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Monitoring large scale wine fermentations with infrared spectroscopy

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Abstract

Negative effects on wine quality and productivity caused by stuck and sluggish fermentations can be reduced significantly, if such problems are detected early through periodic chemical analysis. Infrared spectroscopy (IR) has been used successfully for monitoring fermentations, since many compounds can be measured quickly from a single sample without prior treatment. Nevertheless, few applications of this technology in large scale winemaking have been reported, and these do not cover the entire fermentation from must to finished wine. In this work, we developed IR calibrations for analyzing the fermenting must at any stage of fermentation. The calibration model was obtained with multivariable partial least squares and proved effective for analyzing Cabernet Sauvignon fermentations for glucose, fructose, glycerol, ethanol, and the organic acids; malic, tartaric, succinic, lactic, acetic, and citric. Upon external validation we found an average relative predictive error of 4.8%. Malic acid showed the largest relative predictive error (8.7%). In addition, external validation found that insufficient data for these calibrations made the analysis of fermenting musts using other grape varieties less reliable.

Keywords: Cabernet sauvignon; Winemaking; Sugars; Organic acids; Glycerol; PLS

1. Introduction

In a highly competitive market wineries need to invest more in technology, to increase productivity and improve average quality, to remain competitive. By reducing stuck and sluggish fermentations, which in turn requires a monitoring system that can detect and classify them early, less wine will be lost or downgraded. Artificial intelligence techniques have been applied to predict the result of other fermentation processes early on (Kamimura [1,2]; Stephanopoulos [3]) by analyzing significant variables over time. Several compounds play a key role in problematic wine fermentations, such as sugars, nitrogen substrates and organic acids (Bisson [4,5]; Boulton [6]; Pszczólkowski [7]). As conventional chemical analysis is both time consuming and expensive, currently these compounds are not measured frequently enough. This problem is more acute in large wineries that operate hundreds of fermentation tanks simultaneously.

Infrared spectroscopy offers an alternative to conventional chemical analysis. This analytical technique has been applied successfully in other kinds of bioprocess such as the production of antibiotics and the cultivation of mammalian cells. In these processes infrared spectroscopy has been used for monitoring alanine, glucose, glutamine, leucine, lactate and ammonium (Riley [8–10]; Rhiel [11]; Vaccari [12]; Vaidyanathan [13,14]). Although, we have found many applications of IR to wine analysis, such as for controlling denominations of origin, monitoring wines during the aging process (Palma [15]), classification of red-wine dried-extracts according to their geographic origin (Picque [16]) and discrimination among red wines based on the analysis of their phenolic extracts (Edelman [17]),

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we have not found any reference to the monitoring of large scale wine fermentations. One report though, does describe the application of IR to study the effect of fermentation temperature over time on significant metabolites such as glucose, fructose, glycerol and ethanol during winemaking in a lab scale fermentor (10 L), using artificial musts (Fayolle [18]) with different initial sugar concentrations. Infrared spectroscopy has also been applied to analyze musts for 40 different wine varieties from three different regions of the world for determining glucose, fructose, glycerol, ethanol, total acidity, volatile acidity, malic acid, acetic acid, tartaric acid, lactic acid, pH and sucrose (Dubernet [19,20]).

In this work we use IR analysis and develop specific calibrations for monitoring glucose, fructose, glycerol, ethanol, and the organic acids malic, tartaric, succinic, lactic, acetic, and citric during large scale wine fermentations of Cabernet Sauvignon.

2. Materials and methods

2.1. Sampling and analysis

Samples of 100 mL were collected every 8 h from large scale fermentation tanks (40 and 60 m^3) in a winery from Chile's Central Valley in 2002. Four fermentation runs of Cabernet Sauvignon and one fermentation run each of Syrah, Pinot Noir, Merlot and Carmenere, were followed until they finished. Each run includes between 30 and 35 samples. A total of 273 samples were collected, frozen and stored at −20 °C until they were analyzed.

