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Changes in Nutritive Value and Digestion Kinetics of Canola Seed Due to Microwave Irradiation

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ABSTRACT : This study aimed to evaluate effects of 800 W microwave irradiation for 2, 4 and 6 min on chemical composition, anti-nutritional factors, ruminal dry matter (DM) and crude protein (CP) degradability, and *in vitro* CP digestibility of canola seed (CS). Nylon bags of untreated or irradiated CS were suspended in the rumen of three bulls from 0 to 48 h. Protein subfractions of untreated and microwave irradiated CS before and after incubation in the rumen were monitored by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Microwave irradiation had no effect on chemical composition of CS ($p>0.05$). There was a linear decrease ($p<0.001$) in the phytic acid and glucosinolate contents of CS as irradiation time increased. Microwave irradiation for 2, 4 and 6 min decreased the phytic acid content of CS by 8.2, 27.6 and 48.6%, respectively. The total glucosinolate contents of CS microwave irradiated for 2, 4 and 6 min decreased by 41.5, 54.7 and 59.0% respectively, compared to untreated samples. The washout fractions of DM and CP and degradation rate of the *b* fraction of CP decreased linearly ($p<0.001$) as irradiation time increased. Microwave irradiation for 2, 4 and 6 min decreased effective degradability (ED) of CP at a ruminal outflow rate of 0.05 h^{-1} by 4.7, 12.3 and 21.0%, respectively. Microwave irradiation increased linearly ($p<0.001$) *in vitro* CP digestibility of ruminally undegraded CS collected after 16 h incubation. Electrophoresis results showed that napin subunits of untreated CS disappeared completely within the zero incubation period, whereas cruciferin subunits were degraded in the middle of the incubation period (16 h incubation period). In 4 and 6 min microwave irradiated CS, napin subunits were degraded after 4 and 16 h incubation periods, respectively, and cruciferin subunits were not degraded untile 24 h of incubation. In conclusion, it seems that microwave irradiation not only protected CP of CS from ruminal degradation, but also increased *in vitro* digestibility of CP. Moreover, microwave irradiation was effective in reducing glucosinolate and phytic acid contents of CS. (**Key Words** : Canola Seed, Microwave Irradiation, Protein Degradation, SDS-PAGE, Glucosinolate)

INTRODUCTION

In rapidly growing ruminants or dairy cows at early lactation, production may be limited by a low dietary rumen undegradable protein. In these conditions microbial protein synthesis is not sufficient to meet the animal's protein requirement (NRC, 2001), making it essential that the diet contain slowly degraded protein with a high potential for rumen escape.

Canola seed (CS) is utilized as an energy and protein source in ruminant diets. It contains approximately 21% protein and 43% oil and has an amino acid composition well-suited for ruminants (Wang et al., 1999). Because the protein of CS is highly degradable by rumen microbes (Madsen and Hvesplund, 1985; Mustafa et al., 2000), treatment of CS to increase rumen escape protein has received attention (Deacon et al., 1988; Wang et al., 1999). On the other hand, anti-nutritional factors, such as phytic acid and glucosinolate in brassica-originated feeds, are of major concern relative to animal originated-feeds (Sarıçiçek et al., 2005; Gharaghani et al., 2008).

Several heat processing methods have been used to enhance nutritive value of whole oilseeds, including extrusion, roasting, toasting and Jet-Sploding (Deacon et al., 1988; Wang et al., 1999; Yu et al., 2004; Chen et al., 2008; Fathi Nasri et al., 2008). Heat treatment decreases the solubility of proteins by creating cross-linkages both within and among peptides chains and to carbohydrates (Deacon et al., 1988; Mustafa et al., 2000).

Microwave has been extensively employed in food engineering and processing (Oliveira and Franca, 2002).

