12 Chemical Methods

Determining of loss on drying (volatile matter) in CBG (INEC-TC)

1 Principle of the method
A known amount of carob bean gum (CBG) is dried at a constant temperature to constant weight. The difference between weight before and after drying, in percent, is defined as loss of drying.

2 Apparatus and auxiliary agents
2.1 Analytical balance, sensitivity 0.1 mg
2.2 Desiccator provided with efficient drying agent (silica gel with hygrometric indicator)
2.3 Drying oven maintaining a constant temperature of 102 to 105 °C, +/- 2 °C
2.4 Non-corrosive, flat dishes about 2 cm deep and about 6-8 cm in diameter with well fitting lids.

3 Procedure
3.1 Dry the dish and place it in the in the oven for 30 minutes
3.2 Allow the dish to cool to room temperature with lid on in the Desiccator and weigh
3.3 Place about 5 g CBG into the dish, cover the dish and weigh.
3.4 Dry the dish in the drying oven for 3 to 5 hours. The lid shall be placed beside the dish
3.5 Cover the dish with the lid, allow to cool to room temperature in the Desiccator and weigh

4 Expression of result
4.1 Method of calculation:
% Loss of weight = \( \frac{\text{weight before drying} - \text{weight after drying}}{\text{weight before drying}} \times 100 \)

4.2 Accuracy of determination
The maximum deviation between duplicate determinations should not exceed 0.05 % of loss of weight.

Determination of loss on drying in carob kernels (INEC-TC)

1 Preparation
Approximately 10g of kernels are cut in halves using side cutting pliers (“Seitenschneider-Zange”) or another suitable device

Cutting in halves with pliers is better than grinding because of the possible loss during drying of other volatile substances than water. The further procedure is the same as with CBG.
**Determination of ash content (JECFA)**

[“Compendium of food additive specifications”, Addendum 1, FAO Food and Nutrition Paper 52 (1992)]

1. **Principle of the method**

A known amount of carob bean gum (CGB) is dried, charred, and ignited. Ignition is continued until constant weight. The weight after ignition constitutes the weight of the ash.

2. **Apparatus and auxiliary agents**

   2.1 Analytical balance, sensitivity 0.1 mg
   2.2 Platinum or porcelain or silica crucible of suitable quality, about 50 mm in diameter and 25 mm deep.
   2.3 Bunsen burner, tripod and pipe-clay triangle
   2.4 Muffle furnace regulated at 800 °C
   2.5 Desiccator with suitable drying agent

3. **Procedure**

   3.1 Dry the crucible in the furnace at 800 °C for 30 min
   3.2 Allow the crucible to cool to room temperature in the desiccator and weigh.
   3.3 Weigh 2.0 to 2.5 g of CGB accurately into the crucible.
   3.4 Char carefully over the Bunsen burner (ignite cautiously to avoid spattering).
   3.5 When the flame ceases, complete ignition in muffle at 800 °C until a white ash remains (for 3 to 4 hours).
   3.6 Allow the crucible to cool in the desiccator and weigh.

4. **Expression of result**

Method of calculation:

\[
\text{% Ash content} = \frac{\text{weight of ash} \times 100}{\text{weight of sample}}
\]

Accuracy of determination: The maximum deviation between duplicate determinations should not exceed 0.15 % ash.
**Determination of acid insoluble (AIR) fibre (JECFA)**

[“Compendium of food additive specifications”, Addendum 1, FAO Food and Nutrition Paper 52 (1992)]

1 **Principle of the method**

A weighed amount of sample is digested for 6 hrs. with 0.4 N H$_2$SO$_4$ at the boiling point. The acid insoluble material is separated, dried and weighed.

