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Rapid screening of the fermentation profiles of wine yeasts by Fourier transform infrared spectroscopy

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Abstract

A rapid screening method for the evaluation of the major fermentation products of *Saccharomyces* wine yeasts was developed using Fourier transform infrared spectroscopy and principal component factor analysis. Calibration equations for the quantification of volatile acidity, glycerol, ethanol, reducing sugar and glucose concentrations in fermented Chenin blanc and synthetic musts were derived from the Fourier transform infrared spectra of small-scale fermentations. The accuracy of quantification of volatile acidity in both Chenin blanc and synthetic must was excellent, and the standard error of prediction was 0.07 g l^{-1} and 0.08 g l^{-1}, respectively. The respective standard error of prediction in Chenin blanc and synthetic musts for ethanol was 0.32% v/v and 0.31% v/v, for glycerol was 0.38 g l^{-1} and 0.32 g l^{-1}, for reducing sugar in Chenin blanc must was 0.56 g l^{-1} and for glucose in synthetic must was 0.39 g l^{-1}. These values were in agreement with the accuracy obtained by the respective reference methods used for the quantification of the components. The screening method was applied to quantify the fermentation products of glycerol-overproducing hybrid yeasts and commercial wine yeasts. Principal component factor analysis of the fermentation data facilitated an overall comparison of the fermentation profiles (in terms of the components tested) of the strains. The potential of Fourier transform infrared spectroscopy as a tool to rapidly screen the fermentative properties of wine yeasts and to speed up the evaluation processes in the initial stages of yeast strain development programs is shown.
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Keywords: Wine yeasts; FT-IR spectroscopy; Principal component factor analysis

1. Introduction

The development of improved yeast strains is important in the food and wine industries. This often involves the screening of large numbers of natural yeast isolates in order to select desirable variants within a population of yeast strains, or alternatively, the evaluation of variants of established yeasts that have been optimized for specific properties (Pretorius, 2000; Dequin, 2001). Projects of this nature are typically very time-consuming requiring large amounts of analytical work. Recently, the wine industry has been under pressure to accelerate the development of improved yeast strains to meet the needs of the current market-orientated transformation of the industry

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(Pretorius, 2000; Pretorius and Bauer, 2002). Therefore, methods and technological innovations that can rapidly evaluate the fermentative properties of yeast strains, at least in the initial stages of strain selection, would be major advantages.

The potential of Fourier transform infrared (FT-IR) spectroscopy to analyze wine has recently attracted attention (Patz et al., 1999; Dubernet and Dubernet, 2000; Gishen and Holdstock, 2000; Kupina and Shrikhande, 2003). This technology is based on the measurement of the absorbance of radiation in the mid infrared region (4000–400/cm) by molecules that contain chemical bonds such as C−C, C−H, O−H, C=O and N−H (Smith, 1999). The concept of calibration, which is widely used in analytical chemistry, also applies to FT-IR spectroscopy, and in order to predict the concentration of a component of interest, a predetermined calibration for the component is required. Due to the complexity of the information contained in the FT-IR spectra, an extensive calibration process that involves multivariate statistical procedures such as principal component analysis, principal component regression and partial least squares regression is required (Eriksson et al., 1999; Esbensen, 2000; Næs et al., 2004). To date, the application of FT-IR spectroscopy as an analytical tool in enology has largely been focused on the routine analysis of wine (Patz et al., 1999; Dubernet and Dubernet, 2000; Gishen and Holdstock, 2000; Kupina and Shrikhande, 2003) and commercial calibrations (referred to as global calibrations) for a limited number of components are provided with the instrument and can be adjusted using multivariate data analysis techniques to provide the best possible quantification accuracy.

This study reports on the use of principal component factor analysis in conjunction with FT-IR spectroscopy to rapidly screen the fermentation profiles of a selection of glycerol-overproducing *Saccharomyces cerevisiae* strains (Prior et al., 1999). The yeast strains were developed in a selective breeding program and the fermentation profiles in Chardonnay juice for some of these hybrid strains showed that increased amounts of glycerol produced by the hybrid strains were accompanied by increased amounts of secondary metabolites. Notably acetic acid, acetaldehyde, succinic acid and 2,3-butanediol were produced in a response to the altered carbon flux in the modified yeasts (Prior et al., 2000). The potential negative impact on wine quality of these increases (especially acetic acid) necessitated extensive analysis and evaluation of the fermentation profiles of the modified strains. Since large numbers of strains are generated in such breeding programs, the method described here provides for a rapid, high throughput screening tool and significantly increases the efficiency of future yeast selection processes.

