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An automatic fermentation nutrition system compared to a traditional one: fermentation performances and composition of white wines

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ABSTRACT

The Avaferm® automatic yeast nutrition system was tested in comparison to manual nutrition management during the fermentation of white wines. To evaluate the two approaches, a factorial experimental design with interaction was established consisting of the two ways of nutrition (Avaferm® and Control) and two white grape varieties (Catarratto and Chardonnay). The experiments were conducted on an industrial scale, ensuring the practical relevance of the findings. The parameters that underwent evaluation encompassed a wide range of aspects, including the fermentation kinetics of sugars, the use of α-amino nitrogen and ammonium, as well as the technological and aromatic volatile composition of the wines.

To study the sugar consumption kinetics during fermentation, a new use of the 5-parameter logistic regression was applied to fit these compounds. The automatic nutrition showed a substantial similarity in the fermentative kinetics and in all cases, we have reached adequate sugar depletion (almost no residual fermentable sugars). Moreover, with regards to the composition of the wines, the two methods were found to be similar, although with a slightly higher concentration of residual YAN in the control nitrogen management. The wines obtained with Avaferm® showed a greater concentration in 3-oxo-α-ionol and a slightly lesser concentration in butanoic hexanoic, octanoic and decanoic acids as well as ethyl hexanoate, ethyl octanoate and ethyl decanoate. The variety factor as expected showed several differences, which can be explained by the chemical composition of the different grape musts.

KEYWORDS: automatic yeast nutrition, industrial winemaking, industrial-scale trials, GC-MS, mathematical model, non-linear regression

INTRODUCTION

Alcoholic fermentation is the fundamental biological process for producing any type of wine. The scientific-technical knowledge of the process has expanded over time, both in terms of the optimisation of the production of primary products of wine fermentation (ethanol and carbon dioxide) and in terms of the secondary products produced by yeast (Englezos *et al.*, 2019; Lola *et al.*, 2023; Nardi *et al.*, 2019; Sablayrolles, 2009; Vicente *et al.*, 2023).

The knowledge and optimisation of the wine fermentation process has allowed for the reduction of stuck and sluggish processes (Nelson & Boulton, 2024), which represent a risk for wine quality due to the action of heterofermentative lactic bacteria that may no longer be contained by the competition of the yeasts (especially *Saccharomyces cerevisiae* (Meyen ex E.C. Hansen, 1883)). These heterofermentative lactic acid bacteria, when not contained by yeast competition, can metabolise hexose sugars which then produce acetic acid, lactic acid, ethanol and carbon dioxide. The production of acetic acid can lead to a deterioration in the quality of the product. The action of acetic bacteria is also possible following fermentation as ethanol is present and the concentration of carbon dioxide tends to decrease over time allowing molecular oxygen to be available for these types of microorganisms (Bambina *et al.*, 2024; Blateyron & Sablayrolles, 2001; Russo *et al.*, 2020; Sommer *et al.*, 2015)

Over time, various yeast nutrition strategies have been developed. These strategies are based, for example, on the supply of organic and inorganic nitrogenous forms, thiamin, minerals and molecular oxygen delivered by dispensers or pumping over the wine; these are essential for the oenological activity of the yeast (Nelson & Boulton, 2024; Schmidt & Henschke, 2015; Torrea *et al.*, 2011; Fornairon-Bonnefond *et al.*, 2002).

The nutrition of the yeast involves not only the difference in the level of depleted fermentable sugars but also in terms of the yeast's secondary metabolites, in general. In particular, the nitrogen nutrition of the yeast affects the production of ethyl esters and acetate esters. Garde-Cerdán and Ancín-Azpilicueta (2008) found a positive correlation between the concentration of amino acids supplied and the concentration of isoamyl acetate, 2-phenylethyl acetate, ethyl hexanoate, ethyl octanoate and ethyl decanoate, while they also found a negative correlation with ethyl acetate and diethyl succinate.

Nitrogen nutrition can influence the concentration of higher alcohols. Rollero *et al.* (2015) found that the initial yeast assimilable nitrogen (YAN) has a negative quadratic relationship with the production of isoamyl alcohol and 2-phenylethanol, while they observed a linear positive relationship between initial YAN and propanol concentration. For amino-acidic nitrogen, Garde-Cerdán and Ancín-Azpilicueta (2008) observed a positive linear correlation, albeit non-significant, between amino acid concentration and isoamyl alcohol. Guittin *et al.* (2021) observed a positive correlation between grape juice lipid content and fusel alcohol concentration. They hypothesised that this outcome stems from an alteration in alcohol acetyltransferase activity in the presence of lipids, affecting the catalysis of higher alcohol transformation into esters (Guittin *et al.*, 2021).

Regarding fusel alcohol 1-propanol, a strong positive correlation has been observed between its concentration and the initial assimilable nitrogen content of the grape juice. For this reason, it can be considered a marker of high or low initial YAN concentration in the musts (Guittin *et al.*, 2021).

Vilanova *et al.* (2012) found that the addiction of diammonium phosphate (DAP) increased the titratable acidity of Albariño wines. They suggested that a greater availability of ammonium ions during fermentation stimulates amino acid synthesis driving the carbon flux of the yeast towards the accumulation of an acidic intermediate of amino acids (most likely pyruvic acid) and a greater fraction of free α-amino acid nitrogen in the media generated by the addiction of DAP, which in turn can affect the titratable acidity.

