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Title: Impact of aspects of the polysaccharide structure of mannoproteins on their interactions with Enological Tannins

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Section 3: Sensory perceptions of professionals and consumers

Section 2: Winemaking processes

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This study used mannoproteins (MPs) with specific differences in the structure of their polysaccharide moiety to understand the influences of common features of the polysaccharide part of MPs when interacting with Grape Seed Tannins (ST).

Employing mannoproteins (MPs) with divergent polysaccharide structures, the aim of this study is to better understand the impact of characteristics of the usual structure of MPs when interacting with Grape Seed Tannins (ST).

Four *Saccharomyces cerevisiae* strains were used to obtain four MP pools: an enological strain LMD47 (presenting high levels of N-glycosylation and O-Mannosylation), a wild-type BY4742 strain (used as reference), and its mutants Δ Mnn4 (with no mannosyl-phosphorylation) and Δ Mnn2 (with a linear N-glycosylation backbone). The extraction method applied, with the exclusive enzymatic activity of Endo- β -1,3-Glucanase of *Trichoderma sp.* (E-LAMSE, Megazym), preserved the indigenous structure of mannoproteins to their utmost extent. Characterizations of the pools confirmed differences among the polysaccharide moieties of the four MPs regarding charge, ratio mannose/glucose, and branching degrees but no differences between their protein moieties.

The colloidal formation and evolution of aggregates due to interactions between MPs and ST at different concentrations were evaluated through Dynamic Light Scattering (DLS), while the number of colloidal aggregates formed and the particle size distribution were assessed by Nanoparticle Tracking Analysis (NTA). The possible differences in the mechanisms of interaction among the four kinds of mannoproteins were analyzed through Isothermal Titration Calorimetry (ITC).

DLS and NTA experiments indicated an immediate formation of colloidal aggregates, in which the final particle size and concentration were dependent on the ratio ST/MP. Whenever the latter was extremely high, a very progressive flocculation related to a reversible aggregation occurred. The kinetics of this instability phenomenon was dependent on the polysaccharide structure of MPs. ITC analysis showed two different kinds of interactions: an intense exothermic one susceptible to temperature, and a much weaker interaction (as for enthalpy release) less thermo-dependent, possibly related to H-bonding and hydrophobic interactions, respectively.

Neither the absence of mannosyl phosphate groups, the absence of ramifications on the outer chains of the N-glycosylation, nor the protein glycosylation overexpression seem to play a decisive role in those interactions. However, these structural differences affected the stability of MP-ST colloids formed at specific concentrations and slightly changed the enthalpy exchange profiles.