FOOD COMPOSITION AND ADDITIVES

Determination of Total Dietary Fiber in Selected Foods Containing Resistant Maltodextrin by Enzymatic-Gravimetric Method and Liquid Chromatography: Collaborative Study

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A method was developed for determination of total dietary fiber (TDF) in foods containing resistant maltodextrin (RMD) which includes nondigestible carbohydrates that are not fully recovered as dietary fiber by conventional TDF methods such as AOAC 985.29 or 991.43. Because the average molecular weight (MW) of RMD is 2000 daltons, lower MW soluble dietary fiber components do not precipitate in 78% ethanol; therefore, RMD is not completely quantitated as dietary fiber by current AOAC methods. The accuracy and precision of the method was evaluated through an AOAC collaborative study. Ten laboratories participated and assayed 12 test portions (6 blind duplicates) containing RMD. The 6 test pairs ranged from 1.5 to 100% RMD. The method consisted of the following steps: (*1*) The insoluble dietary fiber (IDF) and high MW soluble dietary fiber (HMWSDF) were determined by AOAC 985.29. (*2*) Ion exchange resins were used to remove salts and proteins contained in the AOAC 985.29 filtrates (including ethanol and acetone). (*3*) The amount of low MWRMD (LMWRMD) in the filtrates were determined by liquid chromatography. (*4*) The TDF was calculated by summation of the IDF, HMWSDF, and LMWRMD fractions having nondigestible carbohydrates with a degree of polymerization of 3 and higher. Repeatability standard deviations (RSD_r) were 1.33–7.46%, calculated by including outliers, and 1.33–6.10%, calculated by not including outliers. Reproducibility standard deviations (RSD_R) were 2.48–9.39%, calculated by including outliers, and 1.79–9.39%, calculated by not including outliers. This method is recommended for adoption as Official First Action.

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With the elucidation of the physiological attributes of dietary fiber and its relationship to health (1), the importance of dietary fiber in our diets has been well recognized. In many countries, dietary fiber is considered the 6th group of the major nutrients along with proteins, saccharides, fats, vitamins, and minerals. Conversely, the actual intake of dietary fiber is declining every year as result of our changing life style, especially our eating habits. It is thought that the increased consumption of tastier processed foods that replace unprocessed foods has contributed to the decreased consumption of dietary fiber. A solution to this problem is to find more ways of adding dietary fiber to these processed foods to reduce the gap between intake levels and recommended levels.

Dietary fiber has been divided into 2 categories: insoluble and soluble. This division helps explain the use of dietary fiber in food systems and physiological effects in the body (2). However, these types of dietary fiber are not always suitable for all processed foods: some insoluble dietary fiber can have an unpleasant taste and texture, and some soluble dietary fiber provides undesirable viscosity and or gelatinization properties when added to processed foods. Alternatively, new types of low molecular weight (MW) soluble dietary fiber have been developed that can easily be added to various foods to avoid these problems.

One example of a new source of dietary fiber, which can be added to most processed foods, is resistant maltodextrin (RMD; 3). However, because RMD and other sources of dietary fiber that can be added to foods do not completely precipitate in 78% ethanol after the enzyme treatments, they are not measurable as dietary fiber by current AOAC methods (4). The inability of current AOAC methods to reliably determine total dietary fiber (TDF) in foods containing RMD prompted us to develop this method. It is also important that methods accurately measure all forms of dietary fiber. This method is val-

idated and has been used routinely to determine TDF in Japan under the Food Nutrition Standards since May 1996.

The appropriateness of the method was evaluated through an AOAC collaborative study.

Collaborative Study

The detailed procedure for the proposed method was submitted to 10 participating laboratories. Collaborators were provided with 12 test food portions (6 blind duplicates) and 2 practice test portions with known dietary fiber content. A liquid chromatography (LC) guard column and 2 LC separation columns were loaned to collaborators if needed. Two containers of charged ion-exchange resins (Amberlite IRA-67 [OH-type] and Amberlite 200 [H-type]) were supplied to some collaborators to save their time for charging and facilitating completion of the collaborative study. The 12 test samples identified in letters were RMD (A and I), candy (B and G), powdered soup (C and K), juice (D and J), bread (E and H), and Jell-O® (F and L). The test samples were chosen through consideration of the following factors: commercial usage of RMD as a low MW soluble dietary fiber; stability and preservation of test samples during shipment and analysis; a range of low to high content of RMD in test samples; and homogeneity of the distributed test samples.

AOAC Official Method 2001.03
Total Dietary Fiber in Foods Containing Resistant Maltodextrin
Enzymatic-Gravimetric Method and Liquid Chromatography Determination
First Action 2001

[This method is applicable to resistant maltodextrin (RMD) and to foods containing RMD listed in Table **2001.03** at ≥1.4% RMD.]

A. Principle

This method determines total dietary fiber (TDF) value of processed foods containing insoluble dietary fiber (IDF) and high molecular weight soluble dietary fiber (HMWSDF), which are precipitated in ethanol and low molecular weight resistant maltodextrin (LMWRMD), which is soluble in ethanol. This method defines dietary fiber (DF) as consisting of nondigestible carbohydrates having a degree of polymerization with 3 sugar moieties (DP3) or higher after enzymatic hydrolysis (5). All the starches contained in food are converted to glucose after this enzymatic hydrolysis. This method to determine TDF content in processed foods containing RMD is a combination of **985.29** (*see* 45.4.07) for DF and a LC method for LMWRMD. A food is first analyzed for the total quantity of IDF and HMWSDF, precipitated in ethanol, according to **985.29** (*see* 45.4.07). Then an LC determination is conducted on the desalted filtrate to obtain the quantity of LMWRMD not precipitated in the 78% alcohol preparation. These 2 values [(IDF + HMWSDF) + LMWRMD] are summed to obtain the TDF value in the food.

B. Apparatus

(**a**) *Balance*.—Analytical, weighing to 0.1 mg.

(**b**) *Beakers*.—Tall-form, 500 mL.

(**c**) *Water baths*.—To maintain a temperature of 95–100°C and 60°C with ability to shake the containers.

