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IN VITRO RUMEN MICROBIAL FERMENTATION OF DEHULLED AND INDUSTRIALLY SORTED CHICKPEA (*CICER ARIETINUM* L.) USING GAS PRODUCTION TECHNIQUE

E. ABDI GHEZELJEH*, M. BESHARATI

Department of Animal Science, Ahar Faculty of Agriculture and Natural Resources, University of Tabriz, Ahar, Iran

ABSTRACT

Based on the official statistics of the industries and mines organization, about 7500 tons of wastes in chickpea processing plants are produced annually in the East Azarbaijan province of Iran. In order to determine chemical composition, anti-nutritional factors, and metabolizable energy of chickpea (*Cicer arietinum* L.) by-product (including chickpea pre-screening and chickpea hull), classified random sampling from 10 % of plants was performed first. Then amount of dry matter (DM), organic matter (OM), crude protein (CP), crude fibre (CF), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), and antinutrient including total extractable phenolic compounds (TPC) and total tannin (TT) were determined. There were significant differences ($P < 0.01$) between chemical composition and antinutrient amount of chickpea by-product except for DM and OM. An *in vitro* gas production technique was used to determine the rate and extent of gas production and organic matter digestibility. Amounts of gas production were recorded at 2, 4, 8, 12, 24, 36, 48, 72, 96 and 120 h after incubation. Gas production rate constant and value calculated for OMD and ME of chickpea pre-screening were significantly higher than chickpea hull ($P < 0.01$), but the amount of b and lag time of chickpea hull were significantly higher than chickpea pre-screening ($P < 0.01$).

Key words: chickpea pre-cleaning; gas production; metabolizable energy; chickpea hull

INTRODUCTION

Iran is deficient in protein-rich feedstuffs for livestock and relies heavily on soybean meal imports. For this reason, it is trying to develop its own protein crops and the production of grain legumes has been promoted due to their high level of protein. Chickpeas are one of the oldest and most widely consumed legumes in the world, particularly in tropical and subtropical areas. Chickpeas (*Cicer arietinum*) are a yearly leguminous crop belonging to Fabaceae family. Together with other legumes it has long been one of the most important protein sources of the rural population (Sarno and Stringi, 1980); the dry mature seed is traditionally cooked and eaten with cereals. Chickpea seed has high protein content, about 20 % (Aman, 1979; Khan *et al.*, 1979), and is therefore well suited to human and animal nutrition.

Based on seed colour and site of origin, chickpeas are generally classified as either desi (Indian origin) or Kabuli (Mediterranean origin). The desi type has a smaller seed size and a thicker seed coat than the Kabuli type (Gil *et al.*, 1996). The two chickpeas also differ in their nutrient composition, with the Kabuli type having lower fibre, higher starch and higher fat contents than the desi type (Gil *et al*., 1996). Also chickpea seeds (*Cicer arietinum* L.) are usually grown for human consumption, but approximately 20 % of the production is damaged during harvesting and processing, and considered as by-product sold at low prices for livestock feeding (Ulloa *et al.*, 1988).

After screening, dehulling of chickpea resulted in three fractions including seed coat, cotyledon, and embryonic axe. During the processing of chickpea, 13.8, 1.6, and 84.6 % seed coat, embryonic axe, and cotyledon fractions, respectively, are produced. (Sreerama *et al*., 2010). The rapid development and attention is being

***Correspondence:** E-mail: E_abdi2005@yahoo.com
Einollah. Abdi Ghezeljeh, Department of Animal Science, Ahar Faculty of Agriculture and Natural Resources, University of Tabriz, Ahar, Iran

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paid to growing chickpeas in west of Asia. However, as production increases, more chickpeas, which are not suitable for human consumption, will be feed grade and made available as animal feed. Proper utilization of feed grade chickpeas as animal feed will increase the economic returns of growing chickpeas in west of Asia.

The sown area of legume in Iran is over 1.1 and 0.7 million ha is under chickpea cultivation (Sabaghpour *et al.*, 2006). Approximately 7500 ton by-product of chickpea including pre-screening seeds and chickpea hull (known as chickpea by-product) are produced annually in North West Iran. Information on nutritive value of legume in general and chickpea by-product in particular are very low in relation to cereal grains. The aim of the present study was to elucidate the types of nutritional and anti-nutritional compounds and gas production parameters of chickpea by-product.

MATERIAL AND METHODS

Sampling and chemical composition

Dehulling is the first step in the chickpea plants. Dehulling of the legumes results in the production of various types of by-products such as seed coat, embryonic axe fraction, and powder. In the chickpea plants, after dehulling, brown-colored and thick seed coat, powder and embryonic axe fractions were collected by sieving. These fractions were sieved through a 2 mm sieve to collect powder and embryonic axe fractions (passed through the sieve) and seed coats (hulls, remaining on the sieve). The embryonic axe and powder fractions were added to the pre-screening fraction. Samples of chickpea by-product including pre-screening seeds and chickpea hull were collected from 10 % of the chickpea plants of this province according to classified sampling during September to December, 2014.

