Effects of temperature, heating time and particle size on values of rumen undegradable protein of roasted soybean

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Abstract

Roasted soybeans are commonly used in diets for dairy cows, but the processing conditions of roasted soybean and their effects on rumen undegradable protein (RUP) have not been fully understood. In this study, the effect of the processing of roasted soybean on RUP was examined through a factorial arrangement of treatments. The effects of roasting temperature (115 ºC, 130 ºC and 145 ºC), time of roasting (5, 10 and 15 min) and particle size of soybean (whole, coarse and fine) on the protein fractions of roasted soybean were analysed with the Cornell Net Carbohydrate and Protein System (CNCPS) and protein dispersibility index (PDI) methods. The whole, coarsely ground and finely ground soybean had mean particle sizes of 5.30 mm, 1.15 mm and 1.05 mm. The proportion of soluble protein was reduced with increasing temperature and time of roasting. The true protein with intermediate rates of degradation decreased with escalating temperatures, but increased when the time was raised and the particle size reduced. Also, the true protein level with slow rates of degradation was raised with increasing temperature and heating time, but reduced when decreasing the particle size. The undegradable true protein level, on the other hand, was increased by decreasing the particle size. The results of CNCPS and PDI revealed that the recommended method would be to use soybeans with a particle size of 1.15 mm, and to roast them at 115 ºC for 10 minutes. Furthermore, the results showed that different methods of processing used in soybean roasting could strongly affect RUP content.

Introduction

Soybean seeds contain 19.2% oil and 39.2% protein (NRC, 2001). Soybean is therefore regarded as an economical and convenient source of dietary fat and protein (Dhiman *et al.*, 1997). However, raw soybean contains several anti-nutritional factors that reduce its nutritional value, among which are anti-trypsin, urease and hemagglutinins. To eliminate these anti-nutritional substances, soybean seeds are processed. Heat is the most common way of doing so (Reddy *et al.*, 1993). Heating results in cross linking between the peptide chain (free amino acid groups) and carbohydrate (sugar aldehyde groups), thus decreasing protein solubility and increasing the rumen bypass protein (Reddy *et al.*, 1993; Dhiman *et al.*, 1997). The processing temperature must be controlled carefully to ensure that the beans are heated sufficiently to eliminate anti-nutritional factors, but not excessively as this could increase processing costs and reduce protein quality. High temperatures also damage the nutritional value of soybean seeds, because they reduce the degradability of protein in the rumen and small intestine (Reddy *et al.*, 1993).

Extrusion (Annextad *et al.*, 1987), forced-air oven treatment (Faldet *el al.*, 1992b) and roasting (Faldet & Satter, 1991) are among the thermal methods that are used to process soybean seeds. Energy and fixed costs are lower for roasting than for extrusion (Reddy *et al.*, 1993). Socha (1991) reviewed 16 published feeding trials and reported that, compared with soybean meal and raw soybeans, roasted soybeans increased milk production in dairy cows by 1.65 kg/day and fat-corrected milk (FCM) by 1.95 kg/day. There was no effect, however, on dry matter (DM) intake. Roasted soybeans could be considered an excellent source of RUP, but there could be variations in quality. For example, Faldet *et al.* (1991) examined 13 samples of roasted soybeans and found that product quality varied greatly. Rumen undegradable protein

content ranged from 48% to 61% of crude protein (CP). There is little information about the holding times and particle sizes of roasted soybean. Faldet *et al.* (1992b) reported that the optimal heat treatment for soybeans in a forced air oven was 140 ºC for 120 minutes. Dhiman *et al.* (1997) concluded that roasted soybeans in half and quarter sizes are optimal for milk production. The nutritional qualities of soybean proteins are essentially determined by amino acid composition, amino acid availabilities (digestibility) and the contents of biologically active components. Of these factors, the last two are mostly affected by processing conditions (Del Valle, 1981). In general, different conditions of processing could have significant impacts on the amount of bypass protein available in roasted soybeans (Faldet *et al.*, 1991; 1992a).

In vivo, *in vitro* and *in sacco* techniques are employed to estimate the ruminal degradability of protein in feedstuffs (Elwakeel *et al.*, 2007). The best method for evaluating protein source is the *in sacco* approach, but it is expensive, needs cannulated animals and is not practical for the usual evaluation of feeds. On the other hand, it is good for estimating ruminal protein degradation, but soluble and small particles of protein could leave the bags through the pores without complete degradation (Ho *et al.*, 2008). Simple procedures that are suitable for use by commercial feed testing laboratories are required to monitor the quality of an increasing supply of heat-processed soybeans that are intended as a protein and energy supplement for ruminants. The protein dispersibility index (PDI) has been claimed to have the most constant response to heating full-fat soybeans. Another *in vitro* procedure that is used to estimate protein quality is the Cornell Net Carbohydrate and Protein System (CNCPS) (Sniffen *et al.*, 1992).

