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Effects of microwave irradiation on *in vitro* ruminal fermentation and ruminal and post-ruminal disappearance of safflower seed

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Abstract

Effects of microwave irradiation (900 W) for 3 min on fermentation kinetics and ruminal, post-ruminal and total tract dry matter (DM) and crude protein (CP) disappearance of safflower seed were evaluated by *in vitro* gas production, modified three-step method and *in vitro* disappearance method. Cumulative gas production was recorded at 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h of incubation and its kinetic was estimated using model: GP = A (1 – e^{-ct}). The rumen undegradable protein fractions of the raw and microwave treated safflower seed were obtained by ruminal incubation in two cannulated wethers and incubation in protease solution (protease type xiv, streptomyces griseus). The data were analysed using completely randomized design. Results indicate that microwave processing increased cumulative gas production ($p<0.05$). Disappearance of DM and CP were significantly decreased ($P<0.05$) compared with untreated safflower seed as microwave irradiation. Also, microwave irradiation significantly reduced ($P<0.05$) post-ruminal disappearance of DM and CP. According to the results, microwave irradiation can be used for feed processing and reduced feed nutrient availability. Also, these processing methods appear to shift the site of CP digestion from the rumen to the small intestine and increase the amount of CP digested in the small intestine.

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Introduction

A number of different oilseed crops such as oil seed rape, soybean, sunflower, and safflower are grown around the world. Oil seeds are major source of raw materials such as fat, protein, carbohydrate with potential application as nutritional and functional foods (Weiss, 2000). Safflower (*Carthamus tinctorius L.)* is annual oil-seed crops that originated in the eastern Mediterranean area (Knowles, 1976). Safflower is cultivated in 800000 ha in the world with a yield of 650 000 tones. Safflower seed (*Corthamus tinctorius*) was recognized to contain the highest concentration of linoleic acid among all oilseeds (Dubois *et al.,* 2007), but research in this field is a few.

Safflower seed can be used as a protein and energy supplement for ruminants (Bottger *et al.,* 2002). Under certain dietary and production Conditions, ruminant diets must be supplemented with forms of rumen non degraded dietary protein (by pass protein) to increase the efficiency of nutrient utilization and hence production (Quin *et al.,* 1938). Many studies have been performed to decrease the ruminal degradability of protein in oilseed using chemical treatment such as formaldehyde (Sahebi ala *et al.* 2011) and physical and heating treatment such as roasting (Fathi-nasry *et al.*, 2008), micronization (Wang *et al.*, 2007), because suppression of protein degradation in the rumen improves protein utilization (Nishimuta *et al.*, 1974).

Use of microwaves, the non-ionizing electromagnetic waves (Tatke and Jaiswal, 2011), is being investigated in ruminant nutrition (Sadeghi and Shawrang, 2008, Maheri-Sis *et al.,* 2012) because of the less startup time, faster heating, energy efficiency, space savings, precise process control, selective heating and final products with improved nutritive quality (Sumnu, 2001).

The aim of this study was to determine the nutritive value of safflower seed and effect of a microwave treatment of safflower on gas production and its ruminal and post ruminal dry matter and crude protein disappearance.

Materials and methods

Samples preparation and treatments

Safflower seeds were supplied from a local commercial supplier at Hashtrood city, Eastern Azerbaijan, province in northwest Iran. Triplicate 500 g samples of safflower seeds were placed in a Pyrex pan (28×28×6 cm) with 1 to 2 cm height and were subjected to microwave irradiation at a power of 900 W (1.8 w/g microwave energy) for 3 min.

Chemical analysis

Samples of safflower seeds were dried in an oven at 80°C until constant weight and the DM content calculated. Ground samples were analyzed for ash (AOAC, 2005). Determinations of N were conducted using the Kjeldahl method in an automated Kjelfoss apparatus (Foss Electric, Copenhagen, Denmark). Neutral-detergent fiber and ADF were determined by the detergent procedures of Van Soest *et al.* (1991). Ether extract (EE) was determined by extracting the sample with ether (AOAC, 2005).

