Residue Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 86th Meeting 2018

CASSIA GUM

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CASSIA GUM

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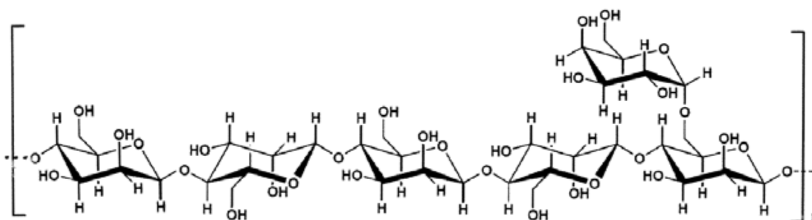
SYNONYMS INS 427

DEFINITION Cassia gum is obtained from the ground purified endosperm of the seeds of *Cassia tora* and *Cassia obtusifolia* (Fam. *Leguminosae*) containing less than 0.05% of *Cassia occidentalis*. It consists mainly of high molecular weight (approximately 200,000-300,000) polysaccharides composed of galactomannans with a mannose:galactose ratio of about 5:1. The seeds are dehusked and degermed by thermal and mechanical treatment followed by milling and screening of the endosperm. The ground endosperm is purified by extraction with isopropanol.

Structural formula



The structure above is provided for illustrative purposes. A specific repeat unit for Cassia gum cannot be defined.

Assay Not less than 75% of galactomannans

DESCRIPTION Pale yellow to off-white, odourless free-flowing powder. Forms colloidal solutions in cold water.

FUNCTIONAL USES Thickener, emulsifier, foam stabilizer, moisture retention agent, and texturizing agent.

CHARACTERISTICS

IDENTIFICATION

Solubility Insoluble in ethanol

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Gel formation with borate	Add sodium borate TS to an aqueous dispersion of the sample to raise the pH above 9; a gel is formed.
Gel formation with xanthan gum	Passes test See description under TESTS
Gum constituents (Vol. 4)	Proceed as directed under 'Gum Constituents Identification' using 100 mg of 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.
Viscosity	Less than 500 $\mathrm{mPa} \times \mathrm{s}$ See description under TESTS
pH (Vol. 4)	5.5-8.0 (1% solution)

PURITY

Loss on drying (Vol. 4)	Not more than 12% (105º , 5 h)
Total ash (Vol. 4)	Not more than 1.2%
Acid-insoluble matter (Vol. 4)	Not more than 2.0%
Protein (Vol. 4)	Not more than 7.0% Multiply percent nitrogen by 6.25.
Crude fat	Not more than 1% See description under TESTS
Starch	To a 1 in 10 dispersion of the sample add a few drops of iodine TS; no blue colour is produced.
Anthraquinones	Not more than 0.5 mg/kg See description under TESTS
Residual solvents (Vol. 4)	Isopropanol: Not more than 1.0%

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See description under TESTS

Lead (Vol. 4) — Not more than 1 mg/kg

Determine using a method 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 (under "General Methods, Metallic Impurities").

Microbiological criteria (Vol. 4) — Total plate count: Not more than 5,000 cfu/g

Yeast and mould: Not more than 100 cfu/g

E. coli: Negative in 1 g

Salmonella: Negative in 25 g

TESTS

IDENTIFICATION TESTS

Gel formation with xanthan gum — Weigh 1.5 g of sample, 1.5 g of xanthan gum and blend them. Add this blend (with rapid stirring) to 300 ml water at 80º in a 400 ml beaker. Stir until the mixture is dissolved and continue stirring for an extra 30 min after dissolution (maintain the temperature above 60º during the stirring period). Discontinue stirring and allow the mixture to cool to room temperature for at least 2 h.

A firm, viscoelastic gel forms after the temperature drops below 40º, but no such gel forms in a 1% control solution of cassia gum or xanthan gum alone prepared in a similar manner.

Viscosity — Add 495 ml of deionized water into a 1L beaker, insert a magnetic stir bar and place the beaker on a magnetic stirrer equipped with a heater. Adjust the stirrer speed to about 750 rpm. Weigh 5 g of sample and quickly add to the beaker. Switch on the heater and heat the beaker to reach 90º and keep it at 90º for 15 min. Cool the solution to room temperature (25º ±1.5º) in a water bath. Measure the viscosity at 25º, after 2 h, using a RVT Brookfield Spindle 1 and 20 rpm speed. Repeat the procedure with a sample of 5 g of carob (locust) bean gum. The viscosity of the cassia gum (150 – 500 $mPa \times s$) must be less than 50% that of carob bean gum (2000 - 3500 $mPa \times s$)

PURITY TESTS

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Residual solvents

Determine residual solvents using headspace gas chromatography (Vol. 4; Method I) under the following conditions.

Internal standard solution
Add 50.0 ml water to a 50 ml vial and seal. Accurately weigh and inject 15 µl of 3-methyl-2-pentanone through the septum and reweigh the vial to within 0.01 mg.

Standard solution
Add 50.0 ml water to a 50 ml vial and seal weigh accurately. Inject 15 µl isopropanol and reweigh the vial.

Blank solution:
Add 5.0 ml of water and pipette 1.0 ml of the internal standard solution into a headspace vial. Seal the vial and mix the contents using a vortex mixer.

Calibration solution:
Add 4.0 ml of water into the headspace vial. Pipette 1.0 ml each of the internal standard solution and the standard solution. Seal the vial and mix the contents using a vortex mixer.

Preparation of sample:
Pipette 5 ml of water and 1 ml internal standard solution into a headspace vial. Accurately weigh 0.500 ± 0.001 g of sample in a small weighing boat and add the sample carefully to prevent clumping of sample at the bottom of the vial. Seal the vial and mix the contents using a vortex mixer. Do not shake the sample vial.

