19 chapter

Carbohydrate Analysis

James N. BeMiller

Department of Food Science,
Purdue University,
West Lafayette, IN 47907-2009, USA
e-mail: bemiller@purdue.edu

S. Nielsen (ed.), *Food Analysis*, Food Science Text Series,
DOI 10.1007/978-3-319-45776-5_19, © Springer International Publishing 2017

19.1 INTRODUCTION

Carbohydrates are important in foods as a major source of energy, to impart crucial textural properties, and as dietary fiber which contributes to overall health. There is interest in analysis of food products and ingredients for the various types of carbohydrates (not only the different structural types but also types differing in physiological effects, e.g., digestible vs. nondigestible, metabolizable vs. non-metabolizable, caloric vs. reduced caloric vs. noncaloric, prebiotic vs. non-prebiotic). However, definitions of the types are not always agreed upon, and analytical methods do not always measure exactly what is included in the definition, which results in controversies about what should be measured and how. This chapter covers analysis of carbohydrates primarily by structural type.

Digestible carbohydrates are converted into monosaccharides, which are absorbed and provide metabolic energy and satiety. Nondigestible polysaccharides (all those other than starch) comprise the major portion of dietary fiber (Sect. 19.6). Carbohydrates also provide other attributes, including bulk, body, viscosity, stability to emulsions and foams, water-holding capacity, stability to freezing and thawing, browning (including generation of flavors and aromas), and a range of desirable textures (from crispness to smooth, soft gels), and they may lower water activity and thereby inhibit microbial growth. Basic carbohydrate structures, chemistry, and terminology can be found in references [1, 2].

Major occurrences of major carbohydrates in foods are presented by structural classes in Table 19.1. Ingested carbohydrates are almost exclusively of plant origin, with milk lactose being the major exception. Of the **monosaccharides** (sometimes called **simple sugars**), only D-glucose and D-fructose are found in other than minor amounts. Monosaccharides are the only carbohydrates that can be absorbed from the small intestine. Higher saccharides (**oligo**- and **polysaccharides**) must first be digested (i.e., hydrolyzed to monosaccharides) before absorption and utilization can occur. (Note: The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) [3] recommend that carbohydrates be classified by molecular size into sugars [degree of polymerization (DP) 1–2], oligosaccharides (DP 3–9), and polysaccharides (DP >9), but carbohydrate chemists (according to international nomenclature rules) consider an oligosaccharide to be a carbohydrate composed of 2 to 10 (or 2–20) sugar (saccharide) units). Polysaccharides usually contain from about 30 to 60,000 or more monosaccharide units. Humans can digest only sucrose, lactose, maltodextrins (maltooligosaccharides), and starch. All are digested with enzymes found in the small intestine.

At least 90% of the carbohydrate in nature is in the form of polysaccharides. As stated above, the starch polymers are the only polysaccharides that humans can digest and use as a source of calories and carbon. All other polysaccharides are nondigestible. **Nondigestible polysaccharides** can be divided into **soluble** and **insoluble** classes. Along with lignin and other nondigestible, nonabsorbed substances, they make up **dietary fiber** (Sect. 19.6.1). As dietary fiber, they regulate normal bowel function, reduce the postprandial hyperglycemic response, and may lower serum cholesterol. However, nondigestible polysaccharides most often are added to processed foods because of the functional properties they impart. Nondigestible oligosaccharides serve as prebiotics and are, therefore, increasingly used as ingredients in functional foods and nutraceuticals. The foods in which dietary fiber components can be used, and particularly the amounts that can be incorporated, are limited because addition above a certain level usually changes the characteristics of the food product. Indeed, as already stated, they are often used as ingredients because of their ability to impart important functional properties at a low level of usage, rather than for a physiological effect.

Carbohydrate analysis is important from several perspectives. Qualitative and quantitative analyses are used to determine compositions of foods, beverages, and their ingredients. **Qualitative analysis** ensures that ingredient labels present accurate compositional information. **Quantitative analysis** ensures that added components are listed in the proper order on ingredient labels. Quantitative analysis also ensures that stated amounts of specific components of consumer interest are proper and that the caloric content can be calculated. Table 19.2 summarizes some of the methods described in this chapter and commonly used for nutrition labeling, quality assurance, or research for food ingredients and/or products. Of increasing importance are analyses to determine authenticity and origin of foods, beverages, and ingredients. Both qualitative and quantitative analysis can be used to authenticate (i.e., to detect adulteration of) food ingredients and products and for quality assurance.

The most commonly used methods of carbohydrate determination are presented here. However, methods often must be made specific to a particular food product because of the nature of the product and the presence of other constituents. Approved methods are referenced, but method approval has not kept pace with methods development; so in some cases, other methods are presented. Methods that have been in longtime use, although not giving as much or as precise information as newer methods, nevertheless may be useful for product standardization in some cases.

In general, evolution of analytical methods for low-molecular-weight carbohydrates has followed the succession: qualitative color tests, adaptation of the color test for reducing sugars based on reduction of Cu(II) to Cu(I) (Fehling test) to quantitation of reducing sugars, qualitative paper chromatography, quantitative paper chromatography, gas chromatography (GC) of derivatized sugars, qualitative and quantitative thin-layer chromatography, enzymic methods, and high-performance liquid chromatography (HPLC). Some older methods are still in use, and multiple official methods for the analysis of mono- and disaccharides in foods are currently approved by AOAC International [4]. Methods employing nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR) spectroscopy (Sect. 19.7.3 and Chap. 8), near-infrared (NIR) spectroscopy (Sect. 19.7.4 and Chap. 8), immunoassays (Chap. 27), fluorescence spectroscopy (Chap. 7), capillary electrophoresis (Sect. 19.4.2.4), and mass spectrometry (MS) (Sect. 19.7.2 and Chap. 11) have been published but are not yet in general use for carbohydrate analysis. Reference [5] also may be consulted for food carbohydrate analysis.

According to the nutrition labeling regulations of the US Food and Drug Administration (FDA) [6] [21 CFR 101.9 (c)(6)(i)–(iv)], the following are details regarding carbohydrates (all declared in relation to a serving, as defined by the FDA):

1. **Total carbohydrate** content of a food must be calculated by subtraction of the sums of the crude protein, total fat, moisture, and ash in a serving from the total weight of the food (i.e., total carbohydrate is determined by difference). (Note that this calculation is not an actual measurement of carbohydrate content. Its accuracy depends on the accuracies of determinations of the other components, but this method is required by US regulations for nutrition labeling. As described in Chap. 3, Sect. 3.2.1.6, caloric content for the label can be calculated with or without taking into account the insoluble dietary content of the food.)
2. **Dietary fiber** (FDA definition given in Table 19.5) content in a serving must also be stated on the label. Declaration of contents of the subcategories **soluble fiber** and **insoluble fiber** is voluntary.
3. **Total sugars** are defined for labeling purposes as the sum of all free monosaccharides and disaccharides (such as glucose, fructose, lactose, and sucrose).
4. **Added sugars**, a required listing on the nutrition label in the 2016 updated regulations, are defined by the FDA as follows: "Added sugars are either added during the processing of foods, or are packaged as such, and include sugars (free, mono- and disaccharides), sugars from syrups and honey, and sugars from concentrated fruit or vegetable juices that are in excess of what would be expected from the same volume of 100 percent fruit or vegetable juice of the same type, except …." The statement continues by stating the exceptions of what is not included in "added sugar."
5. **Sugar alcohols**' declaration on the nutrition label is voluntary, except it is required if a claim is made on the label or in labeling about sugar alcohol or total sugars, or added sugars when sugar alcohols are present in the food. "Sugar alcohols are defined as the sum of saccharide derivative in which a hydroxyl group replaces a ketone or aldehyde group and whose use in the food is listed by FDA (e.g., mannitol or xylitol) or in generally recognized as safe (e.g., sorbitol)." If only one sugar alcohol is present in the food (e.g., xylitol), the specific name of the sugar alcohol may be used in place of "sugar alcohol."

See Table 19.3 [7] for the total carbohydrate, sugar, and total dietary fiber (TDF) content of selected foods.

19.2 SAMPLE PREPARATION

19.2.1 General Information

Sample preparation is related to the specific carbohydrate being determined (because carbohydrates have such a wide range of solubilities) and the specific raw material, ingredient, or food product being analyzed. However, some generalities can be presented (Fig. 19.1).

19.1 table

Occurrences of some major carbohydrates in foods

Carbohydrate	*Source*	*Constituent(s)*
Monosaccharides[a]		
D-glucose (dextrose)	Naturally occurring in honey, fruits, and fruit juices. Added as a component of glucose syrups and high-fructose syrups. Produced during processing by hydrolysis (inversion) of sucrose	
D-fructose	Naturally occurring in honey, fruits, and fruit juices. Added as a component of high-fructose syrups. Produced during processing by hydrolysis (inversion) of sucrose	
Sugar alcohol[a]		
Sorbitol (D-glucitol)	Added to food products, primarily as a humectant	
Disaccharides[a]		
Sucrose	Widely distributed in fruit and vegetable tissues and juices in varying amounts. Added to food and beverage products	D-fructose D-glucose
Lactose	In milk and products derived from milk	D-galactose D-glucose
Maltose	In malt. In varying amounts in various glucose syrups and maltodextrins	D-glucose
Higher oligosaccharides[a]		
Maltooligosaccharides	Maltodextrins. In varying amounts in various glucose syrups	D-glucose
Raffinose	Small amounts in beans	D-glucose D-fructose D-galactose
Stachyose	Small amounts in beans	D-glucose D-fructose D-galactose
Polysaccharides		
Starch[b]	Widespread in cereal grains and tubers. Added to processed foods	D-glucose
Food gums/hydrocolloids[c]	Added as ingredients	[d]
Algins		
Carboxymethylcelluloses		
Carrageenans		
Gellan		
Guar gum		
Gum arabic		
Hydroxypropylmethyl celluloses		
Inulin		
Konjac glucomannan		
Locust bean gum		
Methylcelluloses		
Pectins		
Xanthan		
Cell-wall polysaccharides[c]	Naturally occurring	
Pectin (native)		
Cellulose		
Hemicelluloses		
Beta-glucan		

[a]For analysis, see Sect. 19.4.2
[b]For analysis, see Sect. 19.5.1
[c]For analysis, see Sect. 19.5.2
[d]For compositions, characteristics, and applications, see [1, 2]

19.2 table Summary of carbohydrate analysis methods

To determine:	*Description of method*	*Method measures*	*Advantages/disadvantages*
Total carbohydrate for nutrition label	Grams of carbohydrate per serving is calculated as total grams of serving minus (g of moisture + g of protein + g of lipid + g of ash)	Total carbohydrate by difference	Not an actual measurement of carbohydrate. Depends on accuracy of determinations of other components, but this method is required by US regulations. Should not be used to calculate caloric content because carbohydrate components of dietary fiber, such as cellulose, provide essentially no calories
Total carbohydrate[a]	Spectrophotometric, phenol-sulfuric acid	Measures all carbohydrates except sugar alcohols	Solution must be clear, i.e., carbohydrates must be soluble, so the method may not measure all carbohydrates. Method requires a standard curve made with the same exact mixture of carbohydrates in the same ratio that occurs in the sample
Total reducing sugars[a]	Spectrophotometric. Somogyi-Nelson and related methods	Primarily used to measure glucose/dextrose, maltose, and other low-molecular-weight oligosaccharides in glucose syrups	If carbohydrates are not already in solution, requires extraction. Solution must be clear. Fructose gives some response
Glucose/dextrose[a]	(1) Enzymic assay using GOPOD reagent (spectrophotometric)[b] (2) HPLC	Both methods specifically determine the amount of glucose in a mixture of sugars	Extraction required. Enzymic method can be automated
Fructose[a]	HPLC	Specific determination of fructose in a mixture of sugars	Extraction required
Sucrose[a]	(1) Enzymic assay using GOPOD reagent (spectrophotometric)[b] (2) HPLC	Both methods specifically determine the amount of sucrose in a mixture of sugars	Extraction required. Enzymic method can be automated. For enzymic method, solution must be clear
Lactose[a]	(1) Enzymic assay (spectrophotometric)[b] (2) HPLC. Enzymic assay employs galactose oxidase[b]	Both methods specifically determine the amount of lactose in a mixture of sugars, except that the enzymic method will also measure free galactose (uncommon) or other galactose-containing substances	Extraction required. Enzymic method can be automated
Concentrations of syrups[a]	(1) Measurement of specific gravity using a hydrometer (2) Refractive index using a refractometer	Concentration of solids in the solution	Solutions must be pure and of a single substance
Starch[a]	(1) Hydrolysis of starch to glucose using a mixture of amylases and determination of glucose using the (GOPOD) reagent (2) Hydrolysis of starch with glucoamylase and determination of glucose with glucose oxidase[b]	Specific for starch, including modified starches	Does not measure resistant starch[c]. Sample must be free of glucose or a correction made for it. Amylases must be purified to remove any interfering activities

19.2 table (continued)

To determine:	Description of method	Method measures	Advantages/disadvantages
Pectin[a]	Spectrophotometric. m-Hydroxydiphenyl-sulfuric acid method	Uronic acids	Extraction may be required. Standard curve is required. Other hydrocolloids containing uronic acids will interfere
Dietary fiber	Gravimetric (residue after removal of lipids, digestible starch, and protein and subtraction of ash content)	"Total dietary fiber"	Does not include low-molecular-weight soluble dietary fiber. Is not a measure of the physiological efficacy of the particular dietary fiber. Soluble and insoluble dietary fiber can be determined by specific methods for them

[a]For research and quality assurance. Not required for nutrition labeling
[b]The YSI Life Sciences instrument method uses an electrode that detects hydrogen peroxide
[c]A Megazyme kit includes resistant starch in the measurement

For most foods, the first step is drying, which also can be used to determine moisture content. For other than beverages, drying is done by placing a weighed amount of material in a vacuum oven and drying to constant weight at 55 °C and 1 mm Hg pressure. Then, the material is ground to a fine powder, and lipids are extracted using 19:1 vol/vol chloroform-methanol in a Soxhlet extractor (Chap. 17). (Note: Chloroform-methanol forms an azeotrope boiling at 54 °C with a mole ratio of 0.642:0.358 or a vol/vol ratio of 3.5:1 in the vapor.) Without prior extraction of lipids and other lipid-soluble substances, extraction of water-soluble carbohydrates will likely be incomplete.

