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(Acts whose publication is obligatory)

COMMISSION REGULATION (EEC) No 1822/86

of 30 May 1986

amending Regulation (EEC) No 1061/69 as regards the methods of analysis for the determination of starch in soya protein concentrates and in goods containing such products

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Regulation (EEC) No 97/69 of 16 January 1969 on measures to be taken for uniform application of the nomenclature of the Common Customs Tariff⁽¹⁾, as last amended by Regulation No 2055/84⁽²⁾ and in particular Article 3 thereof;

Whereas Article 1 of Commission Regulation (EEC) No 1061/69⁽³⁾, as last amended by Regulation (EEC) No 419/77⁽⁴⁾, defines the methods of analysis to be used to implement Council Regulation (EEC) No 3033/80 of 11 November 1980 laying down the trade arrangements applicable to certain goods resulting from the processing of agricultural products⁽⁵⁾;

Whereas, in the light of the studies carried out, it is necessary to replace by a more appropriate method the method of analysis laid down for the determination of starch as applied to soya protein concentrates and in goods containing such products;

Whereas the method set out in the Annex is more reliable;

Whereas it is desirable to change certain chemical names;

Whereas the measures provided for in this Regulation are in accordance with the opinion of the Committee on Common Customs Tariff Nomenclature,

HAS ADOPTED THIS REGULATION:

Article 1

Regulation (EEC) No 1061/69 is hereby amended as follows:

⁽¹⁾ OJ No L 14, 21. 1. 1969, p. 1.
⁽²⁾ OJ No L 191, 19. 7. 1984, p. 1.
⁽³⁾ OJ No L 141, 12. 6. 1969, p. 24.
⁽⁴⁾ OJ No L 56, 1. 3. 1977, p. 46.
⁽⁵⁾ OJ No L 323, 29. 11. 1980, p. 1.

1. Article 1 is replaced by the following:

Article 1

1. Where classification of goods referred to in Article 1 of Regulation (EEC) No 3033/80 under one or other of the subheadings of the Common Customs Tariff depends on the starch content by weight, such content shall be determined by reference to the quantity of starch in the anhydrous state contained in such goods.

2. The starch content by weight of soya protein concentrates and of goods containing such products shall be ascertained by the enzymatic method set out in Annex V.

3. The starch content by weight of goods other than those referred to in paragraph 2 shall be ascertained by the modified Ewers method set out in the first chapter of Annex I to the third Commission Directive 72/199/EEC⁽¹⁾.

However, where the goods in question contain starches other than native starches but do not also contain sucrose or invert sugar, the starch content by weight of such goods shall be ascertained by the saccharification method set out in Annex I to the abovementioned Directive.

For the purposes of implementing this paragraph, dextrines shall be treated as starches other than native starches.

⁽¹⁾ OJ No L 123, 29. 5. 1972, p. 6.

2. Article 4 is replaced by the following:

Article 4

The D-mannitol content of products falling within subheadings 29.04 C III and 38.19 T of the Common Customs Tariff, calculated by reference to the D-glucitol (sorbitol) content, shall be ascertained by the methods set out in Annex IV.

3. Annex IV is amended as follows :

(a) The title is replaced by the following :

'DETERMINATION OF THE D-MANNITOL CONTENT OF GOODS FALLING WITHIN SUBHEADINGS 29.04 C III AND 38.19 T OF THE COMMON CUSTOMS TARIFF, CALCULATED ON THE D-GLUCITOL CONTENT' ;

(b) Paragraph I is replaced by the following :

'I. Principle

Gas chromatography is used to determine the D-mannitol content of goods falling within subheadings 29.04 C III and 38.19 T of the Common Customs Tariff, calculated on their D-glucitol content. For that purpose, non-volatile products must first be processed into their acetylated derivatives.' ;

(c) Paragraph III (b) is replaced by the following :

'(b) Conditions required for carrying out chromatography :

Injection temperature : 300 °C ;

Temperature of the column : 210 °C ;

Flow rate of carrier gas stream (e.g. nitrogen) : 25 ml/minute ;

Hydrogen flow rate : 25 ml/minute ;

Quantity injected : 1 microlitre.

