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Effect of Growing Zone and Vintage on the Prediction of Extractable Flavanols in Winegrape Seeds by a FT-NIR Method

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Supporting Information

ABSTRACT: The feasibility of Fourier transform−near-infrared (FT-NIR) spectroscopy for predicting the extractable content of phenolic compounds directly in intact grape seeds was evaluated. NIR calibration models were based on the correlation of spectral data with the phenolic composition determined by reference chemical methods for 40 grape samples. The vintage effect was studied evidencing that the predictive accuracy improved only for spectrophotometric indices when samples of two years were simultaneously considered. The statistical parameters of calibration showed that the models developed are sufficiently robust for quantitative purposes of total flavonoids, proanthocyanidins, low molecular weight flavanols, catechin, epicatechin, procyanidin B_1, and galloylation percentage (standard prediction error (SEP%) < 15, predictive index (RPIQ) > 3.0), but could be also useful for screening of absorbance at 280 nm, total polymers, epicatechin gallate, and procyanidin B_2 (SEP% < 15, RPIQ = 2.7−2.9). Although a calibration model is required for each geographical origin, the results suggest that FT-NIR spectroscopy is a promising analytical technique in this field.

KEYWORDS: *extractable phenols, grape seeds, red grapes, near-infrared spectroscopy, chemometrics, partial least-squares regression*

INTRODUCTION

The most abundant phenolic compounds in grape seeds are flavanols, which include monomeric catechins ((+)-catechin, (−)-epicatechin, and (−)-epicatechin-3-*O*-gallate) as well as their oligomeric and polymeric procyanidins, although gallic acid is also well represented.[1−4] The qualitative and quantitative phenolic composition of the seeds greatly depends on the grape cultivar, but it is also influenced by multiple factors such as environmental conditions, cultural practices, and degree of grape ripeness.[2,4−8] Seed flavanols contribute significantly to wine sensory quality because the large majority of extractable monomeric flavanols and galloylated procyanidins of the grape is located in the seeds.[2] In fact, the decreased content of seed monomer flavanols, particularly (−)-epicatechin-3-*O*-gallate, and the increased content of the polymeric fraction in ripe grapes are sensorially perceived as low astringency and bitterness.[1,9,10] Therefore, the flavanolic profile of the seeds is responsible for the sensations perceived in the mouth.[1]

The most commonly used analytical methods for the determination of phenolic compounds in grape seeds require a preliminary step of solvent extraction.[11−13] Therefore, sample preparation is one of the most time-consuming steps for qualitative and quantitative analyses. The use of microwave-assisted extraction permits partial reduction of solvent consumption and shortened extraction times.[14] Nevertheless, the necessity of real-time decision-making in the enological sector has promoted the development of rapid methods of analysis with minimal sample preparation or on even intact samples.

Near-infrared (NIR) spectroscopy is an accurate, fast, versatile, nondestructive, simple, environmentally friendly, and economically reasonable analytical technique that has been widely used in the grape sector to monitor the ripening and dehydration processes or to discriminate varieties according to different parameters such as titratable acidity, pH, and the content of soluble solids, reducing sugars, moisture, and organic acids.[15−18] With regard to phenolic compounds, some studies have been published on the determination of these compounds in grape homogenates.[19−21] Nowadays, the research is more focused on the direct analysis of intact grape berries, and the determination of total anthocyanins,[17−19] extractable anthocyanins (at pH 1.0 and 3.2), and total phenols[22] has been attempted. Ferrer-Gallego et al. have recently determined the concentration of the main families of phenolic compounds (flavanols, anthocyanins, flavonols, and phenolic acids) and their total content in grape skins and intact red grapes during ripening.[23] In intact grape seeds, only two works have assessed the possible use of NIR spectroscopy to determine phenolic compounds. In the first one, total phenol content and extractability were evaluated.[24] In the other one, flavanols were determined.[25] However, a detailed study focused on the effect of the growing zone and vintage on the robustness of the predictions for the phenolic composition has not been performed to date, particularly in winegrape seeds. Another important advantage of NIR spectroscopy is the feasibility of quickly and simultaneously evaluating several metabolites (metabolic fingerprinting) in a large number of samples.[26,27]

The aim of this work was to develop a method based on Fourier transform−near-infrared (FT-NIR) spectroscopy for the direct determination of the extractable content of phenolic compounds, particularly flavanols, in intact grape seeds. To

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obtain a sufficiently robust predictive model, a high-variability calibration data set was used by sampling grapes from multiple vineyards at different ripening stages during two vintages. Furthermore, this work evaluated the vintage effect on the robustness of the predictive model. Its adaptability to different growing zones was also checked. The study was carried out on Nebbiolo winegrapes usually used for the production of renowned red wines that are commercialized worldwide and of recognized international prestige. Therefore, this work also aimed to contribute to the assessment of the phenolic composition of Nebbiolo seeds during ripening while shorting the time involved in the chemical analysis methodologies commonly used for determining the extractable content of phenolic compounds in grape seeds.

MATERIALS AND METHODS

Grape Samples. A total of 40 grape samples of the Nebbiolo red cultivar (*Vitis vinifera* L.) were used for the development of the FT-NIR method. The bunches (n = 25) were randomly harvested from vines selected in 2010 and 2011. In the two years, the samples were collected at different ripening stages in 10 commercial vineyards with a total range of soluble solids content between 21.6 and 25.5 °Brix. These vineyards are located in Valtellina (Sondrio province, Lombardy, northern Italy) and confined over nearly 50 km, with elevations ranging from 300 to 700 m asl, along the southern slope of a typical valley of an alpine glacial. This is pedologically divided into four landscape units (alluvial fans, terraces, mountainside glacial tills, and valley scarp), but the vineyards are mainly located in the valley scarp landscape.[28] The geographical distribution of the vineyards selected in the Valtellina valley is shown in Figure S1 in the Supporting Information. In 2011, another six samples were harvested at different ripening stages (total range of soluble solids content from 19.5 to 23.9 °Brix) from vineyards located in Piedmont (Cuneo province, northwestern Italy) and Trentino (Trento province, northeastern Italy) with altitudes of 239 and 262 m asl, respectively, and were used to evaluate the production zone effect. The location and geographical coordinates of the different vineyards studied are shown in Table S1 in the Supporting Information. On the other hand, the climatic conditions of each vintage in terms of temperature, relative humidity, and precipitations are represented in Figure S2 in the Supporting Information.

The analyses were carried out separately on the samples collected at each date, vineyard, and year. Once in the laboratory, a subsample of approximately 0.5 kg of grapes (ca. 350−400 berries) was randomly selected by picking berries from different positions in the cluster. For each subsample, two sets of 30 berries were randomly selected. The first set was subdivided into three replicates of 10 grape berries that were weighed. The seeds were carefully separated from the pulp, cleaned with adsorbent paper, and weighed before determining extractable phenolic compounds by spectrophotometric and chromatographic methods, which were taken as the reference (n = 3). In the second set, the seeds were also separated and cleaned before FT-NIR analysis. In this last case, one seed per berry was used to cover a wider variation range with the same number of seeds. The FT-NIR spectrum was individually acquired in these 30 intact grape seeds (n = 30). Finally, the remaining berries of each initial subsample, distributed into three replicates, were used for determining the technological ripeness parameters in the grape must obtained by manual crushing and centrifugation.

Chemical Analysis. Solvents of HPLC gradient grade and all other chemicals of analytical reagent grade were purchased from Sigma (Milan, Italy). The solutions were prepared in deionized water produced by a Purelab Classic system (Elga Labwater, Marlow, UK). Among phenol standards, (+)-catechin (C), (−)-epicatechin (EC), and (−)-epicatechin gallate (ECG) were obtained from Sigma, and cyanidin chloride and procyanidins B_1 and B_2 were purchased from Extrasynthèse (Genay, France). Phloroglucinol was supplied by Aldrich (Steinheim, Germany).

