

# Isolation, Identification and Partial Characterization of Bacteria Responsible for Hydrolysis of Tamarind Kernel Powder Based Gum

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**ABSTRACT:** Tamarind kernel powder, popularly known as TKP, is a natural polysaccharide derived from the seeds of *Tamarindus indica* Linn. Tamarind is a very important tree commonly found in India and South East Asia. The gum prepared from the seeds of TKP finds wide applications in textile sizing and printing, paper binding, as additives in food and pharmaceutical products. In the textile industries this TKP based thickener is widely used in printing application. This TKP based gum is hydrolyzed after 48-72 hours. The hydrolysis is primarily attributed to the growth of bacteria and fungi. Bacterial isolates are isolated by various isolation methods. Isolates were identified by Gram staining, Biochemical tests and hydrolytic activity of the isolates on proteins, lipids and carbohydrates.

**KEYWORDS:** Tamarind seeds, Tamarind kernel powder, Industrial Thickener, Hydrolysis, Isolation, Identification, Biochemical tests, characterization.

## I. INTRODUCTION

Tamarind, botanical name: *Tamarindus indica* Linn, consists of fruit pulp and a seed or kernel. Tamarind seed is an under-utilized bi-product of the tamarind pulp industry [1, 2]. Tamarind gum is obtained from endosperm of seeds of the tamarind tree, which is a seed gum with potential industrial applications. The powder prepared by pulverizing the kernel is known as TKP and is a rich high molecular weight polysaccharide. The proportion of Glucose : Xylose : Galactose in TKP is in the ratio of 3:2:1 [3, 4, 5].

TKP is also known as Xyloglucan, which is a food-grade starch which mainly substitutes high molecular weight (720-880 kDa) polysaccharides by imparting viscosity to the solution when dissolved in water. It serves as a polysaccharides gum extracted from plant material [3, 6, 7].

The manufacturing or processing of TKP is done by the following different steps like:

- Harvesting
- Sorting
- Roasting
- Stripping
- Grinding, and
- Screening [8]

The powder thus obtained is ready for preparing TKP thickener. TKP obtained from tamarind seeds, is also used in textile industry as a sizing agent and in dyeing as a anti-migrating agent and as printing thickener. It also finds applications in paper making, jute industries, food industry and as a binding agent in pharmaceuticals [9, 14].

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## II. COMPOSITION OF TKP

The major chemical constituents of TKP are:

Moisture	7.1	%
Polysaccharide	51.0	%
Total Ash	3.9	%
Crude fiber	1.2	%
Protein	7.6	% [8]

TKP disperses rapidly in cold water on constant stirring at approximately 4000 rpm. A uniform solution having maximum viscosity is quickly obtained on heating, by cooking the mixture in a hot water bath at 70 - 80 °C for 25-30 minutes with intermittent manual stirring [10, 13].

The thickener is a crucial component of printing process. Its main role is to act as a carrier of the unfixed dyes in place, first on to the fabric, which are subsequently after drying, fixed in to the fabric uniformly and evenly, generally by high temperature loop ager steaming, wherein the dye is released from the thickener and is diffused into the fiber. It also holds each color in place so that one color paste can be applied adjacent to the other without the colors bleeding onto and into the other [10].

## III. IMPORTANCE IN PRINTING INDUSTRIES

- TKP has the potential for commercial application in the textile printing as a thickening agent [12].
- TKP based gum is a natural environmental friendly biodegradable product. Its production cost is low, and is abundantly available as well as economically viable [11, 12, 15].

## IV. METHODOLOGY

### Materials:

TKP used in this research was procured from Spectrum Auxi-Chem Private Limited, Block 307/1, NH 48, Baleswar, Palsana, District Surat.

### Preparation of Thickening Agent:

Thickening agent was prepared according to the following steps:

- 40 grams of TKP is added in 500 ml of water with constant stirring. The concentration of the prepared paste is maintained at 8% w/v.
- The dispersion mixture is vigorously stirred for 15 minutes by mechanical stirrer at 4000 rpm at room temperature.
- Maximum and uniform viscosity is quickly obtained on cooking the mixture in a hot water bath at 70 - 80 °C for 25-30 minutes.
- The mixture is then allowed to cool to room temperature.
- After 24 hours of stabilizing, this TKP is used for further research [3].

