Chapter

NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS) EVALUATION OF THE NUTRITIVE VALUE OF LEAF AND GREEN PRUNING RESIDUES OF GRAPEVINE (*VITIS VINIFERA* L.)

Pier Giorgio Peiretti* [1*], *Giorgio Masoero* [2,3] *and
***Sonia Tassone* [3]**

[1] Institute of Sciences of Food Production, National Research Council, Grugliasco, Italy
[2] Academy of Agriculture of Torino, Torino, Italy
[3] Department of Agriculture, Forestry and Food Sciences, University of Torino, Grugliasco, Italy

ABSTRACT

The aim of this study has been to investigate the efficiency of NIR scanning to detect differences related to the chemical composition, gross energy, *in vitro* apparent digestibility (DMD) and relative feed value (RFV) of leaves and green pruning residues (GPRs) of eleven red grapevine cultivars (Barbera, Cabernet Sauvignon, Cabernet Franc, Canaiolo Nero, Carignan Noir, Grenache, Lambrusco Salamino, Nebbiolo, Pinot Noir, Sangiovese and Syrah) and five white grapevine cultivars (Malvasia Bianca, Moscato Bianco, Sauvignon Blanc, Verdicchio and Vernaccia). Vibrational analyses were performed on lyophilized samples in reflectance mode using an NIR-SCÏO^{TM} molecular sensor, that is, a miniaturized web-based device that operates over the 740-1070 nm NIR range. The present study demonstrates that the RFV of the considered grape leaves is 22.5% higher than that of the grape GPRs. This feed value may be predicted by means of NIR spectroscopy of the lyophilized samples; however, such information could also easily be approximated through a rapid NIR tomoscopy of adequate samples of intact leaves. Foliar moisture could be predicted by means of NIR tomoscopy of intact leaves, after the grape dataset has been enlarged appropriately. A concerted elaboration of the chemical and digestibility analyses leads to a significant compositional fingerprint of the sixteen cultivars studied here. NIR tomoscopy can be used to rapidly classify the phenotypes, since other physico-chemical information that is not revealed by means of the usual analyses are incorporated in the electromagnetic spectrum. Other key biological properties (polyphenols, antioxidants, stress reaction, etc.) that are prospected for precision agriculture purposes could be revealed by a rapid NIR scan and perhaps even through remote NIR sensing.

---

*Corresponding author: piergiorgio.peiretti@ispa.cnr.it

Keywords: grapevine, leaves, green pruning residues, nutritive value, vibrational spectroscopy, NIR-SCÏO™.

INTRODUCTION

The Near Infrared Reflectance Spectroscopy (NIRS) technique has been used extensively as a rapid and eco-friendly analysis system for the quality evaluation of agricultural products and forage crops (Cozzolino and Moron, 2004; Locher et al., 2005; Masoero et al., 2007; Montes et al., 2009) and for the prediction of animal intake and digestibility from the spectra of forage samples (Decruyenaere et al., 2009; Asekova et al., 2016). Even though NIRS fiber optics instruments have reached a satisfactory degree of portability, very few researchers have used NIRS as a tomoscopy instrument to predict *in vitro* digestibility and the crop maturity index of different forages (Tassone et al., 2014) or to determine other parameters in agricultural products (Masoero et al., 2018a, 2018b).

Viticulture generates a huge amount of grapevine (*Vitis vinifera* L.) by-products (Velázquez-Martí et al., 2011), which could be proposed for use in ruminant feeding (Sanchez et al., 2002; Peiretti et al., 2017) and are generally browsed by sheep and goats (Heuzé et al., 2017). Leaves and green pruning residues (GPRs) can constitute an alternative source of forage for these animals, when the quantity and quality of pasture is limited during drought conditions (Romero et al., 2000; Kamalak, 2005; Gurbuz, 2007). However, very little information is available on the antioxidant activity (Acquadro et al., 2018; Peiretti et al., 2019) or on the nutritive value of leaves and GPRs (Kok et al., 2007; Peiretti and Tassone, 2019).

Generally, forage quality is evaluated considering different indexes to assess, rank and compare them. The most recent index is the relative forage quality index (Moore and Undersander, 2002). However, in our study, the leaves and GPRs have been ranked with a relative feed value (RFV), as it is the most frequently used index in marketing and educational programs in the United States (Moore and Undersander, 2002).

The first aim of this study was to investigate the efficiency of NIR scanning for the detection of differences related to the chemical composition and quality, gross energy (GE) and *in vitro* apparent digestibility (DMD) of leaves or GPRs of eleven red grapevine and five white grapevine cultivars. As a second objective, this research has proposed the study of direct scanning (tomoscopy) of the fresh leaves, a strand that is in the riverbed of a precision agriculture roadmap.

MATERIAL AND METHODS

Plant material and environmental conditions

The trials were carried out on plots located in an experimental field in the North-West of Italy (45°06′N 7°59′E) at an altitude of 290 m above sea level. Samples of the leaves and GPRs of eleven varieties of red grapevine (Barbera, Cabernet Franc, Cabernet Sauvignon, Canaiolo Nero, Carignan Noir, Grenache, Lambrusco Salamino, Nebbiolo, Pinot Noir, Sangiovese and Syrah) and five varieties of white grapevine (Malvasia Bianca, Moscato Bianco, Sauvignon Blanc, Verdicchio and Vernaccia) were collected in duplicate from standard vertical trellises using edging shears. Sampling was done in the morning, after dew had evaporated, during June and September, for the GPR and leaf samples, respectively. Sampling was only conducted in favorable weather conditions and after the disappearance of dew. Fresh samples of the GPRs and leaves were immediately frozen and freeze-dried using a lyophilizer (5 Pascal, Trezzano sul Naviglio, Italy). They were then ground in a Cyclotec mill (Tecator, Herndon, VA, USA), to pass through a 1 mm screen, and were stored for further analyses.

Chemical analysis and *in vitro* digestibility

An aliquot of 200 g of each collected sample was used, in duplicate, to determine the dry matter (DM) in a forced draft air oven at 105 °C overnight. The freeze-dried samples were analyzed to determine the total nitrogen content (AOAC, 1990). Acid detergent fiber (ADF), neutral detergent fiber (NDF) and lignin (ADL) were determined using an Ankom 200 Fiber Analyser (Ankom Technology Corp., Macedon, NY, USA), according to the Van Soest et al. (1991) method. Gross energy (GE) was determined using an adiabatic calorimeter bomb (IKA C7000, Staufen, Germany).

