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Chemical Composition, Fatty Acids Profile and Biological Evaluation of Tannins of Selected Date Seeds Grown in Iran

Research Article

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ABSTRACT

This study was conducted to evaluate the chemical composition and fatty acids profile of six predominant date seed cultivars grown in Iran including Barhi, Estamaran, Mazafati, Khasi, Kharak and Zahedi and the biological effects of their tannins based on *in vitro* gas production. Organic matter digestibility (OMD), metabolizable energy (ME) and concentration of short chain fatty acids (SCFA) were estimated after 24 h of incubation without or with inclusion of polyethylene glycol (PEG). Results showed that date seeds of all cultivars contained high amount of neutral detergent fiber (NDF), ether extract (EE) and total phenolics (TP), which ranged from 689 to 782, 82 to 118 and 41 to 110 g/kg DM, respectively. However, they had low levels of crude protein (CP) (50 to 69 g/kg DM) and ash (10 to 26 g/kg DM). Gas chromatography revealed that the major unsaturated fatty acid was oleic acid (40.13 to 46.35 g/100 g fatty acids), while the main saturated fatty acid was lauric acid (20.96 to 26.25 g/100 g fatty acids). Except for Estamaran, all cultivars had low OMD (<334 g/kg DM) and ME (<4.1 MJ/kg DM). Inclusion of PEG increased gas volume (GV), OMD, ME and SCFA ($P<0.05$) suggesting the inhibitory effect of date seed tannins on microbial fermentation. Total tannins were negatively correlated with nutritive value (OMD and ME) of date seeds. It can be concluded that despite low digestibility and ME, date seed may be considered as an alternative feed because of high amount of structural carbohydrates and EE.

KEY WORDS date seed, fatty acids, gas production, phenolics.

INTRODUCTION

Iran with an annual production of more than 1 million tons is the second producer of date (*Phoenix dactylifera*) around the world, playing an important role in the economic and social life of Iranian people (FAO, 2013). The date fruit is composed of a fleshy pericarp and seed (pit) providing nutritional needs of domestic animals as an alternative feed (Hossain *et al.* 2014). However, before incorporating less-known feedstuffs in the ruminant ration, it is important to have preliminary information on chemical and mineral compositions of feeds because some chemical compounds such as tannins may negatively affect the bioavailability of other nutrients in the diet (Al-Farsi *et al.* 2008). Furthermore, physical properties of dietary fiber (e.g., particle size, lignification, gravity, etc.) may modulate the rumination process and consequently the rumen health and animal productivity (NRC, 2000). The potential dysregulation of rumen fermentation under heat stress in hot climates (Hall, 2009) may be more intensive due to use of high tannin-containing feeds in the ration. Determination of chemical composition along with reaction properties of organic matter of feedstuffs under laboratory assessments such as *in vitro* gas production have been widely used to evaluate the nutritive value of feedstuffs in ruminant studies since 1979 (Menke *et al.* 1979) to now. This technique could be more

efficient than other *in vitro* methods to evaluate tannin-containing feeds (Getachew *et al*. 2002). Moreover, inclusion of polyethylene glycol (PEG) in the gas production technique can reveal the biological effect of tannins on organic matter digestibility during the fermentation (Makkar *et al*. 1995) and the volume of produced gas in this method is closely related to organic matter digestibility and energetic value of substrate (Menke and Steingass, 1988). Another source of variation in *in vitro* studies can be the rumen liquor of donor animal because of diversity of rumen microbial population (Menke and Steingass, 1988). In this research, we used an exceptional beef cattle native to the southern-east of Iran called Sistani cow that is very resistant to harsh conditions in tropical regions and even long-term drought. It has been suggested that the high ability of Sistani cow to adapt with rough climate may be partly due to its rumen ecosystem and resident microbiota (Mansoori *et al*. 2006).

Therefore, the aim of this study was to evaluate the chemical and mineral composition and lipid profile of six date palm seeds cultivated in Iran and to determine the biological effects of their tannins on organic matter fermentation.

