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Chemical composition, including secondary metabolites, and rumen fermentability of seeds and pulp of Californian (USA) and Italian grape pomaces

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ABSTRACT

This study determined the chemical composition and metabolizable energy (ME) value for ruminants of seeds and pulps from grape pomaces (GP), which were produced from white and red *Vitis vinifera* cultivars in Italy and California (USA). Six Italian (*i.e.*, three white and three red) and five California red cultivars were collected after the crush of grape juice had been completed and were ensiled in micro-silos for 45 d. Fresh and ensiled samples were manually sieved to separate seeds from the pulp plus skin fraction and an overall inventory of 44 samples was obtained from the 24 Italian and 20 California samples (*i.e.*, seeds and pulp fractions, fresh or ensiled from 6 or 5 cultivars, respectively). Both in seeds and pulp, the red Italian samples had higher organic matter ($P<0.01$), ether extract ($P<0.05$), neutral detergent fiber (aNDF, $P<0.01$), acid detergent fiber (ADF, $P<0.01$), lignin(sa, $P<0.01$) and Cu ($P=0.02$ and $P<0.01$, respectively) relative to the California red samples. In addition, K ($P<0.01$), Fe ($P<0.01$) and Zn ($P=0.03$ and $P<0.01$, respectively in seeds and pulps) were higher in red samples from California *versus* those from Italy. Italian white samples had higher saponins in pulp (SAP, $P<0.05$) and tended to have higher total extractable phenolics both in seeds and pulps ($P=0.07$ and $P=0.10$, respectively)

Abbreviations: ADF, acid detergent fiber; AF, aqueous fraction of lectins, polypeptides, starch; ALK, alkaloids; CP, crude protein; DM, dry matter; EE, ether extract; Lignin(sa), sulphuric acid lignin; NDF, neutral detergent fiber; SAP, saponins; SC, secondary compounds; TA, total anthocyanins; TP, total extractable phenolics.

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than did Italian reds. Both in seeds and pulps, ensiling increased concentrations of aNDF (P<0.01 and P=0.08, respectively), ADF (P<0.01) and lignin(sa) (P<0.01 and P=0.03, respectively), had no impact on mineral levels and decreased concentrations anthocyanins (P=0.05) and SAP (P=0.01), respectively in seeds and pulp. Samples were fermented in diluted rumen fluid to measure *in vitro* rumen fermentability in terms of gas production, which was used to predict ME. Gas production from seeds of different origin were similar, with the exception of higher values at 48 h in red California cultivars with respect to those from Italy (19.2 ml *versus* 18.0 ml, P<0.05). In pulps, Italian reds did not differ from Italian whites, but had lower gas production than the red California samples (9.2 ml *versus* 12.2 ml, 16.6 ml *versus* 21.3 ml, 26.3 *versus* 32.7, P<0.01, at 4, 24 and 48 h respectively). Ensiling reduced gas production in seeds at 24 and 48 h of incubation (P<0.01) and in pulps at 48 h (P=0.09). Seeds from Italian red samples were lower (P<0.01) in ME than Italian whites (6.23 MJ/kg DM *versus* 6.89 MJ/kg DM, P<0.01) and higher (P<0.01) than the California samples (5.58 MJ/kg DM). There were no differences between ME of the groups of pulps, but ensiling reduced estimated ME content in seeds (5.94 MJ/kg DM *versus* 6.54 MJ/kg DM, P<0.01), but not in pulps. On the basis of our results, and in agreement with other recent papers and/or textbook data, the potential to use de-seeded GP as a feed in diets of high producing ruminants is limited.

1. Introduction

Annual world production of wines has ranged between 267 and 300 million hectolitres in recent years (O.I.V., 2005). The largest wine productions are in Italy, France and Spain (54, 52 and 36 million hectolitres/year), where vineyard acreage is almost stable. However in other parts of the world, such as the USA, Argentina, China and Australia (~64 million of hectolitres/year) wine yields have consistently expanded (+12, +29, +25 and +94%, respectively) in recent years (2005 *versus* the 1996 to 2000 average).