The freeze-dried samples were also analyzed to determine their DMD, using a Daisy^{II} Incubator (Ankom Technology Corp., Fairport, NY, USA), according to Robinson et al. (1999). DMD was calculated using the following equation:

$$\text{DMD (g/kg DM)} = \text{DM}_{\text{wt1}} - \text{DM}_{\text{wt2}} / \text{DM}_{\text{wt1}} * 1000$$

where DM_{wt1} is the DM weight before the incubation and DM_{wt2} is the DM weight after the incubation.

RFV is an index that is used to indicate forage quality (Van Dyke and Anderson, 2002; Moore and Undersander, 2002; Hackmann et al., 2008) and it was calculated using neutral detergent fiber (to show the intake potential) and acid detergent fiber (to represent DM digestibility) as follows:

$$\text{RFV} = [(88.9-0.779*(\text{ADF}/10))]*120/(\text{NDF}/10)/1.29$$

where ADF is the digestible acid detergent fiber as a % of dry matter, and NDF is the neutral detergent fiber as a % of dry matter. RFV has a base of 100 (for full bloom alfalfa) and higher values indicate better forage quality.

NIRS

Vibrational analyses were performed, in reflectance mode, using a smart SCÏO$^{\text{TM}}$ molecular sensor (Consumer Physics Inc., Tel Aviv, Israel), a new miniaturized web-based wireless device, over the 740-1070 nm NIR range, with 331 absorbance points. The upper page of the fresh leaves was scanned using an 8 mm spacer height, and considering 20 leaves per cultivar. The freeze-dried samples were scanned in triplicate from bottom up, placing a quartz petri dish directly onto the instrument, without any spacer. The powdery sample that was to be analyzed was poured into this petri dish.

Statistical analysis

Multivariate analyses utilized the principal component explorative tool (StatBOX v. 6.5, Grimmer-Soft, Paris) as well as linear regression in order to enucleate the essential relationships between the constituents and feed values pertinent to the two sources.

The chemometric elaborations were conducted on the Lab-SCÏO$^{\text{TM}}$ collections of spectra, which were exported from the repository to Excel, and were then imported into WinISI 1.04 software. Calibration and cross validation were developed by means of the modified Partial Least Squares method. The freeze-dried sub-sample spectra were calibrated to the chemical parameters. The spectra were averaged by the cultivars and then fitted, by the PLS software, to the chemical analyses.

A qualitative discrimination of the fresh leaves sampled from six cultivars was performed using the Lab-SCÏO$^{\text{TM}}$ random forest software, as described in Giovannetti et al. (2019). The "As Known As" (AKA) matrices were tested through the online MedCalc free software against the random threshold of 1/6. Moreover, in order to compare the classification ability of NIRS and the chemical methods, a PLS-discriminant analysis (PLS-DA) was performed on the seven properties of the six cultivars.

RESULTS AND DISCUSSION

Nutritive value and RFV

Table 1 shows the mean values of the chemical composition of the leaves and some considerations can be made. The red grapevine was on average higher in NDF, ADF, ADL and protein than the white one (404.8 *vs* 374.4; 310.7 *vs* 282.4; 72.0 *vs* 68.1; 114.0 *vs* 105.7 g/kg, respectively), and its digestibility (572.1 *vs* 621.0 g/kg) and RFV (197 *vs* 222) were consequently lower. The highest fiber content among the red grapevine leaves was found in: Sangiovese (NDF=461.7 g/kg), Nebbiolo (ADF=386.9 g/kg) and Pinot Noir (ADL=84.5). The Lambrusco Salamino leaves were the most proteic (CP=121.4 g/kg), while the Carignano ones were the most digestible (631.7 g/kg). However, Barbera had the best RFV (250.1) (Figure 1), with a chemical composition of: 404.1 g/kg of NDF, 256.4 g/kg of ADF, 62.8 g/kg of ADL, 120.1 g/kg of CP, a gross energy value of 17.7 MJ/kg and DMD of 607.0 g/kg. The highest fibrous values of the white grapevine leaves were found in Vernaccia (NDF=423 g/kg) and Moscato Bianco (ADF=301.1 g/kg and ADL=74.5 g/kg). The Verdicchio leaves had the highest CP content (127.0 g/kg) and Moscato Bianco the highest energy (GE=18.0 MJ/kg). Vernaccia resulted to be the most digestible and to have the highest RFV (664.7 and 242.6, respectively).

As far as the GPRs are concerned (Table 2), it is possible to observe that they were more fibrous than the leaves (NDF: 545.2 *vs* 389.6; ADF 388.7 *vs* 296,6; ADL 124.4 *vs* 70.0 g/kg, respectively), and were consequently less digestible (482.3 *vs* 596.5 g/kg) with a lower RFV (141 *vs* 209). However, the pruning residues were characterized by a high protein content, that is, on average 130 g/kg. Very few differences emerged in the nutritional value between the red and white GPRs. The red grapevine had a higher ADF (394.7 *vs* 382.7 g/kg) and CP (134.5 *vs* 125.2 g/kg) than the white grapevine. The red and white grapvines had similar GE and DMD (on average 17 and 482, respectively), but a lower RFV (137 *vs* 145). Sangiovese had the highest RFV (149) of the red GPRs, but the most digestible was Barbera (DMD=544.4 g/kg). The Nebbiolo GPRs were rich in protein (CP=163.0 g/kg). The white grapevine Verdicchio GPRs were interesting, from an animal nutrition point of view, as they had very high RFV (156) and protein contents (CP=159.5 g/kg) and good digestibility (DMD=488.4 g/kg). The most digestible were the Malvasia Bianca GPRs (DMD=528.7 g/kg).

Table 1. Chemical composition and nutritive value (g/kg DM basis) of the leaves.

