iVES OENO One vine & wine

DOI: https://doi.org/10.20870/oeno-one.2024.58.3.7955

OPEN ACCESS

ORIGINAL RESEARCH ARTICLE

Effect of different yeast-derived products on a red wine polyphenolic composition: influence of the wine age

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Associate editor:
Benoit Divol

Received:
22 January 2024

Accepted:
19 July 2024

Published:
29 August 2024

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ABSTRACT

The name “yeast-derived products” (YDPs) refers to different preparations obtained from yeast cells through various processes. These YDPs have different characteristics and applications in oenology, one being the improvement of the sensory properties of wines. This work aimed to identify the impact of YDPs obtained from different yeast strains and through different downstream inactivation processes on polyphenols in a red wine at different aging times. At an oenological dosage of 40 mg/L in a young wine, the addition of YDPs resulted in a variable decrease in total polyphenol index (TPI) (2 to 9 % depending on the YDPs) and a significant decrease in the precipitation of tannins (15 to 50 %) and high molecular weight (HMW) pigments (19 to 85 %) were obtained in the BSA assay, thus demonstrating the involvement of tannins and HMW pigments in interactions with the studied yeast-derived products. Pigment involvement led to a change in colour indices ($L*a*b*$ and colour intensity), but in most cases this change was not visible to the human eye at the oenological dosage tested (40 mg/L). Experiments conducted on both the young wine and the same wine aged for three months using the same yeast-derived products, dosages and aging conditions revealed the influence of the chemical evolution of polyphenols on their interactions with YDPs. The significant variation observed in this study highlights the critical role of yeast selection and the yeast fragmentation downstream process on YDP efficiency. The high-pressure homogenisation (HPH) yeast fractions were shown to be more efficient than the corresponding whole yeast cells. Increasing the contact surface area between yeast cell constituents and wine matrix enabled by the HPH process may be a key strategy for enhancing the effectiveness of yeast derivatives in red wines, and in aged wines in particular, in which the phenolic compounds underwent changes and exhibited less interaction with the studied yeast-derived products. This research has contributed to increasing our understanding of and the potential for apply these processing aids in oenology.

KEYWORDS: Yeast-derived products, aging, red wine, tannins, pigments

INTRODUCTION

Nowadays, one of the main targets of the wine sector is to propose wines that satisfy consumer demand for products of constantly high quality. Aging on lees is one of the traditional techniques for giving specific sensory characteristics to wines and improving their overall quality. It has been shown to have a potential impact on wine characteristics via different mechanisms:

- the autolysis of dead yeasts, which induces the release of yeast-derived components in wines; for example, polysaccharides (especially mannoproteins), oligosaccharides, free amino acids and peptides and fatty acids (Fornairon-bonnefond *et al.*, 2001);

- the adsorption of wine components in yeast cell walls (Vasserot *et al.*, 1997; Mazauric and Salmon, 2005; Merida *et al.*, 2005);

- the reduction of polyphenols oxidation in wines (Marquez *et al.*, 2009);

The positive effects attributed to this winemaking technique are the improvement of wine mouthfeel (Escot *et al*.,2001), aromatic profile (Bautista *et al*., 2007; Chalier *et al*., 2007; Ramírez *et al*., 2004), colour and colloidal stability (Dupin *et al*., 2000; Moine-ledoux and Dubourdieu, 2002).

However, the release of yeast compounds during aging on lees is slow and costly, and it increases the risks of microbiological spoilage. Hence, some alternatives are being studied to obtain the aforementioned positive effects without the latter disadvantages. In recent years, a large variety of commercial products derived from yeast has been developed and is proposed to winemakers for different applications. These yeast-derived products (YDPs) are obtained via different physical and/or biochemical processes and can be classified as inactivated yeasts, yeast autolysates, yeast cell walls, mannoproteins and yeast extracts (Pozo-Bayón *et al*., 2009). They can be used at different stages of the winemaking process, depending on the winemaker's objectives (Guadalupe and Ayestarán, 2008; Guadalupe *et al.*, 2010), and some are proposed to improve mouthfeel and/or as alternatives to wine aging on lees. However, it is not clear how exactly they act on wine composition and which are the active compounds/fractions. Indeed, YDPs are usually complex products, including both soluble and insoluble fractions, and are not always accurately characterised. They may act through either an intake of components that have a direct impact on the sensory properties of wine or interactions that occur between soluble/insoluble fractions and wine components of organoleptic interest. The evolution of the wine matrix may also influence interactions between YDPs and wine compounds, especially in the case of phenolics During fermentation, maceration and subsequent wine aging modifications to tannin structures occur, thus much wine chemistry research focuses on the identification of reactions that lead to structure modifications (Mouls and Fulcrand, 2012; Mouls *et al.*, 2011; Vernhet *et al*., 2014); for example, direct condensation reactions produce tannin-anthocyanin (T-A) adducts, resulting in pigmented polymers (Drinkine *et al*., 2007), while indirect condensation reactions involve the mediation of condensed tannin polymerisation via oxidation products in the wine matrix, such as acetaldehyde, resulting in tannins with modified interflavan bonds. Some resulting compounds, such as ethyl-linked polymers, are unstable and undergo further reactions (Haslam, 1980; Monagas *et al*., 2005). Stable pigments, such as pyranoanthocyanins, also formed from anthocyanins, including malvidin 3-glucoside (Haslam, 1980), can be incorporated into the tannin structure (Cheynier *et al*., 2006). In addition, continued gradual oxidation over time is thought to alter the structure of tannins (Cheynier *et al.*, 2006). All these chemical changes to polyphenols are likely to influence their interactions with yeast-soluble (proteins for instance) or yeast-insoluble (cell walls) fractions.

