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Automated red microvinification (1kg) adapted to the needs of varietal innovation

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Abstract. The development of disease-resistant grape varieties adapted to climate change is crucial for the future of the viticulture and winemaking industry. Currently, the selection of these varieties mainly relies on genetic resistance markers and agronomic criteria. However, incorporating oenological criteria at the early stages of selection could accelerate the process and make the selection more efficient. This requires vinifying small quantities of grapes (1 kg) at high throughput, in a repeatable and robust manner. The automated Vinimag system, along with the developed microvinification process, meets this need. It enables the simultaneous online monitoring of 60 fermentations through weight measurements and color assessment using reflectance. Temperature is controlled individually for each fermentor with a precision of +/- 0.3°C. This system allows for the calculation of fermentation and color extraction rates and can perform up to 1,000 deferred fermentations annually through grape freezing. Comparative trials with traditional vinification methods have validated the effectiveness of this process. This approach offers various applications, from varietal selection to yeast studies, allowing for the assessment of the oenological profile of new grape varieties.

1. Introduction

In a context of digital transformation and climate change, having well-controlled, high-throughput winemaking tools and methods is essential for research in oenology and viticulture. Winemaking suitability is an essential criterion in the varietal selection process as well as disease-resistant and adapted to climate change grape varieties. However, winemaking is the last step in the long varietal selection process because it requires a high quantity of grapes. Accelerating this process by integrating relevant oenological quality markers requires robust and automated winemaking tools and methods adapted to small volumes (1kg/1L). These small-scale winemaking equipments enable high-throughput winemaking and contributes to the oenological phenotyping of grape varieties, called "Oenotyping." Currently, variety evaluations are conducted during 100L vinifications, designed to be representative of industrial-scale winemaking. This experimental scale requires a sufficient availability of grapes and significant labor. Winemaking setups at the laboratory scale, below 100L in liquid phase, suitable for white and rosé wines, are already available [1-2]. Numerous publications [1-9] reflect the interest in reducing the scale of vinification to increase the throughput and number of fermentations needed to set up research programs in varietal innovation, viticulture and oenology. Studies have been carried out to compare polyphenol extractions during vinification of small volumes (250mL, 1L, 1.5L, 4L and 20L) compared with larger volumes (360L and 780L) [7-8]. During these studies, various containers were used as fermenters: beakers, glass jars, piston coffee makers or plastic buckets [4, 6, 8]. The use of piston coffee maker [4] makes it easy to immerse the cap and 'punch it down' once or twice a day, without having to open the fermenter [6]. In other studies, the cap is kept immersed throughout fermentation by means of a plastic plate [7-8]. In some experiments, measurements are taken only at the end of fermentation to confirm the absence of residual sugars [5, 8]. Finally, when

fermentation is complete, the solid and liquid phases are separated. This separation is easy for coffee makers with a plunger, where the plunger is held in the lowered position. In other cases, the cap can be filtered [7], or pressed by hand, using a fiberglass sieve [8-9]. In all cases, the musts are yeasted and the fermenters are placed in temperature-controlled rooms at around 25°C-30°C. These various experiments demonstrate the feasibility and benefits of using small-volume fermentations, but these tools are not automated. Moreover, the various stages in the whole process limit the number of fermentations carried out in parallel, and therefore the possibility to test many varieties or get reliable results.

Designing a complete process centered on an automated system for conducting small-scale (<1 kg) vinifications suitable for studying red wines (pre-fermentation phases, heterogeneous phase fermentation, pressing) was a real technical challenge. The aim of this article is to showcase the performance of the automated winemaking system for red wines.