(**d**) *Filtering crucibles*.—Coarse, ASTM, 40–60 μm pore size, Pyrex, 50 mL.

(**e**) *Glass or plastic columns*.—To hold ion exchange resins, 75 cm × 15 mm id; a shorter (40–75 cm × 15 mm id) column can also be used.

(**f**) *Liquid chromatograph (LC)*.—With oven to maintain a column temperature of 80°C and a 20 μL injection loop. Column operating conditions are: Temperature, 80°C; mobile phase, distilled water, **C**(**d**); flow rate, 0.5 mL/min.

(**g**) *Guard column (or precolumn)*.—TSK® guard column PWXL, 6.0 mm id × 4 cm (Tosoh Corp., distributed by TosoHaas, Montgomeryville, PA, USA; www.tosohbiosep.com) or equivalent.

(**h**) *LC columns*.—Two LC columns connected in series, TSK-GEL® G2500PWXL, 7.8 mm id × 30 cm (Tosoh Corp.), or equivalent.

(**i**) *Detector*.—Refractive index (RI); maintained at 40°C.

(**j**) *Data integrator or computer*.—For peak area measurement.

(**k**) *Filters for disposable syringe*.—0.2 μm membrane, 13 mm.

(**l**) *Filters for water*.—0.2 μm, 47 mm.

(**m**) *Filter apparatus*.—To hold 47 mm, 0.2 μm filter, (**l**); to filter larger volumes of water, **C**(**d**).

Table 2001.03. Interlaboratory results for the determination of total dietary fiber in selected foods containing resistant maltodextrin by enzymatic-gravimetric method and liquid chromatography

Food	$\bar{x}$, %	No. labs[a(b)]	s_r	RSD_r, %	s_R	RSD_R, %
Resistant maltodextrin	95.36	8(0)	1.63	1.71	2.37	2.48
Hard candy	37.99	7(1)	0.58	1.53	0.68	1.79
Chicken and vegetable soup	25.41	8(0)	0.74	2.89	1.18	4.65
Grapefruit juice	1.38	8(0)	0.02	1.33	0.04	3.20
White bread	9.60	8(0)	0.33	3.41	0.64	6.66
Strawberry Jell-O	9.91	8(0)	0.60	6.10	0.93	9.39

[a(b)] a = Number of laboratories retained after eliminating outliers; b = number of laboratories removed as outliers.

(**n**) *Glass rods*.—With fire-polished ends, ca 20 cm long.

(**o**) *Syringes*.—10 mL, plastic disposable.

(**p**) *Pasteur pipet*.

(**q**) *Volumetric pipet*.—10 mL.

(**r**) *Volumetric flasks*.—10, 50, 250, and 1000 mL.

(**s**) *Top loading balance*.—4000 g capacity.

(**t**) *Tubing*.—PVC, 2.79 mm id (for ion exchange columns).

(**u**) *Glass LC syringe*.—50 μL.

(**v**) *Teflon scraping rod*.—Use in place of glass stirring rod to scrape precipitate from tall-form beaker.

(**w**) *Rotary evaporator*.—R-3000VW "Student" (Büchi, Switzerland; www.buchi.com) or equivalent.

C. Reagents

(**a**) *Ethanol*.—95%. Technical grade, used at 60°C.

(**b**) *Ethanol*.—78%. Place 207 mL water in 1 L volumetric flask and dilute to volume with 95% ethanol, (**a**).

(**c**) *Acetone*.—Reagent grade.

(**d**) *Distilled water*.

(**e**) *Sodium phosphate dibasic.*

(**f**) *Sodium phosphate monobasic.*

(**g**) *Phosphate buffer*.—0.08M, pH 6.0. Dissolve 1.400 g Na_2HPO_4 (or 1.753 g dihydrate) and 9.68 g $NaH_2PO_4 \cdot H_2O$ (or 10.94 g dihydrate) in ca 700 mL water, (**d**). Dilute to 1 L with water, (**d**), and verify pH with pH meter.

(**h**) *Heat stable α-amylase solution (Termamyl)*.—No. 120L (activity: 12 units/mg protein; Novo Laboratories, Inc., 59 Danbury Rd, Wilton, CT 06897, USA), or equivalent (should not contain glycerol).

(**i**) *LC retention time standard*.—Standard source of the distribution of oligosaccharides (DP ≥3) in the LMWRMD fraction of RMD, corn syrup solids (DE 25; Matsutani Chemical Industry Co., Ltd., Itami City, Hyogo, Japan; www.matsutani.com), analyzed by LC (Figure **2001.03A**) as in **D**.

(**j**) *Protease*.—No. P-3910 or P-5380 (activity: 7–15 units/mg protein; Sigma Chemical Co., St. Louis, MO, USA), or equivalent (should not contain glycerol). Prepare protease stock solution just before use by adding 100 mg protease enzyme to a 10 mL volumetric flask and bringing to volume with water, (**d**), (amount is sufficient for ≥9 test portions in duplicate).

(**k**) *Amyloglucosidase*.—No. A-9913 (activity: 400 units/mg protein; Sigma Chemical Co.), or equivalent (should not contain glycerol).

(**l**) *Celite*.—No. C-8656 (Sigma Chemical Co.) or No. C-211, acid washed (Fisher Scientific Co., Fair Lawn, NJ, USA), or equivalent.

(**m**) *Mixed-bed ion exchange resins for each test portion*.—(*1*) *m-1*.—25 g Amberlite IRA-67 (OH-type; Organo Corp., Tokyo, Japan, www.bioscorpio.com/organo_corp.htm), or equivalent.

