ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

STUDY OF MICROBIAL HYDROLYSIS OF TAMARIND KERNEL POWDER BASED THICKENER USING FTIR AND ITS PREVENTIVE MEASURES

Bhavini I Surati*[1], Farida P Minocheherhomji[1]

Department of Microbiology, B. P. Baria Science Institute, Navsari, Gujarat, INDIA

**Corresponding author: bdsolanki87@gmail.com*

ABSTRACT

Tamarind Kernel Powder (TKP) is a polysaccharide obtained from the endosperm of the tamarind seed of the tamarind tree. Botanical name of Tamarind is *Tamarindusindica Linn*. This TKP based gum is a valuable thickener and stabilizer, used in different industries. TKP is abundantly available and has a good economic viability. TKP is also known as Xyloglucan. This TKP based thickener is hydrolyzed within 48-72 hours. The hydrolysis is primarily attributed to the growth of bacteria and fungi. These microorganisms are isolated by various isolation methods. Isolates were identified by Gram staining, Biochemical tests and hydrolytic activity of the isolates on proteins, lipids, carbohydrates and 16S rRNA sequencing. Viscosity was monitored without preservatives and with preservatives at different concentration and temperature. Fourier Transforms Infrared Spectroscopy was performed to check any major or minor changes in the TKP based thickener with and without preservatives.

Keywords: Tamarind Kernel Powder, Hydrolysis, Isolation, Characterization

1. INTRODUCTION

Tamarind (*Tamarindus Indica Linn.*), is a member of the dicotyledonous family fabaceae (Leguminosae). It grows in more than 50 countries of the world and the major areas of production are Asian globe [1]. It consists of fruit pulp, seeds and kernel. Tamarind seeds are anunder-utilized bi-product of the tamarind pulp industry [2, 3]. 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. [1, 4]. Tamarind gum is obtained from endosperm of seeds of the tamarind tree, which is a seed gum with potential industrial applications. The manufacturing of TKP is done by the steps such as harvesting, sorting, roasting, stripping, grinding and screening [5]. TKP disperses rapidly in cold water on constant stirring at approximately. A uniform solution having maximum viscosity is quickly obtained on heating, by cooking the mixture in a hot water bath at specific temperature for several minutes with intermittent manual stirring [6]. TKP having enormous significance like thickening, suspending, emulsifying, film forming and gel forming properties because of having physico-chemical stability in terms of viscosity, compatibility, drying [1, 7].

2. EXPERIMENTAL DESIGN

2.1. Material

Nutrient Agar, Rose Bengal agar, Milk agar, Tributyrine agar plate, 1% cellulose and 1% xylan containing nutrient agar, p-Chloro m-Cresol. All media were procured from Hi-Media Pvt. Ltd

2.2. Methodology

2.2.1. Preparation of TKP thickener

Forty (40) grams of TKP was added in 500 ml of water in a steel container under constant mechanical stirring at 4000 rpm at room temperature. Stirring is continued for 15 minutes. Till this mixture became smooth without any lumps. The concentration of the prepared paste is maintained at 8% w/v. The dispersion is then heated in a waterbath maintained at 70-80 °C for 25-30 minutes in order to attain a uniformly viscous solution with manual stirring [8]. Mixture is then allowed to cool to room temperature before use. The viscosity of the cooled solution is in the range of 18000±500 cps. After 24 hours of stabilizing, this TKP paste is used for further research. The prepared paste is diluted with normal water before use to lower down the viscosity to match the desired printable parameters [4]. Disperse dyes require a pH of 4.0-4.5 for getting their optimum color or tinctorial value. Hence, nonvolatile organic acids like

citric acid or tartaric acid and certain loop accelerator agents are added with the disperse dyes before printing. The overall pH of the printing paste is thus acidic, which makes the unused paste more prone to microbial and fungal hydrolysis [6].

