Oenological attributes of the yeast *Hanseniaspora vineae* and its application for white and red winemaking

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Abstract. Flavour and some compounds associated with wine colour are known to be yeast strain-dependent. These metabolites are important for the sensory quality of wines, studies searching for increase aroma and color are a key area today in winemaking. The aim of this work was to study the oenological potential of the two main strains of *Hanseniaspora vineae*, native to Uruguay to better understand their successful application at winery level. It is known that these strains contribute with extracellular proteases and β-glucosidase enzyme activities that might increase cell lysis and flavor depending in grape varieties. Application and nutrient management of the process of these strains in production of white wines (Chardonnay, Macabeo and Petit Manseng) and red wine Tannat are discussed. Wines were evaluated to determine the volatile compounds composition and their effect compared to conventional processes. Low production of short and medium chain fatty acids and ethyl esters, and high production of acetate esters and isoprenoids are found compared to *S. cerevisiae* strains. The most outstanding characteristic of the species *H. vineae* was the production of benzenoids, phenylpropanoids and acetate esters. This behavior was reflected in the sensory evaluation, where all the fermentations performed with *H. vineae* were considered superior compared to *Saccharomyces cerevisiae* wine strains.

1. Introduction

Wine markets continue growing in brands quantity, and the challenge of product style differentiation is always more competitive and difficult to attract consumers attention. It is considered that regional consumption will be the main alternative for the many new brands that appear in the market. This is also in agreement with an opposite situation that is happening in the distribution channels, as every year there is a reduction in distribution companies in the main importer countries, such as the USA, UK and Germany. The use of non-conventional yeasts is a strategy to create unique wine profiles within an extensive market, where region identity is the challenge [1].

Returning to low input winemaking strategies to develop particular characteristics of the “terroir” that might differentiate their wines, is one of the ways that was followed by some winemakers in the last 20 years [2,3]. New sites and soil selection, minimal handling of the grapes and fertilizers addition, decrease irrigation, old vines selection, are some of the topics that are being discussed in wine quality definition in the last two decades searching for complexity [4]. One of the key components of the terroir concept are the native yeast associated with the mature grapes of a particular region. In this presentation we showed our experience with a native grape yeasts of the apiculate group, *Hanseniaspora vineae* [5]. Since 2009 we have used this species for commercial wine production, with many interesting results firstly in white wines [6,7], and now for red Tannat. Two strains genomes that were applied of this species were sequenced so as to understand metabolic differences with the conventional *S. cerevisiae* strains [8]. Although there is an increase interest in non- *Saccharomyces* yeast application, there are still very limited commercial strains available for the winemaker [9,10]. We discuss here how we have applied small quantity production of liquid ferments that will improve non-conventional yeasts availability in particular regions, and a protocol for successful vinifications of these strains.

2. Materials and methods

2.1 Yeast strains

The commercial wine yeast strain used was *S. cerevisiae* ALG 804 (DSM, Denmark). The apiculate NS strain used was isolated from Tannat wine fermentation, *H. vineae* T02/5AF.

*H. vineae* was prepared by Lage y Cia in liquid sterile bags of 3 liters for inoculation at the winery to obtain an initial cell concentration of 5 × 10⁵ cells/ml in triplicate 400 liters bins for Tannat and 225 L barrels for white grapes Petit Manseng wines. Final population inoculated was checked by microscope counting and by plating in WLN medium where green dark colonies can be clearly associated with *Hanseniaspora*.

The commercial strain, *S. cerevisiae* ALG 804 was hydrated as instructed by the manufacturer and subsequent to microscope counting, the appropriate dilution of the rehydrated wine yeast was inoculated (at time 0 or 6 days after inoculation with strain *H. vineae* T02/5AF in sequential co-inoculations) to obtain an initial cell concentration of 1 × 10⁶ cells/ml.

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2.2. Fermentation conditions

The grape of Petit Manseng must contained 178 mg N/L yeast assimilable nitrogen (YAN), 248 g/L total sugars, 5.5 g/L of total acidity was measured as sulphuric acid at pH 3.3. Grapes were crushed, cooled by refrigeration to 12 °C and pressed with the addition of 3 g/Hl of SO2, allowed to settle for 12 h and then transferred to three 2251 French oak barrels for each yeast treatment. Red grapes of Tannat were crushed, and no SO2 was added, so immediately were inoculated with the corresponding yeast treatments. Tannat grape contained 148 mg N/L of YAN, 225 g/L of total sugars, 5.8 g/L of total acidity and pH 3.3.

