

Effect of sequential inoculation (*Torulaspora delbrueckii*/*Saccharomyces cerevisiae*) in the first fermentation on the foam properties of sparkling wine (Cava)

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Abstract. In a previous study we reported that sequential inoculation of *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* during the first fermentation increased the protein concentration and improved the foaming properties of a base wine. Since effervescence and foam of sparkling wines are key quality factors, the interest of this practice for sparkling wine industry is obvious. In this paper we study whether the foaming properties of the sparkling wines produced from the base wines obtained by sequential inoculation with *T. delbrueckii* and *S. cerevisiae* remains better than those of their controls produced from base wines fermented only with *S. cerevisiae*. The obtained results confirmed that sequential inoculation in the production of the base wine originated sparkling wines with significantly higher maximum heights of foam than conventional inoculation, probably because autolysis of the *T. delbrueckii* cells in the base wine released higher amounts of proteins, especially of the low molecular weight fraction.

1. Introduction

The quality of sparkling wines depends on several factors. Among them, effervescence and foam are probably some of major determinants of the quality of this kind of wines [1]. For that reason, one of the main concerns of sparkling wine industry is find new procedures to improve the foamability and the foam persistence. The foaming properties of sparkling wines depend largely on its chemical composition which, is closely related to their varietal origin, grape maturity and winemaking conditions [2,3]. In this sense, it has been described that foam stability is favored by the presence of surfactant agents such as proteins, mannoproteins and polysaccharides which stabilize the bubble's interface because of their surface properties [4,5]. In fact, it has been reported that all winemaking treatments that decrease protein concentration affect drastically the foam properties of the wine [6,7]. Consequently, sparkling wine industry use to be very careful with all the factors that affect to protein levels of musts, base wines and sparkling wines.

It is also well know that the development of some non-*Saccharomyces* yeast such as *Torulaspora delbrueckii* during alcoholic fermentation can improve wine quality as well as their complexity [8,9]. Recently, our research group has proposed the use of *T. delbrueckii* as a tool for improving the foam properties in sparkling wine production [10]. The use of a sequential inoculation of *T. delbrueckii* and *S. cerevisiae* during the first fermentation increased the protein concentration as well as the maximal height of the foam (HM) of the base wine by the method Mosalux [11]. However, it has not been

verified yet if this effect remains in the sparkling wine after the second fermentation. For that reason, the aim of this work was studying if the sparkling wines elaborated from the base wines obtained by sequential inoculation with *T. delbrueckii* and *S. cerevisiae* present better foaming properties than its corresponding controls elaborated from base wines fermented only with *S. cerevisiae*.

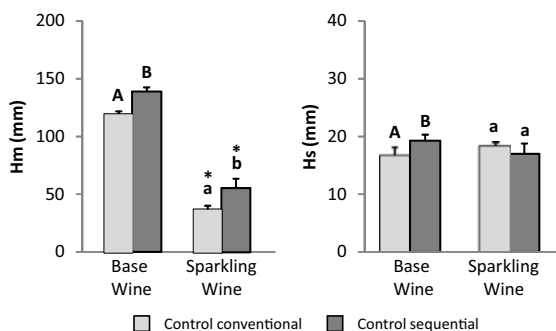
2. Materials and methods

Sparkling wine production (Cava) was carried out with both base wines (conventional inoculation and sequential inoculation) six months after alcoholic fermentation was finished following the traditional method. Nine months later all sparkling wines were disgorged, analyzed and tasted. Foam properties were measured with the Mosalux [9] method. Proteins were measured by HRSEC-DAD [12]. Polysaccharides were determined by HRSEC-RID [13].

3. Results and discussion

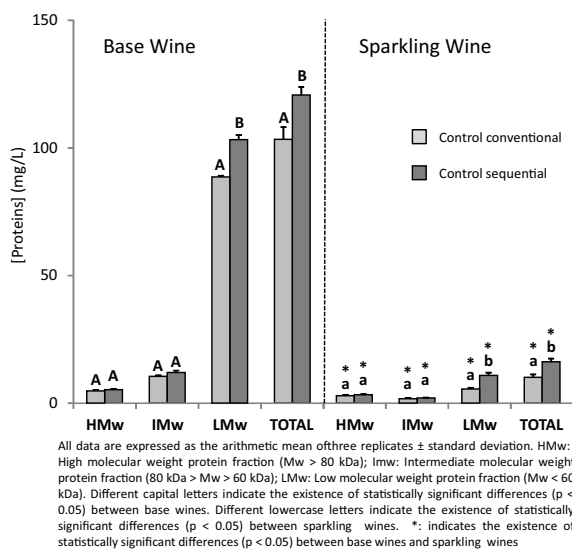
Figure 1 shows the foam properties of the base and sparkling wines. As expected, the maximal height of the foam (HM) was significantly lower in both sparkling wines than in their corresponding base wines. This diminution, which has previously been reported [14], can be attributed to the increase in ethanol content, which exerts a negative effect on wine foamability, and to the absorption of proteins by bentonite added as a riddling agent [7].