The CNCPS is one of the schemes that have been developed to fractionate protein in feeds. In this system, feed protein is partitioned into three fractions: non protein nitrogen (fraction A), true protein (fraction B) and undegradable true protein (fraction C). True protein, based on its rates of rumen degradation, is divided into three subfractions (B_1, B_2 and B_3). The rumen degradation rates of B_1, B_2 and B_3 fractions are 120% to 400% /h, 3% to 16% /h, and 0.06% to 0.55% /h, respectively (Schwab *et al.*, 2003). Fractions A and B_1 are soluble and rapidly degraded in the rumen (soluble protein). The fractions of protein in CNCPS system are A (NPN, soluble in borate-phosphate buffer, but not precipitated with trichloroacetic acid); B_1 (rapidly degradable true protein; soluble in borate-phosphate buffer and precipitated with trichloroacetic acid); B_2 (moderately degradable true protein, calculated as the difference between total CP and the sum of the other four CP fractions); B_3 (slowly degradable true protein, calculated as the difference between neutral detergent insoluble CP [NDICP] and acid detergent insoluble CP [ADICP]); and C (undegradable true protein, measured as ADICP) (Licitra *et al.*, 1996; Schwab *et al.*, 2003).

The use of roasted soybeans in the diet of dairy cows is common. However, factors such as temperature, heating time and particle size could influence the quality. The most suitable conditions for roasting are unknown and need further research. The main objective of this study was to use the CNCPS system and PDI method to estimate the effects of temperature, roasting time and particle size of soybean on protein fractions and bypass protein.

Materials and Methods

First, soybean seeds were prepared from the same batch in three particle sizes (whole, coarsely ground and finely ground). The coarse-grounds and fine-ground soybeans were obtained by passing whole soybean through a hammer mill (model 55, Gehl Bros Mfg Co, West Bend, Wis) using 9 mm and 6 mm screen sizes, respectively. The geometric mean particle size was then calculated with a dry-sieving technique (ASAE, 1995). For this evaluation, 100 g of each experimental soybean was sieved in duplicate in a column with a series of eight sieves, with 6.35, 4.75, 3.35, 2.36, 2.00, 1.70, 0.85 and 0.60 apertures, in a vertical oscillator for 10 minutes. The mean particle size (MPS) was calculated by fitting the data to a log normal distribution (Ensor *el al.*, 1970). The average geometric mean particle sizes were 5.30 mm, 1.15 mm and 1.05 mm for whole, coarsely ground and finely ground soybean, respectively. The distribution of soybean particles on the sieves is shown in Table 1.

Soybeans in various particle sizes were roasted at 115, 130 and 145 ºC (Gem Roaster, Winona, Minn) and held at that temperature for 5, 10 and 15 minutes. The soybeans were allowed to cool immediately after being removed from the roaster. Subsequently, in this trial the researchers had 27 treatments, three particle sizes (whole, coarse and fine), three roasting temperature (115 ºC, 130 ºC and 145 ºC) and three holding times (5, 10 and 15 min). Dry matter content of soybean samples was determined after drying at 60 ºC in a forced-air oven for 48 hours, and ground to pass through a 1-mm screen (Wiley mill, Arthur H. Thomas Co, Philadelphia, Phil). The ground samples were analysed for neutral detergent fibre (NDF) through heat stable alpha-amylase (100 µL/sample) (Van Soest *et al.*, 1991), ash, CP (AOAC, 2000) and acid detergent fibre (ADF) (Van Soest *et al.*, 1991). Extra samples of the NDF and ADF components were processed for their acid detergent insoluble CP (ADICP) and neutral detergent insoluble CP (NDICP). Nitrogenous fractions of diets were partitioned into five fractions, A, B_1, B_2, B_3 and C,

according to CNCPS (Sniffen *et al.*, 1992), using standardization of procedures for nitrogen fractionation, as described by Licitra *et al.* (1996) (three replicates).

