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Effects of chemical processing on the nutritional value of green pea (*Pisum sativum*) residues

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Abstract This study was conducted to investigate the effects of processing with water (H_2O, 2.5 l/kg), calcium oxide (CaO, 160 g/kg), hydrogen peroxide (H_2O_2, 57 mL/kg), and sodium hydroxide (NaOH, 50 g/kg) on the nutritional value of green pea (*Pisum sativum*) residues (GPR). Chemical composition of GPR samples was determined using the standard methods of AOAC. Ruminal degradability trial was carried out using nylon bag technique. Gas production test was performed to estimate *in vitro* fermentation parameters. *In vitro* digestibility of the samples was determined by batch culture method. Results demonstrated that the chemical composition of GPR was affected by the processing method ($P<0.05$). Ash content was increased by CaO, H_2O_2, and NaOH treatments compared to the control treatment. All treatments led to a decrement in crude protein (CP) content with the least CP amount in CaO treatment. Acid detergent fiber (ADF) content of CaO treated samples was also lower than other treatments. The treatments, except CaO, caused an increase in effective rumen degradability (ERD) of dry matter (DM) at rumen outflow rates of 0.02, 0.05, and 0.08 h^{-1} ($P<0.05$). The greatest ERD was observed in H_2O_2 treatment. The potential of gas production (b fraction) was increased by processing with H_2O_2 and NaOH as compared to the control ($P<0.05$). Processing with NaOH and H_2O_2 increased ($P<0.05$) the concentration of short chain fatty acids (SCFAs) and metabolizable energy (ME) content. The DM digestibility (DMD) and organic matter digestibility (OMD) rates were greater in H_2O_2, NaOH, and CaO treatments as compared to the control ($P<0.05$). All treatments, except H_2O, increased the partitioning factor (PF) and efficiency of microbial biomass (EMB) of GPR samples ($P<0.05$). In conclusion, considering nutritional value and *in vitro* degradability parameters, processing GPR with NaOH and H_2O_2 was more beneficial compared to the CaO and control treatments.

Keywords: chemical compositions, green pea residues, nutritional value, processing

Introduction

The paucity of feed ingredients and the high cost of some feedstuff 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 feed shortage (Wadhwa et al., 2013).

Agriculture residues are the parts of the plant that are left in the farm after harvesting and mainly consist of structural carbohydrates (Nie et al., 2020). Lack of nutrient balance and high lignin rate have restricted the usage of these products in animal diets. Lignin makes some internal bonds with cellulose, hemicellulose and some other macromolecules. These bonds cannot be hydrolyzed in normal biological situations (Nie et al., 2020). Therefore, the

voluntary intake and ruminal degradability of agriculture residues are usually low (Brodie et al., 2019).

Nutritional value of lignocellulosic compounds can be improved using proper methods of processing. The aim of processing is delignification and breaking lignocellulosic bonds of the cell wall components, so that, cellulose and hemicellulose would be easily available for ruminal degradation and consequently, the voluntary intake as well as the digestibility would be increased. Different physical processing procedures (chopping, grinding, soaking, pelleting, pressure steaming, cooking and radiation), as well as chemical treatments (use of alkaline, acidic and oxidative agents), or biological methods (microbial factors, fungi and commercial enzymes) have been used to improve the nutritional value of agriculture residues (Bouchard et al., 2006; Sarnklong et al., 2010). Amongst the different mentioned methods, chemical processing seems to be more practical at the farm level. Chemical compounds inexpensive and relatively easy to apply (Sheikh et al., 2018).

Green pea (*Pisum sativum*), a crop belonging to *Fabaceae* (*Leguminosae)*, is proper plant for the regions with a cold and relatively wet climate which would be favorable to the winter cultivation in warm regions (Summerfield and Roberts, 1985). Post harvesting residues of legumes are of a higher ME and lower neutral detergent fiber (NDF) compared to the cereal straws (Sultan et al., 2011). Thus, to meet some part of the livestock feed requirements and reducing the production costs, it is important to pay attention to the premium use of these residues (especially the processed residues).

