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ORIGINAL RESEARCH ARTICLE

Impact of using yeast derivatives on the development of atypical aging in wines

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ABSTRACT

Yeast derivatives (YDs) are commercial products obtained from yeast colonies grown in bioreactors then inactivated and dried. They are employed in winemaking to supply nitrogen (N), enhance yeast growth and remove unwanted compounds. Since glucose is the primary carbon and energy source for yeast growth, agricultural byproducts are commonly used in their production.

Atypical aging (ATA) is a wine aroma defect arising from the oxidation of indole-3-acetic acid (IAA) to 2-aminoacetophenone (AAP). It entails the appearance of unpleasant odours as well as the rapid loss of varietal aromas. As IAA is one of the main plant hormones and YDs are often manufactured using material of plant origin, it can be assumed that AAP precursors are contained in those commercial formulations. If this is the case, the use of YDs could potentially increase the risk of obtaining ATA-tainted wines. To explore this hypothesis, 28 YDs were screened for the presence of AAP-related compounds, as well as their amino acidic content. Since several ATA precursors were detected, fermentation experiments were carried out in which different amounts of YD were added to different grape musts. Interestingly, depending on the nature of the commercial formulation, AAP development was observed to be either enhanced or diminished. More specifically, as well as concentration of AAP and precursors, the presence of cell walls within the YDs seemed to have an influence on the development of this defect. Furthermore, as diammonium phosphate (DAP) is also used to provide grape must with N, the effect of supplementing must with DAP was investigated. The addition of increasing doses of DAP did not significantly affect the accumulation of AAP. These results are very promising for both winemakers and YD producers, and they highlight the need for further research on the use of YDs in oenology.

KEYWORDS: Atypical aging, yeast derivative, wine, fermentation, autolysate

INTRODUCTION

Yeast derivatives (YDs) are obtained from colonies of *Saccharomyces* and/or non-*Saccharomyces* spp, which are grown on sugar-rich media and thermally inactivated before autolytic, plasmolytic and hydrolytic techniques and subsequent drying are applied in order to produce commercial formulations (Münch & Schieberle, 1998; Nagodawithana, 1992). YDs represent a heterogeneous family of products and, depending on how they are produced, are grouped into different classes: inactivated yeast (thermally or pH degraded and dried), yeast autolysate (from biomass autolysis), yeast cell wall (insoluble part without the cytoplasmic portion) and yeast protein extracts (soluble fraction, mainly cytoplasm) (Ángeles Pozo-Bayón *et al*., 2009; Rigou *et al*., 2021). While this classification reflects the variety of products that can be obtained from the bacterial biomass, each manufacturer has its own formulation, which can include one or more classes along with other compounds, such as vitamins and minerals. Furthermore, the compositional parameters of each product are highly influenced by the yeast strain used, the conditions in which the culture is grown and the manufacturing process itself (Guilloux-Benatier & Chassagne, 2003; Klis *et al*., 2006; Rigou *et al*., 2021).

YDs are generally employed to provide assimilable nitrogen, stimulate yeast growth and prevent stuck fermentations (Ángeles Pozo-Bayón *et al*., 2009). Moreover, they can provide protection against chromatic and sensorial faults (Andújar-Ortiz *et al*., 2014) and enhance colloidal stability due to their mannoproteins content (Morata *et al*., 2019; Pérez-Magariño *et al*., 2015). Additionally, their ability to bind unwanted molecules and their capacity for delivering volatile compounds to the wine have recently been demonstrated (Comuzzo *et al*., 2006). Industrially, YDs are produced in bioreactors, where yeast colonies are grown in large amounts and transformed into the final products. As glucose is the main carbon and energy source required for the growth of yeasts, the substrates utilised for biomass production typically include agricultural and food waste by-products. More specifically, starch, molasses, wood, fruits and vegetable waste are among the most common raw materials employed in their manufacture (Bekatorou *et al*., 2006; Jay, 1992).

Atypical aging (ATA) is a sensory defect that occurs in young white wines and is described as a loss of varietal aroma and the advent of unpleasant notes reminiscent of naphthalene, dirty cloth and acacia. Although other compounds might be involved in ATA formation, research has identified 2-aminoacetophenone (AAP) as the main chemical and sensory descriptor (Schneider, 2014). AAP arises from the oxidation of indole-3-acetic acid (IAA), a natural hormone present in plants. As IAA is responsible for regulating several biological mechanisms, its availability is controlled via methylation and/or binding with amino acids and sugar moieties. During vinification, the bound IAA contained in must is freed up by yeast, which uses the amino acids for its metabolism (Simat *et al*., 2004). Subsequently, free IAA is released into the wine and upon sulphoration a cascade of chemical reactions can lead to the formation of AAP (Hoenicke *et al*., 2002b). Additionally, yeast has recently been demonstrated to be capable of the *de novo* synthesis of IAA and AAP (Álvarez-Fernández *et al*., 2020).

Considering that YDs can be manufactured using byproducts of plant origins as raw material, it is plausible that they contain both free and bound IAA. Moreover, the production of YDs may involve thermal treatments; thus, the IAA present could be converted into AAP (Christoph *et al*., 1999). If this is the case, using YDs to enhance the quality of wine would also increase the likelihood of obtaining ATA-tainted products. Hence, the adoption of YDs in winemaking might represent a double-edged sword. To test this hypothesis, 28 commercially available YDs were specifically screened for the presence of AAP and some ATA precursors. The samples were also profiled according to amino acid (AA) content and compared for their yeast available nitrogen (YAN) contribution. In order to understand the effect of using YDs on ATA formation, fermentations were carried out using varying amounts of YDs and measuring the AAP content in the finished wines. Additionally, the effects of supplementing different grape musts with YD and the use of diammonium phosphate (DAP) prior to fermentation were also evaluated in relation to AAP development.

MATERIALS AND METHODS

1. Analysis of AAP and ATA precursors

The AAP and ATA precursors were quantified using a method developed by Nardin *et al*. (2022). In brief, an ultra-high performance liquid chromatographer (UHPLC; Thermo Ultimate R3000, Thermo Scientific; Waltham, MA, USA) coupled to a high-resolution mass spectrometer (HRMS; Q-Exactive; Thermo Scientific; Waltham, MA, USA) equipped with a heated electrospray ionisation chamber (HESI-II) was operated in positive ionisation mode. Details regarding the standards are given in Supplementary Table S1.

AAP and ATA precursors present in both the YDs and the wines at the end of the alcoholic fermentation (AF) were quantified. For each of the YDs, 1 g of dry powder was put in a falcon tube and dissolved by adding 50 ml of deionised water (Arium Pro Lab Water System; Sartorius AG, Germany). Then, after placing the tube in a mechanical shaker (Multi Reax; Heidolph, Germany) for 30 min, it was centrifuged (5000 rpm, 5 min, 4 °C) and the content was filtered directly into HPLC vials using 0.45 µm PTFE filters. For each YD, the extraction process was performed in triplicate. The samples of the wines were centrifuged and then filtered following the same procedure used for the YDs.

2. Amino acids quantification in YD samples

The method developed by Delaiti *et al*. (2024) was used to measure the AAs content of the YDs. Briefly, the method entailed the use of a UHPLC-HRMS system with an ESI-II interface. The column was a Raptor Polar X (Bellefonte, US) and the mobile phase consisted of water (Arium® 98

Pro Lab Water System; Sartorius AG, Goettingen, Germany) with 0.5 % formic acid (Honeywell; Charlotte, NC, USA) and acetonitrile (Honeywell; Charlotte, NC, USA). Samples were prepared following the same procedure reported above. Details regarding the standards used are given in Supplementary Table S2.

3. Fermentation experiments

3.1. Experimental set-up for the study of the effect of pre-fermentative YD addition on AAP production

Two yeast derivatives (YD1 and YD2) were chosen to carry out the vinifications. Each fermentation was carried out in triplicate: 90 L of non-varietal grape must (21.5° Brix, pH 3.3, 14.7 NTU) was divided into 18 tanks of 5 L. Following the inoculation with 200 mg/L of Blastosel FR 95 yeast (Perdomini-IOC; VR, Italy), DAP (200 mg/L, n = 3; DAl Cin, MB, Italy), YD2 (400 mg/L, n = 3), YD1 (200 mg/L, n = 3), YD1 (400 mg/L, n = 3) and YD1 (600 mg/L, n = 3) were added to separate tanks. Three tanks were left untreated (i.e., no YD was added) for the control.