(*2*) *m-2*.—25 g Amberlite 200 CT(HG)H (H-type; Organo Corp.), or equivalent, are mixed and packed in column for analysis of each test portion. The converted resin should satisfy the following specifications: (*a*) Total ion exchange capacity: 1.74 meq/mL (min); (*b*) Effective ion exchange capacity (R-H exchange capacity): 1.6 meq/mL (min); (*c*) pH: 4–7. Before mixing and packing the 2 resins into a column, wash each resin with H_2O to obtain a pH value of 7–8.8 for *m-1* and 4–7 for *m-2*. If Amberlite 200CT(HG)H cannot be obtained, Amberlite 200 (Na-type; Sigma Chemical Co.) or Amberlite 200CT (Organo Corp.) can be used by converting it to "H-type" by the following procedure:

Fill column (100 cm × 40 mm id), **B**(**e**), with 600 mL (500 g) Amberlite 200 "Na-type" resin and determine approximate resin volume. Wash resin with 2 volumes of water, (**d**), at the rate of 60 mL/min. Pass 2 volumes of 10% HCl (1 + 3, w/w) through the resin at the rate of 60 mL/min. Remove HCl with 3 volumes of water, (**d**), passed through the resin at the rate of 60 mL/min. Add 3–6 volumes of additional water, (**d**), at the rate of 120 mL/min. The column is adequately washed of HCl when a pH value of 4–7 is obtained. (It takes 2–3 h to charge and rinse these resins.)

(**n**) *Sodium hydroxide*.—0.275M; reagent grade. Dissolve 11.0 g NaOH in ca 700 mL water, (**d**), in a 1 L volumetric flask. Dilute to volume with water, (**d**).

(**o**) *Hydrochloric acid*.—0.325M; reagent grade. Dilute stock solution of known titer, e.g., 325 mL 1M HCl, to 1 L with water.

(**p**) *Glycerol (LC standard)*.—10 mg/mL. For stock solution: weigh 10 g glycerol >99.5% purity into a small beaker. Quantitatively transfer to 1 L volumetric flask with repeated washes with water, (**d**), and dilute to volume. It is important to measure and record the exact weight of the glycerol, weighing as close to 10 g as possible. Take purity and weight of glycerol into consideration when calculating concentration of final glycerol LC standard.

(**q**) *Glycerol (for dextrose–glycerol standard)*.—100 mg/mL. Weigh 10 g high purity glycerol into a small beaker, transfer to a 100 mL volumetric flask with water, (**d**), and dilute to volume.

(**r**) *Ammonium sulfate*.—Reagent grade; standard to test micro-Kjeldahl procedure.

(**s**) *Dextrose*.—LC grade, high purity >99.5%.

(**t**) *Silver nitrate solution*.—0.1M. Dissolve 1.70 g $AgNO_3$ in ca 70 mL water, (**d**), in a 100 mL volmetric flask, and dilute to volume with water, (**d**).

D. Determination

(**a**) *Enzymatic hydrolysis and filtration*.—Weigh 1.0 g test portion (crushed, sieved to 10 mesh, fat extracted if >10% fat, and dried) into a 500 mL previously weighed tall-form beaker, **B**(**b**). Prepare in duplicate with 2 blank digestion determinations. Disperse in 50 mL 0.08M phosphate buffer, **C**(**g**), and sonicated to ensure complete hydration. Add 100 μL heat stable α-amylase, **C**(**h**), and cover beaker with aluminum foil. Place beaker in shaker water bath and hold at 95°C for 30 min with shaking. Cool to room temperature, and adjust the pH of the solution to pH 7.5 ± 0.1 with 0.275M NaOH, **C**(**n**). Add 0.5 mL protease solution, **C**(**j**), and digest solution for 30 min at 60°C. Cool solution to room temperature (25°C), and adjust pH to 4.5 ± 0.2 with 0.325M HCl, **C**(**o**). Add 0.3 mL amyloglucosidase, **C**(**k**), and digest at 60°C for 30 min. Upon completion of the 3 enzyme digestion sequence, add 4 volumes of 95% ethanol, **C**(**a**), by weight, previously heated to 60°C. Use the top loading balance to weigh beaker with diges-

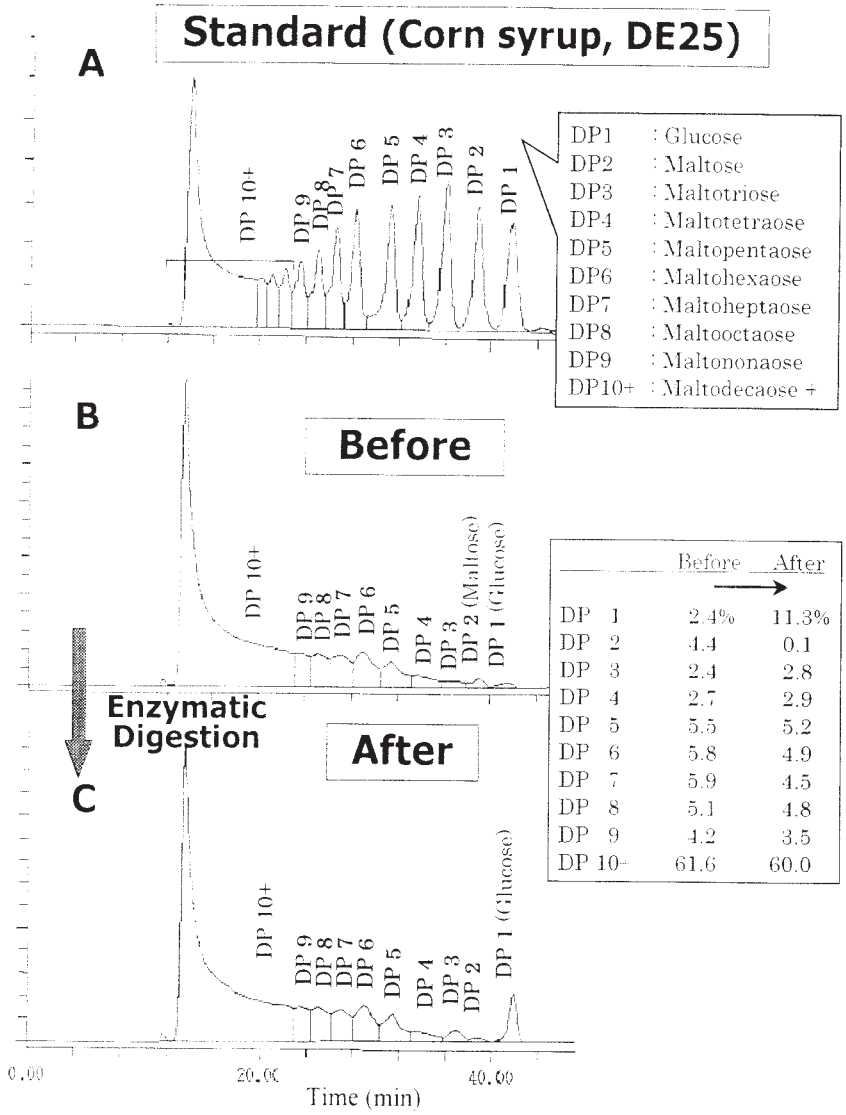

Figure 2001.03A. LC chromatogram of DE-25 corn syrup (A), resistant maltodextrin before treatment with hydrolytic enzymes (B), and resistant maltodextrin after treatment with hydrolytic enzymes (C).

