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Test-tube vinification: a methodology for high throughput oenotyping of white wines obtained from grapes harvested from single vines

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Grape aroma has been a key factor in the selection of grapevine varieties for centuries[1] and remains a crucial determinant of wine quality. However, understanding grape composition alone is not sufficient to accurately predict the quality and characteristics of the resulting wine. While numerous studies have explored the genetic basis of grape aroma biosynthesis[2], and despite growing knowledge about wine composition, the genetic basis of wine aroma compounds diversity remains relatively unexplored. To investigate the relationship between genetic variation and aroma compounds across a large number of grape genotypes, we developed a small-scale, high-throughput test-tube vinification (TTV) method to produce white wines from single-vine stocks.

Background and aims of the study

Experimental wines are typically produced in 10 to 25 litre carboys, but this scale is not workable for genetic studies involving hundreds of genotypes. A simple, standardised method that can be applied on a much smaller scale is therefore needed in order to enable high-throughput oenotyping of white wines. To address this issue, we developed a specific protocol for producing white wines from less than 100 ml of must. We evaluated the TTV method on commercial grapevine varieties and genotypes from a bi-parental progeny, covering a wide aromatic palette. The wines were analysed using standard oenological parameters and GC-MS profiling of volatile compounds.

Small-scale and high-throughput vinification of white wine

One to 2.5 kg of grapes were harvested from a selection of commercial grapevine varieties (Riesling, Gewurztraminer, Chardonnay, Chasselas, Floreal and Muscat à Petits Grains Blancs) and four genotypes selected from the progeny of a bi-parental cross between Riesling and Gewurztraminer[3], representing a wide aromatic palette. The juices were obtained after pressing the berries using an Automatic Sieve C80® (ROBOT COUPE SNC, Vincennes, France) and 6 g SO_2/hl were added before centrifugation (3500 rpm, 10 min). After 24 h of decantation at 10 °C and racking, the clarified musts were supplemented with i) rectified concentrated must to obtain a final 12.5% alcohol concentration, and ii) diammonium phosphate to obtain 200 mg/l of assimilable nitrogen. The musts were inoculated with the yeast strain Levulia® GC (AEB-France, Sigolsheim, France). The must of each variety was then distributed among five 85 ml tubes for the fermentation phase, which was performed in a temperature-controlled environment at 22 °C (Figure 1). At the end of fermentation, the wines obtained were stabilised with 8 g SO_2/hl and stored in a cold room at 10 °C before analysis.

An area of 0.3 m² was required to run 50 TTVs. Two people handled the pre-fermentation operations, including destemming, crushing, juice extraction, settling and yeasting. The vinification process was monitored by one person alone.

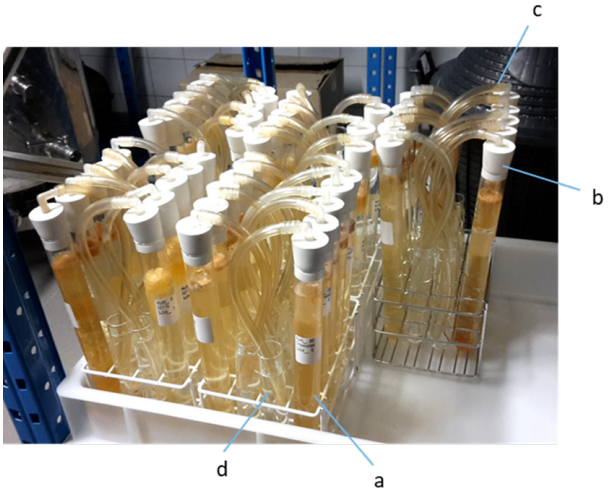


FIGURE 1. Experimental set-up of test-tube vinification. 85 ml of must were transferred to 100 ml glass tubes (a) sealed with a rubber stopper (b) fitted with a flexible bubble tube (c) the end of which was immersed in a tube of water (d) to prevent oxidation of the wine during fermentation.

Evaluation of oenological parameters

All the fermentations carried out using the TTV method almost reached dryness within 25 days. The values of the oenological parameters (pH, residual sugar and alcohol content) of the resulting wines were in line with the usual oenological standards (Table 1).

TABLE 1. Means ± standard deviations (n = 5, except for Muscat, n = 3) for oenological parameters of the wines obtained by test-tube vinification (TTV).

Variety or Genotype	pH	Glucose + Fructose (g/l)	Alcohol (% v/v)
Chardonnay	$3.30^{d*} \pm 0.02$	$1.44^{bcd} \pm 1.52$	$13.8^{ab} \pm 0.25$
Floreal	$3.53^{b} \pm 0.02$	$0.44^{cd} \pm 0.31$	$12.6^{ab} \pm 0.88$
Gewurztraminer	$3.65^{a} \pm 0.04$	$0.94^{cd} \pm 0.68$	$15.0^{a} \pm 1.27$
Riesling	$2.82^{g} \pm 0.02$	$4.42^{a} \pm 2.28$	$13.0^{ab} \pm 0.85$
Chasselas	$3.39^{c} \pm 0.02$	$0.30^{d} \pm 0.00$	$11.0^{b} \pm 1.67$
Muscat à petits grains blancs	$3.03^{e} \pm 0.01$	$1.43^{bcd} \pm 1.04$	$14.2^{ab} \pm 0.86$
0074E	$3.31^{d} \pm 0.02$	$3.26^{abc} \pm 1.41$	$15.0^{a} \pm 1.22$
0211E	$3.05^{e} \pm 0.02$	$4.02^{ab} \pm 2.4$	$15.1^{a} \pm 0.72$
0238E	$2.92^{f} \pm 0.02$	$1.04^{cd} \pm 1.26$	$13.2^{ab} \pm 2.87$
4095G	$2.90^{f} \pm 0.02$	$0.78^{cd} \pm 0.69$	$15.1^{a} \pm 0.47$

*Different letters indicate significant differences at $p \leq 0.05$, according to Tukey's honest significant difference test.

