SLOVENSKI STANDARD
oSIST prEN ISO 22579:2020

01-februar-2020

Hrana za dojenčke in prehranska dopolnila za odrasle - Določevanje fruktanov - Anionska izmenjevalna kromatografija z visoko ločljivostjo s pulzno amperometrijsko detekcijo (HPAEC-PAD) po encimski obdelavi (ISO/DIS 22579:2019)

Infant formula and adult nutritionals - Determination of fructans - High performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) after enzymatic treatment (ISO/DIS 22579:2019)

Säuglingsnahrung und Nahrungsergänzungsmittel für Erwachsene - Bestimmung von Fructanen - Hochleistungs-Anionenaustausch-Chromatographieverfahren mit gepulster amperometrischer Detektion (HPAEC-PAD) nach enzymatischer Behandlung (ISO/DIS 22579:2019)

Formules infantiles et produits nutritionnels pour adultes - Détermination de la teneur en fructanes - Chromatographie déchange danions à haute performance couplée à la détection par ampérométrie pulsée « CEAHP-DAP » après traitement enzymatique (ISO/DIS 22579:2019)

Ta slovenski standard je istoveten z: **prEN ISO 22579**

ICS:

67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products

oSIST prEN ISO 22579:2020 **en**

DRAFT INTERNATIONAL STANDARD

ISO/DIS 22579
IDF 241

ISO/TC **34**/SC **5**

Secretariat: **NEN**

Voting begins on:
2019-12-12

Voting terminates on:
2020-03-05

Infant formula and adult nutritionals — Determination of fructans — High performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) after enzymatic treatment

ICS: 67.050

This document is circulated as received from the committee secretariat.

ISO/CEN PARALLEL PROCESSING

Reference numbers
ISO/DIS 22579:2019(E)
IDF 241:2019(E)

COPYRIGHT PROTECTED DOCUMENT

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

International Dairy Federation
Silver Building • Bd Auguste Reyers 70/B
B-1030 Brussels
Phone: +32 2 325 67 40
Fax: +32 2 325 67 41
Email: info@fil-idf.org
Website: www.fil-idf.org

Published in Switzerland

Contents

Page

Forewords

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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), in collaboration with AOAC INTERNATIONAL.

It is being published jointly by ISO and IDF and separately by AOAC INTERNATIONAL. The method described in this document is equivalent to the AOAC Official Method 2016.14: *Fructans in Infant Formula and Adult Nutrition*.

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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. IDF shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www .iso .org/ patents).

Any trade name used in this document is information given for the convenience of users and does not constitute and endorsement.

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 on C41 the *Standing Committee on Analytical Methods for Composition* under the aegis of its project leader Mr. S. Austin (CH).

Infant formula and adult nutritionals — Determination of fructans — High performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) after enzymatic treatment

WARNING — The method described in this document employs corrosive (sodium hydroxide, acetic acid) and toxic (sodium azide) chemicals. Refer to the materials safety data sheets and take appropriate additional safety precautions for handling and waste disposal.

1 Scope

This document specifies a method for the determination of inulin-type fructans (including oligofructose, fructooligosaccharides) in infant formula and adult nutritionals (both powder and liquid) containing 0,03 g/100 g to 5,0 g/100 g of fructans in the product as prepared ready for consumption.

The method has been validated in a multi laboratory study[1] with reconstituted Standard Reference Material (SRM) infant/adult nutritional formula at a level of 0,204 g/100 g, adult nutritionals Ready-To-Feed (RTF) at levels of 1,28 g/100 g and 2,67 g/100 g, infant formula RTF at a level of 0,300 g/100 g, reconstituted follow-up-formula at levels of 0,209 g/100 g to 0,275 g/100 g, reconstituted infant formula at levels from 0,0308 g/100 g to 0,264 g/100 g. During the single laboratory validation study,[2] spike-recovery experiments have been performed up to 5 g/100 g in reconstituted infant formula powders (milk based, partially hydrolysed milk based and soy based), adult nutritional RTF and reconstituted adult nutritional powders.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
adult nutritional
nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment, made from any combination of milk, soy, rice, whey, hydrolysed protein, starch and amino acids, with or without intact protein.

3.2
infant formula
breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding.

[SOURCE: Codex Standard 72-1981]

3.3
follow-up-formula (including child formula and toddler formula)
A food intended for use as a liquid part of the weaning diet for the infant from the 6th month , and for young children up to the age of 3 years.

