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Use of non-*Saccharomyces* yeasts and oenological tannin in red winemaking: Influence on colour, aroma and sensorial properties of young wines

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ABSTRACT

Today, many non-*Saccharomyces* strains have been verified can be positive for the development of wine anthocyanin and aroma in different fermentation scenarios. Moreover, oenological tannins are widely used in wine industry to improve the colour profile and aroma complexity. The aim of this work is to analyze the fermentation characters of non-*Saccharomyces* strains and investigate the effects of pre-fermentative addition of oenological tannins on the wine components as well as sensory properties. For this purpose, five selected non-*Saccharomyces* strains and grape seed tannin were used to carry out the different fermentation trials. As a result, the grape seed tannin were less likely to influence growth kinetics of non-*Saccharomyces* strains. *Schizosaccharomyces pombe* has been proved can be effective to reduce the malic acid content while increase the level of vinylphenolic pyranoanthocyanin, which is positive for wine colour stability. Pre-fermentative use of oenological tannin was verified could be beneficial for the wines fermented with non-*Saccharomyces* regarding the improvement of wine colour, anthocyanin composition and the complexity of volatile compounds. Nevertheless, sensory analysis showed that oenological tannin could be less effective to modify the aroma impression of non-*Saccharomyces* wines.

1. Introduction

Wine fermentation is a complex biochemical process in which yeasts play an important role during their transformation of sugar into ethanol, carbon dioxide and hundreds of other secondary products (Ciani et al., 2010). Some studies have been carried out to determine the impact of yeasts on wine composition, sensory properties and final flavours (Lencioni et al., 2007; Benito et al., 2014, 2016; Ciani and Comitini, 2011). At present, it is known that the ecology of fermentation process is more complex than previous investigations, and some non-*Saccharomyces* yeasts are also playing relevant roles in the metabolic impact, anthocyanin composition and aroma complexity of the final products (Pretorius, 2000). Recently, there has been a growing demand of new and improved wine yeasts that adapted to different fermentation scenarios (Jolly et al., 2006). In order to improve chemical composition and sensory properties of red wine, the combined use of non-*Saccharomyces* and *Saccharomyces* has been proposed as a tool to take advantage of spontaneous fermentation as well as avoid the risk of stuck fermentations (Fleet, 2008).

Traditionally, many non-*Saccharomyces* strains were considered as a kind of spoilage yeast in terms of the production of undesirable metabolites that result in negative sensory impacts on wine quality (Batt and Tortorello, 2014). Moreover, most of the non-*Saccharomyces* wine-related species showed limited fermentation aptitudes, such as low fermentation kinetics and low sulfur dioxide (SO_2) resistance (Ciani and Comitini, 2011). However, in mixed fermentations, some negative oenological characters of non-*Saccharomyces* may not be expressed or could be modified by *Saccharomyces cerevisiae* (*S. cerevisiae*) (Bely et al., 2008). Many experimental evidences have highlighted the positive role of non-*Saccharomyces* in

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the analytical composition of wine (Comitini et al., 2011). For instance, *Schizosaccharomyces* not only can improve the fermentation behaviour of yeast starter cultures and aroma complexity but also produce a high amount of pyruvic acid which contributes to the formation of vinylphenolic pyranoanthocyanins (Benito et al., 2016; Morata et al., 2007); *Lachancea thermotolerans* (*L. thermotolerans*) can produce more L-lactic acid while limit the concentration of malic acid. It has been specially used to modify the wine acidity (Kapsopoulou et al., 2007); *Torulaspora delbrueckii* (*T. delbrueckii*) can produce high amount of higher alcohols and terpenes (Chen and Liu, 2015). Nevertheless, some researches indicate that the yeast growth kinetics could be reduced by addition of grape tannins. It is due to yeast cell wall interact with tannins, hence, the material exchange with must could be inhibited (Mekoue et al., 2015).

Nowadays, oenological tannins have been commonly used into different fermentation scenarios in order to modify aroma complexity, prevent colour loss and enhance well-rounded taste (Chen et al., 2016a,b). Particularly, the pre-fermentative addition of grape seed tannin which contains large amount of oligomeric proanthocyanidin (a set of bioflavonoid complexes that can perform the high antioxidant activity while lower astringency) has been widely reported as a practical tool to improve wine colour stability as well as aroma complexity (Chen et al., 2016a,b). During alcoholic fermentation (AF), tannin treatment could influence the development of pyruvic acid and acetaldehyde, which can directly link with malvidin-3-*O*-glucoside to form high stable pyranoanthocyanins by cycloaddition, such as vitisin A (Vit A) and vitisin B (Vit B). These pyranoanthocyanins are strongly resistant to SO_2 bleaching effect and can protect the wine from oxidation (Morata et al., 2007). With pre-fermentative addition of oenological tannin, the effects of non-*Saccharomyces* on wine components and sensory properties can be regarded as a valuable research since it has been scarcely ever reported.

Above all, the aim of this work is to analyze the interactions between selected non-*Saccharomyces* strains and oenological tannins during AF, meanwhile, the effects of oenological tannin on wine colour, aroma and sensory properties were also studied.