In vitro gas production

For *in vitro* gas production, samples including both untreated and microwave treated safflower seed for 3 min were ground in a Wiley Mill to pass a 2 mm screen (Arthur H. Thomas, Philadelphia, PA, USA) and weighed (300 mg) into 50 ml glass vials. Mc Dougall (McDougall, 1948) buffer solution was prepared and placed in a water bath at 39°C. Rumen liquor samples were obtained from the two cannulated adult Ghezel wethers that were fed on a diet comprising (DM basis), 550g Kg^{-1} alfalfa hay, 400g Kg^{-1} barely grain, 48 g Kg^{-1} wheat bran and 2 g kg^{-1} lime stone at maintenance level (NRC, 1985). Rumen fluid was collected after the morning feeding. Rumen fluid was pumped with a manually operated vacuum pump and transferred into pre-warmed thermos flask, combined, filtered through four layers of cheesecloth and flushed with CO_2. Each feed sample was incubated in five reapplications with 20

ml of rumen liquor and buffer solution (1:2). Five vials containing only the rumen fluid/buffer solution and no feed sample was included with each test and the mean gas production value of these vials was termed the blank value. The vials were sealed immediately after loading and were affixed to a rotary shaker platform and housed in an incubator at 39°C. Gas production was measured in each vial after 2, 4, 8, 12, 16, 24, 36, 48, 72 and 96 h of incubation using a water displacement apparatus (Fedorak and Hrudey, 1983).

Ruminal and post-ruminal disappearance

This experiment was carried out according to procedure of Gargallo *et al.* (2006). Approximately, 5 g of each sample (raw and microwave treated safflower seeds) was weighed into a 5×10 cm Dacron polyester nylone bag with 50 µm pore size (four bags per each sample) and suspended in the rumen two cannulated wethers for 12 h. Bags were then removed and washed with washing machine and dried in a force air oven at 55 °C for 48 h and weighed. Samples from each bag were taken for N analysis using Kjeldahl method (Kjeltec 2300 Autoanalyser, Foss Tecator). After weighing, residue (0.5 g) was placed in an in situ bag (5×5 cm with pore size 50 µm) and placed in an ANKOM daisy incubator for determination of post-ruminal digestibility. Briefly, samples were incubated in pepsin/HCl solution for 1 h in a Daisy incubator, followed by incubation in a panctratin/HK_2PO_4 solution for 24 h. After 24 h, bags were washed with washing machine. Bags were then dried in a forced air oven at 55 °C for 48 h. The dry weights of the samples and bags were recorded and bags were opened and contents were pooled by sample for crude protein analysis.

In vitro dry matter and crude protein disappearance procedure

In vitro determination of dry matter and crude protein digestibility was conducted according to the method described by McNiven *et al.* (2002). Briefly, samples (raw and microwave treated safflower seeds) were weighed into small nylon bags (5×5 cm with pore size 50 µm) and maximum of 25 bags were place in 2.4 l bottle containing borate-phosphate buffer at 39 °C. After 1 h, protease solution (protease type xiv, streptomyces griseus) was added. After 4 h, the bags were removed and rinsed thoroughly. Half of the bags were dried at 55 °C for 48 h and the dry weight was recorded. The rest of the bags were incubated for 1 h in pepsin solution, then NaOH and pancreatin solution were added and the bags were incubated for 24 h at 39 °C. Bags were rinsed and dried at 55 °C for 48h. The dry weight of the bags was recorded and the bag residue was analyzed for crude protein.

Calculations and statistical analysis

Rate and extent of gas production was determined for each feed by fitting gas production data to the one component McDonald model: $Y = A\,(1 - e^{-ct})$, where y is the volume of gas produced at time t, A the potential gas production (ml g^{-1} DM), and c the fractional rate of gas production. Parameters A and c were estimated by an iterative least square method using a non-linear regression procedure of the statistical analysis systems (SAS, 1999).

The metabolizable energy (MJ.kg^{-1} DM) content of feeds was calculated using equation of Getachew *et al.* (2002) as:

ME (MJ.kg^{-1}DM) = 1.06 + 0.157GP + 0.084CP + 0.22CF – 0.081CA

The short chain fatty acid (SCFA) and organic matter digestibility (OMD) for feeds were calculated using equations of Menke *et al.* (1979) as:

SCFA mmol. 200 mg^{-1} DM = 0.0222 GP - 0.00425

OMD (%) = 14.88 + 0.889 GP + 0.45 CP + CA

Where, GP is 24 h net gas production (ml/200 mg DM); CP, CF and CA are crude protein, crude fat and crude ash (% DM), respectively.

