Institute of Medicine
Food and Nutrition Board
Committee on Food Chemicals Codex

Revised Monograph - Pectins

Please send comments to the Committee on Food Chemicals Codex, National Academy of Sciences, FO 3042, 2101 Constitution Avenue, N.W., Washington, DC 20418 or email them to fcc@nas.edu. All comments must be received by December 15, 1996, for consideration for the First Supplement.

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Pectins

INS: 440 CAS: [9000-69-5]

DESCRIPTION

Pectins consist mainly of the partial methyl esters of polygalacturonic acid and their sodium, potassium, calcium, and ammonium salts. It is obtained by extraction in an aqueous medium of appropriate edible plant material, usually citrus fruits or apples. No organic precipitants shall be used other than methanol, ethanol, and isopropanol. In some types, a portion of the methyl esters may have been converted to primary amides by treatment with ammonia under alkaline conditions. It usually occurs as a white, yellowish, light grayish, or light brownish powder. The commercial product is normally diluted with sugars for standardization purposes. In addition to sugars, pectins may be mixed with suitable food-grade salts required for pH control and desirable setting characteristics.

> Note: The following REQUIREMENTS and TESTS apply to the Pectins as supplied, whether standardized or not, except for specifications covering amide substitution and the weight percent of total galacturonic acid in the Pectin component, in which cases the test procedures provide for removing the sugars and soluble salts before analysis of the Pectin component.

Functional Use in Foods Gelling agent; thickener; stabilizer; emulsifier.

REQUIREMENTS

Labeling Indicate the presence of sulfur dioxide if the residual concentration is greater than 10 mg/kg.

Identification

A. To a 1 in 100 aqueous solution of the sample add an equal volume of alcohol. A translucent, gelatinous precipitate is formed (most gums will not form such a precipitate).

~~B. To 10 mL of a 1 in 100 aqueous solution of the sample add 1 mL of thorium nitrate solution (1 in 10), stir, and allow to stand for 2 min. A stable precipitate or gel forms (most gums will not form such a precipitate).~~

B. To 5.0 mL of a 1 in 100 aqueous solution of the sample add 0.1 mL of a 0.125 M solution of calcium chloride or calcium acetate (equivalent to 10 mg of calcium per gram of sample) and 1 mL of 1 N sodium hydroxide, and allow to stand at room temperature for 15 min. A gel or semi-gel forms (tragacanth and other gums will not form such a precipitate).

C. ~~D.~~ Acidify the gel formed from Identification Test B with 1 mL of 2.7 N hydrochloric acid, and shake well. If necessary, continue adding the acid dropwise until the mixture is acid to litmus. A voluminous, colorless, gelatinous precipitate forms, which, upon boiling, becomes white and flocculent (pectic acid).

Acid-Insoluble Ash Not more than 1.0%.

Arsenic (as As) Not more than 3 mg/kg.
Degree of Amide Substitution Not more than 25% total carboxylic groups.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 12.0%.
Methanol, Ethanol, and Isopropanol Not more than 1.0% total.
Sodium Methyl Sulfate Not more than 0.1%.
Sulfur Dioxide Not more than 0.005%.
Total Galacturonic Acid in Pectin Component Not less than 65.0%, calculated on the ash-free, dried basis.

TESTS

Acid-Insoluble Ash Determine as directed in the general method, Appendix IIC.
Arsenic A Sample Solution prepared as directed for organic compounds meets the requirements of the Arsenic Test, Appendix IIIB.
Degree of Amide Substitution and Total Galacturonic Acid in the Pectin Component Weigh 5 g of the sample to the nearest 0.1 mg, and transfer to a suitable beaker. Stir for 10 min with a mixture of 5 mL of 2.7 N hydrochloric acid, and 100 mL of 60% ethanol. Transfer to a fritted-glass filter tube (30- to 60-mL capacity), and wash with six 15-mL portions of the same hydrochloric acid–60% ethanol mixture, followed by 60% ethanol until the filtrate is free of chlorides. Finally, wash with 20 mL of ethanol, dry for 2.5 h in an oven at 105°, cool, and weigh. Transfer exactly one-tenth of the total net weight of the now ash-free, dried sample (representing 0.5 g of the original unwashed sample) to a 250-mL conical flask, and moisten the sample with 2 mL of ethanol. Add 100 mL of recently boiled and cooled distilled water, stopper, and swirl occasionally until a complete solution is formed. Add 5 drops of phenolphthalein TS, titrate with 0.1 N sodium hydroxide, and record the results as the initial titre (V_1).

