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MECHANICAL BEHAVIOR OF POLYSACCHARIDE BASED BIOPOLYMER SYNTHESIZED FROM THE SEED KERNEL OF TAMARINDUS INDICA L

ABSTRACT

Biopolymer carboxymethyl tamarind seed kernel polysaccharide (CMTSP) was synthesized by the reaction of tamarind kernel powder (TKP) of *Tamarindus indica* L. with monochloroacetic acid by an improved method. The synthesis was conducted in presence of sodium hydroxide at optimized conditions of time, temperature, concentrations of TKP, MA, sodium hydroxide. Tamarind seed polysaccharide (TSP) was also extracted from TKP by boiling distilled water. The chemical structure of TKP, TSP and CMTSP were analyzed by the ATR-FTIR. When TKP, TSP, and CMTSP's comparative physico-mechanical properties were examined and compared, CMTSP performed better due to increase in viscosity, water solubility and tensile properties.

Keywords: *tamarind seed;* ***b****iopolymer; carboxymethyl tamarind kernel polysaccharide*

INTRODUCTION

Tamarind (*Tamarandus indica* Linn.) is very common and commercially most important large evergreen tree that is grown abundantly in the South East Asian countries [1-5]. TKP is a crude extract of tamarind seed and it has major industrial use as an important sizing material for jute and textile industries [6]. All parts of the tamarind tree can contribute to the development of green and sustainable products such as leaves, shell, pulp, fiber and seed apart from its domestic use finds application in food industry, pharmaceutical industry, biofuel industry, water industry, electrochemical industry, textile industry and composite industry [7]. TSP or tamarind gum polysaccharide (TGP) is extracted from the TKP. TSP is a biodegradable and biocompatible natural polymer which has advantages over synthetic and semi-synthetic excipients because of their lack of toxicity, fewer side effects, soothing action and non irritant nature [2, 8]. TSP is called ‘ageing free starch’ because its property is similar to starch but is more stable [2]. TSP is chemically inert, non-toxic, noncarcinogenic, less expensive, biodegradable, widely available and also possesses property like high viscosity, broad pH tolerance, adhesiveness, high drug holding capacity, and high thermal stability and [1, 8]. These properties led to its application as a controlled and targeted drug delivery in pharmaceutical industries, as thickener and gelling agent in food industries, as solid electrolyte in electrochemical industries and in the form of hydrogel in agricultural industries [1, 7]. They are also used in cosmetics, textiles, paints and paper making [8]. In food and pharmaceutical industries, TSP is used as gelling agent, thickener, stabilizer and binder [1, 6, 7]. Tamarind seed consists of testa (20%–30% dry mass) and kernel (70%–75% dry mass) and TSP is a galactoxyloglucan (50-58 %) consisting of glucose, xylose and galactose in the molar ratio 3.1:1.7:1 [7]. Today, the whole world is increasingly interested in natural drugs and excipients like TSP due to their diverse pharmaceutical applications such as diluent, binder, disintegrant in tablets, thickeners in oral liquids, protective colloids in suspensions, gelling agents in gels and bases in suppositories [8]. It requires chemical modification because of its faster biodegradability and to eliminate certain drawbacks of basic polymer and can be further explored as an excipient in novel drug delivery systems [7-8].