Wine grapes are typically processed by crushing to extract variable amounts of juice, depending upon the wine being made, leaving a residue referred to as grape pomace (GP) that is mainly seeds, skin and pulp (~18–20 kg/100 kg of grapes). Traditional uses of GP have largely been restricted to land application due to its low nutritive value for ruminants (Alipour and Rouzbehan, 2007; Baumgärtel et al., 2007; Bocqué et al., 1984; Larwance et al., 1985). A major limitation of use of GP as a ruminant feed is the presence of grape seeds which are high in lignified fiber (D'Urso and Nicolosi Asmundo, 1983) and are often largely undigested in bovines because few of the seeds are broken open during eating or rumination thereby preventing the grape seed oil from being digested. In addition, grape seeds are high in secondary compounds, such as phenolics and anthocyanins (Makris et al., 2007), which can have potentially negative effects on rumen fermentation. In contrast, the skin and pulp fraction has a lower fiber level (D'Urso and Nicolosi Asmundo, 1983), with less lignin, as well as generally lower levels of secondary compounds (Makris et al., 2007).

If the seeds could be separated from the pulp and skin fraction, the latter may have potential as a ruminant feed while allowing the former to be used for other purposes. Grape seed processing to obtain oil for cooking, aromatic additives for lotions and cremes, and nutraceuticals for new health products (Yilmaza and Toledo, 2006; Diamond, 2000; Fuhrman et al., 2005; Goodman, 2001; Zhang et al., 2007) has enhanced the value of seeds and led to commercial technologies that separate seeds from the pulp and skin fraction in both Italy and California.

In this study, the chemical composition and rumen fermentability of seeds and pulps from GP have been determined with the aim to identify a fraction of grape (*i.e.*, pulp plus skin) to be used as conventional feed in ruminants and the other (*i.e.*, seed) as a potential supplier of nutraceutical

substances. The study examined samples from different grape cultivars (*i.e.*, white and red *Vitis vinifera* cultivars), obtained in Italy and in California (USA), as fresh material or after ensiling.

2. Materials and methods

2.1. Sample origin and collection

Grape pomace samples (*Vitis vinifera* sp.) were collected in the Friuli Venezia Giulia Region of north-eastern Italy and in the Suisun Valley area of north central California (USA). The six Italian samples were from three white (*i.e.*, Sauvignon blanc, Tokay, Verduzzo) and three red (*i.e.*, Merlot, Cabernet sauvignon, Refosco) cultivars. The five California samples were all from red cultivars (*i.e.*, Pinot noir, Cabernet sauvignon, Red zinfandel and two Syrahs). All samples were collected immediately after the crush of grape juice had been completed.

2.2. Sample ensiling and manipulation

Each sample, about 10 kg of fresh material, was divided immediately after collection into four equal amounts, two of which were stored in mini-silos while the other two were used for pH measurement and then dried in a forced air oven at 40 °C. In Italy, each micro-silo was a cylindric plastic bottle (10 cm diameter × 20 cm high), that was filled with pressed pomace, packed manually exerting force to an iron disc (4 cm diameter) welded to a metal rod. Mini-silos had a gas release valve in the lid and were stored upright at ambient temperature (*i.e.*, 15–25 °C) in closed paper boxes. In California, each mini-silo was a cylindric plastic container (30 cm diameter × 50 cm high), that was filled with manually pressed pomace, covered with an airtight plastic lid with a small hole for gas release and then stored upright at ambient temperature (*i.e.*, 15–25 °C). After 45 d, all silos were opened and the ensiled samples were used for pH measurement and then dried in a forced air oven at 40 °C.

Each dried sample of fresh and ensiled pomace was manually sieved to separate seeds from the pulp plus skin fraction (hereafter referred to as 'pulp') and the two samples obtained from each fraction were weighed and bulked. The 24 Italian samples (*i.e.*, seed and pulp fractions, fresh or ensiled from 6 cultivars) were shipped to the Department of Animal Science in Davis (CA, USA), where they were added to the 20 California samples (*i.e.*, seed and pulp fractions, fresh or ensiled from 5 cultivars) to create a single inventory of 44 samples. These samples were ground to pass a 1 mm screen on a model 4 Wiley Mill and divided into three sub-samples. One set was shipped to the Department of Animal Science in Alexandria (Egypt) for secondary compound analysis, one was retained in Davis for primary compound analysis, and one was shipped back to the Department of Animal Science in Udine (Italy) for *in vitro* gas test analysis.

