SYNTHESIS AND PHYSIO-CHEMICAL PROPERTIES OF SULPHATED TAMARIND (*Tamarindus indica L.*) SEED POLYSACCHARIDE

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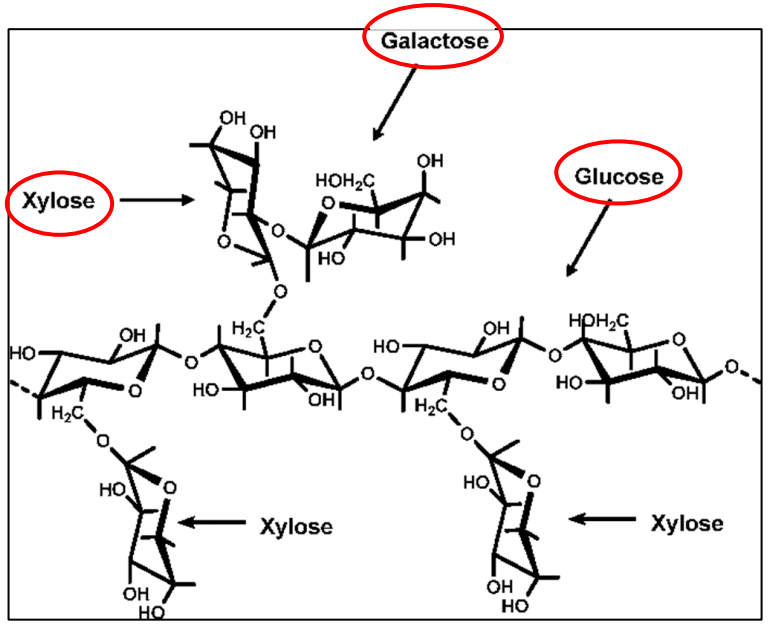
TAMARIND SEED POLYSACCHARIDE (TSP)

- Derived from the seeds of Tamarindus indica
- Neutral and watersoluble polysaccharide
- Formed by glucose, xylose and galactose (4:3:1)
- Used in the food industry as a thickener, gelling agent, stabilizer and binder
- TSP has demonstrated significant mucomimetic, mucoadhesive, and bioadhesive properties



AIM OF THE PROJECT

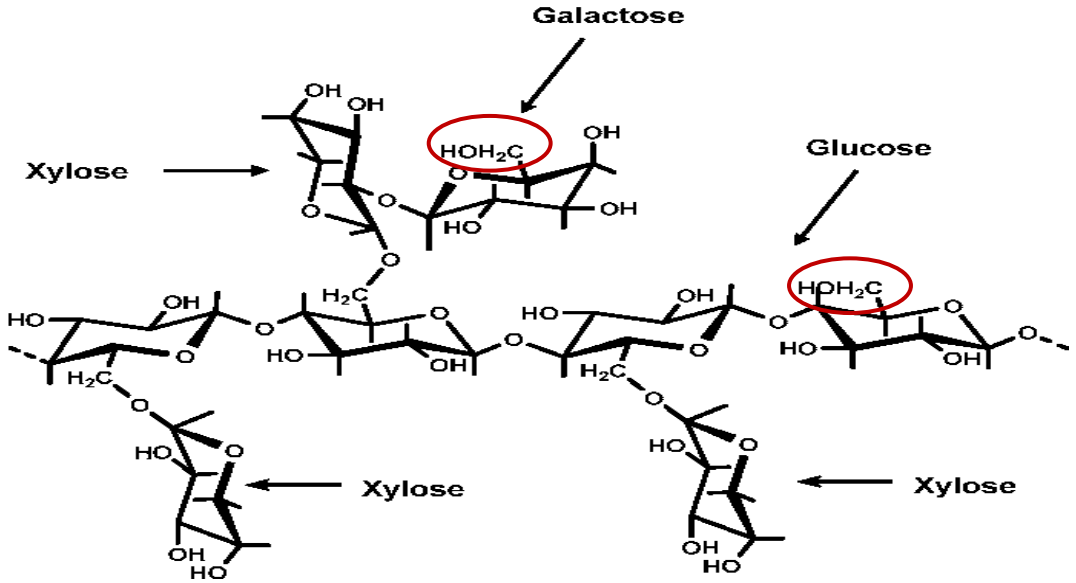
Modification of TSP adding sulphated groups

