Monitoring of alcoholic fermentation using NIR and MIR spectroscopy

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Introduction

Effective fermentation monitoring is a growing need, due to the rapid pace of change in the wine industry, which calls for fast methods providing real time information in order to assure the quality of the product at all stages of the process.

During wine fermentation it is important to measure both substrate and product concentrations (e.g. sugars, ethanol, phenolic compounds). However, the analysis of these compounds by traditional means requires sample preparation and in some cases several steps of purification are needed.

Infrared spectroscopic techniques, in both near and mid regions, have proven to be successful analytical methods for quantitative and qualitative monitoring of food production processes.[1,2] Some important applications come from the modern wine industry, in which NIR and MIR spectroscopy have been used as interesting tools for wine quality assessment.[3,4]

The objective of this work was to apply IR spectroscopy in both near and mid regions, combined with different chemometric strategies, to monitor time-related changes that occur during red wine fermentation.

Material and methods

Fermentation trials and sampling

Fifteen micro-fermentation trials were carried out using stainless steel vessels (capacity 100 L). Each trial was conducted during the 2008 vintage, using grapes Nebbiolo, ecotype Chiavennasca, from different vineyards situated in the Valtellina viticulture area (Northern Italy).

Chemical analyses

Glucose, fructose, ethanol and glycerol were determined by High-performance liquid chromatography (HPLC) analysis. Total phenolic content was determined according to the Folin-Ciocalteau method.[5]

Total anthocyanins and total flavonoids were determined spectrophotometrically as described by Di Stefano *et al*.[6]

Near and mid infrared spectroscopy

NIR spectral data were collected in transmission mode (12500 cm^{-1} to 3600 cm^{-1}; resolution 8 cm^{-1}) using a flow cell of 1 mm path length with an FT-NIR spectrometer (MPA, Bruker Optics, Ettlingen, Germany). MIR spectra (4000 cm^{-1} to 700 cm^{-1}; resolution 16 cm^{-1}) were collected by using a spectrometer (VERTEX 70, Bruker Optics, Ettlingen, Germany) equipped with a germanium crystal ATR with a multiple reflection.

NIR and MIR data processing

Principal Component Analysis (PCA)[7] was applied to standardised spectral data to uncover molecular modifications during fermentation process.

Linear Discriminant Analysis (LDA)[8] was carried out on spectral data after having applied the algorithm stepwise decorrelation of the variables (SELECT).[9] The data analysis was performed

Table 1. Average values and the relative standard deviation for glucose, fructose, ethanol and glycerol, measured at the beginning of fermentation (initial time) and at the end of the process (30–35 days).

Fermentation trials	Glucose g L^{-1}		Fructose g L^{-1}		Ethanol g L^{-1}		Glycerol g L^{-1}	
	T_i	T_f	T_i	T_f	T_i	T_f	T_i	T_f
1	130.4 ± 0.4	nd	141.4 ± 0.4	nd	nd	125.9 ± 2.4	nd	10.2 ± 0.2
2	109.5 ± 6.6	nd	116.5 ± 7.3	nd	nd	121.7 ± 1.3	nd	9.7 ± 0.1
3	110.6 ± 0.6	nd	119.5 ± 0.6	nd	nd	115.5 ± 2.3	nd	9.5 ± 0.2
4	110.5 ± 0.7	nd	119.8 ± 1.0	nd	nd	112.9 ± 1.9	nd	9.5 ± 0.2
5	114.2 ± 4.1	nd	121.7 ± 4.3	nd	nd	117.1 ± 1.1	nd	9.5 ± 0.1
6	124.6 ± 0.6	nd	135.4 ± 0.6	nd	nd	120.8 ± 2.4	nd	10.0 ± 0.2
7	96.5 ± 1.8	nd	106.0 ± 2.2	nd	nd	99.4 ± 1.0	nd	7.2 ± 0.1
8	106.1 ± 1.0	nd	119.4 ± 0.8	nd	nd	121.3 ± 4.8	nd	9.8 ± 0.4
9	109.8 ± 1.5	nd	119.8 ± 1.8	nd	nd	117.9 ± 1.5	nd	9.2 ± 0.5
10	126.3 ± 0.8	nd	133.0 ± 0.6	nd	nd	126.7 ± 4.1	nd	9.7 ± 0.3
11	105.7 ± 0.1	nd	116.6 ± 0.9	nd	nd	112.7 ± 7.1	nd	8.9 ± 0.4
12	121.1 ± 1.9	nd	130.8 ± 2.3	nd	nd	126.3 ± 2.0	nd	10.4 ± 0.2
13	119.3 ± 1.7	nd	127.0 ± 1.3	nd	nd	127.0 ± 1.8	nd	10.5 ± 0.3
14	111.9 ± 1.6	nd	120.7 ± 1.6	nd	nd	120.6 ± 3.3	nd	9.8 ± 0.3
15	119.4 ± 2.1	nd	126.7 ± 2.2	nd	nd	116.1 ± 1.8	nd	9.4 ± 0.2

T_i = initial time; T_f = final time; nd = not detectable (below limit of quantification (LOQ); LOQ glucose = 4 mg L^{-1}; fructose = 47 mg L^{-1}; ethanol = 55.3 mg L^{-1}; glycerol = 114.8 mg L^{-1}).

by using V-PARVUS package.[1] For the classification analysis, the samples belonging to different fermentation stages were divided into four classes: "step 1" (initial time), "step 2" (two days), "step 3" (five to seven days) and "final step" (between 30 and 35 days).

