

Microwave irradiation of whole soybeans in ruminant nutrition: Protein and carbohydrate metabolism *in vitro* and *in situ*

Sara Golshan¹, Rasoul Pirmohammadi², Hamed Khalilvandi-Behroozyar^{2*}

¹ MSc Graduate, Department of Animal Science, Faculty of Agriculture, Urmia University, Urmia, Iran; ² Department of Animal Science, Faculty of Agriculture, Urmia University, Urmia, Iran.

Article Info	Abstract
Article history: Received: 15 August 2017 Accepted: 07 November 2017 Available online: 15 December 2019	<p>Whole soybeans serve as one of the main sources of protein in ruminant nutrition. Different processing methods have been employed for ruminal protein protection. The present study was conducted to determine the effects of microwave irradiation [900 W; 2, 4 and 6 min] on quality, ruminal degradability and estimated <i>in vitro</i> intestinal digestibility of availability soybean crude protein. This experiment was performed in a completely randomized design with seven treatments including control (no processing), along with 2, 4 and 6 min of microwave irradiation on whole and ground soybeans. Protein and carbohydrate fractions were determined according to Cornell Net Carbohydrate and Protein System (CNCPS). Triplicates of the samples were incubated in the rumen of three cannulated Holstein steers for up to 48 hr. Microwave irradiation increased neutral detergent insoluble nitrogen, metabolizable protein content and resulted in a lower effective rumen degradability and <i>in vitro</i> gas production. Nevertheless, longer processing time led to higher unavailable protein and carbohydrate fractions. In the main, microwave irradiation of ground samples for 4 min increased metabolizable protein content, without negative effects on protein and carbohydrate availability.</p>
Key words: Heat treatment Metabolizable protein Undigested crude protein	

© 2019 Urmia University. All rights reserved.

Introduction

Whole soybeans are in use as one of the main feedstuffs for high producing dairy cows to meet protein requirements.^{1,2} However, some difficulties may be raised with the high dietary inclusion of raw soybeans such as certain anti-nutrients or anti-nutritional factors and low bypass protein.³ Different processing methods such as extrusion and roasting have been used to decrease rumen protein degradability using accelerating cross-linkages between free amino acids and aldehyde groups as a result of heating.² Nevertheless, environmental impacts of fossil fuels, high energy and instrumental costs, difficulties in efficient processing and negative effects of extra heating either on intestinal availability of bypass protein, or increase in processing costs should be considered. Microwave irradiation has been reported as a quick feed processing method with simple heat control.^{4,5} Efficiency of microwave irradiation on the grains and oilseed meals

was extensively investigated.^{4,5} However, a few reports are now available about the effects of microwave irradiation on ruminal nutrient degradability of nutrients in whole oilseeds such as soybeans.⁶ The main purpose of this study is to evaluate the effects of microwave irradiation on *in situ* degradability, *in vitro* gas production and metabolizable protein parameters of the whole soybeans.

Materials and Methods

Sample collection, processing, and laboratory analysis. Whole soybeans were supplied by a commercial feed processing factory and ground through a 2.00 mm screen (Wiley Mills, Thomas Scientific, Swedesboro, USA) while some remained whole. The moisture content of the sample was reached to 250 g kg⁻¹ with deionized water⁴⁻⁶ and irradiation was done by a commercial microwave supplying 900 W of power, for 2, 4 and 6 min with gentle agitation in every 15 sec. This experiment was performed

*Correspondence:

Hamed Khalilvandi-Behroozyar. PhD
Department of Animal Science, Faculty of Agriculture, Urmia University, Urmia, Iran
E-mail: h.khalilvandi@urmia.ac.ir



This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License which allows users to read, copy, distribute and make derivative works for non-commercial purposes from the material, as long as the author of the original work is cited properly.

in a completely randomized design with seven treatments including control (no processing), along with 2, 4 and 6 min of microwave irradiation on whole and ground soybeans.

After processing, dry matter content was determined by drying at 105 °C overnight, and samples of different treatments were ashed by ignition in a muffle furnace (AOAC 2000, ID 942.05).⁷ The ether extract content was determined by BUCHI automated apparatus (Büchi Labortechnik AG, Flawil, Switzerland; AOAC 2000, ID 920.39).⁷ The total nitrogen (N) content was measured by the Kjeldahl method (Behr Labor-Technik GmbH, Dusseldorf, Germany) and CP was calculated as $N \times 6.25$. The neutral and acid detergent fiber contents (NDF and ADF) were determined by using an automated Ankom Fiber apparatus (Ankom200; Ankom Technology, Macedon, USA) according to the report by Van Soest *et al.*⁸ Moreover, protein contents of NDF and ADF were measured and considered as neutral and acid detergent insoluble protein (NDICP and ADICP) respectively. In the case of CNCPS and metabolizable protein fractionation, sodium sulphite was not included in a neutral detergent solution (NDS).⁹ The chemical analysis of the samples from each of the treatments were carried out in triplicates.