2. Materials and methods

2.1. Yeast strains and fermentation conditions

The yeast strains used in this study are listed in Table 1. The isolates were stored at −80 °C and subcultured on YPD agar (2% glucose, 2% peptone, 1% yeast extract, 2% agar, Biolab, Midrand, South Africa). For fermentation studies, Chenin blanc and synthetic musts were used. Chenin blanc must contained 106.53 g l^{-1} glucose and 100.16 g l^{-1} fructose (pH 3.34) and was supplied by ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa. The synthetic must consisted of 20% glucose, 2% bacteriological peptone, 0.2% yeast extract and 0.1% K2HPO4 (pH 3.20; Radler and Schütz, 1982). Chenin blanc juice was treated with 2-methyl dicarbonate (Velcorin^R, Bayer) at a final concentration of 0.2 ml l^{-1}. The sterility of treated juice was verified through plate counts on YPD agar prior to inoculation. Overnight cultures of the yeast strains in YPD broth (BioLab) were prepared at 30 °C and used to inoculate aliquots of the fermentation media (130 ml in 150 ml bottles) at an inoculum level of 1 to 6×10^6 cfu ml^{-1}. Triplicate independent fermentations were conducted at 20 °C±2 °C and the weight loss indicative of CO_2 production was monitored on a daily basis. The time course for glycerol production in Chenin blanc and synthetic musts was monitored with a test set of fermentations inoculated with *S. cerevisiae* VIN13. For this purpose, the fermentation bottles were sampled aseptically on a daily basis and the samples were stored at −20 °C until analyzed.

Table 1
Yeast strains used in this study

Species	Strain	Source/ reference
Saccharomyces cerevisiae	WE14, WE372,VIN13, VIN7, NT116, NT7, NT50, NT112, 228	Commercial wine yeasts [a]
S. cerevisae var. *bayanus*	N96	Commercial wine yeast [a]
S. cerevisiae	Enoferm Bordeaux red, D47, 71B	Commercial wine yeasts [b]
Strains used in breeding program (University of California, Berkeley)		
Parental strains		
S. cerevisiae	Premier Cuvée Ba25, Prise de Mousse UCB2	16, 17
Hybrid strains obtained through breeding		
S. cerevisiae	XPB3 (n=19)	16, 17

[a] Anchor Yeast, Cape Town, South Africa.
[b] Lallemand Inc., Montréal, Canada.

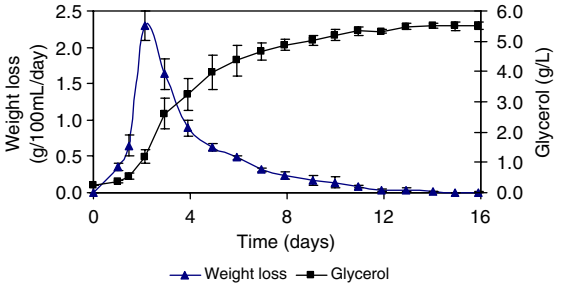

Fig. 1. Glycerol production (■) and weight loss rate (▲; indicative of CO_2 release) by *S. cerevisiae* VIN13 in Chenin blanc must at 20 °C ± 2 °C. Error bars indicate standard deviations of independent triplicate fermentations.

2.2. Reference methods

Glycerol concentration was determined enzymatically (Roche, catalogue number 148270) with a 100 µl total assay volume in microtitre plates and measured spectrophotometrically at 340 nm. Similarly, ethanol (Roche, catalogue number 0176290) and glucose (Roche, catalogue number 716251) were determined enzymatically. Acetic acid in wine is usually expressed as volatile acidity and corresponds to the total fatty acid content in wine. Volatile acidity was determined by steam distillation and reducing sugar by the Rebelein method (Ough and Amerine, 1988). All the assays were done in duplicate. The accuracy of the reference method was expressed as the standard error of laboratory (SEL) and calculated as:

$$\mathrm{SEL} = \sqrt{\frac{\sum(y_1 - y_2)^2}{2n}}$$

where y_1 and y_2 are the values from duplicate determinations and n is the number of samples.

2.3. FT-IR spectroscopy and quantification of chemical components

Fermentation broths were degassed by filtration *under vacuum* prior to analysis. FT-IR spectra of the fermentation broths were generated in the wavenumber region 5011–929 cm^{-1} using a spectrometer (WineScan FT 120 spectrometer, Foss Analytical, Denmark; http://www.foss.dk). that employs a Michelson interferometer was used to generate the FT-IR spectra. Samples (~30 ml) were pumped through the CaF_2-lined cuvette (optical path length 37 µm) housed in the heater unit of the instrument where the sample temperature was brought to 40 °C. Samples were scanned from 5011–929 cm^{-1} at 4 cm^{-1} intervals (i.e 1056 data points per spectrum). The frequencies of the infrared beam transmitted by each sample were recorded at the detector and used to generate an interferogram. The latter is calculated from a total of 10 scans before being processed by Fourier transformation and corrected for the background absorbance of water to generate a single beam transmittance spectrum. The transmittance spectra were finally converted into linearised absorbance spectra. Background absorbance in the wine samples (which included the absorbance of water) was corrected through the use of Zero Liquid S-6060 that was scanned prior to the wine sample under exactly the same conditions as described for the sample (WineScan FT 120 Type 77110 and 77310 Reference Manual, 2001; Foss Analytical).

