Techniques used in carbohydrate analysis
(monosaccharides and oligosaccharides)

1. Chromatography

- Thin layer chromatography (TLC)
- Gas chromatography (GC)
- High performance liquid chromatography (HPLC)

2. Electrophoresis

Carbohydrates can also be separated by electrophoresis after they have been derivitized to make them electrically charged, *e.g.*, by reaction with borates. A solution of the derivitized carbohydrates is applied to a gel and then a voltage is applied across it. The carbohydrates are then separated on the basis of their size: the smaller the size of a carbohydrate molecule, the faster it moves in an electrical field.

3. Chemical Methods

(a) Titration method

The Lane-Eynon method is an example of a tritration method of determining the concentration of reducing sugars in a sample. A burette is used to add the carbohydrate solution being analyzed to a flask containing a known amount of boiling copper sulfate solution and a methylene blue indicator. The reducing sugars in the carbohydrate solution react with the copper sulfate present in the flask. Once all the copper sulfate in solution has reacted, any further addition of reducing sugars causes the indicator to change from blue to white. The volume of sugar solution required to reach the end point is recorded. The reaction is not stoichemetric, which means that it is necessary to prepare a calibration curve by carrying out the experiment with a series of standard solutions of known carbohydrate concentration.

(b) Gravimetric method

- Carbohydrates are oxidized in the presence of heat and an excess of copper sulfate and alkaline tartrate under carefully controlled conditions which leads to the formation of a copper oxide precipitate:
- ������������ reducing sugar + Cu^{2+} + base ⟶ oxidized sugar + CuO_2 (precipitate)
- The amount of precipitate formed is directly related to the concentration of reducing sugars in the initial sample. The concentration of precipitate present can be determined gravimetrically (by filtration, drying and weighing), or titrimetrically (by redissolving the precipitate and titrating with a suitable indicator). This method suffers from the same disadvantages as the Lane-Eynon method, neverthless, it is more reproducible and accurate.

(c) Colorimetric method

- The Anthrone method is an example of a colorimetric method of determining the concentration of the total sugars in a sample. Sugars react with the anthrone reagent under acidic conditions to yield a blue-green color. The sample is mixed with sulfuric acid and the anthrone reagent and then boiled until the reaction is completed. The solution is then allowed to cool and its absorbance is measured at 620 nm. There is a linear relationship between the absorbance and the amount of sugar that was present in the original sample. This method determines both reducing and non-reducing sugars because of the presence of the strongly oxidizing sulfuric acid. Like the other methods it is non-stoichemetric and therefore it is necessary to prepare a calibration curve using a series of standards of known carbohydrate concentration.
- The Phenol - Sulfuric Acid method is an example of a colorimetric method that is widely used to determine the total concentration of carbohydrates present in foods. A clear aqueous solution of the carbohydrates to be analyzed is placed in a test-tube, then phenol and sulfuric acid are added. The solution turns a yellow-orange color as a result of the interaction between the carbohydrates and the phenol. The absorbance at 420 nm is proportional to the carbohydrate concentration initially in the sample. The sulfuric acid causes all non-reducing sugars to be converted to reducing sugars, so that this method determines the total sugars present. This method is non-stoichemetric and so it is necessary to prepare a calibration curve using a series of standards of known carbohydrate concentration.

4. Enzymatic methods

D-Glucose/D-Fructose

This method uses a series of steps to determine the concentration of both glucose and fructose in a sample. First, glucose is converted to glucose-6-phosphate (G6P) by the enzyme hexokinase and ATP. Then, G6P is oxidized by $NADP^+$ in the presence of G6P-dehydrogenase (G6P-DH)

G6P + $NADP^+$ → gluconate-6-phosphate + NADPH + H^+

The amount of NADPH formed is proportional to the concentration of G6P in the sample and can be measured spectrophotometrically at 340nm. The fructose concentrationn is then determined by converting the fructose into glucose, using another specific enzyme and repeating the above procedure.

Maltose/Sucrose

The concentration of maltose and sucrose (disaccharides) in a sample can be determined after the concentration of glucose and fructose have been determined by the previous method. The maltose and sucrose are broken down into their constituent monosaccharides by the enzyme a-glucosidase:

maltose + H_2O → 2 glucose

sucrose +H_2O → glucose + fructose

The concentrations of glucose and fructose can then be determined by the previous method. The major problem with this method is that many other oligosaccharides are also converted to monosaccharides by a-glucosidase, and it is difficult to determine precisely which oligosaccharides are present. This method is therefore useful only when one knows the type of carbohydrates present, but not their relative concentrations.

5. Physical methods

Polarimetry

- Molecules that contain an asymmetric carbon atom have the ability to rotate plane polarized light. A polarimeter is a device that measures the angle that plane polarized light is rotated on passing through a solution. A polarimeter consists of a source of monochromatic light, a polarizer, a sample cell of known length, and an analyzer to measure the angle of rotation. The extent of polarization is related to the concentration of the optically active molecules in solution by the equation a = [a]lc, where a is the measured angle of rotation, [a] is the optical activity (which is a constant for each type of molecule), l is the pathlength and c is the concentration.
- A calibration curve of a versus concentration is prepared using a series of solutions with known concentration, or the value of [a] is taken from the literature if the type of carbohydrates present is known. The concentration of carbohydrate in an unknown sample is then determined by measuring its angle of rotation and comparing it with the calibration curve.

Density

The density of a material is its mass divided by its volume. The density of aqueous solutions increases as the carbohydrate concentration increases. Thus the carbohydrate concentration can be determined by measuring density, *e.g.*, using density bottles or hydrometers. This technique is routinely used in industry for determination of carbohydrate concentrations of juices and beverages.

Infra-red

- A material absorbs infrared due to vibration or rotation of molecular groups. Carbohydrates contain molecular groups that absorb infrared radiation at wavelengths where none of the other major food constituents absorb consequently their concentration can be determined by measuring the infrared absorbance at these wavelengths. By carrying out measurements at a number of different specific wavelengths it is possible to simultaneously determine the concentration of carbohydrates, proteins, moisture and lipids. Measurements are normally carried out by measuring the intensity of an infrared wave reflected from the surface of a sample: the greater the absorbance, the lower the reflectance. Analytical instruments based on infrared absorbance are non-destructive and capable of rapid measurements and are therefore particularly suitable for on-line analysis or for use in a quality control laboratory where many samples are analyzed routinely.

6. Immunoassays

- Applications in food industry for the qualitative and quantitative analysis of food products
- Extremely sensitive, specific, easy to use and rapid
- Developed by attaching the carbohydrate of interest to a protein, and then injecting it into an animal. With time the animal develops antibodies specific for the carbohydrate molecule. These antibodies can then be extracted from the animal and used as part of a test kit for determining the concentration of the specific carbohydrate in foods.

https://people.umass.edu/~mcclemen/581Carbohydrates.html