In previous studies, the improvement in nutritional value of some legume residues was reported as a result of chemical processing (Alaei et al., 2020; Babayi et al., 2016; SoltaniNaseri et al., 2018). The aim of this study was to investigate the effects of chemical processing on the nutritional value of GPR under *in vitro* and *in situ* conditions.

Materials and methods

Preparation and processing of green pea (Pisum sativum) residues

Samples of GPR were collected from industrial farms around Minoodasht county (north east of Golestan province, Iran) and chopped into 5cm pieces.

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, each kg of the residues was mixed with 2 L of distilled water. Then, 160 g of CaO as powder was poured on it and mixed well for one hour (Chaudhry, 2000). In order to process the residues by H_2O, 2.5 L of H_2O was used for 1 kg of DM. In processing with H_2O_2, the samples were first pretreated by NaOH. Then 30 min later, 57 mL of H_2O_2 (35%) was dissolved in 0.5 L distilled water and added to 1 kg of the residues DM (Bouchard-et al., 2006). The samples were kept in two layers 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 and CP was determined according to the standard methods of AOAC (2005). Measurements of NDF and ADF were performed by Van Soest (1991) method.

Animals and diet

Three rumen fistulated Dallagh sheep (45 ± 2.5 kg) housed in individual pens (1 × 1.50 m) 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

The degradability trial was performed using nylon bag technique (Mehrez and Orskov, 1997). All experimental samples were ground by laboratory hammer mill to 3 mm. Then 3 g of each sample was 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 and incubated for 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h. All bags were inserted in the rumen at the same time, just before the morning feeding. At the end of each incubation time, 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 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 were estimated using *Fit Curve* software. The exponential model of Ørskov and McDonald (1979) was used for fitting DM degradability data:

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

where, ‘P’ is DM degradability at time t (h), ‘a’ is the washout (soluble) fraction (%), ‘b’ is the potentially degradable fraction (%), ‘e’ is Euler's number, and ‘c’ is the degradation rate (h^{-1}) of b fraction.

Using the fractional outflow rate from the rumen, k, the 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.

a' is the washout (soluble) fraction (%), 'b' is the potentially degradable fraction (%), 'and 'c' is the degradation rate (h^{-1}) of b fraction.

Gas production test

Gas production of the experimental samples was measured according to the standard method (Menke et al., 1979). Rumen fluid was collected through rumen fistula before the morning feeding and transferred to the laboratory immediately. Artificial saliva and the filtered rumen fluid were poured into the special balloon in a ratio of 2:1 (volume of artificial saliva to rumen fluid.). Then, CO_2 gas was injected into the balloon and kept in a 39°C water bath. Finally, 30 mL of the mixture was poured into the glass vials including 200 mg of the sample. The vials were sealed with rubber caps and an aluminum cover, and put in a 39°C water bath immediately. During this step, the glass vials were being shacked at definite intervals. The volume of gas production was calculated cumulatively at the time intervals of 2, 4, 6, 8, 12, 24, 36, 48, 72, and 96 h after the incubation. Gas production parameters were estimated according to following equation by SAS software (Ørskov and MacDonald, 1979):

$$y= b (1- e^{-ct})$$

where, 'y' is gas produced at time t (mL/g of DM), 'b' is gas production from the fermentable insoluble fraction (mL), 'e' is Euler's number, 'c' is gas production rate for b fraction (mL/ h), and 't' is the incubation time (h).

The ME, organic matter digestibility (OMD), and short chain fatty acids (SCFA) of the samples were estimated using Getachew et al. (1998) and Menk and Steingass (1988) equations:

$$ME= 2.20+ 0.136\ GP+ 0.057\ CP+ 0.0029\ CF$$
$$OMD= 14.88+ 0.889\ GP+ 0.45\ CP+ 0.0651\ XA$$
$$SCFA= 0.0222\ GP- 0.00425$$

where, ME is metabolizable energy (Mj/kg of DM), GP is net gas production after 24 h incubation (mL/200 mg of DM), CP is crude protein (% of DM), CF is crude fiber (% of DM), OMD is organic matter digestibility (%), SCFAs is short chain fatty acids (mmol/200 mg DM), and XA is ash amount (% of DM).

