Research Note

Electrophoretic analysis of the polypeptide composition during berry development

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Introduction: The concentration of proteins in wine varies typically from 15 to 230 mg·l^{-1}. Proteins are of considerable technologic and economic importance because they may precipitate, causing haze or sediment in bottled wine, thereby reducing its commercial value or making it unacceptable for sale. Haze formation is traditionally prevented by protein removal (bentonite adsorption). However, because this procedure alters the organoleptic characteristics of wines (Miller *et al.* 1985) and leads to a considerable loss of wine volume (bentonite lees), it is important to develop alternative removal techniques.

The proteins in wines derive almost entirely from the grape pulp but most of them are lost during vinification (Ferreira *et al.* 2000). In fact, proteins that end up in wines are highly resistant to proteolysis and to low pH values (Waters *et al.* 1992). Immunological and N-terminal sequencing experiments have shown that these polypeptides are structurally related. They are essentially homologous to pathogenesis-related (PR) proteins, including chitinase, thaumatin and osmotin. It is suggested that they have a possible physiological role in grapes as "bio-control agents", *i.e.* in the protection against biotic and abiotic stresses (Monteiro *et al.* 2001*)*.

In the present work, *Vitis vinifera* L. (cv. Moscatel) grapes were harvested during berry development; berry proteins were extracted, quantified and studied by denaturing electrophoresis.

Material and Methods: Grapes (*Vitis vinifera* L. cv. Moscatel) were harvested in 2000 at José Maria da Fonseca, Palmela, Portugal. An area of the vineyard was randomly chosen. Grapes showing the same state of development and similar sun exposure were picked weekly from healthy vines (from 03/07/2000 to 14/09/2000). Moscatel wine was produced by a conventional microvinification procedure, according to the classical white wine technology. Grapes and wine were stored at -80 ºC until required.

E x t r a c t i o n   o f   t h e   t o t a l   p r o t e i n f r o m   g r a p e s : Protein from grapes was extracted following the procedure of Tattersall *et al.* (1997). Only berry pulp was used. In the case of fruit harvested during the first 5 weeks after berry set, frozen berries were cut in halves and the seeds and skins were removed. Berry pulp (4 g) was ground in liquid nitrogen and homogenized in 8 ml of 500 mM Tris-HCl buffer, pH 8.0, containing 5 % (w/v) SDS, 10 mM dithiothreitol and 10 mM sodium diethyldithiocarbamate. The homogenate was incubated at 95 ºC for 5 min and centrifuged at 12,000 x g for 5 min. The total protein was precipitated with trichloroacetic acid (10 % w/v final), incubated for 15 min at 0 ºC and centrifuged at 12,000 x g for 15 min. The resulting pellet was washed twice with an ice-cold solution of ethanol:ethyl acetate (2:1 v/v). The pellet was dried under nitrogen, resuspended in a solution containing 7 M urea, 2 M thiourea, 2 % (v/v) NP-40 and 1 % (w/v) dithiothreitol, solubilized in a sonicator and desalted in NAP-10 columns (Amersham Pharmacia) previously equilibrated with water. After lyophilization, the dried residue was solubilized in the same solution after adding 0.5 % (v/v) IPG-buffer pH 3-10 (Amersham Pharmacia).

E l e c t r o p h o r e s i s : Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described earlier (Ferreira *et al.* 2000).

P r o t e i n   d e t e r m i n a t i o n : Protein concentrations were measured by the modified Lowry method (Bensadoun and Weinstein 1976).

Results and Discussion: During berry development proteins of the pulp were extracted and quantified (Table). The pulp protein content increased dramatically during berry development starting with 12.37 µg·g Fw^{-1} just after berry set to 245 µg·g Fw^{-1} at maturity. Berry weight increased from the 1st (03/07/2000) to the 5th week (02/08/2000) (stage I of berry growth), remaining constant thereafter. We were unable to detect an increment in berry weight during stage III, which was probably due to the se-

T a b l e

Protein content of the Moscatel grapes

Week post flowering (date)	Total soluble protein (mg·g-1 fresh weight)
Berry set	
1st (03/07/2000)	12.4
3rd (17/07/2000)	16.9
5th (02/08/2000)	41.3
Veraison	
7th (17/08/2000)	60.0
9th (31/08/2000)	102.0
11th (14/09/2000)	245.0

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vere water shortage that is so typical of southern Portugal. Berry color changed between the 5th to the 7th (17/08/2000) week in stage II of berry growth. These results show that at veraison (between the 2nd to the 17th of August) the protein content of the pulp, *i.e.* the PR proteins increased.

