Evaluation of Nutritive Values of Agro-Industrial By-Products (AIBPS) and Effect of Their Nano Structural Characteristics on the Kinetics of Biogas Production and Methane Emission

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Abstract

Over the past decades, the agro-industrial by-products (AIBP) has received considerable attention. With this motivation, the aim of this study was to investigate the effect of AIBP as a source of non-fiber carbohydrates on biogas production kinetic, methane emission and fermentation characteristics. Experimental treatments were (1) Sugar beet pulp (SBP) (control), (2) Apple pomace (AP), (3) Orange pulp (OP, (4) 33% AP + 66% OP, (5) 50% AP + 50% OP and (6) 66% AP + 33%OP. For this work, we analyzed the data collected from the kinetics of digestion through biogas production, ex-vivo methane emission, in-vitro digestibility of dry matter and fermentation parameters. Field emission scanning electron microscope was used to show the nano structural differences of the AIBPs. Our results demonstrated the significant differences of the crude protein among the treatments ($P < 0.05$). Biogas production and methane emission were significantly higher in SBP and OP treatments ($P < 0.05$). The most eminent and the lowest amounts of acetate were observed for AP and OP (61.84 mmol/L, 58.15 mmol/L), respectively. More broken edges were obvious in OP images. particle size was rather smaller in SBP. Images of AP showed a sleek surface which may act as a shield preventing more digestion. Overall, beside reducing environmental contamination by AIBP, our results showed a positive effect of AIBPs on degradation and biogas kinetics, methane emission and in vitro fermentation parameters describing that they can be used as a good source of non-fiber energy sources.

Statement Of Novelty

Agro-industrial by-products (AIBPs) are contaminating environment and causing serious health problems. Understanding AIBPs biogas production, methane emission and fermentation characteristics can lead us to a precise bio-recycling method.

Monitoring biodegradation of AIBPs as a source of non-fiber carbohydrates by ruminal anaerobic microorganisms seems a better way to reduce contamination beside introducing a new feedstuff composited by different amount of AIBPs.

1. Introduction

Sustainable agriculture collectively is farming using least damaging techniques to provide food and energy for increasing population without compromising the ecosystem and the environment. Sustainable agriculture is one of the main loops of food security chain [1], Broadly speaking, approximately, more than thirty percent of produced food is being lost or wasted in total amount of food chain [2]. Therefore, better knowledge to reduction of food waste is essential to provide a solution to the problem. Not only that AIBP enforces negative effect on the environment, human health and resources beside but also is a threat to the food security [3–7]. food production is responsible for 19–29% of global greenhouse gas emissions by is beside the resources impacts of food waste which assume as serious problem[8]. In the recent decades, many studies have focused on the bioeconomy of treated agro-industrial by-products (AIBP) recycling and its bioconversion in the livestock. High nutrient content of AIBP makes it an ideal candidate as feedstuff. Bioeconomy is trying to promote the added value to AIBP thus introducing new marketplaces and technologies for the future. AIBP can be a substitute to traditional feedstuff such as grains or protein resources. Inclusion of AIBP as feedstuff has positively affected the animal while reducing the agricultural wastes as the environmental contaminators[9].

A natural barrier and concern for applicability of AIBP regarding livestock feed is its high problematic moisture content and presence of contaminants. According to literature there is a lack of data on the digestibility of food waste in livestock nutrition.

Studying AIBP as feedstuff sources provide insights into the methods for reduction of cost of formulation of feed, milk and beef production. Although ruminants can be assumed as a huge biorefineries of agro-industrial by-products, but it is well established that farmed ruminants are responsible for a substantial amount of anthropogenic methane emissions worldwide [2]. Microbial consortium including archaea, bacteria, protozoa and fungi living in the rumen, can convert vast amount of bio polymers by their enzymatic systems in to volatile fatty acids (VFA) and microbial protein that can be used by the ruminants for maintenance, growth and production [10]. Beside VFA production, molecular hydrogen (H_2), carbon dioxide (CO_2) and some other gases are also produced. In the rumen, as an anerobic chamber, microbial consortium can produce methane (CH_4) by using H_2 for reducing CO_2 into methane (CH_4) [6, 11–14].

Ruminants due to their rumen microbial consortium can use AIBP to meet their requirements of growth, reproduction and production. Utilizing locally available AIBP beside ruminant’s potential seems a practical alternative.

