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Comparing nutritive value of ensiled and dried pomegranate peels for ruminants using *in vitro* gas production technique

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ABSTRACT

This study was conducted to determine the chemical composition and estimation of nutritive value of ensiled and dried pomegranate peel using in vitro gas production technique. The experimental samples were incubated in vitro with rumen liquor taken from three fistulated Iranian native (Taleshi) steers at 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h. The results showed that although there is significant differences between gas production volume of dried and ensiled samples at early incubation times (2, 4, 6 and 8 h), the significant differences was not observed at further incubation times. The gas volume at 24 h incubation, were 45.71 and 45.17 ml/200mg DM for dried and ensiled pomegranate peels, respectively. The organic matter digestibility (OMD), metabolizable energy (ME), net energy for lactation (NEL) and short chain fatty acid (SCFA) of both treatments were similar (57.29%, 8.61 MJ/kg DM, 4.67 MJ/kg DM, 1.03 mmol for dried samples and 57.18%, 8.58 MJ/kg DM, 4.83 MJ/kg DM, 1.02 mmol for ensiled samples, respectively). In conclusion, it can be suggest that both preservation methods (drying and ensiling) have similar effect on nutritive value of pomegranate peel for ruminants.

Key words: nutritive value, gas production, ensiling, pomegranate peel, ruminants.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is an important fruit crop of tropical and subtropical regions of the world. Pomegranate fruit is consist of three parts: the seeds (about 3% of the weight of the fruit); the juice (about 30% of the fruit weight); and the peels which include the husk and interior network membranes [19].This fruit is either consumed fresh or used in the juice industries. Increasing agro-industrial units for producing pomegranate juice leads to the accumulation of a new by-product, namely, pomegranate peel [21]. Usually huge amounts of this by-product produced in pomegranate producing regions and countries. Annual production of this by-product exceeds 120,000 metric tons in Iran [15]. If it can not used by farmers and industries as well as medical activities cause serious environmental problems.

Health benefits of pomegranate peel (antioxidant, antimicrobial, anti inflammatory, anticancer and other biological activities), recently reviewed by Prakash and Prakash [19]. Presence of chemical compounds such as Hydroxybenzoic acids (Gallic acid, Ellagic acid), Hydroxycinnamic acids (Caffeic acid, Chlorogenic acid, *p*-Coumaric acid), Cyclitol carboxylic acids (Qunic acid), Flavon-3-ols/Flavonoids and their glycosides (Catechin, Epicatechin, Epigallocatechin-3-gallate, Quercetin, Kaempferol, Luteolin, Rutin, Kaempferol-3-O-glycoside, Kaempferol-3-O-rhamnoglycoside, Naringin), Anthocyanins (Cyanidin, Pelarginidin, Delphinidin), Ellagitannins (Punicallin, Punicalagin, Corilagin, Casuarinin, Gallagyldilacton, Pedunculagin, Tellimagrandin, Granatin A, Granatin B) and Alkaloids (Pelleteriene); [19], in particulate tannins [7] can be the main reasons of its bioactive functions.

In spite of sufficient knowledge on biological effects of pomegranate peel in human and in some case in animal health; there is a little information on its nutritive value for ruminant animals [4, 15, 17, 21]. Shabtay et al., [21] reported that using pomegranate peel up to 20% in feedlot calves diet, not only does not possess adverse effects on fattening performance but also because of its palatability, feed intake and consequently average daily gain were increased. They are suggested that tannins are considered to have both adverse and beneficial effects in ruminant animals. High concentrations of tannins may reduce feed intake, digestibility of protein and carbohydrates and animal performance via their negative effects on palatability and digestion. Low and moderate (2-4.5%) concentrations of condensed tannins in the diet improved production efficiency in ruminants, by increasing the flow of non-ammonia nitrogen and essential amino acids from the rumen. Mirzaei-Aghsaghali et al. [15] concluded that pomegranate pulp may be a potentially fair to good food-industrial by-product for ruminant nutrition.

Pomegranate pulp can be fed in fresh, dried and/or ensiled forms to ruminants. Drying and ensiling are common preservation methods of wet feeds for further using on farm conditions. Each of these methods has itself advantages and disadvantages. Nutritionally, it is important that which method is the better for preservation of pomegranate peel (which is rich in tannins). In the season that pomegranate is harvested, consumed and processed sun drying of processing by-products is difficult and farmers prefer to ensile them.