Inoculation of the must was done immediately except for the spontaneous trials. Treatments were as follows: coferm- H. vineae (initially inoculated with H. vineae and then inoculated six days later with strain ALG 804), Commercial (inoculation with strain ALG 804 at time 0) and Spontaneous (without inoculation). Twenty-four hours after inoculation, 0.3 g/L thiamine was added to all barrels. A supplementation of 100 mg/L with diammonium phosphate (DAP) and an extra 0.3 g/L of thiamine and 1 g/L of yeast extract were added when ALG 804 was added at day 6 to the H. vineae treatment. Fermentation activity was measured by juice density every day, together with temperature and room temperature was maintained at 20 °C. Procedures for wine chemical analysis and YAN levels by the formaldehyde method were described previously [11]. Samples for analysis were taken once a day, for cell growth measurement, using an improved Neubauer chamber and the numbers of dead cells were counted by the methylene blue-staining technique. Upon completion of alcohol fermentation, chemical and sensory analysis was done. Subsequently, malolactic fermentation (MLF) was done using Oenococcus oeni VP41 (Lallemand, Montreal). All barrels were maintained at 18–22 °C and malic and lactic acid production during MLF was analysed by thin-layer chromatography [12]. After MLF completion 50 mg/L SO2 was added followed by 2 months barrel ageing before bottling and final sensory analysis. All samples for analysis were filter sterilised (0.45 μm membrane) and the free SO2 content was then adjusted to 35 mg/L, before and after MLF.

2.3. Sensory analysis

Duplicate sensory analysis was performed on fifteen samples, comprising three wines from each of the white wine treatments and two red Tannat wines. The three treatments as shown, are coded as follows. Spontaneous, Sacch and H. vineae, this last one refers to sequential co-fermentation. Sensory aroma description was made by a panel of eight established winemakers. Wines were presented in individual testing booths with normalised red lighting, to eliminate the colour perception of the white wine samples in flavour descriptions. Samples of 60 ml were served at 18 ± 1 °C in 250 ml clear, tulip shaped wine glasses (ISO 3591, 1977) covered with a watch glass, and were identified with three digit random codes. Two samples were evaluated for aroma characteristics in each session. Panelists were required to rate secondary and tertiary tier terms using a 10-point intensity scale [13].

2.4. GC and GC–MS analysis

Aroma volatile compounds extraction of aroma compounds was done by adsorption and subsequent elution and separation from an Isolute (IST Ltd., Mid Glamorgan, U.K.) ENV+ cartridge packed with 1 g of highly, cross-linked, styrene-divinyl benzene (SDVB) polymer. Sample preparation and GC analysis was described previously [14]. Wine aroma components were identified by comparison of their Linear Retention Indices, with pure standards or data reported in the literature. Comparisons were also made with MS fragmentation patterns obtained with those in databases. GC-FID and GC–MS methods with an internal standard (1-heptanol) were used for quantitative purposes.

2.5. Statistical analysis

ANOVA of chemical and volatile compound analysis was done for the different fermentation treatments. ANOVA for sensory descriptors was done for different treatment and panel assessors’ effect. Mean rating and Least Significant Differences (LSD) for each treatment were calculated from each analysis of variance with Statistica 7.1.

3. Results and discussion

In Table 1 we showed the basic wine quality parameters of Tannat red wines produced as an average of three processes with two yeast treatments for the Cerro Chapeu region. As it is shown wines obtained with H. vineae treatment give similar results in terms of final alcohol, volatile

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**Table 1. Analytical parameters of Tannat wines produced with H. vineae compared to a conventional commercial yeast Saccharomyces cerevisiae ALG 804 (Sacch).** Data showed are before and after malolactic Fermentation MLF, and during barrel and bottle aging. ND not determined.