The D-mannitol peak appears first, then the D-glucitol peak.

To determine the D-mannitol content of the goods under analysis, calculated on their D-glucitol content, simply calculate the ratio of the areas of the two peaks.'

4. The Annex to this Regulation is added as Annex V.

Article 2

This Regulation shall enter into force on the 21st day following its publication in the *Official Journal of the European Communities*.

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

Done at Brussels, 30 May 1986.

For the Commission

COCKFIELD

Vice-President

ANNEX**ANNEX V****1. Purpose and field of application**

The method permits the determination of the starch content by weight of soya protein concentrates and of goods containing soya protein concentrates.

2. Definition

"Starch content" in the products referred to in paragraph 1 is defined as value "T" as calculated in paragraph 7 of the present method.

3. Principle

The sample is washed with 40 % ethanol to eliminate soluble sugars and soluble products of starch decomposition. The residue is broken down by means of sodium hydroxide and the starch divided into glucose units with amyloglucosidase. The glucose determination is performed by the enzymatic route.

4. Reagents

(Use double-distilled water.)

4.1. Ethanol 40 % vol. in water.

4.2. 0,5 N Sodium hydroxide solution (0,5 mol/l).

4.3. 96 % (minimum) glacial acetic acid.

4.4. Solution of amyloglucosidase :

Immediately before use, dissolve approximately 10 mg of amyloglucosidase (EC 3.2.1.3) (6 U per mg) in one ml of water (1).

4.5. Triethanolamine buffer solution :

Dissolve 14,0 g of triethanolamine hydrochloride [tris(2-hydroxyethyl)ammonium chloride] and 0,25 g of magnesium sulphate $MgSO_4 \cdot 7H_2O$ in 80 ml of water, add approximately 5 ml of 5 N sodium hydroxide solution (5 mol/l) and adjust the pH to 7,6, using a 1 N sodium hydroxide solution (1 mol/l). Make up to 100 ml with water. This buffer solution can be kept for at least four weeks at 4 °C.

4.6. NADP (nicotinamide adenine dinucleotide phosphate, disodium salt) solution :

Dissolve 60 mg of NADP in 6 ml of water. This solution can be kept for at least four weeks at 4 °C.

4.7. ATP (adenosine-5'-triphosphate, disodium salt) solution :

Dissolve 300 mg of $ATP \cdot 3H_2O$ and 300 mg of sodium hydrogen carbonate ($NaHCO_3$) in 6 ml of water.

This solution can be kept for at least four weeks at 4 °C.

4.8. Suspension of HK/G6P-DH [Hexokinase (EC 2.7.1.1) and glucose-6-phosphate-dehydrogenase (EC 1.1.1.49)] :

suspend 280 U HK and 140 U of G6P-DH in 1 ml of 3,2 M ammonium sulphate solution. This suspension can be kept for at least one year at 4 °C.

5. Apparatus

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

5.2. 100 ml glass centrifuge tubes.

5.3. Magnetic stirrer with water bath at 60 °C.

5.4. Magnetic rods.

5.5. UV spectrophotometer with 1 cm optical cells.

5.6. Pipettes for enzymatic analysis.

(1) U is the international unit of enzyme activity.

6. Method

6.1. *The sample is washed with ethanol, digested in sodium hydroxide and the starch subjected to enzymatic hydrolysis:*

6.1.1. Select the weight of sample as follows, according to the presumed starch content (the starch content must not exceed 0,4 g per sample) as follows:

Presumed starch content of product in g/100 g	Approximate weight of sample in g (p)	Volume of graduated flask in ml	Dilution factor up to 1 litre (f)
> 70	0,35 — 0,4	500	2
20 — 70	max. 0,5	500	2
5 — 20	max. 1	250	4
< 5	max. 2	200	5