***In situ* degradability.** An *in situ* experiment was designed for evaluating the effects of treatments on dry matter (DM), organic matter (OM), CP, and NDF degradability, according to Vanzant *et al.*, using three ruminally fistulated Holstein steers (mean body weight, 510 ± 20.00 kg).¹⁰ The animals were kept individually, handled and sampled according to FASS¹¹ and fed two equal meals (8:00 am and 6:00 pm) of a balanced ration (4.00 kg of Alfalfa hay, 3.00 kg of corn silage and 2.00 kg of a commercial concentrate mix) to achieve 10.00% more than maintenance energy requirements.¹²

The samples were ground to pass a 2.00 mm screen (Wiley mill) and sieved to remove particles less than 50.00 μm .¹⁰ Samples (5.00 g) were weighed into nylon bags (100×200 mm) with 50.00 μm pore size, to create sample size: surface area of 0.125 mg per mm^2 .¹⁰ Triplicates of the bags were incubated for 2, 4, 8, 12, 24 and 48 hr in the ventral rumen, just before the morning meal. Once removed, the bags were washed by agitation in cold tap water, until the rinsed water stayed clear and dried at 60.00 °C for 48 hr in a forced air oven and then weighed. To obtain the washing loss values, triplicates were washed with water at 39.00 °C for 20 min.¹⁰ Aliquots of bag residues were used for OM, CP and NDF determination and degradation profiles were calculated by fitting the data on the nonlinear model using the NLIN PROC of SAS statistical package (version 9.1; SAS Institute, Cary, USA).

Fractioning of CNCPS and metabolizable protein system, intestinal protein digestibility and PDI. The CNCPS carbohydrate fractions were determined following the procedure described by Lanzas *et al.*¹³ The A1, A2 and A3 fractions were considered to be zero and were not

measured.¹³ Protein fractions of the samples were determined in triplicate, according to a standardized procedure where fraction A was determined using trichloroacetic acid solution (10.00%, w/v, in water).¹³ The rumen degradable protein (RDP) and rumen undegradable protein (RUP) values were computed using National Research Council (NRC) equations.¹² The rates of passage (kp) were considered equal to 0.05 and 0.08 percent per hour and degradation rates (kd) for B1, B2, and B3 were taken to be 150, 5.00 and 0.18% per hr, respectively.¹²

The metabolizable protein (MP) values of treated samples were calculated according to *in situ* CP degradability data as stated by Agriculture and Fisheries Research Council (AFRC).¹⁴ The intestinal protein digestibility was measured using three-step *in vitro* assay with 12 hr of ruminal incubation.¹⁵ In addition, protein dispersibility index (PDI) was measured.¹⁶

Statistical Analysis. All data except for *in situ* and gas production kinetics were analyzed with GLM procedure of SAS, with the following model:

$$Y_{ij} = \mu + G_i + I_j + GI_{ij} + e_{ij}$$

In the statistical model, Y_{ij} is the mean of the each of measurements, μ is the overall mean, G_i and I_j represent the fixed effects of grinding and irradiation time, respectively. GI_{ij} is the interaction of the main effects and e_{ij} is the error term. The least square (LS) means were adjusted and compared with Tukey and PDIF options, respectively. Data were shown as the LS means and corresponding SEM. The $p < 0.05$ considered that differences were significant.

Results

The effects of microwave irradiation on the chemical composition of soybeans are shown in Table 1. The organic matter, CP and total fat content of dry matter were affected neither by microwave irradiation time nor by grinding. However, the dry matter content increased with microwave irradiation compared with the control group, without significant difference between different irradiated groups. The contents of NDF, ADF and their associated nitrogenous fractions increased with longer treatment time. In addition, grinding amplified the effects of microwave irradiation on NDF, ADF, NDIN and ADIN. Also, Microwave irradiation decreased soluble protein as shown in Tables 2, 3 and 4.

The effects of microwave irradiation on ruminal degradation parameters of DM, OM, CP, and NDF are shown in Table 2. In general, microwave irradiation declined the ultimate disappearance of the measured nutrients from the nylon bags. Irradiation of the ground samples did not develop a significant difference in DM degradation kinetics compared with the whole samples,

but in the case of OM, CP, and NDF, the ground samples had lower ruminal degradability. Unlike CP, degradation kinetics for NDF did not show linear responses to heating time. In initial incubation times, NDF degradability was higher for short time irradiated samples.

As shown in Table 2, microwave irradiation for 2 min increased the potentially degradable fraction of DM, OM, and CP. On the other hand, a longer irradiation time caused lower fraction A and B, and increased indigestible fraction C.

Table 1. Chemical composition of full fat soybeans (g 100 g⁻¹ DM) among different treatment groups.

Parameters	Control	M2		M4		M6		<i>p</i> values			Contrasts, <i>p</i> value			
		M	W	M	W	M	W	SEM	Mic	Mill	Control vs. Processed	M2 vs. M4	M4 vs. M6	M2 vs. M6
DM	89.27	94.10	93.85	95.09	92.89	96.01	94.24	1.12	0.0069	0.65	0.001	0.9856	0.3291	0.3377
OM	93.91	94.33	92.79	93.67	92.31	93.29	92.55	1.13	0.9172	0.81	0.7456	0.619	0.9497	0.5759
CP	36.08	36.78	36.10	36.55	35.66	36.36	35.89	0.44	0.7098	0.48	0.4261	0.4529	0.9526	0.4885
EE	18.19	18.60	18.44	18.34	18.42	18.47	18.33	0.22	0.9096	0.57	0.8113	0.5333	0.9303	0.5913
NDF	18.12	19.46	18.29	20.61	21.15	25.30	23.66	0.23	<0.0001	0.02	<0.0001	<0.0001	<0.0001	<0.0001
ADF	14.16	14.53	14.28	15.55	14.83	15.37	15.25	0.17	<0.0001	0.21	0.0009	0.0004	0.5151	0.0001
NDICP	7.10	11.33	10.66	11.91	11.31	13.37	12.69	0.12	<0.0001	0.00	<0.0001	0.0001	<0.0001	<0.0001
ADICP	4.86	5.17	5.02	5.23	5.11	5.38	5.18	0.06	0.0004	0.08	0.0002	0.2669	0.0824	0.0084

DM: Dry matter; OM: Organic matter; CP: Crude protein; EE: Ether extract; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; NDICP: Neutral detergent insoluble crude protein; ADICP: Acid detergent insoluble crude protein; M2: Microwave 2 min; M4: Microwave 4 min; M6: Microwave 6 min; M: Milled; W: Whole; Mic: Microwave irradiation; Mill: Milling.