	NDF	**ADF**	**ADL**	**DMD**	**CP**	**GE (MJ/kg)**	**RFV**[1]
Red grapevines							
Barbera	404.1	256.4	62.8	607.0	120.1	17.7	250.1
Cabernet Franc	368.8	284.7	67.3	619.8	113.3	19.0	218.0
Cabernet Sauvignon	393.8	288.4	66.6	611.7	112.6	17.9	214.3
Canaiolo Nero	434.0	345.1	79.4	516.1	111.6	17.1	167.2
Carignan Noir	367.0	265.5	62.1	631.7	104.2	18.4	239.0
Grenache	409.4	297.8	58.9	628.3	119.9	17.8	205.2
Lambrusco Salamino	432.8	343.0	76.9	538.6	121.4	17.3	168.6
Nebbiolo	408.9	386.9	74.6	534.9	107.5	17.9	141.3
Pinot Noir	417.4	334.8	84.5	492.8	111.2	18.1	174.5
Sangiovese	461.7	302.9	83.2	526.8	115.5	17.6	200.6
Syrah	355.4	312.3	75.5	586.0	116.9	17.8	192.3
White grapevines							
Malvasia Bianca	374.6	267.2	58.0	639.7	105.5	16.1	237.0
Moscato Bianco	319.7	308.1	74.5	560.7	86.4	18.0	195.9
Sauvignon Blanc	353.1	301.3	73.2	591.1	92.3	17.9	202.0
Verdicchio	401.6	273.1	65.8	648.3	127.0	16.8	230.3
Vernaccia	423.0	262.5	68.8	664.7	117.4	17.1	242.6

[1] RFV = [(88.9-0.779*(ADF/10)]*120/(NDF/10)/1.29

Table 2. Chemical composition and nutritive value (g/kg DM basis) of the green pruning residues.

	NDF	ADF	ADL	DMD	CP	GE (MJ/kg)	RFV[1]
Red grapevines							
Barbera	495.4	387.2	91.3	544.4	131.6	16.4	141.1
Cabernet Franc	528.6	398.0	112.5	493.4	108.8	17.2	135.3
Cabernet Sauvignon	522.2	375.4	126.5	492.5	144.7	16.7	147.8
Canaiolo Nero	588.9	390.3	127.0	448.2	106.2	16.9	139.4
Carignan Noir	554.6	402.9	111.1	503.6	105.9	17.2	132.8
Grenache	492.8	384.9	138.3	489.5	158.6	16.7	142.4
Lambrusco Salamino	580.7	434.4	129.2	446.5	128.0	17.2	117.9
Nebbiolo	541.8	424.8	141.7	449.5	163.0	17.3	122.2
Pinot Noir	497.7	385.3	126.7	493.9	144.5	16.9	142.2
Sangiovese	582.5	373.8	130.5	452.3	136.4	17.5	148.8
Syrah	537.4	385.1	125.5	489.3	151.8	16.5	142.3
White grapevines							
Malvasia Bianca	567.8	361.7	114.0	528.7	133.6	17.0	156.2
Moscato Bianco	598.1	435.8	134.2	446.1	74.2	16.9	117.3
Sauvignon Blanc	570.0	361.6	128.9	464.5	120.0	16.8	156.2
Verdicchio	466.2	356.3	122.1	488.4	159.5	17.1	159.6
Vernaccia	557.7	398.0	126.2	484.7	138.5	17.3	135.3

[1] RFV = [(88.9-0.779*(ADF/10)]*120/(NDF/10)/1.29

A difference of 35% (Figure 1) was found for the RFV between the lowest (Nebbiolo) and the highest (Carignan Noir). But the correlation was poor (r 0.36).

In general, the standardized differences between the two sources were very high. As can be seen in Figure 2, the GPRs had higher fiber (ADL: +1.82; NDF: +1.78 and ADF: +1.65 of standard deviate) and protein (+1.05 of standard deviate) contents. Their GE, DMD and RFV diminished.

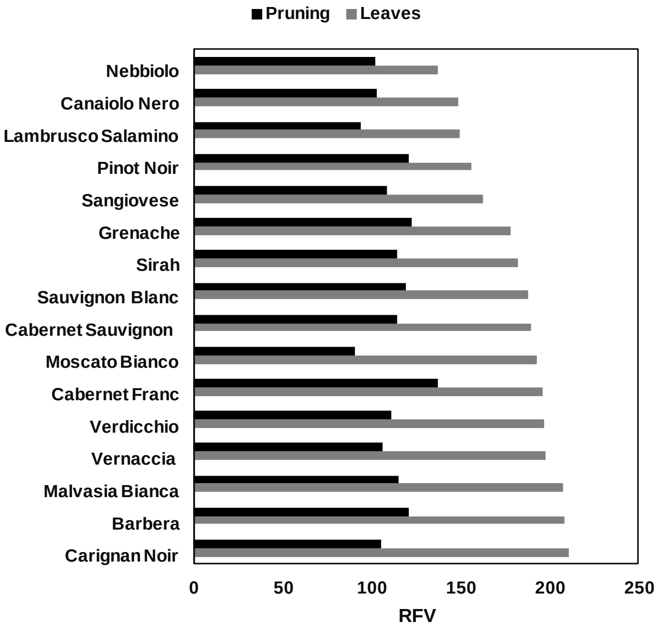

Figure 1. Histogram of the relative feed value (RFV) of the green pruning residues and leaves of the sixteen cultivars, ordered according to the leaf-RFV values (r=0.36).

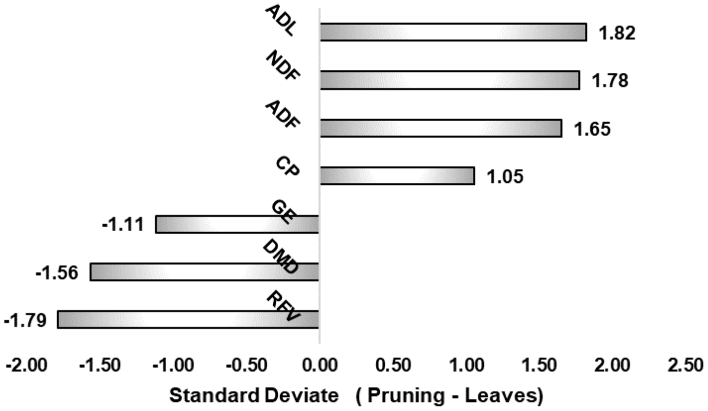

Figure 2. Histogram of the Standard Deviate differences of the green pruning residues *vs* leaves for the seven variables from the sixteen cultivars, ordered according to the values.

