MONITORING OF MANNOPROTEIN RELEASE DURING WINE AGING ON LEES: DEVELOPMENT OF A SIMPLE ENZYMATIC METHOD•

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1. INTRODUCTION

Mannoproteins are polysaccharides released by *Saccharomyces cerevisiae* yeast during alcoholic fermentation or by enzymatic action during aging on yeast lees (autolysis). Significant amounts of these polymers can be found in wines, and their concentration also depends on the yeast strain and on the wine-making process. Mannoproteins play a major role in wine characteristics processing, namely, in the tartaric stabilization and protein haze prevention; moreover, they improve color stability and reduce astringency (Fornairon-Bonnefond *et al*., 2002). The precipitation by ethanol, used to quantify total colloids, is not specific and reproducible. The methodologies commonly used for polysaccharide analysis, which include glycosidic bond hydrolysis, and derivatization for producing volatile sugar derivatives (determined by gas chromatography) are time consuming and expensive. The aim of this work is developing a rapid and simple method for routine analysis in laboratories for the determination of mannoprotein release from yeast cell walls during wine aging on lees.

2. MATERIALS AND METHODS

2.1. Method description

The wine was centrifuged at 4.000 rpm on a Centrifuge 5810R (Eppendorf, Milano, Italy) for 10' in two 250 mL tubes. 500 mL of wine was pre-concentrated on a tangential flow filter Vivaflow 200 (Sartorius, Göttingen, Germany), with a cut-off of 10 KDa. 10 mL of concentrate was hydrolyzed with 830 µL of sulfuric acid 96 % at 100 °C for 90'. The hydrolyzate was cooled at room temperature and then neutralized at pH 7 with NaOH 2N.

Quantitative analysis of glucose and mannose after hydrolysis was conducted by an enzymatic method (Megazyme International, Ireland). This method consists in the spectrophotometric dosage of NADPH, obtained from the oxidation of glucose-6-phosphate, respectively before and after the action of two different enzymes: a phosphomannose and a phosphoglucose isomerase (Dupin *et al*., 2000). The polymeric

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mannose and glucose quantification was carried out both on the concentrate and on the permeate. The repeatability of the method was determined by 8 replicate analyses of a commercial white wine, with a medium mannose content of 90 mg L^{-1}. The described method was used to quantify the amount of the polymeric forms of glucose and mannose (>10 KDa) in 36 commercial white wines obtained respectively from Arneis (16 wines), Cortese (16), Sauvignon (2), Chardonnay (1) and Pignoletto (1).

2.2. Statistical data analysis

The results were subjected to one-way ANOVA and graphically represented as box-plots. The SPSS 15.0 program for Microsoft Windows was used (2004, Spss inc. Chicago, Illinois). The box-plots show, separately for year and type of wine, the value of the central tendency (median) of the variables. The width of the rectangle represents the interquartile range, that includes 50 % of the data. In the same graphic, the ends of the whiskers represent the minimum and maximum of all the data.

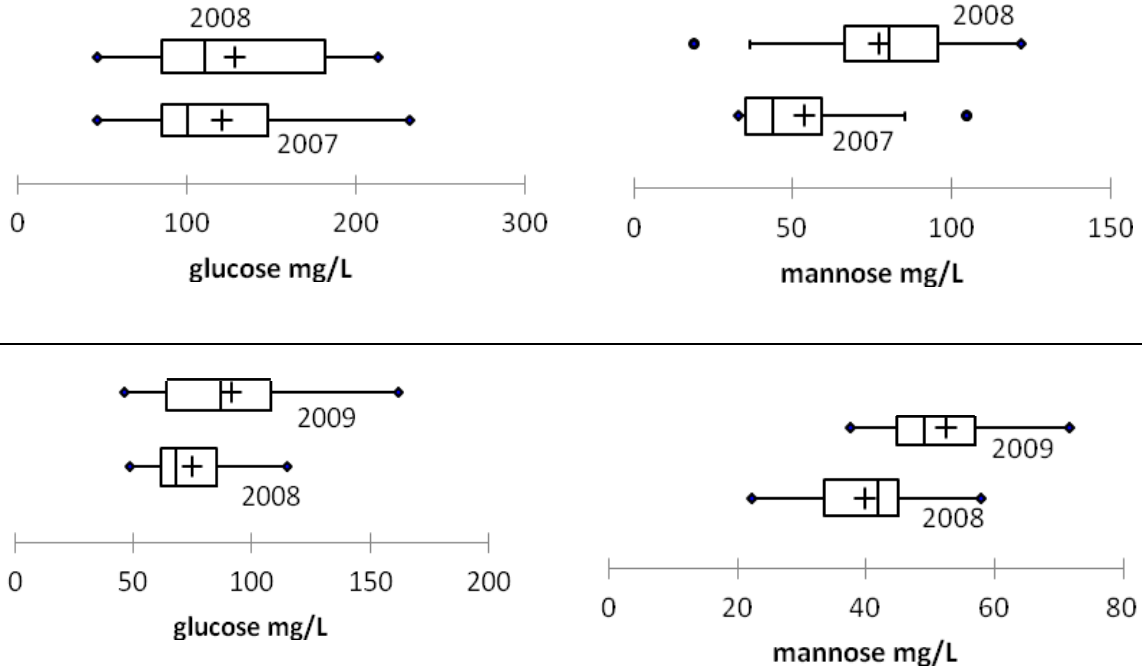

Fig. 1 - Graphic representation (box-plot) of glucose and mannose content in Cortese (above) and Arneis (below) wines in both vintages.