Data on gas production parameters and ruminal-postruminal dry matter and crude protein disappearance were subjected to one-way analysis of variance using the analysis of variation model (ANOVA) of SAS (1999). Multiple comparison tests used Duncan's multiple-range test (Snedecor and

Cochran, 1989).

Result and discussion

Chemical composition

The chemical components (Table 1) are similar to those reported by Alizadeh *et al.* (2010) and Dschaak *et al.* (2011). Observed difference for CP, NDF and ADF content of safflower seed in literature can be due to variation in varieties, cultivating and environmental conditions of feeds that are used this and other study.

Table 1. Chemical composition of safflower seed (% DM) [†].

Dry matter	94.1
Crude protein	16.8
Organic matter	93.2
Neutral detergent fiber	43.5
Acid detergent fiber	33.7
Hemicellulose	9.8
Ether extract	26.1

Hemicellulose=neutral detergent fiber-acid detergent fiber.

[†]Three samples analyzed for safflower seed.

In vitro gas production

The cumulative gas production data of 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h incubation times for both untreated and microwave treated sorghum grain and gas parameters are presented in Table 2. We have preferred 96 h of incubation period for amplification of kinetics' data (Orskov and McDonald, 1979). Cumulative gas production of safflower seed was increased ($P<0.05$) as microwave irradiation treatment, but it was not affected until 12 h of incubation, as it can be seen.

Table 2. Effects of microwave treatments on *in vitro* gas production of safflower seed.

	Gas production (ml. g^{-1} DM)											Gas production constants	
Feeds	2 h	4 h	6 h	8 h	12 h	16 h	24 h	36 h	48 h	72 h	96h	*A*	*c*
RSS	37	67	89	102	116	128^{b}	141^{b}	151^{b}	155^{b}	157^{b}	158^{b}	155^{b}	0.11^{a}
MSS	36	65	88	101	120	137^{a}	155^{a}	167^{a}	174^{a}	175^{a}	177^{a}	175^{a}	0.09^{b}
SEM (n=5)	1.5	1.9	2.2	2.2	2.0	1.9	1.9	2.2	2.3	2.6	2.4	3.53	0.004

RSS= Raw Safflower Seed, MSS= Microwave treated Safflower Seed

a, b Means within a column with different subscripts differ ($P<0.05$).

c: fractional rate of gas production (h^{-1}); *A*: potential gas production (ml.g^{-1} DM).

When feeds such as oil seeds enter the rumen as a substrate for microorganisms, the inherent physicochemical properties of the seeds on the one hand and its availability to fermentation on the other hand play important roles in fermentability and producing end products during ruminal digestion. Wang *et al.* (1997) reported that micronization (like microwave) increased canola seed gas production by damaging seed coat and increasing its availability.

A critical difference between microwave and traditional dry or wet heat treatments is that the latter use external sources to heat the seed from outside, whereas microwave generates heat from inside the seed. During microwave, rapid internal heating

vaporizes the water inside the seed, increasing pressure inside the seed until the seed coat ruptures. The conditions of high temperature and pressure existing within the seed immediately prior to seed coat rupture are likely crucial for effecting the changes observed in microwave treated safflower seed.

Parnian *et al.* (2014) reported that microwave irradiation increases cumulative gas production of cereal grain, probably by improving starch utilization through disruption of the protein matrix surrounding the starch granules (Rooney and Pflugfelder, 1986), and enhances their susceptibility to enzymatic degradation.

Ruminal and post-ruminal dry matter and crud protein disappearance

Values for DM and CP disappearance determined by modified three step procedure and *in vitro* procedure (McNiven procedure) shown in table 3.