2. Materials and methods

2.1. Grapes and fermentation conditions used in the study on the effect of freezing

Two *Vitis vinifera* red grape varieties, Grenache noir and Carignan, were harvested from vineyards of the INRAE experimental domain of Pech Rouge, Gruissan, France at an average potential alcohol of 12% vol., in 2018. The berries were sorted as described earlier [10] then split into homogeneous and heterogeneous groups. Two criteria were selected: the first one was the volume of the berry, low or high; the second one was the sugar content, sorted by their apparent density to obtain low or high alcoholic degrees. The small-lot fermentations were performed from an adaptation of the AWRI method [4] using “French Press” coffee plunger. The pilot scale fermentations were performed in 100L stainless fermenters. A total of 9 batches of Grenache noir and Carignan grapes covering a wide range of maturity levels (acidity, alcohol) were vinified and compared in triplicate before and after freezing (52 samples).

2.2. Grapes and fermentation conditions used for the comparison of the 1kg/100kg processes

Grapes from *Vitis vinifera* var. Grenache (GRE1, GRE2), Syrah (Syr), Merlot (Mer) and Cabernet Sauvignon (CabS) grown in southern France (30 samples) were used for this study. Each variety was harvested at two stages of maturity (M1 and M2). For each modality, around one hundred kilograms of grapes were destemmed, crushed and distributed into 100L stainless steel tanks in triplicate. 900 grams of the destemmed grapes were sampled and kneaded by a lab blender (Jumbomix, Interscience). The small-lot were frozen and fermentations were performed in triplicate from an adaptation of the AWRI method [4] using “French Press” coffee plunger. The same yeast was used for all fermentations conducted in triplicate.

2.3. Automated microvinification (1kg) for red wines

2.3.1. Sampling and storage of grapes

During the harvest, the grapes were destemmed (Cube, Socma) and batches of 1 kg of grapes were frozen in 6 hours in a rapid cooling system, then vacuum-packed (figure 1) and stored at -20°C until vinification. Rapid freezing leads to the creation of small ice crystals, thereby reducing damage to the cell walls grapes. As a result, this method produces better quality grapes than slow freezing.

Figure 1. Grapes inside the freezing cell (left) and vacuum-packed after freezing (right).

Together, these batches made up a Grape Bank, enabling vinification to be deferred throughout the year, for up to 1,000 fermentations a year.

2.3.2. Pre-fermentation stages

Figure 2. Grapes kneaded in a bag (left) then transferred to a fermenter (right).

After defrosting, grapes were kneaded (Jumbomix, Interscience) and transferred directly to the fermenters (figure 2). 60 musts were then rapidly prepared and vatted (figure 3) in fermenters adapted to our automated fermentation system.

Figure 3. Fermenters after vatting 1kg of kneaded grapes.

2.3.3. Fermentation and automated monitoring on Vinimag

Figure 4. Vinimag : Automated fermentation system.

Vinimag (ISP Aquitaine, Figure 4) is the central tool for the kilogram-scale vinification process. This collaborative robot monitors the vinification of 1 kg of grapes in 60 fermenters. The 60 stations are temperature-controlled by 3 infra-red sensors positioned at three levels of the fermenter (top, middle, bottom). They are independently regulated by the cold of the room and their heating belt.

Figure 5. Vinimag measuring station: weighing and colour.

Fermentation kinetics are monitored by weighing and extracting colour by reflectance in L*, a* b* at one-hour intervals (Figure 5). An automated addition station allows the incorporation of inputs as fermentation progresses. Juice/skin homogenisation is achieved by means of a piston that keeps the pomace submerged during fermentation.

2.3.4. Pressing

A pressing device (Nano'Press, Socma) with 9 stations and an ingenious double piston system have been developed, enabling 9 batches to be pressed *in situ* in the fermenters at the same time and in a reproductible manner (Figure 6).

Figure 6. Nano'Press : 9-station pressing system.

The 60 fermenters can be pressed in less than 4 hours, with a pressing yield (volume of wine/weight of grapes) between 60 and 65%.

2.4. Analysis of wines at the end of alcoholic fermentation

2.4.1. Standard oenological analyses

Measurements of oenological parameters (alcohol, pH, total acidity), of extraction and color indices (anthocyanins, total polyphenol index (TPI), color intensity (CI)) were carried out on wines at the end of alcoholic fermentation following the official methods [11].

