Are you Made of Sugar?

by Stephanie Kappes and Dr Gabriele Zierfels
Metrohm AG, Ionenstrasse, 9000 Herisau, Switzerland
ska@metrohm.com

Carbohydrates constitute the biggest part of the biomass on Earth. They are produced by photosynthesis and are present in all plants and plant-based materials. The amount and composition of carbohydrates in a sample can reveal a wide range of different information, depending on the context. As a result, they are subject to analysis in various industries.

Carbohydrates are Everywhere

In the food industry, carbohydrate and sugar content are key factors in determining the nutritional value of food and beverages. In environmental analysis – to mention but one example – the anhydrosugar levoglucosan, which is produced by the pyrolysis of cellulose and acts as a tracer for biomass combustion, is determined in aerosols. These are just two of the many applications of carbohydrate analysis.

Carbohydrates are composed of one or more monosaccharide units, each of which has a carbonyl group (aldehyde or ketone group) and several hydroxyl groups [1]. Because mono-, di-, and oligosaccharides are water-soluble, ion chromatography, which is performed in the aqueous phase, is particularly suitable for analysing them [2,3]. Determination can thus be performed directly – without prior extraction to the organic phase. However, a high-capacity column is necessary because sugars are relatively large molecules which are in many cases similar in structure (e.g., glucose and galactose; see Figure 1). If the column capacity is too low, similar sugars elute together and cannot be detected separately.

Sugars in Foods

From December 2016, the European Union (EU) requires that nutritional values are indicated on all foodstuffs, with the exception of unprocessed products and products sold loose (regulation no. 1924/2006). What is already an established practice, i.e., indicating the calorific value and certain nutrients including sugar and carbohydrates, is now set to become mandatory.

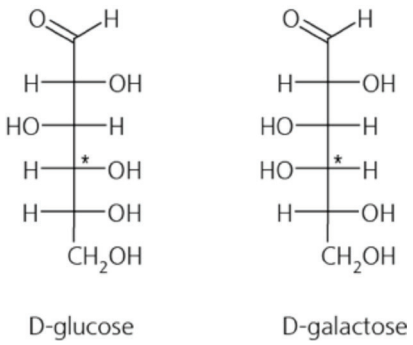

Figure 1: Structural formulae of glucose and galactose. The molecules differ only in the position of the OH group at the C4 atom (highlighted with an asterisk).

Along with starch, which is a polymer of glucose, the usable carbohydrates found in foodstuffs are largely in the form of sugars. According to the EU definition, this includes all mono- and disaccharides with the exception of polyvalent alcohols. The majority of sugars in foodstuffs is made up of the monosaccharides glucose, fructose, galactose, and the disaccharides sucrose, lactose, and maltose. Table 1 shows a multitude of sugars and related compounds that can be analysed using ion chromatography. The table indicates the matrices, i.e., foodstuffs and beverages, in which the compounds can be determined, as well as the necessary sample preparation steps.

Table 1: Sugars and related compounds that can be analysed by ion chromatography. The plus sign indicates the matrices in which each analyte can be determined.

Matrix	Sample preparation	Propylene glycol	Inositol	Glycerol	Xylitol	Sorbitol	Mannitol	Ribose	Xylose	Arabinose	Mannose	Glucose	Fructose	Galactose	Maltose	Lactose	Lactulose	Sucrose	Cellobiose	Maltotriose	Raffinose	Maltotetraose	Maltopentaose	Maltohexaose	Maltoheptaose	Steviolglycoside (Stevia)
Apple juice	F, D											+	+					+	+							+
Baby Food	Dialysis											+	+			+		+								
Beer	U, D	+																								
Beer wort	F, D											+			+	+		+		+						
Candies	E, D, F											+	+		+	+		+								
Chocolate	E, D, F		+	+	+	+	+		+	+		+	+			+		+								
Cola	D											+	+					+								
Corn syrup	D											+			+					+						
Dairy beverages	Dialysis		+		+					+	+	+	+	+		+	+	+								
Dairy products	Dialysis		+		+					+	+	+	+	+		+		+								
Diet cola	D			+			+		+			+														
Food extracts	P, D											+	+		+	+		+								
Functional food	P, D, F			+								+	+		+	+		+								
Instant coffee	E, F, D						+		+	+	+	+	+	+				+								
Instant tea	F, D											+	+			+		+								
Malt extract	D											+			+					+		+	+	+	+	
Maple syrup	D											+	+					+								
Orange juice	F, D		+									+	+					+								+
Potato extract	P, D, F											+	+					+								
Red beet extract	D		+			+	+	+		+		+	+	+				+			+					
Sugar-free chewing gum	E, D, F				+	+	+					+														
Tomato juice	F, D											+	+													
Vodka	D											+	+					+								