TABLE 1. Commercial yeast derivatives (YDs) under examination.

Sample no.	Composition (as stated on label)	
1	ammonium phosphate, yeast hulls, thiamine, alpha cellulose	
2	yeast derivatives, cellulose	
3	inactivated yeast, thiamine	
4	yeast autolysate	
5	yeast autolysate	
6	yeast autolysate, thiamine	
7	inactivated yeast, yeast autolysate	
8	yeast derivatives	
9	inactivated yeast, thiamine	
10	yeast autolysate	YD1
11	inactivated yeast, mineral salts, vitamins	
12	inactivated yeast, autolysed yeast	
13	yeast autolysate	
14	yeast autolysate, inactivated yeast, chitosan	
15	inactivated yeast (80 %), yeast autolysate	
16	yeast autolysate, yeast hulls, thiamine	
17	inactivated yeast, DAP, thiamine	
18	inactivated yeast, mineral salts, vitamins	YD2
19	diammonium phosphate, inactivated yeast, yeast autolysate, thiamine	
20	yeast autolysate	
21	inactivated yeast, diammonium phosphate, thiamine	
22	yeast autolysate	
23	diammonium phosphate, cellulose, thiamine	
24	yeast autolysate	
25	yeast autolysate, inactivated yeast	
26	yeast hulls	
27	-	
28	-	

YD1 and YD2: yeast derivatives used in the fermentation experiments.

The alcoholic fermentation (AF) was carried out at a constant temperature of 18 – 19 °C and monitored by checking the sugar levels (° Brix readings) every three days. At the end of the AF, 70 mg/L of SO_2 were added to each tank and 50 ml of each sample were stored at - 20 °C until analysis.

3.2. Experimental set-up for the study of the impact of DAP supplementation and the matrix effect on AAP production.

Ten grape musts obtained from different grape varieties were selected and fermented in triplicate. 30 L of each matrix were divided into three batches, inoculated with 200 mg/L of Vin 13 (Oenobrands; Montferrier-sur-Lez, France), and supplemented with 100 (n = 10), 250 (n = 10) and 400 (n = 10) mg/L of DAP. Fermentation was carried out at a constant temperature of 18 – 19 °C and monitored by checking the sugar levels (° Brix readings). At the end of the AF, 70 mg/L of SO_2 were added to each batch and 50 ml of each sample were stored at - 20 °C until analysis.

To study the matrix effect, another experiment was set up. Ten grape musts from different grape varieties were selected and fermented in triplicate. 30 L of each matrix were inoculated with 200 mg/L of Vin 13 (Oenobrands; Montferrier-sur-Lez, France), divided into three batches and then supplemented with 400 mg/L of YD1. Fermentation was carried out at a constant temperature of 18 – 19 °C and monitored by checking the sugar levels (° Brix readings). At the end of the AF, 70 mg/L of SO_2 were added to each batch and 50 mL of each sample were stored at - 20 °C until analysis.

4. Data processing and statistical analysis

Data were processed using XLSTAT 2021.5 (Addinsoft, NY, USA), Microsoft Excel 2013 (Redmond, Washington, USA) and R (R Foundation for Statistical Computing, Vienna, Austria) version 4.0.3 in RStudio (Rstudio 2021.09.0, Inc., Boston, MA).

5. Optical densities measurements

To assess the differences in the cell wall content of the YDs, optical density measurements were carried out. Eight solutions containing increasing doses (200, 400, 600 and 800 mg/L) of two distinct YDs (YD1 and YD2) were prepared by accurately weighing the dry powders and diluting them in distilled water. Their optical densities were measured using a spectrophotometer (UV1700; Yoke Instrument, Shanghai, China) set at 660 nm.

RESULTS AND DISCUSSION

1. YD screening for AAP and ATA precursors

28 YDs (Table 1) were screened for the presence of AAP and some ATA precursors. As shown in Figure 1 and Supplementary Table S3, considerable amounts of those compounds were found in most of the samples under examination (Figures 1A and 1C). Furthermore, great compositional variability was observed (Figures 1B and 1C). TRP and TRH were the most abundant compounds detected in the samples. With a mean value of 13356 mg/kg, TRP concentrations ranged from 1.74 to 41491 mg/kg and in 13 of the 28 samples they were lower than 7000 mg/kg. Regarding TRH, more than half (17 out of 28) of the YD samples contained less than 7 mg/kg, and just one sample contained a conspicuously high concentration (462 mg/kg, sample 15). The IAN values ranged between 0 and 56.26 mg/kg; however, most (20 out of 28) of the YD samples contained less than 10 mg/kg. Meanwhile, regarding ILA, the vast majority of the samples (23 out of 28) showed variable amounts of the compound, but they were all below 2 mg/kg. While TAM concentrations ranged between 0 and 18.89 mg/kg, they were lower than 1 mg/kg in 21 of the 28 YD samples. Only trace amounts of IAM were detected (< 0.37 mg/kg). In contrast to the bound and methylated forms of IAA, which were found in minute concentrations, unbound IAA was detected in 23 samples, with concentrations ranging from 0.07 to 4.48 mg/kg and a mean value of 1.89 mg/kg. Finally, low amounts of AAP (0.003 - 0.05 mg/kg) were also found in all but two of the YD samples; the presence of those compounds can be ascribed to normal yeast metabolism (Álvarez-Fernández *et al*., 2020; Simat *et al*., 2004). Furthermore, AAP might result from the YD production process, which can entail a thermal treatment (e.g., in the case of inactivated yeast) leading to the degradation of IAA to AAP (Münch *et al*., 1997).

2. Amino acid profiling

The 28 YDs (Table 1) were screened for their amino acid (AAs) and ammonium (NH_4) content (Supplementary Table S4). As shown in Figure 2, high variability among the samples was found: this was as expected, as the processing techniques and commercial formulation vary for each company and product.

With a mean value of 26828 mg/kg, NH_4 concentrations ranged from 66 to 178863 mg/kg. However, while only trace amounts were observed in most of the YDs, NH_4 was the most abundant compound in samples 1, 11, 17, 18, 19, 21 and 23, constituting around 65 % of the dry weight (DW).

As regards AAs, on average, the YDs analysed mostly contained glutamic acid (glu), leucine (leu), valine (val) and alanine (ala), with concentrations representing about 10 % of the DW, followed by isoleucine (ile), ornithine (orn) and phenylalanine (phe), constituting 5 % of the DW. The other amino acids were present in varying but small quantities.

The nitrogen (N) fraction available for use by the yeast (known as the yeast available nitrogen (YAN)) was calculated from the results of the AA screening (Verdenal *et al*., 2021). The computation was carried out multiplying the ratio of the concentration of each primary amine and ammonium ion over their corresponding atomic mass unit by the atomic weight of N. The trendline in Figure 2B reports the concentrations of YAN calculated for each YD.