Various modifications of TSP which have been executed till date include carboxymethylation, acetylation, hydroxyl-alkylation and thiolisation, polymer grafting etc which can compete with the available synthetic excipients [3, 8]. Carboxymethylation of TSP into carboxy methyl TSP (CMTSP) resulted in amplifying swelling capacity, in situ gelations, wide pH tolerance, high drug holding efficiency, stability, release kinetics, and hydrophilicity. So CMSTP has extensively been used in the field of drug delivery systems via developing various forms like nanoparticles, composites, films, hydrogels and pellets etc. broader applications in a number of industries [9]. Industrial discarded fruit waste fibres of *Tamarindus Indica. L* were investigated and found as a potential eco-friendly bio-reinforcement for polymer composite [10-11]. In this work, industrial discarded fruit waste seed kernel of *Tamarindus Indica. L* will be modified to CMTSP which may be used as potential reinforcement for polymer composites. The carboxymethylation of TSP by monochloroacetic acid was done to use for a wider range of applications by minimizing the drawbacks of TSP, such as its dull color, disagreeable odor, poor solubility in cold water, presence of water insoluble components [12-13]. CMTSP also exhibited enhanced penetrability and better screen ability in the printing paste [7]. Carboxymethylation of TSP disrupted the polysaccharide structure, enabled an anionic nature to the polymer and exposed the network of hydration which made higher viscosity and lower biodegradability than TSP so enhanced the shelf life of CMSTP by increasing the resistant toward enzymatic attack [14-15].

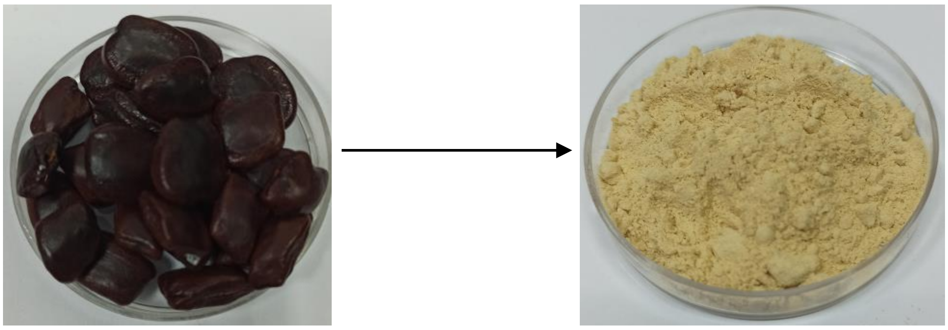
The objective of the present study was to develop a simple, improved method for producing CMTSP utilizing TKP in 100 % aqueous media reducing the heating time. CMTSP was produced in methanol or aqueous methanol and heated at 70 °C for one hour [12, 14, 16-17]. This study also aimed to evaluate the effectiveness of CMTSP by comparing its physico-mechanical characteristics to those of TSP and TKP. According to the literature, no research has been published to far comparing the mechanical behavior of TKP, TSP, and CMTSP.

MATERIALS AND METHODS

Tamarind seeds were collected from the local market in Dhaka. The chemicals were purchased from Merck, Germany. Distilled water was produced in the laboratory.

Preparation of TKP from tamarind seeds

Tamarind seed (Fig. 1a) was taken to make TKP (Fig.1b). Tamarind seeds (187.5 g) were taken in the vessel, washed and dried in oven for 2-3 hours at 105°C to make the seed coating or husks brittle and friable. The husks (33% approx.) of the dried seeds were decorticated using grinder. Separated husks were removed by air blowing. The tamarind seed's endosperm, or white kernel (67% approx.) (125 g) were taken to make fine powder in the grinder. The ground powder was sieved through 100 mesh to obtain fine powder. The sieving and grinding processes were repeated to collect all TKP. Finally the TKP (125 g) was stored in a dry place in moisture-proof containers.



(a) (b)

Fig. 1. (a) Tamarind seed and (b) TKP

Analysis of the chemical constituents of TKP

The moisture content of the TKP was determined by calculating the initial weight before drying and the final weight after drying, which was done in an oven. The dried TKP was then extracted with n-hexane for 12 hours to remove the fat. The defatted TKP were taken to isolate protein and polysaccharide according to method described in the literature [6]. In order to determine the amount of ash in the TKP, it was first ignited in a crucible using a gas burner and then heated to 650 °C in a muffle furnace (model: CWF1200, UK). By calculating the difference, the polysaccharide content was finally determined.