Other sample preparation schemes may be required. For example, the AOAC International [4] method for presweetened, ready-to-eat breakfast cereals calls for removal of fats by extraction with petroleum ether (hexane) rather than the method described above and extraction of sugars with 50 % ethanol (AOAC Method 982.14), rather than the method described below.

19.2.2 Extraction and Cleanup for Determination of Mono- and Oligosaccharides

Food raw materials and products and some ingredients are complex, heterogeneous, biological materials. Thus, it is quite likely that they may contain substances that interfere with measurement of the mono- and oligosaccharides present, especially if a spectrophotometric method is used. Interference may arise either from compounds that absorb light of the same wavelength used for the carbohydrate analysis or from insoluble, colloidal material that scatters light, since light scattering will be measured as absorbance. Also, the aldehydic or keto group of the sugar can react with other components, especially amino groups of proteins, a reaction (the **nonenzymatic browning** (**Maillard**) **reaction**) that simultaneously produces color and destroys the sugar. Even if chromatographic methods, such as HPLC (Sect. 19.4.2.1), are used for analysis, the mono- and oligosaccharides must be isolated from the other components of the food before chromatography.

For determination of any mono- (glucose, fructose), di- (sucrose, lactose, maltose), tri- (raffinose), tetra- (stachyose), or other oligosaccharides (e.g., **maltodextrins**) present, the dried, lipid-free sample (Sect. 19.2.1) is extracted with **hot 80 % ethanol** in the presence of precipitated calcium carbonate to neutralize any acidity (AOAC Method 922.02, 925.05) (Fig. 19.1). Some of the higher oligosaccharides from added maltodextrins or **fructooligosaccharides** (FOS) may also be extracted. Most carbohydrates (especially those of low molecular weight) are soluble in hot 80 % ethanol. However, much of the composition of a food (other than water) is in the form of polymers, and almost all polysaccharides and proteins are insoluble in hot 80 % ethanol. Thus, this extraction is rather specific. Extraction is done by a batch process. Refluxing 1 h, cooling, and filtering is standard practice. (A Soxhlet apparatus cannot be used because aqueous ethanol undergoes azeotropic distillation as 95 % ethanol.) Extraction should be done at least twice to check for and ensure completeness of extraction. If the foodstuff or food product is particularly acidic, for example, a low-pH fruit, neutralization may be necessary to prevent hydrolysis of sucrose, which is particularly acid labile; thus, precipitated calcium carbonate is routinely added.

The 80 % ethanol extract will contain components other than carbohydrates, in particular ash, pigments, organic acids, and perhaps free amino acids and low-molecular-weight peptides. Because the mono- and oligosaccharides are neutral and the contaminants are

19.3 table Total carbohydrate, sugars, and total dietary fiber contents of selected foods

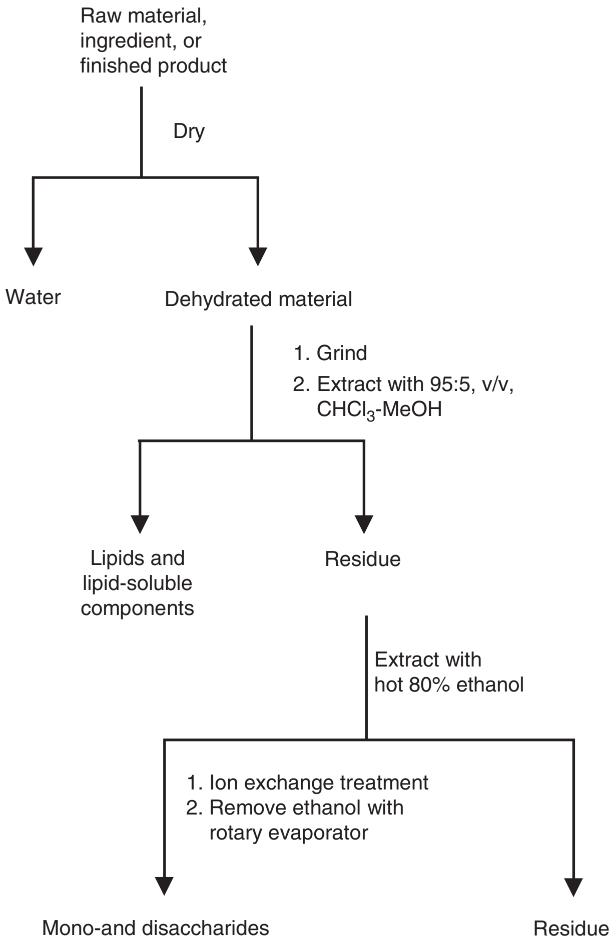
Food	*Approximate percent total carbohydrate (wet weight basis)*	*Sugars, %*	*TDF[a], %*
Cereals, bread, and pasta			
Bagels, plain	53	NR[b]	2.3
Bread, white	49	5.7	2.7
Macaroni, dry, enriched	75	NR	4.3
Macaroni, cooked	27	1.1	4.3
Ready-to-eat cereals			
Cheerios	73	4.4	9.4
Corn flakes	84	9.5	3.3
Dairy products			
Ice cream, soft serve, chocolate	22	21	0.7
Ice cream, light chocolate	23	20	0
Milk, reduced fat (2%)	4.8	5.1	0
Milk, chocolate, commercial	1.0	9.5	0.8
Yogurt, plain, low fat (12 g protein/8 oz)	7.0	7.0	0
Fruits and vegetables			
Apples, raw, with skin	14	10	2.4
Apples, raw, without skin	13	10	1.3
Applesauce, canned, sweetened	20	NR	12
Broccoli, raw	6.6	1.7	2.6
Broccoli, cooked	8.8	3.5	3.0
Carrots, raw	9.6	4.7	2.8
Carrots, cooked	8.2	3.5	3.0
Grapes, raw	18	16	0.9
Potatoes, raw, with skin	18	1.0	2.1
Tomato, juice	4.1	2.9	0.8
Meat, poultry, and fish			
Bologna, beef	4.3	2.1	0
Chicken, broilers or fryers, skinless, boneless breast	0	0	0
Chicken, breast, tenders, cooked	18	0	NR
Fish sticks, frozen, prepared	22	1.7	1.5
Other			
Beer, regular	3.6	0	0
Beer, light	1.6	0.1	0
Carbonated beverage, cola, regular	10	9.9	0
Carbonated beverage, cola, diet	0.1	0	0
Cream of mushroom soup	6.8	0.4	0.7
Honey	82	81	0.2
Salad dressing, ranch	5.9	4.7	0
Salad dressing, reduced fat	21	3.8	1.1
Salad dressing, fat free	27	5.6	0.1

From US Department of Agriculture, Agricultural Research Service (2016) USDA National Nutrient Database for Standard Reference. Release 28. Nutrient Data Laboratory Home Page, http://ndb.nal.usda.gov

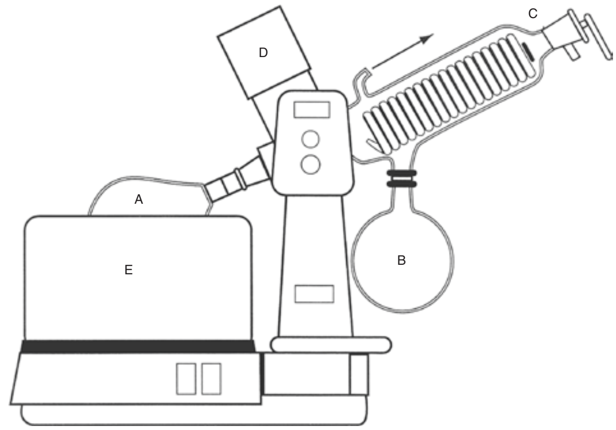
[a]Total dietary fiber

[b]Not reported

charged, the contaminants can be removed by **ion-exchange** techniques. Because reducing sugars can be adsorbed onto and be isomerized by strong anion-exchange resins in the hydroxide (OH^-) form, a weak anion-exchange resin in the carbonate (CO_3^{2-}) or hydrogencarbonate (HCO_3^-) form is used. [**Reducing sugars** are those mono- and oligosaccharides that contain a free carbonyl (aldehydic or keto) group and, therefore, can act as reducing agents; see Sect. 19.4.1]. Because sucrose and sucrose-related oligosaccharides



19.1 figure Flow diagram for sample preparation and extraction of mono- and disaccharides



19.2 figure Diagram of a rotary evaporator. The solution to be concentrated is placed in the round-bottom flask (*A*) in a water bath (*E*) at a controlled temperature. The system is evacuated by means of a water aspirator or vacuum pump; connecting tubing is attached at the arrow. Flask A turns (generally slowly). Because of the reduced pressure, evaporation is relatively rapid from a thin film on the inside walls of flask A produced by its rotation, the large surface area, and the elevated temperature. *C* is a condenser. *D* is the motor. Condensate collects in flask B. The stopcock at the top of the condenser is for releasing the vacuum

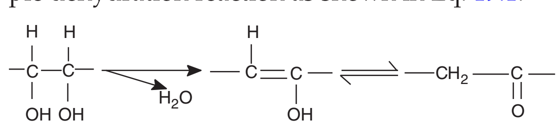
are very susceptible to acid-catalyzed hydrolysis, the anion-exchange resin should be used before the cation-exchange resin. However, because the anion-exchange resin is in a carbonate or hydrogencarbonate form, the cation-exchange resin (in the H^+ form) cannot be used in a column because of CO_2 generation. Mixed-bed columns are not recommended for the same reason. AOAC Method 931.02 reads basically as follows for cleanup of ethanol extracts: Place a 50-mL aliquot of the ethanol extract in a 250-mL Erlenmeyer flask. Add 3 g of anion-exchange resin (OH^- form) and 2 g of cation-exchange resin (H^+ form). Let stand 2 h with occasional swirling.

The aqueous ethanol is removed from the extract under reduced pressure using a **rotary evaporator** (Fig. 19.2) and a temperature of 45–50 °C. The residue is dissolved in a known, measured amount of water. Filtration should not be required, but should be used if necessary. Some methods employ a final passage through a hydrophobic column such as a Sep-Pak C18 cartridge (Waters Associates, Milford, MA) as a final cleanup step to remove any residual lipids, proteins, and/or pigments. However, this should not be necessary if the lipids and lipid-soluble components were properly removed prior to extraction.

19.3 TOTAL CARBOHYDRATE: PHENOL-SULFURIC ACID METHOD

19.3.1 Principle and Characteristics

Carbohydrates are destroyed by strong acids and/or high temperatures. Under these conditions, a series of complex reactions takes place, beginning with a simple dehydration reaction as shown in Eq. 19.1.



(19.1)

Continued heating in the presence of acid produces various furan derivatives that react with various phenolic compounds, such as phenol, resorcinol,

orcinol, α-naphthol, and napthoresorcinol, and with various aromatic amines, such as aniline and *o*-toluidine, to produce colored compounds [1]. The most often used condensation is with phenol itself [8]. This widely used method is simple, rapid, sensitive, accurate, and specific for carbohydrates. The reagents are inexpensive, readily available, and stable. Virtually all mono-, oligo-, and polysaccharides can be determined using the phenol-sulfuric acid method. (Oligo- and polysaccharides react because they undergo hydrolysis in the hot, strong acid, releasing monosaccharides.) However, neither sorbitol nor any other **sugar alcohol** (**alditol**, **polyol**, **polyhydroxyalcohol**) gives a positive test. A stable color is produced, and results are reproducible. Under proper conditions, the phenol-sulfuric method is accurate to ±2 %.