Table 2. Ruminal nutrient degradability parameters of full fat soybeans among different treatment groups.

Parameters	Contr ol	M2		M4		M6		SEM	<i>p</i> values		Control vs. Processed	Contrasts, <i>p</i> value		
		M	W	M	W	M	W		Mic	Mill		M2 vs. M4	M4 vs. M6	M2 vs. M6
Dry matter														
A (%)	45.43	21.32	35.93	23.05	30.27	22.33	29.15	1.94	<0.0001	0.01	<0.0001	0.0786	0.0748	0.9787
B (%)	49.26	77.21	56.90	56.82	46.24	47.87	54.52	3.71	0.0009	0.00	0.0298	0.0009	0.9292	0.0008
UD (%)	5.31	1.47	7.17	20.13	23.49	29.8	16.33	1.30	<0.0001	0.04	<0.0001	0.0174	0.0003	<0.0001
C per hr (%)	0.11	0.09	0.10	0.06	0.11	0.05	0.07	0.06	0.5571	0.33	0.7731	0.9887	0.2358	0.2307
ED 0.03 (%)	79.77	70.61	72.39	65.74	64.63	56.46	66.06	1.66	<0.0001	0.04	<0.0001	0.0019	0.0332	<0.0001
ED 0.05 (%)	73.11	63.58	62.53	60.01	57.98	50.50	59.27	1.17	<0.0001	0.10	<0.0001	0.0038	0.0035	<0.0001
ED 0.08 (%)	66.01	56.17	52.83	54.50	50.84	44.12	52.50	0.88	<0.0001	0.59	<0.0001	0.0567	0.0002	<0.0001
Organic matter														
A %	42.83	22.94	30.14	24.95	36.33	18.65	33.94	2.06	<0.0001	0.579	<0.0001	0.0663	0.0532	0.9066
B %	51.59	74.40	57.94	57.85	46.95	55.16	49.04	3.97	0.0062	0.03	0.1252	0.0038	0.9398	0.0033
UD %	5.58	2.66	11.92	17.2	16.72	26.19	17.02	1.20	<0.0001	<0.0001	<0.0001	0.2773	0.0011	<0.0001
C per hr (%)	0.13	0.11	0.09	0.07	0.08	0.09	0.13	0.02	0.4312	0.27	0.1693	0.7677	0.5611	0.3852
ED 0.03 (%)	81.31	72.02	73.45	67.25	67.79	58.63	68.52	1.44	<0.0001	0.01	<0.0001	0.0029	0.0166	<0.0001
ED 0.05 (%)	75.19	65.03	63.91	61.44	61.01	52.12	62.07	0.99	<0.0001	0.01	<0.0001	0.0057	0.001	<0.0001
ED 0.08 (%)	68.42	57.57	54.25	55.78	53.57	45.23	55.18	0.732	<0.0001	0.05	<0.0001	0.1135	<0.0001	<0.0001
Crude protein														
A (%)	48.24	35.31	37.91	30.68	32.34	28.75	34.83	2.59	0.0063	<0.0001	0.0572	0.0019	0.0906	0.0656
B (%)	46.47	58.22	58.03	62.09	55.68	58.85	52.70	8.66	0.0063	0.04	0.0454	0.0435	0.1525	0.0022
UD (%)	5.29	6.47	4.06	7.23	11.98	12.4	12.47	1.54	<0.0001	0.03	<0.0001	0.0481	<0.0001	<0.0001
C per hr (%)	0.06	0.04	0.04	0.04	0.04	0.03	0.04	0.017	0.0465	0.06	0.1112	0.2438	0.0167	0.1557
ED 0.03 (%)	70.40	62.14	66.68	63.59	64.15	45.22	57.64	2.32	0.0041	<0.0001	0.0631	0.1498	0.0227	0.0011
ED 0.05 (%)	61.97	53.53	58.71	56.43	56.73	39.58	54.79	1.40	0.0069	<0.0001	0.2105	0.4734	0.0076	0.0018
ED 0.08 (%)	54.57	44.71	52.24	50.21	53.99	33.37	52.31	0.71	0.0012	<0.0001	0.69	0.0387	0.0001	0.0096
Neutral detergent fiber														
A (%)	39.93	40.85	41.31	47.46	41.03	31.97	40.73	1.85	0.0033	0.26	0.6485	0.0017	0.0008	0.6993
B (%)	54.37	48.31	46.72	36.83	40.68	41.91	43.25	4.70	0.0033	0.06	0.0539	0.0014	0.43	0.0068
UD (%)	5.70	10.84	11.97	15.71	18.29	26.12	16.02	3.56	<0.0001	0.04	<0.112	0.374	0.345	<0.0001
C per hr (%)	0.11	0.09	0.12	0.08	0.13	0.09	0.09	0.18	0.8097	0.45	0.8788	0.4583	0.8927	0.3833
ED 0.03 (%)	77.15	73.38	76.29	70.01	69.92	62.17	69.68	1.49	<0.0001	0.02	<0.0001	0.0056	0.017	<0.0001
ED 0.05 (%)	70.07	67.42	67.69	65.36	64.81	57.29	64.16	1.02	<0.0001	0.03	<0.0001	0.0298	0.0008	<0.0001
ED 0.08 (%)	62.72	61.25	59.59	61.01	59.36	52.21	58.65	0.71	<0.0001	0.14	<0.0001	0.744	<0.0001	<0.0001

