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Research article

Nutritive value of several raisin by-products for ruminants evaluated by chemical analysis and *in situ* ruminal degradability

Yari M [1,4], Manafi M[1], Hedayati M[1], Khalaji S[1], Mojtahedi M[2], Valizadeh R[3] and Hosseini Ghaffari M[3]

[1]Department of Animal Science, College of Agriculture, Malayer University,Malayer 65719–95863, Iran; [2]Department of Animal Science, College of Agriculture, Birjand University, Iran; [3]Department of Animal Science, College of Agriculture, Ferdowsi University of Mashhad, Iran

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Abstract

Feeding ruminants with agro-industrial by-products can help to fill feed shortages in dry period. The objectives of current study were to determine the nutritive value of several sun dried grapevine (raisin) by-products (RBPs) for ruminants using chemical composition analysis and *in situ* ruminal degradability. Several RBPs of sun dried treated grapevine cluster (*Vitis vinifera L. cv. Sultana*) include 1) outer layer of flesh and skin and pedicle of berries (RBP1); 2) rejected raisins mostly un-ripped berries with their pedicles (RBP2) and 3) peduncles and rachises with their lateral branches of clusters (RBP3). Results showed that the RBP1 had lower neutral detergent fiber, lignin (sa.) and nitrogen to organic matter ratio while these components were higher for RBP3, with RBP2 intermediate ($P<0.05$). The RBP1 and RBP3 had higher total tannin concentration compared with RBP2 (75.8 and 69.2 respectively versus 34.6 g/kg DM; $P<0.05$). The RBP1 had higher *in situ* ruminal degradation and lower undegradable fraction (predicted by *in situ* and DVE/OEB 1994 model) for dry matter (DM) and organic matter (OM) compared with RBP3, with RBP2 intermediate ($P<0.01$). In conclusion, the RBPs could be considered as alternative feed in ruminants feeding during dry periods; however, their tannin and lignin (sa) content should be taken into consideration for decision making.

Keywords: *In situ* ruminal degradation; raisin by-products; tannin

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Introduction

Grapevine is an important agricultural product in most countries. During post harvest processing of grapevine cluster, several products are produced. Sun drying of grapevine in the field is one post harvest processing method to produce sun dried grapevine or raisin. Raisin has several health benefits in human nutrition mostly due to its phenolic compounds (Williamson and Craughi, 2010). During raisin production different by-products are produced which may have potential to fill the shortages of feed for ruminants during dry period in semi-arid and temperate climate condition. However, these by-products have usually unknown nutrient profile and availability and sometimes may have anti- nutrients factors (Valizadeh and Sobhanirad, 2009; Besharati and Taghizadeh, 2011).

According to Food and Agriculture Organization data, Iran ranked ninth for grapevine production with

***Corresponding author:** MojtabaYari, Department of Animal Science, Malayer University, Malayer 65719–95863, Iran; E-mail: m.yari@malayeru.ac.ir; Tell: +98 9124479406

2150000 metric tons while China and U.S.A ranked first to second respectively (FAO, 2012). In Iran grapevine is mostly used for fresh consumption and raisin and juice production. Among countries producing raisin, Iran ranked third while U.S.A and Turkey ranked first and second (FAO, 2012). During post harvest grapevine processing, some waste is produced that its magnitude is considerable. The production of grapevine by-products in Iran is ~2.87 million tons per year (Alipour and Rouzbehan, 2007; Besharati and Taghizadeh, 2011; Saremi et al., 2014). About one fourth of total Iranian sun dried raisin is produced in Malayer Town (Hamedan Province) from white Sultana grapevine (*Vitisvinifera L.*; IMA, 2009). In general, after harvest the fresh grapevine cluster is soaked with conventional solution (90 g/kg K_2CO_3 + 1.5 g/kg olive oil) and left for sun drying in the field. Drying process usually takes 7 to 12 days for pre-treated grapes, depending on the relative humidity and the temperature of ambient air under the field (Pala et al., 1993). Available different raisin by-products (RBPs) which are produced during machinery cleaning, sorting and the packing of sun dried treated grapevine cluster are 1) some outer layer of flesh and skin and pedicle of berries (RBP1); 2) un-ripped and abnormal berries (low quality raisin; RBP2), and 3) peduncles and rachises with their lateral branches of grapevines.

