IJAS Iranian Journal of Applied Animal Science

Nutritional Value, Fourier Transform Infrared Spectroscopic Molecular Structures, Mycotoxines and Heavy Metals Concentration of Un-Ripe, Ripe and Sun-Dried Fruit from 'Sultana' Grapevine for Ruminants

Research Article

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Received on: 27 Sep 2016
Revised on: 22 Feb 2017
Accepted on: 15 Mar 2017
Online Published on: Sep 2017

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Online version is available on: www.ijas.ir

ABSTRACT

Grapes and grape-derived products have worldwide importance due to its consumption by human, however, they may also be used for ruminant feeding when their price or quality is low. The objectives of current study were to determine the nutritive value, in terms of chemical composition, *in vitro* rumen gas production kinetics and predicted nutrient supply, Fourier Transform Infrared Spectroscopic (FTIR) molecular structures and mycotoxin and heavy metal contaminants in un-ripe, ripe and sun-dried (raisin) fruits of 'Sultana' grapevine for ruminants. Un-ripe fruit had higher nitrogen to total carbohydrate ratio and total phenol and tannin concentration ($P<0.05$) than ripe and sun-dried grapevine, and tended to have higher vibration spectroscopy peak area related to phenolic compounds and related to structural carbohydrates than ripe and sun dried fruit. Raisin had higher *in vitro* cumulative gas production at 24 h of incubation ($P<0.05$). Aflatoxin B1, B2, G1 and G2 and Ochratoxin A were not detectable in un-ripe and ripe grapevine fruit, while low concentrations were found in raisin ($P<0.05$). The lead concentration was lower in un-ripe fruit and raisin than in ripe fruit ($P<0.05$). In conclusion, phenolic compounds and tannins, mycotoxins and heavy metal concentration in un-ripe and ripe fruits and raisin of Sultana' grapevine were lower than toxic level for animal nutrition and nutrient profile and availability of the grapevine products make it a suitable feed to replace forage in the diet of ruminants.

KEY WORDS energy value, mycotoxines, FTIR vibration spectroscopy, grapevine products, *in vitro* gas production kinetics, ruminants.

INTRODUCTION

Grapes were among the first fruit species to be domesticated and today is the most economically important fruit crop in the world (Keller, 2010). Grapevines are planted on approximately 7.3 million hectares worldwide producing approximately 67 million metric tons of fruit in 2007. Iran is one of the major grapevine growers in the world with 215000 ha producing 2.15 million tons of fruit (FAO, 2009) and Malayer is the leading grapevine growing areas (Hamedan province) of Iran, with about 11200 ha grapevines (Karimi and Ershadi, 2014). 'Sultana' (synonym White Kishmish) is one of main grapevine cultivars planted in Malayer, which produces seedless fruit and raisins that are mainly exported as raisins or locally sold fresh for human consumption (Karimi and Ershadi, 2014). The over-

supply of grapevine products (e.g. fruit) or low quality or contaminated (e.g. mycotoxins and heavy metals) grapevine and raisin products not suitable for the human market are usually used for domestic ruminant feeding (Besharati Taghizadeh, 2009). However, no information was found in literature regarding nutritive value, plant secondary compounds and contaminants in un-ripe, ripe and sun-dried (raisin) 'Sultana' grapevines for ruminants. This information is essential for ration formulation and for safe feeding of these products to ruminants. Nutritive value and plant secondary compounds in grapevine products likely change with phenological stage, berry development and fruit ripening. Berry development and ripening in grapevine consist of three main phases being early fruit development, lag phase and berry ripening. The duration of the early fruit development phase is specific to individual cultivars and this phase ends simultaneously with the end of the herbaceous phase followed by a lag phase without fruit growth. Then a second growth phase takes place, with the onset of ripening indicated by a change in grape berry skin colour called 'véraison' in French. The largest changes in grape berry composition occur during this ripening phase (Boss and Davies, 2001). Chemical composition, molecular features and nutritive value of feeds for ruminants can be analysed by wet-chemical methods, *in vitro* gas production technique and Fourier Transform Infrared (FTIR) vibration spectroscopy.

Contamination of fruit with mycotoxins (secondary metabolites of moulds) and heavy metals can occur during berry development, ripening and sun drying (to produce raisins) through management practices, weather conditions and ground contamination, which lower the product quality for local consumption and export. Mycotoxins and heavy metals can be hazardous to animal health (Besharati Taghizadeh, 2009), therefore, their levels need to be determined. The objectives of current study were to determine the nutritive value, in terms of chemical composition, *in vitro* gas production kinetics, predicted nutrient supply, FTIR molecular structures, and mycotoxin and heavy metal contaminants in un-ripe, ripe and sun-dried (raisin) fruits of 'Sultana' grapevine. Our hypothesis was that the chemical composition, nutritive value, FTIR features and presence of mycotoxines and heavy metals in un-ripe, ripe and sun-dried fruits of 'Sultana' grapevine would change with phenological stage and during product processing for raisin production.

