PROCESSED EUCHEUMA SEAWEED

Prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), superseding specifications prepared at the 57th JECFA (2001)and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI "not specified" for carrageenan and processed Eucheuma *seaweed was established at the 57th JECFA (2001).*

SYNONYMS PES, PNG-carrageenan, semi-refined carrageenan; INS No. 407a

DEFINITION A substance with hydrocolloid properties obtained from either *Eucheuma cottonii* or *E. spinosum* (from the *Rhodophyceae* class of red seaweeds). In addition to carrageenan polysaccharides, processed eucheuma seaweed may contain up to 15% of insoluble algal cellulose and minor amounts of other insoluble matter. Articles of commerce may include sugars for standardization purposes or salts to obtain specific gelling or thickening characteristics. It is distinguished from carrageenan (INS No. 407) by its higher content of cellulosic matter and by the fact that it is not solubilized and precipitated during processing.

The functional component of the product obtained from *E. cottonii* is kappa-carrageenan (a copolymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose). From *E. spinosum* it is iota-carrageenan (a copolymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose-2-sulfate).

Processing consists of soaking the cleaned seaweed in alkali for a short time at elevated temperatures. The material is then thoroughly washed with water to remove residual salts followed by purification, drying, and milling to a powder. Alcohols that may be used during purification are restricted to methanol, ethanol, and isopropanol.

DESCRIPTION Light tan to white coarse to fine powder

FUNCTIONAL USES Thickener, gelling agent, stabilizer, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Forms cloudy viscous suspensions in water; insoluble in ethanol
A 1 g sample disperses and partially dissolves in 100 ml of water at 80º giving a cloudy opalescent solution. (The sample 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. Heat to boiling, cool to room temperature, and add 3 ml of barium chloride TS and 5 ml of hydrochloric acid, dilute TS. Filter the mixture. Boil the filtrate for 5 min. A white,

	crystalline precipitate appears.
Test for galactose and anhydrogalactose (Vol.4)	Proceed as directed in Volume 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 80º, with constant stirring until dissolved. Replace any water lost by evaporation, and allow the solution to cool to room temperature. The solution 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. A compliant ("elastic") gel indicates a predominantly iota-type.
Infrared absorption	Passes test See description under TESTS
PURITY	
Loss on drying (Vol. 4)	Not more than 12% (105º to constant weight)
pH (Vol. 4)	Between 8 and 11 (1 in 100 suspension)
Viscosity	Not less than 5 cp at 75º (1.5% solution) See description under TESTS
Sulfate	Not less than 15% and not more than 40% (as SO_4^{2-}) on the dried basis See description under TESTS
Total ash	Not less than 15% and not more than 30% 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
Acid-insoluble matter (Vol. 4)	Not less than 8% and not more than 15% on the dried basis Use 2 g of sample obtained from part (a) of the procedure for sulfate determination
Residual solvents (Vol. 4)	Not more than 0.1% of ethanol, isopropanol, or methanol, singly or in combination See description under TESTS
Microbiological criteria (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 homogenizing the mixture in a high speed blender. Total (aerobic) plate count: Not more than 5000 cfu/g

Salmonella spp.: Negative per test
E. coli: Negative in 1 g

Arsenic (Vol. 4) — Not more than 3 mg/kg
Determine by the atomic absorption hydride technique. Use Method II for sample preparation.

Lead (Vol. 4) — Not more than 5 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").

Cadmium (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 method described in Volume 4 (under "General Methods, Metallic Impurities").

Mercury (Vol.4) — Not more than 1 mg/kg
Determine by the cold vapour atomic absorption technique

TESTS

IDENTIFICATION TESTS

Infrared absorption — Prepare a 0.2% aqueous solution of the sample. Cast films of 0.5 mm thickness (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).

Iota- and kappa-carrageenan have strong, broad absorption bands, typical of all polysaccharides, in the 1000 to 1100 cm^{-1} region. 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
1220-1260	ester sulfate	0.6-1.0	0.9-1.2
928-933	3,6-anhydrogalactose	0.3-0.6	0.2-0.6
840-850	galactose-4-sulfate	0.3-0.5	0.2-0.4
800-805	3,6-anhydrogalactose-2-sulfate	0.0-0.2	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 105º 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 and 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. 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 charred sample into a muffle furnace and ignite at about 550º 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 80º is reached (20-30 min). Add 7.5 g of diatomaceous earth or perlite filter aid.

Stir for two minutes. Add water to adjust for loss by evaporation. Filter the solution through a Büchner funnel (pre-heated with hot water to 80º) equipped with a coarse filter paper. Place the filter assembly in a vacuum receiver bottle.

Filter 200 ml of solution. Cool to 76-77º, and heat in a constant temperature bath at 75º. Pre-heat the bob and guard of a Brookfield LVF viscometer to approximately 75º 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.

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 Method1.

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 60º for 10 min and shake vigorously for 10 sec.