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Exploring the influence of *S. cerevisiae* mannoproteins in wine astringency and the impact of their polysaccharide structure

RÉPUBLIQUE FRANÇAISE
Liberté Égalité Fraternité

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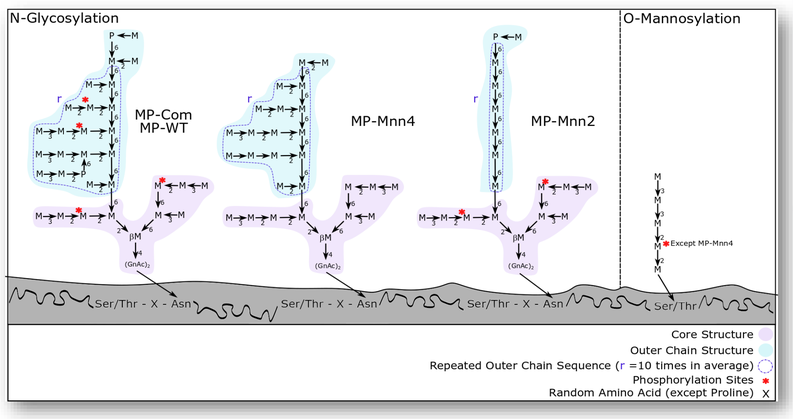
Context and Objectives

Mannoproteins are an important group of carbohydrate macromolecules in wines. Numerous functional properties are attributed to these bio-copolymers which are related to the stability and sensory characteristics of wines[4]. However, neither the exact mechanisms involved in these properties nor the impact of mannoprotein structural characteristics are well known. Focusing on mannoproteins property of modulating astringency, the objective of this work was to evaluate mannoproteins capacity to affect tannin-protein interactions and/or the consequent particle formation. In the mouth, tannin interactions with salivary proteins are associated to the puckering sensation and the lost of lubricity; characteristic of the astringent mouth-feel[5]. Mannoproteins differing in their polysaccharide structure were employed to also elucidate the impact of particular features of the generic polysaccharide structure of mannoproteins (mannosyl-phosphorylation and molecular compactness). They were extracted through an specific and exclusive enzymatic activity, purified and thoroughly characterized to preserve to uttermost extent the native structure of the macromolecule[1].

Materials and Methods

Yeast strains and their mannoproteins polysaccharide structure:
- Yeast-Com: LMD47 Enological strain;
- Yeast-WT: BY4742 – Wild-type strain;
- Yeast-Mnn4: BY4742 ΔMnn4p – Absence of mannosyl-phosphorylation
- Yeast-Mnn2: BY4742 ΔMnn2p - no branches in the α(1,6)-linked backbone

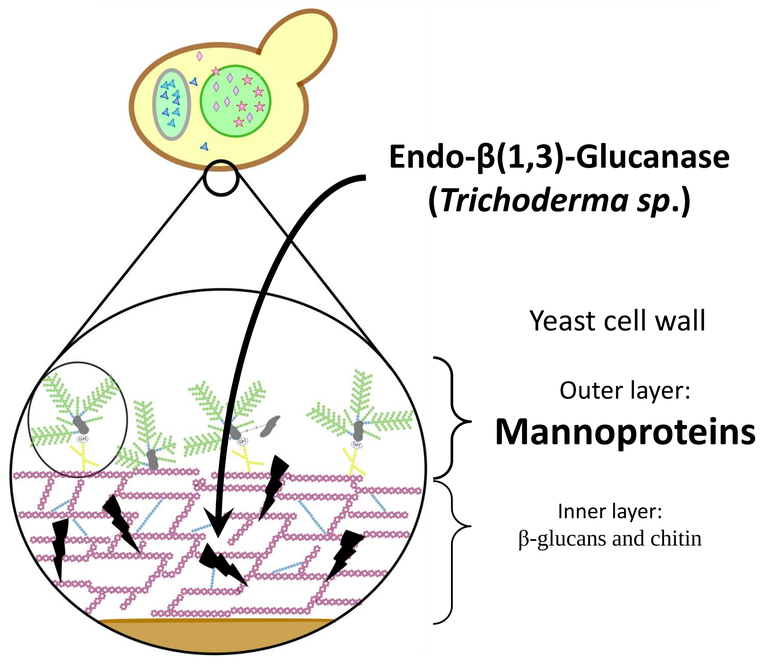


Purification :
- Ion Exchange Chromatography (IEC)
- Ultrafiltration (30kDa cut-off membranes)