Figure 2 shows the protein fractions of the base wines and the sparkling wines.



All data are expressed as the arithmetic mean of three replicates ± standard deviation. Hm: maximal height of the foam; Hs: stable height of the foam. Different capital letters indicate the existence of statistically significant differences ($p < 0.05$) between base wines. Different lowercase letters indicate the existence of statistically significant differences ($p < 0.05$) between sparkling wines. *: indicates the existence of statistically significant differences ($p < 0.05$) between base wines and sparkling wines

Figure 1. Influence of sequential inoculation (*Torulaspora delbrueckii/Saccharomyces cerevisiae*) in the first fermentation on the foam properties of base wine and sparkling wine.



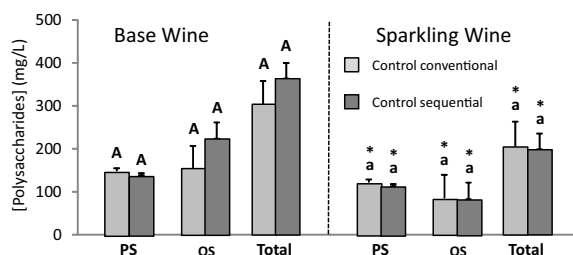
All data are expressed as the arithmetic mean of three replicates ± standard deviation. HMw: High molecular weight protein fraction (Mw > 80 kDa); IMw: Intermediate molecular weight protein fraction (80 kDa > Mw > 60 kDa); LMw: Low molecular weight protein fraction (Mw < 60 kDa). Different capital letters indicate the existence of statistically significant differences ($p < 0.05$) between base wines. Different lowercase letters indicate the existence of statistically significant differences ($p < 0.05$) between sparkling wines. *: indicates the existence of statistically significant differences ($p < 0.05$) between base wines and sparkling wines

Figure 2. Influence of sequential inoculation (*Torulaspora delbrueckii/Saccharomyces cerevisiae*) in the first fermentation on the protein fraction of base wines and sparkling wines.

As expected, the total protein contents of the base wines were far higher than those of the sparkling wines. This sharp decrease in the total protein content of the sparkling wines was significant in both (conventional and sequential) wines and all molecular weight fractions, especially the low molecular weight fraction (LMW). It has previously been reported and largely attributed to the absorption of proteins by the bentonite used as a riddling agent [7]. Also, it is probably one of the main reasons why HM is lower in sparkling wines than in their corresponding base wines.

Figure 3 shows the polysaccharide fraction of base wines and sparkling wines. Again the concentration of polysaccharides in sparkling wines was significantly lower than in their corresponding base wines and this was true in all molecular weight fractions.

Similar results have been reported by other authors [15,16], who have attributed this decrease to precipitation. Since ethanol decreases the solubility of some polysaccharides, the increase in ethanol concentration caused by the second fermentation may explain this phenomenon.



All data are expressed as the arithmetic mean of three replicates ± standard deviation. PS: Polysaccharides (MW > 5 kDa); OS: Oligosaccharides (MW: < 5 kDa). Different capital letters indicate the existence of statistically significant differences ($p < 0.05$) between base wines. Different lowercase letters indicate the existence of statistically significant differences ($p < 0.05$) between sparkling wines. *: indicates the existence of statistically significant differences ($p < 0.05$) between base wines and sparkling wines

Figure 3. Influence of sequential inoculation (*Torulaspora delbrueckii/Saccharomyces cerevisiae*) in the first fermentation on the polysaccharides and oligosaccharides of base wine and sparkling wine.

Another possible explanation is the absorption of some polysaccharides by the bentonite used as riddling agent or even by the dead yeast cells.

It can therefore be concluded that sequential inoculation with *T. delbrueckii* and *S. cerevisiae* may be useful for obtaining sparkling wines with better foaming properties. Specifically, sequential inoculation produced base wines with significantly higher maximum heights of foam (HM) than conventional inoculation, probably because autolysis of the *T. delbrueckii* cells in the base wine released higher amounts of proteins, especially of the low molecular weight fraction. This trend of higher protein concentration and better HM was maintained in the sparkling wines from sequential inoculation though, logically, both values were reduced by the *prise de mousse*.

