Chapter

NUTRITIVE VALUE OF LEAVES AND PRUNING RESIDUES OF RED AND WHITE GRAPEVINE (*VITIS VINIFERA* L.) VARIETIES

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ABSTRACT

Interest in the green pruning residues of grapevine (GPR), harvested in spring, and in grapevine leaves (GL), harvested in autumn, as a feedstuff, has been increasing due to their nutritive value. The aim of this study has been to investigate the differences between the chemical composition, gross energy (GE) and *in vitro* apparent digestibility (DMD) of the GPR and GL of five varieties of red grapevine (Cabernet franc, Canaiolo nero, Carignan noir, Lambrusco salamino, and Sangiovese) and of five varieties of white grapevine (Malvasia bianca di Candia, Moscato bianco, Sauvignon blanc, Verdicchio and Vernaccia di S. Gimignano). The dry matter, acid and neutral detergent fibre, GE and DMD were found to

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differ ($P < 0.01$) between cultivars for both GPR and GL, while no significant differences were observed between the cultivars, in terms of their mean acid detergent lignin content for GPR and crude protein content for GL, respectively. In conclusion, the results show that both the GL and GPR obtained from red and white grape varieties originating from Italy are suitable dietary sources for ruminant feeding, even though GL has a lower fibrous content than GPR and, consequently, a higher DMD.

Keywords: by-product, chemical composition, digestibility, fibrous content, ruminant

INTRODUCTION

Viticulture and the production of wine constitute one of the most important and most widespread agro-economic activities in the world, with more than 7 million ha cultivated worldwide in 2016 (FAOSTAT, 2016). Moreover, they constitute an important traditional activity, mainly in Southwestern Europe, with 3.2 million ha being cultivated as vineyards (EUROSTAT, 2014) and 0.7 million ha in Italy in 2011 (ISTAT, 2014). Grapevines (*Vitis vinifera* L.) generate huge amounts of by-products (Velázquez-Martí et al., 2011). Pruning is the most important operation that growers perform on the plants during spring, and it is estimated that about 1.4 million tons of potential residues are derived from vine cultivation in Europe per year (Duca et al., 2016). Most of these residues are made up of green pruning residues (GPR) and, to a lesser extent, of grapevine leaves (GL), which may be collected through selective removal operations. These residues are usually left in the fields or destined for composting, with the potential risk of causing environmental problems (Rondeau et al., 2013). Furthermore, several wine production by-products, such as seeds and peels, have been studied as sources of natural bioactive compounds (Moure et al., 2001), and have been proposed as health promoters (Teixeira et al., 2014).

There is a growing demand for green materials and renewable nutrient and bioactive compound sources for the feed/food, pharmaceutical and cosmetic sectors in order to reduce the environmental impact of winery activities (Spanghero et al., 2009). These by-products could also constitute a source of alternative feedstuff for ruminants, in particular for sheep (Gurbuz, 2007) and for monogastric animals, thus enhancing the oxidative stability of their meat (Brenes et al., 2016). Although numerous studies have been conducted on the chemical composition of grape pomace, as a promising source of compounds that show good nutritional properties for herbivorous animals (Baumgärtel et al., 2007; Pirmohammadi et al., 2007; Zalikarenab et al., 2007; Besharati and Taghizadeh, 2009; Spanghero et al., 2009; Abarghuei et al., 2010; Bahrami et al., 2010; Basalan et al., 2011; Deng et al., 2011), little information is available on the nutritive value of GPR and GL generated from the annual pruning of vineyards (Romero et al., 2000; Kok et al., 2007; Gurbuz, 2007; Peiretti et al., 2017).

The valorisation of grapevine by-products is currently of great interest, due to their health promoting benefits and their environmental impact, and this study is part of a research project that is aimed at enhancing the value of viticulture by-products, in particular, at characterising the phenolic content of GPR and GL, in order to establish whether they are a valid source of antioxidants with nutritional properties and biological potential, so as to increase their economic value and, at the same time, limit their waste and impact on the environment (Acquadro et al., 2018). The aim of this study has been to investigate the differences in chemical composition, gross energy (GE) and *in vitro* apparent digestibility (DMD) of the GPR and GL of various *Vitis vinifera* cultivars, cultivated in Italy, to produce some of the most prestigious wines sold and appreciated throughout the world.