Within the framework of red wine aging, this work aimed to determine the impact of 8 YDPs obtained through different yeast strains and different downstream inactivation processes on red wine polyphenolic composition. Wine aging with YDPs was implemented in the same young and aged wine to assess how the impact of the studied YDPs varies with wine age.

MATERIALS AND METHODS

1. Winemaking process and treatments

The study was carried out using a blend of Petit Verdot grape variety (55 %) and Cabernet-Sauvignon grape variety (45 %). According to the Vitis International Variety Catalogue (VIVC), the variety number for the two cultivars used are VIVC 12974 and VIVC 1929 for Petit Verdot and Cabernet Sauvignon respectively. The grapes were harvested manually on the optimum harvest date and vinification was carried out at the INRAE Pech Rouge experimental winery (Gruissan, France) by applying the traditional red winemaking process. The initial must characteristics were: 217.2 g/L sugar, 12.91 % alcohol potential, 3.11 g/L H_2SO_4 total acidity and a pH of 3.3. The must was inoculated with a selected wine yeast under the form of active dry yeast "Anchor NT202" (200 mg/L, Oenobrands), and a specific autolysate "GO-Ferm Protect™ (300 mg/L, Lallemand) was added as a rehydration protectant. Alcoholic fermentation was performed at 25 °C. Free-run and press wines were blended, and the malolactic fermentation was done spontaneously. The wine was racked, sulfited (50 mg/L potassium disulfite) and clarified by cross-flow microfiltration on a Vaslin Buscher FM20 filter equipped with 0.2 µm organic membranes. The filtrated wine was split into two 300 L tanks for two aging processes with YDPs. One of the two tanks was used to carry out the first aging process with the young wine directly after the microfiltration step. The wine was split into different 30 L tanks in which the different YDPs were added. The experiments comprised the control wines without the addition of any products, and wines to which eight different yeast-derived products (YDPs) were added. Table 1 shows the characteristics of the different products studied.

TABLE 1. Characteristics of yeast-derived products (YDPs).

YDPs	Characteristics	Solubility (%)
IY1	Thermally inactivated whole yeast	20
IY2	Thermally inactivated whole yeast	20
DY_{HS}	HPH fully disrupted yeast cells	80
DY_{MS}	HPH moderately disrupted yeast cells	60
DY_{LS}	HPH low disrupted yeast cells	40
YCW	HPH yeast cell walls	10
YE	HPH yeast protein extract	90
MP	Mannoprotein preparation	100

Two inactivated yeasts (IY1 and IY2) were used in this work: they both underwent the same inactivation process (achieved by holding the yeast cream at 70 °C for 15 min), but were each derived from two different *Saccharomyces cerevisiae* yeast strains (1 and 2 respectively). The aim of using different strains was to study the impact of yeast strain on the potential interaction of inactivated yeasts with polyphenols. The biomass from yeast strain 2 also underwent an inactivation process through High-Pressure Homogenisation (HPH). HPH is a mechanical process that involves applying high pressure to yeast cream to break down the cells. The process is carried out at a low temperature (4 °C) to preserve macromolecules. The resulting homogenate consists of cell walls and constituents of the cytoplasm made soluble by the HPH process. This process can increase interactions between the yeast and the compounds in wine by significantly increasing the contact surfaces. The parameters and operation were refined to modulate the grinding intensity: intense grinding with extraction of almost all the cytoplasm (DY_{HS}), medium grinding for moderate cellular disruption (DY_{MS}), and light grinding for limited extraction of intracellular compounds (DY_{LS}). The aim was to identify the impact of yeast disruption intensity on the efficiency of the obtained fractions. Furthermore, part of the intensely disrupted cells (DY_{HS}) underwent centrifugal separation to separate the insoluble fraction (mainly yeast cell walls (YCW)) from the soluble yeast protein extract fraction (YE). The impact of a mannoprotein-enriched fraction (MP) was also studied. All fractions were provided by Lallemand (Montreal, Canada) and used in solid forms (powder), apart from the mannoprotein enriched preparation (in liquid form). The solubility of each YDP powder was measured in the laboratory to assess the impact of the applied process on yeast destructuration and the ability to release soluble compounds into the wine. To this end, they were dispersed at a final concentration of 15 g/L in a model ethanolic solution composed of 12 % ethanol (v/v) in water with 50 mM ionic strength adjusted with NaCl, the pH of which was adjusted to 3.5 with HCl. After stirring for 24 h the suspensions were centrifuged (15000 g, 15 min), and the supernatants and pellets were collected separately and freeze-dried. Soluble fractions were evaluated by weighing (Table 1).