Sugar beet pulp (SBP) Apple pomace (AP) Orange pulp (OP) are three agro-industrial by-products as potential alternative feedstuff. Also, replacing this AIBP with a part of a conventional diet ingredient due to its energy and protein not only contribute to environmental contamination cut but also develop bio-economy by bio-recycling of these AIBPs. Handful studies have investigated the use of apple pomace silage in ruminant diets [15, 16].The results of a study showed that feeding dairy cows with apple pomace silage containing 10% wheat straw, 10% alfalfa hay, and 10% rice hulls resulted in an increase in milk yield and milk protein content[17]. Another study revealed that apple pomace silage could be added to the diet of lactating cows up to 30% [18]. However, the combination of apple pomace silage and other types of silage has not been studied adequately [19]. Other agro-industrial by-products could also be used as a feedstuff (e.g., apple pomace and orange pulp, by-products of juice extraction industry). These products with high fermentable nutrients used whether as fresh, dry, or ensiled can be effectively fermented by ruminal microorganisms [20–24]. Per report of Paya et al. (2012) nutritive values of dry apple pomace (DAP) and dry orange pulp (DOP) were; crude protein (CP): 7.2% – 7.9%, ether extract (EE): 2.9%- 1.8%, neutral-detergent fiber (NDF):43.3% – 22.4%, acid detergent fiber (ADF): 32.3% – 15.3% and metabolizable energy (ME): 10.8–8.3 MJ/Kg, These values represent suitable capacity of these processed wastes as feedstuff which is not in competing with human food. Not only the nutritive value, but also economically being suitable as an alternative for feedstuff make these agro-industrial by-products useful [19, 25]. The hypothesis that we followed in the present study was that; biorefining of agro-industrial by-products can decrease environmental contaminators beside producing the food for human.

Additional investigation of AIBP would further our understanding on behavior and pattern of AIBP as a source of non-fiber carbohydrates on degradation and biogas kinetics, methane emission and in vitro fermentation parameters.

2. Material And Methods

2.1. Chemical analyses

Chemical analyses were conducted according to AOAC (2005) in which three Samples of each treatment were dried in an oven to calculate dry matter (AOAC, 2005; Method 930. 15). Heated samples in a furnace for about 600°C for 2h were used to determine ash (AOAC, 2005; Method 942.05). Kjeldahl was utilized to determine N content of samples (AOAC, 2005; Method 984.13). Ether extract (EE) of samples were determined using diethyl ether for extraction (AOAC, 2005; Method 920. 39). Van Soest et al. (1991) assay was used to determine acid detergent fiber (ADF) and neutral detergent fiber (NDF) of samples.

2.2. Animals and experimental treatments

Experimental animals were treated according to the Iranian Council on Animal Care guidelines and recommendations (1995). Data for this study were collected using three ruminally fistulated adult Ghezel indigenous castrated male sheep having live weights of 48 ± 3 kg (initial mean ± SE), utilized for rumen liquor donators. During experiment wethers fed two times a day with a TMR (total mixed ration) balanced by CNCPS Sheep version 1.0.21. Roughage section of TMR was included alfalfa hay and barley straw and concentrate section was a mixture of grounded corn grain, dry-rolled barley grain, wheat bran, soybean meal, salt and mineral vitamin premix, feedings were in the same portion at every meal at the maintenance level. Animals were free to have fresh water. Experimental treatments were (1) Sugar beet pulp (SBP) (control), (2) Apple pomace (AP), (3) Orange pulp (OP) (4) 33% AP + 66% OP, (5) 50% AP + 50% OP and (6) 66% AP + 33%OP

2.3. Field emission scanning electron microscope (FESEM) procedure

A 4% glutaraldehyde solution was used to fixed samples at 4°C for overnight, then samples were washed out with a buffer solution (4% Glutaraldehyde in 2M Sodium cacodylate) for 15 min. Serial dehydration method using 30%, 50%, 70%, 80%, 90% and 100% acetone solutions (AS) for 15 min at each dilution were utilized, 100% AS for 15 min was repeated [29, 30]. Samples were oven dried at 38°C for 30 min. Gold-platinum coating were used after drying the samples, they were placed in sputter coater (process current 10 mA for 2 min) and then FESEM (MIRA3 TESCAN) images were taken.

