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Nutritive Value Evaluation of Processed Chickpea (*Cicer arietinum*) Residues with some Chemicals Based on *in vitro*, *in situ* and X-Ray Diffraction (XRD) Techniques

Research Article

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ABSTRACT

This research was conducted to investigate the the effect of sodium hydroxide (NaOH, 50 g/kg DM), calcium oxide (CaO, 160 g/kg DM), hydrobromic acid (HBr, 60 mL/kg DM) and hydrogen peroxide (H_2O_2, 57 mL/kg DM) processings on the nutritive value of chickpea (*Cicer arietinum*) residues. The chemical composition of the samples was determined using the standard methods. Degradability trial was done using nylon bag technique. *In vitro* digestibility of the samples was determined by the batch culture procedure. X-ray diffraction method (XRD) was used to determine the crystallininty degree of the samples. Treatments of NaOH, CaO and H_2O_2 increased the ash content (P<0.0001). The ether extract (EE) was reduced by the NaOH and H_2O_2 treatments (P=0.0006). Except CaO, the other treatments reduced (P<0.0001) the neutral detergent fiber (NDF). Processing with HBr increased (P=0.0014) total digestible nutrients (TDN), net energy for lactation (NEl), and net energy for gain (NEg). CaO, HBr, and H_2O_2 treatments increased the effective ruminal degradability (ERD) of dry matter at ruminal outflow rates of 0.02, 0.05 and 0.08 h^{-1} (P=0.0074, P<0.0001). Except CaO (P<0.0001), the other treatments had no positive effect on the samples *in vitro* digestibility. The treatments increased the efficiency of microbial biomass at the end of 24 h incubation (P<0.0001). Chemicals reduced the crystallinity degree of chickpea residues compared to the control. The least crystallinity percentage was observed in CaO treated samples. Totally, based on the *in vitro* and *in situ* results, treatments, especially HBr, had a positive effect on nutritional value of chickpea residues. However, these results must be confirmed or invalidated by *in vivo* tests.

KEY WORDS chemical processing, chickpea (*Cicer arietinum*) residues, cristallinity, *in situ* degradability, *in vitro* digestibility, nutritive value.

INTRODUCTION

The paucity of feed ingredients and high prices some of them are the major obstacles in development of animal husbandry sector. Proper use of unconventional feeds and agro-industrial by-products seems to be a good strategy to compensate for part of the shortages of main feed sources and decrease in feed costs (Wadhwa and Bashi, 2013).

Crop residues are the fibrous parts of the plants that are remained in the farm after the harvesting. They have a good potential for using in the ruminant diets as a source of energy (Jami *et al.* 2014). These materials help to reduce feed costs and the environmental pollution as a result of their accumulations (Ferro *et al.* 2018). The main limitations for using the crop residues as ruminant feedstuffs are the nutritional imbalances and the high rate of their natural lignin.

Lignin alongside cellulose and hemicellulose makes insoluble polymers which could be bonds with the other polymers. These later bonds are not hydrolysable in the normal biologic conditions. So, as a result the voluntary intake of dry matter (DM) and digestibility of crop residues are always low (Nie *et al*. 2020).

Improving the nutritional value of the agricultural by-products such as crop residues is one of the important investigation goals in ruminant nutrition. Different physical processing procedures (chopping, grinding, soaking, pelleting, pressure steaming, cooking and radiation), as well as chemical treatments (use of alkaline, acidic and oxidative solutions), or biological methods (microbial factors, fungi and commercial enzymes) have been applied for breaking the lingo-cellulosic bonds and increasing the bioavailability of the crop residues (Nie *et al*. 2020; Tauqir *et al*. 2022). Many of the physical methods are not applicable in small-scale livestock farms, because they need complicated machines and costly industrial processing techniques. So, these treatments are not economically affordable in any cases. Despite some advantages of biological methods, they are very time consuming and sometimes may result into nutritional wastages. Chemical treatments seem to be the most applicable methods for enhancement of nutritional values of agricultural residues and industrial by products at the farm level. Chemical compounds are relatively less expensive and easy to use (Sheikh *et al.* 2018).