The YDP doses added to the wine were 400 mg/L. The products were first dispersed in deionised water (100 g/L) before being added to the wine. During treatments, two *battonage*s (lees stirring) were performed monthly, and the temperature was maintained at 15 °C ± 3 °C. All treatments lasted 3 months, the treated and control wines were then racked, sulfited (addition of 50 mg/L SO_2), filtrated (cross-flow microfiltration, 0.2 µm), bottled and analysed. The second aging process was carried out 3 months later on the initial wine that had been left in the other 300 L tank to determine how the impact of YDPs on polyphenols varies with wine age. The wine was split into different 30 L tanks in which the different YDPs were added following the same protocol implemented for the first aging. All treatments lasted 3 months, after which the treated and control wines were racked, filtrated (cross-flow microfiltration, 0.2 µm), bottled and analysed.

2. Wine analysis

2.1 Enological analyses

Conventional oenological parameters were analysed at the INRAE experimental unit (Pech Rouge): alcoholic content, pH, volatile and total acidity, free and total sulfur dioxide, glucose, fructose, L-malic acid and L-lactic acid. Analyses were performed according to the Vine and Wine International Organization (OIV) methods.

2.2 Polyphenols analysis by UV-visible Spectrophotometry

UV-visible absorbance measurements were performed with a Safas UV-visible spectrophotometer. Total polyphenol index (TPI) and total red pigments index

(TRPI) were measured at 280 and 520 nm respectively (Vernhet *et al.*, 2020). Absorbance was measured 30 min after adequate dilution of samples in 1 M HCl, using 1 cm path length cells (TPI = A280 x dilution factor and TRPI = A520 x dilution factor). Colour intensity (CI) was determined by absorbance measurements using 1 mm path length cells, the values being converted to an optical path of 1 cm (CI = [A420 + A520 + A620]. Tristimulus values X, Y, and Z were determined from the visible absorbance spectra of samples using the Ayala *et al.* method (Ayala *et al.*, 1997). CIELAB colour parameters (lightness L^*, red/green colour component a^* and blue/yellow component b^*) were calculated from X, Y, and Z using the CIE standard illuminant D65 and the CIE 1964 standard observer (10 º visual field) as references. Total colour differences (ΔEab^*) between samples were calculated using the CIELAB formula:

$$\Delta Eab^* = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$$

For red wines, it is generally considered that the human eye cannot distinguish between two samples if ΔEab^* is less than 3 units (Martínez *et al.*, 2001).

2.3 BSA precipitation assay

BSA precipitable tannins and polymeric pigments were determined according to the procedure described by Boulet *et al.* (Boulet *et al.*, 2016). The procedure was adapted from the Adam-Harbertson tannin essay (Harbertson *et al.*, 2002). Briefly, 0,5 mL of wine was divided between two different tubes and diluted twice with a model wine composed of 5 g/L potassium hydrogen tartrate and 12 % ethanol; the pH was adjusted to 3.3 by adding 5 M HCl. One sample (test) was then mixed with 1 mL of a 1 g/L BSA solution in a sodium acetate buffer at a pH of 4.9 (200 mM acetic acid, 170 mM NaCl, pH adjusted to 4.9 by adding 5 M NaOH), and the other with the same buffer but without protein (blank). After 1 h incubation at room temperature, samples were centrifuged for 5 min at 13500 g to pellet tannin-protein precipitates and the supernatants recovered. Supernatants and controls were diluted tenfold in HCl 2 % and absorbance values at 280 and 520 nm were recorded after 30 min. Total precipitable tannins and pigments were calculated from the difference between blank and test samples. These differences (P280 and P520) were multiplied by the dilution factor (40) in the present work. Experiments were performed in triplicate.

3. Statistical analysis

Data are expressed as the average of three measurements. Analysis Of VAriance (ANOVA) was carried out using Minitab 17 software (Minitab® 17.3.1). Multiple factors ANOVA was performed to evaluate the effects of each YDPs on different variables. The difference among mean values was determined using Tukey's test and results were considered significant at $p < 0.05$.

RESULTS

Overall, no statistically significant differences were found between the control and samples in ethanol content, volatile and total acidity, pH, L-malic and L-lactic acids (Tables 2 and 3) in the young and aged wines. SO_2 and residual sugar seemed to be slightly modified in the presence of YDPs. As shown in Table 1, most of these YDPs had a higher or lower soluble fraction; these results show that their addition to wine does not lead to a change in the acid/base balance, nor does it increase the amount of residual sugar. The consumption of the free SO_2 decreased in the presence of certain YDPs, reflecting a possible protective effect of the latter on oxidation.

TABLE 2. Standard chemical parameters of wines after early aging with YDPs (addition of YDPs directly after fermentation and 3 months of contact).