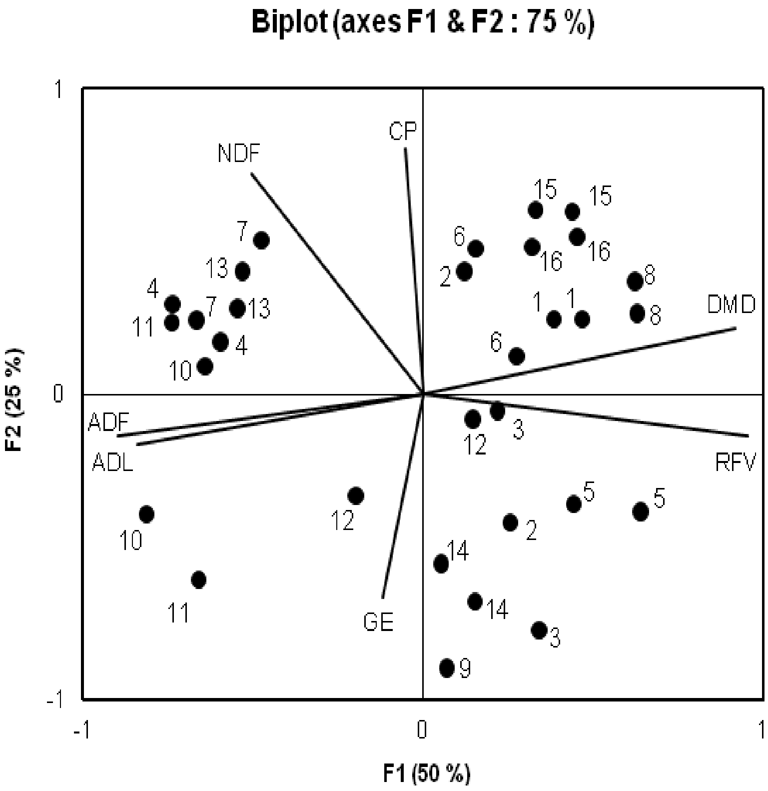

Figure 3. Principal component analysis (PCA) plots of the lyophilized leaf analyses: crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin (ADL), gross energy (GE), *in vitro* apparent digestibility (DMD) and relative feed value (RFV). Cultivars: [(1) Barbera, (2) Cabernet Sauvignon, (3) Cabernet Franc, (4) Canaiolo Nero, (5) Carignan Noir, (6) Grenache, (7) Lambrusco Salamino, (8) Malvasia Bianca, (9) Moscato Bianco, (10) Nebbiolo, (11) Pinot Noir, (12) Syrah, (13) Sangiovese, (14) Sauvignon Blanc, (15) Verdicchio and (16) Vernaccia].

The total variance of the seven variables in the principal component analysis can be condensed into new variables (F1, F2). It showed that the variation is principally explained by the first principal component (F1 50%). The second F2 explain 25% of variation, with a cumulative percentage of 75% (Figure 3). The first principal component is defined by the DMD, RFV, ADF and ADL. The DMD and RFV were placed to the right in the loading plot and resulted to be negatively correlated with ADF and ADL.

It is possible to observe, in Figure 3, that the best grapevine leaves and GPRs as highly digestible feeds are: Barbera, Grenache, Malvasia Bianca, Verdicchio and Vernaccia. Instead, the best leaves and GPRs for a high RFV are: Cabernet Franc, Carignan Noir, Moscato Bianco, Syrah and Sauvignon Blanc. Cabernet Sauvignon has a high DMD and high RFV. The grapevines placed to the left in the loading plot are more fibrous (Canaiolo, Lambrusco Salamino, Nebbiolo, Pinot Noir, Syrah, Sangiovese).

The Pearson correlations (Table 3) testify that the RFV in the leaves is significantly opposed to the fiber components, especially to the ADF ($r=-0.93$), and it is substantially linked with DMD (0.79), but independent of CP and GE. As far as the GPRs are concerned, similar negative correlations linked RFV to ADF (-0.82) and NDF (-0.80), but less so to ADL. It may be observed that the correlation between ADF and ADL is significant in the leaves (0.63) but not significant in the GPRs. Moreover, it may be noted that GE is independent of all the other variables.

The relationship between DMD and ADF was studied by means of a linear regression. When the leaves and GPRs were considered separately (Figure 4), the following equations were obtained: $y=-1.126x+92.73$ and $y=-0.539x+69.58$ with an R^2 of 0.610 and 0.206, respectively. When the two sources were pooled together, the prediction equation ($y=-1.062x+90.33$) showed a greater precision ($R^2=0.764$).

Table 3. Pearson Correlation of the seven variables considered separately within the leaves and within the green pruning residues.

		NDF	ADF	ADL	DMD	CP	GE	RFV	
					Leaves No. 32				
NDF		1	0.24	0.3	-0.32	0.50	-0.29	**-0.55**	
ADF		**0.33**	1	**0.63**	**-0.78**	-0.05	0.13	**-0.93**	Leaves
ADL	Pruning	0.21	0.29	1	**-0.82**	-0.06	0.18	**-0.67**	
DMD		**-0.51**	**-0.45**	**-0.69**	1	0.16	-0.19	**0.79**	
CP		**-0.55**	**-0.33**	0.22	0.17	1	-0.22	-0.14	
GE		0.26	0.2	0.25	-0.28	0	1	-0.01	
RFV		**-0.80**	**-0.82**	**-0.30**	**0.58**	**0.54**	-0.27	1	
		Green pruning residues No. 44							

Leaves, if $|r| > 0.49$, Prob < 0.05; green pruning residues if $|r| > 0.29$, Prob < 0.05.

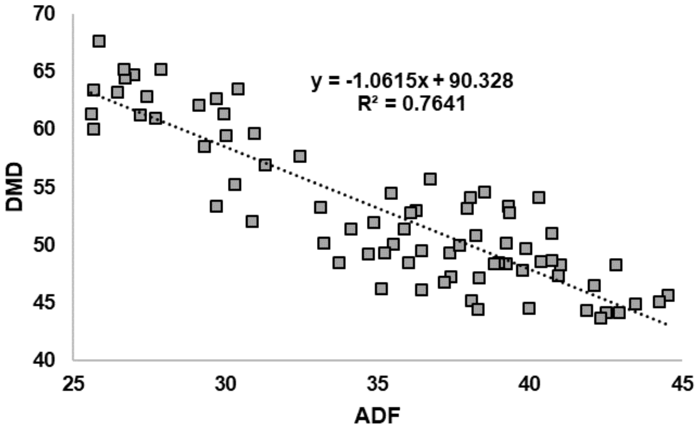

Figure 4. Regression of the DMD on the ADF pooled for the two sources.

