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In vitro Gas Production and Dry Matter Digestibility of Irradiated Pomegranate (*Punica granatum*) Seeds

Research Article

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ABSTRACT

The objective of this study was to determine the effect of irradiation of pomegranate seed (PS) on chemical composition, digestibility and kinetic of gas production. Pomegranate seeds were exposed to gamma ray (GR) and electron beam (EB) at doses of 5, 10, 15 and 20 kGy. Three ruminally fistulated rams were used for obtaining ruminal fluid for *in vitro* digestibility and gas production measurements. Irradiation had no effect on chemical composition of PS. Orthogonal contrast did not show any significant effect for EB irradiation on neutral detergent fiber % (NDF%), but GR irradiation of PS at low doses (i.e.; 5 and 15 kGy) increased NDF percentage (P<0.05). Irradiation decreased condensed tannin (CT) content of PS at all doses (P<0.01). Gas production potential (*b*) and gas production rate (*c*) of PS were decreased; but EB irradiation at doses of 5 and 20 kGy did not effect on gas production potential of PS. Irradiation treatment did not affect partitioning factor. Ionizing radiation decreased PS digestibility, but EB irradiation at a dose of 20 kGy and 5, 15 and 20 kGy did not change *in vitro* dry matter digestibility and organic matter digestibility. In conclusion, the result of this study suggests that ionizing radiation processing, especially EB irradiation, can be regarded as an efficient method in decreasing CT of pomegranate seeds.

KEY WORDS condensed tannin, digestibility, irradiation, pomegranate seed.

INTRODUCTION

Pomegranate (*Punica granatum*) is an important fruit crop in tropical and subtropical regions of the world as well as in Mediterranean countries with moderate temperatures (Taher-Maddah *et al.* 2012). The fruits are globally consumed fresh, in processed forms as juice, jam and oil and in extract supplements (Prakash and Prakash, 2011). This has led to development of advanced industrial technologies which provide consumers with “ready to eat” pomegranate grains and fresh fruit juices. These products have led to production of high quantities of pomegranate byproduct biomass. Pomegranate seeds constitute about 3% of the weight of the fresh fruit. Feizi *et al.* (2005) demonstrated that pomegranate seeds can be used in animal nutrition. Although protein and oil content of pomegranate seed are considerable (Mirzaei-Aghsaghali *et al.* 2011), it has a high amount of cell wall content and is rich in antinutritional factors, particularly tannins (Parakash and Parakash, 2011). Removal of these undesirable components is essential to improve the nutritional quality of pomegranate seeds as animal feed. There is scant information on the nutritive value of pomegranate seeds for ruminants (Feizi *et al.* 2005; Shabtay *et al.* 2008; Modarresi *et al.* 2010; Mirzaei-Aghsaghali *et al.* 2011). The previous methods introduced by researchers (Chen *et al.* 1995; Duodu *et al.* 1999; Parker *et al.* 1999) to deactivate the antinutrients compounds in feeds did not necessarily completely eliminate or reduce the amounts of them; instead in some cases the methods gave the adverse results and led to the reduced nutritive value of the feeds.

There is no information on the effect of gamma ray (GR) and electron beam (EB) irradiation on the nutritive value of pomegranate seeds. Therefore, the major aim of the present study was to evaluate the impacts of gamma and electron irradiation on the nutritional and antinutritional components, *in vitro* digestibility and rumen fermentation characteristics of pomegranate seeds.

MATERIALS AND METHODS

Samples preparation and irradiation treatments

Pomegranate seeds (PS) were obtained from the Neyriz Green Farm pomegranate factory, in Fars, Iran, during the pomegranate harvest season and were air dried before it was used in this study.

The experimental treatments were: pomegranate seeds treated by gamma ray (GR) and electron beam (EB) irradiation at doses of 5, 10, 15 and 20 kGy and the control group without irradiation. Irradiations of samples were done in Radiation Applications Research School, Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran.