Extraction of TSP from TKP

Endosperm or white kernel (67% approx.) of tamarind seed was taken to make tamarind seed polysaccharide (TSP). Tamarind kernel powder (TKP) or crushed pieces of tamarind kernel (36 g) was added to distill water (2500-3000g) and boiled to make 3.5% tamarind seed kernel solution. When TKP was used then it was dissolved in boiling water by stirring for 2-3 hours. However, when crushed tamarind kernel pieces were used, the seed kernels were removed after boiling in water for 2-3 hours and then pressed in a mortar and pestle to make a thick, viscous tamarind seed kernel solution. The solution was boiled with stirring for a further thirty minutes to an hour. In order to allow the protein and crude fiber to precipitate and settle out, the solution was filtered through a clean, white cloth, and the resulting solution (1000g) was then left overnight in a glass vessel. The supernatant liquid (850 g) was separated out by simple decantation. The supernatant liquid was boiled to concentrate until the volume reduced to half of its initial volume. Solution was cooled down at room temperature and equal volume ethanol (96%) was poured into the solution by continuous stirring. The precipitate was washed with ethanol (96%) and dried in oven at 70-80 °C for 6 hours. To obtain fine TSP powder, the dry material was pulverized, and then the powdered material was sieved through a 100 mesh screen (Fig. 2). The percentage of TSP obtained from the seed kernel was 54 % (approx.).

Fig. 2. Extracted TSP from TKP

Synthesis of CMTSP using TKP

TKP (20 g) was dissolved in 500 ml distilled water. Sodium hydroxide solution (10%) (21 g) was added into TKP solution with stirring at room temperature 30°C. After 30 minutes, monochloroacetic acid (MA) (3.05 g) was added into the TKP solution slowly with stirring at room temperature 30°C. Again after 30 minutes, the solution mixture was heated for 15 minutes at 70°C. The solution had a pH of 3.5. The solution was cooled and then CMTSP was precipitated (ppt) out of the solution by adding ethanol (96%). The precipitate of CMTSP was washed with ethanol (96%), filtered, collected and then dried first at room temperature then in an oven at 40°C for 6 hours. Finally, the CMTSP was stored in a dry place in moisture-proof containers.

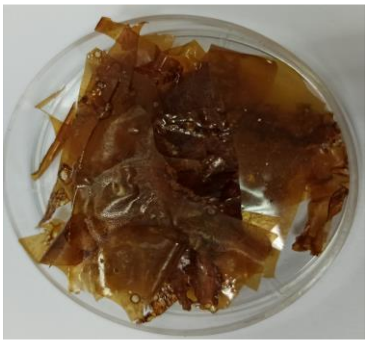
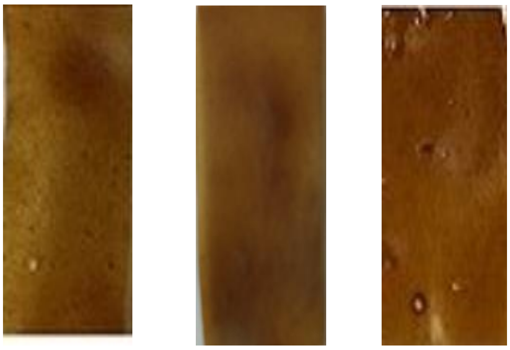


Fig. 3. Synthesized CMTSP from TKP

Solid sheet formation of TKP, TSP and CMTSP

TKP and TSP were dissolved in hot distilled water in a separate vessel then poured into the petridish. The petridishes were then allowed to air dry for 24 hours at 30°C in order to form solid sheets. Similarly, CMTSP was dissolved in cold distilled water and solid sheet was made in petridish using the same solution casting technique. The dried sheets were peeled off from the petridish and used to measure mechanical properties (Fig. 4 a-c).



a. b. c.