2.3. Primary compound analysis

Samples were assayed for analytical DM by gravimetric loss of free water from heating to 105 °C for 2 h (Reuter et al., 1986). Neutral detergent fiber analyses (aNDF) utilized ND containing sodium sulfite and a heat stable amylase (Van Soest et al., 1991) and data are expressed inclusive of residual ash. Acid detergent fiber (ADF) and lignin(sa) were determined according to AOAC (1997, method 973.18). Total N in samples and in ADF residues (ADICP) were determined with an N gas analyzer using an induction furnace and thermal conductivity (LECO FP-528, AOAC, 1997, method 990.03). Ash was determined as gravimetric residue after incineration at 550 °C for 3 h and ether extract (EE) by ether extraction (AOAC, 1997, method 984.13). The Ca, P, Mg, S, Zn, Mn, Fe and Cu levels were determined by microwave acid digestion and dissolution of the sample and quantitative determination by AAS or ICP-AES (Meyer and Keliher, 1992; Sah and Miller, 1992). Total K was determined using a 2 g/kg acetic acid extraction, then quantitatively by AES (Johnson and Ulrich, 1959). Glucose, fructose, sucrose and starch (after enzymatic hydrolysis, Smith, 1969) were determined by HPLC (Johansen et al., 1996).

2.4. Secondary compound analysis

Assays of secondary compounds (SC) followed the procedure of Salem et al. (2006), with the following modifications. Samples, 20 g, were extracted in 40 ml of methanol/acetone/water (60/30/10, v/v/v) for 24 h at room temperature followed by 60 min at 39–40 °C in a water bath. The solution was filtered and evaporated to obtain the crystallized extract (CE) to be used for further secondary compound assay.

Total extractable phenolics (TP) were assayed by partitioning dissolved CE in an equal volume of ethyl acetate. The ethyl acetate-soluble fraction contained the phenolic compounds and it was it was followed by colorimetric measurement using Folin-Denis reagent with a recovery ranging from 95 to 106% (Xu and Diosady, 1997).

Saponins (SAP) were assayed according to Ahmad et al. (1990). After dissolving sub-samples from the CE extract, it was mixed with an equal volume of *n*-butanol. The butanolic extract was separated and evaporated under reduced pressure to create a saponin containing *n*-butanol fraction.

Alkaloids (ALK) were assayed according to methods of Yamauchi et al. (1990) and Cannell (1998). Sub-samples of CE was dissolved in the same solvent used for CE extraction and treated with an equal volume of 10 ml/l aqueous HCl. Alkaloids were then precipitated by drop-wise addition of 100 g/l NH_4OH. The precipitate was collected by centrifugation (5000 × *g* at 4 °C for 30 min) and washed with 10 g/l NH_4OH. The residue was dissolved in a few drops of chloroform to obtain the chloroform fraction that contained the precipitated alkaloids. After separation of TP, SAP and ALK from the CE, the aqueous fraction (AF) of lectins, polypeptides and starch (see review of Cowan, 1999) was determined according to Hussein et al. (1999).

Total anthocyanins (TA) in the CE were determined by measuring absorbance at 535 nm against a blank of 10 ml/l HCl in distilled water according to methods described by Luque-Rodriguez et al. (2007). A calibration curve with solutions of 5, 10, 15, 20, 25, 30, 35 and 40 mg of malvidin-3-glycoside (Mv3G)/l in 10 ml/l HCl in distilled water was prepared and all the CE extracts were diluted with 10 ml/l HCl in distilled water until the absorbance was within the calibration limits. Results are expressed as the equivalent to mg of Mv3G/gm of skin (*i.e.*, mg Mv3GE/g).

2.5. Gas production and metabolizable energy content

Samples were incubated in diluted rumen fluid to measure rumen fermentability in terms of gas production according to Menke and Steingass (1988). Approximately 220 mg of each dried sample was weighed into a graduated 100 ml glass syringe, which was filled with 30 ml of diluted rumen fluid. The rumen inoculum was collected at a slaughter house from the rumen of four lactating dairy cows. These cows were selected to have been from herds located within 40 km of the slaughter facility, had been transferred within 12 h of slaughter, were not slaughtered for health reasons and had a live weight between 650 and 750 kg. Each rumen was opened within 20 min of slaughter and wet rumen contents, sampled from several different points, were individually squeezed to obtain a pooled mixed rumen liquid for each animal. About 300 ml of rumen fluid from each cow was transferred to a pre-warmed (39 °C) thermos and flushed with CO_2 saturated gas for 1 min.

Syringes were placed vertically in a water bath at 39 °C and three syringes without substrate were used as blanks. Gas production was measured at 4, 24 and 48 h of incubation and the net gas production (NGP) of syringes with substrate was calculated by subtraction of the respective average gas production value measured for blanks. A total of 47 syringes were used in each fermentation run and the gas test was repeated thrice.