Technological Ripeness Parameters. Total soluble solids concentration (°Brix, as SSC) was measured with an Atago 0−32 °Brix temperature compensating refractometer (Atago Corp., Tokyo, Japan), and pH was determined by potentiometry using a Crison electrode (Carpi, Italy). Titratable acidity (TA), expressed as grams per liter tartaric acid, was estimated using the International Organization of Vine and Wine (OIV) method.[29] Organic acids (malic acid and tartaric acid) were quantified (as g L^{-1}) according to the methodology proposed by Giordano et al.[30]

Seed Phenol Extraction and Determination. In the reference method, the berry seeds, after immersion in 50 mL of a hydroalcoholic buffer at pH 3.2 containing 5 g L^{-1} tartaric acid, 2 g L^{-1} $Na_2S_2O_5$, and 12% (v/v) ethanol, were placed in a controlled-temperature room at 25 °C for 1 week.[31,32] The extracts were filtered through a 0.20 μm filter, bottled, and stored at 4 °C until their analysis. Spectrophotometric methods were used to measure seed absorbance at 280 nm ($A_{s,280}$)[33] and to determine the extractable content of seed total flavonoids (TF_s, mg (+)-catechin kg^{-1} grape or g^{-1} seed), seed proanthocyanidins (PRO_s, mg cyanidin chloride kg^{-1} grape or g^{-1} seed), and seed flavanols reactive to vanillin (FRV_s, mg (+)-catechin kg^{-1} grape or g^{-1} seed).[31,34] Proanthocyanidins were determined after acid hydrolysis with warming (Bate−Smith reaction) using a ferrous salt ($FeSO_4$) as catalyst. An UV-1800 spectrophotometer (Shimazdu Corp., Kyoto, Japan) was used.

Individual flavanols were determined by reversed-phase liquid chromatography before and after acid-catalyzed degradation of polymeric proanthocyanidins in the presence of phloroglucinol. Phloroglucinolysis was carried out according to the method proposed by Kennedy and Jones,[35] although slightly modified as follows. One milliliter of the hydroalcoholic extract was evaporated to dryness under reduced pressure at 35 °C. The residue was redissolved in 0.5 mL of phloroglucinol reagent consisting of 50 g L^{-1} phloroglucinol and 10 g L^{-1} ascorbic acid in methanol containing 0.1 M hydrochloric acid. After reacting for 20 min at 50 °C, the reaction was stopped by adding 0.5 mL of 200 mM aqueous sodium acetate. The final extracts (taken before and after phloroglucinolysis) were filtered through 0.20 μm PTFE filters (VWR International, Radnor, PA, USA) into LC vials and immediately injected into a HPLC-DAD system. This assay provides information on the extractable content of total polymer flavanols (TP), the mean degree of polymerization (mDP), and the percentage of galloylation (G%).

An Agilent 1260 Infinity HPLC system (Milford, MA, USA) equipped with a diode array detector (DAD) was used to determine individual flavanols. The chromatographic separation was carried out at 25 °C on a LiChroCART analytical column (250 mm × 4.0 mm i.d.) purchased from Merck (Darmstadt, Germany), which is packed with LiChrospher 100 RP-18 (5 μm) particles supplied by Alltech (Deerfield, IL, USA). The injection volume was 20 μL. The mobile phases consisted of 1% aqueous acetic acid (mobile phase A) and methanol (mobile phase B). They were filtered through a 0.20 μm filter. A linear gradient, at a flow rate of 0.8 mL min^{-1}, was used for the separation of flavanols in 55 min, starting at 5% B for 10 min and increasing to 20% B in 20 min and to 40% B in 25 min. The column was then washed with 90% B for 10 min and equilibrated with 5% B for 5 min prior to each analysis.[35] The UV−vis spectra were acquired from 230 to 400 nm, and the detection wavelength was set at 280 nm. The identification of monomer and dimer flavanols was achieved by comparing their absorption spectra and retention times with those of pure standards. The quantification (mg kg^{-1} grape or mg g^{-1} seed) was carried out by the external standard method. The phloroglucinol adducts were identified on the basis of their retention times and quantified as equivalents of their respective free flavanols (external standard method), assuming the same molar absorptivity between each free flavanol and its corresponding phloroglucinol adduct.[36] The mDP value was calculated as the molar ratio of the sum of all flavanol units produced by phloroglucinolysis (phloroglucinol adducts plus monomers) to the sum of monomer flavanols. The G% value was calculated as the ratio of the sum of galloylated flavanols to the sum of all flavanols. All analyses were performed in duplicate.

Table 1. Spectral Pretreatments, Number of Principal Components (PC), and Wavenumbers Selected in the Development of NIR Calibration Models for Absorbance at 280 nm and Extractable Content of Phenolic Compounds in Intact Nebbiolo Seeds Using Different Numbers of Vintages

chemical parameter	spectral pretreatments[a]	PC	wavenumber (cm^{-1})
	2010 Vintage		
$A_{s,280}$ kg^{-1} grape	mf	6	8500−11520
TF_s (mg kg^{-1} grape)	mf, dg1	7	6000−7200
PRO_s (mg kg^{-1} grape)	SNV, dg1	5	6000−6800
FRV_s (mg kg^{-1} grape)	SNV, db1	5	6000−6800
C (mg kg^{-1} grape)	SNV, db1	2	7400−10600
EC (mg kg^{-1} grape)	SNV, db1	2	7400−10600
ECG (mg kg^{-1} grape)	mf	4	6000−10600
TM (mg kg^{-1} grape)	SNV, db1	1	7400−10600
B_1 (mg kg^{-1} grape)	sa3, ncl, db1	5	7400−11520
B_2 (mg kg^{-1} grape)	db1, ncl	6	6000−6800
TP (mg kg^{-1} grape)	db1, ncl	3	8500−11520
mDP	SNV	2	7400−11520
G%	db1, ncl	4	6000−6600, 7800−11520
$A_{s,280}$ g^{-1} seed	mf, dg2	2	8500−11520
TF_s (mg g^{-1} seed)	mf, dg2	2	6000−11520
PRO_s (mg g^{-1} seed)	dg1, mf	2	7400−11520
FRV_s (mg g^{-1} seed)	mf, dg1	5	8500−11520
C (mg g^{-1} seed)	sa3, ncl, db1	2	6000−10600
EC (mg g^{-1} seed)	mf	2	6000−10600
ECG (mg g^{-1} seed)	mf	3	6000−10600
TM (mg g^{-1} seed)	dg1, nle	2	8500−11520
B_1 (mg g^{-1} seed)	sa3, ncl, db1	6	8500−10600
B_2 (mg g^{-1} seed)	mf	4	6000−10600
TP (mg g^{-1} seed)	dg1, nle	2	6000−11520
	2011 Vintage		
$A_{s,280}$ kg^{-1} grape	ncl	2	6000−6600, 7800−11520
TF_s (mg kg^{-1} grape)	dg1, SNV	7	8500−10600
PRO_s (mg kg^{-1} grape)	ncl, db1	4	6000−10000
FRV_s (mg kg^{-1} grape)	SNV	5	6000−6800
C (mg kg^{-1} grape)	mf	4	6000−10600
EC (mg kg^{-1} grape)	dg2, SNV	4	7400−11520
ECG (mg kg^{-1} grape)	mf, dg2	3	8500−11520
TM (mg kg^{-1} grape)	ncl, db1	6	8500−11520
B_1 (mg kg^{-1} grape)	SNV, dg1	2	6000−6800
B_2 (mg kg^{-1} grape)	SNV	2	6000−9800
TP (mg kg^{-1} grape)	ncl, db1	4	6000−6800
mDP	db1, ncl	2	7400−11520
G%	dg1, mf	6	7400−11520
$A_{s,280}$ g^{-1} seed	mf	3	7400−11520
TF_s (mg g^{-1} seed)	dg2, SNV	6	8500−10600
PRO_s (mg g^{-1} seed)	SNV	2	6000−7200
FRV_s (mg g^{-1} seed)	SNV, dg2	6	6000−11520
C (mg g^{-1} seed)	mf, dg2	4	6000−11520
EC (mg g^{-1} seed)	mf, dg2	2	8500−11520
ECG (mg g^{-1} seed)	n01, db1	8	8500−11520
TM (mg g^{-1} seed)	ncl	6	6000−10600
B_1 (mg g^{-1} seed)	SNV, dg2	3	6000−6600, 7800−11520
B_2 (mg g^{-1} seed)	mf	2	8500−10600
TP (mg g^{-1} seed)	mf, dg2	2	6000−6800
	2010 and 2011 Vintages		
$A_{s,280}$ kg^{-1} grape	SNV, dg2	5	6000−7100, 7400−10000
TF_s (mg kg^{-1} grape)	db1, ncl	6	6000−9200
PRO_s (mg kg^{-1} grape)	dg2, SNV	6	6000−7100, 7400−9600
FRV_s (mg kg^{-1} grape)	dg1, mf	10	6000−10000
C (mg kg^{-1} grape)	mf, db1, nle	6	7400−10600
EC (mg kg^{-1} grape)	sa3, ncl, db1	2	6000−11520
ECG (mg kg^{-1} grape)	dg2, SNV	4	6000−10000
TM (mg kg^{-1} grape)	db1, SNV	4	8500−11520