Fig. 4. Solid sheet of (a) TKP, (b) TSP and (c) CMTSP

Physical parameter analyses of TKP, TSP and CMTSP

TKP, TSP and CMTSP were separately dissolved in distilled water to make a 2% solution. At 30°C, the pH of the TKP, TSP and CMTSP solutions were measured using Jenway pH & Conductivity Meter, (model-3540, UK). The densities of the three products were also determined at 30°C using a Density and Refractometer (DMA 5000, Anton Paar, Austria). Using a Falling Ball Viscometer, the viscosities of the three products were determined at 30 °C.

Characterization of TKP, TSP and CMTSP by ATR-FTIR analyses

The ATR-FTIR spectra of TKP, TSP and CMTSP were recorded at the range of frequency 4000–650 cm^{-1} by the ATR-FTIR spectrophotometer (PerkinElmer-FTIR/NIR Model-Frontier, USA).

Mechanical properties of TKP, TSP and CMTSP

Mechanical properties of all sheets of TKP, TSP and CMTSP were carried out to investigate the tensile properties following ASTM D 3039/D 3039M-00 (2002) standard method using a Universal Testing Machine (UTM), model: 1410-Titan^5, Load cell: 5000 N, U.K. Cross-head speed of the test was 10 mm/min and gauge length was 50.00 mm. The average values of tensile strength (MPa) and elongation at break (%) were determined using the test results of five specimens.

RESULTS AND DISCUSSION

Chemical constituent of TKP

Tamarind seed is a waste and byproduct of tamarind pulp industries. In this research, tamarind kernel powder was used to extract TSP and it was modified with chloroacetic acid to make biopolymer CMTSP. The TKP was analysed and the ingredients found in TKP are presented in Table 1. The composition of tamarind seed varies widely with variety and the extent of maturity and it is about 30% of the whole fruit [15]. The kernel of the tamarind contains 50–57% carbohydrates [4, 15]. The percentage of polysaccharides or poly carbohydrates in this work is found to be around 60% (Table 1).

Table 1. *Chemical constituents of TKP*

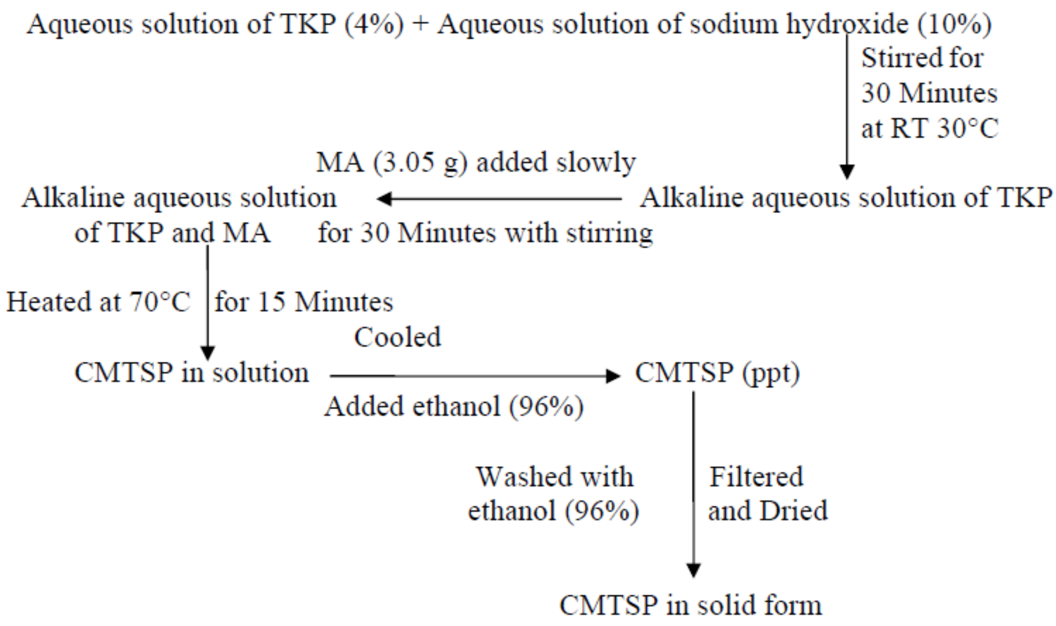
S. No.	Parameters	Tamarind seed kernel
1	Moisture content	7 % MAX
2	Ash Content	4 % MAX
3	*Polysaccharide content	60 % MAX
4	Protein content	15.5 % MAX
5	Fat/oil content	9.5 % MAX
6	Crude fiber and other constituents	4 % MAX

