PROCESSED EUCHEUMA SEAWEED

Prepared at the 57th JECFA (2001) and published in FNP 52 Add 9 (2001), superseding specifications prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998)). 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 80^{o} 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 — Proceed as directed under Gum Constituents Identification (FNP 5), 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. 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% (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 SO_4^{2-}) on a dry weight basis See description under TESTS
Total ash	Not less than 15% and not more than 30% on a dry weight basis See description under TESTS
Acid-insoluble ash (Vol. 4)	Not more than 1%
Acid-insoluble matter (Vol. 4)	Not less than 8% and not more than 15% on a dry weight 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 (Method II)
Lead (Vol. 4)	Not more than 5 mg/kg Determine using an atomic absorption 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, "Instrumental Methods."

Cadmium — Not more than 2 mg/kg
See description under TESTS

Mercury — Not more than 1 mg/kg
See description under TESTS

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 8 g sample of commercial product into 400 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 10-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 and Acid-insoluble matter.

(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) x 100 x 0.4116.

Total ash

Accurately weigh 2 g of the dried sample (W1) 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 (W2) 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: (W2/W1) x 100. Retain the ash for the Acid-insoluble ash test.

Viscosity

Transfer 7.5 g of the sample 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

Standard Alcohol Solution:
Transfer 500 mg each of chromatographic quality methanol, ethanol, and isopropanol into a 50 ml volumetric flask. Dilute to volume with water, and mix. Pipet 10 ml of this solution into a 100-ml volumetric flask. Dilute to volume with water and mix.

TBA Standard Solution:
Transfer 500 mg of chromatographic quality tertiary-butyl alcohol into a 50-ml volumetric flask. Dilute to volume with water, and mix. Pipet 10 ml of this solution into a 100-ml volumetric flask. Dilute to volume with water and mix.

Mixed Standard Solution:
Pipet 4 ml each of the Standard Alcohol Solution and of the TBA Standard Solution into a 125-ml graduated Erlenmeyer flask. Dilute to about 100 ml with water and mix. This solution contains approximately 40 µg of each alcohol per ml.

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 foam does not enter the column. Add 4.0 ml of TBA Standard Solution to the distillate to obtain the Sample Preparation.

Procedure:
Inject 5 µl of the Mixed Standard Solution into a suitable gas chromatograph equipped with a flame-ionization detector and a 1.8-m x 3.2-mm stainless steel column packed with 80-100-mesh Porapak QS or equivalent. The carrier is helium flowing at 80 ml per min. The injection port temperature is 200°; the column temperature is 165°; and the detector temperature is 200°. The retention time of isopropanol is about 2 min, and that of tertiary-butyl alcohol about 3 min.

Measure the areas of the methanol, ethanol, isopropanol, and TBA peaks. Calculate each response factor, f_i, by the formula A_i/A_{TBA}, in which A_i is the area of each alcohol peak (i=methanol, ethanol, or isopropanol). Similarly, inject 5 µl of the Sample Preparation, and measure the peak areas, recording the area of each alcohol peak as A_i, and that of the tertiary-butyl alcohol peak as A_{TBA}. Calculate the concentration of each alcohol (mg/kg) in the sample taken, by the formula:

$$A_i \cdot 4000 / f_i \cdot A_{TBA} \cdot W$$

where W is the weight of the sample taken (grams).

Lead (Vol. 4)

Principle:
The sample is wet-ashed with nitric and perchloric acids and analysed using flame atomic absorption spectrophotometry (Vol. 4).

Equipment:
Atomic absorption spectrophotometer
Reagents:
Nitric acid, concentrated, Reagent Grade
Perchloric acid, concentrated, Reagent Grade
Hydrochloric acid, concentrated, Reagent Grade
Lead standard solution (certified)

Solutions:
Stock solution (1 mg/ml): Dilute an appropriate volume of certified reagent lead standard solution with distilled and deionized water (D/D water) to make one liter.