The RDP and RUP values were computed using the following equations:

$$RDP = A + B_1 [k_d B_1 / (k_d B_1 + k_p)] + B_2 [k_d B_2 / (k_d B_2 + k_p)] + B_3 [k_d B_3 / (k_d B_3 + k_p)]$$
$$RUP = B_1 [k_p / (k_d B_1 + k_p)] + B_2 [k_p / (k_d B_2 + k_p)] + B_3 [k_p / (k_d B_3 + k_p)] + C$$

The rate of passage (k_p) was equal to 0.08% /h and the rates of degradation (k_d) for parts B_1, B_2 and B_3 were taken to be 150%, 5% and 0.18% per hour, respectively.

Table 1 Least squares means of distribution of soybean particles on the sieves

Item	Roasting temperature, ºC			Holding time, min			Particle size, mm			Raw seed
	115	130	145	5	10	15	WS	CS	FS	
Mean geometric particle size, mm										
	2.52[a]	2.51[a]	2.47[b]	2.50[a]	2.53[ab]	2.47[b]	5.30[a]	1.15[b]	1.05[c]	5.69
Sieve opening, mm										
6.35	17.10[a]	17.55[a]	15.88[b]	17.05	17.25	16.23	49.51[a]	0.25[b]	0.76[b]	67.36
4.75	16.57[a]	15.53[ab]	15.72[b]	15.66	16.46	15.70	43.90[a]	2.95[b]	0.97[c]	29.81
3.35	4.76	4.81	4.66	4.78[ab]	5.01[a]	4.44[b]	4.68[b]	8.14[a]	1.40[c]	2.17
2.36	12.83[a]	13.12[a]	10.76[b]	12.23	12.29	12.18	1.36[c]	22.56[a]	12.80[b]	0.46
2.00	6.50	6.79	6.22	6.71	6.19	6.61	0.10[c]	8.49[b]	10.92[a]	0.09
1.70	11.02[a]	10.02[ab]	9.78[b]	9.88	10.90	10.03	0.10[c]	13.03[b]	17.67[a]	0.08
0.85	25.89[b]	25.73[b]	30.35[a]	27.35[b]	25.38[c]	29.24[a]	0.18[c]	34.03[b]	47.76[a]	0.00
0.60	2.90	2.58	2.91	3.17[a]	2.77[ab]	2.45[b]	0.04[c]	4.72[a]	3.63[b]	0.00
pan	3.22[ab]	3.79[a]	2.80[b]	3.12[ab]	3.72[a]	2.98[b]	0.02[c]	5.73[a]	4.06[b]	0.03

[a,b,c] Row means with different superscripts differ significantly at *P* <0.05.
WS: whole soybean; CS: coarse-ground soybean; FS: fine-ground soybean.

The PDI procedure involved blending 20 g of the ground soybean sample with 300 mL distilled water for 10 min in a Waring blender, which operated at 8500 rpm and 21 ºC. After blending, the mixture was centrifuged at 1118 x g (2500 rpm) (Beckman 12-21 centrifuge with JA-14 rotor; Beckman Instruments, Inc., Palo Alto, Calif) for 10 min. The supernatant was analysed for Kjeldahl-N to obtain a PDI such that PDI = (total supernatant CP/total CP in blended soybean sample) x 100 (AOCS, 1985). Hsu & Satter (1995) concluded that the ideal range for PDI was 9% to 11%. A PDI between 11% and 14% indicated marginal heating, and underheated soybeans have a PDI greater than 14% (Satter *et al.*, 1993). PDI values above 14% corresponded with being under-processed, whereas values below 9% were related to over-processing (Hsu & Satter, 1995). The data were subjected to ANOVA with the general linear models (GLM) procedure proposed by the SAS Institute, Inc. (SAS, 2001). Results were analysed according to a 3 × 3 × 3 factorial design (27 treatments with three replicates), with raw soybeans being studied as controls. The main sources of variation were particle size, roasting temperature and holding time. Sums of squares for treatments were partitioned to provide linear (L) and quadratic (Q) contrasts. Significant differences between individual means were identified using the Duncan test. Significance was set at *P* <0.05, and a trend toward significance was taken at *P* <0.10 unless stated otherwise. The basic model was:

$$Y_{ijk} = \mu + P_i + T_j + H_k + e_{ijk}$$

where Y_{ijk} = all dependent variables represented; μ = overall mean; P_i = the average effect of particle size i; T_j = the average effect of roasting temperature j; H_k = the average effect of holding time temperature k; e_{ijk} = random residual assumed to be normally distributed.

The Pearson correlation coefficients were estimated using the CORR procedure of SAS (2001).