|                      | Before MLF March 2013 | After MLF (May 2013) | barrel aging Oct. 1st | Aug 1st 2014 and Bottled | Jul–15 |
|----------------------|-----------------------|----------------------|-----------------------|--------------------------|--------|
|                      | Sacch                 | H. vineae            | Sacch                 | H. vineae                | Sacch  |
| Free SO2 mg/L        | ND                    | ND                   | 11                    | 12.4                     | 17     |
| Total SO2 mg/L       | 7                     | 9.8                  | 26.52                 | 23.2                     | 51     |
|                      |                       |                      | 45                    | 46                       | 66     |
|                      |                       |                      | 53                    | 60                       | 48     |
| Volatile Ac. g/L in Sulf | 0.255         | 0.27                | 0.5                   | 0.48                     | 0.34   |
|                      |                       |                      | 0.4                   | 0.6                      | 0.49   |
|                      |                       |                      | 0.49                  | 0.59                     | 0.44   |
| Total Ac. g/L in Sulf | 4.65                 | 5.1                  | 4.1                   | 4.3                      | 4.3    |
|                      |                       |                      | 4.3                   | 4.2                      | 4.1    |
|                      |                       |                      | 4.1                   | 4.2                      | 4      |
| pH                   | 3.5                   | 3.4                  | 3.81                  | 3.65                     | ND     |
|                      |                       |                      | ND                    | 3.67                     | 3.64   |
|                      |                       |                      | 3.64                  | 3.72                     | 3.67   |
| Sugars g/L           | 1.9                   | 2.4                  | 1.9                   | 2.3                      | ND     |
|                      |                       |                      | 2.3                   | 1.25                     | 1.4    |
|                      |                       |                      | ND                    | ND                       | ND     |
| Alcohol % Vol.        | 13.2                  | 13                   | 13.2                  | 13                       | ND     |
|                      |                       |                      | 13                    |ND                       | ND     |
|                      |                       |                      | 13                    | ND                       | ND     |
|                      |                       |                      | 13                    | ND                       | ND     |
| OD 420nm             | 9.415                 | 9.44                 | 7.86                  | 7.5                      | 7.04   |
|                      |                       |                      | 6.51                  | 6.8                      | 6.3    |
|                      |                       |                      | 6.3                   | 7.1                      | 7.9    |
| OD 520nm             | 22.075                | 21.84                | 15.4                  | 15.17                     | 12.73  |
|                      |                       |                      | 11.78                 | 10.7                     | 9.7    |
|                      |                       |                      | 9.7                   | 9.3                      | 7.8    |
| OD 620nm             | 4.035                 | 3.985                | 3.53                  | 3.12                     | 2.96   |
|                      |                       |                      | 2.56                  | 3                       | 2.5    |
|                      |                       |                      | 2.5                   | 3                       | 2.1    |
| CI                   | 35.55                 | 35.25                | 26.79                 | 25.79                     | 22.73  |
|                      |                       |                      | 20.85                 | 20.5                     | 18.5   |
|                      |                       |                      | 18.5                  | 19.3                     | 15.9   |
| Total Anthocyanin mg/L | 1710                | 1563.5               | 1061                 | 931                       | ND     |
|                      |                       |                      | 931                   | 577                      | 628    |
|                      |                       |                      | 628                   | 386                      | 351    |
| Polyphenol Index      | 88                    | 88                   | 84.8                  | 81.7                      | 80     |
|                      |                       |                      | 76.2                  | 75                       | 74     |
|                      |                       |                      | 74                    | 69                       | 67     |
Table 2. Aroma compounds produced by *H. vineae* and *S. cerevisiae* of Tannat grapes of two different regions. Results are the average of triplicates and SD. *, ** indicate significance at $p < 0.05$, $p < 0.01$ between the mean.