2 **Reagents**

The following reagent is required:

2.1 Sulphuric acid, H$_2$SO$_4$, 96.0 %

3 **Apparatus**

3.1 Glass beaker, of capacity 400 ml

3.2 Balance, capable of weighing to an accuracy of 0.1 mg

3.3 Stirring aids, a glass rod and rubber policeman

3.4 Watch glass

3.5 Filter aid, Celite (washed)

3.6 Tarred filter crucible with fritted disc (e.g. Buchner or Schott No. IG 3)

3.7 Drying oven maintaining a constant temperature of 105-110°C

3.8 Desiccator provided with efficient drying agent (silica gel with hygrometric indicator)

4 **Procedure**

4.1 Transfer about 2 g of the gum to a 400 ml beaker and record the weight of sample to the nearest 0.001 g. Add 150 ml of 0.4 N H$_2$SO$_4$ (diluting 22.2 ml of 96% H$_2$SO$_4$ to 2000 ml with distilled water, while stirring with a rubber-tipped glass stirring rod), mark the level of the liquid in the beaker.

4.2 Cover beaker with a watch glass and place on a boiling water bath for 6 hours.

4.3 Stir the solution about every hour and rub down wall of beaker, add hot distilled water if necessary to replace evaporated liquid.

4.4 After 6 hours stir in exactly 0.500 g of Celite (filter aid) and filter by suction the hot solution through a tarred filter crucible with fritted disc

4.5 Wash beaker and residue on crucible with hot distilled water until filtrate is free of acid.

4.6 Dry filter crucible at 105-110°C (about 3 hours) to constant weight, cool and weigh

5 **Expression of result**

5.1 Calculation of acid insoluble residue (A.I.R):

\[
\% \text{A.I.R.} = \frac{(A - B) \times 10}{\text{weight of sample}}
\]

where

A= Weight of crucible + 0.500 g Celite + residue (A.I.R.)

B=Weight of crucible + 0.500 g Celite

6 **Precision**

6.1 Repeatability:

6.2 Reproducibility:
1 Scope
Applicable to nitrate-free samples; also to flour, wheat and other grains, cereal adjuncts, yeast foods, and animal feeds.

2 Apparatus
2.1 Kjeldahl flasks, Pyrex or equiv., 800 ml capacity; used for both digestion and distillation (dissn.)
2.2 Digestion heaters, 600 W (more or less, depending on voltage). Heater unit should bring to vigorous boil 250 ml water at 25°C in 5 min with hot burners.
2.3 Digestion unit; consists of electric heaters, large lead tube, and plastic fume stack with suction fan capable of exhausting toxic fumes to outside air.
2.4 Distn unit (see footnote); to consist of Iowa State-type connecting bulbs (traps) 36 x 100 mm, Pyrex glass condenser tubes, pure gum-rubber stoppers and tubing, electric heating units (600 W), and condenser tubes capable of being kept cool with adequate amounts of cool water during distn and with thermo-water control on stills. Upper ends of bulbs connect with high-quality rubber tubing to condenser tubes; lower ends with rubber stoppers to 800 ml distn flask. Lower ends of condenser tubes have rubber-connected glass or polyethylene tubes that lead to:
2.5 Receiving bottles or flasks, 300 ml capacity.
2.6 Proper burettes for dispensing (a) conc. H₂SO₄, (b) caustic soda, (c) 0.1 N H₂SO₄, and (d) 0.1 N NaOH. (See 6.1)

3 Reagents
3.1 H₂SO₄, conc. (95-98%, nitrogen-free; sp gr 1.84).
3.2 Catalyst; 15 g potassium sulphate, 0.04 g anhyd. CuSO₄, 0.5-1.0 g Alundum granules (see 6.2).
3.3 Anti-bumping agent; zinc metal, 20 mesh; pumice stone or Alundum, 8-14 mesh. Can be combined in catalyst mixt.
3.4 NaOH, pellets or soln., nitrate-free. For soln., dissolve approx. 450 g solid NaOH in 1 L water. Sp gr of soln should be 1.36 or more.
3.5 Methyl red indicator. Dissolve 1 g in 200 ml alc. (95%). Other indicators may be used satisfactorily. (See 6.3).
3.6 Std NaOH, 0.1 N. Weigh 73 g NaOH per 18 L water and stdze. May be stdze by titration against pure acid potassium phthalate (NBS SRM for acidimetry 84 is recommended) dissolved in CO₂-free water, using phenolphthalein as indicator; 0.5108 g will neutralise 25 ml 0.1000 N NaOH. Other recognised stdze methods may be used. (See 6.4).

