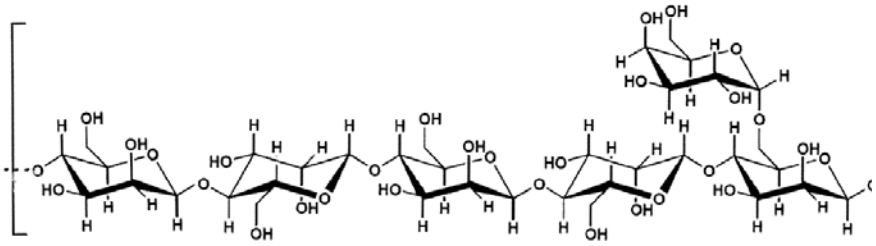
CASSIA GUM

Prepared at the 73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010), superseding tentative specifications prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009). An ADI "not specified" was established at the 71st JECFA (2009).

SYNONYMS INS 427

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

Structural formula



Assay Not less than 75% of galactomannan

DESCRIPTION Pale yellow to off-white, odourless free-flowing powder

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

CHARACTERISTICS

IDENTIFICATION

Solubility
Insoluble in ethanol
Disperses well in cold water forming colloidal solutions.

Gel formation with borate
Add sufficient amounts of sodium borate TS to an aqueous dispersion of the sample sufficient to raise the pH to 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 (Vol. 4) 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 mPas (25º, 2h) (1% solution) See description under TESTS
pH (Vol. 4)	5.5-8.0 (1%)
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% Proceed as directed under Nitrogen Determination (Kjeldahl Method; Vol. 4). The percent of nitrogen in the sample multiplied by 6.25 gives the percent of protein in the sample.
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	Isopropanol: Not more than 1.0% See description under TESTS
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES 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 (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 the sample and 1.5 g of xanthan gum and blend them. Add this blend with (rapid stirring) into 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 process). Discontinue stirring and allow the mixture to cool at 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

Weigh 5 g of the sample in a plastic dish and 495 g of distilled water at 20º in a 1000 ml beaker. Add a magnetic bar and place the beaker on the agitation plate. Adjust the speed of agitation to 750 rpm. Introduce quickly the 5 g of sample in the water and cover the beaker with a watch glass. Keep the temperature at 90º for 15 min. Cool the solution at 25º (the cooling must be ±1.5º) in a water bath and measure the viscosity after 2 h at 25º using a RVT Brookfield Spindle 1, speed 20 rpm. Repeat the procedure with a sample of 5 g of carob (locust) bean gum.

(Note: The viscosity of the cassia gum (150 - 500 mPas) must be less than 50% that of carob bean gum (2000 - 3500 mPas))

PURITY TESTS

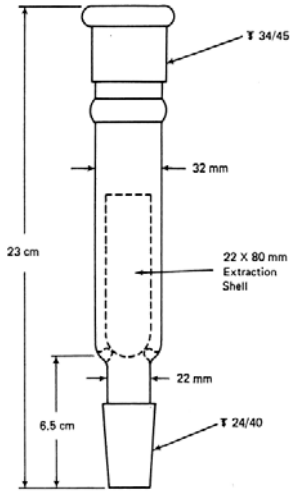
Crude fat

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 15-cm filter paper, roll the paper tightly around the sample, and place it in a suitable extraction shell. Plug the top of the shell with cotton previously extracted with hexane, and place the shell 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 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 odor 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 the Food Chemicals Codex, 6th Edition, 2008, p. 1163.
Reprinted with permission from the US Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD USA 20852.

Anthraquinones

Principle

The antraquinones are extracted with acetonitrile and determined by High Performance Liquid Chromatography (Vol. 4) using the conditions below.
NOTE: Samples and standards should be protected from light.

Standards

Emodin (EMO), Aloe-emodin (AEM), Physcion (PHY) or 1,8-dihydroxy-3-methoxy-6-methyl-anthraquinone, Rhein (RHE) and Chrysophanic acid (CHR).
Internal standard: Danthrone (DAN) or 1,8-dihydroxy anthroquinone.

Use HPLC grade methanol for the solutions.

Stock standard solutions (100 mg/l): For each of the specific anthraquinone standards and for the internal standard: accurately weigh about 1 mg (±0.01 mg) of the standard. Transfer to 10 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).

Mixed standard solution (10 mg/l):
Pipette 1 ml of each of the specific anthraquinone stock standard solutions into a 10 ml volumetric flask and dilute to volume with methanol.

Working standard solutions: To each of five 10 ml volumetric flask pipette 5, 2, 1, 0.5 and 0 ml respectively of the Mixed standard solution, pipette 1 ml of the Internal standard stock solution to each flask, mix and dilute to volume with methanol.

Sample preparation

Accurately weigh about 0.40 g of the sample into a 50 ml round-bottom flask. Add 20 ml trifluoroacetic acid and reflux at 70º for 4 hours. Cool the sample to ambient temperature and evaporate to dryness using a rotary evaporator. Add 3 ml of acetonitrile/$NaHCO_3$ (0.2%) (60:40 v/v) and sonicate for 30 min. Transfer the solution in a centrifuge tube and run it at 5000 rpm for 30 min. Filter the supernatant solution through an Extrulet column (Merck, NT1 or equivalent) previously neutralized with a pH 9.0 buffer. Pipette 900 µl of this filtered sample solution into a 2.5 ml volume vial and add 100 µl of the Internal standard stock solution and mix thoroughly.

Chromatographic conditions

Column: Hypersil C18 (250 mm x 4.6 mm ID, 5 µm) or equivalent
Mobile phase:
(A): 0.1% trifluoroacetic acid in water
(B): Acetonitrile (HPLC grade)
Injection volume: 50 µl
Run Time: 60 min
Gradient:

Min	% (A)	% (B)
0	86	14
10	86	14
15	80	20
25	80	20
55	20	80
60	0	100

Flow rate: 1 ml/min
Detector: Photodiode Array Detector. Quantification is performed at 435 nm

Standard curves

Inject 50 µl of each working standard solution and internal 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 (mg/l).

Procedure

Inject 50 µl of the Sample solution and the internal standard solution. Calculate the ratios of the peak areas of each specific anthraquinone / internal standard, and obtain the concentration (C) of each specific anthraquinone from the standard curves.

Calculate the percentage of each specific anthraquinone from:

$$\text{Anthraquinone (mg/kg)} = C \times 3 \times 1000 / (100 \times 0.9 \times W)$$

where
C is the concentration of specific anthraquinone (mg/l); and

W is weight of sample (g).

METHOD OF ASSAY The difference between 100 and the sum of the percent Loss on Drying, Total Ash, Acid-Insoluble Matter, Protein and Crude Fat represents the percent *Galactomannans*.