tion mixture when adding ethanol (obtain tare weight of beaker before adding test portion). Assay the 2 blank digestions (i.e., 2 beakers and 2 crucibles) in an identical manner.

Let solutions stand overnight to form a precipitate. Filter by suction, using a water aspirator or vacuum pump, through 1.0 g Celite layered on a Pyrex glass crucible filter that previously has been dried to constant weight. Wash the 500 mL tall-form beaker and the residue 3 times with 20 mL 78% ethanol, **C**(**b**), 2 times with 10 mL 95% ethanol, **C**(**a**), and 2 times with 10 mL acetone, **C**(**c**).

Quantitatively transfer filtrate and washings to a 1 L round bottom flask. Dry residue in an air oven at 105°C overnight and record weight. This residue weight, minus the protein, ash, and blank residue weights represents the weight of the dietary fiber (IDF + HMWSDF) recovered by the AOAC method.

(**b**) *Filtrate recovery, desalting, and LC analysis.*—Evaporate with a rotary evaporator to near dryness. Dissolve the residue with a minimum amount of water, **C**(**d**), and transfer quantitatively to a 50 mL volumetric flask. Add 10 mL of 10 mg/mL glycerol LC standard and dilute to volume with water, **C**(**d**). Transfer contents of the 50 mL volumetric flask to a column (75 cm × 15 mm id) containing 25 g each, thoroughly mixed, of Amberlite IRA-67 (*m-1*) and Amberlite 200CT(HG)H (*m-2*) prepared just before use. Wash extract through the column with 250 mL water, **C**(**d**), at the rate of 0.8 mL/min.

Collect 250 mL eluant from the ion exchange column and quantitatively transfer into a 500 mL round bottom flask. Evaporate to near dryness and quantitatively transfer to a 10 mL volumetric flask and dilute to volume with water, **C**(**d**). Transfer the contents of the 10 mL volumetric flask to a 10 mL disposable syringe, **B**(**o**), and filter through a 0.2 μm filter,

B(**k**). Use a 50 µL LC glass syringe, **B**(**u**), to fill the 20 µL injection loop on the LC, **B**(**f**).

(**c**) *Determining the response factor for dextrose; dextrose is equivalent to RMD in LC response*.—Each chromatogram must be evaluated or standardized for the RI response of RMD. This is accomplished using glycerol standard, **C**(**q**). The peak areas, representing concentration, obtained by LC analysis of equal amounts of RMD and dextrose are equivalent. Glycerol is used as the internal standard but its peak area compared to the peak area for an equal amount of dextrose or RMD is not equivalent. A glycerol standard curve is therefore prepared to obtain a "response factor" to calculate the exact amount of RMD in a chromatogram of each test portion.

Prepare 3 solutions in individual 100 mL volumetric flasks containing the same amount of glycerol and 3 levels of dextrose. It is important to know and use the reported content (i.e., >99.5% purity) of both glycerol and dextrose standards as reported by suppliers. Accurately weigh 0.5, 1.0, and 2.0 g dextrose into 3 separate 100 mL volumetric flasks, respectively. To each flask add 10 mL of the 100 mg/mL glycerol standard, **C**(**q**). Dilute each flask to volume with water, **C**(**d**). These 3 flasks represent the standard solutions to calculate the "response factor" for dextrose that is used to determine the amount of RMD as displayed in LC chromatograms.

Use a 50 µL LC syringe, **B**(**u**), to fill the 20 µL injection loop for each standard glycerol–dextrose solution. Obtain the values for the peak areas of dextrose and glycerol from the 3 chromatograms. The reciprocal of the slope obtained by comparing the ratio of peak area of dextrose/peak area of glycerol (y-axis) to the ratio of the weight of dextrose/weight of glycerol (x-axis) is the "response factor." The average "response factor" among laboratories is 0.82, varying slightly in each laboratory.

$$\text{Response factor} = 1 / (\text{PA-dex} / \text{PA-gly}) \cdot (\text{Wt-gly} / \text{Wt-dex})$$

where PA-dex = peak area dextrose; PA-gly = peak area glycerol; Wt-dex = weight of dextrose in standard; Wt-gly = weight of glycerol in standard.

A flow diagram for a combined enzymatic-gravimetric method and LC determination is shown in Figure **2001.03B**.

E. Calculation

All values used in calculations are in mg, except for percent (%) values.

Assay each test portion in duplicate, resulting in 2 test portion weight values, test portion weight and test portion weight′ (prime); 2 crucibles for each blank and test portion, blank and blank′ (prime); and test portion and test portion′ (prime).

(**a**) *Calculate average % (IDF + HMWSDF) as follows*.—(*1*) Blank ash (Ab**)** = (ash + Celite + blank crucible) – (Celite + blank crucible).

(*2*) Blank residue weight (BRW) = ((BR + BR′) / 2) – (Pb + Ab) where Pb = blank protein, determined by micro-Kjeldahl procedure; BR = weight of first blank crucible with residue; BR′= weight of second blank crucible with residue; Ab = weight of blank ash from step (**a**)(*1*).

(*3*) Test portion residue weight (SR) = (residue + Celite + test portion crucible) – (Celite + test portion crucible). Duplicate test portion residue weight (SR′) = (residue′ + Celite′ + test portion crucible′) – (Celite′ + test portion crucible′).

(*4*) Test portion ash weight (As) = (ash + Celite + crucible) – (Celite + crucible).

