FINDING NEW PROTEINS RESPONSIBLE OF "NATURAL" PROTEIN INSTABILITY IN WHITE AND ROSE' WINES

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INTRODUCTION

Wine proteins may have different origins (Dambrouck *et al.*, 2003): the grape, from which the majority is originated, the yeast, the bacteria and the enological treatments, as in the case of over fining.
Although proteins are minor components of the wine, protein instability of white and rose' wines is a real problem and it is quite diffused. In fact, proteins can be the cause of turbidity and sediment in bottle that became a economical lost for the wine producer. In order to prevent the problem, the winemakers are almost systematically treating all the wines with bentonite.

Fig 1

Fig 2

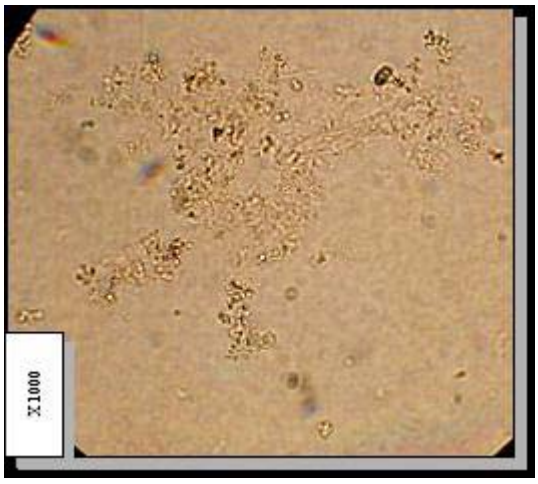


Fig. 1: *Protein casse in bottle: after few months at room temperature, a wine rich in proteins (bottle on the left) shows the haze. The wine that does not contain proteins instead (bottle on the right) is clear.*

Fig. 2: Optical microscopy observation (1000 magnification) of a protein casse. The protein sediment appears very often like a blob and is translucent.

Bentonite is a clay capable to adsorb proteins that unfortunately removes also many aromatic and color compounds from the wine (Ribéreau-Gayon *et al.*, 1998).
Scientific literature (Ribéreau-Gayon *et al*., 1998) and experience show that protein casse is influenced by numerous factors: favorable (polyphenols, Fe2+, Cu2+, heat etc.) and inhibiting (mannoprotein, colloids, etc). Other than the proteins, the main factors necessary to provoke haze are the polyphenols. In the presence of a cork closure that release tannins, for instance, a white wine or a rose' rich in proteins develops almost always protein casse.
Some study carried out some time ago, showed that grape defense proteins – chitinase and taumatine-like – are involved in phenomena of the turbidity caused by exposure to heat (Waters *et al.*, 1996).

Using analytical techniques such as SDS Polyacrylamide Gel Electrophoresis (SDS- PAGE) and Western blot (Manteau *et al.*, 2003) proteins involved in the spontaneous (not triggered by heat) protein precipitations in bottle were studied.

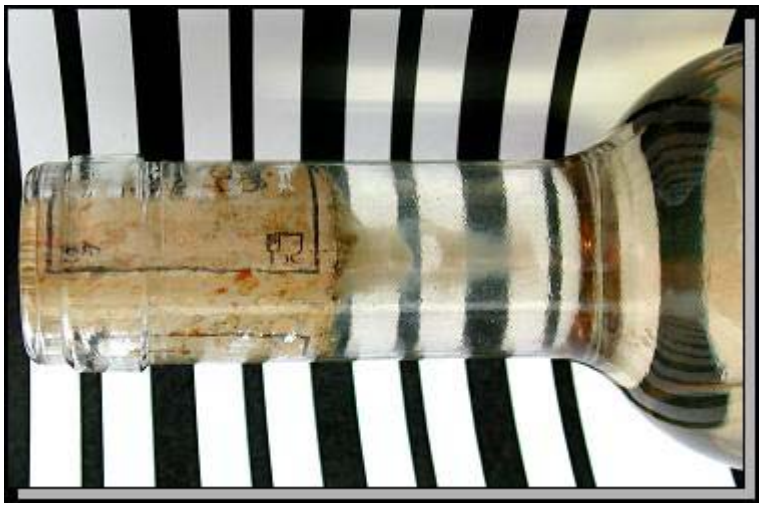


Fig. 3: Protein casse caused by a cork that release tannins in a protein rich wine.