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Table 1. continued

chemical parameter	spectral pretreatments[a]	PC	wavenumber (cm^{-1})
	2010 and 2011 Vintages		
B_1 (mg kg^{-1} grape)	dg2, SNV	2	6000−9200
B_2 (mg kg^{-1} grape)	SNV, dg2	3	8500−10600
TP (mg kg^{-1} grape)	db1, SNV	6	6000−7200
mDP	SNV, db1	2	6000−7200
G%	db1, SNV	8	7400−11520
$A_{s,280}$ g^{-1} seed	ncl	4	6000−11520
TF_s (mg g^{-1} seed)	dg1, SNV	8	6000−8800
PRO_s (mg g^{-1} seed)	dg1, nle	7	7400−11520
FRV_s (mg g^{-1} seed)	dg2, SNV	7	6000−9600
C (mg g^{-1} seed)	dg1, mf	3	8500−10600
EC (mg g^{-1} seed)	dg2, SNV	2	6000−7144, 7404−11520
ECG (mg g^{-1} seed)	SNV, db1	6	8500−11520
TM (mg g^{-1} seed)	dg2, SNV	3	6000−10600
B_1 (mg g^{-1} seed)	mf	3	6000−9800
B_2 (mg g^{-1} seed)	dg2, SNV	2	6000−6600, 7800−11520
TP (mg g^{-1} seed)	dg1, SNV	3	8500−11520

[a]mf, full multiplicative scatter correction; dg1, first-derivative Savitzky−Golay (9 points); SNV, standard normal variate; db1, first-derivative BCAP; sa3, smooth average (3 points); ncl, normalization by closure; dg2, second-derivative Savitzky−Golay (9 points); nle, normalization to length unit; n01, normalization between 0 and 1.

Spectral and Chemometric Analysis. Intact berry seeds were individually scanned using a NIRFlex N500 spectrophotometer equipped with a Solids Transmittance module (Buchi, Flawil, Switzerland). The FT-NIR spectra were collected in transmittance mode within the 4000−12000 cm^{-1} wavenumber range at 4 cm^{-1} resolution using the NIR-Operator software (Buchi). For each seed, a total of 64 scans were acquired on the lateral side and were averaged for each transmittance spectrum.[24] All NIR analyses were carried out at room temperature (20 ± 1 °C).

All spectra were randomly subdivided into the calibration set (about two-thirds) and the validation set (about one-third), the two sets being associated with comparable ranges of phenolic compounds. Principal component analysis (PCA) was performed before regression to provide information on the latent structure of the spectral data. This permits possible grouping samples to be seen and the presence of outliers to be detected. The FT-NIR spectra were pretreated, and a spectral region was selected to minimize the standard error of prediction (SEP).[37] Table 1 shows the spectral pretreatments applied, the number of principal components (PCs), and the wavenumbers selected using the NIRCAL 5.2 software (Buchi). Calibration models based on the phenolic composition determined by the reference method and the NIR spectra were performed by partial least-squares (PLS) regression using the NIRCAL 5.2 software.

PCA was an effective tool in addressing the classification of samples according to the growing zone, verifying whether unknown Piedmont and Trentino samples do not belong to the spectral space created by the Valtellina samples involved in the development of the regression equations. Other statistical analyses were performed using the SPSS software package version 19.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to establish significant differences in the chemical parameters or loadings of principal components among growing zones.

RESULTS AND DISCUSSION

Chemical Analysis. Nebbiolo winegrapes from Valtellina were sampled at different dates to consider the natural variability in the ripening stage that influences both the technological ripeness parameters and the phenolic composition. Furthermore, the samples were collected in 10 vineyards to consider agroclimatic variability (Supporting Information Table S1). In this way, a wider compositional range of the grape was covered. Figure 1 shows the variation range of the technological ripeness parameters for Nebbiolo winegrapes from Valtellina in the two vintages studied (2010 and 2011). In 2010, the results obtained for all of the technological ripeness parameters evidenced higher variability than in 2011, as can be observed in the variation range and in the interquartile amplitude. The values represented in Figure 1 vary within the range expected for Nebbiolo winegrapes from Valtellina[28] and practically cover the variability during ripening in Piedmont growing areas.[38]

Because of little scientific contributions currently available regarding the phenolic composition of Nebbiolo seeds, a comparison was established with other winegrape varieties with the aim of contributing to a better characterization of the Nebbiolo cultivar. At the last harvest date, the extractable content of total flavonoids ranged from 70.9 to 103.8 mg g^{-1} seed in 2010 (2697−4047 mg kg^{-1} grape) and from 32.4 to 54.7 mg g^{-1} seed in 2011 (1240−1802 mg kg^{-1} grape). The vintage effect was also observed on the content of total flavanols in Graciano seeds, the variation range being 25.3−40.9 mg g^{-1} seed during 2005−2008.[1] The Nebbiolo cultivar seems to evidence higher concentrations of total flavonoids in the seeds than the Graciano cultivar. Other works also reported lower concentrations of total flavanols for Cencibel, Cabernet Sauvignon, Merlot, and Shiraz seeds (330−870 mg kg^{-1} grape),[4] whereas values of total flavonoids of ca. 50 mg g^{-1} seed were found in Vranec and Merlot seeds.[39]

The extractable concentration of high molecular weight proanthocyanidins, determined by spectrophotometry and expressed as PRO_s, for Nebbiolo winegrapes at harvest varied between 20.5 and 33.7 mg g^{-1} seed in 2010 (837−1351 mg kg^{-1} grape), and between 12.5 and 29.3 mg g^{-1} seed in 2011 (492−999 mg kg^{-1} grape). These are comprised between the values reported for Carménère, Merlot, Cabernet Franc, and Cabernet Sauvignon seeds (6.6−16.2 mg g^{-1} seed)[7] and the found for Malbec seeds (99.2−125.2 mg g^{-1} seed).[5] On the other hand, the extractable content of simple monomer flavanols together with low molecular weight oligomer flavanols, determined by spectrophotometry and expressed as FRV_s,[40] in Nebbiolo seeds at the harvest date ranged from 8.8 to 13.4 mg g^{-1} seed in 2010 (314−610 mg kg^{-1} grape), and

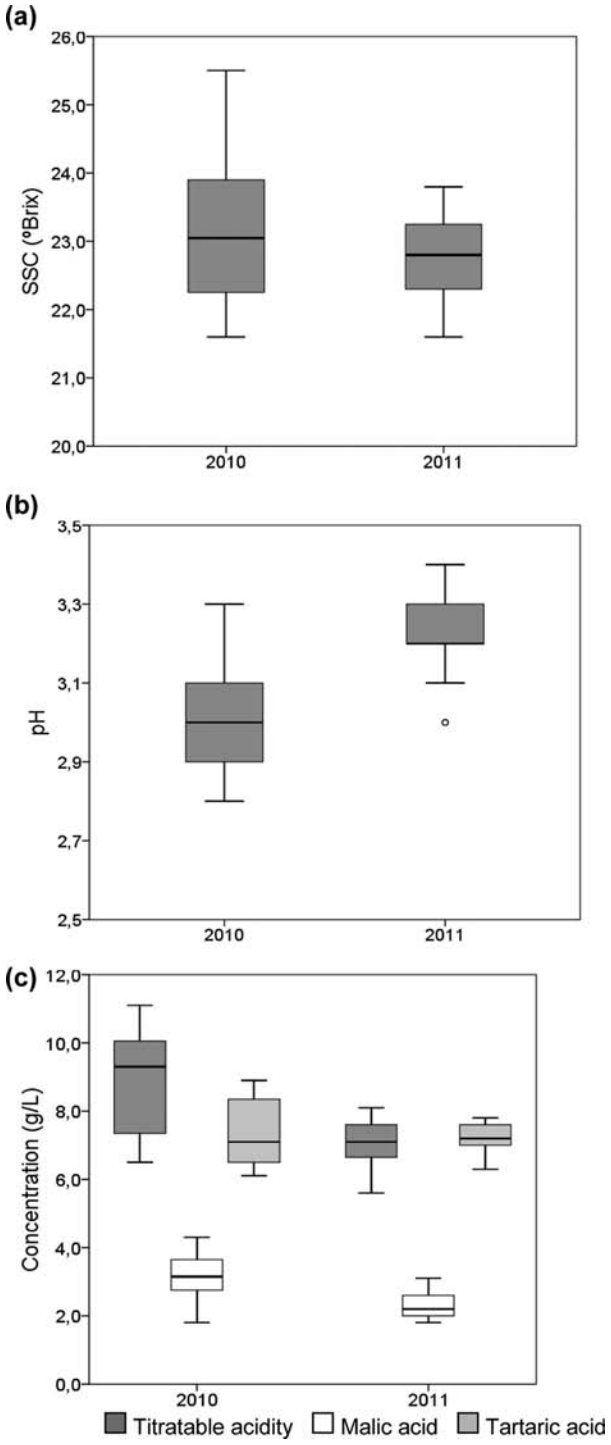

Figure 1. Technological ripeness parameters for Nebbiolo winegrapes from 10 vineyards at different ripening stages in 2010 and 2011: total soluble solids concentration (a); pH (b); titratable acidity, malic acid concentration, and tartaric acid concentration (c).

