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Effects of a β-glucanase enzymatic preparation on yeast lysis during aging of traditional sparkling wines

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ABSTRACT

Scientific researches on characterization of the commercial enological preparation of Lallzyme MMX® containing β-glucanase and its influence on autolysis of different yeast strains typically employed in the production of sparkling wine are lacking. The aim of the present work was to plug a gap in this field, studying the β-glucanase activity of Lallzyme MMX® and its interactions with BCS103® and EC1118® yeast strains. The results showed that β-glucanase was slightly inhibited by ethanol, but its residual activity at wine pH was sufficient for the purposes. Kinetic parameters showed a better enzyme–substrate complex formation for the EC1118® strain. The influence on yeast lysis during 12 months of bottle-aging was monitored, demonstrating that enzyme addition did not substantially influence either the content and progression of total proteins, or foam characteristics. However, scanning and transmission electron microscopy images and free amino acid analysis indicated β-glucanase improved cell wall degradation of both selected yeasts, evidencing a lower autolytic capacity of the BCS103® strain. Our study demonstrated that addition of β-glucanase catalyzed cell disorganization and promoted release of yeast components into sparkling wine, with strain-dependent effects. Therefore, employment of β-glucanase rich Lallzyme MMX® might effectively accelerate some aging characteristics of traditional sparkling wines.

1. Introduction

The production of sparkling wine by the traditional (champenoise) method is a long process that follows many steps, most of them requiring long time and expensive skilled labor (Torresi, Frangipane, & Anelli, 2011). It involves the production of the base wine, which undergoes a second fermentation in the bottle during which CO_2 and alcohol content increase, followed by aging on yeast lees for several months. The death and autolysis of yeasts which occur during aging is essential for the final characteristics of the product and improves its organoleptic properties (Pérez-Serradilla & Lauque De Castro, 2008).

Over the years many attempts to simplify this complex production process have been made, while at the same time trying to keep the quality and the characteristics unaltered. Some new approaches in this area include the improvement of the yeasts used in bottle secondary fermentation and the design of procedures to accelerate the natural process of yeast autolysis (Pozo Bayón, Martínez-Rodríguez, Pueyo, & Moreno-Arribas, 2009). Among the various factors one must consider in the selection of strains for second fermentation, their autolytic capacity is one of the most important. In fact, a good autolytic strain will produce a better quality sparkling wine compared to a strain in which this characteristic is not present (Martínez-Rodríguez, Carrascosa, Barcenilla, Pozo Bayón, & Polo, 2001). Many researchers have addressed their studies towards the selection of autolytic yeast strains for secondary in bottle fermentations (Gonzalez, Martínez-Rodríguez, & Carrascosa, 2003; Todd, Fleet, & Henscheke, 2000).

The cell wall of *Saccharomyces* is made of mannoproteins (high molecular weight polysaccharides conjugated with proteins) crossed by fibers of glucan and chitin (Pretorius, 2000). Its rupture after yeast death involves the action of β-glucanases, classified as endo- and exo-glucanases, which can hydrolyze the β-O-glycosidic linkage of β-glucan chains, leading to the release of glucose and oligosaccharides (Dubourdieu, Villetaz, Desplanques, & Ribéreau Gayon, 1981). As a consequence of the cell wall structure breakdown, several cytoplasmic and parietal compounds are released into the sparkling wine, which can modify its organoleptic and foaming properties with positive effects on the product characteristics (Alexandre & Guilloux Benatier, 2006; Pozo Bayón et al., 2009).

Autolysis needs several months to occur; low wine aging temperature causes a low death rate and low enzymatic reaction rates, thus accounting for the slowness of the process. For years on end, two methods have been available to accelerate the autolysis during sparkling wine production: adding yeast autolysates to the wine and increasing the

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aging temperature (Charpentier & Feuillat, 1993). However, both techniques caused organoleptic defects in the final product, often described as "toasty". Lately, some producers employ commercially available inactive yeasts just before the secondary fermentation; however, despite their practical use, very few scientific papers report specific results about their real effects on sparkling wine.

The endogenous enzymes of grape, yeasts and other microorganisms usually present in must and wine are often unable to sufficiently catalyze the different bio-transformations that occur during winemaking. These activities may, however, be enhanced by the use of exogenous enzymatic preparations. Exogenous enzymes in winemaking are extensively used to improve clarification and the release of varietal compounds, to reduce the formation of ethyl carbamate, and to lower alcohol levels (Van Rensburg & Pretorius, 2000). Another important application of commercial enzymatic preparations concerns the improvement of the yeast lysis, that may contribute to enhancing the release of intracellular and parietal compounds from yeast cells, thus improving the quality of wines aged on lees. The products useful for promoting yeast lysis are a mix of β-glucanase and other collateral enzymes such as pectinases, whose positive effect on the characteristics of red and white wines has been evidenced by several studies (Masino, Montevecchi, Arfelli, & Antonelli, 2008; Palomero et al., 2009).

Yeast autolysis improvement may be a promising tool to enhance sparkling wine quality and reduce production times and costs. Some manufacturers suggest employing β-glucanase enological preparations both in wines and in sparkling wine production (see datasheet and technical documents for Lallzyme MMX®), however their effective utility on sparkling wines is only supposed, because the scientific knowledge on their effect on these peculiar wines really falls short.