[SOURCE: Codex Standard 156-1987]

4 Principle

Samples are reconstituted in water (if required) and further diluted until the concentration of fructans in solution is such that after hydrolysis the fructose and glucose concentration will be within the range covered by the standard curve. The diluted sample is treated with a mixture of a highly specific sucrase and α-glucanases to hydrolyse sucrose and α-glucooligosaccharides to their constituent monosaccharides. The sample is passed through a solid phase extraction (SPE) column packed with graphitized carbon. Salts and monosaccharides pass through and are washed away, while the fructans are retained. Fructans are released from the column using an acetonitrile solution. The released fructans are hydrolysed with a fructanase mixture, and the released glucose and fructose are analysed by high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The fructan content is calculated by summing the glucose and 0,9 times the fructose contents measured. In some matrices, a blank correction may be necessary and can be applied.

5 Chemicals and reagents

Use only reagents of recognized analytical grade, unless otherwise specified. Solvents shall be of quality for HPLC analysis, unless otherwise specified.

5.1 Deionized water, purified with resistivity ≥18 MΩ.

5.2 Maleic acid, purity ≥ 99,0 %.

5.3 Acetonitrile.

5.4 Acetic acid, glacial 100 %, anhydrous.

5.5 Potassium hexacyanoferrate(II)trihydrate, optional.

5.6 Zinc acetate, optional.

5.7 Trifluoroacetic acid (TFA).

5.8 Hydrochloric acid, substance concentration c = 1 mol/l.

5.9 Sodium acetate anhydrous, purity ≥ 99,0 %, only when using columns B (6.13.2) for HPAEC-PAD.

5.10 Sodium hydroxide solution, 50 % (w/w).

5.11 Sodium hydroxide pellets.

5.12 Sodium chloride.

5.13 Sodium azide, optional.

5.14 D-(-)-fructose, purity ≥ 99,0 % (dry weight basis).

5.15 D-(+)-glucose, purity ≥ 99,5 % (dry weight basis).

5.16 N,N'-diacetylchitobiose, purity > 90 %.

5.17 Mixture of highly purified sucrase, β-amylase, pullulanase and maltase, from Fructan Assay Kit K-FRUC[1)] (Megazyme International Ireland Ltd or equivalent). 200 µl of enzyme mixture working solution (5.19.10) should be able to completely hydrolyse 2 mg of sucrose under the conditions described in the method (90 min at 40 °C) without hydrolysing any fructans.

5.18 Mixture of highly purified recombinant exo- and endo-inulinases and recombinant endo-levanase, from Fructan Assay Kit K-FRUC[1] (Megazyme International Ireland Ltd or equivalent). 100 µl of enzyme mixture working solution (5.19.11) should be able to hydrolyse 70 µg of fructans under the conditions described in the method (40 min at 40 °C).

5.19 Preparation of reagents

5.19.1 Sodium hydroxide solution, c = 2 mol/l.

Dissolve 40 g ± 1 g of sodium hydroxide pellets in 250 ml of deionized water in a 500 ml volumetric flask. After cooling down to room temperature, make up to the mark with deionized water and mix well. This solution is stable for 6 months at room temperature.

5.19.2 Sodium maleate buffer solution, c = 0,100 mol/l, pH = 6,5.

Into a large beaker (> 500 ml) weigh 5,8 g of maleic acid and dissolve with 450 ml of deionized water using a magnetic stirrer. Adjust to pH = 6,5 with sodium hydroxide solution (5.19.1). Transfer the solution to a 500 ml volumetric flask and make up to the mark with deionized water. This solution is stable for 3 months at 4 °C.

5.19.3 Sodium acetate buffer solution, c = 0,100 mol/l, pH = 4,5.

Into a large beaker (> 500 ml) containing 450 ml of deionized water, pipette 2,9 ml of glacial acetic acid. Adjust to pH = 4,5 with sodium hydroxide solution (5.19.1). Transfer the solution to a 500 ml volumetric flask and make up to the mark with deionized water. This solution is stable for 3 months at 4 °C.

5.19.4 N,N'diacetylchitobiose internal standard solution, mass concentration ρ = 600 µg/ml.

Into a 25 ml volumetric flask weigh 15 mg of N,N'diacetylchitobiose and make up to the mark with deionized water. This solution is stable for 1 year at -20 °C.

5.19.5 Glucose stock solution, ρ = 5 mg/ml.

Into a 25 ml volumetric flask weigh 125 mg of glucose and make up to the mark with deionized water. This solution is stable for 1 year at -20 °C.

5.19.6 Fructose stock solution, ρ = 10 mg/ml.

Into a 25 ml volumetric flask weigh 250 mg of fructose and make up to the mark with deionized water. This solution is stable for 1 year at -20 °C.

1) This is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of the product named. Equivalent products may be used if they can be shown to lead to the same results.