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Advantages of microwave irradiation compared to conventional heating are faster heating rates, shorter processing times and energy efficiency, since most of the electromagnetic energy is converted to heat (Fakhouri and Ramaswamy, 1993; Oliveira and Franca, 2002). Recently, treatment of oilseed meals with microwave irradiation was successful in reducing degradation of CP by rumen microorganisms and increasing CP digestibility (Sadeghi and Shawrang, 2006, 2007), but effects of microwave irradiation on nutritive value of oilseeds have not been evaluated.

The main objectives of this study were to determine effects of microwave irradiation on chemical composition, anti-nutritional factors, ruminal CP and DM degradability and *in vitro* CP digestibility of CS, and to monitor the ruminal fate of true proteins of untreated and irradiated CS by using SDS-PAGE methodology.

MATERIALS AND METHODS

Sample preparation and treatments

The CS sample (*Okapi* variety) was obtained from Oilseed Developing and Cultivation Company (Tehran, Iran). The DM of CS was determined by oven drying a 1 g sample in duplicate at 55°C for 48 h. Then sufficient water was added to the sample to increase the moisture content to 25%. Three samples (500 g each) were subjected to microwave irradiation (Butane Co. Model 245, Iran) at a power of 800 W for 2, 4 and 6 min. The CS samples were ground to pass a 2 mm screen for the *in situ* ruminal study.

Animals and diet

Three ruminally fistulated bulls (approximately 416±18 kg body weight) were fed 8 kg DM of a total mixed ration containing 70% of DM as forage (70% alfalfa hay and 30% wheat straw on DM basis) and 30% of DM as concentrate. The concentrate consisted of ground barley grain, canola meal, ground CS, cottonseed meal, wheat bran, dicalcium phosphate, and a vitamin+mineral premix (53, 13, 16, 4, 12, 1 and 1% on DM basis, respectively). Water and salt lick were available *ad libitum*. The diet was formulated according to National Research Council (NRC, 1996) beef guidelines to contain 13.9% CP and was fed twice daily at 08:00 and 16:00 h.

In situ ruminal degradability

The nylon bag technique (Ørskov and McDonald, 1979) was used to measure the kinetics of DM and CP degradation of treated and microwave irradiated CS. Nylon bags (10 cm ×20 cm; 45 μm pore size) were filled with approximately 6 g of sample (size: bag surface area of 15 mg/cm^2). Duplicate bags were incubated in the rumen for 0, 2, 4, 8, 16, 24 and 48 h. All bags were simultaneously placed in the rumen, just before the bulls were offered their first meal (i.e., 08:00 h). The bags were removed from the rumen and washed with tap water until the rinsing water was clear. The same procedure was applied to two bags to obtain the 0 h value. The residues were oven dried (at 55°C for 48 h) and analyzed for DM and CP to determine degradation kinetics of CS.

In vitro crude protein digestibility

The three-step *in vitro* procedure of Calsamiglia and Stern (1995) was used to estimate digestibility of rumen undegraded CP. Samples of the ruminal undegradable fraction collected after the 16 h incubation period containing approximately 15 mg N were incubated for 1 h in 10 ml HCl solution (0.1 N) containing 1 g/L of pepsin (Sigma P-7012, Sigma Chemical, St. Louis, MO). Following incubation, the pH was neutralized with 0.5 ml of 1 N NaOH and 13.5 ml of phosphate buffer (pH 7.8) containing 37.5 mg of pancreatin (Sigma P-7545, Sigma Chemical, St. Louis, MO) were added. Samples were incubated for 24 h at 38°C, and then undigested protein was precipitated using trichloroacetic acid (3 ml TCA). Samples were centrifuged at 10,000×g for 15 min, and then supernatants were analyzed for soluble N. *In vitro* CP digestibility was calculated as soluble N divided by amount of initial sample N (i.e., nylon bag residues).