For each sample, its metabolizable energy (ME) content was calculated using the equation of Menke and Steingass (1988) as:

$$ME\ (MJ/kg\ DM) = 1.06 + 0.157*NPG_{24} + 0.0084*(N*6.25) + 0.022*EE - 0.0081*ash$$

where N, EE and Ash are g/kg of DM and NPG_{24} are ml of gas after 24 h of incubation.

2.6. Statistical analysis

Data were analyzed (PROC GLM of SAS, 1999) separately for seeds and pulp according to a factorial model which considered the effect of varieties (*i.e.*, the three groups: Italian whites, Italian reds and California reds), effect of ensiling (*i.e.*, fresh and ensiled) and the appropriate interaction:

$y = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$

where μ = overall mean; α = fixed effect of group of varieties ($i = 1, 3$); β = fixed effect of ensiling ($j = 1, 2$). In the absence of a statistically significant interaction, differences among groups were tested by orthogonal contrasts (*i.e.*, two degrees of freedom) that compared Italian whites to Italian reds as well as Italian reds to Californian reds. Statistical significance was accepted if P<0.05 and a trend to a difference if P<0.10.

3. Results

The proportion of seeds in GP averaged 483 g/kg (Table 1), but it was highly variable among cultivars (*i.e.*, RSD = 134 g/kg). The pH did not differ between the Italian samples and increased slightly after ensiling (from 3.92 to 4.20, P=0.02).

3.1. Primary compounds

White Italian seeds had (Table 1) higher EE (P=0.02) than Italian red seeds, which had higher OM (P<0.01), EE (P=0.04), as well as higher aNDF, ADF and lignin(sa) (P<0.01) *versus* Californian red seeds. White and red Italian pulps had similar primary compound levels, with the exception of ADICP, which tended (P<0.07) to be lower in Italian samples. Red Italian pulps had higher OM (P<0.01), EE (P=0.03), as well as aNDF, ADF and lignin(sa) (P<0.01) *versus* pulps from California.

Ensiling increased (P<0.01) the concentrations of ADICP, aNDF, ADF and lignin(sa) in seeds while, in pulps, ensiling increased the concentrations of ADICP (P<0.01), ADF (P<0.01) and lignin(sa) (P<0.05), and tended (P=0.08) to increase aNDF.

Both in seeds and pulps (Table 2), K (P<0.01), Fe (P<0.01) and Zn (P=0.03 and P<0.01, respectively for seeds and pulps) were higher in red samples from California *versus* reds from Italy, while Cu (P<0.01) was lower. White Italian seeds had lower Ca (P=0.03) than did red Italian seeds and Mn (P<0.01) was lower in red samples from California *versus* red from Italy. Both in seeds and pulps, ensiling generally had no impact on mineral levels.

3.2. Secondary compounds

In contrast to the low variation within primary compounds, the RSD values for all SC were very high (Table 3), and this caused several large numerical differences among groups and, due to ensiling, to not differ statistically. The reasons for this high variability are likely due to actual variability among cultivars within secondary compounds, as this analytical procedure typically leads to low variability (*i.e.*, Salem et al., 2006).

White Italian samples had higher SAP (P<0.05) in pulps and tended to have higher TP both in seeds and pulps (P=0.07 and P=0.10, respectively) than did Italian reds. While ensiling numerically decreased concentrations of most of secondary compounds in both seeds and pulp, it only reached statistical significance for TA (P=0.05) and SAP (P=0.01) respectively in seeds and pulps, while SAP in seeds, and TA in pulps, tended (P=0.10 and P=0.09, respectively) to be lower after ensiling.

3.3. Gas production and ME content

There were no differences in gas production between seeds of different cultivars (Table 4), with the exception of the higher gas production at 48 h of incubation in California reds *versus* those from Italy (19.2 ml *versus* 18.0 ml, P<0.05). In pulps, Italian reds did not differ from Italian whites, but had lower gas production than California reds (9.2 ml *versus* 12.2 ml, 16.6 ml *versus* 21.3 ml, 26.3 ml *versus* 32.7 ml, P<0.01, at 4, 24 and 48 h respectively).

Table 1
Impact of cultivar and ensiling on dry matter, seed proportion, pH of whole pomace and chemical composition[a] of grape seeds and pulp.

