



RAPID ASSESSMENT OF NEBBIOLO BERRY VOLATILE COMPOUNDS BY NEAR-INFRARED SPECTROSCOPY

C. Pagliarani, A. Carlomagno, M. Cremonini Bianchi, A. Schubert*, A. Ferrandino

*corresponding author: andrea.schubert@unito.it



Department of Plant, Forestry and Food Sciences – University of Torino, via L. da Vinci, 44 – I-10095 Grugliasco (TO)

INTRODUCTION

Aroma is one of the main components of grape and wine sensory quality. The term "aromatic compounds" refers a group of organic compounds with low molecular weight, therefore volatile, and able to stimulate the human olfactory system. Berry aromatic profile depends on genotype and pedo-climatic conditions. These compounds are produced, stored and lost during berry ripening, and they are mainly synthesised in the berry skin, and in a lower concentration in the berry flesh. Under the chemical point of view, the main varietal and pre-fermentative volatile compounds can be classified in: terpenes, C13-norisoprenoids, C6 alcohols and aldehydes, esters, benzene derivatives and lactones (Sefton et al., 1992, AJEV; Salinas et al., 2004, JAFAC).

In this study, a rapid quantification method of volatiles was developed and tested, based on the use of an FT-NIR apparatus, with the aim of setting up a faster and cheaper than traditional analysis technique.

Whole berries of *Vitis vinifera* cv 'Nebbiolo' were collected during the growing season, from véraison to harvest. Non-destructive quantifications of volatiles were performed on intact berry samples by means of FT-NIR spectroscopy. After NIR measurements, berry volatiles were extracted from skins and pulps and analysed. The classes of volatile compounds studied were: aliphatic and aromatic aldehydes, aliphatic alcohols, terpenes, benzene derivatives, norisoprenoids and lactones (io i lattoni li toglierei!). As primary analysis technique, we used GC-MS coupled with SBSE (Stir Bar Sorptive Extraction).

MATERIALS AND METHODS

- Year of trial: 2011, in two *Vitis vinifera* L. cv Nebbiolo vineyards.

- Vineyard locations: Piedmont, North-West Italy, at Barolo (44° 37' 41" N, 7° 56' 72" E, 240 m a.s.l., Langhe area) and at Monteu Roero (44° 46' 48" N, 7° 58' 34" E, 230 m a.s.l., Roero area).

- Three field replicates of 20 vines each were established and 250-300 berries were collected from each replicate for volatile analysis and for NIR measurements.

NIR measurements: NIR spectra (Fig.2 a) were acquired on samples of 200 intact berries each by means of a Fourier Transform NIR spectrometer based on a polarisation interferometer (NIRFlex N500; Büchi Labortechnik AG, Flawil, Switzerland) in the 4000-10000 cm⁻¹ range at 8 cm⁻¹ resolution. Both spectra acquisition and chemometric analyses were carried out using the chemometric software NIRCal v.5.2 (Büchi Labortechnik AG, Flawil, Switzerland). Average spectra (Fig.2 b) were calculated every 200 acquired spectra, to properly correlate NIR predicted values with each GC-MS analysis. Mathematical pre-treatments were applied on average spectra (Fig.2 c,d), in order to minimise any internal sources of variation. Then, calibrations were built by using the partial least squares regression (PLS) modelling coupled with the internal validation method (C-set/V-set).

Sample preparation and GC-MS analysis: berries were crushed for 2 min without breaking seeds; 10 g of homogenised grapes were diluted with distilled water and 20 mL of the aqueous grape extract were stirred with a PDMs-coated stir bar (Twister®, Gerstel, Mulheim and der Ruhr, Germany) for 6 hours at room temperature. The Twister® was transferred into a TDU-CIS injector system for thermal desorption of volatiles and for consequent separation and identification by means of GC/MS (Agilent Technologies, GC 7890A, MS 5975C).



Fig. 1 Total volatile accumulation trend from véraison to harvest in two Nebbiolo vineyards of Piedmont.