The key relationship of the experiment is the degree of dependency of the DMD on the ADF. As expected, the DMD decreased when the ADF increased, that is, by about -1.06% per ADF unit variation (r^2= 0.76; Figure 4). However, as shown in Figure 5, the relationship was higher (-1.13) and closer (r^2 0.61) in the leaves than in the GPRs (-0.54 and r^2 0.21).

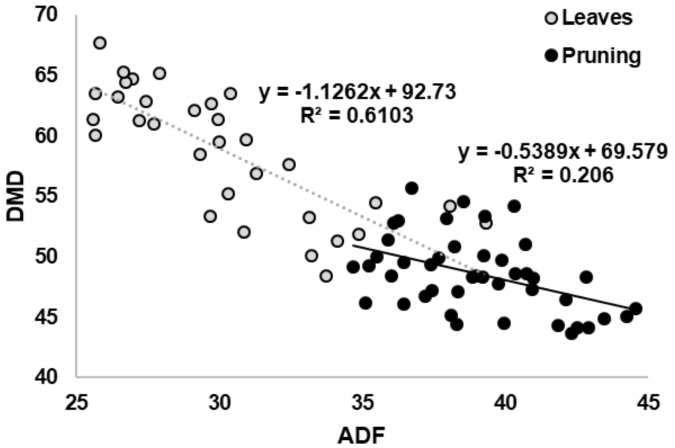

Figure 5. Separate regression of the DMD on the ADF according to the sources.

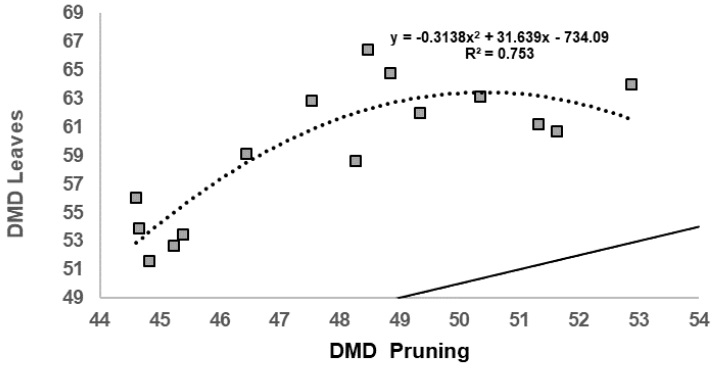

Figure 6. Regression of the DMD of the leaves on the DMD of the green pruning residues.

The DMD of the two sources are related, that is, with r=0.87 (Figure 6), but in a parabolic sense, with a plateau of 65-67 % in the leaves and at 49% in the GPRs. Barbera and Cabernet Sauvignon, which showed the most digestible GPRs, did not show the most digestible leaves. The Pearson correlation was +0.36.

Bumb et al (2018) studied the forage quality of leaves and stems in a rangeland system for herbivores, and observed that combining feed values with litter quality for decomposers were two key plant properties that affected the ecosystem carbon and nutrient cycling. The fiber concentration and dry matter content can be considered as good predictors of both digestibility and decomposability, and in this case showed an overall correlation of 0.73 for the leaves and 0.76 for the stems.

NIRS

The reflectance spectra of the lyophilized samples appeared less uniform in the leaves (Figure 7) than in the GPRs (Figure 8). Moreover, the distribution appeared quite scattered in the fresh leaves (Figure 9), showing a broad water flexus at 975 nm.

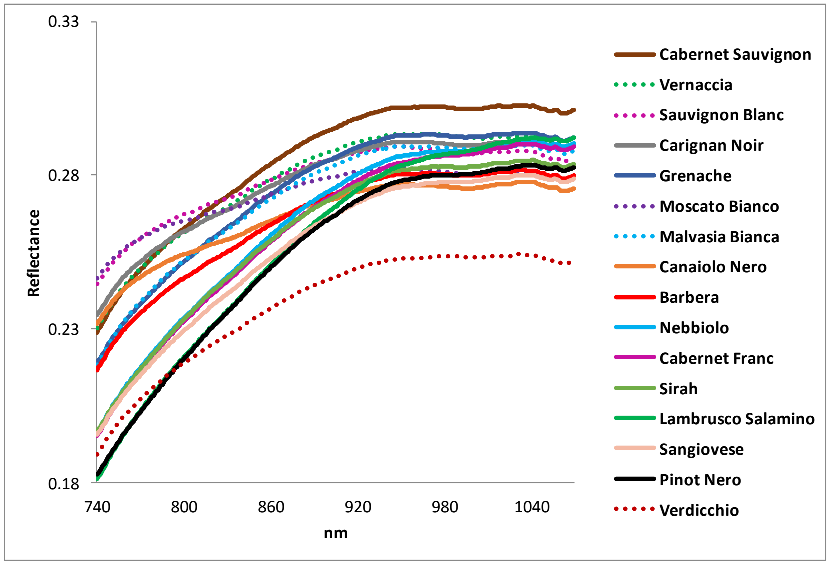

Figure 7. NIR-SCÏO^{TM} reflectance spectra of the lyophilized leaves of sixteen cultivars ordered according to the decreasing mean reflectance values.

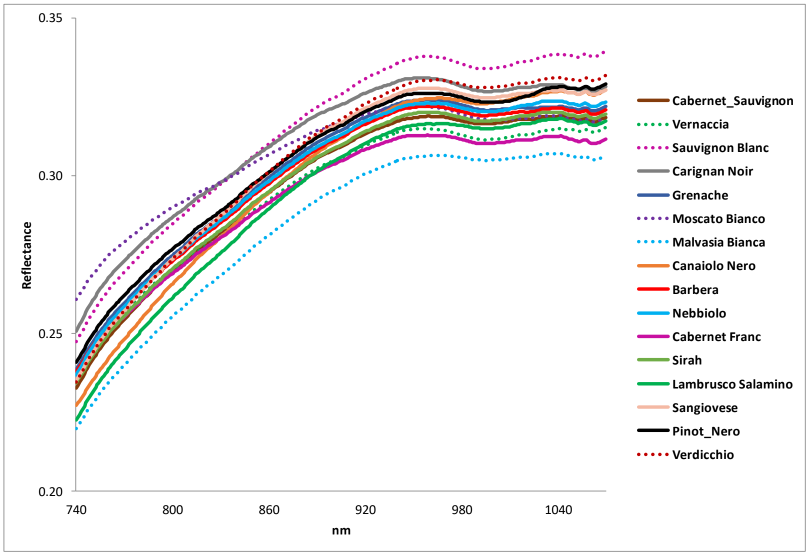

Figure 8. NIR-SCÏO^{TM} reflectance spectra of the lyophilized green pruning residues of sixteen cultivars, ordered according to the decreasing mean reflectance values of the lyophilized leaves.