A: Soluble fraction; B: Potentially degradable fraction; UD: Undegradable fraction; C: Degradation rate; ED: Effective degradability in different rumen outflow rates; M: Milled; W: Whole; Mic: Microwave irradiation; Mill: Milling; M2: Microwave 2 min; M4: Microwave 4 min; M6: Microwave 6 min; M: Milled; W: Whole; Mic: Microwave irradiation; Mill: Milling.

Table 3. Metabolizable protein parameters of full fat soybeans (g 100 g⁻¹ CP) among different treatment groups.

Parameters	Control	M2		M4		M6		SEM	<i>p</i> values		Contrasts, <i>p</i> value			
		M	W	M	W	M	W		Mic	Mill	Control vs. Processed	M2 vs. M4	M4 vs. M6	M2 vs. M6
QDP	47.99	34.89	38.50	30.01	32.31	28.47	34.46	0.69	<0.0001	<0.0001	<0.0001	<0.0001	0.6905	<0.0001
SDP	34.64	32.12	26.43	28.27	24.09	21.85	23.32	0.52	<0.0001	<0.0002	<0.0001	<0.0002	<0.0003	<0.0004
RDP	82.63	67.01	64.93	58.29	56.41	50.33	57.72	1.21	<0.0001	0.8351	<0.0001	<0.0001	0.0149	<0.0001
ERDP	73.03	60.03	57.23	52.29	49.94	44.63	50.84	1.07	<0.0001	0.6696	<0.0001	<0.0001	0.0064	<0.0001
UDP	16.91	32.75	36.27	42.89	44.31	49.45	41.67	0.92	<0.0001	0.3909	<0.0001	<0.0001	0.0496	<0.0001
DUP	13.72	27.97	31.19	36.95	38.32	42.84	35.91	0.79	<0.0001	0.4041	<0.0001	<0.0001	0.045	<0.0001
MP	60.46	67.35	67.32	73.13	70.75	71.74	68.81	1.58	<0.0001	0.4544	<0.0001	0.0102	0.3081	0.0818

QDP: Quickly degradable protein; SDP: Slowly degradable protein; RDP: Rumen degradable protein; ERDP: Effective rumen degradable protein; UDP: Undegradable protein; DUP: Digestible undegradable protein; MP: Metabolizable protein; M2: Microwave 2 min; M4: Microwave 4 min; M6: Microwave 6 min; M: Milled; W: Whole; Mic: Microwave irradiation; Mill: Milling.

Table 4. The CNCPS protein fractions and RDP and RUP content of full fat soybeans (g 100 g⁻¹ CP) among different treatment groups.

Parameters	Control	M2		M4		M6		SEM	<i>p</i> values		Contrasts, <i>p</i> value			
		M	W	M	W	M	W		Mic	Mill	Control vs. Processed	M2 vs. M4	M4 vs. M6	M2 vs. M6
A	22.89	17.06	17.88	14.32	16.56	10.81	13.73	0.382	<0.0001	<0.0001	<0.0001	<0.0002	<0.0003	<0.0004
B	65.47	73.16	71.31	79.65	73.60	76.84	74.21	1.674	<0.0001	0.0923	<0.0001	0.0185	0.5205	0.067
B1	45.17	10.24	12.99	9.71	11.25	7.29	9.79	0.396	<0.0001	<0.0001	<0.0001	0.0111	0.0002	<0.0001
B2	14.19	49.65	45.71	53.95	47.84	52.38	47.85	1.066	<0.0001	0.0002	<0.0001	0.0082	0.4765	0.0363
B3	6.11	13.27	12.60	15.99	14.51	17.17	16.56	0.319	<0.0001	0.0119	<0.0001	<0.0001	0.0001	<0.0001
C	11.64	11.63	11.54	12.36	11.70	13.06	12.54	0.268	0.0021	0.2466	0.1761	0.1177	0.0107	0.0003
RDP 0.08	71.36	46.17	48.08	44.64	45.96	38.25	41.79	1.103	<0.0001	0.0107	<0.0001	0.1174	0.0002	<0.0001
RUP 0.08	28.64	55.68	52.66	61.69	55.90	62.46	58.68	1.245	<0.0001	0.0043	<0.0001	0.0019	0.1732	<0.0001
Digestibility	88.23	88.87	88.71	87.43	85.80	85.76	86.09	0.994	0.0308	0.6753	0.1419	0.044	0.4939	0.0107
PDI %	53.11	13.39	14.96	12.28	12.75	9.33	10.32	0.413	<0.0001	0.0103	<0.0001	0.001	<0.0001	<0.0001

A: Non protein nitrogen soluble fraction; B1: Soluble true protein; B2: Potentially rumen degradable true protein; B3: Rumen undegradable fraction; C: Indigestible fraction, RDP: Rumen degradable protein in rumen outflow rate of 0.08, RUP: Rumen undegradable protein in rumen outflow rate of 0.08; PDI: Protein dispersibility index; M2: Microwave 2 min; M4: Microwave 4 min; M6: Microwave 6 min; M: Milled; W: Whole; Mic: Microwave irradiation; Mill: Milling.