2.4. Biogas production test

Each of the treatment samples was ground in a Wiley Mill (2 mm screening) and 0.3 gr of grounded materials were weighed into glass phials (50 ml volume). Synthetic saliva prepared as stated by McDougall (1948). Ruminal inoculum of three fistulated wethers were strained through 4-layer cheese cloth to a flask that warmed already at 39°C, and immediately were taken to the laboratory. Ruminal fluids were mixed at 39°C with the McDougall buffer (1:2 v/v) to form digestion medium. Six labeled glass phials for each treatment were loaded (20 ml) by digestion medium. Blank phials were loaded by only digestion medium in six replicates. Packed phials placed in the shaker (39°C,120 rpm)[29]. Gas production data were documented at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h of incubation for each phial [32].

2.5. Ex-vivo methane emission measurement

Beside gas production test, 0.2 gr of ground (2mm) samples of each treatment were poured into graduated syringes (100 mL) in three replicates and filled with prepared digestion medium (20 ml), then three syringes loaded with only digestion medium as blank and set in a shaker platform (39°C,120 rpm) for 24 h. The cumulative gas production of samples was documented and 5 ml of upper gas phase were injected into a gas chromatograph equipped with a semi capillary column [33]. Quantitative methane values were determined by external standard.

2.6. In-vitro digestibility of dry matter

In vitro digestibility of dry matter was determined, along with gas production test. Triplicate vials were loaded (20 ml) with same inoculum used in gas production test for each feedstuff for incubation times of 2,4,8, 12, 24 and 48 h. In order to correct the inoculum effect on in-vitro dry matter disappearance three vials of just digestion medium were loaded for each incubation time. Unlike gas production test the produced gas was emptied during incubation using needles being installed on caps vials. Each set of vials for each incubation hour were removed and frozen at -20°C till further analyses. Thawed vials (39°C) contents were transferred into pre-weighed falcon tubes (50 ml), then centrifuged (10000×g for 10 minutes at 4°C). Falcon tube contents were washed completely by saline buffer. Remaining solid phase of falcon tubes were dried at 55°C for 48 h and weighed. The in vitro disappearance of DM was calculated for each incubation time of each feedstuff [34].

2.7. Fermentation parameters

A calibrated laboratory pH meter was used to determine the pH of digestion medium of each glass phials after 12 hours and digestion medium samples of glass phials were strained through 4-layer cheese cloth and sub-samples were taken. Sub-samples were acidified with 50% H_2SO_4 (5:1 v/v) for NH_3 analyses [35] and for VFA analyses another sub-sample was acidified with 25% metaphosphoric acid (5:1 v/v) [36] and frozen at − 20°C till further analyses.

2.8. Calculations and statistical analyses

Degradation kinetics of DM was calculated according to the following model.

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

Where A is the gas production from the degradable fraction (mL); c is the constant rate (mL/h) of gas production from the degradable fraction; t is the incubation time (h); and Y is the volume (mL) of gas produced at time t.

The Metabolizable Energy (ME) (MJ/kg DM) was calculated according to Getachew et al. (2002) for DOM (Digestible Organic Matter), and SCFA (Short Chain Fatty Acid) suggested equations of Menke et al. (1979) were used.

ME (MJ/kg DM) = 1.06 + 0.157 GP + 0.084 CP + 0.220 CF − 0.081 CA

DOM (% DM) = 9.00 + 0.9991 GP + 0.0595 CP + 0.0181 CA

SCFA (m mol/200 mg DM) = 0.0222GP − 0.00425

GP is the 24 h net gas production (ml/200 mg DM); CP, CF and CA are crude protein, crude fat and crude ash (% DM), respectively.

Statistical analyses were performed by using the complete randomized design (CRD) applying a significance level of 0.05, utilizing SAS software (version 9.2, the ANOVA procedure, Duncan's multiple range test).

3. Results

3.1. Chemical analyses

We describe the results of dry matter content of samples, which varied from 95.07% for the treatment 33% AP + 66% OP to 97.10% for AP ($P < 0.05$). Further analyses of CP showed that, there was a significant difference between the experimental treatments. In detail, SBP and OP indicated the highest (8.57% DM) and lowest (3.82 % DM) amount of CP respectively($P < 0.05$). Additionally, the AP had the highest value of NDF and ADF (38.46 % DM,28.40% DM) while the OP was the lowest (20.26% DM, 15.26% DM) compared to other treatments, respectively ($P < 0.05$). Furthermore, our results demonstrated the lowest value of EE for SBP (3.03% DM) while its ash was highest (5.60% DM) ($P < 0.05$) (Table 1).

