Polydextrose (Litesse®) provides physiological effects consistent with dietary fiber. However, AOAC methods for measuring total dietary fiber (TDF) in foods include an ethanol precipitation step in which polydextrose and similar carbohydrates are discarded and therefore not quantitated. This study describes a method developed to quantitate polydextrose in foods. The new method includes water extraction, centrifugal ultrafiltration, multienzyme hydrolysis, and anion exchange chromatography with electrochemical detection. Six foods were prepared with 4 levels of polydextrose to test the ruggedness of the method. Internal validation demonstrated the ruggedness of the method with recoveries ranging from 83 to 104% with an average of 95% (%(n=24) and relative standard deviation of recoveries ranging from 0.7 to 13% with an average of 3.3% (n=24). The value is added to that obtained for dietary fiber content of foods using the AOAC methods, to determine the TDF content of the food.

Physiological Effects of Polydextrose

Human clinical, animal clinical, and in vitro studies on polydextrose have demonstrated physiological effects associated with dietary fiber (3–11). Upon reaching the lower intestine, polydextrose is partially fermented by colonic bacteria to short-chain fatty acids, especially acetate and butyrate (7–9). Polydextrose increases fecal bulking, reduces transit time, softens stools, and lowers the fecal pH (4–7, 9). It modulates intestinal microflora, increasing beneficial bacteria such as Lactobacillus and Bifidobacterium and decreasing detrimental species such as Clostridium (7, 8). In addition, polydextrose fermentation reduces the concentration of certain putrefactive/carcinogenic substances (e.g., indole and p-cresol) in the colon (7). Polydextrose also aids blood glucose homeostasis because of its low glycemic index (15% compared to glucose; 11). A new large scale human clinical study further confirms these findings (12).

Dietary Fiber Measurement

The original AOAC Method (985.29) for determining TDF in food is an enzyme-gravimetric method that is accepted in most countries. We evaluated this method, along with several outside laboratories, and determined that polydextrose (alone or in foods) gives essentially no TDF value (1–1%; n = 16). In Method 985.29, the sample is defatted in petroleum ether to remove lipids, and a series of enzyme incubations removes starch, starch dextrins, and protein to mimic human digestion. The ethanol step is designed to precipitate nondigested polysaccharides and leave the simple sugars and other small molecules in solution. However, the supernatant contains ROs, and other RPs, preventing them from being measured in the TDF content of foods. As the use of ROs in foods increases, this becomes a more significant problem. Furthermore, studies have indicated that other polysaccharides (pectin, arabinan, and arabinogalactan) are not quantitatively precipitated by 80% ethanol (13–15). Concentrations of ethanol and other solvents were studied in an attempt to quantitate polydextrose, but none worked quantitatively without interferences from sugars or other small molecules (1).
**Methods of Polydextrose Analysis**

The first method developed to measure polydextrose in food involved an aqueous extraction of polydextrose, followed by colorimetric assay (16). The method uses acid hydrolysis to break the glycosidic bonds; this step is followed by dehydration and derivatization of dextrose by phenol and sulfuric acid. The derivatized dextrose is measured spectrophotometrically at 490 nm. This method is cumbersome and has only satisfactory precision. Its accuracy is also affected by other carbohydrates that may be present in a food.

Several liquid chromatography (LC)-based methods have been published (17–20). An assay currently used in Japan quantitates polydextrose as a fiber in foods (17). As with the AOAC dietary fiber method, enzymes are used to degrade starch and maltodextrins in food. The sample is membrane-filtered, deionized, and injected into the LC system (Ultron PS-80 N column and refractive index [RI] detection). Noffsinger et al. (18) used a sulfonated polystyrene divinylbenzene (calcium form) column (Bio-Rad, Richmond, CA; Aminex HPX-87C) and RI, but no enzyme step. Arrigoni and Amado (19) used a sulfonated polystyrene divinylbenzene (lead form) column (Bio-Rad Aminex HPX-87P) and RI with a different enzyme. These methods work well for simple foods such as clear beverages. However, the sample preparation does not remove many resistant oligomers or polymers, leading to potential interferences. Also, as a universal detector, RI is not specific to carbohydrates (including polydextrose).