The presence of sulphate groups may allow interaction with proteins or receptors

Sulphated polymers possess important biological activities such as antioxidants, antitumors, immunoregulators and anticoagulants

The presence of negatively charged groups could maintain or increase the mucoadhesive properties of TSP

The presence of negative charges could promote a better solubilization of TSP in water

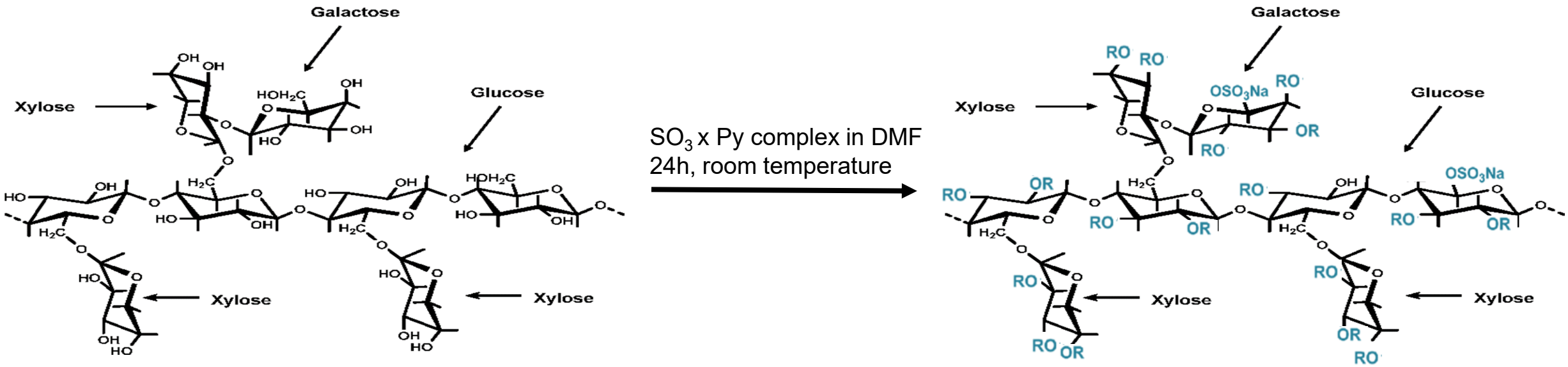


METHODS OF CHARACTERIZATION

NMR and FTIR	DLS	Titratror
Structural composition	Zeta Potential	Substitution degree

HP-SEC-TDA	Rheometer	LC-MS
Distribution of molecular weight	Viscosity	Finger print

