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Original Article

Effect of Roasting and Oil Extraction Method on the Nutritive Value of Soybean Seed Cake

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ABSTRACT

This study was conducted to evaluate the effect of roasting and method of oil extraction on the chemical composition, Metabolizable energy (ME), and *in vitro* gas production of soybean seed cake: from un-roasted or roasted (at 164.5 °C for 10 minutes) soybean seeds oil was extracted mechanically or by solvent (hexane). A complete randomized design was used with 2×2 factorial arrangements (un-roasted × mechanical extraction, un-roasted × solvent extraction, roasted × mechanical extraction, and roasted × solvent extraction). The results revealed that although roasting had no significant effect on the chemical composition of the seed cake, it resulted in a significant ($P<0.05$) reduction in total gas production throughout the different incubation periods. More oil was retained in mechanically extracted soybean cake (24.51%) compared with solvent extracted cake (5%). The high oil content of mechanically extracted soybean cake was reflected in high ME content (14.1 MJ/kg DM) compared with solvent extracted cake (11.2 MJ/kg DM). Crude protein content was higher in solvent extracted cake (48.8%) compared with mechanically extracted cake (35.1%). The study concluded that the heat or roasting reduced the fermentation process resulting in less *in vitro* gas production, and the same trend was observed for mechanically extracted cake. Solvent extraction increased crude protein content and enhanced the fermentation process.

Keywords: Soybean Seed Cake, Roasting, Extraction, Nutritive value

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INTRODUCTION

The first domestication of soybean has been traced to the eastern half of North China. According to early authors, soybean production was localized in China until after the Chinese-japans war of 1894-1895. The production of soybean seeds was about 260 million tons in season 2009/2010 in the world (Rynek, 2010). In Sudan recently kenana Sugar Company cultivated soybean seeds, the production of soybean seeds about 800 kg/Feddan in season 2011. The majority of the soybean crop is processed into oil and meal. Oil extracted from soybeans is made into shortening, margarine, cooking oil, and salad dressings. Soybeans account for 80 percent or more of the edible fats and oils consumed in the United States. Soy oil is also used in industrial paint, varnishes, caulking compounds, linoleum, printing inks, and other products (Lance and Garren, 2005). Dehulled soybean meal has a higher composition of crude protein, amino acids, and Metabolizable energy than soybeans having hulls (NRC, 1994). Soybean contains above 40% of crude protein (Warnick and Anderson, 1968). Soybean meal is considered the gold standard among intact protein sources used in the feed industry (Cromwell, 1999). Soybean meal is a major source of protein used in Animal feed. The poultry and swine industries are major consumers of soybean meal where over half of the soybeans processed for livestock feed is fed to poultry, about one-quarter is fed to swine, and the rest is used for beef cattle, dairy cattle, and pet food. Soybean meal contains anti-nutritional compounds, these anti-nutritional include trypsin inhibitors, lectins, flatulence producing compounds, and much other allergenic protein (Kim and Baker, 2003; Baker, 2000; Dunsoford *et al*., 1989). Soybean meal contains trypsin inhibitors which cause enlargement of pancreases in chicks (Hittle, 1975 and Leiner, 1981). These anti-nutritional compounds can be denatured by fermentation (Feng *et al*., 2007), or subjected to heat treatment before use as the animal to destroy the anti-nutritional factors (Ewan, 1975). The objectives of this study were to evaluate the effect of roasting and method of oil extraction on the chemical composition of soybean seed cake, Metabolizable energy (ME), and in vitro gas production of soybean seed cake.

MATERIALS AND METHODS

Study location

The experiment was conducted in Animal Nutrition Laboratory at the University of Khartoum, Faculty of Animal Production - Department of Animal Nutrition.

Source of seeds

Soybean Seeds were obtained from Kenana Sugar Company, Research and Development Department.

Sample preparation

Soybean seeds were divided into four parts, two samples of soybean seed were roasted in an oven at 164.5°C for 10 minutes in Research and Development Department (Kenana) and the other two samples were unroasted, two samples were extracted mechanically and the other two samples were extracted by solvent (Hexane) (Roasted and UN Roasted) in Savola Company for edible oil production (Soba).