Results and discussion

Chemical analysis

Average values and the relative standard deviation for the parameters involved during the alcoholic fermentation (glucose, fructose, ethanol and glycerol), measured at the beginning of fermentation (initial time) and at the end of the process (30–35 days), are reported in Table 1.

The initial value of glucose and fructose ranged from 97 g L^{-1} to 130 g L^{-1} and from 106 g L^{-1} to 141 g L^{-1}, respectively. Ethanol concentration at the end of the process ranged from 99 g L^{-1} and 127 g L^{-1} and glycerol content was about 10 g L^{-1}.

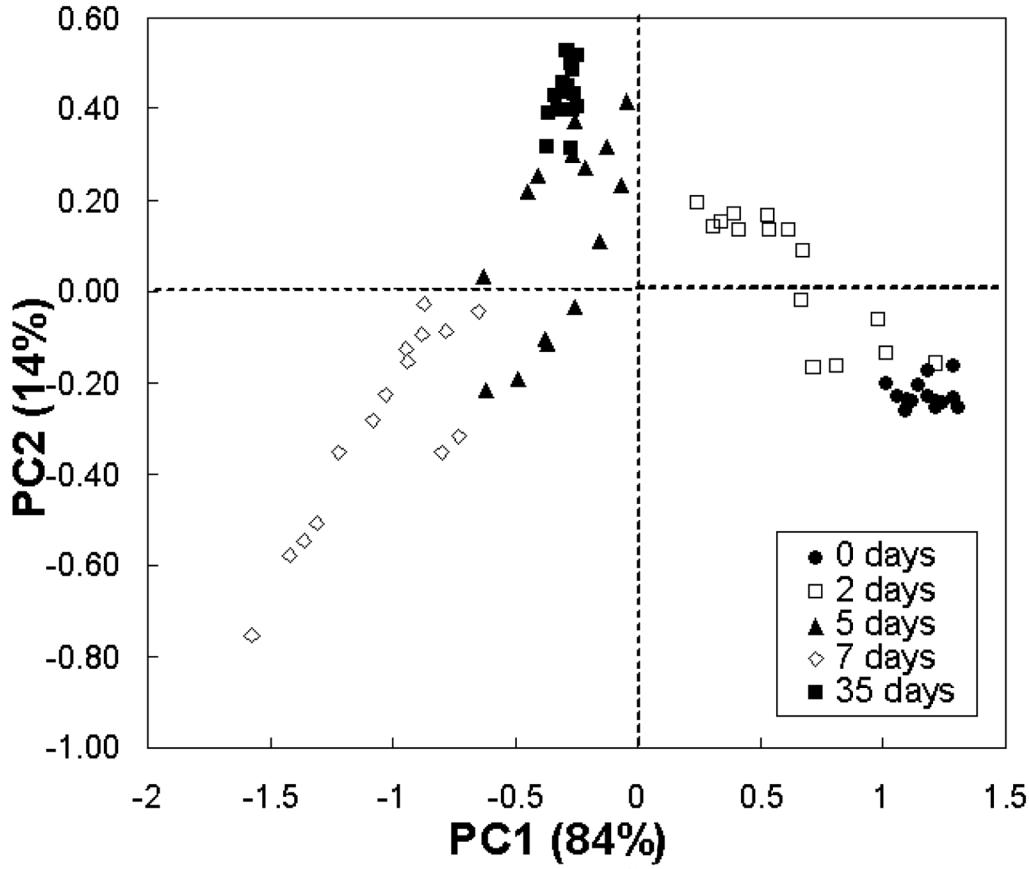

Figure 1. PCA applied to the standardised FT-NIR spectra of samples during alcoholic fermentation.

The concentrations of phenolic compounds (total phenolics, total antocyanins and total flavonoids) increase during fermentation due to their extraction from grape skins by the hydroalcoholic medium.

NIR and MIR spectroscopy

Both for NIR and MIR spectra, PCA was performed as preliminary data examination. Examining the score plots, obtained by applying PCA to NIR (Figure 1) and MIR spectra (Figure 2), in the area defined by the first two principal components, a satisfactory sample distribution was found according to the fermentation stages.