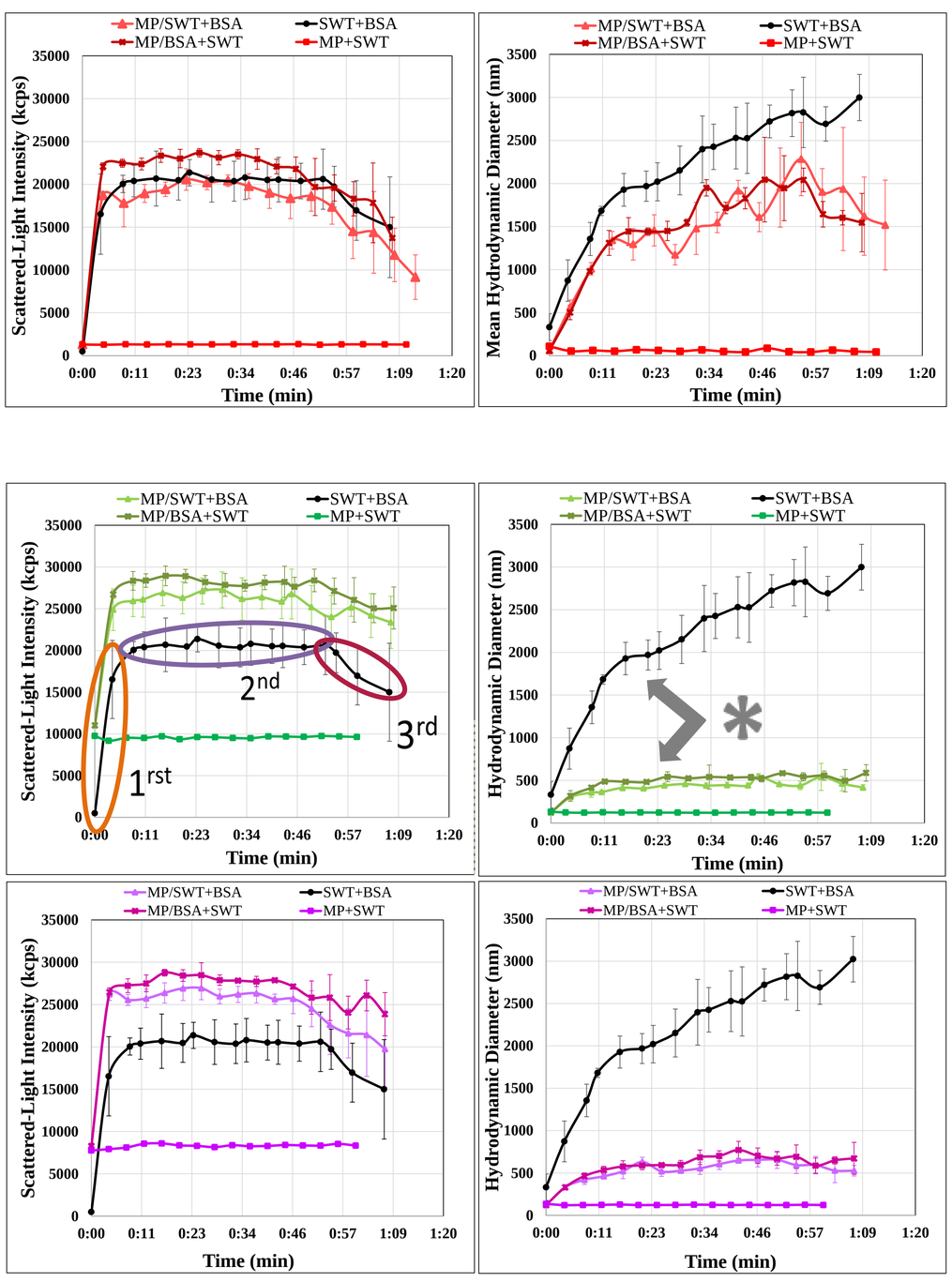
Characterization (Assunção Bicca *et al.*, 2022, DOI: 10.1016/j.carbpol.2021.118758) :
- Neutral sugars composition (GC-FID of the alditol acetates);
- Glycosyl linkage composition of the polysaccharide moiety (GC-MS of the partially methylated alditol acetates);
- Amino acid composition (IEC after hydrolysis, Biochrom);
- Net charge (PCD02, titration with standard polyelectrolytes associated with streaming potential measurements);
- Real Molecular size distribution and Molecular Conformation (HPSEC-MALS).

Interaction studies – Particle Formation Kinetics from Syrah-Wine-Tannins/BSA interactions
- Different addition steps to consider a possible competition mechanism
  - **SWT** + MP ← **BSA**
  - **BSA** + MP ← **SWT**
- Dynamic Light Scattering measurements over 1 h (DLS, Nano ZS, Malvern)
- Quantification of precipitated **SWT** after 48h (HPSEC-DAD)

