COMMISSION REGULATION (EU) No 118/2010

of 9 February 2010

amending Regulation (EC) No 900/2008 laying down the methods of analysis and other technical provisions necessary for the application of the arrangements for imports of certain goods resulting from the processing of agricultural products

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Council Regulation (EEC) No 2658/87 of 23 July 1987 on the tariff and statistical nomenclature and on the Common Customs Tariff [1], and in particular Article 9 thereof,

Whereas:

(1) Commission Regulation (EC) No 900/2008 [2] lays down the formulas, procedures and methods to be used for the determination of starch/glucose for applying Annexes II and III of Commission Regulation (EC) No 1460/96 of 25 July 1996 establishing the detailed rules for implementing the preferential trade arrangements applicable to certain goods resulting from the processing of agricultural products, as provided for in Article 7 of Council Regulation (EC) No 3448/93 [3].

(2) Regulation (EC) No 900/2008 has been examined by a group of experts, with a view to assessing whether that Regulation takes account of the scientific and technological evolution of the methods laid down in that Regulation. Studies and tests carried out in the framework of that examination indicate that the determination of the starch/glucose content by solubilisation by means of sodium hydroxide (prior to enzymatic degradation to glucose) and the measurement of the total glucose content using the enzymatic method with spectrophotometry as prescribed for most goods now, do not meet any longer the current technical requirements and are therefore to be updated.

(3) It is therefore appropriate to provide that the degradation of starch/glucose is to be carried out in an enzymatic way by amylase and amyloglucosidase and that the total glucose content is to be determined using high performance liquid chromatography (HPLC) and to specify how the enzymatic method is to be carried out.

(4) Regulation (EC) No 900/2008 should therefore be amended accordingly.

(5) The measures provided for in this Regulation are in accordance with the opinion of the Customs Code Committee,

HAS ADOPTED THIS REGULATION:

Article 1

Annex I to Regulation (EC) No 900/2008 is replaced by the text set out in the Annex to this Regulation.

Article 2

This Regulation shall enter into force on the 20th day following its publication in the *Official Journal of the European Union*.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 9 February 2010.

For the Commission
The President
José Manuel BARROSO

[1] OJ L 256, 7.9.1987, p. 1.
[2] OJ L 248, 17.9.2008, p. 8.
[3] OJ L 187, 26.7.1996, p. 18.

ANNEX

'ANNEX I

Enzymatic determination of starch and its degradation products including glucose in food products using high performance liquid chromatography (HPLC)

1. **Scope**

This method describes the determination of the content of starch and its degradation products including glucose in food products for human consumption hereafter referred to as "starch". The starch content is determined from the quantitative analysis of glucose by high-performance liquid chromatography (HPLC) after enzymatic conversion of starch and its degradation products into glucose.

2. **Definition of the total glucose content and of the total glucose content expressed as starch**

The total glucose content means the value Z as calculated in point 7.2.1 of this Annex. It represents the content of starch and all its degradation products, glucose included.

The starch/glucose content as defined in Annex III to Regulation (EC) No 1460/96 shall be calculated on the basis of the total glucose content Z and as set out in Article 2 point 1 of this Regulation.

The starch (or dextrin) content as referred to in column 3 of Annex IV to Commission Regulation (EC) No 1043/2005 [1] shall be calculated on the basis of the total glucose content Z as set out in Article 2 point 2.1 of Commission Regulation (EC) No 904/2008 [2].

The Starch content referred to in point 1 of this Annex means the value E, as calculated in point 7.2.2 of this Annex. It is expressed in % (m/m). It is equivalent to the total glucose content Z, expressed as starch. This value E does not interfere in the above mentioned calculations.

3. **Principle**

The samples are homogenised and suspended in water. The starch and its degradation products, present in the samples, are enzymatically converted into glucose in two steps:

1. Starch and its degradation products are partially converted into soluble glucose chains using thermostable alpha-amylase at 90 °C. For effective conversion it is necessary that the samples should be completely solved or should be present in the form of a suspension containing very small solid parts

2. The soluble glucose chains are converted into glucose using amyloglucosidase at 60 °C.

Products containing a high content of proteins or fat are clarified and filtrated.

The determination of sugars is performed by HPLC analysis.

Because a partial inversion of sucrose may occur during the enzymatic treatment, the determination of free sugars is also performed by HPLC analysis to calculate the corrected glucose content.

4. **Reagents and other materials**

Use reagents of recognised analytical grade and demineralised water.