Stumm and Baltes (20) used an enzyme step followed by an anion exchange column (Dionex, Sunnyvale, CA) PA1, mobile phase gradient, and pulsed amperometric detection. This system provided more selectivity for carbohydrates but is a more complex technique. The method described here is based on the Stumm and Baltes method, but uses an improved sample preparation step.

**METHOD**

**A. Principle**

Figure 1 shows a schematic of the method; Figure 2 shows a sample chromatogram.

Polydextrose is extracted from food with hot water, and centrifuged. The supernatant then passes through a centrifugal ultrafilter to remove high molecular weight interferences. The filtrate is treated with an enzyme mix (isoamylase, amyloglucosidase, and fructanase) to remove any oligosaccharide interferences, mainly maltosglomers and fructans. Polydextrose standards undergo the same treatment. High-pressure anion exchange chromatography with electrochemical detection (HPAEC–ED) is used to detect and quantify a high molecular weight fraction of polydextrose.

**B. Apparatus**

(a) **Water baths** (2).—One set at 80 ± 2°C (increased to boiling); the second set at 50 ± 2°C.

(b) **High speed centrifuge and rotors**.—Capable of 6000 × g, preferably to 38 000 × g with 50 mL (holds ca 38 mL) centrifuge tubes; 9200 × g with 1.7 mL microcentrifuge tubes; and 6400 × g with 15 mL centrifuge tubes, e.g., Beckman (Fullerton, CA); J2-21 centrifuge with JA-17, JA 18.1, and JA-25.15 rotors.

(1) **Analytical balance**.—0.1 mg accuracy.

(2) **Bottles/tubes**.—Containers with screw caps (250 mL) capable of withstanding 80°C water. 50 mL centrifuge tubes (holds ca 38 mL), capable of withstanding 38 000 × g (e.g., Nalgene [Rochester, NY]), and microcentrifuge tubes (1.7 mL) capable of withstanding 10 000 × g.

(3) **Vortex mixer**.

(4) **Pipettors**.—Adjustable microliter pipet (20–200 µL); adjustable microliter pipet (200–1000 µL).

(5) **Filters**.—PTFE syringe filter, 0.2 µm pore size, 13 mm diameter.

(6) **Centrifugal ultrafiltration devices**.—100 000 nominal molecular weight limit, polyethersulfone membrane, 2 mL capacity, e.g., Millipore (Bedford, MA) Ultrafree-CL PBHK BIOMAX-100.

(i) **Liquid chromatography and detectors**.—HPAEC–ED; LC system capable of producing ca 3000 psi (200 bar); gradient pump (capable of handling NaOH eluents), and pulsed integrated electrochemical detector (with gold electrode), e.g., Dionex DX 500 basic gradient carbohydrate system.

(j) **Liquid chromatograph column**.—Microporous substrate (10 µm) agglomerated with a microbead quaternary ammonium functionalized latex, 250 × 4 mm column (e.g., CarboPak PA1 [Dionex, Sunnyvale, CA]) with guard column (e.g., CarboPak PA1 Guard, 23 × 3 mm).

**C. Reagents**

(a) **Solvents**.—Deionized water (resistance ≥18 megohm/cm).

(b) **Sodium hydroxide**.—ACS grade, or equivalent (50%; carbonate free).

(c) **Acetic acid**.—ACS grade or equivalent. Prepare 0.2 M solution.

(d) **Sodium acetate trihydrate**.—ACS grade or equivalent. Prepare 0.2 M solution.

(e) **Acetate buffer (pH 4.5)**.—Mix 28 mL acetic acid (0.2 M) with 22 mL sodium acetate (0.2 M); then dilute to 100 mL with deionized water.

(f) **Fructanase**.—667 U/mL (Megazyme [Bray, Ireland] or equivalent). One unit is amount of enzyme required to release 1 µmol fructose-reducing sugar equivalents from kestose/min under standard assay conditions (10 mM kestose; pH 4.5; 40°C). Or 200 U/mL (p-np-β-maltoside). One unit is amount of enzyme required to release 1 µmol p-nitrophenol from p-nitrophenol β-maltoside (in the presence of excess β-glucosidase; 10 mM pNP β-maltoside; pH 4.5; 40°C). (Megazyme E-AMGDF or equivalent.)
Figure 1. Schematic of determination of polydextrose in foods.
**Isoamylase.**—200 U/mL (Megazyme E-ISAMY or equivalent). One unit is amount of enzyme required to release 1 μmol glucose-reducing sugar equivalents from oyster glycogen/min under standard assay conditions (10 mg/mL oyster glycogen; pH 3.5; 40°C).