The nitrogen nutrition of the yeast can also influence the production of the undesirable aroma of hydrogen sulfide and other reductive sulfur compounds (RSC). Jiranek *et al.* (1995) proved that in nitrogen-deficient conditions and in the presence of sulphate ions or sulfur dioxide, *S. cerevisiae* overproduces hydrogen sulfide through the sulfur assimilation pathway. However, for *S. cerevisiae*, the relationship between nitrogen concentration and hydrogen sulfide is not uni-directional, as shown by Ugliano *et al.*, 2011), who reported an increase in the production of this compound due to the addition of DAP, dependent on the yeast strain. A different behaviour was observed for *Saccharomyces bayanus* (Sacc.) where the production of hydrogen sulfide is inversely proportional to the initial content of yeast assimilable nitrogen (Ugliano *et al.*, 2009). De Guidi *et al.* (2024) proved that copper used as a treatment in vineyards selects *S. cerevisiae* strains with a high tendency to produce hydrogen sulfide, which is relevant, especially for fermentation that does not use industrial yeast starters.

Another crucial fermentation variable is temperature, which significantly influences the concentration of volatile compounds in wines. Mouret *et al.* (2014) illustrated how temperature affects the ratio between fermentative esters and fusel alcohols. Higher temperatures result in a lower ratio of these compounds compared to fermentation conducted at lower temperatures. Regarding the effect of temperature on the ethyl ester biosynthesis, Beltran *et al.* (2006) revealed that the genes associated with fatty acid activation via acetyl-CoA (MCT1, ETR1, OAR1) exhibit heightened expression under lower temperatures, while Godillot *et al.* (2023) suggest that under these conditions, there could be a high availability of acyl-CoA precursors, leading to better synthesis of the respective ethyl esters.

In addition to yeast nutrition and fermentation temperature, several factors significantly impact the composition of wines. These include the cultivar, soil, climate, agronomical practices, grape ripeness level, winemaking techniques, yeast strains or species and grape juice turbidity. All of which play a pivotal role in the wine's features. Below, we will provide some illustrative examples of the oenology mentioned above.

Pollon *et al.* (2023) reported that Nero d'Avola showed a higher concentration of 3-hydroxy-β-damascone, 3-oxo-α-ionol, blumenol C, and vomifoliol compared to Syrah. Furthermore, they observed that sur lies élevage increased the levels of ethyl hexanoate, ethyl octanoate, ethyl decanoate and ethyl dodecanoate along with their respective fatty acids when compared to the control wines. Additionally, the sur

lies treatment led to a reduction in quercetin (aglycone) concentration by approximately 68 % compared to the untreated wines, with sur lies wines exhibiting a decrease of about 42–43 % in total flavonoids relative to control wines.

Nicolini *et al.* (2011) investigated the impact of varying levels of grape juice turbidity (15, 45, 86, 141, 215 and 350 NTU) across three grape varieties (Chardonnay, Pinot gris and Müller-Thurgau). Their study revealed positive correlations between juice turbidity and the concentration of certain compounds in the resultant wines, such as fusel alcohols (excluding propanol), six-carbon alcohols, and acetaldehyde. Additionally, they reported negative correlations between juice turbidity and volatile acidity in the wines, fermentation duration, acetate esters concentration (notably from 45 NTU onwards), glycerol, ethyl decanoate, as well as the fatty acids hexanoic, octanoic, an in their study assessing 10 different *Saccharomyces cerevisiae* strains through either pure fermentation or sequential fermentation with *Starmerella bacillaris* strain FC54, noted substantial variations in esters, fatty acids, higher alcohols, glycerol and volatile acidity.

Corona *et al.* (2010) vinified Grillo variety grapes, cooled down them to a temperature of 8 °C before pressing and added solid carbon dioxide, resulting in a product with a pronounced aroma of grapefruit peel and passion fruit, attributable to thiol compounds. The authors ascribed this outcome to the decrease in pre-fermentative polyphenol oxidase activity. The reduction in polyphenol oxidase activity may have preserved the glutathione and cysteine content of the grape juice, which would have been available for reaction with trans-2-hexenal, forming the respective precursors of 3-sulfanylhexanol.

As a last example, Alti-Palacios *et al.* (2023) reported that cold pre-fermentation maceration on Tempranillo Blanco, Maturana Blanca, Viura and Garnacha Blanca grape varieties resulted in increased concentrations of esters, fatty acids and fusel alcohols compared to traditional winemaking methods. The authors attributed these findings to the enhanced extraction of amino acids from the grape skins. However, a possible role of the extraction of grape lipids could be investigated to explain the increase of both esters and fusel alcohols (personal consideration). It is worth mentioning that an improvement of the volatile profile is generally obtained if the ratio between esters and fusel alcohols does not decrease. Additionally, they found that the titratable acidity of cold-macerated wines was lower than that of control wines, while the pH was higher.

Recently (Giovenzana *et al.*, 2021), the automatic nutrition system Avaferm® (HTS, Marsala (TP), Italy) was tested in comparison to the traditional fermentation nutrition management for the manpower, energy consumption and cleaning water consumption in two different cellars finding a 10.6 % of money savings of the automated system compared to traditional approach in Borgo Molino Vigne & Vini Treviso cellar and 24.7 % in Cantina Forlì Predappio Soc. Agricola Coop Forlì Cesena.