4.1. Glucose, min 99 %.

4.2. Fructose, min 99 %.

4.3. Sucrose, min 99 %.

4.4. Maltose-monohydrate, min 99 %.

4.5. Lactose-monohydrate, min 99 %.

4.6. Solution of thermostable alpha-amylase (1,4-alpha-D-Glucan-glucanohydrolase), with activity about 31 000 U/ml (1U will liberate 1,0 mg of maltose from starch in 3 minutes at pH 6,9 and 20 °C). This enzyme can contain a low amount of impurities (e.g. glucose or sucrose) and other interfering enzymes. Storage at ca. 4 °C. Alternatively, other sources of alpha-amylase may be used yielding a final solution with comparable enzyme activity.

[1] OJ L 172, 5.7.2005, p. 24.
[2] OJ L 249, 18.9.2008, p. 9.

4.7. Amyloglucosidase (1,4-alpha-D-Glucan glucohydrolase) from Aspergillus niger, powder with activity about 120 U/mg or about 70 U/mg (1U will liberate 1 micromol glucose from starch per minute at pH 4,8 and 60 °C). This enzyme can contain a low amount of impurities (e.g. glucose or sucrose) and other interfering enzymes (e.g., Invertase). Storage at ca. 4 °C. Alternatively, other sources of amyloglucosidase may be used yielding a final solution with comparable enzyme activity.

4.8. Zinc acetate dihydrate, p.a..

4.9. Potassium hexacyanoferrate (II) ($K_4[Fe(CN)]_6.3H_2O$), extra pure.

4.10. Sodium acetate anhydrous, p.a..

4.11. Glacial acetic acid, 96 % (v/v) (minimum).

4.12. Sodium acetate buffer (0,2 mol/l). Weigh 16,4 gram sodium acetate (point 4.10) into a beaker glass. Dissolve in water and rinse into a volumetric flask of 1 000 ml. Dilute to the mark with water and adjust the pH to 4,7 with acetic acid (by use of a pH-meter (point 5.7). This solution may be used for max 6 months with storage at 4 °C.

4.13. Amyloglucosidase solution. Prepare a solution of amyloglucosidase powder (point 4.7) by using sodium acetate buffer (point 4.12). The enzyme activity must be sufficient and in accordance with the starch content in the amount of sample (for example, activity about 600 U/ml is obtained from 0,5 g amyloglucosidase powder 120 U/mg (point 4.7) in a final volume of 100 ml for 1 g starch in the amount of sample). Prepare immediately before use.

4.14. Reference solutions. Prepare solutions of glucose, fructose, sucrose, maltose and lactose in water, as conventionally used in the HPLC analysis of sugars.

4.15. Reagent for clarification (Carrez I). Dissolve 219,5 gram zinc acetate (point 4.8) in water in a beaker glass. Rinse into a volumetric flask of 1 000 ml and add 30 ml acetic acid (point 4.11). Mix thoroughly and dilute to the mark with water. This solution may be used for max 6 months while stored at ambient temperature. Other clarification reagents, equivalent to Carrez solution, may be used.

4.16. Reagent for clarification (Carrez II). Dissolve 106,0 gram potassium hexacyanoferrate (II) (point 4.9) in water in a beaker glass. Rinse into a volumetric flask of 1 000 ml. Mix thoroughly and dilute to the mark with water. This solution may be used for max. 6 months while stored at ambient temperature. Other clarification reagents, equivalent to Carrez solution, may be used.

4.17. HPLC Mobile phase. Prepare a mobile phase which is conventionally used in the HPLC analysis of sugars. In case of using an aminopropyl silicagel column, e.g., a common mobile phase is a mixture of HPLC grade water and acetonitrile.

5. **Apparatus**

5.1. Standard laboratory glass ware.

5.2. Fluted filters, e.g., 185 mm.

5.3. Syringe filters, 0,45 µm, suitable for aqueous solutions.

5.4. Sample vials suitable for the HPLC autosampler.

5.5. 100 ml volumetric flasks.

5.6. Plastic syringes, 10 ml.

5.7. pH-meter.

5.8. Analytical balance.

5.9. Water bath with thermostat, adjustable to 60 °C and 90 °C.

5.10. HPLC Apparatus suitable for analysis of sugars.

6. **Procedure**

6.1. *Preparation of the sample for several types of products*

The product is homogenised.

6.2. *Sample portion*

The amount of sample is estimated from the ingredient declaration and the conditions of the HPLC analysis (concentration of the glucose reference solution), and shall not exceed:

$$\text{amount of sample (g)} = \frac{\text{volume of volumetric flask (e.g., 100ml)}}{\text{estimated starch content (\%)}}$$

Weigh the sample to 0,1 mg accuracy.