Table 1

Chemical composition of agro-industrial by-product samples. (% DM) (P < 0.05).

Feedstuff †	**DM**	**CP**	**NDF**	**ADF**	**EE**	**Ash**
SBP	96.90^{a}	8.57^{a}	25.60^{d}	13.46^{f}	3.03^{c}	5.60^{a}
AP	97.10^{a}	6.18^{b}	38.46^{a}	28.40^{a}	8.63^{a}	2.6^{b}
OP	95.20^{dc}	3.82^{e}	20.26^{e}	15.26^{e}	6.90^{b}	2.92^{b}
33%AP + 66%OP	95.07^{d}	4.22^{de}	25.20^{d}	18.33^{d}	7.96^{a}	2.86^{b}
50%AP + 50%OP	95.33^{c}	4.98^{dc}	32.46^{b}	23.40^{b}	7.94^{a}	2.86^{b}
66%AP + 33%OP	96.10^{b}	5.29^{c}	28.60^{c}	20.86^{c}	8.23^{a}	2.66^{b}
SEM	0.07	0.27	0.20	0.22	0.27	0.13
P Value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Means within a column with different superscripts differ ($P < 0.05$).

†Sugar beet pulp (SBP) (control), Apple pomace (AP), Orange pulp (OP), 33% AP + 66% OP (33% Apple pomace + 66% Orange pulp), 50% AP + 50% OP (50% Apple pomace + 50% Orange pulp), and 66% AP + 33%OP (66% Apple pomace + 33% Orange pulp).

3.2. Field emission scanning electron microscope (FESEM) procedure

Microscopic figures of a suitable area of the feedstuffs imaged by MIRA3 TESCAN with the magnification of 1.5kx, SEM HIGH VOLTAGE of 5kv, View field of 84–85 μm and the scale of 20 μm by the Secondary detector. To show more detailed area, SEM MAG: 10kx, View field 12–13 μm, SEM HV: 5kv, Det: SE and the scale of 2 μm at the Working distance of 9.8 mm were selected. More broken edges or in another word more particle sizes were obvious in OP than the other two feedstuffs, these small pieces of the feedstuff, not only provide more surface areas for microorganisms to inhabit but also allows them to develop colonies and digest the feedstuff. Unlike other two treatments images of the AP show a smooth and sleek surface which is something like a cover that prevent more digestion by microorganisms. It seems that there is less amount of particle size in SBP compared to two other treatments, but the surface is not as sleek as AP (more rigid). Also, some cracked areas are vivid in the picture of SBP which may let the microorganisms to inhabit more and develop colonies easily.

3.3. Biogas production test

Table 2 presents outputs of gas production data. A significant difference of produced gas among the treatments was observed from the beginning hours of incubation, OP showed the highest value (67.80 ml/g DM) while AP was the lowest (42.87 ml/g DM), respectively($P < 0.05$). The incubation pattern was continued as same as the initial hours, but cumulative gas production data of 24h showed that the treatments 33%AP + 66%OP and 50%AP + 50%OP had no significant differences compared to OP ($P < 0.05$) being the highest, whereas the lowest values at the same incubation hour (24h) were for the treatments 66%AP + 33%OP and AP ($P < 0.05$). At the end of the incubation (96h) the cumulative data of gas production showed that the treatments SBP, OP and 33%AP + 66%OP were the highest, however OP was the greatest numerically, and the treatment AP was the lowest.

Table 2

Cumulative biogas production of agro-industrial by-product samples (ml/g of DM) (P < 0.05).