CHEMICAL MODIFICATION

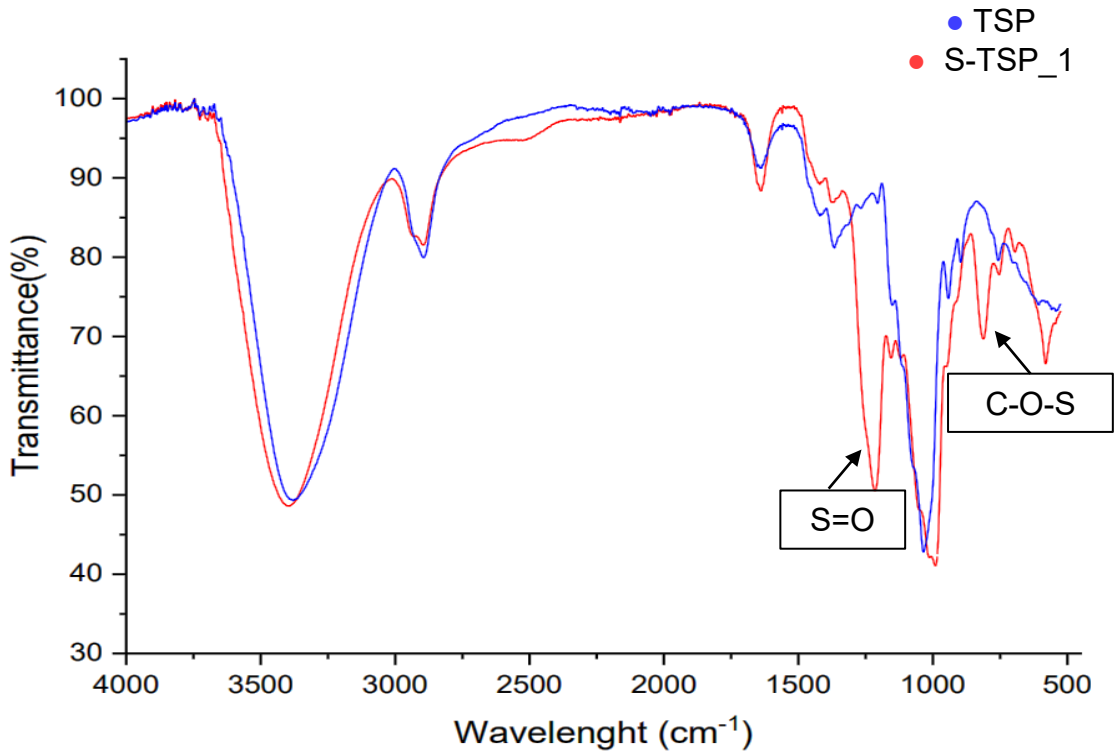


Samples	Req= TSP: SO_3 x Py
S-TSP_1	1:1
S-TSP_2	1:1.5
S-TSP_3	1:2

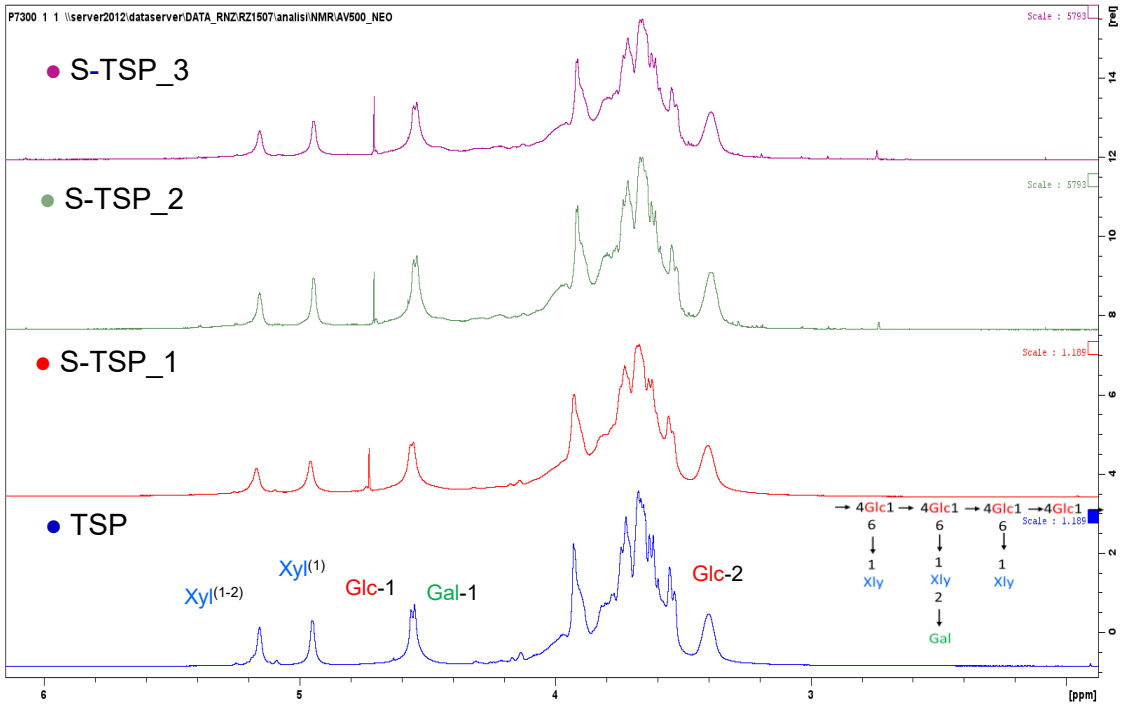
R= H or OSO_3Na
(according to the DS)

