XANTHAN GUM

Prepared at the 53rd JECFA (1999) and published in FNP Add 7 (1999), superseding specifications prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998). ADI "not specified", established at the 30th JECFA in 1986.

SYNONYMS INS No. 415

DEFINITION A high molecular weight polysaccharide gum produced by a pure-culture fermentation of a carbohydrate with *Xanthomonas campestris*, purified by recovery with ethanol or isopropanol, dried and milled; contains D-glucose and D-mannose as the dominant hexose units, along with D-glucuronic acid and pyruvic acid, and is prepared as the sodium, potassium or calcium salt; its solutions are neutral.

C.A.S. number 11138-66-2

Assay Yields, on the dried basis, not less than 4.2% and not more than 5.4% of carbon dioxide (CO_2), corresponding to between 91.0% and 117.0% respectively of xanthan gum.

DESCRIPTION Cream-coloured powder

FUNCTIONAL USES Thickener, stabiliser, emulsifier, foaming agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water; insoluble in ethanol

Gel formation To 300 ml of water, previously heated to 80° and stirred rapidly with a mechanical stirrer in a 400-ml beaker, add, at the point of maximum agitation, a dry blend of 1.5 g of the sample and 1.5 g of carob bean gum. Stir until the mixture goes into solution, and then continue stirring for 30 min longer. Do not allow the water temperature to drop below 60° during stirring. Discontinue stirring, and allow the mixture to cool at room temperature for at least 2 h. A firm rubbery gel forms after the temperature drops below 40°, but no such gel forms in a 1% control solution of the sample prepared in the same manner but omitting the carob bean gum.

PURITY

Loss on drying (Vol. 4) Not more than 15% (105°, 2.5 h)

Ash (total) (Vol. 4) Not more than 16% after drying

Pyruvic acid Not less than 1.5%
See description under TESTS

Nitrogen (Vol. 4) Not more than 1.5%

Proceed according to the Kjeldahl method

Ethanol and isopropanol
Not more than 500 mg/kg, 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
Yeasts and moulds: Not more than 500 cfu/g
See also description under TESTS

TESTS

PURITY TESTS

Pyruvic acid

Sample preparation
Weigh 600 mg of the sample to the nearest 0.1 mg and dissolve in sufficient water to make 100 ml. Transfer 10.0 ml of the solution into a 50-ml glass-stoppered flask. Pipette 20 ml of N hydrochloric acid into the flask, weigh the flask, and reflux for 3 h, taking precautions to prevent loss of vapours. Cool to room temperature, and add water to make up for any weight loss during refluxing. Pipette 1.0 ml of a 1 in 200 solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid into a 30-ml separatory funnel, then add 2.0 ml of the sample solution, mix, and allow to stand at room temperature for 5 min. Extract the mixture with 5 ml of ethyl acetate, and discard the aqueous layer. Extract the hydrazone from the ethyl acetate with three 5-ml portions of sodium carbonate TS, collecting the extracts in a 50-ml volumetric flask. Dilute to volume with sodium carbonate TS and mix.

Standard preparation
Weigh 45 mg of pyruvic acid, to the nearest 0.1 mg, and transfer into a 500-ml volumetric flask. Dilute to volume with water, and mix. Transfer 10.0 ml of this solution into a 50-ml glass-stoppered flask, and continue as directed under "Sample preparation", beginning with "Pipette 20 ml of N hydrochloric acid into the flask".

Procedure
Determine the absorbance of each solution with a suitable spectrophotometer in 1-cm cells at the maximum of about 375 nm, using sodium carbonate TS as the blank. The absorbance of the "Sample preparation" is equal to or greater than that of the "Standard preparation".

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 stopwatch (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

: Using aseptic technique, disperse 1 g of sample in 99 ml of Lactose broth using either a Stomacher, shaker or stirrer to fully dissolve the sample. Limit the dissolving time to about 15 min and then lightly seal the container and incubate the broth for 18-24 h at 35±1°. Using a sterile pipette, inoculate 1 ml of the incubate into a tube containing 10 ml GN broth. Incubate for 18-24 h and then streak any GN broths showing positive growth or gas production onto duplicate plates of Levine EMB agar. Incubate the plates for 24±2 h at 35±1° and then examine for colonies typical of E. coli i.e. showing strong purple growth with dark centre and a green metallic sheen sometimes spreading onto the agar. Record any typical E. coli colonies as presumptive positive, otherwise negative. Streak any well isolated suspect colonies onto a plate of PCA and incubate for 18-24 h at 35±1°. Perform a Gram stain on any growth to confirm it is Gram negative. If so, disperse any colony growth into a small volume of 0.85% saline and perform chemical tests to confirm the identity of the bacterial growth. This can most conveniently be done by 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.

Media
GN Broth (Gram Negative Broth)
Peptone 20.0 g
Dextrose 1.0 g
Mannitol 2.0 g
Sodium citrate 5.0 g
Sodium deoxycholate 0.5 g
Potassium phosphate (dibasic) 4.0 g
Potassium phosphate (monobasic) 1.5 g
Sodium chloride 5.0 g
Make up to 1 litre with distilled or de-ionised water, pH 7.0±0.2 at 25°

Salmonella determination
: Using aseptic technique, disperse 5 g of sample into 200 ml of sterile lactose broth using either a Stomacher, shaker or stirrer to maximise dissolution over a 15 min period. Loosely seal the container and incubate at 35±1° for 24±2 h.

Tighten lid and gently shake incubated sample mixture; transfer 1 ml mixture to 10 ml selenite cystine broth and another 1 ml mixture to 10 ml tetrathionate broth. Incubate 24±2 h at 35°. Mix (vortex, if tube) and streak 3-mm loopful incubated selenite cystine 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 3-mm loopful of tetrathionate 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
: 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 15-20 ml of Potato dextrose agar (either acidified or containing antibiotic) previously tempered to 44-46°. Mix well by alternate rotation and back and forth motion of the plates, and allow the agar to solidify. Invert the plates and incubate for 5 days at 20-25°.
After incubation, count the growing colonies visible on each plate using a colony counter and record the number of colonies. Separate the yeasts from the moulds according to their morphology and count them separately. 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.

METHOD OF ASSAY

Proceed as directed in the test for Carbon Dioxide Determination by Decarboxylation (Volume 4) using 1.2 g of the sample accurately weighed.