Chemical Analysis

The nutrients composition of soybean cake (Roasted-Solvent), (Roasted-Mechanical), (UN Roasted-Solvent), and (UN Roasted-Mechanical) were determined according to the methods of AOAC (1990). ME values of the feeds for poultry and ruminants were estimated according to the following equation;

ME for poultry (MJ/Kg) = 1.549 + 0.102 CP + 0.275 EE + 0.148 NFE – 0.034 CF according to (MAFF, 1975).
ME for ruminants (MJ/Kg) = 0.12 CP + 0.31 EE + 0.05 CF + 0.14 NFE according to (MAFF, 1975).

Determination of Trypsin Inhibitors

Tris-Buffer

Tris (hydroxymethyl) aminomethane (1.21g) and 0.59g of $CaCl_2$ $2H_2O$ were dissolved in 180 ml of distilled water. The pH was adjusted to 8.2 with 1 *N* HCl (10-15 drops) and made up to 200 ml with distilled water. This solution was pre-warmed to 37°C for Benzoyl-DL-arginine-p-nitroanalide hydrochloride (BAPA) formulation, was stable up to 8 hours.

Benzoyl- DL-arginine-p-nitroanalide hydrochloride (BAPA)

Benzoyl-DL-arginine-p-nitroanalide hydrochloride (BAPA) (0.080g) was dissolved in 2 ml of dimethyl sulfoxide and diluted to 200 ml with tris buffer. The solution was stable for up to 4 hours.

Trypsin solution

Trypsin (0.0040g) was weighed into a 200 ml volumetric flask and diluted to 200 ml with 0.001 *N* HCl. Although the solution may be stored for as long as a month at 5-10°C, a fresh solution was made with each run.

Sample Extract

A soy sample (1.00g) was extracted with 50 ml of 0.01 *N* NaOH (the pH adjusted, when required to 8.4 – 10.0) for 3 hours. Stirring sufficient to keep the sample in suspension was maintained. This suspension was then diluted so that 2 ml of the sample extract inhibited 40- 60% of the trypsin used as a standard in the analysis. The appropriate dilutions were determined from either a pre-knowledge of the heat treatment of the sample or from urease analysis, this is a reflection of the heat treatment of the soy product. If the value obtained did not fall within the specified range of inhibition, the analysis was repeated with the correct dilution (Hamerstrand *et al.,* 1980).

Procedure

To each of four test tubes, 2 ml aliquots of the diluted sample extract were added with a wide-tip pipette. A fifth tube was prepared for the trypsin standard by adding 2 ml of distilled water. To three of the four tubes containing the sample extract and the tube containing distilled water, 2 ml of the trypsin solution was added, and the tubes were placed in a constant temperature bath (37°C) for 10 minutes. Five milliliters of BAPA solution (pre-warmed to 37°C) was rapidly blown into each tube. The contents were stirred immediately on a vortex mixer, and the tubes were replaced in the constant temperature bath. The reaction was determinate exactly 10 minutes later by blowing in 1 ml of 30% acetic acid with immediate mixing with a vortex mixer. A sample blank (the fourth tube Containing sample extract) was prepared by the same procedure except that the trypsin solution was added after the reaction was terminated by the addition of acetic acid (Hamerstrand *et al.,* 1980). The absorbance of each solution was determined at 410 nm against the sample blank. Values obtained from each of the three sample extracts were subtracted from the trypsin standard. These values were averaged, and the trypsin inhibitor content was determined from the following relationship:

$$\text{TI, mg/g of sample} = \left(\frac{differential\ absorbance}{(0.019\times 1000)}\right)\times dilution\ factor$$

In-vitro gas production test

In-vitro gas production test was measured using the technique of (Menke and Steingus, 1988).

Rumen Fluid Collection

The rumen fluid was collected in the early morning before feeding. The liquor was collected from the calf using 800 ml beaker for collecting the liquor through the rumen fistula while squeezing by hand. The coarse particles of food were removed; the rumen liquor was transferred through two layers of cheesecloth into a warm flask of about 500 ml volume filled with CO_2. The flask was sealed with a rubber stopper and placed in a water bucket preheated to 39°C.