MATERIALS AND METHODS

Plant materials and grapevine sampling

In this study, un-ripe and ripe fruit of grapevine were sampled from 14 years old own-rooted 'Sultana' grapevines (*Vitis vinifera*) grown at four different vineyards (blocks) in the Malayer region (Malayer, Iran; lat. 34˚ 30´ N, long. 48˚ 85´ E, alt. 1550 m). All vines were spaced 2.5 m apart in north–south-orientated rows that were 3 m wide. The vines were pruned when fruit spurs were at 5-6 buds, which occurred in the middle of March, and vines were irrigated every two weeks.

The sample collection was performed during the 2012 growing season (dates and weather conditions in Table 1) at berry véraison (colour change and maturation nascent; i.e. un-ripe fruit), grape harvest (grape maturity; i.e. ripe fruit) and postharvest after sun drying (i.e. raisin). In each vineyard, 3 rows with 12 vines were used as replicates and about 36 grapevine clusters (at least 3 representative clusters per vine) were randomly collected from different parts of grapevine: un-ripe (4×3×12×3), ripe (4×3×12×3) and sun-dried (4×3×12×3) harvest. The grapevine clusters from vines within each row were pooled to produce 3 samples (3 rows) per vineyard. Fresh samples were packed in polyethylene bags and placed on ice in styrofoam boxes and shipped to the Laboratory.

Raisin production process

For raisin preparation, part of the ripe grapevine clusters harvested were dipped into a solution of potassium carbonate and olive oil (90 g/kg K_2CO_3+1.5 g/kg olive oil). This operation preserves the vitamins and minerals in the grape, accelerates the grape drying process and gives the raisins the golden yellow colour. The treated grapevines were spread directly on the ground in the sun to dry for approximately 7-12 days according to the heat intension (Pala *et al.* 1993). After drying, raisins were separated from the stalks and stored in fruit boxes.

Chemical composition analysis

Before chemical composition analysis and *in vitro* ruminal fermentation measurements, all samples (12=4 vineyards×3 rows within vineyard) were dried at 50 ˚C to constant weight and ground to pass a 1 mm screen (Ghods miller, Ghods Company, Iran). Standard procedures described by the Association of Official Analytical Chemists (AOAC, 10) were used to determine dry matter (DM; method 930.15), ash (AOAC method 942.05), crude protein (CP; AOAC method 984.13) and ether extract (EE; AOAC, method 954.02).

Neutral detergent fibre (NDF), assayed with heat stable alpha-amylase, and acid detergent fibre (ADF) were determined according to Van Soest *et al.* (1991) with the ANKOM A200 Filter Bag technique (Ankom Technology, Fairport, NY, USA). Sodium sulfate was used for NDF and ADF determination to remove nitrogen attached to cell wall structure.

Table 1 Weather conditions during sampling of un-ripe, ripe and sun-dried (raisin) Sultana grapevine in 2012

Items	Grapevine product[1]		
	Un-ripe (n=12)	Ripe (n=12)	Sun-dried (n=12)
Sampling date	July 26	Sep 5	Sep20
Max. temperature (˚C)	35.00	32.00	27.00
Min. temperature (˚C)	18.00	12.00	10.00
Relative humidity (%)	10.00	15.00	25.00
Day length (h)	14:01	13:01	12:14

[1] n= 12; (4 vineyards×3 rows within vineyard).

Acid detergent lignin was determined by soaking the ADF filter bag residue in 72% sulphuric acid for 3 h followed by washes with water (method 973.18; 10). All chemical analyses were performed in duplicate and repeated if error was higher than 5%. Non-fiber carbohydrates (g/kg DM; NFC= 1000 - (NDF+CP+EE+Ash)) and total carbohydrates [g/kg DM; CHO=1000-(CP+EE+Ash)] were calculated according to NRC (2001).

Before total phenolics and tannin analysis, samples (4 vineyards×3 rows within each vineyard=12) were ground through a 0.5 mm screen (Makkar, 2000). Total phenolic compounds were extracted from 200 mg dried samples in 10 ml aqueous acetone water (700:300, v/v) at 4 ˚C overnight.

The samples were then centrifuged at 3000 g at 4 ˚C for 15 min, and the supernatant (i.e. extract) used in the colorimetric Folin–Ciocalteu assay, as described by Singleton and Rossi (1965) with tannic acid (Merck GmbH, Darmstadt, Germany) as a standard. Total tannin was estimated indirectly after binding tannins in the supernatant of the phenolic extraction to insoluble polyvinyl-polypyrrolidone followed centrifugation at 3000 g at 4 ˚C for 15 min, and supernatant again used in the Folin–Ciocalteu assay. Concentration of total tannin was then calculated by subtracting phenolic compounds remaining in the supernatant after the polyvinyl-polypyrrolidone precipitation of tannins from total phenolic compounds (Singleton and Rossi, 1965).

