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COMMISSION REGULATION (EEC) No 4154/87

of 22 December 1987

laying down the methods of analysis and other technical provisions necessary for the implementation of Regulation (EEC) No 3033/80 laying down the trade arrangements applicable to certain goods resulting from the processing of agricultural products

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laying down the methods of analysis and other technical provisions necessary for the implementation of Regulation (EEC) No 3033/80 laying down the trade arrangements applicable to certain goods resulting from the processing of agricultural products

THE COMMISSION OF THE EUROPEANCOMMUNITIES,

Having regard to the Treaty establishing the European Economic 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] as amended by Regulation (EEC) No 3985/87 [2] and in particular Article 9 thereof,

Whereas Commission Regulation (EEC) No 1061/69 [3], as last amended by Regulation (EEC) No 1822/86 [4], defined the analytical methods to be used for the application of Council Regulation (EEC) No 1059/69 [5]; whereas Regulation (EEC) No 1059/69 was repealed and replaced by Council Regulation (EEC) No 3033/80 [6], as last amended by Regulation (EEC) No 3743/87 [7];

Whereas the procedures for implementing Regulation (EEC) No 3033/80 for imports, were prescribed by Council Regulation (EEC) No 3034/80, of 11 November 1980, fixing the quantities of basic products considered to have been used in the manufacture of goods covered by Regulation (EEC) No 3033/80 [8] amending Regulation (EEC) No 950/68 on the Common Customs Tariff, as last amended by Regulation (EEC) No 4091/87 [9];

Whereas in order to take account of the scientific and technological evolution of analytical methods and to continue to ensure the uniform treatment on import into the Community of goods covered by Regulation (EEC) No 3033/80, it is appropriate to repeal and replace Regulation (EEC) No 1061/69;

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

HAS ADOPTED THIS REGULATION:

Article 1

This Regulation lays down the methods of analysis necessary for the implementation of Regulations (EEC) No 3033/80 (as far as imports are concerned) and (EEC) No 3034/80, or, in the absence of a method of analysis, the nature of the analytical operations to be carried out or the principle of a method to be applied.

Article 2

In accordance with the definitions set out in Annex III to Regulation (EEC) No 3034/80 concerning:

— starch/glucose, content, and

— sucrose/invert sugar/isoglucose content

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[1] OJ No L 256, 7. 9. 1987, p. 1.
[2] OJ No L 376, 31. 12. 1987, p. 1.
[3] OJ No L 141, 12. 6. 1969, p. 24.
[4] OJ No L 158, 13. 6. 1986, p. 1.
[5] OJ No L 141, 12. 6. 1969, p. 1.
[6] OJ No L 323, 29. 11. 1980, p. 1.
[7] OJ No L 352, 15. 12. 1987, p. 29.
[8] OJ No L 323, 29. 11. 1980, p. 7.
[9] OJ No L 382, 31. 12. 1987, p. 27.

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and for the purpose of implementing Annexes II and III to that Regulation, the following formulae, procedures and methods shall be used:

1. *Starch/glucose content:*

   expressed as 100 % anhydrous starch content of the goods as presented)

   (a) (Z − F) × 0,9,

   if the glucose content is not less than the fructose content; or

   (b) (Z − G) × 0,9,

   if the glucose content is less than the fructose content

   where:

   Z is the glucose content determined by the method set out in Annex I of this Regulation;

   F is the fructose content determined by HPLC (high performance liquid chromatography);

   G is the glucose content determined by HPLC.

   In case 1 (a), where the presence of a lactose hydrolysate is declared and/or quantities of lactose and galactose are detected, a glucose content equivalent to the galactose content (determined by HPLC), shall be deducted from the glucose content (Z) before any other calculation is made.

2. *Sucrose/invert sugar/isoglucose content:*

   (expressed as sucrose content of the goods as presented)

   (a) S + (2F) × 0,95,

   if the glucose content is not less than the fructose content

   (b) S + (G + F) × 0,95,

   if the glucose content is less than the fructose content

   where:

   S is the sucrose content determined by HPLC;

   F is the fructose content determined by HPLC;

   G is the glucose content determined by HPLC.

