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It's time to pop a cork on champagne's proteome!☆

Clara Cilindre[a,b,*], Elisa Fasoli[c], Alfonsina D'Amato[c], Gérard Liger-Belair[a,b], Pier Giorgio Righetti[c,**]

[a]Equipe Effervescence, Champagne et Applications, GSMA UMR CNRS 7331, Université de Reims Champagne Ardenne, Moulin de la Housse, BP 1039, 51687 Reims Cedex 2, France
[b]Laboratoire d'oenologie et chimie appliquée, URVVC EA 4707, Université de Reims Champagne Ardenne, Moulin de la Housse, BP 1039, 51687 Reims Cedex 2, France
[c]Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Via Mancinelli 7, 20131 Milano, Italy

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ABSTRACT

Champagne is a world-renowned French sparkling wine, which undergoes many steps (fermentation, aging …) for its elaboration. Various compounds might evolve during this winemaking process and thus modify its final quality. Here, we report the first proteome analysis of two standard commercial Champagne wines, using the powerful Combinatorial Peptide Ligand Library (CPLL) technique. Indeed, wine proteins are present in small amounts but they are key compounds, likely to impact on both foam quality and aroma behavior. Forty-three unique gene products were retrieved in a single-varietal champagne and a blended champagne. Several proteins from *Vitis vinifera* together with seven yeast proteins were undoubtedly identified in these Champagne wines.

Biological significance
The main advantage of CPLLs was the detection of low abundance proteins despite the absence of purification or pre-concentration step. It is an important fact to take into account, since Champagne wines generally contain a low amount of proteins (5–10 mg/L) that implies to usually concentrate wine proteins before 1D or 2D electrophoresis. Most Champagne proteins are grape and yeast glycoproteins which are considered as good foam "promoters". Some of these proteins might also interact with wine aromas, and thus contribute to the overall quality of Champagne wines.
This article is part of a Special Issue entitled: Proteomics of non-model organisms.

1. Introduction

Champagne, the world-renowned French sparkling wine, is a multicomponent hydroalcoholic system holding a huge quantity of various compounds. Proteins are not the major components of wine, although they are essential compounds, contributing to many organoleptic characteristics. Indeed, wine proteins are implied in the foaming properties of Champagne and sparkling wines [1–3], the interaction with wine volatile compounds [4], the stabilization of tartaric salts [5], the decrease in wine astringency [6] and, unfortunately, the formation of haze in white wines [7].

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* *Correspondence to:* C. Cilindre, Equipe Effervescence, Champagne et Applications, GSMA UMR CNRS 7331, Université de Reims Champagne Ardenne, Moulin de la Housse, BP 1039, 51687 Reims Cedex 2, France. Tel: + 33 3 26 91 32 58.
** *Corresponding author.*
E-mail addresses: clara.cilindre@univ-reims.fr (C. Cilindre), piergiorgio.righetti@polimi.it (P.G. Righetti).

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Since the end of the 90s, a broad range of methods for the separation of specific or total proteins have been developed and applied to wine [8], including chromatographic techniques [9–12], capillary gel electrophoresis [13], one-dimensional polyacrylamide gel electrophoresis [1,7,11], or two-dimensional electrophoresis [3,7,15–17]. Despite these complementary approaches applied to depict the wine proteome, only few different protein species – from 4 to 15 – were identified in still wines, but none were reported in Champagne wines. In 2006, Okuda et al. [17] have undergone successive steps to extract the soluble proteins from a Chardonnay wine. Though more than 300 spots were visualized on Coomassie Brilliant Blue (CBB-R250) stained 2D-gels, only 4 different grape protein species were identified by N-terminal sequencing. In 2008, 14 different protein species originating mainly from *Vitis vinifera*, from *Saccharomyces cerevisiae* and *Botrytis cinerea* were undoubtedly identified in a Champagne Chardonnay base wine, by 2D-nano-LC–MS/MS and Western blot analysis [16]. Most of these identified grape proteins were also isolated in a Semillon wine by hydrophobic interaction chromatography [11]. More recently, Sauvage et al. (2010) [7] employed 2D electrophoresis to monitor the impact of enological treatments (bentonite) on the protein content of a Chardonnay wine. Only ten spots were displayed, corresponding to seven protein species from grape origin exclusively. These previous studies illustrate the complexity of wine protein extraction and analysis, owing to the numerous methods available. On the other hand, various factors might explain the qualitative and quantitative changes of the wine protein content, as for example: the grape variety [14,15], the infection of grape berries by the widespread phytopathogen *B. cinerea* [3,16], the yeast strain employed for alcoholic fermentation [18], the aging on lees [19], and also various fining treatments applied during the winemaking process [1,7,9]. It might be thus important to prefer a short extraction protocol suitable for a low protein content (as this is the case for wine) that allows the identification of as many proteins as possible without causing degradation and/or chemical modification.