In vitro gas production and predicted nutrient supply in ruminants

In vitro rumen incubations were performed using the semi-automated gas production technique (Theodorou *et al.* 1994; Rogerio *et al.* 1999) with buffered rumen fluid prepared according to Menke and Steinglass (1988). Rumen fluid was collected before the morning feeding from four ruminally fistulated steers (482.5±22.5 kg, body weight) fed 9 kg DM/d (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).

Feeding and animal husbandry of the steers were according to procedures of the Iranian Council on Animal Care (ICAC; 1995) guidelines. After collection, ruminal contents were strained through four layers of cheese cloth, to eliminate large feed particles, and transported to the laboratory at Ferdowsi University of Mashhad in a pre-warmed thermos.

Each sample [3 samples per vineyard × 4 vineyards × 3 products (un-ripe, ripe and sun-dried berries)= 36] was incubated in triplicate vials (125 mL) with 10 mL of rumen liquid and 20 mL of buffer (Menke and Steinglass, 1988) for 96 h at 37.5 ˚C.

Three vials with buffered rumen medium, without sample, were incubated to correct for gas release from the inoculum.

Gas accumulated in the head-space of the vial was determined using a pressure transducer (Razi Instruments, Mashhad, Iran) and head-space gas volume (Gp) was predicted by Boyle's Gas Law from pressure measurements as:

$$GP= (Vh/Pa) \times Pt$$

Where:
Vh: represents head-space volume (95 ml).
Pa: represent atmospheric pressure (14.692 psi; Meteorological Office, Mashhad, Iran).
Pt: represents pressure transducer reading (psi) (Theodorou *et al.* 1994; Rogerio *et al.* 1999).

Head-space pressure readings were taken at 0, 2, 4, 8, 12, 20, 24, 48, 72 and 96 h after the start of incubation. All incubations were repeated in two runs. The rate and extent of gas production were determined for each sample by fitting gas production data over time to a nonlinear equation:

$$Y= b(1-\exp^{-ct})$$

Where:
Y: volume of gas produced at time t.
b: asymptotic gas production.
c: fractional rate of gas production (Ørskov and McDonald, 1979).

Parameters b and c were calculated using the NLIN (nonlinear) procedure of SAS using iterative least-squares regression (SAS, 2003). Linear regression equations (Menke and Steinglass, 1988) were used to estimate organic matter digestibility (OMD, g kg^{-1})= 14.88 + 0.8893 × gas + 0.0448 × CP + 0.0651 × ash and net energy for lactation (NE_L; MJ kg DM^{-1}= -0.22 + 0.1062 × gas + 0.0048 × CP + 0.0132 × fat based on cumulative gas volume at 24 h of incubation (gas) and sample CP, fat and ash concentrations.

Molecular spectroscopic study and spectral features

All samples (4 vineyards×3 rows within vineyard=12 per grapevine product) were scanned by FTIR vibration spectroscopy using a JASCO FTIR-ATR-4200 (JASCO Corporation, Tokyo, Japan). Spectra were generated in the mid-infrared spectra range from 4000 to 700 cm^{-1} in transmission mode. Quantitative analyses (peak area and height) of the molecular spectral were performed using JASCO software. Typical spectra of the three grapevine products with different functional groups is presented in Figure 1. The spectral peak area, height and centre were identified for phenolic compounds at 3000-3730 cm^{-1} and 1700-1830 cm^{-1}, (Fernaandez and Agosin, 2007; Musingarabwi, 2015), for lipids at *ca.* 2790-3000 cm^{-1}, for proteins at 1546-1703 cm^{-1}, for total carbohydrates at 920-1188 cm^{-1}, and for structural carbohydrates at 1188-1496 cm^{-1} (Wetzel *et al.* 1998; Yu *et al.* 2004).

Mycotoxines and heavy metals analysis

Mycotoxines, aflatoxin B1, B2, G1 and G2 were determined according to ISIRI (No. 6872; Ref. 2012; ISIRI, 2012a) and for Ocratoxin A (No. 9238; 2012; ISIRI, 2012b) according to AOAC (1995) and Romer *et al.* (1978) using HPLC method and immunoaffinity column clean up-Test method. Heavy metals were analysed according to procedures in ISIRI standards (No. 12968; Ref. 2012; ISIRI, 2012) based on AOAC (1995). Number of samples used for mycotoxines and heavy metal analysis were 12 (4 vineyards×3 rows within each vineyard).