Gamma-irradiation was completed by using a cobalt-60 irradiator at 20 ˚C. The dose rate determined by Fricke dosimetry (Holm and Berry, 1970) was 0.36 Gy/s. Three-paper packages of samples were irradiated to total doses of 15, 30 and 45 kGy in the presence of air. After irradiation and prior to sealing the plastic bags, samples were allowed to air equilibrate for 2 h. The pomegranate seed samples were irradiated under various doses of 5, 10, 15 and 20 kGy. Three poly-ethylene packages of samples were exposed to 10 MeV electron beam of a Rhodotron accelerator model TT-200 (IBA Co., Belgium) at various doses (5, 10, 15 and 20 kGy) in Radiation Applications Research School of Atomic Energy Organization of Iran. All irradiations were performed at room temperature in air, with 4 mA beam of 10 MeV electrons. Regarding the low thickness of the samples packages, single sided irradiation has been used. The required doses were delivered to the samples by adjusting the conveyer speed when each of the sample batches passed under the beam.

Chemical analysis

The dry matter (DM) of pomegranate seed was determined by drying at 60 ˚C for 48 h. After drying, the samples were ground through a 1 mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, USA), and DM, crud protein (CP) (948.13), ether extract (EE) (954.02) and ash (924.05) were analyzed according to AOAC (1995).

Neutral detergent fiber (NDF), acid detergent fiber (ADF) were analyzed according to the method of Van Soest *et al.* (1991). Condensed tannins were analyzed using the vanillin-HCl procedure according to Galyean (1997) and results are expressed as catechin equivalents (mg of catechin equivalents/g of dry sample).

In vitro study

In vitro digestibility

Two-step digestion technique (Tilly and Terry, 1963) was used to determine *in vitro* digestibility of un-irradiated and irradiated PS. Allocation of treatments into *in vitro* experimental units was done as a completely randomized design. Samples (1 g) were weighed and placed into tubes. Subsequently, 12 mL of McDougall's buffer and 8 mL of rumen fluid were added. The tube was closed with a rubber cap and incubated anaerobically for 24 h in an automatic shaker water bath, maintained at 39 ˚C during the process. After 24 h, the cap was opened, 0.2 mL of $HgCl_2$ was added, centrifuged at 10000 rpm for 10 min and the supernatant was removed. The residue was combined with 20 mL of 0.2% pepsin under acidic condition, and further incubated for 24 h.

The remaining sample after the two-stage *in vitro* incubation procedure was filtered with a Whatman paper no. 41 for determination of *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD).

Values of IVDMD and IVOMD were presented as percentage (%) of digested substance from their initial amounts prior to incubation. Blanks (rumen fluid and buffer only without sample substrate) were incubated as described above and served as a correction factor to the DM and organic matter (OM) contents of residuals. The incubation was done in three replicates according to the treatments (n=3) and each replicate was represented by two tubes.

In vitro gas production

The method used for gas production measurements was as described by Theodorou *et al.* (1994). Three ruminally fistulated Sanjabi rams, as rumen fluid donors, were fed at 8:15 and 17:15 h daily with a diet of lucerne hay and whole barley (70:30, DM basis) at the maintenance requirements. Rumen fluid was collected at the morning before feeding and strained into a pre-warmed thermos flask. Pomegranate seeds were ground to pass a 0.2 mm screen. Approximately 125 mg of each substrate was weighed into 25 mL serum bottles and incubated in a water bath at 39 ˚C with 5 mL strained rumen fluid and 10 mL of medium (McDougall, 1948) in order to determine rate and extent of gas production by reading gas production at 2, 4, 6, 8, 10, 12, 24, 48, 72 and 96 h post-inoculation.

Each sample was incubated in three replicates. Cumulative gas production data were fitted to the model of Orskov and McDonald (1979) as follows:

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

Where:
b: potential extent of gas production.
c: gas production rate constant.
t: incubation time.
y: gas produced at time "t".

In a separate run of gas production, the method of Blummel *et al.* (1997) was adopted to determine the partitioning factor.