Legumes are the most important products of the *Fabaceae* (Leguminosae) and have a significant role in providing valuable by-products for the animal nutrition (Joshi and Parthasarathy Rao, 2016). Common bean (*Phaseolus vulgaris*), green pea (*Pisum sativum*), and chickpeas (*Cicer arietinum*) are the most important legumes cultivated in many countries especially in arid and semi-arid areas (Heidarvand and Maali-Amiri, 2013). Several by- products of chickpea cultivation and processing are used in livestock feeding. Low quality seeds, bran, hay and agricultural residues (stem/straw) are some examples of these by- products. In fact, chickpea straw has a higher nutritional value than the cereals straw (44 to 46% total digestible nutrients and 4.5 to 6.5% crude protein/DM). Metabolizable energy (ME) in chickpea straw is 7.70 MJ/kg DM while the wheat straw has a ME of 5.60 MJ/kg DM. Ruminal DM digestibility and degradability (DMD) of the chickpea is 10 to 42% more than the wheat straw which represents it can be used as an alternative forage source in ruminant diets. Considering the fact that it is more palatable than the wheat straw, too (Tassoni *et al*. 2020).

In previous studies, the improvement in nutritional value of some legume residues has been reported as a result of chemical processing.

Alaei *et al.* (2020) observed that sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) treatments improved the effective ruminal degradability (ERD) of DM and *in vitro* efficiency of microbial biomass (EMB). In the study of Soltani *et al*. (2018), processing with NaOH increased the Ash and crude protein (CP), and decreased the cell wall content of chickpea residues. They also reported that DM and organic matter (OM) digestibility, microbial biomass (MB) and its efficiency (EMB) were also increased by alkali treatment. Babayi *et al.* (2015) observed that treatments of hydrobromic acid (HBr) and H_2O_2 improved the gas production parameters, as well as increased *in situ* degradability of vetch residues. This study was conducted to investigate the effects of some chemical compounds on nutritional value of chickpea residues under *in vitro* and *in situ* conditions.

MATERIALS AND METHODS

Preparation and processing of chickpea residues

Chickpea residues were collected from industrial farms around Naniz-Rabour county, Kerman province, Iran, and chopped into 5 cm pieces. The residues were then processed by NaOH, Cao, HBr, and H_2O_2.

For the NaOH processing, a solution 50 g/L of the chemical in distilled water was sprayed on 1 kg DM of GPR, and mixed well for one hour. For processing with CaO, at first, each kg of the residues was mixed well with 2 L of distilled water. Then, 160 g of CaO as powder was poured on and mixed well for one hour (Chaudhry, 2000). In order to process the residues by HBr, 6 mL of HBr diluted in 25 mL of distilled water was used for 100 g of DM. In processing with H_2O_2, the samples were first pretreated by NaOH. 30 min later, 57 mL of H_2O_2 (35%) was dissolved in 0.5 L distilled water and added to each 1 kg of the residues DM (Bouchard *et al*. 2006). The samples were kept in two-layer vacuumed nylon bags. After the processing time, the bags including different samples were opened and air dried.

Chemical analysis

Chemical composition of the samples including DM, Ash, ether extract (EE) and CP was determined according to the standard methods of AOAC (2005). Measurements of NDF and ADF were performed by Van Soest (1991). Total digestible nutrients (TDN), net energy for lactation (NEl) and net energy for gain (NEg) were estimated using the following equations (NRC, 2001):

TDN (g/kg DM)= 81.38 + (CP×0.36) - (ADF×0.77)
NEl (Mj/kg)= (0.1224×TDN) - 0.12
NEg (Mj/kg)= (0.0229×TDN) - 1/01

Animals and diet

Three rumen fistulated Dallagh sheep (45±2.5 kg) housed in individual 1 × 1.50 m pens were used for *in situ* and *in vitro* trials. The animals were fed a total mixed ration (TMR) according to the standard of the experiments on maintenance level (Table 1) twice daily in equal meals at 08:00 h and 17:00 h and also had free access to drinking water.

***In situ* ruminal degradability trial**

Degradability trial was performed using the nylon bag technique (Mehrez and Orskov, 1997). Un-treated and chemical treated chickpea residues were ground by laboratory hammer mill to 3 mm. Then 3 g of samples were put in polyester bags (10 cm×21 cm; 45 mm pore size). Two bags were prepared for each sample at each incubation time per sheep. Ruminal incubation times of nylon bags containing samples were 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h. All bags were inserted into the rumen at the same time, just before the morning feeding. At the end of each incubation time, bags containing. Residual materials were removed from rumen by fistula and then were immediately washed by cold water to avoid fermentation and remove debris from outside of the bags. This was done until the rinse water was clear. Then the bags were placed in a washing machine with cold water for 30 min. Washed bags were dried in forced-air oven at 60 ˚C for 48 h and then weighed. To estimate the disappearance rate of feedstuffs at time 0, the nylon bags including samples were washed without ruminal incubation.