In a recent study, Rodriguez-Nogales, Fernández-Fernández, and Vila-Crespo (2012) found that the addition of an enzyme preparation rich in β-glucanase seemed to increase the aging characteristics of traditional sparkling wines. In another recent work, the same researchers found that β-glucanase can be an excellent coadjuvant to enhance the antioxidant properties of sparkling wines (Rodriguez-Nogales, Fernández-Fernández, Gómez, & Vila-Crespo, 2012). These results are encouraging but not conclusive and further studies aimed at investigating the action and effects of β-glucanase on sparkling wines are essential.

To better understand the behavior of β-glucanase and to maximize its effects and benefits on sparkling wine elaboration, the present work focuses on the characterization of a commercial enzymatic preparation (Lallzyme MMX®) rich in this enzyme, and on its application in sparkling wine produced with the méthode champenoise. To our knowledge, studies on the activity of this enzymatic preparation and its effect on different yeast strains are not currently available, oriented at sparkling wine production. Therefore, the β-glucanase activity was first studied in different buffers and in model wine solutions. Both pH and ethanol content effects were also considered, at the typical values of base wine and sparkling wine. Moreover, interactions between the enzyme and two different yeast strains (*Saccharomyces cerevisiae* BCS103® and EC1118®), suitable for the second fermentation of sparkling wines, were evaluated. Subsequently, sparkling wines were produced employing these two strains, both in the presence and absence of the enological preparation, and the influence on yeasts autolysis process during 12 months of bottle aging was investigated, by direct (scanning and transmission electron microscopy) and indirect (free amino acids, total proteins, foam height and stability) methodological approaches.

2. Materials and methods

2.1. Enzymatic preparation stock solution

The enological enzymatic preparation used for all experiments was Lallzyme MMX® (Lallemand Inc., Canada), sourced from *Trichoderma* spp. and *Aspergillus niger*. In the technical data sheet provided by the manufacturer, β-glucanase activity is not reported. Other declared collateral enzymatic activities are: polygalacturonase, 1840 IU g^{-1}; pectinesterase, 545 IU g^{-1}; pectin-lyase, 25 IU g^{-1}. A stock solution of Lallzyme MMX® at 1 mg ml^{-1} was prepared daily in deionized water.

2.2. Yeast stock solution

The two different yeast strains used were: *S. cerevisiae* BCS103® (Fermentis, Division of S.I. Lesaffre, France) and *S. cerevisiae* Lalvin EC1118® (Lallemand Inc., Canada). A fresh stock solution of each yeast at 1 mg ml^{-1} was prepared daily in deionized water.

2.3. β-Glucanase substrate stock solution

The selected substrate for β-glucanase enzymatic activity determinations was laminarin (Humbert Goffard et al., 2004; Vázquez Garcidueñas, Leal Morales, & Herrera Estrella, 1998), a β(1 → 3)-glucan from *Laminaria digitata*. A stock solution of laminarin at 1 mg ml^{-1} was freshly prepared daily in deionized water.

2.4. β-Glucanase enzymatic activity evaluation

The enzymatic assays were performed incubating at 25 °C the substrate laminarin stock solution (0.4 ml), Lallzyme MMX® stock solution (0.08 ml) and 5.92 ml of McIlvaine buffer (McIlvaine, 1921), both in the presence (1.6 ml of the stock solution) and the absence (1.6 ml of deionized water) of yeast. Blank assays without enzyme or Laminarin were also performed. After 45 min of incubation, the reaction was stopped by heat inactivation (100 °C, 2 min) (Humbert Goffard et al., 2004). The glucose released was determined after centrifugation, through the enzymatic test combination D-glucose/D-fructose kit (R Biopharm, Germany), following the instructions of the manufacturer. The test is based on the NADPH amount formation that is stoichiometric with the amount of D-glucose; NADPH was measured by absorbance increase at 340 nm by using a Perkin Elmer Lambda 25 UV/VIS spectrophotometer (Beaconsfield, UK). Since the laminarin substrate is a polysaccharide having a variable molecular weight, the enzymatic activity was expressed as the amount of enzyme that releases 1 mg l^{-1} min^{-1} of glucose instead of International Unit (μmol min^{-1} of laminarin); substrate concentration was expressed as mg ml^{-1} instead of mM. Enzyme activity (U ml^{-1}) was then determined as described in the manufacturer's instructions, adequately modified as follows:

$$\mathrm{U\ ml^{-1}} = \left[\Delta A/\text{incubation time(min)} \times 180.16\left(\mathrm{g\ mol^{-1}}\right) \times \text{final volume(ml)}\right]/6.3\left(\mathrm{mM^{-1}\ cm^{-1}}\right) \times \text{sample volume(ml)}$$

where: ΔA was obtained by subtracting from each sample the absorbance of the blank assays; 180.16 (g mol^{-1}) is the D-glucose molecular weight; 6.3 (mM^{-1} cm^{-1}) is the extinction coefficient of NADPH at 340 nm.

Moreover, the enzyme activity per milligram of total proteins (Specific Activity) was evaluated at maximal pH, and expressed as $mg_{glucose}\ l^{-1}\ min^{-1}\ mg^{-1}_{protein}$.

2.5. Effect of pH on β-glucanase enzymatic activity

The effect of pH on the β-glucanase activity of Lallzyme MMX® was determined in McIlvaine buffer (McIlvaine, 1921), both in the presence and the absence of yeast cells (EC1118® or BCS103® strains). This buffer solution was employed for all the experiments because of its capacity to cover a pH range from 2.2 to 8.0, thus avoiding drawbacks caused by the use of different buffer solutions. Samples were tested at different pH (2.60, 3.20, 4.00, 5.00, 6.00 and 7.00), and the enzyme activity was determined as described above.