The reaction is not stoichiometric, and the extent of color formation is, in part, a function of the structure of the sugar. Therefore, a standard (calibration) curve (Chaps. 4 and 6) must be used. Ideally, the standard curve will be prepared using mixtures of the same sugars present in the same ratio as they are found in the sample being analyzed. If this is not possible (e.g., if a pure preparation of the sugar being measured is not available or if more than one sugar is present either as free sugars in unknown proportions or as constituent units of oligo- or polysaccharides or mixtures of them), D-glucose is used to prepare the standard curve. In these cases, accuracy is a function of conformity of the standard curve made with D-glucose to the curve that would be produced from the exact mixture of carbohydrates being determined. In any analysis requiring a standard curve, the concentrations used to construct the standard curve must cover a range that begins below the lowest carbohydrate concentration of the samples and extends above the highest concentration of the samples and must be within the limits reported for sensitivity of the method.

19.3.2 Outline of Procedure

1. A clear, aqueous solution of carbohydrate(s) is transferred using a pipette into a small tube.
2. An aqueous solution of phenol is added, and the contents are mixed.
3. Concentrated sulfuric acid is added rapidly to the tube so that the stream produces good mixing. The tube is agitated. (Adding the sulfuric acid to the water produces considerable heat.) A yellow-orange color results.
4. Absorbance is measured at 490 nm.
5. The average absorbance of blanks (sample alone and reagents alone) is subtracted, and the amount of sugar is determined by reference to a standard curve.

19.4 MONO- AND OLIGOSACCHARIDES

19.4.1 Total Reducing Sugar

19.4.1.1 *Somogyi-Nelson Method*

19.4.1.1.1 Principle

Oxidation is a loss of electrons; reduction is a gain of electrons. Reducing sugars are those sugars that have an aldehydic group (aldoses) which acts as a reducing agent by giving up electrons to an oxidizing agent, which is reduced by receiving the electrons. Oxidation of the aldehydic group produces a carboxylic acid group (Eq. 19.2).

$$R-\overset{\overset{\large O}{||}}{C}-H + 2Cu(OH)_2 + NaOH \longrightarrow R-\overset{\overset{\large O}{||}}{C}-O^{-}Na^{+} + Cu_2O + 3H_2O \quad (19.2)$$

The most often used method to determine amounts of reducing sugars is the Somogyi-Nelson method [9], also at times referred to as the Nelson-Somogyi method. This and other reducing sugar methods (Sect. 19.4.1.2) can be used in combination with enzymic methods (Sect.19.4.2.3) for determination of oligo- and polysaccharides. In enzymic methods, specific hydrolases are used to convert the oligo- or polysaccharide into its constituent monosaccharide or repeating oligosaccharide units, whose total amounts are measured using a reducing sugar method. The Somogyi-Nelson method is based on **reduction of Cu(II) to Cu(I) ions by reducing sugars**. The reaction is conducted in an alkaline solution containing tartrate or citrate ions, which function to keep the copper ions in solution. The Cu(I) ions then reduce an arsenomolybdate complex prepared by reacting ammonium molybdate [$(NH_4)_6Mo_7O_{24}$] and sodium arsenate (Na_2HAsO_7) in sulfuric acid. Reduction of the arsenomolybdate complex produces an intense, stable blue color that is measured spectrophotometrically. The extent of color formation is, in part, a function of the sugars present, so the method must be used with a standard curve (Chaps. 4 and 6) of the sugars in the same ratio as they are found in the sample being analyzed or D-glucose (if a constituent sugar is available or the constituents are unknown).

19.4.1.1.2 Outline of Procedure

1. A solution of copper(II) sulfate and an alkaline buffer solution are added by pipettes to a solution of reducing sugars(s) (prepared as per the sample preparation procedure described in Sect. 19.2.2) and a water blank.
2. The resulting solution is heated in a boiling water bath.
3. A reagent prepared by mixing solutions of acidic ammonium molybdate and sodium arsenate is added.

4. After mixing, dilution, and remixing, absorbance is measured at 520 nm.
5. After subtracting the absorbance of the reagent blank, the A_{520} is converted into glucose equivalents using a standard plot of μg of glucose vs. absorbance.

19.4.1.2 *Other Methods*

An alternative method to the Somogyi-Nelson method that is also based on **reduction of Cu(II) ions in alkaline solution to Cu(I) ions** is the **Lane-Eynon method** (AOAC Method 945.66). To perform the Lane-Eynon method, the solution to be analyzed is added (using a burette) to a flask containing a boiling, alkaline solution of cupric sulfate of known concentration containing potassium sodium tartrate and methylene blue. Any reducing sugars in the solution being analyzed reduce Cu(II) ions to Cu(I) ions. When all the Cu(II) ions have been reduced, further addition of reducing sugars results in the indicator losing its blue color. The volume of the solution required to reach the end point is used to calculate the amount of reducing sugar present in the sample. Again, because this reaction is not stoichiometric and because each reducing sugar reacts differently, this method must be used with a standard curve (Chap. 4).

A keto group cannot be oxidized to a carboxylic acid group, and thus ketoses are not reducing sugars. However, under the alkaline conditions employed, ketoses are isomerized to aldoses [1] and, therefore, are measured as reducing sugars. Because the conversion is not 100 %, the response is less with ketoses, so a standard curve (Chap. 4) made with D-fructose as one of the sugars in the mixture of sugars should be used if it is present.

The **dinitrosalicylic acid method** [10] will measure reducing sugars naturally occurring in foods or released by enzymes, but is not much used. In this reaction, 3,5-dinitrosalicylate is reduced to the reddish monoamine derivative.

19.4.2 Specific Analysis of Mono- and Oligosaccharides

Determination of contents of specific mono- and oligosaccharides is often done chromatographically. The most commonly used method is high-performance liquid chromatography (HPLC) (Sect. 19.4.2.1). The method is simple and determines sugar alcohols in addition to reducing sugars. Gas chromatography (GC) (Sect. 19.4.2.2) is more time consuming in that it requires derivatization of the sugars. In GC, sugars are determined as their reduced forms (sugar alcohols).

19.4.2.1 *High-Performance Liquid Chromatography*

19.4.2.1.1 Overview

HPLC (Chap. 13) is the method of choice for analysis of mono- and oligosaccharides in foods and can be used for analysis of polysaccharides after hydrolysis (Sect. 19.5.2.2) to their constituent monosaccharides. HPLC gives both qualitative analysis (identification of the carbohydrate) and, with peak integration, quantitative analysis. HPLC analyses are rapid, can tolerate a wide range of sample concentrations, and provide a high degree of precision and accuracy. HPLC requires micron-filter filtration prior to injection. Complex mixtures of mono- and oligosaccharides can be analyzed. The basic principles and important parameters of HPLC (the stationary phase, the mobile phase, and the detector) are presented and discussed in Chap. 13. Some details related to carbohydrate analysis are discussed here. The use of HPLC to determine food and other carbohydrates has been reviewed many times; some recent reviews can be found in references [11–20]. Specific details of methods of analysis of specific food ingredients or products should be obtained from the literature. Sample preparation for HPLC analysis is discussed in references [17, 19]. Various column packing materials and detectors have been used. Only the most often employed column packing material and detector are presented here. The reviews should be consulted for other columns and detectors.

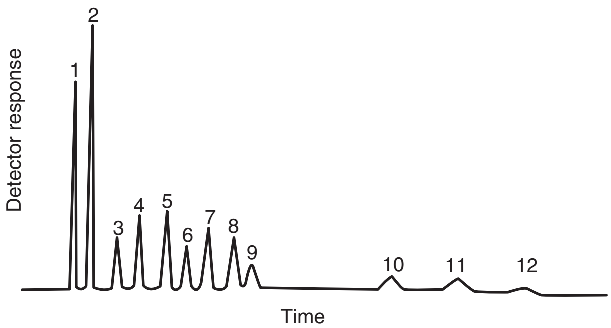
19.4.2.1.2 Anion-Exchange HPLC

Separation of carbohydrates by HPLC is most often done using anion-exchange (AE-HPLC) columns. Carbohydrates have pKa values in the pH range 12–14 and are, therefore, very weak acids. In a solution of high pH, some carbohydrate hydroxyl groups are ionized, allowing sugars to be separated on columns of anion-exchange resins. Special resins have been developed for this purpose. The general elution sequence is sugar alcohols (alditols), monosaccharides, disaccharides, and higher oligosaccharides.

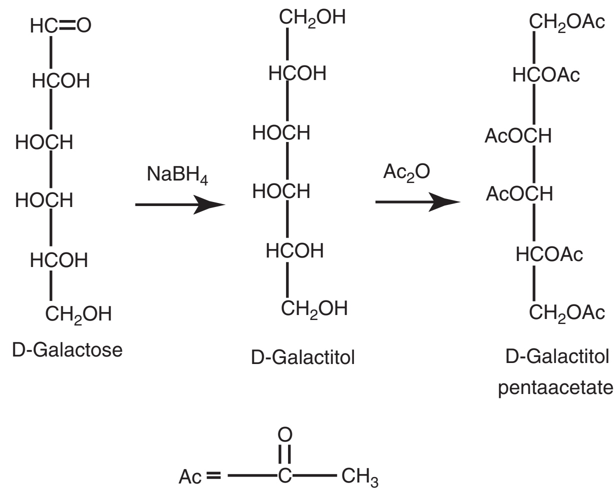
19.4.2.1.3 Pulsed Electrochemical Detection

The **pulsed electrochemical detector** (ECD) [formerly called a **pulsed amperometric detector** (PAD)], which relies on oxidation of carbohydrate hydroxyl and aldehydic groups, is universally used with anion-exchange chromatography [11–19, 21–25]. ECD requires a high pH. Both gradient and graded elutions can be used. The solvents employed are simple and inexpensive (sodium hydroxide solutions, with or without sodium acetate). (Water may be used, but when it is, post-column addition of a sodium hydroxide solution is required.) The detector is suitable for both reducing and nonreducing monosaccharides. Lower detection limits are approximately 1.5 ng for monosaccharides and 5 ng for di-, tri-, and tetrasaccharides. ECD responses vary from sugar to sugar and change continuously, so standards must be run and response factors calculated at least daily.

AE-HPLC coupled to an ECD has been used to examine the complex oligosaccharide patterns of



19.3 figure High-performance liquid chromatogram of some common monosaccharides, disaccharides, alditols, and the trisaccharide raffinose at equal wt/vol concentrations separated by anion-exchange chromatography and detected by pulsed electrochemical detection. Peak *1* glycerol, *2* erythritol, *3* L-rhamnose, *4* D-glucitol (sorbitol), *5* mannitol, *6* L-arabinose, *7* D-glucose, *8* D-galactose, *9* lactose, *10* sucrose, *11* raffinose, *12* maltose



19.4 figure Modification of D-galactose in preparation for gas chromatography

many food components and products. The method has the advantage of being applicable to baseline separation within each class of carbohydrates (Fig. 19.3) and of providing separation of homologous series of oligosaccharides into their components [21, 26]. A newer detector with increasing use is the evaporative light-scattering detector [27].

19.4.2.1.4 Other HPLC Methods

There are other HPLC methods for carbohydrate analysis. Among them is what is called normal-phase chromatography, which is also rather widely used. In normal-phase chromatography, the stationary phase is polar, and elution is effected by employing a mobile phase of increasing polarity. Silica gel that has been derivatized with one or more of several reagents to incorporate amino groups is used. These so-called amine-bonded stationary phases are generally used with acetonitrile-water as the eluent. The elution order is monosaccharides and sugar alcohols, disaccharides, and higher oligosaccharides [28]. Amine-bonded silica gel columns have been used successfully to analyze the low-molecular-weight carbohydrate contents of foods [29].

19.4.2.2 *Gas Chromatography*

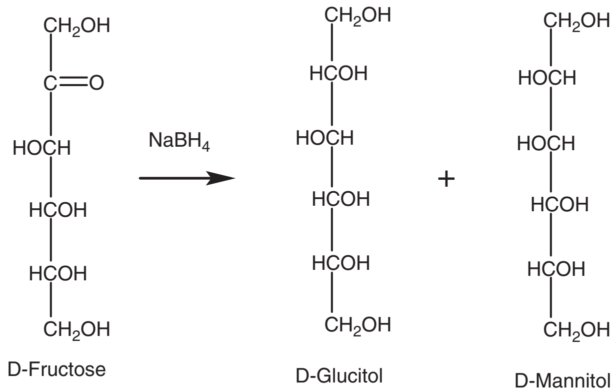
19.4.2.2.1 Overview

GC (**i.e., gas-liquid chromatography,** GLC) (Chap. 14), like HPLC, provides both qualitative and quantitative analysis of carbohydrates [17, 30]. For GC, sugars must be converted into volatile derivatives. The most commonly used derivatives are the alditol peracetates [31, 32]. These derivatives are prepared as illustrated in Fig. 19.4 for D-galactose. A **flame ionization detector** (FID) is the detector most often used for peracetylated carbohydrate derivatives, but mass spectrometers are increasingly used as detectors. A mass spectrometric (MS) detector lowers detection limits, and an MS/MS detector lowers them even more [33]. A technique known as gas chromatography-combustion-isotope ratio mass spectrometry has been applied to determine the origins and adulterations of food and food ingredients [34].