from 10.3 to 29.7 mg g^{-1} seed in 2011 (405−828 mg kg^{-1} grape). These values agreed with those reported for Vranec and Merlot seeds (16.7 and 18.4 mg g^{-1} seed, respectively)[39] and for Malbec ones (10.8−11.9 mg g^{-1} seed).[5]

With regard to monomer and dimer flavanols, determined by HPLC, the extractable concentration in the seeds of Nebbiolo winegrapes at harvest ranged from 1.50 to 3.15 mg g^{-1} seed, from 1.04 to 1.57 mg g^{-1} seed, from 0.04 to 0.09 mg g^{-1} seed, from 0.43 to 0.66 mg g^{-1} seed, and from 0.36 to 0.55 mg g^{-1} seed in 2010 for (+)-catechin (C), (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), and procyanidins B_1 and B_2, respectively (60.6−126.6, 42.1−63.3, 1.6−3.6, 17.5−29.9 and 14.6−23.7 mg kg^{-1} grape, respectively), and from 1.52 to 2.97 mg g^{-1} seed, from 0.92 to 1.59 mg g^{-1} seed, from 0.03 to 0.05 mg g^{-1} seed, from 0.35 to 0.73 mg g^{-1} seed, and from 0.37 to 0.76 mg g^{-1} seed in 2011 for C, EC, ECG, and procyanidins B_1 and B_2, respectively (59.7−109.1, 28.9−57.8, 1.1−1.6, 14.1−21.0 and 14.5−23.5 mg kg^{-1} grape, respectively). This supposes a variation range for total monomer flavanols (TM) of 2.60−4.81 mg g^{-1} seed in 2010 and 2.47−4.33 mg g^{-1} seed in 2011 (104.6−193.5 and 97.1−163.0 mg kg^{-1} grape, respectively). Nebbiolo seeds accounted for an extractable content of total polymer flavanols (TP) in the range of 6.81−10.26 mg g^{-1} seed in 2010 and 5.93−11.79 mg g^{-1} seed in 2011 (291.1−449.0 and 223.3−416.3 mg kg^{-1} grape, respectively). The extractable content of individual monomer flavanols in Nebbiolo seeds agreed with that reported for other autochthonous and international red winegrape varieties grown in Trentino.[2] At harvest, the most abundant monomer flavanol in Nebbiolo seeds was C (58−73%), followed by EC (26−41%). Furthermore, the extractable content of polymeric proanthocyanidins agreed with the values published for Nebbiolo seeds at the last ripening stages, EC-phloroglucinol being the more representative adduct in the extension units.[41] On the other hand, the values of the mean degree of polymerization (mDP) and the galloylation percentage (G%) (4.0−7.2 and 5.3−12.1%) were slightly lower than others published for Nebbiolo seeds at the last ripening stages (9.5−10.7 and 13.1−14.7%).[41] This can be supported by a poorer extraction of large proanthocyanidins in the wine-like solution used and/or by possible depolymerization as a consequence of the acidity of the hydroalcoholic solution buffered at pH 3.2.[2]

Figures 2 and 3 show the variation range for the measurement of $A_{s,280}$ and for the extractable content of phenolic compounds in the seeds from Nebbiolo winegrapes collected, in Valtellina, at different stages of maturity considering each year separately. The median was higher in 2010 for $A_{s,280}$, TF_s, PRO_s, ECG, and procyanidin B_1, but lower for FRV_s, C, EC, TM, procyanidin B_2, and TP. The lowest variation range and interquartile amplitude were associated with 2010, except for TF_s. The vintage effect was particularly evident on the extractable content of total flavonoids because of the completely different variation range for the two years studied. This variability in the phenolic composition of Nebbiolo seeds was considered suitable for developing NIR calibrations. In fact, the variation range covers the most typical values reported for the extractable content of total flavonoids, polymeric proanthocyanidins, and low molecular weight flavanols in the seeds of Nebbiolo winegrapes, at harvest, from several production zones located in Piedmont.[41,42]

FT-NIR Analysis. Tables 2 and 3 show the mean, minimum, and maximum values in both the calibration and validation sets, respectively, for the measurement of $A_{s,280}$ and the extractable content of phenolic compounds in the seeds determined by the reference method (extraction combined with UV−visible spectrophotometry or liquid chromatography). The performance of PLS calibration models was assessed from the correlation coefficient of calibration (R_c) and the standard error of calibration (SEC). The standard error of calibration was also standardized (SEC%) by rating its value to the mean of the calibration population and is related to the mean error of

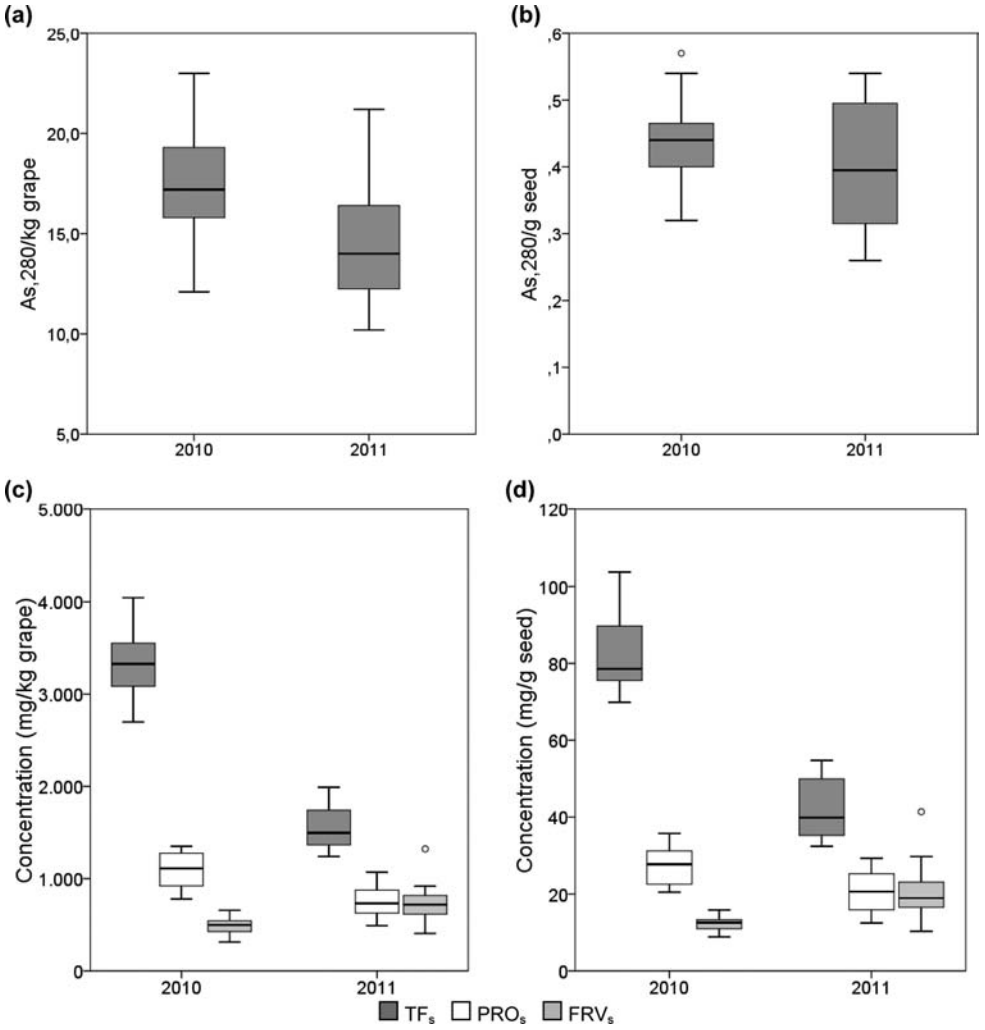

Figure 2. $A_{s,280}$ (a, b) and extractable content of TF_s, PRO_s, and FRV_s (c, d) per weight of grape (a, c) and seed (b, d) for Nebbiolo seeds from 10 vineyards at different grape ripening stages in 2010 and 2011.