The main wave-numbers responsible for the separation of the samples belonging to the different fermentation times were associated in the near-infrared region with the C-H bonds and in the medium region with the C-O and C-C of ethanol and carbohydrates.[2,11]

In order to delete no useful information from the spectra, the stepwise decorrelation of the variables (SELECT) was applied on the NIR and MIR matrices.

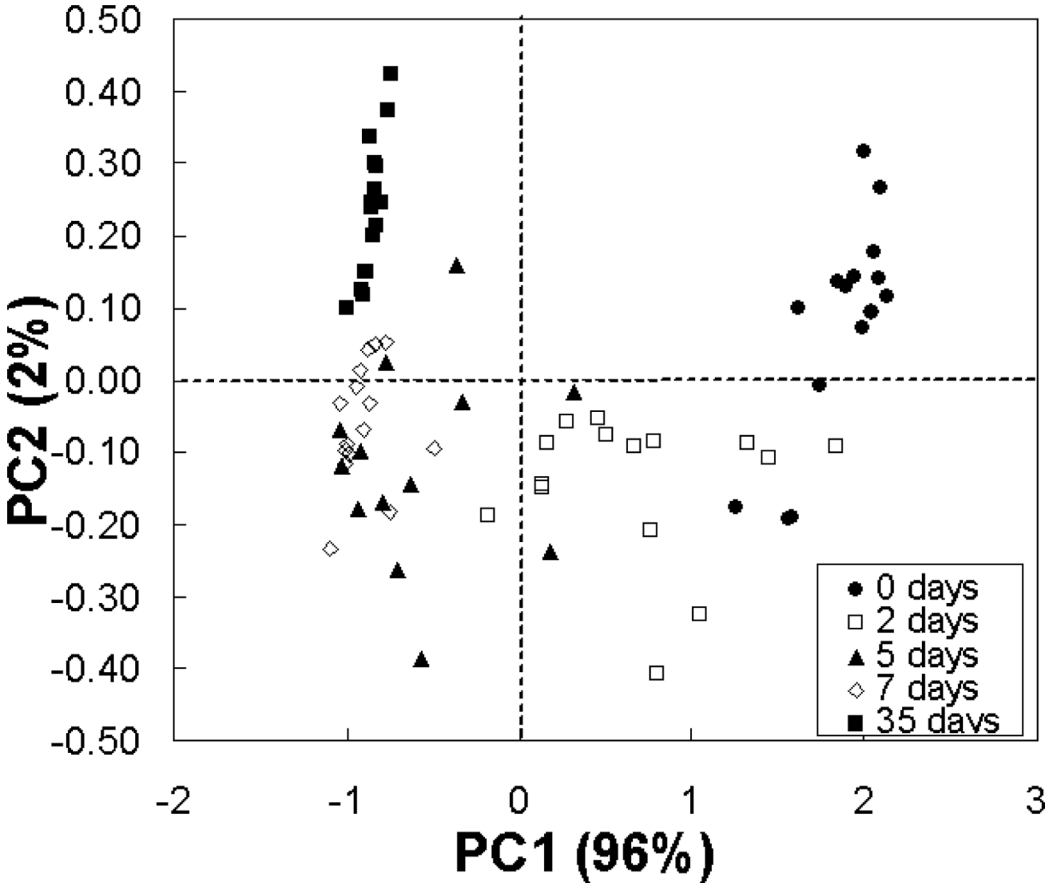

Figure 2. PCA applied to the standardised FT-IR spectra of samples during alcoholic fermentation.

Table 2. LDA results after feature selection. Cross-validation performed with five cancellation groups.

NIR	% samples correctly classified (step 1)	% samples correctly classified (step 2)	% samples correctly classified (step 3)	% samples correctly classified (final step)	average
Calibration	100	85.7	85.0	100	91.1
Prediction	92.9	85.7	78.6	100	87.1
MIR	% samples correctly classified (step 1)	% samples correctly classified (step 2)	% samples correctly classified (step 3)	% samples correctly classified (final step)	average
Calibration	100	100	100	100	100
Prediction	100	100	100	100	100

step 1 = initial time; step 2 = two days; step 3 = five-seven days; final step = between 30 and 35 days.

LDA results, performed on these reduced data matrices, were characterised by a high percentage of correct classification in prediction for both NIR and MIR spectroscopy. The average prediction ability obtained using the NIR data (Table 2) was 87.1 %, correctly classified.

Best results were achieved by MIR spectroscopy, where 100% of all samples belonging to the different fermentation steps were correctly classified.

Conclusions

The results obtained in this work show that the spectroscopic methods could be valid and simple tools, able to provide real time information during a fermentation process in order to assure the quality of wine. Although the measurements were carried out in on-line mode, a probe will be used in future works to allow the process to be monitored in-line

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