In vitro gas production technique is a useful as well as cost and time effective tool for estimating nutritive value of feedstuffs particularly for tannin containing feeds and developing countries [9, 10, 11].

The aim of the this study was to comparing the nutritive value of dried and ensiled pomegranate peels including chemical composition, *in vitro* gas production characteristics, organic matter digestibility (OMD), metabolisable energy (ME), net energy for lactation (NEL) and short chain fatty acids (SCFA) using *in vitro* gas production technique.

MATERIALS AND METHODS

Samples collection and treatments

Fresh pomegranate peel samples (after taking juice) were collected from the traditional (local) pomegranate juice producers, in Tehran, Iran. After mixing, some of samples were air-dried (7 days in room conditions) and ground (1mm and 5mm screen) and remained samples were ensiled in experimental level in P.V.C tubes for 45 days. Chemical analysis and *in vitro* gas production evaluated at the laboratories of Animal Science Research Institute in Karaj, Iran.

Chemical Analysis

Dry matter (DM) was determined by drying the samples at 105 ^{o}C overnight and ash by igniting the samples in muffle furnace at 525 ^{o}C for 8h and nitrogen (N) content was measured by the Kjeldahl method [2] . Crude protein (CP) was calculated as N × 6.25. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by procedures outlined by Van Soest et al. [22]. Non-Fibrous Carbohydrate (NFC) is calculated using the equation of NRC [16]; NFC = 100 – (NDF + CP + EE + Ash).

In vitro gas production procedure

Fermentation of dried and ensiled pomegranate peel samples were carried out with rumen fluid obtained from three fistulated Iranian native steers (Taleshi) fed twice daily a diet containing alfalfa hay (60%) and concentrate mixture (40%) following the method described by Menke and Steingass [13]. Both solid and liquid rumen fractions were collected before the morning feeding, placed in an insulated plastic container, sealed immediately and transported to the laboratory. Approximately 200 mg (on dry matter basis) of each samples were weighed into the glass syringes of 100 ml. The fluid-buffer mixture (30 ml) was transferred into the glass syringes of 100 ml. The glass syringes containing samples and rumen fluid-buffer mixture were incubated at 39 ^{o}C. The syringes were gently shaken 30 min after the start of incubation. The gas production was determined at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h of incubation. All samples were incubated in triplicate with three syringes containing only rumen fluid-buffer mixture (blank). The net gas productions of samples were determined by subtracting the volume of gas produced in the blanks. Gas production data were fitted to the model of Ørskov and McDonald [18]:

$$Y = a + b\,(1\text{-}e^{-ct})$$

Where Y is the gas production at time t, a the gas production from soluble fraction (ml/200mg DM), b the gas production from insoluble but fermentable fraction (ml/200mg DM), c the gas production rate constant (ml/h), $a + b$ the potential gas production (ml/200mg DM) and t is the incubation time (h).

The ME, NEL and OMD of pomegranate peels were calculated using equations of Menke and Steingass [13] as:

ME (MJ /kg DM) = 2.20 + 0.136 × GP + 0.0057 × CP
NEL (MJ/kg DM) = 0.115 × GP + 0.0054 × CP + 0.014 × EE - 0.0054 × CA - 0.36
OMD (g/kg DM) = 14.88 + 0.889 × GP + 0.45 × CP + 0.0651 × CA

Where, GP is 24 h net gas production volume (ml/200 mg DM), and CP, EE, CA are crude protein, ether extract and crude ash (g/kg DM), respectively.

Short chain fatty acids (SCFA) are calculated by equation of Getachew et al. [5];

SCFA (mmol) = -0.0601 + (0.0239 × GP)

Where, GP is 24 h net gas production volume (ml/200mg DM).

Statistical analysis:
All of the data (with three replicates) were analyzed using software of SAS [20] and means of two sample groups were separated by independent-samples t-test [12].