Reference analyses for glucose and fructose were performed by HPLC using a Waters high performance carbohydrate cartridge (Waters Corporation, USA), while for organic acids, glycerol and ethanol a BioRad HPX-87H column was used.

2.2. Infrared equipment and software

Acquisition of the samples' spectra was performed with Fourier transform infrared (FT-IR) multispec equipment (module FT-IR AVATAR 360 NICOLET) equipped with a DTGS KBr detector. The spectral resolution was 0.5 cm^{-1}. Triplicate spectra were acquired in two spectral ranges, 200–740 nm and 1350–28500 nm, for each sample and the spectra averaged. This equipment can handle between 60 and 80 samples per hour. The liquid sample (15 mL) needs to be centrifuged or filtered prior to acquiring the spectra. *Bacchus Acquisition* software was used to define measurement parameters.

Spectra processing (first or second derivative, centered media, and reduced variance) were carried out with *Bacchus Quantification* software, which develops calibrations for several compounds simultaneously, using either multiple linear regression (MLR) or partial least squares (PLS). Both packages were developed by CETIM, France (http://perso.wanadoo.fr/cetim2/gb/cetlab_index_gb.html).

2.3. Model development

In this work, we applied PLS for model calibration since this method has been successfully applied in many bioprocesses for this type of problem (Riley [8,10]; Vaidyanathan [13,14]; Fayolle [18]). PLS has shown strong predictive capacity for unknown samples (Martens [21]; Burns [22]). These are reduced dimension multivariable linear models. Their structure is defined by the number of PLS factors which is the same for input and output spaces.

Internal cross validation is used to establish the optimum number of PLS factors. Here, samples used for calibration are also employed for validation. We used the "leave and out" algorithm, where the calibration is repeated several times omitting a subgroup of samples each iteration. The whole procedure is repeated for different PLS factor numbers and the best model structure is determined by the lowest standard error of cross validation (SECV). An optimum number of PLS factors was found for each compound, although we tried to limit the number to 15 factors. Ideally, the selected model structure should have a correlation coefficient larger than 0.985. When spectra were pre-treated, its first derivative was taken. Up to 10% of samples were deleted since they were outside the working range.

In the calibration step, the model parameters were fitted using the optimal number of PLS factors, using the sum of squares of the prediction errors (PRESS) as optimization criteria.

Finally, an external validation was used to test the predictive power of the model. Here, samples not included in the model calibration were used to compute the prediction absolute error and prediction relative error. The absolute error is the average of the difference between the predicted value provided by the FT-IR equipment and the reference value given by the HPLC for the external validation set. Relative error is the absolute error divided by the measurement span, expressed as a percentage.

Table 1
Results of CR calibration

Compound	Calibration			Concentration range (g L^{-1})
	r^2	PLS factors	SECV (g L^{-1})	
Glucose	0.994	3	3.4	0–125
Fructose	0.994	9	4.9	0–133
Alcoholic degree	0.99	9	1.1[a]	0–15.4[a]
Glycerol	0.988	9	0.66	0–11
Malic acid	0.985	13	0.32	0–4.57
Tartaric acid	0.987	12	0.24	0–2.62
Succinic acid	0.982	9	0.67	0–10.97
Citric acid	0.985	10	0.08	0–0.85
Lactic acid	0.989	13	0.12	0–1.03
Acetic acid	0.988	14	0.18	0–2.3

[a] In % (v/v).

We called the calibration obtained with the above procedure complete range (CR) and its performance using the validation set is compared with two default calibrations that are included in the Bacchus equipment, must and wine, both obtained using many samples of unfermented must and finished wines from all over the world.

2.4. Experimental methodology

The 273 samples collected from the winery were divided into two different sets, destining 200 for calibration (73%) and internal cross validation, and 73 for external validation (27%). On average, 22 samples of each run were used for calibration and eight samples for external validation. The samples for the two sets were selected at random, taking care to ensure that each set included samples, that covered the entire range of concentration for each compound (as shown in Table 1).