MATERIALS AND METHODS

Date seed samples

Six fully ripened date seed cultivars including Barhi, Estamaran, Mazafati, Khasi (Khasui), Kharak and Zahedi were obtained from different farms located in southern provinces of Iran. Samples of Barhi, Estamaran and Khasi cultivars were collected from Khuzestan province and samples of Mazafati, Kharak and Zahedi cultivars were collected from Kerman, Sistan and Baluchestan and Yazd provinces, respectively. Air dried samples were ground to pass a 1 mm sieve by a cyclic mill.

Chemical analysis

Proximate analysis

Dry matter content of date seed samples was determined by drying in an oven at 100 ˚C to a constant weight (method 934.01) (AOAC, 2005). Ash (method 942.05), ether extract (method 920.39), acid detergent fiber (ADF) and lignin (method 973.18) were determined according to AOAC (2005). Crude protein (Kjeldahl N×6.25) was determined by the Block Digestion Method (method 2001.11) on Kjeltec 2300 Autoanalyzer, Foss Tecator AB, Hoganas, Sweden) as described in AOAC (2005). Neutral detergent fiber (NDF) was determined by the procedure of Van Soest *et al*. (1991). The sodium sulphite and α-amylase were not used and both NDF and ADF were expressed exclusive of residual ash.

Phenolic compounds and tannins

For total phenolics (TP) and total tannin (TT) measurement, dried samples were ground to pass a 1 mm sieve and then 0.5 mm sieve. Before tannin extraction, fat was removed from dried samples by extracting with diethyl ether. Approximately 200 mg of samples were extracted in 10 mL 70% aqueous acetone (v/v) with four replicates overnight at room temperature (25 ˚C). After centrifugation (3000×g, 4 ˚C, 10 min) the supernatant was collected and kept in refrigerator (4 ˚C). Folin-Ciocalteu reagent was used for quantification of TP and TT and tannic acid (Merck GmbH, Darmstadt, Germany) was used as a standard (Makkar, 2000). The values of TP and TT were expressed as tannic acid equivalent.

Mineral composition

Potassium contents of ground samples were determined using the Sherwood model 410 Flame Photometer (Sherwood Science Single Channel Flame Photometer) after ashing and treating with concentrated HCl. Other minerals including P, Na, Ca, Mg, Zn and Fe were measured on Varian SpectrAA-400 plus Atomic Absorption Spectrometer.

Oil extraction

The oil extraction was conducted with Twisselmann apparatus. About 15 g of the grounded date seeds were extracted with petroleum ether, 40-60 ˚C (Merck, Darmstadt, Germany) for 6 h. The solvent was removed by a rotary evaporator (Heidolph Laborota 4000) at 40 ˚C. The seeds oil was transferred into screw capped test tubes and drained with nitrogen and stored at –20 ˚C until further analysis.

Fatty acids composition

The fatty acid composition was determined according to ISO standard ISO 5509:2000, (ISO, 2000) procedure. The FAs of the oil samples were converted into methyl esters (FAMEs). FAMEs were identified on a Shimadzu Gas chromatography (GC)-17A with a capillary column, Supelco SP^{TM}-2330 (30 m×0.32 mm×0.20 µm film thickness). The column temperature was programmed from 60 to 190 ˚C at 10 ˚C/min and held for 2 min, then heated to 220 ˚C at 2 ˚C/min. The injector and detector temperatures were set at 250 ˚C. The carrier gas was high pure nitrogen. The peak areas were computed by the integration software and percentages of FAMEs were obtained as weight percent by direct internal normalization. Two replicates were injected for each sample.

In vitro tannin bioassay

To investigate the biological effects of tannins, *in vitro* gas production was carried out using PEG as described by Makkar (2000). Briefly, 500 mg dry weight of date seed

samples were incubated without and with 1 g PEG (MW=6000) and filled with 40 mL rumen liquor and buffer mixture in triplicate. Rumen fluid was obtained from three fistulated Sistani cows fed with corn silage and concentrate with a ratio of 70:30, before the morning feeding. Culture bottles were placed in a water bath at 39 ˚C and gas production in the head space of each bottle was read from the display unit (Theodorou *et al*. 1994) at 2, 4, 6, 8, 10, 12 and 24 h of incubation.