Table 3. Ruminal, post-ruminal and total tract DM and CP disappearance (%).

	modified three step procedure			*in vitro* procedure		
	ruminal	Post-ruminal	Total tract	ruminal	Post-ruminal	Total tract
Dry Matter						
RSS	28.0[a]	30.3[b]	58.3	24.5	29.6	54.1
MSS	26.9[b]	33.1[a]	60.3	23.6	30.6	53.6
SEM	0.89	0.98	0.98	0.54	1.13	0.88
Crude protein						
RSS	25.8[a]	25.6[b]	51.5	25.1	31.3	56.4
MSS	22.5[b]	29.9[a]	52.4	24.7	33.3	58.0
SEM	0.98	0.98	1.23	0.54	1.12	0.69

RSS= Raw Safflower Seed, MSS= Microwave Treated Safflower Seed

a, b Means within a column with different subscripts differ (P<0.05).

The disappearance of ruminal DM and CP of microwave treated safflower seeds was significantly (p<0.05) reduced compared to the raw seed (for DM 28 and 26.9 percent and for CP 25.8 and 24.5 percent) but postruminal disappearance of DM and CP was increased. Despite the reduction of ruminal disappearance of DM and CP of microwave treated seeds increased in postruminal disappearance of DM and CP were recorded and total tract disappearance of DM and CP was higher than raw seeds. No significant difference (p>0.05) between the two raw and microwave treated safflower seeds in total tract DM and CP disappearance.

The significant reduction in the mount of disappearance of CP by microwave irradiation showed it appeared to increase by-pass protein from soybean and thereby increase the amount of available CP in the small intestine, provided that the digestibility of by-pass protein does not decrease with microwave irradiation. Previous studies also reported that heat processing reduced the rate and amount of disappearance of DM and CP of whole soybean (Hsu and Satter, 1995, Aldrich *et al.*, 1997), cotton seed (Pena *et al.*, 1986) and Canola seed (Wang *et al.*, 1997 and McKinnon *et al.*, 1995).

Jahani-Azizabadi *et al.* (2010) remarked that heat treatment and heat-xylose treatment effectively impacted reduction of DM and CP ruminal disappearance and increased ruminaly undegradable protein fraction of guar meal's protein. Moreover Fathi Nasri *et al.* (2008) reported that heat processing of soybean, declined its DM and CP

ruminal degradability as well as increased intestinal digestibility. In agreement with other reports, Broderick *et al.* (2006) proposed that heat treatment of linseed meal decreased ruminal CP and DM disappearance and increased bypass protein amounts (rumen undegradable protein) and its intestinal digestibility. They concluded that such increment of bypass protein in response to heat treatment might be result of blocking active and reaction sites of feed with microbial proteolytic enzymes in the rumen.

Microwave irradiation (for 3 min) of whole safflower seed reduced ruminal degradation of CP and DM and increased small intestinal disappearance of them (DM and CP). Consequently, these processing methods appear to shift the site of CP digestion from the rumen to the small intestine and increase the amount of undegraded CP digested in the small intestine. Steeping improved the small intestinal and total tract digestibility of CP beyond the effects of microwave treatment. The determination of DM and CP ruminal and post-ruminal disappearance of raw and microwave processed whole safflower seed in this study may be useful when formulating diets for ruminants.

Table 4. Evaluated SCFA, ME and OMD by *in vitro* gas production results.

	According to *in vitro* gas production data		
Feedstuffs	SCFA mmol. 200mg^{-1} DM	ME MJ.kg^{-1} DM	OMD %
RSS	0.623^{b}	12.10^{b}	38.37^{b}
MSS	0.684^{a}	12.53^{a}	41.12^{a}
SEM	0.008	0.062	0.397

RSS= Raw Safflower Seed, MSS= Microwave Treated Safflower Seed

SCFA = Short Chain Fatty Acid, ME = Metabolizable Energy and OMD = Organic Matter Digestibility

a, b Means within a column with different subscripts differ (P<0.05).

Metabolizable energy (ME) and Short chain fatty acid (SCFA)

According to studies that reported by Menke *et al.* (1979) and Getachew, *et al.* (2002), SCFA, ME and OMD could be evaluated by 24 h *in vitro* gas production data. These results are shown in Table 4.

Low content of raw safflower seed's SCFA, metabolizable energy and organic matter digestibility can be resulted from its low rate of gas production, extent of gas production at 24 h and high availability of nutrients in microwave treated safflower seed.

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