



Evaluation of a specific inactivated yeast developed as an alternative to lees: Effect on polysaccharide and phenolic content, colour and astringency

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Introduction

Yeast derivatives are products that have been subject of an increasing number of studies due to the positive attributes they produce in wine, during and after the alcoholic fermentation process. They are mainly used in winemaking to improve the technological processes and the sensory properties of wine, in addition to removing any undesirable compounds in the wine (POZO-BAYÓN et al., 2009). For many years, these products have been put forward as alternatives to the traditional process of ageing on lees, since they have the same benefits but without (or with a reduced number of) the disadvantages (DEL BARRIO-GALÁN et al., 2010). The improved sensory qualities of the wine are mainly attributed to the mannoproteins that are released by the yeast derivatives during the wine ageing process (POZO-BAYÓN et al., 2009; GUADALUPE et al., 2010; DEL BARRIO-GALÁN et al., 2012). Generally speaking, yeast derivatives used in wine making are classified into 5 groups – depending on the process used to produce them, their composition and degree of purification: dry inactive yeast, yeast autolysates, yeast cell walls, yeast protein extract (POZO-BAYÓN et al., 2009) and purified mannoproteins.

Specific inactivated dry yeasts are products that are rendered inactive using a number of methods – be they thermal or physicochemical – before being subjected to a drying process. Yeast autolysates are obtained following a period where yeast biomass is incubated in order to release enzymes out of the intracellular content, and then it is inactivated and dried. Finally, yeast cell walls are obtained from yeast autolysates in a later step of autolysis centrifugation (POZO-BAYÓN et al., 2009).

Several studies have shown that the release of polysaccharides (mannoproteins in particular), brought about by yeast derivatives, can enhance certain taste characteristics found in red wine, such an increase in volume and structure and a reduction in astringency and bitterness (DEL BARRIO-GALÁN et al., 2011a; DEL BARRIO-GALÁN et al., 2015; GONZÁLEZ-ROYO et al., 2013). This effect is namely brought about by the interaction between the mannoproteins in the yeast derivatives and the phenolic compounds in wine, thereby limiting tannin self-aggregation (MEKOUÉ et al., 2016) and creating more stable polymers. The polymers therefore do not react with the protein found in the saliva in the oral cavity and thus create a smoother, fuller bodied taste and a considerably longer-lasting and more complex palate (VIDAL et al., 2004). Recent studies have demonstrated that some of these products are capable of adsorbing and interacting with the phenolic compounds in wine, and can therefore change sensory qualities in wine (MEKOUÉ-NGUELA et al., 2015a and b). Currently, there are a number of studies that have put forward a series of mechanisms that explain astringency. Given that astringency is perceived as a tactile sensation, some authors have highlighted that this phenomenon could be attributed to reduced or a modified lubrication of the oral cavity (ROSSETTI et al., 2008; ROSSETTI et al., 2009). As BENNICK (2002) sets out, proteins secreted by the parotid gland are seemingly more capable of binding themselves to phenolic compounds. This is where polysaccharides stemming from yeast derivatives can have a lubricating effect and thus palliate the aforementioned tactile sensation.

What is more, the interaction between polysaccharides and phenolic compounds can also help to stabilise the colour of wine, since the formation of more stable polymeric pigments could prevent or reduce oxidation in wine (DEL BARRIO-GALÁN et al., 2012; ESCOT et al., 2001; FRANCOIS et al., 2007).

Abstract

The use of yeast derivatives has increased in recent years due to the positive effects that they can produce in the winemaking technological processes and the sensory characteristics of wine, in addition to eliminating certain undesirable compounds.

The objective of this work was to evaluate the application effect, in post-fermentation, of a specific inactivated yeast in the polysaccharide and phenolic content, the color and the astringency of a Chilean red wine during 2 consecutive vintages.

The addition of the specific inactivated yeast (Optilees™) in a red wine increased the different polysaccharide fractions concentration and, therefore, the total polysaccharide content, producing a decrease in the duration of the wine astringency. However, it did not produce a modification of the phenolic compounds content studied as well as the color parameters.

Key words: Red wines, Yeast derivatives, Polysaccharides, Astringency, Phenolics, Color.

