

CARRAGEENAN

Prepared at the 79th JECFA (2014) and published in FAO JECFA Monographs 16 (2014), superseding specifications prepared at the 68th JECFA (2007), published in FAO JECFA Monographs 4 (2007). A group ADI “not specified” for carrageenan and processed *Eucheuma* seaweed was established at the 57th JECFA (2001).

SYNONYMS

Irish moss gelose (from *Chondrus* spp.); Eucheuman (from *Eucheuma* spp.); Iridophycan (from *Iridaea* spp.); Hypnean (from *Hypnea* spp.); Furcellaran or Danish agar (from *Furcellaria fastigiata*); INS No. 407.

DEFINITION

A substance with hydrocolloid properties obtained from certain members of the class *Rhodophyceae* (red seaweeds).

The principal commercial sources of carrageenans are the following families and genera of the class of *Rhodophyceae*:

Furcellariaceae such as *Furcellaria*
Gigartineae such as *Chondrus*, *Gigartina*, *Iridaea*
Hypnaeaceae such as *Hypnea*
Phylloporaceae such as *Phyllophora*, *Gymnogongrus*, *Ahnfeltia*
Solieriaceae such as *Eucheuma*, *Anatheca*, *Meristotheca*.

Carrageenan is a hydrocolloid consisting mainly of the ammonium, calcium, magnesium, potassium and sodium sulfate esters of galactose and 3,6-anhydrogalactose polysaccharides. These hexoses are alternately linked α -1,3 and β -1,4 in the copolymer. The relative proportions of cations existing in carrageenan may be changed during processing to the extent that one may become predominant.

The prevalent polysaccharides in carrageenan are designated as kappa-, iota-, and lambda-carrageenan. Kappa-carrageenan is mostly the alternating polymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose; iota-carrageenan is similar, except that the 3,6-anhydrogalactose is sulfated at carbon 2. Between kappa-carrageenan and iota-carrageenan there is a continuum of intermediate compositions differing in degree of sulfation at carbon 2. In lambda-carrageenan, the alternating monomeric units are mostly D-galactose-2-sulfate (1,3-linked) and D-galactose-2,6-disulfate (1,4-linked).

Carrageenan is obtained by extraction from seaweed into water or aqueous dilute alkali. Carrageenan may be recovered by alcohol precipitation, by drum drying, or by precipitation in aqueous potassium chloride and subsequent freezing. The alcohols used during recovery and purification are restricted to methanol, ethanol, and isopropanol. Articles of commerce may include sugars for standardization purposes, salts to obtain specific gelling or thickening characteristics, or emulsifiers carried over from drum drying processes.

C.A.S. number 9000-07-1

DESCRIPTION

Yellowish or tan to white, coarse to fine powder that is practically odourless.

FUNCTIONAL USES Thickener, gelling agent, stabilizer, glazing agent.

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in ethanol; soluble in water at a temperature of about 80°, forming a viscous clear or slightly opalescent solution that flows readily; disperses in water more readily if first moistened with alcohol, glycerol, or a saturated solution of glucose or sucrose in water.

Test for sulfate Dissolve a 100-mg sample in 20 ml of water (with heating if necessary), and add 3 ml of barium chloride TS and 5 ml of hydrochloric acid, dilute TS; filter if a precipitate forms. Boil the solution or the filtrate for 5 min. A white, crystalline precipitate appears.

Test for galactose and anhydrogalactose (Vol. 4) Proceed as directed in Vol.4 (under "General Methods, Organic Components, Gum Constituents Identification") using the following as reference standards: galactose, rhamnose, galacturonic acid, 3,6-anhydrogalactose, mannose, arabinose and xylose. Galactose and 3,6-anhydrogalactose should be present.

Identification of hydrocolloid and predominant type of copolymer Add 4 g of sample to 200 ml of water, and heat the mixture in a water bath at 80°, with constant stirring, until dissolved. Replace any water lost by evaporation, and allow the solution to cool to room temperature. It becomes viscous and may form a gel. To 50 ml of the solution or gel add 200 mg of potassium chloride, then reheat, mix well, and cool. A short-textured ("brittle") gel indicates a carrageenan of a predominantly kappa type, and a compliant ("elastic") gel indicates a predominantly iota type. If the solution does not gel, the carrageenan is of a predominantly lambda type.