STRUCTURAL COMPOSITION

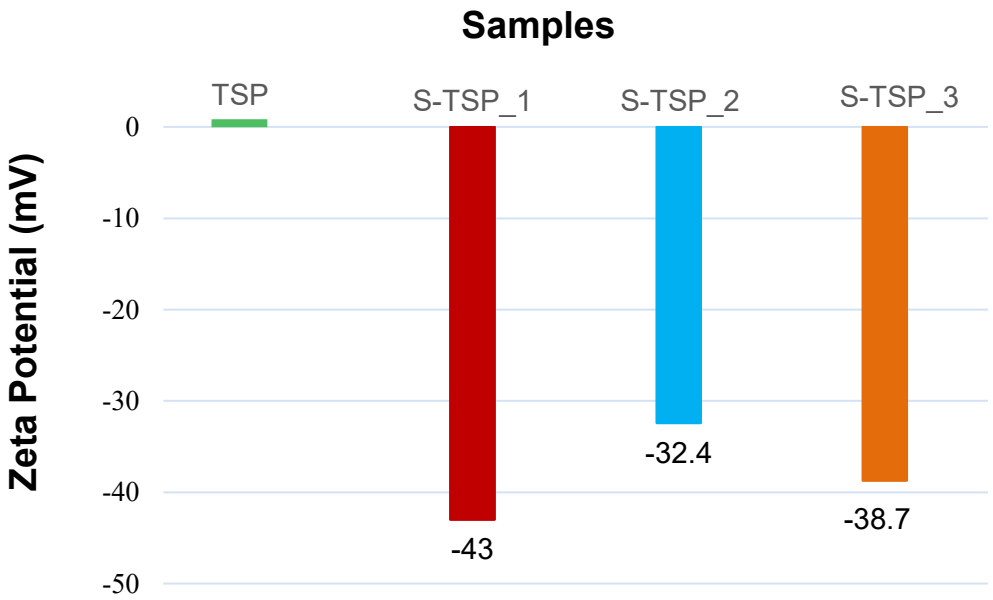
FTIR



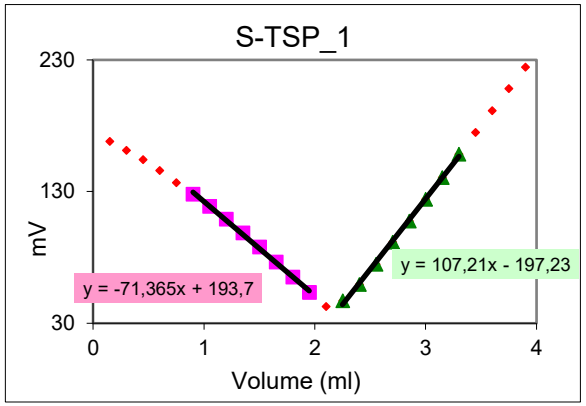
^{1}H



ZETA POTENTIAL



SUBSTITUTION DEGREE

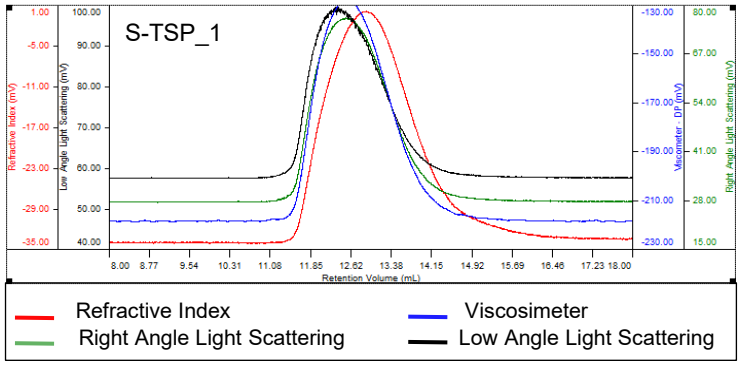


Samples	Sulphate groups for repetitive unit
S-TSP_1	1.76
S-TSP_2	2.10
S-TSP_3	2.62

$$DS_{(mole)} = \frac{mmole\ NaOH}{mg\ TSP - \frac{(mmole\ NaOH\ x\ 80\ g/m\,ole)}{1207\ g/m\,ole}}$$

Samples were analyzed at the concentration of 2 mg/ml in deionized water at 25°C