Electrophoresis (Figure) revealed a relatively complex polypeptide pattern with some alterations in the protein composition between the 5th and the 8th week. The most distinct feature was the accumulation of proteins with molecular masses ranging from 24 to 32 kDa, which was particularly notorious in stage III of berry development. These results were confirmed by two-dimensional electrophoresis (data not shown) indicating that from berry set to maturity, the polypeptide pattern becomes less complex concomitant with a rise in protein concentration. At the onset of berry development, grapes are characterized by a low concentration of a very wide range of distinct polypeptides, while at maturity, berry protein concentration is maximal but the number of distinct polypeptides is reduced to a minimum.
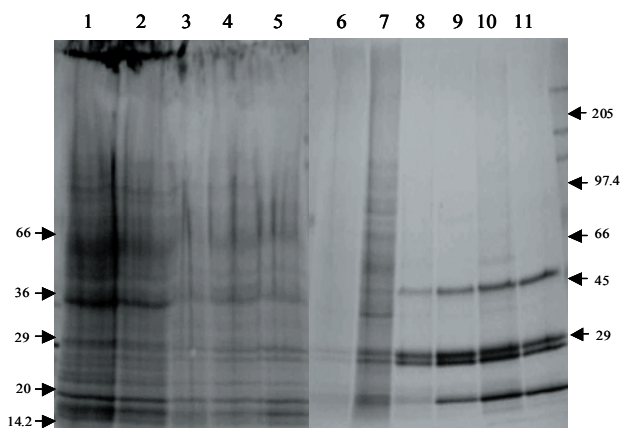

Figure: Protein profiles of grape berries at different stages of development. Total protein was extracted from berries harvested during 11 consecutive weeks (marked on the top of the gels), resolved by SDS-PAGE and stained with Coomassie brilliant blue. Each lane contained 100 μg of protein. The molecular mass standards (kDa) are shown on the left and right margins of the gel.

TATTERSALL *et al.* (1997), using whole berries, found an identical protein profile at different stages of development. WATERS *et al.* (1996) *re*ported that the major haze-forming proteins in commercial white wines have molecular masses of 24 and 32 kDa and that they are pathogenesis-related (PR) proteins.

Wine proteins were found almost entirely in the grape pulp and important changes were noted to occur during vinification (FERREIRA *et al.* 2000).

A simple denaturing electrophoretic analysis of wine suggested that the total protein fraction is mainly composed of only a few polypeptides (MONTEIRO *et al.* 2001). However, a more detailed examination reveals the presence of a very large number of polypeptides, with similar molecular masses but subtle differences in electric charge.

Immunological and N-terminal sequencing experiments revealed that these polypeptides are structurally related, possibly deriving from one or a few common precursors synthesized during grape maturation, to generate the variety of distinct polypeptides present in wines (MONTEIRO *et al.* 2001). However, the data presented in this study do not support a previous hypothesis on the existence of one or a few common precursors to the wide diversity of structurally similar wine proteins. A comparison of the N-terminal sequences of the polypeptides isolated from Moscatel wine with proteins from other sources revealed a high degree of homology to PR proteins (MONTEIRO *et al.* 2001). The wine proteins, in particular the 24 kDa protein, are resistant to proteolysis and this resistance is not due to the presence of inhibitors or wine polysaccharides acting as protective colloids (WATERS *et al.* 1992).

The stability of the PR proteins to low pH and their very high resistance to proteolytic attacks (LINTHORST 1991) means that during winemaking the PR proteins are selectively extracted, releasing vacuolar acids and hydrolytic enzymes which precipitate and degrade most of the other grape proteins. Subsequent fermentation of the must further augments the proteolytic pool (LAGACE and BISSON 1990). The combination of low pH (3.0 to 3.8) and proteolytic activity ensures that only proteins resistant to these conditions, survive winemaking and become troublesome proteins of wines.

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