Results

The distribution of soybean particles on the sieves is shown in Table 1. The particle size decreased with increased roasting temperatures and holding times, because soybeans roasted at 145 ºC or held for 15 min in the roaster had lower MPS compared with other roasting temperatures and holding times.

The effects of processing on DM, organic matter (OM), CP, NDF, NDICP, ADF and ADICP are shown in Table 2. Dry matter was increased linearly with increasing time and temperature (P <0.01), and coarse-ground soybean had the highest DM (96.8%). Roasting temperature had no effect on OM (P >0.05), whereas with increasing the roasting time and particle size of soybeans, OM level was raised linearly (P <0.01). Crude protein percentage was not influenced by treatments. ADF level was not significantly changed by the roasting time either. A quadratic response occurred with roasting temperature for ADF; the roasting temperature of 130 had the lowest ADF concentration (14.28%). However, the NDF level increased linearly with the time and temperature of roasting (P <0.05). There was a linear increase in ADF and NDF levels as the particle size of the roasted soybean was decreased (P <0.01). The ADICP and NDICP levels of roasted soybeans followed the same trend as ADF and NDF levels. In general, DM, OM and NDICP were increased and NDF and ADICP were decreased in the roasted soybean, in comparison with the raw soybean.

Table 2 Least squares means of processing effect on different parts of roasted soybeans (% dry matter)

Item	Roasting temperature, ºC			Holding time, min			Particle size, mm			Raw seed
	115	130	145	5	10	15	WS	CS	FS	
DM	94.7[c]	96.4[b]	97.8[a]	95.6[c]	96.3[b]	97.1[a]	96.5[b]	96.8[a]	95.5[c]	88.5
OM	93.6	93.8	93.8	93.5[b]	93.8[a]	93.9[a]	94.2[a]	93.7[b]	93.6[c]	92.5
CP	39.82	39.91	40.08	39.84	39.74	40.23	39.69	39.77	40.35	38.62
ADF	15.57[a]	14.28[b]	15.00[a]	15.00	15.03	14.82	16.98[a]	14.82[b]	13.04[c]	16.94
ADICP	3.87[ab]	3.80[b]	4.31[a]	4.01	4.04	3.93	4.96[a]	3.83[b]	3.19[c]	5.08
NDF	21.95[b]	22.14[b]	26.24[a]	22.68[b]	23.21[ab]	24.44[a]	25.96[a]	23.49[b]	20.88[c]	21.27
NDICP	8.90[c]	10.03[b]	15.03[a]	10.46[b]	11.13[b]	12.37[a]	12.91[a]	11.65[b]	9.40[c]	6.26

[a,b,c] Row means with different superscripts differ significantly at P <0.05.
WS: whole soybean; CS: coarse-ground soybean; FS: fine-ground soybean.
DM: dry matter; OM: organic matter; CP: crude protein; ADF: acid detergent fibre; NDF: neutral detergent fibre assayed with a heat stable amylase and expressed inclusive of residual ash; NDICP: neutral detergent insoluble CP;
ADICP: acid detergent insoluble CP.

The effects of processing on different fractions of protein are presented in Table 3. The non-protein nitrogen (NPN, A fraction) declined linearly with increasing roasting time and temperature and particle size of soybean (P <0.05). True protein (including B_1, B_2 and B_3 fractions) was increased linearly with increasing the time and temperature of roasting (P <0.01). Also, a quadratic effect of particle size of soybean in true protein was observed (P = 0.07). The effect of roasting temperature on different fractions of protein (%CP) is presented in Figure 1.

True protein was higher for coarse-ground soybeans, compared with whole and finely ground (32.8% vs. 31.7% and 31.8% DM, respectively). Rapidly degradable true protein (B_1 fraction) declined linearly with increasing the time and temperature of roasting and grinding the soybean (P <0.05). Moderately degradable true protein (B_2 fraction) also showed a quadratic effect of roasting temperature (P <0.01). Soybeans roasted at 130 ºC had higher B_2 compared with those roasted at 115 and 145 ºC (an average of 23.5% vs. 22.5% and 19.6% DM, respectively). The effects of holding time on different fractions of protein (%CP) are presented in Figure 2.