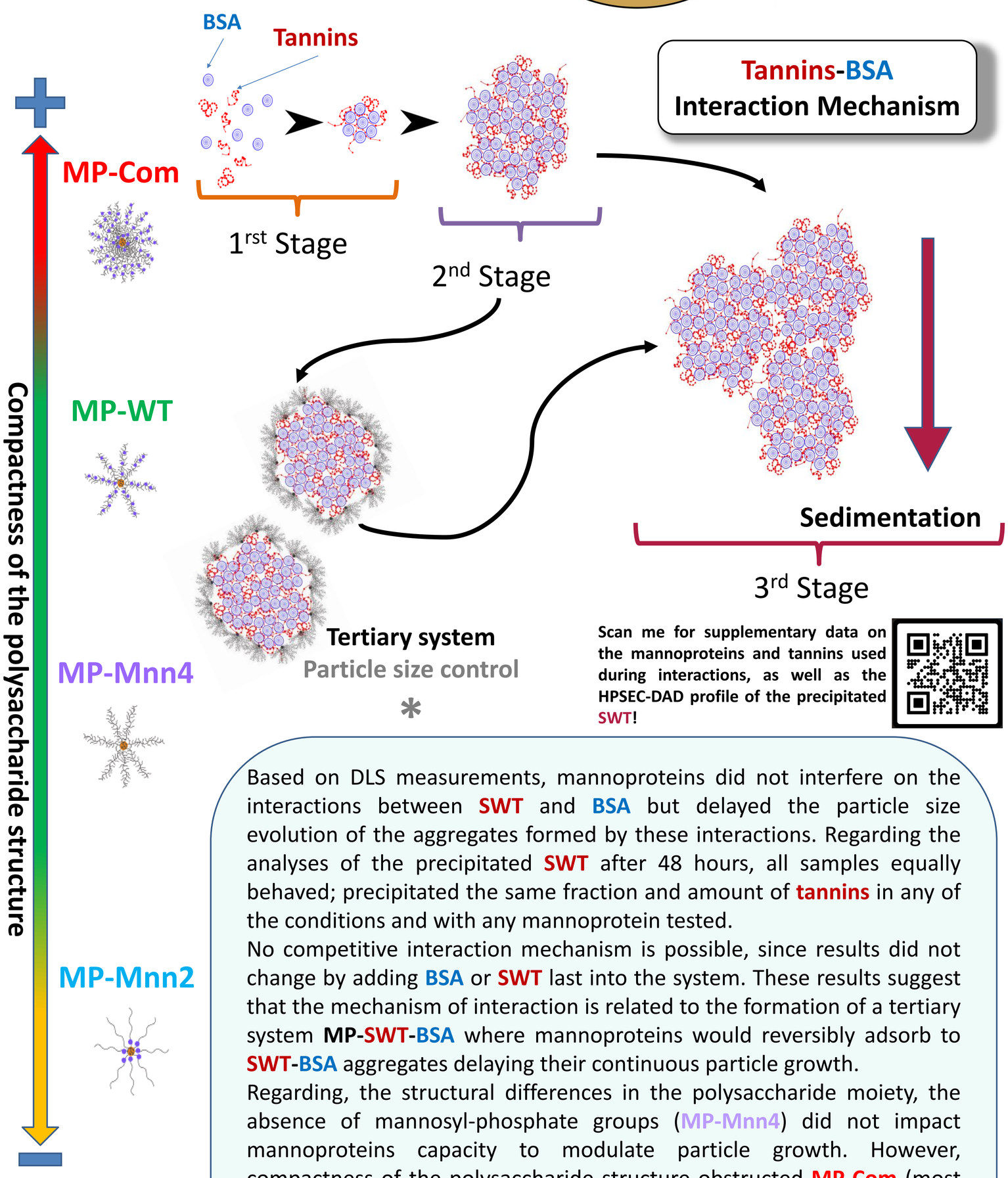
Mannoproteins Enzymatic extraction



DLS Particle Growth Kinetics



Compactness of the polysaccharide structure



Tannins-BSA Interaction Mechanism

Scan me for supplementary data on the mannoproteins and tannins used during interactions, as well as the HPSEC-DAD profile of the precipitated SWT!

Left: Total Scattered-Light Intensity of the particles formed over interaction time. *Right*: Mean Hydrodynamic Diameter measurements of the particles formed over time. Profiles in Black: control sample (**SWT** to which were added **BSA** right before measurement). Colored profiles: respective color of the mannoprotein used according to the schematic drawing on the right. Graphs present kinetics of **MP**+**SWT** samples to which were added **BSA** last and **MP**+**BSA** samples to which were added **SWT** last, as well as **MP**+**SWT** samples (without **BSA** addition).

Based on DLS measurements, mannoproteins did not interfere on the interactions between **SWT** and **BSA** but delayed the particle size evolution of the aggregates formed by these interactions. Regarding the analyses of the precipitated **SWT** after 48 hours, all samples equally behaved; precipitated the same fraction and amount of **tannins** in any of the conditions and with any mannoprotein tested.

No competitive interaction mechanism is possible, since results did not change by adding **BSA** or **SWT** last into the system. These results suggest that the mechanism of interaction is related to the formation of a tertiary system **MP-SWT-BSA** where mannoproteins would reversibly adsorb to **SWT-BSA** aggregates delaying their continuous particle growth.

Regarding, the structural differences in the polysaccharide moiety, the absence of mannosyl-phosphate groups (**MP-Mnn4**) did not impact mannoproteins capacity to modulate particle growth. However, compactness of the polysaccharide structure obstructed **MP-Com** (most compact mannoprotein) from adsorbing to **SWT**/**BSA** aggregates while **MP-Mnn2** (least compact) formed larger and less stable tertiary systems.

This study elucidates the impact of mannoproteins in tannin/protein aggregation process but further studies are needed to confirm their capacity to modulate the astringent mouthfeel.

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