The most serious problem with GC for carbohydrate analysis is that two preparation steps are involved: reduction of aldehydic groups to primary hydroxyl groups and conversion of the reduced sugars (alditol) into volatile peracetate esters; and of course, for the analysis to be successful, each of these steps must be 100% complete. The basic principles and important parameters of GC (the stationary phase, temperature programming, and detection) are presented and discussed in Chap. 14.

19.4.2.2.2 Neutral Sugars: Outline of Procedure [31]

1. **Reduction to Alditols**. Neutral sugars from the 80% ethanol extract (Sect. 19.2.2) or from hydrolysis of a polysaccharide (Sect. 19.5.2.2) are reduced at 40 °C with an excess of sodium or potassium borohydride dissolved in dilute ammonium hydroxide solution. After reaction, glacial acetic acid is added to destroy excess borohydride. The acidified solution is evaporated to dryness. A potential problem exists: If fructose is present, either as a naturally occurring sugar, from the hydrolysis of inulin, or as an additive [from a high-fructose syrup (HFS), invert sugar, or honey], it will be reduced to a mixture of D-glucitol (sorbitol) and D-mannitol (Fig. 19.5).



19.5 figure Reduction of D-fructose to a mixture of alditols

2. **Acetylation of Alditols**. Acetic anhydride and a catalyst are added to a dry mixture of alditols. After 10 min at room temperature, water and dichloromethane are added. After mixing, the dichloromethane layer is washed with water and evaporated to dryness. The residue of alditol peracetates is dissolved in a polar organic solvent (usually acetone) for chromatography.
3. **GC of Alditol Peracetates**. Alditol peracetates may be chromatographed isothermally and identified by their retention times relative to that of inositol hexaacetate, inositol being added as an internal standard prior to acetylation. It is essential to run standards of the alditol peracetates of the sugars being determined with inositol hexaacetate as an internal standard to determine elution times and relative responses.

19.4.2.3 *Enzymic Methods*

19.4.2.3.1 Overview

Enzymic methods (Chap. 26) generally have great specificity for the carbohydrate being determined, do not require high purity of the sample being analyzed, have very low detection limits, do not require expensive equipment, and are easily automated [35, 36]. However, the methods are spectrophotometric and thereby require clear solutions, so extraction and cleanup is required (Sect. 19.4.2.3.2).

The method of choice for the determination of starch employs a combination of enzymes in sequential **enzyme-catalyzed reactions** and is specific for starch, as long as purified enzyme preparations are used (Sect. 19.5.1.1). Other enzymic methods for the determination of carbohydrates have been developed (Table 19.4). They are often, but not always, specific for the substance being measured. Kits for several enzymic methods have been developed and marketed. The kits contain specific enzymes, other required reagents, buffer salts, and detailed instructions that must be followed because enzyme concentration, substrate concentration, concentration of other required reagents, pH, and temperature all affect reaction rates and results. A good description of a method will point out any interferences from other substances and other limitations.

19.4 table Selected enzymic methods of carbohydrate analysis

Carbohydrate	*Reference*	*Kit form*[a]
Monosaccharides		
Pentoses		
L-arabinose	[35, 36]	
D-xylose	[35, 36]	
Hexoses		
D-fructose	[35, 36]	x
D-galactose	[35, 36]	x
D-galacturonic acid	[35]	
D-glucose		
Using glucose oxidase	[36], Sect. 19.4.2.3.3	x
Using glucose dehydrogenase	[35, 36]	
Using glucokinase (hexokinase)	[35, 36]	x
D-mannose	[35, 36]	
Monosaccharide derivatives		
D-gluconate/D-glucono-δ-lactone	[35, 36]	x
D-glucitol/sorbitol	[35, 36]	x
D-mannitol	[35, 36]	
Xylitol	[35, 36]	x
Oligosaccharides		
Lactose	[35, 36]	x
Maltose	[35, 36]	x
Sucrose	[35, 36]	x
Raffinose, stachyose, verbascose	[35, 36]	x
Polysaccharides		
Amylose, amylopectin (contents and ratio)		x
Cellulose	[35, 36]	
Galactomannans (guar and locust bean gums)	[35]	
β-Glucan (mixed-linkage)	[35]	x
Glycogen	[35, 36]	
Hemicellulose	[35, 36]	
Inulin	[35, 36]	x
Pectin/poly (D-galacturonic acid)	[35, 36]	
Starch	Sect. 19.5.1.1" [35, 36]	x

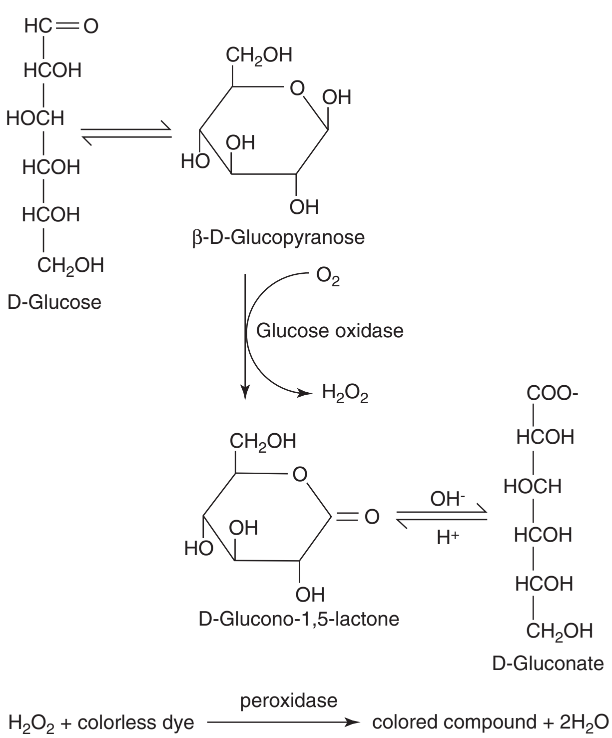
[a]Available in kit form from companies such as R-Biopharm, Megazyme, and Sigma-Aldrich

19.4.2.3.2 Sample Preparation

It is sometimes recommended that the **Carrez treatment** [37], which breaks emulsions, precipitates proteins, and absorbs some colors, be applied to food products prior to determination of carbohydrates by enzymic and other methods. The Carrez treatment involves addition of a solution of potassium ferrocyanide ($K_4[Fe(CN)_6]$, potassium hexacyanoferrate), followed by addition of a solution of zinc sulfate ($ZnSO_4$), followed by addition of a solution of sodium hydroxide. The suspension is filtered, and the clear filtrate is used directly in enzyme-catalyzed assays. Carrez solutions are commercially available.

19.4.2.3.3 Enzymic Determination of D-Glucose (**Dextrose**)

Glucose oxidase oxidizes D-glucose quantitatively to D-glucono-1,5-lactone (glucono-delta-lactone), the other product being hydrogen peroxide (Fig. 19.6). To measure the amount of D-glucose present, **peroxidase** and a colorless compound (a leuco dye) that can be oxidized to a colored compound are added. In the reaction catalyzed by peroxidase, the leuco dye is oxidized to a colored compound, which is measured spectrophotometrically. Various dyes are used in commercial kits. The method using this combination of two enzymes and an oxidizable colorless compound is known as the **glucose oxidase/peroxidase/dye** (GOPOD) **method**.



19.6 figure Coupled enzyme-catalyzed reactions for the determination of D-glucose

YSI Life Sciences makes a commercial instrument that utilizes the glucose oxidase enzyme immobilized between two membranes and an electrode that measures the released hydrogen peroxide. Results are obtained in less than 60 s. Using other immobilized enzymes, the instrument will determine amounts of D-galactose (using galactose oxidase), sucrose (using invertase and glucose oxidase), lactose (using galactose oxidase), and starch (using glucoamylase/amyloglucosidase and glucose oxidase).

Another **coupled-enzyme** enzymic method, also available in kit form, but less often used, involves reaction of D-glucose with ATP in the presence of hexokinase to form glucose 6-phosphate (G6P) + ADP. The reaction mixture also contains glucose 6-phosphate dehydrogenase (G6PDH) and $NADP^+$. G6PDH catalyzes the oxidation of G6P to D-gluconate 6-phosphate and reduction of $NADP^+$ to NADPH, so the amount of NADPH formed is equivalent to the amount of D-glucose that was present. The amount of NADPH formed is determined by measuring its absorbance at 340 nm (a wavelength which $NADP^+$ does not absorb).

19.4.2.4 *Capillary Electrophoresis*

Capillary zone electrophoresis (see also Chap. 24, Sect. 24.2.5.3) has also been used to separate and measure carbohydrates, but because carbohydrates lack chromophores, pre-column derivatization and detection with a UV or fluorescence detector are required [11, 16, 38–43].

19.5 POLYSACCHARIDES

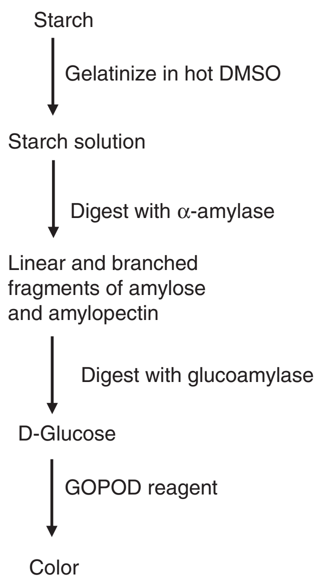
19.5.1 Starch

Starch is second only to water as the most abundant component of food. Starch is found in all parts of plants (leaves, stems, roots, tubers, seeds). A variety of commercial starches are available worldwide as food additives. These include corn (maize), waxy maize, high-amylose corn (amylomaize), wheat, rice, potato, tapioca (cassava), yellow pea, sago, and arrowroot starches. In addition, starch is the main component of wheat, rye, barley, oat, rice, corn, mung bean, and pea flours and certain roots and tubers such as potatoes, sweet potatoes, and yams.

19.5.1.1 *Total Starch*

19.5.1.1.1 Principle and Procedure

The only reliable method for determination of total starch is based on complete conversion of the starch into D-glucose by purified enzymes specific for starch and determination of the D-glucose released by an enzyme specific for it (Sect. 19.4.2.3.3). In the proce-



19.7 figure Flow diagram for determination of total starch

dure outlined in Fig. 19.7, **α-amylase** catalyzes hydrolysis of unbranched segments of 1,4-linked α-D-glucopyranosyl units, forming primarily maltooligosaccharides composed of three to six units. **Debranching enzymes** (both **pullulanase** and **isoamylase** are used) catalyze hydrolysis of the 1,6 linkages that constitute the branch points of starch polysaccharide molecules and molecules of maltooligosaccharides derived from starch polysaccharide molecules and thereby produce short linear molecules. **Glucoamylase** (**amyloglucosidase**) acts at the nonreducing ends of starch oligo- and polysaccharide chains and releases D-glucose, one unit at a time; it will catalyze hydrolysis of both 1,4 and 1,6 α-D-glucopyranosyl linkages. In the assay, glucose and a starch low in protein and lipid content (such as potato starch) are used as standards after determining their moisture contents.

19.5.1.1.2 Potential Problems

Starch-hydrolyzing enzymes (amylases) must be purified to eliminate any other enzymic activity that would release D-glucose (e.g., cellulases, invertase, sucrase, β-glucanase) and catalase, which would destroy the hydrogen peroxide on which the enzymic determination of D-glucose depends. The former contamination would give false high values and the latter, false low values. Even with purified enzymes, problems can be encountered. The method may not be quantitative for a high-amylose or another starch at least partially resistant to enzyme-catalyzed hydrolysis after cooking. **Resistant starch** (RS), by definition, is composed of starch and starch-degradation products that escape digestion in the small intestine [44]. Four types of starch are generally considered to be resistant to digestion in the small intestine or so slowly digested that they pass essentially intact through the small intestine, i.e., without conversion into D-glucose:

1. Starch that is physically inaccessible to amylases because it is trapped within a food matrix, even though it is gelatinized (**RS1**)
2. Starch that resists enzyme-catalyzed hydrolysis because it is uncooked, i.e., not gelatinized (**RS2**)
3. Retrograded starch (i.e., starch polymers that have recrystallized after gelatinization of the granule (**RS3**); cooled cooked potatoes and other starchy foods, such as pasta, contain resistant starch)
4. Starch that has been modified structurally in such a way as to make it less susceptible to digestion (**RS4**)

RS is at best only partially converted into D-glucose by this method; rather, most of it is included in the analysis for dietary fiber (Sect. 19.6). Methods for the specific determination of RS have been reviewed [45].

