Wine instability. I. The importance of the wine proteins

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INTRODUCTION

The presence of a residual amount of unstable protein in wines assumes great economical significance. These polymers can come out of solution and precipitate during storage, affecting the stability and clarity of the wines. This precipitation is due to the insolubilization and subsequent aggregation of the proteins, leading to the formation of an unattractive and unacceptable haze or sediment in the wine. For this reason, proteins are regarded as the most important nitrogenous compounds present in wines. Despite the significant advances in wine protein research and the large number of studies published on wine proteins, there is an incomplete understanding about the factors involved in turbidity formation.

In a recent study, Ferreira *et al.* (1999) used immunological methods to demonstrate that at least the vast majority of the proteins present in wines are derived from the grapes.

A number of factors, such as the total amount of protein, the nature of the proteins, the difference between wine pH and the protein pI, the wine pH and the presence of compounds such as flavonols and metal ions, are known to affect protein stability in wines (Koch and Sajak, 1959; Moretti and Berg, 1965; Krug, 1968). Some reports state that the wine instability is related to its protein content. Other studies indicate that the protein instability does not correlate well with the wine total protein

content. In this hypothesis, the molecular properties of each protein influence its natural tendency to precipitate. However, the nature of the protein(s) responsible for wine turbidity remains unclear.

A small number of studies have been performed on the effect of polysaccharides on protein instability in wines.

The present work consisted in the removal of protein from six Portuguese varietal wines (Fernão Pires, Assario, Tamarez, Verdelho, Arinto and Moscatel) by bentonite fining and subsequent haze induction using the back-addition technique of the total protein from the Fernão Pires wine. A comparison between the pattern of variation of protein instability with increasing temperature, characteristic of each varietal wine with that of the bentonite-treated wine back-added with the total protein isolated from the Fernão Pires wine allowed us to evaluate the role of the proteins on the formation of turbidity.

MATERIALS AND METHODS

Preparation of wine.–Ripened grapes were harvested and processed into wine by a conventional microvinification procedure, according to the classical white wine technology. Bentonite was not added during fermentation. After each bottle was opened, the wine was divided in several aliquots and stored at –70°C until used. To avoid repeated freezing and thawing, a new aliquot was used for each experiment.

Purification and concentration of the wine soluble proteins.–Wine aliquots were thawed and centrifuged at 15,800 g for 5 min, and the supernatant desalted at 4°C on prepacked PD-10 Sephadex G-25M columns (Pharmacia/LKB, Uppsala, Sweden), previously equilibrated with water (Milli-Q plus, Millipore, Bedford, USA). The protein samples (105 ml) were subsequently lyophilised (Edwards Micro Modulyo freeze drier, Crawley, Sussex, England) and the dried residue solubilized in 9 ml of 20 mM citrate- NaOH buffer, pH 2.5. A sample, 2 ml, containing the wine total protein was purified by cation exchange chromatography on a Mono S HR5/5 column (Pharmacia/LKB) previously equilibrated in the same buffer. The bound proteins were eluted with a step gradient (0-1 M) of NaCl.

Heat stability tests.–Wine aliquots were thawed and centrifuged at 4°C and 15,800 g for 10 min. The heat stability of the wines was subsequently determined by the procedure recommended by Pocock and Rankine (1973). All measurements were made in triplicate.

Bentonite fining.–To determine the minimum amount of bentonite required to remove the total soluble proteins from each wine, a bentonite solution (2% w/w) was prepared in warmed Milli-Q plus water at least 24h before being used. Bentonite fining was performed at 4°C. To 10 ml wine, increasing amounts of bentonite solution were added and the mixture was gently agitated during 30 min. The treated wine samples were held for 24 h and subsequently centrifuged at 4°C and 15,800 g for 10 min. The precipitate was discarded.

Protein determination.–The wine total soluble protein was measured by a modification of the Lowry method (Bensadoun and Weinstein, 1976).