	Alcohol (% vol)	Volatile acidity (g/L H_2SO_4)	pH	Total acidity (g/L H_2SO_4)	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Glucose + Fructose (g/L)	L-malic acid (g/L)	L-lactic acid (g/L)
Control	12.15 a	0.35 a	3.63 a	3.14 a	9.00 a	27.00 a	0.77 a	0.02 a	2.09 a
IY1	12.20 a	0.36 a	3.59 a	3.38 a	11.00 b	27.00 a	0.06 b	0.03 a	2.08 a
IY2	12.05 a	0.36 a	3.59 a	3.35 a	9.00 a	21.00 c	0.05 b	0.02 a	2.08 a
DY_{HS}	11.70 a	0.34 a	3.62 a	3.13 a	9.00 a	22.00 c	0.10 b	0.02 a	2.01 a
DY_{MS}	12.20 a	0.39 a	3.60 a	3.33 a	13.00 b	31.00 b	0.06 b	0.03 a	2.09 a
DY_{LS}	12.20 a	0.36 a	3.60 a	3.35 a	12.00 b	33.00 b	0.07 b	0.03 a	2.08 a
YCW	12.20 a	0.35 a	3.61 a	3.30 a	12.00 b	32.00 b	0.06 b	0.03 a	2.09 a
YE	12.15 a	0.35 a	3.60 a	3.36 a	9.00 a	26.00 a	0.10 b	0.03 a	2.02 a
MP	12.05 a	0.35 a	3.61 a	3.22 a	11.00 b	33.00 b	0.71 a	0.03 a	2.08 a

All data express the arithmetic average of three replicates with standard deviation. Different letters indicate statistically significant ($p < 0.05$) differences among quantitative variables in a same column according to the ANOVA and Tukey tests.

Polyphenol content was significantly impacted after early aging with YDPs (Table 4). These results are in line with several previous studies (Guadalupe and Ayestarán, 2008; Guadalupe *et al.*, 2010; Del Barrio-Galán *et al.*, 2012; Mekoue Nguela *et al.*, 2015; Mekoue Nguela *et al.*, 2016). The addition of the YDPs led to a 2 to 9 % decrease in TPI, depending on the YDP used (Table 4). A similar decrease in total red pigments (TRPI) was also observed. We grouped the YDPs according to their impact on TPI and TRPI:

- Group 1: very low decrease in TPI and TRPI (2 % and 7 % respectively): MP, IY1, DY_{MS}, and DY_{LS}.

- Group 2: low decrease in TPI and TRPI (4 % and 7 % respectively): YCW, YE.

- Group 3: moderate decrease in TPI and TRPI (6 % and 9 % respectively): IY2 & DY_{HS}.

- Group 4: high decrease in TPI and TRPI (10 % and 9 % respectively): DY_{HS}.

The decrease in TRPI was accompanied by a drop in colour intensity. Colour intensity was less impacted by DY_{HS} than expected considering the decrease in TRPI it caused; this indicates a different interaction mode with pigments to that of the corresponding whole inactivated yeast (IY2).

TABLE 3. Standard chemical parameters of wines after late aging with YDPs (addition of YDPs in the 3 months-old wine and after 3 months of contact).

	Alcohol (%)	Volatile acidity (g/L H_2SO_4)	pH	Total acidity (g/L H_2SO_4)	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Glucose + Fructose (g/L)	L-malic acid (g/L)	L-lactic acid (g/L)
Control	12.1 a	0.40 a	3.65 a	3.14 a	18.0 a	43.0 a	0.35 a	0.02 a	1.54 a
IY1	11.9 a	0.42 a	3.63 a	3.22 a	18.0 a	36.0 b	0.38 a	0.02 a	1.55 a
IY2	11.8 a	0.41 a	3.63 a	3.17 a	15.0 b	33.0 b	0.35 a	0.01 a	1.51 c
DY_{HS}	12.0 a	0.40 a	3.65 a	3.13 a	13.0 b	32.0 b	0.44 b	0.01 a	1.53 a
DY_{MS}	11.9 a	0.40 a	3.64 a	3.19 a	22.0 c	50.0 c	0.44 b	0.03 a	1.54 a
DY_{LS}	11.7 a	0.42 a	3.63 a	3.12 a	21.0 c	45.0 a	0.41 b	0.01 a	1.52 c
YCW	11.9 a	0.41 a	3.64 a	3.14 a	23.0 c	52.0 c	0.47 b	0.00 a	1.53 a
YE	12.1 a	0.40 a	3.64 a	3.21 a	20.0 c	46.0 a	0.45 b	0.01 a	1.53 a
MP	12.1 a	0.41 a	3.64 a	3.19 a	15.0 b	36.0 b	0.35 a	0.02 a	1.58 b

All data express the arithmetic average of three replicates with standard deviation. Different letters indicate statistically significant ($p < 0.05$) differences among quantitative variables in the same column according to the ANOVA and Tukey tests.

TABLE 4. UV-visible spectrophotometric analyses of wines after early aging with YDPs (addition of YDPs directly after fermentation and 3 months of contact). CI = Colour Intensity, TPI = Total Polyphenol Index, and $L^*a^*b^*$ = CIELab parameters. TRPI = Total Red Pigments Index, NBP = Non-Bleachable Pigments, and ΔEab^* = Total colour differences.