Statistical analyses

Experimental data was analyzed using general linear model (GLM) and significant differences among means from a triplicate analysis at ($P<0.05$) were determined by LSD test using the SAS (2002) software. Orthogonal contrasts were used to detect significant differences among the treatment means.

RESULTS AND DISCUSSION

Effects of irradiation on chemical composition

Orthogonal contrast of chemical composition of PS before and after irradiation are shown in Table 1. Irradiation had no effects on DM, EE, CP, OM, ADF and nonfiber carbohydrates (NFC) percentages of pomegranate seeds ($P>0.05$). Orthogonal contrast indicated that the GR irradiation at low doses (i.e.; 5 and 15 kGy) significantly increased the NDF content (as %) of pomegranate seeds compared to the control group. The effect of EB irradiation on the NDF content of pomegranate seeds was no significant. Condensed tannin content of PS was significantly reduced by irradiation compared to the control. Significant differences were observed between GR and EB irradiation effects on condensed tannin reduction ($P<0.05$).

Chemical composition of untreated and irradiated pomegranate seed are shown in Table 2. Gamma ray and EB irradiation at a dose of 10 kGy significantly increased NDF content of PS but the effect of electron beam irradiation was not significant. Irradiations significantly decreased condensed tannin. Reduction in condensed tannin in response to irradiations was dose dependent (Table 2).

In vitro study

Gas production, kinetic analysis of gas production

Cumulative gas production of irradiation treatments and the estimated parameters of gas production are presented in Figure 1 and in Tables 3 and 4, respectively. In total, irradiation decreased the gas production parameters.

As can be seen from Table 4, rate constant of gas production (*c*) was decreased but gas production potential of PS did not significantly affected by EB irradiation at doses of 5 and 20 kGy.

In vitro digestibility

In vitro digestibilities of dry matter and organic matter of untreated and irradiated PS are presented in Tables 3 and 4. *In vitro* dry matter digestibility and *in vitro* organic matter digestibility of pomegranate seeds decreased by GR and EB irradiation with the exception of 5 and 20 kGy EB irradiation.

In spite of non significant effect of irradiations on the partitioning factor, there was a difference in GV_{24} between untreated and irradiated PS (Table 6). Mean average of partitioning factor was 5.62 mg DM truly degraded/mL gas produced in 24 h. Partitioning factor ranged from 4.61 to 6.90. Orthogonal contrast showed that irradiation affected metabolizable energy, but there was no difference between gamma and electron radiation (Table 5).

The correlation coefficients of chemical composition and experimental parameters of gamma and electron irradiated PS are presented in Tables 7 and 8.

Effects on chemical composition

DM, EE, CP, OM, ADF and NFC content of irradiated pomegranate seeds was not significantly different from the control, which are in agreement with previous works (Shawrang *et al.* 2011; Bhat and Sridhar, 2008; Ebrahimi *et al.* 2009; Taghinejad *et al.* 2009; Farag, 1998). Although gamma irradiation was effective and led to a significant increase in NDF content of PS compared to the control (Table 2), the effect for electron beam irradiation was not significant. In common, irradiation treatment showed to be effective on the structure of cellulosic raw materials (Dela Rosa *et al.* 1983). However, there are some discrepancies regarding the effects of irradiation on fiber content of feeds. Electron beam irradiation in high doses (50, 100 and 150 kGy) decreased NDF and ADF content of soybean and cotton seed meal in Tahan *et al.* (2012) study, but Ebrahimi-Mahmoudabad and Taghinejad-Roudbaneh (2011) did not indicate any significant effect of EB irradiation (at doses of 15, 30 and 45 kGy) on NDF content of whole cottonseed, soybean and canola seeds. Ebrahimi *et al.* (2009) also did not find any significant effect for GR irradiation on chemical composition and fiber content of feeds. In our study EB irradiation at a dose of 10 kGy significantly increased NDF content of PS. Similarly, Khosravi *et al.* (2012) reported that EB irradiation significantly increased ADF content of pomegranate seeds without any significant effect on NDF.