6.1.2. Weigh the sample in a centrifuge tube (5.2) exactly to 0,1 mg and add 50 ml of ethanol, 40 % vol. (4.1).

6.1.3. Stir in a magnetic stirrer for 20 minutes at ambient temperature.

6.1.4. Leave the magnetic rod in the tube and centrifuge for five minutes.

6.1.5. Aspirate carefully and remove the liquid phase (using a water-jet pump or a Pasteur pipette).

6.1.6. Wash the residue by stirring with 25 ml of ethanol, 40 % vol. (4.1).

6.1.7. Centrifuge, aspirate carefully and remove the liquid phase.

6.1.8. Carry out this series of aspirations (6.1.6 and 6.1.7) at least once more.

6.1.9. Add to the residue 50 ml of 0,5 N sodium hydroxide solution (4.2) and stir continuously for 30 minutes in the water bath (5.3) with a magnetic stirrer at 60 °C.

6.1.10. Add a few ml of concentrated acetic acid (4.3) and bring the pH to 4,6 to 4,8.

6.1.11. Place the centrifuge tube in the water bath with the magnetic stirrer (5.3) at 60 °C, add 1,0 ml of enzyme solution (4.4) and allow to react whilst stirring continuously for 30 minutes.

6.1.12. After cooling, transfer quantitatively to a graduated flask (6.1.1) and make up to the mark with water.

6.1.13. If necessary, filter through a fluted filter.

6.2. *Quantitative determination of glucose:*

6.2.1. The test solution must contain 100 to 1 000 mg of glucose per litre, which corresponds to a ΔE_{340} of between 0,1 and 1,0.

The absorbance of the test solution in a 1 + 30 dilution with water must not exceed 0,4 (measured against air), at 340 nm.

6.2.2. Bring the buffer solution (4.5) to ambient temperature (20 °C).

6.2.3. The temperature of the reagents and of the sample must be between 20 and 25 °C.

6.2.4. Measure the absorbance at 340 nm against air (i. e. with no optical cell in the reference path).

6.2.5. Proceed in accordance with the pipetting chart below :

Pour into the optical cells	Control (ml)	Test (ml)
Buffer (reagent 4.5)	1,00	1,00
NADP (reagent 4,7)	0,10	0,10
ATP (reagent 4.7)	0,10	0,10
Test solution (6.1.12 or 13)	—	0,10
Double-distilled water	2,00	1,90

Mix and, after about three minutes, measure the absorbance of the solutions (E_1). Initiate the reaction by adding :

HK/G6P.DH (reagent 4.8)	0,02	0,02
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Mix, allow the reaction to proceed to completion (approximately 15 minutes) and measure the absorbance of the solutions (E_2). If the reaction has not stopped after 15 minutes read absorbances at five-minute intervals until the rate of increase is constant. Then extrapolate backwards to the time of addition of suspension (4.8).

6.2.6. Calculate the absorbance differences for reagent blank and sample ($E_2 - E_1$).

Subtract absorbance difference of the reagent blank (ΔE reagent blank) from that of the sample (ΔE sample);

$$\Delta E = \Delta E_{\text{sample}} - \Delta E_{\text{reagent blank}}$$

This difference gives the glucose content of the test solution :

Glucose content contained in test solution, g/l

$$G = \frac{3,22 \times 180,16}{6,3 \times 1 \times 0,1 \times 1000} \times \Delta E_{340} = 0,921 \times \Delta E_{340}$$

The molecular weight of glucose is 180,16 (g/mol).

6.2.7. If, for any reason, a measurement cannot be made at 340 nm, the measurement may be made at a wavelength of 365 nm or 334 nm, the figure 6,3 in the formula for G above should be replaced by the figure 3,5 or 6,18 respectively.

7. Calculation and expression of results

$$T = \text{Starch content in g/100 g}$$

$$T = \frac{100 \times 0,9 \times G}{p \times f}$$

where

G = glucose in g/l (6.2.6)

f = dilution factor (6.1.1)

p = weight of sample in g

0,9 = glucose conversion factor for starch.