Preparation of reagents:
Prepare five different solutions as media and mix with rumen liquor. The composition of the solutions are as follows:

Solution A (Micro mineral)

13.2 g calcium chloride ($CaCl_2.2H_2O$)

10.0 g manganese chloride ($MnCl_2.4H_2O$)

1.0 g cobalt chloride ($CoCl_2.6H_2O$)

8.0 g iron chloride ($FeCl_3.6H_2O$)

Made up to 100 ml with distilled water.

Solution B (Buffer solution)

39.0 g sodium hydrogen carbonate ($NaHCO_3$)

Made up to 1 liter with distilled water

Solution C (Macro mineral)

5.7 g disodium hydrogen phosphate (Na_2HPO_4)

6.2 g potassium dihydrogen phosphate (KH_2PO_4)

0.6 g magnesium sulfate ($Mg SO_4.7H_2O$)

Made up to 1 liter with distilled water.

Resazurin solution

100 mg resazurin made up to 100 ml with distilled water.

Reducing solution

4 ml sodium hydroxide (1N NaOH)

625 mg sodium sulphide ($Na_2S.9H_2O$)

Carbon dioxide gas is passed through a submerged tube while the reduction solution is being added. The rumen liquor was added when the indicator change to colorless. The ratio of rumen fluid to buffer medium was 1:3(v: v). the mixture was kept under CO_2 in water at 30°C and stirred by a magnetic stirrer.

Estimation of gas production

Fermentation was carried out in the glass, syringe (piston – pipette, 100 ml, graduated, with a capillary attachment which can be shut off with a plastic clip). The piston fitted precisely and was lubricated using Vaseline. The sample (200mg DM) of soybean cake first was introduced into the syringe. It was incubated in duplicates. Forty ml of the rumen liquor medium mixture was pipetted into each syringe pre-warmed to 39°C. Any gas bubbles in the syringe were removed, the plastic chip in the tube was closed, and the position of the piston recorded and the syringe placed in the incubation apparatus (water bath) at 39±0.5°C. All incubations were started in the morning.

The position of the piston was read at a different time (3, 6, 12, 24, 48, 72, and 96 hours).

The data formed were processed in a way similar to those obtained with the nylon bag technique.

The following model is fitted to the data:

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

Where P = represent gas volume (ml) at time t.

a = the gas produced from soluble fraction (ml).

b = the gas produced from insoluble but fermentable fraction (ml)

a + b = the potential gas production (ml).

t = Incubation time.

c = Constant rate.

Statistical Analysis

Data were subjected to analysis of variance according to Completely Randomized Design with 2×2 factorial arrangements, using Statistics software version 8. Means were separated by the least significant difference (LSD) test.

RESULTS AND DISCUSSION

Chemical composition of soybean cake

As shown in Table (1) except for DM there were significant ($p<0.05$) differences of CP, EE, CF, Ash, NFE, ME/P, and ME/R due to extraction methods, this agreement with data of (Poultry Feeding Standard,2005), Found that extraction had a beneficial effect on the chemical composition of soybean cake. The solvent extracted soybean cake contained significantly high ($p<0.05$) crude protein 48.8% compared to 35.1%