	CI	TPI	L^*	a^*	b^*	TRPI	NBP	ΔEab^*
Control	15.2 a	53.5 a	2.8 a	18.8 a	4.91 a	26.0 a	3.39 a	0.00 a
IY1	14.8 c	52.8 c	3.1 c	20.4 c	5.36 b	24.9 a	2.33 a	1.76 e
IY2	15.0 a	50.5 d	2.9 d	18.9 d	4.95 c	23.5 c	2.31 a	0.17 a
DY_{HS}	13.8 b	48.4 b	3.4 b	22.5 b	5.93 b	21.9 b	2.13 a	3.88 c
DY_{MS}	14.5 c	52.5 c	3.2 c	21.1 c	5.53 b	24.7 a	2.31 a	2.42 d
DY_{LS}	14.6 c	52.5 c	3.2 c	21.1 c	5.53 b	24.6 a	2.29 a	2.44 d
YCW	14.5 c	51.5 c	3.2 c	21.3 c	5.59 b	24.1 a	2.28 a	2.68 d
YE	14.6 c	51.3 c	3.2 c	20.8 c	5.46 b	24.1 a	2.27 a	2.17 d
MP	15.3 a	52.6 a	2.9 a	19.0 a	4.97 a	24.2 a	2.41 a	0.28 b

All data express the arithmetic average of three replicates with standard deviation. Different letters indicate statistically significant ($p < 0.05$) differences among quantitative variables in the same column according to the ANOVA and Tukey tests.

The two different strains had significantly different impacts. Furthermore, when comparing strain IY2 and fractions of the same strain from different processes, we also noted a difference in effect. These results showed the impact of strain and process on TPI modulation.

All these changes resulted in a marked evolution of the colour parameters *L*a*b**, which all changed during aging (Table 4). The increase in *L** was consistent with the loss in CI. At the same time, colour evolved towards a more orange shade, with an increase in yellow (*b**) and red (*a**). As observed with CI, the IY2 exhibited a low change in the *L*a*b** colour parameters, despite the high decrease in TPI observed. On the other hand, the DY_{HS} led to a considerable change in colour parameters following the CI and TRPI results (Table 4). The proportion of derived non-bleachable pigments decreased with all YDPs. The delta E*ab** results showed that for almost all YDPs the decrease in colour was not visible to the eye, except for DY_{HS} which had a delta E*ab** value greater than 3.

Compared to the control wine, the BSA test revealed lower precipitation of total tannins (P280; 15 to 50 % lower) and HMW pigments (P520; 19 to 85 % lower) with the addition of YDP (Figure 1A and Figure 1B). This confirms the involvement of HMW polyphenols in the interactions. The extent of precipitation differed depending on the YDP: tannin precipitation with the mannoprotein-rich fraction (MP) did not decrease as much as with the other YDPs (especially DY_{HS}).

