Polydextrose in Foods by Ion Chromatography

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Objective

This method measures the polydextrose content of foods. Polydextrose is a 1 kcal/g randomly bonded polysaccharide used as a food ingredient, and has physiological benefits consistent with dietary fiber. The value obtained from this polydextrose assay may be added to the values from the enzyme-gravimetric methods without concern for double counting. 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 maltooligomers and fructans. Polydextrose standards undergo the same treatment. High-pressure anion-exchange chromatography with electrochemical detection (HPAEC-ED) is used to detect and quantitate the high molecular weight fraction of polydextrose.

Apparatus

1. Water baths, two, one set at 80 ± 2° (increased to boiling), the second set at 50 ± 2°.
2. High-speed centrifuge and three rotors capable of
   a. 6000 × *g* (preferably up to 38,000 × *g*), with 50-ml (holding ~38 ml) centrifuge tubes.
   b. 9200 × *g* with 1.7-ml microcentrifuge tubes.
   c. 6400 × *g* with 15-ml centrifuge tubes.
3. Analytical balance, 0.1 mg accuracy.
4. Bottles and tubes
   a. containers with screw caps (250-ml) capable of withstanding 80° water.
   b. centrifuge tubes, 50-ml (holding ~38 ml), capable of withstanding 38,000 × *g*.
   c. microcentrifuge tubes (1.7 ml) capable of withstanding 10,000 × *g*.
5. Vortex mixer.
6. Pipets, adjustable, 20–200 µl and 200–1000 µl.
7. Filters, PTFE syringe filter, 0.2-µm pore size, 13-mm diameter.
8. Centrifugal ultrafiltration devices, with 100,000 nominal molecular weight cutoff, 2-ml polyethersulfone membrane.
9. Liquid chromatograph (LC) and detectors**,** HPAEC-ED.
   a. LC capable of producing ~3,000 psi (200 bar).
   b. gradient pump (capable of handling NaOH eluents).
   c. pulsed integrated electrochemical detector (with gold electrode).
10. LC column, microporous substrate (10-µm) agglomerated with a micro-bead quaternary ammonium functionalized latex, 250 × 4 mm column, with guard column.

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Reagents

1. Deionized water (resistance ≥18 MΩ-cm).
2. Sodium hydroxide, 50% (w/w), carbonate free.
3. Acetic acid, 0.2*M*. Bring 1.20 g acetic acid to volume in a 100-ml volumetric flask with water.
4. Sodium acetate trihydrate, 0.2*M*. Bring 2.72 g sodium acetate trihydrate to volume in a 100-ml volumetric flask with water.
5. Acetate buffer, pH 4.5. Mix 28 ml acetic acid (0.2*M*) with 22 ml sodium acetate trihydrate (0.2*M*); bring up to 100 ml with water.
6. Fructanase (exoinulinase), 667 U/ml, 8000 U dissolved in 12 ml acetate buffer. One unit is amount of enzyme required to release 1 µmol of fructose reducing sugar equivalents from kestose per min under standard assay conditions (10 m*M* kestose, pH 4.5, 40°). Fructanase contains approximately 1% of endoinulinase.
7. Amyloglucosidase, 3,260 U/ml for soluble starch, 200 U/ml for *p*-nitrophenol-β-maltoside (pNP-β-maltoside). One unit is amount of enzyme required to release 1 µmol of glucose from soluble starch per min under standard assay conditions (starch substrate: 10 mg/ml starch, pH 4.5, 40°). One unit is amount of enzyme required to release 1 µmol of *p*-nitrophenol from pNP-β-maltoside (in presence of excess -β-glucosidase) per min under standard assay conditions (10 n*M* pNP-β-maltoside, pH 4.5, 40°).
8. Isoamylase, 200 U/ml. One unit is amount of enzyme required to release 1 µmol of glucose reducing sugar equivalents from oyster glycogen per min under standard assay conditions (10 mg/ml oyster glycogen, pH 3.5, 40°).
9. Buffered enzyme mix. Combine fructanase (2 ml; 1,324 U), amyloglucosidase (84 µl; 274 U), and isoamylase (84 µl; 16.8 U). Dilute in acetate buffer to 20 ml in a volumetric flask. Make up fresh each day, and store at 4°.
10. HPAEC mobile phases:
    a. Sodium hydroxide, 0.15*M*. Take 12.00 g sodium hydroxide (50%, w/w) and add slowly to ~900 ml water with stirring in a 1-liter volumetric flask. Bring to 1 liter volume with water.
    b. Sodium acetate trihydrate, 0.5*M*. Take 68.04 g sodium acetate trihydrate and bring to volume in a 1-liter volumetric flask with water.

Procedure

Analytical HPAEC conditions

Mobile phase: A = 0.15*M* sodium hydroxide; B = 0.15*M* sodium hydroxide + 0.5*M* sodium acetate. See Note.

Gradient: a) 0–10 min of 70% A/30% B; b) at 10 min, change to 100% B; c) at 15 min, change to 70% A/30% B for 10 min.

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Flow rate: 1.2 ml/min.

TABLE I
Detector Voltage Settings (Dionex ED 40)

Time (sec)	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

Detector voltage settings are shown in Table I.