Feedstuff†	Hours								
	2	**4**	**6**	**8**	**12**	**24**	**48**	**72**	**96**
SBP	43.35^{c}	70.59^{b}	97.37^{bc}	122.48^{b}	165.58^{b}	205.75^{b}	215.60^{bc}	223.45^{bc}	232.72^{abc}
AP	42.87^{c}	70.51^{b}	94.64^{c}	114.22^{c}	152.46^{c}	194.23^{c}	202.76^{d}	208.16^{d}	216.09^{d}
OP	67.80^{a}	102.76^{a}	129.13^{a}	156.42^{a}	180.00^{a}	214.98^{a}	224.44^{a}	234.23^{a}	239.42^{a}
33%AP + 66%OP	49.28^{b}	77.65^{b}	100.83^{b}	122.88^{b}	162.79^{b}	211.75^{ba}	221.93^{ab}	230.18^{ab}	236.98^{ab}
50%AP + 50%OP	48.34^{b}	76.32^{b}	98.89^{bc}	119.67^{bc}	158.98^{bc}	207.28^{ba}	219.87^{abc}	225.13^{bc}	230.39^{bc}
66%AP + 33%OP	46.95^{bc}	75.85^{b}	95.83^{bc}	115.35^{c}	154.33^{c}	195.30^{c}	212.34^{c}	221.07^{c}	226.33^{c}
SEM	1.58	2.30	1.72	2.23	2.37	2.63	2.67	2.39	2.25
P Value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Means within a column with different superscripts differ (P < 0.05).

†Sugar beet pulp (SBP) (control), Apple pomace (AP), Orange pulp (OP), 33% AP + 66% OP (33% Apple pomace + 66% Orange pulp) 50% AP + 50% OP (50% Apple pomace + 50% Orange pulp), and 66% AP + 33%OP (66% Apple pomace + 33% Orange pulp).

3.4. Estimated biogas production parameters and kinetics

Estimated values for ME, SCFA, DOM, *A* and *c* are presented in Table 3. The highest amount of ME was calculated for the treatments AP, OP, 33%AP + 66%OP and 50%AP + 50%OP, however 33%AP + 66%OP were numerically higher, and the lowest amount was for SBP (P < 0.05). Our estimations exhibit no significant difference in the amount of SCFA of treatments SBP, OP, 33%AP + 66%OP and 50%AP + 50%OP, but a significant difference was observed with the treatments AP and 66%AP + 33%OP (P < 0.05). The lowest estimated value of DOM was for AP and 66%AP + 33%OP (48.92% DM,48.58%DM), respectively (P < 0.05). The highest and the lowest amount of parameter A were estimated for treatments 33%AP + 66%OP and AP, respectively (P < 0.05), but the parameter c were the highest for OP and the lowest for 50%AP + 50%OP (P < 0.05).

Table 3

Estimated biogas production parameters, kinnetics and methane emission of agro-industrial by-product samples (P < 0.05).

Feedstuff†	Parameters					Methane
	ME[1] (MJ/kg DM)	SCFA[2] (m mol/200 mg DM)	DOM[3] (% DM)	A[4] (ml/g DM)	c[5] (h^{-1})	(ml/g DM 24h)
SBP	8.65[c]	0.94[a]	52.00[a]	230.39[ab]	0.098[b]	12.36[a]
AP	9.47[ab]	0.87[b]	48.92[bc]	221.29[b]	0.092[bc]	10.48[b]
OP	9.36[ab]	0.94[a]	51.91[a]	223.45[b]	0.132[a]	11.96[a]
33%AP + 66%OP	9.62[a]	0.94[a]	51.84[a]	244.88[a]	0.083[bc]	10.54[b]
50%AP + 50%OP	9.47[ab]	0.91[a]	50.60[ab]	240.95[a]	0.082[c]	10.68[b]
66%AP + 33%OP	9.26[b]	0.87[b]	48.58[c]	221.82[b]	0.089[bc]	10.48[b]
SEM	0.09	0.012	0.548	5.35	0.004	0.21
P Value	0.0001	0.0016	0.0015	0.040	0.0001	0.0001

Means within a column with different superscripts differ (P < 0.05).

†Sugar beet pulp (SBP) (control), Apple pomace (AP), Orange pulp (OP), 33% AP + 66% OP (33% Apple pomace + 66% Orange pulp), 50% AP + 50% OP (50% Apple pomace + 50% Orange pulp), and 66% AP + 33%OP (66% Apple pomace + 33% Orange pulp).

[1] Metabolizable Energy calculated as ME (MJ/kg DM) = 1.06 + 0.157 GP + 0.084 CP + 0.220 CF − 0.081 CA

[2] Short Chain Fatty Acid calculated as SCFA (m mol/200 mg DM) = 0.0222GP − 0.00425

[3] Digestible Organic Matter calculated as DOM (% DM) = 9.00 + 0.9991 GP + 0.0595 CP + 0.0181 CA

[4]A (a + b) = asymptotic gas production (ml/g DM incubated);

[5]c = fractional rate of fermentation (h^{-1})

Table 4

Invitro disappearance of dry mater pattern of agro-industrial by-product samples (%DM) (P < 0.05).