Determination of in vitro digestibility

Digestibility of samples was determined using the batch culture method (Theodorou et al., 1994). Ruminal fluid was collected of fistulated sheep before morning feeding, and 500 mg of each sample and a mixture of artificial saliva and ruminal fluid (at a ratio of 2 to 1) were transferred into a glass vial. The gaseous CO_2 was blown 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 of the vials were taken off the water bath and transferred to an ice container. The content of the vial was sieved by a special cloth and the indigested contents were separated from the liquid phase and dried in an over at 60°C for 48 h. The sample apparent digestibility, and pH of the liquid phase, were measured.

Estimation of rumen fermentation parameters

The ammoniacal nitrogen (NH_3-N) concentration of the samples was determined using the phenol-hypochlorite method (Broderick and Kang 1980). Microbial biomass (MB) was estimated using the following equation (Makkar, 2010).

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

where, MB is microbial biomass (mg/g DM), GY is net gas yield after 24 h of incubation (mL/g DM), and PF is partitioning factor (mg/mL). The PF is the mg of digested real organic matter (OM) to mL of net gas production. MB efficiency (EMB) was calculated as the MB divided by the fermentable real OM at the end of incubation time (24 h).

Statistical analysis

Data analysis was done using the ANOVA procedure of the SAS (9.1, 2002) software. The statistical model was:

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

where, Y_{ij} is the value of each observation, μ is total mean, T_i is treatment effect, and e_{ij} is the random residual error.

Mean comparison was done using the least significant difference (LSD) test.

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*	5
Chemical composition (DM basis)	
Metabolizable energy, (Mj/kg DM)	2.48
Crude protein (%)	10.90
Calcium (%)	0.73
Phosphorus (%)	0.35

* Mineral–vitamin premix contained per kg 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; and NaCl, 250 g; vitamin A, 300000 IU; vitamin D3, 60000 IU; and vitamin E, 0.5 g

Results

Comparison of chemical composition of treatments indicated there were significant effect (P<0.05) among chemical treatments on DM, ash, OM, CP, NDF and ADF of GPR (Table 2). The amount of DM in H_2O_2 treatment was less than control (94.44 % versus 97.17 %). The CaO, H_2O_2, and NaOH treatments on GPR increased the ash content (24.16, 20.91, and 13.58 % of DM, respectively) and decreased OM content (75.83, 79.08, and 86.41 % of DM, respectively) compared with their amounts in control group (9.66 and 90.33 % of DM, respectively). All processed treatments decreased the amount of CP content of GPR. The least CP content was observed in the CaO treatment (5.16 percent of DM). The CaO treatment on GPR also decreased ADF content compared with control group (36.00 % of DM versus 47.33 % of DM).

Table 2. Effects of sodium hydroxide, calcium oxide, hydrogen peroxide and water on chemical composition green pea residues

	Chemical composition (DM%)[2]					
Treatments	DM	Ash	OM	CP	NDF	ADF
Unprocessed	97.17[a]	9.66[d]	90.33[a]	8.49[a]	62.66[ab]	47.33[ab]
NaOH[1]	96.55[a]	13.58[c]	86.41[b]	7.35[b]	70.66[a]	50.00[a]
CaO[1]	96.55[a]	24.16[a]	75.83[d]	5.16[d]	50.66[b]	36.00[c]
H_2O_2[1]	94.44[b]	20.91[b]	79.08[c]	5.78[c]	56.66[b]	43.33[b]
H_2O[1]	96.41[a]	11.58[cd]	88.41[ab]	7.17[b]	68.00[a]	49.33[a]
SEM[3]	0.287	0.963	0.963	0.152	2.748	1.712
P-value	0.0001	0.0001	0.0001	0.0001	0.0025	0.0010

[1] NaOH: Sodium hydroxide; CaO: Calcium oxide; H_2O_2: Hydrogen peroxide; and H_2O: Water
[2]DM: Dry matter; OM: Organic matter; CP: Crude protein; NDF: Neutral detergent fiber; and ADF: Acid detergent fiber
[3]SEM: Standard error of the mean
[a,b:] Within columns, means with common superscript are not different (P>0.05)