CP for mechanically extracted soybean cake, this agreement with data of (Poultry Feeding Standard, 2005), in which CP of solvent extracted soybean cake 40% to 49% and CP of mechanically extracted soybean cake 32% to 43.6%. This result could be due to high protein extracted by chemical method (solvent-hexane). The mechanically extracted soybean cake contained high ($p<0.05$) EE 24.5% compared to 5% of EE for solvent-extracted soybean cake, this agreement with data of (Poultry Feeding Standard, 2005), where EE of mechanically extracted soybean cake about 15.5% to 24.7%. This result could be due to more extraction oil by solvent (hexane) from soybean seed cake. The mechanically extracted soybean cake contained high ($p<0.05$) CF 11.3% compared to 7.9% CF for solvent-extracted soybean cake, this result could be due to breaking down the cell wall of soybean seed cake by solvent. The solvent extracted soybean cake contained high ($p<0.05$) Ash 7.25% compared to 5% Ash for mechanically extracted soybean cake, this in agreement with data of (Poultry Feeding Standard, 2005); found that Ash content from 4.5% to 6.4% for mechanically extracted soybean cake. The solvent extracted soybean cake contained a high ($p<0.05$) of NFE 24.6% compared to 17.4% of NFE for mechanically extracted soybean cake. This result could be due to the low-fat content for solvent-extracted soybean compared to the high-fat content for mechanically extracted soybean cake. The mechanically extracted soybean cake contained high ($p<0.05$) of ME for poultry and ruminants (14.2 and 14.7) MJ/Kg, respectively compared to (11.3 and 11.2) MJ/Kg, of ME for poultry and ruminants for solvent-extracted soybean cake respectively, this result showing high ME/P and ME/R of mechanically extracted soybean cake could be due to high-fat content of mechanically extracted soybean cake. The result in Table (1) showed that there were no significant differences ($P>0.05$) in DM, CP, EE, CF, Ash, NFE, ME/P, and ME/R of Soybean cake due to heat treatment (Roasting). This in agreement with data of Hossain and Becker, (2001*)*, who found that Heat treatment had no effect on chemical composition, and disagree with data of Neshim and Carlich, (1975) who found that heat treatment had a beneficial effect on protein quality.

In-vitro gas production of soybean cake

Gas production of soybean cake subjected to roasting and different extraction methods was presented in Figure (1), Figure (2), and Figure (3). In Figure (1), the solvent-extracted soybean cake in time 3 contained a high ($p<0.05$) volume of gas production (5.25) compared to mechanically extracted soybean cake in the same time (4.25). In time 12, there was no significant ($p<0.05$) difference between mechanical extracted and solvent extracted soybean cake showed (15.8) for mechanical extracted, compared to (15.2) for solvent-extracted soybean cake.

Table 1. Effect of roasting and extraction methods on chemical composition of soybean cake

Treatments \ Parameters	DM%	CP%	EE%	CF%	Ash%	NFE%	ME/P MJ/Kg	ME/R MJ/Kg
ES×UT	93.00[a]	48.50[a]	5.25[b]	8.25[b]	6.25[ab]	24.75[a]	11.40[b]	11.35[b]
EM×UT	93.25[a]	35.20[b]	22.50[a]	10.50[a]	5.25[b]	19.80[ab]	14.05[a]	14.05[a]
ES×HT	94.00[a]	49.00[a]	4.75[b]	7.50[b]	8.25[a]	24.50[a]	11.20[b]	11.10[b]
EM×HT	93.00[a]	35.05[b]	26.50[a]	11.75[a]	4.75[b]	14.95[b]	14.45[a]	15.05[a]
SEM	0.39	0.35	2.08	0.56	1.06	3.01	0.36	0.4

Extraction methods

Treatments \ Parameters	DM%	CP%	EE%	CF%	Ash%	NFE%	ME/P MJ/Kg	ME/R MJ/Kg
ES	93.50[a]	48.75[a]	5.00[b]	7.88[b]	7.25[a]	24.62[a]	11.30[b]	11.23[b]
EM	93.13[a]	35.13[b]	24.50[a]	11.13[a]	5.00[b]	17.37[b]	14.25[a]	14.76[a]
SEM	0.28	0.25	1.47	0.39	0.75	2.13	0.25	0.28

Heat Treatment

Treatments \ Parameters	DM%	CP%	EE%	CF%	Ash%	NFE%	ME/P MJ/Kg	ME/R MJ/Kg
UT	93.13[a]	41.85[a]	13.86[a]	9.38[a]	5.75[a]	22.27[a]	12.72[a]	12.92[a]
HT	93.50[a]	42.03[a]	15.63[a]	9.63[a]	6.50[a]	19.72[a]	12.82[a]	13.07[a]
SEM	0.28	0.25	1.47	0.39	0.75	2.13	0.25	0.28

DM= Dry matter , CP= Crude protein , EE= Ether extract , CF= Crude fiber, Ash= Ash content , NFE=Nitrogen free extract , ME/P=Metabolizable energy for poultry, ME/R= Metabolizable energy for ruminants , ES×UT=solvent extraction soybean cake – UN treated, EM×UT= mechanical extraction soybean cake – UN treated, ES×HT= solvent extraction soybean cake – heat treated, EM×HT= mechanical extraction soybean cake – heat treated, SEM = Standard error of mean, a-b means values with different superscripts in the same column were significantly different ($P<0.05$).