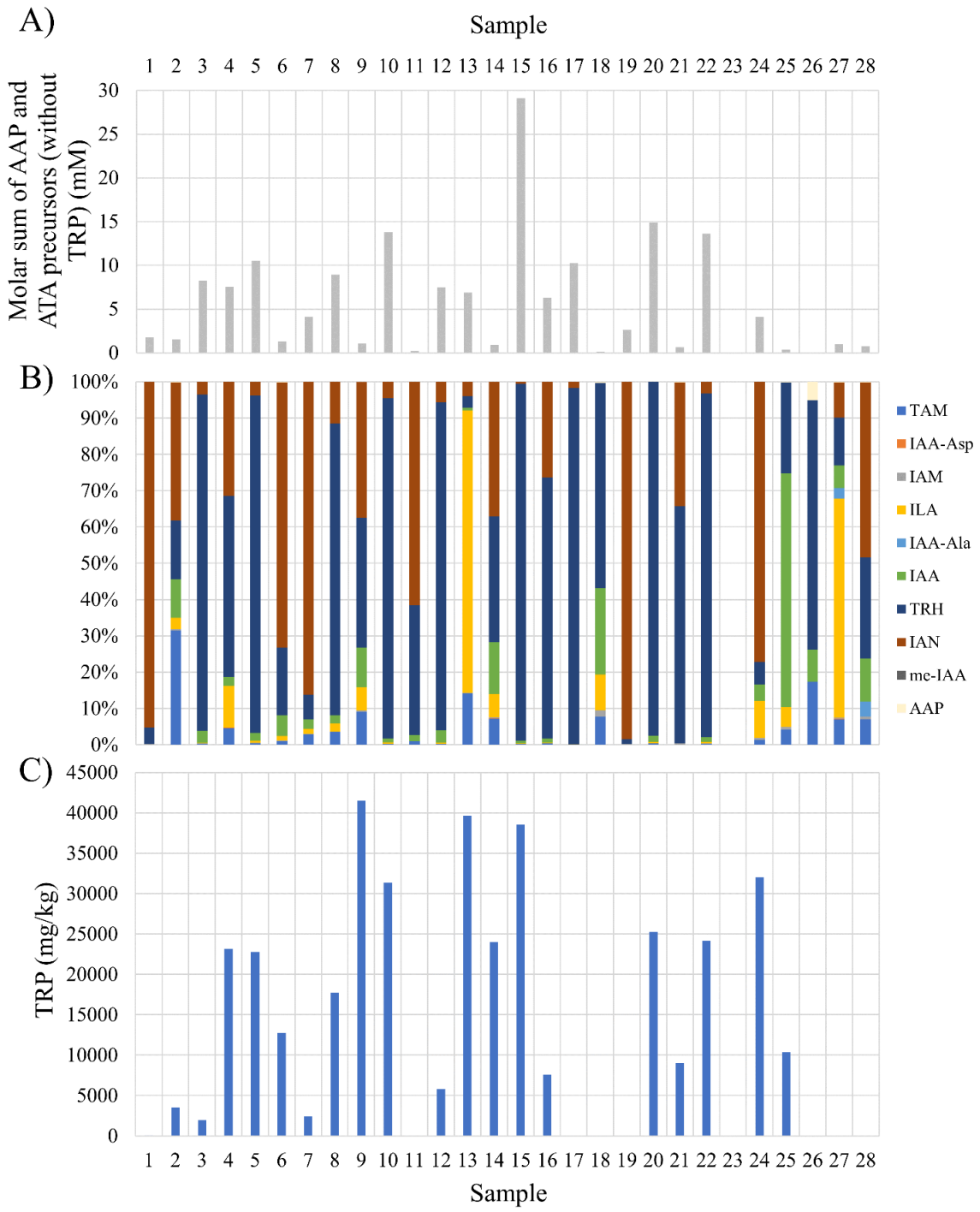

FIGURE 1. A) Molar sum of the 2-aminoacetophenone (AAP) and atypical aging (ATA) precursors (mM), B) compositional variability of AAP and ATA precursors, and C) tryptophan (TRP) amounts (mg/kg) contained in the yeast derivatives (YDs) under examination.

IAA = indole-3-acetic acid; IAA-Ala = Indole-3-acetyl-L-alanine; IAA-Asp = indole-3-acetyl-aspartate; me-IAA = 1-Methyl-3-indoleacetic acid; ILA = indole-3-lactic acid; IAM = Indole-3-acetamide; IAN = Indole-3-acetonitrile; TRH = tryptophol; TAM = tryptamine; TRP = tryptophan; AAP = 2-aminoacetophenone.

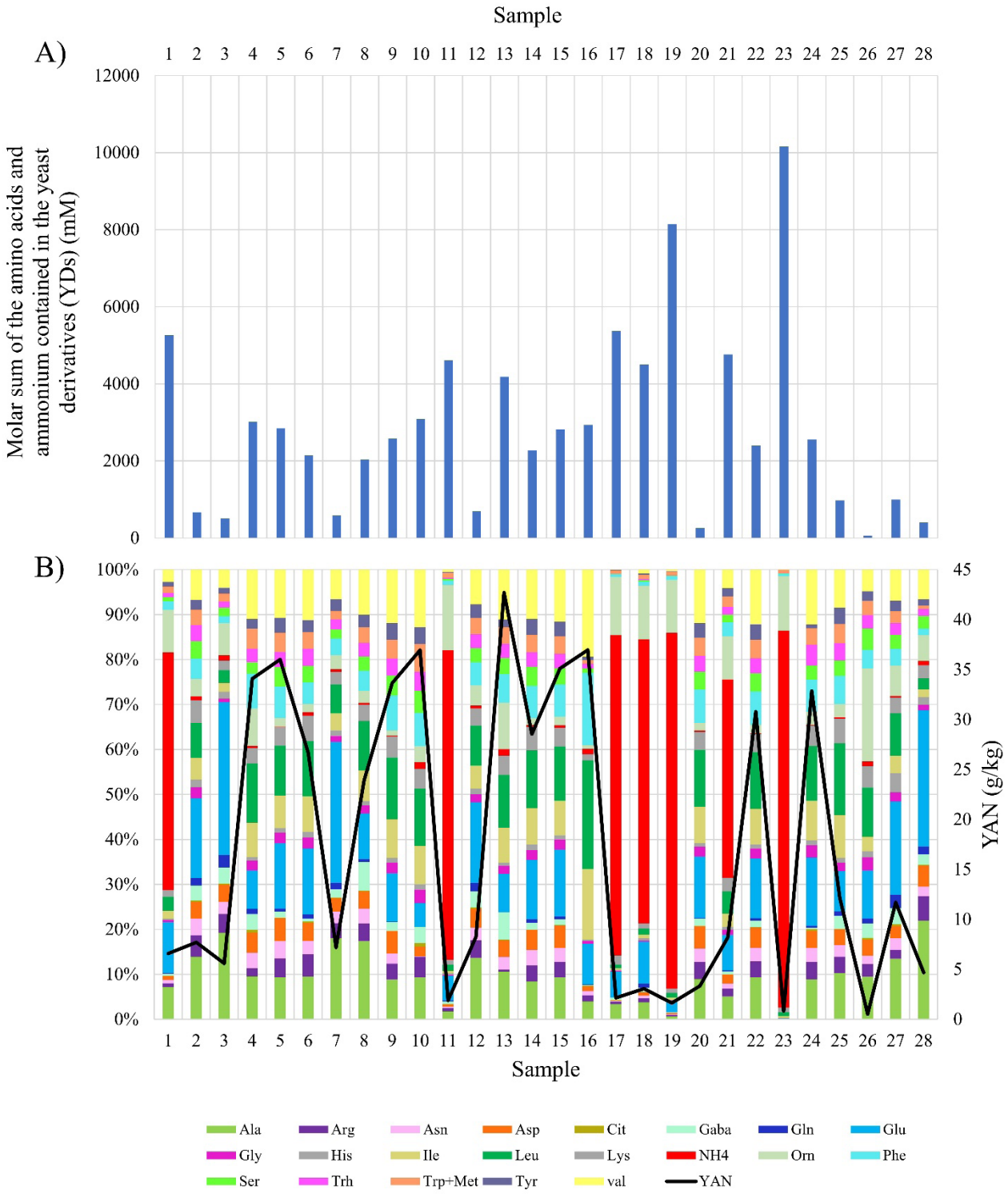

FIGURE 2. A) Molar sum, and B) compositional variability of the amino acids (AAs), ammonium (NH_4) and yeast available nitrogen (YAN) contained in the yeast derivatives (YDs) under examination.

Ala = alanine; Arg = arginine; Asn = asparagine; Asp = aspartic acid; Cit = citrulline; Gaba = aminobutyric acid; Gln = glutamine; Glu = glutamic acid; Gly = glycine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; NH_4 = ammonium ion; Orn = ornithine; Phe = phenylalanine; Ser = serine; Thr = tryptophol; Trp = tryptophan; Met = methionine; Tyr = tyrosine; Val = valine; YAN = yeast available nitrogen.