One method of starch analysis (AOAC Method 969.39, AACCI Method 76-13.01) overcomes these problems. In it, the starch is dispersed in dimethyl sulfoxide (DMSO) and then is converted quantitatively to D-glucose by first treating the solution with a **thermostable α-amylase** to effect depolymerization and solubilization of the starch (Fig. 19.7). Addition of **glucoamylase** (**amyloglucosidase**) then effects complete conversion of the fragments produced by the action of α-amylase into D-glucose. D-Glucose is determined with a GOPOD reagent (Sect. 19.4.2.3.3). The method determines total starch. It does not reveal the botanical source of the starch or whether it is a native starch or a modified food starch. The botanical source of the starch may be determined by microscopic examination (Chap. 32, Sect. 32.2.2.4) of the material being analyzed before it is cooked.

19.5.2 Non-starch Polysaccharides (Hydrocolloids/Food Gums)

19.5.2.1 *Overview*

A starch or starches may occur naturally in a fruit or vegetable tissue, in addition to being used as an ingredient in a food product, either as isolated starch or as a component of a flour. Other polysaccharides are almost always added as ingredients, although there are exceptions. These added polysaccharides, along with the protein gelatin, comprise a group of ingredients known as **hydrocolloids** or **food gums**. The non-starch polysaccharides used as additives in food products are obtained from land plants, seaweeds (marine algae), and microorganisms and by chemical derivatization of cellulose. Their use is widespread and extensive.

Analytical methods are required for these polysaccharides to enable both suppliers and food processors to determine the purity of a hydrocolloid product, to ensure that label declarations of processors are correct, and to confirm that hydrocolloids have not been added to standardized products in which they are not allowed. In addition, it may be desirable to determine such things as the **β-glucan** content of an oat or barley flour or a breakfast cereal for a label claim of a specific dietary fiber or the **arabinoxylan** content of a wheat flour to set processing parameters for bakery products.

Determination of polysaccharides classified as hydrocolloids is problematic because polysaccharides have a variety of chemical structures, solubilities, and molecular weights. Unlike proteins and nucleic acids, the structures of molecules of a single polysaccharide preparation from a plant or microorganism, with very few exceptions, vary from molecule to molecule. In addition, the average structure can vary with the source and the environmental conditions under which the plant or microorganism was grown. Some polysaccharides are neutral; some are anionic. Some contain ether, ester, and/or cyclic acetal groups in addition to sugar units, either naturally or as a result of chemical modification. Some are soluble only in hot water; some are soluble only in room-temperature or colder water; some are soluble in both hot and cold water, and some require aqueous solutions of acids, bases, or metal ion-chelating compounds to release them from plant tissues. And all polysaccharide preparations are composed of a mixture of molecules with a range of molecular weights; so while all molecules of food and beverage components such as D-glucose, D-fructose, maltose, and sucrose have identical structures and molecular weights, each molecule of a polysaccharide preparation probably differs from all other molecules in that sample in structure and/or molecular weight. This structural diversity complicates determination of both the types and amounts of polysaccharides in a food product [46]. As a result, no single approach that will determine all hydrocolloids, either qualitatively or quantitatively, is available. Other potential problems are that hydrocolloids are usually added to foods in very small amounts (0.01–1 %), and blends of hydrocolloids are often used to extend functionalities.

Current methods depend on extraction of the hydrocolloid(s), followed by deproteinization of the extract and precipitation of the hydrocolloids by addition of ethanol, acetone, or 2-propanol (isopropanol), but low-molecular-weight (low-viscosity-grade) hydrocolloids may not be precipitated. Because blends of hydrocolloids are often used in food products, fractionation may be required. Fractionation, like extraction and precipitation, invariably results in some loss of material. Most often, an isolated polysaccharide is identified by identifying and quantitating its constituent sugars after acid-catalyzed hydrolysis. However, sugars are released from polysaccharides by hydrolysis at different rates and are destroyed by hot acids at different rates, so even the exact monosaccharide composition of a polysaccharide preparation may be difficult to determine and may not be achieved. A hydrolytic enzyme specific for the polysaccharide being determined (if available) is useful if the specific hydrocolloid present is known. Analytical strategies for and problems associated with the determination of hydrocolloids in foods have been reviewed [46, 47].

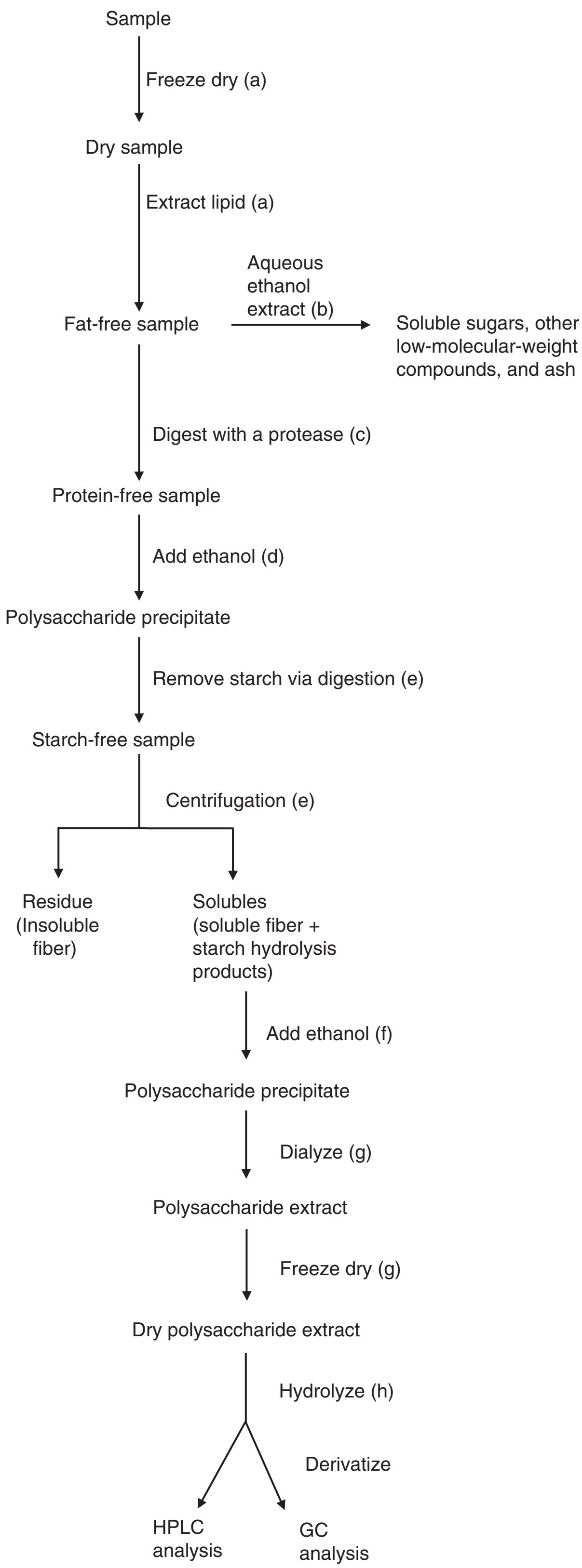
19.5.2.2 *Hydrocolloid Content Determination*

Most schemes for analysis of food products for food gums are targeted to a specific group of food products, as it is difficult, perhaps impossible, to develop a universal scheme. A general scheme for isolation and purification of non-starch, water-soluble polysaccharides is presented in Fig. 19.8 [48]. Letters in the parentheses below refer to the same letters in Fig. 19.8:

(a) It is usually difficult to extract polysaccharides quantitatively when fats, oils, waxes, and proteins are present. Therefore, lipid-soluble substances are removed first. Before this can be effected, the sample must be dried. Freeze-drying is recommended. If the dried material contains lumps, it must be ground to a fine powder. A known weight of dry sample is placed in a Soxhlet apparatus, and the lipid-soluble substances are removed with 19:1 vol/vol chloroform-methanol. (See note in Sect. 19.2.1) (*n*-Hexane has also been used.) Solvent is removed from the sample by air-drying in a hood and then by placing the sample in a desiccator, which is then evacuated.

(b) Although not in the published scheme, soluble sugars, other low-molecular-weight compounds, and ash can be removed at this point using hot 80 % ethanol as described in Sect. 19.2.2.

(c) Protein is removed by enzyme-catalyzed hydrolysis. The cited procedure [48] uses papain as the protease. However, bacterial alkaline proteases are recommended to prevent the action of contaminating carbohydrases – all of which have acidic pH optima. (Essentially all commercial enzyme preparations, especially those from bacteria or fungi, have carbohydrase activities in addition to proteolytic activity.) In this procedure, proteins are denatured for easier digestion by dispersion of the sample in sodium acetate buffer containing sodium chloride and heating the mixture.



19.8 figure Flow diagram for isolation and analysis of polysaccharides

(d) Any solubilized polysaccharides are precipitated by addition of sodium chloride to the cooled dispersion, followed by addition of four volumes of absolute ethanol. The mixture is centrifuged.

(e) The pellet is suspended in acetate buffer. To this suspension is added a freshly prepared solution of glucoamylase in the same buffer. This suspension is then incubated. Just as in the analysis of starch, highly purified enzyme must be used to minimize hydrolytic breakdown of other polysaccharides. [This step may be omitted in future analyses of the same product if no glucose is found in the centrifugate (supernatant) from step f, indicating that no starch is present.] Centrifugation after removal of starch polysaccharides isolates insoluble dietary fiber (IDF) (Sect. 19.6).

(f) Solubilized polysaccharides are reprecipitated by addition of sodium chloride and four volumes of absolute ethanol to the cooled dispersion. The mixture is centrifuged. The precipitate (pellet) of water-soluble polysaccharides (often added hydrocolloids) is soluble dietary fiber (SDF) (Sect. 19.6).

(g) The pellet is suspended in deionized water, transferred to dialysis tubing, and dialyzed against frequent changes of sodium azide solution (sodium azide used to prevent microbial growth). Finally, dialysis against deionized water is done to remove the sodium azide. The retentate is recovered from the dialysis tubing and freeze-dried.

(h) Polysaccharide identification relies on hydrolysis to constituent monosaccharides and identification of these sugars. For hydrolysis, polysaccharide material is added to a Teflon-lined, screw-capped vial. Trifluoroacetic acid solution is added (usually 2 M), and the vial is tightly capped and heated (usually for 1–2 h at 121 °C) (49). After cooling, the contents are evaporated to dryness in a hood using a stream of air or nitrogen. Sugars are determined by HPLC (Sect. 19.4.2.1) or GC (Sect. 19.4.2.2). If GC is used, inositol is added as an internal standard. Qualitative and quantitative analysis of the polysaccharides present can be determined by sugar analysis. For example, guaran, the polysaccharide component of guar gum, yields D-mannose and D-galactose in an approximate molar ratio of 1.00:0.56.

The described acid-catalyzed hydrolysis procedure does not release uronic acids quantitatively. The presence of **uronic acids** can be indicated by the ***m*-hydroxydiphenyl (3-phenylphenol) assay** [50–52]. This and similar methods are based on the same principle as the phenol-sulfuric acid assay (Sect. 19.3), i.e., condensation of dehydration products with a phenolic compound to produce colored compounds that can be measured quantitatively by means of spectrophotometry. If present, specific uronic acids can be identified by a specific GC procedure for them.

19.5.2.3 *Pectin*

19.5.2.3.1 Nature of Pectin

Even though **pectin** is a very important food hydrocolloid, no official method for its determination has been established. What few methods have been published basically involve its precipitation (by addition of ethanol) from jams, jellies, etc. in which it is the only polysaccharide present.

Even the definition of pectin is somewhat ambiguous. What may be called "pectin" in a native fruit or vegetable is a complex mixture of polysaccharides whose structures depend on the source, including the stage of development (i.e., the degree of ripeness) of the particular fruit or vegetable. Generally, much of this native material can be described as a main chain of α-D-galactopyranosyluronic acid units (some (usually many) of which are in the methyl ester form) interrupted by L-rhamnopyranosyl units (1, 2). Many of the rhamnosyl units have polysaccharide (arabinan, galactan, or arabinogalactan) chains attached to them. Other sugars, such as D-apiose, also are present. In the manufacture of commercial pectin, much of the neutral sugar part is removed. Commercial pectin is, therefore, primarily poly(α-D-galacturonic acid methyl ester) with various degrees of esterification, and sometimes amidation.

Enzyme action during development/ripening or during processing can partially de-esterify and/or depolymerize native pectin. These enzyme-catalyzed reactions are important determinants of the stability of fruit juices, tomato sauce, tomato paste, apple butter, etc. in which some of the texture is supplied by pectin.