Determination of protein subunits

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970) to fractionate protein subunits of untreated and irradiated CS. All the ruminal undegradable fractions from each incubation period were dried, well ground and replicates were pooled. Samples (20 mg) of untreated or irradiated CS were placed into 750 μl SDS-PAGE sample buffer. After 30 min of mixing (i.e., vortex and inverse), samples were immersed at 90°C for 3 min, and then centrifuged at 10,000×g for 1 min. A 30 μl aliquot of each sample was loaded into the sample cell of the gel. Electrophoresis of proteins was on 14% acrylamide running gel (1.0 mm×140 mm×190 mm) with 3.75% acrylamide stacking gel. The gels were kept at a constant current of 30 mA until the bromophenol blue marker dye reached the bottom of the gel. Protein fixation and staining were completed simultaneously using a solution of Coomassie brilliant blue. Gel destaining was accomplished by using a 30% methanol and 7% acetic acid solution. A standard protein mixture was used which included β-galactosidase (116 kDa), bovine plasma albumin (66.0 kDa), ovalbumin (45.0 kDa), lactate dehydrogenase (35.0 kDa), restriction endonuclease B*sp*981 (25 kDa), β-lactoglobulin (18.4 kDa) and lysozyme (14.4 kDa).

Table 1. Effects of microwave irradiation on chemical composition and anti-nutritional factors of canola seed

Parameters	Untreated CS	Microwave irradiated CS			Regression equations [1]		R^2	p
		2 min	4 min	6 min	Intercept (SE)	Slope (SE)		
Dry matter (g/kg)	918	912	916	908	971.1 (2.91)	-1.2 (0.78)	0.09	0.148
Crude protein (g/kg)	219	219	224	221	219.3 (2.38)	0.58 (0.64)	0.04	0.376
Ether extract (g/kg)	419	420	418	417	419.8 (1.67)	-0.32 (0.45)	0.02	0.486
Neutral detergent fiber (g/kg)	167	170	169	174	167.1 (2.22)	1.0 (0.59)	0.12	0.096
Acid detergent fiber (g/kg)	109	110	115	116	108.8 (2.42)	1.25 (0.64)	0.15	0.066
Ash (g/kg)	45.0	45.5	45.3	45.7	44.9 (0.40)	0.14 (0.11)	0.07	0.201
Phytic acid (g/kg)	25.7	23.6	18.6	13.2	26.6 (0.65)	-2.13 (0.17)	0.87	0.001
Glucosinolate (μmol/g)	23.4	13.7	10.6	9.6	21.0 (0.98)	-2.2 (0.26)	0.76	0.001

[1] Regression analysis with chemical composition and anti-nutritional factors of canola seed as the dependent variables and times of microwave irradiation (0, 2, 4 and 6 min) as the independent variable.

Chemical analyses

The DM content was determined at 55°C for 48 h in feed samples and nylon bag residues. The N in feed samples and residues after rumen and *in vitro* incubation was determined according to AOAC (Method 984.13; AOAC, 1995). Ash was determined by burning duplicate 2 g samples at 600°C for 2 h in a muffle furnace (Method 942.05; AOAC, 1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to the method of Van Soest et al. (1991), using an automatic fiber analyzer (Fibertec System M, Tecator, Sweden). A standard method was used to determine ether extract (AOAC, 1995; Method 920.39). The DM, CP, EE, NDF, ADF and ash contents of CS (6 replicates) before and after irradiation and DM and CP of the residues remaining after ruminal incubation were measured. Phytic acid was determined by the methods of de Boland et al. (1975). Total glucosinolate was determined spectrophotometrically according to the procedure described by Clifford and Smith (2006).

Data fitting and statistical analysis

Digestion kinetics of DM or CP were determined according to the equation of Ørskov and McDonald (1979) as:

$$P = a+b(1-e^{-ct})$$

Where, P is the amount degraded at a time, a the washout fraction, b the potentially degradable fraction, c the constant rate of disappearance of b, t the time of incubation (h). Effective degradability (ED) was calculated as:

$$ED = a+bc/(c+k)$$

where estimated ruminal outflow rates (k) of 0.02, 0.05 and 0.08 h^{-1} were used.