This study aimed to test the oenological influence of the automatic fermentation nutrition system Avaferm® (HTS, Marsala (TP), Italy) on white wines in comparison to a manual fermentation nutrition strategy.

To better reach the goal, it was introduced also a new application of a fermentation kinetic model based on a 5-parameter logistic regression (Gottschalk & Dunn, 2005) between fermentation time and sugar concentration to help us to objectively describe the performances of the yeast and compare different fermentations.

MATERIALS AND METHODS

1. Fermentation trials

Eight fermentations were carried out on an industrial scale. Two varieties were used (Catarratto and Chardonnay) with two types of nitrogen nutrition (manual and through Avaferm®) and the combination of variety:nutrition was vinified twice. Catarratto was harvested in Sambuca di Sicilia (Italy, Sicily, Agrigento province) and *Chardonnay* was harvested in Marsala (Italy, Sicily, Trapani province). For both grape varieties, a standard winemaking process was used as described in the applied protocol below.

The grapes were destemmed, crushed and sent to the pneumatic press. After pressing, the grape juice was added to 100 mg L^{-1} of $K_2S_2O_5$ (HTS, Marsala (TP), Italy) and 20 mg L^{-1} of Hzym® Clarification FCE G (HTS, Marsala (TP), Italy). The free run juice and the pressed fraction were collected in a single tank until 1.2 bar of pressure was reached, where it was settled by gravitational process overnight at 4 °C. The liquid was divided into four different tanks for fermentation. For Catarratto (CA), four wines were made, each with 190 hL of juice, in four stainless steel tanks at 400 hL each, maintaining the temperature at 15 °C. The yeast used was SafOeno™ EF 85 (HTS, Marsala (TP), Italy) inoculated at 25 g hL^{-1} at a temperature of 15 °C adding also Hnutrix® B-Energia (HTS, Marsala (TP), Italy) at a concentration of 25 g hL^{-1}. The nitrogen nutrition of the yeast was done following the specific protocol automatically (Avaferm trials) or manually (Control trials): on day 0 after the yeast inoculation and the addition of Hnutrix® B-Energia, no ammonium or organic nitrogen was added. On day 1, 4.2 mg L^{-1} of organic nitrogen was supplied, with no ammonium added. On day 2, 4.7 mg L^{-1} of organic nitrogen and 14.0 mg L^{-1} of ammonium were introduced. On day 3, 1.0 mg L^{-1} of organic nitrogen and 3.4 mg L^{-1} of ammonium were added. On day 4, 2.3 mg L^{-1} of organic nitrogen and 3.4 mg L^{-1} of ammonium were provided. On day 5, 2.3 mg L^{-1} of organic nitrogen and 3.2 mg L^{-1} of ammonium were supplied. On day 6, 2.4 mg L^{-1} of organic nitrogen was added, without any ammonium. On day 7, 1.0 mg L^{-1} of organic nitrogen was supplied, with no ammonium added. From day 8 to day 13, no further ammonium or organic nitrogen was introduced. The inorganic nitrogen was supplied by Hnutrix® DAP Liquid (HTS, Marsala (TP), Italy) and the organic nitrogen by ViniLiquid® (HTS, Marsala (TP), Italy). The initial reductive sugar concentration for Catarratto grape juice was 185 g L^{-1} and the initial YAN was 251 mg L^{-1} as N (196 mg L^{-1} of α-amino acids and 55 mg L^{-1} as N).

For Chardonnay (CH), four 170 hL wines were made in four 400 hL stainless steel tanks maintaining the temperature at 15 °C. SafOeno™ EF 85 (HTS, Marsala (TP), Italy) yeast was inoculated at 25 g hL^{-1} at a temperature of 15 °C using also Hnutrix® B-Energia (HTS, Marsala (TP), Italy) at a concentration of 25 g h L^{-1}. As for Catarratto, the nitrogen nutrition was done following the specific protocol automatically (Avaferm trials) or manually (Control trials). As follows, the protocol is reported: on day 0 after the yeast inoculation

and the addition of Hnutrix® B-Energia, no ammonium or organic nitrogen was added. On days 1 and 2, no additions of ammonium or organic nitrogen were made. On day 3, 6.8 mg L^{-1} of organic nitrogen and 9.7 mg L^{-1} of ammonium were introduced. On day 4, 6.4 mg L^{-1} of organic nitrogen and 3.0 mg L^{-1} of ammonium were added. On day 6, 5.7 mg L^{-1} of organic nitrogen and 2.9 mg L^{-1} of ammonium were supplied. On day 7, 3.3 mg L^{-1} of organic nitrogen was supplied without ammonium, followed by 3.0 mg L^{-1} of organic nitrogen on day 8, also without ammonium. On day 9, 2.0 mg L^{-1} of organic nitrogen was added with no ammonium. From day 10 to day 22, no further nitrogen additions were made in either form. The inorganic nitrogen was supplied by Hnutrix® DAP Liquid (HTS, Marsala (TP), Italy) while the organic nitrogen was from ViniLiquid® (HTS, Marsala (TP), Italy).

The initial reductive sugars concentration for Chardonnay grape juice was 220 g L^{-1} and the initial YAN was 194 mg L^{-1} as N (147 mg L^{-1} of α-amino acids and 47 mg L^{-1} of ammonium).