Degradability amount of DM at different incubation times in the rumen was calculated as the difference between the initial feed weight and the portion remaining after incubation in the rumen. The DM degradability parameters of Un-processed and processed chickpea residues were estimated using Fit Curve software. The exponential model of Ørskov and Mc Donald (1979) was used for fitting DM degradability data:

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

Where:
P: DM degradability at time t (h).
a: washout (soluble) fraction (%).
b: potentially degradable fraction (%).
c: degradation rate (h^{-1}) of b fraction.

Using the fractional outflow rate from the rumen, k, the effective ruminal degradability (ERD) of DM was calculated as:

$$ERD = a + \left[\frac{b \times c}{c + k}\right]$$

where estimated solid outflow rates (k) of 0.02, 0.05 and 0.08 h^{-1} were used (Tuncer and Sacakli, 2003).

Determination of *in vitro* digestibility

Digestibility determination of different treatments was done using the batch culture method (Theodorou *et al.* 1994). To do so, the samples were first grinded to 1 mm and then dried. Ruminal fluid was collected by fistula before the morning feeding. Artificial saliva was prepared by the method according to Menke *et al.* (1979). 500 mg of each sample was poured in each glass vial and 50 ml of a combination of artificial saliva and ruminal fluid with a ratio of 2 to 1 was added to each vial. Then CO_2 gas was inserted into each glass vial for 10 seconds, and the vials were then sealed by a rubber cap and aluminum cover. The vials were then put in a warm water bath at 39 ˚C. After 24 hours, all the vials were taken off the water bath and transferred to the ice container. The samples of each vial were sieved by a special cloth and the indigested contents were separated from the liquid phase and dried in 60 ˚C oven for 48 h. Apparent digestibility of the samples was then calculated. The pH of the liquid phase of samples was measured.

Estimation of rumen fermentation parameters

The ammoniacal nitrogen rate of the samples was determined using the phenol-hypochlorite method (Broderick and Kang, 1980). A spectrophotometer device at 630 nm wave length was used to read the light absorption. Calculating the produced microbial biomass was done using the following equation (Makkar, 2010).

$$MB= GP \times (PF-2.2)$$

Where:
MB: produced microbial biomass (mg/g of DM).
GP: net gas production after 24 h incubation (mL/g of DM), and PF is partitioning factor (mg/mL). Partitioning factor is the mg of digested real OM to mL of net gas production. Microbial biomass efficiency was calculated as the produced microbial biomass divided by the fermentable real OM at the end of incubation time (24 h).

Statistical data analysis

Statistical data analysis was done using completely randomized design (CRD). The statistical model was as below:

$$Y_{ij}= \mu + T_i + e_{ij}$$

Where:
Y_{ij}: amount of each observation, μ is total mean.
T_i: treatment effect.
e_{ij}: random residual error.

Data processing was done using the SAS software (SAS, 2003) and ANOVA process. Mean comparison was done using the least significant difference test (LSD).

Table 1 Ingredients and chemical composition of diet

Ingredient	g/kg of total diet DM
Alfalfa hay	200
Corn silage	500
Barley grain	120
Wheat barn	175
Mineral–vitamin premix[1]	5
Chemical composition	
Metabolizable energy	2.48
Crude protein	10.90
Calcium	0.73
Phosphorus	0.35

[1] Mineral–vitamin premix contained per kg dry matter (DM): Ca: 170 g; P: 60 g; Mg: 50 g; Fe: 3 g; Cu: 2 g; Mn: 4 g; Zn: 6 g; Co: 0.1 g; I: 0.25 g; Se: 0.03 g; NaCl: 250 g; vitamin A: 300000 IU; vitamin D_3: 60000 IU and vitamin E: 0.5 g.

Crystalline analysis by x-ray diffraction

The crystallinity of the samples was determined using x-ray diffraction test. Samples were ground to 70 µm and analyzed by x-ray diffraction (XRD). XRD analyses were performed on a Philips Xpert-pro model using Co Kα1 (1.789010 Å) radiation, monochromator on secondary optics, 40-kV power, and 35-mA current at Iranian Mineral Processing Research Center. Interpretation and determination of crystallinity percentage in XRD graphs was done by Xpert High score Plus software (Driscoll *et al.* 2009).