Degradability data were analyzed as a completely randomized block design according to the linear regression procedure of SAS (1996) Chemical composition and anti-nutritional factor data were analyzed as a completely randomized design according to the linear regression procedure of SAS (1996). Times of microwave irradiation (0, 2, 4 and 6 min) were considered as an independent variable and rumen degradation parameters, *in vitro* CP digestibility, chemical composition and anti-nutritional factors of CS were considered as dependent variables.

RESULTS

Effects of microwave irradiation on chemical composition and anti-nutritional factors of CS

The effects of microwave irradiation times on chemical composition and anti-nutritional factors of CS are shown in Table 1. Irradiation had no effect on chemical composition of CS ($p>0.05$). There was a linear decrease ($p<0.001$) in the phytic acid and glucosinolate contents of CS as irradiation time increased. Microwave irradiation for 2, 4 and 6 min decreased the phytic acid content by 8.2, 27.6 and 48.6%, respectively. The glucosinolate content of CS irradiated for 2, 4 and 6 min decreased by 41.5, 54.7 and 59.0%, respectively.

Effects of microwave irradiation on DM and CP degradability and *in vitro* CP digestibility

Ruminal degradability parameters of DM and CP and *in vitro* CP digestibility of untreated and irradiated CS are given in Table 2. There was a linear decrease in the washout fraction and a linear increase in the potentially degradable fraction of DM ($p<0.001$). Microwave irradiation for 2, 4 and 6 min decreased the washout fractions of DM by 13.5, 26.1 and 36.8%, respectively, and increased the potentially degradable fraction of DM by 7.4, 13.9 and 18.7%, respectively, compared to the untreated sample. The washout fraction of CP decreased linearly ($p<0.001$) and the potentially degradable fraction of CP increased linearly ($p<0.001$) as irradiation time increased. The washout fraction of CP decreased by 11.1, 20.9 and 37.5% and the potentially degradable fraction of CP increased by 6.6, 11.5 and 21.2% in samples irradiated for 2, 4 and 6 min,

Table 2. Rumen degradation parameters of dry matter and crude protein and *in vitro* crude protein digestibility of undegraded protein of untreated and microwave-irradiated canola seed

Parameters	Untreated canola seed	Microwave-irradiated canola seed			Regression equations[1]		R^2	p
		2 min	4 min	6 min	Intercept (SE)	Slope (SE)		
Dry matter								
a (g/kg)	390.0	337.2	288.2	246.6	387.4 (7.34)	-24.0 (1.96)	0.87	0.001
b (g/kg)	545.6	589.3	634.0	671.0	544.0 (10.33)	22.3 (2.76)	0.74	0.001
a+b (g/kg)	935.6	926.5	922.1	917.7	929.9 (11.08)	0.13 (2.96)	0.05	0.964
c $(h)^{-1}$	0.102	0.075	0.057	0.055	0.097 (0.0028)	-0.008 (0.0007)		
Effective rumen degradation (g/kg)								
0.02 h^{-1}	846.0	802.0	755.0	735.7	834.4 (7.74)	-17.8 (2.07)	0.76	0.001
0.05 h^{-1}	755.0	690.7	623.3	594.8	747.6 (7.55)	-27.1 (2.02)	0.89	0.001
0.08 h^{-1}	695.3	622.8	549.8	517.2	886.9 (7.74)	-30.1 (2.07)	0.90	0.001
Crude protein								
a (g/kg)	451.3	401.1	356.8	281.9	451.7 (8.48)	-26.0 (2.26)	0.85	0.001
b (g/kg)	539.6	577.5	609.6	684.9	534.0 (8.1)	23.1 (2.16)	0.83	0.001
a+b (g/kg)	990.9	978.6	966.3	966.9	985.7 (6.94)	-2.9 (1.85)	0.06	0.131
c $(h)^{-1}$	0.112	0.099	0.076	0.060	0.113 (0.0026)	-0.009 (0.0007)	0.87	0.001
Effective rumen degradation (g/kg)								
0.02 h^{-1}	909.5	875.5	838.0	791.3	917.3 (4.16)	-19.0 (1.11)	0.93	0.001
0.05 h^{-1}	824.8	785.5	723.0	651.5	837.5 (6.22)	-27.5 (1.66)	0.92	0.001
0.08 h^{-1}	766.5	722.0	652.5	572.3	781.2 (6.97)	-30.8 (1.86)	0.92	0.001
In vitro CP digestibility (g/kg)								
	579.3	623.5	686.8	701.0	560.2 (8.38)	27.4 (2.24)	0.87	0.001

