Rapid Measurement of Sugars in Food, Feed and Pet Food Products

The Expertise Centre CCC (Eurofins Food, Feed & Water Testing Netherlands) is proud to announce the availability of the method for analyses of sugars for product labeling as a validated routine test for food, pet food and feed products according to ISO 17025. The method is based on the Eurofins best practice method and EN-ISO 22184 & IDF 244.
The method is available at CCC as test codes HEC3S, (Milk, dairy, IFT) HEC3Z (Food) and HEC3T (Feed).

Introduction

Carbohydrates are key components in the diet. Carbohydrates are found in a various food products like bakery products (bread, cookies), beans, pasta's, dairy products (milk, infant formula, yoghurt, dessert), popcorn, potatoes, spaghetti and soft drinks and they are present in a variety of forms. The most common and abundant forms are sugars, fibers, starches, non-starch polysaccharides, dietary fibre and prebiotics. Starchy carbohydrates provide an important source of energy, and fiber is important for digestive health.
The WHO 2015 recommended to limit all free sugar consumption to less than 10% of daily calories. The nutrition labels on food products need to specify the amount of total carbohydrates and sugars present in the product. In food and feed products various mono- and di-saccharides are expected, e.g. glucose, fructose, galactose, lactose, sucrose and maltose.

Regulations

Correct nutrition labeling of total sugars in products is mandatory from EC legislation and other authorities, f.i. FDA (Nutrition Labeling requirements as laid down in the Nutrition Labeling and Education Act NLEA). The new EU law on food information to consumers (EU No. 1169/2011), making it compulsory to provide nutrition information, is officially applying as of 2016 (Directive 90/496/EEC concerns nutrition labelling of foodstuffs for the final consumer). According to the Codex Alimentarius Guidelines on Nutrition Labelling (CAC/GL 2 -1985 last rev. 2017) nutritional labelling of food products requires listing of sugars content. 'Sugars' means all monosaccharides and disaccharides present in food, but excludes (acc. EC) polyols, isomaltulose and tagatose.

Claims like Sugar Free, Low or Reduced/Less are also regulated by law (f.i. Codex Alimentarius Nutrition and Health Claims (CAC/GL 23-1997 and EC Regulation 1924/2006).

Method

Currently, no standard protocols (IDF (international dairy federation), ISO, CEN, AOAC, ...) are available for the determination of a complete combination of all relevant mono- and disaccharides in the different dairy matrices in one analysis. Various HPLC based methods only determine glucose, fructose, lactose, sucrose and maltose. ISO/IDF standards are only available for lactose and lactulose, applying either classical chromatography or enzymatic methods. No other sugars are quantified though. Various enzymatic methods are available for individual sugars depending on specificity of enzymes used. This could lead to incorrect values in the presence of saccharides like galactose, lactulose, trehalose, palatinose and allo-lactose.

CCC developed a powerful technique to help you identify and quantify sugars for labeling in your products. The method is an in-house developed method based on the Eurofins best practice method and the EN-ISO 22184 / IDF 244 using high-pH anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) being developed by CCC[1,2]. The method overcomes some of the problems associated with older methods.

Principle

The method consists of an aqueous ethanol extraction of the sugars in the food sample, followed by clarification with Carrez reagents. The clarified filtrate is diluted and then directly introduced in the HPAEC-PAD system for quantification of the sugars.

Applications & Use

The test is applicable to food and feed matrices, covering retail products and labeling requirements. The method reports 6 sugars as well as the sum of the 6 sugars (glucose, fructose, galactose, lactose, sucrose and maltose).

Method principle incl. chromatogram and separation

The sample is weighed and ethanol is added to eliminate any micro-organisms. Then the sample is suspended in water. After clarification with Carrez and filtration, the clear sample solution is diluted and then injected onto a HPAEC-PAD, which is a suitable configuration for the determination of these carbohydrates. For identification, the retention times are compared with those of a standard solution. For the quantitative determination, the peak areas are used in combination with an internal standard.