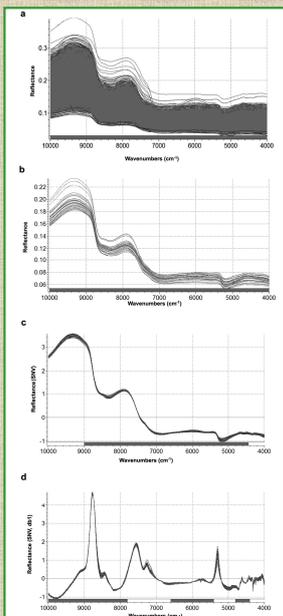


Fig. 2 Spectral features: a) variability of raw absorption spectra acquired on a sample constituted by 200 intact grape berries from the same Nebbiolo vineyard; b) average spectra calculated on a group of 36 samples (200 berries each); c, d) examples of spectra pre-processing treatments: standard normal variate normalization (SNV) and first derivative transformation (dS1), respectively.

Parameters	data set	mean	min	max	se
Total volatiles	All (n=36)	2.1421	0.6280	3.6862	±0.1297
	Calibration (n=27)	2.1754	0.6280	3.6860	±0.7704
	Validation (n=9)	2.0420	0.7670	3.3920	±0.8401
Aliphatic aldehydes	All (n=36)	1.8941	0.3717	3.3936	±0.1316
	Calibration (n=27)	1.9329	0.3720	3.3940	±0.8062
	Validation (n=9)	1.7777	0.4720	2.9450	±0.7712
Aromatic aldehydes	All (n=36)	0.0483	0.0086	0.1092	±0.0040
	Calibration (n=27)	0.0505	0.0086	0.1092	±0.0245
	Validation (n=9)	0.0419	0.0112	0.0799	±0.0222
Aliphatic alcohols	All (n=36)	0.0980	0.0489	0.1832	±0.0056
	Calibration (n=27)	0.0988	0.0489	0.1832	±0.0344
	Validation (n=9)	0.0958	0.0570	0.1553	±0.0329
Terpenes	All (n=36)	0.0374	0.0095	0.0923	±0.0036
	Calibration (n=27)	0.0372	0.0095	0.0923	±0.0217
	Validation (n=9)	0.0381	0.0121	0.0738	±0.0217
Benzene derivatives	All (n=36)	0.0492	0.0122	0.1136	±0.0057
	Calibration (n=27)	0.0482	0.0122	0.1134	±0.0202
	Validation (n=9)	0.0520	0.0248	0.1111	±0.0279
Norisoprenoids	All (n=36)	0.0098	0.0000	0.0199	±0.0005
	Calibration (n=27)	0.0096	0.0000	0.0199	±0.0056
	Validation (n=9)	0.0105	0.0045	0.0169	±0.0046
Lactones	All (n=36)	0.0052	0.0000	0.0156	±0.0006
	Calibration (n=27)	0.0050	0.0000	0.0156	±0.0037
	Validation (n=9)	0.0061	0.0037	0.0090	±0.0018

Table 1. Volatile content range measured by GC-MS (reference method) in *Vitis vinifera* cv 'Nebbiolo'. Data are expressed as kg Kg⁻¹ of fresh berries (min = minimum; max = maximum; se = standard error; n = number of samples).

Parameters	Calibration set (n=27)				Validation set (n=9)			
	mean	min	max	se	mean	min	max	se
Total volatiles	2.170	0.569	3.462	±0.746	2.052	0.737	3.213	±0.829
Aliphatic aldehydes	1.933	0.298	3.289	±0.778	1.771	0.453	2.916	±0.738
Aromatic aldehydes	0.050	0.010	0.105	±0.023	0.041	0.003	0.075	±0.040
Aliphatic alcohols	0.098	0.043	0.184	±0.034	0.096	0.067	0.172	±0.032
Terpenes	0.037	0.010	0.084	±0.019	0.042	0.022	0.086	±0.019
Benzene derivatives	0.048	0.024	0.109	±0.018	0.052	0.023	0.094	±0.024
Norisoprenoids	0.010	0.000	0.019	±0.005	0.010	0.004	0.014	±0.003
Lactones	0.005	0.000	0.016	±0.003	0.006	0.003	0.009	±0.002

Table 2. Volatile content range predicted by NIR spectrometer in intact grape berries of *Vitis vinifera* cv 'Nebbiolo'. Data are expressed as kg Kg⁻¹ of fresh berries (min = minimum; max = maximum; se = standard error; n = number of samples).