Global calibrations for the quantification of ethanol, volatile acidity, reducing sugar and glucose were available and the prediction accuracy of these algorithms were evaluated by validation sample sets for each of the respective components. The multivariate statistical procedures required for the establishment of new calibrations and/or the validation of existing calibrations were used (Eriksson et al., 1999; Esbensen, 2000; Næs et al., 2004). The most pertinent wavenumbers at which glycerol absorbs were identified by partial least squares regression 1 (PLS1) of the reference values for glycerol (as determined with the enzymatic method) and absorbance at all wavenumbers (Næs et al., 2004) using the Advanced Performance Software Module of the instrument (WineScan FT 120 Type 77110 and 77310 Reference Manual, 2001). With the

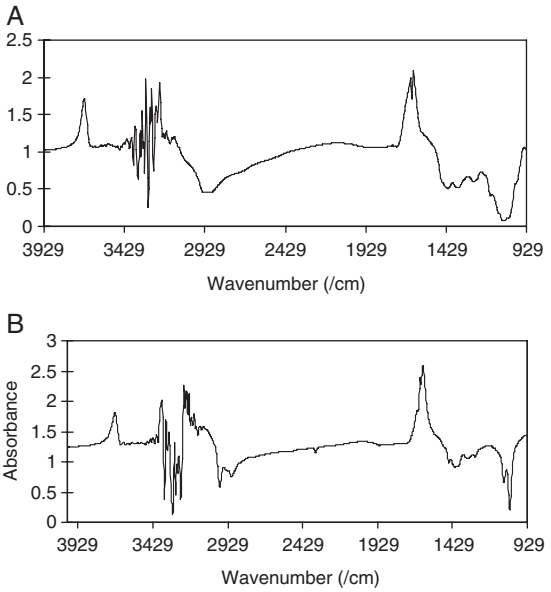

Fig. 2. FT-IR spectra (wavenumbers 3929 to 929/cm) of Chenin blanc must at the start of the fermentation (A) and after a 16-day fermentation period (B) at 20 °C ± 2 °C by *S. cerevisiae* VIN13. Spectra have been corrected for the absorbance of water (see Materials and methods section FT-IR spectroscopy).

Table 2
Validation statistics for the prediction of volatile acidity, ethanol, reducing sugar and glucose in small-scale yeast fermentations in Chenin blanc and synthetic musts using FT-IR spectroscopy

Fermentation medium/component	Volatile acidity	Ethanol (% v/v)	Reducing sugar (g l^{-1})	Glucose (g l^{-1})
Chenin blanc				
Number of samples	19	15	15	
Concentration range [a]	0.19–0.85	9.50–12.96	0.1–5.10	
Mean±S.D. [a b]	0.65±0.28	11.51±1.63	2.57±2.09	
SEP [c]	0.07	0.32	0.56	
r [d]	0.98	0.99	0.97	
Mean bias	0.001	0.10	0.15	
SEL [e]	0.05	0.29	0.20	
RPD [f]	4.0	5.0	3.7	
Synthetic must				
Number of samples	14	14		32
Concentration range [a]	0.24–1.098	7.69–12.36		0–8.62
Mean±S.D. [a b]	0.58±0.43	10.83±2.52		4.68±2.29
SEP [c]	0.08	0.31		0.39
r [d]	0.98	0.98		0.99
Mean bias	0.006	−0.082		0.09
SEL [e]	0.05	0.20		0.15
RPD [f]	5.4	8.1		7.8

[a] Determined with the reference method.
[b] Standard deviation.
[c] Standard error of prediction.
[d] Correlation coefficient.
[e] Standard error of laboratory.
[f] Residual predictive deviation.

WineScan FT120 instrument, a maximum of 15 "filters" (wavenumbers or small groups of wavenumbers) were defined for calibration purposes. In order to exclude noise being introduced into the spectral data, only three regions, 964–1543 cm^{-1}, 1716–2732 cm^{-1} and 2434–2970 cm^{-1}, were used for wavenumber selection.

2.4. Evaluation of the prediction accuracy of the calibration models

The statistical indicators for evaluating the accuracy of the predictive abilities of the calibration models included 1) bias, 2) the standard error of cross validation (SECV), when based on the calibration sample sets, and 3) the standard error or prediction (SEP), when based on independent validation sample sets.

$$\text{bias} = \frac{1}{n}\sum_{i=1}^{n}\left(y_i - \hat{y}_i\right);\ \text{SECV or SEP}$$
$$= \sqrt{\frac{\sum_{i=1}^{n}\left(y_i - \hat{y}_i - \text{Bias}\right)^2}{n-1}}$$

where y_i is the reference value for the ith sample; $\hat{y}_i$ is the predicted value for the ith sample; n is the number of samples (Eriksson et al., 1999; Næs et al., 2004). The predictive abilities of the calibration models were evaluated by the residual predictive deviation (RPD), which was defined as the ratio of the standard deviation of the reference data to the standard error of predicted data for the population tested (Pink et al., 1998). PRD values greater than three was considered to be desirable for prediction purposes (Pink et al., 1998).