6.3. *Blank determination*

The blank is determined by performing a complete analysis (as described in point 6.4), without adding sample. The result of the blank determination is used in the calculation of the starch content (point 7.2).

6.4. *Analysis*

6.4.1. Preparation of the samples

Homogenise the sample by shaking or stirring. The chosen test portion (point 6.2) is weighed into a volumetric flask (point 5.5) and about 70 ml warm water is added.

After dissolving or suspending, add 50 microliter of thermostable alpha-amylase (point 4.6) and heat at 90 °C for 30 min in a water bath (point 5.9). Cool as quick as possible to 60 °C in a water bath, and add 5 ml of a solution of amyloglucosidase (point 4.13). For samples which could influence the pH of the reaction solution, control the pH and adjust it to 4,6 to 4,8, if necessary. Allow to react for 60 min at 60 °C. Cool the samples to ambient temperature.

6.4.2. Clarification

For samples with a high content of proteins or fat, clarification is necessary by adding 1 ml Carrez I (point 4.15) to the sample solution. After shaking, 1 ml Carrez II (point 4.16) is added. Shake the sample again.

6.4.3. Processing for HPLC analysis

The sample in the volumetric flask is diluted to the mark with water, homogenised and filtered through a fluted filter (point 5.2). Collect the sample extract.

Filter the extracts through a syringe filter (point 5.3) with a syringe (point 5.6) that has been preflushed with the extract. Collect the filtrates in vials (point 5.4).

6.5. *Chromatography*

HPLC is performed as conventionally for analysis of sugars. If the HPLC analysis shows traces of maltose, then the starch is incompletely converted, which results in a too low recovery for glucose.

7. **Calculation and expression of results**

7.1. *Calculation of the HPLC results*

For the calculation of the starch content, the results of two HPLC analysis are necessary, namely sugars present in the sample before ('free sugars') and after enzymatic treatment (as described in this method). Also a blank determination has to be performed to be able to correct for sugars present in the enzymes.

In the HPLC analysis, the peak area is determined after integration and the concentration is calculated after calibration with reference solutions (point 4.14). From the glucose concentration (g/100 ml) after enzymatic treatment, the concentration of glucose (g/100 ml) in the blank is subtracted. Eventually the content (g sugar/100 g sample) of sugars is calculated using the weighted amount of sample, which results in:

1. HPLC analysis before enzymatic treatment, giving the content (g/100 g) of free sugars:

   — glucose G

   — fructose F

   — sucrose S

2. HPLC analysis after enzymatic treatment, giving the content (g/100 g) of sugars:

   — glucose after correction for the blank ($G_{e\ cor}$)

   — fructose F_e

   — sucrose S_e

7.2. *Calculation of the starch content*

7.2.1. Calculation of total glucose "Z"

If the amount of fructose after enzymatic treatment (F_e) is higher than the amount of fructose before enzymatic treatment (F), then the sucrose, present in the sample, is partly converted into fructose and glucose. This means that a correction shall be made for the liberated glucose ($F_e - F$).

Z, final glucose content after correction in g/100g:

$$Z = (G_{e\ cor}) - (F_e - F)$$

7.2.2. Calculation of the total glucose content expressed as starch

E, "starch" content in g/100g:

$$E = [(G_{e\ cor}) - (F_e - F)] \times 0{,}9$$

8. **Precision**

Details of an inter laboratory test relating to precision data of the method performed on 2 samples are given in this point. They reflect the performance requirements for the method described in this annex.

Results of an interlaboratory test (Informative)

An inter laboratory test was carried out in 2008 with the participation of the European Customs laboratories.

The evaluation of precision data was performed according to the "Protocol for the design, conduct and interpretation of method-performance studies", W. Horwitz, (IUPAC technical report), Pure & Appl. Chem., Vol. 67, N° 2, PP.331-343, 1995.

The precision data are given in the table below.

Samples **1 : chocolate and biscuit bar** **2 : biscuit**	**Z** **sample 1**	**Z** **sample 2**
Number of laboratories	41	42
Number of laboratories after eliminating outliers	38	39
Mean (%, m/m)	29,8	55,0
Repeatability standard deviation sr (%, m/m)	0,5	0,5
Reproducibility standard deviation sR (%, m/m)	1,5	2,3
Repeatability limit r (%, m/m)	1,4	1,4
Reproducibility limit R (%, m/m)	4,2	6,6'