According to published studies, grapevine by-products including pomace and RBPs have low nutrient availability for ruminants probably as a result of their higher phenolic compounds like tannins (Abel and Icking, 1984). In some cases feeding sheep with grapevine pomace and RBPs as the only diet ingredient or including partially in diet reduced total tract *in vivo* diet digestibility and animal performance (Abel and Icking, 1984; Tabatabaei et al., 1992; Lu and Yeap-Foo, 1999; Baumgartel et al., 2007; Moghaddam et al., 2013; Saremi et al., 2014). Improved nutrient availability of RBPs in ruminants were reported when polyethylene glycol and polyvinyl-pyrrolidone used for the deactivation of adverse effects of their tannin (Alipour and Rouzbehan, 2007; Besharati and Taghizadeh, 2011). Tannins could modify ruminal microorganism's populations which may change ruminal microbial protein yield, by-pass protein and volatile fatty acids profile and total tract nutrient digestibility with potential effects on animal metabolism and performance (Makkar et al., 1989). However, low nutrient availability of RBPs for ruminants may be due to other chemical compositions and these may not be the same in different RBPs.

To our knowledge, there is no information on different RBPs nutrient composition and availability in ruminants. The objectives of current study were to investigate the nutritive value of RBPs using crude wet chemical analysis and *in situ* ruminal degradability.

Materials and Methods

Raisin by-products sources

Different RBPs samples were collected from two factories (Malayer Town, Hamedan province, Iran; 34°20′N, 48°45′E) processing terminals for chemical composition analysis and *in situ* ruminal degradability. Therefore, in the current study there were three RBPs with two blocks which resulted in six different RBPs based on a randomized complete blocks design.

Chemical composition analysis and predicted energy contents

For chemical composition analysis, the RBPs were ground to pass a 1 mm screen (Retsch ZM-1, Brinkmann Instruments LTD, ON, Canada) and for *in situ* ruminal incubation the RBPs were ground to pass a 2 mm screen (Laboratory Hammer Mill, Christry and Norris LTD, England). Standard procedures described by the Association of Official Analytical Chemists (AOAC, 1990) were used to determine dry matter (DM; method 930.15; AOAC, 1990), ash (method 942.05; AOAC, 1990), crude protein (CP; method 984.13; AOAC, 1990) and ether extract (EE; AOAC, method 954.02; AOAC, 1990). Neutral detergent fibre (NDF) was assayed with heat stable alpha amylase and acid detergent fibre (ADF) were determined with the ANKOM A200 Filter Bag technique (Ankom Technology, Fairport, NY, USA) according to Van Soest et al. (1991). In determination of NDF and ADF, sodium sulfate was used to remove nitrogen attached to cell wall structure. Lignin (sa.) was determined by soaking the ADF filter bag residue in sulphuric acid for 3 h followed by washes with water (method 973.18; AOAC, 1990). All chemical analysis was performed in duplicate. Non-fibre carbohydrates (g/kg DM; NFC= 1000-(aNDF+CP+EE+Ash)) and total carbohydrates [g/kg DM; CHO=1000-(CP+ EE+Ash)] were calculated according to NRC dairy program (NRC, 2001).

For tannin assay, samples were dried at 40°C to constant weight to minimize changes in tannin content and activity, and dried samples were ground through a 0.5 mm screen before analysis (Makkar, 2000). Phenolic compounds were extracted using 200 mg of dried samples. The extraction process involved the sample being made up to 10 ml with aqueous acetone water (700:300, v/v), and the extraction was left at 4°C overnight. The extracts were centrifuged at 3000 *g* at 4°C for 15 min, and the supernatant was obtained and used in the following assay. The concentration of total phenolic compounds was determined using the Folin–Ciocalteu assay as described by Singleton and Rossi (1965) and the regression equation of tannic acid (Merck GmbH, Darmstadt, Germany) standard. Total tannin was estimated indirectly after being absorbed to insoluble polyvinyl-polypyrrolidone. Concentration of

total tannin was calculated by subtracting the total phenolic compounds remaining after the polyvinyl-polypyrrolidone treatment in the assay mixture (Singleton and Rossi, 1965).