a, the washout fraction (g/kg); b, the potentially degradable fraction (g/kg); c, the rate of degradation.

[1] Regression analysis with rumen degradation parameters and *in vitro* crude protein digestibility of canola seed as the dependent variables and times of microwave irradiation (0, 2, 4 and 6 min) as the independent variable.

respectively. Microwave irradiation decreased degradation rates of the *b* fraction of DM and CP ($p<0.001$). Irradiation for 2, 4 and 6 min decreased the effective degradability (ED) of DM at a ruminal outflow rate of 0.05 h^{-1} by 8.5, 17.4 and 21.2%, respectively. ED of CP decreased as irradiation time increased ($p<0.001$). Irradiation for 2, 4 and 6 min decreased ED of CP at a ruminal outflow rate of 0.05 h^{-1} by 4.7, 12.3 and 21.0%, respectively. Microwave irradiation for 2, 4 and 6 min increased *in vitro* CP digestibility of CS by 7.1, 15.6 and 17.4%, respectively.

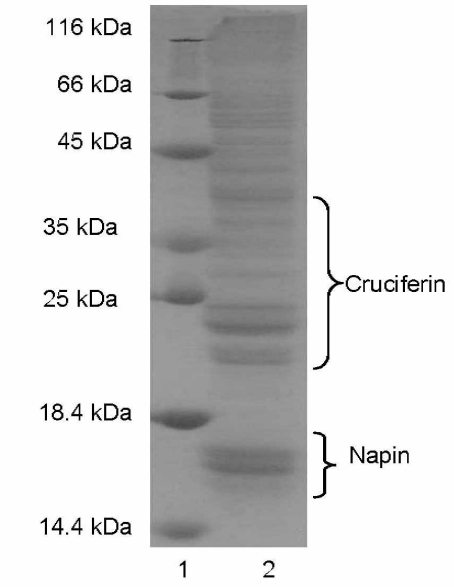

Figure 1. Molecular weights of standard protein (lane 1) and canola seed protein subunits (lane 2).

Effects of microwave irradiation on electrophoretic profiles of CS protein subunits

The SDS-PAGE analysis of standard protein and protein subunits of CS is presented in Figure 1. The two major protein components in CS are cruciferin (12S globulin) with four subunits, and napin (2S albumin) with two subunits. The approximate molecular weights of the cruciferin subunits were 40.2, 38.4, 23.9 and 21.1 kDa, and of napin subunits were 16.3 and 15.2 kDa. The SDS-PAGE gels of untreated, 2, 4 and 6 min microwave-irradiated CS proteins are presented in Figure 2. Each line represents the hours of incubation (i.e., 0, 2, 4, 6, 8, 16, 24 and 48 h of incubation in the rumen). Electrophoretic analysis of untreated CS proteins (Figure 2A) incubated in the rumen showed that the napin subunits disappeared at zero incubation time, whereas the cruciferin was resistant to degradation until 16 h of incubation. From the SDS-PAGE patterns (Figure 2C, D), napin subunits of 4 and 6 min microwave-irradiated CS were degraded after 4 and 16 h of incubation in the rumen, respectively, whereas the cruciferin subunits of microwave-irradiated CS were not degraded completely until 24 h of incubation.