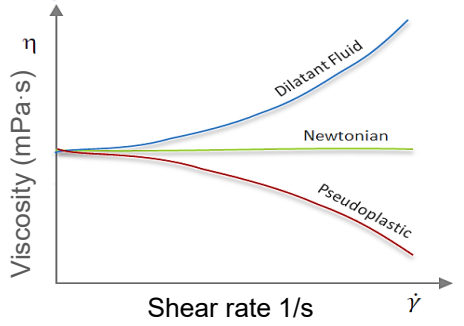
MOLECULAR WEIGHT DISTRIBUTION

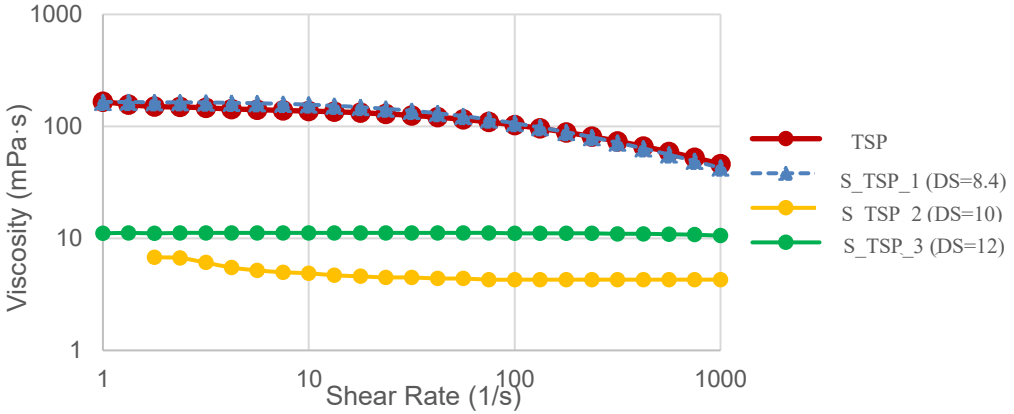


Sample	Mw (kDa)	Mn (kDa)	Mw/Mn	μ (dl/g)	Rh (nm)
TSP	**620**	**405**	**1.53**	**5.1**	**35**
S-TSP_1	744	470	1.58	3.8	34
S-TSP_2	168	81	2.07	1.2	14
S-TSP_3	343	191	1.79	1.9	21

Temperature: 40°C
Flow: 0.6 ml/min
Mobile phase : AcONa 0.3M/NaN_3 0.05% pH=8.11
dn/dc= 0.139 (TSP) and (S-TSP) 0.125