Polyols, sugar alcohols, monosaccharides, disaccharides, oligosaccharides, steviol glycosides
E: extraction, F: filtration, U: ultrasonic treatment, D: dilution

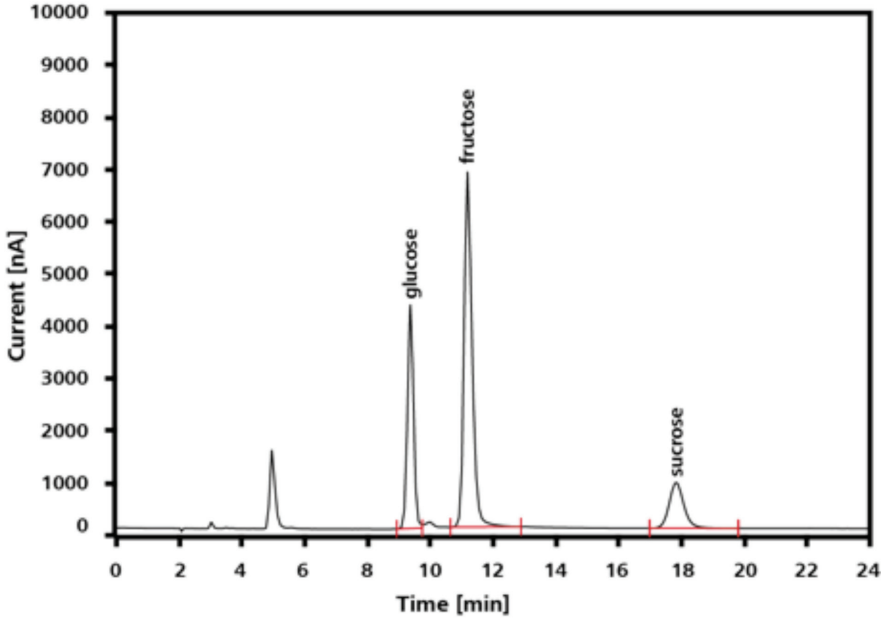

Figure 2: Determination of glucose, fructose, and sucrose in apple juice. Except for simple dilution, no sample preparation is required. Column: Metrosep Carb 2 - 150/4.0; eluent 100 mmol/L sodium hydroxide / 10 mmol/L sodium acetate; column temperature: 30°C; sample volume: 20 µL; flow rate: 0.5 mL/min; amperometric detector: PAD mode; working electrode (WE): Au, reference electrode (RE): Pd, working potential: 50 mV; dilution 1:1000.