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References

- [1] Cilindre C, Liger-Belair G, Villaume S, Jeandet P, Marchal R (2010) Foaming properties of various Champagne wines depending on several parameters: Grape variety, aging, protein and CO₂ content. *Anal. Chim. Acta*, **660**, 164–170
- [2] Coelho E, Reis A, Domingues, M.R.M, Rocha S.M, Coimbra M.A (2011) Synergistic Effect of High and Low Molecular Weight Proteins in the Foamability and Foam Stability of Sparkling Wines. *J. Agric. Food Chem.*, **59**, 3168–3179
- [3] Kemp B, Alexandre H, Robillard B, Marchal R (2015) Effect of Production Phase on Bottle-Fermented Sparkling Wine Quality. *J. Agric. Food Chem.*, **63**, 19–38
- [4] Brissonnet F, Maujean A (1993) Characterization of foaming proteins in a Champagne base wine. *Am. J. Enol. Vitic.*, **44**, 297–301
- [5] Vanrell G, Esteruelas M, Canals JM, Zamora F (2005) Influence du type de clarification du vin de base et des adjuvants de tirage sur la qualité de la mousse des vins effervescents. *Revue des Œnologues*, **114**, 28–30

- [6] Pocock K.F, Salazar F.N, Waters E.J (2011) The effect of bentonite fining at different stages of white winemaking on protein stability. *Aust. J. Grape Wine Res.*, **17**, 280–284
- [7] Vanrell G, Canals R, Esteruelas M, Fort F, Canals JM, Zamora F (2007) Influence of the use of bentonite as a riddling agent on foam quality and protein fraction of sparkling wines (Cava). *Food Chem.*, **104**, 148–155
- [8] Jolly N.P, Varela C, Pretorius I.S (2014) Not your ordinary yeast: non-Saccharomyces yeasts in wine production uncovered. *FEMS Yeast Res.*, **14**, 215–237
- [9] Benito S, Hofmann T, Laier M, Lochbühler B, Schüttler A, Ebert K, Fritsch S, Röcker J, Rauhut D (2015) Effect on quality and composition of Riesling wines fermented by sequential inoculation with nonSaccharomyces and Saccharomyces cerevisiae. *Eur. Food Res. Technol.*, **241**, 707–717
- [10] GonzálezRoyo E, Pascual O, Kontoudakis N, Esteruelas M, EsteveZarzoso B, Mas A, Canals JM, Zamora F (2015) Oenological consequences of sequential inoculation with non-Saccharomyces yeasts (*Torulaspota delbrueckii* or *Metschnikowia pulcherrima*) and *Saccharomyces cerevisiae* in base wine for sparkling wine production. *Eur. Food Res. Technol.*, **240**, 999–1012
- [11] Maujean A, Poinssaut P, Dantan H, Brissonet F, Cossiez E (1990) Étude de la tenue et de la qualité de mousse des vins effervescents II. Mise au point d'une technique de mesure de la moussabilité, de la tenue et de la stabilité de la mousse des vins effervescents. *Bulletin de l'OIV*, **711–712**, 405–426
- [12] Canals JM, Arola L, Zamora F (1998) Protein fraction analysis of white wine by FPLC. *Am. J. Enol. Vitic.*, **49**, 383–388
- [13] Ayestarán B, Guadalupe Z, León D (2004) Quantification of major grape polysaccharides (Tempranillo V.) released by maceration enzymes during the fermentation process. *Anal. Chim. Acta*, **513**, 29–39
- [14] Esteruelas M, González-Royo E, Kontoudakis N, Orte A, Cantos A, Canals JM, Zamora F (2015) Influence of grape maturity on the foaming properties of base wines and sparkling wines (Cava). *J. Sci. Food Agric.*, **95**, 2071–2080
- [15] Moreno-Arribas V, Pueyo E, Nieto F.J, Martín-Alvarez P.J, Polo M.C (2000). Influence of the polysaccharides and the nitrogen compounds on foaming properties of sparkling wines. *Food Chem.*, **70**, 309–317
- [16] Martínez-Lapuente L, Guadalupe Z, Ayestarán B, Ortega-Heras M, Peírez-Magarino S (2013). Changes in polysaccharide composition during sparkling wine making and aging. *J. Agric, Food Chem.*, **61**, 12362–12373