Feedstuff†	Hours					
	2	4	8	12	24	48
SBP	20.12[b]	27.11[ab]	37.08[ab]	47.64[b]	59.88[ab]	60.73[b]
AP	18.45[c]	23.99[c]	33.54[b]	44.83[c]	55.55[c]	57.66[c]
OP	21.91[a]	28.65[a]	38.68[a]	50.78[a]	60.83[a]	64.15[a]
33%AP + 66%OP	21.31[ab]	26.63[ab]	38.66[a]	48.45[ab]	59.29[ab]	63.44[ab]
50%AP + 50%OP	20.64[ab]	26.12[abc]	38.21[a]	48.31[ab]	57.65[bc]	62.15[ab]
66%AP + 33%OP	19.91[bc]	25.82[bc]	38.44[a]	46.74[bc]	56.81[c]	61.73[ab]
SEM	0.506	0.780	1.275	0.793	0.698	0.943
P Value	0.0065	0.0255	0.0933	0.0046	0.0014	0.0053

Means within a column with different superscripts differ (P < 0.05).

†Sugar beet pulp (SBP) (control), Apple pomace (AP), Orange pulp (OP), 33% AP + 66% OP (33% Apple pomace + 66% Orange pulp), 50% AP + 50% OP (50% Apple pomace + 50% Orange pulp), and 66% AP + 33%OP (66% Apple pomace + 33% Orange pulp).

3.5. Ex-vivo methane emission

Methane emission data were recorded along with gas production procedure. The treatments SBP and OP showed the highest values (12.36 ml/gr DM in 24h, 11.96 ml/gr DM in 24h) compared to other test feeds.

3.6. In vitro digestibility of dry matter

In vitro disappearance of dry matter for test feeds at the earlier hours of incubation (2h,4h) were continued by being the highest amount for OP and the lowest for AP, noticeably at the 8h of incubation we found no significant differences among the test feeds except the AP, which were the lowest (33.54% DM) ($P < 0.05$). The later hours of incubation were continued with the disappearance of the OP as the highest amount (64.15% DM) and the AP as the lowest (57.66% DM) ($P < 0.05$).

3.7. Fermentation parameters

Table 5 displays the inoculum fermentation parameters of the feedstuff. The highest pH were recorded 6.7 for SBP, and the highest ammonia-N were for AP and SBP (10.06,10.96 mg/dL), while the lowest was for OP (6.09 mg/dL) ($P < 0.05$).Not only the total VFA, but also the propionate and the butyrate highest amount were measured for SBP ($P < 0.05$). The highest and the lowest amounts of acetate were for AP and OP (61.84 mmol/L, 58.15 mmol/L), respectively ($P < 0.05$). the highest valerate and isovalerate was for OP (1.21 mmol/L, 0.95 mmol/L). The highest calculated Acetate: propionate was for 66%AP + 33%OP (2.64) and the lowest (2.15) was SBP ($P < 0.05$).

Table 5

Inoculum fermentation parameters of agro-industrial by-product samples ($P < 0.05$).

Feedstuff†	Parameters								
	pH	Ammonia-N (mg/dL)	Total VFA (mmol/L)	Acetate (mmol/L))	Propionate (mmol/L)	Butyrate (mmol/L)	Valerate (mmol/L)	Isovalerate (mmol/L)	Acetate: propionate
SBP	6.40^{dc}	10.96^{a}	91.39^{a}	59.40^{c}	27.55^{a}	2.83^{a}	0.95^{b}	0.66^{bc}	2.15^{c}
AP	6.7^{a}	10.06^{a}	89.37^{bc}	61.84^{a}	23.82^{dc}	1.76^{c}	1.07^{ab}	0.87^{ab}	2.60^{a}
OP	6.23^{d}	6.09^{d}	89.41^{bc}	58.15^{d}	26.83^{ab}	2.26^{abc}	1.21^{a}	0.95^{a}	2.17^{c}
33%AP + 66%OP	6.24^{d}	7.35^{c}	88.75^{bc}	59.06^{dc}	26.02^{ab}	2.02^{bc}	1.00^{b}	0.65^{c}	2.27^{bc}
50%AP + 50%OP	6.46^{bc}	7.91^{c}	89.76^{b}	60.29^{bc}	25.19^{bc}	2.49^{ab}	1.02^{b}	0.75^{abc}	2.39^{b}
66%AP + 33%OP	6.63^{ab}	9.08^{b}	88.16^{c}	60.97^{ab}	23.09^{d}	2.22^{abc}	1.04^{a}	0.83^{abc}	2.64^{a}
SEM	0.065	0.309	0.445	0.385	0.541	0.196	0.051	0.063	0.057
P Value	0.0012	0.0001	0.0049	0.0002	0.0005	0.0337	0.0540	0.0336	0.0002

