

12º Encontro de Química dos Alimentos

**Composição Química, Estrutura e Funcionalidade:
A Ponte Entre Alimentos Novos e Tradicionais**

12th Meeting on Food Chemistry

**Bridging Traditional and Novel Foods:
Composition, Structure and Functionality**

Extended Abstracts

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Bridging Traditional and Novel Foods: Composition, Structure and Functionality

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Influence of structural features of mannoproteins in white wine protein stabilization

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ABSTRACT

Wine haze formed in white wines due to protein instability is a visual defect. Therefore this instability affects considerably its commercial acceptance. Several processes are used to prevent the occurrence of protein wine instability, however with several limitations, such as modification of physicochemical and sensory characteristics. Mannoproteins could be an alternative for wine protein stabilization. These products are mainly obtained from *Saccharomyces cerevisiae* cell walls, being composed mostly by backbone of $\alpha(1,6)$ -linked mannose residues, containing short side chains attached to the *O*-3 or *O*-2 position of the main chain mannose residues.

The objective of this work was to evaluate the effectiveness of commercial mannoproteins on white wine protein stabilization, based on their structural features. Eleven mannoproteins were characterized concerning their sugar profile and concentration, and total protein content. Results showed, that preparations rich in mannoproteins, presented a different performance on white wine protein stabilization and this could be related to their high mannose to glucose ratio. Thus, mannoproteins could be an effective alternative to wine protein stabilization, sometimes increasing also the wine sensorial quality.

1. INTRODUCTION

Proteins are present in wine at low concentration; however these compounds could be responsible for colloidal instability and haze of wines [1, 2]. In white wine this issue is of great importance, since limpidity is an essential quality feature required by consumers. The most important proteins related with white wine instability are pathogenesis related proteins of *Vitis vinifera*, these include chitinases and thaumatin-like proteins [3, 4], that survive throughout the winemaking process, because they are highly resistant to proteolysis and to the low must and wines pH [5]. These proteins slowly denaturant and aggregate during wine

storage, giving a light dispersing haze [6]. Protein instability is currently prevented by removing proteins using fining agents. Bentonite addition is the most commonly used process to avoid protein instability in white wine, by using the right dose, determined by stability tests [7]. However, bentonite fining could affect wine quality under some conditions, like the removal of colour, flavour and aroma compounds [8, 9] changing in this way wine sensory characteristics. Thus, the main objective of this study was to evaluate white wine stabilization effectiveness using different mannoproteins and to assess their effects on phenolic compounds.

2. MATERIAL AND METHODS

Wine samples: A white wine from Douro Valley 2011 vintage, was used with the main characteristics as follows: Alcohol content (% v/v) 14.2, specific gravity (20°C) (g/mL) 0.9890, titratable acidity (g/L tartaric acid) 5.5, pH 3.29, volatile acidity (g/L acetic acid) 0.31, protein stability heat test 7.09 NTU.

Protein stability experiments: Eleven types of mannoproteins (Mp1 - Mp11) were used at highest concentration according to the manufacture's specifications (Table 1). Assays were done in 375 mL flasks at 20°C for 7 days. Wine without any treatment was used as a control. Protein stability was assessed by heat test [10].

Table 1. Major commercial characteristics and recommended dosage of mannoproteins used.

Characteristics	Codes	Dosage (g/hL)
Yeast cell wall	Mp1	30
Yeast cell wall polysaccharides/peptides	Mp2	1 – 5
Yeast cell wall	Mp3	5 – 10
Yeast cell wall	Mp4	10 – 40
Yeast cell wall (MW 150 KD)	Mp5	40
Yeast cell wall and mannoproteins	Mp6	5 – 10
Yeast cell wall	Mp7	5 – 40
Yeast cell wall (MW 20 KD)	Mp8	40
Specific yeast cell wall	Mp9	5 – 40
Polysaccharides from yeast cell wall	Mp10	0.5 – 5
Yeast cell wall	Mp11	40

Commercial mannoproteins characterization: Sequential acid hydrolysis was performed, with and without Saeman hydrolysis, in order to obtain the amount of insoluble polysaccharide. Sugar composition was obtained by anion-exchange chromatography with pulsed amperometric detection. Total nitrogen was determined by the Kjeldahl method and total protein content was determinate as Kjeldahl nitrogen multiplied by 6.25 [11, 12].

Browning potential and phenolic profile: The browning potential was determined according to the methodology proposed by Singleton and Kramling [13]. Phenolic acids and flavonoid profile were performed by HPLC with photodiode-array detector. Compounds were presented as percentage of area.