Generally, solvent-extracted soybean cake contained significantly high ($p<0.05$) volume gas (ml/200mg DM) at different times (24, 48, 72, and 96 hours), compared to mechanically extracted soybean cake, this agreed with data of Goelema et al., (1998), who found that pressure (mechanical) and toasting decrease rumen degradation. As shown in Figure (2), the UN treated soybean cake contained a high ($p<0.05$) volume of gas production, compared to heat-treated soybean cake of all reading times, this agreement with data of Lord (2000), who found that UN treated soybean highly soluble protein. And agree with the data of stern *et al*., (1985), who found that heat treatment reduced protein degradation in the rumen. This result could be due to heat or roasting reduced the fermentation process resulting in less *in vitro* gas production.

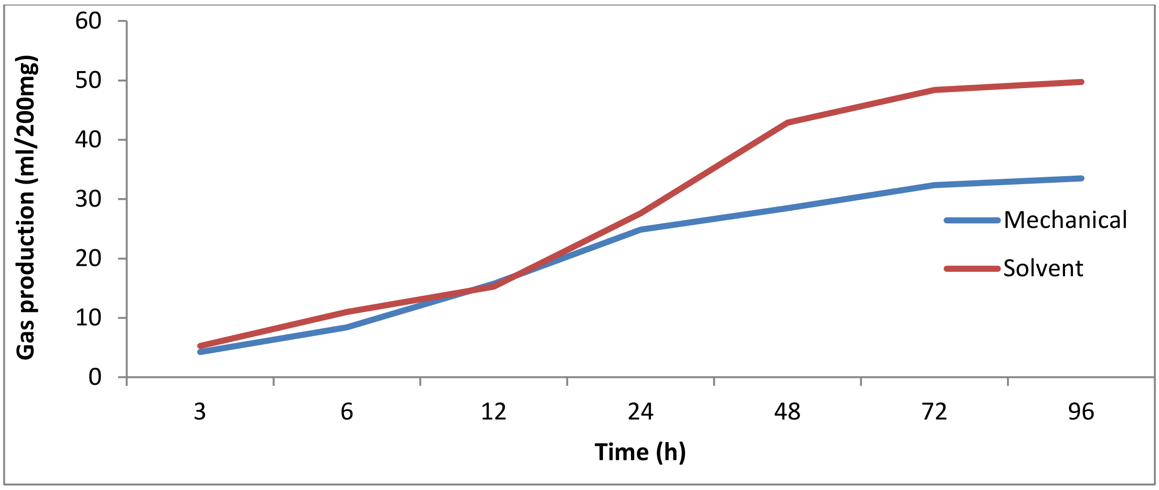

Figure 1. Effect of extraction methods on gas production volume (ml/200mg) DM / (h) for soybean cake