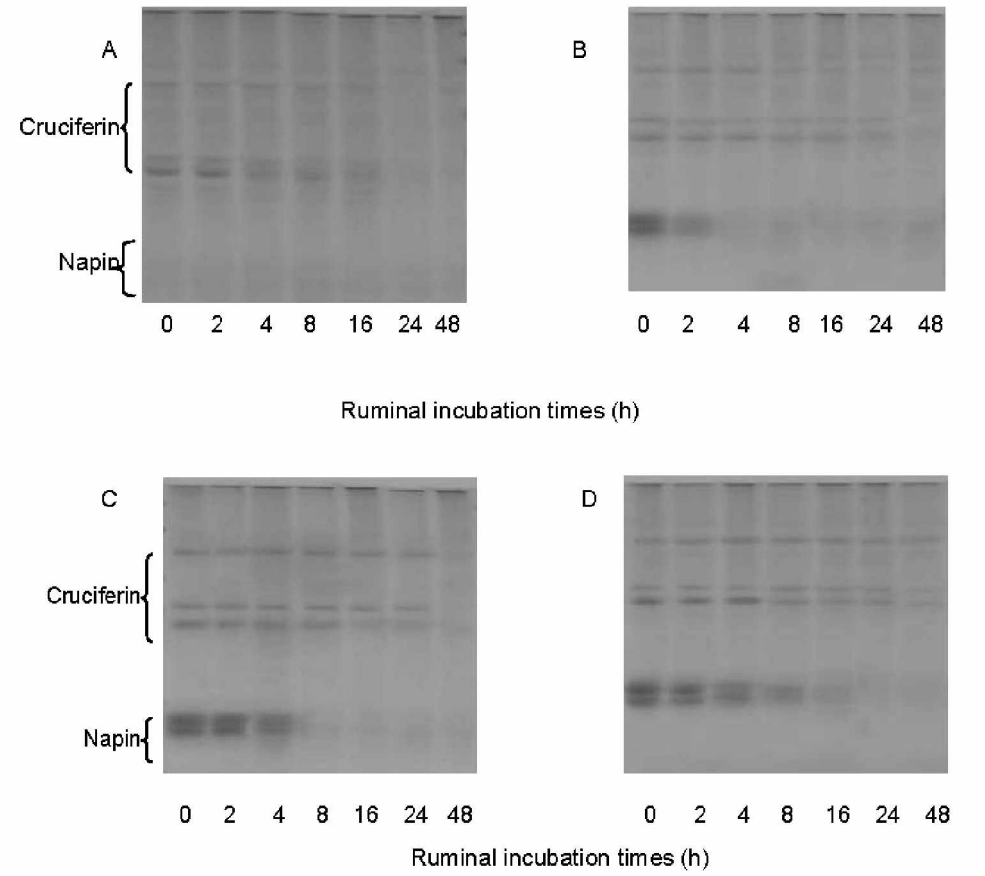

Figure 2. Electrophoretic patterns of untreated (A), 2 min (B), 4 min (C) and 6 min (D) microwave-irradiated canola seed protein incubated in the rumen.

DISCUSSION

Chemical composition and anti-nutritional factors of CS

Microwave irradiation had no effect on chemical composition of CS, which is consistent with results of other studies (Khatoon and Prakash 2004; Dong et al., 2005; Alajaji and El-Adawy, 2006). The glucosinolate content (23.4 μmol/g) of untreated CS in this study was reduced by microwave irradiation. Similarly, Maheshwari et al. (1980) showed that microwaving of rapeseed meal (98.7% DM) for 2.5 min at 2,450 MHz decomposed glucosinolate by 37%. Vallejo et al. (2002) reported that microwaving of broccoli (15% moisture) for 5 min at 1,000 W decreased glucosinolate content by 74%. Reduction in glucosinolate content of rapessed meal with heat treatment has been variously reported (Fenwick et al., 1986; Huang et al., 1995; Jensen et al., 1995). The micronization process of rapeseed meal for 90 s at 195°C and wet extrusion (150°C, 200 rpm) of high rapeseed meal were effective in reducing glucosinolate content (Fenwick et al., 1986; Huang et al., 1995). Major deleterious effects of glucosinolate ingestion in animals are reduced palatability, decreased growth and reduced production. Glucosinolate reduction in CS by irradiation is due to inactivating myrosinase enzyme, glucosinolate degradation and removal of breakdown products such as isothiocyanates and oxazolidinethione (Fenwick et al., 1986; Huang et al., 1995; Oerlemans et al., 2006).