3.7 Std H₂SO₄, 0.1 N. Add 50.4 ml H₂SO₄ (reagent grade, sp gr 1.84) to 18 L water. Titrate against std NaOH and adjust as necessary, using methyl red as indicator. Other recognised stdze procedures may be used. (See 6.4 and 6.5).

4 Procedure
4.1 Weigh quickly and accurately well-mixed and finely ground sample. Bread, 2 g prep’d by Method 6205; yeast foods, 0.5 g; wheat and other grains, feeds and feed stuff, 1.0 g. Place in digestion flask. (Sample may be placed in nitrogen-free paper to prevent clinging to sides of flask.) Add catalyst (reagent 3.2) and 20 ml conc. H₂SO₄ to flask. Add addnl 1.0 ml H₂SO₄ for each 0.1 g fat or 0.2 g other organic matter if sample wt is over 1.0 g. Heat flask at specified rate until dense white fumes clear bulb of flask, swirl gently, continue heating addnl 90 min. (See Note 6.2) Remove and cool but do not allow to crystallise.
4.2 Add 25 ml std acid to 300 ml bottle or flask, dil to 50 ml, add indicator, and immerse tip of condenser tube in this receiver soln. Add 250-275 ml tap water to cool digestion flask. Add 2-3 drops of tributyl citrate to distn flask to reduce foaming; add another 0.5-1.0 g Alundum granules. Gently add 50 ml conc. NaOH, connect to condenser with tight-fitting rubber stopper, and swirl. Boil until all ammonia has distilled (at least 150 ml of distillate) and then set receiving bottle down so that condenser tube is completely drained.
4.3 Titrate distillate with std NaOH soln to neutrality, using methyl red indicator.
4.4 Run blank detn using all ingredients except sample.

5 Calculations
\[
\text{% Protein} = \frac{(B - S) \times N \times 1.4007 \times f}{\text{sample weight(g)}}
\]
where
\( B = \text{ml alk back-titration of blank} \)
\( S = \text{ml alk back-titration of sample} \)
\( N = \text{normality of alk} \)
\( f = 5.7 \) for bread, wheat, and wheat flour, \( f = 6.25 \) for other grains, \( f = 6.38 \) for milk products, and \( f = 6.25 \) for samples of unknown source.

Crude protein – improved Kjeldahl method, copper catalyst modification
6 Notes and Precautions

6.1 In routine testing of large no. of samples, use large dispensing burettes for conc. acid and alk and for receiver acid, which may contain indicator.

6.2 As a catalyst, copper sulphate is recommend-ed as less hazardous than either mercury or selenium, or their compounds. Kane (Ref. 4) stresses that specific parameters of time, heat input, and salt-acid ratio are important. Adequate exhaust ventilation must be pro-vided in digestion-distn area.

6.3 Mixed indicator consisting of 0.75 g methyl red and 0.625 g Guinea green per L or 0.75 g methyl red and 0.5 g methylene blue (Ref. 9) dissolved in 300 ml alc may be used. Any indicator used should have sharp end point and distinct color change.

6.4 Rodkey (Ref. 9) has successfully applied tris (hydroxymethyl) aminomethane as a convenient primary std for direct stdze of acid solns.

6.5 Reeder and Patton (Ref. 8) suggested use of reagent-grade sodium acid sulphate (NaHSO₄ • H₂O) in water to make std soln equiv. to std H₂SO₄, 13.81 g/L will give 0.1000 N soln.

6.6 To check entire protein method, digest 0.1 g pure ammonium oxalate (monohydrate) with 1 g pure sugar using regular procedure. Resulting protein should be 11.24 % calcd as follows: (NH₄)₂C₂O₄ • H₂O with mol wt of 142.12 contains 28.016 g nitrogen or 19.713 %; 19.713 % x factor 5.7 : 10 (0.1g sample) = 11.24 % protein.