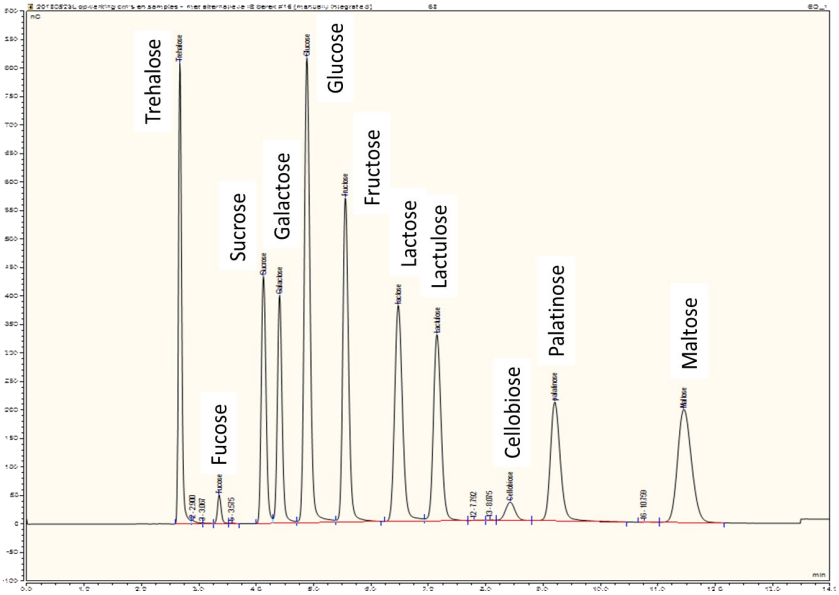

Figure: HPAEC-PAD. A base line separation is achieved for all main food or dairy sugars (6) with no interference from most other relevant sugars, e.g. trehalose, lactulose, isomaltulose (palatinose™) & sugars from FOS, GOS, MOS, ...

Literature:

1. ISO/CD 22184 Milk and milk products — Determination of the sugar contents — High performance anion exchange chromatographic method (HPAEC-PAD). Technical Committee: ISO/TC 34/SC 5
2. J AOAC Int. 2017 Vol 100, May 11. p. 1577. The Determination of Sugars in Dairy Products: Development of a New Standard Method for the Intern. Dairy Federation and the Intern. Organization for Standardization by Sanders, Ernste-Nota, Visser, van Soest, Brunt
3. J AOAC Int., 21 July 2020: Results MLT ISO/CD 22184 – IDF/WD 244: Milk and milk products – Determination of the sugar contents – High performance anion exchange chromatography method with pulsed amperometric detection (HPAEC-PAD) Brunt, Sanders, Ernste Nota, van Soest.

Our tests at a glance

Test code	Matrix	Analytical method (HPAEC-PAD)
HEC3S (glc,fru,lac,suc,mal,gal)#	Dairy: milk (products), IFT	Method equivalent to ISO-EN / IDF
HEC3Z (glc,fru,lac,suc,mal,gal)	Food	Method based on ISO-EN / IDF
HEC3T (glc,fru,lac,suc,mal,gal)	Feed	Method based on ISO-EN / IDF
HEC3L (Dairy) / HEC3M (Foods)*	All foods incl. dairy	Conform ISO-EN / IDF (fru,lac,suc,mal,gal,glc)

Method replaces packages PHECB & PHECE incl. tests codes HEC2F and HEC2H and HEC3X/HEC36. Sum totals and reducing sugars are included in report. *Method specially designed for complex products, e.g. dairy & infant formula, as new ISO, CEN and IDF standards. - Methods are suitable for determination trehalose, palatinose, lactulose, maltotriose: please contact CCC for options.

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Performance characteristics: Sugars according ISO-EN & IDF & AOAC