Table 1 Orthogonal contrast of pomegranate seeds before and after irradiation (means square)

Treatments	df	OM	CP	EE	NDF	ADF	NFC	CT
Irradiation *vs.* control	1	0.001	0.14	0.04	101.28	35.14	148.08	10.44**
GR *vs.* control	1	0.001	0.09	0.01	200.82*	55.47	234.35	8.88**
5 and 10 GR *vs.* control	1	0.04	0.01	0.003	166.20	21.38	177.84	3.66**
15 and 20 GR *vs.* control	1	0.01	0.18	0.01	158.99	74.29	235.96	12.44**
5 and 10 *vs.* 15 and 20 GR	1	0.17	0.13	0.01	0.63	26.34	15.40	3.90**
EB *vs.* control	1	0.01	0.15	0.08	22.43	13.39	65.99	9.93**
5 and 10 EB *vs.* control	1	0.01	0.09	0.05	16.37	26.93	44.39	4.07**
15 and 20 EB *vs.* control	1	0.002	0.15	0.09	18.93	1.77	66.73	13.95**
5 and 10 *vs.* 15 and 20 EB	1	0.01	0.01	0.01	0.10	19.92	3.40	4.42**
GR *vs.* EB	1	0.04	0.01	0.10	165.93	22.91	134.02	0.07*
5 and 10 GR *vs.* 5 and 10 EB	1	0.17	0.04	0.05	87.14	1.87	60.75	0.01
15 and 20 GR *vs.* 15 and 20 EB	1	0.01	0.00	0.04	79.56	66.22	73.28	0.06

GR: gamma ray; EB: electron beam; df: degree of freedom; OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; NFC: non fibrous carbohydrates and CT: condense tannin (mg of CE/g of dry sample).
* (P<0.05) and ** (P<0.01).

Table 2 Chemical compositions of irradiated pomegranate seeds (as g/100 g dry matter)

Treatments	OM	CP	EE	NDF	ADF	NFC	CT
Control	97.61	11.12	10.92	52.72^{bc}	39.18	28.29^{ab}	4.10^{a}
Gamma							
5 kGy	97.76	11.19	10.97	59.48^{ab}	45.50	17.24^{ab}	3.03^{b}
10 kGy	97.74	10.81	10.96	68.30^{a}	40.87	9.53^{ab}	1.85^{c}
15 kGy	97.42	10.66	11.05	65.95^{ab}	46.56	9.96^{ab}	1.41^{de}
20 kGy	97.61	10.83	11.03	63.08^{ab}	47.93	10.60^{ab}	0.69^{f}
Electron							
5 kGy	97.44	11.01	11.06	42.71^{c}	40.41	34.78^{a}	3.12^{b}
10 kGy	97.58	10.67	11.16	70.30^{a}	48.33	5.48^{b}	1.59^{d}
15 kGy	97.55	10.53	11.17	57.28^{abc}	42.13	18.77^{ab}	1.21^{e}
20 kGy	97.60	10.97	11.19	56.30^{abc}	38.26	17.80^{ab}	0.52^{f}
LSD	0.79	1.02	1.13	15.36	12.67	27.32	0.26
P-value	0.9892	0.7475	0.9997	0.0438	0.3887	0.2660	0.0001
SEM	0.07	0.08	0.09	2.40	1.20	2.67	0.27

GR: gamma ray; EB: electron beam; df: degree of freedom; OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; NFC: non fibrous carbohydrates and CT: condense tannin (mg of CE/g of dry sample).
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.
LSD: least significant difference.