Follow the procedure described in Vol. 4 for the determination of residual solvents.

Crude fat

Apparatus
The apparatus consisting of a Butt-type extractor, as shown below, having a standard-taper 34/45 female joint at the upper end, to which is attached a Friedrichs- or Hopkins-type condenser, and a 24/40 male joint at the lower end, to which is attached a 125-ml Erlenmeyer flask.

Procedure
Transfer about 10 g of the sample, previously ground to 20-mesh or finer and accurately weighed, to a cellulose thimble or a 15-cm filter paper (roll the paper tightly around the sample), and place it in a suitable extraction shell). Plug the top of the thimble or the extraction shell with cotton previously extracted with hexane, and place it in the extractor. Attach the extractor to a dry 125-ml Erlenmeyer flask containing about 50 ml of hexane and to a water-cooled condenser, apply heat to the flask to

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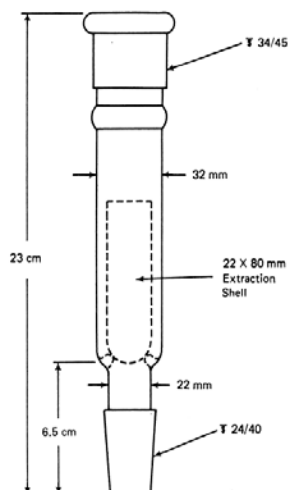
produce 150 to 200 drops of condensed solvent per min, and extract for 16 h. Disconnect the flask, and filter the extract to remove any insoluble residue. Rinse the flask and filter with a few ml of hexane, combine the washings and filtrate in a tared flask, and evaporate on a steam bath until no odour of solvent remains. Dry in a vacuum for 1 h at 100°, cool in a desiccator, and weigh.



Butt-Type Extractor for fat determination.

NOTE: The method for crude fat is referenced from Appendix X: Crude fat in the Food Chemicals Codex, 11th Edition, 2018. Reproduced from USP-NF with permission from The U.S. Pharmacopeial Convention (USP)

<u>Anthraquinones</u>

Principle

Anthraquinones are extracted with chloroform and determined by High Performance Liquid Chromatography (Vol.4) using the conditions below.

NOTE: Anthraquinones are photosensitive. Samples and standards shall be protected from light and all manipulations shall be carried out under the subdued light.

Standards and Reagents:

Emodin, Aloe-emodin, Physcion (1,8-dihydroxy-3-methoxy-6-methyl-anthraquinone), Rhein and Chrysophanic acid (>99%).
Internal standard: Danthrone (1,8-dihydroxy-anthraquinone, >99%)

Methanol, acetonitrile, deionized water, chloroform, trifluoroacetic acid, sulfuric acid and sodium hydrogen carbonate

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Individual stock standard and internal standard solutions (100 µg/ml)

Accurately weigh about 10 mg of the standards and internal standard, transfer to 100 ml volumetric flasks with about 5 ml of methanol, sonicate for 15 min and dilute to volume with methanol.
Store these solutions in amber coloured bottles at 4° (the solutions are stable for 2 weeks under these conditions).

Internal spike standard solution (20 µg/ml)

Dilute 2 ml of internal standard stock solution to 10 ml with methanol.

Mixed standard solution (10 µg/ml)

Transfer 1 ml of each of the anthraquinones stock standard solution into a 10 ml volumetric flask and dilute to volume with methanol.

Working standard solutions

To each of five 10 ml volumetric flasks transfer 0, 0.5, 1, 2 and 5 ml respectively of the mixed standard solution, and 1 ml of the internal spike standard solution (20 µg/ml), dilute to volume with methanol and mix.

Sample preparation:

Accurately weigh about 4.0 g of the sample into a 250 ml Erlenmeyer flask. Add 80 µl of internal standard solution (100 µg/ml), and 100 ml 2N H_2SO_4 to the flask. Stopper the flask using a PTFE stopper and heat at 103° for 3.5 hours in an oven. After cooling to room temperature, add 100 ml of chloroform and shake well. Allow phase separation. Evaporate 50 ml of the chloroform layer to dryness in a rotary evaporator at 68°. Dissolve the residue in 2 ml of methanol. Filter the solution through a PTFE membrane syringe filter.

Chromatographic conditions:

Column: Hypersil ODS C18 (250 mm x 4.6 mm ID, 5 µm)
Mobile phase:
(A) 0.1 % trifluoroacetic acid in water
(B) Acetonitrile
Injection volume: 20 µl

Gradient:

Time, min	% (A)	% (B)
0	86	14
10	86	14
15	80	20

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25	80	20
55	20	80
60	0	100
66	86	14

Flow rate: 1 ml/min
Detector: Photodiode Array/UV Detector operated at 435 nm.

Procedure

Inject individual standard solutions and internal standard solution (dilute, if required) and record retention times.

Construction of standard curves

Inject 20 µl of each working standard solution. Construct the standard curves by plotting the ratios of the peak areas of each of the specific anthraquinone / internal standard against the concentrations of each working standard solution (µg/ml).

Inject 20 µl of the Sample solution. Calculate the ratios of the peak areas of each anthraquinone / internal standard, and obtain the amount (A) of each anthraquinone from the respective standard curve.

Concentration of
anthraquinone in the sample $(\mu g/g) = (A \times 4)/W$

Where
A = the amount of each anthraquinone (µg) obtained from the standard curve
W = Mass of sample (g)
4 = Dilution factor for sample

METHOD OF ASSAY

$$\%\ \text{Galactomannans} = 100 - (L + A + I + P + F)$$

L % Loss on Drying
A %Total Ash
I %Acid-Insoluble Matter
P %Protein
F %Crude Fat

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