RESULTS AND DISCUSSION

The chemical compositions of ensiled and dried pomegranate peels are presented in table 1. Although there is no more differences between CP, EE and ash content of ensiled and dried peels; NDF and ADF content of pomegranate peels were increased and NFC content decreased by drying. In other word, dried peels have higher fiber content than ensiled forms. Chemical compositions of dried peels were approximately in line with findings of Mirzaei-Aghsaghali et al., [15]. They are reported that DM, CP, EE, NDF, ADF and NFC content of pomegranate peels were 96.20, 3.60, 0.61, 20.80, 15.10 and 69.57%, respectively. There are some differences between chemical composition of pomegranate by-product in current study comparing those reported by Feizi et al., [4] and Shabtay et al., [21]. These variations in chemical composition of by-products may be due to different original materials, growing conditions (such as geographic, seasonal variations, climatic conditions and soil characteristics), and extent of foreign materials, impurities, varieties, different processing and measuring methods. It is clear that, any variation in chemical composition can be resulted in different nutritive value; because chemical composition is one of the most important indices of nutritive value of feedstuffs [1, 9, 10].

Table 1: Chemical composition of dried and ensiled pomegranate peels (%)

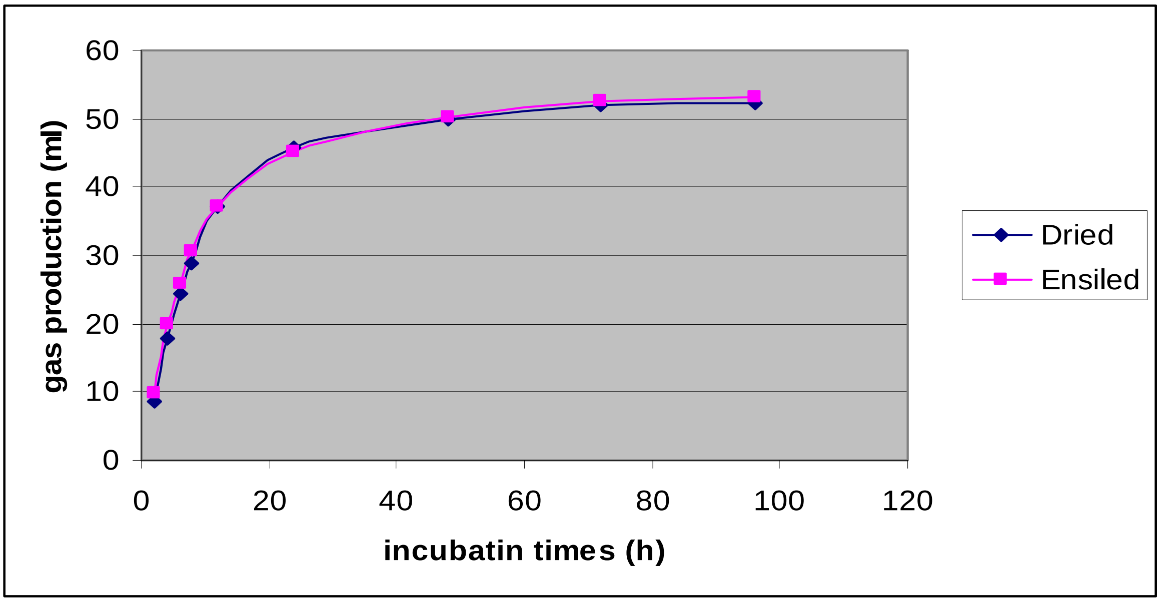
Items	DM	CP	EE	Ash	NDF	ADF	NFC
Dried	94.76	3.37	0.70	4.00	18.20	12.60	73.73
Ensiled	48.36	4.19	0.50	4.00	13.60	8.60	77.71

DM: dry matter, CP: crude protein, EE: ether extract, NDF: neutral detergent fiber, ADF, acid detergent fiber, NFC: non fibrous carbohydrate.

Amounts of gas produced (ml/200mg DM) in different incubation times illustrated in table 2 and figure 1. The results of *in vitro* gas production showed that although there is significant differences between gas production volume of dried and ensiled samples at early incubation times (2, 4, 6 and 8 h), the significant differences was not observed at further incubation times. The gas volume at 24 h incubation, were 45.71 and 45.17 ml/200mg DM for dried and ensiled pomegranate peels, respectively. Amount of gas production at 24 h incubation is important because of its high positive correlation by energetic value of feedstuffs [6, 9].