Combinatorial Peptide Ligand Libraries (CPLLs) have been first proposed, in 2010, to depict the proteome of red and white Italian still wines [20,21]. This technology has been successfully applied to various beverages and foods (from animal and plant sources) and allows the detection of low abundance protein species in comparison with classical methods (as mentioned earlier) [22,23]. Among the main advantages of this new technology, CPPLs allowed the detection of exogenous wine proteins in commercial wines, such as: milk allergen proteins [20] or fungal proteins linked to a potentially contaminated harvest [21]. In white wines, Cereda et al. [20] have detected trace amounts of casein, a fining agent, with a limit of detection equal to 1 μg/L for casein, thus 250 times more sensitive than official ELISA methods (as recommended by OIV for routine controls). Anyway, this method permitted the identification of more than 100 unique gene products in a white wine (not treated with fining agents, though) [22] and around 25 proteins in a Valpolicella red wine [21], much more than in previous studies.

While proteins are generally considered as being important sparkling wine components for the stabilization of their foaming properties, no studies have clearly identified, to date and to our knowledge, the proteins from champagne. Indeed, most studies were dedicated to Champagne base wine proteins [1,3,16]. Champagne wines (and some other French and foreign sparkling wines) are elaborated through the traditional method, which consists in two major yeast-fermented steps, to transform sugars into ethanol and gaseous CO_2: (i) a first alcoholic fermentation (from grape must to base wine), and (ii) a second alcoholic fermentation in sealed bottle, the so-called "prise de mousse" (from base wine to champagne). The second alcoholic fermentation (6–8 weeks) and the maturation on yeast lees (which may last from 12 months up to several years) both induce various quantitative and qualitative changes in the wine through the action of yeast, as listed hereafter: (1) development of aromas during aging on lees, (2) release of nitrogen compounds during autolysis, and (3) release of macromolecules (polysaccharides, lipids, nucleic acids) in wine [24]. Champagne wines are elaborated through a long winemaking process, leading to an unavoidable loss of proteins. In 2003, Manteau et al. [25] demonstrated, by SDS-PAGE and Western blot, the complete extinction of proteins through the winemaking process of a single-varietal champagne elaborated with the Pinot Noir variety. Nevertheless, these results were not confirmed by Le Bourse et al. [26] who revealed by SDS-PAGE the presence of two bands at 60 and 18 kDa in a champagne made with Chardonnay. Unfortunately, these authors did not identify these champagne proteins.

In the present study, we applied the powerful CPLL technique to provide the first insight of the low-abundance proteome from two representative types of Champagne wines made from either 100% Chardonnay (single-varietal champagne, "*Blanc de blancs*") or a blend of three grape varieties (Chardonnay, Pinot noir and Pinot Meunier). The main advantage of CPLLs, as compared to previous extraction procedures, is the detection of low abundance proteins despite the absence of purification or pre-concentration step. It is an important fact to take into account, since Champagne wines generally contain a low amount of proteins (5–10 mg/L) that implies to usually concentrate wine proteins before 1D or 2D electrophoresis.

2. Material and methods

2.1. Champagne wines

Two commercial Champagne wines elaborated, in 2011, at the Nogent l'Abbesse Cooperative (Marne, France) were used for this set of experiments. These Champagne wines underwent the same traditional winemaking but differed from their grape variety content. A first type of champagne, a single-varietal champagne (also named "*Blanc de Blancs*") was only elaborated with base wines from white Chardonnay grapes, whereas the second batch, a blended champagne ("*Brut non-vintage*") was a mixture of base wines from all three grape varieties: Chardonnay (32%), Pinot Noir (19%) and Pinot Meunier (49%). These two types of Champagne wines are the most representative, though *Blanc de Blancs* has a higher consumer preference. Both champagnes underwent malolactic fermentation. No fining treatment was applied during the vinification process, except two riddling agents containing sodium bentonite and tannins added to the