Ruminal degradation trend of different treatments (Figure 1) showed that processing with NaOH and H_2O_2 increased DM degradability of the samples at different incubation times. But in CaO processed samples, degradability was decreased after 48 h incubation time. Mean comparisons for different parameters of DM ruminal degradability and ERD of DM in different samples of GPR are shown in Table 3. All processed treatments on GPR increased the washout fraction (a) compared to the control (P<0.05). The highest amount of this trait was observed in the samples processed with H_2O_2 (26.65 % compared to 11.76 %). Except CaO, the other treatments increased ERD of DM at rumen outflow rates of 0.02, 0.05, and 0.08/h (P<0.05). The highest ERD at the mentioned outflow rates was observed in H_2O_2 (44.36, 37.50 and 34.53 %, respectively).

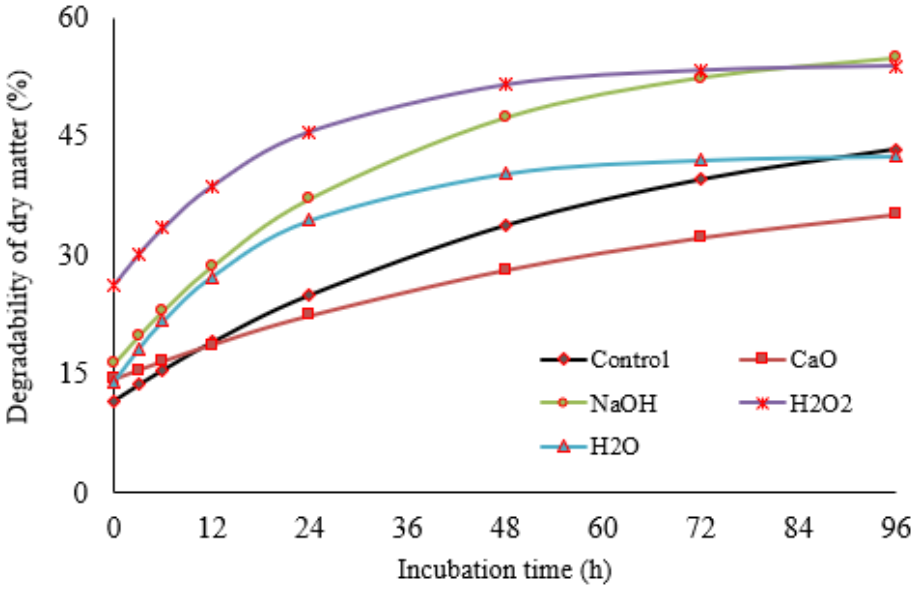

Figure 1. Degradability trend of chemically-treated green pea residues. NaOH: Sodium hydroxide; CaO: Calcium oxide; H_2O_2: Hydrogen peroxide; H_2O: Water

Table 3. Effects of sodium hydroxide, calcium oxide, hydrogen peroxide and water on ruminal degradability parameters and effective degradability of green pea residues

	Ruminal degradability parameters [2]				Effective degradability (%) at rumen outflow rates of:		
Treatments[1]	a (%)	b (%)	a+b (%)	c (h^{-1})	0.02 (h^{-1})	0.05 (h^{-1})	0.08 (h^{-1})
Unprocessed	11.67^{c}	39.54^{ab}	51.21^{ab}	0.017^{ab}	29.86^{d}	21.83^{d}	18.76^{d}
NaOH[1]	16.87^{b}	41.79^{a}	58.65^{a}	0.026^{ab}	40.23^{b}	31.03^{b}	27.03^{b}
CaO[1]	14.39^{b}	28.93^{c}	43.32^{b}	0.013^{b}	26.00^{e}	20.53^{d}	18.56^{d}
H_2O_2[1]	26.65^{a}	32.02^{bc}	58.68^{a}	0.028^{a}	44.36^{a}	37.50^{a}	34.53^{a}
H_2O[1]	15.63^{b}	34.34^{b}	49.97^{b}	0.019^{ab}	32.50^{c}	25.26^{c}	22.36^{c}
SEM[3]	0.493	1.437	1.443	0.002	0.429	0.282	0.287
P-value	<0.0001	0.0716	0.0245	0.0839	<0.0001	<0.0001	<0.0001