Infrared absorption Passes test
See description under TESTS

PURITY

Loss on drying (Vol. 4) Not more than 12% (105° to constant weight)

pH (Vol. 4) Between 8 and 11 (1 in 100 suspension)

Viscosity Not less than 5 cp at 75° (1.5% solution)
See description under TESTS

Sulfate Not less than 15% and not more than 40% (as SO₄²⁻) on the dried basis
See description under TESTS

Total ash Not less than 15% and not more than 40% on the dried basis
See description under TESTS.

Acid-insoluble ash (Vol. 4) Not more than 1%
Use the ash from the Total ash test

<u>Acid-insoluble matter</u> (Vol. 4)	Not more than 2% Use 2 g of sample obtained from part (a) of the procedure for sulfate determination.
<u>Residual solvents</u> (Vol. 4)	Not more than 0.1% of ethanol, isopropanol, or methanol, singly or in combination See description under TESTS
<u>Microbiological criteria</u> (Vol. 4)	Initially prepare a 10 ⁻¹ dilution by adding a 50-g sample to 450 ml of Butterfield's phosphate-buffered dilution water and homogenising the mixture in a high-speed blender. Total (aerobic) plate count: Not more than 5000 cfu/g <i>Salmonella</i> spp.: Negative per test <i>E. coli</i> : Negative in 1 g
<u>Arsenic</u> (Vol. 4)	Not more than 3 mg/kg Determine using an AAS (Hydride generation technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").
<u>Lead</u> (Vol. 4)	Not more than 5 mg/kg Determine using an AAS (Electrothermal atomization technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").
<u>Cadmium</u> (Vol. 4)	Not more than 2 mg/kg Determine using an AAS (Electrothermal atomization technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").
<u>Mercury</u> (Vol.4)	Not more than 1 mg/kg Determine using AAS (Cold vapour generation technique). The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

IDENTIFICATION TESTS

<u>Infrared absorption</u>	Obtain infrared absorption spectra on the gelling and non-gelling fractions of the sample by the following procedure: Disperse 2 g of the sample in 200 ml of 2.5% potassium chloride solution, and stir for 1 h. Let stand overnight, stir again for 1 h, and transfer into a centrifuge tube. (If the transfer cannot be made because the dispersion is too viscous, dilute with up to 200 ml of the potassium chloride solution.) Centrifuge for 15 min at approximately 1000 x g. Remove the clear supernatant, resuspend the residue in 200 ml of 2.5% potassium chloride solution, and centrifuge again. Coagulate the
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combined supernatants by adding 2 volumes of 85% ethanol or isopropanol (NOTE: Retain the sediment for use as directed below). Recover the coagulum, and wash it with 250 ml of the alcohol. Press the excess liquid from the coagulum, and dry it at 60° for 2 h. The product obtained is the non-gelling fraction (lambda-carrageenan).

Disperse the sediment (retained above) in 250 ml of cold water, heat at 90° for 10 min, and cool to 60°. Coagulate the mixture, and then recover, wash, and dry the coagulum as described above. The product obtained is the gelling fraction (kappa- and iota-carrageenan).

Prepare a 0.2% aqueous solution of each fraction, cast films 0.5 mm thick (when dry) on a suitable non-sticking surface such as Teflon, and obtain the infrared absorption spectrum of each film. (Alternatively, the spectra may be obtained using films cast on potassium bromide plates, if care is taken to avoid moisture).

Carrageenan has strong, broad absorption bands, typical of all polysaccharides, in the 1000 to 1100 cm^{-1} region. Absorption maxima are 1065 and 1020 cm^{-1} for gelling and non-gelling types, respectively. Other characteristic absorption bands and their intensities relative to the absorbance at 1050 cm^{-1} are as follows:

Wave number (cm^{-1})	Molecular Assignment	Absorbance relative to 1050 (cm^{-1})		
		Kappa	Iota	Lambda
1220-1260	ester sulfate	0.3-1.4	1.2-1.7	1.4-2.0
928-933	3,6-anhydrogalactose	0.2-0.7	0.2-0.4	0-0.2
840-850	galactose-4-sulfate	0.2-0.5	0.2-0.4	-
825-830	galactose-2-sulfate	-	-	0.2-0.4
810-820	galactose-6-sulfate	-	-	0.1-0.3
800-805	3,6-anhydrogalactose-2-sulfate	0-0.2	0.2-0.4	-

PURITY TESTS

Sulfate

Principle

Hydrolysed sulfate groups are precipitated as barium sulfate.

Procedure

(a) Disperse an accurately weighed 15 g sample of commercial product into 500 ml of 60% w/w isopropanol/water at room temperature. Stir gently for 4 h. Filter through ash-free filter paper. Discard the filtrate. Wash the material remaining on the filter paper with two 15-ml portions of 60% isopropanol/water. Dry the material at 105° to constant weight. Approximately 1 g of the dried matter is to be used for part (b). The remainder should be retained for determination of Total ash, Acid-insoluble matter, and viscosity.