2. Technological parameters

Reducing sugars, titratable acidity, pH and volatile acidity were determined through FT-IR spectrometry with a Foss WineScan (Foss, Hillerød, Denmark).

3. Inorganic and α-amino nitrogen

Inorganic and α-amino nitrogen were determined through enzymatic reaction and spectrophotometry using the Megazyme Ammonia Assay Kit (Megazyme, Wicklow, Ireland) and Megazyme Primary Amino Nitrogen Assay Kit (Megazyme, Wicklow, Ireland).

4. Volatile compounds

Volatile compounds were determined as described by Corona (2010), with some modifications. In brief: 25 mL of sample were spiked with 0.25 mL of internal standard (1-heptanol, 35.05 mg L^{-1} in 10 % v v^{-1} of absolute ethanol), diluted in distilled water 3 times and passed into a 1 g ISOLUTE® C18 cartridge (Biotage, Uppsala, Sweden), previously activated with 12 mL of methanol and washed with 24 mL of distilled water. After the passage of the sample, the cartridge was washed with 24 mL of distilled water.

The lipophilic fraction was recovered with 12 mL of dichloromethane (Sigma-Aldrich, St. Louis, Missouri). Then, this fraction was dried with anhydrous sodium sulphate (Sigma-Aldrich, St. Louis, Missouri) and reduced manually to 500 µL of volume. 1 µL of the dichloromethane extracts were injected in splitless mode in a 6890 GC (Agilent, Santa Clara, USA) coupled with a 5973N mass spectrometer (Agilent, Santa Clara, California).

GC was equipped with a DB-WAX column (Agilent Technologies; 30 m, 0.250 mm, 0.25 µm). The oven thermal program was 40 °C for 2 min, 30 °C/min until 60 °C, 2 °C/min until 190 °C, 5 °C/min until 230 °C and 230 °C for 15 min. The temperature of the transfer line was 230 °C.

The m/z acquisition range was 30-350. The integration was done in total ion current mode expressing the concentration of analytes in the equivalent of 1-heptanol for volume of wine.

5. Data analysis

The fermentation kinetics were determined by modelling the reducing sugars through a 5-parameter logistic regression (5PLR) with R 4.0.3 (R Core Team) using the drc package (Ritz *et al.*, 2015) as follows:

$$S = c + \frac{d - c}{\left(1 + e^{b(t-\varepsilon)}\right)^f} \quad (1)$$

where:

S = reducing sugar concentration in g L^{-1}

t = time of day

Six standard time points were determined to compare different sugars fermentation kinetic: 1 g L^{-1} fermented, 25 %, 50 %, 75 %, 99 % and 100 % of initial sugars fermented.

The fermentation inflexion was determined by placing equal to zero on the second derivative of S with respect to t, which is equivalent to solving equation 2 with the parameters fitted from equation 1, as follows:

$$\varepsilon - \frac{ln(f)}{b} = t_{\frac{d^2S}{dt^2}=0} \quad (2)$$

After the fitting of 5PLR on data the inverse function of equation 1 was used to determine the six times (t) points plugging into equation 3 the sugar concentration correspondent to the t point

$$t = \frac{b\varepsilon + \ln\left(\left(\frac{c-d}{c-S}\right)^{\frac{1}{f}} - 1\right)}{b} \quad (3)$$

Then the time points, the inflection points, and the maximum rates of sugar consumption (minimum dS/dt) were treated as variables.

All the data were subjected to factorial ANOVA with interaction using as independent variables the variety and the nutrition using R 4.0.3 (R Core Team). In case of a p-value lower than 0.05 and more than two levels of factors, the independent variable was submitted to Tukey's HSD post-hoc test with a total α value of 0.05 using the R package *agricolae* (De Mendiburu, 2020).

RESULTS

1. Effect on fermentation kinetic

The automated nutrition system exhibited no influence on the time at which the fermentative process began to decelerate, and there were no disparities in the maximum daily sugar consumption (Table 1). Furthermore, the fermentation kinetics displayed no significant distinction between the Avaferm® and Control nutrition protocols. For the duration needed to consume 100 % of the initial sugars, no distinction was observed between these treatments (Table 1).

Analysing the obtained results, we can observe that for Catarratto the fermentation process starts to slow down about 2 days later than for *Chardonnay*, and in general, the speed of Catarratto fermentation is greater than Chardonnay, which seems related to the lower initial concentration of fermentable sugars of Cataract grape juice (Table 1), and the different amount of YAN of the two grape juices.

Considering the fermentation kinetic parameters within the context of the interaction between variety and nutrition type, no discernible additive or subtractive effects on fermentation kinetics are observable when both factors are simultaneously applied (Table 2).

TABLE 1. Fermentation kinetic parameters for the main factors nutrition and variety.