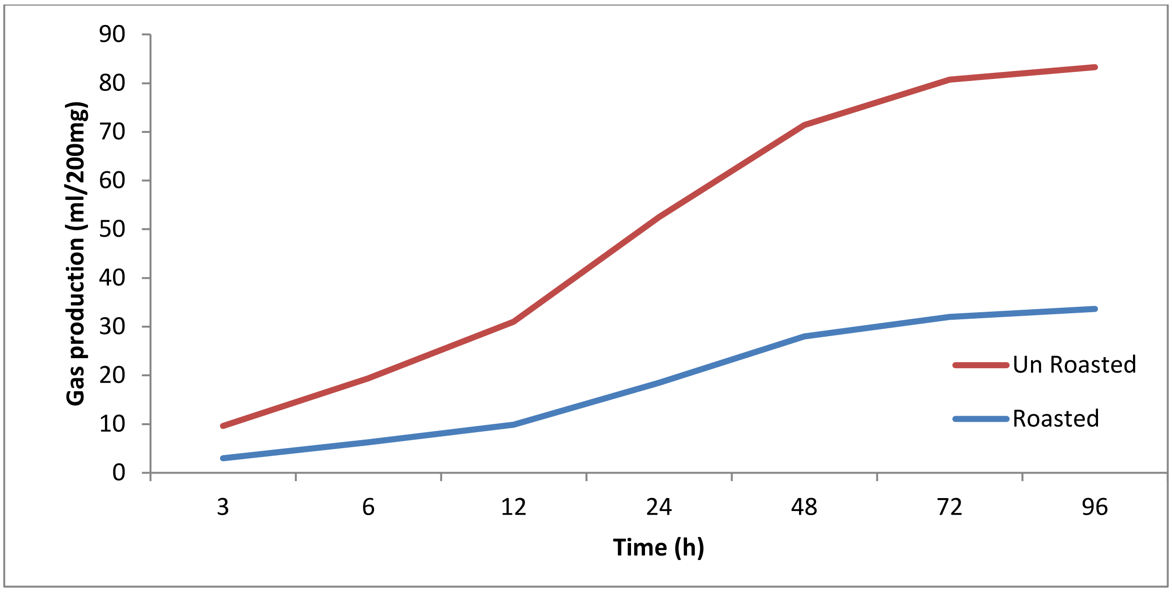

Figure 2. Effect of heat treatment on gas production volume (ml/200mg) DM / (h) for soybean cake