Preparation of standards

1. Polydextrose. Food Chemical Codex grade of known moisture content determined by the Karl Fischer method.

2. Stock standard (5000 µg/g polydextrose for DX 500 system). Weigh 500 ± 0.1 mg (dry basis at room temperature) polydextrose into a preweighed 250-ml glass container with screw cap. Make sure cap is on container when weighing, and record weight in g. Add ~100 g of water preheated to 80°. Screw cap on tightly, and vortex for 30 sec. Place container in a water bath at 80° for 10 min; vortex for 30 sec at 5 and 10 min to solubilize the polydextrose. Remove container from water bath; allow cooling to room temperature (25°); and weigh (accurate to 0.01 g). Make sure cap is on container when weighing.

3. Intermediate standards. Serially dilute the 5000 µg/g standard to make 2500, 2000, 1500, 1250, 1000, 750, 500, and 250 µg/g polydextrose standards in water. This range is for the DX 500 system; some systems may require a different range. These standards will be diluted fivefold during the procedure described in a later section (*Treatment of food and polydextrose standards*) and are considered the working standards (500, 400, 300, 250, 200, 150, 100, and 50 µg/g polydextrose). An eight-point calibration curve should give a polynomial regression with a correlation coefficient ≤0.995.

4. Standards are stable at 4° for 1 month.

Preparation of test sample (extraction of polydextrose from food)

1. Grind or cut food into small particles. Store in sealed containers to prevent

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moisture changes. If approximate amount of polydextrose in food test sample is known, weigh a quantity of food that will yield an amount of polydextrose within the range of the calibration curve. If
amount of polydextrose in test sample is not known, estimate amount of polydextrose and choose a test sample weight based on that estimate.

2. Weigh an appropriate amount of food test sample (accurate to 0.01 g) and add to a preweighed container with screw cap. Record weight of food test sample in g. Add ~100 g hot water (80°) and immediately replace cap tightly. Vortex for 30 sec to disperse food. Place container in a water bath at 80° for 10 min. Vortex for 30 sec at 5 and 10 min to solubilize polydextrose. Remove container from water bath; allow cooling to room temperature (25°). Record total weight in g, and calculate weight in g of water. Take an aliquot (~25 g) and place in a 35-ml centrifuge tube. Centrifuge mixture 38,000 × *g* for 10 min to separate solids from supernatant.

Treatment of food and polydextrose standards

1. Take an aliquot (2 ml) of polydextrose intermediate standard or an aliquot (2 ml) of supernatant from the "*Preparation of test sample*" section (freeze remaining solution). Transfer into a centrifugal ultrafiltration device. Centrifuge at 5,000 × *g* (6,400 rpm) for 45 min. Record the weight in g of a 1.7-ml microcentrifuge tube (accurate to 0.1 mg).
2. Take a 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. Record final weight in g (accurate to 0.1 mg). Calculate weight of 0.2-ml aliquot and weight of 0.8-ml buffer enzyme mix.
3. Secure cap and vortex to mix thoroughly.
4. Incubate at 50° for 60 min. After incubation, place in boiling water bath for 10 min to denature enzymes. Then cool in an ice bath or freezer until enzyme precipitates (~5 min in ice).
5. Centrifuge at 9,200 × *g* (10,000 rpm) for 10 min. Use supernatant within 72 hr of preparation.

Determination

1. Filter supernatant through a 0.2-µm syringe filter into an autosampler vial.
2. Inject 25 µl through high-pressure anion-exchange chromatograph. Analyze each test sample supernatant in triplicate, and determine mean detector response.
3. Determine peak area for high molecular weight component of polydextrose at retention time of ~12 min; % relative standard deviation should be ≤5%. If not, repeat HPAEC analysis. Compare this area with calibration curve. Polydextrose concentration should be in range of calibration curve. If it is not, rerun assay, adjusting concentration of initial test sample supernatant.

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Calculations

1. Stock standard concentration (µg/g):

$$\text{Stock standard, } \mu g/g = \frac{\text{mg polydextrose (dry basis)}}{\text{g water}} \times 1000$$

where, 1000 is the conversion factor of mg to µg.

2. Working standard concentration (µg/g):

$$\text{Working standard, } \mu g/g = \left(\frac{A_1}{A_1 + A_2}\right) \times \text{intermediate standard concentration}$$

where, A_1 = weight (in g) of aliquot (0.2 ml) of intermediate standard and A_2 = weight (in g) of aliquot (0.8 ml) of buffered enzyme mix.

3. Polydextrose (%) determination:

$$\%\ \text{Polydextrose} = P \times \left(\frac{A_1 + A_2}{A_1}\right) \times \left(\frac{F + W}{F}\right) \times\ 0.0001$$

where, P = polydextrose concentration (µg/g), 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) of diluted food (g); A_2 = weight of aliquot (0.8 ml) of buffered enzyme mix (g); and 0.0001 is the conversion factor from µg/ml to %.

Note

It is very important to degas the mobile phase and store under inert gas.

Reference

AOAC International. 2001. Official Methods of Analysis of AOAC International, 17th ed., 1st rev. Method 2000.11. The Association, Gaithersburg, MD.