

Use of immunological methods in the study of the wine proteins

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INTRODUCTION

Monteiro *et al.* (1999) produced antibodies against the total proteins and against individual polypeptides from a Portuguese wine.

The antibodies were shown to be highly specific for the wine proteins. Furthermore, the antibodies produced specifically against a highly purified wine polypeptide recognised the other major wine polypeptides, raising the possibility of structure similarity between different wine proteins.

In the present work these antibodies were used to detect the presence of identical or similar proteins in distinct wines or in wines prepared from different grape varieties.

MATERIALS AND METHODS

Preparation of grape juices and wines.—Musts were prepared under laboratory conditions from white (Assario, Cercial, Borrado de Mosca) and red (Tinta Pinheira) grape varieties.

The mature grapes were harvested at the Dão region, Portugal. Most exper-

iments were performed with the white wine prepared from the single grape variety Assario.

The Assario wine (Dão region, Portugal, 1994) was prepared according to the classical white wine technology. Bentonite was not added during fermentation. Encruzado (Dão, 1995), Borrado de Mosca (Dão, 1995) and Arinto (Bucelas, 1995) wines were prepared using the same methodology. When appropriate, the Arinto wine was treated with bentonite or with bentonite plus casein. Four other single grape variety white wines of commercial origin were used: Alvarinho (Monção, vinho verde, 1995), Roupeiro (Alentejo, 1994), Sauvignon (Ribatejo, 1994) and Chardonnay (Ribatejo, 1994).

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.

Isolation and concentration of the soluble proteins from grape juices and wines.—Grape juice and wine aliquots were thawed and centrifuged for 5 min at 15,800 g and the supernatant desalted at 4°C on a PD-10 column, previously equilibrated with water (Milli-Q plus, Millipore, Bedford, USA). The protein samples were, subsequently, lyophilized (Edwards Micro Modulyo freeze dryer, Crawley, Sussex, England) and the dried residues resuspended and solubilized in 20 mM citrate-NaOH buffer, pH 2.5, or directly in electrophoresis buffer.

Protein measurement.—Protein content was determined by a modification of the Lowry method (Bensadoun and Weinstein, 1976).

Preparation of antibodies specific for the total or individual wine proteins.—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).

RESULTS, DISCUSSION, CONCLUSIONS

Immunodetection of the Assario wine proteins in grape juices of other varieties.—Immunological methods were used to determine if distinct grape varieties possess essentially the same complement of proteins. To this end, a number of grape varieties, both white (Assario, Verdelho and Borrado de Mosca) and red (Tinta Pinheira) were selected. To minimise the effect of "terroir" on the protein content, the grapes from all varieties were grown in the same year and region, Dão, of Portugal. The protein content ranged from $110 \mu\text{g}\cdot\text{g}^{-1}$ fresh weight (Tinta Pinheira) to $60.3 \mu\text{g}\cdot\text{g}^{-1}$ weight (Verdelho).

The SDS-PAGE presented in Fig. 1A shows that the different grape varieties analysed possess polypeptides with a similar range of molecular masses, regardless of their protein content. Furthermore, a comparison between Fig. 1A, lane 1 (total

soluble protein from the Assario wine) and Fig. 1A, lane 2 (total soluble protein from the Assario grape juice) indicates that the pattern of total polypeptides is markedly altered during vinification.

When the polypeptides of the selected grape varieties were blotted onto a nitrocellulose membrane and probed with the appropriate antibodies (Fig.1B) it was observed that the grapes from different varieties, white or red, grown under similar soil and climate (terroir) conditions, contain an identical set of polypeptides.

