Fate of anthocyanins in the presence of inactivated yeasts and yeast cell walls during simulation of wine aging

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Abstract In the present research, two inactivated yeast strains (W13 and BM45) and a commercial yeast cell wall preparation (YCW) already tested for their ability to removal ochratoxin A were used to simulate the wine aging. During the simulated aging, the concentrations of the main 4 anthocyanins decreased in both the control wine and the wines added with yeasts, although at rates depending on the type of yeast and on the nature of anthocyanins. Peonidin-3-*O*-glucoside decreased by about 20% in the control wine and by ~ 50% in the wines added with yeast strains or the commercial yeast preparation. Malvidin-3-*O*-glucoside decreased by about 80% in the control wine and in the wine added with YCW and by about 96% in the wines added with W13 and BM45 strains. Cyanidin-3-*O*-glucoside decreased by 47% in the control wine, by 65–66% in the wines added with W13 and BM45 strains, and by 73% in the wine added with YCW. Delphinidin-3-*O*-glucoside decreased by 100% already after 21–28 days of aging in all the wines.

Keywords Anthocyanins · Cell wall · Yeasts · Wine aging

Introduction

The aging of red wines on lees is a traditional oenological practice, which involves the contact of the wine after alcoholic fermentation with the resting dead yeast cells

(Juega et al. 2015) and the so called ‘batonnage’, consisting in stirring the settled lees back into the wine. Lees include mainly yeast, and, in a minor proportion, tartaric and inorganic matter (Perez-Serradilla and de Castro 2008). The reasons for which the aging on lees is performed are related to the release of protective colloids such as polysaccharides and mannoproteins that occurs during yeast autolysis. Among the effects of these compounds, the following ones should be considered: reduction of astringency and bitterness; improvement of wine body, structure and roundness; obtainment of more aromatic complex wines; improvement of colour stability; protection of wine from oxidation (due to the oxygen consumed by the dead yeasts).

Techniques alternative to the aging on lees have been developed in order to obtain the improvements provided by this practice without its disadvantages. They include: addition of β -glucanase to the lees; use of different yeast commercial preparations; use of non-toasted oak chips; and aging on lees combined with micro-oxygenation (Del Barrio-Galán et al. 2011).

Currently, an increasing interest concerns the use in wine aging of yeasts, yeast lees, and inactivated yeast fractions. The reason is the ability of some strains in adsorption/removal of ochratoxin A (OTA), a mycotoxin produced by several toxigenic moulds belonging to *Aspergillus* and *Penicillium* species (Petruzzi et al. 2014a, b, c, 2015; Núñez et al. 2008).

Although it is well known that wine polyphenols interact with yeast lees only to a limited extent, such interactions can have larger effects on the wine sensory qualities (mainly on colour and taste). For these reasons, it is important to study the chemical composition of phenolic compounds that remain in solution or that are adsorbed on yeast lees. Mazauric and Salmon (2005) found that wine

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polyphenols adsorption on yeast lees follows a biphasic kinetic with an initial and rapid fixation followed by a slow, constant, and saturating fixation that reaches its maximum after about 1 week. Furthermore, only very few monomeric phenolic compounds remained adsorbed on yeast lees and the anthocyanin adsorption on yeast lees is unrelated to its polarity. Morata et al. (2003) studied the desorption of anthocyanins by yeast cell walls during fermentation and highlighted that acetyl and *p*-coumaryl derivatives of anthocyanins were more strongly adsorbed than nonacyl derivatives. The results of this differential adsorption are reflected by an increase in the yellow colour and a reduction in the blue colour of the wine.

The aim of the present research was to investigate the fate of the main anthocyanins in the presence of inactivated yeasts and yeast cell walls during simulation of wine aging.

Materials and methods

Yeast strains and yeast cell preparation

Two inactivated yeast strains and a yeast cell wall preparation were used to simulate the wine aging.