(b) Accurately weigh a 1 g sample (W_1) obtained from part (a). Transfer the sample to a 100-ml long-neck round-bottom flask. Add 50 ml of 0.2 N hydrochloric acid. Fit a condenser, preferably one with at least 5 condensing bulbs, to the flask and reflux for 1 h. Add 25 ml of a 10% (by volume) hydrogen peroxide solution and resume refluxing for about 5 h or until the solution becomes completely clear.

Transfer the solution to a 600-ml beaker, bring to a boil, and add dropwise 10 ml of a 10% barium chloride solution. Heat the reaction mixture for 2 h on a boiling water bath. Filter the mixture through ash-free slow-filtration filter paper. Wash with boiling distilled water until the filtrate is free from chloride. Dry the filter paper and contents in a drying oven. Gently burn and ash the paper at 800° in a tared porcelain or silica crucible until the ash is white. Cool in a desiccator.

Weigh the crucible containing the ash. Calculate the percentage sulfate from the weight in g (W_2) of the ash (barium sulfate) using the formula:

$$(W_2/W_1) \times 100 \times 0.4116$$

Total ash

Accurately weigh 2 g of the dried sample (W_1) obtained from part (a) under the procedure for sulfate determination above. Transfer to a previously ignited, tared silica or platinum crucible. Heat the sample with a suitable infrared lamp, increasing the intensity gradually, until the sample is completely charred; continue heating for an additional 30 min. Transfer the crucible with the charred sample into a muffle furnace and ignite at about 550° for 1 h. Cool in a desiccator and weigh. Repeat the ignition in the muffle furnace until a constant weight (W_2) is obtained. If a carbon-free ash is not obtained after the first ignition, moisten the charred spot with a 1-in-10 solution of ammonium nitrate and dry under an infrared lamp. Repeat the ignition step.

Calculate the percentage of total ash of the sample:

$$(W_2/W_1) \times 100$$

Retain the ash for the Acid-insoluble ash test.

Viscosity

Transfer 7.5 g of the dried sample obtained from part (a) under the procedure for sulfate determination into a tared, 600-ml tall-form (Berzelius) beaker, and disperse with agitation for 10 to 20 min in 450 ml of deionized water. Add sufficient water to bring the final weight to 500 g, and heat in a water bath with continuous agitation, until a temperature of 80° is reached (20 - 30 min). Add water to adjust for loss by evaporation, cool to 76-77°, and heat in a constant temperature bath at 75°.

Pre-heat the bob and guard of a Brookfield LVF or LVT viscometer to approximately 75° in water. Dry the bob and guard, and attach them to the viscometer, which should be equipped with a No. 1 spindle (19 mm in diameter, approximately 65 mm in length) and capable of rotating at 30 rpm. Adjust the height of the bob in the sample solution, start the viscometer rotating at 30 rpm and, after six complete revolutions of the viscometer, take the viscometer reading on the 0-100 scale.

If the viscosity is very low, increased precision may be obtained by using the Brookfield UL (ultra low) adapter or equivalent. (Note. Samples of some types of carrageenan may be too viscous to read when a No. 1 spindle is used. Such samples obviously pass the specification, but if a viscosity reading is desired for other reasons, use a No. 2 spindle and take the reading on the 0-100 scale or on the 0-500 scale.)

Record the results in centipoises, obtained by multiplying the reading on the scale by the factor given by the Brookfield manufacturer.

Residual solvents (Vol.4) See Method 1 under Vol. 4. General Methods, Organic Components, Residual Solvents.

Prepare standard, blank, and calibration solutions as directed under Method 1.

Sample Preparation

Disperse 1 ml of a suitable antifoam emulsion, such as Dow-Corning G-10 or equivalent, in 200 ml of water contained in a 1000-ml 24/40 round-bottom distilling flask. Add about 5 g of the sample, accurately weighed, and shake for 1 h on a wrist-action mechanical shaker. Connect the flask to a fractionating column and distil about 100 ml, adjusting the heat so that the foam does not enter the column. Quantitatively transfer the distillate to a 200-ml volumetric flask, fill to the mark with water and shake the flask to mix. Weigh accurately 8.0 g of this solution into an injection vial. Add 1.0 ml of the internal standard solution. Heat at 60° for 10 min and shake vigorously for 10 sec.