2.4.2. Statistical analysis

Statistical analysis of data discrepancies and sampling robustness are assessed using the Root Mean Square Error (RMSE), the Mean Absolute Error (MAE) and the Mean Absolute Percentage Error (MAPE).

3. Results and discussion

3.1. Process performance

3.1.1 Comparison 1kg/100kg

Trials on several varieties (5) harvested at 2 ripening dates in triplicate (30 samples), were compared after vinification conducted at 1kg and 100kg scales. As presented in Table 1, differences between oenological parameters (alcohol, pH, total acidity) obtained at the 2 scales were low. This result showed that the 1kg sampling was correct and representative of the 100 kg scale.

Table 1. Root Mean Square Error (RMSE), Mean Absolute Error (MAE) and Mean Absolute Percentage Error (MAPE) calculated on the data (pH, total acidity and alcohol) of the wines from the 2 processes (1kg/100kg).

Number of samples	30		
	Alcohol	pH	Total Acidity
RMSE (Root Mean Square Error)	0.42	0.18	0.42
MAE (Mean Absolute Error)	0.36	0.11	0.32
MAPE (Mean Absolute Percentage Error)	2.6%	2.9%	8.3%

Between the two scales, the parameters CI (colour intensity), Anthocyanins and TPI (total polyphenol index) analysed at the end of alcoholic fermentation (figure 6.a,b,c) are strongly correlated (R^2=0.95, 0.99, 0.94 respectively). Red microvinification (1kg) makes it possible to classify wines from different varieties and maturities in the same order as the ranking obtained for pilot-scale red wine fermentation (100kg) in regard to the parameters of extraction.