MATERIAL AND METHODS

Plant Material and Environmental Conditions

The trials were carried out in the western Po Valley (Italy) in June and September 2017. The GPR and GL of five varieties of red grapevine

(Cabernet franc, Canaiolo nero, Carignan, Lambrusco salamino and Sangiovese) and of five varieties of white grapevine (Malvasia bianca di Candia, Moscato bianco, Sauvignon blanc, Verdicchio and Vernaccia di S. Gimignano) were cut from each variety, with edging shears, on two plots randomly located in an experimental field at an altitude of 290 m above sea level (45°06′N 7°59′E). Sampling was only conducted in favorable weather conditions and after the disappearance of dew.

Chemical Analysis

An aliquot of 200 g of each of the collected GPR and GL samples was used, in duplicate, overnight in a forced draft air oven at 105°C to determine the dry matter (DM). Another aliquot of 200 g was immediately refrigerated, freeze-dried, and then brought to air temperature, ground in a Cyclotec mill (Tecator, Herndon, VA, USA) to pass through a 1-mm screen, and then stored for analyses, which were performed in duplicate. The samples were analysed to determine the total N content (AOAC, 1990). Acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) were determined using an Ankom 200 Fibre 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).

In Vitro Digestibility

The GPR and GL samples were also analysed, using a DaisyII Incubator (Ankom Technology Corp., Fairport, NY, USA), to determine their DMD, according to Robinson et al. (1999). Freeze-dried samples (0.25 ± 0.01 g) were double-weighed in F57 Ankom bags, with a pore size of 25 µm, heat-sealed and then placed into an incubation jar. Each jar was a glass recipient with a plastic lid provided with a single-way valve, which prevented the accumulation of fermentation gases, and it was filled with 2

L buffered rumen fluid, under anaerobic conditions. The jar was introduced into the incubator. The rumen liquor was collected, at a slaughterhouse, from the rumen content of cattle fed a fibre-rich diet (Spanghero et al., 2010). The heat (39°C) and agitation were maintained constant and uniform in the controlled chamber by means of continuous rotation. After 48 h of incubation, the jars were emptied and the bags were rinsed gently. DMD was calculated using the following equation:

$$\text{DMD (g/kg DM)} = \text{DMwtante- DMwtpost/DMwtante} * 1000$$

where DMwtante is the DM weight before the incubation and DMwtpost is the DM weight after the incubation.

Statistical Analysis

The variability in the chemical composition and the digestibility of the samples was analysed to establish their statistical significance, by means of an analysis of variance (ANOVA), using SPSS version 25 for Windows (SPSS Inc., Chicago, IL, USA) to test the effect of the cultivars. Multiple comparisons of the means were conducted using a Post Hoc (Tukey test) procedure to establish any differences among cultivars. Differences were considered significant at the $P < 0.01$ level. The principal component analysis (PCA) was laid out using SPSS version 25 for Windows.

RESULTS AND DISCUSSION

The correlation matrix indicated correlation coefficients between parameters measured in this study (Table 1). Further, PCA showed that principal component 1 (F1) described 66.35% of total variation and principal component 2 (F2) described 14.76% (Figure 1) with a cumulative percentage of 81.11%. Results obtained from the chemical composition of the leaves and of the green pruning residues of grapevine showed that NDF, ADF and ADL were positively correlated, while DM and DMD were negatively correlated. Figure 2 show that the GPR was on average more fibrous and less digestible than GL, but with similar mean values of crude protein (CP) and GE.

