 1 L round-bottom flask were evaporated with a rotary evaporator to near dryness. The residue was dissolved with a minimum amount of water and transferred quantitatively to a 50 mL volumetric flask. A known amount of glycerol should be added as LC standard here; however, it was omitted in this work, because an adequate amount of glycerol was already contained in enzyme preparations as stabilizer. The test solution diluted to volume with water was transferred onto a column filled with the resin mixture for desalting, and the extract was washed through the column with 200 mL of water at the rate of 0.8 mL/min.

Approximately 250 mL of eluent from the ion-exchange column was collected and quantitatively transferred into a 500 mL round-bottom flask. The eluent evaporated to near dryness was quantitatively transferred to a 20 mL volumetric flask and diluted to volume with water. After filtrating with a 0.2 μm membrane filter attached to a disposable syringe, 20 μL of the aliquot was served for LC analysis.

LC analysis to determine low-molecular-weight NDO, soluble nondigestible carbohydrates having a degree of polymerization with three monosaccharides (DP3) or higher after enzymatic hydrolysis, was conducted by following AOAC Official Method 2001.03. Each chromatogram was standardized for RI response of low-molecular-weight NDO, and glycerol contained in enzyme solution was used as internal standard for calculation. The glycerol amount in the test solution of the 50 mL volumetric flask was determined by the glycerol assay kit. In case there is no glycerol contained in any regents, the known quantity of glycerol is simply added to the test solution as internal standard.

The weight of glycerol, peak area for glycerol, and peak area for NDO lead the weight of NDO. The peak areas representing concentration obtained by LC analysis of equal amounts of dextrose and NDO were equivalent; however, the peak area for glycerol was not equivalent to the peak areas for an equal amount of dextrose or NDO. A glycerol standard curve was prepared to obtain a "response factor" to calculate the amount of NDO in a chromatogram of each test solution. As the average response factor for glycerol, 0.82 has been reported. AOAC Official Method 2001.03 was referred to for detailed calculations.

Determination of Resistant Starch (AOAC Official Method 2002.02). AOAC Official Method 2002.02 was independently performed to secure the resistant starch amount by the widely recognized official method.

RESULTS AND DISCUSSION

Selection of NDC. The following food ingredients were chosen as the source of nondigestible carbohydrates (NDC): cellulose and wheat bran as insoluble DF (IDF); gum arabic as high-molecular-weight soluble DF (HMWSDF); resistant maltodextrin (RMD), polydextrose, fructooligosaccharide (FOS), and galactooligosaccharide (GOS) as low-molecular-weight soluble DF, i.e., nondigestible oligosaccharides (NDO). The major objective of this study was to develop a series of assays for all forms of nondigestible carbohydrates (NDC) in foods to include NDO and RS. While this study did not deal with any one NDC, but the measurement of foods containing all forms of NDC collectively, including the accurate measurement of RS was an important objective of this study.

Applicability of LC Measurement from AOAC Official Method 2001.03 to NDC. The results reported here on NDO measurement by LC applied from AOAC Official Method 2001.03 demonstrate the quantitative utility of LC not only for the measurement of RMD but also for polydextrose, FOS, and GOS and any combination of these NDOs. **Figure 2** illustrates chromatograms obtained from the analyses of NDC ingredients, used for the quantitative measurement of NDOs in these ingredients. Peak areas were measured, and glycerol was successfully used as the internal standard. IDF (cellulose and wheat bran), high-molecular-weight SDF (gum arabic), and RS did contain little or no low-molecular-weight NDOs by LC measurement, as also shown in the "NDO" column in **Table 4**.

Assessment of LC Internal Standard Substances. To use glycerol as LC internal standard according to AOAC Official Method 2001.03, the glycerol amount derived from the enzyme preparation was checked by a glycerol assay kit. To simplify the procedure of this assay in future, we also reviewed the applicability of other substances as LC internal standard in these aspects: (1) nonfood ingredient to avoid the contamination from food samples, (2) good peak isolation from the other components such as glucose, and (3) full recovery after the evaporation procedure in sample preparation—high boiling point in vacuum. We had conducted LC analyses and vacuum evaporation tests on ethylene glycol, propylene glycol, diethylene glycol (di-EG), and triethlyene glycol (tri-EG) as possible nonfood substances. Among these substances, di-EG and tri-EG had isolated LC peaks at the current LC conditions and were fully recovered by evaporation at 60 °C (data not shown). In order to avoid the second assay for glycerol amount, it would be effective to select another substitutable internal standard such as di-EG or tri-EG.