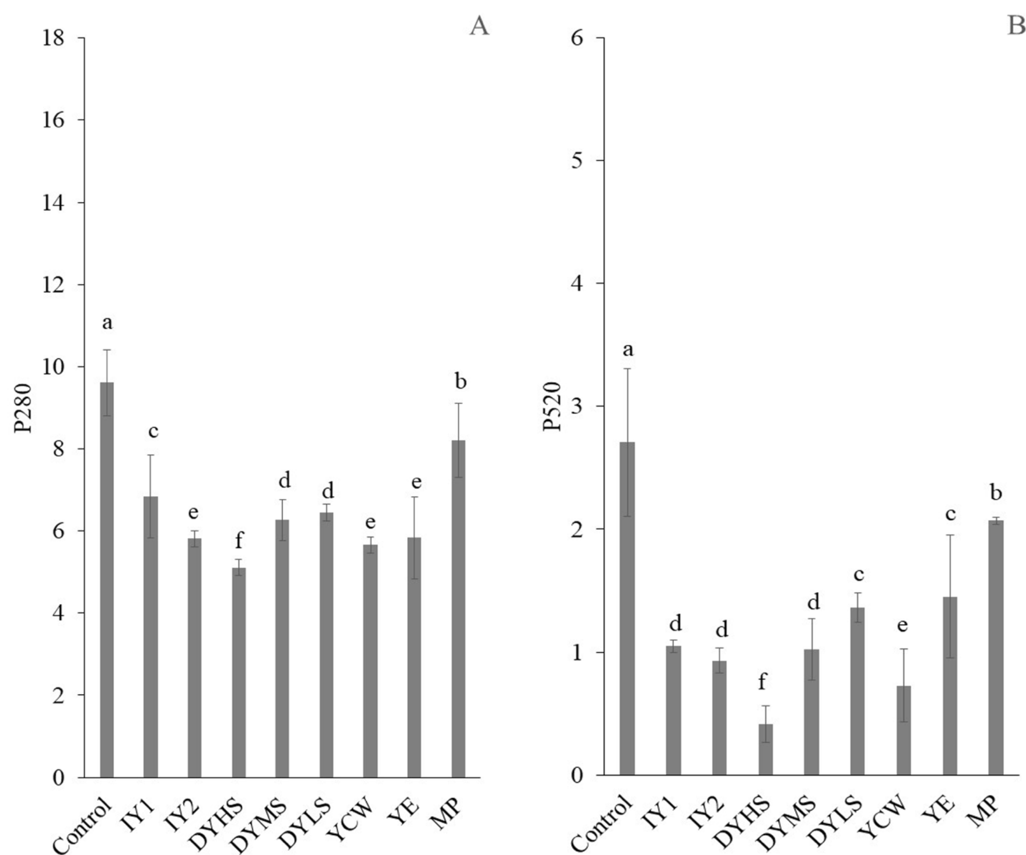

FIGURE 1. BSA precipitation assays after early aging with YDPs (addition of YDPs directly after fermentation and with 3 months of contact). A) Total precipitable tannins and pigments evaluated at 280 nm, and B) Total precipitable pigments evaluated at 520 nm. Different letters indicate statistically significant ($p < 0.05$) differences among samples according to the ANOVA and Tukey tests.

Overall, the HPH fully disrupted yeast (DY_{HS}) led to the highest decrease in polyphenol content: TPI, TRPI and HWM tannins and pigments. In early aging, the choice of yeast strain and the subsequent downstream process appeared to influence the interactions between yeast and polyphenols, with the downstream process having a more pronounced effect.

In the second part of this study, wine was aged for 3 months before adding YDPs to determine the impact of polyphenol chemical changes on their interactions with YDPs. UV-visible parameters measured in the aged wine after the addition of the YDPs are shown in Table 5. The total polyphenol index and colour intensity of the two control wines (early and late aging) were not significantly different, but there was a significant decrease in total red pigments and non-bleachable pigments, as well as a change in *L*a*b** colour parameters. These results highlight an evolution in wine composition over time.

The modification of wine colour could be a result of pigment chemical changes or pigment precipitation.

TABLE 5. UV-visible spectrophotometric analyses of wines after late aging with YDPs (addition of YDPs to the 3 month-old wine and after 3 months of contact). CI = Colour Intensity, TPI = Total Polyphenol Index, and *L*a*b** = CIELab parameters. TRPI = Total Red Pigments Index after ethanol correction, NBP = Non-Bleachable Pigments, and Δ E*ab** = Total colour differences.

	CI	TPI	*L**	*a**	*b**	TRPI	NBP	Δ E*ab**
Control	14.2 a	54.0 a	9.6 a	38.2 a	16.5 a	22.5 a	2.93 a	0.00 a
IY1	14.1 a	53.0 b	10.0 b	38.6 a	17.1 a	22.4 a	2.77 a	0.83 f
IY2	13.7 a	53.0 b	10.1 b	38.7 a	17.3 a	22.0 a	2.72 a	1.03 f
DY_{HS}	14.2 a	53.0 b	9.8 a	38.5 a	16.8 a	22.2 a	2.70 a	0.45 c
DY_{MS}	13.1 b	53.0 b	11.1 b	40.1 a	19.1 b	22.7 a	2.72 a	3.57 e
DY_{LS}	12.9 b	52.0 c	11.4 b	40.4 a	19.6 b	22.2 a	2.59 a	4.23 d
YCW	12.6 b	53.0 b	11.7 b	40.8 a	20.1 b	22.4 a	2.65 a	4.89 d
YE	13.2 b	52.0 c	11.1 b	40.1 a	19.0 b	22.6 a	2.69 a	3.48 e
MP	14.9 a	54.0 a	8.9 a	37.4 a	15.3 a	22.3 a	2.91 a	1.60 b

All data express the arithmetic average of three replicates with standard deviation. Different letters indicate statistically significant ($p < 0.05$) differences among quantitative variables in a same column, according to the ANOVA and Tukey tests.