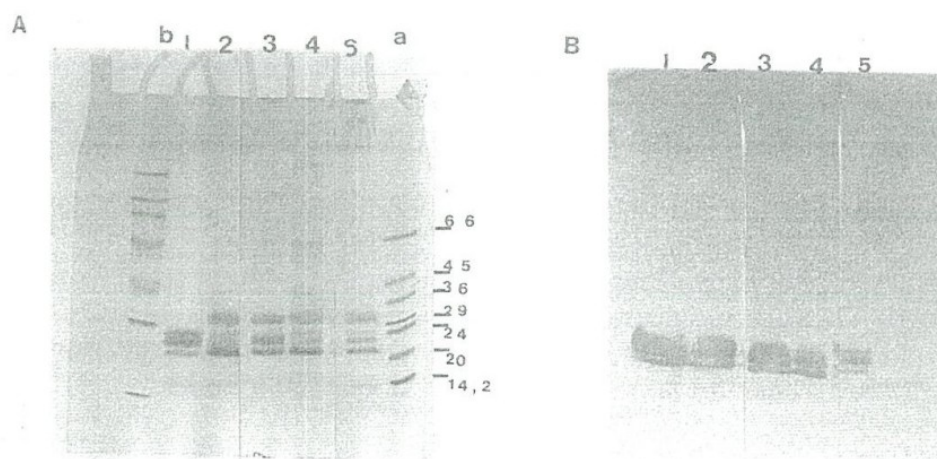


Figure 1.—Search for identical proteins in distinct grape varieties. The total soluble protein from the Assario wine (control, lane 1), from the juices of white grape varieties (Assario, lane 2; Verdelho, lane 3; Borrado de Mosca, lane 4) and from the juice of a red grape variety (Tinta Pinheira, lane 5) were isolated, subjected to SDS-PAGE (A) or probed with anti-polypeptide 5 antibodies (B). Protein loaded in each lane: 150 μ g (A), 20 μ g (B). Lanes a,b: molecular mass standards (kDa).

Immunodetection of the Assario wine proteins in other wines.—Immunological methods were also used in the analysis of proteins from a range of different wines. To this end we have selected single grape variety white wines produced in distinct regions of Portugal and prepared by microvinification techniques or of commercial origin. As expected the protein content of the wines under study varied widely (results not shown). However, when a fixed amount of protein from each wine was analysed by SDS-PAGE, Fig. 2A, the different wines produced in different years, in distinct regions of Portugal and from different grape varieties are composed by polypeptides of similar size. Furthermore, immunoblot analysis of the blotted wine proteins using anti-polypeptide 5 antibodies (Fig.2B) revealed that all wines tested contain structurally identical or very similar polypeptides, regardless of grape variety, year or region.

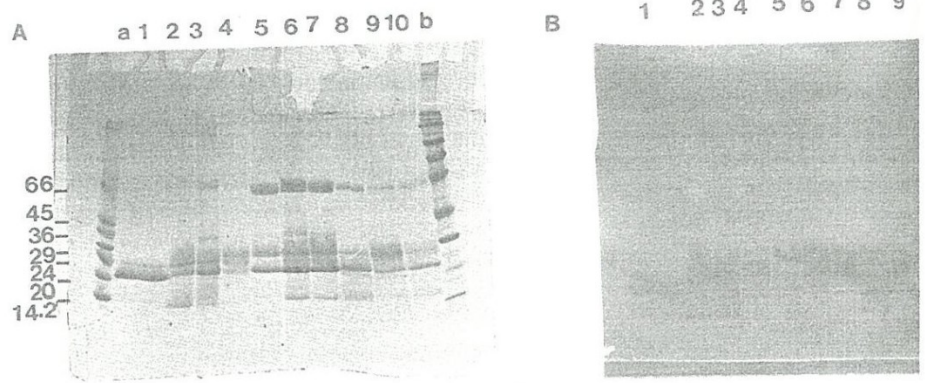


Figure 2.—Search for identical proteins in wines produced from different grape varieties, distinct years and in different regions. The total soluble protein from each of the wines mentioned in the Materials and Methods section was isolated, subjected to SDS-PAGE (A) and probed with anti-polypeptide 5 antibodies (B), as described in the Methods section. Protein loaded in each lane: 150 μ g (A), 30 μ g (B). Lanes a, b: molecular mass standards (kDa). Lanes 1,2,3,4,5,6,7,8,9,10: total protein from the Assario, Encruzado, Borrado de Mosca, Alvarinho, Arinto, Arinto (treated with bentonite), Arinto (treated with bentonite and casein), Roupeiro, Sauvignon and Chardonnay, respectively.

Immunology is an expanding area of biological research. The analyses performed indicate:

a) different white or red grape varieties possess an identical set of polypeptides;

b) wines prepared by microvinification techniques or of commercial origin from grapes of distinct varieties grown under different climate and/or soil conditions also contain an identical set of polypeptides.

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