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DETERMINATION OF NUTRITIVE VALUE AND ANTI-METHANOGENIC POTENTIAL OF TURKISH GRAPE POMACE USING *IN VITRO* GAS PRODUCTION TECHNIQUE

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ABSTRACT

Grape pomace from the wine industry has been used in ruminant nutrition to meet the requirements of animals during shortages of conventional feed in most parts of the world. The aim of the current study was to screen Turkish grape pomaces (GPs) collected from various sites for chemical composition and anti-methanogenic potential using an *in vitro* gas production technique. Source had a significant effect on the chemical composition, gas production, methane (CH_4) production, metabolizable energy (ME), and organic matter digestibility (OMD) of some Turkish GPs. Crude ash (CA), ether extract (EE), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), condensed tannin (CT) contents, gas production, CH_4 production, ME, and OMD values of the GPs were in the ranges of 3.90-9.37%, 3.07-7.87%, 2.65-13.50%, 26.60-58.80%, 24.98-53.88%, 1.99-16.43%, 42.75-113.50 mL, 5.57-13.38 mL, 5.36-8.69 MJ/kg DM, and 39.87-61.27%, respectively. The *in vitro* experiment showed that most of the GP samples studied have low CH_4 mitigation potential. However, there is a need for *in vivo* experiments to test the mitigating potential of GP samples.

Key words: Grape pomace, chemical composition, digestibility, methane production.

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INTRODUCTION

After the processing of grape fruits in the wine industry, a considerable amount of a byproduct called grape pomace (GP) becomes available in most parts of the world. GP has been used in ruminant nutrition to meet the requirements of animals during shortages of conventional feed, otherwise being a wasted product. It consists of seeds, pulp, skin, and stalk. The chemical composition of GP is variable and depends on the method of wine production, type of grape (Ruberto *et al*., 2008; Basalan *et al*., 2011), and relative ratios of components of the pomace (Baumgartel *et al*., 2007). It was also reported that there was a significant variation in condensed tannin (CT) contents of GP (Hixon *et al*., 2016). All these variations in chemical composition are likely to play a role in the nutritive value and anti-methanogenic potential of GP. In addition to its nutritive value, GP also contains considerable amounts of CT with potential anti-methanogenic activity for ruminants (Hixson *et al*., 2016). It is well known that significant dietary energy loss occurs through enteric fermentation, which is one of the main contributors to greenhouse gasses (Johnson and Johnson, 1995). Recently, the inclusion of tannin-containing feeds in ruminant diets has been employed as a promising CH_4 mitigation strategy (Bhatta *et al*., 2013; Bodas *et al*., 2012).

In vivo and *in vitro* experiments have recently indicated that feeding dairy cows in late lactation with GP with high tannin contents decreased methane (CH_4) production by approximately 20% without a concomitant reduction in dry matter (DM) intake (Pellikaan *et al*., 2011; Moate *et al*., 2014). Although considerable research has been carried out to determine the nutritive value of GP, including its chemical composition, metabolizable energy (ME), and organic matter digestibility (OMD), less attention has been paid to the anti-methanogenic potential of GP obtained from different sources.

The aim of the present study was to screen GP collected from various sources for anti-methanogenic potential using an *in vitro* gas production technique to determine the relationship between chemical composition and CH_4 production.

MATERIALS AND METHODS

Grape pomace collection: The GP samples for the current experiment were obtained from 8 different companies in Turkey and dried in the shade (Table 1).

Chemical analysis of grape pomace: The GP samples were analyzed separately in the laboratory of the Animal Science Department, Faculty of Agriculture, Kahramanmaraş Sütçü İmam University, Turkey, in 2018. The GP samples were analyzed for DM, crude ash (CA), and ether extract (EE) contents (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents of the GP samples were analyzed with the method suggested by Van Soest *et al.* (1991). The CT contents of GP samples were analyzed with the butanol-HCl method (Makkar *et al.*, 1995). The water-soluble DM (WSDM) content of GP was estimated using nylon

bags containing 2 g of GP samples washed with a washing machine for 25 min (Ly and Preston, 1997).

Table 1. Grape pomace samples obtained from different companies.