In situ degradation kinetics of raisin by-products

For *in situ* incubations, four individually housed ruminal fistulated Holstein Frisian steers (500±10 kg body weight and 3±0.02 years) were used. The steers were fed 9 kg DM/day (in g/kg DM; total mixed ration with 556 g barley silage, 300 g alfalfa hay, and 144 g dairy cow concentrate) twice daily in equal portions at the experimental farm of the Ferdowsi University of Mashhad (Mashhad, Iran) as described by Yari et al. (2014) in detail. The animals were cared for according to Iranian Council on Animal Care guidelines (ICAC, 1995).

In situ ruminal degradation kinetics was determined as described by Yari et al. (2014), using number-coded nylon bags (5 cm×10 cm, pore size 40). Approximately 5 g of RBP samples was placed into each bag. Filled bags were randomly assigned to four individually housed steers and incubated in the rumen for 4, 8, 12, 36 and 72 h (2, 2, 3, 4 and 6 bag per RBP sample respectively). Immediately after retrieval from the rumen, bags were manually washed and oven dried at 60ºC for 48 h. The two bags per each RBP for zero time washed out and oven dried by similar procedure. The ruminal incubation was run for one and two factories (blocks) were used as replicates. Incubation residues from the treatment bags were combined within time per block.

The residues in the bags analyzed for DM (method 930.15 AOAC, 1990) and ash (method 942.05; AOAC, 1990). The rumen degradation characteristics were calculated for DM and organic matter (OM). Three fractions were determined for each component) a rapidly degradable washable fraction (W) which consists of material that escapes from the bag after manually washing in cold tap water; a potentially degradable fraction (D) and a truly undegradable fraction (U) which was determined in g/kg as 1000-W-D. The first order kinetic degradation model, D(t)=W+D×(1-e-kdt) was used to calculate the fractional rate of degradation (kd) and D fraction with D(t) is degradable amount of incubated material after t h of rumen incubation (Ørskov and Mcdonald, 1979; Yari et al., 2014). The first order kinetic model parameters were calculated using the NLIN (nonlinear) procedure of SAS using iterative least-squares regression (Gauess-Newton method) (SAS, 2003).

The rumen effective degradability (ED) for DM and OM were calculated as $ED=W+(D\times K_d)/(K_d+K_p)$ (Ørskov and Mcdonald, 1979), assuming a passage rate (kp) of 0.045/h (Tamminga et al., 1994). The undegradable fraction of DM and OM content of RBPs calculated based on *in situ* and DVE/OEB 1994 model.

Statistical analysis

Data of current study was analyzed using PROC MIXED of SAS 9.2 (SAS, 2003) with the following statistical model:

$$Y_{ij}=\mu+T_i+B_j+e_{ij}$$

Where Y_{ij} is the observation of the dependent variable ij; μ is the fixed effect of population mean for the variable; T_i is the fixed effect of RBPs (i=3; RBP1, RBP2 and RBP3); B_j is the random effect of block (k= 2) and e_{ij} is the random error associated with the observation ij. For all analysis, experimental replicates were block for RBP (n=2). The Fisher's protected least significant difference test was used for multiple treatment comparisons using the LSMEAN statement of SAS. For the different statistical tests, significance was declared at $P\leq0.05$.

Results

Chemical composition

The RBP1 had lower ADF, lignin (sa), ash, NDF, nitrogen to organic matter ratio (N:OM) and N to total carbohydrate ratio (N:CHO) while these chemical components were higher for RBP3, and with intermediate for RBP2 ($P<0.05$; Table 1). The RBP1 and RBP2 had higher NFC and lower CP content compared with RBP3 ($P<0.05$). The RBP1 had higher CHO and RBP3 had lower CHO content with RBP2 intermediate ($P<0.05$; Table 1).