the model. A good calibration model should have high R_c and low SEC and SEC%. With the exception of $A_{s,280}$ per weight of seed, the best statistical parameters of calibration for the spectrophotometric indices were obtained when the calibration sets corresponding to the two years studied were simultaneously considered ($R_c > 0.80$, SEC% < 16) (Table 2). With regard to flavanolic compounds determined by HPLC, the best statistical parameters of calibration for individual monomer flavanols, total monomers, and galloylation percentage were associated with the 2011 vintage, whereas those for dimer flavanols, mDP, and total polymeric proanthocyanidins were obtained in the 2010 vintage ($R_c \geq 0.52$, SEC% $\leq$ 19) (Table 2). The statistical parameters evidenced that PLS calibration models could be used with quantification purposes for TF_s, FRV_s, PRO_s, individual flavanols, and G%. In fact, very good calibration models between the FT-NIR spectra and the content of phenolic compounds determined by the reference method were obtained in 2010 for TF_s per weight of grape and procyanidin B_1 per weight of seed; in 2011 for FRV_s and ECG per weight of seed; and in 2010 and 2011 together for FRV_s per weight of grape ($R_c > 0.95$, SEC% < 9). On the other hand, satisfactory calibration models were found in 2010 for TF_s per weight of seed and procyanidin B_2 per weight of grape; in 2011 for TF_s, FRV_s, and EC per weight of grape, C and procyanidin B_1 per weight of seed, and G%; and in 2010 and 2011 together for PRO_s per weight of grape, TF_s per weight of grape or seed, FRV_s and ECG per weight of seed, and G% (R_c = 0.86−0.95, SEC% $\leq$ 15). Because of the goodness of the statistical parameters for the Nebbiolo cultivar, the usefulness of FT-NIR spectroscopy to predict the flavanolic composition of the seeds for different red winegrape cultivars was also investigated. However, inadequate values of R_c were obtained, which coincided with excessively high SEC% values (data not shown). Therefore, the grape variety strongly affects the usefulness of PLS calibration models.

An external validation was performed to assess the robustness of PLS calibration models using a sample set that did not belong to the calibration set. The calibration equations obtained were applied to the validation set, and the chemical parameters determined in the seeds by the reference method were compared with those predicted by the NIR calibration (Table 3). The predictive accuracy of PLS calibration models was evaluated from the correlation coefficient of validation (R_v) and SEP. The goodness of the prediction ability requires high R_v and low SEP. Bellon-Maurel et al. referred to the increase in the SEP value when the measurement range, or the mean of this range, increases.[43] Therefore, another three statistical parameters were used to standardize the predictive accuracy of

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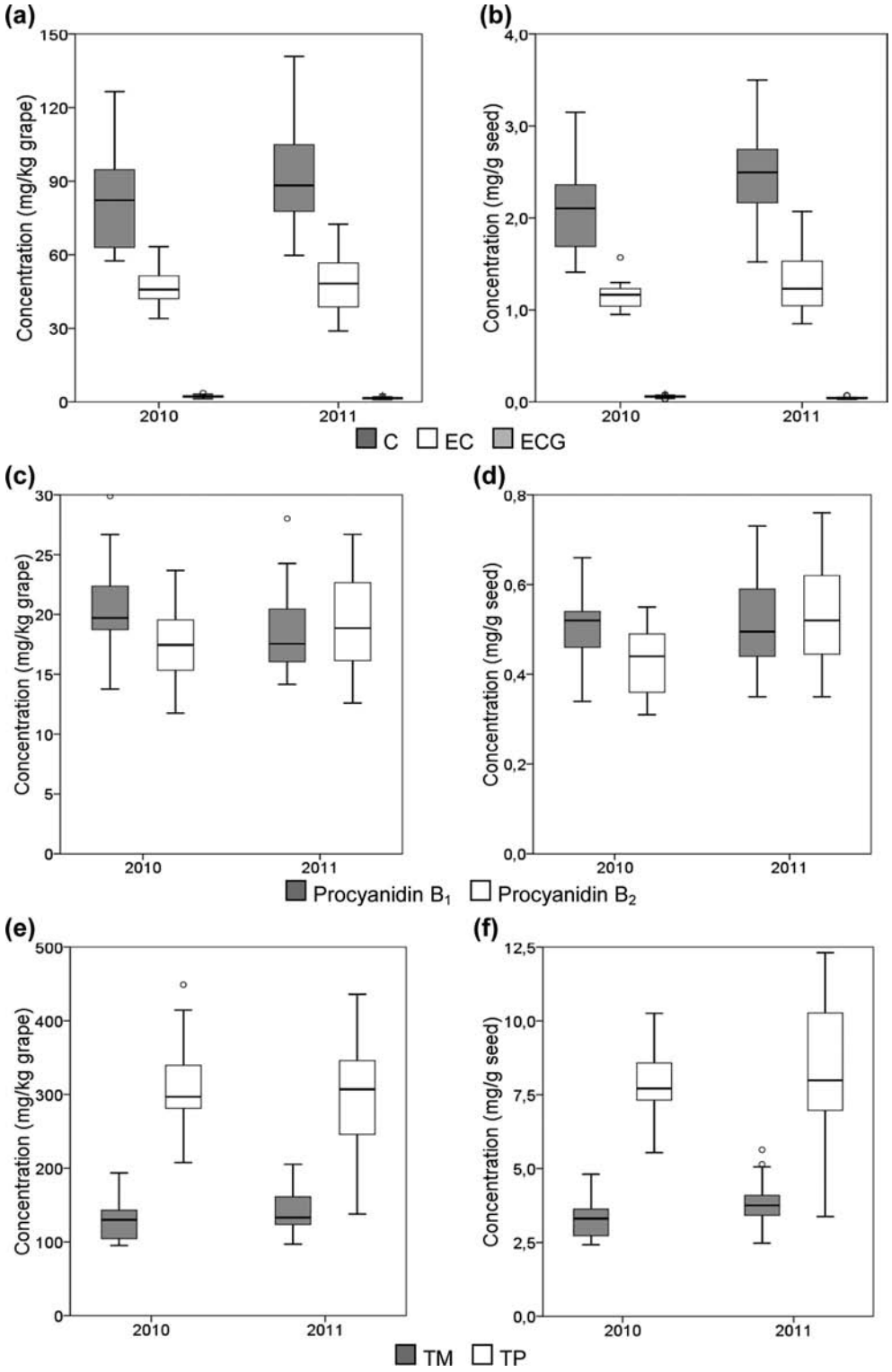

Figure 3. Extractable content of C, EC, and ECG (a, b), procyanidins B_1 and B_2 (c, d), and TM and TP (e, f) per weight of grape (a, c, e) and seed (b, d, f) for Nebbiolo seeds from 10 vineyards at different grape ripening stages in 2010 and 2011.

each calibration model, removing any variation range effect. The coefficient of variation (SEP%) was calculated as the ratio of the SEP value to the mean of the validation population. The residual predictive deviation (RPD) was defined as the ratio between the standard deviation (SD) of the validation set and the SEP value. RPD is the most commonly used statistical index to account for model reliability. However, a new index, RPIQ, based on quartiles, better represents the population spread, regardless of the distribution.[43] This was calculated as the ratio of the interquartile amplitude of the validation population to the SEP value.

SEP% values <20 are considered acceptable for most analytical purposes,[20] which evidence the suitability of the NIR calibration models developed to predict the phenol

Table 2. Statistical Parameters of NIR Calibration Models Developed for Absorbance at 280 nm and Extractable Content of Phenolic Compounds in Intact Nebbiolo Seeds Using Different Numbers of Vintages