Intermediate solutions: (a) 100 µg/ml. Pipet 10 ml of the stock solution into a 100-ml volumetric flask and dilute to volume with D/D water. (b) 10 µg/ml. Pipet 10 ml of the 100 µg/ml solution into a 100-ml volumetric flask and dilute to volume with D/D water.

Working solutions: Assemble four 100-ml volumetric flasks and transfer to them (pipet), respectively, 1, 5, 10, and 20 ml of intermediate lead solution (b). Dilute to volume with D/D water to make solutions containing 0.1, 0.5, 1, and 2 µg Pb/ml.

Sample preparation:
(CAUTION: This procedure employs concentrated oxidizing acids and results in evolution of noxious gases. Perform operations in a fume hood.) Accurately weigh 7.5 grams of a representative dry powdered test sample into a 250-ml Erlenmeyer flask. Set up a reagent blank and carry through the same operations as performed on the test sample. Wet the test sample with ca. 10 ml of D/D water and add 25 ml of nitric acid. Heat gently on a hot plate (100o - 150o) until most of the dark fumes are evolved (ca. one hour); swirl the flask occasionally. Cool and add 5 ml of perchloric acid; particles become visible at this stage. Heat gently (hot plate, 100o - 150o) to concentrate until the solution turns yellowish or colourless (ca. one hour). Midway during the heating, if the solution darkens, slowly add 2-3 ml portions of nitric acid as necessary until the desired colour is achieved; do not let the solution go to dryness. Cool the digest and wash the sides of the flask with ca. 5 ml of D/D water and swirl. Add 2 ml of hydrochloric acid. Heat again until all brown fumes are evolved and the solution is white to yellowish in colour; do not let the solution go to dryness. Cool the solution and wash the sides of the flask with ca. 10 ml of D/D water. Transfer the slightly viscous solution to a 50-ml volumetric flask and dilute to volume with D/D water. Filter using two layers of filter paper (Whatman no. 5 or equivalent).

Determination: Set the spectrophotometer to previously established optimum conditions at 283.3 nm using an air/acetylene oxidizing flame. Measure the absorbance of the sample, blank, and working solutions. Prepare a standard curve by plotting absorbance against :g Pb/ml for the blank and working solutions. Determine the concentration of lead in the sample solution from the standard curve. The concentration of lead in the test sample (mg Pb/kg) is:

$$[Pb] = FxA/B$$

where A is the concentration of lead in the sample solution (µg /ml), B is the weight of the test sample (grams), and F is the dilution factor (50 ml).

Cadmium

Proceed as directed above for the determination of lead, using 228.8 nm as the analysis wavelength. Intermediate and working solutions are prepared from certified reagent cadmium standard solution as follows:
Intermediate solutions: (a) 100 µg/ml. Pipet 10 ml of the stock solution (1mg/ml) into a 100-ml volumetric flask and dilute to volume with distilled and

deionized (D/D) water. (b) 10 µg/ml. Pipet 10 ml of solution (a) into a 100-ml volumetric flask; dilute to volume with D/D water. (c) 1 µg/ml. Pipet 1 ml of solution (a) into a 100-ml volumetric flask; dilute to volume with D/D water.
Working solutions: Assemble five 50-ml volumetric flasks and transfer to them (pipet), respectively, 0.5, 2.5, 5.0, 10, and 20 ml of intermediate solution (c). Dilute to volume with D/D water to make solutions containing 0.01, 0.05, 0.1, 0.20, and 0.40 µg Cd/ml.
The concentration of cadmium in the test sample (mg Cd/kg) is:

$$[Cd] = FxA/B$$

where A is the concentration of cadmium in the sample solution (µg /ml), B is the weight of the test sample (grams), and F is the dilution factor (50 ml).

Mercury (Vol. 4)

Principle:
The sample is wet-ashed with nitric and perchloric acids and analysed using hydride-generation atomic absorption spectrophotometry (Vol. 4).

Equipment:
Atomic absorption spectrophotometer equipped with a hydride vapour generator. Integral to the generator is a reactor tube or coil and a peristaltic pump with dual tubing channels: one channel for the sample solution and one for the two reagent solution tubes. Flow control is determined by tubing size and tubing clamps. Flow rates are measured at the exit of the hydride generator.