Microwave irradiation decreased the phytic acid content of CS. Mubarak (2005) showed that phytic acid of mung bean seeds was reduced by microwave irradiation at 2,450 MHz for 15 min. Also, Alajaji and El-Adawy (2006) reported that microwave irradiation of chickpea at 2,450 MHz for 15 min and autoclave processing (121°C) were effective in reducing phytic acid content. Since phytic acid chelates minerals and protein, forming insoluble complexes, which leads to reduced bioavailability of trace minerals and reduced digestibility of protein in the small intestine (Duodu et al., 1999; Debnath et al., 2005; Taghinejad et al., 2009), its reduction with microwave irradiation may enhance the nutritional value of CS. The reduction of phytic acid is probably due to chemical degradation of phytate to lower inositol phosphates and inositol or cleavage of the phytate ring itself (De Boland et al., 1975; Duodu et al., 1999; Chen and Betty, 2003).

Ruminal degradation of irradiated CS

Maximum potential degradability (a+b) of DM and CP were 935.6 and 990.9 g/kg, respectively, for untreated CS, indicating that CS was highly degradable in the rumen. Microwave irradiation decreased the washout fraction and increased the potentially degradable fraction of CP. These results were supported by electrophoretic analyses of the true protein of bag residues. Increasing the amount of dietary protein available for digestion in small intestine is nutritionally benefical for high-producing animals such as lactating cows and rapidly growing calves (Wang et al., 1999). Microwave irradiation decreased ED of DM and CP in agreement with Sadeghi and Shawrang (2006) and showed that microwave irradiation for 2, 4 and 6 min decreased the effective CP degradability of canola meal at a ruminal outflow rate of 0.05 h^{-1} by 21, 24 and 29%, respectively. It is well known that unfolding and denaturation of proteins with heat processing can break bonds that stabilize the three-dimensional structure of proteins. If hydrophobic groups are exposed, this will result in reduced solubility of proteins and reduce their ruminal degradabilty (Voragen et al., 1995; Fathi Nasri et al., 2008). Similar effects of heat treatment on ruminal CP degradation characteristics of CS have been reported (Deacon et al., 1988; Wang et al., 1999). Wang et al. (1999) reported that micronization of CS reduced the soluble fraction and increased the potentially degradable fraction of both total amino acids and essential amino acids. Other reactions that proceed with longer heat processing time, such as iso-peptide cross-links involving amino acids, such as lysine and serine (Finley, 1989), asparagines and glutamine (Hurrell and Carpenter, 1977), and methionine and tryptophan (Broderick et al., 1991), may explain the additional effects of heat processing time on ED of CP. These effects were mainly attributed to microwave heating, but Banik et al. (2003) have suggested that there are non-thermal microwave effects in terms of the energy required to produce various types of protein transformation and alteration, and proposed that the microwaves either caused ions to accelerate and collide with other molecules or caused dipoles to rotate and line up rapidly with an alternating (2,450 million times/s) electric field, resulting in a change in secondary and tertiary structures of protein. When secondary and tertiary structures of a protein are unfolded, proteins may be converted to high molecular weight aggregates due to generation of inter-protein cross-linkages, hydrophobic and electrostatic interactions, and formation of disulfide bonds (Lee Maire et al., 1990; Guan et al., 2006), thereby reducing ruminal CP degradability.