RESULTS AND DISCUSSION

Mean comparison of the chemical composition in unprocessed (control) and chemically processed chickpea residues (Table 2) showed significant differences between the treatments (P=0.0014, P<0.0001).

In NaOH, CaO and H_2O_2 treatments, the amount of ash increased compared to the control (14.76, 27.33 and 33.15% of DM *vs.* 12.95% of DM). Similar to the present study, processing of vetch, chickpea and faba bean residues with NaOH, CaO, and H_2O_2 has increased the ash amount in samples (Babayi *et al.* 2016; Soltani Naseri *et al.* 2018; Alaei *et al.* 2020). It seems as the increased ash content in NaOH and CaO processed samples is for sedimentation of Na and Ca included in these chemicals on chickpea residues (Baytok *et al.* 2005). Also, as in H_2O_2 processing, NaOH is first added to the substance (to keep the pH in range of 11.5), the ash percentage would be increased in the processed sample. Reduced OM of the mentioned treatments also occurred in consequence of increased ash, as there is a reverse relationship between these two parameters. The decrease in OM is the result of sugar substances dilution and consequently increased Ash (Ghiasvand *et al.* 2011).

Similarly, Chaudhry (2000) indicated that NaOH and NaOH+ H_2O_2 treatments increased the Ash content of wheat straw through adding Na and reducing the cell wall. The amount of EE and CP in the control group were 2.73 and 10.03% of DM, respectively. NaOH and H_2O_2 treatments increased EE (4.67 and 3.63% of DM, respectively), and HBr and H_2O_2 reduced CP content (8.32 and 7.32% of DM respectively). In previous studies, processing of crop residues with chemical compounds such as CaO, NaOH and HBr has increased the EE of the samples (Aslanian *et al.* 2015; Babayi *et al.* 2016; Zhao *et al.* 2016). The increase in EE as a result of chemical treatments has been related to the decreased cell wall components including cellulose and hemi-cellulose and consequently increased proportion of this nutrient (Zhao *et al.* 2016).

Aslanian *et al.* (2015) and Alaei *et al.* (2020) reported reduction of CP in soybean straw and faba bean residues processed with CaO. The reason for the decrease in the CP content of processed lignocellulosic residues with chemical compounds is the separation of nitrogen from the components of these residues (Khorvash *et al.* 2010).

Except for CaO (48.58% of DM), the other treatments reduced NDF compared to the control (50.50% of DM). The greatest reduction was observed in HBr (31.75% of DM). ADF content decreased from 35.58% of DM in the control to 23.08% of DM in HBr. The other treatments had no effect on this trait. Decreasing cell wall content (cellulose, hemicellulose, lignin, and pectin) and consequently increasing their digestibility are the important goals of processing lignocellulosic compounds. In the previous studies, decreased crude fibers (CF), NDF, ADF, and lignin were reported for agricultural by- products as a result of chemical processing (Harun and Geok, 2016; Zhao *et al.* 2016; Kim, 2018; Zhang *et al.* 2020; Manokhoon and Rangseesuriyachai, 2020). Chemical substances, especially alkaline compounds, are very effective in separation of lignin from the biomass. They lead to the destruction of cell wall components including NDF, ADF, hemi-cellulose, as well as lignin, and so reduce them (Zhang *et al.* 2020). Alkaline compounds are able to break the bonds between lignin and polysaccharides and so dissolve the hemicellulose included in straws. Therefore, the amounts of cellulose and NDF are reduced in these materials (Polyorach and Wanapat, 2015). It has been observed that pretreatment of soybean straw and husk with NaOH, destroyed cell wall structure and solubility of lignin and hemicellulose (Qing *et al.* 2017). In a similar way, reduced CF content of vetch wastes processed with H_2O_2 has been reported (Babayi *et al.* 2016). In one study, pretreatment with H_2O_2 decreased lignin content and so NDF in palm fibers (Shengqiang *et al.* 2018).