**Buffered enzyme mix.**—Combine fructanase (f) (2 mL; 1324 U), amyloglucosidase (g) (84 μL; 274 U), and isoamylase (h) (84 μL; 16.8 U). Dilute in acetate buffer (e) to 20 mL. Make fresh each day. Store at 4°C.

**D. Analytical HPAEC Conditions**

**Mobile phase.**—A = 0.15M sodium hydroxide; B = 0.15M sodium hydroxide + 0.5M sodium acetate (it is very important to degas the mobile phase and store under inert gas). Gradient: (a) 0–10 min of 70% A/30% B; (b) at 10.1 min, change to 100% B; (c) at 15.1 min, change to 70% A/30% B for 10 min. Flow rate: 1.2 mL/min.

**Detector voltage settings (Dionex ED 40).**—Time (S); potential (V) integration: 0.00, 0.05; 0.20, 0.05 begin; 0.40, 0.05 end; 0.41, 0.75; 0.60, 0.75; 0.61, –0.15; 1.00, –0.15.

**E. Preparation of Standards**

**Polydextrose.**—FCC grade/Litesse® of known moisture content determined by the Karl Fisher method.

**Stock standard (5000 ppm for DX 500 system).**—To prepare 5000 ppm standard, weigh polydextrose (500 mg dry basis, accurate to 0.01 g) into preweighed glass container with screw cap (250 mL). Be sure cap is on container when weighing. Record weight. Add ca 100 g deionized water preheated to 80°C. Screw cap on tightly, and Vortex mix 30 s. Place container in water bath at 80°C for 10 min, and Vortex mix 30 s at 5 and 10 min to solubilize polydextrose. Remove container from water bath, let cool to room temperature, and weigh (accurate to 0.01 g). Be sure cap is on container when weighing.

**Intermediate standards.**—Serially dilute 5000 ppm standard to make 2500, 2000, 1500, 1250, 1000, 750, 500, and 250 ppm standards in water. This range is for the DX 500 system. Some systems may require a different range. These standards will be diluted 5-fold during the procedure described in G and are considered the working standards (500, 400, 300, 250, 200, 150, 100, 50 ppm). An 8-point calibration curve should give a polynomial regression with a correlation coefficient not less than 0.995.

**Stability.**—Standards are stable at 4°C for 1 month.

**F. Preparation of Samples**

**Sugar cookie.**—Sugar cookie dough (1000 g) was prepared as follows: Crisco shortening (240.7 g) and sugar (288.9 g) were creamed in a large mixing bowl at low speed for 4 min. A preblend of salt (4.0 g), eggs (104.9 g), water (21.9 g), and vanilla extract (9.9 g) were added to the creamed ingredients and mixed for 3 min at medium speed. Flour (321.0 g), baking powder (5.8 g), and baking soda (2.9 g) were added and mixed at low speed for 1.5 min. Sides of the mixing bowl were scraped, and mixing continued for an additional 1.5 min. An ice cream scoop (No. 40) was used to deposit 15 cookies on a parchment-lined tray. Cookies were baked in a 350°F oven for 15 min and cooled on a wire rack for 6 min before final weight was recorded (tray wt, initial wt of cookies, and final wt of cookies were recorded for yield determinations). Sugar cookies containing 5.0, 10.0, 14.9, and 20.4% polydextrose were prepared as above. Polydextrose replaced some of the sugar at the creaming stage (shortening, sugar, and polydextrose).

**Soft jellied candy.**—Soft jellied candy (400 g) was prepared as follows: Lycasin (222.84 g), sodium citrate (2.0 g), and water (64.92 g) were added to a 500 mL round bottom flask. The flask was placed in an oil bath set at 170°C. Ingredients were heated to 100°C. A starch suspension (water, 74.8 g; and starch, 33.2 g) was added slowly with stirring. Ingredients were cooked to a yield of 67% and removed from oil bath. Color (0.24 g of an FD&C Yellow No. 5 in 10% aqueous solution) and citric acid (2.0 g of 5% aqueous solution) were added and mixed thoroughly. Ingredients were cooked to a yield of 67% and removed from oil bath. The candy was then deposited into starch molds. Soft jellied candy containing 10, 20, 14.9, and 20.4% polydextrose were prepared as above. Polydextrose replaced some of the Lycasin.