3. Results and discussion

The results of CR calibration and its validation, for Cabernet Sauvignon and for the other varieties grouped together (Merlot, Pinot Noir, Syrah and Carmenere), are shown and discussed in this section. In addition, the performance of CR calibration at monitoring full fermentations of Cabernet Sauvignon and Syrah musts is illustrated and compared

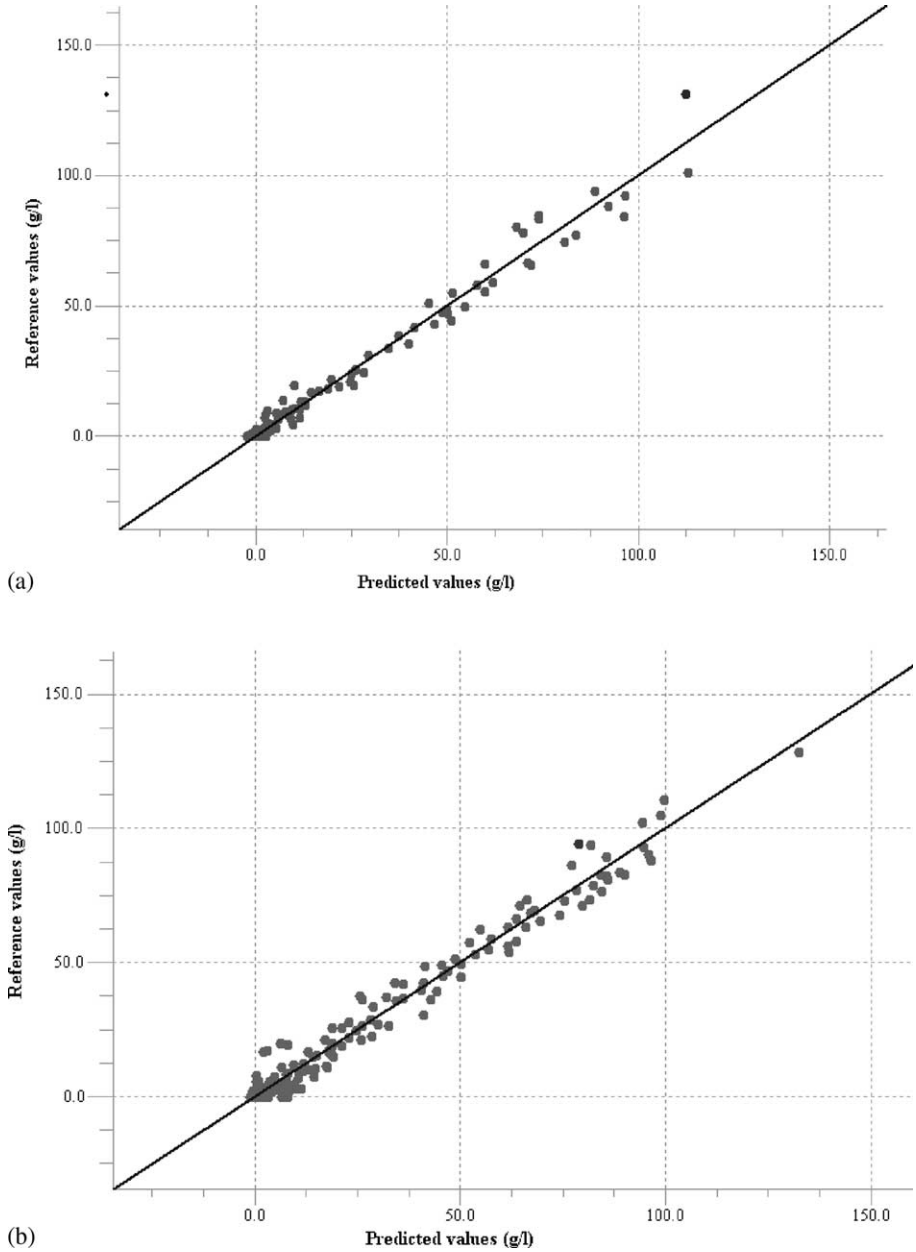

Fig. 1. Concentration correlation plots for CR calibration for: (a) glucose and (b) fructose in Cabernet Sauvignon samples.

with the default calibrations from must and wine and with the reference analysis.