3. Effects of using YDs on fermentation kinetics

To determine the effects of using a YD on the development of AAP, a fermentation trial was set up. Of the 28 previously analysed samples (Table 1), two were chosen that met the criteria of the presence of large and small quantities of AAP and ATA precursors: sample 10 (YD1) and sample 18 (YD2). While the former contained relatively high concentrations of IAA (2.36 µg/kg), TRP (31326 µg/kg), TRH (209 µg/kg) and AAP (34.19 µg/kg), the latter contained lower concentrations (0.52 µg/kg of IAA, 46.12 µg/kg of TRP, 1.25 µg/kg of TRH and 6.15 µg/kg of AAP).

Upon inoculation with yeast and YD supplementation, the fermentation kinetics were monitored. They were found to vary depending on the treatment (Figure 3). Fermentation was slower in the control sample (with no YD present) than in the other treatments. This was expected, as supplementing grape must with YAN is known to improve fermentation performance (Seguinot *et al*., 2020), and the grape must used for the experiment was N deficient (YAN: 94 mg/L) (Verdenal *et al*., 2021). Furthermore, this confirms the results of Simat *et al*. (2004), which showed that nutritional supplements added to must accelerate fermentation.

Although the use of DAP slightly improved fermentation rate, it was not observed to be as effective as using YDs, both YD1 and YD2 being found to dramatically speed up fermentation. Aside from the yeast cell content and cell walls, YDs may also contain other compounds (such as vitamins and minerals) which can affect yeast metabolism and thus the metabolites formed during fermentation. In contrast to YD2, fermentation with YD1 at different concentrations led to a higher pace in the vinification process. Additionally, the fermentations rates increased with increasing amounts of YD1.

When the same dose (400 mg/L) of two different YDs was used, the kinetic rates were significantly different: YD1 addition resulted in a much faster fermentation than YD2 addition. Besides the distinct AA composition and NH_4 content, it was noted that the YAN supplemented with YD1 was higher (36.9 g/kg) than that provided by YD2 (3.06 g/kg). However, the N contribution of the two YDs was lower than that of DAP. This highlights that despite AAs, NH_4 and YAN play an important role in fermentation (Gobert *et al*., 2019; Torrea *et al*., 2011), they are not the only responsible for the nutritional capacity of yeast and other factors might be involved. In this regard, supplementation with fatty acids (Pinu *et al*., 2019; Redón *et al*., 2009), as well as the bioavailability of certain metal ions (Birch *et al*., 2003) - both potentially influenced by YD addition (Rigou *et al*., 2021) - have been shown to have an impact on fermentation performance.

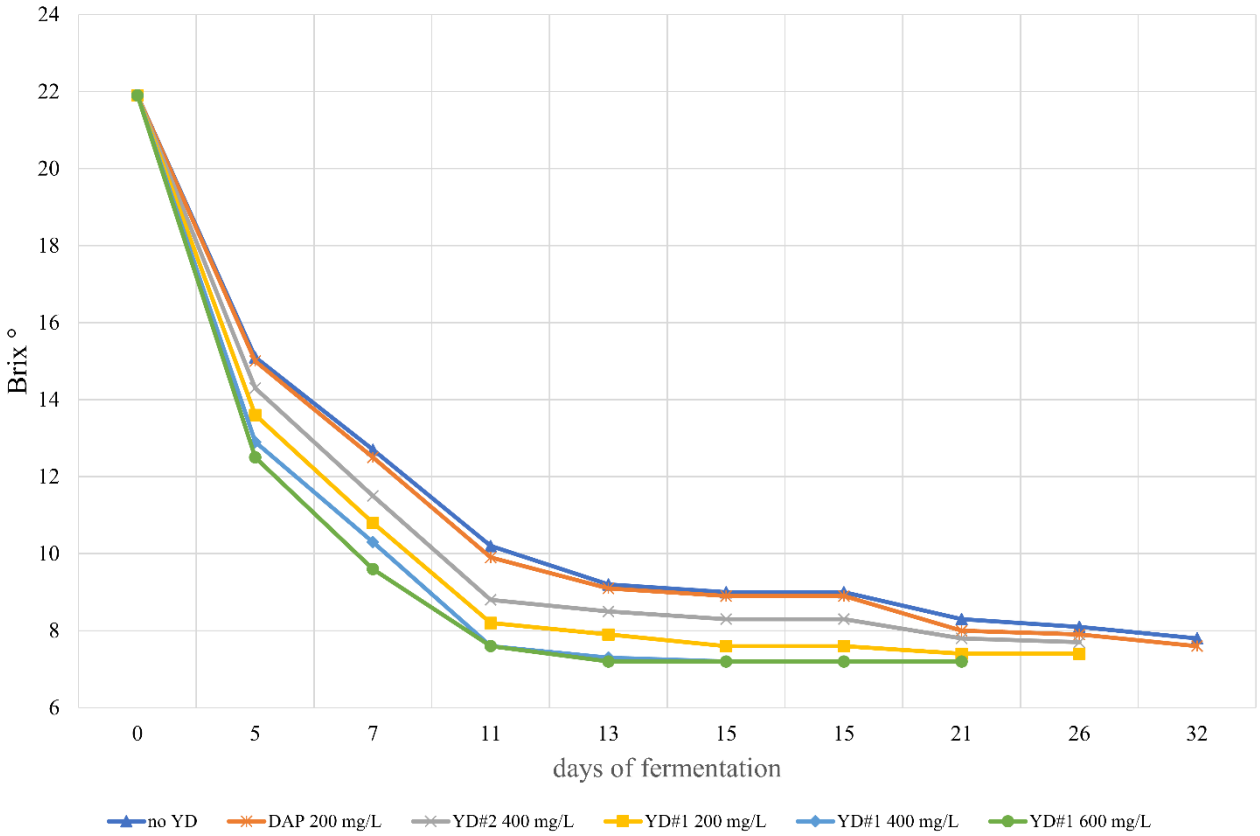

FIGURE 3. Fermentation kinetics of a non-varietal grape must inoculated with different concentrations of two selected yeast derivatives (YDs) and diammonium phosphate (DAP).

4. Effects of the use of YDs on AAP formation

Figure 4 is a graphical representation of the mean and standard deviations of the AAP amounts detected in the wines at the end of the AF. Adding the same dose (400 mg/L) of two different YDs before fermentation led to the formation of inconsistent amounts of AAP. Indeed, the addition of YD2 was observed to produce significantly lower concentrations of this compound compared to YD1 (0.103 ± 0.009 vs. 0.173 ± 0.018 µg/L). While this agrees with the higher amounts of AAP and ATA precursors delivered with YD1, it also highlights the significance of the YD composition on the development of this sensorial defect.

Moreover, the formed AAP was found to increase linearly with increasing doses of YD1, and a positive correlation ($R^2 = 0.98$) was observed. This underlines how the addition of increasing amounts of a YD rich in AAP and ATA precursors is closely correlated with AAP development during AF. Compared to not using YDs, must supplemented with 400 mg/L of YD2 was not found to have any consequences in terms of AAP accumulation (0.103 ± 0.009 vs. 0.111 ± 0.002 µg/L). It was assumed that the AAP and ATA precursors contained in this preparation were not sufficient to produce a consistent effect on the buildup of AAP in the finished wines. The quantity of AAP in the wine made from must supplemented with DAP (200 mg/L) did not differ from that obtained using YD1 at the same concentration (0.131 ± 0.002 and 0.132 ± 0.015 µg/L respectively). Additionally, a double dose of YD2 (400 mg/L) led to an accumulation of significantly lower amounts of AAP compared to the use of DAP (0.103 ± 0.009 vs. 0.131 ± 0.002 µg/L respectively). Finally, Figure 4 also shows the YAN supplemented with each treatment. Interestingly, AAP formation was not found to be dependent on the N status of the grape must prior to fermentation.

