Synthesis and physio-chemical properties of sulphated tamarind (*Tamarindus indica L.*) seed polysaccharide

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INTRODUCTION

Tamarind Seed Polysaccharide (TSP) is a neutral, water-soluble biopolymer with a ramified structure: a backbone of glucose is linked with xylose and disaccharide units of xylose-galactose. The three monomers are present in a molar ratio of 4:3:1. TSP is already applied in the industrial fields, due to its physical, chemical, and biological properties (1). A chemical modification of this biopolymer is carried out adding sulphated groups on the TSP chain. The presence of negatively charged groups may allow a better solubilization of polysaccharide and also specific binding to proteins or receptors, giving new biological properties to TSP (2).

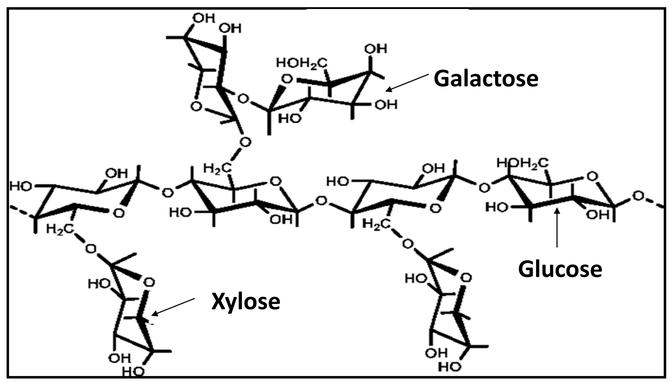


Fig. 1 Ripetitive unit of TSP

CHEMICAL REACTION

The sulfation reaction of TSP was performed in one-step process, using dimethylformamide as a solvent, and sulfur trioxide pyridine complex as reagent at different mole/residue ratios of TSP/SO_3-py (1:1, 1:1.5 and 1:2). The reaction was carried out for 24 h at room temperature. The sulphated products (S-TSP) were precipitated with ethanol 70%, centrifugated and then dialyzed in water (c. o. = 6-8 KDa).

RESULTS

CHARACTERIZATION SULPHATED PRODUCTS

Chemical and physical proprieties of S-TSP and TSP samples were characterized.

Table 1 Comparison of different analytical results of TSP and S-TSP samples

Samples	Req= TSP:SO_3xPy	(1)DS (%)	(2)Mw (KDa)	(3)Zp (mV)	(2)Size (nm)
TSP	-	-	650	0	45
S-TSP_1	1:1	8.4	911	-40	39
S-TSP_2	1:1.5	28.8	128	-63	9
S-TSP_3	1:2	33.6	247	-60	12

(1) Potenziometric titratation
(2) High Perfomace Size Exclusion Chromatography withTriple Detector Array (HP-SEC-TDA)
(3) Dynamic Light Scattering (DLS)

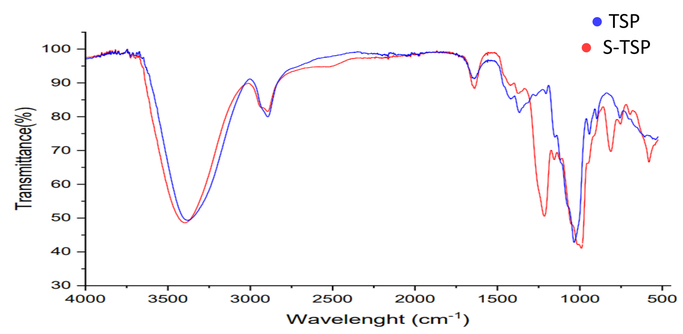


Fig. 2 FTIR spectra of TSP and S-TSP_1

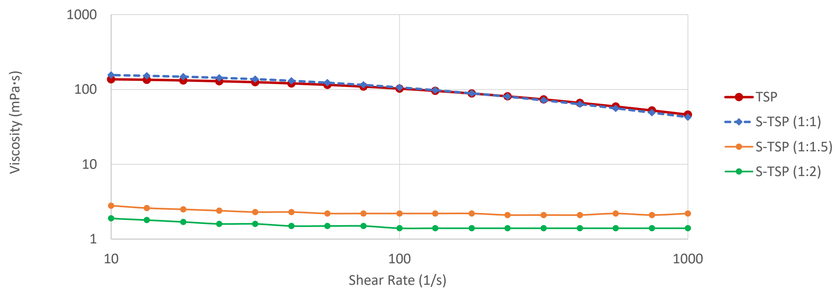


Fig. 3 Viscosity of TSP and S-TSP samples at 20°C obtained by Modular Compact Rheometer (Double Gap)

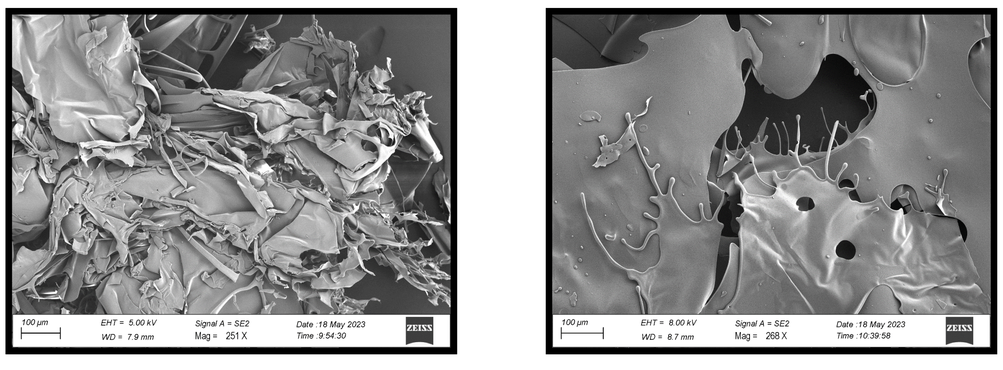


Fig. 4 SEM images of TSP and S-TSP_3

ENZYMATIC DEGRADATION

The distribution of sulphated groups on TSP is studied by NMR (Fig.5). Due to signals broadening the interpretation is difficult ; so, sulphated products were hydrolyzed by cellulase and/or xyloglucanase and characterized by NMR and LC-MS (Fig. 6-7-8).