Nutrition (n = 4)			
Fermentation parameter	Control	Avaferm	Sign.
Inflection point	4.5 ± 0.4	4.6 ± 0.5	ns
dZ/dt minimum	-30 ± 1	-29 ± 1	ns
1 g L-1 fermented	0.5 ± 0.2	0.6 ± 0.1	ns
25 % fermented	3.8 ± 0.2	4.1 ± 0.1	ns
50 % fermented	5.6 ± 0.2	6.1 ± 0.3	ns
75 % fermented	8.1 ± 0.7	9.7 ± 0.9	ns
99 % fermented	15 ± 3	18 ± 3	**
100 % fermented	18 ± 3	20 ± 4	ns
Variety (n=4)			
Fermentation parameter	CA	CH	Sign.
Inflection point	5.2 ± 0.2	3.9 ± 0.1	*
dZ/dt minimum	–31 ± 1	–28.3 ± 0.9	ns
1 g L-1 fermented	0.33 ± 0.07	0.80 ± 0.07	*
25 % fermented	3.8 ± 0.2	4.1 ± 0.1	ns
50 % fermented	5.4 ± 0.2	6.2 ± 0.2	*
75 % fermented	8 ± 1	9.8 ± 0.3	ns
99 % fermented	11.8 ± 0.5	22 ± 1	***
100 % fermented	12.8 ± 0.4	24.8 ± 1.4	***

Data are reported as mean plus and minus standard error of the mean. n = number of measurements. Sign. = ANOVA significance; ns = not significant; * = 95 % of significance; ** = 99 % of significance; *** = 99.9 % of significance. dZ/dt minimum is expressed in g L^{-1} day^{-1}, all the other parameters are expressed in day. CA = Catarratto, CH = Chardonnay. Inflection point = time point in days from which fermentation speed starts to decline. dZ/dt minimum = maximum daily sugar consumption modelled by 5 parameters logistic model. 1 g L^{-1} fermented = modelled time to ferment 1 g L^{-1} of sugars from the initial concentration; 25 %, 50 %, 75 %, 99 %, 100 % fermented = modelled time to ferment a specific mass percentage of initial amount of sugars.

Regarding the results of the nitrogen dynamics during fermentation and at the end of the process, the "alcohol minimum YAN" represents the alcoholic concentration in which we have noted no decrease in YAN concentration but a release of it in wine and hence it is the minimum concentration of YAN detected in the samples. This parameter was obtained by reporting the alcoholic concentration corresponding to the minimum YAN measured. At around 1.5 % v v^{-1} of alcohol concentration, no more ammonia was detected in all fermentations (Table S2). However, the fermentative kinetic of α-amino-acidic nitrogen, or rather the concentration of α-amino-acidic nitrogen observed depending of the fermentation time, showed a double behaviour in all the trials, or rather we assisted the depletion in concentration by a decreasing sigmoidal curve until a minimum was reached and then we have noted an increasing of the amino acid nitrogen concentration (Table S2). Regarding the alcohol minimum YAN, we can see from Table 3 that the nutrition systems have the same influence on this variable showing no statistical differences.

TABLE 2. Fermentation kinetic parameters for the interaction between variety and nutrition.

Interaction (n = 2)					
Fermentation parameter	CA: Control	CA: Avaferm	CH: Control	CH: Avaferm	Sign.
Inflection point	5.1 ± 0.3	5.3 ± 0.4	3.9 ± 0.1	3.9 ± 0.3	ns
dZ/dt minimum	-31.1 ± 2.4	-31 ± 2	-29.2 ± 0.6	-27.5 ± 1.8	ns
1 g L^{-1} fermented	0.2 ± 0.1	0.42 ± 0.02	0.8 ± 0.2	0.84 ± 0.08	ns
25 % fermented	3.5 ± 0.2	4.2 ± 0.2	3.98 ± 0.04	4.1 ± 0.2	ns
50 % fermented	5.18 ± 0.04	5.7 ± 0.3	6.0 ± 0.1	6.5 ± 0.3	ns
75 % fermented	6.87 ± 0.01	9 ± 2	9.4 ± 0.3	10.3 ± 0.5	ns
99 % fermented	11.0 ± 0.2	12.6 ± 0.1	19.9 ± 0.7	23.6 ± 0.8	ns
100 % fermented	12.2 ± 0.4	13.4565 ± 0.0008	23.2 ± 2.4	26.4 ± 0.8	ns

Data are reported as mean plus and minus standard error of the mean. n = number of measurements. Sign. = ANOVA significance; ns = not significant; * = 95 % of significance; ** = 99 % of significance; *** = 99.9 % of significance. dZ/dt minimum is expressed in g L^{-1} day^{-1}, all the other parameters are expressed in day. CA = Catarratto, CH = Chardonnay. Inflection point = time point expressed in days from which fermentation speed starts to decline. dZ/dt minimum = maximum daily sugar consumption modelled by 5 parameters logistic model. 1 g L^{-1} fermented = modelled time to ferment 1 g L^{-1} of sugars from the initial concentration; 25 %, 50 %, 75 %, 99 %, 100 % fermented = modelled time to ferment a specific mass percentage of initial amount of sugars.

The variety showed a statistically significant influence at 10 % on the alcohol minimum YAN (Table 3). In particular, the amino-acidic concentration starts to increase at an alcohol concentration less than CA of more than 1.5 % v v^{-1}, this phenomenon can be attributable to the different chemical compositions of the grape juices between the initial amount of sugars and YAN. No interaction effect between nutrition and variety was noted regarding alcohol minimum YAN and residual YAN (Table 3).