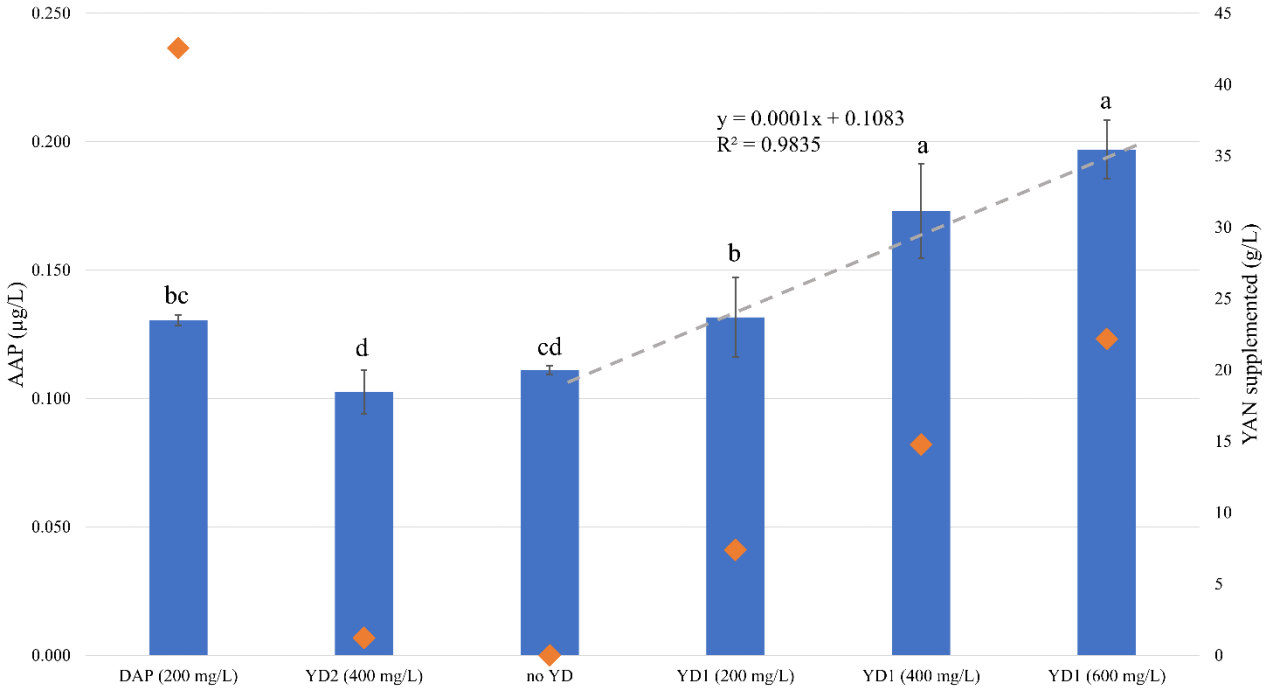

FIGURE 4. Statistical distribution (ANOVA test) of the 2-aminoacetophenone (AAP) content and yeast available nitrogen (YAN, orange squares) added to grape musts fermented with and without the addition of diammonium phosphate (DAP) and different concentrations of two yeast derivatives (YDs).

The trendline represents the correlation between the increasing amount of YD1 and the AAP detected in the finished wines.

5. Effects of DAP supplementation on AAP development

Together with YDs, the addition of diammonium phosphate (DAP) represents one of the most common tools in winemaking when N deficiency poses a threat to fermentation (Bell & Henschke, 2005). To date, research on the impact of the pre-fermentative addition of DAP on ATA development has only been touched upon, and no clear effect has yet been demonstrated. While some authors (Bach, 2005; Schwab *et al.*, 1996) have not found any effects, Rauhut *et al.* (2003) established that DAP supplementation can actually diminish the formation of AAP. Schneider *et al.* (2014) hypothesised that this is due to the quenching of oxygen radicals through the action of metabolites generated during the fermentation process; if this is the case, the extent of the effect would be dependent on the dose of DAP added. These results are not conclusive and they emphasise the importance of further investigating the impact of DAP on ATA development. In the first fermentation trial of this study, compared to the control, the use of DAP slightly increased the production of AAP (0.131 ± 0.002 vs. 0.111 ± 0.002 µg/L; Figure 4). However, the increase was very limited (non-significant ANOVA test).

Given the uncertainty associated with the use of DAP, another fermentation experiment was set up. As can be seen in Figure 5 (and Supplementary Table S5), increasing doses of DAP (100, 250 and 400 mg/L) added to 10 different grape juices prior to fermentation did not produce any effect on the accumulation of AAP in the finished wines.

6. Comparison of added versus detected AAP concentrations

As reported above, AAP was detected in most of the analysed YDs (Figure 1, Supplementary Table S3). This indicates that, even though the amounts are limited, supplementing the grape must with such products poses a considerable risk of producing ATA-tainted wines. Additionally, AAP can originate from the oxidation reaction of IAA (Hoenicke *et al*., 2002a), and yeast is capable of a *de novo* formation of both compounds (Álvarez-Fernández *et al*., 2020; Liu *et al*., 2016).

To investigate the AAP generated solely during fermentation, aside from the AAP contained within the YD itself, the theoretical and actual concentrations were considered.

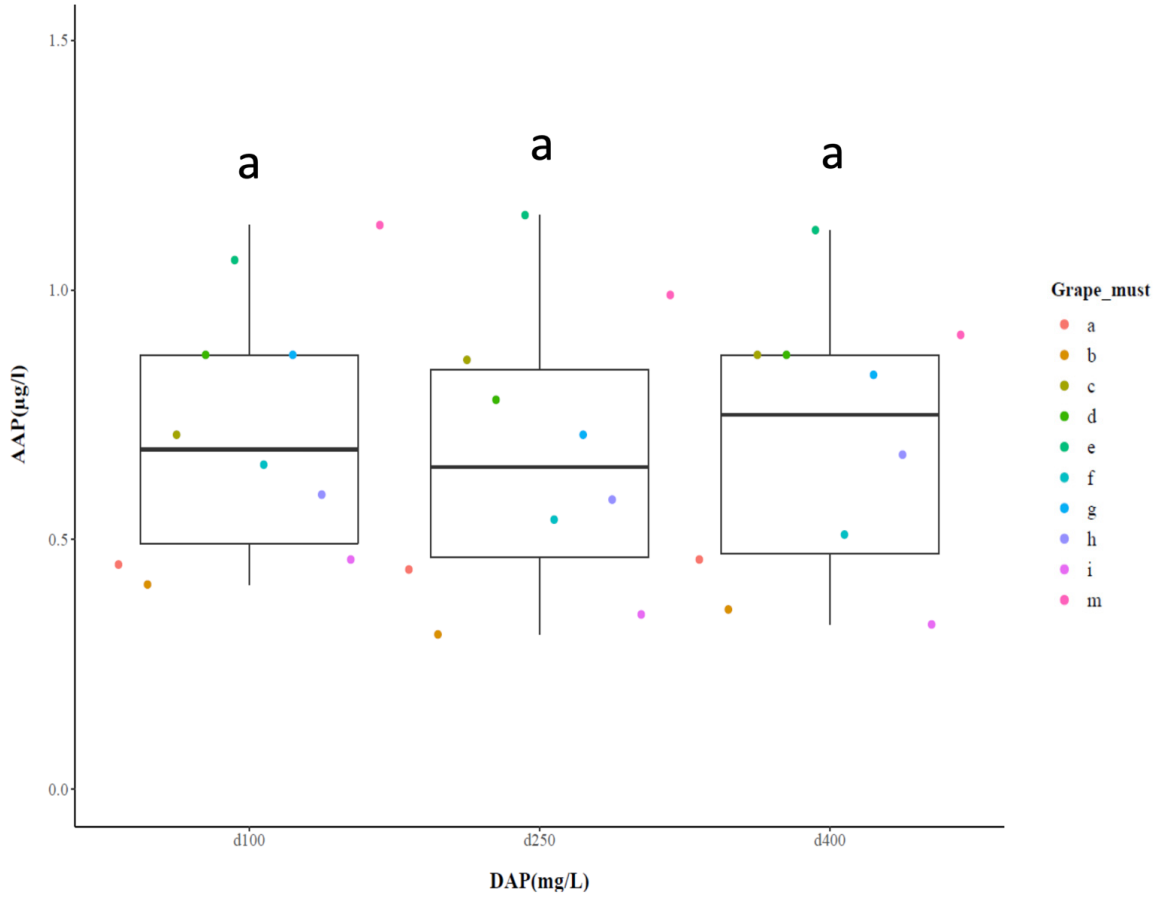

FIGURE 5. Statistical distribution (ANOVA) of the 2-aminoacetophenone (AAP) content of 10 finished wines obtained from different grape juices fermented with increasing amounts of diammonium phosphate (DAP).