RESULTS, DISCUSSION, CONCLUSIONS

To perform the present studies six Portuguese varietal wines were selected: Fernão Pires, Assario, Tamarez, Verdelho, Arinto, and Moscatel. Moscatel is the richest wine as far as the concentration of protein is considered (334.3 mg ml^{-1}), followed by Fernão Pires (141.0 µg ml^{-1}), Assario (114.7 µg ml^{-1}), Arinto (87.0 µg ml^{-1}), Tamarez (64.5 µg ml^{-1}) and Verdelho (30.9 µg ml^{-1}).

Each of the six wines exhibits a characteristic response when exposed to increasing temperatures. The results presented (Fig. 1) show the increase in turbidity detected when each wine is incubated at temperatures varying from 30 to 80ºC.

The patterns of turbidity variation vary from sigmoidal-like (Fernão Pires, Fig. 1A) to exponential-like (Assario and Tamarez, Fig. 1B and 1C), being intermediate in the cases of Verdelho (Fig. 1D) and Arinto (Fig. 1E.). Moscatel showed, always, a strange pattern. Furthermore, a positive correlation between the turbidity at 80ºC and the protein concentration can be observed.

The different wines analysed differ widely in their response to bentonite treatment (results not shown). The amount of bentonite (µg ml^{-1} of wine) required to achieve maximal removal of protein varies from 1600 µg ml^{-1} (Moscatel) to 50 µg ml^{-1} (Verdelho).

For wines containing low levels of protein there is a linear relationship between the total wine protein content and the amount of bentonite added to obtain maximum removal of protein. This was observed with all the wines studied, except Moscatel, a protein rich wine.

Fernão Pires wine was selected for the experiments involving back-addition of proteins. Its total protein was purified as described under Materials and Methods.

The five wines, Fernão Pires (control), Assario, Tamarez, Verdelho and Arinto were treated with bentonite. To each protein- free wine an amount of lyophilised Fernão Pires protein equivalent to the protein originally present in that wine was added. The amount of Fernão Pires soluble protein in each of the five wines was subsequently determined. The results obtained indicated that the treated Fernão Pires ended up with 104 µg protein ml^{-1} (74% of its original value), Assario with 120 µg protein ml^{-1} (105% of its original value), Tamarez with 43 µg protein ml^{-1} (66% of its original value), Verdelho with 28 µg protein ml^{-1} (91% of its original value) and Arinto with 78 µg protein ml^{-1} (90% of its original value).

Each of the five treated wines (depleted of their own protein and back-added with Fernão Pires protein) was subsequently incubated at temperatures varying from 30 to 80ºC. The results presented (Fig.1) clearly show that the wine characteristic response to increasing temperatures does not depend on the proteins themselves: each wine exhibits similar patterns of turbidity variation regardless of containing equivalent amounts of its own protein or of another wine protein. This result may be explained if different wines contain the same set of proteins or a set of structurally related proteins, as suggested by Ferreira *et al.* (1999).