3.1. Calibration

Table 1 summarizes the results of the CR calibration obtained through cross validation and includes the concentration range for each component.

All components present a good fit showing low standard deviations and good correlation, even though a relatively high number of PLS factors were needed. Only glucose required a small number of PLS factors and incidentally also showed the best fit.

The concentration correlation plots of the CR calibration are presented for glucose and fructose in Fig. 1. Both sets of measurements provide good agreement between predicted values and reference values. The other components showed similar trends.

3.2. Validation

To address the accuracy of measurements, the results of validation are expressed in terms of absolute average error (AAE), maximum absolute error (MAE), average relative error (ARE) and maximum relative error (MRE). Tables 2 and 3 summarize the validation results using CR calibration for samples of Cabernet Sauvignon and the other varieties, respectively.

In Cabernet Sauvignon samples the lowest ARE prediction occurred with glucose, fructose and tartaric acid, while glucose, fructose and citric acid presented the smallest MRE prediction. Although the models for fructose and glucose present a good predictive power and both sugars used the same concentration range, the AAE for fructose is larger than the AAE for glucose. The latter is probably due to some spectral interference between fructose and another component in wine.

For the remaining components except for ethanol, glycerol, succinic and malic acids, the average prediction errors

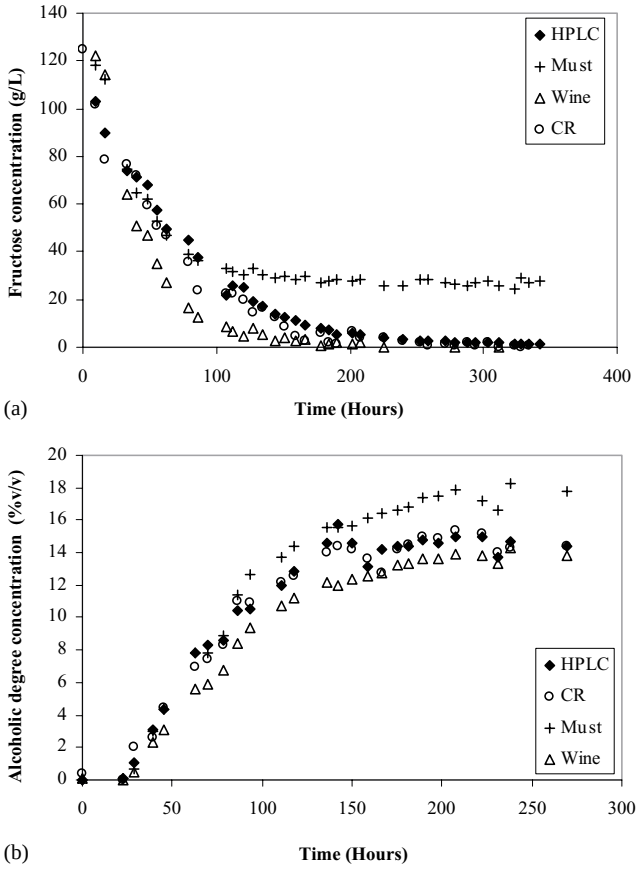

Fig. 2. Comparison between CR, must and wine calibrations, with the reference analysis for: (a) fructose, (b) alcohol content in Cabernet Sauvignon.

are good (<5%). Maximum deviations are reasonable also, with values lower than 14%.