MATHERIAL AND METHODS

SDS-PAGE and protein staining

Before to be loaded on a polyacrilamide gel (PAGE), the protein extract is mixed with Laemli buffer. The latter contains anionic tensioactive, sodium dodecyl sulfate (SDS), that allow to negatively charge all the proteins. Acrylamide gel forms a net that act like a molecular strainer. Under the action of the electric field, the proteins negatively charged are pulled to the positive pole. A protein of a well defined molecular mass will form a well defined band of revelation after staining or Western blot.

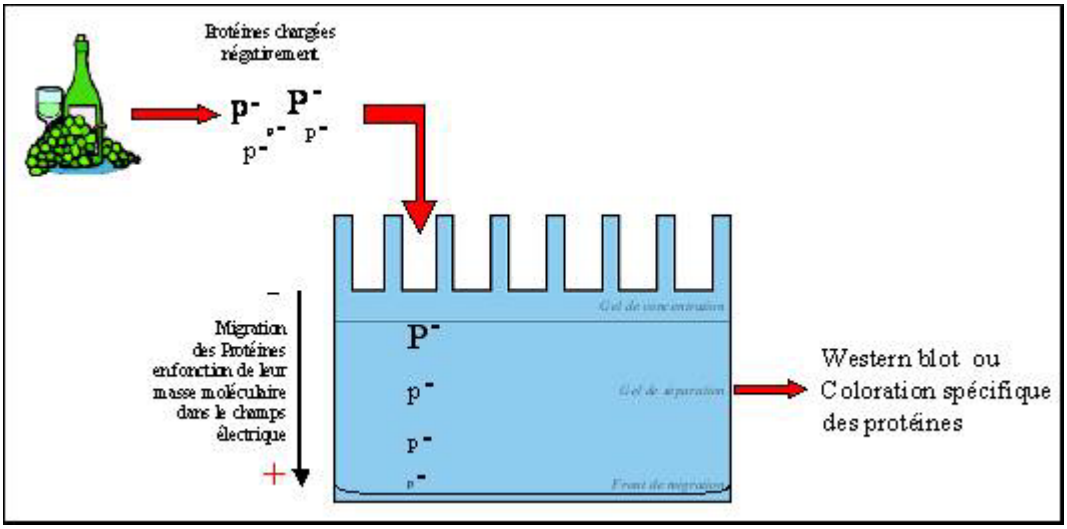
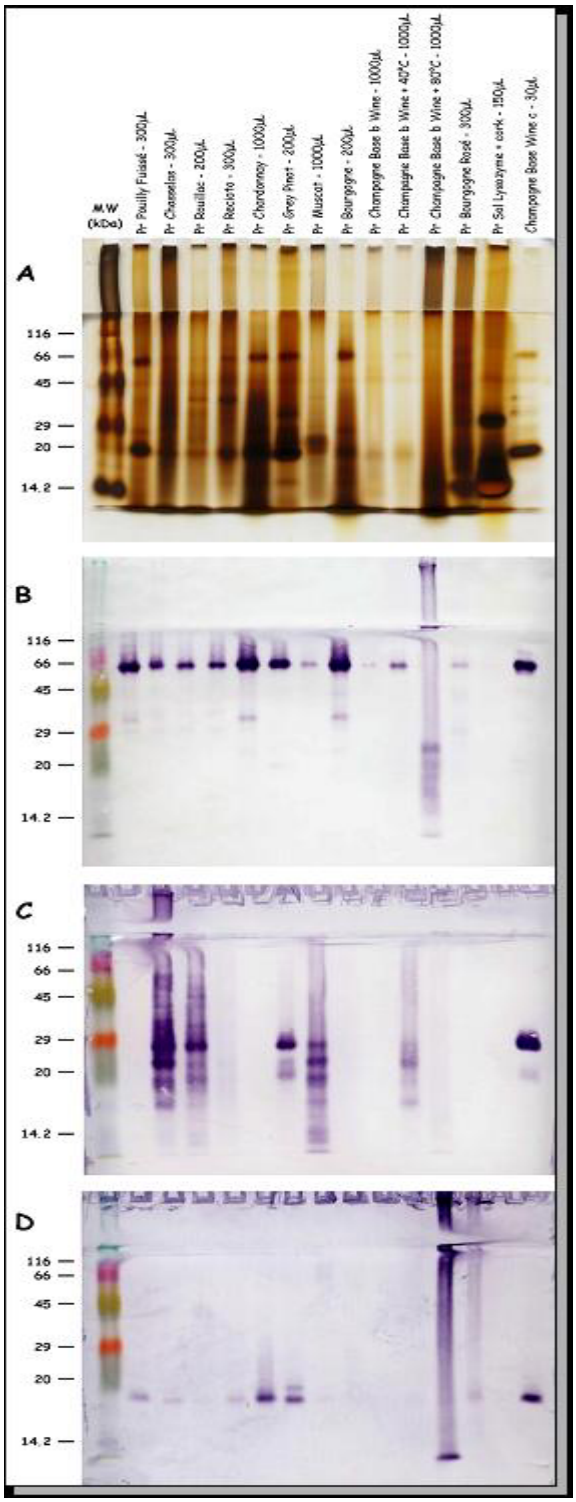