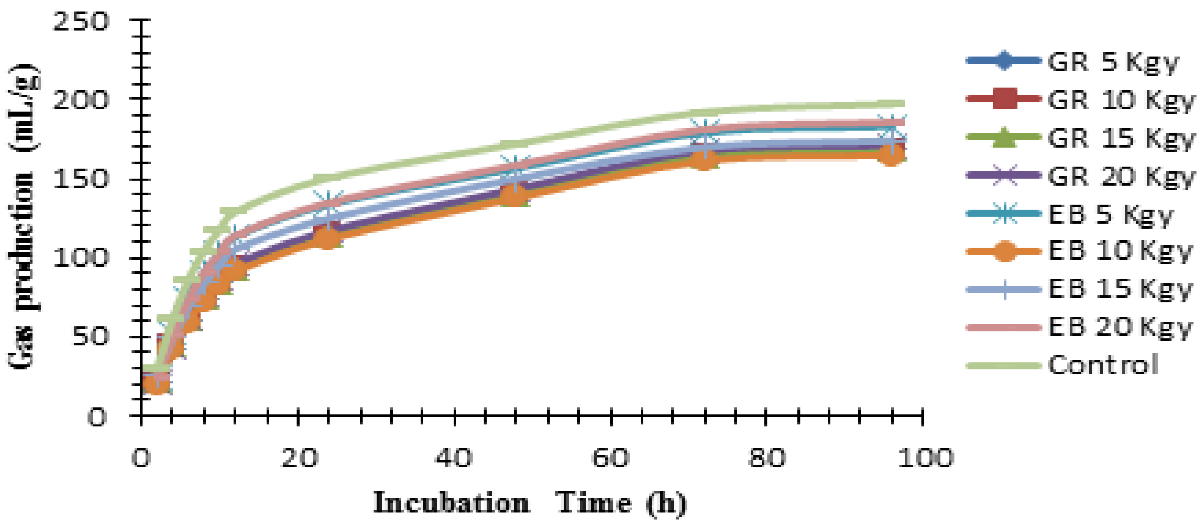

Figure 1 Cumulative gas production curve of gamma and electron beam irradiated pomegranate seed

Table 3 Orthogonal contrast of *in vitro* gas production parameters and digestibility of GR and EB irradiated pomegranate seeds (means square)

Treatments	df	Parameters		DMD (%)	OMD (%)
		b	*c*		
Irradiation *vs*. control	1	1106.90**	0.0016**	107.25**	117.58**
GR *vs*. control	1	1275.03**	0.0022**	170.45**	165.79**
5 and 10 GR *vs*. control	1	1114.07**	0.0018**	171.49**	153.36**
15 and 20 GR *vs*. control	1	1012.20**	0.0020**	115.36**	111.64**
5 and 10 *vs*. 15 and 20 GR	1	3.66ns	0.00ns	8.31ns	6.17ns
EB *vs*. control	1	751.75**	0.0008**	43.48**	55.31*
5 and 10 EB *vs*. control	1	812.17**	0.0008**	114.71**	107.06**
15 and 20 EB *vs*. control	1	464.82**	0.0005**	1.76ns	10.44ns
5 and 10 *vs*. 15 and 20 EB	1	72.22ns	0.00 *	132.0**	75.95**
GR *vs*. EB	1	171.78ns	0.0009**	104.37**	73.60**
5 and 10 GR *vs*. 5 and 10 EB	1	35.70ns	0.0003**	8.53ns	11.0ns
15 and 20 GR *vs*. 15 and 20 EB	1	157.76ns	0.0006**	132.86**	80.42**

GR: gamma ray; EB: electron beam; df: degree of freedom; *b*: gas production potential (mL/g DM); *c*: rate constant of gas production (mL/h); DMD: dry matter digestibility and OMD: organic matter digestibility.
* (P<0.05) and ** (P<0.01).
NS: non significant.

Table 4 *In vitro* gas production parameters and digestibility of pomegranate seeds before and after irradiation

Treatments	Parameters		DMD (%)	OMD (%)
	b	*c*		
Control	185.73a	0.09a	47.41a	47.38a
Gamma				
5 kGy	162.93b	0.06d	39.30de	38.54bc
10 kGy	161.33b	0.06d	37.01e	37.31c
15 kGy	162.35b	0.05d	38.65de	38.19bc
20 kGy	164.12b	0.06d	40.99cd	41.02bc
Electron				
5 kGy	172.73ab	0.08b	42.50bc	42.75ab
10 kGy	158.43b	0.06d	37.18e	37.38c
15 kGy	165.66b	0.07c	44.49b	42.98ab
20 kGy	175.31ab	0.07b	48.45a	47.21a
LSD	17.40	0.006	2.86	4.83
P-value	0.006	0.0001	0.0001	0.0011
SEM	1.99	0.002	0.83	0.88

b: gas production potential (mL/g DM); *c*: rate constant of gas production (mL/h); DMD: dry matter digestibility and OMD: organic matter digestibility.
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.
LSD: least significant difference.