Table 2 Effect of chemical treatments on chemical composition, total digestible nutrient and net energy for gain and lactation of chickpea residues

Treatments	Chemical composition (% DM)									
	DM	Ash	OM	EE	CP	NDF	ADF	TDN (g/kg DM)	NEg (MJ/kg)	NEl (MJ/kg)
Control	94.23^{b}	12.95^{d}	87.05^{a}	2.73^{c}	10.03^{a}	50.50^{a}	35.58^{a}	575.90^{b}	1.29^{b}	0.66^{b}
NaOH	95.50^{a}	14.76^{c}	85.24^{b}	4.67^{a}	10.49^{a}	46.58^{bc}	33.83^{a}	591.00^{b}	1.33^{b}	0.70^{b}
CaO	90.74^{a}	27.33^{b}	72.67^{c}	2.67^{c}	10.07^{a}	48.58^{ab}	32.50^{a}	599.80^{b}	1.35^{b}	0.73^{b}
HBr	95.20^{a}	12.76^{d}	87.24^{a}	2.60^{c}	8.32^{b}	31.75^{d}	23.08^{b}	666.00^{a}	1.51^{a}	0.92^{a}
H_2O_2	91.51^{c}	33.15^{a}	66.85^{d}	3.63^{b}	7.32^{b}	46.25^{c}	32.83^{a}	587.30^{b}	1.32^{b}	0.69^{b}
SEM	0.201	0.334	0.334	0.251	0.342	0.644	1.401	1.111	0.027	0.032
P-value	< 0.0001	< 0.0001	< 0.0001	0.0006	0.0002	< 0.0001	0.0007	0.0014	0.0014	0.0014

NaOH: sodium hydroxide; CaO: calcium oxide; HBr: hydrobromic acid and H_2O_2: hydrogen peroxide.
DM: dry matter; OM: organic matter; EE: ether extract; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; TDN: total digestible nutrient; NEg: net energy for growth and NEl: net energy for lactation.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

CaO has been a strong treatment in delignification and reduces NDF, ADF, hemicellulose, as well as lignin (Trach *et al.* 2001). Changes in straw cell wall and improved solubility of cellulose and hemicellulose have been indicated as the reason for the decreased NDF and ADF of rice straw processed with urea (Ma *et al.* 2020).

The amount of TDN, NEg and NEl in the control group were 575.90 g/kg DM, 1.29 and 0.66 MJ/kg, respectively. HBr increased these traits to 666.00 g/kg DM, 1.51 and 0.92 MJ/kg, respectively. TDN indicates available nutrients for the livestock and depends on the density of ADF in hays. So, by the increase in density of ADF, TDN will be decreased. In other words, the livestock will not be able to use the nutrients included in the hay (Lithourgidis *et al.* 2006).

In the present study, through the reduction in NDF and ADF contents as a result of chemical treatments, TDN was increased. NEl and NEg are both affected by TDN (NRC, 2001). So, in chemical treatments, by the increase indicated in TDN, these parameters were increased too. In the study by Hosseinzadeh *et al.* (2020), increased TDN as well as NEl and NEg in processed barley grains were related to increased CP content and reduced density of cell wall components in the processed grains. In the study by Soltani Naseri *et al.* (2018), there were observed no effect of chemical treatments including H_2O_2, NaOH and H_2O_2 on NDF, ADF and consequently on TDN, NEl, as well as, NEg.

Degradability parameters and ERD of DM

Degradability trend of different treatments during 96 h of rumen incubation (Figure 1) showed that chemical processing increased DM ruminal degradability of chickpea residues. The greatest increase was observed in CaO treatment. Mean comparison of degradability parameters and ERD of DM for unprocessed and chemically processed samples is shown in Table 3.

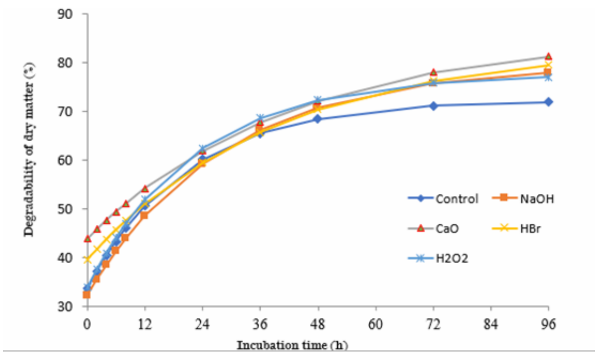

Figure 1 Degradability trend of untreated and treated chickpea residues with chemicals compounds

