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## **Tamarind Seed Polysaccharide**

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## TAMARIND SEED POLYSACCHARIDE

*New specifications prepared at the 84<sup>th</sup> JECFA (2017) and published in FAO JECFA Monographs 20 (2017). An ADI of 'not specified' was established at the 84<sup>th</sup> JECFA (2017).*

### SYNONYMS

Tamarind seed gum, tamarind gum, tamarind xyloglucan, tamarind seed xyloglucan, tamarind galactoxyloglucan

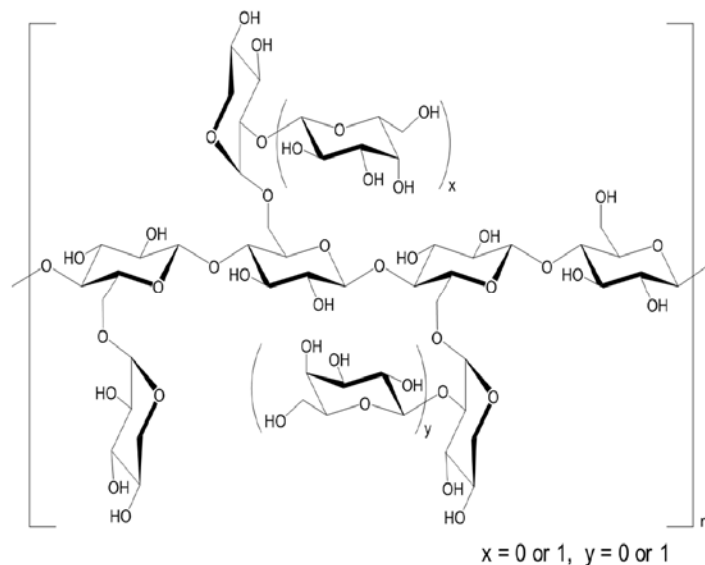
### DEFINITION

Tamarind seed polysaccharide is a high molecular weight (400-6000 kDa) polysaccharide. It is produced from the kernel of tamarind seed by husking and pulverizing the seeds of *Tamarindus indica* L. It is composed of a linear chain of D-glucose units linked by  $\beta(1-4)$  glycosidic bonds. Single D-xylose units are attached to about 75% of these D-glucose units via  $\alpha(1-6)$  bonds. Single D-galactose units are attached to some of the D-xylose units through  $\beta(1-2)$  bonds. The molar ratio of D-glucose: D-xylose:D-galactose is about 4:3:1.

Tamarind seed polysaccharide is obtained from tamarind kernel powder by treating it with an aqueous solution of methanol, followed by sodium hydroxide or sulphuric acid to wash and adjust pH. The insoluble polysaccharide is separated from the supernatant (containing protein, fat and minerals) by centrifuging. The material is dried, pulverized, sieved and mixed with food-grade bulking agents to standardize the product. Depending on the pH adjustment, and further alkali treatment and/or purification with methanol or 2-propanol, tamarind seed polysaccharide products with varying viscosity can be manufactured.

### C.A.S. Number

39386-78-2

*Structural formula*

## Assay

Not less than 75% on the dried basis

**DESCRIPTION**

White to light brown powder, nearly odourless

**FUNCTIONAL USES**

Thickener, stabilizer, emulsifier and gelling agent

**CHARACTERISTICS**

## IDENTIFICATION

Solubility (Vol. 4)

Soluble in hot water (75°); insoluble in ethanol

Precipitate and Colour  
FormationPasses the tests.  
See description under TESTS

## PURITY

Loss on drying (Vol. 4)

Not more than 14.0% (105°, 5 h)

Ash (Total) (Vol. 4)

Not more than 1.0% on the dried basis

Protein (Vol. 4)Not more than 3.0%  
Weigh accurately 1.0 g tamarind seed polysaccharide, and  
proceed as directed under Nitrogen determination (Kjeldahl)

Method, Method 1) in Volume 4 (under “General Methods, Inorganic components”). Multiply %N with 6.25 to get % protein.

Residual solvents (Vol. 4) Methanol: Not more than 200 mg/kg  
2-propanol: Not more than 1,000 mg/kg

See description under TESTS.

Lead (Vol. 4) Not more than 2 mg/kg  
Determine using a method 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 under Metallic Impurities in Volume 4 (under “General Methods, Inorganic components”).

Microbiological criteria  
(Vol. 4) Total plate count: Not more than 5,000 cfu/g  
*Escherichia coli*: Negative in 1 g  
Salmonella: Negative in 5 g  
Yeasts & moulds: Not more than 500 cfu/g

## TESTS

### IDENTIFICATION TEST

Precipitate and colour formation (Vol. 4) Reagents:  
- Sodium hydroxide  
- Iodine TS  
- Sodium sulfate

Sodium hydroxide solution  
Dissolve 22 g of sodium hydroxide in water, and dilute up to 1000 mL with deionized water in a volumetric flask. Store in a polyethylene bottle.

Sodium sulfate (saturated)  
Dissolve 20 g of sodium sulfate and make up to 100 mL of deionized water in a volumetric flask. Store in a polyethylene bottle.

Preparation of sample solution (20 mg/mL)  
Add gradually 2 g of Tamarind Seed Polysaccharide sample to 100 mL of sodium hydroxide solution, and dissolve by vigorous stirring.

To 5 mL of the sample solution, add 3 mL of saturated sodium sulfate. White lumps are produced. Observe and record.