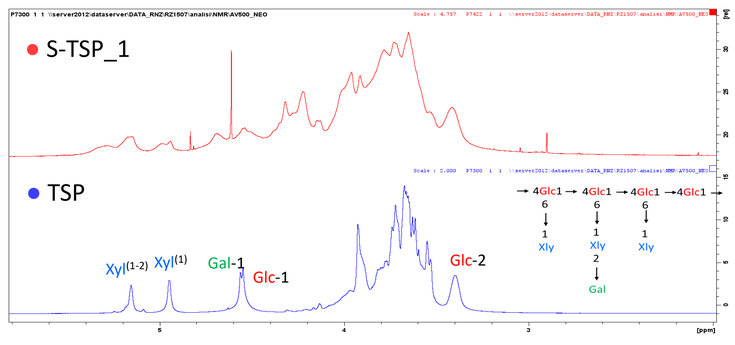


Fig. 5 ^{1}H spectra of TSP and S-TSP

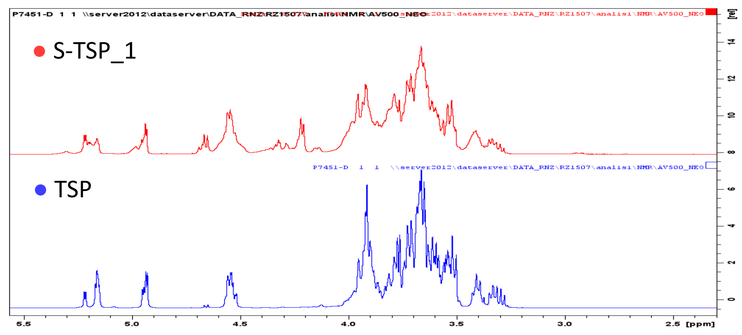
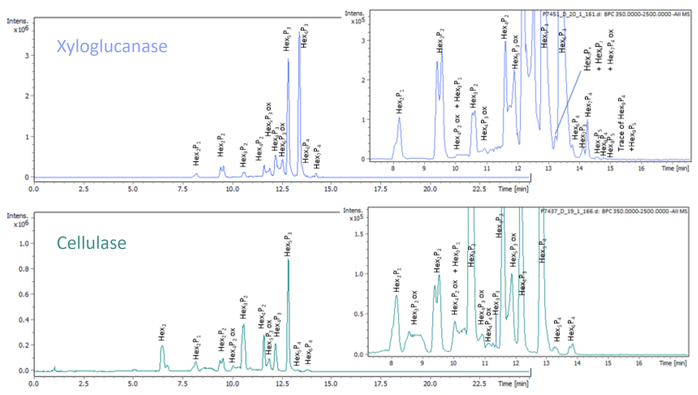
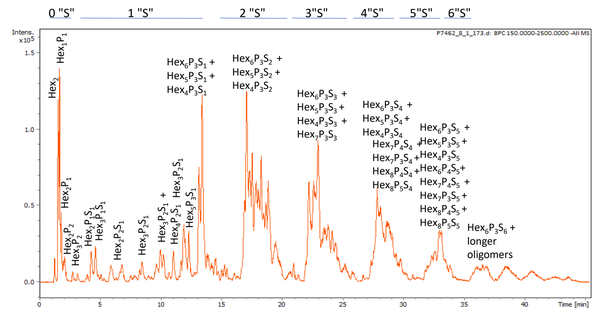


Fig. 6 ^{1}H spectra of TSP and S-TSP hydrolyzed with xiloglucanase



Column HILIC XBridge Amide 100x2.1 1.7 um Waters
Eluent A: 5 mM ammonium acetate in water
Eluent B: acetonitril
Flow 0.15 ml min

Fig. 7 HILIC/ESI-MS of TSP hydrolyzed with Xyloglucanase and Cellulase (P - pentose, Hex - Glc o Gal)



Column: Kinetex C18 100x2.1. 2.6 um Phenomenex
Eluent A: 10 mMdibuthylamine 10 mM acetic acid in water
Eluent B: 10 mM dibuthylamine 10 mM acetic acid in methanol
Flow 0,15 mlmin

Fig. 8 IPRP-HPLC/ESI-MS of S-TSP hydrolyzed with Xyloglucanase and Cellulase

FUTURE PERSPECTIVE

- Sulphation with higher substitution degrees will be carried out again, to obtain products with higher molecular weight.
- More NMR studies will be performed on hydrolyzed products.
- Different analyzes of optics and optometry will be employed to verify the behavior of the polymers in a physiological environment and the positive effects that these may have once compared to base polymers.
- The tolerability, toxicity and efficacy will be evaluated by one-dimensional cell and three-dimensional cell culture.

REFERENCES

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