Furthermore, other studies suggest that yeast derivatives can help to prevent or reduce oxidation in white wines (RAZMKHAB et al., 2002; LÓPEZ-TOLEDANO et al., 2006; DEL BARRIO-GALÁN et al., 2011B; SIECZKOWSKI col. 2016)), and improve tartaric (MOINE-LEDOUX AND DUBOURDIEU 2002; BOWYER AND MOINE-Ledoux 2007) and protein (DUPIN et al., 2000; GONZÁLEZ-RAMOS et al., 2008; LOMOLINO Y CURIONI 2007) stability in white wines. Moreover, studies indicate that they can alter the volatility of aromatic compounds in wine (COMUZZO et al., 2006; MAHADEVAN AND FARMER 2006; CHALIER et al., 2007; COMUZZO et al., 2011), remove undesirable compounds in wine, such as Ochratoxin A (PETRUZZI et al., 2015), and improve the effervescent qualities of sparkling wines (NÚÑEZ et al., 2006, MEDINA-TRUJILLO et al., 2017).

For all these reasons, the objective of this work was to evaluate how, in post-fermentation, the use of a specific inactivated dried yeast would affect the physical, chemical and sensory properties of a Carménère-variety Chilean red wine over the course of two consecutive grape harvests.

Material and methods

Wine production and experimental design

The pilot study used a Carménère-variety wine over the course of two consecutive grape harvests (2015 and 2016), which used grapes that were harvested in the same vineyard in both years. Alcoholic fermentation was conducted in 300 hl stainless steel tanks. The wine making process was carried out using the routine work conditions established by the cellar. The bunches of grapes were harvested, destemmed and crushed and then the grape pulp was inoculated with 20g/hl of Uva-germ HPS® *Saccharomyces cerevisiae*.

The classic oenological parameters of both harvests of the fermented wines can be found in Table 1.

Table 1. Classic oenological parameters of red wines after malolactic fermentation.

	2015	2016
Alcohol strength (% vol.)	13,7	14,10
pH	3,82	3,98
TA* (g/L)	3,40	3,10
VA** (g/L)	0,38	0,37

* TA: Total acidity expressed in g/L of sulphuric acid

** VA: Volatile acidity, expressed in g/L of acetic acid

Once malolactic fermentation had been completed, the level of free sulphur dioxide was adjusted to 30 ppm and the wine was distributed into food-grade plastic tanks of 25 litres in capacity. Different ageing processes were then used in duplicates (4 x 25 L tanks).

The following treatments were used in the red wines: control wines (wines with no yeast derivative added (C)); wines treated with a specific dry inactivated yeast (Optilees™), which was chosen on account of its high autolytic capability and capacity to release polysaccharides of different molecular weights during the wine ageing process. Treatment took place over

Reactives used

two months and was carried out at a constant temperature ($15 \pm 3^\circ\text{C}$). A medium-high dosage (30g/hL) of the yeast derivative was added, based on standard recommendations (a recommended dosage of 20–40g/hL). During the treatment period, a weekly 'bâttonage' took place in the first month and then a fortnightly 'bâttonage' took place in the second month. After the treatment period, the wines were filtered (without any clarifying treatment) and then bottled and stored at a constant temperature ($15 \pm 3^\circ\text{C}$) for three months.

Gallic acid, catechin, malvidin 3-glucoside, dextrans and pectins were supplied by Sigma-Aldrich (St. Louis, MO, USA). The $0.45\ \mu\text{m}$ and $0.22\ \mu\text{m}$ pore size polyethylene filters were supplied by EMD Millipore (Billerica, MA, USA). The ammonium formate was supplied by Merck (Darmstadt, Germany).

Methods of analysis

Traditional oenological parameters were analysed in accordance with the official methods set out by the International Organisation of Vine and Wine (OIV). The colour of the wines were assessed using the coordinates of the CIELab space, measured using the MSCV[®] (simplified method for measuring the colour of wines), developed by the colour group laboratory of the University of La Rioja (NAGUERUELA et al., 2001).

