

Wine instability. III. The influence of pH

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INTRODUCTION

A major cause of white wine turbidity is due to the instability of the grape proteins.

They naturally denature which leads to their aggregation and subsequent precipitation, forming an amorphous sediment, or flocculation, producing an unattractive haze.

Mesquita *et al.* and Piçarra-Pereira *et al.* (in the previous publications, this same issue) showed that the proteins are not the main factor controlling their own solubility in wines under high temperatures.

In the present work the influence of pH on wine stability was investigated.

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.

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

RESULTS, DISCUSSION, CONCLUSIONS

The strategy used in this work to improve our understanding of the factors responsible for wine turbidity involved the determination of the pattern of variation of protein instability with increasing temperature (from room temperature to 80°C).

The ultimate evidence that the proteins are not the main factor controlling their own solubility under high temperatures is illustrated in Fig. 1.

In this experiment, original Fernão Pires wine (containing 141.0 µg protein ml⁻¹), 20 mM citrate-NaOH buffer, pH 2.5 (containing 123.3 µg of total Fernão Pires protein ml⁻¹), water with pH adjusted to 5.5 (containing 143.4 µg of total Fernão Pires protein ml⁻¹) and 20 mM Tris-HCl buffer, pH 7.5 (containing 134.6 µg of total Fernão Pires protein ml⁻¹) were incubated under different temperatures and the resulting turbidity measured spectrophotometrically at 540 nm.

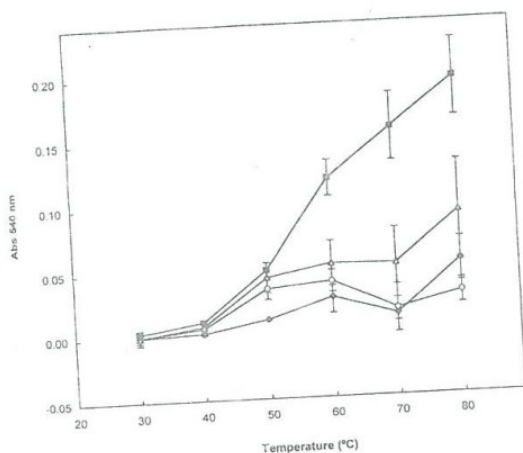


Figure 1.—Changes in turbidity (detected by measuring the absorbance at 540 nm) observed after incubation at different temperatures of the total Fernão Pires protein at distinct pH values. (■): original Fernão Pires wine (containing 141.0 mg protein ml⁻¹); (Δ): 20 mM citrate-NaOH buffer, pH 2.5 (containing 123.3 µg total Fernão Pires protein ml⁻¹); (◆): water with pH adjusted to 5.5 (containing 143.4 µg total Fernão Pires protein ml⁻¹); (○): 20 mM Tris-HCl buffer, pH 7.5 (containing 134.6 µg total Fernão Pires protein ml⁻¹). Vertical bars represent the standard deviations.

The results obtained clearly indicate that the total Fernão Pires protein is increasingly heat-stable when the pH of the solution in which the proteins are dissolved gradually increases from the wine pH to 7.5.

From the data presented it is possible to conclude that although the presence of proteins is a prerequisite for the formation of haze when wines are heated at high temperatures, the development of turbidity in wines does not depend on the protein characteristics. It seems to be controlled by a combination of non-protein factors such as the presence of polysaccharides and the low pH that is characteristic of wines.

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