19.5.2.3.2 Pectin Content Determination

Conditions for extraction of pectin from various plant tissues, followed by its precipitation, have been studied for many years and continue to be investigated – not for analytical interests, but because of pectin's commercial value. Several different extractants, extraction conditions, precipitants, and precipitation conditions (with variations of each) have been investigated and optimized. Product characteristics vary with the source material, isolation conditions, and in the case of fruit sources, the degree of ripeness. The constant, but not the sole, constituent of pectins is D-**galacturonic acid** as the principal component (often at least 80 %). However, glycosidic linkages of uronic acids are difficult to hydrolyze, so methods involving acid-catalyzed hydrolysis are generally not applicable. Therefore, pectins are often determined using the ***m*-hydroxydiphenyl method** [50–52], following isolation of crude pectin. For reviews of methods for the determination of pectin, see references [53, 54]. A procedure involving methanolysis followed by reverse-phase HPLC has been published [55]. Interference by other hydrocolloids in the determination of pectin has been reviewed [56].

19.6 DIETARY FIBER

19.6.1 Definition

Because labeling of food products for dietary fiber content is required, an official analytical method(s) for its determination is required. The first step in adopting a method must be agreement on what constitutes dietary fiber. Then, there must be a method that measures what is included in the definition. However, no single definition of **dietary fiber** has been agreed upon by all domestic and international organizations that need, or would like, a definition of it [57]. One hurdle is that dietary fiber not only needs a chemical definition for development of an assay procedure, but a measurement of it is important because of its positive physiological effects, which differ in effectiveness from source to source.

Four official definitions are given in Table 19.5. AACC International was the first organization to develop an official definition for dietary fiber [58, 59]. According to the AACC International definition, and that of others, dietary fiber is essentially the sum of the nondigestible components of a food ingredient or product. No polysaccharide other than starch is digested in the human small intestine; so all polysaccharides other than nonresistant starch are included in this definition. Of the oligosaccharides, only sucrose, lactose, and those derived from starch (maltodextrins) are digested. **Analogous carbohydrates** are defined as those carbohydrate-based food ingredients that are nondigestible and nonabsorbable but are not natural plant components. Wax (suberin and cutin) is included within associated substances. The definition also includes some of the health benefits known to be associated with ingestion of dietary fiber. Since adoption of the AACC International definition of dietary fiber, modified versions have been adopted by both governmental and nongovernmental organizations around the world, such as the US Institute of Medicine [60], the FDA, and the Codex Alimentarius Commission [61] (Table 19.5). Definitions adopted by some other regulatory bodies, commissions, and organizations can be found in reference [62].

Formulating a definition acceptable to all is difficult because dietary fiber from different sources is often composed of different mixtures of nondigest-

19.5 table Definitions of dietary fiber

AACC International	"Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fiber promotes beneficial physiological effects, such as, laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation" [58, 59]
US Institute of Medicine	"Dietary fiber consists of nondigestible carbohydrates and lignin that are intrinsic and intact in plants. Functional fiber consists of isolated, nondigestible carbohydrates that have beneficial physiologic effects in humans. Total fiber is the sum of dietary fiber and functional fiber" [60]
Codex Alimentarius Commission	"Dietary fiber denotes carbohydrate polymers with 10 or more monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belonging to the following categories: edible carbohydrate polymers naturally occurring in the food consumed; carbohydrate polymers obtained from food raw materials by physical, enzymatic or chemical means; synthetic carbohydrate polymers" [61] (Note: Published definition contains footnotes)
Food and Drug Administration	"Dietary fiber is defined as non-digestible soluble and insoluble carbohydates (with 3 or more monomeric units), and lignin that are instrinsic and intact in plants; isolated or synthetic non-digestible carbohydrates (with 3 or more monomeric units) determined by FDA to have physiological effects that are beneficial to human health" [6]

19.6 table Components of dietary fiber

Insoluble dietary fiber
Cellulose, including microcrystalline and powdered cellulose added as ingredients
Lignin
Insoluble hemicelluloses and soluble hemicelluloses entrapped in the lignocellulosic matrix
Resistant starch
Soluble dietary fiber
Soluble hemicelluloses not entrapped in the lignocellulosic matrix
Native pectin (most)
Hydrocolloids (most)
Nondigestible oligosaccharides, such as those derived from inulin (FOS)

ible and nonabsorbable carbohydrates and other substances with different effects on human physiology. However, there is general agreement that dietary fiber consists of oligo- and polysaccharides, lignin, and other substances not acted on by the digestive enzymes in the human stomach or small intestine and that most, but not all, dietary fiber is plant cell-wall material (cellulose, hemicelluloses, lignin) and thus is composed primarily of polysaccharide molecules. Because only the amylose and amylopectin molecules in cooked starch are digestible, all other polysaccharides are components of dietary fiber. Some are components of **insoluble fiber**; some make up **soluble fiber**. Major components of soluble and insoluble dietary fiber are listed in Table 19.6. **Total dietary fiber** (TDF) is the sum of insoluble and soluble dietary fiber.

19.6.2 Methods

19.6.2.1 *Overview*

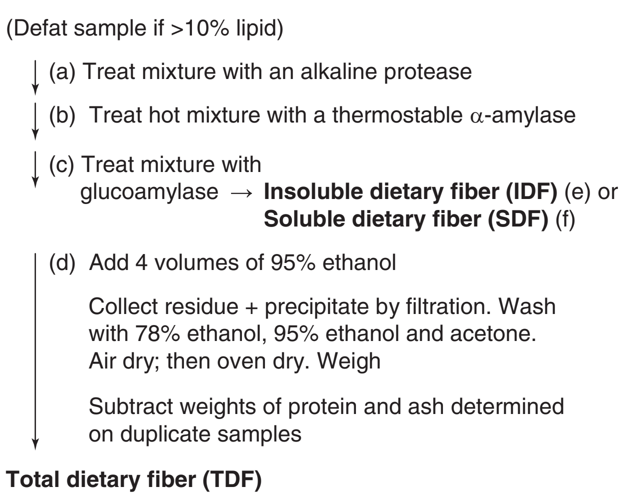
Measurement of insoluble fiber is important not only in its own right, but also for calculating the caloric content of a food. According to nutrition labeling regulations, one method allowed to calculate calories involves subtracting the amount of insoluble dietary fiber from the value for total carbohydrate, before calculating the calories based on protein, fat, and carbohydrate content (approximately 4, 9, and 4 cal per gram, respectively) (Chap. 3). This scheme ignores the fact that soluble fiber, like insoluble fiber, is also essentially noncaloric. [Fiber components can contribute some calories via absorption of products of fermentation (mostly short-chain fatty acids) from the colon.]

The food component that may be most problematic in fiber analysis is **starch**. In any method for determination of dietary fiber, it is essential that all digestible starch be removed, since incomplete removal of digestible starch increases the residue weight and inflates the estimate of fiber. All fiber methods include a heating step (e.g., 95–100 °C for 35 min) to **gelatinize starch granules** and make them susceptible to hydrolysis, which is effected using a thermostable α-amylase and glucoamylase (Sect. 19.5.1.1.1). Resistant starch granules and/or molecules (Sect. 19.5.1.1.2) remain essentially intact and, therefore, are components of dietary fiber, but some nondigestible products made from starch may not be determined as dietary fiber by approved methods.

Nondigestible oligosaccharides such as those derived from inulin (a fructan), certain maltodextrins designed to be nondigestible, and partially hydrolyzed guar gum [63] may be problematic in an analytical sense since they are in the soluble portion that is not precipitated with 78% ethanol. They should be measured in AOAC Method 2009.01 (AACCI Method 32-45.01) and AOAC Method 2011.25 (AACCI Method 32-50.01). Methods for determination of fructans in certain products have been reviewed [64].

It is essential that either all digestible materials be removed from the sample so that only nondigestible components remain or that a correction be applied for any remaining digestible contaminants. **Lipids** are removed easily from the sample with organic solvents (Sect. 19.2) and generally do not pose analytical problems. **Protein** and **salts/minerals** that are not removed from the sample during the solubilization steps should be corrected for by Kjeldahl nitrogen analysis (Chap. 18) and by ashing (Chap. 16) on other samples of the fiber residue.

The scheme presented in Fig. 19.9 is designed to separate non-starch, water-soluble polysaccharides from other components for quantitative and/or qualitative analysis. The residue from the filtration step (e) is insoluble fiber, and those components precipitated from the supernatant with alcohol [step (f)] constitute soluble fiber.



19.9 figure Flow diagram of AOAC Method 991.43 (AACCI Method 32-07.01)

19.6.2.2 *Sample Preparation*

Measures of fiber are most consistent when the samples are low in fat (less than 10% lipid), dry, and finely ground. If necessary, the sample is ground to pass through a 0.3–0.5-mm mesh screen. If the sample contains more than 10% lipid, the lipid is removed by extraction with 25 parts (vol/wt) of petroleum ether or hexane in an ultrasonic water bath. The mixture is then centrifuged, and the organic solvent is decanted. This extraction is repeated. The sample is air-dried to remove the organic solvent. It may then be dried overnight in a vacuum oven at 70 °C if a measure of lipid and moisture content is required. Loss of weight due to fat and moisture removal is recorded, and the necessary correction is made in the calculation of the percentage dietary fiber value determined in the analysis.

If samples contain large amounts of soluble sugars (mono-, di-, and trisaccharides), the samples should be extracted three times with 80% aqueous ethanol in an ultrasonic water bath at room temperature for 15 min. The supernatant liquid is discarded, and the residue is dried at 40 °C.

A variety of methods have been developed and used at different times for different products. *AOAC Official Methods of Analysis* [4] and *AACC International Approved Methods* [65] are listed in Table 19.7. It is obvious from the list that methods are generally specific for the type of fiber or the fiber component desired to be measured. Several methods are available in kit form.

19.6.2.3 *Enzymic-Gravimetric Method*

Dietary fiber is most often determined **gravimetrically** after digestible carbohydrates, lipids, and proteins are selectively solubilized by chemical reagents or removed by enzyme-catalyzed hydrolysis. After such treatments, non-solubilized and/or undigested materials are collected by filtration, and the fiber residue is recovered, dried, and weighed.

19.6.2.3.1 Total, Soluble, and Insoluble Dietary Fiber

AOAC Method 991.43 (AACCI Method 32-07.01) determines total, insoluble, and soluble dietary fiber in cereal products, fruits, vegetables, processed foods, and processed food ingredients. It contains the features of a general analytical method for dietary fiber:

1. **Principle**. Starch and protein are removed from a sample by treating the sample sequentially with a thermostable α-amylase, a protease, and glucoamylase. The insoluble residue is recovered and washed [**insoluble dietary fiber** (IDF)]. Ethanol is added to the soluble portion to precipitate soluble polysaccharides [**soluble dietary fiber** (SDF)]. To obtain **total dietary fiber**

19.7 table Some official methods of analysis for dietary fiber in food ingredients and products

AOAC Official Method No. (4)	*AACCI Approved Method No. (65)*	*Description of method and measured substance*
994.13	32-25.01	TDF determined as neutral sugar and uronic acid monomer units plus Klason lignin by a gas chromatographic-spectrophotometric-gravimetric method
993.21		Nonenzymic-gravimetric method for TDF applicable to determination of >10% TDF in foods and food products with <2% starch
985.29	32-05.01	Enzymic-gravimetric method for TDF in cereal grains and cereal grain-based products
	32-06.01	A rapid gravimetric method for TDF
991.42, 992.16		Enzymic-gravimetric method for insoluble dietary fiber in vegetables, fruits, and cereal grains
993.19		Enzymic-gravimetric method for soluble dietary fiber
991.43	32-07.01	Enzymic-gravimetric method for total, soluble, and insoluble dietary fiber in grain and cereal products, processed foods, fruits, and vegetables
2002.02	32-40.01	Enzymic method for RS2 and RS3 in food products and plant materials
	32-21.01	Enzymic-gravimetric method for insoluble and soluble dietary fiber in oats and oat products
	32-32.01	Enzymic-spectrophotometric method for total fructan (inulin and FOS) in foods applicable to FOS
999.03		Enzymic-spectrophotometric method for fructan (inulin) in foods (not applicable to FOS)
997.08	32-31.01	AE-HPLC method for fructan in foods and food products applicable to the determination of added inulin in processed foods
2000.11	32-28.02	AE-HPLC method for polydextrose in foods
	32-22.01	Enzymic method for β-glucan in oat fractions and unsweetened oat cereals
	32-23.01	Rapid enzymic procedure for β-glucan content of barley and oats
2001.03	32-41.01	Enzymic-gravimetric and HPLC method for dietary fiber containing added resistant maltodextrin
2001.02	32-33.01	HPLC method for *trans*-galactooligosaccharides (TGOS) applicable to added TGOS in selected food products
2009.01	32-45.01	Determines high-molecular-weight and low-molecular-weight soluble dietary fiber by an enzymic-gravimetric method and HPLC
2011.25	32-50.01	Determines insoluble, soluble, and total dietary fiber according to the Codex Alimentarius definition by an enzymic-gravimetric method and HPLC

(TDF), alcohol is added after digestion with glucoamylase, and the IDF and SDF fractions are collected together, dried, weighed, and ashed.

2. **Outline of Procedure**. A flow diagram outlining the general procedure for the method is given in Fig. 19.9. Letters in the parentheses refer to the same letters in Fig. 19.9:

(a) To samples devoid of significant lipid solvent-soluble substances is added a basic buffer containing an alkaline protease.