	Cultivars			Ensiling(E)		P[b]			RSD
	White(W)	Red(R)		None	45 days	E	WI *versus* RI	RI *versus* RC	
	Italy(I)	Italy(I)	California(C)						
Whole pomace									
Dry matter (g/kg)	470	470	329	–[c]	–[c]	–[c]	ns	<0.01	59.8
Seeds (g/kg DM)	426	567	454	479	486	ns	ns	ns	134
pH	3.98	4.13	–[d]	3.92	4.20	0.02	ns	–[d]	0.16
Seeds (g/kg DM)									
Organic matter	962	961	942	955	955	0.99	0.83	<0.01	5.4
Crude protein (CP)	126	125	119	122	125	0.73	0.89	0.58	18.7
Ether extract	145	117	95	127	111	0.07	0.02	0.04	19.4
Neutral detergent fiber (aNDF)	519	542	506	503	542	<0.01	0.09	<0.01	22.7
Acid detergent fiber (ADF)	495	514	467	466	519	<0.01	0.10	<0.01	18.7
CP bound to ADF[e]	134	143	140	121	157	0.01	0.58	0.84	28.5
Lignin(sa)	416	437	371	387	428	<0.01	0.12	<0.01	22.6
Pulp (g/kg DM)									
Organic matter	934	920	863	908	904	0.56	0.15	<0.01	16.1
Crude protein (CP)	99	106	110	107	103	0.61	0.33	0.64	13.5
Ether extract	101	90	59	85	81	0.74	0.45	0.03	24.5
Neutral detergent fiber (aNDF)	423	402	350	379	404	0.08	0.27	<0.01	30.8
Acid detergent fiber (ADF)	401	380	312	343	386	<0.01	0.22	<0.01	28.7
CP bound to ADF[e]	275	214	183	170	278	<0.01	0.07	0.28	54.8
Lignin(sa)	261	242	175	206	246	0.03	0.39	<0.01	38.6

[a] Starch and free sugars (*i.e.*, sucrose, glucose, fructose) were also determined, but values were generally less than the detection levels of 5 mg/kg DM for starch and less than the detection levels of 2 mg/kg DM for each sugar.

[b] No interaction of cultivar and ensiling was $P<0.05$.

[c] Dry matter not measured after ensiling.

[d] pH not measured.

[e] Expressed as g/kg of CP.

Table 2
Impact of cultivar and ensiling on mineral composition of grape seeds and pulp.

	Cultivars			Ensiling(E)		P^a			RSD
	White(W)	Red(R)		None	45 days	E	WI *versus* RI	RI *versus* RC	
	Italy(I)	Italy(I)	California(C)						
Seeds (g/kg DM)									
Ca	5.0	5.9	5.3	5.5	5.3	0.66	0.03	0.08	0.70
P	3.1	3.2	3.2	3.2	3.0	0.13	0.56	0.55	0.29
K	8.0	7.0	15.4	9.8	10.5	0.51	0.42	<0.01	2.14
Mg	1.3	1.2	1.2	1.3	1.2	0.05	0.84	0.65	0.14
S	1.3	1.4	1.5	1.4	1.4	0.64	0.51	0.15	0.13
Zn[b]	13	14	17	15	14	0.58	0.24	0.03	1.8
Mn[b]	19	23	17	20	19	0.59	0.06	<0.01	3.8
Fe[b]	58	64	174	101	96	0.84	0.85	<0.01	48.5
Cu[b]	49	40	16	34	36	0.82	0.44	0.02	18.3
Pulp (g/kg DM)									
Ca	3.9	4.9	4.1	4.2	4.4	0.64	0.09	0.15	0.93
P	3.0	2.7	2.9	2.7	2.9	0.16	0.16	0.36	0.31
K	23.1	28.0	45.2	31.5	32.7	0.65	0.17	<0.01	5.98
Mg	1.1	0.9	1.0	0.9	1.0	0.02	0.05	0.40	0.15
S	1.7	1.7	1.8	1.7	1.7	0.59	0.85	0.55	0.29
Zn[b]	12	12	18	14	14	0.81	0.89	<0.01	2.1
Mn[b]	17	17	13	15	16	0.65	0.98	0.15	4.9
Fe[b]	111	117	398	194	223	0.39	0.89	<0.01	75.4
Cu[b]	102	124	23	83	84	0.97	0.56	<0.01	63.9

[a] No interaction of cultivar and ensiling was P<0.05.
[b] Expressed as mg/kg DM.

