IMMUNOTEST $^{\pi}$: A NEW TEST FOR THE DETERMINATION OF PROTEIC STABILITY IN WHITE AND ROSÉ WINES[•]

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1. INTRODUCTION

Proteic haze is a problem which may occur in all fruit base drinks (apple, pear, banana, orange, etc.) and fermented beverages (beer, cider, wine).

In white or rosé wines, proteic haze phenomenon can occur in bottle when there are residual proteins. When it happens, the economic loss is important because the wine producer has to remove all his bottles of the business to handle again the wine in order to make it stable.

To avoid this, there are numerous tests which estimate the proteic stability of wines. However, these tests are not specific to unstable proteins. In wine, Pocock and Waters (2006), counted more than 17 different tests for the determination of wine proteic stability, among which 14 are based on wine heating. These tests can give results that are not always coherent for the same wine (Esteruelas *et al*., 2009), and the interpretation of their results often leads to an overdose during the bentonite treatment.

As none of these tests seems to completely satisfy the users, we compared them. This allowed us to demonstrate that some of these tests gave false negative results and other gave false positive results and did not seem to be reliable (Manteau *et al*., 2006).

Secondly, we were thus brought to characterize the natural proteic haze and the involved unstable proteins. Proteins, which cause this instability in bottle, come exclusively from grapes and are defence proteins of the grape-berry. Those proteins, which seem to be markers of the proteic haze, had been identified as chitinase and thaumatin-like (Manteau *et al*., 2006; Calegari *et al*., 2007).

From these elements, immunology theory was then used to develop a new test for the determination of proteic stability of wine: ImmunoTest $^{\pi}$.

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2. MATERIALS AND METHODS

2.1 Antibody production

In previous work (Manteau *et al*., 2003; 2006), we have demonstrated that grape's defence proteins (chitinase and thaumatin-like) are present in wines and in spontaneous or natural proteic haze. After purification of these proteins, specific rabbit antibodies against chitinase and thaumatin-like proteins were produced in partnership with the Bio-Rad company.

2.2. Comparative study of protein assays

Electrophoresis (SDS-PAGE) and usual protein tests used in enology are described by Manteau *et al*. (2007). SDS-PAGE electrophoresis is used in all biochemistry labs. This technique is very sensitive and the results are easy to read by a simple observation. By this method, proteins can be identified and their quantities can be determined. Unfortunately, electrophoresis is very slow and the products used are very dangerous.

3. RESULTS AND DISCUSSION

Although a large number of protein determination tests in wines are available (Pocock, Waters, 2006; Esteruelas *et al*., 2009), their results often do not satisfy the winemakers. We developed the ImmunoTest$^{\pi}$, based on an immunological method (dot-blot) that is very easy to implement, sensitive, reliable and used in all biochemistry labs throughout the world. The ImmunoTest$^{\pi}$ does not require expensive equipments to be read. By a simple observation of the spot's colour, the quantity of unstable proteins in wine can be estimated (fig. 1).