(*5*) Final test portion residue weight (FSR) = SR – Ps – As – BRW = FSR where Ps = protein, determined by micro-Kjeldahl procedure; SR = final test portion residue weight from step (**a**)(*3*); As = test portion ash weight from step (**a**)(*4*); BRW= blank residue weight from step (**a**)(*2*). Repeat this calculation for FSR′ using SR′ – Ps – As – BRW (using values from duplicate test portion weights).

(*6*) Percent final test portion residue weight (% FSR) = (FSR / SW) × 100 = % FSR where FSR = final test portion residue weight from step (**a**)(*5*); SW = test portion weight. Repeat this calculation for % FSR′ using FSR′ and SW′.

(*7*) % (IDF + HMWSDF) = average % FSR = (% FSR + % FSR′) / 2 where % FSR = percent final test portion residue weight; % FSR′ = percent final duplicate test portion residue weight.

(**b**) *Calculate average % LMWRMD as follows*.—(*1*) LMWRMD = (peak area of LMWRMD / peak area of glycerol) × (glycerol standard, mg × response factor).

(*2*) % LMWRMD = (LMWRMD / SW) × 100 where LMWRMD = weight of LMWRMD from step (**b**)(*1*); SW = test portion weight. Repeat calculations for % LMWRMD′ using LMWRMD′ and SW′.

(*3*) % ALMWRMD = average % LMWRMD = (%LMWRMD + % LMWRMD′) / 2 where % LMWRMD = percent LMWRMD for test portion from step (**b**)(*2*); % LMWRMD′ = percent LMWRMD for duplicate test portion from step (**b**)(*2*).

(**c**) *Calculate average % total dietary fiber (TDF) as follows*.—Percent (%) TDF = % (IDF + HMWSDF) + % ALMWRMD where % (IDF + HMWSDF) = average percent IDF + HMWSDF from step (**a**)(*7*); % ALMWRMD = average percent LMWRMD from step (**b**)(*3*).

F. Resistant Maltodextrin

The commercially available U.S. GRAS status RMD is a source of dietary fiber. Resistant maltodextrin is certified as an approved dietary fiber ingredient for the Program for Foods for Specific Health Use (FOSHU) in Japan (6). Dietary fiber supplements prepared simply by packaging RMD (or agglomerated RMD) in sachet forms and labeled as RMD have been on the market. Fibersol®-2, RMD, is manufactured and was supplied by Matsutani Chemical Industry Co., Ltd. (Itami City, Hyogo, Japan). The moisture content of the product is 2.7% and DE is 10.5. The RMD is produced by the pyrolysis and subsequent enzyme treatment of corn starch. It is an aggregate of glucose polymers with the MW distribution of 180 (DP-1) to >10 000 (DP-62) daltons, but the average MW is 2000 daltons. It contains α 1–4 and α 1–6 glucosidic bonds, which originate from starch and 1–2 and 1–3 glucosidic bonds that are created by transglucosidation during pyrolysis (3).

Internal utilization of RMD by in vitro and in vivo tests show that <10% is digested and absorbed in the small intestine (7). Ap-

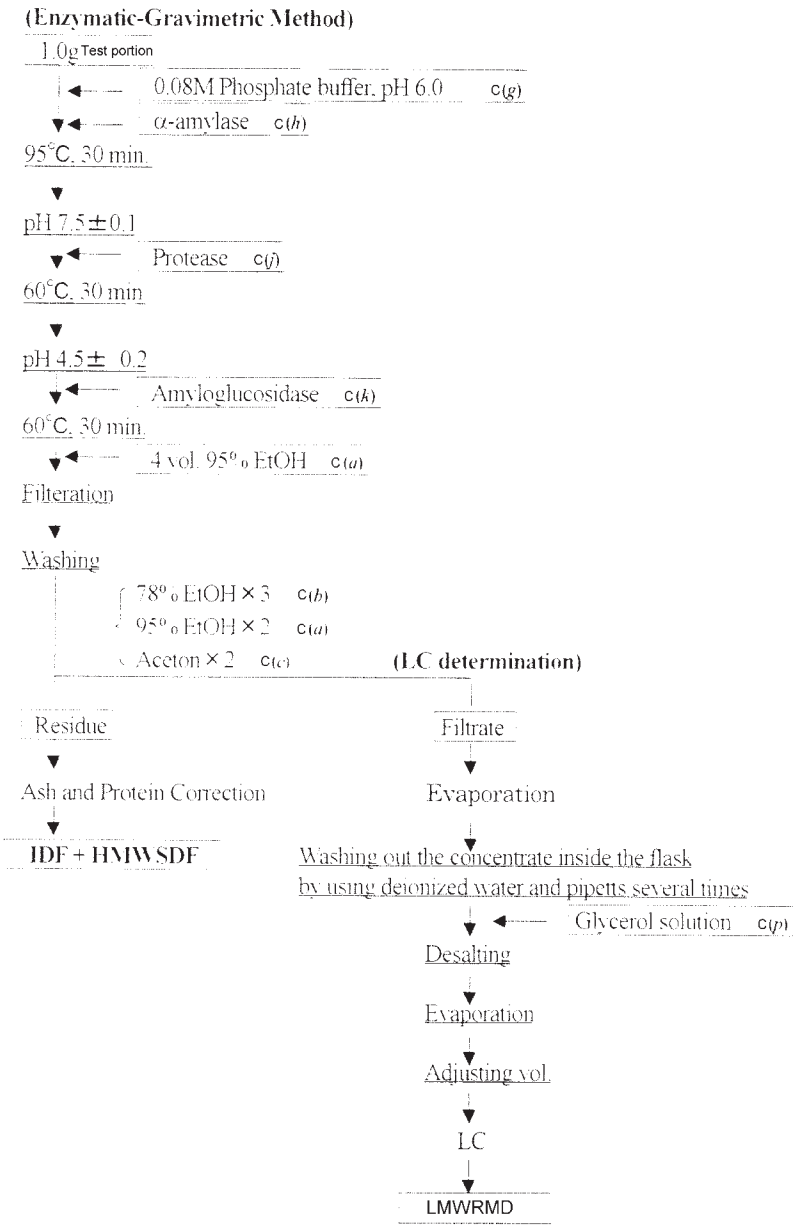

Figure 2001.03B. Flow diagram for a combined enzymatic-gravimetric method and LC determination.

proximately 50% of the products are fermented in the large intestine and ca 40% of the products are excreted into the feces (7). In order to distinguish this substance from conventional maltodextrin (digestible), the term "resistant" is added and used to describe this compound.