Validation of the samples from other varieties returned poorer results. Although the average error is not much higher than for the Cabernet Sauvignon- due to smaller errors for ethanol, glycerol and malic acid- for most compounds average errors were over 5%. Maximum errors proved far higher

Table 2
Results of external validation for Cabernet Sauvignon samples

Component	Average absolute error (AAE) ($g\,L^{-1}$)	Maximum absolute error (MAE) ($g\,L^{-1}$)	Average relative error (ARE) (%)	Maximum relative error (MRE) (%)
Glucose	2.0	4.7	1.5	3.6
Fructose	3.0	12	2.3	9.0
Alcoholic degree (%, v/v)	0.91	2.1	5.8	13.4
Glycerol	0.74	1.5	6.7	13.4
Malic acid	0.34	0.52	8.7	13.3
Tartaric acid	0.11	0.34	3.4	10.9
Succinic acid	0.73	1.6	6.6	13.7
Citric acid	0.12	0.24	4.6	9.5
Lactic acid	0.07	0.25	4.2	14.2
Acetic acid	0.15	0.36	4.8	11.6
Average			4.8	11.3

Table 3
Results of external validation for other varieties samples

Component	Average absolute error (AAE) ($g\,L^{-1}$)	Maximum absolute error (MAE) ($g\,L^{-1}$)	Average relative error (ARE) (%)	Maximum relative error (MRE) (%)
Glucose	3.1	16.3	2.3	12.3
Fructose	4.2	17.7	3.2	13.6
Alcoholic degree (%, v/v)	0.82	1.9	5.2	11.8
Glycerol	0.64	1.7	5.8	15.2
Malic acid	0.29	0.56	7.4	14.5
Tartaric acid	0.21	0.40	6.8	13.0
Succinic acid	0.83	1.6	6.9	13.5
Citric acid	0.10	0.25	4.0	9.6
Lactic acid	0.11	0.23	6.4	13.1
Acetic acid	0.21	0.46	6.6	14.9
Average			5.5	13.2

in the other varieties, and particularly for glucose and fructose. Even glycerol and malic acid present larger maximum errors than in Cabernet Sauvignon samples.

3.3. Monitoring the entire fermentation process

Fig. 2 shows the performance of three calibrations (must, wine and CR), as compared with the reference analysis during a whole fermentation of 340 h for the Cabernet Sauvignon variety. For fructose and alcohol content, good agreement was achieved between CR estimation and the reference analysis; however, the default calibrations (must and wine) performed badly. Fig. 2a shows that must calibration only provides a reasonable estimation for fructose over the first 100 h of fermentation. On the other hand, the wine calibration just yields good estimates over the final 160 h. Only the CR calibration provides good estimates of fructose from 100 to 200 h of fermentation. The *must* calibration works well only at the beginning (up to 90 h), while the wine calibration consistently under predicted alcohol content even at the end of the fermentation (Fig. 2b). Estimations of the remaining components studied in Cabernet Sauvignon behaved like the ones shown in the figure.

CR calibration performance of glycerol (a) and succinic acid (b) in a Syrah fermentation is illustrated in Fig. 3. Syrah presented the best performance compared with the other varieties (excluding Cabernet Sauvignon) in this study. On average, there is good agreement between CR estimates and reference analysis; however, in some points, highlighted with circles in the graphs, differences between CR estimations and reference values are large. These large differences can also be observed in the external validation set of the other varieties group (Table 3) for almost all components. The small number of samples of these wines used in the calibration can explain these large errors. Of the 200 samples used for calibration, 90 samples corresponded to Cabernet Sauvignon (four fermentation runs) while the other varieties contributed with less than 30 samples each (one fermentation run). According to Fayolle et al. [18] and Rhiel et al. [11], four fermentation runs (100 samples approximately) do provide enough data to obtain good calibration. In addition, Riley et al. [9] suggest that the number of calibration samples required should be approximately six times the number of varying components and for monitoring biological processes, between 70 and 100 samples for calibration are recommended. Therefore, CR calibration can be applied with confidence to Cabernet Sauvignon wines only.