2.5. Analysis of variance and principal component factor analysis

The mean values of data obtained from triplicate independent fermentations were used for all the statistical calculations using Statistica release 6 program (Microsoft Corporation, USA). A significance level of 5% was used in all cases. For the purposes of ANOVA and principal component factor analysis, the yeast isolates were classified into two groups. One group, referred to as 'hybrid strains', consisted of the segregant strains obtained through the breeding experiments (Prior et al., 1999, 2000) and the second group, referred to as 'commercial wine yeasts', consisted of the commercial strains which included the two parental strains used in the breeding experiments (see Table 1). One-way ANOVA of

Table 3
Calibration and validation statistics for the estimation of glycerol in Chenin blanc and synthetic musts using FT-IR spectroscopy

Chenin blanc			
Calibration set		Validation set	
Sample number	35	Sample number	19
Number of PLS factors	4	Mean bias	0.13
Number of filters	15	r [a]	0.96
SECV [b] ($g\ l^{-1}$)	0.24	SEP [c] ($g\ l^{-1}$)	0.38
Concentration range ($g\ l^{-1}$)	3.43–20.65	Concentration range ($g\ l^{-1}$)	5.46–14.40
Mean±S.D. [d] ($g\ l^{-1}$)	12.77±5.86	Mean±S.D. [d] ($g\ l^{-1}$)	7.92±2.72
SEL [e] ($g\ l^{-1}$)	0.19		
		RPD [f]	7.1
Synthetic must			
Calibration set		Validation set	
Sample number	41	Sample number	18
Number of PLS factors	7	Mean bias	0.19
Number of filters	15	r [a]	0.95
SECV [b]	0.32	SEP [c]	0.32
Concentration range ($g\ l^{-1}$)	4.12–9.87	Concentration range ($g\ l^{-1}$)	4.34–16.29
Mean±S.D. [d] ($g\ l^{-1}$)	6.16±2.57	Mean±S.D. [d] ($g\ l^{-1}$)	6.98
SEL [e] ($g\ l^{-1}$)	0.17		
		RPD [f]	8.5

[a] Correlation coefficient.
[b] Sandard error of cross validation.
[c] Standard error of prediction.
[d] Standard deviation.
[e] Standard error of laboratory.
[f] Residual predictive deviation.

glycerol, ethanol, volatile acidity, reducing sugar (for fermentations conducted in Chenin blanc must) and glucose (for fermentations done in synthetic must) was performed to compare the two groups of yeasts. Principal component factor analysis (using a Varimax rotation to determine the factor loadings) was used to determine the common factors responsible for the correlation-structure among the variables volatile acidity, reducing sugar, ethanol and glycerol (Johnson and Wichern, 1992).

3. Results and discussion

3.1. Small-scale fermentations and time course for glycerol production

Fermentation of Chenin blanc must by *S. cerevisiae* VIN13 showed that CO_2 (judged by weight loss) and glycerol production most rapidly occurred between day 1 and 3 and that the fermentation was complete after 16 days when glycerol attained a maximum concentration of 5.5 $g\ l^{-1}$ (Fig. 1). This fermentation pattern was typical for the various yeast strains in both Chenin blanc and synthetic musts (data not shown) and is similar to the pattern of small-scale fermentations previously reported (Radler and Schütz, 1982). The pattern of glycerol production by *S. cerevisiae* was ascribed to the need for redox balancing during the early stages of fermentative metabolism (Bakker et al., 2001). For the purposes of comparison, a 16-day period was used as standard fermentation conditions before samples were harvested for analysis by FT-IR spectroscopy.

3.2. FT-IR spectroscopy

A comparison of the FT-IR spectra at the start and at the end of the fermentation of Chenin blanc by *S. cerevisiae* VIN13 showed that major changes occurred in the chemical composition of musts suggesting that several novel compounds were formed (Fig. 2). The spectra represented the accumulated information of all the IR active components in the medium, including yeast cells in the case of the fermented broths. The peaks in the wavenumber regions of 3626–2970 cm^{-1} and 1716–1543 cm^{-1} were ascribed to the absorbance by water (Smith, 1999). The absorbance in the region 1800–929 cm^{-1} corresponded to the vibrations of several chemical bonds, including C − O, C − C and C − H.

3.3. Validation of the commercial calibrations for ethanol, reducing sugar, glucose and volatile acidity

Table 2 summarizes the validation statistics for the quantification of volatile acidity, ethanol, reducing sugar and glucose in musts from small-scale fermentations using the commercial calibrations supplied with the spectrometer. Preliminary results for the quantification of glucose in synthetic must were not satisfactory (SEP = 1.54 $g\ l^{-1}$, mean bias = 1.06 $g\ l^{-1}$). An independent FT-IR validation spectral sample set was therefore established and used to adjust the slope and intercept of the regression line of the commercial glucose calibration, to provide a better fit to the data. The samples in the independent validation set were selected on the basis of their respective glucose concentrations (as determined by the reference method) in order to span the complete glucose concentration range over which predictions in future samples were to be done (ca. 0–8.62 $g\ l^{-1}$, see Table 2).