Parameters	Spectral pre-treatments	PLS factors	Calibration set (n=27)			Validation set (n=9)				
			R ²	slope	SEC	R ²	slope	SEP	BIAS	RPD
Total volatiles	SNV	16	0.94	0.94	0.1882	0.96	0.96	0.1782	0.005	4.65
Aliphatic aldehydes	nd, log	14	0.93	0.93	0.2096	0.92	0.93	0.2075	0.007	3.56
Aromatic aldehydes	db1, SNV	6	0.89	0.89	0.0080	0.93	0.90	0.0072	0.001	3.04
Aliphatic alcohols	ds2	7	0.95	0.95	0.0080	0.96	0.92	0.0093	0.003	3.56
Terpenes	log, dg1, SNV	4	0.81	0.81	0.0096	0.86	0.82	0.0089	0.002	2.27
Benzene derivatives	dg1, SNV	9	0.84	0.84	0.0081	0.85	0.80	0.0110	0.000	2.21
Norisoprenoids	ds2	8	0.83	0.83	0.0023	0.83	0.77	0.0016	±0.005	2.01
Lactones	nd	15	0.93	0.93	0.0010	0.83	1.45	0.0015	0.001	1.32

Table 3. Calibration and internal validation statistical descriptors for contents of different classes of volatiles measured by NIR spectrometer in intact Nebbiolo berries. Data are expressed as kg Kg⁻¹ of fresh berries. n = number of samples; R² = coefficient of determination; SEC = standard error of calibration; SEP = standard error of prediction; RPD = ratio performance deviation of internal validation (SNV). Pre-treatments: SNV = Standard Normal Variate; nd = Normalization by closure; log = Absorbance Log₁₀(1/X); db1 = First Derivative; ds2 = Second Derivative Smoothing; dg1 = First Derivative Savitzky-Golay 9 Points; dg2 = Second Derivative Savitzky-Golay 9 Points.

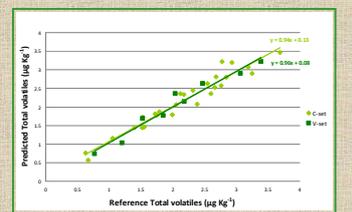


Fig. 3 Correlation between total volatiles (µg Kg⁻¹) contents measured by GC-MS as reference method, and predicted by using NIR spectroscopy in intact grape berries of Nebbiolo. For the calibration (C-set, in light green) and validation sets (V-set, in dark green).

RESULTS AND DISCUSSION

- In both growing areas, volatiles were already present in the early stages of véraison. In both vineyards, volatile accumulation peaked about 10 days post véraison (27/8/2011, Julian day - Fig. 1).

- Comparing the statistics obtained for the reference method (Table 1) with those resulting from NIR analysis (Table 2) for the volatile content range, we can observe a good level of correspondence between the two sets of data, since, especially considering standard error values, NIR measurements are particularly precise.

- Looking at the calibration statistics (Table 3), very good results in terms of prediction accuracy were obtained for total volatiles (R²=0.96; SEP<0.18 µg Kg⁻¹; RPD>4.5), aliphatic aldehydes (R²=0.92; SEP<0.207 µg Kg⁻¹; RPD>3.5) and aliphatic alcohols (R²=0.96; SEP<0.009 µg Kg⁻¹; RPD>3.5), thus giving a first evidence of the possibility to apply NIR spectroscopy also for the assessment of berry aromatic molecules.

Nevertheless, NIR spectrometer seemed to be less powerful in detecting contents of other volatile compounds, which are present in very low concentrations in the berry. This is, for instance, the case of norisoprenoids and lactones calibrations, where RPD values are lower than 2.5 and the main statistics reported for the two sets (C-set and V-set), in particular the R² and slope of the internal validation (V-set), indicate a less level of precision of the NIR measurement (Table 3). Further work is thus needed to optimise NIR calibration for the quantification of these aromatic compounds, which strongly contribute to the aroma of wines.

CONCLUSION

Good calibration models have been obtained for the determination of total volatiles, aliphatic aldehydes, aliphatic alcohols, aromatic aldehydes and terpenes, thus demonstrating the good prediction capacity of NIR spectrometer in detecting aromatic compounds. To our knowledge, these results represent one of the first attempts to evaluate the NIR predictive ability in quantifying the content of different classes of volatiles in fresh grapes by intact berry analysis, an application only reported in a few previous studies for other quality parameters, such as total anthocyanins (Cozzolino et al., JNIRS 2004; Guidetti et al., Trans. Am. Soc. Agric. Biol. Eng. 2010) and other polyphenols (Ferrer-Gallego et al., Food Sci. Technol. 2011).

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