The sugar, oligosaccharide, and polysaccharide composition of the LMWRMD fraction of the RMD has been determined before and after hydrolytic enzyme treatments and is shown in Figure **2001.03A**. The distribution of these oligosaccharides is not significantly changed when RMD is treated with hydrolytic enzymes. To assess the oligosaccharide moieties and their distribution in the LMWRMD of RMD, corn syrup solids were used as a standard source of these oligosaccharides (Figure **2001.03A**). The nondigestible portions of RMD consists of DP units of 3 (DP-3) and above (Figure **2001.03A**). These nondigestible oligosaccharides and polysaccharides constitute >90% of RMD. Approximately 60% of RMD consist of polymers having >10 DP. Complete information on the composition RMD is available in ref 8.

Refs.: *J. AOAC Int.* **83**, 1013–1019(2000); **85**, 436–440(2002)

Test Samples

Hard candy.—Candy containing 40% RMD was prepared as follows: 200 g RMD was added to 200 mL water; then 300 g sugar was added and stirred thoroughly. The ingredient mixture was transferred into a copper pan and boiled down. When the temperature reached 170°C, the mixture was cooled to 100°C, and a trace amount each of citric acid and

lemon flavor was added and mixed completely. The obtained 500 g candy containing 40% RMD was poured into molds and cooled to 25°C. The candy was crushed and used as an analytical sample.

Chicken and vegetable soup.—Powdered soup containing 22.2% RMD was prepared as follows: 60 g RMD (equivalent to 20% of the total amount of ingredients) was added to 240 g commercial powdered chicken and vegetable soup and mixed thoroughly. The fat contained in the powdered soup was removed by ether in Soxhlet extraction equipment, and dried; 10.0% fat was extracted from the mixture as a result. The addition of RMD to the fat extracted mixture was calculated to be 22.2%. The original powdered soup contained a certain amount of dietary fiber as starch, maltodextrin, lactose, sugar, sweet corn, and vegetable powder as source of carbohydrate.

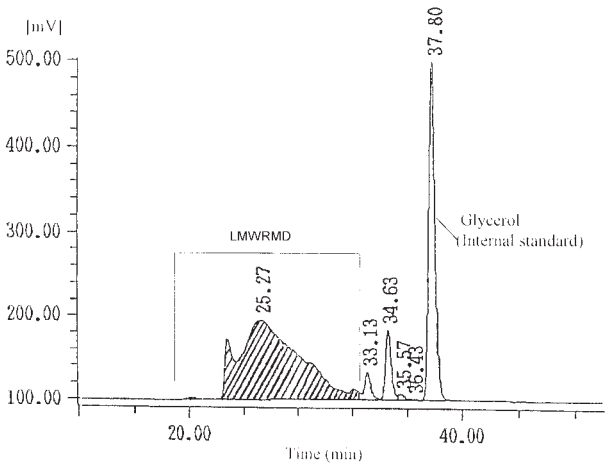

Figure 1. LC profile of LMWRMD.

Table 1. Collaborative results (blind duplicate) of determination of IDF + HMWSDF % by enzymatic-gravimetric method[a]

Sample	Resistant maltodextrin		Hard candy		Chicken and vegetable soup		Grapefruit juice		White bread		Srawberry Jello®	
Sample code	A	I	B	G	C	K	D	J	E	H	F	L
Collaborator												
1	44.84	41.69	13.40	13.45	10.25	10.91	0.21	0.24	6.08	7.00	1.50	2.75
2	36.86	36.42	10.29(G)	10.07(G)	9.10	9.93	0.19	0.23	6.09	5.91	1.96	1.59
3	34.73	48.02	10.85(G)	8.63(G)	10.34	10.63	0.30	0.31	6.42	5.89	—	2.62
5	45.13	44.10	12.20	13.94	9.90	10.61	0.25	0.30	4.80	4.38	2.64	2.87
8	39.53	40.00	11.51	12.72	9.82	10.88	0.32	0.34	5.43	5.58	2.50	2.23
9	44.77	36.04	14.00	11.71	11.34	10.40	0.32	0.24	6.62	6.83	2.95	2.23
10	41.08	40.36	12.96	12.90	10.60	10.48	0.28	0.27	4.89	5.61	2.77	2.87
11	39.54	41.62	13.20	11.86	11.57	12.27	0.30	0.29	5.98	5.24	2.40	2.66
12	46.37	46.38	13.04	13.19	10.65	11.77	0.25	0.27	5.22	6.07	2.00	2.74
13	40.90	41.69	13.25	12.33	10.62	10.68	0.27	0.28	6.38	6.61	2.59	2.94
			(a)	(b)								
No. labs	10		10	8	10		10		10		10	
No. outliers	0		0	2	0		0		0		0	
Mean	41.50		12.28	12.85	10.64		0.27		5.85		2.47	
s_r	3.67		0.93	0.88	0.52		0.03		0.40		0.41	
s_R	3.75		1.43	0.88	0.73		0.04		0.72		0.44	
RSD_r	8.85		7.60	6.85	4.92		9.19		6.88		16.77	
RSD_R	9.04		11.68	6.85	6.85		14.50		12.31		17.71	
r	10.28		2.61	2.47	1.47		0.07		1.13		1.16	
R	10.50		4.02	2.47	2.04		0.11		2.02		1.23	

[a] Abbreviations: C = Cochran (repeatability) outlier (p = 2.5%); G = Grubbs (reproducibility) outlier (p = 2.5%); No. labs = number of laboratories included in calculations; No. outliers = number of outlier laboratories; s_r = repeatability standard deviation; s_R = reproducibility standard deviation; RSD_r = repeatability relative standard deviation (%); RSD_R = reproducibility relative standard deviation (%); r = repeatability value = $2.8s_r$; R = reproducibility value = $2.8s_R$.