Statistical analysis

Data was analysed using PROC MIXED of SAS (2003) with the following statistical model:

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

Where:

Y_{ij}: observation of the dependent variable ij.

μ: fixed effect of population mean for the variable.

T_i: fixed effect of treatment (i=3; un-ripe, ripe and sun dried fruit of grapevine).

B_j: random effect of block (vineyard; j=4).

e_{ij}: random error associated with the observation ij.

For gas production values, triplicate vials per run per samples were averaged and the effect of run was included in the model as random effect. For all analysis, experimental replicates were three samples per block (i.e. vineyard) for each treatment (3 samples×4 vineyards×3 treatments=36). 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 $P \leq 0.05$ and trend at $P \leq 0.15$.

RESULTS AND DISCUSSION

Chemical composition

Raisin samples had higher dry matter (DM) concentration than ripe grapevine, which both had higher DM concentration than un-ripe grapevine (P<0.01) (Table 2). Nitrogen to CHO ratio (N:CHO), N to organic matter ratio (N:OM), total phenolics and total tannin (P<0.05) were higher in un-ripe grapevine compared with ripe grapevine, and these constituent were lowest in raisin(P<0.05) (Table 2). None-fibre carbohydrate and CHO concentrations were lower and ADF and acid detergent lignin (ADL) concentration were higher in un-ripe and ripe grapevine compared with raisin (P<0.05) (Table 2). Ripe grapevine had higher NDF concentration than un-ripe grapevine, which had higher NDF than raisin (P<0.01). Ether extract was similar among grapevine products.

***In vitro* gas production and estimated nutrient supply in ruminants**

In vitro potential gas production (i.e. b) and rate of gas production (i.e. c) were similar among the three grapevine products (Table 3).

Cumulative gas production after 24 h of incubation was higher for un-ripe grapevine than for raisins, which were both higher than for ripe grapevine (P<0.05). Unripe grapevine tended (P≤0.10) to have a lower predicted organic matter digestibility and net energy for lactation than ripe and sun-dried grapevine.

FTIR spectroscopic features

The three grapevine products had similar FTIR molecular structures related to amides, aromatic compound bonds and total carbohydrate (Table 4). Ripe grapevine had lower peak area related to CH_2 and CH_3 symmetric and anti-symmetric stretching compared with raisin and un-ripe grapevine (P<0.05). Un-ripe grapevine tended to have higher area related to phenolic compounds and tannins (P=0.15) compared with ripe grapevine.

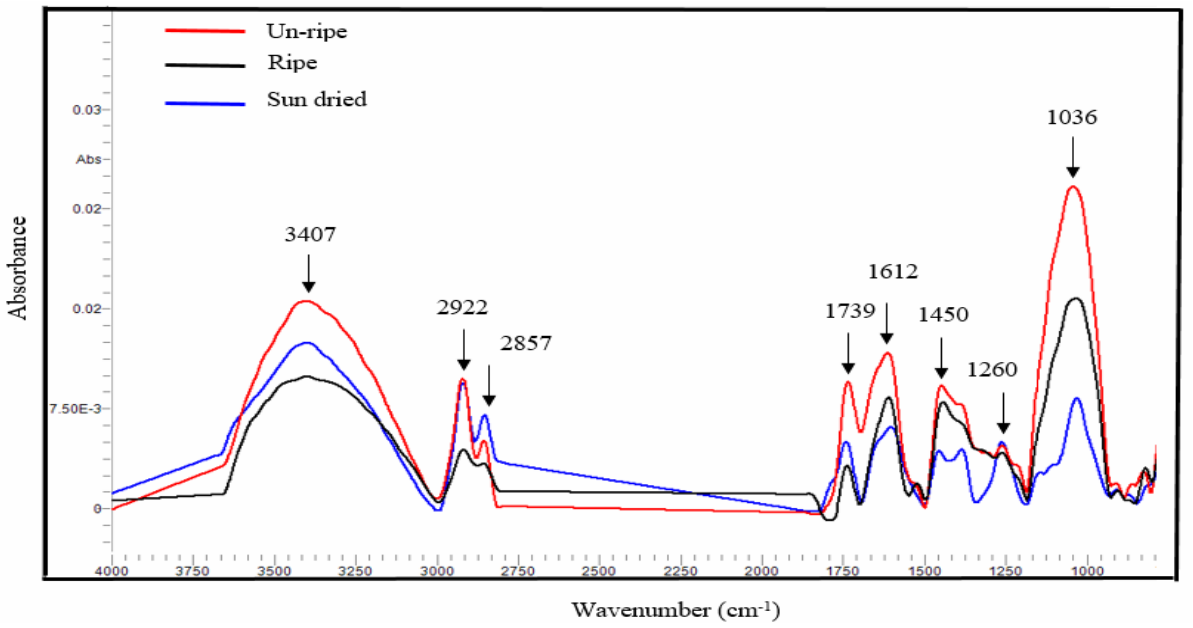

Figure 1 Typical FTIR graph for un-ripe, ripe and sun-dried grape vine after baseline correction and normalization scale