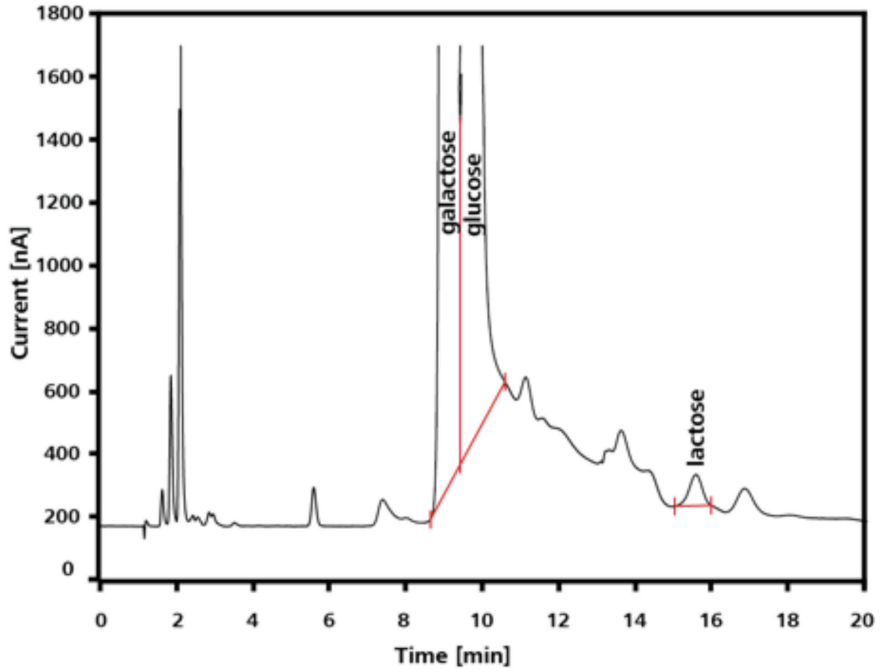

Figure 3: Determination of lactose traces in milk declared lactose-free, diluted 1:100 and spiked with 100 mg/L lactose, after Inline Dialysis. Column: Metrosep Carb 2 - 150/4.0; eluent: 5 mmol/L sodium hydroxide / 2 mmol/L sodium acetate; column temperature 40°C; sample volume 20 µL; flow 0.8 mL/min, amperometric detector: PAD mode; WE: Au; RE: Pd; working potential: 50 mV.

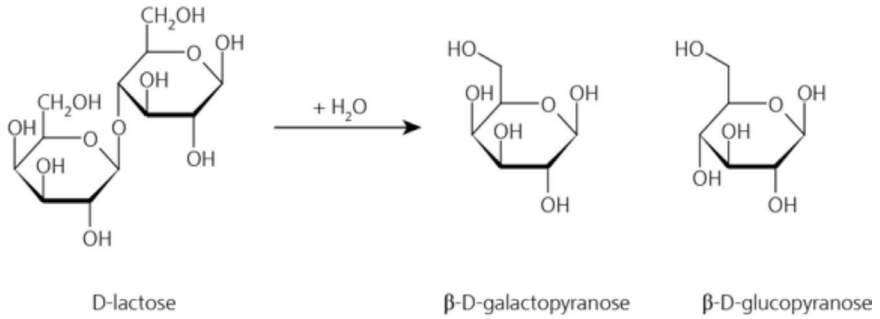

Figure 4: Lactose is composed of the monosaccharides galactose and glucose. The hydrolysis of lactose illustrated here is catalysed by the enzyme lactase.

Apple Juice Analysis

The chromatogram in Figure 2 results from the injection of 20 µL of apple juice which was diluted with ultra-pure water. Depending on the sample, it is advisable to filtrate the sample in addition, to avoid introducing particles to the column and the detector. The alkaline eluent (100 mmol/L sodium hydroxide / 10 mmol/L sodium acetate) ensures that the sugars are present in dissociated form (as anions) and can therefore be separated in the column by the ion exchanger.

Because carbohydrates are electrochemically active, they can be detected amperometrically. During amperometric detection, the analytes are oxidised to a working electrode by applying a potential to the latter. This results in an electrical current that reveals the concentration. Over time, however, carbohydrates form deposits on the working electrode when a continuous potential is applied. The amperometric detector is therefore operated in PAD (pulsed amperometric detection) mode. Here, a three-stage cyclic potential ensures that after measuring the current, i.e., after the determination stage, the electrode is cleaned from the adsorbed molecules and eventually conditioned [4].

Residual Lactose in 'Lactose-Free' Products

A key part of the quality control of products declared lactose-free is the determination of residual lactose. The ion chromatogram in Figure 3 illustrates the determination of lactose in 'lactose-free' milk to which 100 mg/L lactose was added. Again, the separation takes place under strongly alkaline conditions (eluent of 5 mmol sodium hydroxide / 2 mmol sodium acetate) and the analyte is detected by pulsed amperometry. The high concentrations of galactose and glucose illustrated in the chromatogram are a result of the enzymatic breakdown of lactose into these very monosaccharide constituents (Figure 4). Because of its fat- and protein-rich matrix, milk must undergo dialysis before being analysed. With the Metrohm Inline Sample Preparation, which includes an Inline Dialysis option, this is a fully automated process, and therefore does not involve any additional effort.

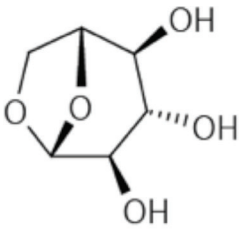

Figure 5: Levoglucosan (1,6-anhydro-β-D-glucopyranose) is produced in the pyrolysis of cellulose and is therefore commonly used as an indicator for biomass combustion.