Table 2: Gas production volume (ml/200mg DM) of dried and ensiled pomegranate peels at different incubation times (h)

Incubation times (h)	Dried	Ensiled	*P* value	S.E.M
2	8.75	9.67	0.0167	0.1670
4	17.81	19.81	0.0004	0.1309
6	24.38	25.90	0.0180	0.2779
8	28.90	30.73	0.0227	0.3596
12	37.11	37.05	0.9176	0.4071
24	45.71	45.17	0.4052	0.4086
48	50.00	50.24	0.6099	0.2988
72	52.11	52.50	0.5662	0.4215
96	52.27	53.12	0.2501	0.4494



Gas production parameters (*a*, *b*, *c*) and estimated values of OMD, ME, NEL and SCFA of dried and ensiled pomegranate peels are presented in Table 3. Although gas production of soluble fraction (*a*) in ensiled samles was significantly higher than that of dried peels; there are no significant differences about insoluble but fermentable fraction (*b*), potential gas production (*a*+*b*) and rate constant of gas production (c) of treatments. Blummel and Becker [3] stated that the soluble fraction (*a*) of feed makes it easily attachable by rumen microorganisms and leads to much gas production. Thus higher soluble fraction in ensiled pomegranate peels in current study cause faster starting of fermentation than samples dried. In other hand, the gas volume at asymptote (b) is an important index for predicting feed intake. So it can be concluded that ruminants should consume same amount of ensiled and dried pomegranate peels. High rate of gas production possibly affected by carbohydrate fractions which readily available to the microbial population.

The organic matter digestibility (OMD), metabolizable energy (ME), net energy for lactation (NEL) and short chain fatty acid (SCFA) of both treatments, also were similar (57.29%, 8.61 MJ/kg DM, 4.67 MJ/kg DM, 1.03 mmol for dried samples and 57.18%, 8.58 MJ/kg DM, 4.83 MJ/kg DM, 1.02 mmol for ensiled samples, respectively). Mirzaei-Aghsaghali et al., [15] found that amounts of gas produced at 24 from *in vitro* incubation of dried pomegranate peels was 47.42 ml/200 mg and for *a*, *b*, *a*+*b* and *c* were 6.72, 47.39, 54.12 ml/200 mg and 0.078 ml/h, respectively. They are also reported that estimated amounts of OMD, ME, NEL and SCFA of dried pomegranate peels were 59.00%, 8.85 MJ/kg DM, 5.09 MJ/kg DM and 1.048 mmol, respectively.

Table 3: *In vitro* gas production parameters and estimated metabolisable energy (ME), net energy for lactation (NEL), Organic matter digestibility (OMD) and short chain fatty acids (SCFA) of dried and ensiled pomegranate peels

Items	Dried	Ensiled	*P* value	S.E.M
a (ml)	0.33	1.82	0.0096	0.2258
b (ml)	51.66	49.78	0.0559	0.4978
a+*b* (ml)	52.00	51.60	0.6231	0.3782
c (ml/h)	0.1050	0.1040	0.7747	0.0023
ME (MJ/ Kg DM)	8.61	8.58	0.7519	0.0555
NEL (MJ/ Kg DM)	4.67	4.83	0.5778	0.0412
OMD (%)	57.29	57.18	0.8372	0.3634
SCFA (mmol)	1.03	1.02	0.4014	0.0001

a: the gas production from soluble fraction (ml/200mg DM), b: the gas production from insoluble but fermentable fraction (ml/200mg DM), c: rate constant of gas production during incubation (ml/h), a + b: the potential gas production (ml/200mg DM)

It is predictable that variation in chemical components of same feeds in different studies such as starch, NFC, OM, CP, NDF and soluble sugars contents can be result in variation of in vitro gas production volume [9]. In ruminant animals, short chain fatty acids (SCFAs) such as acetic, propionic, butyric, isobutyric, valeric and isovaleric which are produced in rumen by microbial fermentation of dietary nutrients (e.g. fiber), supply up to 80% of their maintenance energy requirements. Acetate, propionate and butyrate, the dominant SCFAs, are readily absorbed and assimilated as a nutrient source by the ruminant. The SCFA account for between 50-70% of digestible energy intake. Thus it can be said that, higher SCFA production in gas production technique is the reliable index of gas production and energy content of tested materials [15].