Fraction B_2 was increased with the time of roasting and grinding level (P <0.05). Slowly degradable true protein (B_3 fraction) increased with expanding particle size, time and temperature of roasting (P <0.05). Generally, processing reduced NPN, and B_1 and C fractions and increased B_2 and B_3 parts in the roasted soybeans, compared with the raw ones.
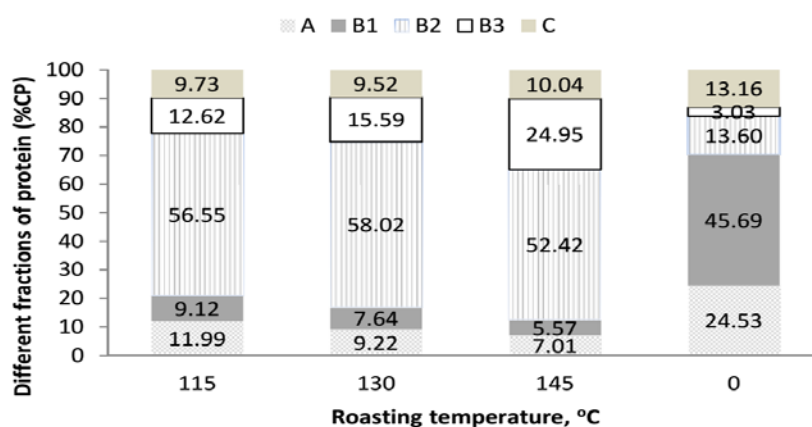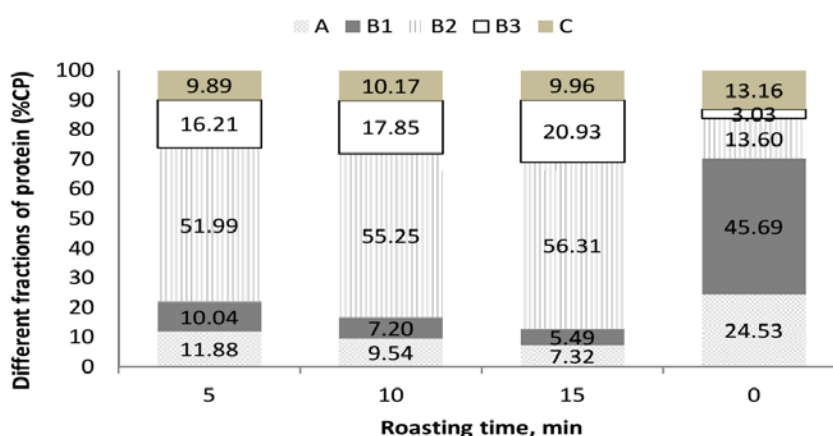
Table 3 Least squares means of processing effect on protein fractions of roasted soybeans (% dry matter)

Item	Roasting temperature ºC			Holding time min			Particle size mm			Raw seed
	115	130	145	5	10	15	WS	CS	FS	
A	4.77^a	3.68^b	3.01^b	4.72^a	3.79^{ab}	2.95^b	3.01^b	3.13^b	5.32^a	9.47
B	31.17^b	32.42^a	32.75^a	31.10^c	31.91^b	33.33^a	31.70^b	32.80^a	31.83^b	24.07
B_1	3.63^a	3.05^{ab}	2.39^b	3.99^a	2.86^b	2.21^b	4.61^a	2.49^b	1.97^b	17.64
B_2	22.50^a	23.15^a	19.63^b	20.66^b	21.95^a	22.68^a	19.15^b	22.48^a	23.65^a	5.25
B_3	5.02^c	6.22^b	10.71^a	6.44^b	7.09^b	8.43^a	7.94^a	7.82^a	6.20^b	1.17
C	3.87^{ab}	3.80^b	4.31^a	3.93	4.04	4.01	4.96^a	3.83^b	3.19^c	5.08

[a,b,c] Row means with different superscripts differ significantly at $P < 0.05$.
WS: whole soybean; CS: coarse-ground soybean; FS: fine-ground soybean.
A: non-protein nitrogen; B: true protein; C: un-degradable true protein; B_1: rapidly degradable true protein;
B_2: moderately degradable true protein; B_3: slowly degradable true protein.

Figure 1 Effects of roasting temperature on different fractions of protein (% crude protein (CP)).

Figure 2 Effects of holding time on different fractions of protein (% crude protein (CP)).