The analysis of total phenol content (expressed in mg/L of gallic acid), total anthocyanins (expressed in mg/L of malvidin 3-glucoside) and total tannins (expressed in g/L of catechin) were analysed in accordance with the methods set out in RIBÉREAU-GAYON et al., (2006).

Extraction, identification and quantification (in mg/L) of the polysaccharide fractions, in accordance to their molecular weight, were conducted following the method set out by AYESTARÁN et al., (2004). High-resolution size-exclusion chromatography and refractive index detector (HRSEC-RID) was used to achieve this.

Sensory analysis of the wines was conducted by a selected and trained panel, consisting of 14 people belonging to the Agroindustry and Oenology Department, Faculty of Agronomical Sciences, University of Chile. The study assessed astringency. The methods used to identify the astringency perception threshold, assessment training and defining the attribute of astringency were the same as those used in MEDEL-MARABOLÍ et al., (2017).

The wines were assessed in a random order in individual tasting booths, which had a controlled temperature of $18\text{--}19^\circ\text{C}$. Each glass was filled with 20 ml of wine, which had a three-digit code. The wines were analysed using FIZZ software (Biosystemes, France) – a visual tool that allows for different methods of sensory analysis, automation, collection and processing of data (LAWLESS AND HEYMANN 2010). The 'Time Intensity' method was used, which is a dynamic sensory analysis technique that allows for the change of a specific sensory attribute to be observed over a given time (PINEAU et al., 2009). The astringency intensity was evaluated using a 15 cm unstructured scale, where 0 indicated a perceived absence of astringency and 15 a perceived maximum astringency. The assessment period for each wine lasted 90 seconds.

This method could be very useful in order to study the temporal perception of a specific sensory quality in a wine. Generally speaking, according to literature on the subject, the 'Time Intensity' method can be used for a small number of attributes or on a limited range of products, and only one attribute can be tested at any time (PINEAU et al., 2009).

Statistical analysis

The statistic analysis was carried out using the statistic programme Info-Sat version 2012p (FCA National University of Córdoba, Argentina). The data was subjected to analysis of variation (ANOVA), followed by testing (LSD-Fisher) that identified significantly statistic differences between averages with a 95 % significance level ($p < 0.05$).

Results and discussion

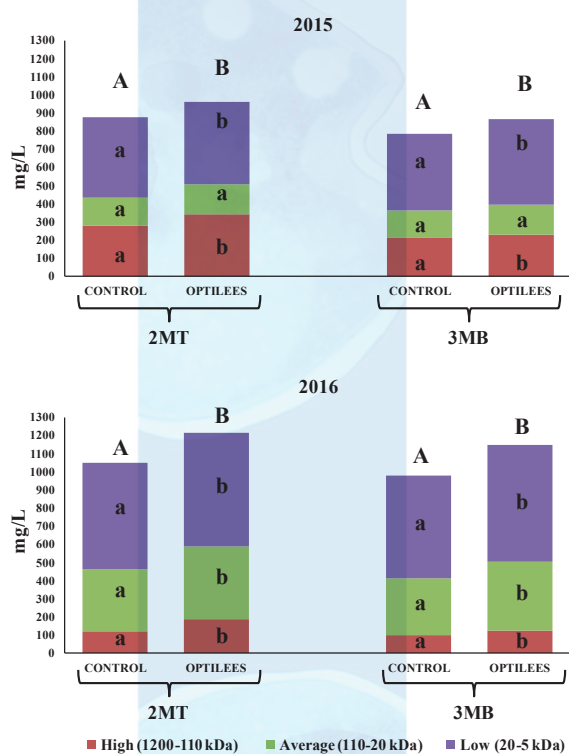


Figure 1. Concentration of total polysaccharides and the various identified and quantified fractions, ordered in terms of their molecular weight of the wines from the 2015 and 2016 grape harvests.

