Characteristics of the wine proteins

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INTRODUCTION

With the purpose of studying the proteins present in wines and the reason why they precipitate, during storage, causing a haze which renders these beverages of low commercial value, experiments are under way to determine the structural factors responsible for this phenomenon. In addition, a detailed structural analysis of the wine proteins will certainly help in developing methods for the specific removal of these polymers.

In the present work, a single grape variety wine, Moscatel, was used.

MATERIALS AND METHODS

Preparation of wine.–The white wine used was prepared from the single grape variety, Moscatel. Ripened Moscatel grapes were harvested in 1997, in Portugal, and processed into wine by a conventional microvinification procedure, according to the classical white wine technology. Bentonite was not added during fermentation. After opening each bottle, 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.

Protein purification by FPLC ion exchange chromatography.–Wine aliquots

were thawed and centrifuged at 15,000g 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 were subsequently lyophilised (Edwards Micro Modulyo freeze drier, Crawley, Sussex, England) and the dried residue solubilized in 20 mM citrate-NaOH buffer, pH 2.5.

A sample containing the wine total proteins was purified or fractionated by FPLC cation exchange chromatography on a Mono S HR5/5 column (Pharmacia/LKB) previously equilibrated in 20 mM citrate-NaOH buffer, pH 2.5. The bound proteins were eluted with a step gradient (0/1 M, for the isolation of total proteins) or a continuous gradient (0 to 1 M, for the fractionation of the individual wine proteins) of NaCl.

Each of the peaks obtained with the continuous gradient was subsequently desalted in PD-10 Sephadex G-25M columns previously equilibrated with water, lyophilised, dissolved in 20 mM piperazine buffer, pH 9.8 and fractionated by FPLC anion exchange chromatography on a Mono Q HR5/5 column (Pharmacia/LKB) previously equilibrated in the same buffer. The bound proteins were eluted with a continuous gradient (0 to 1M) of NaCl.

For the fractionation of the total Assario proteins a wine sample was subjected to FPLC cation exchange chromatography on the Mono S HR5/5 column (Pharmacia/LKB) previously equilibrated in 20 mM citrate-NaOH buffer, pH 2.5. The bound proteins were eluted with a continuous gradient (0 to 1M) of NaCl. The major Assario polypeptides were further purified by preparative SDS-PAGE.

Preparation of antibidies specific for the total or individual wine proteins.– The antibodies were prepared as described by Monteiro *et al.*, 1999.

Electrophoresis.–Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by a modification (Christy *et al.*, 1989) of the method described by Weber & Osborn (1969) and by Laemmli (1970). The molecular mass polypeptide standards used ranged from the 205 kDa subunit of rabbit muscle myosin to the 14.2 kDa of bovine milk α-lactalbumin.

Western Blotting and Immunoblotting.–The techniques used are described by Monteiro *et al.*, 1999.

Protein measurement.–Protein content was measured using a modification of the Lowry method (Bensadoun & Weinstein, 1976).

RESULTS, DISCUSSION, CONCLUSIONS

It was observed by SDS-PAGE electrophoresis (results not shown) that the purified Moscatel wine proteins revealed the presence of only a few polypeptides ranging in molecular mass from 15 to 30 kDa.

However this very low diversity is only apparent. Purification of the individual Moscatel proteins by FPLC cation exchange chromatography (Mono S column, pH 2.5) showed ten major components (Fig. 1). The wine components with no positive charge at pH 2.5 did not bind to the column and appeared as a large A_{280} peak (peak 1), whereas the components with positive charge were eluted with a continuous

gradient (0 to 1M) of NaCl (peaks 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12). Peak 1 contains polysaccharides and peaks 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 revealed the presence of protein. Each of the ten major protein peaks was subsequently desalted, lyophilised and further fractionated by FPLC anion exchange chromatography on the Mono Q column of the FPLC, at pH 9.8, into four major distinct peaks with a continuous gradient of NaCl (0 to 1M).

These experiments indicate that Moscatel wine contains a huge number (over 40) of polypeptides with different pI's but similar molecular masses. SDS-PAGE electrophoresis (results not shown) of each of the peaks eluted from the Mono Q column shows that each can still be resolved into several different polypeptides.