Table 2. Collaborative results (blind duplicate) of determination of LMWRMD % by LC determination[a]

Sample	Resistant maltodextrin		Hard candy		Chicken and vegetable soup		Grapefruit juice		White bread		Strawberry Jell-O®	
Sample code	A	I	B	G	C	K	D	J	E	H	F	L
Collaborator												
1[b]												
2[c]												
3	56.88	48.70	26.89(C)	18.21(C)	17.37	15.09	1.12	1.12	4.52	4.54	7.52	9.27
5	47.82	48.07	25.79	23.52	14.15	14.34	1.08	1.04	4.23	4.25	7.05	6.70
8	58.18	57.64	26.98	26.00	15.19	14.16	1.12	1.12	4.56	4.41	6.80	7.42
9	50.98	62.08	23.45	26.81	13.49	15.39	1.02(C)	1.16(C)	3.00	2.90	6.04	7.89
10	56.24	54.03	25.35	24.00	13.52	13.80	1.04	1.07	3.73	3.69	5.64	6.90
11	57.72	56.15	24.33	26.39	15.03	15.72	1.08	1.07	3.57	3.75	6.74	6.61
12	46.45	45.93	24.63	23.64	14.49	13.36	1.11	1.09	4.02	3.82	7.12	6.82
13	55.20	53.35	25.70	26.39	14.98	13.92	1.12	1.13	3.27	3.33	9.11	7.48
			(a)	(b)			(a)	(b)				
No. labs	8		8	7	8		8	7	8		8	
No. outliers	0		0	1	0		0	1	0		0	
Mean	53.46		24.88	25.21	14.63		1.09	1.09	3.85		7.19	
s_r	3.55		2.50	1.33	0.90		0.04	0.01	0.08		0.84	
s_R	4.97		2.50	1.33	1.04		0.04	0.03	0.56		0.96	
RSD_r	6.64		10.06	5.29	6.13		3.45	1.36	2.16		11.70	
RSD_R	9.29		10.06	5.29	7.12		3.52	2.88	14.61		13.36	
r	9.94		7.01	3.74	2.51		0.11	0.04	0.23		2.36	
R	13.90		7.01	3.74	2.91		0.11	0.09	1.58		2.69	

[a] Abbreviations: C = Cochran (repeatability) outlier (p = 2.5%); G = Grubbs (reproducibility) outlier (p = 2.5%); No. labs = number of laboratories included in calculations; No. outliers = number of outlier laboratories; s_r = repeatability standard deviation; s_R = reproducibility standard deviation; RSD_r = repeatability relative standard deviation (%); RSD_R = reproducibility relative standard deviation (%); r = repeatability value = $2.8s_r$; R = reproducibility value = $2.8s_R$.

[b] Values generated by the collaborator were out of line because of incomplete removal of salts. All results from them were not used for further statistical analyses.

[c] Analyses were not performed because collaborator did not have appropriate LC equipment.

Grapefruit juice.—Juice containing 1.5% RMD was prepared as follows: 360 g RMD (equivalent to 1.5% of the total amount of ingredients) was added to 23.64 kg commercial grapefruit juice, which contains 2% grape fruit juice and crystallized fructose. The obtained RMD fortified juice sample was packed into 500 mL PET bottles and sterilized at 80°C for 30 min.

White bread.—Bread containing 4.5% RMD was prepared by the following formula: Sponge: strong flour 27.05%, yeast food 0.06%, yeast 1.38%, water 23.29%; dough: strong flour 15.88%, white sugar 3.17%, salt 1.06%, skim milk powder 1.06%, water 11.12%, shortening 3.18%, RMD 2.75%.

During the baking process, 10% moisture was evaporated, and 460 g white bread was obtained. At this point, RMD was added so that it would constitute 3% of the whole bread prepared. The obtained bread sample was sliced and dried at 70°C for 15 h in a vacuum drier. By the drying process, the moisture, equal to 33.3% of the total, was evaporated. Hence, the addition of RMD to the dried bread sample was calculated to be 4.5%. Further, the dried bread sample was crushed by coffee miller and passed through a screen mesh of 0.5 mm. The original dried bread sample contained a certain amount of dietary fiber.

Strawberry Jell-O®.—Jell-O containing 10% RMD was prepared as follows: 40 g RMD (equivalent to 10% of the total amount of ingredients) was added to 360 g commercial strawberry flavored Jell-O and mixed thoroughly. The original Jell-O powder contained sucrose as a source of carbohydrate.

Results and Discussion

Before conducting the collaborative study, we asked all collaborators to practice the method by using 2 practice test portions. However, the results from most of the collaborators were lower than expected. The problem was traced to the incomplete removal of test portion from the tall-form beakers

Table 3. Collaborative results (blind duplicate) of determination of TDF % (IDF + HMWSDF and LMWRMD) by Combination of enzymatic-gravimetric method and LC determination[a]

Sample	Resistant maltodextrin		Hard candy		Chicken and vegetable soup		Grapefruit juice		White bread		Strawberry Jell-O®	
Sample code	A	I	B	G	C	K	D	J	E	H	F	L
Collaborator												
1[b]												
2[c]												
3	91.61	96.72	37.74(C)	26.84(C)	27.71	25.72	1.42	1.43	10.94	10.43	NR	11.89
5	92.95	92.17	37.99	37.46	24.05	24.95	1.33	1.34	9.03	8.63	9.69	9.57
8	97.71	97.64	38.49	38.72	25.01	25.04	1.44	1.46	9.99	9.99	9.30	9.65
9	95.75	98.12	37.45	38.52	24.83	25.79	1.34	1.40	9.62	9.73	8.99	10.12
10	97.32	94.39	38.31	36.90	24.12	24.28	1.32	1.34	8.62	9.30	8.41	9.77
11	97.26	97.77	37.53	38.25	26.60	27.99	1.38	1.36	9.55	8.99	9.14	9.27
12	92.82	92.31	37.67	36.83	25.14	25.13	1.36	1.36	9.24	9.89	9.12	9.56
13	96.10	95.04	38.95	38.72	25.60	24.60	1.39	1.41	9.65	9.94	11.70	10.42
			(a)	(b)								
No. labs	8		8	7	8		8		8		8	
No. outliers	0		0	1	0		0		0		0	
Mean	95.36		37.27	37.99	25.41		1.38		9.60		9.91	
s_r	1.63		2.78	0.58	0.74		0.02		0.33		0.60	
s_R	2.37		2.85	0.68	1.18		0.04		0.64		0.93	
RSD_r	1.71		7.46	1.53	2.89		1.33		3.41		6.10	
RSD_R	2.48		7.65	1.79	4.65		3.20		6.66		9.39	
r	4.57		7.78	1.63	2.06		0.05		0.92		1.69	
R	6.62		7.98	1.90	3.31		0.12		1.79		2.60	