| Aromatics | Tannat Chapeu | Tannat Melilla |
|-----------|---------------|---------------|
| **Alcohols** | H. vineae + Sc | Sc | H. vineae + Sc | Sc | B | Sc | B |
| 1-propanol | 40290 | 38070 | 2380 | 68600 | 980 | 28100 | 3570 |
| 2-methyl-1-propanol | 113280 | 65340 | 1400 | 47180 | 380 | 42060 | 240 |
| 1-butanol | 129 | 6 | 140 | 0.3 | 251 | 10 | 283 | 10 |
| 3-methyl-1-butanol | 370520 | 263210 | 33350 | 269180 | 3640 | 259340 | 1310 |
| 1-hexanol | 966 | 59 | 920 | 6 | 1585 | 13 | 1478 | 15 |
| 2-ethyl-1-hexanol | 245 | 3 | 22 | 1 | 18 | 1 | 15 | 1 |
| 3-ethyl-1-propanol | 151 | 10 | 435 | 5 | 407 | 6 | 1113 | 6 |
| benzyl alcohol | 80 | 14 | 94 | 4 | 203 | 5 | 165 | 5 |
| 2-phenethyl alcohol | 35076 | 41685 | 2113 | 23844 | 80 | 23298 | 120 |
| methanol | 59510 | 54290 | 2090 | 100310 | 3040 | 84020 | 80 |
| methionol | 919 | 159 | 977 | 238 | 330 | 14 | 401 | 10 |
| **Ethyl Esters** | H. vineae + Sc | Sc | H. vineae + Sc | Sc | B | Sc | B |
| ethyl isobutyrate | 50 | 3 | 33 | 0.03 | 22 | 2 | 37 | 1 |
| ethyl butyrate | 85 | 4 | 72 | 1 | 157 | 4 | 159 | 5 |
| ethyl hexanoic | 175 | 16 | 161 | 2 | 195 | 6 | 239 | 9 |
| ethyl octanoic | 170 | 10 | 132 | 3 | 125 | 3 | 158 | 12 |
| ethyl decanoic | 45 | 3 | 33 | 1 | 43 | 1 | 46 | 1 |
| ethyl lactate | 18610 | 321 | 17079 | 324 | 10211 | 118 | 10450 | 120 |
| ethyl pyruvate | nc | nc | 4 | 1 | 24 | 1 | 40 | 1 |
| diethyl succinate | 3649** | 680 | 1245 | 131 | 616 | 17 | 662 | 16 |
| ethyl succinate | 48837 | 2876 | 59456 | 1431 | 25660 | 380 | 24605 | 200 |
| 2-hidroxi-glutarato de dietilo | 392 | 25 | 388 | 1 | 375 | 12 | 375 | 12 |
| **Acetate Esters** | H. vineae + Sc | Sc | H. vineae + Sc | Sc | B | Sc | B |
| 2-phenylethyl acetate | 134 | 25 | 133 | 21 | 433 | 5 | 193 | 6 ** |
| isobutyl acetate | 60 | 0 | 40 | 0 | 65 | 1 | 74 | 1 |
| isovaleric acid | 788 | 56 | 553 | 52 | 2050 | 45 | 2281 | 43 |
| ethyl acetate | 35810 | 2280 | 32610 | 7630 | 57440 | 1000 | 32930 | 360 ** |
| 1,3-propanediol diacetate | 465 | 78 | 970 | 16 | 467 | 2 | 772 | 20 ** |
| **Acids** | H. vineae + Sc | Sc | H. vineae + Sc | Sc | B | Sc | B |
| butanoic acid | 214.13 | 26.10 | 232.10 | 3 | 354 | 6 | 494 | 7 * |
| hexanoic acid | 664.89 | 97.35 | 755.32 | 47 | 840 | 3 | 956 | 12 * |
| octanoic acid | 845.06 | 143.38 | 950.54 | 20 | 988 | 21 | 1089 | 21 * |
| decanoic acid | 119.85 | 16.64 | 81.42 | 7 | 174 | 13 | 148 | 10 * |
| isobutanoic acid | 1660.65 | 220.26 | 1017.56 | 14 | 954 | 7 | 1466 | 8 |
| isovaleric acid | 619.35 | 13.56 | 979.22 | 50 | 548 | 7 | 637 | 9 |
| **Lactones** | H. vineae + Sc | Sc | H. vineae + Sc | Sc | B | Sc | B |
| γ-butyrolactone | 761 | 47 | 1019 | 112 | 328 | 3 | 441 | 3 |
| pantolactone | 66 | 7 | 80 | 1 | 47 | 2 | 94 | 4 |
| Others | H. vineae + Sc | Sc | H. vineae + Sc | Sc | B | Sc | B |
| 2,3-Butanondione | 152 | 3 | 224 | 12 | 206 | 4 | 250 | 10 |
| 2,3-Pentanondione | 67 | 5 | 65 | 3 | 170 | 3 | 168 | 15 |
| 3-hidroxi-2-butanone | 102 | 12 | 222 | 52 | 98 | 5 | 183 | 14 |
| acetaldelyde | 5250 | 810 | 3330 | 1380 | 8600 | 600 | 2370 | 310 ** |
| guaiaol | 102 | 16 | 116 | 8 | 220 | 25 | 88 | 6 ** |
| 4-vinyl-guaiaol | 119 | 12 | 225 | 41 | 133 | 3 | 103 | 10 |
| 2,6-dimethoxy phenol | 379 | 16 | 592 | 16 | 867 | 51 | 438 | 15 |
| methyl vanillylther | 123 | 11 | 166 | 6 | 158 | 2 | 156 | 12 |
| 4-(4-hidroxy-3-methoxy-phenyl)butan-2-ol | 11 | 0 | 76 | 65 | 17 | 1 | 18 | 1 |
| zingerone | 3 | 0 | 4 | 0 | 12 | 0 | 10 | 0 |
| ethyl-b-(4-hidroxy-3-methoxy-phenyl-propionate | 7 | 0 | 10 | 1 | 28 | 0 | 19 | 1 |
| 3-oxo-α-ionol | 29 | 2 | 30 | 0 | 51 | 0.20 | 37 | 3 * |
| vanilidol | 38 | 9 | 58 | 5 | 55 | 1 | 43 | 2 * |
Table 3. Aroma compounds produced by the 3 treatments of Petit Manseng barrel fermented. Results are the average of triplicates and SD. *, ** indicate significance at *\(p < 0.05\), **\(p < 0.01\) between the mean.