chemical parameter	mean	min	max	R_c[a]	SEC[b]	SEC%[c]
			2010 Vintage[d]			
$A_{s,280}$ kg^{-1} grape	17.8	12.1	23.0	0.77	2.0	11.5
TF_s (mg kg^{-1} grape)	3304	2776	4047	0.96	121	3.6
PRO_s (mg kg^{-1} grape)	1122	781	1351	0.81	113	10.1
FRV_s (mg kg^{-1} grape)	491	359	610	0.78	48	9.7
C (mg kg^{-1} grape)	81.0	57.6	126.6	0.28	20.0	24.6
EC (mg kg^{-1} grape)	46.0	34.1	63.3	0.46	7.1	15.4
ECG (mg kg^{-1} grape)	2.3	1.3	3.6	0.70	0.5	22.2
TM (mg kg^{-1} grape)	130.0	95.3	193.5	0.18	27.3	21.0
B_1 (mg kg^{-1} grape)	20.5	13.8	29.9	0.70	3.3	16.2
B_2 (mg kg^{-1} grape)	17.9	11.7	23.7	0.87	1.9	10.5
TP (mg kg^{-1} grape)	316.9	207.4	449.0	0.63	51.9	16.4
mDP	5.9	4.9	7.5	0.56	0.6	10.8
G%	6.8	5.4	8.9	0.49	0.8	12.2
$A_{s,280}$ g^{-1} seed	0.436	0.324	0.567	0.85	0.038	8.7
TF_s (mg g^{-1} seed)	82.9	69.9	103.8	0.86	5.7	6.9
PRO_s (mg g^{-1} seed)	28.1	21.1	35.7	0.68	3.3	11.8
FRV_s (mg g^{-1} seed)	12.3	9.6	15.9	0.56	1.5	11.9
C (mg g^{-1} seed)	2.05	1.41	3.15	0.66	0.41	19.7
EC (mg g^{-1} seed)	1.16	0.96	1.57	0.19	0.16	13.7
ECG (mg g^{-1} seed)	0.06	0.03	0.09	0.61	0.01	22.9
TM (mg g^{-1} seed)	3.35	2.42	4.81	0.66	0.49	14.5
B_1 (mg g^{-1} seed)	0.50	0.34	0.66	0.99	0.01	2.5
B_2 (mg g^{-1} seed)	0.44	0.31	0.55	0.57	0.07	14.9
TP (mg g^{-1} seed)	7.81	5.54	10.26	0.52	1.02	13.0
			2011 Vintage[d]			
$A_{s,280}$ kg^{-1} grape	14.8	10.2	21.2	0.49	2.8	19.1
TF_s (mg kg^{-1} grape)	1538	1240	1990	0.90	101	6.6
PRO_s (mg kg^{-1} grape)	732	492	1072	0.48	149	20.3
FRV_s (mg kg^{-1} grape)	731	405	1322	0.86	110	15.0
C (mg kg^{-1} grape)	91.7	59.7	140.8	0.73	14.4	15.7
EC (mg kg^{-1} grape)	48.6	28.9	72.4	0.93	4.8	10.0
ECG (mg kg^{-1} grape)	1.5	0.9	2.8	0.79	0.3	19.0
TM (mg kg^{-1} grape)	140.9	97.1	205.2	0.61	23.6	16.8
B_1 (mg kg^{-1} grape)	18.8	14.1	28.0	0.45	3.2	16.9
B_2 (mg kg^{-1} grape)	19.4	12.6	26.7	0.73	2.9	15.1
TP (mg kg^{-1} grape)	299.5	137.8	436.1	0.60	67.7	22.6
mDP	5.3	4.4	6.1	0.27	0.5	8.9
G%	7.4	4.3	11.4	0.94	0.6	8.8
$A_{s,280}$ g^{-1} seed	0.389	0.241	0.542	0.55	0.087	22.3
TF_s (mg g^{-1} seed)	41.9	32.4	54.7	0.80	4.9	11.6
PRO_s (mg g^{-1} seed)	21.1	12.5	29.3	0.27	5.2	24.9
FRV_s (mg g^{-1} seed)	21.0	10.3	41.4	0.98	1.6	7.6
C (mg g^{-1} seed)	2.51	1.52	3.50	0.91	0.24	9.5
EC (mg g^{-1} seed)	1.31	0.85	2.07	0.40	0.30	22.7
ECG (mg g^{-1} seed)	0.04	0.03	0.07	0.99	0.00	4.9
TM (mg g^{-1} seed)	3.86	2.47	5.64	0.81	0.47	12.3
B_1 (mg g^{-1} seed)	0.52	0.35	0.73	0.88	0.06	10.8
B_2 (mg g^{-1} seed)	0.53	0.35	0.76	0.46	0.12	21.7
TP (mg g^{-1} seed)	8.20	3.37	12.31	0.35	2.34	28.5
			2010 and 2011 Vintages[e]			
$A_{s,280}$ kg^{-1} grape	16.2	10.2	23.0	0.85	1.8	11.3
TF_s (mg kg^{-1} grape)	2308	1240	3796	0.94	327	14.2
PRO_s (mg kg^{-1} grape)	923	492	1351	0.89	118	12.7
FRV_s (mg kg^{-1} grape)	630	359	1322	0.97	51	8.2
C (mg kg^{-1} grape)	87.2	57.6	126.6	0.53	16.5	18.9
EC (mg kg^{-1} grape)	47.4	28.9	72.4	0.10	11.1	23.5
ECG (mg kg^{-1} grape)	1.9	0.9	3.6	0.72	0.5	23.8
TM (mg kg^{-1} grape)	134.8	95.3	205.2	0.35	27.3	20.2
B_1 (mg kg^{-1} grape)	19.5	13.8	29.9	0.63	3.1	15.9

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Table 2. continued

chemical parameter	mean	min	max	R_c[a]	SEC[b]	SEC%[c]
2010 and 2011 Vintages[e]						
B_2 (mg kg^{-1} grape)	18.5	11.7	26.7	0.63	3.1	16.4
TP (mg kg^{-1} grape)	302.3	137.8	449.0	0.67	51.7	17.1
mDP	5.6	4.4	6.9	0.43	0.6	10.1
G%	7.2	4.3	11.4	0.87	0.8	10.5
$A_{s,280}$ g^{-1} seed	0.420	0.241	0.567	0.65	0.072	17.1
TF_s (mg g^{-1} seed)	62.3	33.0	103.8	0.95	7.2	11.5
PRO_s (mg g^{-1} seed)	23.9	12.5	35.7	0.83	3.6	15.1
FRV_s (mg g^{-1} seed)	15.4	9.6	23.9	0.93	1.7	11.1
C (mg g^{-1} seed)	2.29	1.41	3.50	0.73	0.40	17.4
EC (mg g^{-1} seed)	1.22	0.85	2.07	0.44	0.26	21.5
ECG (mg g^{-1} seed)	0.05	0.03	0.09	0.90	0.01	13.8
TM (mg g^{-1} seed)	3.59	2.42	5.64	0.65	0.61	16.9
B_1 (mg g^{-1} seed)	0.52	0.34	0.73	0.43	0.09	17.3
B_2 (mg g^{-1} seed)	0.49	0.31	0.76	0.40	0.11	22.3
TP (mg g^{-1} seed)	7.88	5.48	11.30	0.62	1.14	14.4

[a]R_c, correlation coefficient of calibration. [b]SEC, standard error of calibration. [c]SEC% = (SEC/mean) × 100. [d]n = 42. [e]n = 84.

content in intact berry seeds. The differences found between the reference values and those predicted by NIR calibration models were smaller ($R_v \geq 0.85$, SEP% < 15) in 2010 for TF_s, PRO_s, and procyanidin B_2 per weight of grape; in 2011 for TF_s and EC per weight of grape, C per weight of seed, G%, and FRV_s per weight of grape or seed; and in 2010 and 2011 together for PRO_s per weight of grape and ECG per weight of seed, as well as TF_s and FRV_s per weight of grape or seed (Table 3). A better standardization of the SEP value is provided by the RPD and RPIQ indices. If the SEP value is small compared to the population spread of a certain chemical parameter, a relatively high index is obtained. Therefore, the higher the RPD value, the greater the predictive accuracy. Chang et al. established that RPD values >2.0 are very satisfactory for prediction purposes, whereas values ranging between 1.4 and 2.0 are indicative of fair models.[44] This would permit the use of the NIR calibration models developed, using simultaneously the data sets corresponding to the two years studied, for the quantification of spectrophotometric indices related to total flavonoids per weight of grape or seed and to proanthocyanidins and low molecular weight flavanols per weight of grape, as well as for screening of $A_{s,280}$ and (−)-epicatechin gallate per weight of grape or seed and galloylation percentage (Table 3). On the other hand, the NIR calibration models were sufficiently reliable for quantification or screening purposes only if constructed from the data set corresponding to the 2010 vintage for the determination of total polymer flavanols per weight of grape and procyanidin B_2 per weight of grape or seed, or those of the 2011 vintage for the determination of (−)-epicatechin per weight of grape and (+)-catechin and procyanidin B_1 per weight of seed (Table 3). However, according to standards proposed by other authors (RPD = 2.4−4.9),[45] the NIR calibration models developed could be fair and recommended only for screening purposes of the extractable content of total flavonoids in both 2010 and 2011 vintages together or of the extractable content of (+)-catechin per weight of seed and galloylation percentage in the 2011 vintage. No statistical basis was used to determine these thresholds, and some researchers have begun to criticize this statistical index.[46] As above-mentioned, the use of the RPIQ index provides a better evaluation of the predictive ability of NIR calibration models. Using simultaneously the data of the two years studied, the RPIQ values indicated that the predictive accuracy was very good for total flavonoids per weight of grape or seed (RPIQ ≥ 5.0), satisfactory for the determination of spectrophotometric indices related to proanthocyanidins and monomer together with oligomer flavanols per weight of grape (RPIQ = 3.0−5.0), and unreliable for quantitative purposes of $A_{s,280}$ per weight of grape and ECG per weight of grape or seed but acceptable for screening (RPIQ = 2.4−3.0). Furthermore, satisfactory predictive accuracy for (−)-epicatechin per weight of grape, procyanidin B_1 per weight of seed, and galloylation percentage and good robustness for the quantitative prediction of (+)-catechin per weight of seed were achieved only in the 2011 vintage. Instead, the only use of the data set corresponding to the 2010 vintage enabled the screening of procyanidin B_2 per weight of grape and total polymer flavanols per weight of seed.