Treating with CaO and HBr increased washout fraction (a) from 33.52% to 43.79 and 39.96%, respectively, and processing with NaOH and HBr increased the potentially degradable fraction (b) from 38.90% to 47.28 and 48.08%, respectively (P<0.0001, P=0.0098). ERD of DM at rumen outflow rates of 0.02, 0.05 and 0.08 /h in un-treated samples was 61.00, 52.70 and 48.33%, respectively. Treatments of CaO, HBr and H_2O_2 at outflow rate 0f 0.02/h (66.53, 64.43 and 63.97%, respectively), treatments of CaO and H_2O_2 at outflow rate 0f 0.05/h (57.40 and 54.43%, respectively) and CaO treatment H_2O_2 at outflow rate 0f 0.08/h (53.60%) increased ERD of the samples (P=0.0074, P<0.0001). Ruminal degradability of lignocellulosic agricultural by- products is considered as a main challenge in ruminants. In lignocellulosic substances, cellulose which is a linear polymer of glucose, bonds with hemicellulose and is surrounded by lignin. Lignin is a three-dimensional complex of the poly-aromatic matrix that prevents accessibility of enzymes to some parts of the cellulose polymer (Zhao *et al.* 2016). Processing of lignocellulosic substances is mainly aimed to increase accessibility to the surface of cellulose and converting cellulose to glucose (Kucharska *et al.* 2018).

Table 3 Effect of chemical treatments on ruminal degradability parameters and effective degradability of chickpea residues

Treatments	Ruminal degradability parameters			Effective degradability (%) at rumen outflow rates		
	a (%)	b (%)	c (h^{-1})	0.02 (h^{-1})	0.05 (h^{-1})	0.08 (h^{-1})
Control	33.52^{c}	38.90^{b}	0.049^{a}	61.00^{c}	52.70^{cd}	48.33^{bc}
NaOH	32.18^{c}	47.28^{a}	0.035^{b}	62.37^{bc}	51.83^{d}	46.83^{c}
CaO	43.79^{a}	42.79^{ab}	0.023^{c}	66.53^{a}	57.40^{a}	53.60^{a}
HBr	39.96^{b}	48.08^{a}	0.022^{c}	64.43^{ab}	54.07^{bc}	49.93^{b}
H_2O_2	33.86^{c}	43.71^{ab}	0.044^{ab}	63.97^{b}	54.43^{b}	49.50^{b}
SEM	0.697	2.406	0.003	0.766	0.547	0.570
P-value	< 0.0001	0.0098	< 0.0001	0.0074	< 0.0001	< 0.0001

NaOH: sodium hydroxide; CaO: calcium oxide; HBr: hydrobromic acid and H_2O_2: hydrogen peroxide.
a: washout fraction; b: potentially degradable fraction and c: degradation rate of "b" fraction.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Chemical reagents could be absorbed into the cell wall of lignocellulosic residues and chemically break down the ester bonds between lignin and hemicellulose or cellulose. These reactions would result in larger reaction area between rumen microbes and residues, and consequently make rumen microbes more readily digest structural carbohydrates (Zhao *et al.* 2016). In a study, ammoniation degraded the side chains of esters and glycosides leading to structural modification of lignin, cellulose swelling, cellulose decrystallization, and hemicellulose solvation and increased the degrading activity of the microbes (Ma *et al.* 2020). In accordance with the present study, Chaudhry (2000) reported increased DM ruminal degradability of wheat straw processed by NaOH and H_2O_2.

Polyorach *et al.* (2018) reported a linear increase in degradability parameters and ERD of Ca $(OH)_2$ treated rice straw. In another study, processing with CaO (7 and 10% of DM) increased the degradability parameters of Bahiagrass (*Paspalum notatum*) hay (Ciriaco *et al.* 2021).

In vitro digestibility and fermentation parameters are

The effects of chemical processing on *in vitro* digestibility of DM and OM, and fermentation parameters of chickpea residues are shown in Table 4. The amount of DMD and OMD in the control group were 60.00 and 63.00%, respectively. HBr increased DMD (66.00%), but it had no effect on the OMD (65.00%). These traits were lower than control in other treatments (P<0.0001). There is an inverse relationship between NDF and ADF with the digestibility of feedstuff (Nazem *et al.* 2008). Thus, increasing the *in vitro* digestibility of HBr-treated samples may be related to the reduction of their cell wall content. Similarly, Babayi *et al.* (2015) reported an increase of DMD and OMD in vetch wastes treated with HBr. The reason for reduced digestibility in samples treated with NaOH, H_2O_2 and CaO can be the deposition of sodium (Na) and calcium (Ca) in the samples, because these elements replace part of the fermentable substrate in the samples.