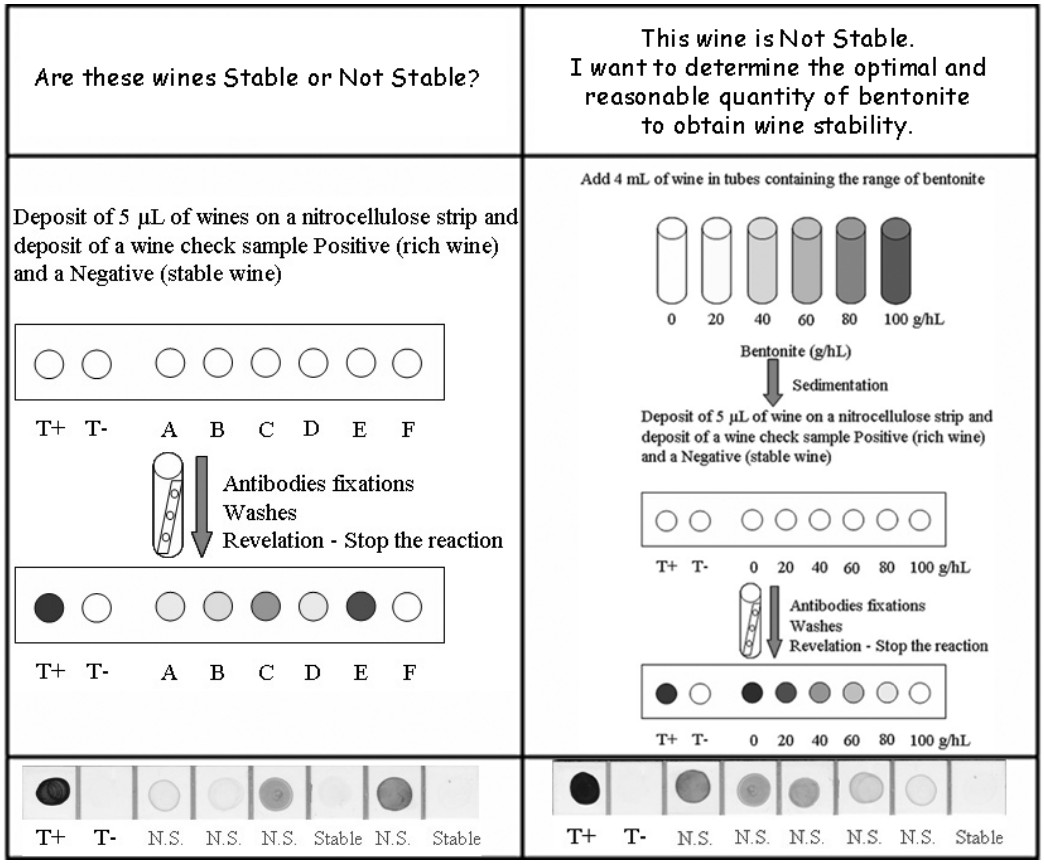

Fig. 1 - How to use the ImmunoTest $^{\pi}$

We applied the ImmunoTest$^{\pi}$ on more than 200 white and rosé wines and on a very large number of proteic haze cases. On the same samples we applied different wine protein tests.

Sometimes enological proteic tests gave weak negative results for wines rich in proteins, or wrongfully positive results for protein-lacking wines. Like electrophoresis, the ImmunoTest $^{\pi}$ is on the contrary very sensitive and reproducible, and the results are very easy to read since a coloured spot shows that the wine still contains proteins and is not stable (fig. 2).

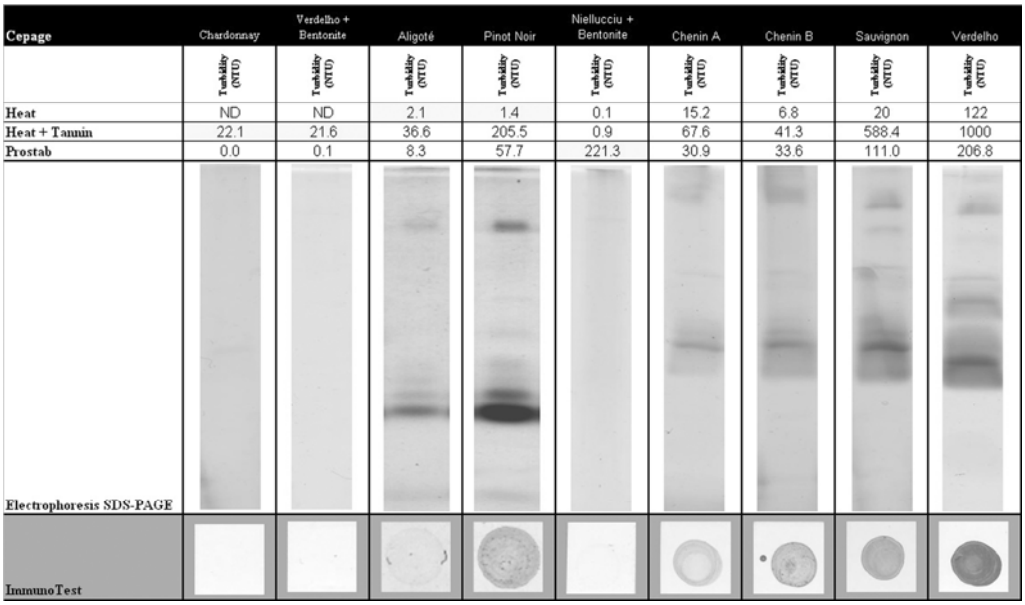

Fig. 2- Comparative study of protein assays used in enology. Electrophoresis SDS-PAGE and these usual proteic tests used in enology are described by Manteau *et al*. (2007).

4. CONCLUSION

This ImmunoTest$^{\pi}$ is a new test to check wine proteic stability and is totally reliable. It seems not to be sensitive neither to products used in oenology nor to wine turbidity (data not shown). ImmunoTest $^{\pi}$ is presented as a kit. A bentonite solution can be associated to it. Thanks to the specific antibodies and the range of bentonite used by the winemakers, this kit allows to determine the optimal and tailored quantity of bentonite to obtain wine stability.

We hope that this ImmunoTest $^{\pi}$ will become the reference test for the determination of proteic stability of white and rosé wines.

Abstract

Proteic haze is a problem which may occur in all fruit-based beverages and fermented juices (beer, cider, wine). When it occurs, the economic loss is important. To avoid this, there are numerous tests to estimate the proteic stability of white and rosé wines. However, these are not specific towards unstable proteins. As none of these tests seems to satisfy completely the users, we compared them. We then used immunology to develop a new test for the determination of proteic stability of wine: ImmunoTest $^{\pi}$. This immunological test is a new test to check wine proteic stability. It seems to be sensitive neither to products used in oenology nor to wine turbidity. ImmunoTest $^{\pi}$ is presented as a kit and a range of bentonite can be associated to it. Thanks to the specific antibodies and

to the range of bentonite used by the winemaker, this kit allows determining the optimal and reasonable quantity of bentonite needed to obtain wine stability.

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