[1] NaOH: Sodium hydroxide; CaO: Calcium oxide; H_2O_2: Hydrogen peroxide; and H_2O: Water
[2]a: Washout (soluble) fraction, b: Potentially degradable fraction, and c: Degradation rate of b fraction.
[3]SEM: Standard error of the mean
[a,b]: Within columns, means with common superscript are not different (P>0.05)

The gas production trend of GPR processed with chemical compounds during 96 h incubation (Figure 2) showed that after 12 h incubation time, NaOH and H_2O_2 treatments led to more gas production. Mean comparisons of gas production and estimated parameters of different treatments are shown in Table 4. The potential of gas production (b) in H_2O_2 and NaOH processed samples was increased (P<0.05) compared to the control (228.13 mL and 227.09 mL versus 189.28 mL, respectively). The amounts of SCFAs, ME, OMD in unprocessed (control) GPR were 0.46 mmol/200 mg DM, 9.95 Mj/kg and 33.74 %, respectively. Processing with NaOH increased (P<0.05) concentration of SCFAs (0.55 mmol/200 mg DM). The amounts of ME and OMD were also increased (P<0.05) by NaOH and H_2O_2 treatments (9.83 and 9.70 MJ/ kg; 37.26 and 36.99 %, respectively).

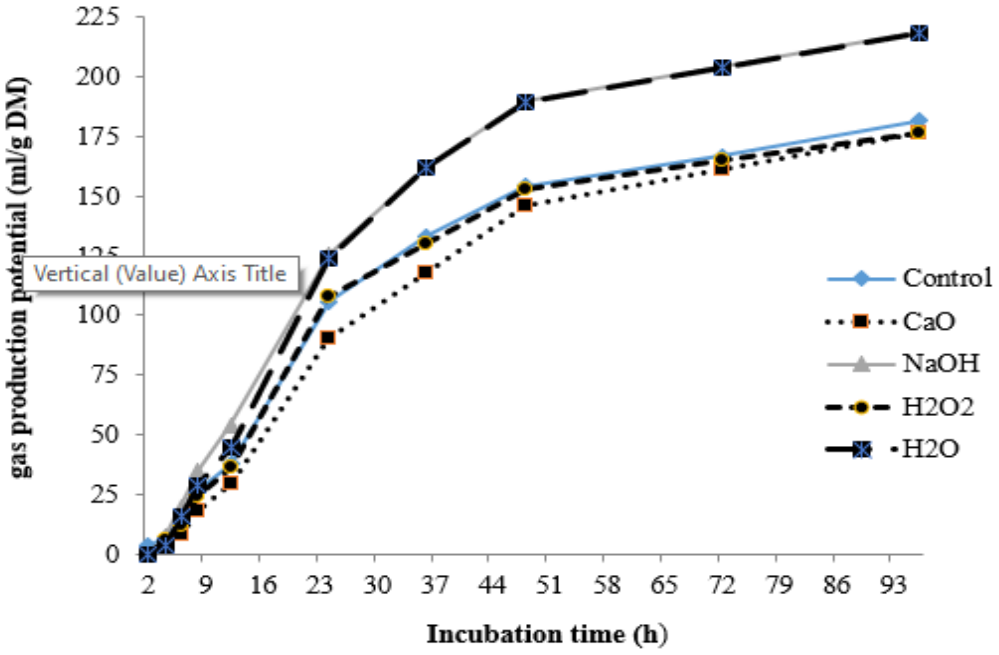

Figure 2. Gas production trend of chemically-treated green pea residues. NaOH: Sodium hydroxide; CaO: Calcium oxide; H_2O_2: Hydrogen peroxide; H_2O: Water

Table 4. Effects of sodium hydroxide, calcium oxide, hydrogen peroxide and water on gas production and estimated parameters green pea residues