2.2.2. Measurement of viscosity

The viscosities for all tested samples were scanned using well-standardized viscometer suspended vertically (FungiLab, VL101002, viscosity measurement range is 100-130,000,000 cP). A perfect horizontal level to the plane of the instrument was maintained at by adjusting the leveling screw. Instrument electrical power supply was then switched on to display the 'Home Page' on the screen. Instructions was displayed on the home page then sequentially followed. Spindle was removed and 'Enter' key is pressed/selected to initialize 'Auto Test' wherein the following three options were displayed: Instrument Profile, Measurement, and Calibration

2.2.3. For measurement of viscosity

'Measurement' menu was selected followed by Appropriate Spindle Number & RPM.'ON' key was then pressed to commence the measurement of viscosity. Torque was kept in the range of 15-95 %.On stabilization of the display, the measurement was noted, followed by again pressing the 'ON' key to stop the rotor/motor. Finally the instrument switch off procedure was initiated by pressing the 'QUIT' key, taking the test sample from the platform and carefully clean the instrument parts.

2.2.4. Isolation of microorganism

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 for bacterial isolates. Other plates maintained at 25 °C at room temperature for 48-72 hours for fungal isolates. Different types of bacterial growth were observed on the Nutrient agar plate and fungal growth were on the Rose Bengal agar plates. Further isolation was carried out for purification of microorganisms. The purified and isolated bacteria were checked for Gram reaction, motility, and biochemical reaction and wet mounting for fungal isolates, hydrolysis of Protein, Lipid, Carbohydrate by using Milk agar plate, Glycerol tributyrine agar plate, 1% cellulose containing nutrient agar plate and 1% xylan containing nutrient agar plate and Rose Bengal agar plates respectively.

2.2.5. Identification of microorganisms

Identification was carried out for those isolates which show maximum hydrolysis. The 16S r RNA for bacteria and 18 S ITS region for fungi sequencing method was used.

2.2.6. Incorporation of synthetic preservative in TKP thickener

Incorporation was carried out by the addition of various concentration of p-Chloro m-Cresol as a preservative in the TKP thickener. For the stabilization of TKP thickener, container was kept for 24 hours. Viscosity of stabilized TKP thickener was checked, and noted down. Viscosity was checked every day until the thickener was hydrolyzed. Incorporation of preservatives and viscosity check was proceeded for different concentration of preservatives until satisfactory result were obtained.

2.2.7. p- Chloro m- Cresol as a preservative

It is used as an Antiseptic and preservative at 0.1 % to 0.2 % concentration [9]. PCMC is approved for use an indirect food additive [10]. Other than food or medical uses of PCMC like packaging materials Glues, paints, textile furnishes, Leather and tanning agents and industrial oils and emulsions [11].

The use of different concentration for most non cosmetics applications of PCMC ranges from 0.05 % to 0.5 % [12]. Parenteral uses of aqueous drugs are sometime preserved with 0.05 % to 0.1% concentration of PCMC [13]. Enzymatic activity or the use of amino acid by the PCMC which is inhibits the germination of most of Bacillus species [14]. The initiation of germination of Bacillus species in medium and in the phosphate buffer, peptidase enzyme is inhibited by PCMC. The activity of the enzyme is not only primary site of inhibition but amino acid inhibited by PCMC which is initiated germination of bacteria. The concentration of PCMC is 0.02 % is required to showing antimicrobial activity against gram positive, gram negative, including tubercle bacilli and fungi [15]. 0.01 % of PCMC concentration is sufficient to inhibit the growth at 106 organism/ gramm of *Candiida albicans* and *Apergillus niger* [16].

2.2.8. Characterization: fourier transforms infrared spectroscopy

Native and hydrolyzed TKP gum samples were studied by Fourier transform infrared spectroscopy (FTIR). IR-

spectral studies were performed at CENTER OF EXCELLENCE, VAPI. Spectrometer under dry air at room temperature using KBr pellets. KBr was supplied with FTIR unit. The TKP gum samples were pressed directly on to attenuated reflectance KBr crystal into the sampling unit. Spectra were scanned between 4000 and 400 cm^{-1}.

3. RESULTS AND DISSCUSION

Experiments carried out for viscosity of TKP gum, Hydrolysis determine based on dropping of Viscosity. Identification of responsible microorganism, Incorporation of p- Chloro m-Cresol were incorporated to prevent hydrolysis from microorganism. This is confirmed by the FTIR wave number showing variation in functional group.

