SLOVENSKI STANDARD

SIST EN 15086:2006

01-september-2006

Živila - Določevanje izomalta, laktitola, maltitola, manitola sorbitola in ksilitola v živilih

Foodstuffs - Determination of isomalt, lactitol, maltitol, mannitol, sorbitol and xylitol in foodstuffs

Lebensmittel - Bestimmung von Isomalt, Lactit, Maltit, Mannit, Sorbit und Xylit in Lebensmitteln

Produits alimentaires - Dosage de l'isomalt, du lactitol, du maltitol, du mannitol, du sorbitol et du xylitol dans les produits alimentaires

Ta slovenski standard je istoveten z: EN 15086:2006

ICS:

67.050

SIST EN 15086:2006 **en**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 15086

March 2006

ICS 67.050

English Version

Foodstuffs - Determination of isomalt, lactitol, maltitol, mannitol, sorbitol and xylitol in foodstuffs

Produits alimentaires - Dosage de l'isomalt, du lactitol, du maltitol, du mannitol, du sorbitol et du xylitol dans les produits alimentaires

Lebensmittel - Bestimmung von Isomalt, Lactit, Maltit, Mannit, Sorbit und Xylit in Lebensmitteln

This European Standard was approved by CEN on 3 February 2006.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: rue de Stassart, 36 B-1050 Brussels

Ref. No. EN 15086:2006: E

Contents

Page

Foreword

This European Standard (EN 15086:2006) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by September 2006, and conflicting national standards shall be withdrawn at the latest by September 2006.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

1 Scope

This European Standard specifies an HPLC-method for the determination of isomalt and other polyols such as lactitol, maltitol, mannitol, sorbitol and xylitol in foodstuffs. Chemically isomalt is described as a mixture of 6-O-α-D-glucopyranosyl-D-sorbitol (1,6-GPS) and 1-O-α-D-glucopyranosyl-D-mannitol (1,1-GPM).

The method has been validated in a collaborative study for isomalt (sum of GPS and GPM) on cookies, chewing gum, chocolate and on hard candies. Validation data for GPS and GPM are given in Clause 8, Annex A, Tables A.1 and A.2

The determination of the other sugar alcohols has been validated in a further collaborative study using the same method. The samples were pudding (lactitol, mannitol, xylitol), cookies (lactitol, maltitol, mannitol, sorbitol and xylitol), hard candies (lactitol, mannitol, xylitol, sorbitol) and chewing gum (maltitol, mannitol, sorbitol). Validation data are given in Clause 8 and Annex A, Tables A.3 to A.7.

2 Normative references

The following referenced documents are indispensable for the application of this European Standard. 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 (ISO 3696:1987)*

3 Principle

The sample is diluted, dissolved or extracted with water and possibly filtered. If necessary the sample is clarified using modified Carrez solutions. The polyols are separated by HPLC on cation exchanger with Ca^{++} or Pb^{++} counter ion using high purity water at 60 °C to 80 °C, detected using a refractive index detector (differential refractometer, RI-detector), and determined by the external standard method [1].

4 Reagents

4.1 General

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and water of at least grade 1 according to EN ISO 3696 or use distilled water.

4.2 Standard substances

4.2.1 General

When using standard substances containing constitutional water, this content has to be taken into account.

Example: molar mass of GPS = 344,32 g/mol, GPM dihydrate = 380,32 g/mol. The exact water contents of the standard substances are determined by Karl-Fischer titration.

Alternative to the calibration with pure substances, an isomalt reference sample with exactly known mass concentrations of GPM and GPS can be used.

4.2.2 6-O-α-D-Glucopyranosyl-D-sorbitol (1,6 GPS), without constitutional water.

4.2.3 1-O-α-D-Glucopyranosyl-D-mannitol (1,1-GPM)[1)], crystallizes with 2 mole of water (water content approximately 10 %).

4.2.4 Lactitol, crystallizes with 1 mole of water (water content approximately 5 %).

4.2.5 Maltitol

4.2.6 Mannitol

4.2.7 Sorbitol

4.2.8 Xylitol

4.3 Standard solutions

Dissolve appropriate amounts of polyol standard substances (4.2.2 to 4.2.8) in water and dilute this solution again with water to obtain standard solutions with a total mass concentration of approximately 2 g/100 ml for the sum of all components.

This solution may be stored for 6 weeks in a refrigerator set at +4 °C. Alternatively it is possible to store the standard solution deep frozen (-18 °C) for up to 1 year.

4.4 Carrez solution I, modified

Dissolve 53,45 g of potassium hexacyanoferrate(II) ($K_4[Fe(CN)_6] \cdot 3\ H_2O$) in water, mix well and dilute to 500 ml with water. Store in a brown bottle and replace it regularly.