Samples were then ground to pass through a 1 mm sieve (Retsch Muhle mill, Retch EPP 15X20, Germany), and then were used for chemical analysis and gas production technique. Dry matter content of each sample was determined using a forced-air oven at 105 °C for 24 h. Nitrogen content was determined using the Kjeldahl method (Kjeltec 2300 Autoanalyzer, Foss Tecator AB, Hoganas, Sweden) and CP was calculated as N × 6.25. Ash-free neutral detergent fibre (NDF) was determined, using thermo stable alpha amylase (Sigma A-3306), without sodium sulphite in the ND, according to Van Soest *et al*. (1991). Acid detergent fibre [(AOAC, 2000), ID 973.18] was determined and expressed exclusive of residual ash. Samples were also analyzed for ether extract ([AOAC, 2000], ID 920.39), crude fibre [(AOAC, 2000), 962.09], and ash [(AOAC, 2000), ID 942.05] concentrations. Total carbohydrate was calculated by subtracting the amount of crude protein, ether extract, moisture, and ash from the 1000. Total extractable phenolic compounds (TPC) and total tannin (TT) were determined using procedures of Julkunen-Titto (1985) and Makkar *et al.* (1992), respectively.

Gas production

Three fistulated adult Balochi male sheep (49.5 ± 2.5 kg) were used as rumen liquor donor for gas production technique. Animals were fed a diet to meet their maintenance requirement (NRC, 1985). Sheep were fed a total mixed ration consisting of 0.8 kg DM alfalfa hay and 0.5 kg DM concentrate consisting of 50 % barley grain, 20 % sugar beet pulp, 12 % soybean meal, 15 % wheat barn and 3 % vitamin and minerals (165 g $CP.kg^{-1}$ of DM). The ration was fed twice daily at 08:00 and at 15:00 h (Bilik and Lopuszanska-Rusek, 2010).

Equal volumes of ruminal fluid (about 350 ml) from each sheep were collected via a vacuum pump through fistula before the morning feeding and combined. Rumen fluid was strained through 4 layers of cheesecloth into a pre-warmed CO_2-filled flask. All laboratory handling of rumen fluid was carried out under a continuous flow of CO_2. Incubation of the samples was done using a manual pressure transducer technique.

Incubation of the samples was done using calibrated glass syringes following the procedures of Menke and Steingass (1988). Approximately, 200 mg DM of each sample that were ground to pass through a 2-mm screen in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) was weighed into a glass vial (n = 4). Vials were pre-warmed at 39 °C before the injection of 30 ml rumen fluid-buffer mixture (pH = 6.8) into each bottle followed by incubation in a water bath at 39 °C. Following inoculation, during gas pressure reading, vials were briefly and gently rolled to facilitate mixing and to maximize contact of the inocula with the samples, which exhibited a slight tendency to adhere to the glass above or below the gas-liquid interface. Gas pressure measurement were made with a digital pressure gauge (model SEDPGB0015PG5 sensor unit, SenSym, Milpitas, Calif.) having a 0.01 lb/in^2 (or psi; 1 psi = 0.06805 atm) sensitivity. Measurements of the pressure and gas production were done at 2, 4, 6, 8, 12, 24, 36, 48, 72, 96 and 120 h after the incubation. Volume was measured by a graduated syringe (20 mL) also with a coupled needle (0.6 mm). Immediately after inoculation, the initial reading was performed with the aim to standardize the pressure and discard the volume of gas in all bottles. From insertion of the needle into the synthetic rubber stopper, the pressure produced inside the vials was verified in the digital reader. After pressure reading, the volume of gases was determined by pulling the plunger until the transducer pressure returned to zero.

Total gas volumes were corrected for a blank incubation which contained only the buffered rumen

fluid without any barley samples and weight of sample according to the equation proposed by Valentin *et al.* (1999):

$$GP\ (ml.200\ mg.DM^{-1}) = \frac{200\ (V_t - V_0 - V_b)}{W}$$

Where; GP is corrected gas volume (ml), V_t is gas volume recorded in the vial containing sample (ml) at time t (h), V_0 is volume in the vial with sample at 0.0 h of incubation, V_b is gas volume in the vial without sample, and W is weight of sample (mg). Corrected cumulative gas production data were fitted to the exponential model of McDonald (1981): $Y = A(1-e^{(-c(t-lag))})$, where A is the asymptotic gas production (ml); c is the fractional rate of gas production (/h); lag is the initial time delay in the onset of gas production (h) and t is the gas reading time (h). The model allowed for the estimation of a lag phase (Lag) before rapid gas production began.