| Alcohols | Spontaneous SD | Sacch SD | H. vineae SD |
|----------|----------------|----------|--------------|
| 1-propanol | 181 43.9 | 220 13.5 | 188 26.1 |
| 2-methyl-1-propanol | 3412 42.0 | 4507 10.8 | 2649 16.1 ** |
| 1-butanol | 360 42.8 | 376 13.9 | 395 15.5 |
| 3-methyl-1-butanol | 71093 28.7 | 67586 13.2 | 65990 9.2 |
| 3-hydroxy-2-butane | 235 56.6 | 145 47.6 | 119 16.8 ** |
| 1-hexanol | 785 21.0 | 831 8.0 | 795 7.2 |
| 3-hexen-1-ol | 29 19.8 | 33 9.9 | 32 5.0 |
| 2-propanol | 20 14.4 | 16 25.7 | 20 11.8 |
| 2-ethyl-1-hexanol | 48 13.2 | 37 21.9 | 52 39.6 |
| 2-furanmethanol | 24 21.7 | 35 14.6 | 34 42.0 |
| benzyl alcohol / benzenemethanol | 49 8.8 | 44 22.2 | 40 23.5 |
| benzeneethanol / Phenethyl alcohol | 14757 11.3 | 12810 15.9 | 12135 15.3 |
| tyrosol | 4546 16.2 | 4738 22.3 | 2747 21.5 ** |
| 3-methylthio-1-propanol | 340 16.9 | 324 14.0 | 300 30.8 |
| Esters | | | |
| ethyl isobutirate | 84 43.6 | 108 13.9 | 109 22.0 ** |
| acetate 3-methyl-1-butanol | 1248 33.9 | 987 34.9 | 953 20.2 |
| hexanoic acid ethyl ester | 248 13.1 | 245 16.2 | 283 9.4 |
| hexyl ester acetic acid | 84 28.7 | 54 63.1 | 52 25.4 |
| 2-hydroxy-propanoic acid | 23660 18.2 | 20447 30.2 | 24202 5.7 |
| ethyl ester octanoic acid | 528 14.1 | 549 4.9 | 625 7.7 |
| ethyl ester 3-hydroxybutanoic acid | 125 18.7 | 139 8.4 | 93 12.0 |
| ethyl ester decanoic acid | 228 11.8 | 222 12.8 | 247 12.3 |
| diethyl succinate | 3222 22.1 | 3239 16.4 | 2099 24.0 ** |
| acetato de etilo | 17960 17.7 | 27820 22.1 | 42077 23.3 ** |
| 1,3-propanediol acetate | 958 13.0 | 742 13.8 | 1065 12.9 ** |
| ethyl hidroxy butyrate | 4429 11.5 | 4041 19.6 | 3357 14.9 ** |
| 2-phenethyl acetate | 281 18.0 | 259 25.2 | 229 22.1 |
| butanedioic acid , hydroxy diethyl ester | 600 12.3 | 633 21.3 | 614 15.1 |
| ethyl succinate | 58273 21.4 | 52686 11.5 | 32247 18.0 |
| Acids | | | |
| acetic acid | 726 23.9 | 711 14.8 | 1211 13.3 ** |
| propanoic acid | 39 16.1 | 27 16.5 | 38 22.8 |
| isobutanoic acid | 451 5.7 | 476 10.0 | 365 9.4 |
| butanoic acid | 532 12.8 | 564 10.8 | 576 7.0 |
| isovaleric acid | 348 9.6 | 363 9.6 | 336 11.5 |
| hexanoic acid | 3786 19.5 | 3685 13.6 | 3629 13.6 |
| octanoic acid | 7649 11.7 | 7373 16.5 | 6444 12.9 |
| decanoic acid | 2578 18.2 | 2530 25.6 | 2421 19.0 |
| dodecanoic acid | 613 26.7 | 415 10.2 | 418 48.1 |
| other | | | |
| g-butyrolactone | 565 22.4 | 521 20.9 | 541 16.1 |
| Pantolactone | 91 11.6 | 80 13.0 | 96 22.8 |
| Sum of aroma compounds | | | |
| Alcohols | 76268 29.1 | 73929 12.6 | 70421 9.2 * |
| Esters | 7131 3.6 | 6465 10.0 | 5855 8.8 * |
| Fatty acids | 15196 10.7 | 14594 15.2 | 13525 12.6 * |
Figure 1. Malolactic fermentation is stimulated by *H. vineae* fermentations in Tannat. Similar results were obtained with barrel fermentation of Petit Manseng.