*Polysaccharide content was calculated by difference

Comparison of extracted TSP, CMTSP and TKP

TSP is a biopolymer extracted from TKP in aqueous medium, as stated in the experimental section. It is used in pharmaceutical excipients, as thickener in food industries, as solid electrolyte in electrochemical industries and as hydrogel in agricultural industries [7].

In this work, TKP was modified with MA to synthesize biopolymer CMTSP by an easy method. Reaction parameters such as concentration of TKP, monochloroacetic acid, and sodium hydroxide were optimized with reaction time and reaction temperature. According to the literature, CMTSP was synthesized in methanol or aqueous methanol and heated at 70 °C for one hour [12, 14, 16-17]. In this work, an improved method for producing CMTSP has been developed by reacting the TKP with MA in 100 % aqueous media in the presence sodium hydroxide reducing the heating time (Scheme 1). The percentage yield of CMTSP was about 45% (Table 2). The specifications of TKP, TSP and CMTSP were analyzed and the results are summarized in Table-2. It is found from Table 2 that the carboxymethylation of TKP increased its solubility in cold water and higher viscosity of solution. The results indicated that the CMTSP is a suitable candidate than the TSP to sustain the drug release in the colonic region [14]. The drawback of TKP and TSP is microbial contamination and modification of TKP can eliminate the drawbacks [8].



Scheme 1. Synthesis of CMTSP from TKP

***Table 2.** Specifications of TKP, TSP and CMTSP*

Sl. No.	Properties	TKP	TSP	CMTSP
01	Physical state	Solid powder	Solid powder	Solid powder
02	Color	Creamy white	Light brown	Dark brown
03	Solubility in water	Soluble in hot water	Soluble in hot water	Soluble in cold water
04	Moisture content	7 %	7.2%	10.6 %
05	Ash content	4 %	3.5%	6.9 %
06	pH at 30 °C	6.32	6.53	5.49
07	Density at 30 °C	1.034 g/cc	1.011 g/cc	1.098 g/cc
08	Viscosity at 30 °C	158.37 m Pa.s	140.20 m Pa.s	169.86 m Pa.s
09	Percentage yield	67%	54%	45%

ATR-FTIR characterization of TKP, TSP and CMTSP

To determine the purity and chemical structure of TKP, TSP, and the biopolymer CMTSP, ATR-FTIR spectra were conducted. Spectra of TKP, TSP and CMTSP are presented in Fig. 5, 6 and 7 respectively. It is found from Fig. 5, 6 and 7 that the peaks of TKP, TSP and CMTSP are almost similar in the ranges. Same observation was reported in the literature [14]. The main groups present in TKP are carboxylic acid group (-COOH) and hydroxyl groups (-OH) [3]. The characteristic peak of TKP, TSP and CMTSP in Fig. 5-7 exhibited broad strong peaks at 3500–3000 cm-1 belonging to stretching vibration of –OH groups present in glucose, xylose and galactose units in the polysaccharide and water involved in hydrogen bonding [17-20]. The peaks at 2800-2950 cm^{-1}, and 1600-1734 cm^{-1}in all spectra of Fig. 5-7 are associated with asymmetric stretching of CH and carbonyl groups (C=O) stretching [17-18, 20]. A strong peak at 1000-1025 cm^{-1} ranges in all spectra are attributed to the CH_2-O-CH_2 stretching [14, 18-20]. The C=C stretching observed at 1600-1640 cm^{-1} in TKP and TSP [19]. The prominent peak at 1420.61 cm^{-1} in Fig. 7 revealed the presence of carboxyl groups in CMSTP. The carboxymethylation is confirmed by the appearance of characteristic C=O and –COO bands at 1637.88 cm^{-1} and 1420.61 cm^{-1} respectively [21].