Effects on electrophoretics profile of protein subunits of irradiated CS

In untreated CS, the napin subunits disappeared at zero incubation time (Figure 2A), but cruciferin subunits were more resistant to degradation (Figure 2A). Napin (2S albumin) is a soluble protein containing high levels of hydrophilic amino acids (i.e., glutamic acid, cysteine). Rumen degradability of napin was more rapid than cruciferin (12S globulin), a globular protein rich in hydrophobic amino acid (methionine). This result is consistent with studies of PrestlØkken (1999) and Fathi Nasri et al. (2008), who reported that hydrophobic amino acids, such as leucine, isolecine, phenylalanine, methionine, valine, alanine and tyrosine, were degraded to a lesser extent than more hydrophilic amino acids, such as histidine, arginine, lysine, cysteine, glutamic acid, glycine and serine. Napin subunits of irradiated CS did not disappear with water washing (Figure 2B, C and D); irradiation induced denaturation of this protein (Finley, 1989; Voragen et al., 1995) and reduced solubility and disappearance with water washing.

The cruciferin subunits of untreated CS represented a large proportion of the protein remaining in the bag residue. Microwave irradiation for 2 min had a slight effect in decreasing ruminal CP degradability of CS, but microwave irradiation for 4 and 6 min decreased ruminal CP degradability. This electrophoretic pattern regarding cross-linking of proteins was also observed in the study of Sadeghi and Shawrang (2006) by microwaving of canola meal. Heat treatment of protein will transform the proteins to a more enzyme-resistant structure (Englard and Seifter, 1990). In addition, chemical reactions such as the Maillard reaction, denaturation of proteins occurring during heat processing, and cross-linkages occurring both within and among peptide chains and to carbohydrates, may be responsible for reduction in ruminal CP degradation (Deacon et al., 1988; Finley, 1989; Voragen et al., 1995).

Effects on *in vitro* protein digestibility of irradiated CS

The CP digestibility value of untreated CS was similar to that obtained by Gonzalez et al. (2003) and higher than that reported by Azarfar et al. (2008) for rapeseed. The value of 680 g/kg was reported by Shawrang et al. (2008) for intestinal CP digestibility of canola meal. Discrepancies in reported CP digestibility values may be due to differences in methods of estimating CP digestibility and in the variety of seed. Microwave irradiation increased *in vitro* CP digestibility of CS. Our results are consistent with the results of Sadeghi and Shawrang (2006, 2007), who showed that microwave irradiation of canola and cottonseed meals for 4 min increased CP digestibility. Also, Alajaji and El-Adawy (2006) reported that microwave irradiation increased *in vitro* CP digestibility of chickpea. Similar results have been demonstrated by heat treatment of canola meal and full-fat soybean (Moshtaghi Nia and Ingalls, 1992; Fathi Nasri et al., 2008). Wang et al. (1999) showed

micronization of CS increased intestinal disappearance of amino acids. Microwave irradiation may induce unfolding of the protein and its denaturation, thereby exposing hydrophobic amino acids (especially aromatics) that are positional groups for the active sites of pepsin and trypsin enzymes (Negi et al., 2001; Murray et al., 2003; Alajaji and El-Adawy, 2006). Moreover, the improvement in CP digestibility may be attributed to reduction of phytic acid (Negi et al., 2001; Alajaji and El-Adawy, 2006).

IMPLICATIONS

The microwave irradiation of CS was effective in decreasing ruminal CP degradability with a positive effect on *in vitro* CP digestibility that is benefical for high producing dairy cows and rapidly growing ruminants. Also, microwave irradiation may be an effective method for decreasing glucosinolate and phytic acid contents of CS. Electrophoretic analysis of untreated CS proteins incubated in the rumen showed that the main rumen undegradable true protein was cruciferin alone and for microwave-irradiated CS protein, it was both napin and cruciferin. The results suggest that microwave irradiation may be a useful method for improving the nutritive value of CS. However, further study is needed to evaluate the cost of this process, as well as the technical feasibility of setting up an industrial process.

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