Calculation and statistical analysis

The OM digestibility (OMD) and metabolizable energy (ME) were estimated from the net 24 h gas volume without and with PEG by the following equations according to Menke and Steingass (1988):

OMD (g/100 g)= 148.8 + 8.893 GV + 0.448 CP + 0.651 Ash

ME (MJ/kg DM)= 0.72 + 0.1559 GV + 0.0068 CP + 0.0249 Ash

Where:

GV: net gas volume produced at 24 h fermentation (mL/200 mg of DM).

CP: g/kg DM.

Ash: g/kg DM.

Short chain fatty acids were calculated using the equation of Getachew *et al*. (2002):

SCFA (mmol/40 mL)= -0.00425 + 0.0222 × Gas (mL)

The difference in the *in vitro* gas production without and with PEG was considered as a biological effect of tannin (Makkar, 2000).

Data from chemical, mineral and lipid profile composition were analyzed statistically using GLM procedure of SAS (2003) as a completely randomized design. Means were separated by Tukey test when a significant ($P<0.05$) effect of treatment was observed. For tannin bioassay, data were analyzed as a completely randomized design with a 6 × 2 factorial arrangement of treatment. The model included fixed effects of date seed cultivar, PEG level (without and with) and interaction between date seed cultivar and PEG.

RESULTS AND DISCUSSION

Chemical composition

Chemical composition and phenolic content of date seeds are presented in Table 1. Among date seed cultivars, Estamaran had the highest content of ash (26.0 g/kg DM) and CP (69.0 g/kg DM).

The highest concentration of ADF and EE were observed in Barhi with the content of 577.7 and 118.0 g/kg DM, respectively. The highest amount of NDF was observed in Mazafati (782.3 g/kg DM). The content of lignin among cultivars ranged from 101.4 to 133.5 g/kg DM.

Of the cultivars studied, significant differences in TP and TT contents of date seeds were observed (Table 1). The concentration of TP and TT ranged from 41 (Barhi) to 110.2 g/kg DM (Kharak) and from 14.0 (Khasi) to 63.9 g/kg of DM (Kharak) as tannic acid equivalent, respectively.

Estamaran cultivar had the highest amount of K, Na, Mg, Ca and Fe, while the lowest amount of Na and Mg was observed in Kharak (Table 2). Khasi contained the highest content of P, but the lowest content of Fe. Zinc was highest in Khasi and lowest in Estamaran, Mazafati and Zahedi.

Fatty acid composition

Fatty acid composition of the six date seed cultivars is presented in Table 3. Among fatty acids presented, the most important fatty acids were oleic C18:1 (with the average concentration of 42.78 g/100 g FA) followed by lauric C12:0 (22.88 g/100 g FA), myristic C14:0 (11.82 g/100 g FA), palmitic C16:0 (10.30 g/100 g FA) and linoleic C18:2 (7.13 g/100 g FA). Oleic was the predominant fatty acid in date seeds oil ranging from 40.13 to 46.35 g/100 g FA. Major saturated fatty acids (SFA) were lauric, myristic and palmitic while linoleic and linolenic were the major polyunsaturated fatty acids (PUFA) ranged from 6.73 to 8.00 and 0.85 to 0.95 g/100 g FA. The average relative percentage of total unsaturated fatty acid of date seeds oil was 50.80%.

Fatty acid composition of date seed oils vary among cultivars. Barhi contained the highest amount of caprylic, capric, lauric and behenic acids and the lowest amount of palmitic acid. The highest content of SFA was detected in Barhi and Khasi cultivars; Kharak had the highest content of oleic acid (46.35 g/100 g FA) and consequently the highest content of total unsaturated fatty acids (54.22 g/100 g FA).

Tannin bioassay

Table 4 presents cumulative GV, percentage increase in GV, nutritive value of date seeds (OMD and ME) and concentration of SCFA after 24 h of incubation without and with inclusion of PEG. There was a significant effect ($P=0.001$) of date seeds on all *in vitro* parameters studied. In the absence of PEG, Mazafati and Estamaran showed the lowest and the highest GV, OM, ME and SCFA, respectively. Addition of PEG to the medium significantly increased GV and estimated parameters ($P=0.001$). The percentage increase in gas production varied from 33 to 180%.