Ensiling reduced gas production at 24 and 48 h of incubation in seeds (11.2 ml *versus* 12.9 ml and 17.3 ml *versus* 19.4 ml, respectively, P<0.01) and at 48 h in pulps (26.4 ml *versus* 29.4 ml, P=0.09). Seeds from Italian reds had lower ME contents than Italian whites (6.23 MJ/kg DM *versus* 6.89 MJ/kg DM, P<0.01) and higher (P<0.01) than samples from California (5.58 MJ/kg DM). There were no differences between the ME contents of the groups of pulps. Ensiling reduced

Table 3
Impact of cultivar and ensiling on the secondary compound levels of grape seeds and pulp.

	Cultivars			Ensiling(E)		P^a			RSD
	White(W)	Red(R)		None	45 days	E	WI *versus* RI	RI *versus* RC	
	Italy(I)	Italy(I)	California(C)						
Seeds (g/kg DM)									
Phenolics	90	51	64	66	71	0.76	0.07	0.45	33.9
Saponins	40	47	48	54	37	0.10	0.63	0.89	22.0
Aqueous fraction[b]	56	59	72	67	58	0.49	0.85	0.42	31.2
Alkaloids[c]	15	13	14	14	13	0.74	–	0.74	7.6
Anthocyanins[d]	3	99	85	103	21	0.05	0.08	0.77	90.0
Pulp (g/kg DM)									
Phenolics	62	31	42	38	53	0.30	0.10	0.51	38.0
Saponins	47	26	32	44	25	0.01	0.03	0.56	20.6
Aqueous fraction[b]	22	44	28	36	26	0.33	0.13	0.20	24.0
Alkaloids[c]	11	11	9	11	9	0.66	–	0.64	3.8
Anthocyanins[d]	6	44	34	45	10	0.09	0.16	0.69	44.3

[a] No interaction of cultivar and ensiling was P<0.05.
[b] The sum of lectins, polypeptides and starch.
[c] Contrast WI *versus* RI not estimable for missing missing values.
[d] Expressed as mg/kg DM.

Table 4
Impacts of cultivar and ensiling on *in vitro* gas production and metabolizable energy content of grape seeds and pulp.

	Cultivars			Ensiling(E)		P^a			RSD
	White(W)	Red(R)		None	45 days	E	WI *versus* RI	RI *versus* RC	
	Italy(I)	Italy(I)	California(C)						
Seeds									
Gas volume (ml/0.2 g DM)									
- 4 h incubation	5.0	5.0	5.5	5.4	4.9	0.17	0.99	0.23	0.72
- 24 h incubation	12.1	11.9	12.2	12.9	11.2	<0.01	0.79	0.66	0.72
- 48 h incubation	17.8	18.0	19.2	19.4	17.3	<0.01	0.69	0.04	1.08
Metabolizable energy (MJ/kg DM)[b]	6.89	6.23	5.58	6.54	5.94	0.01	<0.01	<0.01	0.36
Pulp									
Gas volume (ml/0.2 g DM)									
- 4 h incubation	7.8	9.2	12.2	9.7	9.7	0.98	0.11	<0.01	0.92
- 24 h incubation	14.8	16.6	21.3	18.1	17.1	0.22	0.11	<0.01	1.86
- 48 h incubation	24.6	26.3	32.7	29.4	26.4	0.09	0.46	<0.01	3.78
Metabolizable energy (MJ/kg DM)[b]	5.89	5.88	5.52	5.91	5.61	0.16	0.97	0.16	0.47

[a] No interaction of cultivar and ensiling was P<0.05.
[b] Calculated by the equation of Menke and Steingass (1988, see Section 2).

the estimated ME content in seeds (5.94 MJ/kg DM *versus* 6.54 MJ/kg DM, P<0.01), but not in pulps.

4. Discussion

The low pH of fresh grapes is due their high content of organic acids (mainly malic and tartaric acids, Ribèreau-Gayon et al., 1998), which are metabolized during ensiling and this probably explains the slight pH increase of ensiled grapes.

4.1. Primary compounds

Grape seeds are not accurately characterized as ruminant feeds by conventional chemical analyses such as ash, CP, fat and NDF/ADF. Indeed, based upon those values 40–60 g/kg ash, 120–125 g/kg CP, 100–140 g/kg ether extract and only about 500 g/kg aNDF, their feeding value might be considered similar to a high oil corn silage. However because most seeds are not broken upon during eating or rumination, little of the grape seed oil is actually digested. However addition of the lignin(sa) assay reveals the highly lignified nature of the aNDF (from 750 to 800 g lignin(sa)/kg aNDF) and its low feeding value.