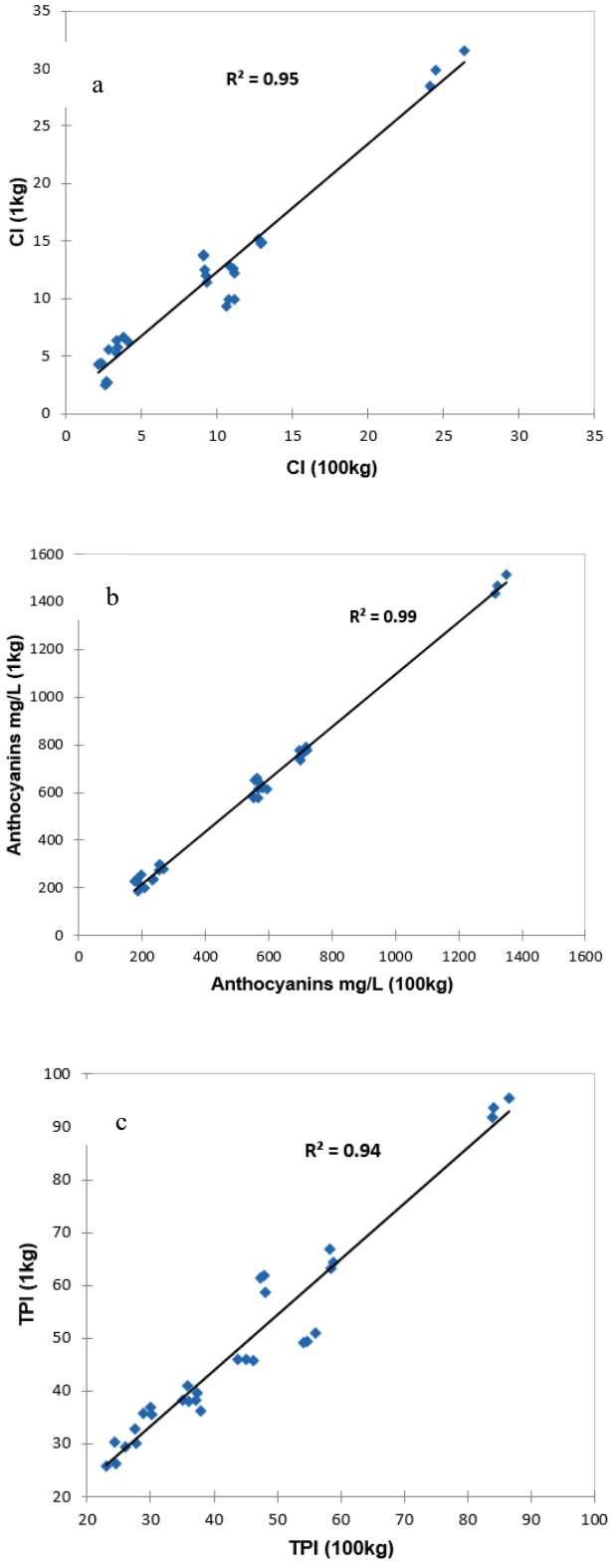

Figure 6. Correlation of colour intensity (a), anthocyanins (b) and total polyphenol index (c) between wines from 1kg and 100kg fermentations.

3.1.1. Impact of freezing

For the parameters CI (colour intensity), Anthocyanins and TPI (total polyphenol index) analysed at the end of alcoholic fermentation (figure 7.a,b,c), strong correlations (R^2=0.99, 0.83, 0.82 respectively) were noted between

fresh and frozen conditions. Correlations are lower for pH and total acidity than for alcohol content. This observation can be explained by the fact that freezing induced precipitation of tartaric salts impacting the total acidity of the wine produced.

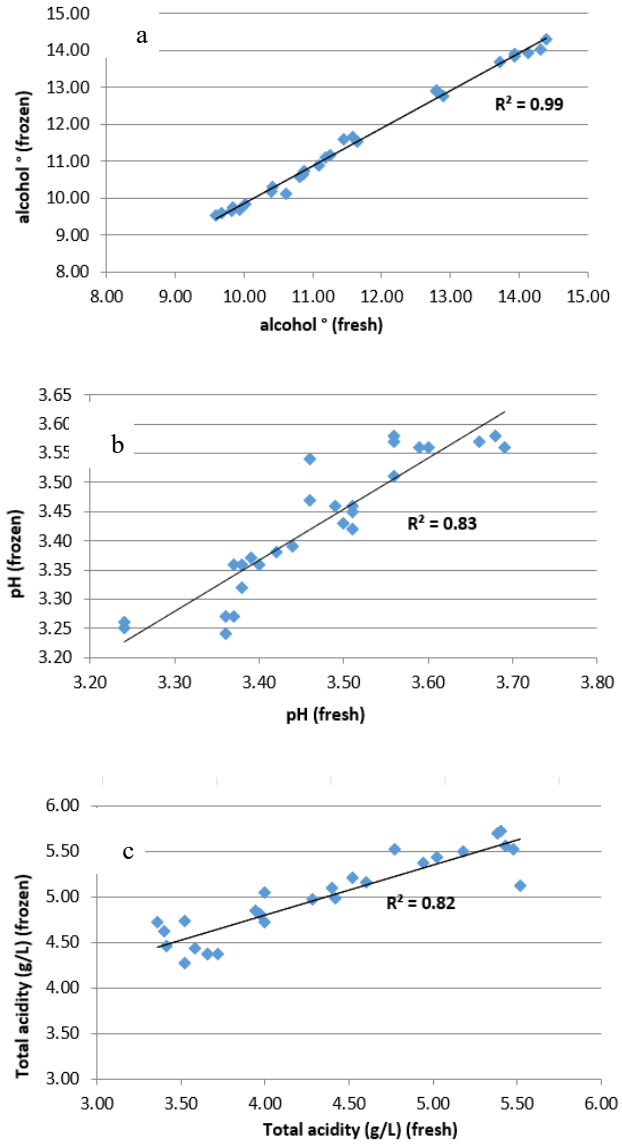

Figure 7. Correlations of the parameters alcohol (a), pH (b) and total acidity (c) between wines obtained from fermentation of frozen and fresh grapes.