The degradation rate was not affected by the treatments, except for CP. Processing of the soybeans with microwaves significantly increased rumen undegradable protein fraction subjected to irradiation time.

The effects of microwave irradiation on metabolizable protein profile are shown in Table 3. Irradiation for 2 and 4 min significantly increased rumen undegradable protein (UDP), digestible undegradable protein (DUP) and metabolizable protein (MP), but longer processing led to a worse ratio of MP and DUP to UDP.

The CNCPS protein fractions, PDI and the intestinal protein digestibility were represented in Table 4. Fraction A and also total B fractions were affected by the treatments, but the unavailable protein was not affected. Microwave irradiation reduced calculated RDP and caused higher RUP, as a function of processing time. The processing for 6 min, decreased PDI to a desirable point but negatively affected intestinal digestibility.

Microwave irradiation significantly changed protein dispersibility index (PDI) *in vitro* but intestinal protein digestibility was not affected (Table 4). The measured PDI was 13.39, 12.28 and 9.33 for ground and 14.96, 12.75 and 10.32 for whole samples, respectively after 2, 4 and 6 min of irradiation.

Discussion

As shown in Table 1, microwave irradiation increased the DM content of the samples, which may indicate microwave ability to decrease the moisture-holding capacity of the soybeans. Another Similar study has shown the higher DM content for microwave irradiated feeds.¹⁷ The crude protein, OM and ether extract content of the samples were not affected by microwave irradiation, as the results were reported for cottonseed.^{17,18} Nevertheless, contradictory results were reported by Thongprajukaew *et al.* regarding soybean meal.¹⁹ This inconsistency may be due to the fatty acid content, irradiation time and applied temperature. Fundamentally, irradiation can induce the release of unsaturated fatty acids, makes oxidative conditions and may cause the formation of secondary oxidative products.²⁰

The major fiber fractions measured as NDF and ADF were affected by heating and NDF was increased as a function of irradiation time. Increased crude fiber and NDF content in heated samples had been reported previously.²¹⁻²³ Instead, researchers reported no changes or even reduced fiber content in the heat-treated feed samples.^{16,23}

Microwave irradiation decreased soluble protein as shown in Tables 2, 3 and 4. This effect is comparable to the findings of soluble protein reduction of camelina seeds (52.73 to 20.41% CP), soybeans (43.38 to 11.35% CP) and flaxseeds (51.88 to 8.82% CP) as a result of moist heating at 120 °C for 60 min. Recovered nitrogen in NDF and ADF increased with microwave irradiation and extended treatment, led to greater values. Comparable results were reported by researchers who observed the same results in the higher cell wall bound CP with conventional heating methods.¹⁶ The higher cell wall associated CP could be related to Maillard reactions.^{8,24}

In the present study, NDCIP was increased more than ADICP, which was in agreement with the previous findings.^{21,23} A decrease in the soluble protein of the samples (Tables 2, 3 and 4) in this study, can be used to explain more increase in NDCIP than ADCIP in the heated samples. Commonly, heat treatment may increase NDICP, but its effect on ADICP can be variable, depending on the differences in ADF and NDF content and composition, feed CP content and fractions, soluble protein content, along with the type and duration of heating.²¹ Some reports revealed the recovery of less soluble and heat denatured proteins in NDF, but, protein recovery in ADF fraction may require higher heat input than what is need for increase in NDICP.^{22, 24}

In vitro and *in situ* experiments have shown that the rate and the extent of ruminal degradation of protein may be reduced by conventional heat treatment such as roasting, extruding or moist heating techniques.²⁴ When degradation rate (K_d) remains unchanged, the lower effective ruminal degradability might be related to the lower soluble and the higher potentially degradable fractions of the CP in the treated samples (Tables 2 and 4). In contrast to the present study, many reports revealed the lower K_d for microwave treated feed samples.^{4,5,25} This inconsistency may be due to the lower K_d of untreated samples in this study compared with control groups reported in the literature. Different factors such as pre-treatment processing, heating extent, and duration, amount of moisture, applied pressure, energy input and finally plant variety and growing conditions, may explain the miscellaneous results.^{12,23} Protein degradability was affected more than DM, OM and NDF parameters by microwave irradiation. This may be attributed to the non-thermal effects of microwave irradiation in the excitation of the protein reconstruction.²⁶ The mechanisms behind the protection of protein against the ruminal degradation in the heat-treated feedstuffs are complex. However, the development of chemical reactions during heat processing can be used to explain part of the results.^{27,28} Blockage of the reactive sites for microbial proteolytic enzymes as a result of heating was suggested as an explanation for the reduced ruminal CP and DM degradability.²⁹ Besides, Van Soest declared an increase in CP escape through heating as

a result of CP denaturation and reduction in solubility and degradation rate.²³ Moreover, cross-linking of the polypeptide chains, and protein aggregation,^{30, 31} unfolding of protein structures and increasing the surface hydrophobicity by exposing non-polar groups,³² electrostatic interactions and formation of disulfide bonds^{33,34} may be aid to explain. In line with *in situ* results, microwave irradiation decreased *in vitro* ruminal gas production which may be due to the unavailable nutrients created by heating.^{4,5,25}