The evaluation of all statistics permitted verification that the most reliable PLS calibration models for the prediction of the spectrophotometric parameters were obtained when the sample sets of the two different vintages were considered, and the reference values were expressed as content or absorbance per weight of grape. Nevertheless, the equations corresponding to $A_{s,280}$ could be useful only for screening purposes in the seeds. With regard to the flavanolic composition, the most robust calibration models corresponded to the individual use of the sample set of 2011 vintage.

The statistical parameters obtained for the prediction of the content of total flavonoids in seeds by FT-NIR spectroscopy were compared with the reported values for total flavanols by Ferrer-Gallego et al. for samples harvested in two vineyards located in the same growing zone (SEP = 4.9 mg g^{-1} seed).[25] Although a worse value of SEP was found (8.8 mg g^{-1} seed), the standard deviation of the sample set was also 1.7 times larger. Therefore, comparable predictive accuracy is expected. Also, the extractable contents of (+)-catechin, (−)-epicatechin, procyanidins B_1 and B_2, and total monomer flavanols were compared, and lower values of SEP were obtained in the present work, except for procyanidin B_1 with slightly higher values than reported by Ferrer Gallego et al. as a function of vintage (0.05−0.09 instead of 0.06 mg g^{-1} seed).[25] With regard to the measurement of $A_{s,280}$ per weight of seed, the statistics obtained were quite similar to those previously provided by

Table 3. Validation of NIR Calibration Models Developed for Absorbance at 280 nm and Extractable Content of Phenolic Compounds in Intact Nebbiolo Seeds Using Different Numbers of Vintages

chemical parameter	mean	min	max	R_v[a]	SEP[b]	SEP%[c]	RPD[d]	RPIQ[e]
2010 Vintage[f]								
$A_{s,280}$ kg^{-1} grape	17.3	13.6	21.0	0.75	1.8	10.4	1.5	2.7
TF_s (mg kg^{-1} grape)	3345	2826	3901	0.94	95	2.8	4.0	6.1
PRO_s (mg kg^{-1} grape)	1167	871	1310	0.88	119	10.2	1.5	2.6
FRV_s (mg kg^{-1} grape)	502	398	597	0.84	46	9.1	1.8	3.5
C (mg kg^{-1} grape)	88.0	57.6	124.0	0.29	20.9	23.7	1.0	1.8
EC (mg kg^{-1} grape)	47.5	34.1	57.7	0.35	6.4	13.5	1.0	1.6
ECG (mg kg^{-1} grape)	2.3	1.3	2.8	0.71	0.3	12.6	1.3	2.1
TM (mg kg^{-1} grape)	137.6	100.0	184.2	0.42	30.1	21.9	1.0	1.7
B_1 (mg kg^{-1} grape)	19.9	13.8	23.8	0.66	2.8	14.1	1.0	1.2
B_2 (mg kg^{-1} grape)	17.2	12.6	23.1	0.86	1.9	11.0	1.9	2.9
TP (mg kg^{-1} grape)	305.4	263.5	386.5	0.81	33.9	11.1	1.4	1.9
mDP	5.9	5.4	6.7	0.42	0.5	8.6	1.1	1.5
G%	7.2	5.9	8.5	0.55	0.8	10.9	1.1	2.0
$A_{s,280}$ g^{-1} seed	0.451	0.402	0.523	0.71	0.039	8.6	1.3	2.2
TF_s (mg g^{-1} seed)	83.8	74.4	97.2	0.82	6.2	7.4	1.4	2.6
PRO_s (mg g^{-1} seed)	29.1	22.1	33.3	0.81	3.0	10.2	1.5	2.6
FRV_s (mg g^{-1} seed)	12.4	9.9	14.7	0.72	1.5	11.8	1.3	2.3
C (mg g^{-1} seed)	2.12	1.41	2.46	0.63	0.37	17.3	0.9	1.6
EC (mg g^{-1} seed)	1.14	0.96	1.30	0.01	0.13	11.8	1.0	1.7
ECG (mg g^{-1} seed)	0.05	0.03	0.07	0.68	0.01	16.7	1.3	2.4
TM (mg g^{-1} seed)	3.16	2.47	4.02	0.61	0.52	16.5	1.1	1.6
B_1 (mg g^{-1} seed)	0.51	0.34	0.60	0.76	0.05	8.9	1.3	1.9
B_2 (mg g^{-1} seed)	0.44	0.31	0.53	0.70	0.06	12.6	1.4	2.1
TP (mg g^{-1} seed)	7.73	5.76	9.79	0.74	1.05	13.6	1.3	2.8
2011 Vintage[f]								
$A_{s,280}$ kg^{-1} grape	14.2	11.9	18.3	0.46	2.2	15.3	1.1	2.0
TF_s (mg kg^{-1} grape)	1577	1357	1777	0.88	105	6.7	1.7	3.4
PRO_s (mg kg^{-1} grape)	763	553	926	0.74	107	14.1	1.3	2.3
FRV_s (mg kg^{-1} grape)	744	600	845	0.91	98	13.2	1.0	1.9
C (mg kg^{-1} grape)	92.5	61.7	120.8	0.68	16.9	18.2	1.3	2.4
EC (mg kg^{-1} grape)	46.4	34.5	57.8	0.91	4.1	8.9	2.3	4.3
ECG (mg kg^{-1} grape)	1.6	1.1	2.3	0.77	0.3	18.9	1.4	2.7
TM (mg kg^{-1} grape)	142.9	103.6	195.5	0.65	25.5	17.8	1.3	2.3
B_1 (mg kg^{-1} grape)	18.4	14.8	24.2	0.45	3.1	17.0	1.1	1.8
B_2 (mg kg^{-1} grape)	19.2	15.1	25.3	0.49	3.5	18.2	1.1	1.9
TP (mg kg^{-1} grape)	313.5	223.3	396.1	0.61	49.2	15.7	1.3	2.0
mDP	5.4	4.4	6.0	0.28	0.4	7.9	1.0	2.2
G%	7.3	5.0	8.6	0.95	0.6	8.0	2.4	3.9
$A_{s,280}$ g^{-1} seed	0.409	0.294	0.519	0.53	0.078	19.0	1.2	2.5
TF_s (mg g^{-1} seed)	44.2	35.1	53.4	0.76	5.1	11.5	1.5	2.7
PRO_s (mg g^{-1} seed)	19.8	15.1	26.4	0.23	4.0	20.1	1.0	1.7
FRV_s (mg g^{-1} seed)	18.6	14.0	23.5	0.91	1.8	9.9	1.7	2.3
C (mg g^{-1} seed)	2.49	1.87	3.25	0.93	0.18	7.1	2.8	5.8
EC (mg g^{-1} seed)	1.30	0.96	1.85	0.57	0.29	22.3	1.2	2.3
ECG (mg g^{-1} seed)	0.04	0.03	0.05	0.84	0.01	16.2	1.3	2.5
TM (mg g^{-1} seed)	3.83	2.80	5.15	0.68	0.59	15.3	1.3	2.3
B_1 (mg g^{-1} seed)	0.49	0.38	0.68	0.80	0.07	15.1	1.4	3.1
B_2 (mg g^{-1} seed)	0.52	0.38	0.73	0.39	0.11	21.4	1.1	1.8
TP (mg g^{-1} seed)	8.75	5.93	11.79	0.53	1.83	20.9	1.2	1.6
2010 and 2011 *vintages*[g]								
$A_{s,280}$ kg^{-1} grape	15.7	11.9	21.0	0.82	1.8	11.2	1.7	2.7
TF_s (mg kg^{-1} grape)	2556	1357	4047	0.94	309	12.1	3.0	6.0
PRO_s (mg kg^{-1} grape)	958	568	1279	0.89	121	12.6	2.1	4.3
FRV_s (mg kg^{-1} grape)	604	395	828	0.87	74	12.3	2.1	3.2
C (mg kg^{-1} grape)	84.8	60.6	115.8	0.51	16.7	19.7	1.2	2.3
EC (mg kg^{-1} grape)	47.1	38.1	57.7	0.10	6.5	13.8	1.0	1.7
ECG (mg kg^{-1} grape)	1.8	1.1	2.8	0.69	0.4	23.4	1.4	2.4
TM (mg kg^{-1} grape)	141.9	102.3	195.5	0.36	27.5	19.4	1.1	1.5
B_1 (mg kg^{-1} grape)	19.3	14.8	26.7	0.52	3.1	16.1	1.1	1.8