Add exactly 20 mL of 0.5 N sodium hydroxide, stopper, shake vigorously, and let stand for 15 min. Add exactly 20 mL of 0.5 N hydrochloric acid, and shake until the pink color disappears. Titrate with 0.1 N sodium hydroxide to a faint pink color that persists after vigorous shaking; record this value as the saponification titre (V_2).

Quantitatively transfer the contents of the conical flask into a 500-mL distillation flask fitted with a Kjeldahl trap and a water-cooled condenser, the delivery tube of which extends well beneath the surface of a mixture of 150 mL of carbon dioxide-free water and 20.0 mL of 0.1 N hydrochloric acid in a receiving flask. To the distillation flask add 20 mL of a 1 in 10 sodium hydroxide solution, seal the connections, and then begin heating carefully to avoid excessive foaming. Continue heating until 80 to 120 mL of distillate has been collected. Add a few drops of methyl red TS to the receiving flask, titrate the excess acid with 0.1 N sodium hydroxide, and record the volume required, in mL, as S. Perform a blank determination on 20.0 mL of 0.1 N hydrochloric acid, and record the volume required, in mL, as B. Record the amide titre (B – S) as V_3.

Transfer exactly one-tenth of total net weight of the dried sample (representing 0.5 g of the original unwashed sample), and wet with about 2 mL of ethanol in a 50-mL beaker. Dissolve the Pectin in 25 mL of 0.125 M sodium hydroxide. Let the solution stand for 1 h, with agitation, at room temperature. Transfer quantitatively the saponified Pectin solution to a 50-mL volumetric flask, and dilute to volume with distilled water. Transfer 25.0 mL of the diluted Pectin solution to a distillation apparatus, and add 20 mL of Clark's solution, which consists of 100 g of magnesium sulfate heptahydrate and 0.8 mL of sulfuric acid and distilled water to a total of 180 mL. The distillation apparatus consists of a steam generator connected to a round-bottom flask to which a condenser is attached. Both steam generator and the round-bottom flask are equipped with heating mantles. Start the distillation by heating the round-bottom flask containing the sample. Collect the first 15 mL of distillate separately in a measuring cylinder. Then start the steam supply and continue distillation until 150 mL of distillate has been collected in a 200-mL beaker. Quantitatively combine the distillates, titrate with 0.05 M sodium hydroxide to pH 8.5, and record the volume required, in mL, as S.

Perform a blank determination using 20 mL of distilled water. Record the required volume, in mL, as B. Record acetate ester titre (S – B) as V_4.

Calculate the degree of amidation (as the percent of total carboxyl groups) by the formula

$$100 - [V_3/(V_1 + V_2 + V_3 - V_4)].$$

Calculate mg of galacturonic acid by the formula

$$19.41 (V_1 + V_2 + V_3 - V_4).$$

The mg of galacturonic acid obtained in this way is the content of one-tenth of the weight of the washed and dried sample. To calculate the percent galacturonic acid on a moisture- and ash-free basis, multiply the number of mg obtained by 1000/x, in which x is the weight, in mg, of the washed and dried sample.

> Note: If the Pectin is known to be of the nonamidated type, only V_1 and V_2 need to be determined, and V_3 may be regarded as zero.

Heavy Metals Prepare and test a 1-g sample as directed in Method II under the Heavy Metals Test, Appendix IIIB, using 20 µg of lead ion (Pb) in the control (Solution A).