7 References

Crude protein – Kjeldahl method, boric acid modification

1 Scope
Applicable to wheat and flour mill products only.

2 Apparatus
2.1 Kjeldahl flasks, Pyrex, 800 ml capacity; used for both digestion and distillation (distn).
2.2 Digestion heaters, 600 W (more or less, depending on voltage). Heater unit should boil 250 ml water starting at 25°C in 5 min with hot burners.
2.3 Digestion unit; consists of electric heaters, large lead tube, fume stack (plastic), and suction fan capable of exhausting toxic fumes to outside air.
2.4 Distn unit (see footnote): to consist of Iowa State type connecting bulbs (traps) 36 x 100 mm, Pyrex glass condenser tubes, pure gum-rubber stoppers and tubing, electric heating units (600 W), condenser tubes capable of being kept cool with adequate amounts of cool water during distn and with thermo-water control on stands. Upper ends of bulbs or traps connect with high quality rubber tubing to condenser tubes and lower ends with rubber stoppers to 800 ml distn flask. Lower ends of condenser tubes have rubber-connected glass or polyethylene tubes that lead to:
2.5 Receiving bottles or flasks, 300 ml capacity.
2.6 Proper burettes for dispensing (a) conc. H₂SO₄, (b) conc. NaOH, (c) boric acid-indicator soln, and (d) class A burette for dispensing 0.1000 N H₂SO₄. (See 5.1).

3 Reagents
3.1 H₂SO₄, conc., 93-98 %, nitrogen-free.
3.2 Catalyst. Polyethylene packets contg 15 g potassium sulphate, 0.7 g mercuric oxide, and approx. 0.10 g pumice stone. (See 5.2).
3.3 Anti bumping agent. Either zinc metal, 20 mesh, or pumice stone (if pumice is not already combined in catalyst mix).
3.4 NaOH, pellets or soln, nitrate-free. For soln, dissolve approx. 450 g solid NaOH in 1 L water. (Sp gr of soln should be 1.36 or more.) Since mercury is used as catalyst, add 80 g sodium thiosulphate per L to NaOH soln to ppt mercury.
3.5 Methyl red-methylene blue indicator. Mix 2 parts 0.2 % alc methyl red soln with 1 part 0.2 % alc methylene blue soln. Other indicators may be used satisfactorily. (See 5.3).
3.6 Std H₂SO₄, approx. 0.1 N but accurately stdze (See 5.4).
3.7 Boric acid-methyl red-methylene blue receiver soln. Add 360 g boric acid (crystals) and 48 ml methyl red-methylene blue indicator (reagent 3.5) to 18 L water. (See 5.5).

4 Procedure
4.1 Weigh quickly and accurately 1 g finely ground sample. Place in digestion flask. (Sample may be placed in nitrogen-free paper to prevent clinging to sides of flask.) Add polyethylene packet of catalyst, or equiv., and 25 ml conc. H₂SO₄ to flask (reagent 3.1). Digest till soln is clear and then 30 min longer; remove and cool but do not allow to crystallise.
4.2 Place 300 ml bottle or flask contg 50 ml boric acid-methyl red-methylene blue indicator soln (reagent 3.7) under condenser tube with tip of condenser tube immersed under surface of soln. Add to original flask that is cooling 250-300 ml tap water and anti bumping agent, if not previously added. Gently add 50 ml conc. NaOH (reagent 3.4), connect to condenser with tight fitting rubber stopper, and swirl. Boil until all ammonia has dist (at least 150 ml of distillate), and then set receiving bottle down so that condenser tube is completely drained.
4.3 Titrate distillate to neutrality with std 0.1 N H₂SO₄, using boric-methylene blue indicator soln (reagent 3.7) under condenser tube with tip of condenser tube immersed under surface of soln. Add to original flask that is cooling 250-300 ml tap water and anti bumping agent, if not previously added. Gently add 50 ml conc. NaOH (reagent 3.4), connect to condenser with tight fitting rubber stopper, and swirl. Boil until all ammonia has dist (at least 150 ml of distillate), and then set receiving bottle down so that condenser tube is completely drained.
4.4 Run blank detn periodically, using all ingredients except sample. Corr burette reading for nitrogen in reagents as shown by blank.