Assessment of RS Correction Procedure. In this method, RS is measured separately using AOAC Official Method 2002.02

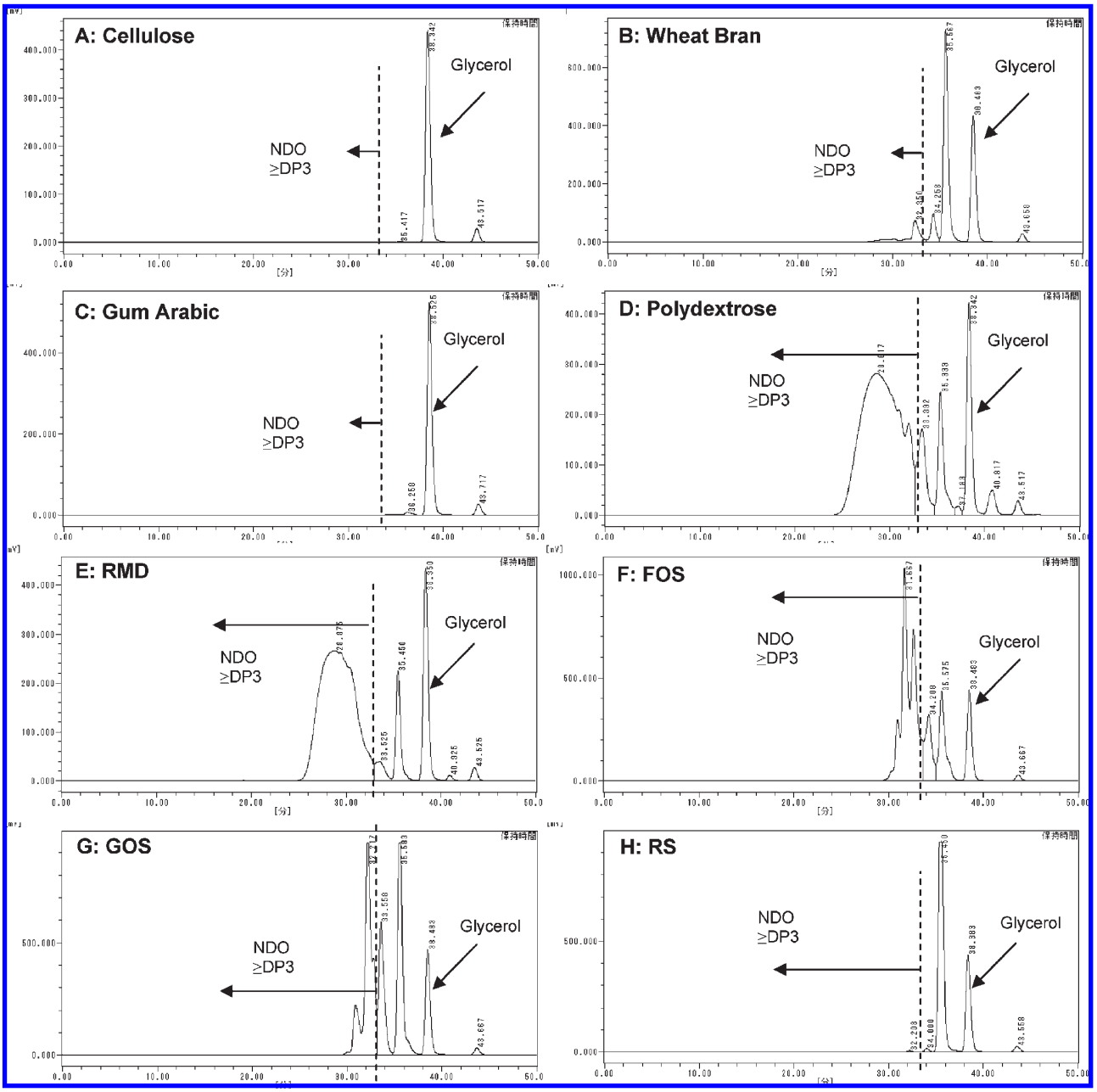

Figure 2. LC chromatograms of test ingredients to determine low-molecular-weight soluble nondigestible carbohydrates: (A) cellulose, (B) wheat bran, (C) gum arabic, (D) polydextrose, (E) resistant maltodextrin, (F) fructooligosaccharide, (G) galactooligosaccharide, and (H) resistant starch.

