CAROB BEAN GUM (TENTATIVE)

Prepared at the 67th JECFA (2006) and published in FAO JECFA Monographs 3 (2006), superseding specifications prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999) and in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI "not specified" was established at the 25th JECFA (1981).

Information required on gum content, solubility in water and a test method to determine ethanol and isopropanol using capillary gas chromatography.

Note: The tentative specifications will be withdrawn unless the required information is received before the end of 2007.

SYNONYMS Locust bean gum, carob gum, INS No. 410

DEFINITION Primarily the ground endosperm of the seeds from *Ceratonia siliqua* (L.) Taub. (Fam. *Leguminosae*) mainly consisting of high molecular weight (approximately 50,000-3,000,000) polysaccharides composed of galactomannans; mannose:galactose ratio is about 4:1. The seeds are dehusked by treating the kernels with dilute sulfuric acid or with thermal mechanical treatments, followed by milling and screening of the peeled seeds to obtain the endosperm (native carob bean gum). The gum may be washed by ethanol or isopropanol to control the microbiological load (washed carob bean gum).

C.A.S. number 9000-40-2

Structural formula



DESCRIPTION White to yellowish white, nearly odourless powder

FUNCTIONAL USES Thickener, stabilizer, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in ethanol
Gel formation	Add small amounts of sodium borate TS to an aqueous dispersion of the sample; a gel is formed.
<u>Viscosity</u>	Transfer 2 g of the sample into a 400-ml beaker and moisten thoroughly with about 4 ml of isopropanol. Add 200 ml of water with vigorous stirring until the gum is completely and uniformly dispersed. An opalescent, slightly 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 an appreciable increase in viscosity.
Gum constituents (Vol. 4)	Proceed as directed under Gum Constituents Identification using 100 mg of the sample instead of 200 mg and 1 - 10 μ I of the hydrolysate instead of 1 - 5 μ I. Use galactose and mannose as reference standards. These constituents should be present.
Microscopic examination	Disperse a sample of the gum in an aqueous solution containing 0.5% iodine and 1% potassium iodide on a glass slide and examine under a microscope. 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 14.0% (105°, 5 h)
<u>Total ash</u> (Vol. 4)	Not more than 1.5%
Acid-insoluble matter (Vol. 4)	Not more than 4.0%
<u>Protein</u> (Vol. 4)	Not more than 7.0% Proceed as directed under nitrogen determination (Kjeldahl Method). The percentage of nitrogen determined multiplied by 6.25 gives the percent of protein in the sample
<u>Starch</u>	To a 1 in 10 dispersion of the sample add a few drops of iodine TS; no blue colour is produced
Ethanol and isopropanol	Not more than 1%, singly or in combination See description under TESTS
<u>Lead</u> (Vol. 4)	Not more than 2 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 methods described in Volume 4.
Microbiological criteria	Total plate count (Vol. 4): Not more than 5,000 CFU/g

E. coli: Negative in 1g See description under TESTS

Salmonella (Vol.4): Negative in 25 g

Yeasts and moulds (Vol. 4): Not more than 500 CFU/g

TESTS

PURITY TESTS

<u>Ethanol and isopropanol</u> Information required on a method using capillary gas chromatography to replace the method below.

Principle

The alcohols are converted to the corresponding nitrite esters and determined by *headspace gas chromatography.*

Sample preparation

Disperse 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.

<u>Standard alcohol solution</u> 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 dispersion, 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: 250°; column: 150° 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 E. coli determination The use of mannan endo-1,4-betamannosidase (EC 3.2.1.78) 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% mannosidase solution (1 g mannan endo-1,4-betamannosidase to 99 ml water) and sterilize by filtration through a 0.45 µm membrane. (The mannosidase 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% mannosidase 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 confirmation test for coliforms on the presumptive positive (gassing) result, according to the procedure in Volume 4.