In Situ ruminal degradability

In situ ruminal DM and OM degradation kinetics of RBPs are shown in Table 2. The RBP1 had higher *in situ* washable fraction and effective degradability for DM and OM while these parameters were lower for RBP3, with RBP2 intermediate ($P<0.01$). The RBP3 had higher *in situ* ruminal degradable fraction and calculated *in situ* undegradable fraction for DM and OM and predicted *in situ* undegradable fraction for DM and OM by DVE/OEB 1994 model while these parameters were lower for RBP1, with RBP2 intermediate ($P<0.01$).

Discussion

Chemical composition

In the current study different raisin by-products had not similar chemical compositions. Vivin et al. (2003) studied grapevine samples for two consecutive years and reported that chemical compositions varied among structural and reproductive organs in which ash and N content were higher in lateral stems than fruits. Increasing the concentration of soluble carbohydrates in ripening berries, may dilute other compounds like N

Table 1: Basic chemical composition, total phenol and total tannin of raisin by-products

Item	Raisin by-products (RBP)[d]			SED	P-values
	RBP1	RBP2	RBP3		
Basic chemical composition (g/kg dry matter)[e]					
DM	963.7	960.5	962.1	1.65	0.52
ADF	100.9^{c}	199.0^{b}	355.4^{a}	25.46	<0.01
NDF	126.1^{c}	252.8^{b}	530.7^{a}	34.54	<0.01
ash	28.7^{c}	56.1^{b}	76.8^{a}	5.64	0.05
CP	45.7^{b}	54.4^{b}	83.4^{a}	1.98	<0.01
EE	39.3	27.7	22.9	3.71	0.15
CHO	886.3^{a}	861.8^{b}	816.8^{c}	3.68	0.01
NFC	760.2^{a}	609.0^{a}	286.1^{b}	78.81	0.05
N:OM (g/kg)	7.52^{c}	9.22^{b}	14.46^{a}	0.33	<0.01
N:CHO (g/kg)	8.24^{c}	10.10^{b}	16.35^{a}	0.38	<0.01
Lignin (sa)	88.3^{c}	142.2^{b}	280.9^{a}	10.28	<0.01
Phenolic compounds (g/kg dry matter)					
Total phenol	87.4^{a}	36.6^{b}	74.3^{a}	6.13	0.05
Total Tannin	75.8^{a}	34.6^{b}	69.2^{a}	5.61	0.05

[d]Raisins by-products were 1) some outer layer of flesh, skin and pedicle of berries (RBP1); 2) rejected raisins mostly un-ripped berries with their pedicles (RBP2), and 3) stalks, rachises and pedicles of grapevines; SED, standard error of difference; Means with different letters (a, b and c) within the same row differ (P<0.05); [e]DM, dry matter content; ADF, acid detergent fiber; NDF, neutral detergent fiber; CHO, total carbohydrate calculated as 1000-(CP+EE+Ash) (NRC, 2001); N:CHO, ratio between nitrogen and total CHO; NFC, non-fiber carbohydrates calculated as 1000-(CP+EE+Ash+aNDF) (NRC, 2001); N:OM, ratio between nitrogen and organic matter.

Table 2: *In situ* ruminal dry matter and organic matter degradation kinetics of raisin by-products using first order kinetics digestion model

Item	Raisin by-products (RBP)[d]			SED	P-value
	RBP1	RBP2	RBP3		
In situ dry matter (DM) degradation characteristics (g/kg)[e]					
W_{DM}	712.5^{a}	556.6^{b}	334.5^{c}	26.71	<0.01
D_{DM}	182.8^{c}	268.5^{b}	362.0^{a}	33.17	<0.01
kd (/h)	0.069	0.075	0.059	0.01	0.59
ED_{DM}	823.1^{a}	724.9^{b}	539.1^{c}	9.54	<0.01
$U_{DM}^{in\text{-}situ}$	104.6^{c}	174.8^{b}	303.5^{a}	15.52	<0.01
$U_{DM}^{DVE/OEB\text{-}1994}$	114.1^{c}	184.5^{b}	297.0^{a}	23.45	<0.01
In situ organic matter (OM) degradation characteristics (g/kg OM)					
W_{OM}	717.0^{a}	559.4^{b}	323.6^{c}	25.63	0.02
D_{OM}	188.6^{c}	270.0^{b}	369.3^{a}	30.35	0.02
kd (/h)	0.067	0.078	0.063	0.01	0.59
ED_{OM}	830.5^{a}	731.1^{b}	539.1^{c}	1.23	<0.01
$U_{DM}^{in\text{-}situ}$	94.4^{c}	170.6^{b}	307.1^{a}	1.65	<0.01
$U_{DM}^{DVE/OEB\text{-}1994}$	113.1^{c}	182.5^{b}	294.3^{a}	23.36	<0.01