VISCOSITY





T=20°C
C=10 mg/ml
Double Gap

ENZYMATIC DEGRADATION

To determine the position on the sugar chain of sulphated groups by LC-MS analysis

Four different enzymes were used:

- Hyaluronidase
- **Cellulase**
- Lysozyme
- **Xyloglucanase**

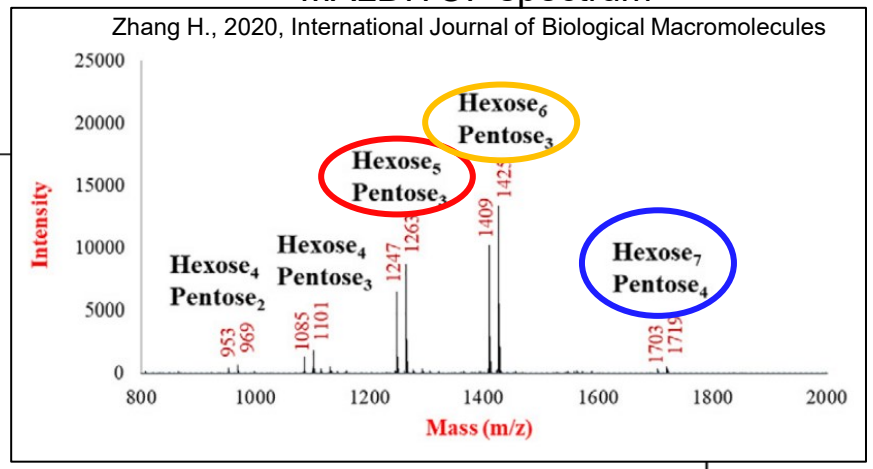
The samples were collected after different times of digestion, heated at 100°C for ten minutes, filtrated or precipitated with ethanol 70% and then lyophilized

LC-MS TSP

Hex=Glc or Gal
P= Xyl

MALDITOF spectrum

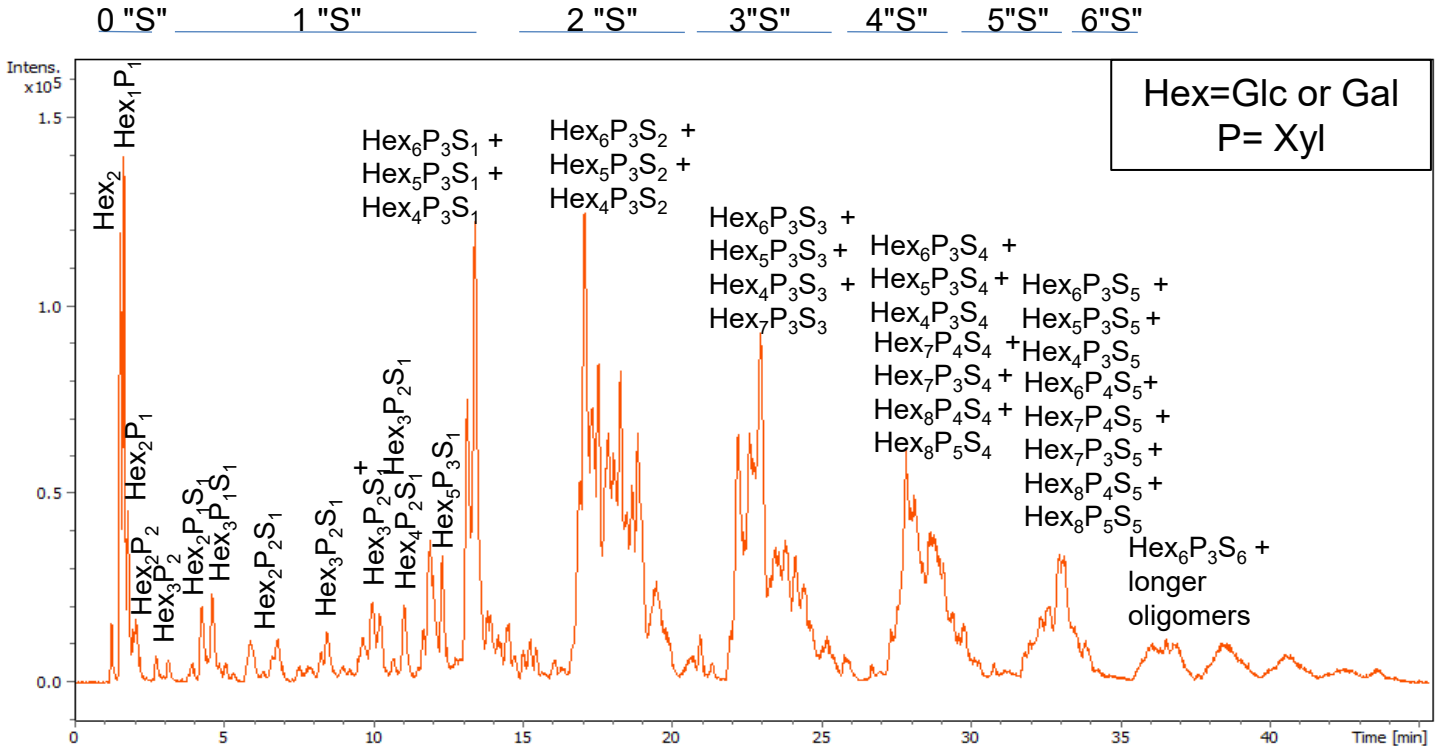
Zhang H., 2020, International Journal of Biological Macromolecules