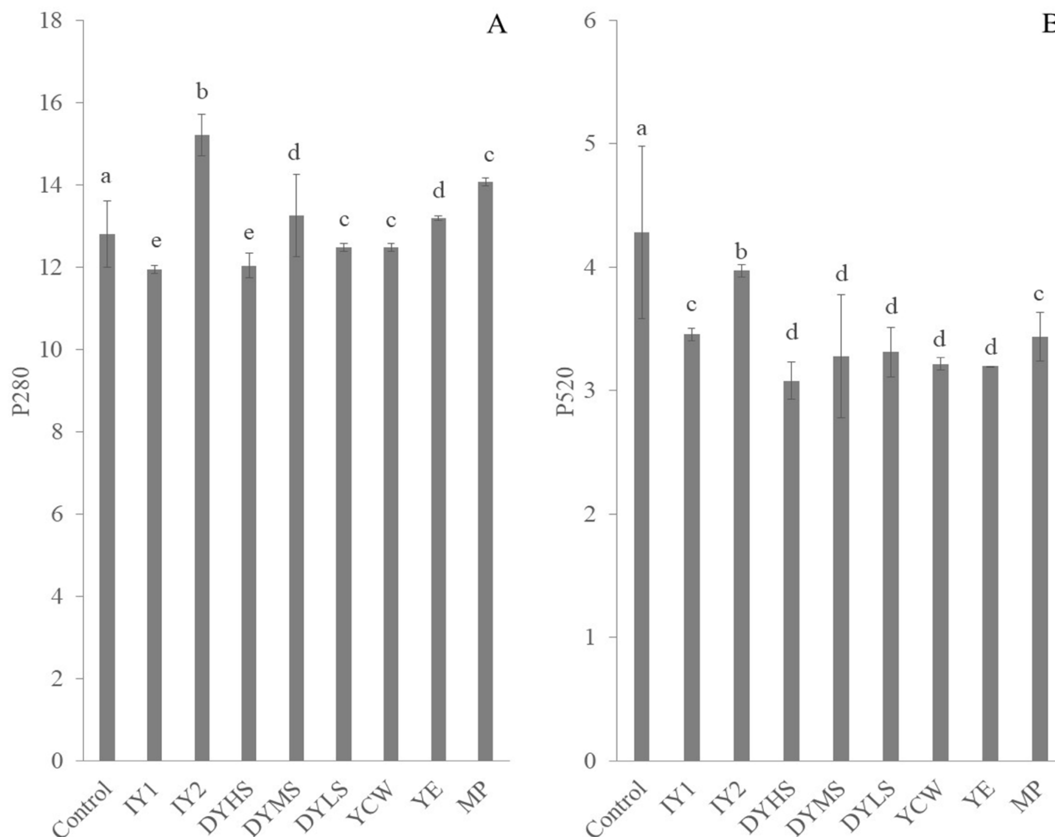

FIGURE 2. BSA precipitation essays after late aging with YDPs (addition of YDPs in the 3 month-old wine and after 3 months of contact). A) Total precipitable tannins and pigments evaluated at 280 nm, and B) Total precipitable pigments evaluated at 520 nm. Different letters indicate statistically significant ($p < 0.05$) differences among samples.

The addition of YDPs in aged wine impacted polyphenol composition to a lower extent than that observed in the young wine; only a slight decrease of TPI (2 to 4 %) was observed. We grouped the YDPs according to their impact on TPI:

- Group 1: No significant impact on TPI: MP.

- Group 2: Low decrease in TPI (2 %): DY_{HS}, YCW, DY_{MS}, IY1, IY2.

- Group 3: Moderate decrease in TPI (4 %): YE and DY_{LS}.

The most effective products in the young wine in terms of TPI reduction (DY_{HS} and IY2) became almost ineffective in the aged wine. TRPI and NBP were not significantly modified regardless of the YDP used (Table 5). These results showed fewer interactions between YDPs and wine polyphenols in the case of aged wine. The addition of YDPs did not lead to a significant decrease in colour in the young wine (except DY_{HS}) (Table 4), whereas a significant decrease in colour intensity and an increase in L^* a^* b^* parameters visible to naked eye (Delta Eab^* > 3) was observed with most of the HPH fractions in the aged wine (Table 5). This demonstrated once again a different mode of action of these products depending on the polyphenol chemical status of the wine.

Interactions between BSA and tannins were different with the addition of YDPs (Figure 2), with an overall decrease in tannin precipitation relative to the control. However, they had a much lower impact on aged wine than on young wine. The maximum decrease in precipitation of total tannins (P280) and HMW pigments (P520) was 8 % and 30 % respectively (Figure 2A and Figure 2B) with the DY_{HS}. We even observed an increase in tannin precipitation with some YDPs (MP and IY2). These results evidenced a decrease in interactions between tannins and YDPs with wine age.

As in the case of early aging, the impact of strain and the downstream process appeared to have a significant impact on the interactions of YDPs with polyphenols. There was a clear difference between IY1 and IY2, as well as between IY2 and the different fractions obtained after HPH treatment of this strain. These results could be explained by the fact that the physical treatments applied have broken up the yeast, thereby increasing the contact surface between the yeast constituents and the wine compounds.