The yeasts used in this study were: (a) *Saccharomyces cerevisiae* W13 (Accession number: KC542799), a yeast strain belonging to the Culture Collection of the Laboratory of Predictive Microbiology (Department of the Science of Agriculture, Food and Environment, University of Foggia, Foggia, Italy) already selected for its OTA-removal ability in model wine systems (Petruzzi et al. 2014a, b, c); (b) *S. cerevisiae* BM45, a commercial mannoprotein-overproducing yeast strain (Lallemand Inc., Montreal, QC, Canada), commonly used in the over-lees aging of red wines and already studied for its OTA-removal ability in a model wine system (Petruzzi et al. 2015). The inactivated yeasts were prepared according to Petruzzi et al. (2015).

The commercial yeast cell wall preparation (YCW, Biolees; Laffort, Bordeaux, France), already studied for its ability to remove OTA in a model wine system (Petruzzi et al. 2015), was included in the experiment.

Simulation of wine aging

It was performed according to Petruzzi et al. (2015) in an experiment focused on the removal of ochratoxin A by two inactivated yeasts and a commercial yeast cell wall preparation. Briefly, 100 mL-wine samples inoculated with the yeast strains and the yeast cell wall preparation were kept for 84 days in darkness at 18 °C with a weekly stirring (inversion and shaking for 15 s) to simulate the enological batonnage. All the experiments were done in triplicate.

Analytical determinations

The concentrations of 4 anthocyanins (malvidin-, peonidin-, delphinidin-, and cyanidin-3-*O*-glucoside) were determined by High Pressure Liquid Chromatography coupled to a Diode Array Detector (HPLC–DAD). The chromatographic analyses were performed according to the method described by Revilla and Ryan (2000) with some modification. Samples were pre-filtered, injected into a Zorbax SB C18 column (100 mm 9 4.6 mm, 1.8 μ m; Agilent, Santa Clara, CA), protected by a guard column, and eluted at a flow rate of 0.5 mL/min. Solvent A was water/acetonitrile (95:5 v/v) adjusted to pH 1.8 with perchloric acid, while solvent B was water/acetonitrile (50:50 v/v) adjusted to pH 1.8 with perchloric acid. The gradient programme of solvent A was as follows: from 95 to 90% in 4.8 min, to 80% in 12 min, to 70% in 4.8 min, to 60% in 9.6 min, to 55% in 9.6 min, to 0% in 7.2 min, then remaining at 0% for 10 min, to 95% in 2 min, and finally remaining at 95% for 20 min. Compounds identification was achieved by combining different information: elution pattern and UV–Vis spectra. Quantitative determinations were made using the external standard method with commercial standards (cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, peonidin-3-*O*-glucoside; Extrasynthèse, Genay, France). The calibration curves were obtained by injection of standard solutions under the same conditions of the samples analysed, over the range of concentrations observed.

Analysis was replicated at least three times and the average values are reported in figures. The kinetics of anthocyanin decay was also determined using the package Statistica for Windows ver. 10 (Statsoft Inc., Tulsa, OK).

Results and discussion

Figures 1–4 show the changes of concentration of peonidin-, delphinidin-, malvidin-, and cyanidin-3-*O*-glucoside in wine with and without the presence of inactivated yeasts or yeast cell walls during simulation of wine aging. The concentrations of the four anthocyanins decreased in all the wines (included the control) but the rate of change depended on both type of yeast and anthocyanins. This behaviour confirms that several mechanisms could affect such changes: adsorption by yeast; degradation and oxidation reactions; precipitation of anthocyanins with proteins, polysaccharides or condensed tannins; formation of more complex and stable anthocyanin derived pigments (He et al. 2012).

The behaviour of peonidin-3-*O*-glucoside during the simulated aging can be describe as a succession of decreasing concentrations alternated to phases of constant

Fig. 1 Changes of concentration of peonidin-3-*O*-glucoside during the simulated aging of the control wine (CT) and the wines added with W13 or BM45 strains, or cell wall preparation (YCW)

values (Fig. 1). In the control wine, starting from the 28th day of aging, the concentration remained constant at about 70% of the initial value. Instead, in the wines added with the yeast strains or the yeast cell wall preparation, the concentrations remained constant at about 50% of the initial value from the 56th day of aging.