The nutrient content of the GPRs is given in Table 2. The chemical composition of the different GPRs was highly variable in the present study, except for ADL which was similar for all the cultivars, with a mean value of 123 g/kg. The CP content ranged from 74 to 159 g/kg, with the lowest value in Malvasia bianca di Candia GPR and the highest in Verdicchio GPR. As far as the fibrous component content is concerned, the NDF was generally high (528÷598 g/kg), except for the Verdicchio GPR (466 g/kg), while the ADF content ranged from 356 to 436 g/kg. The digestibility results were influenced by the chemical composition, and in particular by the ADF content, in agreement with Romero et al. (2000). The Malvasia bianca di Candia and Lambruscio salamino GPRs, which showed the highest ADF content and the lowest CP content, were in fact the least digestible GPRs. The Sangiovese GPR was the most digestible, and showed a low ADF content and a high CP content. However, although the Sauvignon blanc GPR had a similar chemical composition to the Sangiovese GPR, it resulted less digestible. The Verdicchio GPR had the highest CP content, albeit of a generally low quality, and although it was not particularly fibrous, it resulted in a lower digestibility than the Sangiovese GPR. Finally, the GE content ranged from 16.8 MJ/kg in the Sauvignon blanc GPR to 17.5 MJ/kg in the Moscato bianco GPR.

Table 1. Correlation matrix (Pearson) analysis. Results obtained from the chemical composition of the leaves and of the green pruning residues of grapevine

Variables	DM	CP	NDF	ADF	ADL	DMD	GE
DM	1						
CP	- 0.340	1					
NDF	- 0.827	0.238	1				
ADF	- 0.739	0.081	0.852	1			
ADL	- 0.923	0.262	0.917	0.890	1		
DMD	0.748	- 0.140	- 0.826	- 0.904	- 0.908	1	
GE	0.418	- 0.147	- 0.434	- 0.282	- 0.311	0.244	1

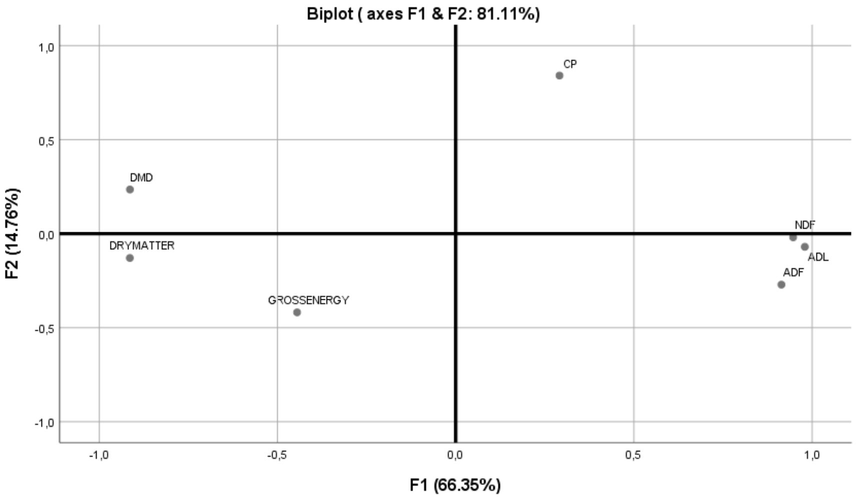

Figure 1. Loading plots of principal components 1 and 2 of the PCA. Results obtained from the chemical composition of the leaves and of the green pruning residues of grapevine.