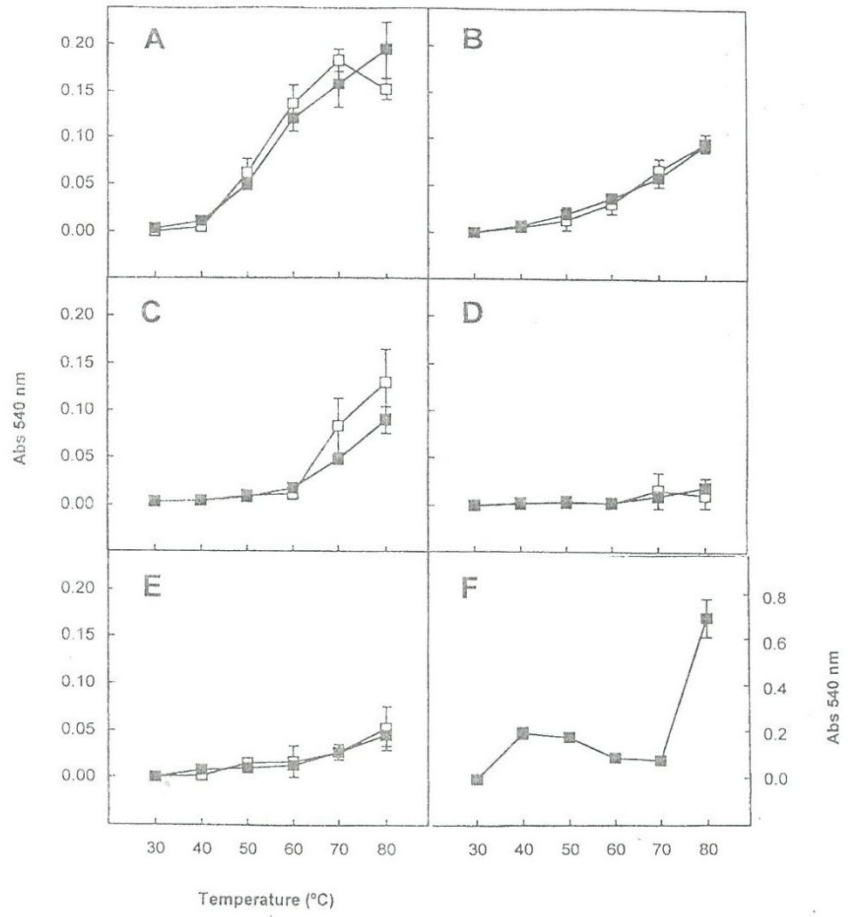

Figure 1.–*Changes in turbidity* (detected by measuring the absorbance at 540 nm) *observed after incubation of each original wine (■) or treated wine (□) at different temperatures.* Treatment of each wine was performed by bentonite fining to remove its own protein followed by back addition of an equivalent amount of Fernão Pires protein. A, B, C, D, E and F: Fernão Pires, Assario, Tamarez, Verdelho, Arinto and Moscatel wines, respectively. Vertical bars represent the standard deviations.

Due to its unusual pattern of turbidity variation when exposed to increasing temperatures (Fig. 1F), the total protein from Moscatel wine was purified according to the method described for the Fernão Pires wine (Materials and Methods) and back-added to a protein free Assario wine. Original Moscatel and Assario wines exhibit distinct and typical patterns of response to increasing temperature (Fig.1B, 1F). Protein-free (bentonite-treated) Assario wine shows no haze formation when treated to temperatures up to 80°C. However, bentonite treated Assario wine back-added with different amounts of Moscatel wine protein behaves, in what turbidity is concerned, like the original Assario wine and unlike Moscatel wine (Fig. 2).

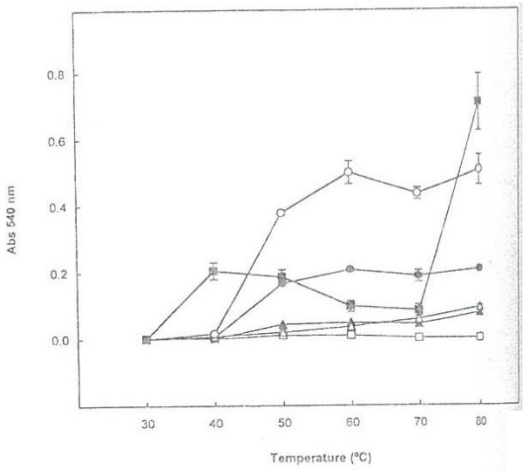

Figure 2.–*Changes in turbidity* (detected by measuring the absorbance at 540 nm) *observed after incubation of the wine at different temperatures.* (■): Moscatel wine; (Δ): Assario wine; (□): protein-free, bentonite-treated, Assario wine; (▲, ●, ○): bentonite-trated Assario back-added with total Moscatel wine protein containing 98.5 µg, 159.2 µg and 240.0 µg of Moscatel wine protein, respectively. Vertical bars represent the standard deviation.

These results suggest that it is the wine, not the proteins themselves, that determine the pattern of turbidity formation with increasing temperatures. This pattern is determined by the other components of the wine and needs to be investigated. Nevertheless, the amount of protein present in the wine correlated positively with the intensity of turbidity formation at any given temperature.

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