Table 4. Result of Analysis for Total Nondigestible Carbohydrates (NDC) in Each Test Ingredient Incorporated into Bread for Insoluble Dietary Fiber (IDF), High-Molecular-Weight Soluble Dietary Fiber (HMWSDF), Low-Molecular-Weight Soluble Nondigestible Oligosaccharides (NDO), and Resistant Starch (RS) Contents[a]

ingredient	IDF + HMWSDF + part of RS[b]	NDO[c]	−RS[d] (third crucible)	RS (AOAC 2002.02)[e]	total NDC (wt %, dry basis)[f]
cellulose	99.75	0	(0)	0.18	99.33
wheat bran	39.67	4.86	(0.54)	0.40	44.39
gum arabic	98.39	0	(0)	0.19	98.58
polydextrose	0.47	76.54	(0)	0	77.01
resistant maltodextrin	24.69	68.47	(0)	0	93.16
fructooligosaccharide	0	68.64	(0)	0	68.64
galactooligosaccharide	0	46.13	(0)	0	46.13
resistant starch	63.44	0.26	(63.22)	43.48	43.96
bakery flour	4.32	1.66	(0.55)	1.21	6.64

[a] Mean of duplicate measurements, wt %, dry basis. [b] Determined with AOAC Official Method 991.43. [c] Determined by LC with AOAC Official Method 2001.03. [d] Subtracted RS value determined from third crucible. [e] Determined with AOAC Official Method 2002.02. [f] Total NDC = (IDF + HMWSDF + part of RS) + NDO − RS (third crucible) + RS (AOAC 2002.02).

Table 5. Result of Analysis for Total Nondigestible Carbohydrates (NDC) in Sample Breads with Different Combinations of NDCs[a] and Comparison with Theoretical Value

					total NDC (wt %, dry basis)	
bread[b]	IDF + HMWSDF + part of RS[c]	NDO[d]	−RS[e] (third crucible)	RS (AOAC 2002.02)[f]	analytical value[g]	theoretical value[h]
1	7.77	1.68	(0.79)	1.35	10.01	8.98
2	7.00	1.57	(0.87)	1.27	8.97	9.11
3	7.47	1.10	(0.94)	1.22	8.85	8.91
4	7.38	1.45	(0.91)	1.23	9.15	8.75
5	7.44	2.61	(0.67)	1.29	10.67	10.29
6	6.33	1.87	(0.66)	0.90	8.44	8.11
7	6.77	1.56	(0.61)	0.94	8.66	8.24
8	6.68	1.73	(0.76)	1.03	8.68	8.04
9	6.52	1.05	(0.65)	0.92	7.84	7.87
10	7.70	2.73	(0.43)	0.98	9.98	9.49
control	4.21	1.12	(0.51)	1.06	5.88	5.22
			correlation coeff R^2 (analytical vs theoretical values) = 0.9271			

[a] Mean of duplicate measurements, wt %, dry basis. [b] See **Table 2** for bread composition. [c] Determined with AOAC Official Method 991.43. [d] Determined by LC with AOAC Official Method 2001.03. [e] Subtracted RS value determined from third crucible. [f] Determined with AOAC Official Method 2002.02. [g] Total NDC = (IDF + HMWSDF + part of RS) + NDO − RS (third crucible) + RS (AOAC 2002.02). [h] Calculated values determined on the basis of bread formulations (**Table 2**) and composition of each nondigestible carbohydrate (**Table 4**).

because it is the sole official method for RS; however, in order to avoid double counting of RS in a sample, we found a means to subtract the amount of RS remaining in the combined IDF and HMWSDF residue, thus, the use of a third crucible. If the residue in the third crucible contained any distribution of IDF and high-molecular-weight SDF components along with the RS, only RS could be dissolved with KOH, washed from the residue, and hydrolyzed with amyloglucosidase, and the resulting glucose was measured to calculate the amount of RS. This is the procedure used in AOAC Official Method 2002.02. Any double counting of RS in a sample is thus avoided. By simply testing an iodine−starch reaction on the residue in the third crucible for the presence of RS, we could check the need to run the following process for RS determination.