4.5 Carrez solution II, modified

Dissolve 148,75 g of zinc nitrate ($Zn(NO_3)_2 \cdot 6\ H_2O$) in water, mix well and dilute to 500 ml with water. Store in a brown bottle and replace it regularly.

5 Apparatus

5.1 General

Usual laboratory apparatus and, in particular, the following:

5.2 Membrane filter, for filtering the sample test solution, with pore size of e.g. 0,45 µm**.**

NOTE Filtering of the mobile phase as well as of the sample solution through a membrane filter prior to use or injection is supposed to increase longevity of the columns.

[1)] For the availability of the standard substance, contact your National Standardization Institute.

5.3 Magnetic stirrer

5.4 HPLC system, consisting of a pump, a sample injecting device, a refractive index (RI) detector with variable temperature setting, a column oven and an evaluating system.

5.5 Analytical separation column, e.g. 300 mm x 7,8 mm, filled with ion exchange resin with Ca^{++} or Pb^{++} counter ion. To protect the analytical column, it is recommended to use a pre-column of similar characteristics. Shorter columns (e.g. 100 mm x 7,8 mm) may cause insufficient separations.

6 Procedure

6.1 Preparation of the test sample

Homogenize the test sample. This can be achieved by grinding solid samples as hard candies or chocolate with an appropriate mill at low temperatures, and homogenizing subsequently by mixing the ground sample. Deep freeze chewing gum before grinding. Homogenize semi solid samples e.g. ice cream by melting and stirring.

Treat turbid solutions which can occur when preparing e.g. cakes and pastries with modified Carrez solutions.

NOTE Although it has not been tested in the inter-laboratory study, it is recommended to de-ionize sample test solutions, if treated with Carrez solutions in order to protect the separation column. Ions introduced by these reagents may be eliminated by means of appropriate ion exchange resins (e.g. de-ashing systems, cartridges) prior to the injection on the analytical column.

6.2 Preparation of the sample test solution

6.2.1 Soluble samples (e.g. hard candies, compressed products)

Weigh in 2 g of the homogenized sample in a 100 ml volumetric flask and dissolve with water, mix and dilute to the mark. Filter turbid solutions through a membrane filter (5.2) or clarify with modified Carrez solutions (4.4) (4.5).

6.2.2 Incompletely soluble samples (e.g. pastries, chewing gum, chocolate)

Weigh in 5 g to 10 g of the homogenized sample in a 100 ml volumetric flask and mix with 50 ml of water, stir for 30 min at 40 °C to 60 °C with a magnetic stirrer and clarify with modified Carrez solutions. Let the flask stand to reach room temperature and dilute to the mark with water. When the precipitate has settled, filter the supernatant aqueous phase through a membrane filter (5.2). Fat containing sample solutions may sometimes need to be filtered a second time through a membrane filter of smaller pore size.

6.3 HPLC determination

6.3.1 HPLC conditions

The separation and the quantification have proven to be satisfactory if following experimental conditions are followed:

Mobile phase:	Water
Flow rate:	0,5 ml /min
Injection volume:	20 µl
Column temperature	60 °C to 80 °C

6.3.2 Identification

Inject the same appropriate volumes, e.g. 20 µl of the standard test solution (4.3) as well as of the sample test solution (6.2) into the HPLC-system.

Identify the sugar alcohols by comparison of the retention time of the peak in the chromatograms obtained with the sample test solution and with the standard solution. Peak identification can also be performed by adding small amounts of the appropriate standard solutions to the sample test solution.

6.3.3 Determination

To carry out the determination by external calibration, integrate the peak areas of the sample and compare the results with the corresponding values for the standard substance or use a calibration graph. Check the linearity of the calibration graph.

Note that e.g. peaks of GPM and fructose (Pb^{++}-column), GPS and maltitol (Ca^{++}-column), maltitol and lactitol or xylitol and sorbitol (Ca^{++}-column) may overlap under certain circumstances (see chromatograms in Annex C). In these cases the chromatographic conditions should be optimized. Chromatographic resolution is mainly influenced by the type of counter-ion Pb^{++} to Ca^{++} and the temperature of the column.

7 Calculation

Calculate the mass concentration, ρ, of each polyol as water free substance in g/100 g or the mass fraction *w*, in g/100 ml of the sample using equation (1):

$$w \text{ or } \rho = \frac{A_1 \cdot V_1 \cdot m_1}{A_2 \cdot V_2 \cdot m_0} \cdot 100 \qquad (1)$$

where

A_1 is the peak area for the polyol concerned obtained with the sample test solution (6.2), in units of area;

A_2 is the peak area for the polyol concerned obtained with the standard test solution (4.3), in units of area;

V_1 is the total volume of sample test solution (6.2), in millilitres;

V_2 is the total volume of standard test solution (4.3), in millilitres;

m_1 is the mass of polyol (calculated as dry substance) contained in V_2, in grams;

m_0 is the sample mass, in millilitres or grams.