Table 2 Chemical composition of un-ripe, ripe and sun-dried (raisin) fruits Sultana grapevine

Items	Grapevine product[1]			SEM	P-value
	Un-ripe (n=12)	Ripe (n=12)	Sun-dried (n=12)		
Basic chemical composition (% of dry matter)					
Dry matter	13.45^{c}	18.16^{b}	84.95^{a}	0.401	< 0.01
Crude protein	7.65^{a}	4.94^{b}	3.72^{c}	0.188	< 0.01
Ether extract	2.60	2.02	1.92	0.622	0.71
Acid detergent fiber	30.23^{a}	33.64^{a}	3.75^{b}	1.72	0.01
Aacid detergent lignin	29.92^{a}	32.74^{a}	2.3^{b}	1.84	< 0.01
Neutral detergent fiber	45.75^{b}	59.43^{a}	4.8^{c}	2.05	< 0.01
Non-fiber carbohydrates	37.02^{b}	26.01^{b}	85.02^{a}	2.36	< 0.01
Total carbohydrates	82.77^{b}	85.44^{b}	89.82^{a}	0.757	0.03
Nitrogen to carbohydrate (CHO) and organic matter ratio (g/kg)					
N:carbohydrates	14.78^{a}	9.26^{b}	6.62^{c}	0.41	< 0.01
N:organic matter	13.15^{a}	8.56^{b}	6.24^{c}	0.399	< 0.01
Phenolic compounds (g/100 g of dry matter)					
Total phenol	6.51^{a}	2.85^{b}	0.87^{c}	0.414	0.02
Total tannin	5.33^{a}	2.34^{b}	0.57^{c}	0.232	< 0.01

[1] n= 12; (4 vineyards×3 rows within vineyard).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Un-ripe grapevine tended to have higher peak height related to structural carbohydrate compared with raisin (P=0.15).

FTIR molecular ratios were similar among grapevine products, except for CH_2 and CH_3 symmetric and anti-symmetric functional groups related to lipids to phenolic compounds and tannins ratio, which tended to be higher for raisin compared with un-ripe and ripe grapevine (P=0.07) (Table 4).

Mycotoxines and heavy metals

Concentration of aflatoxin B1, B2, G1 and G2 and Ocratoxin A were lower in un-ripe and ripe grapevine than in raisins (P<0.01) (Table 5).

All three grapevine product had similar cadmium concentrations, while lead concentration was lower in un-ripe grapevine and raisin than in ripe grapevine (P<0.05).

Chemical composition

Un-ripe and ripe grapevine and raisin are mostly used for human consumption, however, they may be used for animal feeding if fruit is physically damaged, contaminated with mycotoxins or other pollutant, or if the price is low. The chemical composition of 'Sultana' grapevine differed greatly among un-ripe, ripe and sun dried grapevine in this study.

Table 3 Kinetics of ruminal fermentation of un-ripe, ripe and sun-dried (raisin) fruit of sultana grapevine measured by the *in vitro* gas production technique

Items	Grapevine treatment[1]			SEM	P-value
	Un-ripe (n=12)	Ripe (n=12)	Sun-dried (n=12)		
Kinetics of gas production[1]					
b (mg/0.2 g DM)	230.80	245.25	256.30	7.668	0.26
c (/h)	0.1591	0.1409	0.1040	0.0088	0.09
gas 24 (mg/0.2 g DM)	39.73^{c}	44.60^{b}	47.28^{a}	0.780	< 0.01
Potential of nutrient supply in ruminants					
OMD (% DM)	58.19	61.70	61.56	0.754	0.06
NE_L (MJ/kg DM)	4.71	5.02	5.23	0.090	0.10

[1] n= 12; (4 vineyards×3 rows within vineyard).
b: total potential gas production and c: rate of gas production.
DM: dry matter; OMD: organic matter digestibility and NE_L: net energy for lactation.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Fourier Transform Infrared Spectroscopic (FTIR) spectroscopy molecular structures in un-ripe, ripe and sun-dried (raisin) fruit of sultana grapevine[1]

