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SUGAR, SUGAR ANALYSIS

Sugar analysis includes the analysis of sucrose and other sugars in sugar processing and commercial trade, and in sugar-containing foodstuffs. In sugar production and sugar trading, sugar analysis also includes the determination of other components present in the sugar.

Since the early 1980s, several important developments have taken place in the field of sugar analysis. (*1*) Worldwide efforts at harmonization of methods have led to greater definition and standardization of the methods, with the subsequent discarding of many obsolete methods; (*2*) polarimetry of sugar solutions has been extended to the higher wavelength range of 880 nm; (*3*) lead abatement has resulted in new clarification agents for polarimetry; (*4*) the $100^{\circ}S$ point for sucrose has been redefined and is now called the $100^{\circ}Z$ point; (*5*) chromatographic methods have been improved to the point where they are widely accepted as official methods; and (*6*) near-infrared spectroscopic analysis is developing rapidly as an alternative method for many tests.

1. Standards and Definitions

The trend toward international standardization and harmonization of methods used in trading has had a significant impact on the methods used for sugar analysis. The Codex Alimentarius Commission was established in 1962 by FAO/WHO of the United Nations to develop an international compilation of food and commodity standards, which includes those pertaining to sugar and many sugar-containing products. In Europe, the European Union (EU) is carrying out a similar function for its member nations. The Nutrition and Labeling Act (NLEA) passed by the U.S. Congress in 1990 has provided some impetus toward developing more accurate analytical methods for sugars.

The International Commission for Uniform Methods of Sugar Analysis (ICUMSA) promulgates official methods of sugar analysis for the cane and beet sugar industry by the standardization and validation of methods through collaborative testing (1) (see Sugar, cane sugar; Sugar, beet sugar). The Corn Refiners Association (CRA) establishes methods used in the corn sugar industry (2). The Association of Official Analytical Chemists (AOAC) reviews methods of sugar analysis along with a vast array of other methods, many of which are required by the U.S. Food and Drug Administration (FDA) for setting standards of identity for foodstuffs and for labeling purposes (3).

At least six specifications of standards for granulated sugar quality are applicable in the United States. These include *Codex Alimentarius*, *Food Chemicals Codex* (FCC) (4), *U.S. Pharmacopeia* (USP) and *National Formulary* (NF) (5), National Soft Drink Association (6), National Canners Association, and Military Standard-900 for white sugar. These standards are intended to set limits on various components, including, but not necessarily limited to, polarization, invert or reducing sugar, ash, moisture, color, sulfur dioxide, arsenic, lead, and copper.

Sugar trading is controlled by contractual agreements between buyers and sellers. The contracts set specifications and limits and detail the required tests that must be conducted. In the United States, three parties participate in the analyses of raw sugar: the buyer, the seller, and the New York Sugar Trade Laboratory

(NYSTL). The settlement value for polarization is determined as the average of the two closest results; if all three results are equidistant, all three results are averaged.

2. Physical Methods of Sugar Analysis

The concentration of a pure sugar solution is determined by measurements of polarization (optical rotation), refractive index, and density.

2.1. Polarimetry

Polarimetry, or polarization, is defined as the measure of the optical rotation of the plane of polarized light as it passes through a solution. Specific rotation [α] is expressed as $[\alpha] = \alpha / lc$, where α is the direct or observed rotation, l is the length in dm of the tube containing the solution, and c is the concentration in g/mL. Specific rotation depends on temperature and wavelength of measurement, and is a characteristic of each sugar; it may be used for identification (7).

Polarization is the most common method for the determination of sugar in sugar-containing commodities as well as many foodstuffs. Polarimetry is applied in sugar analysis based on the fact that the optical rotation of pure sucrose solutions is a linear function of the sucrose concentration of the solution. Saccharimeters are polarimeters in which the scales have been modified to read directly in percent sucrose based on the normal sugar solution reading 100%.

The normal sugar solution corresponds to 26.000 g of pure sugar dissolved in water at 20.000°C to a final volume of 100.000 mL. The International Sugar Scale is calibrated in °Z. The 100°Z point is the optical rotation of the normal solution of pure sucrose at the wavelength of the green line of the mercury isotope 198 Hg (546.2271 nm *in vacuo*) at 20.00°C in a 200.000-mm tube and is established as 40.777 angular degrees (on the old °S scale, this value was 40.765). For other wavelengths, the Bünnagel formula for the rotatory dispersion of sucrose solutions is used. For quartz wedge instruments, the effective wavelength has been fixed at 587.0000 nm, and the 100°Z point is established as 34.934 (on the old °S scale, this value was 34.924). For the double line of yellow sodium light, the mean effective wavelength has been fixed at 589.4400 nm, and the 100°Z point is established as 34.626 (on the old °S scale, this value was 34.616) (1). Because of the changeover of the sugar scale from °S to °Z in 1988, some saccharimeters are calibrated in both scales, as some contracts continue to call for the older scale.

