12. Chemical Methods

Determination of specks content in carob bean gum (DBG) – draft

0 Introduction

The method is adopted from the procedure for determination of scorched particles in caseins and caseinates (FIL/IDF Int. Standard 107A: 1995)

1 Scope and field of application

This standard specifies a method for the determination of the speck particles of carob bean gum (CBG).

2 Definition

For the purpose of this standard, the following definition applies.

Speck content of CBG: The amount of coloured residue, per 0.2 g of sample, which is insoluble in a sodium acetate solution containing Gamanase, as determined and classified by the procedure specified in this standard.

3 Principle

Dissolution of a test portion in sodium acetate buffer at 60°C, addition of Gamanase , incubation at 60°C for 1 hour, filtration of the solution through a filtering disk, and visual comparison of the dried disk with speck standard disks.

4 Reagents

The reagents shall be of recognised analytical quality. The water used in the procedure shall be distilled water.

4.1 0.1 M sodium acetate buffer, pH 5.0: Dissolve 8.2g sodium acetate in 500 ml distilled water, adjust pH with acetic acid to pH 5.0, dilute to 1000 ml.

4.2 Gamanase 1.5L (1,500,000 VHCU/g.) from Novo Nordisk

5 Apparatus

Usual laboratory equipment and, in particular, the following.

5.1 Balance, accurate to 0.01g.

5.2 Conical flask, of capacity of 200 ml.

5.3 Measuring cylinder, 50 to 100 ml.

5.4 Pipette, 200µl

5.5 Water bath, capable of being controlled at 60°C±1°C

5.6 Filtering disks: diameter of 55 mm (Schleicher & Schuell filter paper 5892, 82 g/m^2, or equivalent, suitable for use in the filtering device (5.7).

5.7 Filtering device, aspirator or pressure type, with a filtering area of diameter 36 mm.

5.8 Speck standard disks, indicating increasing speck content by the classification letters A, B, C, D, E, and F, respectively. (Instructions for the preparation of these standard disks are given in Annex A)

6 Procedure

6.1 Preparation of test sample: Add 0.2g CBG sample to 100 ml 0.1 M sodium acetate buffer (4.1) heated in the water bath (5.4), controlled at 60°C until the test portion is dissolved, add 50µl Gamanase 1.5L (4.2) to the solution, stir periodically during the 1 hour incubation at 60°C, cover the flask.

Filtration: Filter the test solution through filtering disk (5.6) mounted in the filtering device (5.7).

Rinse the flask with 2 successive 50 ml portions of water, allowing the rinsing to run down the walls of the filtering device.

Remove the filtering disk and allow it to dry, or dry it at 30-40°C, protected from dust.

7 Expression of Results

7.1 Evaluation: Compare the test disk with the speck standard disk, and assign the appropriate classification letter to the test disk.
A test disk falling between two standard disks shall be assigned the classification letter corresponding to the higher specks content.

7.2 Repeatability: Two single results obtained on identical test material by one analyst using the same apparatus within a short time interval, shall indicate the same classification.

8 Test report

The test report shall show the method used and the result obtained. It shall also mention any operating conditions not specified in this standard method as well as any circumstances that may have influenced the results.

The report shall include all details required for complete identification of the sample.

Annex A

Preparation of speck standard disks

A.1 Materials

A.1.1 Sodium acetate buffer (see 4.1).

A.1.2 Gamanase 1.5L (see 4.2).

A 1.3 CBG powder.

A 2 Apparatus

Usual laboratory equipment and, in particular, the following.

A.2.1 Balance, accurate to 0.0001 g.

A.2.2 Desiccator, provided with an efficient desiccant.

A.2.3 Measuring cylinders, capacity of 100 and 500 ml, respectively.

A.2.4 Conical flask, of capacity 1000 ml.

A.2.5 Pipette,(see 5.4)

A.2.6 Filtering disks (see 5.6)

A.2.7 Filtering device (see 5.7)

A.2.8 Filtering glass Buchner funnel G3, ∅14 cm

A.2.9 Grinding device, ceramic mortar for laboratory use.

A 2.10 Sieve, of Nylon cloth, nominal aperture size of 200µm.

A.3 Procedure

A.3.1 Collection of specks particles: Add 1 g CBG sample to 1000 ml 0.1 M Sodium acetate buffer (4.1) heated to 60°C in a conical flask, stir the solution, heat in the water bath, controlled at 60°C until the test portion is dissolved, add 200µl Gamanase 1.5L (4.2) to the solution, stir periodically during the 1 hour incubation at 60°C, cover the flask.

Filter the CBG solution through the Buchner funnel (A.2.8), mounted in the filtering device (5.7).

Rinse the flask with 2 successive 100 ml portions of water, allowing the rinsing to run down the walls of the filtering device.

Allow the funnel to dry at 35°C, protected from dust, collect the dried specks.

Grind the dried specks and pass them through the sieve (A.2.10), collect the passing through fraction and store it in the desiccator.

A.3.2 Standard dispersion with specks: Weigh portion of 5mg, 10mg, 20mg, 30mg, 40mg and 50mg of dried specks (A.3.1), respectively. Disperse each of the portion in 100 ml sodium acetate buffer (4.1).

A.3.3 Standard disks: In turn, sonicate each of the dispersions prepared as described in A.3.2 until the dispersion looks homogeneous and immediately filter through a filtering disk (5.6) mounted in the filtering device (5.7). Rinse the vessel in which the dispersion was prepared, passing the rinsing through the filter disk. Dry the disks at room temperature.

A.3.3.1 If difficulties are encountered in passing the solution through a disk, or if a significant quantity of gelatinous material appears on this disk, repeat the relevant procedure by adding 50µl of Gamanase (4.2) to the dispersion, incubate it at 60°C for 1 hour.

iccator provided with efficient drying agent (silica