PECTINS

Prepared at the 57th JECFA (2001) and published in FNP 52 Add 9 (2001), superseding specifications prepared at the 39th JECFA (1992) and published in FNP 52 Add 1 (1992). A group ADI "not specified" was established for pectins and amidated pectins, singly or in combination at the 25th JECFA in 1981.

SYNONYMS INS No. 440

DEFINITION Consists mainly of the partial methyl esters of polygalacturonic acid and their sodium, potassium, calcium and ammonium salts; 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. Sulfur dioxide may be added as a preservative.

The commercial product is normally diluted with sugars for standardization purposes. In addition to sugars, pectins may be mixed with suitable food-grade buffer salts required for pH control and desirable setting characteristics. The article of commerce may be further specified as to pH value, gel strength, viscosity, degree of esterification, and setting characteristics.

C.A.S. number 9000-69-5

DESCRIPTION White, yellowish, light greyish or light brownish powder

FUNCTIONAL USES Gelling agent, thickener, stabilizer, emulsifier

CHARACTERISTICS

IDENTIFICATION

Test for pectins

Passes test
See description under TESTS

Test for amide group

Passes test (amidated pectins only)
Add 2 ml of hydrochloric acid and 50 ml of 60% ethanol to 0.5 g of the sample, and stir well for 20 min. Transfer to a fritted glass filter tube wash with six 10 ml portions of the HCl-60% ethanol mixture. Dissolve in 100 ml distilled water; it may be necessary to add a few drops 0.1 mol/L sodium hydroxide to achieve solution. Transfer 4 ml of this solution into a test tube (recommended dimensions 15.5 mm inner diameter and 146 mm length). Add 1 ml 5 mol/L sodium hydroxide and mix. The mixture will form a gel. Fill a small glass tube (recommended dimensions 7.8 mm inner diameter and 79 mm length) with 2.5 ml boric acid TS and let glide into the test tube. Close with parafilm and incubate overnight at 30^{o}. In case of presence of amide groups the indicator changes its colour from red to green, due to release of ammonia.

PURITY

Loss on drying (Vol. 4)	Not more than 12% (105°, 2 h)
Sulfur dioxide	Not more than 50mg/kg See description under TESTS
Methanol, ethanol and 2-propanol	Not more than 1% singly or in combination See description under TESTS
Acid-insoluble ash (Vol. 4)	Not more than 1%
Total insolubles	Not more than 3% See description under TESTS
Nitrogen content (Vol. 4)	Not more than 2.5% after washing with acid and ethanol
Galacturonic acid	Not less than 65% calculated on the ash-free and dried basis See description under TESTS
Degree of amidation	Not more than 25% of total carboxyl groups of pectin See description under TESTS
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.”

TESTS

IDENTIFICATION TESTS

Test for Pectins

Moisten 0.05 g of the sample with 2-propanol. Add 50 ml of water on a magnetic stirrer. Adjust pH to 12 using 0.5 mol/L sodium hydroxide and let the solution remain without stirring for 15 min. Reduce pH to 7.0 with 0.5 mol/L hydrochloric acid. Adjust to 100.0 ml with water. Make up samples in 1 cm quartz cuvettes as follows:

	Buffer pH 7.0 *)	Sample soln	Water	Enzyme soln **)
Enzyme blank	0.5 ml	1.0 ml	1.0 ml	-
Sample blank	0.5 ml	-	1.5 ml	0.5 ml
Sample	0.5 ml	1.0 ml	0.5 ml	0.5 ml

*) Dissolve 6.055 g of tris(hydroxymethyl)aminomethane (e.g. TRIZMA Base, Sigma) and 0.147 g of calcium chloride dihydrate in water to 1 L. Adjust pH to 7.0 with 1 mol/L hydrochloric acid

**) Dilute pure pectate lyase 1:100 with buffer pH 7.0
Shake the solutions well, and measure the absorbance at 235 nm at 0 and 10 min.

Calculations

A_0 = absorbance at 0 min = Sample - (enzyme blank + sample blank)
A_{10} = absorbance at 10 min = Sample - (enzyme blank + sample blank)

The amount of unsaturated product produced is proportional to the change in absorbance (A_{10} - A_0). This value should be greater than 0.023. This distinguishes pectins from other gums, which show essentially no change.

PURITY TESTS

Sulfur dioxide

Suspend 100 g of the sample in 500 ml of methanol in a 1000-ml round-bottom flask, which is provided with a gas inlet tube reaching almost the bottom and connected to the neck with a reflux condenser. Prepare a glass joint connection from the condenser to an absorption flask or U-tube containing 10 ml of 3% hydrogen peroxide solution neutralized to methyl red TS. Connect the gas inlet tube with an oxygen-free source of carbon dioxide or nitrogen, and maintain a gas stream so as to cause steady bubbling. As soon as the apparatus is flushed free of air, pour 30 ml of hydrochloric acid solution (10 ml conc. HCl + 20 ml H_2O) into the reflux condenser, and immediately connect the absorption flask or U-tube. Heat slowly until methanol starts refluxing, and reflux gently for 2 h. Disconnect the apparatus and titrate the hydrogen peroxide solution against methyl red TS with 0.01 mol/L sodium hydroxide. Each ml of 0.01 mol/L sodium hydroxide corresponds to 0.32 mg of SO_2.