Lead (Note: Use deionized water throughout this procedure.)

Diluted Standard Lead Solution (2 *m*g Pb/mL) Immediately before use, pipet 0.10 mL of a certified commercially available 1000 ppm (1000 µg/mL) Pb stock solution into a 50-mL volumetric flask containing 30 mL of water and 4 mL of 20% v/v hydrochloric acid and 4 mL of 0.1 M EDTA. Dilute to volume with water, and mix.

Control Lead Solution (0.4 *m*g Pb/mL) Pipet 5.0 mL of the Diluted Standard Lead Solution into a 25-mL volumetric flask containing 10 mL of water, 2 mL of 20% v/v hydrochloric acid, and 2 mL of 0.1 M EDTA. Dilute to volume with water, and mix.

Standard Lead Blank Solution Add 30 mL of water, 4 mL of 20% v/v hydrochloric acid, and 4 mL of 0.1 M EDTA into a 50-mL volumetric flask. Dilute to volume with water, and mix.

Sample Preparation Transfer 2.0 g of the sample into a clean 100-mL glass beaker, add 25 mL of nitric acid (70% v/v), cover with a watch glass, and heat at low to moderate heat on a hot plate in a fume hood for 2 h. Remove watch glass, and continue to heat until the sample is dry with no visible fumes. Add 0.5 mL of nitric acid, and heat to dryness. Cool to room temperature and add 2 mL of 20% v/v hydrochloric acid and 2 mL of 0.1 M EDTA. Transfer quantitatively to a 25-mL volumetric flask, then dilute to volume with water, and mix.

Procedure Set up the inductively coupled plasma emission spectrometer according to manufacturer's instructions, using the Pb emission line at 220.35 nm. Calibrate the instrument using the Standard Lead Blank Solution and the Diluted Standard Lead Solution. Then analyze the Sample Preparation and the Control Lead Solution. The sample passes the test if the lead concentration found in the Sample Preparation is equal to or less than that in the Control Lead Solution.

Loss on Drying, Appendix IIC Dry at 105° for 2 h.

Methanol, Ethanol, and Isopropanol The alcohols are converted to their nitrite esters, and their levels are determined by headspace gas chromatography.

Internal Standard Solution Dissolve 50 mg of n-propanol in 1 L of water.

Sample Solution Dissolve 100 mg of the sample in 10 mL of water, and as necessary, use sodium chloride as a dispersing agent.

Standard Alcohol Solution Using a micropipet, transfer 50 mg each of methanol (corresponding to 39.55 µL), ethanol (corresponding to 39.47 µL), and isopropanol (corresponding to 39.28 µL) into a 1000-mL volumetric flask, dilute to volume with water, and mix.

Sodium Nitrite Solution Dissolve 250 g of sodium nitrite in 1000 mL of water.

Chromatographic System Use a suitable gas chromatograph equipped with a flame-ionization detector. Use a 90-cm × 4-mm id glass column with the first 15 cm packed with Chromopack (or equivalent) and the remainder packed with Poropak R 120- to 150-mesh (or equivalent). The operating conditions of the gas chromatograph are as follows: the injection port temperature is 250°, and the column temperature is 150° isothermal. Use nitrogen as the carrier gas with a flow rate of 80 mL/min.

Procedure Weigh 200 mg of urea, and place it in a 25-mL amber-glass vial (Reacti-Flasks or equivalent). Purge with nitrogen for 5 min, add 1 mL of saturated oxalic acid solution, close with a rubber stopper, and swirl. Add 1 mL of Sample Solution and 1 mL of Internal Standard Solution, and simultaneously start a stopwatch (t = 0). Swirl the vial, and recap it with an open screw cap fitted with a silicone rubber septum. Swirl the vial until t = 30 s. At t = 45 s, inject through the septum 0.5 mL of Sodium Nitrite Solution. Swirl until t = 70 s, and at t = 150 s,

withdraw through the septum 1.0 mL of the headspace using a pressure lock syringe (Precision Sampling Corporation, or equivalent). Inject the 1.0 mL into the injection port of the gas chromatograph. Repeat this procedure, but use 1 mL of the Standard Alcohol Solution instead of the Sample Solution.