Table 5 Orthogonal contrast of PF, GV_{24} and ME of pomegranate seeds (means square)

Treatments	PF	GV_{24}	ME
Irradiation *vs*. control	0.22	2337.87**	1.16*
GR *vs*. control	0.05	2965.95**	1.23*
5 and 10 GR *vs*. control	0.32	2521.92**	1.48*
15 and 20 GR *vs*. control	1.0	2421.84**	0.63*
5 and 10 *vs*. 15 and 20 GR	3.72	1.51	0.15
EB *vs*. control	0.44	1389.80**	0.80*
5 and 10 EB *vs*. control	0.002	1525.36**	0.73*
15 and 20 EB *vs*. control	1.35	841.45**	0.50
5 and 10 *vs*. 15 and 20 EB	1.84	151.44*	0.04
GR *vs*. EB	0.46	737.92**	0.05
5 and 10 GR *vs*. 5 and 10 EB	0.58	186.91*	0.05
15 and 20 GR *vs*. 15 and 20 EB	0.03	612.32**	0.009

GR: gamma ray; EB: electron beam; PF: partitioning factor (mg DM/mL gas); GV_{24}: gas volume at 24 hour (mL) and ME: metabolizable energy (MJ/kg DM).
* (P<0.05) and ** (P<0.01).

Table 6 Effect of irradiation on partitioning factor, gas volume and metabolizable energy of pomegranate seeds before and after irradiation

Treatments	PF	GV_{24}	ME
Control	5.36[ab]	150.38[a]	7.21
Gamma			
5 kGy	5.29[ab]	114.77[c]	6.20
10 kGy	4.61[b]	114.97[c]	6.10
15 kGy	6.90[a]	114.27[c]	6.22
20 kGy	5.23[ab]	116.90[c]	6.70
Electron			
5 kGy	4.72[b]	133.83[b]	6.71
10 kGy	6.07[ab]	111.70[d]	5.98
15 kGy	5.82[ab]	124.86[bc]	6.38
20 kGy	6.54[ab]	134.88[b]	6.70
LSD	2.12	13.67	0.95
P-value	0.3536	0.0001	0.1458
SEM	0.24	2.59	0.11

PF: partitioning factor (mg DM/mL gas); GV_{24}: gas volume at 24 hour (mL) and ME: metabolizable energy (MJ/kg DM).
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.
LSD: least significant difference.

Table 7 Correlation coefficient (*r*) of the relationship between chemical composition and experimental parameters of gamma irradiated pomegranate seeds

	DMD	OMD	*b*	*c*	GP_{96}	GP_{24}	ME	PF
DMD	1	0.93*	0.88*	0.87*	0.38	0.88*	0.93*	-0.16
OMD	0.93*	1	0.86*	0.92*	0.41	0.91*	0.92*	-0.15
CT	0.47	0.54	0.69*	0.73*	-0.01	0.73*	0.25	0.07
NDF	-0.55	-0.61	-0.73*	-0.66	-0.40	-0.71*	-0.23	-0.43
ADF	-0.31	-0.29	-0.59	-0.56	-0.31	-0.59	-0.20	0.48
NFC	0.37	0.61	0.65	0.71*	0.67	0.70	-0.38	0.39

DMD: dry matter digestibility; OMD: organic matter digestibility; *b*: gas production potential (mL/g DM); *c*: rate constant of gas production (mL/h); GP_{96}: gas production at 96 hour; GP_{24}: gas production at 24 hour (mL); ME: metabolizable energy (MJ/kg DM); PF: partitioning factor (mg DM/mL gas); CT: condense tannin (mg of CE/g of dry sample); NDF: neutral detergent fiber; ADF: acid detergent fiber and NFC: non fibrous carbohydrates and.
* (P<0.05).