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

LC-MS S-TSP
XYLOGLUCANASE AND CELLULASE

DS= 2 sulphated groups on the repetitive unit

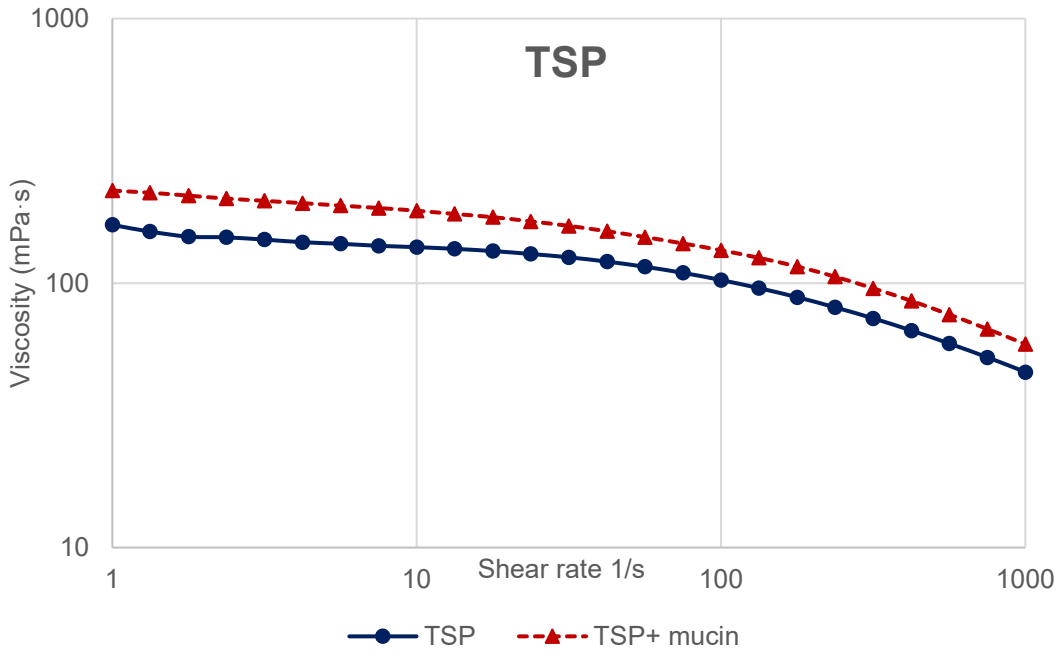


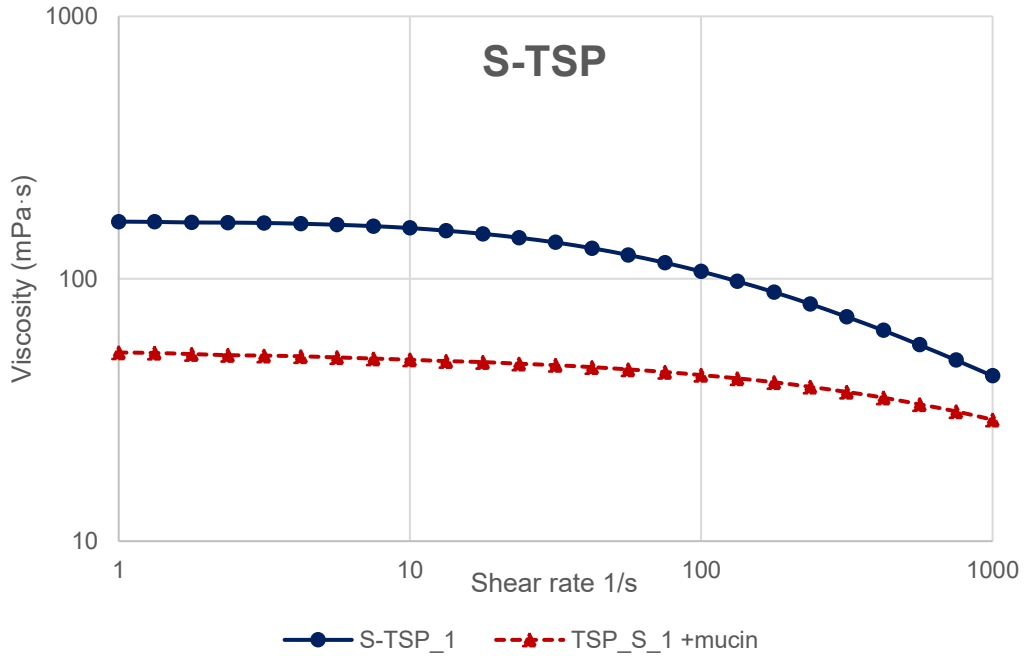
Column: Kinetex C18 100x2.1. 2.6 um Phenomenex
Eluent A: 10 mMdibuthylamine 10 mM acetic acid in water
Eluent B: 10 mM dibuthylamine 10 mM acetic acid in methanol
Flow 0.15 ml/min

MUCHOADHENSION

- Muchoadhension is the ability of polysaccharides to adhere to epithelial tissues
- Mucin is a glycoprotein that represents the main component of the secretion product of epithelial tissues
- The mucoadhesive of polymers is influenced by its physicochemical properties, by environment and by physiological variables
- To evaluate the muchoadhension, a rheological approach was used compared the viscosity of solution of TSP and S-TSP in water and in solution in water with mucin

MUCHOADHENSION





T=20°C
C=10 mg/ml (TSP) and mucina 2.5%
Double Gap

CONCLUSIONS

- Sulphation of TSP was achieved, but a depolymerization of the products happened with higher concentration of reagent
- Methods of characterization of the sulphated products were developed
- Probably the galactose is the residue preferably sulphated
- TSP and S-TSP show different viscose and mucoadhesive properties

FUTURE PERSPECTIVE

- NMR studies will be carried out to determine the pattern of sulphation
- The tolerability, toxicity and efficacy will be evaluated by one-dimensional cell and three-dimensional cell culture
- The biological effects of the sulphated products will be evaluated, especially in the ophthalmic field

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THANKS FOR THE ATTENTION!