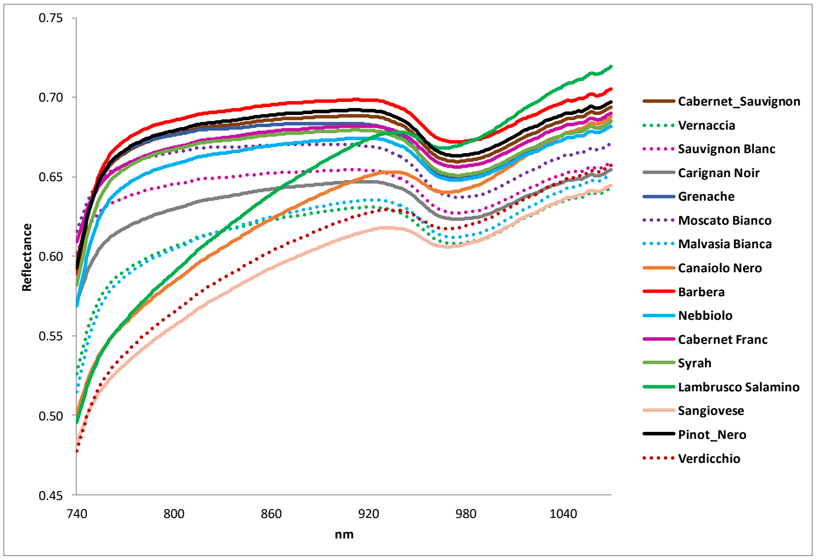

Figure 9. NIR-SCÏO^{TM} reflectance spectra of the fresh leaves of sixteen cultivars ordered according to the decreasing mean reflectance values of the lyophilized leaves.

The NIRS performances in the lyophilized GPRs (Table 4) showed reliable results with RPD values > 2 in NDF, ADF and GE.

Table 4. NIR-SCÏO^{TM} performances in calibration and cross validation for seven properties of the lyophilized green pruning residues of sixteen cultivars (No. 74).

	Mean	**SD**	**SEC**	**RSQ**	**SECV**	**1-VR**	**RPD**
NDF	54.44	3.61	1.05	0.92	1.25	0.88	2.89
ADF	39.05	2.51	0.68	0.93	1.15	0.79	2.19
ADL	12.58	0.77	0.49	0.59	0.71	0.14	1.08
DMD	47.79	2.77	1.21	0.81	2.09	0.43	1.33
CP	13.48	1.81	1.07	0.65	1.16	0.58	1.56
GE	17.00	0.32	0.12	0.85	0.15	0.79	2.17
RFV	107.80	10.25	3.35	0.89	5.67	0.69	1.81

The NIRS performance for the lyophilized leaves (Table 5) showed reliable results with RPD values > 2 in NDF and CP.

Table 5. NIR-SCÏO$^{\text{TM}}$ performances of the calibration and cross validation of seven properties of lyophilized leaves from sixteen cultivars (No. 67).

	Mean	**SD**	**SEC**	**RSQ**	**SECV**	**1-VR**	**RPD**
NDF	40.15	2.07	0.71	0.88	0.89	0.82	2.33
ADF	29.40	2.82	1.69	0.64	2.25	0.36	1.25
ADL	7.13	0.83	0.49	0.65	0.59	0.49	1.40
DMD	58.93	4.59	1.46	0.90	2.70	0.66	1.70
CP	11.23	1.10	0.35	0.90	0.53	0.78	2.10
GE	17.67	0.46	0.28	0.63	0.40	0.26	1.17
RFV	181.99	22.20	7.40	0.89	12.53	0.69	1.77

The NIRS performance, after pooling the GPRs and lyophilized leaves (Table 6), increased the reliable results, with RPD values > 2 in NDF, ADF, ADL, and especially in RFV (2.55). It should be noted that the RPD value for the type of source (GPRs *vs* leaves) was the highest (3.04).

The results achieved with the smart NIR-SCIO$^{\text{TM}}$ are even better than the predictions obtained from restricted ranges of other instruments. Tassone et al. (2014) showed that an IR range of 2501-3333 nm performed the best in freeze-dried samples (RPD 3.07), while the short NIR range showed average RPD values of 1.54 or 1.22, for two instruments, which are clearly below the average 2.19 RPD values for properties of pooled sources shown in Table 6. The RPD of 2.3 for the NDF featured here is higher than the values of 1.6 and 1.2 of the cited work.

Table 6. NIR-SCÏO$^{\text{TM}}$ performances in calibration and cross validation for seven properties of lyophilized green pruning residues and leaves from sixteen cultivars (No. 141).

	Mean	**SD**	**SEC**	**RSQ**	**SECV**	**1-VR**	**RPD**
NDF	46.70	7.50	2.91	0.85	3.25	0.81	2.30
ADF	34.21	5.33	2.05	0.85	2.31	0.81	2.31
ADL	9.40	2.81	0.86	0.91	1.03	0.87	2.73
DMD	53.65	6.47	3.05	0.78	3.40	0.72	1.90
CP	12.25	1.70	0.88	0.73	0.92	0.71	1.85
GE	17.19	0.42	0.22	0.72	0.25	0.66	1.72
RFV	143.69	40.14	14.32	0.87	15.73	0.85	2.55

Pruning residues *vs* leaves	1.53	0.50	0.15	0.91	0.17	0.89	3.04

Table 7. NIR-SCÏO™ performances in calibration and cross validation of the seven properties of fresh leaves, based on the average spectrum of the sixteen cultivars (No. 16).