Reagents:
Nitric acid, concentrated, Reagent Grade
Perchloric acid, concentrated, Reagent Grade
Hydrochloric acid, concentrated, Reagent Grade
Sodium borohydride, >98%
Sodium hydroxide, Reagent grade
Mercury standard solution (certified)

Solutions:
Nitric acid-perchloric acid (1:1): Mix equal volumes of the two acids.
Hydrochloric acid, 5M: Dilute 417 ml concentrated hydrochloric acid to 1 liter with deionized water.
Sodium borohydride solution, 0.4% (Prepare immediately before use.): First, dissolve 2.5 g sodium hydroxide in deionized water. Then, add and dissolve 2.0 g sodium borohydride. Dilute to 500 ml.
Stock solution (1 mg/ml): Dilute an appropriate volume of certified reagent mercury standard solution with distilled and deionized water (D/D water) to make one liter.
Intermediate solutions: (a) 10,000 µg/l. Pipet 1 ml of the stock solution into a 100-ml volumetric flask and dilute to volume with D/D water. (b) 100 µg/l. Pipet 1 ml of the 10,000 :g/l solution into a 100-ml volumetric flask and dilute to volume with D/D water.
Working solutions: Assemble five 100-ml volumetric flasks and transfer to them (pipet), respectively, 1, 5, 10, 15, and 20 ml of intermediate solution (b). To each, add 10 ml of 1:1 nitric acid-perchloric acid and dilute to volume with D/D water to make solutions containing 1, 5, 10, 15, and 20 µg Hg/l.

Sample preparation**:**
(CAUTION: This procedure employs concentrated oxidizing acids and results in evolution of noxious gases. Perform operations in a fume hood.)
Accurately weigh 5 grams of a representative dry powdered test sample into a 250 ml Erlenmeyer flask. Set up a reagent blank and carry through the same operations as performed on the test sample. Wet the test sample with 5 ml of D/D water and then add 10 ml of 1:1 nitric acid-perchloric acid. Heat gently on a hot plate (100^{o}-150^{o}) until all of the dark fumes are evolved and the solution turns yellowish or colourless; swirl the flasks occasionally. Do not let the solution go to dryness. Cool and wash the sides of the flask with a small amount of D/D water. (Some particles may be visible.) Cover the flask lightly and let the slightly viscous solution stand overnight. Transfer the solution to a 50-ml volumetric flask and dilute to volume with D/D water. Filter using 2 layers of Whatman no. 5 (or equivalent) filter paper into a 100-ml Erlenmeyer flask. Immerse the flask in an ultrasonic bath and sonicate it for 10 minutes or until bubbles no longer form on the surface of the solution.

Determination:
Calibrate (using water) the peristaltic pump to provide a flow rate of the sample solution of 8 ml/min and a combined flow rate for the two reagent solutions (sodium borohydride and 5M hydrochloric acid) of 2 ml/min. (The combined flow rate is achieved with a single pump setting.)
Set the spectrophotometer to previously established optimum conditions at the mercury lamp wavelength of 253.7 nm.
Transfer suitable quantities of the two reagent solutions into separate graduated cylinders. Insert separate aspirator tubing leading from the peristaltic pump into each reagent solution and into the sample flask.
Start the flow of argon carrier gas (tank outlet pressure: 3.2±0.2 kg/cm^2) through the hydride vapour generator of the spectrophotometer. Start the pump to initiate flow of the three solutions into the hydride generator manifold where they are mixed and pass into the reactor coil to generate atomic mercury, which is carried into the absorbance cell of the spectrophotometer. Measure the absorbance for the sample. Repeat for the blank solution and each of the working standards.
Prepare a standard curve by plotting absorbance against µg Hg/l for the blank and working solutions. Determine the concentration of mercury in the sample solution from the standard curve. The concentration of mercury in the test sample (mg Hg/kg) is:

$$[Hg] = FxA/1000B$$

where A is the concentration of mercury in the sample solution (µg /l), B is the weight of the test sample (grams), and F is the dilution factor (50 ml).