SLOVENSKI STANDARD
oSIST prEN ISO 22184:2019

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Mleko in mlečni proizvodi - Določanje vsebnosti sladkorja - Anionsko izmenjevalna kromatografska metoda z visoko ločljivostjo (HPAEC-PAD) (ISO/DIS 22184:2019)

Milk and milk products - Determination of the sugar contents - High performance anion exchange chromatographic method (HPAEC-PAD) (ISO/DIS 22184:2019)

Milch und Milcherzeugnisse — Bestimmung des Zuckergehalts — Hochleistungs-Anionenaustausch-Chromatographieverfahren (HPAEC-PAD) (ISO/DIS 22184:2019)

Lait et produits laitiers - Détermination de la teneur en sucre - Chromatographie d'échange d'anions haute performance couplée à la détection par ampérométrie pulsée (HPAEC-PAD) (ISO/DIS 22184:2019)

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Milk and milk products — Determination of the sugar contents — High performance anion exchange chromatographic method (HPAEC-PAD)

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Forewords

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products* and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

IDF (the International Dairy Federation) is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/food control authorities.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

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This document was prepared by the IDF *Standing Committee on Analytical Methods for Composition* and ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by ISO and IDF.

The work was carried out by the IDF/ISO Action Team C22 of the *Standing Committee on Analytical Methods for Composition* under the aegis of its project leader Mr. H. Cruijsen (NL).

Milk and milk products — Determination of the sugar contents — High performance anion exchange chromatographic method (HPAEC-PAD)

1 Scope

The document specifies the quantitative liquid chromatographic determination of specific sugars (galactose, glucose, fructose, sucrose, lactose, and maltose) in various milk and milk products, applying arabinose as internal standards. The method is applicable for the following different dairy matrices: milk, milk powder, cheese, whey powder, infant formula, milk dessert and yogurt.

Soy containing dairy products are excluded. The determination of the lactose content in low lactose milk products is excluded.

High performance anion exchange chromatographic method in combination with pulsed amperometric detection (HPAEC-PAD) is applied.

With this method the following thirteen different mono- and disaccharides can be separated: fucose, arabinose, galactose, glucose, fructose, sucrose, lactose, lactulose, maltose, melibiose, trehalose, isomaltulose (e.g. palatinose™) and maltotriose.

The method is especially meant for labelling purposes of the six most important sugars which can be present by nature or by addition in milk and milk products. The method is not applicable for sugar contents less than 0,1%.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 3696, *Water for analytical laboratory use — Specification and test methods*

3 Principle

The sugars present in the sample are extracted with an aqueous ethanol buffer solution in order to inhibit possible probiotic activities. The obtained extract is deproteinized with Carrez clarification. After clarification the solution is diluted and the sugars present are separated and quantified by high performance anion exchange chromatography (HPAEC). In order to improve the sensitivity and stability of the pulsed amperometric detector (PAD), post-column a sodium hydroxide solution was added to the HPAEC effluent with pulsed amperometric detection (HPAEC-PAD). GOS and fructans don't interfere. Arabinose is applied as internal standard for the quantitation of the sugars.

4 Reagents

Use only reagents of recognized analytical grade and water according to EN ISO 3696, unless otherwise specified.

4.1 Water, complying with EN ISO 3696, grade 3.

4.2 Sodium hydroxide (NaOH) pellets.

4.3 Aqueous sodium hydroxide solution, substance concentration c = 1 mol/l

Add to a 1 000 ml volumetric flask 40 g ± 1 g NaOH pellets (4.2), dissolve in about 500 ml of water and after cooling down, dilute with water to the mark and homogenize.

4.4 Aqueous sodium hydroxide solution, c = 4 mol/l

Add to a 1 000 ml volumetric flask 160 g ± 1 g NaOH pellets (4.2), dissolve in about 500 ml of water and after cooling down, dilute with water to the mark and homogenize.

4.5 Sodium hydroxide solution, mass fraction w(NaOH) = 33 % in water.

4.6 Sodium hydroxide solution, w(NaOH) = 50 % in water.

The reagent should contain the minimum amount of carbonate and mercury. Do not shake or stir the solution before use.

4.7 Concentrated hydrochloric acid, 36 % to 38 %

4.8 Aqueous hydrochloric acid solution, c = 1 mol/l

Add to a 1 000 ml volumetric flask (5.2) 500 ml of water followed by 83 ml of concentrated HCl (4.7) and after cooling down, dilute with water to the mark and homogenize.

4.9 Acetonitrile

4.10 Acetonitrile in water, 5 % in water

Add to a 1 000 ml volumetric flask (5.2) 50 ml of acetonitrile (4.9), dilute with water to the mark and homogenize.

4.11 Anhydrous sodium acetate (CH_3COONa)

4.12 Eluent 1 (E1), aqueous solution of sodium acetate (CH_3COONa), c = 1,0 mol/l.

Add to a 1 000 ml volumetric flask (5.2) about 800 ml of degassed water (eluent 3, 4.14) followed by 82 g sodium acetate (4.11). Then dilute the aqueous solution with degassed water (eluent 3, 4.14) to the mark and homogenize. Store the eluent under inert atmosphere.