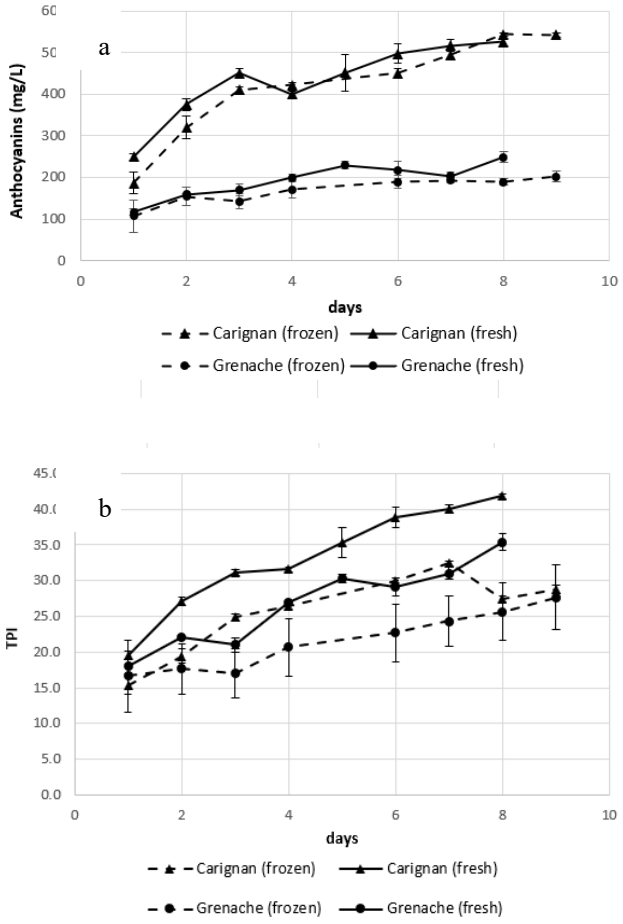

Figure 8. Monitoring of anthocyanins (mg/L) (a) and TPI (b) during alcoholic fermentation of Grenache and Carignan must from frozen (- -) and fresh (-) grapes.

Anthocyanin and TPI monitoring (figure 8) exhibited the impact of freezing during alcoholic fermentation. Indeed, TPI showed lower values after freezing due to the oxidative degradation of polyphenols [13]. Nevertheless, the ranking of modalities has been maintained between the two scales, with higher values for Carignan than Grenache.

3.2. Automated device performance

3.2.1. Temperature control and fermentation monitoring

During red fermentation, the fermenters have a heterogeneous phase, unlike white and rosé vinification, which takes place in a homogenous, liquid medium. The three temperature sensors on Vinimag provide a better understanding of the temperature differentials between marc and wine during the different phases of fermentation. The system provides independent, robust regulation within +/-0.3°C (example figure 9a). The speed of movement of the arm enables the weight and colour of each of the 60 fermenters to be measured every 70 minutes. This frequency of measurement is sufficient and necessary (after smoothing the weight data) to calculate the release of CO_2 (example figure 9a), and the instantaneous fermentation rate calculated by the derivation of the production of CO_2. Then, the fermentation kinetics can be

plotted (example figure 9b) with a good repeatability like those of the liquid phases according to the method of Sablayrolles and Barre [14].

3.2.2. Colour monitoring

Colour extraction is mainly monitored by measuring the tristimular coordinates L*a*b* (L*, from white to black, a* from green to red, b* from blue to yellow) reflecting the perception of colour by the human eye. In particular, the a* coordinate reflects the red component of the wine's colour. The curve for a* is marked by punching down (arrow) and a sharp decrease during fermentation, before stabilising at the end of fermentation, on the example shown in Figure 9c. We are currently working on statistical processing of the a* signal curve (red component) to establish a colour extraction rate as a function of alcohol production during fermentation.