Figure 2. Sample chromatogram of extraction of polydextrose from food.
Grape jelly.—Grape jelly (700 g) was prepared as follows: Grape concentrate (108.7 g), water (540.75 g), and calcium chloride (35.0 g of 1% aqueous solution) were combined in a 1000 mL beaker. The beaker was placed in an oil bath set at 110°C. Pectin (11.05 g) and sodium benzoate (0.91 g) were added slowly. The ingredients were heated to 100°C. Aspartame (1.4 g) was added and stirred thoroughly; and citric acid (2.03 g) was added. Ingredients were cooked with stirring for an additional 8 min. The beaker was removed from the oil bath and final weight was recorded for the yield. Grape jelly containing 5.4, 10.0, 15.6, and 19.9% polydextrose were prepared as above. Polydextrose replaced some of the water.

Milk chocolate candy.—Milk chocolate candy (250 g) was prepared as follows: Chocolate liquor (112.5 g), lecithin (0.75 g), and milk fat (25.0 g) were combined in a 400 mL metal beaker. The beaker was placed in an oil bath set at 52°C. Ingredients were mixed continuously at low speed with an overhead stirrer (motor equipped with 3-blade stir shaft). A preblend of confectioners sugar (98.625 g), vanillin (0.25 g), and inulin (12.5 g) was added over 15–20 min, with stirring for an additional 20 min. The beaker was then transferred to a water bath set at 33°C, and the paste was mixed continuously at low speed with the overhead stirrer while cooking. When the paste temperature reached 33°C, stirring was continued for another 10 min. The paste was then cooled to 30°C and stirring continued for another 10 min. Seed crystals (milk chocolate; 0.375 g) were added and ingredients were stirred for an additional 10 min at 30°C. The beaker was removed from the water bath and the chocolate was poured into molds. Final weight was recorded for yield calculations. Milk chocolate candy containing 5.0, 10.0, 15.0, and 20.0% polydextrose were prepared as above. Polydextrose replaced some of the confectioners sugar.

Iced tea.—A commercial lemon-flavored iced tea (control) was transferred into 30 cc glass jars. Iced tea containing 2.1, 4.2, 6.3, and 8.3% polydextrose were prepared by adding polydextrose to the iced tea and mixing completely.

Powdered drink mix.—A 16 g amount of commercial powdered drink mix (control) was weighed directly into a 1 oz glass jar. Powdered drink mixes containing 7.81, 15.62, 23.44, and 31.25% polydextrose were prepared by weighing polydextrose and powdered drink mix directly into a 1 oz jar and shaken thoroughly. The polydextrose replaced some of the powdered drink mix.

Figure 3. Typical calibration curve for polydextrose.
Extraction of polydextrose from food.—Grind or cut food into small particles. Store in sealed containers to prevent moisture changes. If approximate amount of polydextrose in food sample is known, weigh a quantity of food that will yield an amount of polydextrose within the range of the calibration curve. If the amount of polydextrose in the sample is not known, estimate the amount of polydextrose and choose a sample weight based on that estimate.

Weigh an appropriate amount of food sample (accurate to 0.01 g) and add to a preweighed container (250 mL) with screw cap. Record weight of food sample. Add ca 100 g hot deionized water (80°C) and immediately replace cap tightly. Vortex mix 30 s to disperse food. Place container in a water bath at 80°C for 10 min. Vortex mix 30 s at 5 and 10 min to solubilize polydextrose. Remove container from water bath, and let cool to room temperature. Record total weight and calculate weight of water. Place aliquot (ca 25 g) in 35 mL centrifuge tube. Centrifuge mixture (at least 6000 x g, preferably 38,000 x g) to separate solids from supernatant.