Fig. 4: SDS-PAGE: The proteins negatively charged will migrate to the negative pole according to their molecular mass and bands with specific molecular mass will be observed after Western blot or after applying protein specific staining

RESULT & DISCUSSION

SDS-PAGE is the technique the most used in research laboratories to analyze proteins. The polyacrilamide gel form a kind of molecular strainer and the protein migrate more or less, in function of its dimension, under the influence of the electric field. Therefore a protein of a well defined mass will form a specific band.



To reveal the protein bands two techniques have been used: silver nitrate staining and Western blot. Silver nitrated staining of a SDS-PAGE highlights not only the proteins but also polyphenols. These latter one are actually a mix of compound of different molecular size that produce a smear along the migration path of the proteins. The use of Western blot, together with specific antibody, instead, allows to specifically highlight invertates, chitinases and taumatine-like compounds.

In this study, more than 70 wines of several origins have been analyzed. After SDS-PAGE it is possible to observe up to 12 different proteins in the white and in the rose' wines (data not published). None of the analyzed wines showed the presence of bacterial or yeast in the protein precipitate. Therefore this sample can be considered representative of the natural protein casse in bottle.

Fig. 5: SDS-PAGE profile of proteins involved of protein casse
SDS-PAGE protein profile of Champenois base wine and of some other sediment generated from different wines.
A: SDS-PAGE and silver nitrate staining; B: Western blot with grape invertase specific antibody; C: Western blot with grape chitinase specific antibody; D: Western blot with grape taumatine-like specific antibody.
After wine centrifugation, the sediment is washed with a water-ethanol solution at pH3, and dissolved in Laemmli buffer. The "Wine equivalent volume" is showed in the top of the figure.
A comparison among protein casse of the Champagne Base Wine b and the one induced by heating at 40°C (Champagne Base Wine b + 40°C) and at 80°C (Champagne Base Wine b + 80°C) was also showed.
The next to the last column on the right shows the profile of a protein sediment obtained after put in contact a lisozyme solution and a cork closure. The last column on the right is the profile of a base wine Champenois (Champagne Base Wine c).

As is possible to see in the electrophoresis picture, in order to have a similar revelation with silver nitrate staining, sediment correspondent to different volume of wine were used. This means that where the protein casse occurred, the different wines did not contain the same amount of sediment.

The use of heat to induce haze denatures and strongly modifies the proteins in the sediment. In fact, after heating at 80°C the protein bands are not well defined and the invertase and the chitinase form a smear.

The observation of the protein profile shows also that in the natural protein casse several proteins are involved.

On the contrary to what has been found in the scientific literature (Waters *et al*., 1996), the defense proteins of the grape berry, chitinase and taumatine-like, are not systematically involved in the protein casse in bottle. Throughout the course of this work, we were able to show that proteins like lisozyme (non clarified wine) and more than a dozen of other proteins may be involved in a protein casse in bottle (more than 10 band of different molecular size were observed by SDS PAGE with silver nitrate staining). It is important to notice that in all the protein hazes investigated in this study, the Western blot, has always revealed the presence of inveratse, usually considered a stabilizing protein.
Further investigations are necessary, in order to define the role in the protein casse of white and rose' wines of all the detected proteins, in particular of the mannoprotein like the invertase..

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