GUAR GUM

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995). ADI "not specified", established at the 19th JECFA in 1975.

SYNONYMS Gum cyamopsis, guar flour; INS No. 412

DEFINITION Primarily the ground endosperm of the seeds from *Cyamopsis tetragonolobus* (L.) Taub., (Fam. *Leguminosae*) consisting mainly of polysaccharides of high molecular weight (50,000-8,000,000), composed of galactomannans; mannose: galactose ratio is about 2:1. The gum may be purified by washing with ethanol or isopropanol or dispersing in boiling water, followed by filtering, evaporation and drying.

C.A.S. number 9000-30-0

DESCRIPTION White to yellowish-white, nearly odourless, free-flowing powder

FUNCTIONAL USES Thickener, stabilizer, emulsifier

CHARACTERISTICS

IDENTIFICATION

Gel formation — Add small amounts of sodium borate TS to an aqueous dispersion of the sample; a gel is formed.

Viscosity — Transfer 2 g of the sample into a 400-ml beaker and moisten thoroughly with about 4 ml of isopropanol. Add, with vigorous stirring, 200 ml of water and continue the stirring until the gum is completely and uniformly dispersed. An opalescent, viscous solution is formed. Transfer 100 ml of this solution into another 400-ml beaker, heat the mixture in a boiling water bath for about 10 min and cool to room temperature. There is no substantial increase in viscosity (differentiating guar gum from carob bean gum).

Gum constituents (Vol. 4) — Proceed as directed under GumConstituents Identification (FNP 5) using 100 mg of the sample instead of 200 mg and 1 - 10 µl of the hydrolysate instead of 1 - 5 µl. Use galactose and mannose as reference standards. These constituents should be present.

Microscopic examination — Place some ground sample in an aqueous solution containing 0.5% iodine and 1% potassium iodide on a glass slide and examine under microscope. Guar gum shows close groups of round to pear formed cells, their contents being yellow to brown. (Carob bean gum contains long stretched tubiform cells, separated or slightly interspaced. Their brown contents are much less regularly formed than in guar gum).

PURITY

Loss on drying (Vol. 4) — Not more than 15.0% (105^{o}, 5 h)

Borate	Not detectable Dissolve 1 g of the sample in 100 ml of water. The solution should remain fluid and not form a gel on standing. Mix 10 ml of dilute hydrochloric acid with the solution, and apply one drop of the resulting mixture to turmeric paper. A brownish red colour, which upon drying becomes intensified and changes to greenish black when moistened with ammonia TS, should not be formed.
Total ash (Vol. 4)	Not more than 1.5%
Acid-insoluble matter (Vol. 4)	Not more than 7.0%
Protein (Vol. 4)	Not more than 10.0% Proceed as directed under Nitrogen Determination (Kjeldahl Method; FNP5). The percent of nitrogen in the sample multiplied by 6.25 gives the percent of protein in the sample
Ethanol and isopropanol	Not more than 1%, singly or in combination See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
Microbiological criteria (Vol. 4)	Total plate count: Not more than 5,000 cfu/g *E. coli:* Negative by test Salmonella: Negative by test Yeast and mould: Not more than 500 cfu/g

TESTS

PURITY TESTS

Ethanol and isopropanol

Principle
: The alcohols are converted to the corresponding nitrite esters and determined by *headspace gas chromatography* (see Volume 4).

Sample preparation
: Dissolve 100 mg of sample in 10 ml of water using sodium chloride as a dispersing agent if necessary.

Internal standard solution
: Prepare an aqueous solution containing 50 mg/l of n-propanol.

Standard alcohol solution
: Prepare an aqueous solution containing 50 mg/l each of ethanol and isopropanol.

Procedure

: Weigh 200 mg of urea into a 25-ml "dark vial" (Reacti-flasks, Pierce, Rockford, IL, USA, or equivalent). Purge with nitrogen for 5 min and then add 1 ml of saturated oxalic acid solution, close with a rubber stopper and swirl. Add 1 ml of sample solution, 1 ml of internal standard solution, and simultaneously start a stop watch (T=0). Swirl the vial and recap with an open screw cap fitted with a silicone rubber septum. Swirl until T=30 sec. At T=45 sec inject through the septum 0.5 ml of an aqueous solution of sodium nitrite (250 g/l). Swirl until T=70 sec and at T=150 sec withdraw through the septum 1 ml of the headspace using a pressure lock syringe (Precision Sampling Corp., Baton Rouge, Louisiana, USA, or equivalent.