DISCUSSION

The purpose of the present study was to evaluate the effects of eight different yeast-derived products (YDPs) produced using various yeast strains and a downstream inactivation process, on the polyphenolic composition of a red wine. Wine aging with YDPs was conducted in both young (directly after fermentation and filtration) and mature wines (3 months after fermentation) to explore how the influence of these YDPs changes depending on the age of the wine. In the young wine, the two YDPs that led to a greater decrease in polyphenols were either the whole inactivated yeast IY1 or the HPH-fully disrupted yeast mixture (DY_{HS}). This showed that, in these YDPs, the compounds that interact with polyphenols can be insoluble or soluble. Thus, the decrease may have several origins: i) adsorption by YDP insoluble fractions (whole cells, cell walls, insoluble cytoplasm components, such as proteins or organelles) (Mekoue Nguela *et al.*, 2015), and ii) aggregation and precipitation with soluble yeast macromolecules, such as proteins and peptides (Mekoue Nguela *et al.*, 2016). In both cases, the interactions involved pigments as was observed with the concomitant loss of TRPI. Our previous work has shown that polyphenols that are involved in interactions with yeasts (Whole cells, cell walls, or proteins) (Mekoue Nguela *et al.*, 2016; Mekoue Nguela *et al*., 2015; Vernhet *et al.*, 2020) are mostly in forms of high molecular weight (HMW): tannins and HMW derived pigments. The extent of precipitation differed depending on the YDP: the decrease in tannin precipitation with the mannoprotein-rich fraction was lower than with the other YDPs (especially DY_{HS}). This difference in precipitation does not necessarily reflect a low impact of the mannoprotein fraction on the perception of astringency, but rather different interaction mechanisms. This study evidenced that the majority of the products tested are involved in interactions that lead to a decrease in the concentration of HMW polyphenols in solution (absorption, precipitation), unlike mannoproteins which instead tended to stabilise the tannins in solution. This might reflect low interactions or complexation of tannins in solution with mannoproteins, as shown in our previous studies (Mekoue Nguela *et al*., 2016; Bicca *et al.*, 2022), in which we demonstrated via light scattering techniques the formation of stable complexes between yeast mannoproteins and grape skin or wine tannins. These different modes of interactions may lower the perception of astringency in red wines as a result of a decrease in either the concentration of tannins in solution (adsorption/precipitation) or the accessibility of active tannin sites (complexation with mannoproteins) concerning salivary proteins. These are in agreement with various previous studies that have shown the positive impact of YDPs (Del Barrio-Galán *et al.*,2014; Del Barrio-Galán *et al*.,2012; Del Barrio-Galán *et al*., 2011) and mannoproteins (Guadalupe *et al.*, 2010; Guadalupe *et al*.,2007; Ramos-Pineda *et al*., 2018) on the astringency of red wines.

In the aged wine, the results show an overall decrease in YDP impact on TPI, colour and tannin precipitation, thus indicating a decrease in interactions between polyphenols and YDPs with wine age. These observations are in agreement with McRae *et al*. (McRae *et al*., 2010), who studied the thermodynamics of the interactions between poly- (L-proline) and grape seed and skin tannins or Shiraz wine tannins (2 years old and 9-10 years old): the change in enthalpy associated with hydrophobic interaction and hydrogen bonding decreased with tannin age and the stoichiometry of binding indicated that grape tannins were associated with more proline residues than wine tannins, irrespective of molecular size.

In the two aging trials (early and late), the impact of yeast strain and the downstream process appeared to have a significant impact on the interactions of YDPs with polyphenols. There was a clear difference between IY1

and IY2 in the two trials, as well as between IY2 and the different fractions obtained through the HPH-process of this strain (DY_{HS}, DY_{MS}, DY_{LS}, YCW, YE). Overall, the HPH-process had an amplifying effect on the interactions of yeast cells with wine phenolic compounds in terms of decreasing their concentration in solution, particularly for tannins and high molecular weight pigments. The HPH-fully disrupted cells (DY_{HS}) were the most effective. The observed results might be due to the physical treatments fragmenting the yeast cells and enhancing the interactions between the yeast components and wine compounds by increasing the contact surface area. The disruption intensity has a significant impact on the interaction efficiency: the more fragmented the yeast, the higher the efficiency of the interactions with polyphenols, especially HMW tannins and pigments. Thus, yeast selection and the downstream HPH process could be effective strategies for optimising the impact of inactivated yeast on wine polyphenols, particularly in the context of wine aging, when polyphenol potential interactions decrease. The results obtained for the yeast protein extract fraction (YE), show that it could be used to fine young and aged wine.

CONCLUSION

This work has shown a significant impact of yeast derivatives on red wine polyphenols in the context of aging. The extent of their impact on total polyphenols and wine colour differed depending on the yeastderived product, and was certainly related to their composition (soluble/insoluble, proteins, polysaccharides, etc). The involvement of tannins and high molecular weight pigments was highlighted, in line with previous work. The trials carried out on the young wine and the same 3-month-old wine with the addition of the same yeast derivatives, at the same dosage and under the same aging conditions, allowed us to identify the impact of the chemical evolution of polyphenols on their interactions with YDPs. The wide variability of results obtained in this study highlighted the effectiveness of yeast strain selection and the HPH downstream process in optimising the impact of yeast derivatives in red wines, especially in old wines, in which phenolic compounds have evolved and show a lower potential for interaction. This work brings new advances to oenology, potentially helping optimise the use of these processing aids to improve wine quality. In future research, we intend to characterise in more detail the fractions in terms of macromolecules, such as proteins and polysaccharides, and to test them in several red wine matrices in order to consolidate the results of the present study.

ACKNOWLEDGMENTS

We are grateful to The INRAE Pech Rouge experimental unit (Gruissan, France) for their technical assistance in the setting up and monitoring of aging trials and wine analysis. We would like to thank the whole technical team, in particular Alain Samson, Garcia Emmanuel, and Marie-Agnes Ducasse. We thank Olivier Teuf for his help with the processing of the results.

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