Treatments	Gas production parameters[2]		Estimated parameters[3]		
	b (mL)	C (mL/h)	OMD (%)[3]	ME (Mj/kg)	SCFAs (mmol/200 mg DM)
Unprocessed	189.28^{b}	0.036^{ab}	33.74^{b}	9.95^{a}	0.46^{cb}
NaOH[1]	227.09^{a}	0.036^{ab}	37.26^{a}	9.83^{a}	0.55^{a}
CaO[1]	190.85^{b}	0.030^{b}	31.04^{b}	7.63^{c}	0.39^{c}
H_2O_2[1]	228.13^{a}	0.036^{ab}	36.99^{a}	9.70^{a}	0.54^{ab}
H_2O[1]	183.64^{b}	0.040^{a}	34.09^{b}	8.38^{b}	0.47^{abc}
SEM[4]	0.0025	7.17	1.028	0.172	0.026
P-value	0.0021	0.170	0.0086	<0.0001	0.0095

[1] NaOH: Sodium hydroxide; CaO: Calcium oxide; H_2O_2: Hydrogen peroxide; and H_2O: Water
[2]b: Gas production potential; and C: Gas production rate,
[3]OMD: Organic matter digestibility; ME: Metabolizable energy; and SCFAs: Short chain fatty acids
[4]SEM: Standard error of the mean
[a,b]: Within columns, means with common superscript are not different (P>0.05)

Digestibility, NH_3-N and pH, as well as fermentation parameters for different samples of GPR have been shown in Table 5. Processing of GPR with NaOH, H_2O_2, and CaO increased (P<0.05) DMD and OMD compared to the control (51.00 and 54.00 % versus 40.00 %, respectively; 46.00, 47.00, and 46.00 % versus 40.00 %, respectively). Except H_2O, the other treatments led to an equal decrease (P<0.05) in the NH_3-N concentration. pH was increased (P<0.05) by H_2O_2 and CaO treatments compared to the control (6.92 and 6.97 versus 6.86, respectively). Means of PF, efficiency of gas production at the end of 24 h incubation, MB and EMB in control group were 2.64 mg/mL, 347.06 mL/g of DM, 31.01 mg/g of DM, and 0.16, respectively. PF was increased (P<0.05) by CaO, H_2O_2, and NaOH treatments (8.41, 5.67, and 3.78 mg/g of DM, respectively). Except H_2O, the other treatments increased MB and EMB as well (P<0.05). The greatest of these factors was observed in samples processed with CaO (133.25 mg/ g of DM, and 0.73, respectively).

Table 5. Effect of chemical treatments on dry matter and organic matter digestibility, ammoniacal nitrogen, pH, partitioning factor, gas yield, microbial biomass and efficiency of microbial biomass of green pea residues

Treatments	DMD[2] (%)	OMD[3] (%)	NH_3-N[4] (mg/dL)	pH	PF[5] (mg/mL)	GY[6] (mL/g DM)	MB[7] (mg/g DM)	EMB[8]
Unprocessed	40.00^{cb}	40.00^{b}	3.66^{a}	6.86^{c}	2.64^{d}	347.06^{a}	31.01^{c}	0.16^{d}
NaOH[1]	51.00^{a}	46.00^{a}	2.49^{b}	6.89^{cb}	3.78^{c}	200.84^{c}	81.14^{b}	0.41^{c}
CaO[1]	44.00^{b}	46.00^{a}	2.63^{b}	6.97^{a}	8.41^{a}	98.13^{e}	133.25^{a}	0.73^{a}
H_2O_2[1]	54.00^{a}	47.00^{a}	2.71^{b}	6.92^{b}	5.67^{b}	122.22^{d}	114.06^{a}	0.60^{b}
H_2O[1]	38.00^{c}	37.00^{b}	3.02^{a}	6.88^{cb}	2.71^{d}	316.57^{b}	31.54^{c}	0.18^{d}
SEM[9]	1.15	1.27	0.180	0.010	0.265	6.89	6.69	0.022
P-value	<0.0001	0.0012	0.0069	0.0002	<0.0001	<0.0001	<0.0001	<0.0001

[1] NaOH: Sodium hydroxide; CaO: Calcium oxide; H_2O_2: Hydrogen peroxide; and H_2O: Water
[2]Dry matter digestibility
[3]Organic matter digestibility
[4]NH_3-N: Ammoniacal Nitrogen
[5]PF: Partitioning Factor
[6] GY-24: Gas yield after 24 h incubation
[7]MB: Microbial biomass
[8]EMB: Efficiency of microbial biomass
[9]SEM: Standard error of the mean
[a,b]: Within columns, means with common superscript are not different (P>0.05)