For turbid or colored sugar solutions, such as raw sugar, clarification of the solution is required. Until recently, lead acetate solution was used, but with the growing prominence of health and environmental concerns, as of the mid-1990s lead is being phased out, and use of other clarification agents, usually based on aluminum salts, is being implemented. An alternative to clarification has been to extend the wavelength of determination into a higher wavelength region, namely 880 nm, where a simple filtration to obtain an optically clear solution suffices to obtain a polarimetric reading. Values obtained using different methods of clarification and different wavelengths are not always the same, and issues of equivalence are still being determined.

Polarimetric determination of the sucrose concentration of a solution is valid when sucrose is the only optically active constituent of the sample. In practice, sugar solutions are almost never pure, but contain other optically active substances, most notably the products of sucrose inversion, fructose and glucose, and sometimes also the microbial polysaccharide dextran, which is dextrorotatory. Corrections can be made for the presence of impurities, such as invert, moisture, and ash. The advantage of polarization is that it is rapid, easy, and very reproducible, having a precision of $\pm 0.001^\circ$.

The value obtained as a result of a polarization measurement for sucrose is expressed as “pol” or “polarization” or “degrees pol” and not as percentage of sugar, but it is considered to closely approximate the sugar content.

2.1.1. Double Polarization

The Clerget double polarization method is a procedure that attempts to account for the presence of interfering optically active compounds. Two polarizations are obtained: a direct polarization, followed by acid hydrolysis and a second polarization. The rotation of substances other than sucrose remains constant, and the change in polarization is the result of inversion (hydrolysis) of the sucrose.

2.2. Refractive Index

The refractometric value of sugar solutions is used as a rapid method for the approximate determination of the solids content (also known as dry substance), because it is assumed that the nonsugars present have a similar influence on the refractive index as sucrose. Measurement is usually carried out on a Brix refractometer, which is graduated in percentage of sucrose on a wt/wt basis (g sucrose/100 g solution) according to ICUMSA tables of refractive index at 20.0°C and 589 nm. Tables are available that give mass fraction corrections to refractometric values at temperatures different from 20°C.

ICUMSA (1) has adopted tables showing the relationship between the concentration of aqueous solutions of pure sucrose, glucose, fructose, and invert sugar and refractive index at 20.0°C and 589 nm.

Equations have been developed that determine the relationship of the refractive index of sucrose solutions between 0–85% concentration, 18–40°C, and 546–589 nm.

2.3. Density

Measurement of density is widely used in the sugar industry to determine the sugar concentration of syrups, liquors, juices, and molasses. The instrument used is called a hydrometer or a spindle. When it is graduated in sucrose concentration (percent sucrose by weight), it is called a Brix hydrometer or a Brix spindle. Brix is defined as the percent of dry substance by hydrometry, using an instrument or table calibrated in terms of percent sucrose by weight in water solution. Hydrometers are also graduated in °Baumé, still in use in some industries. The relationship between °Baumé and density, d, in g/cm^3, is °BaumeB°aumé = $145(1 - 1/d)$.

Although spindles are calibrated for pure sucrose, other components are normally present in sugar that contribute to the density, such as ash and invert. Because the densities of these components are not much different from that of sucrose, the spindle value is considered a measure of total dissolved substances.

2.4. Purity

This is a widely used expression in the industry and represents, as a percentage, the proportion between polarization (considered a measure of sucrose) and dry solids (usually obtained by refractometry).

$$\text{purity} = 100 \times \text{sucrose/dry substance}$$

3. The Determination of Reducing Sugars

Many methods exist that utilize the reduction of copper or other compounds by aldose and ketose sugars, the most important being glucose and fructose. In relatively pure samples, it is assumed that the reducing sugars are present in essentially equal quantities of glucose and fructose, which may not be the case. In less pure samples, such as molasses, it is understood that other reducing substances are present, so the test is a general test for reducing substances.

The most common methods for determining reducing sugars are based on the reduction of the copper(II) complex with tartaric acid in alkaline solutions. The differences among them lie mostly in the composition

of the alkaline solution. The choice of the method for reducing sugars depends on the concentration of the reducing sugars as well as the product matrix. Because the reaction is not quite stoichiometric, the reagents and procedures for all copper reduction methods are strictly standardized, and large errors result if deviations from the method occur. The methods for reducing sugars are listed as follows.