Treatment effects on soluble and bypass protein can be seen in Table 4. Soluble protein decreased linearly with increasing the time and temperature of roasting ($P < 0.01$). Particle size had a quadratic effect on soluble protein. Soybeans with 1.15 mm particle size had lower soluble protein than those of 5.30 and

1.05 mm (an average of 5.63% vs. 7.62% and 7.29% DM, respectively). Increasing the time and temperature of roasting linearly enhanced RUP and decreased RDP. There was no difference between whole and coarsely ground soybeans in RUP and RDP content, but the finely ground soybean had lower RUP than whole and coarsely ground soybeans ($P <0.05$). PDI declined linearly with increasing the time and temperature of roasting and increased linearly with decreasing the particle size of soybean grains ($P <0.05$). In general, the roasted soybeans had lower soluble protein and PDI and higher bypass protein than raw ones. The effects of particle size of soybean on different fractions of protein (% CP) are presented in Figure 3. The correlation between RUP and the variable that affected the RUP content of the roasted soybean is presented in Table 5.

Table 4 Least squares means of processing effect on soluble protein, rumen degradable protein and rumen undegradable protein and protein dispersibility index of roasted soybeans (% dry matter)

Item	Roasting temperature, ⁰C			Holding time, min			Particle size, mm			Raw seed
	115	130	145	5	10	15	WS	CS	FS	
SP	8.41[a]	6.73[b]	5.41[b]	8.71[a]	6.66[b]	5.17[c]	7.62[a]	5.63[b]	7.29[a]	27.11
RUP	23.24[c]	24.29[b]	27.00[a]	23.24[c]	24.62[b]	26.25[a]	24.75[b]	25.44[a]	23.92[c]	9.28
RDP	16.99[a]	15.62[b]	13.07[c]	16.60[a]	15.11[b]	13.97[b]	14.93[b]	14.32[b]	16.43[a]	29.38
PDI(%)	13.28[a]	7.10[b]	6.78[c]	10.81[a]	9.49[b]	6.86[c]	6.90[c]	8.29[b]	11.97[a]	54.89

[a,b,c] Row means with different superscripts differ significantly at $P <0.05$.
WS: whole soybean; CS: coarse-ground soybean; FS: fine-ground soybean;
SP: soluble protein; RUP: rumen undegradable protein; RDP: rumen degradable protein;
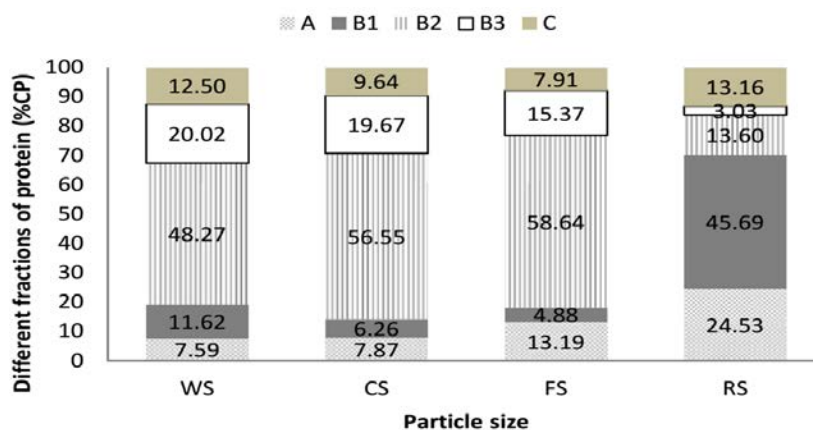PDI: protein dispersibility index.

Figure 3 Effects of particle size of soybean on different fractions of protein, % crude protein (CP) (WS: whole soybean; CS: coarse-ground soybean; FS: fine-ground soybean; RS: raw soybean).

Discussion

Thermal processing reduced the A fraction of protein (NPN) (Hsu & Satter, 1995; Ljukjel *et al.*, 2000; Kafilzadeh *et al.*, 2013). The reduction in the NPN component from heating was presumably because of the loss of amino acids (Ljukjel *et al.*, 2000). One of the first noticeable changes of proteins on heating (even at temperatures around 100 ⁰C) was the loss of labile amino acids such as cysteine and lysine. Lysine is one of the most temperature-sensitive amino acids and destruction of lysine is often 5 to 15 times greater than that occurring with other amino acids (Dakowski *et al.*, 1996).

Heat treatment of mustard meal reduced the B_1 fraction and increased the B_2 and B_3 fractions, compared with the raw mustard meal (Mustafa *et al.*, 1999). The increase in the B_2 and B_3 fractions reduced RDP in heat treatment of mustard meal (Mustafa *et al.*, 1999). Relative to the control, heated mustard meal had lower effective *in vitro* CP degradability (44.5% vs. 74.6% of CP) and a higher ruminal escape CP

(61.5% vs. 12.0% of CP) value (Mustafa *et al.*, 1999). Kafilzadeh *et al.* (2013) reported that thermal processing of canola seed increased B_2 and B_3, and decreased A and B_1 fractions, compared with the untreated varieties.