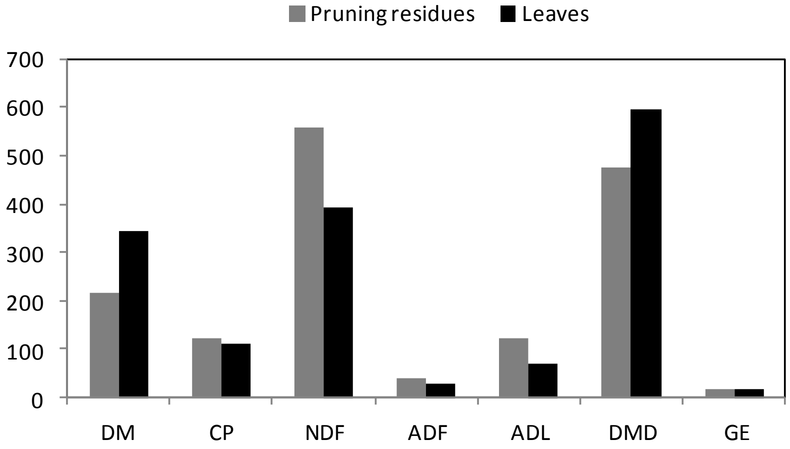

Figure 2. Comparison of the mean chemical composition values (g/kg), *in vitro* apparent digestibility (DMD, g/kg) and gross energy (GE, MJ/kg) of the leaves and of the green pruning residues of grapevine.

Normally, there are high levels of NDF and nitrogen associated with NDF is normally cell-wall bound (Romero et al., 2000). Moreover, tannins are present in vine by-products, at variable levels and in different forms, and they can reduce digestibility (Molina-Alcaide et al., 2008). It is very difficult to compare our results with those present in literature, because the previous studies mainly focused on the potential of vineyard pruning residues for use for energy production (Ntalos and Grigoriou, 2002; Spinelli et al., 2012) and there is a lack of articles on the use of the GPR of *Vitis vinifera* in animal nutrition. In general, the GPRs of this study had a slightly lower nutritional quality than those found by Peiretti et al. (2017). Higher fibre and CP values were found than in other studies (Rebolé and Alvira, 1986).

The fibre values of vine shoots reported by Molina-Alcaide et al. (2008) were also higher than those reported in the present study (NDF 741 g/kg, ADF 518 g/kg, and lignin 166 g/kg), and the DMD value was consequently lower.

The chemical composition, DMD and GE of the GL are shown in Table 3. No differences between cultivars can be observed, in terms of CP, and a mean value of 109 g/kg was reached. The Canaiolo nero, Lambrusco salamino and Moscato bianco GLs were the most fibrous and the least digestible. On the other hand, the GL of the Vernaccia di S. Gimignano cultivar had the lowest ADF content and the highest digestibility. The GE content range was larger for the GL than for the GPR.

The varieties all had a somewhat similar chemical composition to those reported by Feedipedia (Heuzé et al., 2017). The GL digestibility was higher than that obtained by Romero et al. (2000), with a mean value of 594 g/kg vs. 422 g/kg. These authors reported an inverse relationship between high tannin, lignin levels, protein quality and digestibility, while Kamalak (2005) found a negative correlation between *in vitro* DMD and the cell wall content (in particular for the ADF and NDF contents) in grapevine leaves. Gurbuz (2007) confirmed the negative correlation between cell wall content and DM degradation, and determined the potential value of the leaves of four Turkish grapevine cultivars (Ak, Kabarcık, Kıbrıs and Mahrabası), considering their chemical composition, *in situ* DM and CP degradation, and the *in vitro* gas production. The protein content they observed was similar to ours, but the here considered Italian grapevine cultivars resulted more fibrous. Kok et al. (2007) studied the forage and nutritive values of grapevine leaves plus the summer lateral shoots of four cultivars (Cabernet sauvignon, Merlot, Sauvignon blanc and Sémillon) at grape harvest and at two post-harvest dates. By comparing their Sauvignon blanc results with ours, it is possible to observe that the leaves alone had a higher protein content, but also higher levels of NDF and ADF.

