Commission Regulation (EC) No 121/2008 of 11 February 2008 laying down the method of analysis for the determination of starch content in preparations of a kind used in animal feeding (CN code 2309)

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THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

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(1) (a) thereof,

Whereas:

(1) In order to ensure that preparations of a kind used in animal feeding (CN 2309) receive uniform treatment on import throughout the Community, it is necessary that, when laying down analytic methods, account should be taken of the scientific and technological evolution of those methods.

(2) In accordance with the third Commission Directive 72/199/EEC of 27 April 1972 establishing Community methods of analysis for the official control of feedingstuffs[(2)], for the determination of the starch content in preparations of a kind used in animal feeding, the polarimetric method (also called: modified Ewers method), described in point 1 of Annex I to that Directive is to be applied.

(3) In the light of studies carried out by the experts of the customs laboratories of the Member States it is necessary to provide that where the polarimetric method laid down in Directive 72/199/EEC cannot be applied for the determination of the starch content in the mentioned preparations, an enzymatic analytical method should be applied. It is therefore appropriate to specify how that enzymatic method is to be carried out.

(4) The measures provided for in this Regulation are in accordance with the opinion of the Tariff and Statistical Nomenclature Section of the Customs Code Committee,

HAS ADOPTED THIS REGULATION:

Article 1

By derogation from Article 1 of Directive 72/199/EEC, the starch content by weight of preparations of a kind used in animal feeding within the meaning of CN code 2309 shall be determined by the enzymatic analytical method laid down in the Annex to this Regulation in cases where the following feed materials are present in significant amounts:

(a) (sugar) beet products such as (sugar) beet pulp, (sugar) beet molasses, (sugar) beet pulp-molassed, (sugar) beet vinasse, (beet) sugar;

(b) citrus pulp;

(c) linseed; linseed expeller; linseed extracted;

(d) rape seed; rape seed expeller; rape seed extracted; rape seed hulls;

(e) sunflower seed; sunflower seed extracted; sunflower seed, partially decorticated, extracted;

(f) copra expeller; copra extracted;

(g) potato pulp;

(h) dehydrated yeast;

(i) products rich in inulin (e.g. chips and meal of Jerusalem artichokes);

(j) greaves**[**F1;**]**

(k) **[**F2soya products.**]**

Textual Amendments

F1 Substituted by Commission Implementing Regulation (EU) 2017/68 of 9 January 2017 amending Regulation (EC) No 121/2008 laying down the method of analysis for the determination of starch content in preparations of a kind used in animal feeding (CN code 2309).

F2 Inserted by Commission Implementing Regulation (EU) 2017/68 of 9 January 2017 amending Regulation (EC) No 121/2008 laying down the method of analysis for the determination of starch content in preparations of a kind used in animal feeding (CN code 2309).

Article 2

This Regulation shall enter into force on the twentieth day following that of 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.

***Changes to legislation:** There are currently no known outstanding effects for the Commission Regulation (EC) No 121/2008. (See end of Document for details)*

ANNEX

ENZYMATIC METHOD FOR THE DETERMINATION OF THE STARCH CONTENT IN PREPARATIONS USED IN ANIMAL FEEDING USING HIGH PRESSURE LIQUID CHROMATOLOGY (HPLC)

1. Scope

This method describes the enzymatic determination of the starch content in animal feeding. The starch content is derived from the quantitative determination of glucose after enzymatic breakdown of the present starch into glucose. All measured glucose is considered to be derived from the starch, present in the sample.

2. Definitions

In this method, the content of starch and its high molecular mass breakdown products, insoluble in 40 % ethanol, is determined. The starch content is expressed in % (m/m).

3. Principle

The samples are homogenised by milling. The sample is washed with 40 % ethanol to eliminate soluble sugars and soluble products of starch decomposition.

The enzyme thermostable alpha-amylase is added to the suspension. This enzyme breaks down the starch at 100 °C in smaller chains, whether or not the starch is fully in solution.

Large lumps of starch are broken down very slowly. Therefore it is necessary that the samples should be wholly solved or should be present in the form of a suspension containing very small solid parts.

Then the second enzyme amyloglucosidase is added, which hydrolyses the degraded glucose chains into glucose at 60 °C.

After clarification of the liquid whereby the present proteins, fats and residues are discarded after filtration, a clear solution is obtained which may be used for HPLC.

Separation of the present sugars is done by HPLC.

4. Reagents and other materials

Use reagents of recognized analytical grade and demineralized water.

4.1. Ethanol 40 % vol. in water

4.2. Glucose, min 99 %

4.3. Solution of Amyloglucosidase (1,4-alpha-D-Glucan glucohydrolase) from Aspergillus niger (enzyme activity > 5 000 U/ml). Storage at ca. 4 °C

Alternatively, also amyloglucosidase powder can be used.

4.4. Thermostable alpha-amylase (1,4-alpha-D-Glucan-glucanohydrolase). Storage at ca. 4 °C

4.5. Zinc acetate dihydrate, p.a.

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

4.7. Sodium acetate anhydrous, p.a.

Changes to legislation: *There are currently no known outstanding effects for the Commission Regulation (EC) No 121/2008. (See end of Document for details)*

4.8. Glacial acetic acid, 100 % (v/v)

4.9. Sodium acetate buffer (0,2 mol/l).

Weigh 16,4 gram sodium acetate (4.7) 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 (5.11)). This solution may be used for max 6 months with storage at 4 °C.

4.10. Amyloglucosidase solution (enzyme activity > 250 U/ml)

Prepare a solution of 5 ml of amyloglucosidase-solution (4.3) or 660 mg amyloglucosidase powder in a final volume of 100 ml by using sodium acetate buffer (4.9). Prepare instantaneously.

4.11. Reference solution

Prepare solutions of glucose in water, as conventionally used in the HPLC analysis.

4.12. Reagent for clarification (Carrez I)

Dissolve 219,5 gram zinc acetate (4.5) in water in a beaker glass. Rinse into a volumetric flask of 1 000 ml, add 30 ml acetic acid (4.8). Mix thoroughly and make up 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.

4.13. Reagent for clarification (Carrez II)

Dissolve 106,0 gram potassium hexacyanoferrate (II) (4.6) in water in a beaker glass. Rinse into a volumetric flask of 1 000 ml. Mix thoroughly and make up 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.

4.14. Mobile phase

A mobile phase is prepared 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 graded water and acetonitrile.

5. Apparatus

5.1. Standard laboratory glassware

5.2. Centrifuge giving 1 000 g or more (calculated at the centre of the tube)

5.3. Glass centrifuge tubes of 100 ml

5.4. Magnetic stirrer

5.5. Magnetic rods

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

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

5.8. Sample vials suitable for the HPLC autosampler

5.9. 100 ml volumetric flasks