Discussion

The DM decreased in the samples treated with H_2O_2 as using water through processing with chemical compounds leads to a decrease in the DM of the samples (Chaudhry, 2000). Except for H_2O, the other treatments increased the ash contents of the samples. Increased ash content of vetch, faba bean, soybean, and canola residues due to processing with NaOH, CaO, and H_2O_2 also had been reported in previous studies (Babayi et al., 2016; Ghiasvand et al., 2012; Khorvash et al., 2010; SoltaniNaseri et al., 2018). It seems that increased ash content after NaOH and CaO processing is due to sedimentation of Na and Ca on GPR (Baytok et al., 2005). Processing with H_2O_2 also included a preparation step with NaOH (to keep the pH in range of 11.5); therefore, the ash content was expected to increase in this treatment as well. Reduced OM contents in these treatments are probably due to dilution of sugar substances because of the increased ash content (Ghiasvand et al., 2012). Similarly, addition of Na to the

samples were suggested as the factors increasing the ash content in NaOH and NaOH+ H_2O_2 processed wheat straw (Chaudry, 2000). Chemical treatments decreased the CP content in GPR. Ghiasvand et al. (2012) also observed similar results in canola straws processed with NaOH and NaOH+ H_2O_2. The NaOH treatment of straw increased the percentage of ash while decreasing the percentage of CP (because of decreased OM). Aslanian (2015) reported that CaO and NaOH treatments reduced the CP content in soybean straws. Khorvash et al. (2010) reported a reduction in CP content of the CaO treated soybean straw as a consequence of nitrogen detachment from the straw components. In this study, CaO treatment reduced the ADF contents. Chemical substances, especially alkaline compounds, are very effective in detaching lignin from the biomass. They destroy the cell wall components such as NDF, ADF, hemicellulose, and lignin thus reducing their contents (Zhang et al., 2020). CaO is a strong chemical used in delignification. This compound is capable of breaking the chemical bonds between lignin and polysaccharides and so dissolving hemicellulose present in agriculture residues, and thus reducing the cell wall content (Trach et al., 2001). Chaudry (2000) also reported a decrease in the cell wall content of the CaO-treated wheat straws. In the present study, NaOH treatment increased the NDF and ADF contents numerically. Canale et al. (1992) reported increased NDF in NaOH-processed alfalfa, attributing it to a reaction between carbonyl group of carbohydrates with nitrogen (Millard reaction).

In this study, except CaO, the other treatments increased the ERD of DM in samples. Ruminal degradability of lignocellulosic agricultural by-products is considered as a main challenge for their usage in ruminant nutrition. 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. Processing of lignocellulosic substances is mainly aimed to increase enzyme accessibility to the cellulose (Zhao et al., 2016). 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 surface area for microbial enzymes to digest the structural carbohydrates in plant residues (Zhao et al., 2016). In agreement with the present study, Chaudhry (2000) reported increased DM ruminal degradability of wheat straw processed by NaOH and H_2O_2. Similarly, Ghiasvand et al. (2012) showed that degradability parameters (b and c fractions), potential degradability and ERD of DM and OM of canola straw were increased as a result of processing with NaOH+ H_2O_2 and also H_2O, compared to the control.

In the present study, the potential of gas production was increased in samples processed with H_2O_2 and NaOH. Chemical compounds, such as NaOH and H_2O_2, increase microbial attachments through breaking the cell wall and providing more soluble carbohydrates. These compounds reduce cellulose crystallinity and increase the substrate surface porosity (Selim et al., 2004). This way, microbial degradation of cellulose and hemicellulose is improved and consequently gas production resulting from the fermentation process would be increased (Trach, 2000). Previous studies also reported increased gas production due to chemical processing of the lignocellulosic materials (Aslanian et al., 2015; Liu et al., 2002; Trach et al., 2001;). NaOH and H_2O_2 processing increased concentration of SCFAs, ME content, and OMD. There is a positive relation between the volume of gas production and SCFAs, ME, as well as OMD (Aslanian et al., 2015). Thus, the increase of mentioned parameters in NaOH and H_2O_2 treatments (which increased the gas production) seems logical. Moghadam et al. (2012) reported high gas production as an indicator of high ME and also fermentable nitrogen as well as other nutrients required for the activity of microorganisms. Aslanian et al. (2015) and Al-Masri. (2005) reported increased contents of SCFAs, ME, and OMD in some agriculture residues (soybean straw, wheat straw, peanut shell, sunflower nut shell, and olive wood) processed with NaOH, H_2O_2, and hydrobromic acid (HBr).