The commercial calibrations for volatile acidity, ethanol and reducing sugar did not require any adjustments for

Table 4
Mean±S.D. (in parentheses) concentrations of products produced in triplicate small-scale fermentations in Chenin blanc and synthetic musts by commercial wine yeasts at 20±2 °C

Yeast strain	Volatile acidity (g l^{-1})		Glucose/reducing sugar (g l^{-1})		Ethanol (% v/v)		Glycerol (g l^{-1})	
Commercial strain [a]	Chenin blanc must	Synthetic must	Chenin blanc must	Synthetic must	Chenin blanc must	Synthetic must	Chenin blanc must	Synthetic must
228	0.29 (0.03)	0.28 (0.05)	3.82 (0.98)	0.56 (0.29)	11.12 (0.35)	10.84 (0.49)	6.45 (0.35)	5.22 (0.23)
VIN7	0.23 (0.02)	0.42 (0.02)	2.73 (0.71)	0	11.09 (0.38)	10.70 (0.73)	6.11 (0.11)	4.88 (0.30)
N96	0.31 (0.03)	0.25 (0.07)	1.72 (0.62)	1.10 (0.21)	11.37 (0.22)	10.50 (0.54)	5.65 (0.21)	4.42 (0.39)
NT116	0.33 (0.01)	0.29 (0.03)	2.54 (0.38)	0.81 (0.53)	10.91 (0.31)	10.76 (0.58)	7.43 (0.25)	5.20 (0.15)
NT50	0.42 (0.02)	0.27 (0.05)	1.22 (0.19)	0.90 (0.09)	11.70 (0.25)	10.11 (0.09)	6.95 (0.43)	5.72 (0.80)
71B	0.36 (0.04)	0.38 (0.03)	2.49 (1.02)	0	10.64 (0.09)	11.08 (0.42)	5.86 (0.18)	4.63 (0.56)
VIN13	0.29 (0.03)	0.56 (0.05)	1.45 (0.85)	0.21 (0.58)	10.47 (0.17)	11.03 (0.60)	6.76 (0.43)	5.53 (0.09)
NT7	0.28 (0.01)	0.35 (0.03)	2.17 (1.15)	1.42 (0.37)	11.51 (0.14)	10.89 (0.25)	6.01 (0.08)	4.78 (0.60)
WE14	0.42(0.03)	0.25 (0.04)	2.81 (0.76)	0	11.27 (0.08)	10.21 (0.32)	5.00 (0.07)	3.7 (0.73)
Bred [b]	0.26 (0.04)	0.47 (0.04)	1.80 (0.42)	1.09 (0.09)	11.92 (0.51)	11.01 (0.60)	6.56 (0.61)	5.03 (0.56)
WE372	0.30 (0.01)	0.38 (0.03)	2.82 (0.55)	0.98 (0.15)	11.87 (0.46)	10.69 (0.41)	7.77 (0.27)	5.54 (0.26)
D47	0.37 (0.02)	0.19 (0.02)	3.14 (0.39)	0	11.59 (0.38)	11.00 (0.16)	7.42 (0.09)	4.76 (0.45)
Ba25	0.31 (0.02)	0.33 (0.04)	2.31 (0.19)	0	9.9 (0.39)	10.77 (0.43)	4.09 (0.27)	4.59 (0.37)
UCB4	0.27 (0.02)	0.49 (0.03)	2.47 (0.54)	0	9.87 (0.53)	10.36 (0.50)	4.42 (0.22)	4.17 (0.27)
Mean±S.D.	0.32±0.06	0.35±0.11	2.39±0.69	0.54±0.53	11.09±0.66	10.71±0.31	6.18±1.11	4.87±0.55
Range	0.23–0.42	0.19–0.56	1.22–3.82	0.0–1.42	9.87–11.92	10.11–11.08	4.09–7.77	3.77–5.72

[a] Abbreviated strain designations are given; see Table 1 for full details.
[b] Bordeaux Red.

quantification purposes in either Chenin blanc or synthetic musts, and the SEP values were obtained with the unadjusted commercial calibrations. In the interpretation of these results using FT-IR spectroscopy, it should be kept in mind that the commercial calibrations were established for a background matrix of fermented grape must. Therefore

Table 5
Mean±S.D. (in parentheses) concentrations of products produced in triplicate small-scale fermentations in Chenin blanc and synthetic musts by hybrid wine yeasts at 20±2 °C