5 Notes
5.1 In routine testing of large no. of samples, use large dispensing burettes for conc. acid and alk and Schellbach automatic zero burets at titration table.
5.2 Precaution: Copper sulphate is recommended as a less hazardous catalyst than either mercury or selenium, or their compounds (see Ref. 4). Adequate exhaust ventilation must be provided in digestion-distn area. With mercury as catalyst and 40 min digestion time, use polyethylene packets contg 9.9 g potassium sulphate, 0.41 g mercuric oxide, 0.08 g copper sulphate, and approx. 0.10 g pumice stone.
5.3 Mixed indicator consisting of 0.75 g methyl red and 0.625 g Guinea green per L or 0.75 g methyl red and 0.5 g methylene blue (Ref. 8) dissolved in 300 ml alc may be used. Any indicator used should have sharp end point and distinct colour change. Use 35 ml to 18 L bottle.
5.4 Rodkey (Ref. 8) has successfully applied tris (hydroxymethyl) aminomethane as a convenient primary std for direct stdn of acid solns.
5.5 For proteins higher than 22 %, use 720 g (4 %) boric acid crystals and 48 ml methyl red-methylene blue indicator to 18 L bottle of water.
5.6 To check entire protein method, digest 0.1 g pure ammonium oxalate (monohydrate) with 2 g pure sugar using regular procedure. Resulting protein
should be 11.24 % calcd as follows: (NH\textsubscript{4})\textsubscript{2}C\textsubscript{2}O\textsubscript{4} • H\textsubscript{2}O with mol wt of 142.12 contains 28.016 g nitrogen or 19.713 %; 19.713 % x 5.7 = 10(0.1g sample) = 11.25 % protein.

5.7 It is best that Boric Acid modification be used in air-conditioned laboratory (Ref. 5). Ammonia may be lost if contents of receiver flasks exceed 40 °C.

5.8 Use of 0.1253 N H\textsubscript{2}SO\textsubscript{4} simplifies calculation by making % protein equal to the ml of 0.1253 N H\textsubscript{2}SO\textsubscript{4} used for titrating sample, minus the ml used for titrating blank.

Calculation

\[
\frac{\text{% Protein}}{\frac{\text{ml std H}_2\text{SO}_4 \times N \text{ of H}_2\text{SO}_4 \times 1.4007 \times 5.7}{\text{sample wt (g)}}}
\]

where: factor for wheat, flour, and bread = 5.7.

7 References


Viscosity of CBG (LBG)  
(INEC-TC)

1 Principle of the method
A gum solution is prepared at 1% concentration w/w. After completion of the mixing cycle the solution is placed in a water bath at 90°C (for cold water soluble dissolve at 25°C) equipped with a stirring device, 500 rpm. The solution is heated to 86°C, within 15 min and the temperature kept at 86-88°C for 10 min, while being stirred. After cooling to approx. 30°C the solution is placed in constant temperature water bath of 25°C until the solution has reached a temperature of 25°C and is air bubble-free. The viscosity is determined at 20 rpm with a Brookfield viscometer.

2 Reagents
The following reagents are normally required:
2.1 Distilled or de-ionised water, pH 6.0-7.0
2.2 Acetone (isopropanol)

3 Apparatus
3.1 Brookfield viscometer model RVO, RVF or RVT.
3.2 Balance, capable of weighing to an accuracy of ±0.1 mg
3.3 Water bath equipped with 500 rpm stirring device, wing stirrer blade of porcelain, type WETA 4401 or equivalent type of wing stirrer blade (it is important that the edges of the blades are rounded and not sharp).
3.4 Porcelain or stainless steel beaker. Height approx. 20 cm., diameter approx. 8 cm, the weight of beaker should be not less than 125 g.

3.5 Beaker, 600 ml capacity
3.6 Plastic spatula approx. 22 cm long, blade 2 cm wide.
3.7 Watch glass covers.
3.8 Constant temperature water bath or equivalent equipment, capable of maintaining the temperatures of the test solutions at 25°C ± 0.5°C.