CONCLUSION

The results of current study based on chemical composition, OMD, ME, NEL and SCFA indicated that both preservation methods (drying and ensiling) have similar effect on nutritive value of pomegranate peel for ruminants. It can be said that pomegranate peel has a potentially relative nutritive value in ruminants under *in vitro* conditions. However there is need to *in vivo* studies for confirming this result.

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REFERENCES

[1] Aghajanzadeh-Golshani, A., N. Maheri-Sis, A. Mirzaei-Aghsaghali and A. R. Baradaran-Hasanzadeh. *Asian J. Anim. Vet. Adv,* **2010**, 5, 43-51.
[2] AOAC, Official Method of Analysis. 15th edn. Washington DC. USA, Association of Official Analytical Chemists, **1990,** pp. 66-88.
[3] Blummel, M., Becker, K., *Br. J. Nutr*. **1997**, 77, 757-768.
[4] Feizi, R., Ghodrat Nama, A., Zahedifar, M., Danesh Mesgaran, M., Raisianzade, M., *Proceeding of British Society of Animal Science*. **2005,** pp. 223.
[5] Getachew G, Makkar H P S and Becker K, EAAP Satellite Symposium, 18-19 August, **1999,** Wageningen, the Netherlands.
[6] Getachew, G., Robinson, P.H, DePeters, E.J., Taylor, S.J., *Anim. Feed Sci. Technol.* **2004**, 111, 57-71.
[7] Hassanpour S, Maheri-Sis N, Eshratkhah B, Mehmandar F B. *Int. J. Forest, Soil and Erosion,* **2011**, 1, 47-53.
[8] Khoshnam F., A. Tabatabaeefar, M. Ghasemi Varnamkhasti, A.M., Borghei, *Sci. Hortic.* **2007**, 114, 21– 26.
[9] Maheri-Sis, N., Chamani, M., Sadeghi, A.A., Mirza- Aghazadeh, A., Aghajanzadeh-Golshani, A., *Afr. J. Biotechnol*. **2008**, 7, 2946-2951.
[10] Maheri-Sis, N., Chamani, M., Sadeghi, A.A., Mirza-Aghazadeh, A., Safaei, A.A., *J. Anim. Vet. Adv*. **2007,** 6, 1453-1457.
[11] Makkar, H.P.S., *Anim. Feed Sci. Technol*. **2005,** 123, 291-302.
[12] McDonald, J. H. Handbook of Biological Statistics. Sparky House Publishing, Baltimore. **2008.**
[13] Menke, K.H., Steingass, H., *Anim. Res. Dev*. **1988,** 28, 7-55.
[14] Mirzaei-Aghsaghali, A., Maheri-Sis, N., *World J. Zool.* **2008**, 3, 40-46.
[15] Mirzaei-Aghsaghali. A., N. Maheri-Sis, H. Mansouri, M. E. Razeghi, A. Mirza-Aghazadeh, H. Cheraghi and A. Aghajanzadeh-Golshani, *ARPN J. Agr. Biol. Sci,* **2011**, 6, 45-51.
[16] NRC, Nutrient requirements of dairy cattle. (7th Ed.) National Research Council, **2001**, National Academy Press, Washington, DC.
[17] Oliveira, R.A., Narciso, C.D., Bisinotto, R.S., Perdomo, M.C., Ballou, M.A., Dreher, M., *J. Dairy Sci.* **2010**, 93, 4280–4291.
[18] Ørskov, E.R., McDonald, I, *J. Agric. Sci. (Camb.).* **1979**, 92, 499-503.
[19] Prakash, C. V. S. and I. Prakash, *Int. J. Res. Chem. Environ,* **2011**, 1, 1-8.
[20] S.A.S. Institute, SAS for windows, Version 6.0, Ed. **1991**, SAS Institute Inc., Cary, NC.
[21] Shabtay, A., Eitam, H., Tadmor, Y., Orlov, A., Meir, A., Weinberg, P., Weinberg, Z.G., Chen, Y., Brosh, A., Izhaki, I., Kerem, Z., *J. Agric. Food Chem*. **2008,** 56, 10063–10070.
[22] Van Soest, P.J., Robertson, J.B., Lewis, B.A., *J. Dairy Sci*. **1991,** 74, 3583-3597.