   Where the presence of a lactose hydrolysate is declared and/or quantities of lactose and galactose are detected, a glucose content, equivalent to the galactose content (determined by HPLC), shall be deducted from the glucose (G) content before any other calculation is made.

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3. *Milk fat content:*

   (a) Save as otherwise provided in paragraph (b), the milk fat content by weight shall be determined by extraction with light petroleum after hydrolysis with hydrochloric acid.

   (b) Where fats other than milk fats are also declared in the composition of the goods, the following procedure shall be applied:

   — the percentage of weight of the total fats in the goods shall be determined as mentioned in paragraph (a),

   — for the purposes of determining the milk fat content, a method based on extraction with light petroleum, preceded by hydrolysis with hydrochloric acid and followed by gas chromatography of the methyl esters of the fatty acids, shall be used. If the presence of milk fats is detected, the percentage proportion thereof shall be calculated by multiplying the percentage concentration of methyl butyrate by 25, multiplying the product by the total percentage fat content by weight of the goods and dividing by 100.

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4. *Milk protein content:*

   (a) Save as otherwise provided in (b) below, the milk protein content of the goods shall be calculated by multiplying the

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nitrogen content (determined by the Kjeldahl method) by the factor 6,38.

(b) Where components containing proteins other than milk proteins are also declared in the composition of the goods:

(i) the total nitrogen content shall be determined by the Kjeldahl method;

(ii) the milk protein content shall be calculated as mentioned in (a) above by subtracting from the total nitrogen content the nitrogen content corresponding to the non-milk proteins.

Article 3

For the purposes of implementing Annex I to Regulation (EEC) No 3034/80, the following methods and/or procedures shall be used:

1. For the purposes of classifying goods falling within subheadings 0403 10 51 to 0403 10 59, 0403 10 91 to 0403 10 99, 0403 90 71 to 0403 90 79 and 0403 90 91 to 0403 90 99 of the combined nomenclature, the milk fat content shall be determined by the method referred to in Article 2 (3);

2. For the purposes of classifying goods falling within subheadings 1704 10 11 to 1704 10 99 and 1905 20 10 to 1905 20 90 of the combined nomenclature, the sucrose content, including invert sugar expressed as sucrose, shall be determined by an HPLC method; (invert sugar expressed as sucrose means the sum of equal amounts of glucose and fructose multiplied by 0,95);

3. For the purposes of classifying goods falling within subheadings 1806 10 10 to 1806 10 90 of the combined nomenclature, the sucrose/invert sugar/isoglucose content shall be determined in accordance with the formulae, method and procedures set out in Article 2 (2);

4. For the purposes of classifying goods falling within subheadings 3505 20 10 to 3505 20 90 of the combined nomenclature, the starch, dextrin or other modified starch content shall be determined by the method set out in Annex II of this Regulation;

5. For the purposes of classifying goods falling within subheadings 3809 10 10 to 3809 10 90 of the combined nomenclature, the amylaceous substances shall be determined by the method set out in Annex II to this Regulation;

6. For the purposes of classifying goods falling within either subheading 1901 90 11 or subheading 1901 90 19 of the combined nomenclature, the distinction shall be drawn on the basis of the dry extract determined by drying at a temperature of 103 ± 2 ºC to constant weight;

7. For the purposes of classifying goods falling within subheadings 1902 19 10 and 1902 19 90 of the combined nomenclature, the method set out in Annex III to this Regulation shall be used to test for the presence of common wheat flours and semolinas in pasta;

8. The content of mannitol and D-glucitol (sorbitol) of the goods falling within subheadings 2905 44 11 to 2905 44 99 and 3823 60 11 to 3823 60 99 of the combined nomenclature, is determined using a method based on HPLC.

Article 4

1. A test report shall be drawn up.

2. The test report shall include the following particulars:

— all the information necessary for identifying the sample,

— the method used and precise reference to the legal instrument in which it is laid down, or, where appropriate, detailed reference to a method, specifying the nature of the analytical operations to be carried out, or the principle of the method to be applied, as indicated in this Regulation,

— any factors liable to have influenced the results,

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— the results of the analysis, with due regard to the way in which they are expressed in the method used and the means of expression dictated by the needs of the customs or administrative departments that requested the analysis.