(b) After protein digestion, the pH is adjusted to the acid side, a thermostable α-amylase is added, and the mixture is heated at 95–100 °C to gelatinize any starch so that the α-amylase can break it down. After cooling the mixture to 60 °C, an alkaline protease is added, and the mixture is incubated at 60 °C to break down the protein.

(c) Glucoamylase is added, and the mixture is incubated at 60 °C to complete the digestion of any starch.
The next few steps differ depending on whether total, insoluble, or soluble fiber is to be determined.

(d) To determine TDF, four volumes of 95% ethanol are added (to give an ethanol concentration of 78%). The mixture is vacuum filtered through a pre-weighed, fritted crucible containing pre-washed Celite (a siliceous filter aid). The residue in the crucibles is dewatered by washing with 78% ethanol, 95% ethanol, and acetone in that order. Then, the crucibles are air-dried (to remove all acetone), oven-dried at 103 °C, and weighed. Since some protein and salts/minerals are combined with plant cell-wall constituents, protein (Kjeldahl procedure (Chap. 18)) and ash (muffle furnace procedure (Chap. 15)) are determined on separate duplicate samples, and fiber values are corrected for them. If resistant starch in the fiber residue is to be determined separately, it can be determined using AOAC Method 2002.02 (AACC International Method 32-40.01).

(e) To determine IDF, the mixture obtained from step (c) is vacuum filtered through a tared, fritted crucible containing pre-washed Celite. The residue retained by the filter is washed with water, then dewatered by washing in order with 78% ethanol, 95% ethanol, and acetone. The crucibles are air-dried (to remove all acetone), oven-dried at 103 °C, and weighed. SDF is in the filtrate.

(f) To determine soluble dietary fiber, four volumes of 95% ethanol (to give an ethanol concentration of 78%) are added to the filtrate and water washes from step (e) at 60 °C to precipitate soluble fiber. The precipitate is collected by vacuum filtration through tared, fritted crucibles containing pre-washed Celite. The residues are dewatered by washing with 78% ethanol, 95% ethanol, and acetone in that order. Then, the crucibles are air-dried (to remove all acetone), oven-dried at 103 °C, and weighed.

Duplicate reagent blanks must be run through the entire procedure for each type of fiber determination. Table 19.8 is a form used to calculate fiber percentages. Using the equations shown, percent dietary fiber is expressed on a dry weight basis if the sample weights are for dried samples. Representative values obtained using this method are given in Table 19.9.

Note: Neither this method for TDF nor that for SDF determines SDF that does not precipitate in 78% aqueous ethanol, including some or most inulin, polydextrose, digestion-resistant maltodextrins, and partially hydrolyzed guar gum and all fructo-, arabinoxylo-, xylo-, and galactooligosaccharides. AOAC Method 2009.01 (AACCI Method 32-45.01) incorporates the deionization and HPLC procedures of AOAC Method 2002.02 (AACCI Method 32-40.01) to quantitate these lower-molecular-weight, digestion-resistant materials in the filtrate so that all SDF is measured.

19.8 table Dietary fiber data sheet[a]

	SAMPLE				BLANK			
	Insoluble Fiber		*Soluble Fiber*		*Insoluble Fiber*		*Soluble Fiber*	
Sample wt (mg)	m_1	m_2						
Crucible + Celite wt (mg)								
Crucible + Celite + residue wt (mg)								
Residue wt (mg)	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2
Protein (mg) **P**								
Crucible + Celite + ash wt (mg)								
Ash wt (mg) **A**								
Blank wt (mg) **B**[b]								
Fiber (%)[c]								

Adapted with permission from *The Journal of AOAC International*, 1988, 71:1019. Copyright 1988 by AOAC International

$$^{b}\text{Blank}(\text{mg}) = \frac{R_1 + R_2}{2} - P - A$$

$$^{c}\text{Fiber}(\%) = \frac{\frac{R_1 + R_2}{2} - P - A - B}{\frac{m_1 + m_2}{2}} \times 100$$

Table 19.9 Total, soluble, and insoluble dietary fiber in foods as determined by AOAC Method 991.43

Food	Soluble[a]	Insoluble[a]	Total[a]
Barley	5.02	7.05	12.25
High-fiber cereal	2.78	30.52	33.73
Oat bran	7.17	9.73	16.92
Soy bran	6.90	60.53	67.14
Apricots	0.53	0.59	1.12
Prunes	5.07	4.17	9.29
Raisins	0.73	2.37	3.13
Carrots	1.10	2.81	3.93
Green beans	1.02	2.01	2.89
Parsley	0.64	2.37	2.66

Adapted from Official Methods of Analysis, 20th ed. Copyright 2016 by AOAC International
[a]Grams of fiber per 100 g of food on a fresh weight basis

19.6.2.3.2 Dietary Fiber Components as Defined by Codex Alimentarius

The most recent method (AOAC Method 2011.25; AACCI Method 32-50.01) combines aspects of previously approved methods to measure individual components of dietary fiber as defined by Codex Alimentarius. It is outlined in Fig. 19.10.

Note: **SDFP** is dietary fiber that is soluble in water, but insoluble in 78% ethanol; it includes most hydrocolloids and some of such compounds as polydextrose, nondigestible maltodextrins, inulin, and partially hydrolyzed guar gum. **SDFS** is dietary fiber that is soluble in both water and 78% ethanol; it includes various oligosaccharides, such as low-molecular-weight FOS and galactooligosaccharides.

19.7 PHYSICAL METHODS

19.7.1 Measurements of Sugar Concentrations in Solution

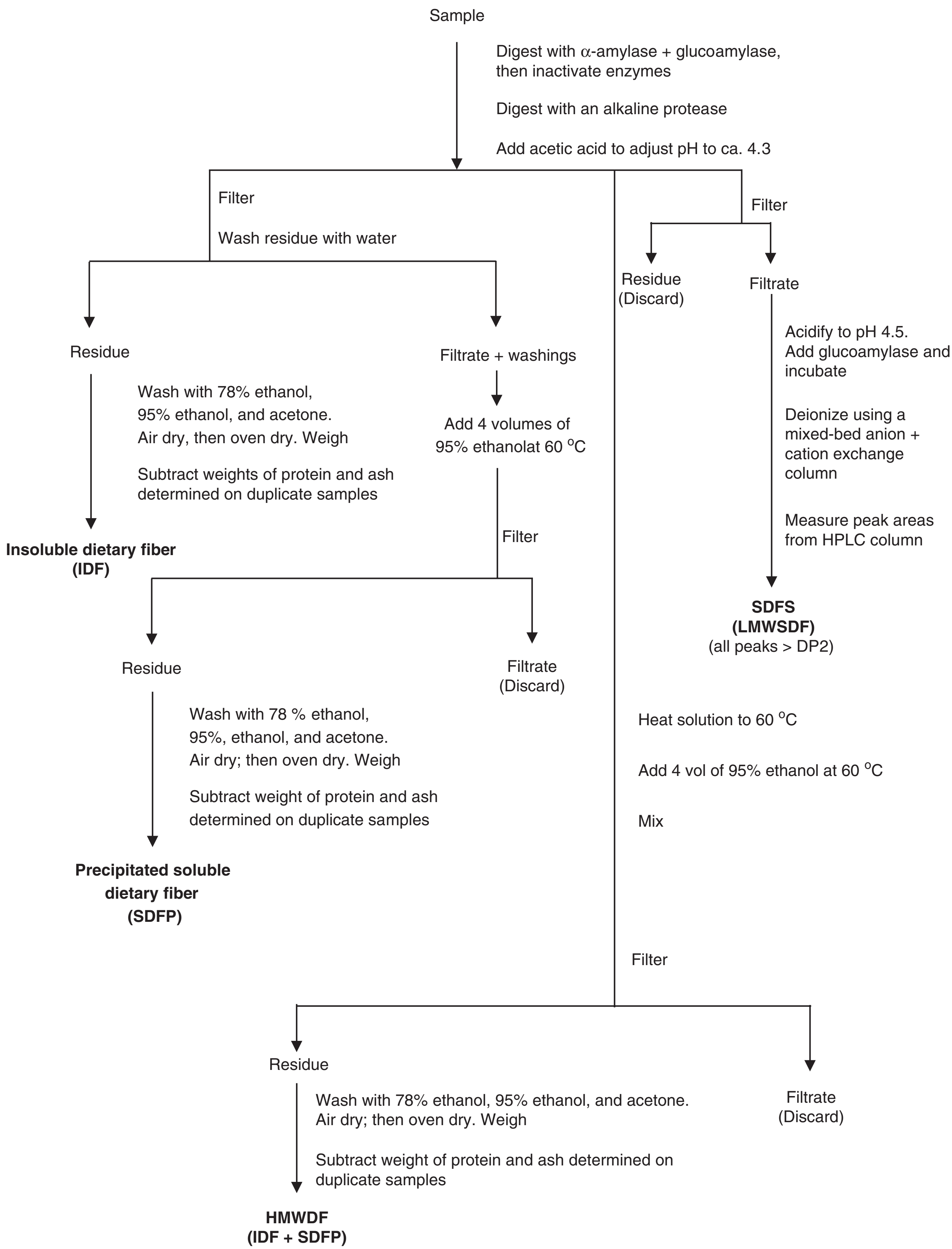
The concentration of a carbohydrate in solution can be determined by measuring the solution's specific gravity, refractive index (Chap. 6), or optical rotation. The **specific gravity** is the ratio of the density of a substance to the density of a reference substance (usually water) both at a specific temperature. By far the most common way to determine specific gravity is the use of a hydrometer calibrated in °**Brix**, which corresponds to sucrose concentrations by weight, or in **Baumé modulus** (°**Bé**) (AOAC Method 932.14). The obtained values are then converted into concentrations using tables constructed for the substance being measured in a pure solution. Measurement of specific gravity as a means of determining sugar concentration is accurate only for pure sucrose or other solutions of a single pure substance, but it can be, and is, used for obtaining approximate values for liquid products for which appropriate specific gravity tables have been constructed (Chap. 6).

When light passes from one medium to another, it changes direction, i.e., it is bent or refracted. The ratio of the sine of the angle of incidence to the sine of the angle of refraction is called the index of refraction, or **refractive index** (RI). RI varies with the nature and concentration of the dissolved compound, the temperature, and the wavelength of light used. By holding the nature of the compound, the temperature, and the wavelength constant, the concentration of the dissolved compound can be determined by measuring the RI (Chap. 6). To determine RI, the solution must be clear. Like determination of specific gravity, the use of RI to determine concentrations is accurate only for pure sucrose or other solutions of a single pure substance. Also like specific gravity, it is used for obtaining approximate sugar concentrations in liquid products. Refractometers that read directly in sucrose concentration units are available.

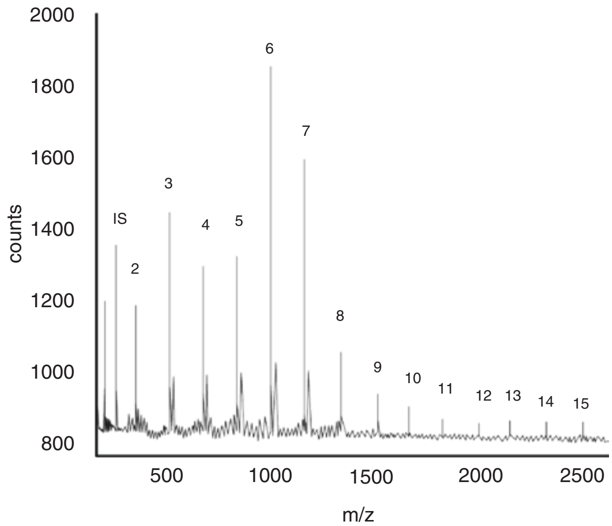
Most compounds that contain a chiral carbon atom have optical activity, i.e., they will rotate the plane of polarization of polarized light. A **polarimeter** measures the extent to which a compound in solution rotates a plane of polarized light. Carbohydrates have chiral carbon atoms and, thus, optical activity. Carbohydrates can rotate the plane of polarized light through an angle that depends on the nature of the compound, the temperature, the wavelength of light, and the concentration of the compound. The concentration of the compound can be determined from a value known as the **specific optical rotation** if all other factors are held constant and if the solution contains no other optically active compounds. Determination of specific optical rotation can be used to measure sucrose concentration (AOAC Methods 896.02, 925.46, 930.37). Determination of sucrose concentration by **polarimetry** requires a clear solution. Instruments are available that read in units of the International Sugar Scale. Determination of specific optical rotation before and after hydrolysis of sucrose into its constituent sugars, D-glucose and D-fructose, a process called **inversion**, can be used to determine sucrose in the presence of other sugars (AOAC Methods 925.47, 925.48, 926.13, 926.14).