Grape Pomace Samples	Company Name
GP1	Aker Şarapçılık Tekirdağ, Turkey
GP2	Bor-Sa Bortaçina Şarap Gıda San. Tic. Ltd. Şti, Balıkesir, Turkey
GP3	Urla Şarapçılık Gıda Turizm Tarım San A.Ş. İzmir, Turkey
GP4	Kalecik Şarap Sanayi Tic. A.Ş, Ankara, Turkey
GP5	Vinero Bağcılık San. Tic. A.Ş., Çanakkale, Turkey
GP6	Tariş Sirke Pekmez İşletmesi, Manisa, Turkey
GP7	Mey Alkollü İçkiler San Tic. A.Ş. Nevşehir, Turkey
GP8	Erol Sahin, Karaman, Turkey

Determination of gas and methane production: The gas and CH_4 production of GP samples was evaluated using the *in vitro* gas production (Menke *et al*., 1979). Rumen fluid was obtained from three Awassi sheep (approximately 50 kg average weight) fed with alfalfa hay (800 g) and barley grain (400 g). Before the morning feeding, an equal amount of rumen fluid from each sheep was taken into a thermo flask and filtered through four layered cheesecloths under flushing with CO_2. The buffered rumen fluid (40 mL, 1:2 V/V) was taken into syringes containing approximately 500 mg of GP samples and standard hay with known gas production in a bath set at 39 °C. The same amount of buffered rumen fluid was transferred into syringes without substrate for blanks. All incubations were carried out in quadruplicate. The gas and CH_4 production of the GP samples was measured after 24 h of incubation.

The ME (MJ/kg DM) and OMD of GP samples were estimated with the equations below (Menke and Steingass, 1988):

$ME\ (MJ/kg\ DM) = 2.20 + 0.1357GP_{24} + 0.057CP + 0.00285EE^2$

$OMD\ (\%) = 14.88 + 0.8893GP_{24} + 0.448CP + 0.651\ CA$

GP_{24}: Gas production (mL) at 24 h of incubation
CP: Crude protein (% of DM)
EE: Ether extract (% of DM)
CA: Crude ash (% of DM)

The CH_4 of total gas production after 24 h of incubation of GP samples was analyzed with an infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) (Goel *et al*., 2008) and presented as mL and percentage.

CH_4 production (mL) = Total gas production (mL) × Percentage of CH_4 (%)

Statistical analyses: One-way analysis of variance (ANOVA) was used to differentiate among the sources of the GP samples. Differences ($P<0.05$) among the means of GP samples were determined with Tukey's multiple range tests. Pearson correlation coefficients were calculated to show the relationship between chemical composition and *in vitro* gas production parameters. All statistical analyses were carried out using SPSS (2011).

RESULTS AND DISCUSSION

The chemical compositions of some Turkish GP samples are presented in Table 2. Source significantly affected the chemical composition of the GP samples. Crude ash contents varied between 3.90% and 9.37%, with the highest values in GP2 and GP6 and the lowest in GP8. Ether extract contents ranged from 3.07% to 7.87%, with the highest values in GP1 and the lowest in GP6. It was found that crude protein contents varied considerably within the GP samples, ranging from 2.65% to 13.50%; the highest values were found in GP7 and the lowest in GP5. There was also significant variation in the NDF contents of the GP samples, ranging from 26.60% to 58.80%, with the highest values in GP1 and GP4 and the lowest in GP8. The ADF contents of the GP samples varied from 24.98% to 53.88%, the highest values being seen in GP1 and the lowest in GP8. The CT contents of the GP samples varied between 1.99% and 16.43%, the highest values being seen in GP1 and GP4 and the lowest in GP6 and GP8. These results are consistent with the findings of Hixson *et al.* (2016), who reported that the CA, EE CP, NDF, ADF, and CT of GP samples were in the ranges of 3.12-8.49%, 1.3-17.4%, 3.2-14.4%, 18.4-61.4%, 16.2-56.1%, and 0.69-13.8%, respectively. As can be seen from Table 2, there is significant variation among the Turkish GP samples in terms of chemical composition. These variations among GP samples are possibly related to wine production method, type of grape, and relative ratios of components of the grape pomace (Zalikarenab *et al*., 2007; Baumgartel *et al*., 2007; Ruberto *et al*., 2008; Basalan *et al*., 2011; Winkler *et al*., 2015).