2. Sugar, alcohol and acid parameters and nitrogen composition of final wines

Starting from the nutrition factor, it is possible to note a greater residual sugar concentration for Avaferm® compared to the Control. No differences in the level of alcohol yield were noted between the two nutrition management protocols, as well as for titratable acidity, pH and volatile acidity (Table 4).

CH showed a greater concentration of residual YAN (amino-acidic) at the end of fermentation compared to CA (Table 4).

3. Volatile composition

The Avaferm® nutrition showed a lesser concentration in acids sum compared to the control (Table 5) and also a statistically significant difference for octanoic acid (Table S3). The same tendency was observed for butanoic, octanoic and decanoic

TABLE 3. Alcohol concentration in which we have recorded the minimum nitrogen concentration.

Nutrition (n = 4)	
Level of factor	Alcohol minimum YAN
Control	9.5 ± 0.5
Avaferm	9.6 ± 0.7
Sign.	ns
Variety (n = 4)	
Level of factor	Alcohol minimum YAN
CA	10.4 ± 0.3
CH	8.7 ± 0.4
Sign.	ns
Interaction (n = 2)	
Level of interaction	Alcohol minimum YAN
CA:Control	10.2 ± 0.8
CA:Avaferm	10.5 ± 0.1
CH:Control	8.9 ± 0.2
CH:Avaferm	8.6 ± 0.9
Sign.	ns

Data are reported as mean plus and minus standard error of the mean. n = number of measurements. Sign. = ANOVA significance; ns = not significant; * = 95 % of significance; ** = 99 % of significance; *** = 99.9 % of significance. CA = Catarratto, CH = Chardonnay. Alcohol minimum YAN = % v v^{-1} of alcohol in which we have registered the minimum concentration in Yeast Assimilable Nitrogen (YAN).

acid but their averages are not statistically different from the control.

Avaferm® nutrition also showed a lower concentration of hexyl acetate compared to the control management (Table S3), a lower concentration in ethyl esters of fatty acids (not significant) and a higher concentration of alcohols (sum of isoamyl alcohol, hexanol, (E)-3-hexenol, 3-ethoxypropanol, (Z)-3-hexenol, methionol and tryptophol) compared to the traditional method, i.e., the means are not statistically different between them.

The varieties, as expected, showed some differences between them, especially regarding acids, benzenoids, C_{13}-norisoprenoids and alcohols (Table 5). Catarratto exhibited a greater concentration of all the classes of compounds mentioned before.

In relation to the outcomes of aroma compound classes calculated for the interaction between variety and nutrition, we observed an additive effect arising from the CA and Control combination concerning acid concentration. However, noteworthy interaction effects were not observed for the remaining classes of aroma compounds (Table 6).

DISCUSSION

1. Fermentation kinetic

Both nutrition management protocols showed good results on fermentation kinetics without stuck or sluggish processes.

TABLE 4. Sugar, alcohol and acid parameters and nitrogen composition of final wines.

Nutrition (n = 4)						
Level of factor	S	Al	TA	pH	VA	Residual YAN
Control	0.3 ± 0.2	12.1 ± 0.6	6.2 ± 0.2	3.37 ± 0.07	0.3 ± 0.1	40 ± 6
Avaferm	2.3 ± 0.3	12.0 ± 0.6	6.2 ± 0.2	3.4 ± 0.1	0.25 ± 0.1	31 ± 5
Sign.	*	ns	ns	ns	ns	ns
Variety (n = 4)						
Level of factor	S	Al	TA	pH	VA	Residual YAN
CA	1.2 ± 0.6	11.03 ± 0.04	5.90 ± 0.05	3.23 ± 0.01	0.060 ± 0.004	28 ± 2
CH	1.4 ± 0.6	13.1 ± 0.1	6.50 ± 0.04	3.53 ± 0.03	0.448 ± 0.003	44 ± 5
Sign.	ns	***	***	***	***	*
Interaction (n = 2)						
Level of factor	S	Al	TA	pH	VA	Residual YAN
CA:Control	0.2 ± 0.1	11.09 ± 0.03	5.82 ± 0.01	3.245 ± 0.005	0.07 ± 0.01	31 ± 4
CA:Avaferm	2.2 ± 0.6	11.0 ± 0.1	6.0 ± 0.0	3.22 ± 0.00	0.06 ± 0.01	25 ± 2
CH:Control	0.5 ± 0.3	13.1 ± 0.1	6.5 ± 0.0	3.50 ± 0.00	0.45 ± 0.01	50 ± 7
CH:Avaferm	2.4 ± 0.6	13.1 ± 0.2	6.5 ± 0.1	3.55 ± 0.05	0.45 ± 0.00	38 ± 7
Sign.	ns	ns	ns	ns	ns	ns

Data are reported as mean plus and minus standard error of the mean. n = number of measurements. Sign. = ANOVA significance; ns = not significant; * = 95 % of significance; ** = 99 % of significance; *** = 99.9 % of significance. S = reductive sugars [g L^{-1}]; Al = alcohol [% v v^{-1}]; TA = titratable acidity [g L^{-1} as tartaric acid]; VA = volatile acidity [g L^{-1} as acetic acid]; residual YAN = residual Yeast Assimilable Nitrogen (YAN) at the end of the fermentation [mg L^{-1} as N].

This kinetic observation proves that an automatic nutrition system for fermentation is possible, and it can help wine cellars be more economical.