3. RESULTS

The repeatability of the method is satisfactory: a coefficient of variation (CV) of 2 % for mannose analysis was obtained. The concentration of mannose in the retentate ranges from 12 to 122 mg L^{-1}, while for glucose varies from 45 to 232 mg L^{-1}. In figure 1 is represented the dispersion of the concentration of polymeric glucose and mannose, separately for each vintage, respectively for Cortese and Arneis (fig. 1). For both wines no

statistically significant differences between the vintages are noticed (ANOVA results). Cortese wines show an average mannose and glucose content significantly higher, as well as a major variability in concentration, than Arneis wines (fig.2).

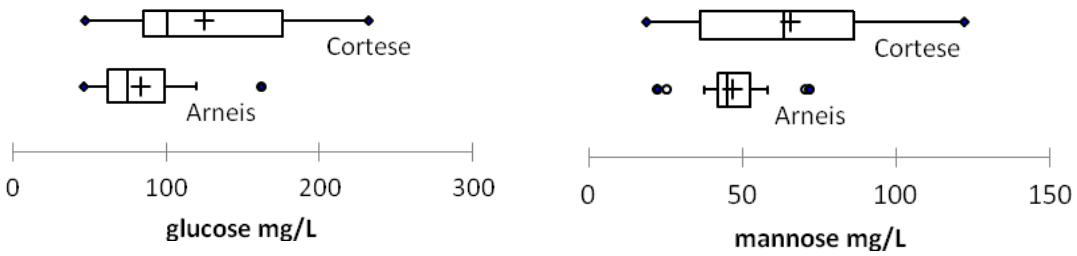

Fig. 2 - Box-plot of the overall average glucose and mannose content in Arneis and Cortese wines.

The dosage of glucose and mannose was then repeated on the permeate fractions. The average glucose and mannose content in the concentrate (>10 KDa) and in the permeate (< 10 KDa), for both vintages, of all the analyzed wines (fig. 3) shows that polymeric mannose is completely retained by the membrane with 10 KDa cut-off and it is present in the permeate only in traces (2.4 mg L^{-1}, on average); moreover, no significant variations of its content between the vintages are observed. The analysis of the concentrate alone allows the complete quantification of mannose polymers in wine. These results agree with a previous work (Guilloux-Benatier, Chassagne, 2003) on a model wine added of yeast cells. The glucose content in the permeate is slightly higher (average content of 43.5 mg L^{-1}), and no statistically significant differences are observed between the wines of different vintages.

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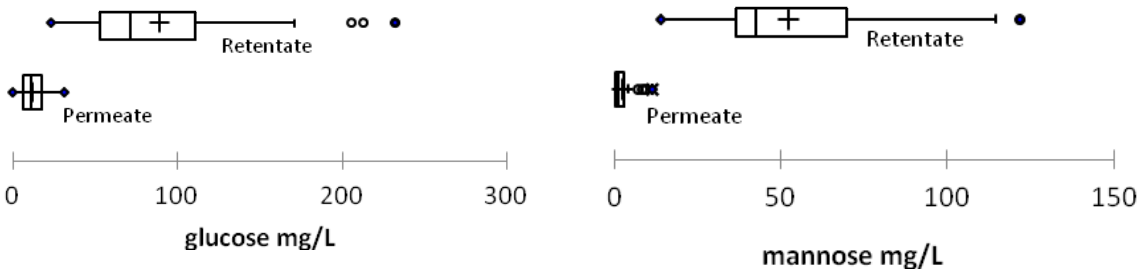

Fig. 3 - Graphic representation (box-plot) of glucose and mannose content in the retentate and in the permeate for all wines.

Abstract

Mannoproteins are polysaccharides released by *Saccharomyces cerevisiae* yeast during alcoholic fermentation or by enzymatic action during aging on yeast lees (autolysis). These molecules play a major role in wine characteristics processing, namely, in the tartaric stabilization and protein haze prevention; moreover, they improve color stability and reduce astringency.

A rapid and simple method for routine analysis in laboratories for monitoring mannoprotein release from yeast cell walls during wine aging on lees was developed. The analytical method for the determination of polymeric glucose and mannose content consists in a pre-concentration of wine, followed by the hot acid hydrolysis of polysaccharides, and the quantitative analysis of monomers by an enzymatic method (dosage of NADPH). The reproducibility of the method is good with a measured CV of 2 %. The use of a membrane with 10 KDa cut-off for concentrating wine during essays of aging on lees, actually involves a negligible permeate mannose recovery. The analytical method was finally applied to 36 commercial white wines for quantifying the average mannose content.

Acknowledgements

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