CT in feedstuffs may have adverse or beneficial effects on animals depending on the amount and chemical structure (Makkar, 2003; Min *et al*., 2003; Mueller-Harvey, 2006). Although low levels of CT (2-3% of DM) may have beneficial effects, preventing protein from

extensive degradation, high CT levels (6% and 10% of DM) were found to reduce the intake and growth of animals (Barry *et al*., 1984). The studied GP samples, except for GP6 and GP8, may have detrimental effects on rumen fermentation and animal performance because of their high CT contents. However, high levels of CT may provide a good opportunity to reduce the supplementation amount of GP in the ruminant diet to make use of the anti-methanogenic potential of the CT.

The gas production, CH_4 production, ME, and OMD of the Turkish GP samples are presented in Table 3. These values ranged from 42.75 to 113.50 mL, 5.57 to 13.38 mL, 5.36 to 8.69 MJ/kg DM, and 39.87% to 61.27%, respectively.

Table 2. The chemical composition of some Turkish grape pomaces obtained from different companies.

Type	DM	CA	EE	CP	NDF	ADF	CT
GP1	92.95^{a}	6.89^{b}	7.87^{b}	11.78^{bc}	57.99^{a}	53.88^{a}	16.43^{a}
GP2	92.39^{ab}	9.32^{a}	4.29^{d}	12.62^{ab}	49.84^{b}	44.47^{c}	14.39^{a}
GP3	92.76^{ab}	7.17^{b}	6.33^{c}	12.18^{bc}	48.21^{c}	43.70^{c}	10.13^{b}
GP4	92.93^{a}	5.87^{c}	9.23^{a}	11.33^{cd}	58.80^{a}	50.06^{b}	16.06^{a}
GP5	91.05^{bc}	4.28^{d}	6.98^{c}	2.65^{f}	37.22^{e}	37.00^{de}	14.08^{a}
GP6	91.57^{ab}	9.37^{a}	3.07^{e}	10.36^{d}	45.60^{d}	39.01d	1.99^{c}
GP7	92.22^{ab}	6.70^{bc}	3.98^{d}	13.50^{a}	49.58^{bc}	35.42^{e}	14.37^{a}
GP8	89.39^{c}	3.90^{d}	3.94^{d}	5.03^{e}	26.60^{f}	24.98^{f}	3.55^{c}
SEM	0.512	0.264	0.185	0.313	0.313	0.641	1.117
Sig.	***	***	***	***	***	***	***

$^{a\,b\,c}$ *Column means with common superscripts do not differ (P>0.05).* ***SEM****: Standard error of the mean.*

Table 3. The gas production, methane production, metabolizable energy, and organic matter digestibility of some Turkish grape pomaces obtained from different companies.

Type	Gas	CH_4 (mL)	CH_4 (%)	ME (MJ/kg DM)	OMD (%)	WSDM (%)
GP1	42.75^{f}	5.57^{d}	13.04	5.36^{f}	39.85^{d}	34.50^{d}
GP2	77.50^{d}	10.82^{bc}	13.96	7.17^{c}	54.16^{b}	35.99^{d}
GP3	67.50^{e}	9.99^{c}	14.84	6.67^{d}	49.01^{c}	43.14^{cd}
GP4	50.50^{f}	6.45^{d}	12.78	5.83^{e}	41.74^{d}	39.33^{cd}
GP5	85.75^{c}	10.57^{c}	12.32	7.14^{c}	49.35^{c}	54.70^{b}
GP6	100.25^{b}	13.38^{b}	13.31	8.25^{ab}	61.27^{a}	46.27^{bc}
GP7	94.75^{b}	12.54^{bc}	13.24	8.15^{b}	58.99^{a}	35.21^{d}
GP8	113.50^{a}	16.08^{a}	14.17	8.69^{a}	60.04^{a}	67.39^{a}
SEM	2.479	0.787	0.760	0.134	0.881	2.942
Sig.	***	***	NS	***	***	***

$^{a\,b\,c}$ *Column means with common superscripts do not differ (P>0.05).* ***SEM****: Standard error of the mean.*

The gas productions and OMD of the GP samples in the current study were comparable to the values previously reported by Moghaddam *et al.* (2013) and Mirzaei-Aghsaghali *et al.* (2011), who found that gas production of GP samples ranged from 56.05 to 63.43 mL and 77.3 mL/500 mg, respectively. However, the gas and CH_4 production of the GP samples in the current study were lower than those reported by Hixon *et al.* (2018), although the percentage values of CH_4 and ME for these GP samples were comparable and fell into the range between 6.6 and 12 MJ/kg DM as reported by Hixon *et al.* (2018). The differences between these two experiments in terms of gas and CH_4 production seem to be related to differences in the chemical composition of the utilized GP samples.