Chemical analysis of grape pulp is remarkably similar to the grape seeds, with the notable exception of a lower level of aNDF and a much lower level of lignification (*i.e.*, ~500–600 g lignin(sa)/kg aNDF). While much of the fat in the pulp is likely to be digested, since it will not be protected by the seed coat, there may be a higher proportion of it that is cuticular wax due to the almost total recovery of skins in this fraction.

4.2. Secondary compounds

The composition of GP major constituents in peels and seeds has been reported by several authors, with high polyphenolic as well as dietary fiber contents (Bravo and Saura-Calixto, 1998; Valiente et al., 1995). All these by-products will have a considerable burden of phenolic components (González-Paramás et al., 2004), depending on the type of grape (*i.e.*, white or red) and the part of the tissue (*i.e.*, skins, pulp or seeds).

The variability in SC levels between the type and location of grape cultivation (*i.e.*, Italy or California and reds or whites) and among the GP fractions (*i.e.*, seeds or pulp) are likely to be due to the biological variability of grape material (*i.e.*, grape variety, factors that affect berry development such as soil, geographical location and weather conditions (Ojeda et al., 2002), as well as stage of maturity), the winemaking process from which they were derived and the production process (*i.e.*, extraction, fractionation, drying) which affect variability between GP types of W and R (Muñoz et al., 2004; Baumgärtel et al., 2007). For example, anthocyanins, the water-soluble pigments responsible for the red color in dark grapes (Camire et al., 2002), form condensed structures which lend stability to grape color (Johnston and Morris, 1996; Mateus et al., 2002). Indeed anthocyanin levels were used as a visual marker in early studies of plant chemotaxonomy, which studied relationships of organisms based on their biochemical constituents (Reinert and Yeoman, 1982). Our variability is consistent with Monagas et al. (2006) who quantified SC in grape seeds, skins, pomace and leaves as well as among production batches. The large variation in the SC composition could also result from varietal characteristics of the selected grape by-products and processing methods.

The most abundant of SC in red grapes are anthocyans, mainly 3-glycosides, 3-acetylglycosides and 3-p-coumaroylglycosides of malvidin, peonidin, delphinidin, petunidin and cyaniding (Wulf and Nagel, 1978), as well as hydroxycinnamic acids, monomeric and dimeric flavanols, flavonols and stilbenes (Rodríguez Montealegre et al., 2006) which may be a potential source of antioxidant polyphenols (Alonso et al., 2002; Louli et al., 2004; Kammerer et al., 2005; Pinelo et al., 2005).

Anaerobic ensiling of the GP samples for 45 d affected the SC contents and this may be due to oxidation of some phenolic compounds, especially tannins (Ben Salem et al., 2005) and/or due to polymerization (Makkar and Singh, 1993) or reduced free tannins (Alipour and Rouzbehan, 2007) which are the more active tannin groups relative to ruminal microbial activity. Kalač et al. (1996) found

that ensiling of alfalfa *(Medicago sativa* L.) for 90 d reduced saponins content by 67–72% depending on their chemical structure (Price et al., 1987; Massiot et al., 1988). In our study, the decrease (Table 3) in the level of intact saponins after ensiling may be due to cleavage of sugar side chains. A similar conclusion was given for the decrease in hemolytic index of ensiled alfalfa leaf concentrate (Szakacs and Madas, 1979). Higher degradation of alkaloids after 45 d of ensiling was consistent by previous studies. For example, Candrian et al. (1984) reported the same results of alkaloids degradation during ensiling some plants such *Amsinckia* and *Cynoglossum* spp.

4.3. In vitro gas production and estimated ME content

The pulp fraction of the GP had a much higher rumen fermentability than seeds (*i.e.*, ~80% higher *in vitro* gas production) at the beginning of the incubation (*i.e.*, after 4 h) likely due to the lower lignified fiber content and possible presence of rapidly fermentable substrates in pulps. During and at the end of fermentation (*e.g.*, after 24 and 48 h) pulps had a higher gas production (*i.e.*, from 40 to 50%) than seeds. However, the fermentability of pulp fraction was low in absolute terms, only 15–21 ml/200 mg DM at 24 h. Indeed these values are lower than reported for some fibrous feedstuffs (*i.e.*, 24–42 ml), such as safflower meal, distillers dried grains and brewers grains (Getachew et al., 2002). Alipour and Rouzbehan (2007) measured *in vitro* gas production on ensiled samples of GP (not fractionated into seeds and pulps) and obtained 24 h values slightly higher than those reported in the present paper (*i.e.*, 21–23 ml/200 mg DM of substrate) for pulps.