Table 1: Viscosity drop

Experimental	Days	Viscosity (cP)	Torque %	pH
1	1	12450	62.0	7.0
	2	8730	43.1	4.7
	3	3210	12.3	3.2
	4	150	01.9	3.0
2	1	13550	60.0	7.2
	2	10182	50.2	4.0
	3	4934	23.1	3.0
	4	119	1.02	3.0

**at rpm 20*

3.1. Identification of microorganisms

Identification of the organisms responsible for hydrolysis was done by 16 s r RNA and 18 S RNA sequencing technique. The three bacterial isolates and three fungal isolates were obtained after the experimental procedures were: Bacterial isolates obtained are: *Bacillus crescens, Bacillus subtilis, Bacillus cereus* and *Penicillum citrinum*. Fungal isolates obtained are *Aspergillus flavus* and *Aspergillus niger*.

Among the different synthetic preservatives, sodium azide showed constant viscosity for several days, where p-chloro m- cresol could maintain higher viscosity for several days.

Table 2: Viscosity with synthetic preservative

Sample	Spindle	RPM	Day 01	Day 02	Day 03	Day 04	Day 05
			Viscosity in Cp				
			Torque in %				
Only Gum	R5	6	27,320	865.3	-	-	-
			73.00	4.30	-	-	-
Gum+0.1% P-Chloro M-Cresol	R5	6	53,700	52,165	51,169	50,154	40,379
			80.00	78.9	76.0	75.2	60.06
Gum+1% P-Chloro M-Cresol	R5	6	60,320	59,335	54,917	54,377	52,525
			58.00	56.10	84.30	81.00	78.80

Table 3: Viscosity with various concentrations of preservatives (37 °C)

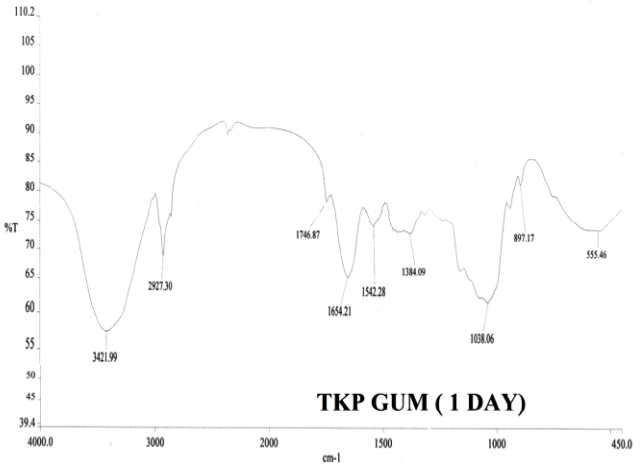
Sample	Spindle	RPM	Day 01	Day 02	Day 03	Day 04	Day 05
			Viscosity in Cp				
			Torque IN %				
Only Gum	R5	6	15,630	13,305	11,862	-	-
			77.8	76.03	59.0	-	-
Gum+0.1% P-Chloro M-Cresol	R5	6	53,700	52,165	51,169	50,154	40,379
			80.00	78.9	76.0	75.2	60.06

Table 4: Viscosity with preservatives: (60º C)

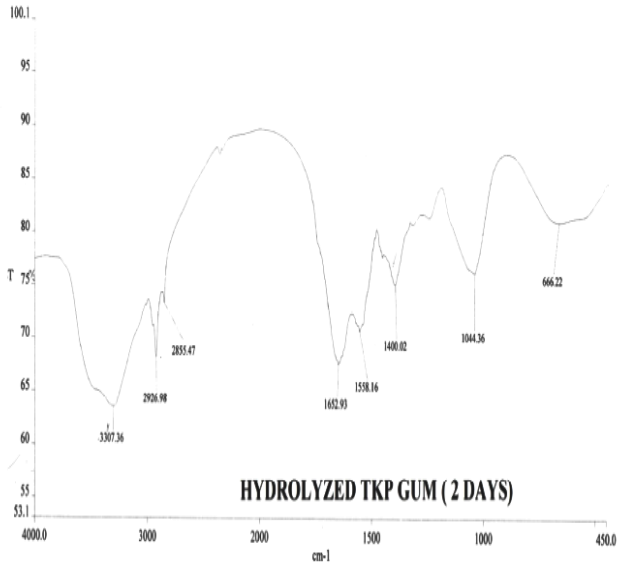
Sample	Spindle	RPM	Day 01	Day 02	Day 03	Day 04	Day 05
			Viscosity in Cp				
			Torque in %				
Gum+0.05 % P- Chloro M-Cresol	R5	6	27,079	26,320	25120	24,920	21,778
			41.1	40.2	38.6	37.4	32.7
Gum+0.03 % P-Chloro m-Cresol	R5	6	21,823	19,325	15,800	11,766	11,100
			32.7	30.30	24.3	17.6	16.00