Regarding early detection of problematic (stuck and sluggish) wine fermentations, the monitoring system using IR spectroscopy provides sufficient precision to distinguish between normal or problematic behavior, as illustrated below (Fig. 4):

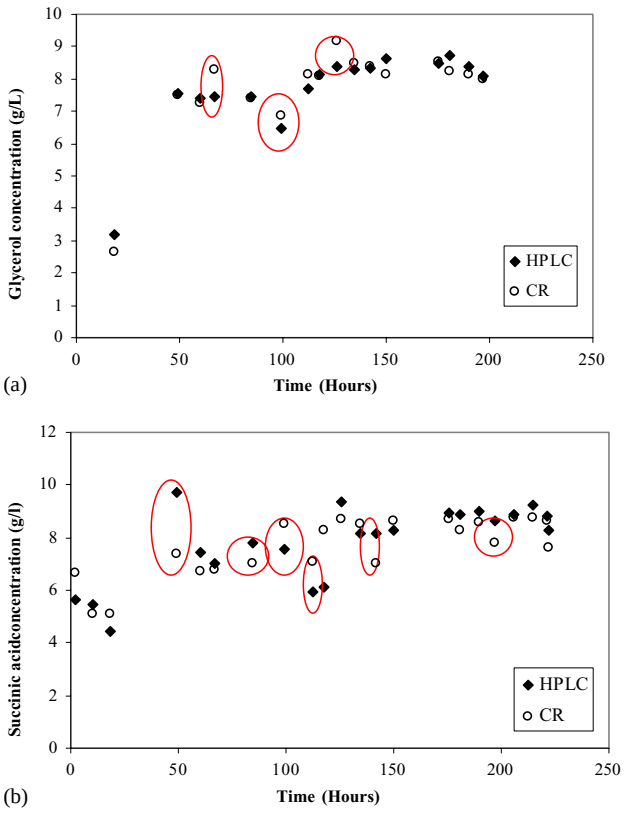

Fig. 3. Comparison between CR calibrations and the reference analysis for: (a) glycerol and (b) succinic acid in Syrah.

