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Influence of *Botrytis cinerea* infection on Champagne wine proteins (characterized by two-dimensional electrophoresis/immunodetection) and wine foaming properties

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Abstract

Proteins are implicated in the foam stabilization of Champagne wines. They may have a grape, yeast, bacteria or fungal origin. *Botrytis cinerea* is a widespread fungal pathogen, which is the causal agent for gray mold. The first part of this work showed the deleterious effect of the presence of this fungus on the foaming properties of a champenois base wine. Foamability and foam stability were reduced, respectively, by 47.7% and 33.3% in the botrytized wine, as compared to the healthy wine. In a second part, SDS–PAGE and two-dimensional electrophoresis (2-DE), coupled with immunodetection, were used to study (thoroughly) the protein patterns of both wines. With 2-DE and silver-staining detection, the disappearance of numerous spots, located in an acidic pH range, was observed. Indeed, the number of spots detected was about two times more abundant in the healthy wine than in the botrytized one, suggesting that a proteolysis occurred. On the other hand, the presence of new proteins, likely fungal proteins, proteins secreted by the plant as a response to *B. cinerea* infection, or even protein fragments resulting from partial proteolysis, was detected in the botrytized wine. All these modifications of the wine protein content were undoubtedly due to the presence of *B. cinerea* and this might be a reason for the loss of foaming properties of Champagne base wines, though no relationship between these two phenomena can be established from the results obtained.

Keywords: Proteins; Wine; Champagne; Foam; *Botrytis cinerea*; Gray mold; 2-DE; Immunoblotting

1. Introduction

In sparkling wines, effervescence and foam are two undissociable criteria and their appearance and behaviour are considered as one of the most important characteristics for consumers. Accordingly, these ephemeral phenomena have been studied in recent years in a number of sparkling wines, particularly in Champagne (Andrés-Lacueva, López-Tamames, Lamuela-Raventós, Buxaderas, & de la Torre-Boronat, 1996; Gallart, Tomás, Suberbiola, López-Tamames, & Buxaderas, 2004; Liger-Belair, 2005; Marchal et al., 2001; Maujean, Poinsaut, Dantan, Brissonnet, & Cossiez, 1990; Pueyo, Martín-Alvarez, & Polo, 1995).

Champagne wines contain surface-active compounds, which have significant effects on bubble stability (Péron et al., 2001), and contribute to the stabilization of foam (Brissonnet & Maujean, 1991; Malvy, Robillard, & Duteurtre, 1994; Senée, Robillard, & Vignes-Adler, 2001).

Abbreviations: SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; MW, molecular weight.

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In this sense, Maujean et al. (1990) reported a positive correlation between the protein concentration and the foamability, which was confirmed by Malvy et al. (1994). Since proteins are present at low levels in sparkling wines, it is of major interest to preserve them from factors that may cause their degradation during the winemaking process. Indeed, fermentations and some enological treatments can lead to a decrease in the protein content of wines (Luguera, Moreno-Arribas, Pueyo, Bartolomé, & Polo, 1998; Marchal, Chaboche, Douillard, & Jeandet, 2002; Martínez-Rodriguez & Polo, 2003), as well as protein degradation, by the action of yeast enzymes (Bayly & Berg, 1967; Moreno-Arribas, Pueyo, & Polo, 1996).

In the Champagne vineyard, *Botrytis cinerea* is a widespread fungal pathogen, responsible for the gray mold disease, which causes considerable economic losses for winemakers. Depending on the vintage, fungal infection rates can reach 15–25% and wines prepared from infected grapes usually exhibit organoleptic defaults, such as an oxidation of the colour or the appearance of typical aromatic notes (''moldy'', ''rotten''), which are not appreciated by consumers (Bocquet, Moncomble, & Valade, 1995, 1996). Marchal et al. (2001) examined the effects of *B. cinerea* infection on the foaming properties of Champagne wines obtained from Chardonnay, Pinot noir and Pinot meunier grape berries. From 20% of infection, foam characteristics of the three Champagne wines were dramatically altered, whereas total protein contents were very similar. These authors concluded that the higher the degree of rot, the more are foaming properties altered. However, they gave no information about the modifications undergone by proteins, and no electrophoretic approach was undertaken in this work.