Article 5

Regulation (EEC) No 1061/69 is hereby repealed.

Article 6

This Regulation shall enter into force on 1 January 1988.

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

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ANNEX I

DETERMINATION OF STARCH CONTENT AND ITS DEGRADATION PRODUCTS INCLUDING GLUCOSE

1. **Purpose and field of application**

(a) The method permits the determination of the starch content, its degradation products including glucose, hereafter referred to as 'starch'.

(b) 'Starch' content referred to in 1 (a) is equal to value E, as calculated in paragraph 6 of the present method.

2. **Principle**

The sample 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.

3. **Reagents**

(Use double-distilled water.)

3.1. 0,5 N Sodium hydroxide solution (0,5 mol/l).

3.2. 96 % (minimum) glacial acetic acid.

3.3. 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].

3.4. Triethanolamine buffer solution:

Dissolve 14,0 g of triethanolamine hydrochloride [tris(2-hydroxyethyl)ammonium chloride] and 0,25 g of magnesium sulphate $MgSO_4 7H_2O$) in 80 ml of water, add approximately 5 ml of 5 N sodium hydroxyde 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 ºC.

3.5. 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 ºC.

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

Dissolve 300 mg of $ATP.3H_2O$ 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 ºC.

3.7. Suspension 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 ºC.

4. **Apparatus**

4.1. Magnetic stirrer water bath at 60 ºC.

4.2. Magnetic rods.

4.3. UV spectrophotometer with 1 cm optical cells.

4.4. Pipettes for enzymatic analysis.

[1] U is the international unit of enzyme activity.

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5. **Method**

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

5.1.1. 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' contentof product in g/100 g	Approximate weight of sample in g (p)	Volume of graduated flask in ml	Dilution factorup 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

5.1.2. Weigh the sample exactly to 0,1 mg.

5.1.3. Add 50 ml of 0,5 N sodium hydroxide solution (3.1) and stir continuously for 30 minutes in the water bath (4.1) with a magnetic stirrer at 60 ºC.

5.1.4. Add a few ml of concentrated acetic acid (3.2) and bring the pH to 4,6 to 4,8.

5.1.5. Place in the water bath with the magnetic stirrer (4.1) at 60 ºC, add 1,0 ml of enzyme solution (3.3) and allow to react whilst stirring continuously for 30 minutes.

5.1.6. After cooling, transfer quantitatively to a graduated flask (5.1.1) and make up to the mark with water.

5.1.7. If necessary, filter through a fluted filter (see Note 1).

5.2. *Quantitative determination of glucose:*

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

5.2.2. Bring the buffer solution (3.4) to ambient temperature (20 ºC).

5.2.3. The temperature of the reagents and of the sample must be between 20 and 25 ºC.

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

5.2.5. Proceed in accordance with the pipetting chart below:

Pour into the optical cells	Control (ml)	Test (ml)
Buffer (reagent 3.4)	1,00	1,00
NADP (reagent 3.5)	0,10	0,10
ATP (reagent 3.6)	0,10	0,10
Test solution (5.1.6 or 5.1.7)	—	0,10
Double-destilled water	2,00	1,90

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

HK/G6P.DH (reagent 3.7)	0,02	0,02

Mix, allow the reaction to proceed to completion (approximately 15 minutes) and measure the absorbence of the solutions (E_2). If the reaction has not stopped after 15 minutes read absorbences at five-minute invervals until the rate of increase is constant. Then extrapolate backwards to the time of addition of suspension (3.7) (see Note 2).

5.2.6. Calculate the absorbence differences for reagent blank and sample (E_2 — E_1).

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Subtract absorbence 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

$$Gl = \frac{3,22 \times 180,16}{6,3 \times 1 \times 0,1 \times 1\,000} \times \Delta E_{340} = 0,921 \times \Delta E_{340}$$

(3,22: volume of the solution to be measured; 1: cell thickness; 0,1: volume of the sample solution; The molecular weight of glucose is 180,16 (g/mol).