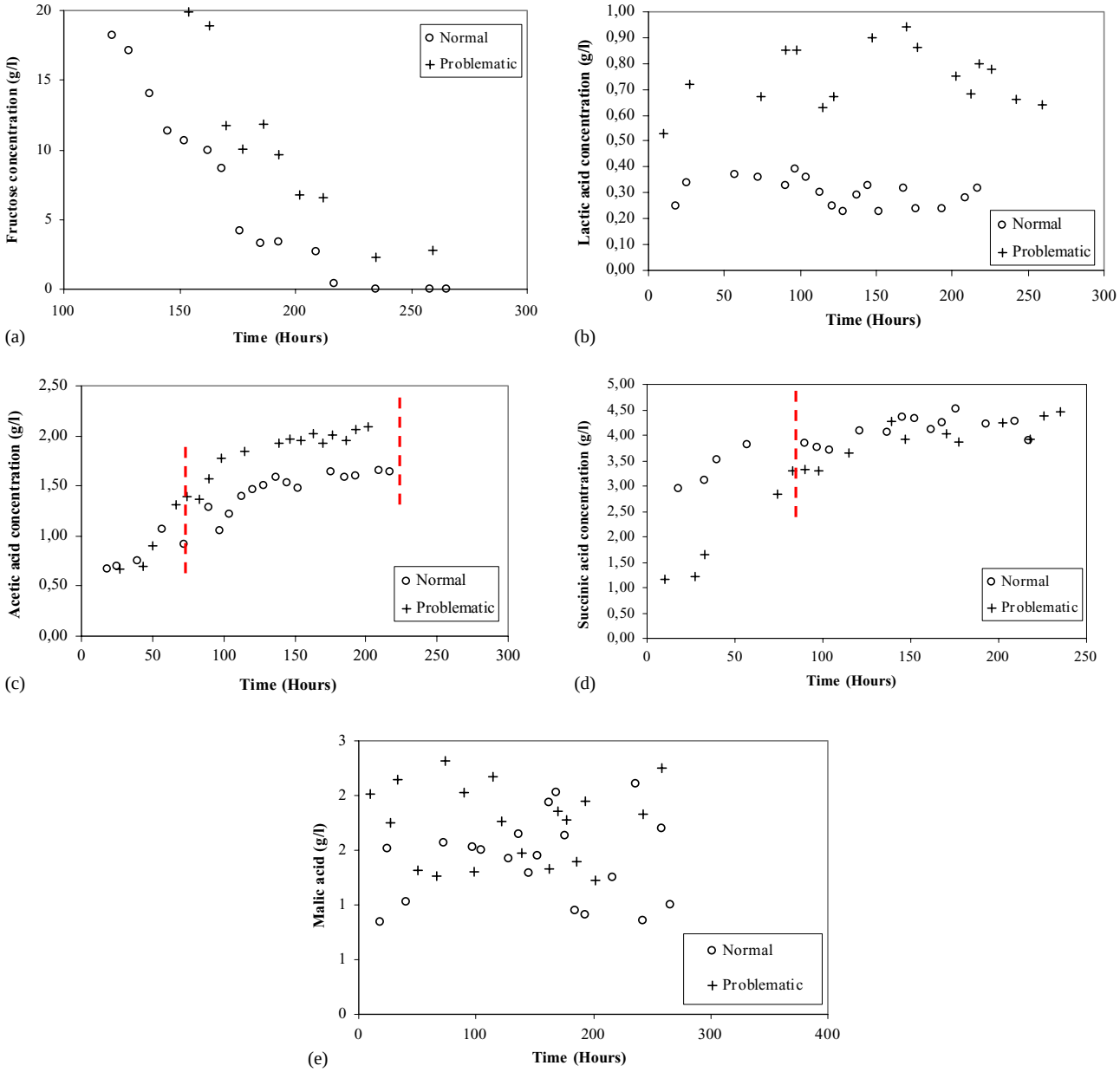

Fig. 4. Wine fermentations of Cabernet Sauvignon using CR calibrations for: (a) fructose, (b) lactic acid, (c) acetic acid, (d) succinic acid, and (e) malic acid.

Fig. 4 shows the evolution of several components during a normal and a problematic fermentation. The latter took two extra days to reach a residual sugar lower than 4 g L^{-1} (Fig. 4a). Clearly, the precision of the CR calibration for the first four components is sufficient to distinguish between both fermentations. Lactic acid (Fig. 4b) presented a different behavior in both fermentations throughout the whole process, while acetic acid (Fig. 4c) behaved differently only after 100 h of fermentation and succinic acid showed a different evolution before 100 h. On the other hand, the precision of the CR calibration for malic acid is not good enough to distinguish between the normal and the problematic behavior.

4. Conclusions

In this study, we verified that FT-IR spectroscopy is a useful analytical tool for monitoring industrial wine fermentations. The developed CR calibration provided good estimations for glucose, fructose, organic acids, glycerol and ethanol during the entire fermentation of Cabernet Sauvignon musts in a given winery located in the Central Valley of Chile. The small estimation errors achieved for most of the components included in this study allowed distinction between a normal and a problematic fermentation. We are currently analyzing the evolution of these components in more than 60 Cabernet Sauvignon fermentations in order to

find common patterns in problematic and normal fermentations.

The calibration obtained was not applicable to Syrah, Merlot, Pinot Noir and Carmenere, since not enough samples of these varieties were available for the calibration, resulting in large estimation errors in many samples. We also determined that the default calibrations, must and wine, were unsuitable for monitoring an entire fermentation.

To generalize the results shown here, it is necessary to significantly increase the number of reference samples, incorporating more varieties and eventually other wineries. Alternatively, a calibration similar to the one developed in this study, can be obtained providing small prediction errors using relatively few samples, although the calibration obtained would be limited to a specific winery and a specific variety.

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