As shown in Table 3, microwave irradiation significantly increased the metabolizable protein content, mainly by reduction of effective rumen degradable protein, and unaffected DUP to UDP ratio. These results are in line with rumen protein degradability data (Table 3). Similarly, DUP:UDP ratios between control and treated soybeans were in line with unchanged *in vitro* intestinal protein digestibility as shown in Table 4 and slightly higher unavailable fraction as measured *in situ*. Carbohydrate fractions were less affected by the treatments than proteins. Microwave irradiation had no effects on starch, soluble and digestible fiber fractions, but sugar content decreased and indigestible fiber fraction increased with 6 min of microwave irradiation. It is possible that the higher temperature resulted in the reaction between sugars and amino acids, and then the sugars were recovered in acid detergent insoluble fraction.

As shown in Table 4, microwave irradiation up to 4 min did not significantly change unavailable protein fraction in the cornel system, which could be possible support for MP system results. Microwave irradiation generally diminished soluble protein (A and B₁) and increased B₂ and B₃ in CNCPS fractionation analogous to *in situ* degradability and MP system. This study is the first to report the effects of microwave irradiation on protein quality of soybeans, but comparable results can be found in the literature about the other heating methods.^{16,21,35,36} Extensive heating around 100 °C was reported to cause the loss of labile amino acids such as cysteine and lysine.^{37,38}

Protein denaturation due to heating served as the main factor contributed to lower protein solubility after applying the heat treatments.²³ The increase in fraction B₂ in microwave treated samples may account in part for losses seen in the fraction B₁. This shift in fractions B, accompanied by a reduction in fraction A can cause slower rumen degradability and then lower effective rumen degradability for treated samples compared to control samples (Table 2). Reduction in the crude protein solubility and subsequently buffer solubility and ruminal degradability as a result of heating is well known and frequently reported.^{36,39} Additionally, changes in the percentage of secondary protein structures such as a reduction in α -helixes and multiplication of β -sheets could be considered as a causal factor in heat-treated samples.⁴⁰ A high proportion of heat-sensitive proteins (globulins and

albumins) and amino acids (cysteine and lysine) in soybean made it prone to heating effects.²³

At least a part of increased B3 fraction can be related to the increased NDICP and no change in ADICP. Increased NDICP is an accepted phenomenon consequent to moderate heating of high protein content of feeds due to the protein denaturation and recovery of denatured and less soluble proteins in NDF.^{35,36,41,42} However, the higher temperature or heating time may be required for amino acid and sugar complexes to be made and recovered as ADICP.²³ The Effect of heating on ADICP seems to be inconsistently higher⁴³, and unchanged³⁵ and even lower amounts¹⁶ of ADIN has been reported for heat-treated feed samples compared to the control group. The availability of reducing sugars, free reactive amino acids, pH, as well as heating extent and durability can affect net results of heating on protein recovery in acid detergent insoluble fraction.²³

In the present study, microwave irradiation up to 4 min did not significantly change ADICP. These results can be interpreted with reported data for *in situ* degradability and MP (Tables 2 and 3). It can be concluded that the processing condition used in the present study is not so harsh to generate unavailable protein. This conclusion can be further supported by unchanged *in vitro* intestinal digestibility of crude protein (Table 4). It seems that microwave had better performance in the case of ground samples to change protein degradability and metabolizability characteristics. This is the first report about the effects of sample pretreatment on microwave irradiation efficiency and nutrient availability of microwave irradiated samples. Rafiee-Yarandi *et al.* revealed that fine grinding of soybeans before roasting was resulted in higher CP availability compared with coarse or whole samples, measured as NDICP, ADICP, and CNCPS protein fractions, but there was no explanation for the results.¹⁶ Carbohydrate fractions did not change over microwave irradiation unless the C (unavailable fraction) increased as a result of irradiation above 2 min (1.68 g 100⁻¹ for control versus 1.72 and 2.12 for 4 and 6 min irradiated samples, respectively).

Microwave irradiation significantly changed protein dispersibility index (PDI) *in vitro* but intestinal protein digestibility was not affected (Table 4). The measured PDI was 13.39, 12.28 and 9.33 for ground and 14.96, 12.75 and 10.32 for whole samples, respectively after 2, 4 and 6 min of irradiation. Particle size reduction before processing, roasting time and the temperature has been reported that they significantly affect the dispersibility index of soybean protein.¹⁶ The ideal range for PDI has been reported to be 9-11, while values between 11 and 14 specified marginal heating. Moreover, suboptimal and over-heated samples will have PDI more than 14 and lower than 9, respectively. The dispersibility index decreased linearly with increased processing time and was in the appropriate range, except for whole beans irradiated for 2 min.

Relevant reports can be found in the literature confirming the lower PDI for higher temperatures or longer heating duration in the case of extruded or roasted feeds.⁴³⁻⁴⁷ The lower PDI for ground irradiated samples in the present study can be attributed to the greater processing extent due to the higher ability of microwaves to penetrate the kernel of the samples with excluding the seed coat. However, the lower PDI for whole compared with ground roasted soybeans has been reported.¹⁶ In a recent study, researchers reported no significant correlation between PDI and unavailable protein fraction in CNCPS.¹⁶ Nevertheless, a significant negative correlation was reported between PDI and rumen undegradable protein.⁴⁴⁻⁴⁷ Conflicting results between experiments could be attributed to the different heating methods.