This study has confirmed that the GPR and GL of *Vitis vinifera* may have a fairly good potential nutritive value for small ruminants and they could be included in a middle-low quality roughage class. We have observed that digestibility is mainly influenced by the cell wall content, and it is probably also affected by a low protein digestibility and tannin content, as reported by Romero et al. (2000). In fact, tannins, which are normally present in grapevine by-products, could interact with bacterial cell walls or with bacterial enzymes (Molina-Alcaide et al., 2008), thereby reducing digestibility. However, tannins may also be beneficial, because of their anti-carcinogenic effect, the protection they offer against bloating and parasites, and their free radical-scavenging abilities (Molina-Alcaide et al., 2008). Another beneficial effect of tannins is to reduce the wasteful protein degradation in rumen through the formation of a protein-tannin complex, as reported by Kamalak (2005). Moreover, according to Spinelli et al. (2012), pruning residues would seem to have a minimum concentration of chemicals, which is below the legal limit for grapes. Therefore, the utilisation of the GPR and GL of *Vitis vinifera* as alternative nutrient sources for animals could play an important role in the use of available resources and in the recycling of by-products, by increasing the efficiency of agricultural and animal production systems.

Table 2. Chemical composition (mean ± standard error) of the green pruning residues of red and white grapevines

		DM (g/kg)	**CP (g/kg)**	**NDF (g/kg)**	**ADF (g/kg)**	**ADL (g/kg)**	**DMD (g/kg)**	**GE (MJ/kg)**
Cabernet	Red	252.8 ± 2.6^{a}	108.8 ± 2.4^{d}	528.5 ± 7.6^{e}	397.9 ± 8.0^{abc}	112.4 ± 5.7	493.4 ± 11.0^{bc}	17.23 ± 0.01^{b}
Canaiolo	Red	217.1 ± 4.1^{b}	106.1 ± 2.5^{d}	588.9 ± 1.3^{ab}	390.3 ± 13.2^{bc}	126.9 ± 2.3	448.2 ± 4.7^{d}	16.89 ± 0.04^{de}
Carignan	Red	253.5 ± 0.4^{a}	105.8 ± 9.5^{d}	554.6 ± 3.4^{d}	402.8 ± 6.0^{ab}	111.0 ± 3.4	503.5 ± 9.0^{ab}	17.20 ± 0.04^{b}
Lambrusco	Red	240.7 ± 1.6^{a}	128.0 ± 1.6^{bc}	580.6 ± 5.2^{abc}	434.3 ± 15.8^{a}	129.2 ± 10.2	446.4 ± 14.4^{d}	17.16 ± 0.06^{bc}
Sangiovese	Red	215.7 ± 3.7^{b}	133.5 ± 3.7^{bc}	567.7 ± 2.1^{cd}	361.7 ± 1.1^{bc}	113.9 ± 8.3	528.7 ± 1.4^{a}	17.02 ± 0.03^{cd}
Malvasia	White	203.6 ± 8.3^{bc}	74.2 ± 3.7^{e}	598.0 ± 3.9^{a}	435.8 ± 9.3^{a}	134.1 ± 2.1	446.0 ± 6.6^{d}	16.92 ± 0.04^{de}
Moscato	White	218.1 ± 7.6^{b}	136.4 ± 1.1^{bc}	582.5 ± 8.2^{abc}	373.7 ± 13.2^{bc}	130.5 ± 6.4	452.2 ± 11.7^{d}	17.46 ± 0.01^{a}
Sauvignon	White	189.7 ± 6.4^{cd}	120.0 ± 3.3^{cd}	569.9 ± 1.3^{bcd}	361.6 ± 14.7^{bc}	128.9 ± 1.1	464.4 ± 4.3^{cd}	16.79 ± 0.03^{e}
Verdicchio	White	178.7 ± 0.5^{d}	159.5 ± 5.4^{a}	466.1 ± 5.2^{f}	356.2 ± 5.6^{c}	122.0 ± 6.6	488.4 ± 5.8^{bc}	17.14 ± 0.07^{bc}
Vernaccia	White	206.1 ± 2.1^{bc}	138.5 ± 0.8^{b}	557.9 ± 5.2^{d}	397.9 ± 13.4^{abc}	126.1 ± 6.0	484.6 ± 1.9^{bc}	17.29 ± 0.04^{b}
SEM		5.5	5.2	8.4	6.5	2.0	6.3	0.05
P		< 0.001	< 0.001	< 0.001	< 0.001	0.022	< 0.001	< 0.001

DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; DMD, *in vitro* apparent digestibility; GE, gross energy.