3.2. Fourier transforms infrared spectroscopy

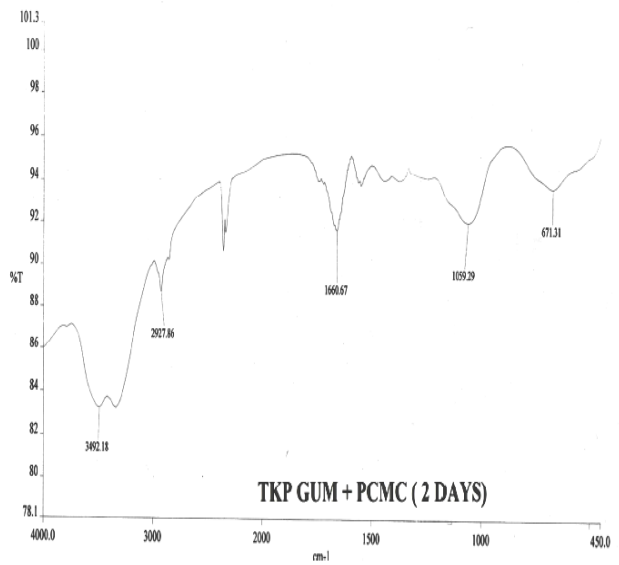
FTIR spectra of native TKP and hydrolyzed TKP were recorded to compare the changes in their chemical structure and characteristic IR wave numberwhich can be observed in table no. 5. Unhydrolyzed TKP gum FTIR spectra are shown in graph no 3 and hydrolyzed TKP gum are shown in graph no. 4. The region of FTIR spectra between 2950 and 2800 cm^{-1} shows C-H stretching frequency [17]. The peak in the spectra around 3400 cm^{-1} was due to O-H stretching frequency of TKP and water involved in hydrogen bonding [18]. The peaks observed in the spectra between 800 and 1200 cm^{-1} represented the highly coupled C-CO, C-OH and C-O-C stretching frequency of TKP. Associated water molecule resulted in the band around 1654 cm^{-1} in thespectra. The region around 1400 cm^{-1} due to CH2 banding modes was also observed [17]. In hydrolyzed TKP gum, sharpening of absorption band around 1652 cm^{-1} shows its increased association with water molecule, which could be a justification of its improved solubility compared tonative TKP gum. Any change in spectra of this region indicates conformational changes [19].



Graph 1: TKP gum at first day



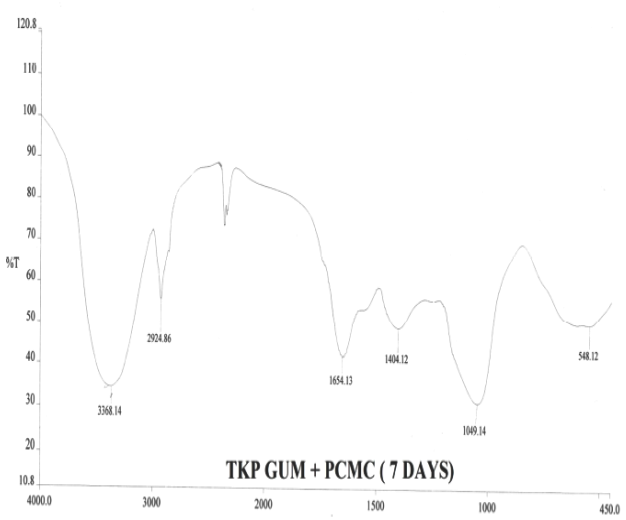
Graph 2: Hydrolysed TKP gum after two days