The parameters A, C and lag time were estimated by an iterative least squares procedure using the NLIN procedure of SAS (2002). Data of 24 h gas production were also used to estimate the organic matter digestibility and metabolizable energy of the samples using the equations of Menke and Steingass (1988) as:

$$ME\ (MJ.kg\ DM^{-1}) = 0.157 \times GP + 0.0084 \times CP + 0.022 \times EE - 0.0081\ CA + 1.06$$

$$OMD\ (\%) = 0.9991 \times GP + 0.0595 \times CP + 0.0181 \times CA + 9$$

Where:

CA is ash in g.kg DM^{-1}; EE is ether extract in g.kg^{-1} and GP is the net gas production (ml.200 mg DM^{-1} in 24 h). Data were statistically analyzed using SAS (1999) software.

RESULTS AND DISCUSSION

Chemical composition of chickpea by-product is presented in Table 1. Nutrient composition and anti-nutritional factors of chickpea pre-screening and chickpea hull, except DM and OM showed significant differences ($P < 0.01$). Chickpea pre-screening contained more crude protein, ether extract but less crude fibre, acid detergent fibre, total carbohydrate, total tannin and total extractable phenolic compounds. Chickpea hull contain higher amount of crude fibre than chickpea pre-screening. Similarly, the ADF, which comprises lignin and cellulose, was also higher in chickpea hull (Table 1). The crude fibre and ADF difference is due to the fact that hull have higher crude fibre and ADF. Dehulling of legumes in general, results in variations in the content of nutrients and anti-nutritional factors in different milled fractions, because the nutrients and anti-nutritional factors in legumes are unevenly distributed in the seed (Shahidi *et al*., 2001).

Results obtained in the present study regarding the chemical composition of chickpea pre-screening confirmed the finding of Abdi and Danesh Mesgaran (2009). These values are similar to the values reported for chickpea (Sreerama *et al*., 2010; Costa *et al*., 2006). Total dietary fibre, was also higher in chickpea hull (Table 1). Ramalho and Portugal (1990) reported the CP values for eight genotypes of chickpea seeds grown at different locations which ranged from 18.2 to 24 % of DM.

The crude protein content of chickpea pre-screening was higher than those reported by Salgado

Table 1: Chemical composition of chickpea by-product (g.kg DM^{-1})

Constituent	Chickpea by-product			
	Chickpea pre-screening	Chickpea hull	s.e.d	P
DM	919	923	4.3	NS
OM	940	927	6.2	NS
CP	279	44	5.4	< 0.01
EE	78	87	1.4	< 0.01
CF	72	178	2.4	< 0.01
Total carbohydrates[a]	502	719	6.5	< 0.01
NDF	351	323	6.6	< 0.01
ADF	96	224	10.1	< 0.01
TT	1	6.5	0.55	< 0.01
TPC	3.4	7.5	0.75	< 0.01

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fibre; NDF, neutral detergent fibre; ADF, acid detergent fibre; TT, total tannin; TPC, total extractable phenolic compounds

[a]By difference as 100 - (moisture + protein + ash + fat). Values are mean ± (standard error of difference); Ns: not significant.

et al. (2001) that showed that the CP level of white and black chickpea seeds were 195.1 and 212.7 g.kg DM^{-1} respectively. This could be due to higher embryonic axe that was produced during chickpea dehulling and added to chickpea pre-screening by product. Sreerama *et al.* (2010) reported that the protein content of embryonic axe fractions in chickpea and horse gram were higher than seed coat fractions. Who also reported that seed coat fractions had the lowest recorded protein content of 7.3 % in chickpea and 9.1 % in horse gram.

Crude fibre content was higher in seed coat fractions than pre-screening fraction. These results are similar to the values reported for chickpea (Sreerama *et al*., 2010) and beach pea (Shahidi *et al*., 2001). The presence of high crude fibre in food material is reported to decrease dry matter digestibility in animals (Devendra, 1995).

The levels of phenolic compounds were higher in seed coat fractions than pre-screening fraction. Similar results were observed by Sreerama *et al*. (2010). These results indicate that phenolic compounds are mostly concentrated in the seed coat fractions and might be easily removed by dehulling. Phenolic compounds, which are abundantly present in the seed coats of legumes, are one of the most important groups of secondary metabolites in plants having anti-nutrient properties (Sreerama *et al*., 2010). Tannins may form a less digestible complex with dietary proteins and may bind and inhibit the endogenous protein, such as digestive enzymes (Kumar and Singh, 1984). Tannin can adversely affect the microbial and enzyme activities (Singleton, 1981; Lohan *et al.*, 1983; Barry and Duncan, 1984; Makkar *et al.*, 1989).