^{abcde} Values with different letters within a column differ.

Table 3. Chemical composition (mean ± standard error) of the leaves of red and white grapevines

		DM (g/kg)	**CP (g/kg)**	**NDF (g/kg)**	**ADF (g/kg)**	**ADL (g/kg)**	**DMD (g/kg)**	**GE (MJ/kg)**
Cabernet	Red	371.8 ± 2.6^{a}	113.3 ± 3.0	368.7 ± 4.6^{e}	284.7 ± 17.7^{cd}	67.2 ± 1.2^{bcde}	619.7 ± 10.0^{bc}	18.96 ± 0.01^{a}
Canaiolo	Red	365.1 ± 4.0^{ab}	111.6 ± 2.1	433.9 ± 11.2^{ab}	345.1 ± 5.4^{a}	79.3 ± 3.2^{ab}	516.1 ± 4.0^{f}	17.12 ± 0.01^{f}
Carignan	Red	353.2 ± 0.4^{bcd}	104.2 ± 0.4	367.0 ± 4.8^{e}	265.5 ± 12.6^{d}	62.1 ± 1.3^{de}	631.7 ± 4.2^{ab}	18.37 ± 0.04^{b}
Lambrusco	Red	358.2 ± 1.6^{abc}	121.3 ± 9.3	432.8 ± 2.0^{abc}	343.0 ± 16.4^{ab}	76.9 ± 5.4^{abc}	538.6 ± 9.1^{ef}	17.29 ± 0.02^{e}
Sangiovese	Red	340.7 ± 3.7^{cdef}	105.5 ± 1.0	374.5 ± 8.4^{de}	267.1 ± 3.7^{cd}	57.9 ± 2.1^{e}	639.7 ± 10.7^{ab}	16.13 ± 0.06^{h}
Malvasia	White	323.1 ± 8.3^{fg}	86.4 ± 0.1	319.6 ± 7.7^{f}	308.0 ± 7.0^{abc}	74.5 ± 2.7^{abcd}	560.6 ± 12.2^{de}	17.97 ± 0.08^{c}
Moscato	White	343.4 ± 7.6^{cde}	115.4 ± 7.0	461.6 ± 1.2^{a}	302.8 ± 8.3^{bcd}	83.1 ± 4.9^{a}	526.7 ± 9.5^{ef}	17.56 ± 0.01^{d}
Sauvignon	White	337.4 ± 6.4^{def}	92.3 ± 1.0	353.1 ± 17.8^{e}	301.2 ± 11.5^{bcd}	73.2 ± 5.4^{abcd}	591.0 ± 8.3^{cd}	17.90 ± 0.01^{c}
Verdicchio	White	315.7 ± 0.5^{g}	127.0 ± 0.1	401.6 ± 1.7^{cd}	273.0 ± 8.4^{cd}	65.7 ± 1.8^{cde}	648.2 ± 5.6^{ab}	16.81 ± 0.01^{g}
Vernaccia	White	328.0 ± 2.1^{efg}	117.4 ± 1.4	422.9 ± 3.5^{bc}	262.5 ± 5.8^{d}	68.7 ± 1.6^{bcde}	664.7 ± 17.0^{a}	17.11 ± 0.01^{f}
SEM		4.1	3.3	9.7	6.9	1.8	12.0	0.18
P		< 0.001	0.078	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; DMD, *in vitro* apparent digestibility; GE, gross energy.

[abcde] Values with different letters within a column differ.

CONCLUSION

The results show that both the GL and GPR obtained from red and white grape varieties originating from Italy are suitable dietary sources for ruminant feeding, even though GL has a lower fibrous content than GPR and, consequently, a higher DMD. However, further studies are needed to determine their tannin content, and ruminant feeding tests should be carried out to assess the palatability and GPR and GL intake to determine the effect of the supplementation of these by-products on the growth performance of ruminants.

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