Reversible self-aggregation of oxidized tannins in model solutions

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REVERSIBLE SELF-AGGREGATION OF OXIDIZED TANNINS IN MODEL SOLUTIONS

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MAIN CONCLUSION

Oxidized polyphenols aggregate and form reversible hazes. Aggregation / dissolution processes in model solution are monitored by DLS in two sets of experiments: 1) temperature ramp and 2) variation of ethanol fraction. The reversible haze appears to be composed of two fractions with well distinguished haze intensities. LC-MS analysis after phloroglucinolysis reveals that aggregates are mostly composed of oxidized procyanidins with intra-molecular oxidative bonds.

INTRODUCTION

Tannins are well known for their self-aggregation and formation of colloidal complexes with biomacromolecules, e.g. proteins and polysaccharides. These physical-chemical phenomena are conditioned by high sensitivity of tannins to oxidation leading to structural and consequent solvation changes. Oxidation of tannins and resulting chemical and physical evolution are determinant for biological, nutritional and organoleptic properties in tannin-rich foodstuffs. For instance, aggregation and oxidation are both involved in haze formation occurring in some fruit-derived alcoholic beverages (Pommeaux, Port wines, …). However, mechanisms for these colloidal instabilities are still not elucidated. The present work focuses on the self-aggregation of well-controlled oxidized tannin oligomers, with emphasis on the reversible aggregation.

MATERIALS AND METHODS

Purified apple procyanidins from dimer to tetramer were oxidized in chemically controlled conditions (concentration 5 g/L, incubation over 40’ at 30°C with periodated resin at controlled [epicatechin]/[periodate] ratio from 8 to 2) in hydroalcoholic model solutions (malate buffer 20 mM, pH 3.8, EtOH 15%). The aggregation / re-dissolution were triggered by (i) decreasing / increasing temperature ramp (-1°C/min. / +5°C/min between 45°C and 5°C) and (ii) rapid decrease / increase of ethanol fraction in the solvent by adding buffer / ethanol to the sample covering the range between ~5% and 21% of ethanol. Evolutions of the haze and of the size distributions of aggregates were monitored by DLS. The impact of oxidation on haze composition and on limpid fractions (obtained by filtration of aliquots on

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0.2 µm PTFE filter) was monitored during the sample ageing by LC-UV/MS coupled to acidolysis in the presence of phloroglucinol. Oxidation markers were followed resolving inter- and intra-molecular oxidation-induced covalent linkages.

RESULTS AND DISCUSSION

Figure 1a shows the light scattering intensity in T-ramp experiments. Maximal magnitude of the haze increases at each cooling-heating cycle indicating the progress of the oxidation. Indeed, all oxidation markers typically evolve over one day in present conditions, see figure 2. The aggregation part (descending T-ramp) of the scattering profiles, figure 1a, exhibits a 2-fold structure, indicating two main aggregating populations, which reminisces the colloidal fractions T1 and T2 found in the model solution of partially oxidized grape seed tannin [1].

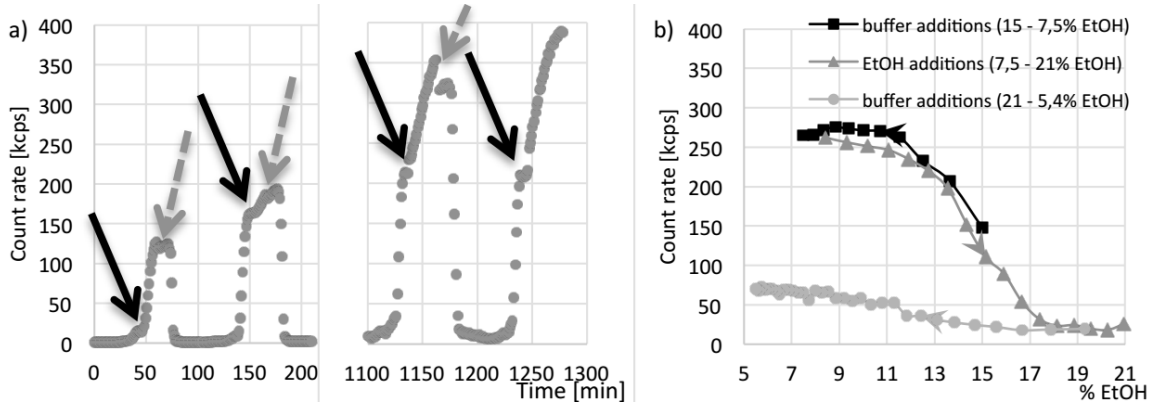

Figure 1 a) Evolution of light scattering intensity upon a series of descending and ascending temperature ramps between 45°C and 5°C. Black arrows indicate the intermediate plateau corresponding to first sub-population. Grey dashed arrows show the moment when the sample was shaken. b) Light scattering intensity evolution after rapid additions of buffer and ethanol. Arrows indicate the order of solvent additions.

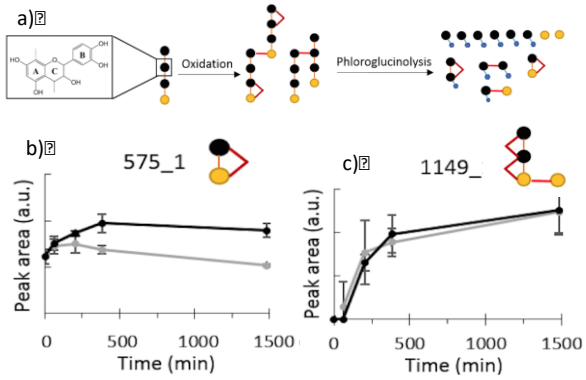

Figure 2 a) Principle of LC-MS analysis of inter- and intra-molecular oxidation markers after phloroglucino-lysis. b) Evolution of typical intra and intermolecular markers in oxidized unfiltered (black) and filtered (grey) samples.

Figure 1b shows the light scattering intensity evolution upon ethanol content variation. The reversible nature of the phenomena is clearly visible and the ethanol range relevant for all relevant haze processes appears to be between about 5% and 21% of ethanol. After our LC-MS analysis of filtered and non-filtered aliquots, see figure 2a, intra-molecular oxidative markers are enhanced in the haze, while no enhancement was observed for the inter-molecular oxidative markers.

REFERENCES

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