Microwave irradiation decreased ruminal protein degradability. Notwithstanding longer irradiation time was accompanied by higher unavailable nutrients, and stunted processing resulted in suboptimal results. Unchanged ADICP and a simultaneous increase in NDICP have been proven as the important features of a non-destructive and efficient processing to maximize digestible undegradable protein.^{22,42,43} Thus, microwave irradiation can serve as an optimal, efficient and affordable processing method for feed.

The optimal condition for microwave irradiation in this study could be defined as irradiation of coarse ground samples up to 4 min with the microwave. The results of this experiment showed that microwave irradiation can serve as an optimal processing method to bypass more protein from the rumen in the case of soybeans without negative effects on intestinal availability. However, it seems that microwave had better performance in the case of ground samples to change protein metabolizability characteristics. More research could be recommended in the case of pre-treatments, processing time and to explore potential differences between different oilseeds.

Acknowledgments

Authors wish to thank Urmia University for the funding, laboratory and technical supports.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Faldet MA, Son YS, Satter LD. Chemical, *in vitro*, and *in vivo* evaluation of soybeans heat-treated by various processing methods. J Dairy Sci 1992; 75 (3): 789-795.
2. McNiven MA, Duynisveld J, Charmley E, et al. Processing of soybean affects meat fatty acid composition and lipid peroxidation in beef cattle. Anim

- Feed Sci Tech 2004; 116 (3-4):175-184.
3. Reddy PV, Morrill JL Bates LS. Effect of roasting temperatures on soybean utilization by young dairy calves. J Dairy Sci 1993; 76 (5): 1387-1393.
 4. Sadeghi AA, Shawrang P. Effects of microwave irradiation on ruminal protein degradation and intestinal digestibility of cottonseed meal. Livest Sci 2007; 106 (2-3): 176-181.
 5. Sadeghi AA, Shawrang P. Effects of microwave irradiation on ruminal dry matter, protein and starch degradation characteristics of barley grain, Anim Feed Sci Tech 2008; 141 (1-2): 184-194.
 6. Taghinejad M, Kazemi-Bonchenari M, Abdelfattah ZMS et al. Influence of roasting, gamma ray irradiation and microwaving on ruminal dry matter and crude protein digestion of cottonseed. Ital J Anim Sci 2016; 15 (1): 144-150.
 7. AOAC. Official methods of analysis. 17th ed. Gaithersburg, MD, USA: AOAC International 2000; 69-88.
 8. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991; 74 (10): 3583-3597.
 9. Licitra G, Hernandez T, Van Soest P. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim Feed Sci Tech 1996; 57 (4): 347-358.
 10. Vanzant ES, Cochran RC, Titgemeyer EC. Standardization of *in situ* techniques for ruminant feedstuff evaluation. J Anim Sci 1998; 76 (10): 2717- 2729.
 11. FASS. Guide for the care and use of agricultural animals in agricultural research and teaching. 3rd ed. Savoy, USA: Federation of Animal Science Societies 2010; 128-138.
 12. NRC. Nutrient requirements of dairy cattle. 7th ed. Washington DC, USA: National Academy Press 2001; 43-104.
 13. Lanzas C, Sniffen CJ, Tedeschi LO, et al. A revised CNCPS feed carbohydrate fractionation scheme for formulating rations for ruminants. Anim Feed Sci Tech 2007; 136 (3-4): 167-190.
 14. Agricultural and food research council. Energy and protein requirements of ruminants. Wallingford, UK: CAB International 1995; 50-78.
 15. Gargallo S, Calsamiglia S, Ferret A. A modified three-step *in vitro* procedure to determine intestinal digestion of proteins. J Anim Sci 2006; 84 (8): 2163-2167.
 16. Rafiee-Yarandi H, Alikhani M, Ghorbani GR, et al. Effects of temperature, heating time and particle size on values of rumen undegradable protein of roasted soybean. S Afr J Anim Sci 2016; 46 (2): 170-179.
 17. Taghinejad M, Kazemi-Bonchenari M, Abdelfattah ZM, et al. Influence of roasting, gamma ray irradiation and microwaving on ruminal dry matter and crude protein digestion of cottonseed. Ital J Anim Sci 2016; 15 (1): 144-150.
 18. Taghinejad M, Shawrang P, Rezapour A, et al. Changes in anti-nutritional factors, ruminal degradability and *in vitro* protein digestibility of gamma irradiated canola meal. J AnimVet Adv 2009; 8 (7): 1298-1304.
 19. Thongprajukaew K, Kovitvadhi U, Chandang Maejo P. Microwave irradiation improves physico-chemical properties of soya meal for economic freshwater fish Karun. Int J Sci Technol 2015; 9 (1): 43-53.
 20. Watabe N, Ishida Y, Ochiai A, et al. Oxidation decomposition of unsaturated fatty acids by singlet oxygen in phospholipid bilayer membranes. J Oleo Sci 2007; 56 (2): 73-80.
 21. Rafiee-Yarandi H, Ghorbani GR, Alikhani M, et al. A comparison of the effect of soybeans roasted at different temperatures versus calcium salts of fatty acids on performance and milk fatty acid composition of mid-lactation Holstein cows. J Dairy Sci 2016; 99 (7): 5422-5435.
 22. Moshtaghi-Nia SA, Ingalls JR. Effect of heating on canola meal protein degradation in the rumen and digestion in the lower gastrointestinal tract of steers. Can J Anim Sci 1992; 72 (1): 83-88.
 23. Van Soest PJ. Nutritional ecology of the ruminant. 2nd ed. Ithaca, USA: Cornell University Press 1994: 476.
 24. Waltz DM, Stern MD, Illg DJ. Effect of ruminal protein degradation of blood meal and feather meal on the intestinal amino acid supply to lactating cows. J Dairy Sci 1989; 72(6): 1509-1518.
 25. Sadeghi AA, Shawrang P. Effects of microwave irradiation on ruminal degradability and *in vitro* digestibility of canola meal. Anim Feed Sci Tech 2006b; 127 (1-2): 45-54.
 26. Banik S, Bandyopadhyay S, Ganguly S. Bio-effects of microwave. Bioresour Technol 2003; 87 (1): 155-159.
 27. Utsumi S, Kinsella JE. Forces involved in soy protein gelation: effects of various reagents on the formation, hardness and solubility of heat-induced gels made from 7S, 11S, and soy isolate. J Food Sci 1985; 50 (10) 1278-1282.
 28. Utsumi S, Kinsella JE. Structure-function relationship in food proteins: subunit interactions in heat-induced gelation of 7S, 11S, and soy isolate. J Agr Food Chem 1985; 33(2): 297-303.
 29. Broderick GA, Craig WM. Effect of heat treatment on ruminal degradation and escape, and intestinal digestibility of cottonseed meal protein. J Nutr 1980; 110 (12): 2381-2389.
 30. Abu JO, Muller K, Duodu KG, et al. Functional properties of cowpea (*Vigna unguiculata* L. Walp) flours and pastes as affected by gamma-irradiation. Food Chem 2005; 93 (1): 103-111.