Report the result with 1 decimal place.

8 Precision

8.1 General

The precision data for the determination of GPM and GPS were established in 2000 by a collaborative study on cookies, chewing gum, chocolate and on hard candies. Additional validation data for the determination of lactitol, maltitol, mannitol, xylitol and sorbitol were established in 2001 by a further collaborative study on cookies, pudding, hard candies and chewing gum. The data derived from this collaborative study may not be applicable to analyte concentration ranges and sample matrices other than those given in Annex A.

8.2 Repeatability

The absolute difference between two single test results found on identical test material by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit r in not more than 5% of the cases. The repeatability is dependent on the concentration level of the analyte in the sample.

The values for GPM are:

cookies	$\bar{x}$ = 12,674 g/100 g	r = 0,237
chewing gum	$\bar{x}$ = 13,409 g/100 g	r = 1,228
chocolate	$\bar{x}$ = 17,911 g/100 g	r = 0,636
hard candies	$\bar{x}$ = 47,058 g/100 g	r = 1,623

The values for GPS are

cookies	$\bar{x}$ = 13,471 g/100 g	r = 0,199
chewing gum	$\bar{x}$ = 15,153 g/100 g	r = 1,230
chocolate	$\bar{x}$ = 18,448 g/100 g	r = 0,987
hard candies	$\bar{x}$ = 49,174 g/100 g	r = 1,456

The values for lactitol are

cookies	$\bar{x}$ = 6,107 g/100 g	r = 0,314
pudding	$\bar{x}$ = 0,664 g/100 g	r = 0,047
hard candies	$\bar{x}$ = 80,529 g/100 g	r = 1,857

The values for maltitol are

cookies	$\bar{x}$ = 1,830 g/100 g	r = 0,128
chewing gum	$\bar{x}$ = 32,288 g/100 g	r = 3,888

The values for mannitol are

cookies	$\bar{x}$ = 4,343 g/100 g	r = 0,154
chewing gum	$\bar{x}$ = 1,669 g/100 g	r = 0,079
pudding	$\bar{x}$ = 1,716 g/100 g	r = 0,094
hard candies	$\bar{x}$ = 3,897 g/100 g	r = 0,382

The values for sorbitol are

cookies	$\bar{x}$ = 3,760 g/100 g	r = 0,161
chewing gum	$\bar{x}$ = 27,238 g/100 g	r = 1,930
hard candies	$\bar{x}$ = 4,720 g/100 g	r = 0,423

The values for xylitol are

cookies	$\bar{x}$ = 3,028 g/100 g	r = 0,139
pudding	$\bar{x}$ = 4,658 g/100 g	r = 0,181
hard candies	$\bar{x}$ = 6,460 g/100 g	r = 0,319

8.3 Reproducibility

The absolute difference between two single test results obtained on identical material reported by two laboratories will exceed the reproducibility limit *R* in not more than 5% of the cases.

The values for GPM are:

cookies	$\bar{x}$ = 12,674 g/100 g	R = 1,601
chewing gum	$\bar{x}$ = 13,409 g/100 g	R = 2,804
chocolate	$\bar{x}$ = 17,911 g/100 g	R = 3,073
hard candies	$\bar{x}$ = 47,058 g/100 g	R = 6,159

The values for GPS are

cookies	$\bar{x}$ = 13,471 g/100 g	R = 1,299
chewing gum	$\bar{x}$ = 15,153 g/100 g	R = 4,813
chocolate	$\bar{x}$ = 18,448 g/100 g	R = 2,111
hard candies	$\bar{x}$ = 49,174 g/100 g	R = 5,077

The values for lactitol are

cookies	$\bar{x}$ = 6,107 g/100 g	R = 1,356
pudding	$\bar{x}$ = 0,664 g/100 g	R = 0,276
hard candies	$\bar{x}$ = 80,529 g/100 g	R = 2,630

The values for maltitol are

cookies	$\bar{x}$ = 1,830 g/100 g	R = 0,663
chewing gum	$\bar{x}$ = 32,288 g/100 g	R = 7,889

The values for mannitol are

cookies	$\bar{x}$ = 4,343 g/100 g	R = 0,437
chewing gum	$\bar{x}$ = 1,669 g/100 g	R = 0,486
pudding	$\bar{x}$ = 1,716 g/100 g	R = 0,153
hard candies	$\bar{x}$ = 3,897 g/100 g	R = 0,477