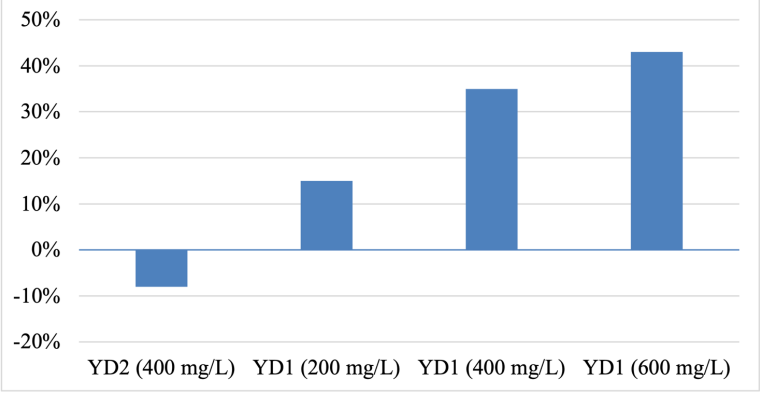

FIGURE 6. Differences between the AAP supplemented with two yeast derivatives (YD1 and YD2) and actual AAP detected in the wines at the end of alcoholic fermentation, expressed as a percentage.

The theoretical concentrations were calculated based on the values reported in Supplementary Table S3 and computed as follows: [AAP] x dose. The differences between the added and measured AAP values, expressed as a percentage, are shown in Figure 6, where 0 % represents the control (i.e., no YD used). Compared to the supplemented AAP, the use of YD1 at different concentrations (200, 400 and 600 mg/L) was associated with a 16 %, 36 % and 44 % (respectively) higher AAP content in the final wines. Furthermore, a positive trend between the amounts of added YD1 and developed AAP was observed. This is in agreement with the generation of AAP as a result of yeast action during fermentation, and the conversion into AAP of ATA precursors present in the juice (Hoenicke *et al*., 2002b) and – as in this case – supplemented with YDs. However, the opposite was observed for YD2: compared to the theoretical supplemented amount, there was an 8 % decrease in the AAP content of the wine. This reduction might be due to the formulation of this commercial product. Indeed, according to the specification sheet of the YDs, YD1 mainly constitutes yeast autolysate and YD2 inactivated yeast. The presence of yeast cell walls in YD2 might have contributed to the adsorption of some of the compounds involved in ATA formation (Lubbers *et al*., 1994; Mazauric & Salmon, 2006). To further explore this hypothesis, an estimation of the number of cells contained in the two previously used YDs was performed. As the most common method used in biotechnology entails the measurement of the optical density (OD 660) of a cell suspension (Hulst & van de Hulst, 1981; Wood & Krieg, 1993), eight solutions containing respectively 200, 400, 600 and 800 mg/L of YD1 (n = 4) and YD2 (n = 4) were prepared and the OD 660 was evaluated. As shown in Figure 7, increasing concentrations of YD2 corresponded to a rise in OD 660 values. Conversely, the OD 660 measured for YD1 did not correspond to the dose. This may indicate that a considerable fraction of YD2 is actually made up of cell walls, thus accounting for the adsorption mechanism involved in the reduction of the AAP content mentioned above. While this might be a promising result regarding finding a solution to counteract the appearance of UTA in wines, further research is needed to better investigate the biochemical mechanisms implicated.

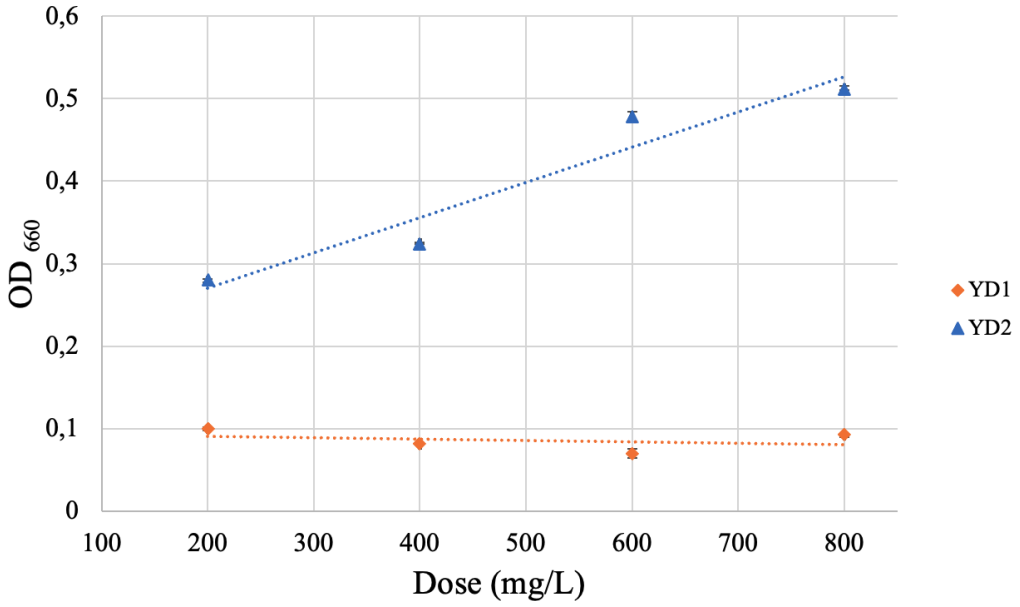

FIGURE 7. Optical densities at 660 nm (OD660) measured for solutions containing increasing doses of yeast derivatives (YDs).

7. Influence of the matrix on AAP development when fermenting with YDs

As ATA formation can occur via the oxidative reaction of the precursors contained in the grape must, the composition of the matrix is a pivotal factor in determining the onset of this defect (Schneider 2014). Indeed, not only do the varietal notes mask this defect (Alpeza *et al*., 2021), but the vintage year also plays a major role in its onset (Delaiti *et al*., 2022).

In this study, it has been demonstrated that the addition of YDs prior to fermentation can affect the rate of AAP formation, sometimes either enhancing or suppressing the formation of this compound. In this regard, it has been speculated that the outcome mainly depends on the type of YD used and its composition. These findings contribute highly to the understanding of ATA development, but as they were obtained using a single grape must, a new fermentation experiment involving the use of different grape juices was carried out to validate the robustness of the results.

Ten different grape musts were inoculated with 400 mg/L of YD1 and the AAP content at the end of the AF was measured. As can be observed in Figure 8 (Supplementary Table S6), the previously obtained results were confirmed: overall, the likelihood of obtaining ATA-tainted wines increased with the pre-fermentation addition of a YD that was rich in AAP and ATA precursors and poor in terms of the cell wall component, regardless of must type used (ANOVA test).