	Mean	**SD**	**SEC**	**RSQ**	**SECV**	**1-VR**	**RPD**
NDF	41.23	1.19	0.71	0.64	0.88	0.48	1.4
ADF	30.81	2.79	2.36	0.29	2.67	0.10	1.0
ADL	7.09	0.72	0.38	0.73	0.45	0.60	1.6
DMD	57.90	3.91	2.18	0.69	2.42	0.60	1.6
CP	11.42	0.42	0.41	0.06	0.47	-0.18	0.9
GE	17.90	0.18	0.17	0.08	0.20	-0.16	0.9
RFV	175.38	21.83	2.94	0.98	9.33	0.83	2.3
Dry Matter	34.99	1.68	0.58	0.88	0.98	0.66	1.7

In the tomoscopy of fresh leaves (Table 7), the NIRS performances for the properties were poor, showing an RPD average of 1.42. This value is in line with the previous reported results of 1.54 or 1.22, which, however, were obtained on freeze-dried samples and not from the NIR tomoscopy of intact leaves. A positive exception concerned the RFV parameter, which reached an RPD of 2.3 and an R^2 cross-validated value of 0.83 (Figure 10). The dry matter showed an RPD of 1.7, but it should be pointed out that the RPD in the work based on foliar pH and tomoscopy of *Sorghum sudanensis* was 2.4 (unpublished).

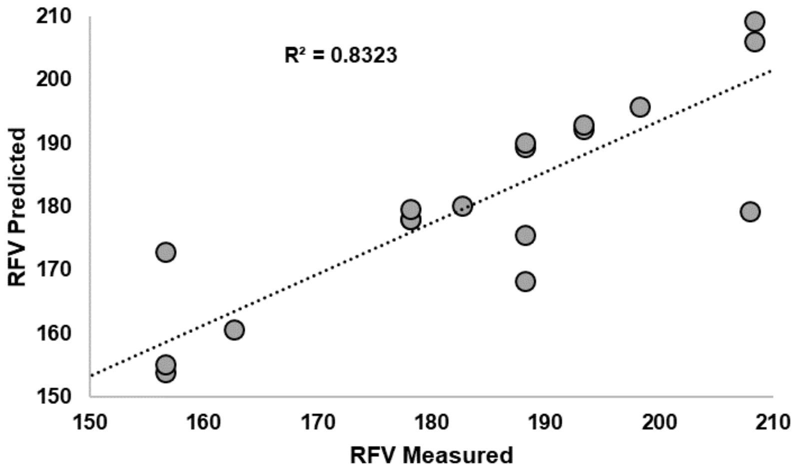

Figure 10. Scatterplot of the measured *vs* predicted RFV of the average spectrum of fresh leaves.

As far as the fingerprinting ability of the six cultivars from the NIR tomoscopy of fresh leaves is concerned (Table 8), the results were highly significant (P<0.0001), except for Syrah (24%; P 0.0495), which was probably mistaken for Barbera (36%).

Table 8. Discriminant classification matrix of the six cultivars from a Random Forest model based on the NIR-SCÏO^{TM} spectra of the fresh leaves (No. 486). Values in percentages, random threshold 16%.

Syrah	2	5	0	0	0	24
Pinot Noir	0	8	4	5	89	4
Nebbiolo	5	11	4	62	0	8
Grenache	0	29	86	5	2	12
Cabernet Sauvignon	2	39	4	11	7	16
Barbera	91	8	2	17	2	36
	Barbera	**Cabernet Sauvignon**	**Grenache**	**Nebbiolo**	**Pinot Noir**	**Syrah**
Prob. Diagonal	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0495

As far as the methodological comparison is concerned, it can be observed, in Table 9, that the fingerprinting ability of the lyophilized samples of the six cultivars was overall significant, at P = 0.014, on the basis of all the information concerted from seven laboratory properties. This procedure is incomparably longer and more difficult than the direct scanning of leaves.

Table 9. Classification matrix of the six cultivars from a PLS-DA based on seven properties of the leaves (No. 12). Values in cases, random threshold 16%.[1]

Syrah						**2**
Pinot Noir				1		
Nebbiolo				**1**	1	
Grenache		1	**1**		1	
Cabernet Sauvignon	1		1			
Barbera	**1**	1				
Prob. Diagonal	**Barbera**	**Cabernet Sauvignon**	**Grenache**	**Nebbiolo**	**Pinot Noir**	**Syrah**
	0.190	0.537	0.190	0.190	0.537	0.0012

[1] Prob 5 hits / 12 cases *vs* 16% P =0.014

CONCLUSIONS

The present study has demonstrated that the RFV of grape leaves is 22.5% higher than that of GPRs. This feed value can be predicted from the NIR spectroscopy of lyophilized samples; however, such information could easily be approximated through a rapid NIR tomoscopy of suitable number of intact leaf samples.

Foliar moisture could be predicted by means of the NIR tomoscopy of intact leaves, after the grape dataset has been enlarged appropriately.

A concerted elaboration of the chemical and digestibility analyses allowed a significant compositional fingerprint of the cultivars studied here to be obtained. NIR tomoscopy can rapidly classify the phenotypes, since other physic-chemical information that cannot be revealed through the usual analyses is embedded in the electromagnetic spectrum. Other key biological properties (polyphenols, antioxidants, stress reaction, etc.) that are prospected for precision agriculture purposes could be revealed by means of a rapid NIR scan and, perhaps even through remote NIR sensing.

ACKNOWLEDGMENTS

The authors would like to thank Mr. T. Strano (Department of Agriculture, Forestry and Food Sciences, University of Torino) for the helpful collaboration in the field operations and Mrs M. Jones for the linguistic revision of the manuscript.

REFERENCES

Acquadro, S., Appleton, S., Marengo, A., Bicchi, C., Sgorbini, B., Gai, F., Peiretti, P. G., Cagliero, C. & Rubiolo, P. (2018). Characterization of the phenolic content of leaves and green pruning residues of sixteen cultivars of *Vitis vinifera* L. In: *Atti del 113° Congresso della Società Botanica Italiana*, Communication N.15. ISBN 978-88-85915-22-0

AOAC. (1990). Official Method of Analysis of the AOAC. AOAC International, Arlington, U.S.A.

Asekova, S., Han, S. I., Choi, H. J., Park, S. J., Shin, D. H., Kwon, C. H., Shannon, J. G. & Lee, J. D. (2016). Determination of forage quality by near-infrared reflectance spectroscopy in soybean. *Turkish Journal of Agriculture and Forestry*, *40*, 45-52.

Bumb, I., Garnier, E., Coq, S., Nahmani, J., Del Rey Granado, M., Gimenez, O., & Kazakou, E. (2018). Traits determining the digestibility–decomposability relationships in species from Mediterranean rangelands. *Annals of Botany*, *121*, 459-469.