In the study of Alaei *et al.* (2020), *in vitro* digestibility of faba bean residues treated with CaO was reduced compared to control samples. Khorvash *et al.* (2010) observed that NaOH and CaO treatments reduced soybean straw cell wall, however they had no effect on its *in vitro* digestibility.

The NH_3-N concentration in CaO and H_2O_2 treatments was higher (P<0.0001) than the control (0.93, 1.10, 0.78, 0.80 and 0.91 mg/dL, respectively). The pH of media culture increased from 6.64 in control samples to 6.82 in CaO treatment, that the difference between them was significant (P=0.0014). In some *in vitro* studies, HBr, NaOH, and H_2O_2 treatments had no effects on NH_3-N density of media culture in vetch and pea wastes (Babayi *et al.* 2016; Soltani Naseri *et al.* 2018). It was observed in a study that using NaOH reduced NH_3-N of the media culture which was similar to the present study (Aslanian *et al.* 2015). Decreased *in situ* concentration of ruminal fluid NH_3-N has been reported in goats receiving alkaline processed wheat straw which the scientists related it partially to increased synthesis of microbial protein and partly to decreased ureolytic and proteolytic activities of ruminal bacteria or ruminal epithelium (Ria and Mudgal, 1996). Similarly, using rice straw treated with alkaline treatments of urea and CaO, increased NH_3-N concentration of ruminal fluid in beef calves (Polyorach and Wanapat, 2015). Increased protein degradability has been indicated as the probable reason of increased NH_3-N concentration in ruminal fluid for the agricultural residues processed with chemicals (Danesh Mesgaran *et al.* 2010). As the NH_3-N concentration increases, the pH of the media culture also increases. On the other hand, the low pH in HBr treatment is due to the acidic nature of this chemical compound.

The amount of PF in CaO treatment increased compared to the control (P<0.0001). The other treatments had no effect on this trait. Processing reduced the amount of gas yield after 24 h of incubation (P<0.0001). In contrast, EMB increased in different treatments compared to the control (P<0.0001).

Table 4 Effect of chemical treatments on dry matter and organic matter digestibility and, ammoniacal nitrogen, pH, partitioning factor, gas yield, microbial biomass and efficiency of microbial biomass of chickpea residues

Treatments	Fermentation parameters							
	DMD (%)	OMD (%)	NH_3-N (mg/dL)	pH	PF (mg/mL)	GY-24 (mL/g DM)	MB (mg/g DM)	EMB
Control	60.00^{b}	63.00^{a}	0.91^{c}	6.64^{b}	3.90^{b}	231.66^{a}	118.76^{c}	0.43^{c}
NaOH	53.00^{c}	44.00^{c}	0.78^{e}	6.61^{bc}	4.30^{b}	141.41^{c}	78.75^{d}	0.48^{b}
CaO	38.00^{d}	51.00^{b}	0.93^{b}	6.82^{a}	8.91^{a}	134.66^{cd}	168.48^{a}	0.75^{a}
HBr	66.00^{a}	65.00^{a}	0.80^{d}	6.52^{c}	4.37^{b}	197.55^{b}	142.22^{b}	0.49^{b}
H_2O_2	57.00^{bc}	41.00^{c}	1.10^{a}	6.71^{ab}	4.41^{b}	109.93^{d}	69.93^{d}	0.50^{b}
SEM	0.017	0.013	0.0014	0.035	0.172	8.72	4.83	0.016
P-value	< 0.0001	< 0.0001	< 0.0001	0.0014	< 0.0001	< 0.0001	< 0.0001	< 0.0001

NaOH: sodium hydroxide; CaO: calcium oxide; HBr: hydrobromic acid and H_2O_2: hydrogen peroxide.
DMD: dry matter digestibility; OMD: organic matter digestibility; NH_3-N: ammoniacal nitrogen; PF: partitioning factor; GY-24: gas yield after 24 h incubation; MB: microbial biomass and EMB: efficiency of microbial biomass.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The highest amount was observed in CaO treatment (0.75). PF is an indicator of the hay quality. It has been defined as the portion of truly disappeared OM (mg) to the volume of gas yield (mL) during the 24 h incubation (Bayatkouhsar *et al.* 2022). The more PF is indicative of the fact that degraded substances have been led to MB production and so the EMB would be more (Blummel and Orskov, 1993). In the present study, the amount of PF was in CaO treatment significantly higher and in other treatments non-significantly higher than control. It means that the EMB has been increased in these treatments and conversely gas yield has been reduced, as the obtained data are indicative of this fact. Similar to the present study, in the previous studies, increased PF and consequently increased MB, as well as reduced gas yield were reported in legume wastes processed with chemical compounds (Soltani Naseri *et al.* 2018; Alaei *et al.* 2020).