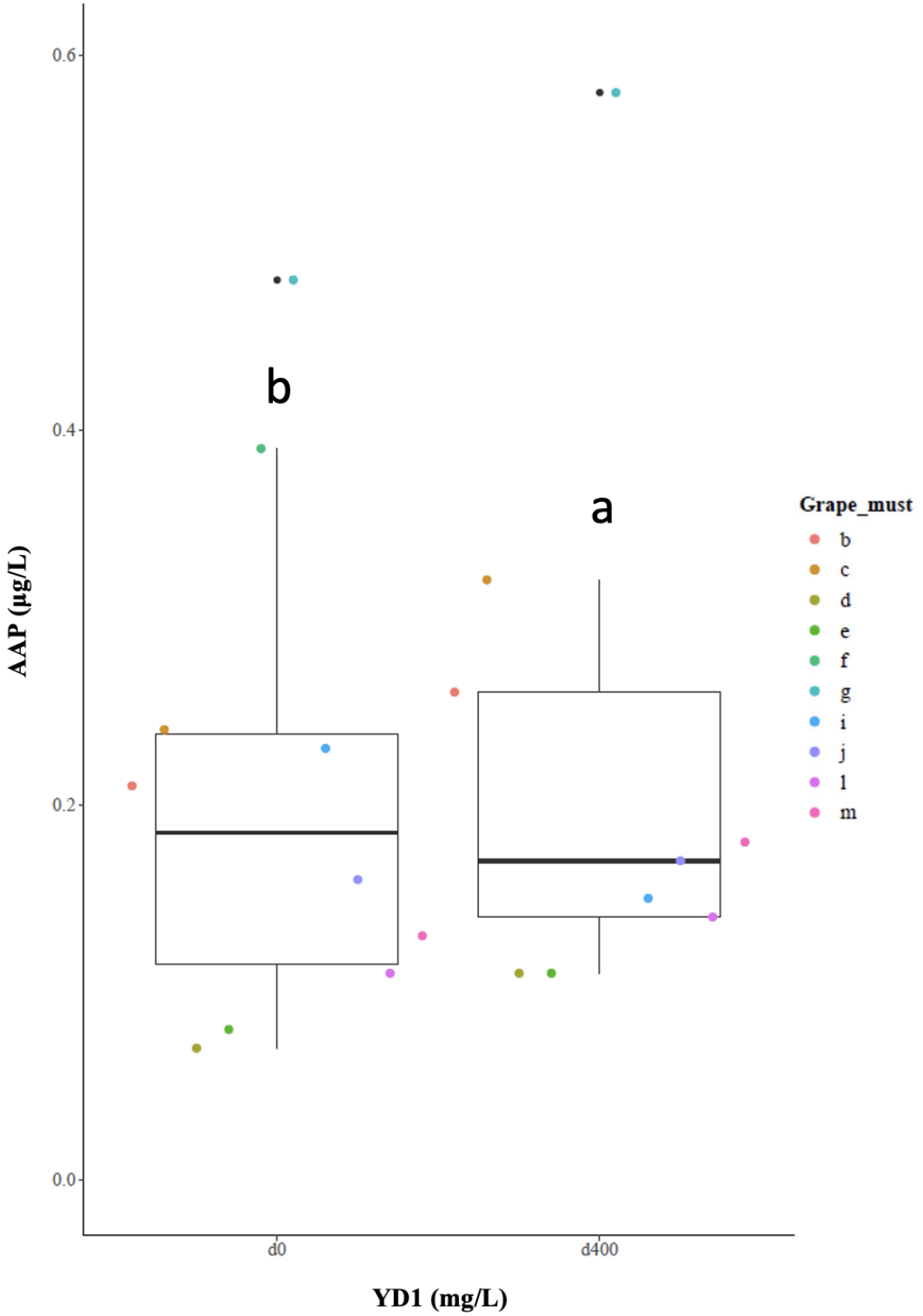

FIGURE 8. Statistical distribution (ANOVA) of the 2-aminoacetophenone (AAP) content of 10 finished wines obtained from different grape juices fermented with and without the addition of YD1 (400 mg/L).

CONCLUSIONS

The aim of this study was to assess the use of YDs in winemaking and the possibility of obtaining ATA-tainted wines. A screening of 28 YDs was first performed, and it was demonstrated that both AAP and ATA precursors are contained in those products in variable amounts. In addition, the impact of using YDs during fermentation was explored and it was found that AAP development can be enhanced or reduced depending on the specific YD used. It was hypothesised that, as well as the AAP and ATA precursors contained in the commercial formulation, the presence of other compounds, such as cell walls, might exert an adsorption effect, thus affecting the AAP content in the finished wine. Nonetheless, a YD rich in ATA precursors but poor in terms of the cell wall component was found to lead to a dose-dependent accumulation of AAP. As grape must composition is known to play a crucial role in the development of the ATA-taint, the applicability of these findings was investigated by fermenting several different grape musts supplemented with the same dose of YD. Interestingly, it was found that regardless of matrix composition, the use of YDs consistently affects the accumulation of AAP. Lastly, as DAP is also used to provide grape musts with N, the effect of DAP supplementation was also studied. The addition of increasing amounts of DAP (up to 400 mg/L) was not found to have a significant effect on the accumulation of AAP.

In conclusion, these findings are of great relevance for both YD producers and winemakers. They prove some of the consequences of YD use and highlight the need for further research on the topic. Indeed, a better understanding of the relationship between the raw material used in YD production and the ATA-precursors contained in the commercial products could aid in manufacturing products less likely to develop sensorial defects. Moreover, given their important role in the onset of ATA, the physio-chemical attributes of YDs should also be thoroughly studied.

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REFERENCES

Alpeza, I., Maslov, L., Linke, I., & Jeromel, A. (2021). Atypical aging off-flavour and relation between sensory recognition and 2-aminoacetophenone in Croatian wines. *Journal of Central European Agriculture*, 22(2), 408–419. https://doi.org/10.5513/jcea01/22.2.3103

Álvarez-Fernández, M. A., Carafa, I., Vrhovsek, U., & Arapitsas, P. (2020). Modulating Wine Aromatic Amino Acid Catabolites by Using *Torulaspora delbrueckii* in Sequentially Inoculated Fermentations or *Saccharomyces cerevisiae* Alone. *Microorganisms*, 8(9). https://doi.org/10.3390/microorganisms8091349

Andújar-Ortiz, I., Chaya, C., Martín-Álvarez, P. J., Moreno-Arribas, M. V., & Pozo-Bayón, M. A. (2014). Impact of Using New Commercial Glutathione Enriched Inactive Dry Yeast Oenological Preparations on the Aroma and Sensory Properties of Wines. *International Journal of Food Properties*, 17(5), 987–1001. https://doi.org/10.1080/10942912.2012.685682

Ángeles Pozo-Bayón, M., Andújar-Ortiz, I., & Moreno-Arribas, M. V. (2009). Scientific evidences beyond the application of inactive dry yeast preparations in winemaking. *Food Research International*, 42(7), 754–761. https://doi.org/10.1016/j.foodres.2009.03.004

Bach, H. P. (2005). Untypischen Alterungston vermeiden. Winzer-Zeitschrift.

Bekatorou, A., Psarianos, C., & Koutinas, A. A. (2006). Production of food grade yeasts. *Food Technology and Biotechnology*, 44(3). https://www.researchgate.net/publication/254613445_Production_of_Food_Grade_Yeasts

Bell, S.-J., & Henschke, P. A. (2005). Implications of nitrogen nutrition for grapes, fermentation and wine. *Australian Journal of Grape and Wine Research*, 11(3), 242–295. https://doi.org/10.1111/j.1755-0238.2005.tb00028.x

Birch, R. M., Ciani, M., & Walker, G. M. (2003). Magnesium, Calcium and Fermentative Metabolism in Wine Yeasts. *Journal of Wine Research / the Institute of Masters of Wine*, 14(1), 3–15. https://doi.org/10.1080/0957126032000114973

Christoph, N., Gessner, M., Simat, T. J., & Hoenicke, K. (1999). Off-flavor compounds in wine and other food products formed by enzymatical, physical, and chemical degradation of tryptophan and its metabolites. *Advances in Experimental Medicine and Biology*, 467, 659–669. https://doi.org/10.1007/978-1-4615-4709-9_85

Comuzzo, P., Tat, L., Tonizzo, A., & Battistutta, F. (2006). Yeast derivatives (extracts and autolysates) in winemaking: Release of volatile compounds and effects on wine aroma volatility. *Food Chemistry*, 99(2), 217–230. https://doi.org/10.1016/j.foodchem.2005.06.049

Delaiti, S., Nardin, T., Roman, T., Pedò, S., Zanzotti, R., & Larcher, R. (2022). Atypical ageing defect in Pinot Blanc wines: a comparison between organic and conventional production management systems. *Journal of the Science of Food and Agriculture*. https://doi.org/10.1002/jsfa.12158

Delaiti, S., Nardin, T., Pedò, S., & Larcher, R. (2024). Development and validation of a fast UHPLC-HRMS method for the analysis of amino acids and biogenic amines in fermented beverages. [Manuscript under review]

Gobert, A., Tourdot-Maréchal, R., Sparrow, C., Morge, C., & Alexandre, H. (2019). Influence of nitrogen status in wine alcoholic fermentation. *Food Microbiology*, 83, 71–85. https://doi.org/10.1016/j.fm.2019.04.008