Table 5 Significant (P <0.05) simple correlations among rumen degradable proteins and variables that affect rumen undegradable protein content of roasted soybean (n = 54)[1]

	RUP	HEAT	TIME	SIZE	A	B_1	B_2	B_3	B	SP	C	NDICP	PDI
RUP	1	0.62	0.43	NS	-0.70	-0.48	NS	0.70	0.59	-0.88	0.29	0.75	-0.51
HEAT		1	NS	NS	-0.31	NS	-0.32	0.71	0.26	-0.39	NS	0.70	-0.47
TIME			1	NS	0.39	-0.44	NS	0.23*	0.36	-0.46	NS	NS	-0.30
SIZE				1	0.39	-0.44	0.43	NS	NS	NS	-0.67	-0.41	0.38
A					1	NS	NS	-0.36	-0.87	0.63	-0.38	-0.46	0.45
B_1						1	-0.55	NS	NS	0.69	NS	NS	NS
B_2							1	-0.58	0.23*	-0.50	-.23*	-0.61	0.21
B_3								1	0.36	-0.35	NS	0.94	-0.53
B									1	-0.60	NS	0.30	-0.40
SP										1	NS	-0.36	0.34
C											1	0.37	NS
NDIN												1	-0.54
PDI													1

[1]RUP and variables data are in percentage of protein. RUP: rumen undegradable protein; HEAT: roasting temperature, ºC; TIME: holding time, min; SIZE: particle size, mm; A: protein fraction A; B_1: protein fraction B_1; B_2: protein fraction B_2; B_3: protein fraction B_3; B: protein fraction B; SP: soluble protein; C: protein fraction C; NDICP: neutral detergent insoluble CP; PDI: protein dispersibility index.
NS: non significant (P >0.10). * Trends toward significance ($0.05 < P$ <0.10).

The increase in the slowly degradable fractions (B_3 and B_2) as a result of heating could be attributed to the denaturation of the proteins as this has been shown to reduce their solubility (Van Soest, 1994). The increase in B_2 fraction on treating may account for loss in the B_1 fraction. Hence, it could be expected that the overall degradation rate would be decreased because of treating as a result of the shift from the rapidly ruminally degradable B_1 fraction to the slowly ruminally degradable B_2 fraction (Kafilzadeh *et al.*, 2013).

The decreased CP solubility of protein supplements as a result of heat treatment is well documented (Ganesh & Grieve, 1990; Moshtaghi Nia & Ingalls, 1992; McAllister *et al.*, 1993; Mustafa *et al.*, 1999; Fathi Nasri *et al.*, 2008). Heat treatment, which decreases rumen protein degradability, can result in a decrease in the percentage of α-helixes and an increase in the percentage of β-sheets (Yu, 2005). The major proportion of protein in soybeans is in the form of globulins, which are followed by albumins, with lesser amounts of glutelins and prolamins. Soybean globulin is apparently extractable by water (Van Soest, 1994). In addition, globulins and albumins are more sensitive to heat denaturation. Thus, rendering them water insoluble could reduce the soluble protein content (Ganesh & Grieve, 1990). Pichard & Van Soest (1977) stated that NDICP roughly represented the slowly degradable and undegradable fractions in plant cell walls.

In general, heat treatment increased NDICP (Mustafa *et al.*, 1999, 2000, 2003; Kafilzadeh *et al.*, 2013), but its effect on the acid detergent insoluble protein is different. In some articles, as a result of heat treatment, an increase in the amount of acid detergent insoluble protein (McKinnon *et al.*, 1995) or no change (Mustafa *et al.*, 1999; 2000; 2003; Kafilzadeh *et al.*, 2013) was observed, and even a decrease (Ganesh & Grieve, 1990; Faldet *et al.*, 1991; Moshtaghi Nia & Ingalls, 1992; Demjanec *et al.*, 1995; Hsu & Satter, 1995). The differences in the ADF and NDF content with increasing heat could be attributed to proteins that are less soluble, and some heat denatured proteins recovered in the NDF fraction (Moshtaghi Nia & Ingalls, 1992). Therefore, the N content of NDF was greatly increased by heating, while ADF required the Maillard reaction or condensation of protein with carbohydrate to render protein recoverable in the ADF fraction (Van Soest, 1994). Heat facilitates the Maillard reaction, or non-enzymatic browning between sugar aldehyde groups and free amino acid groups of protein, yielding an amino-sugar complex. This complex is