X-ray diffraction pattern

X-ray diffraction (XRD) patterns of different treatments are shown in Figure 2. Chemical processing decreased crystallinity of the chickpea residues. Such as in the processed samples, the peak related to cellulose crystallinity was decreased compared to the control. Relative crystallinity of different treatments is shown in Table 5. The highest and lowest percentages of crystallinity were that of the control and CaO treatments (62.00 and 49.50%, respectively). A breakdown in the structure and a reduction in crystallinity have been observed in response to alkaline treatment. This might be responsible for the improved attachment of microbial cellulosome (Nieves *et al.* 2011). Also in theory, reduced cellulose crystallinity leads to increased sensitivity of cellulose in lignocellulosic substances to microbial degradation, and eliminating phenolic monomers from the cell wall matrix increases the availability of structural polysaccharides.

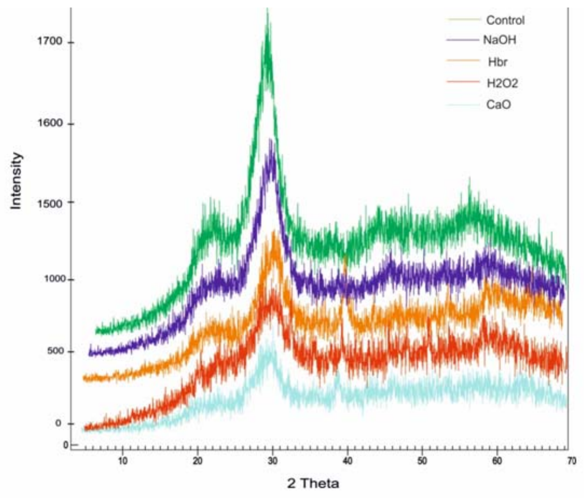

Figure 2 X-ray diffraction pattern of untreated and treated chickpea residues with chemical compounds

Table 5 Effect of chemical treatments on crystallinity degree of chickpea residues

Treatments	Relative crystallinity (%)
Control	62.00
NaOH	60.80
HBr	52.40
H_2O_2	50.20
CaO	49.50

NaOH: sodium hydroxide; CaO: calcium oxide; HBr: hydrobromic acid and H_2O_2: hydrogen peroxide.

Microbial degradation of straw in the rumen depends on elimination of factors that limit the access of microbial enzymes to the structural carbohydrates. Lignin, ferulic acid, and p-coumaric acid are such limiting factors that can be referred to (Kerley *et al.* 1988). It has been revealed that alkaline treatments like H_2O_2 increased degradability of substrate in the rumen through reducing these limiting factors or changing their three- dimensional structures (Kerley *et al.* 1988; Alaei *et al.* 2020).

Chemical compounds lead to the detachment and weakening of lignocellulosic substances through saponification of intermolecular ester bonds that cross link pentoses units of hemicellulose to the compounds like lignin. Also, during chemical treating, cellulose and especially its amorph areas get swelling. So chemical treatment of chickpea residues increases the structural compounds and consequently leads to an increase in cellulose accessibility in samples trough breaking the biomass structure and increasing its porosity, as well as reducing the crystalline structure of cellulose by eliminating the extracting substances (Sun and Cheng, 2002). Xu *et al*. (2007), by using the XRD pattern and Fourier Transform Infrared Spectroscopy (FT-IR), observed that ammonia treatment led to elimination of lignin and reduced the crystallinity of soybean straw. In their study, it was revealed that ammonia increased cellulose density to 70.27%, and reduced hemicellulose and lignin densities to 41.45 and 30.16%, respectively. Zhao *et al*. (2016) stated that alkaline chemicals could break down hydrogen bonding within Bamboo shoot shell and reduce the crystallinity of cellulose to make the structural fibers loose.

CONCLUSION

According to the results of the present study, chemical processing improved the chemical composition as well as ruminal fermentation parameters and reduced crystallinity of chickpea residues. Amongst the treatments, HBr was more effective in improving the nutritional value of the samples. However, these results must be confirmed or invalidated by *in vivo* tests.

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