Our methods for sugar determination fulfill the performance requirements of the official ISO-CEN 22184 – IDF 244 and Specific Method Performance Requirements in the AOAC SMPR 2018.001 (Sugars in Animal Feed, Pet Food, and Human Food: Recovery, repeatability and reproducibility parameters) and AOAC first action method AOAC 2018.16 (Sugar Profile Method by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection in Food, Dietary Supplements, Pet Food, and Animal Feeds).
The method resolves issues of older methods like CEN/TC 15754, HPAEC-PAD sugars determination in animal feeding stuffs and AOAC 980.13 (cacao beans and milk chocolate) and 982.14 (glucose, fructose, sucrose and maltose in presweetened cereals), with their limited applicability and scope. The older methods show no selectivity towards sugars like galactose, lactulose and various fructo- and galacto- and malto-oligosaccharides (FOS, GOS, MOS). The method is suitable for an extended scope of 9 sugars and the matrices food and feed products including milk and milk products.
The method was successfully validated according ISO 17025. All requirements such as robustness, accuracy, detection and quantitation limit, linearity/lack of fit, reproducibility, repeatability and selectivity were met (Validation Report V53050). The validation is done on range of products representative for all foods as well as feed incl. pet food having a broad range of fat, protein, fibre and carbohydrates. The selectivity is studied by determining the separation conditions fulfilling the resolution requirement >1.4. The method show now interferants as defined by AOAC SMPR 2018.001. As such lactulose, isomaltulose and FOS, GOS and MOS do not interfere.
Please be aware that for products containing significant amounts of non-labeling sugars, like trehalose, tagatose, isomaltose, xylose, mannose, lactulose, isomaltulose/trehalulose, allo–/epilactose, melibiose, raffinose, cello–/xylobiose, anhydro-sugars typically found in some insect, soy, beer, dairy, honey, products and caramel, polyol syrups and soluble fiber enriched products separate measurements may be needed to obtain correct total sugar content of products. Contact CCC for more information.

Table 1: General performance characteristics

Sugar	Range (%)	Recovery (%)	$LOD_{,r}$ - LOD_R (%)[#]	LOQ (%)
Trehalose	0.1-100	95-105	0.02 - 0.09	0.1
Sucrose (Suc)	0.1-100	95-105	0.06 - 0.07	0.1
Galactose (Gal)	0.1-100	95-105	0.06 - 0.07	0.1
Glucose (Glu)	0.1-100	95-105	0.04 - 0.09	0.1
Fructose (Fru)	0.1-100	95-105	0.04 - 0.07	0.1
Lactose (Lac)	0.1-100	95-105	0.06 - 0.08	0.1
Lactulose	0.1-100	95-105	0.06 - 0.08	0.1
Isomaltulose	0.1-100	95-105	0.07 - 0.08	0.1
Maltose (Mal)	0.1-100	95-105	0.05 - 0.08	0.1

[#]LOD (repeatability r & reproducibility R), respectively.
The LOD/LOQ was established for non-diluted dairy sample at 3*sd<0.03% (all sugars).

$$d_{rel} = \frac{1}{n}\sum_{i=1}^{n}\left(\frac{x_i - x_{ref}}{x_{ref}}\right)$$

Table 2: Trueness or accuracy (d_{rel}) using broad range of CRM (n=31)

Decade (%)	Number (n)	VC_R (CRM range) (%)	VC_R (CRM average) (%)	d_{rel} (CCC average) (%)
0.1-5.0 (low)	23	2-48	23.0	3.4
5.0-100 (high)	8	2-13	5.0	1.6
0.1-100 (all)	31	2-48	17.2	2.6

CRMs: NIST SRM 3233 (Fortified Breakfast Cereal), NIST SRM 1869a & 1869 & MLT (Infant/Adult Nutritional Formula), MUVA ML2310 (UHT low lactose milk), MUVA-SK-0315 (Processed cheese), MUVA-KI-1106 (Infant food), MUVA-MO-0614 (Whey powder).

Performance characteristics: Sugars according ISO-EN & IDF

The uncertainty data were obtained for 9 sugars for 2 submatrices (food incl. milk (products) and infant formula and feed). The data were compared with the performance characteristics of the official standard for sugars in dairy (ISO-EN / IDF). The method fulfills the requirements of these method for the scope milk, milk products including infant formula (for the sugars suc, gal, glu, fru, lac, mal) according the multi-lab trial, which CCC participated in. The performance requirements also fit with the AOAC 2018.16 for the sugars suc, gal, glu, fru, lac, mal including isomaltulose with the scope food and feed.

The method performance characteristics are shown to be fully aligned with these performance requirements for food and feed matrices as well.