G. Treatment of Food and Polydextrose Standards

Take aliquot (2 mL) of polydextrose intermediate standard from section E, or aliquot (2 mL) of supernatant from section F; freeze remaining solution (see section H). Transfer to centrifugal ultrafiltration device. Centrifuge at 6400 rpm (5000 x g) for 45 min. Record weight of 1.7 mL microcentrifuge tube (accurate to 0.1 mg). Take 0.2 mL aliquot of ultrafiltrate, place in weighed tube, and record weight (accurate to 0.1 mg). Add 0.8 mL buffered enzyme mix C(t). Record final weight (accurate to 0.1 mg). Calculate weight of 0.2 mL aliquot and weight of 0.8 mL buffer enzyme mix. Secure cap. Vortex mix thoroughly. Incubate at 50°C for 60 min. After incubation, place in boiling water bath for 10 min to denature enzymes. Then cool in ice bath or freezer until enzyme precipitates (ca 5 min in ice). Centrifuge at 10,000 rpm (9200 x g) for 10 min. Use sample within 72 h of preparation.

H. Determination

Filter supernatant from G through 0.2 μm syringe filter into autosampler vial. Inject 25 μL through HPAEC system. Analyze each test sample in triplicate and determine mean detector response. Determine peak area for high molecular weight component of polydextrose at retention time ca 12 min. RSD should be ≤5%. If not, repeat HPAEC analysis. Compare this area with calibration curve. The polydextrose concentration should be within the range of the calibration curve. If not, rerun assay, adjusting concentration of initial sample supernatant (F).

I. Calculations

1. Stock standard concentration (ppm):

\[
\frac{\text{mg Polydextrose (dry basis)} \times 1000}{\text{g water}}
\]

where 1000 = the conversion factor of mg to μg.

2. Working standard concentration (ppm):

\[
\frac{A_1}{A_1 + A_2} \times \frac{F + W}{F} \times 0.0001
\]

where \(A_1\) = weight of aliquot (0.2 mL) of intermediate standard (g); \(A_2\) = weight of aliquot (0.8 mL) of buffered enzyme mix (g).

\(P\) = polydextrose concentration (ppm), obtained from the calibration curve, second order polynomial fit; \(F\) = weight of food (g); \(W\) = weight of water (g); \(A_1\) = weight of aliquot (0.2 mL) diluted food (g); \(A_2\) = weight of aliquot (0.8 mL) buffered enzyme mix (g). The 0.0001 is the conversion factor from 100 x 1 mg/1000 g.

Results and Discussion

Several enzyme combinations and incubation conditions were evaluated before those described were selected (data not shown). Precise conditions and type of ultra filtration devices were also determined (data not shown). The step change in LC gradient to the stronger mobile phase creates a consistent peak of high molecular weight polydextrose. A typical calibration curve is shown (Figure 3), and is consistently a second order polynomial fit of \(R^2 \geq 0.998\).

Foods containing various levels of polydextrose were prepared and tested by this method (Table 1). These foods represent a broad cross-section of matrices, with key ingredients (e.g., inulin and pectin) that might have acted as interferences. Our internal validation demonstrated the ruggedness of the method with recoveries ranging from 83 to 104% (average 99.2%). An AOAC collaborative study has been completed, and Method 2000.11 was adopted Official First Action in May 2000.

The method was tested in conjunction with the AOAC TDF method (985.29) to determine whether polydextrose could be quantitated from the ethanol supernatant. An outside laboratory used AOAC Method 985.29 on polydextrose, grape jelly containing polydextrose, and a cookie with polydextrose. They then determined TDF and provided us with the ethanol supernatant, in which we were able to account for all polydextrose. Thus, there was no significant hydrolysis or loss of polydextrose during the AOAC 985.29 procedure. However, because organic solvents such as ethanol can cause changes in detector response, the ethanol was removed from samples to ensure accurate data. We recommend that polydextrose content be determined directly from the food (not from the ethanol supernatant) for more consistent and rapid analysis. This value can then be added to the TDF value determined by the AOAC TDF methods 985.29, 991.43, 992.16, 993.21, and 994.13.
In conclusion, polydextrose is resistant to the enzymes used in the AOAC TDF method and to human digestive enzymes. The physiological effects of polydextrose have been shown in numerous studies to be consistent with dietary fiber. However, the current TDF methods do not quantify polydextrose as dietary fiber. We have developed a new rugged method for determining polydextrose in foods, which is currently undergoing collaborative study. This value can be added to the TDF value determined by current AOAC TDF methods.

References

(11) Pfizer, Inc. (1978) Polydextrose Food Additive Petition No. 9A3441, New York, NY

Table 1. Recovery of polydextrose (PDX) from 6 foods

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<th>RSD, %</th>
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