Items		Grapevine products[2]			SEM	P-value
		Unripe (n=12)	Ripe (n=12)	Sun-dried (n=12)		
FTIR molecular structures						
Total carbohydrates 920 to 1188 cm^{-1}	Height	0.021	0.013	0.015	0.0037	0.48
	Area	2.32	2.97	1.80	0.590	0.50
Structural carbohydrates 1188 to 1496 cm^{-1}	Height	0.0097	0.010	0.0075	0.00102	0.35
	Area	2.03	1.38	1.17	0.209	0.15
Amides 1546 to 1703 cm^{-1}	Height	0.01	0.01	0.01	0.0001	0.95
	Area	0.981	0.976	0.855	0.1772	0.89
Aromatic combination bands 1700 to 1830	Height	0.012	0.007	0.010	0.0022	0.47
	Area	0.513	0.426	0.450	0.1191	0.90
CH_2 and CH_3 symmetric and anti-symmetric 2790 to 3000	Height	0.012	0.008	0.010	0.001541	0.42
	Area	1.261^{a}	0.630^{b}	1.165^{a}	0.1232	0.05
O-H stretching and C-H stretching vibrations (phenolic compounds and tannins)	Height	0.0195	0.0133	0.0150	0.00312	0.51
	Area	8.394	4.713	6.725	0.9945	0.15
Ratio between FTIR molecular structures						
Amides: total carbohydrate		0.505	0.833	0.750	0.1634	0.49
Amides: structural carbohydrate		1.03	1.00	1.50	0.2044	0.35
Structural carbohydrate: total carbohydrate		0.489	0.833	0.500	0.1253	0.18
Lipids: phenolic compounds		0.1495	0.1266	0.1798	0.01173	0.07
Lipids: structural carbohydrate		1.1228	0.7000	1.5000	0.2534	0.20
Lipids: total carbohydrate		0.566	0.700	0.750	0.1833	0.80
Lipids: amides		1.100	0.700	1.000	0.1414	0.25

[1] Lipids, CH_2 and CH_3 symmetric and anti-symmetric 2790 to 3000 cm^{-1}.
[2] n= 12; (4 vineyards×3 rows within vineyard).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The CP concentration decreased with increasing berry ripening, which was consistent with Vivin *et al*. (2003) who suggested that N containing compounds were diluted by the increased concentration of soluble carbohydrates with advancing berry ripening.

Further, with advancing berry ripening, concentration of N commences to decline while it increases in magnitude in rachises with their lateral branches, peduncles and shoots. This translocation of N occurs to replenish the N pool of woody parts of grapevine for the re-growth of next year (Wermelinger, 1991).

The CP of grapevine products in the current study were in a similar range as found for raisin industry by-products (Yari *et al.* 2015a), but more than 50% lower than in wine industry rest-products (Alipour and Rouzbehan, 2007; Spanghero *et al.* 2009).

Changes in FTIR molecular structures related to proteins (amid I+amid II) were previously found to be related to digestibility of proteins in ruminants (Damiran and Yu, 2011; Yari *et al.* 2013).

The FTIR proteins molecular structures were similar among grapevine products, which suggests that the type of protein and digestibility would be similar for the three grapevine products.

This is different than for alfalfa hay where FTIR proteins molecular structures changed with advancing maturity of the forage harvested (Yari *et al.* 2013). The N:CHO and N:OM ratios declined from the un-ripe to ripe and sun-dried grapevine stages as a result of reduction in N and increase in both non-structural and total CHO, and these ratios were in a similar range as found for raisin industry by-products (Yari *et al.* 2015a).

Fibre fractions (NDF and ADF) were lower and NFC higher in raisin compared with un-ripe and ripe grapevine. Fibre fractions and NFC in un-ripe and ripe grapevine were in a similar range as for wine industry rest-products (Alipour and Rouzbehan, 2007; Spanghero *et al.* 2009), while NDF and ADF were lower and NFC higher in raisin of the current study compared with values for raisin industry by-products (Yari *et al.* 2015a).

Plant secondary metabolites

Phenolics like coumaric, caffeic, ferulic and vanillic acids are plant secondary compounds that are relatively simple, while others have more complex polymeric structures such as condensed tannins, which strongly contribute to the mouth feel, antioxidant activity, diet digestibility, ruminal N availability and anti-parasitic activity of grape products fed to animals (Corrales *et al.* 2008; Waghorn, 2008). Total phenolics and total tannins by wet chemistry and FTIR molecular structures related to phenolic compounds and tannins reduced with maturation of grapevine, and with sun drying to generate raisins, in the current study, which was consistent with Vivin *et al.* (2003). Total phenolics and total tannins concentration in un-ripe berries were similar to values previously reported for raisin by-products (Besharati and Taghizadeh, 2009; Yari *et al.* 2015a) and winery rest products (Alipour and Rouzbehan, 2007; Spanghero *et al.* 2009), while these were higher than in ripe berries and raisins in this study. Small berries have higher tannin concentration than large berries because the skin constitutes (fraction which contains most of the tannins) a larger percentage of the berry mass (Wermelinger, 1991), which might explain the higher tannin concentration in un-ripe (i.e. smaller berries) grapevines.