In this study, processing the GPR with NaOH, H_2O_2, and CaO increased both the DMD and OMD. Chemicals expose the hydroxyl agents (-OH) of glucose units in the cellulose molecule to the reaction and lead to a reduction in cellulose crystallinity. This would decrease the covering effect of substances such as lignin and silica on cellulose and increase the cell wall capacity for hydrolysis. Therefore, the inhibitory effect of phenolic acids on the digestion of structural carbohydrates is also omitted or limited by chemical compounds. Hence, there would be a significant improvement in cellulose digestibility of the straw for the samples processed with chemical compounds. It has been indicated that alkaline compounds (such as NaOH, H_2O_2, and CaO) break the bonds between lignin and the cell wall carbohydrates leading to increased digestibility (Yalchi et al., 2012). Similar to the present study, increased *in vitro* DMD and OMD was reported as a result of chemical processing of the agriculture residues (Chaji et al., 2010; Khajeh et al., 2020; Yalchi et al., 2012).

In comparison to the control group, treatments with NaOH, CaO and H_2O_2 on GPR equally reduced NH_3-N concentration in culture media. Also, pH of the culture media in H_2O_2 and CaO treatments was higher than that of the control. In some *in vitro* studies, HBr, NaOH, and H_2O_2 treatments had no effects on NH_3-N concentration in vetch and pea wastes (Babayi et al., 2016; SoltaniNaseri et al., 2018) but Aslanian et al. (2015) reported that NaOH reduced the NH_3-N levels in culture medium. Decreased *in situ* concentration of ruminal fluid NH_3-N has been reported in goats receiving alkaline processed wheat straw. The authors proposed that it can be related to some extent to increased synthesis of micr-

obial protein and partly to decreased ureolytic and proteolytic activities of ruminal bacteria or ruminal epithelium (Ria and Mudgal, 1996). One reason for the higher pH in the samples processed with CaO and H_2O_2 could be the alkaline nature of these compounds (Alaei et al., 2020). The CaO, particles sediment on the residues and because of their alkaline nature, resist against the pH reduction in the culture media due to its buffering capacity. According to Trach et al. (2001), CaO combines with water during the processing, and immediately converts to calcium hydroxide (Ca (OH_2)) which leads to increased pH. In processing with H_2O_2, the samples are first pretreated with NaOH. Van Soest (1994) indicated that NaOH treatment would lead to the combination of Na with straw wall carbons and formation of sodium carbonate (Na_2CO_3) which consequently increased the pH in the fermentation medium. Similar to the present study, increased pH in the culture medium was reported in samples of faba bean residues processed with CaO, NaOH, and H_2O_2 (Alaei et al., 2020).

In this study, except for H_2O, the other treatments increased the PF and EMB values but decreased the gas yield. The PF, an indicator of the hay quality, has been defined as the portion of truly disappeared OM (mg) to the volume of GY (mL) during the 24 h incubation. Higher PF is indicative of the fact that higher portion of degraded substances have been led to MB production and so the EMB would be higher. In the present study, PF in CaO, NaOH and H_2O_2 treatments was higher than the control. It means that the EMB production was increased in these treatments and conversely GY was reduced. Our results are indicative of this fact. Similar to the present study, in the previous studies, increased PF and consequently increased MB, as well as reduced GY were reported in legume wastes processed with chemical compounds (Alaei et al., 2020; SoltaniNaseri et al., 2018).

Conclusions

According to the present *in vitro* and *in situ* trials, processing with NaOH and H_2O_2 improved the nutritional value of GPR. It is suggested that the effects of these treatments be studied under *in vivo* conditions.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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