### Measurement of Viscosity:

#### Instrument Details:

Make & Model:	FUNGILAB, VISCOLEAD ONE R
Serial Code:	VL101002
Viscosity Range:	100 - 130,000,000 cP
Spindles Available:	R2, R3, R4, R5, R6, R7

#### Procedure followed for accurate Viscosity Measurement:

- The leveling screw is adjusted for perfect leveling until the spirit bubble is within the marker circle.
- Instrument electrical power supply is then switched on.
- System will start and the 'Home Page' is displayed on the screen.
- Instructions displayed on the home page are then systematically followed.
- Spindle is removed and 'Enter' key is pressed / selected to initialize 'Auto Test'.
- Options displayed after 'Auto Test' are:

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- 1) **Instrument Profile.**
- 2) **Measurement.**
- 3) **Calibration**

➤ Following steps are adopted for Viscosity Measurement:

- 1) 'Measurement' menu is selected and 'Enter' is pressed.
- 2) Appropriate Spindle Number & RPM are selected.
- 3) 'ON' key is then pressed.
- 4) Viscosity measurement will start.
- 5) Torque is maintained within 15-95 %.
- 6) After stabilization of the display, displayed measurement is noted and the rotor/motor is stopped by again pressing the 'ON' key.
- 7) Finally press 'QUIT' key followed by removing the test sample, carefully cleaning the instrument parts and adopting the specified switching off procedure.

### Isolation of Microbes:

- Hydrolyzed thickener was streaked on to the Nutrient Agar plate.
- Plates were maintained precisely at 37 °C in a standard laboratory incubator for 24 hours.
- Different types of bacterial growth were observed on the Nutrient agar plate.
- Further isolation was carried out for purification of bacteria.
- The purified and isolated bacteria were checked for Gram reaction, hydrolysis of Protein, Lipid, Carbohydrate by using Milk agar plate, Glycerol tributyrine agar plate, 1% cellulose containing nutrient agar plate and 1% xylose containing nutrient agar plate respectively.

## V. RESULTS AND DISCUSSION

### Hydrolysis of TKP leading to Viscosity drop:

- Gum paste was maintained at 37 °C which is generally the ambient temperature in all dyeing and printing units.
- Viscosity and pH were monitored accurately from day 01 to day 04.
- Viscosity was checked regularly for four days at 24 hour intervals

**Table 1: Viscosity Drop:**

	Days	*Viscosity (cP)	RPM	**Torque %	pH
<b>Experimental Procedure 1</b>	1	19357	20	96.3	7.6
	2	18710	20	93.3	7.2
	3	10320	20	40.0	4.0
	4	102	20	0.5	3.5
<b>Experimental Procedure 2</b>	1	18670	20	93.0	7.3
	2	11887	20	58.0	6.4
	3	7325	20	39.3	4.0
	4	109	20	0.8	3.8

**\*Viscosity:** It is a quantity that expresses the magnitude of internal friction in a fluid as measured by a force per Unit-area.

**\*\*Torque:** It is a force that tends to cause rotation.

From Table 1, it can be deduced that:

- Printing thickener starts losing its viscosity due to microbial hydrolysis after 48-72 hours of preparation.
- Viscosity is observed to drop gradually with pH becoming more acidic because of microbial growth leading to hydrolysis.
- The evident drop in viscosity is shown in Table 1.
- The decrease in torque is directly proportional to the loss of viscosity.



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## VI. CONCLUSION

Textile processors are the major consumers of TKP, which is in several tons per month. The positive results of enzymatic hydrolysis prove that the bacterial growth in TKP is primarily responsible for this total loss of viscosity. The binding capacity of TKP is lost due to this viscosity drop, which renders the gum paste useless: a tremendous economical loss to the processor in particular and the overall textile industry as a whole in general. Further detail identification of the organisms can be carried out by Genome sequencing, which will then help in the incorporation of an appropriate preservative in order to retard the particular bacterial growth in TKP directly during its production or during the preparation of the paste.

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