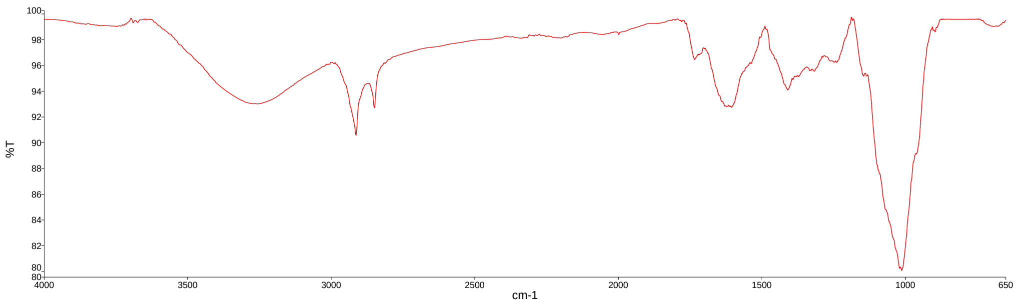


Fig. 5. ATR-FTIR spectrum of TKP

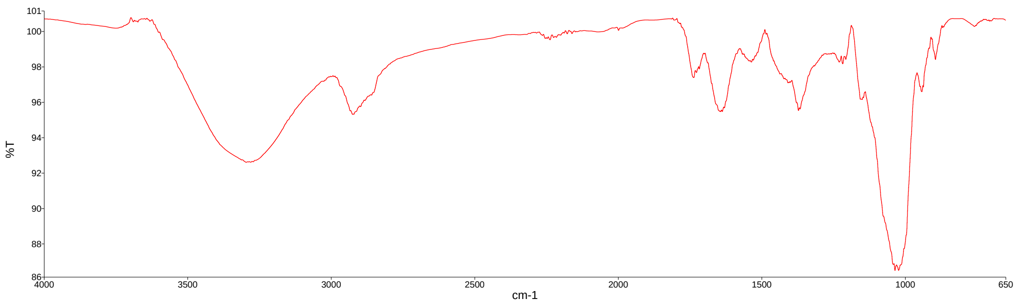


Fig. 6. ATR-FTIR spectrum of TSP

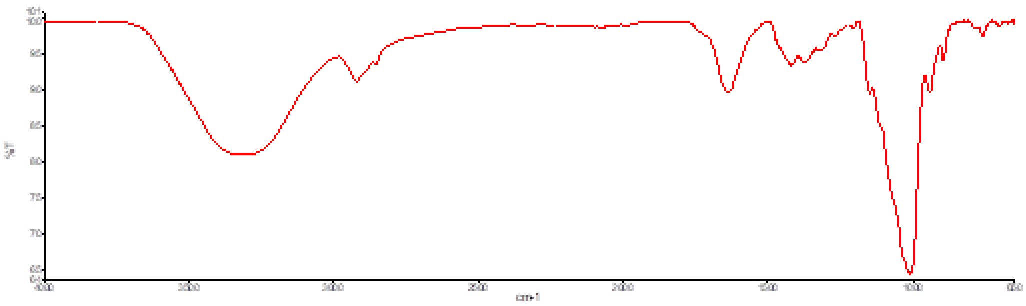


Fig. 7. ATR-FTIR spectrum of CMTSP

Mechanical properties of TKP, TSP and CMTSP

For measurements and comparative analyses of the mechanical properties, solid sheets of TKP, TSP, and CMTSP were used. Tensile strength of TKP, TSP and CMTSP are presented in Fig. 8 and elongation at break of TKP, TSP and CMTSP are presented in Fig. 9 respectively. It is found from Fig. 8 and 9 that CMTSP shows the highest tensile strength and elongation at break compared to TKP and TSP. The tensile strength and elongation at break values of carboxymethyl polysaccharides are higher than those of natural polysaccharides. For example, studies have revealed that carboxymethyl chitosan (CMCh) has higher tensile strength and elongation at break values than pure rice starch, which is a natural polysaccharide [22]. Tamarind seed gum or TKP reinforced sisal fiber composites also attained a high level of tensile strength [23]. Research into the mechanical and physical properties of the tamarind seed particles reinforced epoxy composites revealed improved physico-mechanical properties, allowing the use of the composite material in structural applications, industrial and commercial coating, interior automobile design, and other applications [24]. Tamarind seed gum