4.13 Eluent 2 (E2), aqueous solution of carbonate free sodium hydroxide (NaOH), c = 0,2 mol/l

Add to a 1 000 ml volumetric flask (5.2) about 800 ml of degassed water (eluent 3, 4.14) and purge for 15 min. with helium. Add 16,0 g of sodium hydroxide solution (4.6). Then quickly dilute the aqueous solution with degassed water (eluent 3, 4.14) to the mark, close immediately the bottle and homogenize. Store the eluent under inert atmosphere.

4.14 Eluent 3 (E3), degassed water, stored under inert atmosphere.

4.15 Eluent 4 (E4), aqueous solution of sodium acetate (CH_3COONa), c = 0,025 mol/l

Add to a 1 000 ml volumetric flask (5.2) about 800 ml of degassed water (eluent 3, 4.14) followed by 2.05 g of sodium acetate (4.11). Then dilute the aqueous solution with degassed water (eluent 3, 4.14) to the mark and homogenize. Store the eluent under inert atmosphere.

4.16 Post column reagent, aqueous solution of sodium hydroxide, c = 0,3 mol/l.

Add to a 1 000 ml volumetric flask (5.2) about 800 ml of degassed water (eluent 3, 4.14). Purge for 15 min. with helium. Add 24 g of sodium hydroxide solution (4.6) and quickly fill up to the mark with the degassed water (eluent 3, 4.14), close immediately the flask and homogenize. Store the post column reagent under inert atmosphere.

Important — It is extremely important to remove dissolved carbon dioxide from the eluens and post column reagent prior to use and during use. The eluents and post column reagent are maintained under inert gas during use.

4.17 Mixture of ethanol 96 % (v/v) and 5 % (v/v) methanol

4.18 Potassium hexacyanoferrate (II) trihydrate, $K_4Fe(CN)_6.3H_2O$.

4.19 Zinc acetate dihydrate, $Zn(CH_3COO)_2.2H_2O$.

4.20 Glacial acetic acid

4.21 Carrez reagent I

Weigh 106 g of $K_4Fe(CN)_6.3H_2O$ (4.18) in a 1 000 ml volumetric flask (5.2), dissolve in 800 ml of water (4.1) and dilute with water to the mark. Store the Carrez I reagent in the refrigerator.

4.22 Carrez II reagent

Weigh 220 g of $Zn(CH_3COO)_2.2H_2O$ (4.19) in a 1 000 ml volumetric flask (5.2), dissolve in 800 ml water, add 30 ml of glacial acetic acid (4.20) and dilute with water to the mark. Store the Carrez II reagent in the refrigerator.

IMPORTANT — Do not use Carrez II reagent with zinc sulfate.

4.23 Buffer solution of piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES) (c = 1,5 mol/l and pH = 6,9)

Add 22,5 g of PIPES to an 100 ml Erlenmeyer flask and dissolve in 20 ml of sodium hydroxide solution (4.3). Adjust the pH to pH = 6,9 with NaOH 33 % (4.5) in water. Transfer the PIPES buffer solution quantitatively into a 50 ml calibrated tube and fill up with demineralized water till 50 ml. The pH of the obtained buffer solution shall be within the range of 6,8 to 7,0.

4.24 Arabinose

4.25 Galactose

4.26 Glucose

4.27 Fructose

4.28 Sucrose

4.29 Lactose

4.30 Maltose

4.31 Internal standard stock solution arabinose

Weigh to the nearest mg, approximately 7 g of arabinose (4.24) into a 50 ml volumetric flask (5.2). Add about 30 ml of water and dissolve the arabinose. Add 2,5 ml of acetonitrile (4.9), fill up to the mark with water and homogenize the solution.

4.32 Sugar standard stock solutions

Weigh to the nearest 0,1 mg, approximately 260 mg of the monosaccharide galactose (4.25), glucose (4.26) and fructose (4.27) and approximately 400 mg of the disaccharides sucrose (4.28), lactose (4.29), and maltose (4.30) into a 500 ml volumetric flask (5.2). Add about 200 ml of water and dissolve the sugars. Add 25 ml of acetonitrile (4.9), fill up to the mark with water and homogenize the solution.

4.33 Sugar standard solutions for calibration

Prepare the different dilutions of the sugar calibration standards as specified in Table 1. Dilute the specified volumes of the internal standard stock solution arabinose (4.31) and sugar standard stock solution (4.32) in a 200 ml volumetric flask, add about 50 ml of water and homogenize. Add 10 ml of acetonitrile (4.9), fill up to the mark with water and homogenize.

Table 1 — Preparation of the sugar standard solutions for calibration

Sugar standard solution	Volume of sugar standard stock solution (4.32) ml	Volume of arabinose internal standard stock solution (4.31) ml
1	0,2	0,050
2	1,0	0,050
3	6,0	0,050
4	10,0	0,050
5	20,0	0,050
6	40,0	0,050
7	80,0	0,050
8	100,0	0,050

5 Apparatus

5.1 Analytical balance, capable of weighing to an accuracy of ± 0,1 mg

5.2 Volumetric flask, volume of 50 ml, 500 ml and 1 000 ml

5.3 pH meter

5.4 Black band filter paper

5.5 Glass centrifugation tubes

5.6 Homogenizer[1)]

5.7 Vortex mixer

1) An IKA Ultra turrax with an appropriate probe is an example of a suitable commercially available homogenizer