Guilloux-Benatier, M., & Chassagne, D. (2003). Comparison of components released by fermented or active dried yeasts after aging on lees in a model wine. *Journal of Agricultural and Food Chemistry*, 51(3), 746–751. https://doi.org/10.1021/jf020135j

Hoenicke, K., Borchert, O., Grüning, K., & Simat, T. J. (2002a). "Untypical Aging Off-Flavor" in Wine: Synthesis of Potential Degradation Compounds of Indole-3-acetic Acid and Kynurenine and Their Evaluation as Precursors of 2-Aminoacetophenone. *Journal Of Agricultural And Food Chemistry*, *50*(15), 4303-4309. https://doi.org/10.1021/jf011672r

Hoenicke, K., Simat, T. J., Steinhart, H., Christoph, N., Geßner, M., & Köhler, H. (2002b). 'Untypical aging off-flavor' in wine : formation of 2-aminoacetophenone and evaluation of its influencing factors. *Analytica Chimica Acta*, *458*(1), 29-37. https://doi.org/10.1016/S0003-2670(01)01523-9

Hulst, H. C., & van de Hulst, H. C. (1981). Light Scattering by Small Particles. *Courier Corporation*. https://play.google.com/store/books/details?id=PlHfPMVAFRcC

Jay, J. M. (1992). Modern Food Microbiology. *Springer Netherlands*. https://play.google.com/store/books/details?id=DdDfvLdUC-EC

Klis, F. M., Boorsma, A., & De Groot, P. W. J. (2006). Cell wall construction in *Saccharomyces cerevisiae*. *Yeast, 23*(3), 185–202. https://doi.org/10.1002/yea.1349

Liu, Y.-Y., Chen, H.-W., & Chou, J.-Y. (2016). Variation in Indole-3-Acetic Acid Production by Wild *Saccharomyces cerevisiae* and *S. paradoxus* Strains from Diverse Ecological Sources and Its Effect on Growth. *PloS One, 11*(8), e0160524. https://doi.org/10.1371/journal.pone.0160524

Lubbers, S., Charpentier, C., Feuillat, M., & Voilley, A. (1994). Influence of Yeast Walls on the Behavior of Aroma Compounds in a Model Wine. *American Journal of Enology and Viticulture, 45*(1), 29–33. https://doi.org/10.5344/ajev.1994.45.1.29

Mazauric, J., & Salmon, J. (2006). Interactions between Yeast Lees and Wine Polyphenols during Simulation of Wine Aging : II. Analysis of Desorbed Polyphenol Compounds from Yeast Lees. *Journal Of Agricultural And Food Chemistry*, *54*(11), 3876-3881. https://doi.org/10.1021/jf060037o

Morata, A., Palomero, F., Loira, I., & Suárez-Lepe, J. A. (2019). New Trends in Aging on Lees. *Red Wine Technology* (p. 163-176). https://doi.org/10.1016/b978-0-12-814399-5.00011-6

Münch, P., Hofmann, T., & Schieberle, P. (1997). Comparison of key odorants generated by thermal treatment of commercial and self-prepared yeast extracts: Influence of the amino acid composition on odorant formation. *Journal of Agricultural and Food Chemistry, 45*(4), 1338–1344. https://doi.org/10.1021/jf960658p

Münch, P., & Schieberle, P. (1998). Quantitative Studies on the Formation of Key Odorants in Thermally Treated Yeast Extracts Using Stable Isotope Dilution Assays. *Journal of Agricultural and Food Chemistry, 46*(11), 4695–4701. https://doi.org/10.1021/jf980511t

Nagodawithana, T. (1992). Yeast-derived flavors and flavor enhancers and their probable mode of action. *Food Technology, 46*(11), 138–144. https://cir.nii.ac.jp/crid/1570009750104763264

Nardin, T., Roman, T., Dekker, S., Nicolini, G., Thei, F., Masina, B., & Larcher, R. (2022). Evaluation of antioxidant supplementation in must on the development and potential reduction of different compounds involved in atypical ageing of wine using HPLC-HRMS. *LWT, 154*, 112639. https://doi.org/10.1016/j.lwt.2021.112639

Pérez-Magariño, S., Martínez-Lapuente, L., Bueno-Herrera, M., Ortega-Heras, M., Guadalupe, Z., & Ayestarán, B. (2015). Use of Commercial Dry Yeast Products Rich in Mannoproteins for White and Rosé Sparkling Wine Elaboration. *Journal of Agricultural and Food Chemistry, 63*(23), 5670–5681. https://doi.org/10.1021/acs.jafc.5b01336

Pinu, F. R., Villas-Boas, S. G., & Martin, D. (2019). Pre-fermentative supplementation of fatty acids alters the metabolic activity of wine yeasts. *Food Research International, 121*, 835–844. https://doi.org/10.1016/j.foodres.2019.01.005

Rauhut, D., Shefford, P. G., Roll, C., Kürbel, H., & Löhnertz, O. (2003). Effect on diverse oenological methods to avoid occurrence of atypical aging and related off-flavours in wine. Lonvaud-Funel A, de Revel G, Darriet P (eds) 7th International symposium of oenology, Editions Tec & Doc, Lavoisier, Londres, Paris, 376–379

Redón, M., Guillamón, J. M., Mas, A., & Rozès, N. (2009). Effect of lipid supplementation upon *Saccharomyces cerevisiae* lipid composition and fermentation performance at low temperature. *European Food Research and Technology, 228*(5), 833–840. https://doi.org/10.1007/s00217-008-0996-6

Rigou, P., Mekoue, J., Sieczkowski, N., Doco, T., & Vernhet, A. (2021). Impact of industrial yeast derivative products on the modification of wine aroma compounds and sensorial profile. A review. *Food Chemistry, 358*, 129760. https://doi.org/10.1016/j.foodchem.2021.129760

Schneider, V. (2014). Atypical Aging Defect: Sensory Discrimination, Viticultural Causes, and Enological Consequences. A Review. *American Journal of Enology and Viticulture, 65*(3), 277–284. https://doi.org/10.5344/ajev.2014.14014

Schwab, A., Peternel, M., Köhler, H. J., & Heigel, K. P. (1996). Die „Untypische Alterungsnote“ im Wein, Teil IV: Beeinflussung durch weinbauliche Maßnahmen. *Rebe Wein, 6*, 181–187.

Seguinot, P., Ortiz-Julien, A., & Camarasa, C. (2020). Impact of Nutrient Availability on the Fermentation and Production of Aroma Compounds Under Sequential Inoculation With *M. pulcherrima* and *S. cerevisiae*. *Frontiers in Microbiology, 11*, 305. https://doi.org/10.3389/fmicb.2020.00305

Simat, T. J., Hoenicke, K., Gessner, M., & Christoph, N. (2004). Metabolism of tryptophan and indole-3-acetic acid formation during vinification and its influence on the formation of 2-aminoacetophenone. Mitt. *Klosterneuburg, 54*, 43–55. http://www.weinobstklosterneuburg.at/dam/jcr:5b785738-e130-4941-a628-b3e952f59eb7/43-2004.pdf

Torrea, D., Varela, C., Ugliano, M., Ancin-Azpilicueta, C., Leigh Francis, I., & Henschke, P. A. (2011). Comparison of inorganic and organic nitrogen supplementation of grape juice - Effect on volatile composition and aroma profile of a Chardonnay wine fermented with *Saccharomyces cerevisiae* yeast. *Food Chemistry, 127*(3), 1072–1083. https://doi.org/10.1016/j.foodchem.2011.01.092

Verdenal, T., Dienes-Nagy, Á., Spangenberg, J. E., Zufferey, V., Spring, J.-L., Viret, O., Marin-Carbonne, J., & Van Leeuwen, C. (2021). Understanding and managing nitrogen nutrition in grapevine: a review. *OENO One, 55*(1), 1–43. https://doi.org/10.20870/oeno-one.2021.55.1.3866

Wood, W. A., & Krieg, N. R. (1993). Methods for general and molecular bacteriology. *Am Soc Microbio*l. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC205328/pdf/jbacter00026-0002.pdf.