19.7.2 Mass Spectrometry

There are many different variations of mass spectrometry (MS) (Chap. 11). Most of the techniques applied to carbohydrates are used for structural analysis. MS has been used for determination of carbohydrates, but not in a routine manner. Particularly useful is the **matrix-assisted laser-desorption time-of-flight** (MALDI-TOF) technique for analysis of a homologous series of



19.10 figure Flow diagram of AOAC Method 2011.25 (AACCI Method 32-50.01)



19.11 figure MALDI-TOF mass spectrum of maltooligosaccharides produced by hydrolysis of starch. *Numbers* indicate DP. *IS* internal standard (From [26], used with permission, Copyright Springer-Verlag, 1998)

oligosaccharides (Fig. 19.11). A comparison between anion-exchange HPLC (Sect. 19.4.2.1) (the most used carbohydrate analysis technique today), capillary electrophoresis (Sect. 19.4.2.4), and MALDI-TOF MS for the analysis of maltooligosaccharides led to the conclusion that that the latter technique gave the best results [27].

19.7.3 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR (Chap. 8, Sect. 8.3.1.2) methods are simple and rapid. Detection limits are greater than those required for most other methods. Spectral libraries have been compiled for several hydrocolloids, including κ-, ι-, and λ-carrageenans [66–70], pectin [66, 68, 71], galactomannans [66, 68], and cellulose derivatives [72].

19.7.4 Near-Infrared (NIR) Spectroscopy

NIR (Chap. 8, Sect. 8.4) spectrometry has been used to determine dietary fiber [73] and sugar [74] contents and to identify cellulose derivatives [72].

19.8 SUMMARY

For determination of low-molecular-weight carbohydrates, older colorimetric methods for total carbohydrate, various reducing sugar methods, and physical measurements have largely been replaced by chromatographic methods. Older chemical methods suffer from the fact that different sugars give different results, which makes them particularly problematic when a mixture of sugars is present. Older physical methods suffer from the fact that they work only with pure substances. However, some older methods continue to be used for simplicity, quality assurance, and product standardization. Chromatographic methods (HPLC and GC) separate mixtures into their component sugars, identify each component by retention time, and provide a measurement of the quantity of each component. HPLC is widely used for identification and measurement of mono- and oligosaccharides. Enzymic methods are specific and sensitive, but seldom, except in the case of starch, is determination of only a single component desired.

Polysaccharides are important components of many food products. Yet, there is no universal procedure for their analysis. Generally, isolation must precede measurement. Isolation introduces errors because losses of constituents occur with both extraction, recovery, and separation techniques. Identification is done by hydrolysis to constituent monosaccharides and their determination. An exception is starch, which can be digested to glucose using specific enzymes (amylases), followed by measurement of the glucose released and, therefore, can be specifically measured.

Insoluble dietary fiber, soluble dietary fiber, and total dietary fiber are each composed primarily of nonstarch polysaccharides. Methods for the determination of total dietary fiber and its components rely on removal of the digestible starch using amylases and often on removal of digestible protein with a protease, leaving a nondigestible residue.

19.9 STUDY QUESTIONS

1. Give three reasons why carbohydrate analysis is important.
2. "Proximate composition" refers to analysis for moisture, ash, fat, protein, and carbohydrate. Identify which of these components of "proximate composition" are actually required on a nutrition label. Also, explain why it is important to measure the nonrequired components quantitatively if one is developing a nutrition label.
3. Distinguish chemically between monosaccharides, oligosaccharides, and polysaccharides, and explain how solubility characteristics can be used in an extraction procedure to separate monosaccharides and oligosaccharides from polysaccharides.
4. Discuss why 80% ethanol (final concentration) is used to extract mono- and oligosaccharides, rather than using water. What is the principle involved?
5. What are the principles behind total carbohydrate determination using the phenol-sulfuric acid method? Why is a standard curve employed? Why is a reagent blank used? What are limitations of the method?

6. Define reducing sugar. Classify each of the following as a reducing or nonreducing carbohydrate: D-glucose, D-fructose (Conditions must be described. Why?), sorbitol, sucrose, maltose, raffinose, maltotriose, cellulose, amylopectin, and κ-carrageenan.
7. What is the principle behind determination of total reducing sugars using the Somogyi-Nelson and similar methods?
8. The Somogyi-Nelson and Lane-Eynon methods can be used to measure reducing sugars. Explain the similarities and differences of these methods with regard to the principles involved and the procedures used.
9. Describe the principle behind AE-HPLC separation of carbohydrates.
10. Describe the general procedure for preparation of sugars for GC. What is required for this method to be successful?
11. Why has HPLC largely replaced GC for analysis of carbohydrates?
12. What are the advantages of enzymic methods? What are the limitations (potential problems)?
13. Describe the principles behind the enzymic determination of starch. What are the advantages of this method? What are the potential problems?
14. What is the physiological definition and the chemical nature of resistant starch? What types of foods have relatively high levels of resistant starch?
15. Briefly describe a method that could be used for each of the following:
    (a) To prevent hydrolysis of sucrose when sugars are extracted from fruits via a hot alcohol extraction
    (b) To remove proteins from solution for an enzymic determination of carbohydrates
    (c) To measure total carbohydrate
    (d) To measure total reducing sugars
    (e) To measure glucose enzymically
    (f) To measure simultaneously the concentrations of individual free sugars
16. Describe the principle behind each step in Fig. 19.9. What is the reason for each step?
17. Describe the principles behind separation and analysis of cellulose, water-soluble gums, and starch.
18. Using pectin as an example, explain why the quantitative analysis of hydrocolloids is so difficult.
19. What is a general definition of dietary fiber? Why is the definition of dietary fiber important? How does the definition of dietary fiber affect development of an analytical procedure for it?
20. List the general component classes of dietary fiber that are usually determined for research purposes.
21. List the constituents of dietary fiber.
22. Explain how measurement of dietary fiber relates to calculating the caloric content of a food product.
23. Explain the purpose(s) of each of the steps in the AOAC Method 991.43 for total dietary fiber listed below as applied to determination of the dietary fiber content of a high-fiber snack food:
    (a) Heating sample and treating with α-amylase
    (b) Treating sample with glucoamylase
    (c) Treating sample with protease
    (d) Adding four volumes of 95% ethanol to sample after treatment with glucoamylase and protease
    (e) After drying and weighing the filtered and washed residue, heating one duplicate final product to 525 °C in a muffle furnace and analyzing the other duplicate sample for protein
24. What are differences between AOAC Method 994.13 and AOAC Method 2011.26 with regard to what is measured?
25. Describe the principles behind and the limitations of determining sugar (sucrose) concentrations by (a) specific gravity determination, (b) refractive index measurement, and (c) polarimetry.

19.10 PRACTICE PROBLEMS

1. The following data were obtained when an extruded breakfast cereal was analyzed for total fiber by AOAC Method 991.43 (AACCI Method 32-07.01).

Sample wt, mg	1,002.8
Residue wt, mg	151.9
Protein wt, mg	13.1
Ash wt, mg	21.1
Blank wt, mg	6.1
Resistant starch, mg	35.9

   What is percent total fiber (a) without and (b) with correction for resistant starch determined to the appropriate number of significant figures?
2. The following tabular data were obtained when a high-fiber cookie was analyzed for fiber con-

tent by AOAC Method 991.43 (AACCI Method 32-07.01).

	Sample			
	Insoluble		*Soluble*	
Sample wt, mg	1,002.1	1,005.3		
Crucible + Celite wt, mg	31,637.2	32,173.9	32,377.5	33,216.4
Crucible + Celite + residue wt, mg	31,723.5	32,271.2	32,421.6	33,255.3
Protein, mg	6.5		3.9	
Crucible + Celite + ash wt, mg		32,195.2		33,231.0
	Blank			
	Insoluble		*Soluble*	
Crucible + Celite wt, mg	31,563.6	32,198.7	33,019.6	31,981.2
Crucible + Celite + residue wt, mg	31,578.2	32,213.2	33,033.4	33,995.6
Protein, mg	3.2		3.3	
Crucible + Celite + ash wt, mg		32,206.8		31,989.1

What is the (a) total, (b) insoluble, and (c) soluble fiber content of the cookie determined to the appropriate number of significant figures?

Answers

1. Number of Significant figures = 2 (6.1 mg)

$$\text{(a)}\quad \frac{151.9-13.1-21.1-6.0}{1002.8}\times 100 = 11\%$$

$$\text{(b)}\quad \frac{151.9-13.1-21.1-6.1-35.9}{1002.8}\times 100 = 7.5\%$$

2. (Calculations are done a little differently than those at the bottom of Table 19.8).

Insoluble dietary fiber

$$\text{Number of significant figures} = 2\left(6.5\,\text{mg}, 3.2\,\text{mg}\right)$$
$$\text{Blank residue} = 31{,}578.2-31{,}563.6\,\text{mg} = 14.6\,\text{mg}$$
$$32{,}231.2-32{,}198.7\,\text{mg} = 14.5\,\text{mg}$$
$$\text{Ave.} = 14.6\,\text{mg}$$
$$\text{Blank ash} = 32{,}206.8-32{,}198.7 = 8.1\,\text{mg}$$

$$\text{First sample residue} = 31{,}723.5-31{,}637.2\,\text{mg} = 86.3\,\text{mg}$$
$$\text{ash} = 32{,}195.2-32{,}173.9\,\text{mg} = 21.3\,\text{mg}$$
$$86.3\,\text{mg}\left(\text{residue weight}\right)$$
$$-14.6\,\text{mg}\left(\text{blank}\right)$$
$$-\ 3.3\,\text{mg}\left(\text{protein}, 6.5-3.2\left(\text{blank}\right)\right)$$
$$-13.2\,mg\left(ash, 21.3-8.1\left(blank\right)\right)$$
$$55.2\ \text{mg}$$
$$(55.2\,\text{mg}\div 1{,}002.1[(\text{sample wt.}])\times 100 = 5.5\%$$

$$\text{Second sample residue} = 32{,}271.2-32{,}173.9\,\text{mg} = 97.3\,\text{mg}$$
$$97.3-14.5-3.3-13.2 = 66.3\,\text{mg}$$
$$\left(66.3\div 1005.3\,\text{mg}\left(\text{sample wt.}\right)\right)\times 100 = 6.6\%$$
$$\text{Average of } 5.5\% \text{ and } 6.6\% = 6.1\%$$

$$\text{Second sample residue} = 32{,}271.2-32{,}173.9\,\text{mg} = 97.3\,\text{mg}$$
$$97.3-14.5-3.3-13.2 = 66.3\,\text{mg}$$
$$\left(66.3\div 1005.3\,\text{mg}\left(\text{sample wt.}\right)\right)\times 100 = 6.6\%$$
$$\text{Average of } 5.5\% \text{ and } 6.6\% = 6.1\%$$

Soluble dietary fiber

$$\text{Number of significantfigures figures} = 2\left(3.9\,\text{mg}, 3.3\,\text{mg}\right)$$
$$\text{Blank residue} = 33{,}033.4-33{,}019.6\,\text{mg} = 13.8\,\text{mg}$$
$$33{,}995.6-31{,}981.2\,\text{mg} = 14.4\,\text{mg}$$
$$\text{Ave.} = 14.1\,\text{mg}$$
$$\text{Blank ash} = 31{,}989.1-31{,}981.2\ \ \text{mg} = 7.9\,\text{mg}$$

$$\text{First sample residue} = 32{,}421.6-32{,}377.5\,\text{mg} = 44.1\,\text{mg}$$
$$\text{ash} = 33{,}231.0-33{,}216.4\,\text{mg} = 14.6\,\text{mg}$$
$$44.1\,\text{mg}\left(\text{residue weight}\right)$$
$$-14.1\,\text{mg}\left(\text{blank}\right)$$
$$-0.6\,\text{mg}\left(\text{protein } 3.9-3.3\left(\text{blank}\right)\right)$$
$$-6.7\left(\text{ash}, 14.6-7.9\left(\text{blank}\right)\right)$$
$$22.7\,\text{mg}$$
$$\left(22.7\,\text{mg}\div 1{,}002.1\left(\text{sample wt.}\right)\right)\times 100 = 2.3\%$$

$$\text{Second sample residue} = 33{,}255.3-33{,}216.4\,\text{mg} = 38.9\,\text{mg}$$
$$38.9\,\text{mg}\left(\text{residue weight}\right)$$
$$-14.1\,\text{mg}\left(\text{blank}\right)$$
$$-0.6\,\text{mg}\left(\text{protein}, 3.9-3.3\left(\text{blank}\right)\right)$$
$$-6.7\,\text{mg}\left(\text{ash}, 14.6-7.9\left(\text{blank}\right)\right)$$
$$17.5\,\text{mg}$$
$$\left(17.5\,\text{mg}\div 1{,}005.3\left(\text{sample wt.}\right)\right)\times 100 = 1.7\%$$
$$\text{Average of } 2.3\% \text{ and } 1.7\% = 2.0\%$$

$$\text{Total dietary fiber} = 6.1\%\left(\text{insoluble fiber}\right)$$
$$+2.0\%\left(\text{soluble fiber}\right) = 8.1\%$$

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