Statistical analysis: Analyses of variance (ANOVA) were used to compare data and Tukey test was applied to difference between treatments, using the Statistica 7 software (Statsoft, OK, USA). All the assays and analyses were performed in duplicate.

3. RESULTS AND DISCUSSION

Protein stability test: Heat test, which provides information about protein thermal stability, showed that 2 onto 11 mannoproteins do not stabilized the wine, namely Mp5 and Mp9.

Mannoproteins characterization: To understand previous results, mannoprotein composition, namely protein and sugar composition, was evaluated. Generally, results showed that total sugars accounted for major proportion, comparing to proteins (Figure 1A). Mannose and glucose were the most representative sugars, ranging from 17.4-41.9 g/100 g and from 6.8–41.4 g/100 g, respectively (Figure 1B). The different chemical composition observed among mannoproteins, could be related to industrial extraction process and/or different degrees of purity.

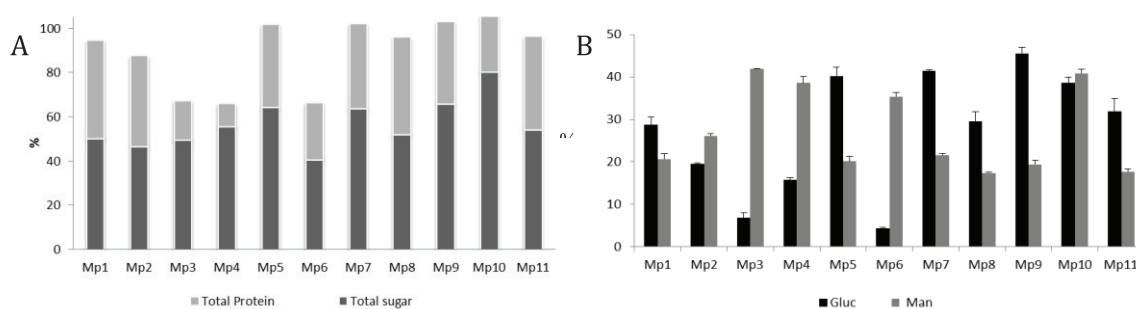


Figure 1. Mannoproteins composition (g/100g): A) total sugar and total protein; B) glucose and mannose content.

Mannose to glucose ratio, allows us to access the relative amount of mannoproteins in each commercial preparations (Figure 2), and beside the different concentrations tested in this work, this ratio remains the same. Mannoproteins which are not able to stabilize wine against protein instability (Mp5 and Mp9) showed low mannose to glucose ratio, suggesting that this feature is implied to their effectiveness. These results are in accordance with Moine-Ledoux and Dubourdiou [14].

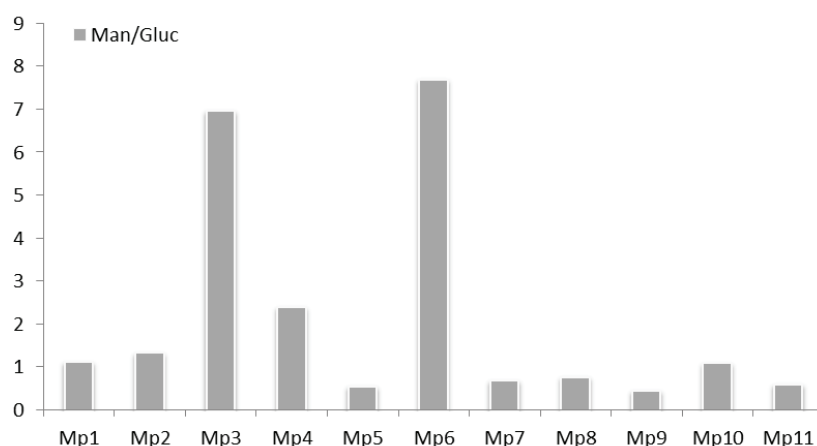


Figure 2. Ratio mannose to glucose (Man/Gluc) quantified in mannoproteins used in this study

All the mannoproteins decreased the wine browning potential; although total phenolic compounds slightly diminished. That could be explained by the fact that mannoproteins stays

in solution and probably interacts with the phenolic compounds, preventing their oxidation. In fact, phenolic profile usually remains unchanged with mannoprotein treatment (Table 2).