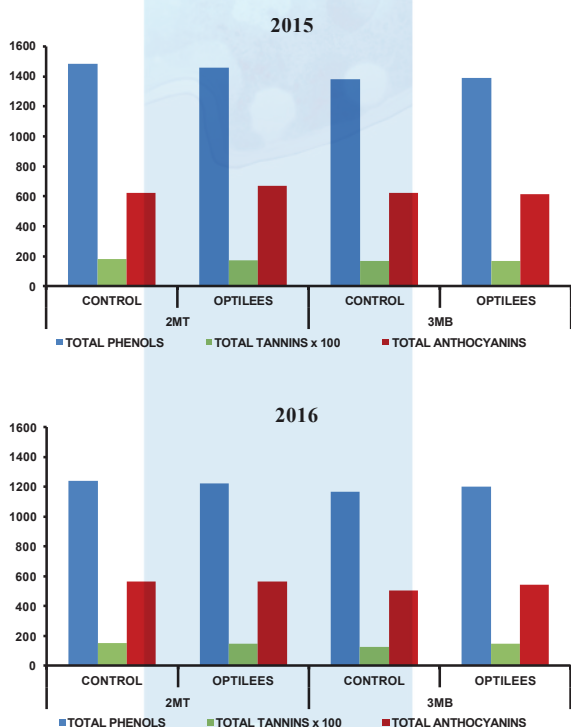


Figure 2. Concentration of total phenols (expressed in mg/L of gallic acid); total tannins (expressed in g/L of catechin x 100) and total anthocyanins (expressed in mg/L of malvidin 3-glucoside) of the wines analysed in the 2015 and 2016 grape harvests.

Figure 1 shows the concentration of total polysaccharides and the various identified and quantified fractions, ordered in terms of their molecular weight after the treatment period (2MT) and a three-month in-bottle ageing period (3MB) in the 2015 and 2016 grape harvests. Three different polysaccharide fractions were identified and quantified. They were then classified according to their molecular weights into high (between 1200 and 100 kDa), average (between 110 and 20 kDa) and low (between 20 and 5 kDa). It was observed that there were different amounts of high and average polysaccharide fractions in each grape harvest. In the 2015 grape harvest, there was a higher amount of fractions with a high molecular weight. In the 2016 grape harvest, there was, however, a higher amount of fractions with an average molecular weight. In all of the studied cases, polysaccharides with a low molecular weight were most prevalent in both all of the cases studied. It was generally observed that the polysaccharide content dropped in the period between the analysis point at the end of treatment and the analysis point after the three-month ageing in bottle period. It is likely that this drop was due to the wine filtering process. In the two studied grape harvests, the wines that were treated with Optilees™ had a higher concentration of total polysaccharides than the control wines after the two-month treatment period and the three-month in-bottle ageing period. The effect of increased polysaccharides in wines after having added yeast derivatives – once alcoholic fermentation had taken place – has already been noted in a number of studies carried out on other varieties of red wines (GUADALUPE et al., 2010; DEL BARRIO-GALÁN et al., 2012; GONZÁLEZ-ROYO et al., 2013; DEL BARRIO-GALÁN et al., 2015).

Figure 2 outlines the results obtained in the analysis of total polyphenols, anthocyanins and tannins in the two studied grape harvests. On the whole, it was observed that the content of the studied phenolic compounds dropped over the course of the study period. No significant statistical differences were observed, however, after two months of treatment and after the bottle-ageing period between the wines treated with Optilees™ and the control wines. Against this background, it can thus be concluded that treatment using this yeast derivative did not alter the phenolic composition of the red wine.

Similarly, use of Optilees™ did not produce any significant statistical differences compared to the control wines when using the CIELab parameters on colour (Figure 3).

Some studies using different yeast derivatives in red wines have shown that the former may alter phenolic content and colour. This is mainly due to the fact that polysaccharides (mannoproteins) are released from the yeast cellular walls. Results, however, often can differ from one study to another. This is likely down to the fact that the composition of the selected wines is different, which in turn, is reflected in the composition and features of the different products used. In some studies, it was observed that yeast-derivative treatments lowered the phenolic content in wines and the total colour content (GUADALUPE et al., 2008 and 2010; DEL BARRIO-GÁLÁN et al., 2012; GONZÁLEZ-ROYO et al., 2017). Other studies, however, showed a slight increase in phenolic composition and an improvement in colour stability (DEL BARRIO-GÁLÁN et al., 2012; AL-CALDA-EÓN et al., 2014).