Calculation Quantify the total methanol, ethanol, and isopropanol present in the sample by the following formula:

$$T = V_{MS} (R_{MU}/R_{MS})0.791 + V_{ES} (R_{EU}/R_{ES})0.7893 + V_{IS} (R_{IU}/R_{IS})0.7855,$$

in which T is the total amount, in mg, of methanol, ethanol, and isopropanol in the sample; the subscripts M, E, and I refer to methanol, ethanol, and isopropanol, respectively; V_S is the volume, in mL, of the corresponding alcohol in the Standard Alcohol Solution; R_S is the ratio of the peak area of the corresponding alcohol in the Standard Alcohol Solution to that of n-propanol in Internal Standard Solution; R_U is the ratio of the peak area of the corresponding alcohol in the Sample Solution to that of n-propanol in the Internal Standard Solution; and 0.791, 0.7893, and 0.7855 are the densities, in g/mL, for methanol, ethanol, and isopropanol, respectively. Calculate the percent methanol, ethanol, and isopropanol present in the sample by the following formula:

$$(T100)/W,$$

in which W is the sample weight, in mg.

Sodium Methyl Sulfate

Mobile Phase Prepare a 0.04 M potassium hydrogen phthalate solution by transferring 16.4 g of potassium hydrogen phthalate into a 2-L volumetric flask, dilute to volume with water, and mix. Filter the solution through a 0.45-µm pore-size filter (Millipore, or equivalent).

Standard Preparation Transfer 10.0 mg of anhydrous sodium methyl sulfate into a 100-mL volumetric flask, dilute to volume with Mobile Phase, and mix.

Assay Preparation Suspend about 1 g of the sample, accurately weighed, in 10.0 mL of 50% (v/v) ethanol solution. Stir for 30 min using a Teflon-coated stirring bar. Allow the suspension to precipitate, and filter. Evaporate a 1.0-mL aliquot to dryness using reduced pressure (10 mm Hg), and heat at 60°. Redissolve the residue in 1.0 mL of the Mobile Phase.

Chromatographic System Use a high-performance liquid chromatograph equipped with a refractive index detector and a 25-cm × 4.6-mm column packed with Nucleosil 10SB (or equivalent) and maintained at 40°. Set the flow rate at 1 mL/min.

System Suitability Three replicate injections of the Standard Preparation show a relative standard deviation of not more than 4.0% for the response factor of the sodium methyl sulfate peak obtained using the formula

$$(A_s/C_s),$$

in which A_s is the peak area response of the Standard Preparation, and C_s is the concentration, in mg/mL, of sodium methyl sulfate in the Standard Preparation.

Procedure Inject 20 µL of the Standard Preparation followed by the Assay Preparation. Determine the peak area in the chromatograms for the Standard Preparation and Assay Preparation. Calculate the quantity in percent of sodium methyl sulfate in the sample by the formula

$$(C_S A_U)/(A_S W),$$

in which C_S is the concentration, in mg/mL, of sodium methyl sulfate in the Standard Preparation; A_U and A_S are the peak area responses obtained from the Assay Preparation and Standard Preparation, respectively; and W is the weight, in g, of the sample taken.

Sulfur Dioxide Determine as directed in the general method, Appendix X, using the following method under Sample Introduction and Distillation: Transfer about 20 g of the sample, accurately weighed, into flask C, and add 20 mL of ethanol to moisten the sample. Add 400 mL of water, swirling vigorously to disperse the sample. Reassemble the apparatus, making sure that the tapered joints are clean and greased with stopcock grease, and

proceed as directed under Sample Introduction and Distillation, beginning with "the nitrogen flow through the 3% Hydrogen Peroxide Solution…."

Packaging and Storage Store in well-closed containers.