more resistant than normal peptides to enzymatic hydrolysis, and reversibility of this reaction is dependent on the temperature and duration of heat exposure (Lin & Kung, 1999). However, no effect on acid detergent-insoluble protein was observed as a result of heating. These results indicated that possibly heat inputs used in the present study were not severe enough to generate unavailable protein via the Maillard reaction. Other researchers reported similar findings when applying modest heat treatments to soybean (Demjanec *et al.*, 1995) and canola meal (Moshtaghi Nia & Ingalls, 1992).

Soya hulls constitute approximately 9% of the total OM of the soybean seed, and contain about 68% NDF, 50% ADF and 2% lignin. Therefore, soya hulls could be regarded as the major contributor of NDF in soybeans. Soya hull loss occurred during grinding, leading to the lower NDF in ground beans (Ganesh & Grieve, 1990). Heating temperature had more effect on slowly degraded protein (B_3 + B_2 fractions) than heating time and grinding. As a result, heating temperature had a greater effect on the RUP of roasted soybeans (Table 4). Petit *et al.* (1999; 2002) reported similar findings.

The PDI for the temperatures of 115, 130 and 145 ºC was 13.28, 7.10 and 6.78, respectively (Table 4). Only PDI at the temperature of 115 ºC is in the appropriate range (9 to 14). Soybean roasted at temperatures of 130 and 145 ºC has inadequate PDI (less than 9). The 5- and 10-minute roasting times had PDI in an appropriate range, but 10-minute roasting had more bypass protein than 5 minutes (62.1% vs. 58.3% CP). Among particle sizes, finely ground particles had acceptable PDI (11.97). However, because the PDI of coarsely ground particles was close to 9 and was shown to have more bypass protein (64.0% vs. 59.4% CP), the particle size of 1.15 mm could be recommended for roasted soybean.

The PDI assay is based on the solubility of soybean protein. Because soluble protein is decreased with increasing time and temperature, the PDI decreased linearly with increasing time and temperature of roasting. In agreement with the results of the current study, PDI decreased linearly with increased extrusion (Zhu *et al.*, 1996; Anuonye *et al.*, 2007; Palic *et al.*, 2011) and roasting (Hsu & Satter, 1995) temperatures. PDI decreased incrementally when heating soybean meal from 0 to 30 minutes (Batal *et al.*, 2000; Caprita *et al.*, 2010a; b). The results of the current experiment showed coarse soybean roasted at 115 ºC for 10 minutes had optimum PDI and high RUP content.

In general, an optimum heat treatment could minimize soluble CP and maximize NDICP without a substantial increase in ADICP (Moshtaghi Nia & Ingalls, 1992; McKinnon *et al.*, 1995; Mustafa *et al.*, 2003).

Correlation matrices for RUP and its variables were shown in Table 5. RUP was correlated (Pearson correlation) positively (P <0.01) with the roasting temperature, holding time and B_3, B and NDICP fraction. The correlation between RUP and C fraction of protein was relatively weaker (P = 0.02). The correlations between A and B_1 fractions and SP and PDI with RUP were significant and negative. The particle size of roasted soybeans had no significant correlation with RUP. In terms of roasting temperature and holding time, temperature had a higher correlation with the RUP content of the roasted soybean.

Conclusions

The results of this experiment showed that different processing conditions (such as particle size, heating temperature and heating time) had significant impacts on different fractions of the protein, implying that the processing condition should be considered in preparing roasted soybeans. Heating temperature had more effect than holding time and particle size on protein fractions. The RUP level was increased linearly with raising the heating temperature, as the highest level was obtained at 145 ºC. The rise in RUP at 145 ºC was accompanied by an increase in the slowly degradable true protein (B_3 fraction) and undegradable true protein (C fraction), while at 130 ºC and 115 ºC, the moderately degradable true protein (B_2 fraction) was increased. The B_2 fraction could therefore be a better indicator of the probable bypass protein value of soybean than the B_3 fraction. Using the results of CNCPS and PDI methods showed that the soybean with the coarsely ground particles, which were roasted at 115 ºC for 10 minutes, could be recommended.

Acknowledgments

Authors' contributions

HRY was in charge of project design and writing of the manuscript. MA, GRG and ASS were in charge of project implementation. All co-authors participated in results, statistics and interpretation of the study.

Conflict of interest declaration

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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