3.1. Lane and Eynon Constant Volume Procedure

Probably the most common test for reducing sugars, this method is based on the reduction of Fehling's solution, Soxhlet's modification. The constant volume modification, a more recent change to the method, has allowed for greater standardization, increased sensitivity, and the use of a simple formula instead of tables to determine the amount of invert. The method determines reducing sugars in the presence of sucrose, and is used for raw cane sugar, cane processing products, and specialty sugars having low levels of invert. This test forms the basis for some molasses purchasing contracts and is required in several standards, including the *National Formulary* and *Food Chemicals Codex*.

3.2. Berlin Institute Method

This method is for determination of invert sugar in products containing not more than 10% invert in the presence of sucrose. It is a copper reduction method that utilizes Müller's solution, which contains sodium carbonate.

3.3. Emmerich Method

This method is for determination of trace amounts of reducing sugars in pure sucrose and white and refined sugars with reducing sugar content up to 0.15%. The test is carried out in a nitrogen atmosphere and is based on the reduction of 3,6-dinitrophthalic acid.

3.4. Knight and Allen

This is a copper reduction method for reducing sugars in white sugar up to 0.02%. It utilizes EDTA to determine excess unreacted copper. Tests undertaken in 1994 to extend the range of this method were unsuccessful. In spite of poor performance in ring tests, it remains an official ICUMSA method.

3.5. Luff Schoorl

This method is for the determination of total reducing sugars in molasses and refined syrups after hydrolysis. It is a copper-reducing method that forms the basis of some molasses purchasing contracts.

3.6. Ofner Method

This method is for the determination of invert sugar in products with up to 10% invert in the presence of sucrose and is a copper-reduction method that uses Ofner's solution instead of Fehling's. The reduced cuprous oxide is treated with excess standardized iodine, which is black-titrated with thiosulfate using starch indicator.

4. Other Methods

4.1. Colorimetric Methods

Numerous colorimetric methods exist for the quantitative determination of carbohydrates as a group (8). Among the most popular of these is the phenol–sulfuric acid method of Dubois (9), which relies on the color formed when a carbohydrate reacts with phenol in the presence of hot sulfuric acid. The test is sensitive for virtually all classes of carbohydrates. Colorimetric methods are usually employed when a very small concentration of carbohydrate is present, and are often used in clinical situations. The Somogyi method, of which there are many variations, relies on the reduction of cupric sulfate to cuprous oxide and is applicable to reducing sugars.

4.2. Enzymatic Methods

Since their earliest use to determine blood glucose, applications of enzyme methods have expanded to include sugar analysis in foodstuffs, beverages, and sugar processing (10). Commercial enzyme analyzers are based on immobilized enzymes embedded in membranes. When the membrane or biosensor contacts a solution of the material to be analyzed, glucose is oxidized by glucose oxidase, releasing hydrogen peroxide, which is then measured electronically, giving an estimation of the amount of glucose present:

$$\beta\text{-D-glucose} + O_2 \xrightarrow{\text{Glc O}_x} \text{glucono-}\delta\text{-lactone} + H_2O_2$$

Three enzymes are required to determine sucrose: invertase to hydrolyze sucrose and produce α-glucose, mutarotase to produce β-glucose, and glucose oxidase for the standard reaction. Enzyme methods have the advantage of being rapid and simple, requiring little sample pretreatment except for solubilization and dilution. The methods require frequent calibration. Enzyme membranes have variable lifetimes and may need to be replaced frequently. Enzyme analyzers are used for quality control in sugar processing, for monitoring wastewater, and in determining sugar in animal feed.

4.3. Chromatographic Methods

These methods are ideally suited for the identification and measurement of individual sugars in many matrices. Chromatographic methods have their widest application in research and in commercial laboratories dealing with food analysis, where both gas liquid chromatography (glc) and high performance liquid chromatography (hplc) are in use. Among the older techniques, paper chromatography is obsolete. Thin-layer chromatography is mostly used for qualitative identification, as quantitation of spots by densitometry has not been widely applied.

Although chromatography offers a more accurate measurement of individual sugars in a sample, it has not supplanted polarization as the method of commerce in the sugar industry. Chromatography lacks the precision of polarimetry, being in the range of 0.5–1.0% for sucrose, about a magnitude higher than polarimetry, and it has longer analysis times. As recently as the early 1980s, the precision of chromatography was in the range of 3–5%. The large improvement in precision is the result of incremental advancements in column technology, flow control, and detection systems. Chromatographic methods have been used to show sources of interference that contribute to inaccuracies in polarimetric measurements (11).