Cozzolino, D. & Moron, A. (2004). Exploring the use of near infrared reflectance spectroscopy (NIRS) to predict trace minerals in legumes. *Animal Feed Science and Technology*, *111*, 161-173.

Decruyenaere, V., Lecomte, P., Demarquilly, C., Aufrere, J., Dardenne, P., Stilmant, D. & Buldgen, A. (2009). Evaluation of green forage intake and digestibility in ruminants using near infrared reflectance spectroscopy (NIRS): Developing a global calibration. *Animal Feed Science and Technology*, *148*, 138-156.

Giovannetti, G., Polo, F., Nutricato, S., Masoero, G. & Nuti, M. (2019). Potency of commercial symbiotic bio-fertilizer consortium for mitigating the Olive Quick Decline Syndrome (OQDS). *Journal of Agronomy Research*.

Gurbuz, Y. (2007). Determination of nutritive value of leaves of several *Vitis vinifera* varieties as a source of alternative feedstuff for sheep using *in vitro* and *in situ* measurements. *Small Ruminant Research*, *71*, 59-66.

Hackmann, T. J., Sampson, J. D. & Spain, J. N. (2008). Comparing relative feed value with degradation parameters of grass and legume forages. *Journal of Animal Science*, *86*, 2344-2356

Heuzé, V., Thiollet, H. & Tran, G. (2017). Grape leaves and vine shoots. Feedipedia, a programme by INRA, CIRAD AFZ and FAO. https://www.feedipedia.org/node/512 (accessed on 7 June 2019).

Kamalak, A. (2005). Chemical composition and *in vitro* dry matter digestibility of leaves of *Vitis vinifera*. *Livestock Research for Rural Development*, *17*, Article 3.

Kok, D., Ates, E., Korkutal, I. & Bahar, E. (2007). Forage and nutritive value of the pruning residues (leaves plus summer lateral shoots) of four grapevine (*Vitis vinifera* L.) cultivars at grape harvest and two post-harvest dates. *Spanish Journal of Agricultural Research*, *5*, 517-521.

Locher, F., Heuwinkel, H., Gutser, R. & Schmidhalter, U. (2005). Development of near infrared reflectance spectroscopy calibrations to estimate legume content of multispecies legume–grass mixtures. *Agronomy Journal*, *97*, 11-17.

Masoero, G., Sala, G. & Peiretti, P. G. (2007). Development of near infrared (NIR) spectroscopy and electronic nose (EN) techniques for analysing the conservation quality of farm silages. *Journal of Food, Agriculture and Environment*, *5*, 172-177.

Masoero, G., Delmastro, M., Cugnetto, A., Giovannetti, G. & Nuti, M. (2018a). NIRS footprint of bio-fertilizers from hay litter-bags. *Journal of Agronomy Research*, *1*, 22-33.

Masoero, G., Peiretti, P. G., Cugnetto, A. & Giovannetti, G. (2018b). Raw pH fall-out as a sign of a mycorrhizal modifier of *Sorghum sudanensis*. *Journal of Agronomy Research*, *1*, 1-11.

Montes, J. M., Mirdita, V., Prasad, K. V., Blummel, M., Dhillon, B. S. & Melchinger, A. E. (2009). A new near infrared spectroscopy sample presentation unit for measuring feeding quality of maize stover. *Journal of Near Infrared Spectroscopy*, *17*, 195-201.

Moore, J. E. & Undersander, D. J. (2002). Relative forage quality: an alternative to relative feed value and quality index. In: *Proceedings 13th Annual Florida Ruminant Nutrition Symposium*, pp: 16–32, Florida, USA.

Peiretti, P. G., Masoero, G. & Tassone, S. (2017). Comparison of nutritive value and fatty acid profile of the pruning residues of six grapevine (*Vitis vinifera* L.) cultivars. *Livestock Research for Rural Development*, *29*, Article 194.

Peiretti, P. G., Barbosa-Pereira, L., Zeppa, G. & Tassone, S. (2019). Nutritive value, total phenolic content and radical scavenging activity before and after digestion of the leaves of six grapevine (*Vitis vinifera* L.) cultivars. In: *Grapevines at a Glance*. Nova Science Publishers, Inc., NY, Chapter X, pp. XX-XX.

Peiretti, P. G. & Tassone, S. (2019). Nutritive value of the leaves and pruning residues of red and white grapevine (*Vitis vinifera* L.) varieties. In: *Grapevines at a Glance*. Nova Science Publishers, Inc., NY, Chapter X, pp. XX-XX.

Robinson, P. H., Campbell, M. & Fadel, J. G. (1999). Influence of storage time and temperature on *in vitro* digestion of neutral detergent fibre at 48 h, and comparison to 48 h *in sacco* neutral detergent fibre digestion. *Animal Feed Science and Technology*, *80*, 257-266.

Romero, M. J., Madrid, J., Hernandez, F. & Ceron, J. J. (2000). Digestibility and voluntary intake of vine leaves (*Vitis vinifera* L.) by sheep. *Small Ruminant Research*, *38*, 191-195.

Sanchez, A., Ysunza, F., Beltran-Garcia, M. J. & Esqueda, M. (2002). Biodegradation of viticulture wastes by Pleurotus: a source of microbial and human food and its potential use in animal feeding. *Journal of Agricultural and Food Chemistry*, *50*, 2537-2542.

Tassone, S., Masoero, G. & Peiretti, P. G. (2014). Vibrational spectroscopy to predict *in vitro* digestibility and crop maturity index of different forages during the growing cycle and after freeze- or oven-drying. *Animal Feed Science and Technology*, *194*, 12-25.

Van Dyke, N. J. & Anderson, P. M. (2002). Interpreting a Forage Analysis. Alabama Cooperative Extension. Circular ANR-890.

Van Soest, P. J., Robertson, J. B. & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*, 3583-3591.

Velázquez-Martí, B., Fernández-González, E., López-Cortés, I., Salazar-Hernández, D.M., 2011. Quantification of the residual biomass obtained from pruning of vineyards in Mediterranean area. *Biomass. Bioenergy*, *35*, 3453-3464.

Wold, J. P., Måge, I., Løvland, A., Sanden, K. W., & Ofstad, R. (2018). Near-infrared spectroscopy detects woody breast syndrome in chicken fillets by the markers protein content and degree of water binding. *Poultry Science*, *98*, 480-490.