Gas production kinetics and predicted nutrient supply in ruminants

Total gas production during *in vitro* incubation provides an indication of the fermentablity, digestibility and energy value of a diet for ruminants (Menke and Steinglass, 1988).

Asymptotic gas production was numerically highest for raisin (Table 3 and Figure 2) among grapevine by-products and rate of gas production tended to be higher for un-ripe and ripe grapevine than for raisin. These values were in a similar range as previously found for raisin by-products (Yari *et al.* 2015b).

The higher gas production in raisin (Figure 2) at different times of incubation and at 24 h may be due to much higher NFC, which is more fermentable than NDF (Menke and Steinglass 1988; Tavendale *et al.* 2005; Tefera *et al.* 2008), than in un-ripe and ripe grapevine. The lower 24 h gas production of un-ripe fruit compared to ripe fruit might have resulted from their higher total phenolic and tannin concentration, which can impair fermentation and gas production. *In vitro* gas production during different times of incubation (Figure 2) where similar for ripe and sun-dried grapevine, while both were higher than in un-ripe grapevine. The FTIR spectroscopy features of ripe and sun-dried grapevine, also, were similar in terms of most FTIR functional groups while being more different compared with unripe grapevine (Figure 1).

Energy values (OMD and NE_L) grapevine products in the current study were higher than of raisin by-products (Yari *et al.* 2015b) and winery rest products (Alipour and Rouzbehan, 2007; Spanghero *et al.* 2009). The energy content of un-ripe grapevine was, however, lower than in ripe grapevine and raisin, which might be due to the higher NDF and total phenolics and tannins concentrations (which all in general reduce feed digestibility and therefore energy value) in un-ripe grapevine. Energy values of the grapevine products in the current study were in a similar range as for alfalfa hay and barley silage (NRC, 2001), which are forages that are commonly fed to ruminants in Iran. Therefore, grapevine products tested in the current study can likely replace part of alfalfa hay or barley silage in the diet of ruminants.

Mycotoxines and heavy metals

Many fungi can grow and infect berries in the vineyard depending on the weather conditions (Pitt, 1993). Fungi most commonly grow on berries when the moisture of air is high, when the ambient temperature ranges between 20 to 30 ˚C (Serra *et al.* 2006) and during maturation of fruit. Temperature and moisture conditions during growth of grapevine in the current study were not ideal for common fungi growth, which might explain why mycotoxines were not detected in un-ripe and ripe grapevine. This is different to Serra *et al.* (2006) who detected mycotoxin ochratoxin A (OTA) concentration in pea berries and OTA were found to differ among different grape cultivars and with advancing maturation stage.

Table 5 Mycotoxines and heavy metal concentrations in un-ripe, ripe and sun dried (raisin) fruits of sultana grapevine

Items	Treatments			SEM	P-value
	Un-ripe (n=12)	Ripe (n=12)	Sun-dried (n=12)		
Mycotoxines (mg/kg of dry matter; ppm)					
Afl-B1	ND	ND	1.775	0.00433	-
Afl-B2	ND	ND	0.2355	0.00087	-
Afl-G1	ND	ND	1.775	0.00433	-
Afl-G2	ND	ND	0.2355	0.00087	-
OTA	ND	ND	1.775	0.00433	-
Heavy metals (mg/kg of dry matter)					
Cadmium	0.01863	0.01347	0.001469	0.01133	0.48
Lead	0.2054[b]	0.5966[a]	0.08548[b]	0.06817	0.0001

[1] n= 12; (4 vineyards×3 rows within vineyard).
Afl: aflatoxine; OTA: Ochratoxin A and ND: not detected.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