Yeast strain	Volatile acidity (g l^{-1})		Glucose/reducing sugar (g l^{-1})		Ethanol (% v/v)		Glycerol (g l^{-1})	
	Chenin blanc must	Synthetic must	Chenin blanc must	Synthetic must	Chenin blanc must	Synthetic must	Chenin blanc must	Synthetic must
XPB3-1A	0.51 (0.03)	0.41 (0.04)	3.68 (1.05)	0	10.53 (0.64)	10.29 (0.38)	7.55 (0.32)	7.66 (0.18)
XPB3-2A	0.54 (0.01)	0.69 (0.05)	3.19 (0.47)	0	10.43 (0.31)	10.64 (0.10)	8.81 (0.130)	8.56 (0.35)
XPB3-3A	0.49 (0.02)	0.32 (0.02)	1.08 (0.91)	0.40 (0.23)	11.10 (0.37)	10.96 (0.39)	9.55 (0.63)	7.87 (0.34)
XPB3-5A	0.83 (0.03)	0.58 (0.03)	1.19 (0.38)	0	10.42 (0.09)	10.03 (0.27)	13.01 (0.18)	11.90 (0.40)
XPB3-1B	0.42 (0.04)	0.71 (0.04)	3.41 (0.15)	1.89 (0.98)	10.45 (0.32)	10.31 (0.26)	7.27 (0.28)	7.52 (0.66)
XPB3-2B	0.88 (0.03)	0.67 (0.06)	2.45 (0.66)	0.37 (0.64)	10.16 (0.59)	10.68 (0.12)	15.13 (0.09)	15.61 (0.70)
XPB3-3B	0.41 (0.02)	0.53 (0.09)	2.39 (1.32)	0	9.98 (0.17)	10.57 (0.46)	7.52 (0.51)	7.90 (0.40)
XPB3-4B	0.52 (0.01)	0.55 (0.06)	1.18 (0.47)	0	10.67 (0.09)	10.51 (0.39)	9.94 (0.14)	9.28 (0.62)
XPB3-5B	0.98 (0.02)	0.81 (0.08)	1.32 (0.61)	1.45 (0.93)	10.17 (0.75)	9.72 (0.21)	19.47 (0.57)	17.43 (1.03)
XPB3-1C	0.62 (0.03)	0.72 (0.06)	1.47 (0.38)	0.51 (0.29)	10.21 (0.41)	10.76 (0.21)	11.29 (0.29)	9.98 (1.07)
XPB3-2C	0.71 (0.04)	0.76 (0.01)	1.09 (0.15)	0.53 (0.91)	10.09 (0.58)	10.44 (0.86)	14.03 (0.42)	13.14 (0.52)
XPB3-3C	0.41 (0.02)	0.58 (0.06)	1.54 (0.48)	0	10.44 (0.23)	10.97 (0.36)	7.93 (0.24)	7.22 (0.41)
XPB3-4C	0.63 (0.05)	0.61 (0.02)	1.88 (0.29)	1.97 (0.12)	10.17 (0.29)	9.91 (1.02)	16.97 (0.21)	17.49 (0.23)
XPB3-5C	0.57 (0.01)	0.66 (0.01)	3.27 (0.91)	0.17 (0.29)	10.60 (0.05)	10.90 (0.58)	11.21 (0.39)	10.74 (0.41)
XPB3-1D	0.70 (0.03)	0.71 (0.01)	3.55 (0.56)	0	10.77 (0.13)	10.51 (0.30)	13.90 (0.62)	14.09 (0.72)
XPB3-2D	0.49 (0.04)	0.58 (0.03)	2.29 (0.27)	0	10.58 (0.21)	10.46 (0.07)	9.16 (0.43)	8.78 (0.43)
XPB3-3D	0.54 (0.05)	0.60 (0.01)	2.95 (0.18)	0.41 (0.85)	9.93 (0.48)	9.85 (0.38)	9.21 (0.27)	8.81 (0.35)
XPB3-4D	0.76 (0.06)	0.61 (0.05)	1.82 (0.40)	0.17 (0.30)	10.14 (0.19)	10.49 (0.25)	14.61 (0.09)	15.40 (0.37)
XPB3-5D	0.61 (0.04)	0.45 (0.06)	1.90 (0.98)	0	10.76 (0.53)	10.91 (0.90)	9.23 (0.51)	9.38 (0.35)
Mean±S.D.	0.61±0.16	0.61±0.12	2.19±0.91	0.41±0.63	10.40±0.31	10.47±0.38	11.36±3.51	10.99±3.48
Range	0.41–0.98	0.32–0.81	1.08–3.68	0–1.98	9.93–11.10	9.72–10.97	7.27–19.47	7.22–17.49