Delphinidin-3-*O*-glucoside (Fig. 2) constantly decreased during the aging and fell under the detection limit between 14 and 21 days of aging in wines added with W13 or BM45 strains, and between 21 and 28 days in control wines and in wine added with YCW. In all the wines, the decrease of delphinidin-3-*O*-glucoside concentration followed a first order kinetic since the logarithm of concentration is a linear function of the time with *R* values of

Fig. 2 Changes of concentration of delphinidin-3-*O*-glucoside during the simulated aging of the control wine (CT) and the wines added with W13 or BM45 strains, or the cell wall preparation (YCW)

0.98, 0.97, 0.99, and 0.97 for control wine, and wines added with W13, BM45, and YCW, respectively.

The changes of malvidin-3-*O*-glucoside concentrations are shown in Fig. 3, which highlights a continuous decrease in all the wines, although, at the end of the simulated aging, the wines added with W13 or BM45 strains showed the lowest concentrations (3–4% of the starting one) while the control wine and the wine added with the yeast cell preparation showed the highest concentration (20%). The reduction of malvidin-3-*O*-glucoside concentration followed a second order kinetic since the reciprocal of concentration is a linear function of the time (*R* equal to 0.97, 0.98, 0.97, and 0.97 for control wine, and wines added with W13, BM45, and YCW, respectively).

The behaviour of cyanidin-3-*O*-glucoside (Fig. 4) was similar to that of peonidin-3-*O*-glucoside. In the control wine, cyanidin-3-*O*-glucoside decreased during 70 days and then remained at about 50% of the starting concentration until the end of the aging. Instead, in wines added with the yeast strains or the cell wall preparation, the concentrations of the anthocyanin decreased until 56 days and then remained at about 27–35% of the starting concentration.

Based on these data, the adsorption operated by the inactivated yeasts and the cell wall preparation strongly contributed to the reduction of the concentrations of peonidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside. Instead, delphinidin-3-*O*-glucoside and malvidin-3-*O*-glucoside were greatly adsorbed by inactivated yeast strains respect to the cell wall preparation. Furthermore, according to Medina et al. (2005), anthocyanin removal was higher for compounds with higher polarity. Nevertheless, while

Fig. 3 Changes of concentration of malvidin-3-*O*-glucoside during the simulated aging of the control wine (CT) and the wines added with W13 or BM45 strains, or the cell wall preparation (YCW)

Fig. 4 Changes of concentration of cyanidin-3-*O*-glucoside during the simulated aging of the control wine (CT) and the wines added with W13 or BM45 strains, or the cell wall preparation (YCW)

the anthocyanin polarity is, in a decreasing order, delphinidin, cyanidin, peonidin, and malvidin (Koh and Mitchell 2008), the rate of decrease observed in the present work is, always in a decreasing order, delphinidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, and peonidin-3-*O*-glucoside. Thus, the anthocyanin removal by yeast can only partially explain the changes of anthocyanins concentrations. Since the molecular weights of the 4 anthocyanins were delphinidin-3-*O*-glucoside > malvidin-3-*O*-glucoside > cyanidin-3-*O*-glucoside > peonidin-3-*O*-glucoside, it could be assumed that the rate of decrease is directly correlated to the anthocyanin molecular weight and that the inactivated yeasts were more able to adsorb anthocyanins having high molecular weights than the cell wall preparation. This means that several factors could be involved in the anthocyanin fate. They include the different affinity of yeasts for anthocyanins and the reactions that can occur during batonnage, such as: oxidation of ethanol to acetaldehyde; reactions involving acetaldehyde with formation of anthocyanin-derived pigments (Fulcrand et al. 2004); reactions of precipitation.

Conclusion

Yeast strain and cell wall addition caused the reduction of the anthocyanin concentration. The decreases of peonidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside were mainly affected by the chemical structure of anthocyanin (the effect of the type of yeast strain or yeast preparation added was less important) while the decreases of delphinidin-3-*O*-glucoside and malvidin-3-*O*-glucoside were accelerated by the addition of W13 and BM45 strains. Delphinidin-3-*O*-

glucoside was the most sensitive anthocyanin since it decreased by 100% in all the wines in about 20–30 days of aging. Peonidin-3-*O*-glucoside was the anthocyanin less sensitive to the addition of yeast strain or yeast cell wall since its concentrations at the end of the simulated aging were about half of the initial concentration.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights This article does not contain any studies with human or animal subjects.

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