Gas chromatography
: Insert syringe needle in the injection port; precompress the sample, then open the syringe and inject the sample.
Use the following conditions:
- Column: glass (4mm i.d., 90 cm)
- Packing: first 15 cm packed with chrompack (or equivalent) and the remainder with Porapak R 120-150 mesh (or equivalent)
- Carrier gas: nitrogen (flow rate: 80 ml/min)
- Detector: flame ionization
- Temperatures: injection port: 250º; column: 150º isothermal

Calculation
: Quantify the ethanol and isopropanol present in the sample by comparing the peak areas with the corresponding peaks obtained by chromatographing the headspace produced by substituting in the procedure 1 ml of Standard alcohol solution for 1 ml of sample solution.

Microbiological criteria (Vol. 4)

Total plate count
: Using aseptic technique, disperse 1 g of sample into 99 ml of phosphate buffer and use a Stomacher, shaker or stirrer to fully dissolve. Limit dissolving time to about 10 min and then pipette 1 ml of the solution into separate, duplicate, appropriately marked petri dishes. Pour over the aliquot of sample in each petri dish 12-15 ml of Plate Count Agar previously tempered to 44-46°. Mix well by alternate rotation and back and forth motion of the plates, allow the agar to solidify. Invert the plates and incubate for 48±2 h at 35±1°.
After incubation count the growing colonies visible on each plate and record the number of colonies. Take the average of both plates, and multiply by the sample dilution factor, 100. Where no colonies are visible, express the result as less than 100 CFU/g.

E. coli determination: The use of cellulase to degrade the gum sample prior to analysis is essential in order to avoid gelling of the gum during its addition to the enrichment broth. Prepare a 1.0% cellulase solution (1 g cellulase to 99 ml water) and sterilize by filtration through a 0.45 µm membrane. (Cellulase solution may be stored at 2-5° for up to two weeks.) Into a sterile tube containing 9 ml of sterile lauryl sulfate tryptose (LST) broth, aseptically add 0.1 ml of the sterile 1% cellulase solution. Add 1g gum sample to the tube and vortex vigorously to disperse the sample. Incubate the tube for 24-48 h at 35±1°. After 24 h, gently agitate the tube and examine for gas production, i.e., effervescence. Reincubate for an additional 24 hours if no gas evolution is observed. Examine a second time for gas. Perform the

following confirmation test on the presumptive positive (gassing) result.

Gently agitate the gassing LST tube and transfer one loopful of the suspension to a tube containing 10 ml of EC broth and a fermentation (Durham) vial. Incubate the EC tube for 24-48 h at 45.5±0.2°. After 24 h, examine for gas production; if negative, examine again at 48 h. Streak a loopful of the suspension from the gassing tube onto L-EMB agar. It is essential that one portion of the plate exhibit well-separated colonies. Incubate 18-24 h at 35°. Examine the plates for colonies typical of *E. coli*, i.e., dark-centered with or without metallic sheen. Select two presumptively positive colonies and transfer them to PCA agar slants for morphological and biochemical tests. Incubate PCA slants for 18-24 h at 35±1°, then perform a Gram stain on the culture.

If the culture is Gram-negative (short rods) perform *either* of the following two biochemical test schemes:

Scheme 1.1 *Tests for IMViC biochemical activity:*
a. Indole production: Inoculate a tube of tryptone broth and incubate 24 h at 35°. Test for indole by adding 0.2-0.3 ml Kovacs' reagent. Appearance of a distinct red colour in the upper layer is a positive test.
b. Voges-Proskauer-reactive compounds: Inoculate a tube of MR-VP broth and incubate 48 h at 35°. Transfer 1 ml to a 13 x100 mm tube. Add 0.6 ml alpha-naphthol solution and 0.2 ml 40% KOH, and shake. Add a few crystals of creatine. Shake and let stand 2 h. Test is positive if eosin pink colour develops.
c. Methyl red-reactive compounds: Incubate the MR-VP tube from the Voges-Proskauer test an additional 48 h at 35°. Add 5 drops of methyl red solution to each tube. A distinct red colour is a positive test. A yellow colour is negative.
d. Use of citrate: Lightly inoculate a tube of Koser's citrate broth; avoid detectable turbidity. Incubate at 35° for 96 h. Development of distinct turbidity is positive reaction.