The kinetics of fermentation were strongly dependent on the varietal factor and this difference is mainly attributable to the initial sugar content (185 g L^{-1} Catarratto and 220 g L^{-1} Chardonnay) with a positive correlation with the duration of the fermentative process. This aspect gains even more prominence during the advanced stages of fermentation, where the time required to completely consume 100 % of the initial sugar concentration is nearly doubled, as modelled by a five-parameter logistic regression. Another important aspect linked with the sugar fermentation kinetic is that we have not observed any interaction effect between nutrition management and variety. The rapid complete disappearance

TABLE 5. Volatile compounds sum for the main factors nutrition and variety.

Nutrition	Control (n = 4)	Avaferm (n = 4)	Sign.
Acids	10790 ± 1396	8865 ± 366	*
Aging compounds	1284 ± 253	1395 ± 388	ns
Benzenoids	9688 ± 1106	10446 ± 635	ns
Acetate esters	3351 ± 151	3311 ± 125	ns
Ethyl esters	2213 ± 468	1662 ± 252	ns
C13-norisoprenoids	286 ± 37	342 ± 69	ns
Alcohols	18382 ± 2522	20733 ± 2598	ns
Terpenes	19 ± 4	21 ± 4	ns
Variety	CA (n = 4)	CH (n = 4)	Sign.
Acids	11030 ± 1259	8625 ± 389	**
Aging compounds	1371 ± 443	1308 ± 142	ns
Benzenoids	11296 ± 404	8837 ± 743	.
Acetate esters	3425 ± 159	3238 ± 86	ns
Ethyl esters	2218 ± 516	1657 ± 120	ns
C13-norisoprenoids	233 ± 22	395 ± 42	**
Alcohols	22990 ± 2460	16126 ± 354	.
Terpenes	17 ± 4	22 ± 4	ns

Data are reported as mean plus and minus standard error of the mean. n = number of measurements. Sign. = ANOVA significance; ns = not significant; . = 90 % significance; * = 95 % of significance; ** = 99 % of significance; *** = 99.9 % of significance. CA = Catarratto, CH = Chardonnay.

TABLE 6. Volatile compounds sum for the interaction between nutrition and variety.

Interaction (n = 2)					
Class	CA: Control	CA: Avaferm	CH: Control	CH: Avaferm	Sign.
Acids	13198 ± 293 a	8862 ± 131 b	8382 ± 46 b	8868 ± 887 b	**
Aging compounds	1349 ± 596	1392 ± 907	1218 ± 148	1397 ± 287	ns
Benzenoids	11153 ± 863	11439 ± 441	8223 ± 1518	9452 ± 500	ns
Acetate esters	3591 ± 115	3258 ± 289	3112 ± 90	3364 ± 67	ns
Ethyl esters	2796 ± 785	1640 ± 563	1631 ± 143	1684 ± 255	ns
C13-norisoprenoids	243 ± 49	223 ± 16	329 ± 46	460 ± 1	ns
Alcohols	20763 ± 5137	25216 ± 62	16001 ± 663	16250 ± 530	ns
Terpenes	20 ± 7	14 ± 4	17 ± 6	27 ± 2	ns

Data are reported as mean plus and minus standard error of the mean. n = number of measurements. Sign. = ANOVA significance; ns = not significant; * = 95 % of significance; ** = 99 % of significance; *** = 99.9 % of significance. Different letters mean different averages for Tukey's HSD *post-hoc* test.

of ammonium after 1 day from the inoculation of CA and after 2 days for CH is in accordance with Boulton *et al.* (1999) and Gobert *et al.* (2019), who list ammonia as a nitrogen compound consumed during the early stage of fermentation.

Regarding the behaviour of amino acid nitrogen, it is well-established that the mechanism of amino acid uptake by yeast during fermentation is reliant on H^+ symporters. This process entails the expulsion of protons from the cytoplasm to maintain pH homeostasis. However, the alcohol concentration in the fermentation environment can disrupt the permeability of cellular structures, leading to the influx of protons into the cytoplasm. This influx necessitates the expulsion of these protons, thereby compromising the entry of amino acids into the cell. This phenomenon effectively obstructs the depletion of amino acid nitrogen from the medium, as highlighted in the work by Leão and van Uden (1984).

Distinctive kinetics in the concentration of amino acid nitrogen was observed. This pattern is characterised by an initial decrease in concentration over time, followed by a subsequent increase. This observed process could potentially be attributed to a dynamic equilibrium between the amino acids acquired by the cells and those subsequently released by the cells themselves. It is worth noting that, in accordance with our protocols, the addition of these compounds consistently concluded prior to reaching the alcoholic concentration at which we observed a notable rise in amino acid nitrogen levels.

2. Sugar, alcohol, acid parameters and nitrogen composition of final wines

Although the Avaferm® nutrition showed a higher concentration of residual sugars (2.3 ± 0.3 g L^{-1} versus 0.3 ± 0.2 g L^{-1}), the recorded values are fully compatible with the technological objective linked to the production of white wines, both from a sensorial and microbiological point of view.

On the other hand, the lower concentration of acetic acid detected in CA wines may be due to a factor linked to the yeast strain used and to a lower initial hypertonicity of CA grape juice due to the lower sugar concentration. However, it cannot be excluded that other factors may have had an influence, such as the lipid composition of the two different musts (Guittin *et al.*, 2021; Moruno *et al.*, 1993) or the different presence of solid suspended of the CA and CH grape juice (Delfini & Costa, 1993; Nicolini *et al.*, 2011).

