Wine instability. II. The influence of several non-proteic components

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INTRODUCTION

Clarity is of vital importance to wine quality because this property makes the first impression on the consumer.

Naturally occurring proteins of grapes have been known for many years to cause clouding or haze in wines.

Mesquita et al. (in the previous publication of this same issue) showed that the wine proteins are not the main factor controlling their own solubility in wines, under high temperatures.

In the present work, the effects on wine protein instability of wine polysaccharides, proteins of non-wine origin and alcohol were also 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.

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 further investigate the compounds that are responsible for the pattern of haze formation that is typical of each wine when exposed to increasing temperatures, the experiments illustrated in Fig.1 were performed.

In Fig. 1A, 1B and 1C, the curves represent the characteristic responses of Fernão Pires wine to increasing temperatures.

This wine was, subsequently, treated with bentonite to remove its own protein. As expected, the protein-free Fernão Pires wine does not develop haze when treated to temperatures up to 80°C (Fig. 1A, curve ◇). To protein-free Fernão Pires wine, bentonite-treated, was added an amount of lyophilised bovine serum albumin (BSA; therefore, protein of non-wine origin) equivalent to the protein originally present in that wine. The concentration of BSA remaining soluble in the wine was 83% of its original value.

When BSA containing wine was incubated at temperatures up to 80°C, the pattern of haze formation is identical to that of the original wine (Fig. 1A, curve □). This result confirms that the typical pattern of protein insolubilization observed in each wine with increasing temperatures is conferred by the non-protein components of the wine.

In search of non-protein wine components that determine the typical pattern of

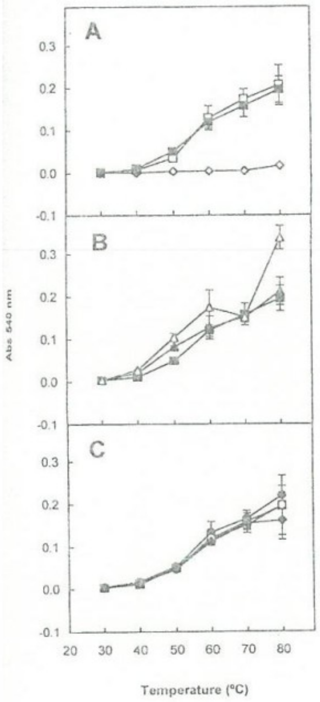

Figure 1.–*Changes in turbidity* (detected by measuring the absorbance at 540 nm) *observed after incubation of treated Fernão Pires wine at different temperatures.* (A) ■: original Fernão Pires wine (141.0 µg protein ml^{-1}); ◇: protein-free, bentonite-treated, Fernão Pires wine (1.5 µg protein ml^{-1}); □: bentonite-treated Fernão Pires wine, back-added with bovine serum albumine (117 µg protein ml^{-1}). (B) ■: original Fernão Pires wine; ▲, △: Fernão Pires wine added with different amounts of polysaccharides previously purified from the wine by FPLC cation exchange chromatography (Monteiro et al., 1999, Fig. 1, peak 1; corresponding to a solution with an absorbance, at 335 nm, of 1 and 2, respectively). (C) ■: original Fernão Pires wine; ●, ○. ◆: Fernão Pires wine added with 0.5%, 1% or 2% (v/v) ethanol, respectively. Vertical bars represent the standard deviations.

haze formation illustrated in Fig. 1 of Mesquita et al. (previous publication, in this same issue), different amounts of polysaccharides (Fig.1B) or ethanol (Fig.1C) were added to the original Fernão Pires wine. While the level of alcohol does not seem to interfere with the solubility of the wine proteins under high temperatures (Fig. 1C), the amount of wine polysaccharides does affect the characteristic pattern of haze formation, increasing protein instability (Fig. 1B).

The presence of protein is necessary for the formation of haze when wines are treated at high temperatures. However, the data presented shows that the turbidity does not depend on the protein characteristics. It is due to the combination of the wine proteins with non-proteic components such as polysaccharides.

REFERENCES

A. Bensadoun., D. Weinstein 1976. Assay of proteins in the presence of interfering materials. Anal. Biochem., 70, 241-250.

P.R. Mesquita, S. Monteiro, M.A. Piçarra-Pereira, V.B. Loureiro, A. Teixeira and R.B. Ferreira. 1999. Wine instability. I- The importance of the wine proteins. This same issue.

S. Monteiro, M.A. Piçarra-Pereira, M.C. Tanganho, J.P. Rente, V.B. Loureiro, A.R. Teixeira and R.B. Ferreira. 1999. Preparation of polyclonal antibodies specific for wine proteins. J. Sci. Food. Agric., 79, 772-779.

K.F. Popock, B.C. Rankine. 1973. Heat test for detecting protein instability in wine. Aust. Wine. Brew. Spirit Rev., 91, 42-43.