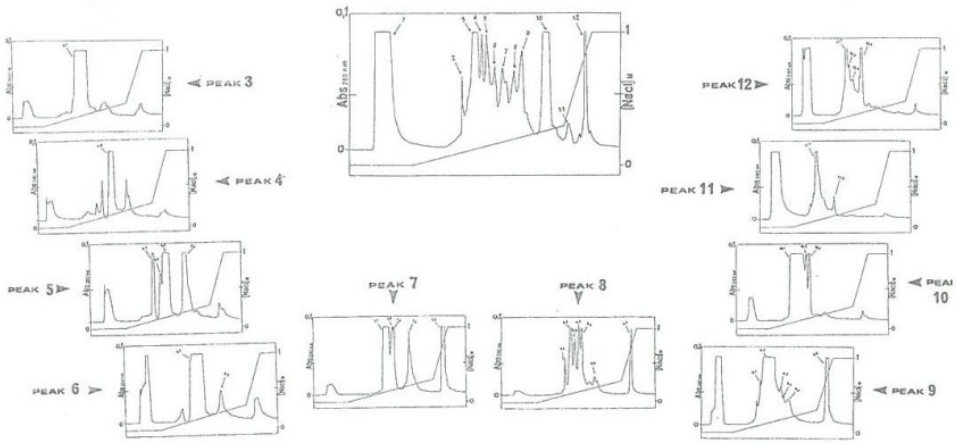

Figure 1.–*Fractionation of the total Moscatel wine proteins by FPLC.* **A wine sample was centrifuged, desalted, lyophilised and fractionated by cation exchange chromatography on the Mono S column of the FPLC, at pH 2.5. The bound proteins were eluted with a continuous gradient of NaCl (0 to 1M). Each of the major protein peaks obtained previously was desalted, lyophilised and further fractionated by anion exchange chromatography on the Mono Q column of the FPLC, at pH 9.8. The bound proteins were eluted with a continuous gradient of NaCl (0 to 1M).**

Probably these polypeptides are structurally similar, differing only by a few amino acid residues. In fact, it was observed that two polypeptides resolved by native electrophoresis at pH 8.8 showed the same molecular mass by SDS-PAGE and the same electrical charge at both pH 2.5 (Mono S column) and 9.8 (Mono Q column).

These polypeptides exhibit identical molecular masses but different electrical charges, pointing to the existence of a common precursor, which could generate all the detected polypeptides by limited proteolysis. To verify this hypothesis, polyclonal antibodies were raised, in rabbits, against a major 20 kDa Assario polypeptide. These antibodies proved to be highly specific, Monteiro *et al.*, 1999.

By immunological methods, immunoblotting, it was observed that when 24 of these distinct Moscatel polypeptides were probed with the anti-20 kDa Assario polypeptide antibodies strong signals were achieved (Figure 2).

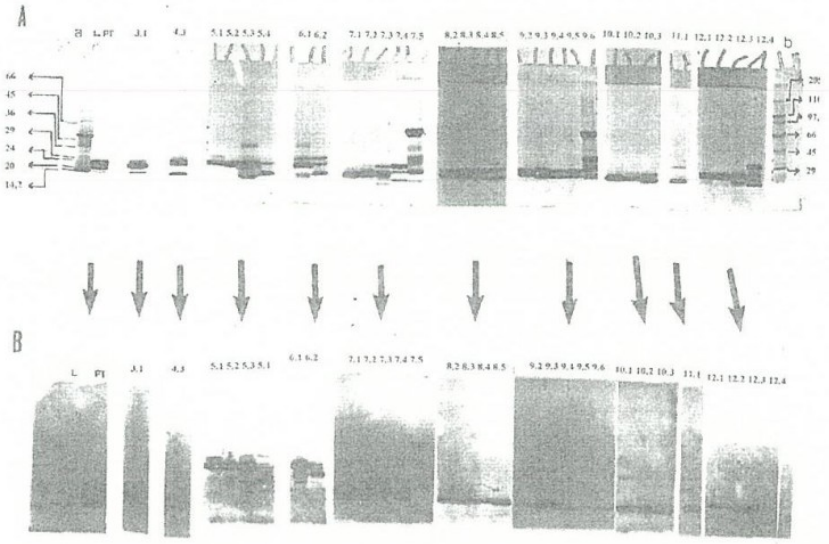

Figure 2.–*Search for identical proteins in different wines.* **The Moscatel proteins were fractionated by FPLC ion exchange chromatography, subjected to SDS-PAGE (A) or probed with anti-20 kDa Assario polypeptide antibodies (B). The protein loaded on each lane was: 100 µg (A), 20 µg (B). Lanes a and b: molecular mass standards (kDa).**

These results suggest not only that there is structural similarity between the Assario polypeptide and the Moscatel polypeptides but also that the 24 selected Moscatel polypeptides are structurally related. The data observed supports the hypothesis of the existence of a common precursor to most or all the Moscatel wine proteins, which subsquently generates a large number of polypeptides by limited proteolysis.

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