It appears clear how the point at which the concentration of amino acids in the medium begins to grow due to spontaneous causes depends on the composition of the fermenting product itself and it is conceivable that there is a negative correlation between the sugar concentration and the maximum alcohol in which the yeast can transport amino acids into the cell. This fact could be due to a greater presence of sugars for the same amount of ethanol and therefore to a greater osmotic stress. The latter could have a concomitant role with the difficulty of expelling protons from the cytoplasm.

An interesting aspect concerns the fact that the fermentation inflection point of the sugars precedes in any case observed the maximum concentration of alcohol in which the amino acid nitrogen decreases. The first aspect linked to sugars

and also to ethanol, as it is one of the products of alcoholic fermentation, could be considered as one of the first indicative moments of fermentation difficulty.

3. Volatile compounds

The general tendency of traditional nutrition to give wines richer in butanoic, hexanoic, octanoic and decanoic acid gives the wines a more favourable esterification balance to the relative ethyl ester, as there is an equilibrium between fatty acid and ethanol between the reactants and ethyl ester and water among the products in which for the most linear fatty acid/ethyl esters a common value of K_{eq} of 4 is accepted (Waterhouse *et al.*, 2016, Usseglio-Tomasset, 1995). At the same time, Avaferm® nutrition reduces the risk of slow fermentation due to the massive presence of fatty acids such as decanoic acid (Bisson, 1999).

Regarding Avaferm®, we have been able to notice a higher concentration of the C_{13}-norisoprenoid 3-oxo-α-ionol. However, to date, information about the variables influencing the enzymatic release of glycosides of this norisoprenoid is very scarce. Regarding the acid-catalysed hydrolysis of 3-oxo-α-ionol glycosides (Slaghenaufi & Ugliano, 2018) during an accelerated ageing test shows that this compound increases in concentration in the free fraction until it reaches a maximum then decreases, they show that simultaneously with the decrease of 3-oxo-α-ionol, there is an increase of megastigmatrienone assuming that the first compound loses a hydroxyl group due to protonation and rearranges itself forming an unsaturation.

According to some authors Slaghenaufi and Ugliano (2018), and Slaghenaufi *et al. (*2014), 3-oxo-α-ionol and the derivative megastigmatrienone are among the compounds that can give wines a tobacco bouquet. Characteristic that depending on the type of wine can be pleasant or not.

As expected, the factor variety showed an effect of different volatile compounds. Starting from fatty acids we can observe (supplementary electronic sheet 3) that wines from CA were richer in hexanoic and octanoic acid compared to CH. For these compounds several aspects able to influence their concentrations were described: yeast strain and species (Englezos *et al.*, 2019), fermentation turbidity effect (Nicolini *et al.*, 2011; Shinohara, 1986), oxygenation (Shinohara, 1986), presence of grape marks (Shinohara, 1986), amino-acidic nitrogen ([Garde-Cerdán & Ancín-Azpilicueta, 2008] for octanoic and decanoic acid). Between these variables, a probable effect could be given by the different yeast used during the winemaking for CA and CH.

Moreover, the differences given by the variety factor about hexyl acetate, isoamyl acetate, ethyl dodecanoate, isoamyl alcohol and tryptophol can be probably partially referred to the different composition of the two types of grape juice and the turbidity factor. A different explanation can be given for the difference in hexanol concentration observed for the variety factor. This compound can be originated after the cellular decompartmentalisation of grape cells and thanks to the activity of lipases on membrane phospholipids with the release of linolenic acid. This latter compound, in the presence of oxygen, can form a hydroperoxide thanks to the catalysis of lipoxygenases this hydroperoxide can undergo a lysis catalysed by a hydroperoxide lyase forming hexanal. The aldehyde can be enzymatically reduced to hexanol in the grape juice or during the fermentation (Dias Araujo, 2017). The greater concentration in CA wines can be attributable to a different phospholipid profile of the grape juice, a different lipoxygenase activity or a different loss of hexanol/hexanol precursors given by the precipitation in winemaking.

From the results obtained and in the light of the bibliography currently available, it is possible to conclude that the automatic management of yeast nutrition during alcoholic fermentation with Avaferm® has led to a substantial compositional similarity of the wines obtained with manual nutrition, paving the way for a greater automation of the processes of the wine industry with qualitative results comparable to a manual process.

The achievement of slightly different fermentation kinetics of the sugars did not significantly impact the final result of the wines at a technological level.

However, a limitation of our experiment was the relatively high YAN concentration of the grape juices. Consequently, nitrogen nutrition might have exerted less influence compared to musts that are severely deficient in this regard. Nonetheless, it is essential to take into consideration that the fermentations occurred industrially and on a large scale, underscoring the significant practical relevance of our experimentation.

The adaptation of mathematical functions to the fermentation kinetics offers numerous advantages, both in terms of scientific methodology and in terms of the optimisation of industrial processes. In the first place, the adaptation of functions always allows different kinetics by choosing the same time point and often allows one to summarise the observations with few indices with an advantage both at the interpretive level of the experimenter and at the communicative level. The advantage in terms of industrial process management concerns the setting of tools based on information collected and synthesised through regressions.

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