4 Procedure
Transfer to 600 ml beaker 4 g of the sample and moisten thoroughly with about 4 ml of isopropanol.
4.2 Add, with vigorous stirring water to a total of 400 g and continue the stirring until the gum uniformly dispersed. An opalescent, slightly viscous solution is formed.
4.3 Heat the mixture in a boiling water bath for about 10 min. Cool again to 25°C.
4.4 Record viscosity with Brookfield viscometer after 30 min at 25°C.

5 Expression of result
5.1 Method of calculation: Use Brookfield instrument instructions
Viscosity in mPa.s = Value read on the scale x factor

6 Precision
The deviation in results of duplicate determinations should not exceed 3% of the arithmetic mean Value.
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 8.2 g 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 0.01 g.

5.2 Conical flask, of capacity of 200 ml.

5.3 Measuring cylinder, 50 to 100 ml.

5.4 Pipette, 200 µl

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/m², 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 0.2 g 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 50 µl Gamanase 1.5 L (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, Ø 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. The desiccator provided with efficient drying agent (silica...
Determination of “Hot”-Viscosity of CBG (LBG)  
(INEC-TC)

1  Principle of the method
A 1% (w/w) gum solution is prepared by dispersing the CBG(LBG) sample with Ethanol, adding distilled water, heating in an boiling water bath and cooling to 25°C. The viscosity of this solution is determined with a Brookfield Viscosimeter after 2 h.

2  Equipment and materials
2.1 Laboratory balance (0.01 g)
2.2 Boiling water bath
2.3 Thermostatised water bath at 25°C
2.4 Mechanical stirrer with INEC stirrer rod (A)
2.5 Brookfield Viscosimeter, at 20 rpm with spindle 3
2.6 Stop watch
2.7 Glass beaker 600 ml, wide form, diameter 9 cm. (A, B)

3  Reagents
3.1 Ethanol 96 – 99.9%
3.2 Deionised or distilled water (pH 6 -7)

4  Procedure
4.1 Weigh the glass beaker with the stirrer rod and note the weight. (A )
4.2 Weigh 5.00 g CBG(LBG) into the tared beaker and moisten with 10 ml ethanol.
4.3 Add 495 ml distilled water of room temperature by shaking the beaker and stirring by hand with the stirrer rod.
4.4 Start the stop watch.
4.5 Place the beaker in the boiling water bath. It is important that, without stirring the solution inside, the beaker and the water of the bath are on the same level. (C)
4.6 After exactly 25 min (on the stop watch) transfer the beaker with the stirrer to the thermostatised 25°C water bath.
4.7 Connect the stirrer rod again and start the stirrer.
4.8 After 105 min (on the stop watch) dry the beaker with the stirrer on the outside and weight it.
4.9 Replace the evaporated quantity with distilled water until the total weight of the content is 500.00 g
10 Put the beaker to the thermostatised water bath again, connect the stirrer rod again and start stirring.
4.11 After 120 min (on the stop watch) stop stirring, take out the beaker from the bath, remove the stirrer rod and measure the Viscosity on the Brookfield Viscosimeter using spindle 3 and speed 20 rpm. Take the reading after 30 seconds.

5  Reporting of the results
If necessary, the viscosity is calculated according to operation manual of the Brookfield Viscosimeter.
The Hot-Viscosity is expressed in mPa*s as 1% solution after 2 h, at 25°C, measured with a Brookfield Viscosimeter at 20 rpm with spindle 3

6  Remarks
A For routine work it is advisable to prepare a separate weight to calibrate the balance for each stirrer rod in use and to tare all the glass beakers and to engrave the weight on the beaker.
B For the heat transfer it is important to work with glass beaker defined under 2.7. In this way the surface in contact with the hot water in the boiling water bath is standardised.
C The position of the beaker is important for the surface in contact with the hot water.
D Select a stirrer speed between 60 rpm and 100 rpm to avoid sprinkling and air bubbles in the solution.
INEC stirring rod