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

6. **Calculation and expression of results**

(a) E = 'Starch' content in g/100 g:

$$E = \frac{100 \times 0,9 \times Gl}{p \times f}$$

(b) Z = 'Glucose' content in g/100 g:

$$Z = \frac{100 \times Gl}{p \times f}$$

where:

Gl = glucose in G/l (5.2.6);

f = dilution factor (5.1.1);

p = weight of sample in g;

0,9 = glucose conversion factor for starch.

Notes:

(1) Where a test solution cannot be filtered according to 5.1.7 appropriate methods should be taken in order to obtain a clear solution.

(2) Where an inhibition of enzymes occurs, it is advisable to apply a method which involves the addition of known amounts of pure starch.

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ANNEX II

DETERMINATION OF STARCHES OR DEXTRINS OR OTHER MODIFIED STARCHES CONTENT INTO GOODS OF SUBHEADINGS 3505 20 10 TO 3505 20 90 OF THE COMBINED NOMENCLATURE AND OF AMYLACEOUS SUBSTANCES CONTENT INTO GOODS OF SUBHEADINGS 3809 10 10 TO 3809 10 90 OF THE COMBINED NOMENCLATURE

I. **Principle**

Starch is converted by acid hydrolysis into reducing sugars which are determined by volume using Fehling's solution.

II. **Apparatus and reagents**

1. 250 ml flask;
2. 200 ml graduated flask;
3. 25 ml graduated burette;
4. Hydrochloric acid at 1,19 density;
5. Potassium hydroxide solution;
6. Decolourizing charcoal;
7. Fehling's solution;
8. Methylene blue solution (1 %).

III. **Method**

Into a 250 ml flask place a sample containing about 1 g of starch. Add 100 ml of distilled water and 2 ml of hydrochloric acid. Bring to the boil and reflux for three hours.

Transfer the contents of the flask and rinsings into a 200 ml graduated flask. Cool and nearly neutralize with potassium hydroxide solution. Add distilled water to 200 ml and filter through a little decolourizing charcoal.

Then pour the solution into a graduated burette and reduce 10 ml of Fehling's solution by the following method:

Into a flat-bottomed flask of about 250 ml pour 10 ml of Fehling's solution (5 ml of solution A and 5 ml of solution B). Shake until clear and add 40 ml of distilled water and a small quantity of quartz or pumice.

Place the flask on a square asbestos plate with a round hole of about 6 cm diameter in the centre, the asbestos in turn resting on a piece of wire gauze. Heat the flask at such a rate that the liquid begins boiling after about two minutes.

From the burette, add to the boiling liquid successive quantities of the sugar solution until the blue colour of the Fehling's solution becomes hardly discernible; then add 2 or 3 drops of methylene blue solution as indicator, and complete the titration by adding further quantities of the sugar solution, drop by drop, until the blue colour of the indicator disappears.

For greater accuracy repeat the titration under the same conditions, but adding without a break almost all the sugar solution required to reduce the Fehling's solution. In this second titration, the reduction of the Fehling's solution should occur within three minutes.

Keep boiling for exactly two further minutes, adding the reagent within one minute drop by drop to the boiling solution until the blue colour disappears.

The percentage by weight of starch in the sample is determined by means of the following formula:

$$\text{starch } \% = \frac{T \times 200 \times 100}{n \times p} \times 0,95$$

where:

T is the quantity in grammes of anhydrous dextrose corresponding to 10 ml of Fehling's solution (5 ml of solution A and 5 ml of solution B). This titer corresponds to 0,04945 g of anhydrous dextrose when solution A contains 17,636 g of copper per litre;

n is the number of ml of the sugar solution used for titration;

p is the weight of the sample amount;

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0,95 is the rate of conversion of anhydrous dextrose into starch

IV. **Preparation of Fehling's solutions**

Solution A: In a graduated flask dissolve 69,278 g of pure crystallized copper sulphate — Analytical Reagent ($CuSO_4 \cdot 5\ H_2O$) — free from iron in distilled water and bring the volume to 1 litre with distilled water. The correct strength of this solution must be verified by a quantitative determination of the copper.

Solution B: In a graduated flask dissolve 100 g of sodium hydroxide and 346 g of double sodium potassium tartrate (Rochelle salt) in distilled water and bring the volume to 1 litre with distilled water.