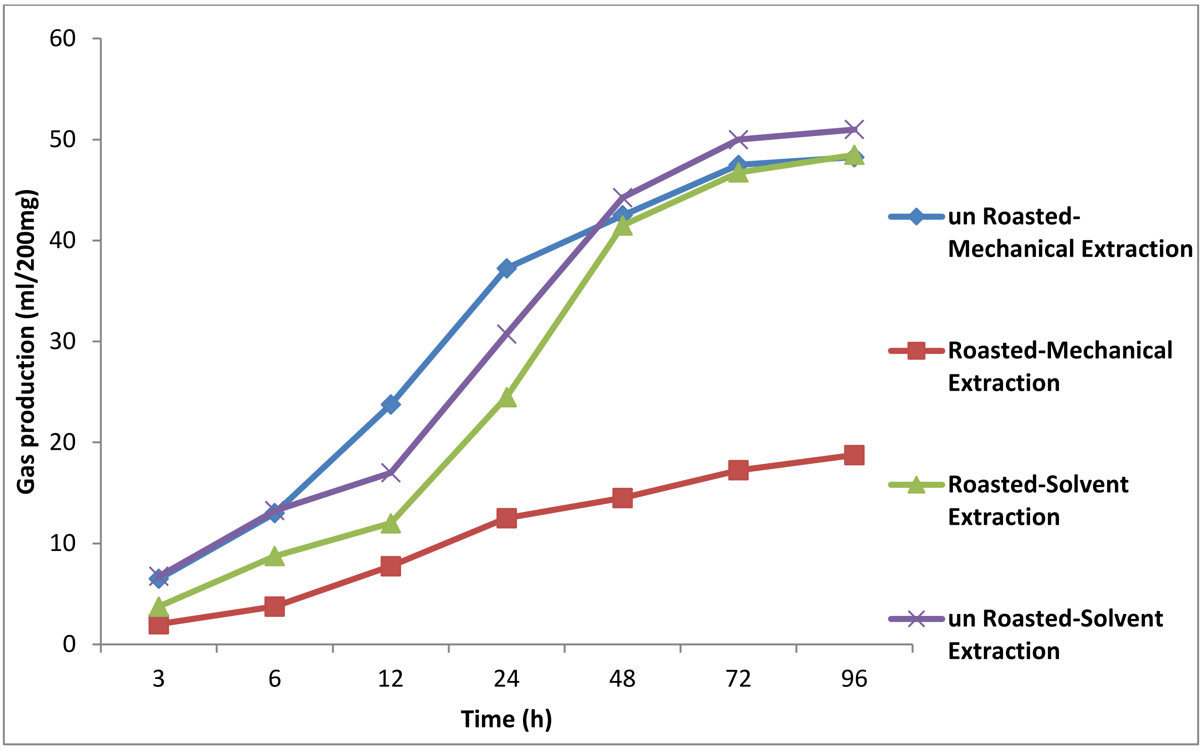

Figure 3. Gas production volume (ml/200mg) DM / (h) for soybean cake

Parameters fraction degradability of soybean cake

Fermentable parameters for roasting and extraction methods of soybean cake were presented in Table (2). The gas volumes from a soluble fraction (a) was significantly (P<0.05) differences for extraction, the highest value recorded by solvent-extracted soybean cake was (0.05) and the lowest value recorded by mechanically extracted soybean cake was (-0.99), but shown there were not significantly (P>0.05) differences due to heat treatment. The gas volumes from a soluble fraction (a) shown also significant (P<0.05) differences due to heat with extraction. The gas volumes from Moderable fraction (b) was significantly (P<0.05) differences due to extraction, solvent-extracted soybean cake contained high (P<0.05) gas volume (53.2) compared to (33.9) for mechanical extracted soybean cake, also UN treated soybean cake contained high (P<0.05) gas volume (50.4) compared to (36.6) for heat-treated soybean cake. There were significant (P<0.05) differences in infraction (b) due to heat with mechanical extraction. There were significant (P<0.05) differences among feeds in the rate of gas production (c) due to extraction, heat, and heat with mechanical extraction. The mechanically extracted soybean cake contained less (P>0.05) gas volume compared to solvent-extracted soybean cake, this agrees with data of Goelema *et al.,* (1998), who found that pressure (mechanical) and toasting decrease rumen degradation.

Table 2. Gas production constants (ml/200mg DM) for different treatments of soybean cake

Treatments \ Degradability	a	b	C	a+b
ES×UT	2.21^{a}	51.49^{a}	0.03^{bc}	53.69^{a}
EM×UT	-1.83^{c}	49.39^{a}	0.06^{a}	47.56^{a}
ES×HT	-1.81^{bc}	54.89^{a}	0.03^{c}	53.71^{a}
EM×HT	-0.17^{b}	18.38^{b}	0.04^{b}	18.22^{b}
SEM	0.53	3.47	5.24	3.28
Extraction methods				
ES	0.52^{a}	53.19^{a}	0.03^{b}	53.70^{a}
EM	-0.99^{b}	33.89^{b}	0.05^{a}	32.89^{b}
SEM	0.37	2.45	3.70	2.32
Heat Treatment				
UT	0.19^{a}	50.44^{a}	0.05^{a}	50.63^{a}
HT	-0.68^{a}	36.64^{b}	0.04^{b}	35.96^{b}
SEM	0.37	2.45	3.70	2.32

a = soluble fraction (ml), b = fermentable fraction (ml), a + b = the potential gas production (ml), c= constant rate, SEM = Standard error of the mean, a, b, c= means with different superscripts in the same row were significantly different (P<0.05).

The UN-treated soybean cake contained high (P<0.05) gas volume compared to heat-treated soybean cake, this agrees with data of Hossain and Becker (2001), who found that heat treatment reduces rumen degradation. And agree with the data of Fetuge *et al.,* (1974), who found that many feedstuffs are exposed to heat during processing can make the protein more resistant to degradation. The gas volumes from potential gas production (a+b) were significantly (P<0.05) different among all treatments (extraction, heat, and heat with extraction). The solvent extracted soybean cake contained high (P<0.05) gas volume (53.7) compared to (32.9) for mechanical extracted soybean cake, this agrees with the data of Goelema et al., (1998), who found that pressure (mechanical) and toasting decrease rumen degradation. The UN treated soybean cake contained high (P<0.05) gas volume (50.6) compared to (36.0) for heat-treated soybean cake, this agrees with Vishnu *et al*. (2001), who found that heat treatment decreased ruminal degradation, and reduced nitrogen disappearances in the rumen. And agree with the data of Broderick et al., (1988), who found that heating decreased the degradation rate or the more slowly degraded fraction and reduce protein solubility. Also, this result agreed with the data of Yong et al., (1993), who found that heat treatment decreased ruminal degradation and reduced protein solubility.

CONCLUSION

The heat or roasting reduced the fermentation process resulting in less in vitro gas production, and the same trend was observed for mechanically extracted cake, Solvent extraction increased crude protein content and enhanced the fermentation process.

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CONFLICT OF INTERESTS

There is no conflict of interest

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