Scheme 1.2 Using the growth from the PCA slants, re-inoculate a tube of LST broth containing a Durham vial and incubate at 35° for 48 h to verify that the isolate has the ability to produce acid and gas from the fermentation of lactose.

Interpretation: Cultures that (a) produce gas as a result of the inoculation of LST broth and subsequent incubation for 24-48 h at 35°, (b) appear as Gram-negative non-spore-forming rods, and (c) give IMViC patterns - (biotype 1) or -+- (biotype 2), are considered to be *E. coli*.

Scheme 2**.** Disperse any colony growth into a small volume of 0.85% saline. Confirmation of the identity of the bacterial growth by chemical tests is conveniently done using API 20E or Micro ID strips or equivalent systems. After completion of the tests, identify the organism from the Identification Manual of the system used and record the final result.

Salmonella determination: The use of cellulase to degrade the gum sample prior to analysis is essential in order to avoid gelling of the gum during its addition to the enrichment medium. Prepare a 1.0% cellulase solution (1 g

cellulase to 99 ml water) and sterilize by filtration through a 0.45 µm membrane. (The cellulase solution may be stored at 2-5° for up to two weeks.) Aseptically weigh 25 g of sample into a sterile beaker (250 ml) or other appropriate container. Into a sterile, wide mouth, screw-cap jar (500 ml) or other appropriate container, introduce 225 ml of sterile lactose broth and 2.25 ml sterile 1% cellulase solution. While vigorously stirring the cellulase/lactose broth with a magnetic stirrer, quickly transfer the 25 g sample through a sterile glass funnel into the cellulase/lactose broth. Cap jar securely and let stand 60 min at room temperature. Loosen the cap and incubate the container at 35±1° for 24±2 h.
Tighten lid and gently shake incubated sample mixture; transfer 1 ml mixture to 10 ml Selenite cystine (SC) broth and another 1 ml mixture to 10 ml Tetrathionate (TT) broth. Incubate 24±2 h at 35°. Mix (vortex, if tube) and streak a 3 mm loopful incubated TT broth on Bismuth sulfite (BS) agar, xylose lysine desoxycholate (XLD) agar, and Hektoen enteric (HE) agar. (Prepare BS plates the day before streaking and store in dark at room temperature until streaked.) Repeat with a 3 mm loopful of SC broth. Incubate plates 24±2 h at 35°. Continue as indicated on pages 221-226 of the Guide to Specifications, FAO Food and Nutrition Paper 5 Revision 2, Rome 1991, "Examine plates for presence of colonies _".

Yeasts and moulds
: Weigh 25 g of sample and add to 2475 ml of sterile 0.1% peptone water (prepared by adding 1 g of peptone to 1 liter of distilled water, mixing to dissolve peptone, and autoclaving at 121° for 15 min) while vigorously stirring with a magnetic stirrer. Stir until the sample is completely dissolved. This is a 1:100 dilution. Aseptically pipette 0.1 ml to each of 10 pre-poured, solidified CCPDA-D plates (see below). Spread inoculum evenly over the surface of the plates using a sterile, bent glass rod. Incubate plates in the upright position at 25° undisturbed for 5 days.

After incubation, count the growing colonies on each plate using a colony counter and record the total number of colonies present on the 10 plates. Separate the yeasts from the moulds according to their morphology and count them separately. Take the total number of colonies present in all 10 plates and multiply by 100 to obtain the CFU/g of sample. If none of the plates shows growth, express the result as less than 100 CFU/g.
CCPDA-D medium: First, prepare a 2% dichloran (2,6-dichloran-4-nitroaniline) stock solution in 95% ethanol. Then, to PDA, add a quantity of the dichloran stock solution sufficient to give a concentration of 2.5 mg/l. Add chloramphenicol to a concentration of 50 mg/l and autoclave at 121° for 15 min. Cool medium to about 50° and just before pouring the plates, add sufficient filter-sterilized chlortetracycline to give a concentration of 50 mg/l.