31. Abu JO, Muller K, Duodu KG, et al. Gamma-Irradiation of cowpea flours and pastes: Effects on functional, thermal and molecular properties of isolated protein. *Food Chem* 2006; 95 (1):138-147.
32. Woods RJ, Pichaev AK. *Applied radiation chemistry. Radiation processing*. New York, USA: John Wiley & Sons 1994: 158-200.
33. Davies KJA, Delsignore ME. Protein damage and degradation by oxygen radicals. III. Modification of secondary and tertiary structure. *J Biol Chem* 1987; 262 (20): 9908-9913.
34. Le Maire M, Thauvette L, De Foresta B, et al. Effects of ionizing radiations on proteins. *Biochem J* 1990; 267 (2): 431-439.
35. Kafilzadeh F, SahebiAla M, Heidary N. The effect of physical and chemical treatments of canola seed varieties on crude protein fractions using CNCPS and *in vitro*. *J Agr Sci Tech* 2013; 9 (6): 1411-1421.
36. Mustafa AF, McKinnon JJ, Christensen DA. Effect of moist heat treatment on *in vitro* degradability and ruminal escape protein and amino acids of mustard meal. *Anim Feed Sci Tech* 1999; 76 (3-4): 265-274.
37. Ljøkjel K, Harstad OM, Skrede A. Effect of heat treatment of soybean meal and fish meal on amino acid digestibility in mink and dairy cows. *Anim Feed Sci Tech* 2000; 84 (1-2): 83-95.
38. Dakowski P, Weisbjerg MR, Hvelplund T. The effect of temperature during processing of rape seed meal on amino acid degradation in the rumen and digestion in the intestine. *Anim Feed Sci Tech* 1996; 58(3-4): 213-226.
39. Fathi-Nasri MH, France J, Danesh-Mesgaran M, et al. Effect of heat processing on ruminal degradability and intestinal disappearance of nitrogen and amino acids in Iranian whole soybean. *Livest Sci* 2008; 113 (1): 43-51.
40. Yu P. Protein secondary structures (α -helix and β -sheet) at a cellular level and protein fractions in relation to rumen degradation behaviors of protein: A new approach. *Br J Nutr* 2005; 94(5): 655-665.
41. Mustafa AF, Christensen DA, McKinnon JJ, et al. Effects of stage of processing of canola seed on chemical composition and *in vitro* protein degradability of canola meal and intermediate products. *Can J Anim Sci* 2000; 80 (1): 211-214.
42. Mustafa AF, Chouinard YP, Ouellet DR, et al. Effects of moist heat treatment on ruminal nutrient degradability of sunflower seed. *J Sci Food Agric* 2003; 83(10): 1059-1064.
43. McKinnon JJ, Olubobokun JA, Mustafa A, et al. Influence of dry heat treatment of canola meal on site and extent of nutrient disappearance in ruminants. *Anim Feed Sci Tech* 1995; 56 (3-4): 243-252.
44. Batal AB, Douglas MW, Engram AE, et al. Protein dispersibility index as an indicator of adequately processed soybean meal. *Poult Sci* 2000; 79(11): 1592-1596.
45. Caprita R, Caprita A, Cretescu I. Protein solubility as quality index for processed soybean. *Anim Sci Biotechnol* 2010; 43(1): 375-378.
46. Anuonye JC, Badifu GIO, Inyang CU. Protein digestibility index and trypsin inhibitor activity of extruded blends of acha/aoybean: A response surface analysis. *Am J Food Tech* 2007; 2 (6): 502-511.
47. Palic D, Modika KY, Oelofse A, et al. The protein dispersibility index in the quality control of heat-treated full-fat soybeans: An inter-laboratory study. *S Afr J Anim Sci* 2011; 41 (4): 413-419.