[d]Raisins by-products were 1) some outer layer of flesh and skin, pedicle of berries (RBP1); 2) rejected raisins mostly un-ripped berries with their pedicles (RBP2), and 3) stalks, rachises and pedicles of grapevines; SED, standard error of difference; means with different superscript letters (a, b and c) within the same row differ (P<0.05); [e]W, washout fraction; D, potentially degradable fraction; U, undegradable fraction (1000-(W+D)); kd, fractional degradation rate of B; ED, effective degradability.

containing compounds which consequently would result to lower CP and higher NFC in the berries compared with rachises and their lateral branches and peduncles (Vivin et al., 2003). Because the grapevine is perennial plant, the mobilization of N from perennial parts to annual parts takes places during growing season. With advancing ripening in berries, concentration of N commences to decline while its magnitude in rachises with their lateral branches, peduncles and shoots increases. This translocation of N occurs to replenish partially the nitrogen pool of woody parts of grapevine for next year re-growth (Wermelinger, 1991).

Generally in grapevine cluster, structural carbohydrates are mostly found in rachises with their lateral branches and peduncles as supportive tissues while non-structural carbohydrates like simple sugar, pectin and starch mostly are presented in the berries (Alipour and Rouzbehan, 2007; Moghaddam et al., 2013). From the point of ruminant nutrition view, simple sugar, pectin and starch are considered as NFC (NRC, 2001). These may be the cause of lower NDF, ADF and lignin (sa) and higher NFC in RBP1 and higher NDF, ADF and lignin (sa) and lower NFC in RBP3, with intermediate for RBP2. Current results for RBP3 chemical composition are comparable with

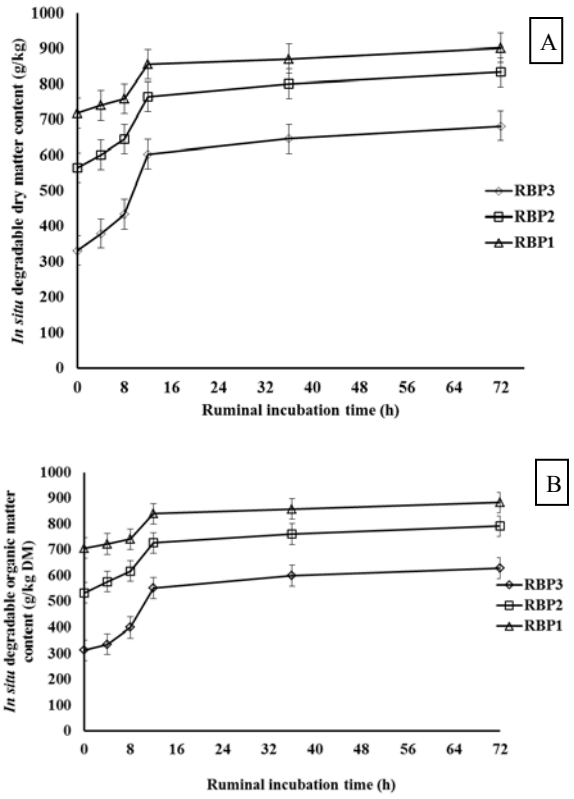

Fig. 1: Pattern of *in situ* ruminal dry matter (DM; a) and organic matter (b) degradable content (lsmeans±standard error of means) of raisin by-products (RBPs) during different time of incubation. Raisins by-products were 1) some outer layer of flesh and skin and pedicle of berries (RBP1); 2) rejected raisins mostly un-ripped berries with their pedicles (RBP2), and 3) stalks, rachises and pedicles of grapevines.

previously reported data (Tabatabaei et al., 1992; Besharati and Taghizadeh, 2009, 2011; Saremi et al., 2014).