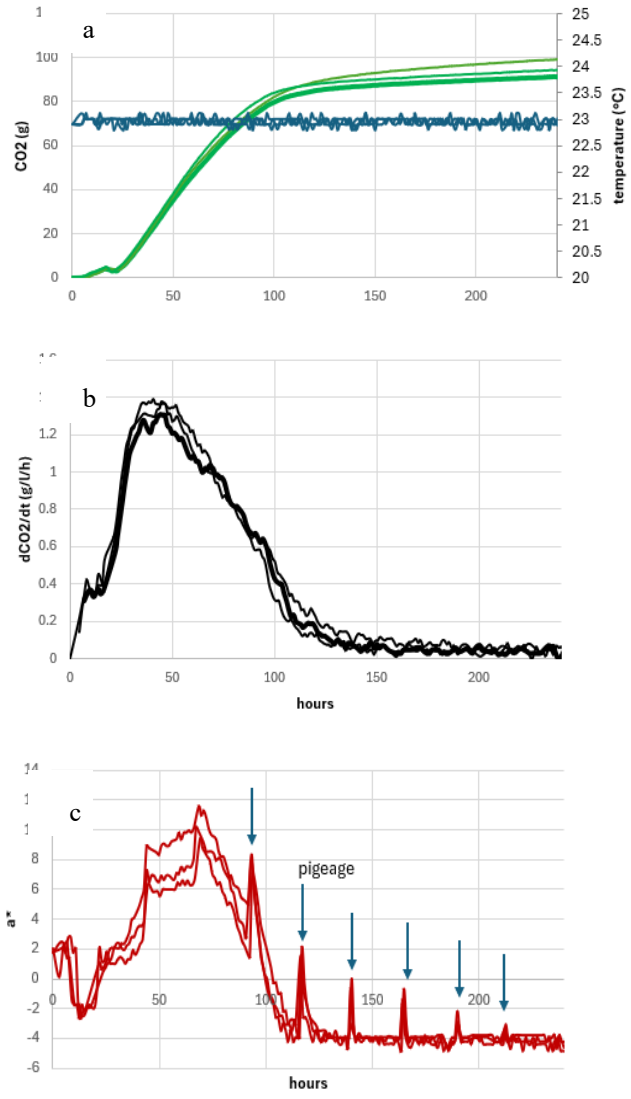

Figure 9. Example of fermentation monitoring (triplicate) during 240 h (10 days) using Vinimag: CO_2 and temperature (a), fermentation kinetics (b), coordinate a* by reflectance (c).

3.3. Applications to the oenological evaluation of varieties

High-throughput analytical methods have also been developed to study the phenolic profile of wines made from new varieties and traditional grape varieties. An untargeted metabolomics approach was conducted to highlight the polyphenol content discriminating the samples. The wine samples from Vinimag fermentations were analyzed by ultra-high performance liquid chromatography-−high-resolution orbitrap mass spectrometry (UHPLC−HRMS), as described by Leborgne and co-workers [14]. MS data were processed by Compound Discoverer software (v. 3.2.2.421, Thermo Scientific, Waltham, MA, USA) to produce features (i.e. variables). Each unique combination of retention time and mass (i.e. deconvoluted mass-to-charge ratio) corresponds to a feature. The features were identified as compounds by comparison with our in-house specialised polyphenol database. Each of the 117 features were integrated for each sample, and a dataset is produced for statistical analysis. Supervised and unsupervised data analysis (PCA, hierarchical clustering…) showed that the expected polyphenol families were grouped into clusters of diglycoside anthocyanins, monoglycoside antocyanins, flavanols and flavonols. An example of an application of our methods (automated red microvinifications and metabolomics) was presented to the OIV congress about the study of the oenological potential of varieties resistant to cryptogamic diseases and drought to anticipate varietal selection in Occitanie [15].

4. Conclusions and perspectives

The applications of the Vinimag are many and varied, ranging from varietal selection to the screening of yeast strains and evaluation of nutrient addition strategies. Knowledge of the ability of new varieties to be transformed into wine will provide the keys to understand what is needed to adapt winemaking conditions to a given raw material. Thanks to their performance and speed in monitoring fermentation parameters (temperature, fermentation kinetics and colour), small-volume red winemaking tools and methods developed provide robustness and confidence in the results obtained from experiments carried out under such controlled conditions. This automated system meets the needs for high throughput and performance that varietal and oenological innovation requires to think about the vines and wines of tomorrow and meet the challenges of the wine sector.

5. Acknowledgments

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