Sensory analysis of the wine was carried out using the 'Time Intensity' methodology, which assessed astringency only. Figure 4 demonstrates the average values of time to appear, duration, end time for astringency perception (estimated in seconds), the average and maximum intensity of the astringency (measured using a 15 cm unstructured scale) in 2015 and 2016. These attributes meant the chart in Figure 5 could show the change in astringency over 90 seconds (the time granted to assess each wine). Significant statistical differences were observed only in wines treated with Optilees™ and the control wine in terms of the duration and final time it took to perceive astringency – although this effect was dependent on the analysis point for the studied grape harvests. It was observed in the wines studied in 2015 that astringency lasted for a shorter time in wines treated with Optilees™ during the 2MT and 3MB periods. This is mainly due to the fact that astringency perception lasted for a shorter time than in the control wines. Effectively, end time for astringency perception happened earlier in wines treated with Optilees™ than in the control wines. However, in the 2016 grape harvest similar results to those of 2015 were observed, although significant statistically different results were observed only after the 2MT period. Therefore meaning, descriptively speaking, astringency intensity was considered greater in the control wines than in those treated with Optilees™.

Although Optilees™ treatment did not cause any changes in the concentration of the studied phenolic compounds, use of it did significantly reduce astringency in red wines. This effect may be due to the interaction between the polysaccharides released by the yeast derivatives and the phenolic compounds in the wine, thus creating macromolecular structures that remain as solid colloids in wine – as previously outlined by some studies (RIOU et al., 2002; PONCET-LEGRAND et al., 2007; MEKOUE et al., 2016). Some other studies demonstrate that polysaccharides and mannoproteins may prevent tannin self-aggregation, and they thus become bonded to the tannins and form more stable aggregates that prevent their polymerisation and future precipitation. Indeed, this could help to maintain lubrication of the oral cavity and reduce the feelings of dryness and irritation that are features common to astringency.

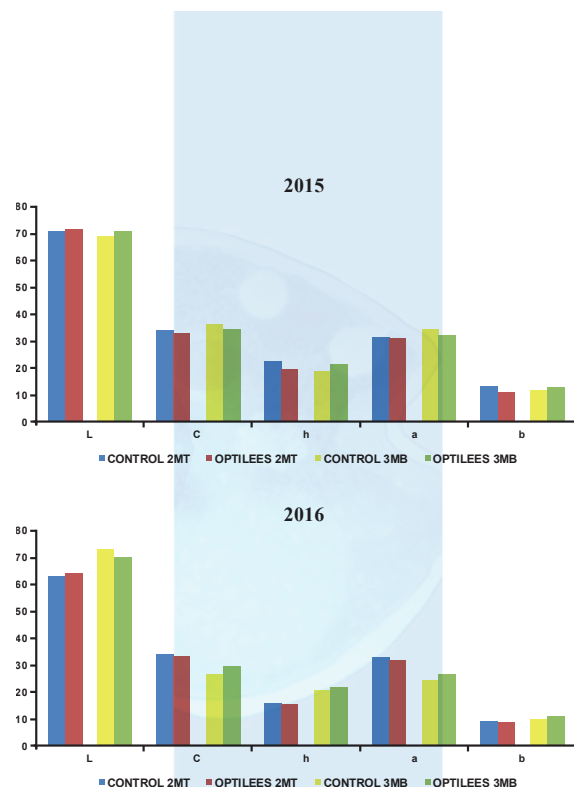


Figure 3. CIELab parameters studied in the wines analysed from the 2015 and 2016 grape harvests