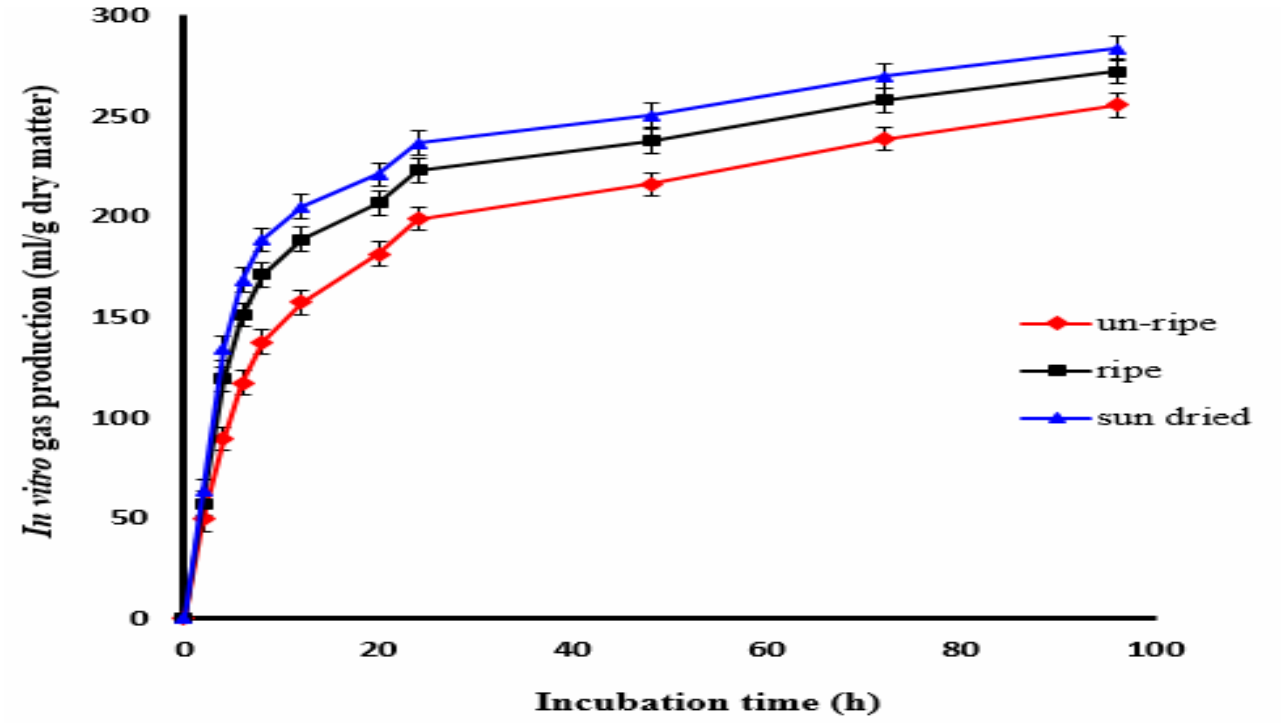

Figure 2 *In vitro* gas production pattern of un-ripe and ripe fruits and sun-dried (raisin) of Sultana grapevine

Accessibility of fruit nutrient due to physical damage of berries during harvest might provide good conditions for fungal growth, which might explain why higher mycotoxines concentrations were found on raisins after sun-drying. Aspergillus and Penicillium species are commonly found on dried fruits, including grape products (Palumbo *et al.* 2011). Raisin samples in the current study had an average OTA concentration of 1.875 ppb, which was lower than average OTA level of over 2 ppb and maximum value up to 100 ppb reported for worldwide dried vine fruits (e.g., sultanas, raisins) (Palumbo *et al.* 2011) and lower than maximum tolerable levels of mycotoxins in barley, maze and legumes for ruminants [respectively, 10, 5 and 5 ppb for aflatoxin B1; 10 ppb for the sum of aflatoxin B1, B2, G1 and G2; 50, 30 and 20 ppb for OTA (ISIRI 2002 confirmed by FAO, 2003; FDA, 2011)]. Mycotoxins are considered less toxic in ruminants than in simple stomach animals because microorganisms in the rumen can partially detoxify mycotoxins.

Contamination of food with toxic heavy metals, such as cadmium, lead and mercury, can occur through for example rainfall, irrigation water, soil, traffic density, atmospheric dust etc., which can be absorbed through the leaf blades (Zaidi *et al.* 2005; Sobukola *et al.* 2010). The concentration of toxic heavy metals differs among plants species, which mainly depends on soil conditions and the ability of plants

to selectively accumulate some metals (Divrikli *et al.* 2006). Levels of lead in the current grapevine samples was in similar range as previously reported for fruits and vegetables bought on the open market (Radwan and Salama, 2006), while cadmium levels were in general higher in samples of the current study than found by Radwan and Salama (2006), but still far below the maximum allowable cadmium level of 1 mg/kg set by the Joint FAO/WHO Expert Committee on Food Additives (FAO/WHO, 1999).

CONCLUSION

Crude protein, non-fibre carbohydrates, structural carbohydrate, lignin, ash, total phenolics, total tannin content and the FTIR spectroscopic molecular structures such as phenolic compounds and tannins, structural carbohydrates and lipids (CH_2 and CH_3 symmetric and antisymmetric functional groups) differed among un-ripe, ripe and sun-dried (raisin) 'Sultana' grapevine. Mycotoxines contamination was detected only in raisin samples, which were lower than hazardous levels set for ruminant feeding. Ripe and sun-dried grapevine (raisins) had higher predicted nutritive value than un-ripe grapevine.

ACKNOWLEDGEMENT

The authors gratefully thank the Iranian Grape and Raising Institute, Malayer University for financial support of this project. The authors also would like to thank Jahad Keshavarzi Malayer for helping to collect samples.

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