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Discrimination of wines based on their volatile fingerprints

In order to investigate the ability of TTV to discriminate between varieties, we performed a profiling of volatile compounds. Volatile compounds were extracted from three ml samples of each wine using headspace solid-phase microextraction (HS-SPME) and analysed by gas chromatography-mass spectrometry (GC-MS). A total of 108 volatile compounds from different chemical families were identified. These included fermentation-derived aroma compounds, such as esters, volatile fatty acids and higher alcohols, as well as varietal compounds, such as monoterpenes, C13-norisoprenoids and C15-sesquiterpenes. Some benzenoids, aldehydes, ketones and lactones were also detected. Volatile thiols were analysed using a specific extraction method described by Thibon *et al*[4].

The results, summarised in Figure 2, demonstrate that the TTV method can discriminate grapevine varieties on the basis of their volatile fingerprint. Intensely flavoured Muscat-like varieties (Muscat, 0074E) show positive loadings on both Axis 1 (Dim1) and Axis 2 (Dim2) (Figure 2A) and show high levels of monoterpenols, such as linalool and α-terpineol (Figure 2B). Genotype 4095G is characterised by a high positive coordinate on Dim1 and a negative coordinate on Dim2, together with high levels of monoterpenols. These varietal compounds contribute to the floral, fruity and citrus characteristics of these wines[5]. Non-Muscat aromatic varieties, such as Riesling and Gewurztraminer, with negative loadings on both Dim1 and Dim2, are separated from non-aromatic grape varieties, such as Chasselas and Chardonnay, with positive loadings on Dim1 and negative loadings on Dim2. Gewurztraminer showed high levels of terpenols, in particular the highest citronellol content of all the varieties studied. Riesling and the 0238E genotype had the highest levels of TDN, a C13-norisoprenoid compound known for its petroleum-like scent or 'wet stone' odour in young Riesling wines, which significantly contributes to the aromatic typicality of this variety[6 7]. High concentrations of 3-sulfanylhexan-1-ol (3SH) were found in Gewurztraminer and 4095G, with particularly high levels in 0074E. In contrast, 4-methyl-4-sulfanylpentan-2-one (4MSP) was only detected in Floreal and 0074E. These volatile thiols are appreciated for their pleasant fruity aromas, reminiscent of blackcurrant buds, passion fruit and grapefruit.

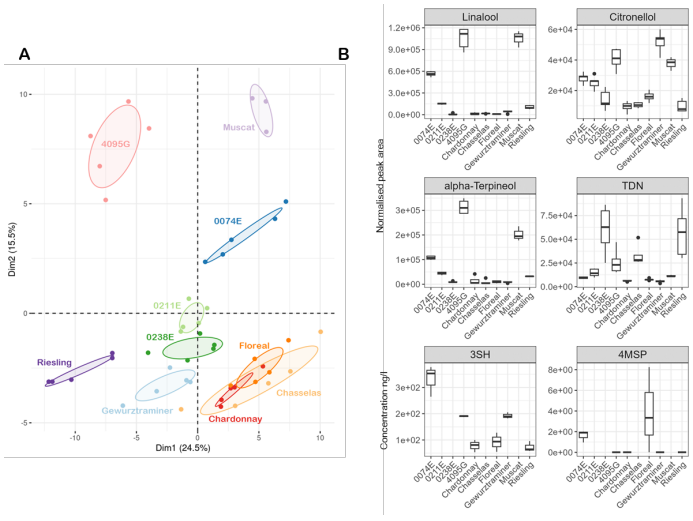


FIGURE 2. Profiling of volatile compounds in wines produced by test-tube vinification (TTV). (A) Principal component analysis (PCA) illustrating the discrimination of wines based on grapevine varieties. (B) Variation of selected significant volatile compounds in the different wine samples. TDN: 1,1,6-trimethyl-1,2-dihydronaphthalene, 3SH: 3-sulfanylhexan-1-ol, 4MSP: 4-methyl-4-sulfanylpentan-2-one.

Conclusion

Our study demonstrates that the TTV method is highly effective for producing white wines on a very small scale (i.e., less than 100 ml), while faithfully reflecting the aromatic potential of different grape varieties. This high-throughput, small-scale vinification method enables the production of hundreds of white wines, allowing the oenological potential of numerous grapevine genotypes to be assessed from the harvest of a single vine. This wine-focused direct phenotyping approach was designed to pave the way toward the scientific genetic determinism of wine aromas, particularly for compounds not directly present in the grapes; it also opens up new avenues for improving the breeding of grape varieties with desirable characteristics.

In addition, this technique offers significant advantages for wine industry professionals, such as winemakers, and R&D departments in wine production, as it provides a rapid and resource-efficient means for evaluating fermentation techniques or treatments on a small scale before large-scale implementation. This method can potentially be used to study fermentation dynamics, explore the oenological potential of yeast strains, assess wine stability and study the impact of different viticultural practices and environmental factors on wine quality. ■

Sources : *Sourced from the research article: 'A test-tube vinification method for high-throughput characterisation of the oenological and aromatic potential of white wines' (OENO One, 2024).*

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