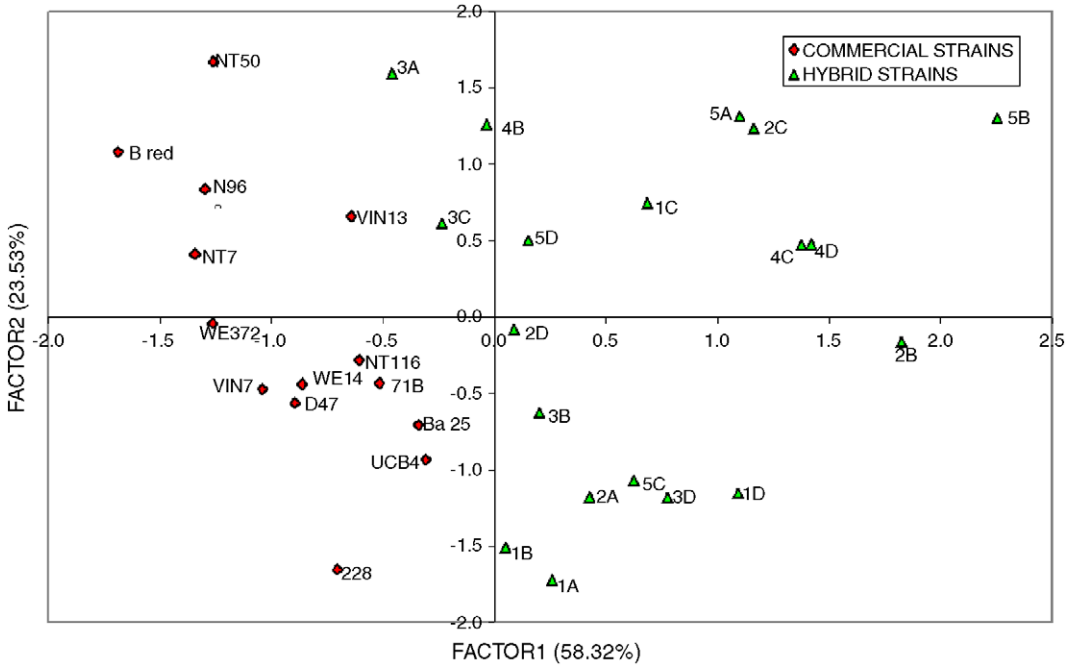

Fig. 3. Principal component factor analysis of the data obtained from the fermentation of Chenin blanc must by commercial wine yeast (♦) and hybrid yeast (▲) strains. The prefix XPB3 of the hybrid strains is omitted for clarity (see Table 1). Factor 1 could be interpreted in terms of the volatile acidity, ethanol and glycerol concentrations and Factor 2 in terms of the reducing sugar content based on the factor loadings.

some calibration models were adjusted for different background matrices. For example, synthetic must consists of many undefined components in the yeast extract and bacteriological peptone that could interfere with the accuracy of prediction. The observation that the calibration of glucose (but not that of volatile acidity, ethanol or reducing sugar) required adjustment could be an indication that interference in the absorbance occurred by compounds present in the matrix at some of the selected wavenumbers. This potential source of error in the prediction data has been described as the 'matrix effect' (Smith, 1999; Esbensen, 2000). From the regression results (Table 2), excellent predictive accuracies were obtained for the quantification of volatile acidity in Chenin blanc and synthetic musts (SEP = 0.07 g l^{-1} and SEP = 0.08 g l^{-1}, respectively) over the concentration ranges tested. The predictive errors for ethanol (SEP = 0.32 g l^{-1} for Chenin blanc, and SEP = 0.31 g l^{-1} for synthetic must) were in agreement with the respective SEL values. FT-IR spectroscopy is an indirect method based on the reference values and therefore the predictive errors (SEP) can never be smaller than those obtained for the reference methods (SEL). The largest predictive errors were found for the quantification of reducing sugar (SEP = 0.56 g l^{-1} for Chenin blanc, and SEP = 0.39 g l^{-1} for synthetic must). Overall, for the purposes of screening, the accuracy of prediction for the concentrations of volatile acidity, ethanol, reducing sugar and glucose were satisfactory.

3.4. Glycerol calibrations

Calibrations for the determination of glycerol in Chenin blanc and synthetic musts were established with PLS1 regression and the relevant statistics are summarized in Table 3. Samples for the respective calibration sets were selected to cover the range in glycerol concentrations expected in future samples. The prediction error based on the calibration set (SECV = 0.24 g l^{-1}) was in agreement with the laboratory error (SEL). The SECV was also in agreement with the prediction error based on independent validation set (SEP = 0.38 g l^{-1}), which provided an indication of the robustness of the glycerol calibration. The samples for the independent validation set were selected to cover the predicted glycerol concentration range of samples (ca. 3–20 g l^{-1}). The highest accuracy (as judged by the lowest SEP values) was obtained by creating a separate glycerol calibration for each matrix, as opposed to adjusting the slope and/or intercept of one calibration. Furthermore the wavenumbers selected for the quantification of glycerol in the two matrices were not similar, although some overlap was observed (data not shown). The accuracy of prediction obtained with the