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Table 3. continued

chemical parameter	mean	min	max	R_v[a]	SEP[b]	SEP%[c]	RPD[d]	RPIQ[e]
			2010 and 2011 *vintages*[g]					
B_2 (mg kg^{-1} grape)	18.5	11.9	25.5	0.67	3.2	17.5	1.2	2.2
TP (mg kg^{-1} grape)	321.8	208.2	436.1	0.65	55.9	17.4	1.2	2.0
mDP	5.4	4.8	6.5	0.48	0.4	8.1	1.1	1.8
G%	7.1	5.0	8.9	0.72	0.9	12.0	1.4	2.1
$A_{s,280}$ g^{-1} seed	0.409	0.294	0.519	0.67	0.048	11.7	1.4	1.6
TF_s (mg g^{-1} seed)	60.6	32.4	88.3	0.91	8.8	14.5	2.4	5.0
PRO_s (mg g^{-1} seed)	25.4	15.7	32.6	0.75	3.7	14.7	1.4	2.2
FRV_s (mg g^{-1} seed)	15.8	11.0	22.7	0.85	2.1	13.4	1.8	2.6
C (mg g^{-1} seed)	2.31	1.50	3.25	0.62	0.38	16.6	1.2	1.9
EC (mg g^{-1} seed)	1.26	1.02	1.57	0.46	0.16	12.9	1.1	1.7
ECG (mg g^{-1} seed)	0.05	0.03	0.07	0.85	0.01	14.7	1.8	2.7
TM (mg g^{-1} seed)	3.57	2.60	5.05	0.56	0.58	16.2	1.2	1.7
B_1 (mg g^{-1} seed)	0.49	0.35	0.68	0.47	0.09	17.8	1.1	1.7
B_2 (mg g^{-1} seed)	0.48	0.31	0.75	0.40	0.10	19.7	1.1	0.9
TP (mg g^{-1} seed)	8.22	5.54	11.31	0.67	1.32	16.1	1.3	1.7

[a]R_v, correlation coefficient of validation. [b]SEP, standard error of prediction. [c]SEP% = (SEP/mean) × 100. [d]RPD, residual predictive deviation (SD/SEP); SD, standard deviation. [e]RPIQ = IQ/SEP; IQ, interquartile amplitude. [f]n = 18. [g]n = 36.

Table 4. Absorbance at 280 nm and Extractable Content of Phenolic Compounds in Intact Nebbiolo Seeds from the Three Growing Zones Studied in 2011[a]

	growing zone			
chemical parameter	Valtellina[b]	Piedmont[c]	Trentino[c]	signif[d]
$A_{s,280}$ kg^{-1} grape	14.6 ± 3.0	20.2 ± 1.8	20.1 ± 1.7	ns
TF_s (mg kg^{-1} grape)	1550 ± 211	1357 ± 152	1261 ± 124	ns
PRO_s (mg kg^{-1} grape)	754 ± 158b	1119 ± 43a	1135 ± 129a	***
FRV_s (mg kg^{-1} grape)	735 ± 185	1119 ± 124	1072 ± 72	ns
$A_{s,280}$ g^{-1} seed	0.400 ± 0.092	0.549 ± 0.021	0.425 ± 0.054	ns
TF_s (mg g^{-1} seed)	42.6 ± 7.8	36.8 ± 0.6	26.7 ± 3.9	ns
PRO_s (mg g^{-1} seed)	20.7 ± 5.0	30.5 ± 2.7	24.1 ± 3.9	ns
FRV_s (mg g^{-1} seed)	20.3 ± 6.6	30.3 ± 0.8	22.7 ± 2.6	ns

[a]All data are expressed as average value ± standard deviation. Different letters within the same row indicate significant differences among growing zones for each chemical parameter (Tukey b test; $p < 0.01$). [b]n = 60. [c]n = 9. [d]Signif: *** and ns indicate significance at $p < 0.001$ and not significant, respectively.

Rolle et al. (R_v = 0.65, SEP% = 7.8)[24] despite the fact the samples were collected in only one vineyard. The main advantage of the NIR calibration models now developed is their applicability to multiple vineyards characterized by very different agroclimatic conditions, despite being located in the same geographical area.

The necessity of developing annual calibration models for the prediction of individual monomer and dimer flavanols, as well as of total polymer flavanols and galloylation percentage, limits the practical applicability of FT-NIR spectroscopy. Therefore, the next step was to study the growing zone effect by means of checking the predictive ability of NIR calibration models only for the spectrophotometric indices, closely related to the phenolic composition of the seeds, in the Nebbiolo cultivar from six vineyards located in other growing zones very environmentally different from Valtellina, such as Piedmont and Trentino (Table S1 in the Supporting Information). The calibration equations developed for Valtellina samples were applied to Piedmont and Trentino samples, and the values predicted for $A_{s,280}$ and the extractable content of TF_s, FRV_s, and PRO_s were compared with the reference data. Although the reference values for Nebbiolo seeds from Piedmont and Trentino agreed with those reported for Valtellina, with very few exceptions as can be seen in Table 4, the differences found between these values and those predicted by the NIR technique ranged from 21.6 to 76.2%, these being significant for most determinations ($p < 0.01$). In an attempt to verify if these differences were due to variations in the NIR spectra, the samples from the three growing zones were classified by applying PCA to the NIR spectral data of the intact seeds. Table 5 shows the loadings of the four principal components for the samples. The two first principal components (91% overall explained variance) permitted satisfactory separation among the three zones, as demonstrated by the significant

Table 5. Loadings of Principal Components (PC) for NIR Spectral Data of Intact Nebbiolo Seeds from the Three Growing Zones Studied in 2011[a]

PC	Valtellina[b]	Piedmont[c]	Trentino[c]	signif[d]
1	−0.0970a	0.7226b	0.4574b	***
2	0.0071b	0.3601c	−0.5134a	***
3	0.0367	0.1148	0.1505	ns
4	−0.0163	−0.1196	−0.1703	ns

[a]Different letters within the same row indicate significant differences among growing zones for each principal component (Tukey b test; $p < 0.01$). [b]n = 40. [c]n = 3. [d]Signif: *** and ns indicate significance at $p < 0.001$ and not significant, respectively.

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differences among loadings ($p < 0.01$). At this moment, the NIR calibration models developed provide only good predictive accuracy of the chemical parameters, usually used in cellars to determine the phenolic ripeness of the seeds, if vineyards belonging to the same production zone are sampled.

FT-NIR spectroscopy is a fast and reliable technique for predicting the extractable content of phenolic compounds in Nebbiolo seeds, particularly from grapes harvested in different vineyards located in the same growing zone. Most of the chromatographic parameters, such as individual monomer and dimer flavanols, total polymer flavanols, and galloylation percentage, can be only satisfactorily predicted by using NIR calibration models developed for each vintage. On the other hand, the robustness of the NIR calibration models to predict spectrophotometric indices, closely related to the phenolic composition of the seeds, increased using simultaneously the sample sets of two years, if compared with that corresponding to each individual year. This work ensured the suitability of the FT-NIR spectra on intact berry seeds as a routine analytical tool for optimizing harvest decisions and selective processing of grapes, as well as assessing and managing the phenols extraction during the winemaking process, depending on the flavanolic characteristics of the seeds. This aspect is particularly important for winegrape cultivars with a high richness in seed flavanols, as observed for the Nebbiolo cultivar if compared with other winegrape varieties. Because the grape variety, vintage, and zone effect were limiting factors on the transferability of the predictive models, further works are in progress covering higher number of years, growing zones, and winegrape varieties to extend the applicability of the method while improving the robustness and accuracy of the calibration models. The FT-NIR method proposed represents a good alternative to the chemical analyses of the seeds most commonly required in cellars.

■ ASSOCIATED CONTENT

Supporting Information

Location and geographical coordinates of the different vineyard zones studied, and climatic conditions of 2010 and 2011 vintages. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

$A_{s,280}$, seed absorbance at 280 nm; TF_s, seed total flavonoids; PRO_s, seed proanthocyanidins; FRV_s, seed flavanols reactive to vanillin; C, (+)-catechin; EC, (−)-epicatechin; ECG, (−)-epicatechin gallate; TM, total monomer flavanols; B_1 and B_2, procyanidins B_1 and B_2; TP, total polymer flavanols; mDP, mean degree of polymerization; G%, galloylation percentage

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