Ensiling caused a reduction in gas production (12–14%) in seeds and pulps, which is consistent with Alipour and Rouzbehan (2007), who reported progressive depression in gas production of samples ensiled for up to 30 d. Since we found no variation in free sugars between fresh and ensiled samples (*i.e.*, contents less than detection levels), gas yields at the beginning of the incubation (*i.e.*, 4 h), which would be most sensitive to differences in fermentable sugar contents, were also similar. Thus the lower gas yields obtained from ensiled samples may be due (Makkar and Singh, 1993; Ben Salem et al., 2005; Alipour and Rouzbehan, 2007) to high release during ensiling of secondary compound (*i.e.*, tannins) from bounds parts (*i.e.*, protein and fiber).

While the pulps from California had the highest fermentability, and the lowest fiber and lignin(sa) contents, no differences in gas yield occurred between Italian red and white types, which were similar in chemical composition.

Textbook ME values for GP range from 4.2 to 5.4 MJ/kg DM (INRA, 2007 and DLG, 1997, respectively) and are lower than those in our pulps and seeds (5.8 and 6.2 MJ/kg DM, respectively). However the calculated ME content of the seeds may have been dramatically overvalued since the calculation assumes that all ether extract is available for digestion, which would clearly not be the case in animals to which it is fed. However, our estimate of the ME content of pulp is very close to that calculated with the same experimental data (*i.e.*, gas production and chemical composition) by Alipour and Rouzbehan (2007) on ensiled grape pomace samples (*i.e.*, 5.9 MJ/kg DM).

Low digestion of GP was reported by Bocqué et al. (1984) and Larwance et al. (1985) and it was confirmed also by a more recent digestibility study with sheep (Baumgärtel et al., 2007) where, on the basis of digestibility, the ME content of a red grape was about 5.8 MJ/kg DM. In the same study, a white grape variety GP, which contained less fiber and had a higher residue of sugars (*i.e.*, 200 g/kg DM), the ME content of a white variety was higher (8.3 MJ/kg DM).

4.4. Implications of secondary compounds in ruminant feeding of GP

The high level of secondary compounds in GP may suggest a potential value relative to its dietary inclusion with the objective of improving the health status of animals rather than to supply them with nutrients. The moderate content of tannins in GP may reduce ruminal CP degradability and thus prevent bloat when cows are fed high concentrate diets (Mangan, 1988). In addition, tannins are known to exert positive anti-helmintic action (Niezen et al., 1995), and this may be positive for cattle in tropical regions. In our study, ensiling of grape pomace considerably reduced concentrations of the SC that have been considered effective in positively influencing digestion in ruminants (Mangan, 1988; Salem et al., 2006).

Another possible motivation for dietary inclusion of GP is related to its high levels of antioxidants (*e.g.*, phenols, flavonoids, anthocyanins) which can prevent animal tissue damage, mainly the unsaturated lipids in membranes (Girotti, 1998), due to reactive oxygen metabolites (*e.g.*, oxidative stress). In a recent study, dietary addition of an extract from grape skins into sheep diets was more effective than other antioxidant sources (*i.e.*, vitamin E and tomato pomace) in inducing specific transcriptional activity of genes involved in oxidant defenses (Sgorlon et al., 2006). However, additional experimental data based on feeding studies are needed to prove a positive impact on dairy cow performance.

Finally, an interesting perspective of GP utilization as a feedstuff for dairy cows comes from the possibility to enrich milk with substances that have health benefits for its consumers. For example, Steinshamn et al. (2008) recently demonstrated a high transfer rate to bovine milk of some phytoestrogens (*i.e.*, isoflavonoid compounds) when cows consuming forages such as red clover (*Trifolium pretense* L.) that are rich in secondary compounds. On the basis of these results, we suggest that also some antioxidants in GP, mainly belonging to the flavonoid family, could be transferred to milk, directly or after metabolic transformation by rumen microbes.

5. Conclusions

Chemical composition and rumen fermentability results indicate that the ME value of GP is low, even if the seeds have been removed. In addition, a substantive proportion of the CP is associated with the ADF fraction, especially when the pomace is ensiled, and likely to have a low digestibility. On the basis of these results, and in agreement with other recent papers and/or textbook data (Alipour and Rouzbehan, 2007; Baumgärtel et al., 2007; INRA, 2007; DLG, 1997) the potential to use de-seeded GP as a feed in diets of high producing ruminants is limited.

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