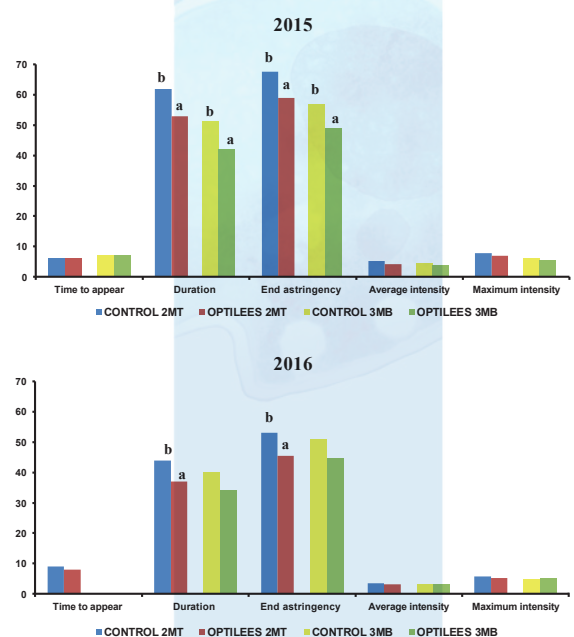


Figure 4. Astringency attributes studied in the wines analysed from the 2015 and 2016 grape harvests. Time to appear, duration and end astringency: measured in seconds (between 0 and 90). Average and maximum astringency intensity: measured using an unstructured scale of 0 to 15 cm (0 = absence of attribute and 15 = maximum intensity of attribute).

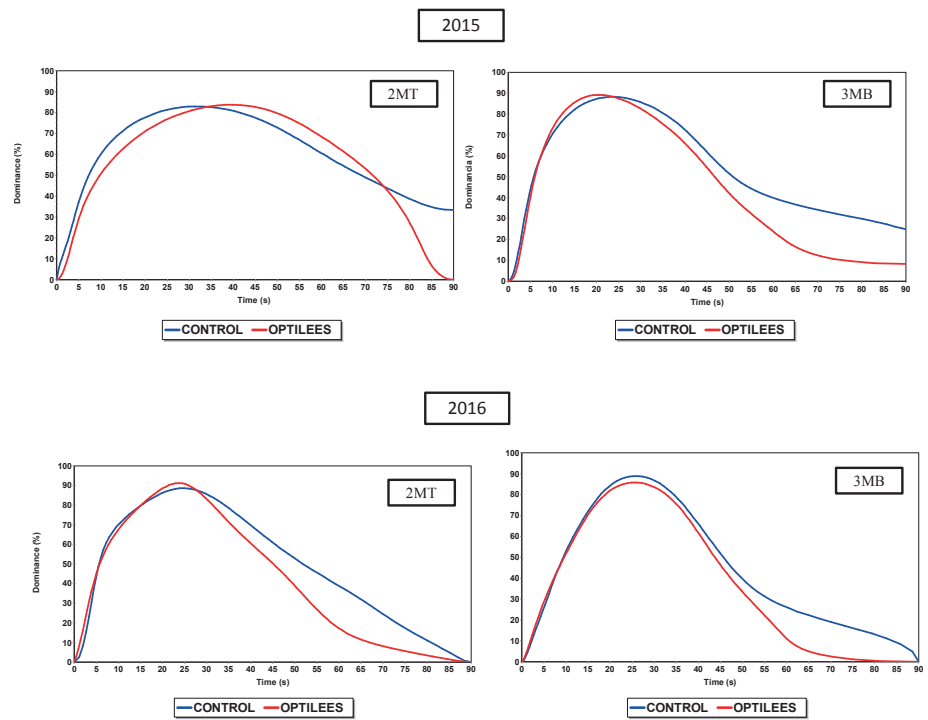


Figure 5. Profile of the change in astringency in the different wines studied from the 2015 and 2016 grape harvests

Conclusions

The addition of a specific inactivated yeast (Optilees™) allowed the concentration of total polysaccharides to increase and a number of fractions were identified and quantified in the red wines. The concentration of the different polysaccharide fractions – in both the control wines and those treated with Optilees™ – were contingent on the grape harvest that was studied. Low-weight polysaccharide fractions were most abundant in both harvests.

The use of the yeast derivative caused no changes in the content of the studied phenolic compounds, or in the colour parameters. Nevertheless, treating the red wine with this yeast derivative did lead to a change in the sensation of astringency – in particular, how long this attribute lasted for in the mouth. When the yeast derivative was added, the sensation of astringency disappeared earlier than in the control wine. The use of Optilees™ led to a reduced sensation of astringency in the mouth and improved the mouthfeel of the wine. This effect appears to be directly linked to the increased release of polysaccharides caused by Optilees™.

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