Table 1 Chemical composition of date seeds from six cultivars (g/kg DM)

Item	Chemical composition								
	DM	Ash	CP	NDF	ADF	Lignin	EE	TP	TT
Barhi	941.9^{d}	10.0^{c}	62.7^{bc}	768.7^{b}	577.7^{a}	123.1^{a}	118.0^{a}	41.0^{d}	31.0^{c}
Estamaran	952.0^{a}	26.0^{a}	69.0^{a}	689.0^{f}	505.0^{f}	118.3^{ab}	102.0^{bc}	63.9^{c}	20.7^{d}
Kharak	941.7^{d}	15.3^{b}	66.7^{ab}	697.0^{e}	563.3^{b}	101.4^{b}	82.2^{d}	110.2^{a}	63.7^{a}
Khasi	937.4^{e}	11.3^{c}	50.0^{e}	728.3^{c}	550.2^{d}	127.1^{a}	99.3^{c}	43.8^{d}	14.0^{d}
Mazafati	945.7^{b}	10.7^{c}	54.7^{d}	782.3^{a}	530.3^{e}	133.5^{a}	105.0^{b}	58.9^{c}	36.2^{bc}
Zahedi	944.6^{c}	14.0^{b}	59.8^{c}	705.0^{d}	555.3^{c}	131.0^{a}	115.2^{a}	71.7^{b}	42.1^{b}
SEM	1.09	1.33	1.67	8.65	5.72	2.58	2.88	5.61	3.99

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
DM: dry matter (g/kg fresh weigh); CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; EE: ether extract; TP: total phenolics and TT: total tannins.
SEM: standard error of the means.

Table 2 Mineral composition of date seeds cultivars (mg/100 g DM)

Item	K	P	Na	Mg	Ca	Fe	Zn
Barhi	156.3^{d}	113.3^{c}	10.67^{b}	60.3^{b}	21.0^{c}	4.03^{c}	1.03^{bc}
Estamaran	255.3^{a}	293.3^{b}	14.04^{a}	90.0^{a}	93.3^{a}	6.00^{a}	1.00^{c}
Kharak	201.7^{b}	133.4^{c}	7.01^{d}	20.0^{e}	33.0^{b}	3.67^{c}	1.17^{b}
Khasi	170.0^{c}	403.4^{a}	12.02^{ab}	53.3^{c}	23.0^{c}	3.00^{d}	1.50^{a}
Mazafati	147.0^{e}	200.0^{bc}	8.07^{cd}	30.0^{d}	25.0^{c}	5.00^{b}	1.00^{c}
Zahedi	205.7^{b}	200.0^{bc}	8.33^{cd}	50.0^{c}	30.0^{b}	2.50^{d}	1.00^{c}
SEM	8.88	24.01	0.637	5.04	6.15	0.290	0.047

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Fatty acids profile of six different date seeds cultivars (g/100 g of total fatty acids)

Item	Barhi	Estamaran	Kharak	Khasi	Mazafati	Zahedi	SEM
Caprylic acid C8:0	0.73^{a}	0.42^{c}	0.39^{cd}	0.53^{b}	0.36^{d}	0.38^{d}	0.031
Capric C10:0	0.68^{a}	0.47^{c}	0.45^{cd}	0.58^{b}	0.40^{e}	0.42^{de}	0.024
Lauric C12:0	26.25^{a}	23.18^{c}	20.96^{d}	24.27^{b}	21.51^{d}	21.12^{d}	0.467
Myristic C14:0	11.58^{b}	12.49^{a}	10.75^{c}	11.59^{b}	12.23^{ab}	12.27^{ab}	0.152
Palmitic C16:0	9.14^{b}	10.17^{a}	10.14^{a}	10.76^{a}	10.62^{a}	10.64^{a}	0.164
Stearic C18:0	2.32^{c}	2.48^{bc}	2.56^{bc}	2.73^{b}	3.20^{a}	3.15^{a}	0.084
Oleic C18:1	41.02^{d}	42.27^{c}	46.35^{a}	40.13^{e}	43.31^{b}	43.59^{b}	0.487
Linoleic C18:2	6.73^{d}	7.19^{b}	6.98^{bc}	8.00^{a}	6.99^{bc}	6.90^{dc}	0.101
α-linolenic acid C18:3 n-3	0.48^{a}	0.46^{abc}	0.44^{abc}	0.43^{bc}	0.42^{c}	0.47^{ab}	0.006
γ-linolenic acid C18:3 n-6	0.37^{c}	0.39^{b}	0.45^{ab}	0.44^{ab}	0.48^{a}	0.48^{a}	0.011
Behenic acid C22:0	0.57^{a}	0.33^{c}	0.38^{b}	0.38^{b}	0.33^{c}	0.40^{b}	0.020
Lignoceric acid C24:0	0.14^{b}	0.15^{b}	0.16^{ab}	0.16a^{b}	0.15^{b}	0.18^{a}	0.004
SAFA	51.40^{a}	49.69^{ab}	45.78^{c}	51.00^{a}	48.80^{b}	48.56^{b}	0.516
MUFA	41.02^{d}	42.27^{c}	46.35^{a}	40.13^{e}	43.31^{b}	43.59^{b}	0.487
PUFA	7.58^{c}	8.04^{b}	7.87^{b}	8.87^{a}	7.89^{b}	7.85^{bc}	0.104

SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids and PUFA: polyunsaturated fatty acids.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The highest biological effect of tannin was observed in Kharak. The correlation coefficients between chemical composition and *in vitro* nutritional characteristics are shown in Table 5. Negative correlation was detected between ash, NDF and ADF contents (P<0.01), while ash was positively correlated with CP (P<0.01).

The percentage increase in GV was positively correlated with TP, TT (P<0.001) and negatively with EE content (P<0.01). Organic matter digestibility, ME and SCFA were negatively correlated with NDF and ADF contents (P<0.01) and positively correlated with ash (P<0.001) and CP (P<0.05). They were also negatively correlated with TT content (P<0.05).

Chemical composition

Neutral detergent fiber was the predominant component in all date seed cultivars, followed by EE, protein and ash. Similar to our results, Hamada *et al.* (2002) reported that NDF and ADF content in three date seeds cultivated in United Arab Emirates ranged from 645 to 688 and from 456 to 506 g/kg of DM, respectively. Values for proximate analysis were within the range of the results previously reported by Habib and Ibrahim (2009) and Ashraf and Hamidi-Esfahani (2011). They found that the ash, CP and fat content of date seeds ranged from 10 to 20, 50 to 70 and 70 to 100 g/kg DM, respectively. However, concentrations of CP (60.5 *vs.* 38.7 g/kg DM) and EE (103.6 *vs.* 53.4 g/kg

DM) of date seeds in this study were higher than those cultivated in Oman (Al-Farsi *et al*. 2007). The average content of chemical composition of date seeds in our study, are also comparable with date seeds of *P. dactylifera* and *P. canariensis* reported by Besbes *et al*. (2004) and Nehdi *et al*. (2010), respectively.

Scarce data is available on TP and TT concentration of date seeds. Ardekani *et al*. (2010) reported that TP content of methanolic extract of fourteen date seed cultivars grown in Iran ranged from 4.6 to 32.8 g/kg DM as gallic acid equivalent. The methanol extract of Algerian date seeds gave TP contents ranging from 2.7 to 3.9 g/kg of fresh weight as caffeic acid equivalent (Messaoudi *et al*. 2013).

Al-Farsi *et al*. (2007) found that TP content in three cultivars of date seeds cultivated in Oman ranged from 31.0 to 44.3 g/kg DM as gallic acid equivalent. Limited data is available regarding TT of date seeds. However, polyphenols are also one of the constituents of date, making up 30 g/kg DM of flesh (Hashempoor, 1999) which are considered as antioxidant agents (Hossain *et al*. 2014).