Table 2. Phenolic profile (% area) of control (C) and treated white wine with mannoproteins (Mp1-Mp11). Different letters for different means ($p < 0.05$)

	C	Mp1	Mp2	Mp3	Mp4	Mp5	Mp6	Mp7	Mp8	Mp9	Mp10	Mp11
Galic acid	40.1±1.1 ^{bc}	36.9±7.2 ^{abc}	41.2±0.5 ^{bc}	41.7±2.6 ^{bc}	41.2±4.2 ^{bc}	44.1±2.9 ^c	40.0±0.9 ^{bc}	39.6±1.4 ^{bc}	40.3±0.0 ^{bc}	41.6±1.5 ^{bc}	41.0±0.6 ^{bc}	41.3±3.4 ^{bc}
Catechin	12.0±0.7 ^{ab}	12.4±3.7 ^{ab}	15.0±4.0 ^{ab}	10.1±1.4 ^{ab}	9.3±0.1 ^a	10.8±0.2 ^{ab}	10.1±0.9 ^{ab}	16.9±0.2 ^{ab}	13.5±2.8 ^{ab}	9.2±3.6 ^a	13.4±2.5 ^{ab}	13.3±3.4 ^{ab}
Trans-caftaric acid	27.4±0.2 ^a	28.5±4.6 ^a	25.1±2.0 ^a	28.1±0.1 ^a	28.9±2.4 ^a	25.4±1.5 ^a	29.1±1.5 ^a	24.4±2.1 ^a	25.4±3.2 ^a	28.2±1.6 ^a	25.8±1.2 ^a	25.1±1.4 ^a
2-S-glutathionyl caftaric acid	9.7±0.4 ^a	10.7±2.9 ^a	8.9±0.7 ^a	9.7±0.2 ^a	10.1±0.5 ^a	9.1±0.3 ^a	10.2±0.7 ^a	9.4±0.1 ^a	9.5±0.1 ^a	10.0±0.2 ^a	9.5±0.8 ^a	9.4±0.4 ^a
Coutaric isomeric acid	3.4±0.1 ^a	3.9±1.8 ^a	3.0±0.3 ^a	3.1±0.4 ^a	3.4±0.8 ^a	3.6±0.7 ^a	3.3±0.1 ^a	3.7±0.1 ^a	3.6±0.4 ^a	3.5±0.1 ^a	3.2±0.3 ^a	3.5±0.5 ^a
Coutaric acid	2.1±0.0 ^a	3.0±1.2 ^a	1.9±0.1 ^a	2.2±0.0 ^a	2.3±0.0 ^a	2.0±0.1 ^a	2.3±0.2 ^a	2.4±0.2 ^a	2.4±0.4 ^a	2.2±0.0 ^a	2.0±0.2 ^a	2.5±0.5 ^a
Caffeic acid	2.3±0.1 ^b	1.6±0.0 ^{ab}	2.1±0.2 ^b	2.0±0.3 ^b	1.8±0.1 ^{ab}	2.1±0.0 ^b	2.0±0.4 ^b	0.8±0.3 ^a	2.3±0.0 ^b	2.3±0.1 ^b	2.2±0.2 ^b	2.2±0.2 ^b
4-hidroxicumaric acid	1.2±0.0 ^a	1.3±0.1 ^a	1.1±0.1 ^a	1.1±0.0 ^a	1.3±0.0 ^a	1.1±0.0 ^a	1.2±0.0 ^a	1.2±0.0 ^a	1.2±0.0 ^a	1.2±0.0 ^a	1.2±0.2 ^a	1.2±0.1 ^a
Ferulic acid	0.4±0.0 ^a	0.4±0.1 ^a	0.4±0.0 ^a	0.4±0.0 ^a	0.4±0.0 ^a	0.4±0.1 ^a	0.4±0.0 ^a	0.4±0.0 ^a	0.4±0.1 ^a	0.4±0.0 ^a	0.4±0.1 ^a	0.3±0.0 ^a
Etil caffeic	0.9±0.0 ^a	0.8±0.1 ^a	0.8±0.1 ^a	0.8±0.1 ^a	0.8±0.0 ^a	0.8±0.0 ^a	0.9±0.0 ^a	0.8±0.1 ^a	0.8±0.2 ^a	0.9±0.0 ^a	0.9±0.1 ^a	0.8±0.1 ^a
Etil coumaric	0.5±0.0 ^a	0.5±0.1 ^a	0.5±0.0 ^a	0.5±0.0 ^a	0.5±0.0 ^a	0.5±0.0 ^a	0.5±0.0 ^a	0.4±0.1 ^a	0.5±0.1 ^a	0.5±0.0 ^a	0.5±0.1 ^a	0.4±0.1 ^a

4. CONCLUSIONS

The chemical composition of commercial mannoproteins affects wine protein stabilization. Mannoproteins with higher mannose to glucose ratio are more effective in achieving a thermal stability of white wine proteins. Additionally, some mannoproteins have a protective effect on the wine colour evolution by decreasing the browning potential. However, more detailed studies are needed to reinforce our results.

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