Table 3: Measurement uncertainty data (NEN 7777 / 7779)[$]

Sugar (%)	Range (%) low/high	$VC_{duplo,\ r}$ (%)	$VC_{duplo,\ R}$ (%)	VC_{inh} (%)	VC_{Rw} (%)*	d_{rel} (%)^	VC_m (%)	$u_{c,rel}$ (%)	U_{rel} (%)
Trehalose	0.1-5/5-100	1.8/1.6	2.9/2.8	- /2.7	5.6/-	0.4/1.2	-	2.9/3.1	5.9/6.2
Sucrose (Suc)	0.1-5/5-100	3.4/1.9	3.4/2.3	2.4/2.3	-/2.4	1.0/0.7	-	3.5/2.4	7.1/4.8
Galactose (Gal)	0.1-5/5-100	2.7/1.8	1.9/2.4	- /4.3	4.3/-	1.7/1.8	-	2.5/3.0	5.1/6.0
Glucose (Glu)	0.1-5/5-100	2.8/1.6	3.4/1.6	-	6.3/-	1.6/4.5	-	3.8/4.8	7.5/9.5
Fructose (Fru)	0.1-5/5-100	2.3/1.6	3.4/2.6	-	5.0/-	0.5/3.1	-	3.4/4.0	6.9/8.0
Lactose (Lac)	0.1-5/5-100	2.6/1.7	2.4/2.8	1.2/5.1	-/2.1	1.8/0.4	-	3.0/2.9	6.0/5.7
Lactulose	0.1-5/5-100	3.4/2.0	3.2/2.7	-	7.7/-	0.6/1.7	-	3.3/3.2	6.5/6.5
Isomaltulose	0.1-5/5-100	4.2/2.6	3.4/2.8	-	5.0/-	0.4/4.1	-	3.4/5.0	6.8/9.9
Maltose (Mal)	0.1-5/5-100	2.8/2.0	2.1/1.4	- /1.2	3.6/-	1.7/1.9	-	2.7/2.4	5.4/4.8
	$VCpooled = \sqrt{[VC_1^2 + VC_2^2 + .. VC_n^2]/n}$^				Requirements official methods (n=6 sugars)				
	Decade (%)	$VC_{r,\ intra}$	$VC_{R,\ intra}$		RSDr AOAC	RSDR AOAC		RSDr ISO	RSDR ISO
Pooled (all/n=6)	0.1-5.0	3.0 / 2.8	3.0 / 2.8		<7	<10		<5	<16
Pooled (all/n=6)	5.0-50	1.9 / 1.8	2.4 / 2.2		<5	<8		<6	<10
Pooled (all/n=6)	50-100	- / 2.1	- / 3.2		<3	<4		<3	<15

$ The uncertainty data fall well within the requirements of the official methods as tested pooled values ($VC_{r/R,\ intra}$)^ all sugars n=9 or labeling sugars n=6 versus the required RSDr and RSDR i.r.t. relevant scopes ISO-EN/IDF (dairy) and AOAC (food/feed) SMPRs and first action method 2018.16. *Control samples are a milk powder (Lac x=34.8±0.8 VC_{Rw}=2.3%) and Cruesli naturel and with added fructans (Glu x=0.57±0.04 VC_{Rw}=6.9%, Suc x=19.6±0.5 VC_{Rw}=2.3%, Mal x=0.56±0.02 VC_{Rw}=3.4%, Fru x=0.38±0.3 VC_{Rw}=6.9%). ^Bias on the basis of recovery (inaccuracy): data fall within range determined on basis CRMs.

@ Please be aware uncertainties may differ for specific products due low as well as high complexity of the compositions.

RSDR & RSDr = relative standard deviation (interlaboratory results);
MU = measurement uncertainty; sd = standard deviation;
LOD = limit of detection; LOQ = limit of quantitation;
VC_r = Variation coefficient under repeatability conditions;
$VC_{duplo,r}$ = Variation coefficient under repeatability duplicates;
$VC_{duplo,R}$ = Variation coefficient under reproducibility duplicates;
VC_R = Variation coefficient under reproducibility conditions;
VC_{Rw} = Variation coefficient under reproducibility conditions control sample;
VC_{inh} = Variation coefficient inhomogeneity;
d_{rel} = uncertainty (precision) or bias;
VC_m = Variation coefficient between-sample variability;
$u_{c,rel}$ = combined measurement uncertainty;
U_{rel} = expanded measurement uncertainty.