Table 8 Correlation coefficient (r) of the relationship between chemical composition and experimental parameters of electron irradiated pomegranate seeds

	DMD	OMD	b	c	GP_{96}	GP_{24}	ME	PF
DMD	1	0.88*	0.68*	0.75*	0.37	0.77*	0.93*	-0.16
OMD	0.88*	1	0.77*	0.71*	0.46	0.82*	0.92*	-0.15
CT	-0.003	-0.001	0.54	0.64*	0.05	0.61	0.50	-0.61
NDF	0.10	0.46	0.40	-0.49	-0.52	-0.56	-0.92	-0.81
ADF	-0.80*	-0.85*	-0.71	-0.72	-0.66	-0.73	-0.67	0.48
NFC	0.48	0.24	0.69	0.85	-0.36	0.76	0.56	-0.27

DMD: dry matter digestibility; OMD: organic matter digestibility; *b*: gas production potential (mL/g DM); *c*: rate constant of gas production (mL/h); GP_{96}: gas production at 96 hour; GP_{24}: gas production at 24 hour (mL); ME: metabolizable energy (MJ/kg DM); PF: partitioning factor (mg DM/mL gas); CT: condense tannin (mg of CE/g of dry sample); NDF: neutral detergent fiber; ADF: acid detergent fiber and NFC: non fibrous carbohydrates and.
* (P<0.05).

These contradictions could be due to differences in doses and types of irradiation, laboratory circumstance in cell wall analysis (Tahan *et al.* 2012), free radicals formation, depolymerization (chain-scission) or cross-linking of cellulose and glucose chain (Polvi and Nordlund, 2014; Khan *et al.* 2006; Pekel *et al.* 2004).

The efficiency of these types of reactions depends mainly on the polymer structure and radiation dose (Charlesby, 1981).

In total, our result did not indicate any beneficial effect of irradiation at low doses on chemical composition of pomegranate seeds.

Effects on tannin content

Despite some differences which exit between GR and EB irradiations for their effects on condensed tannin content of PS, these two techniques significantly (P<0.05) decreased the condensed tannin content of PS compared to control (Table 2).

The decrease in condensed tannin was positively dependent on irradiation dose. Reduction of tannin by irradiation in this study was consistent with some previous studies on gamma irradiation (El-Niely, 2007; Behgar *et al.* 2011; De Toledo *et al.* 2007) and electron irradiation (Bhat and Sridhar, 2008; Shawrang *et al.* 2011).

Irradiation, generally, resulted in the degradation of tannin (Variyar et al. 1998) and a change in its molecular conformation (Topuz and Ozdemir, 2004). Mechanism of gamma action on tannin has been related to generation of the hydroxyl and superoxide anion radicals (Riley, 1994) which indiscriminately attacks neighbouring molecules, but mode of electron beam action on tannins has not been demonstrated.

In vitro study

Gas production profiles

Gas production parameters decreased significantly by irradiation (Table 4). Regarding to the potential gas production, there was no significant difference between gamma and electron radiation techniques.

The rate constant (fraction *c*) of gas production of EB irradiated PS at doses of 5 and 20 kGy decreased but the gas production potential did not affected significantly. The differences between gas parameters of lower (5 and 10 kGy) and upper (15 and 20 kGy) doses of GR and EB were not significant.

Ndlovu and Nherera (1997) reported that rate of gas production was negatively related to ADF, ADL and NDF content, a finding that agrees with our results. A negative correlation between condensed tannins and cell wall content with gas production parameters was reported by several studies (Kamalak et al. 2004; Ndlovu and Nherera, 1997; Nsahlai et al. 1994).

Focusing on the results presented in Tables 7 and 8, condensed tannins of PS showed a high positive correlation with gas parameters both in GR and EB irradiation which could be due to the tanning degradation and free phenolic compounds production that are toxic and suppressed the growth of the cellulolytic microorganism in the rumen (Chesson et al. 1982).