The higher N:OM and N:CHO ratio in RBP3 may be mostly due to its higher N and OM content compared with other two RBPs. In grapevine's rachises with their lateral branches and peduncles, proteins are mostly associated to fiber and lignin and considered as resistant proteins in human nutrition (Llobera and Canellas, 2007). It means that higher fiber and lignin contents might have been resulted in higher CP content. Optimum N:CHO and N:OM ratio for microbial growth in the rumen was reported to be ~32 g N/kg CHO (Sinclair et al., 1991) and ~25 g N/kg OM respectively (Czerkawski, 1986). According to the current data, three RBPs have lower value for them which indicate that these RBPs have lower N per unit of OM and CHO for the optimum growth of ruminal microorganisms when feeding as the only diet ingredient.

The RBP1 and RBP3 had higher total phenol and total tannin concentration compared with RBP2 ($P<0.05$; Table 2). The phenolic substances and tannins are primarily located in the seed and skin in berries of grapevine (Hellman, 2003). The higher total phenol and total tannin content in RBP1 may be as a result of its higher raisin's skin content. The RBP2 was mostly consisted of low quality, un-ripped and small seedless berries which these might have been resulted to its lower total phenol and total tannin content. The RBP3 had higher level of NDF, ADF and lignin (sa). In grapevine's rachises with their lateral branches and peduncles, polyphenols are mostly associated with fiber and lignin and most of the lignin components are considered as a part of total tannin (Llobera and Canellas, 2007). This means higher fibre and lignin (sa) content in RBP3 might have been resulted in higher total phenol and total tannin content in chemical analysis. Current results are in consistence with González-Paramás et al. (2004) who reported that grapevine by-products have a considerable level of phenolic compounds depending on the type and the part of grapevine tissue. Previous studies reported that the skin of grapevine by-products contains the highest amount of polyphenols and in particular of condensed tannins (Makris et al., 2007). Spanghero et al. (2009) reported that the large variation in the phenolic components could also result from varietal characteristics of the selected grapevine by-products and processing methods.

In Situ ruminal degradability

The three *in situ* feed fractions differed among three RBPs in which the ruminal degradability of DM and OM was higher and undegradable fraction was lower for RBP1 compared with RBP3, with RBP2 intermediate. By plotting the degradable DM (g/kg; Fig.1a) and OM (g/kg DM; Fig. 1b) at the different times of *in situ* ruminal incubation, RBP1 had higher degradable content at earlier times of incubation compared with RBP3, with RBP2 intermediate. The patterns of degradation of DM and OM at the later times of incubation were similar between RBP1 and RBP2 but they were greater for RBP1 and RBP2 than RBP3. Based on chemical composition data, RBP1 had lower structural CHO and lignin (sa) content compared with RBP3 treatment, with intermediate for RBP2. Among the different chemical compositions, the structural CHO and lignin (sa) content have been reported to mainly influence the magnitude of *in situ* ruminal degradability of DM and OM (Yari et al., 2012). Higher *in situ* degradability and lower undegradable fraction of RBP1 may be due to its lower lignin (sa) content and higher NFC content. In the RBP1 and RBP2, the washable fraction was greater than degradable fraction while in RBP3 washable and

degradable fraction were mostly equal. These may be related to the different ratio of berry to rachis, pedicle and peduncle in by-products.

Conclusion

Among the three RBPs, RBP1 and RBP2 treatments had greater ruminal degradability. The RBPs could be considered as an alternative feed ingredient to replace partially the forage portion in the diet of ruminants under the semi-arid climate condition. In future studies their potential for providing truly absorbed metabolizable protein in small intestine and healthy impacts on ruminants are required to be investigated.

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