Graph 3: PCMC incorporated TKP gum after two days

Table 5: Characteristic of IR wave numbers of TKP powder, hydrolyzed TKP gum and TKP gum with PCMC preservatives

Characteristic group Wave number	TKP powder	Hydrolyzed TKP	TKP with PCMC	TKP with PCMC
	1DAY	2DAY	2DAY	7DAY
O-H stretching Vibration	3421.99	3307.36	3492.18	3368.14
C -H stretching of CH_2 Group	2927.30	2926.98, 2855.47	2927.18	2924.86
Ring stretching	1654.21	1652.93	1660.67	1654.13
Symmetrical deformations of CH_2	1384.09	1400.02	1404.12	1404.12
C -OH and primary Alcoholic	1038.06	1044.36	1049.14	1049.14



Graph 4: PCMC incorporated TKP gum after seven days

4. CONCLUSION

From the observation of viscosity and Fourier Transforms Infrared Spectroscopy, we can conclude that p-chloro m-cresol has the ability to maintained optimum viscosity for several days. The exact quantity of the p-chloro m-cresol can be optimized by carrying out the same procedure at different concentrations of the p-chloro m-cresol to reduce the cost of the thickener. As per our findings, recommended dosage of PCMC is 0.05 % on the weight of TKP taken to prepare the paste, which leads to a very negligible cost escalation of less than 0.1 %, but at the same time the stability of the paste increase and the processors arebenefitting due to avoidance of monetary losses at an extremely negligible costincrease.

5. REFERENCES

1. Kumar C S, Bhattacharya S. *Crit Rev Food Sci Nutr,* 2008; **48(1):**1-20.
2. Thombare N, Shrivastava S, Chowdhary R, *Science Reporter*, 2012; 32-33,
3. Goyal P, Vineet Kumar, Sharma P, *Carbohydr. Polym,* 2007; **69:**251-255.
4. Klahal K Pikul J, Wancheng, Mongkholrattanasit R *.RMUTP international conference. - [textile and fasion*, 2012; **1:**1-7.
5. Deepak, Umadevi S, Hiremath, Jamuna KV, *J PharmacognPhytochem,* 2018; **7(3):**1113-1118.
6. Freitas RA, Martin S, Santos GL. *Carbohydr. Polym*, 2005; **60(4):**507-514.
7. Shankaracharya NB. *J Food Sci Tech Mys - Mysore,* 1998; **35(3):**193-208.
8. Babel S, Upadhayay H, Gupta R. *Indian J Fibre Text*, 2015; **1(5):**5-8.
9. Rossof IS, Hand book of Veterinary Drugs : A Compendium for Research and Clinical Use . 104. *New York: Springer Publishing Co*. 1974.
10. Rothschild DL. *Washington: Food Chemical News*, 1990.
11. Dooms -Goossens A, Degreef H, Vanhee J , et al., *Contact Derm,*1981; **7:**51-52.
12. Andersen K E, Hamann K. *Contact Derm*, 1984**; 11:**11-20.
13. Cronin E. *Contact Derm,* 1980; 673-674.
14. Sierra G. *Can J Microbiol,* 1970; **16:**51-52.
15. Paulus W, Genth H, Microbiocidal phenolic compounds - A critical examination. In Biodeterioration. eds. T.A. Oxley and S. Barry, 1983 ;(5):701-702. New York: John Wiley & Sons.
16. British Pharmacopoeia. 1982. Efficacy of antimicrobial preservatives in pharmaceutical products. 1980 Addendum. London: *British Pharmacopoeia.*
17. Marta K, Peter SB, Wilson RH, et al., *J Sci Food Agric*, 1998; **77:**38-44.
18. Fringant C, Desbrieres J, Rinaudo M. *Polymer*, 1996; **37 (13):**2663-2673.
19. Mudgil D, Barak S, Khatkar BS. *Int j biol macromol,* 2012; **50:**1035-1039.