[a] Abbreviations: C = Cochran (repeatability) outlier (p = 2.5%); G = Grubbs (reproducibility) outlier (p = 2.5%); No. labs = number of laboratories included in calculations; No. outliers = number of outlier laboratories; s_r = repeatability standard deviation; s_R = reproducibility standard deviation; RSD_r = repeatability relative standard deviation (%); RSD_R = reproducibility relative standard deviation (%); r = repeatability value = $2.8s_r$; R = reproducibility value = $2.8s_R$.

[b] Values generated by the collaborator were out of line because of the incomplete removal of salts. All the results from them were not used for further statistical analyses.

[c] Analyses were not performed because collaborator did not have appropriate LC equipment.

after enzyme treatment and addition of alcohol. Small amounts of RMD adhered to the glass. Hence, in the collaborative study, we gave the following instructions to all collaborators: "The alcohol precipitate of test portions containing RMD is very sticky and will stick to the sides of the glass beaker. To completely remove all RMD from the beaker, it is also necessary to add washing solvents in the beaker and scratch out the precipitate with a spatula (preferably Teflon). It takes considerable time (15–20 min) and patience." After these instructions were given, the results obtained from collaborators on practice test portions were in agreement with the mean values repeatedly obtained in our laboratories.

Before conducting the collaborative study, all collaborators were given detailed instruction on how to read dietary fiber peaks from LC chromatogram (Figure 1) to accurately calculate LMWRMD (%) values.

The RMD and TDF values generated by one of the collaborators were "out of line" for 4 test portions (RMD, candy, bread, and Jell-O) because of incomplete removal of salts. Another collaborator analyzed test portions using the specified AOAC procedure to determine IDF + HMWSDF values, but could not perform further analyses because that collaborator did not have appropriate LC equipment. The results from those 2 collaborators were not used in the statistical analyses. An initial review of data showed that this study has secured the 6 samples supported by 8 reporting valid data and accordingly satisfied the minimum criteria for quantitative study provided in the Harmonization Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis (9).

The results received from the 8 collaborating laboratories for % IDF + HMWSDF, LMWRMD, and TDF are reported in Tables 1–3, respectively. Performance parameters are shown

for the 2 cases of (a) including outliers and (b) without including outliers. For TDF in food test portions analyzed in this collaborative study (Table 3), repeatability standard deviations (RSD_r) ranged from 1.33% for juice to 7.46% for candy, calculated by including outliers, and from 1.33% for juice to 6.10% for Jell-O, calculated without including outliers. Reproducibility standard deviations (RSD_R) ranged from 2.48% for RMD to 9.39% for Jell-O, calculated by including outliers, and from 1.79% for candy to 9.39% for Jell-O, calculated without including outliers. The obtained TDF values for white bread and chicken and vegetable soup were higher than the amounts of RMD added because those 2 food test portions originally contained dietary fiber. For the determination of total dietary fiber (Table 3) only one pair of data from collaborator 3 was a Cochran outlier for candy.

Summary

The importance of dietary fiber in human nutrition and health is continually being justified. Before any nondigestible carbohydrate can be labeled as a source of dietary fiber, it must have an appropriate and reliable method of determination. A nondigestible mixture of soluble oligosaccharides and polysaccharides that contain glucose is commercially available and classified as an RMD. Approximately 45–55% of RMD is not recovered as TDF using AOAC **985.29** because the fiber is not precipitated in 78% ethanol. We have developed a method and tested the feasibility of measuring these nondigestible carbohydrates which are not recovered by the enzymatic–gravimetric method for TDF. The accuracy and precision of this method was evaluated through an AOAC collaborative study. The study involved 10 collaborating laboratories that assayed 12 test portions (6 blind duplicates) containing RMD. The 6 test portion pairs ranged from 1.5 to 100% RMD. RSD_r were 1.33–7.46%, calculated by including outliers, and 1.33–6.10%, calculated by not including outliers. RSD_R were 2.48–9.39%, calculated by including outliers, and 1.79–9.39%, calculated by not including outliers.

Recommendations

This method is designed to determine TDF values for foods containing RMD. It appears reliable and reproducible as verified through an AOAC collaborative study. We recommend that this method for determining TDF in foods containing RMD be adopted Official First Action.

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References

(1) *The Surgeons General's Report on Nutrition and Health* (1988) U.S. Department of Health and Human Services, PHS, DHHS Publ. No. 88-50210

(2) Gordon, D.T. (1989) *Cereal Foods World* **34**, 517–525

(3) Ohkuma, K., Matsuda, I., Katta, Y., & Hanno, Y. (1990) *Dempun Kagaku* **37,** 107–114

(4) *Official Methods of Analysis* (1997) 16th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Methods **985.29**, **991.43**

(5) Cho, S.C., & Prosky, L. (1994) *Cereal Foods World* **39**, 767–768

(6) Tsuji, K. (1995) *Jpn Confect. Res. Cent.* **3**, 1–12

(7) Tsuji, K., & Gordon, D. (1998) *J. Agric. Food Chem*. **46**, 2253–2259

(8) Ohkuma, K., Matsuda, I., Yasuo, K., & Tsuji, K. (2000) *J. AOAC Int.* **83**, 1013–1019

(9) *Official Methods of Analysis* (2000) 17th Ed., Appendix D, Gaithersburg, MD