Due to different methods and different standards used in quantification of TP, it is difficult to compare the values of TP in present study with those obtained by others. However, different factors such as variety, growing condition, maturity stage, geographic origin, fertilizer, soil type, storage conditions and sun drying can elucidate these differences. Potassium and P levels ranged from 147.0 to 255.3 and 113.3 to 403.4 mg/100 g DM, respectively. The amount of P in our study was higher than those reported by Besbes *et al*. (2004) and Rahman *et al*. (2007) which can be due to higher fertilizer used or soil type. The highest concentration of K, Na, Ca, Mg and Fe were observed in Estamaran which resulted in higher ash content, while the highest content of P was detected in Khasi. Evaluation of 18 date seed varieties in United Arab Emirates showed that the concentration of K was the highest, following in descending order by P, Mg, Ca, Na, Fe and Zn (Habib and Ibrahim, 2009).

Minimum requirements of beef cattle for macro-minerals such as K, P, Na, Mg and Ca are 6.0, 1.4, 0.6, 1.0 and 1.7 g/kg DM, respectively and for micro-minerals such as Fe and Zn are 50 and 30 mg/kg DM, respectively (NRC, 2000). All of the date seed cultivars were deficient in macro-minerals (except P). Whilst an adequate amount of Fe was detected in Estamaran and Mazafati cultivars to meet the requirements of beef cattle, all date seed cultivars were deficient in Zn.

Fatty acid composition

The most important fatty acids in date seed oil were oleic, lauric, myristic, palmitic and linoleic acids which composed about 95% of total fatty acids. This result is in agreement with previous studies (Ataye Salehi *et al*. 2010).

Similar to our results, Besbes *et al*. (2004) reported that oleic acid was most abundant fatty acid followed by lauric acid, thus date seed oil may be regarded as oleic-lauric oil. However, date seed oils in *Phoenix canariensis* were oleic-linoleic (Nehdi *et al*. 2010).

The differences in FA composition of seed oils can be ascribed not only to the different cultivars but also to the different regions and different growing conditions (Abramovic and Abram, 2005).

The relative percentage of PUFA in date seeds was much lower than in seed oils such as sunflower or linseed oil. Biohydrogenation of USFA by ruminal microorganisms resulted in formation of stearic acid as an end product. Stearic acid may be converted to oleic acid by tissue Δ^9-desaturase (Bauman *et al*. 2000).

This production of oleic acid along with those escape ruminal biohydrogenation can favorably alter low density lipoprotein cholesterol, triglycerides and factor VII coagulant activity and also has a fundamental role in prevention of cardiovascular diseases (Allman-Farinelli *et al*. 2005).

Moreover, lauric and myristic acids have inhibitory effect on prostatic hyperplasia development (Babu *et al*. 2010). In general, date seed oil may have beneficial effects on animal and human health and is characterized by the presence of five major fatty acids; i.e. oleic, lauric, myristic, palmitic and linoleic.

Tannin bioassay

The difference of GV in the presence and absence of PEG is a measure of tannin effect (Makkar, 2000). The higher the percentage increase in GV, the higher the adverse effect of tannins. The highest biological effect of tannin was observed in Kharak which can be due to its high TT content (63.7 g/kg DM).

It has been demonstrated that tannins can reduce methane production during anaerobic fermentation both *in vivo* and *in vitro*.

Thus, a decrease in GV by increasing TT concentration may be partly due to lower methane production. The increase in gas production and digestion variables obtained on incubation with PEG suggested binding of PEG with tannins, thereby inactivating them (Makkar, 2003) and increasing rumen microbial activity (Makkar, 2005).

Although, the amount of TT in Khasi cultivar was lower than Barhi and Estamaran cultivars, higher percentage increase in gas production may reveal that besides tannin concentration, tannin nature can affect ruminal micro organisms activity (Makkar, 2003).

To our knowledge, no data has been published on biological effect of date seed tannins. However, these results are consistent with those on feeds containing tannin (Getachew *et al*. 2002).