The glc analysis of sugars requires chemical derivatization to produce a volatile molecule. Many derivatization methods for sugars exist, but the simplest and most rapid for routine analysis is silylation to produce trimethylsilyl derivatives. Excess water in the sample interferes and must be carefully controlled. Direct silylation produces single peaks for enantiomerically pure nonreducing sugar, such as sucrose, and multiple peaks for reducing sugars, representing the equilibrium of conformations and anomeric configurations. Reactions are available that produce single peaks, but their use adds to the complexity and time of the analysis. Separation is generally done on methyl silicone or phenyl–methyl silicone phases with detection by flame ionization. Glc

analysis is limited to lower molecular weight carbohydrates, and does not usually exceed the level of tri- or tetrasaccharides.

Sugar analysis by hplc has advanced greatly as a result of the development of columns specifically designed for carbohydrate separation. These columns fall into several categories. (*1*) Aminopropyl-bonded silica used in reverse-phase mode with acetonitrile–water as the eluent. (*2*) Ion-moderated cation-exchange resins using water as the eluent. Efficiency of these columns is enhanced at elevated temperature, ca 80–90°C. Calcium is the usual counterion for carbohydrate analysis, but lead, silver, hydrogen, sodium, and potassium are used to confer specific selectivities for mono-, di-, and oligosaccharides. (*3*) Size exclusion columns packed with sulfonated polystyrene–divinylbenzene copolymer using water alone as eluent. These columns are designed primarily for analysis of corn syrups containing oligomeric materials. Larger molecules elute before smaller ones. (*4*) Pellicular anion-exchange columns using dilute sodium hydroxide eluent having pulsed amperometric detection. This latter technique is known variously as high performance anion-exchange chromatography, ion chromatography, hpaec, hpic, or ic.

4.4. Near-Infrared Spectroscopy

The relatively new technique of near-infrared spectroscopy (nir) is increasingly applied in the sugar industry for several types of analyses (12). Current (ca 1996) feasible applications include sucrose, pol, Brix, and purity. The technique has the advantage of requiring little or no sample preparation, having a great saving in time resulting from multiple determinations being done simultaneously, and the elimination of chemical usage. It is a secondary technique, depending on results from the primary techniques for calibration, hence it can only be as precise and accurate as the primary method used for calibration. It is expected to eventually have wide usage in process control (qv) and has already been accepted as a commercial method for payment purposes in at least one country.

5. Determination of Other Components

In the sugar industry, where the goal is to determine the exact amount of sucrose present, the analysis of other components is essential to determine purity. The most important of these, besides reducing sugars discussed, are moisture, ash, and color. Also relevant are methods used to determine particle-size distribution and insoluble matter.

5.1. Moisture

In relatively pure sugar solutions, moisture is determined as the difference between 100 and Brix. In crystalline products, it is usually determined by loss-on-drying under specified conditions in an oven or by commercial moisture analyzers that have built-in balances. Moisture in molasses and heavy syrups is determined by a special loss-on-drying technique, which involves coating the sample onto sand to provide a greater surface area for oven drying. The result of this test is usually considered dry substance rather than moisture.

Small amounts of moisture (up to about 0.5%) in crystalline sugars can be determined chemically by titration with Karl Fisher reagent. A volumetric Karl Fisher titration procedure for moisture in molasses is accepted by AOAC. Automatic Karl Fisher titrators are available, and as acceptance of pyridine-free reagents increases, their use may increase.

5.2. Ash and Inorganic Constituents

Ash may be measured gravimetrically by incineration in the presence of sulfuric acid or, more conveniently, by conductivity measurement. The gravimetric result is called the sulfated ash. The older carbonate ash method is no longer in use. Ash content of sugar and sugar products is approximated by solution conductivity measurements using standardized procedures and conversion factors.

Tests for elements such as arsenic, lead, and copper are specified in the relevant standards. The methods specified are usually of the colorimetric or atomic absorption types.

5.3. Color

The visual color, from white to dark brown, of sugar and sugar products is used as a general indication of quality and degree of refinement. Standard methods are described for the spectrophotometric determination of sugar color that specify solution concentration, pH, filtration procedure, and wavelength of determination. Color or visual appearance may also be assessed by reflectance measurements.

5.4. Particle-Size Distribution

Particle-size specifications for sugar are not usually a part of the legislated standards, but they are of concern to commercial users and suppliers and are often specified in contracts. Grain-size distribution is determined by using a series of sieves, either hand-sieved or machine-sieved (13).

5.5. Insoluble Matter

Insoluble matter in sugar is determined as the dry weight of material left on a filter or membrane after passage of a sugar solution. This may include bits of sand, filtration medium, plant material, and polymeric material.

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Sugar, Properties; Size measurement of particles; Chromatography