The content of phenolic compounds in chickpea pre-screening fractions of chickpea is lower than those reported for beach pea (Shahidi *et al*., 2001), cowpea, pea, pigeon pea, and chickpea cotyledon fraction (Reddy *et al*., 1985). Phenolic compounds usually form insoluble complexes with protein, thereby interfering with their bioavailability (Liener, 1994). However, these phenolic compounds have been reported to act as antioxidants by preventing oxidative stress that causes diseases such as coronary heart disease, some types of cancer, and inflammation (Tapiero *et al*., 2002). Because the content of phenolic compounds is higher in seed coat and embryonic axe fractions of chickpea and horse gram, they are likely to have antioxidant activity.

Cumulative gas production profiles from the *in vitro* fermentation of chickpea pre-screening and chickpea hull are shown in Figure 1. The cumulative volume of gas production increased with increasing time of incubation. Gas produced after 96 h incubation ranged between 342.43 and 403.98 ml per g of dry matter. Cumulative gas production was comparable to those reported by Maheri-Sis *et al.* (2007). At all incubation times cumulative gas productions (ml) of chickpea pre-screening was significantly ($P < 0.05$) higher than chickpea hull.

Gas production parameter and calculated amount of organic matter digestibility (OMO) and metabolizable

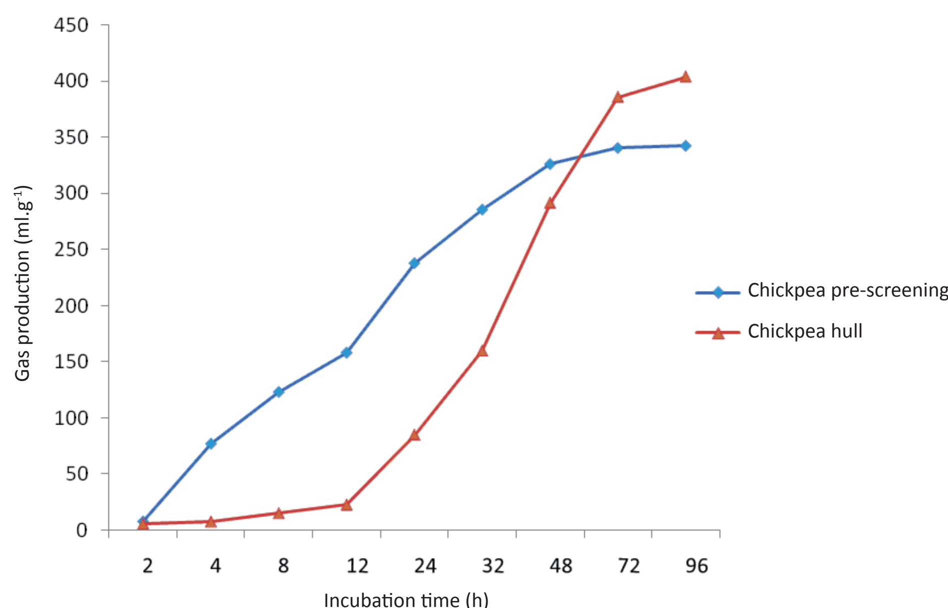

Fig. 1: Gas production of chickpea pre-screening and chickpea hull when incubated with rumen fluid at different incubation times

Table 2: Gas production parameters, organic matter digestibility (OMD)* and metabolizable energy (ME) content of chickpea by-product**

Item	Chickpea pre-screening	Chickpea hull	P
A (ml)	348.0 ± 12.1	467.5 ± 12.5	< 0.01
c ($ml.h^{-1}$)	0.050 ± 0.0022	0.024 ± 0.0023	< 0.01
L (h)	0.7 ± 0.33	5.25 ± 1.3	< 0.01
OMD (%)	59.1 ± 0.42	42.1 ± 1.5	< 0.01
ME ($MJ.kg^{-1}$)	8.95 ± 0.074	6.5 ± 0.2	< 0.01

A, asymptotic gas production (ml); c, fractional rate of gas production ($ml.h^{-1}$); lag, the initial time delay in the onset of gas production (h)
*OMD = 0.9991 Gas + 0.0595 CP + 0.0181 CA + 9
**ME = 0.157 Gas + 0.0084 CP + 0.022 EE – 0.0081 CA + 1.06

energy (ME) are presented in Table 2. The amount of c and value calculated for OMD and ME of chickpea pre-screening were significantly higher than chickpea hull ($P < 0.01$). This might be due to difference in chemical composition and volume of gas production in the first 24 h. Lag time of chickpea hull was significantly higher than chickpea pre-screening ($P < 0.01$).

CONCLUSION

It was concluded that the by-products evaluated in the present experiment had a potential to use as suitable feed in ruminant rations. There is no apparent anti-nutritive factor other than tannins, whose effect must be further tested. However, future feeding trials will be proposed to evaluate the effect of this by-product in ruminant production.

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