Figure 2. Application protocol for vinification in white and red wine with *H. vineae* strains.

Acidity and final SO2. We found that the application of *H. vineae* facilitates the development of native yeast diversity during fermentation and also the malolactic fermentation MLF bacteria are stimulated as shown in Fig. 1. This result is in agreement with our previous work with white wine barrel fermented Chardonnay [6]. Similar results were obtained here also for white Petit Manseng barrel fermented. In Table 2, we present the data for Tannat vinificated in two different regions. Data are the average of triplicates, and from 47 compounds determined, 16 were significantly affected compared to the pure *S. cerevisiae* commercial strain utilized. Here we can find some consistent results with our white wine previous experiences with Chardonnay [6] and Macabeo [7]. Benzyl alcohol, 2-phenylethyl alcohol and ethyl acetate, guaiacol and an increase of norisoprenoids in the Tannat of Melilla were detected. Sensory analysis of the Tannat treatments showed an increase of fruity and caramel descriptors in *H. vineae* compared to the most obvious oak and herbal flavors of the Sacch treatment. In Table 3, the flavor compounds analysis for Petit Manseng barrel fermented is shown and from 42 compounds determined 9 were significantly affected (*p* < 0.01) compared to the conventional yeast treatment. Although it was demonstrated previously that 2-phenylethyl acetate and benzenoids were the main synthetize compounds by *H. vineae* in Chardonnay and Macabeo [6, 7]. Results for Petit Manseng showed a similar behavior for these compounds than with the conventional yeast treatment. Figure 2 shows the application protocol defined as successful to apply in red and white wine winery scale production. Sluggish fermentations are avoided by a
rational nutrient complementation of the co-inoculated strain at day 5 or 6 of the process. This is a simple operation that allowed the Sacch strain to obtain limited nutrients that were removed by *H. vineae* initial activity as it was demonstrated [15].

4. Conclusions

*H. vineae* showed oenological capacities for production of white and red wines, resulting in more complex sensory wines. Its moderate competitive capacity during vinification compared to Saccharomyces strains, help to understand the concept of “friendly yeast” that allowed to increase microbial diversity in the process. The nutrient management when sequential inoculation of *S. cerevisiae* is done should be the key activity to avoid a sluggish fermentation. It was shown that for Tannat wines the vinification process without addition of sulfites before fermentation and with sequential inoculation of *S. cerevisiae* after 6 days, resulted in wines of similar basic quality parameters than conventional vinification methods in terms of alcohol and volatile acidity. We presented an easy vinification protocol for making real wine experiments at the winery with or without commercial strains addition.

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