Behgar et al. (2011) reported that gamma radiation (at 30, 40 and 60 kGy dose) caused a decrease (P<0.05) in potential gas production of pistachio hull compared to the control.

Reductions in gas volume, fraction *b* and rate of gas production were also reported when tannic acid was added to the sunflower meal (Mohmmadabadi et al. 2010). In contrast, the rate of gas production in the study of Kamalak et al. (2004) was not related neither to chemical composition nor condensed tannins. A weak relationship between condensed tannins and gas production of tree leaves during wet and dry season in west Africa was reported by Larbi et al. (1998). A possible reason for these disparities could be due to differences in the nature of tannins between browse species (Jackson et al. 1996).

In vitro digestibility

In vitro dry matter digestibility and organic matter digestibility of PS decreased with the exception of EB at the dose of 20 kGy and 5, 15 and 20 KGy repectively. In a previous study by Ghanbari et al. (2012), the differences between various cottonseed meal treated by irritation (25, 50 and 75 kGy gamma and electron radiation) were not significant statistically.

Shawrang et al. (2011) reported that doses higher than 15 kGy of EB irradiation significantly increased dry matter digestibility of sorghum grains compared to the control. It is well documented that tannins and cell wall content of feedstuffs negatively affect their digestibility (Ndagurwa and Dube 2013; Guimaraes-Beelen et al. 2006; Mohmmadabadi et al. 2010).

These two components also influencing the growth and morphology of rumen microorganisms (O'Donovan and Brooker, 2001).

According to the results presented in Tables 7 and 8, ADF content of EB irradiated PS had significantly negative correlation with PS digestibility. There was no significant correlation between condensed tannin and digestibility of EB irradiated PS. Cell wall contents may be more important than tannins in limiting *in vitro* fermentation (Ndlovu and Nherera, 1997).

The effects of ionizing radiation on nutrients digestibility vary with irradiation dose and chemical structure and concentration of cell wall contents and tannins (Jackson et al. 1996).

The later may be due to differences in the nature of tannins between browse species and degradation of tannin by irradiation and adverse effect of free phenolics on rumen digestion (Chesson et al. 1982).

Irradiation did not change partitioning factor and gas volume at 24 h incubation (GV24) significantly decreased. Mean average of partitioning factor (PF) was 5.62 (mg DM truly degraded/mL gas produced in 24 h). Partitioning factor ranged from 4.61 to 6.90.

The theoretical range for partitioning factor of tannin-free plants suggested by Blummel et al. (1997) was between 2.75 and 4.41 (mg truly degraded substrate/mL gas). PF values greater than 4.41 are not theoretically possible (Makkar et al. 1998) and if this occurred, could simply indicate the inhibition of gas production due to the existence of tannins.

This result agreed with Makkar (2004). Irradiation changed ME of pomegranate seeds, but there is no difference among gamma and electron radiation. The mean of metabolizable energy of pomegranate seed was 6.44 MJ/kg DM, that was higher than Mirzaei-Aghsaghali et al. (2011)

and Taher-Maddah *et al.* (2012) who reported that estimated amounts of ME of dried pomegranate seed was 6.20 and 5.10 MJ/kg DM, respectively.

CONCLUSION

Generally irradiation at low doses could not change in chemical composition of pomegranate seed. Irradiation have the potential to reduce anti-nutritional factors. Condensed tannin content of PS was significantly decrease compared to control. There was a difference between the effects of gamma and electron radiation on reduction of condensed tannin. Electron beam radiation with a higher mean square of difference had more impact on reduction of condensed tannin as compared to gamma ray. Ionizing radiation processing can be used as an efficient method in decreasing condensed tannin content of PS, but it should be noted that potential feed value of PS could be altered by irradiation. So, further studies are needed to evaluate the definite effect of gamma and electron radiation on nutritional value and ruminal metabolism of feedstuff contained anti-nutritional factor.

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