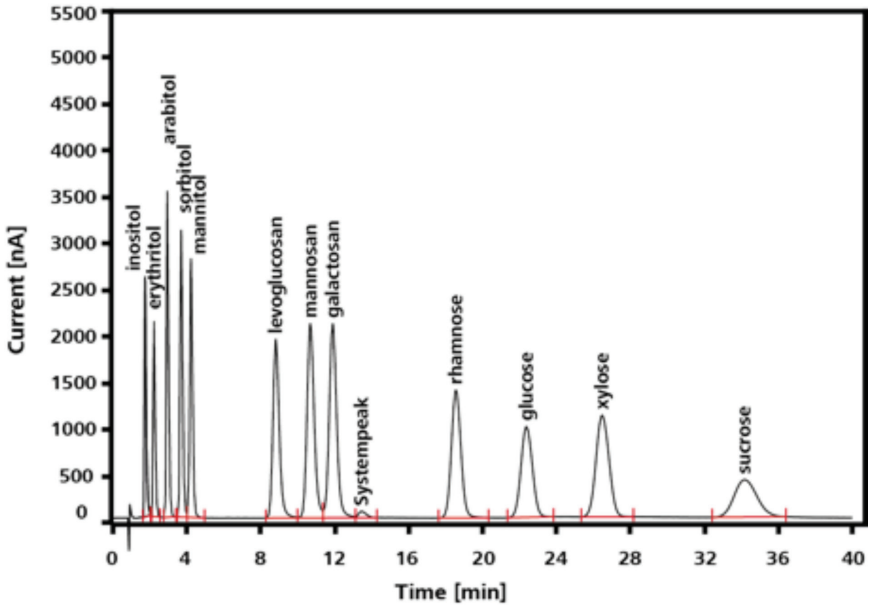

Figure 6: Determination of indicators for wood combustion (levoglucosan, mannosan, and galactosan) and biological sugars and alcohols, which are found in aerosols such as pollen. Column: Metrosep Carb 2 - 150/4.0; eluent: 10 mmol/L sodium hydroxide; column temperature 45°C; sample volume 100 µL; flow 1.0 mL/min; amperometric detector: PAD mode; WE: Au; RE: Pd; working potential: 50 mV

Figure 7: The carbohydrate column Metrosep Carb 2 is available in three models: 250 mm, 150 mm, and 100 mm.

Carbohydrates as Tracers in Environmental Analysis

Fine dust limit values, which are used as health protection measures, are regularly being violated in many places. When looking for the culprit, the usual suspects are traffic and industry, but residential wood burning used for heating, too, has been linked to high fine dust values [5]. The tracer levoglucosan (Figure 5) is often determined in order to detect wood combustion. Figure 6 shows the determination of a standard solution in which, in addition to levoglucosan, mannosan and galactosan – all products of wood combustion – were analysed, as well as several biological sugars, alcohols, etc., which are typically found on aerosol particles. The high-capacity column achieves good separation of all substances which thus can be determined in a single analysis.

A new Carbohydrate Column

The Metrosep Carb 2 chromatography column (Figure 7) excels with its high ion exchange capacity, i.e., with the high number of ion exchange groups contained in its carrier material. This allows clean separation of the various sugars. Applications are found in a wide range of industries: water and environmental analysis, the pharmaceutical and food industries, forensics, the cosmetic industry, and the quality control of biofuels. In addition to carbohydrate analysis, the Metrosep Carb 2 is also suitable for determinations in samples with high salt content where lower-capacity columns fail, e.g., seawater.

References

[1] Ebermann, R., Elmadfa, I. (2008): Lehrbuch Lebensmittelchemie und Ernährung. Vienna: Springer Verlag.

[2] Rocklin, R. D. and Pohl, C. A. (1983) J. Liq. Chromatogr. 6: 1577–1590

[3] De Benedetto, G. E. et al. (2000) Fresenius J. Anal. Chem. 368: 739–758.

[4] Fritz, J. S. and Gjerde, D. T. (2000): Ion Chromatography. Weinheim (Federal Republic of Germany): WILEY-VCH Verlag GmbH.

[5] German Federal Institute for Risk Assessment (2015): «Analyse des quantitativen Einflusses der Holzverbrennung auf die Feinstaubkonzentration in Berlin und Brandenburg anhand des Tracers Levoglucosan (Levoglucosan)». Retrieved on May 18, 2015 from http://www.bfr.bund.de/de/analyse_des_quantitativen_einflusses_der_holzverbrennung_auf_die_feinstaubkonzentration_in_berlin_und_brandenburg_anhand_des_tracers_levoglucosan__levoglucosan_-193056.html.