In order to ascertain the recovery of all RS retained in crucibles after complete enzymatic digestion, four commercial RS samples, FiberStar 70 (MGP Ingredients, Inc., Atchison, KS), RoadStar (Nihon Shokuhin Kako Co., Ltd., Tokyo, Japan), FoodStarch BS-1 (Matsutani Chemical Industry Co., Ltd., Hyogo, Japan), and Matsutani Ayame (Matsutani Chemical Industry Co., Ltd., Hyogo, Japan), were preliminarily assayed. As described in the method for RS correction, residue containing RS in the third crucible was scraped and collected in a beaker together with Celite, and RS was determined after solubilization, hydrolysis, and glucose measurement as described in AOAC Official Method 2002.02. It was confirmed that RS retained on the crucible was not lost during the filtration procedure, showing 96.8% in average recovery of RS to the residue collected by the centrifuge, the original procedure described in AOAC Official Method 2002.02 instead of filtration (data not shown). We also checked the applicability of direct pouring of alkaline solution onto crucibles retaining insoluble residue with Celite for solubilization. The attempt was found to be inapplicable with only 26.6% recovery of resistant starch (data not shown) compared to the centrifuging collection. The result indicated the potential benefits of using a third crucible to extend the utility of AOAC Official Method 991.43.

Analyses of NDC Ingredients, Formulated Bread Samples, and Commercial Food Samples. All eight NDC ingredients incorporated into bread formulations were analyzed for their total NDC content (**Table 4**). "Total NDC" in **Table 4** is the sum of net IDF + HMWSDF + NDO + RS. The RS correction procedure was performed on the RS product, wheat bran, and bakery flour for positive iodine reaction on their third crucible. Most of the substance retained on the third crucible as IDF was RS for RS product. The RS amount on the third crucible did not match the result of independently performed AOAC Official Method 2002.02 analysis, which supports the necessity of independent AOAC Official Method 2002.02 with RS correction. The discrepancy in RS analyses is considered to be caused by different digestive enzymes and temperatures applied in the enzymatic hydrolysis processes of these two methods. Also analyzed was the flour used in the bread formulations. The components known to constitute the DF in flour are arabinoxylan, approximately 2% (*11*), that helps give a degree of elasticity and flow properties to flour-based food products (e.g., bread and pancakes) (*11*, *12*), β-glucan (*12*), cellulose (*12*), and approximately 2% RS (*13*). Since the amount of flour in the bread formula represents about 50% of the total dough weight, a significant amount of NDC was contributed from bread flour. Data reported in **Table 4** were used to calculate the theoretical values of total NDC in breads as formulated in **Table 5**.

Total NDC as the sum of IDF + HMWSDF, NDO, and RS in the prepared bread samples with different combinations (shown in **Table 2**) determined with the same procedure are reported in **Table 5**. In general, analytical and theoretical values in **Table 5** were well matched ($R^2 = 0.92$).

Taking one step further, NDC in other food applications was also determined by using commercial food products such as yogurt, orange juice, nutrition beverage, and cereal products. The result in **Table 3** also reports that the amounts, which the food manufacturers secure on their nutrition labels as dietary fiber and/or indigestible oligosaccharides, have been recovered by this method. Analyzed values tended to be higher than the labeled values, assumed truncation for nutrient value labeled in integers, or partial NDO not measured by the current AOAC Official Methods.

Finally, one comprehensive method for the measurement of all NDC in foods would be convenient, accurate without duplicate counting, and practical for food labeling and regulatory purposes. Results of this study indicate that single measurement of LC to determine all NDO is advantageous, and existing AOAC Official Methods for DF, 991.43 and 2001.03, and for RS, 2002.02, can be combined and extended without modification to accomplish an assay for all forms of NDC in foods. The first advancement of this comprehensive method is the extension of AOAC Official Method 2001.03, which is originally a specific analytical method for total DF in foods containing RMD. AOAC Official Method 2001.03 has employed high-performance LC to determine the low-molecular-weight NDO portion of RMD, not precipitated in ethanol. Theoretically, all NDO, which is not

degraded by α-amylase and glucoamylase used in AOAC Official Methods 985.29, 991.43 and 2001.03, could be measured by the LC procedure as outlined in AOAC Official Method 2001.03 (*1*). The second advancement is the improved accuracy of RS determination. As a further step to simplify the test procedure, we should check whether the preparation of the third crucible can be omitted by sharing the residue on the second crucible for both protein and RS determination.

ABBREVIATIONS USED

NDO, nondigestible oligosaccharide; NDC, nondigestible carbohydrate; RS, resistant starch; LC, liquid chromatography; DF, dietary fiber; IDF, insoluble dietary fiber; HMWSDF, high-molecular-weight soluble dietary fiber; RMD, resistant maltodextrin; FOS, fructooligosaccharide; GOS, galactooligosaccharide; MES, 2-(*N*-morpholino)ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; GOPOD, glucose oxidase−peroxidase; DP, degree of polymerization.

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