Table 4 *In vitro* gas production (ml/500 mg DM), organic matter digestibility (OMD, g/kg DM) and metabolizable energy content (ME, MJ/kg DM) at 24 h of incubation without (–) and with (+) polyethylene glycol of date seeds

Item	GV		Increase (%)	OMD		ME		SCFA	
	–PEG[1]	+PEG		–PEG	–PEG	–PEG	–PEG	–PEG	–PEG
Barhi	41.7	60.3	46.5	331.6	398.0	3.99	5.16	0.92	1.34
Estamaran	76.1	101.4	33.9	467.2	557.5	6.58	8.16	1.68	2.25
Kharak	32.9	92.0	176.4	305.6	516.0	3.61	7.29	0.73	2.04
Khasi	43.8	68.4	55.5	334.4	421.9	4.07	5.61	0.97	1.51
Mazafati	27.1	49.4	87.8	276.8	355.9	3.05	4.44	0.60	1.09
Zahedi	41.1	68.7	75.2	332.0	428.9	4.04	5.76	0.91	1.52
SEM	4.03			0.0149		0.269		0.089	
Significance of effects									
Date seed	0.001			0.001		0.001		0.001	
PEG	0.001			0.001		0.001		0.001	
Date seed × PEG	0.02			0.02		0.02		0.02	

GV: cumulative gas volume at 24 h of incubation; OMD: organic matter digestibility; ME: metabolizable energy; SCFA: short chain fatty acids and PEG: polyethylene glycol.
SEM: standard error of the means.

Table 5 Correlation coefficients (r) of chemical composition, *in vitro* gas production and nutritive value of date seeds

Item	CP	NDF	ADF	EE	TP	TT	GV increase	OMD	ME	SCFA
Ash	0.68**	−0.73**	−0.73**	−0.25	0.28	−0.12	−0.14	0.82***	0.84***	0.79***
CP	-	−0.51*	-0.12	−0.18	0.48*	0.37	0.13	0.48*	0.49*	0.43*
NDF	-	-	0.25	0.40	−0.57**	−0.18	−0.19	−0.55**	−0.56**	−0.52**
ADF	-	-	-	0.13	0.04	0.39	0.31	−0.58**	−0.60**	−0.58**
EE	-	-	-	-	−0.70**	−0.40	−0.65**	0.04	0.03	0.07
TP	-	-	-	-	-	0.85***	0.80***	−0.11	−0.09	−0.16
TT	-	-	-	-	-	-	0.82***	−0.45*	−0.44*	−0.50*

CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; EE: ether extract; TP: total phenolics; TT: total tannins; GV increase (%): percentage of gas volume increase after 24 h of incubation with polyethylene glycol (PEG); OMD: organic matter digestibility; ME: metabolizable energy and SCFA: short chain fatty acids.
* (P<0.05); ** (P<0.01) and *** (P<0.001).

The estimated ME and OMD of date seeds in the absence of PEG was similar to the result of Dayani *et al.* (2012), who found that ME of 4 MJ/kg DM and *in vitro* DM and OM digestibility of 233.9 and 269.8 g/kg DM, respectively.

Negative correlation between ash, NDF and ADF and strong negative relationship were observed between ash, OMD and ME on halophyte browse species (Kumara Mahipala *et al.* 2009).

Kamalak *et al.* (2004) detected a negative relationship between ADF content and GV after 24 h of incubation of some shrub and tree leaves. Kumara Mahipala *et al.* (2009) found a positive correlation between NDF and ADF contents and GV in halophyte browse species, while a negative correlation between ADF content and GV, OMD and ME was detected in leguminous browse species. As expected, a significant positive correlation was observed between percentage increase in gas production and TP and TT concentrations which demonstrated the negative effects of date seeds phenolic compounds specially tannins on ruminal fermentation.

Despite of strong correlation between TP and TT, no relationship was found between TP and nutritive values (OMD and ME) which may suggest that other models such as graphical model (Steffensen *et al.* 2011) can be used for correlations analysis.

CONCLUSION

Results of this study showed that the major constituent of date seeds was NDF, followed by EE, CP, TP and ash. All cultivars of date seed oils also contain high relative percentage of oleic acid. With the exception of Estamaran, these date seeds were poor in digestibility and ME content. Total phenolic compounds and tannins in date seeds significantly decreased GV, OMD, ME and SCFA and may be an inhibitor of methane production. However, regarding high amount of carbohydrates and fat, date seeds could be utilized in ruminant ration in arid and semi arid zones to meet part of their nutritional requirements. However, due to presence of secondary compounds especially tannins, further investigations are needed to evaluate the appropriate levels of date seeds in the diets of ruminant. Moreover, since date seeds are rich in NDF and protein content is relatively low, treatments with urea or ammonia can be of value in this aspect.

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