2. Methods and materials

2.1. Microorganism

Non-*Saccharomyces* strains, *Metschnikowia pulcherrima* (*M. pulcherrima*), *L. thermotolerans*, *T. delbrueckii* as well as two wild *Schizosaccharomyces pombe* (*S. pombe*) strains, 938 and V1 were all selected from the Institute of Industrial Fermentation (IFI, CSIC, Madrid) (Benito et al., 2014). *S. cerevisiae* strain (7VA) (Laboratorio de Tecnología de Alimentos, E.T.S.I. Agronomos, Madrid), which possess high capability to produce pyruvic acid and acetaldehyde (Morata et al., 2006), was carried out in this study as a comparison and supplimentary yeast of sequential fermentation. The yeast suspensions were cultivated at 25 °C for 48 h, until the initial inoculation scale was controlled at 10^6 CFU/mL (Benito et al., 2015a,b,c).

2.2. Micro-vinifications

All fermentations were undertaken by using the juice of Merlot grapes (*Vitis vinifera*) grown at Socuéllamos, Ciudad Real in Castilla la Mancha, Spain. The juice was extracted with classic flash thermovinification from fresh must in order to release more colour and tannins. The process heated grapes to a high temperature (about 82 °C) in few seconds, then immediately pumped the fruit into a vacuum chamber for depressurization. Sugars were amended up to 213 g/L, final pH was 3.2, lactic and acetic acids were less than 0.1 g/L. To facilitate the fermentations, nutrients were added at the level of 0.4 g/L (Nutrient-Vit, Lallemand, Montreal, PQ, Canada). Oenological tannin, Tanicol Vintage (40 g/hL) derived from grape seeds, purchased from Agrovin S.A., Ciudad Real, Spain. Six sets of fermentations were carried out with five strains of non-Saccharomyces and one strain of *S. cerevisiae*. Oenological tannin was added at the beginning of each set of fermentation. To make a comparison, each control was designed to be without exogenous tannin in different fermentations. Every treatment was done in triplicate.

In all treatments, *M. pulcherrima*, *L. thermotolerans* and *T. delbrueckii* were carried out with sequential fermentation, which was inoculated with *S. cerevisiae* (7VA) at the fourth fermentation day. It is required since *S. cerevisiae* must be used as a binding partner to supply these non-*Saccharomyces* stains accomplish AF (Benito et al., 2015a,b,c). *S. pombe* strains, 938 and V1 are capable enough to independently conduct the AF (Benito et al., 2014). Each treatment was fermented in a 150 mL microvessel capped with sulfuric acid (Panreac, Barcelona) filled Müller valve (Alamo, Madrid) to avoid microbial contamination and release carbon dioxide (CO_2). All the fermentation trials were accomplished at 25 °C until no weight loss was detected (12 days). After AF, all wines were centrifuged (5000 rpm, 10 min) and then transferred into 125 mL aseptic brown glass bottles, well-sealed and placed at 4 °C for the following analysis.

2.3. Analysis of physical-chemical parameters

Basic fermentation parameters, such as ethanol, total acidity, pH, volatile acid, L-malic acid, L-lactic acid, glucose, fructose, acetic acid were all detected with a Y15 enzymatic autoanalyzer (Biosystems S.A., Barcelona, Spain). These analyses were performed with the appropriate enzymatic reaction kits, which were purchased from Biosystems enterprise. Prior to detections, the Y15 equipment was calibrated with the external standards, which were technically supported by Biosystems enterprise (www.biosystems.com).

2.4. Analysis of phenolic and colour parameters

An Agilent 8453 UV-Visible spectrophotometer (Santa Clara, CA, USA) was used for the detection of phenolic and colour parameters. Beforehand, samples were analysed by full wavelength range (200–1100 nm) with 1 mm quartz cuvette. Absorbance at 420 nm, 520 nm, and 620 nm was measured and then colour intensity was calculated as the sum of absorbance at the three wavelengths, while hue was calculated as the ratio between the absorbance at 420 nm and 520 nm. Total polyphenols index, total tannin content and total anthocyanin content were carried out based on the methods of Ribereau-Gayon et al. (2006). Four important phenolic parameters were also analysed for each trial. Gelatine index is related to the percentage of tannins which are able to combine with protein and mainly used to detect the level of tannin astringency in wine (Oberholster et al., 2013); HCL index indicates the percentage of polymerized tannins combine with wine polysaccharides and salts; Ethanol index is the percentage of tannins which can combine with wine polysaccharides; Vanillin index expressed with mg/L of catechin reflects the flavonoids react with vanillin to form red pigments (Ribereau-Gayon et al., 2006). In addition, CIELAB scales, L^*, a^*, b^* are the colour scales based on the Opponent-Colour Theory that assumes the receptors in the human eye perceive colour as the following pairs of opposites (Gil-Muñoz et al., 1997). L^* scale: Light vs. dark where a low value (0–50) indicates dark and a high value (51–100) indicates light. a^* scale: Red vs. green where a