The two solutions A and B must be mixed in equal quantities immediately before use. 10 ml of Fehling's solution (5 ml of solution A and 5 ml of solution B) is completely reduced, under the conditions described at III, by 0,04945 g of anhydrous dextrose.

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ANNEX III

DETECTION OF COMMON WHEAT FLOUR OR MEAL IN MACARONI, SPAGHETTI AND SIMILAR PRODUCTS (PASTA)

(by the Young and Gilles method, modified by Bernaerts and Gruner)

I. Principle

An extract of the sample of the pasta for analysis is prepared by using a non-polar solvent.

This extract is chromatographed on a thin layer of silica gel so as to separate the sterols present in various band form fractions.

According to the number of brightly coloured bands it is possible to determine whether the product under examination has been manufactured exclusively from durum wheat or common wheat, or from a mixture of the two. It is also possible to determine whether eggs have been added.

II. Apparatus and reagents

1. Homogenizer or grinder to obtain a grist that will pass through a standard sieve with a 0,200 mm mesh;
2. Standard sieve with a 0,200 mm mesh;
3. Evaporator with a water bath for evaporation under reduced pressure;
4. Glass plate, aluminium sheet or other appropriate backing measuring 20 cm x 20 cm covered with a thin layer of silica gel. If the thin layer has to be prepared, silica gel mixed with about 13 plaster should be used, and it should be applied in a 0,25 mm layer with suitable apparatus in accordance with the manufacturer's instructions;
5. Micropipette for measuring 20 microlitres;
6. Container with lid suitable for the development of chromatograms;
7. Atomizer;
8. Petroleum ether with a boiling point between 40 and 60 ºC, redistilled before use;
9. Anhydrous ethyl ether for analysis;
10. Carbon tetrachloride for chromatography, redistilled before use;
11. Phosphomolybdic acid for analysis;
12. 94º ethyl alcohol.

III. Method

Grind about 20 g of the sample for analysis so that all of it passes through the sieve. Put the sample in an Erlenmeyer flask and cover with 150 ml petroleum ether. Leave at normal temperature until the following day. Shake from time to time.

Then filter on a Buchner funnel fitted with a filtering aid or on a sintered filter. Gradually transfer the clear solution thus obtained into a 100 ml calibrated flask. Evaporate the solvent under reduced pressure by heating the flask in a water bath at 40 ºC to 50 ºC. When the solvent has evaporated, heat under reduced pressure for a further ten minutes.

When the flask has cooled, determine the weight of the extract. Dilute the extract in ethyl ether on the basis of 1 ml ethyl ether per 60 mg of extract.

Activate the thin layers by bringing them to 130 º C for three hours. Leave to cool in a desiccator containing silica gel. Plates which are not used immediately can be preserved in the same desiccator.

Apply, drop by drop, 20 microlitres of the clear solution to form a band of constant width and 3 cm in length on a layer preferably newly activated. Let the solvent evaporate.

Develop the chromatogram under normal temperature with carbon tetrachloride using a chromatographic container the walls of which are covered with filter paper soaked in solvent. After about an hour the solvent will reach a height of 18 cm. Remove the plate and leave the solvent to evaporate in the open. For better separation of the bands, develop the chromatogram a second time. Again let the solvent evaporate in the open.

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Spray the thin layer of silica gel with a solution of 20 % phosphomolybdic acid in ethyl alcohol. The colour of the layer must be a uniform yellow. Develop the bands by the heating the sprayed plate at 110 ºC for five minutes.

IV. **Interpretation of the chromatogram**

If the chromatogram shows a single main brightly coloured band with an Rf of about 0,4 to 0,5, the wheat used for the manufacture of the pasta in question is durum wheat. If, on the other hand, two main bands of equal brightness appear, the raw material used is common wheat. Mixtures of durum wheat and common wheat can be assessed by an evaluation of the relative brightness of the two bands.

If there are three bands (two bands at the height where the main bands for common wheat are to be found, with a further band between them) eggs have been added to pasta. In this case, the raw material used is durum wheat if the middle band is brighter than the upper band. On the other hand, if the upper band is brighter than the middle band, the raw material used is common wheat.