Correlation coefficients (r) of the relationship of chemical composition with *in vitro* gas production and the estimated parameters are given in Table 4. The EE, NDF, ADF, and CT of the GP samples are negatively related to the gas production (mL), CH_4 production (mL), ME, and OMD of GP samples. On the other hand, the WSDM content was significantly correlated with the gas production, CH_4 (mL), and ME of the GP samples. It was reported that cell wall contents and CT contents were negatively related to gas production, OMD, and ME of tannin-containing feedstuffs (Camacho *et al*., 2010; Kaplan, 2011; Rezaeenia *et al.*, 2016). Gas production, including CH_4, depends not only on the amount of the available fermentable substrate but also on the amount and molar proportions of the volatile fatty acids produced during fermentation (Davies *et al*., 2000). Although the contribution of the protein and fat in the diet to gas production is small or negligible when compared with carbohydrate fermentation, high levels of fat in ruminant

diets may have a negative effect on the gas and CH_4 production. The negative relationship between gas production or CH_4 and EE content of the GPs obtained in the current experiment supports this hypothesis. Fat exerts its negative effects on gas and CH_4 production through inhibition of the activity of methanogens and protozoans and the biohydrogenation of fatty acid in oil (Johnson and Johnson, 1995).

Table 4. Relationship of chemical composition with *in vitro* gas production and estimated parameters of some Turkish grape pomaces.

	Chemical composition						
Parameters	CA	EE	CP	NDF	ADF	CT	WSDM
Gas (mL)	-0.140^{NS}	-0.844***	-0.429*	-0.830**	-0.944**	-0.718**	0.632**
CH_4 (mL)	-0.103^{NS}	-0.830**	-0.360^{NS}	-0.830**	-0.938**	-0.759**	0.632**
CH_4 (%)	0.240^{NS}	-0.403^{NS}	0.298^{NS}	-0.218^{NS}	-0.219^{NS}	0.406^{NS}	0.117^{NS}
ME (MJ)	-0.046^{NS}	-0.888**	-0.296^{NS}	-0.752**	-0.914**	-0.711**	0.539**
OMD (%)	0.162^{NS}	-0.956**	-0.128^{NS}	-0.631**	-0.816**	-0.708*	0.393^{NS}

Another factor affecting gas and CH_4 production is the CT contents of the GP samples. As can be seen from Table 4, CT was negatively correlated with gas and CH_4 production since CT has the ability to complex with carbohydrate and protein in the substrate, reducing the availability of fermentable substrate for rumen microorganisms (Patra, 2010; Jayanegara *et al*., 2011). CT contents also have a significant detrimental effect on the activity of microorganisms including bacteria, protozoans, and archaeans, depending on the amount and polymerization of CT (Goel *et al*., 2005; Tavendale *et al*., 2005; McSweeney *et al*., 2011; Galindo *et al*., 2008; Min *et al*., 2014).

A number of reviews indicated that CT and fat had significant enteric CH_4 mitigation potential (Henry and Eckard, 2009; Eckard *et al*., 2010). An *in vivo* experiment with dairy cows in late lactation showed that supplementation of the diet with GP decreased the enteric CH_4 emission by approximately 20% without compromising DM intake. The reduction in CH_4 emission due to GP supplementation is possibly associated with the EE, CT, lignin, and tartaric acid contents of GP (Moate *et al*., 2014). The current experiment clearly showed that several of the GPs tested here contained various amounts of CT and EE, which negatively correlated with CH_4 production.

The CH_4 mitigation potential of feedstuffs has recently been determined using the percentage of CH_4 of gas produced after 24 h of incubation (Lopez *et al*., 2010). The GP samples of the present study, except for GP3, have low CH_4 reduction potential since the percentage of CH_4 in the gas fell into the range of 11-14%, which was categorized as low potential by Lopez *et al.* (2010).

In conclusion, there is a considerable amount of variation among the GP samples studied in the current work in terms of chemical composition, *in vitro* gas production, CH_4 production, ME, and OMD. Most of the GP samples studied here have low CH_4 mitigation potential. Therefore, GP may be included in ruminant diets to mitigate CH_4 emissions. However, there is a need for *in vivo* experiments to test the mitigating effects of GP samples.

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