To 5 mL of the sample solution, add a few drops of Iodine TS. Dark blue-green lumps are produced on the solution surface, and the colour disappears on stirring. Observe and record.

## PURITY TESTS

Residual solvents (Vol. 4) Determine by the method based on headspace gas chromatography as directed under Residual solvents in Volume 4 (under "General Methods, Organic components"). Prepare sample as follows:

Sample solution:

Weigh accurately 0.12 g sample into a headspace vial. Add 5.0 mL water and add 1.0 mL of the internal standard solution, to obtain a final sample concentration of 2%.

## METHOD OF ASSAY

Principle

The assay is based on a solution of xyloglucan reacting with iodine to give a specific greenish color, the intensity of which depends on the concentration of xyloglucan.

Reagents

- Iodine: purity  $\geq 99.8\%$
- Potassium iodide: purity  $\geq 99.5\%$
- Sodium sulfate: purity  $\geq 99.0\%$
- In-house Reference Standard (Not commercially available; prepare following the procedure below)

Preparation of Solutions

Preparation of 0.5% w/v Iodine-1.0% w/v Potassium iodide solution

Place 0.50 g iodine and 1.0 g potassium iodide in a 100 mL glass beaker. Add 75 mL deionized water and put a stir bar to the beaker. Cover the beaker with plastic wrap and then with aluminium foil to protect from light. Stir the mixture for 2 h with a magnetic stirrer. Stir for another 30 min and check for complete dissolution. Transfer to a 100 mL volumetric flask and make up to volume with deionized water. Stir to obtain a homogeneous solution. Label as 0.5 w/v% Iodine-1.0 w/v% Potassium iodide aqueous solution. Store the solution in a refrigerator, and away from light. The solution is stable in a refrigerator, in darkness for several months.

Sodium sulfate (15% w/v) solution

Place 800 mL deionized water in a 1 L glass beaker, put a stir bar

to the beaker, and add 150.0 g sodium sulfate while stirring. Continue stirring until all the sodium sulfate has dissolved. Transfer to a 1000 mL volumetric flask and make up to volume with deionized water. Stir to obtain a homogeneous solution. Label as Sodium sulfate (15% w/v) solution. Store the solution at room temperature.

#### Preparation of In-house Reference Standard

Prepare a 0.5% w/v solution of a reference tamarind seed polysaccharide that has been filtered and alkali treated. Centrifuge to remove impurities, if any. Precipitate by adding 2-propanol. Filter, wash the precipitate with 2-propanol. Repeat precipitation and filtration step. Dry and grind. Label as "In House Reference Standard". Store in house reference standard in a desiccator.

#### Preparation of Sample

Place 170 mL deionized water at room temperature in a beaker. Weigh exactly 1.0 g sample (separately measure its loss on drying[%]), disperse in deionized water while stirring, and dissolve by stirring at room temperature for 15 min. Adjust this solution to exactly 200 g with deionized water and stir to obtain a homogeneous solution. Weigh exactly 1.0 g of this solution; add deionized water to exactly 50.0 g and mix to make the solution homogeneous. Prepare before use in assay.

#### Preparation of Standard Solutions

Place 170 mL deionized water at room temperature in a beaker. Weigh exactly 1.0 g in-house reference standard. Disperse in deionized water while stirring, and dissolve by stirring at room temperature for 15 min. Adjust this solution to exactly 200 g with deionized water and stir to obtain a homogeneous solution. Weigh exactly 4.0 g of this solution; add deionized water to exactly 40.0 g and mix to obtain a homogeneous solution.

Weigh exactly 2.0, 3.0, 4.0, 5.0 and 6.0 g of this solution; add deionized water to exactly 20.0 g and mix to obtain a homogeneous solution. Use the resulting solutions as 0.005, 0.0075, 0.01, 0.0125 and 0.015 w/w% (equivalent to 50, 75, 100, 125, and 150 µg/g respectively) standard solutions, respectively, which should be prepared before use. Calculate the actual concentrations of the in-house reference standard (on a dry matter basis) in each solution prepared.

#### Procedure

Add 1 mL each of deionized water (blank), sample or standard solutions to test tubes. Add 2 mL of 15% w/v Sodium Sulfate Aqueous Solution and 0.25 mL of 0.5% w/v Iodine–1.0% w/v

Potassium Iodide Aqueous Solution to each test tube. Prepare duplicates for each standard, sample, and blank.

After vigorous mixing for a few seconds, close the test tube with a stopper. Place the test tube in a refrigerator and in the dark immediately. Allow to stand for not less than 1 hour to fix iodine, and then for another 30 min in the dark at room temperature. Transfer the blank to a cuvette, mount on a spectrophotometer and use to set the absorbance at 640 nm to zero. Transfer the prepared sample and standard solutions to cuvettes to measure the absorbance of each at 640 nm.

Produce a standard curve using Absorbance vs. Concentration of in-house reference standard using the values obtained for the standard solutions, where the zero point is excluded with a coefficient of correlation of at least 0.99. If the coefficient of correlation is below 0.99, repeat all preparations of standard solutions to generate a new standard curve. Deduce the concentration of tamarind polysaccharide in the sample from the standard curve.

$$T = \frac{D}{W_T \times \left(\frac{100 - L}{100}\right)}$$

Where

- $T$  is %Tamarind Seed Polysaccharide in the sample
- $D$  is concentration of Tamarind Seed Polysaccharide determined in the sample solution,  $\mu\text{g/g}$
- $W_T$  is weight of the sample, g
- $L$  is percent loss on drying of the sample