Total insolubles

Dry a 70 mm glass fiber filter paper (GF/B (Whatman code 1821 070) in an oven with fan set at 105º for about 1 h. Transfer the filter paper to a desiccator containing silica gel and allow to cool. Weigh the paper (M_1). Weigh about 1 g (= S) of the sample into a 250-ml beaker. Add 5 ml of 2-propanol to disperse the sample. While stirring magnetically, add 100 ml of 0.03 mol/L sodium hydroxide containing 0.1% (w/w) ethylene diamine tetra-acetic acid (Na salt), which has been filtered through GF/B paper. Stir for about 30 min at room temperature, then heat to boiling (remove heat if excessive foaming occurs). Filter the hot solution through the glass fiber paper under vacuum using, e.g. a vacuum filtration kit with 3 piece Hartley funnel (70 cm), with heat resistant plate. Rinse the beaker five times and filter the rinsings with 100 ml of warm (about 50º) water that has been filtered through GF/B paper.
Dry the filter paper with the residue at 105º for 1 h. Transfer to desiccator containing silica gel and leave to cool. Weigh the paper (M_2). Calculate the percentage of total insolubles from

$$\text{Total insolubles (\%)} = [(M_2 - M_1)/S] \times 100$$

Galacturonic acid and Degree of amidation

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 hydrochloric acid TS, 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 HCl-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 105º, cool and weigh. Transfer exactly one-tenth of the total net weight of the 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 TS. 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 mol/L sodium hydroxide and record the results as the initial titre (V_1).

Add exactly 20 ml of 0.5 mol/L sodium hydroxide TS, stopper, shake vigorously and let stand for 15 min. Add exactly 20 ml of 0.5 mol/L hydrochloric acid and shake until the pink colour disappears. Titrate with 0.1 mol/L sodium hydroxide to a faint pink colour which 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 mol/L 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 80-120 ml of distillate has been collected. Add a few drops of methyl red TS to the receiving flask, and titrate the excess acid with 0.1 mol/L sodium hydroxide recording the volume required, in ml, as S. Perform a blank determination on 20.0 ml of 0.1 mol/L hydrochloric acid, and record the volume required, in ml, as B. The amide titre is (B - S).

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 ethanol in a 50-ml beaker. Dissolve the pectin in 25 ml 0.125 mol/L sodium hydroxide. Let the solution stand for 1 h with agitation at room temperature. Transfer quantitatively the saponified pectin solution to a 50-ml measuring flask and dilute to the mark with distilled water. Transfer 20.00 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 concentrated sulphuric acid and distilled water to a total of 180 ml. This apparatus consists of a steam generator connected to a round-bottom flask to which a condenser is attached. Both steam generator and 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 have been collected in a 200-ml beaker. Add quantitatively the first 15 ml distillate and titrate with 0.05 mol/L sodium hydroxide to pH 8.5 and record volume required, in ml, as A.

Perform a blank determination on 20 ml distilled water. Record the required volume, in ml, as A_0. The acetate ester titre is ($A - A_0$).
Calculate degree of amidation (as % of total carboxyl groups) by the formula:

$$100\,x\,\frac{B-S}{V_1+V_2+(B-S)\ -(A-A_0)}$$

Calculate mg of galacturonic acid by the formula:

$$19.41\,x\,[V_1 + V_2 + (B - S) - (A - A_0)]$$

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 % galacturonic acid on a moisture- and ash-free basis, multiply the number of mg obtained by 1000/x, x being the weight in mg of the washed and dried sample.

Note 1: If the pectin is known to be of the nonamidated type, only V1 and V2 need to be determined and (B - S) may be regarded as zero.
Note 2: For pectins from apple or citrus (A - A_0) is usually insignificant in calculating galacturonic acid and degree of amidation.
Note 3: If desired, calculate degree of esterification (as % of total carboxyl groups) by the formula:

$$100\,x\,\frac{V_2 - (A - A_0)}{V_1 + V_2 + (B - S) - (A - A_0)}$$

Note 4: If desired, calculate degree of acetate ester (as % of total carboxylic groups from galacturonic acid) by the formula:

$$100\,x\,\frac{A - A_0}{V_1 + V_2 + (B - S) - (A - A_0)}$$

Methanol, ethanol and 2-propanol

Weigh 200 mg of urea into a 25-ml amber glass vial (Reacti-flasks, Pierce, Rockford 3, 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 a sample solution (100 mg of sample in 10 ml of water using sodium chloride as a dispersing agent if necessary), 1 ml of internal standard solution (50 mg n-propanol in 1000 ml of water) 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). This will convert the alcohols to their corresponding nitrite esters. Swirl until T=70 sec and, at T=150 sec, withdraw through the septum 1.0 ml of the headspace using a pressure lock syringe (Precision Sampling Corp., Baton Rouge, Louisiana, USA or equivalent).

Head space gas chromatography:
Insert syringe needle in the injection port; pre-compress the sample, then open the syringe and inject the sample. Use the following conditions:

Column:
- length: 90 cm
- diameter: 4 mm
- material: glass
- 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 type: FID
Temperatures
- injection port: 250°
- column: 150° isothermal

Quantify the methanol, ethanol and 2-propanol 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 an aqueous solution containing 50 mg/l each of methanol, ethanol and 2-propanol for 1 ml of sample solution.