In a previous study (Marchal et al., 1998), it was also reported that the presence of fungal proteins, namely proteases, in a highly botrytized must of Pinot noir, resulted in the complete degradation of the initial protein fraction, as shown by SDS–PAGE and immunodetection analyses. This study gave information about the electrophoretic characteristics of must proteins but no data were presented about what occurs during the winemaking process; moreover, this work did not afford any data concerning wine foaming properties.

According to these results and because proteins have been proven to play a role in wine foaming properties, we have undertaken a thorough study of the proteinic content of two wines prepared with healthy and botrytized grapes.

Proteins, from grape berries, musts, base wines or sparkling wines, have previously been analyzed using various analytical methods (Moreno-Arribas, Pueyo, & Polo, 2002), including chromatographic techniques (Berthier et al., 1999; Luguera et al., 1998; Monteiro, Piçarra-Pereira, Teixeira, Loureiro, & Ferreira, 2003; Waters, Peng, Pocock, & Williams, 1995), polyacrylamide gel electrophoresis (PAGE) (Dambrouck et al., 2003; Marchal, Bouquelet, & Maujean, 1996; Marchal et al., 1998; Moreno-Arribas, Cabello, Polo, Martín-Alvarez, & Pueyo, 1999; Waters, Wallace, & Williams, 1992) and isoelectric focussing (IEF) (Anelli, 1977; Luguera et al., 1998). However, only a few papers concern the use of 2-DE (IEF followed by SDS–PAGE or LDS-PAGE) (Hsu & Heatherbell, 1987a, 1987b, 1987c; Marshall & Williams, 1987; Sarry et al., 2004; Tesnière & Robin, 1992).

In this study, electrophoretic techniques (SDS–PAGE and 2-DE), combined with protein blotting and immunodetection, were applied to study the impact of *B. cinerea* on the protein content of Chardonnay base wines.

2. Materials and methods

2.1. Musts

Musts were prepared from grape berries of the Chardonnay variety. Healthy grapes and grapes infected by *B. cinerea* were hand-harvested in the same vineyard in the Champagne area (France), in September 2003. The rate of infection was expressed as numbers of infected grape berries as a percentage of the total number of grape berries on a bunch. Grapes were pressed using a pneumatic press (pressure between 1.5 and 2 bar). Sulphur dioxide was added to the free run juice as follows: 150 mg/l for polyclonal antiserum production and 60 mg/l for wine production. After static settling at 12 °C for 24 h, musts were centrifuged at 8000*g* for 10 min. Supernatants were separated, filtered through a 3 μm membrane (Schleicher & Schuell) and then through a 0.45 μm membrane (Alltech), and stored at 4 °C prior to use.

2.2. Wines

Wines were made from musts prepared from either healthy grapes or grapes naturally infected with *B. cinerea*. Settled Chardonnay musts were racked and chaptalized with sucrose. The alcoholic fermentation was achieved by *Saccharomyces bayanus* at 18 °C. Malolactic fermentation did not take place in these wines. Wines were centrifuged and filtered through a 0.45 μm membrane and stored at 4 °C.

2.3. Enological analyses

Several parameters, including pH, total acidity, potassium, calcium, gluconic acid content and wine colour, were determined for both healthy and infected wines. An Orion 420A pH meter (Fischer Scientific, Elancourt, France) was used to determine the pH. Total acidity was determined by M/64 NaOH titration, using bromothymol blue as an indicator, and results were expressed as g/l of sulphuric acid. The potassium and calcium contents were determined by atomic absorption spectrophotometry (Varian Spectra 640, Melbourne, Australia). The gluconic acid content, a chemical index currently used to estimate the level of *B. cinerea* infection, was enzymatically assayed and results