reinforced banana fibre composite material has good fire retardant characteristics that can be use as a false roofing material instead of thermocole [25]. The tamarind seed filler (TSF) reinforced vinyl ester (VE) composites are used to fabricate the wheel hubcap of heavy-duty buses, bus seat backrest cover, and silencer guard of the motorcycle [26-27]. Due to tensile characteristics of biopolymer CMTSP, it is a more appropriate and useful material for these applications than TKP and TSP.

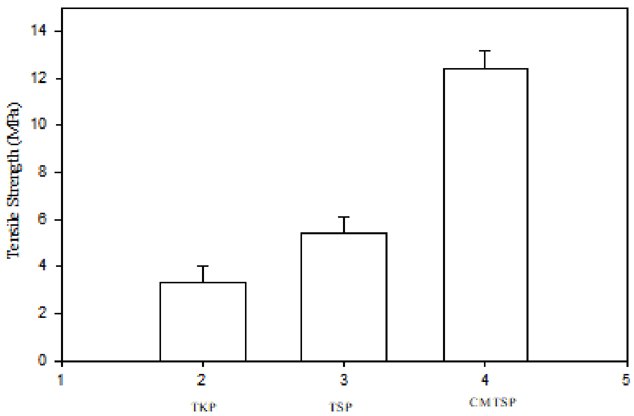


Fig. 8. Tensile strength of TKP, TSP and CMTSP

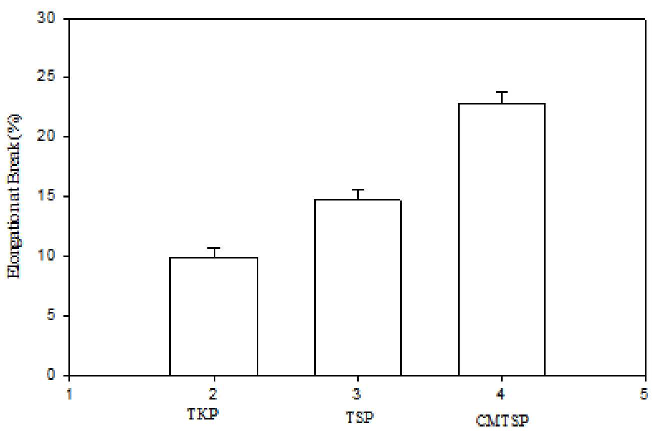


Fig. 9. Elongation at break (%) of TKP, TSP and CMTSP

CONCLUSION

Utilizing the TKP of waste tamarind seeds, the polysaccharide-based biopolymer CMTSP was successfully produced using an improved method. The green biopolymer CMTSP was synthesized by optimizing the reaction conditions of TKP and monochloroacetic acid. ATR-FTIR characterization confirmed the carboxymethylation of TKP. The carboxymethylation of TKP made it soluble in cold water. The viscosity of CMTSP in 2% solutions was higher compared to TKP and TSP. The tensile properties of CMTSP showed higher values than TKP and TSP. Therefore, biopolymer CMSTP can be used in a wide range of industrial applications, such as biomedical, foods, cosmetics, textiles, composite materials, and pharmaceutical products.

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