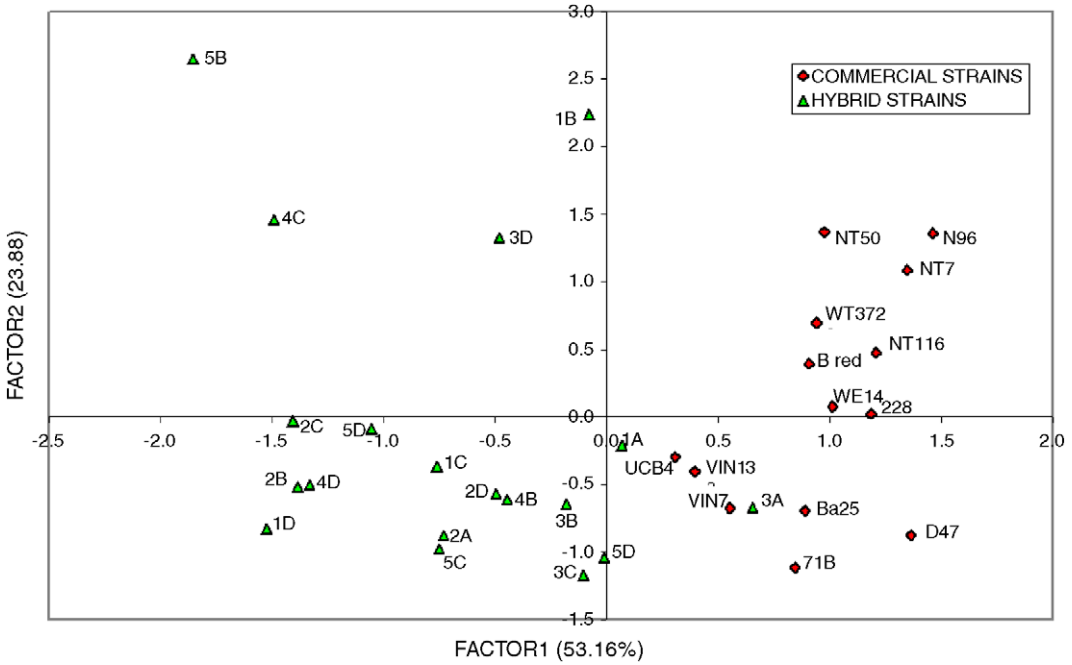

Fig. 4. Principal component factor analysis of the data obtained from the fermentation of synthetic must by commercial wine yeast (♦) and hybrid yeast (▲) strains. The prefix XPB3 of the hybrid strains is omitted for clarity (see Table 1). Factor 1 could be interpreted in terms of the volatile acidity, ethanol and glycerol concentrations and Factor 2 in terms of the glucose content based on the factor loadings.

established glycerol calibrations in Chenin blanc and synthetic musts (over the concentration ranges tested) was satisfactory for screening purposes.

3.5. Fermentation profiles of musts fermented by commercial wine yeasts and hybrid S. cerevisiae strains

FT-IR spectroscopy analysis of fermented musts by commercial wine yeasts (Table 4) and the hybrid strains (Table 5) showed that the mean volatile acidity concentrations formed in Chenin blanc must were similar to those formed in synthetic must, whereas the mean ethanol and glycerol concentrations formed were slightly higher. Wide variations between the different hybrid strains were observed for the volatile acidity and glycerol concentrations. The mean concentrations for these two components were also much higher for the hybrid strains (in both Chenin blanc and synthetic musts) than for the corresponding concentrations of the commercial strains. One-way ANOVA statistical analysis of glycerol, ethanol, volatile acidity, reducing sugar and glucose concentrations in Chenin blanc must showed that in general, the commercial strains and the hybrid strains differed significantly with respect to the mean volatile acidity, ethanol and glycerol concentrations. A similar analysis in synthetic must showed that the two groups differed significantly in the mean volatile acidity and glycerol concentrations, but the differences between the mean ethanol and glucose concentrations, were not significant. Several factors influence the final glycerol concentrations formed by *S. cerevisiae* (Scanes et al., 1998), and care should be taken when extrapolating fermentation data obtained from controlled laboratory fermentations to those of industrial-scale fermentations (Nieuwoudt et al., 2002).

3.6. Principal component factor analysis

Principal component factor analysis of the fermentation data was performed in order to model the relationships between the yeasts with respect to their fermentation profiles. Two factors were extracted (data not shown) and the loadings plots (Factor 1 vs. Factor 2) for the fermentation data obtained in Chenin blanc and synthetic must are shown in Figs. 3 and 4. These common factors respectively explained 81.8% and 79.0% of the variation in the data sets from Chenin blanc and synthetic musts. Factor 1 was interpreted as a combination of the variance in the volatile acidity, ethanol and glycerol concentrations (judged by the high factor loadings for these three variables) and factor 2 was interpreted in terms the reducing sugar concentrations. The loadings plot of the principal component factor analysis of Chenin blanc and synthetic must data showed a clear separation between the commercial strains and the hybrid

yeast strains, with some overlap between the two groups at the centre of the plot. Strains XPB3-5B and XPB3-2B in Chenin blanc must (Fig. 3) and strains XPB3-5B and XPB3-4C in synthetic must (Fig. 4) appeared as extreme members of the hybrid yeast group. These results were not surprising when interpreted in terms of the fermentation data (Tables 4 and 5). These strains had the highest concentration of glycerol, but also very high levels of volatile acidity (up to 0.98 g l^{-1}) and are not suitable for further development as wine yeasts. On the other hand, strains XPB3-3A, XPB3-4B, XPB3-3B and XPB3-2D produced volatile acidity levels below 0.6 g l^{-1} but higher glycerol levels than the mean for the commercial strains. These strains are located close to the commercial strains in the loadings plot indicating that they might be suitable for further development.

4. Conclusions

FT-IR spectroscopy has been extensively used in the quantification of chemical components in food and wine. However to our knowledge, this is the first application of this technique to rapidly screen the fermentation profiles of yeast strains in small-scale laboratory fermentations. Once the calibration has been established using this method, one laboratory technician could screen more than 30 yeast isolates in triplicate in ca. 3 h after the completion of the fermentation. However, if synthetic must is used for the small-scale fermentations, the existing commercial calibrations must be adjusted to obtain accurate prediction data, as the calibrations were initially developed to quantify in the background matrix of grape must. The results of this study also extended the analytical capacity of the instrument through the addition of calibrations for more compounds (like glycerol) to the basic software package. Furthermore, the validation process to establish the accuracy of prediction enlarged the scope of the fermentation profile without increasing the analysis time. Principal component factor analysis of the fermentation data demonstrated that this application may also be a valuable tool in assisting in the interpretation of the analytical data and hence also in the strain evaluation process.

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