Means within a column with different superscripts differ ($P < 0.05$).

†Sugar beet pulp (SBP) (control), Apple pomace (AP), Orange pulp (OP, 33% AP + 66% OP (33% Apple pomace + 66% Orange pulp), 50% AP + 50% OP (50% Apple pomace + 50% Orange pulp), and 66% AP + 33%OP (66% Apple pomace + 33% Orange pulp).

4. Discussion

4.1. Chemical analyses

Our findings about chemical composition of AIBPs are presented in Table 1, variable nutritive values were reported from past to present [9, 22, 23, 25, 39]. Different geographical areas, processing type, cultivation and climate differences beside conservation system seems to be the origin of the variant values.

4.2. Structural differences of AIBP

There are limited data of the surface images of AIBPs. as shown in Fig. 1, the surface of SBP was rather smooth with fewer breakages. This may be due to inclusion of nanofibers with the high pectin content acting like reinforced concrete increasing its tensile strength. As ruminal microorganisms can digest the pectin and fiber, it seems more degradable, but the mentioned surface area can be problematic in the case of the necessary space environment like cutting edge to be embedded by the microorganism's colonies. It is well documented that OP consists of hydro carbons being mostly cellulose, hemicellulose, lignin and pectin, and some other compounds like chlorophyll pigments [40]. Obviously, these biological components displayed a disorganized solid microstructure particle during crushing. These particles are providing a suitable base for microorganisms. fewer colonies can embed on the sleek surface of AP making it rather less digestible by the microorganisms.

4.3. Biogas production test

Commonly more fermentation produces more gas. According to Table 2, OP had higher amount of gas production at 24h, which may be the results of higher amount of soluble fraction of carbohydrate (providing more substrates to inoculum microorganisms) [22, 41, 42], also the combination of OP in the other treatments had positive effect, in comparison to the other test feeds apple pomace had lower amount of gas production which were in line with the findings of García-Rodríguez et al. (2019) these researchers reported 171 ml/g DM at 24 which was less than those reported for beet pulp and orange pulp (273 ml/g DM, 314 ml/g DM), respectively. It seems that combination of more fermentable feedstuff with apple pomace may increase the fermentation level of AP which was clearly reported in Table 2, the combination OP with AP at the levels of 50 and 66 increased gas production level ($P < 0.05$). Münnich et al. (2017) in a meta-analyses study on feeding sugar beet pulp in dairy cows on 36 reports concluded that higher inclusion of BP in diet of dairy cattle which doesn't intake more than 3.5% body weight is beneficial.

4.4. Estimated biogas production parameters and kinetics

Calculated parameters were not in line with those reported by other studies [22, 25] these parameters are affected by different factors, beside GP of 24h such as CP, CF and ash, on the other hand as mentioned factors had a variety of ranges due to conservation and cultivation methods, thus these differences were expectable.

4.5. Ex-vivo methane emission

Methane known as one of the environmental contaminators not only plays an important role on human health, but also being responsible for energy dissipation in ruminants for more than 5% [43]. When bioactive compounds, polyphenols being present in OP and AP, are utilized in the animal feeding system beside reducing methane emission, presence of them in the human food produced by those animals can increase the nutraceutical value such as antioxidants [9].

4.6. In vitro digestibility of dry matter

In a study conducted by Pirmohammadi et al. (2006) for determination of the chemical composition and digestibility of dried AP, reported that after 72h of incubation the digestibility value was 620 g/kg of dry matter, while García-Rodríguez et al. (2019) reported a different value of 566 g/ kg DM after 144h, the later researchers reported the digestibility of SBP and OP as 929 g/kg of DM and 989 g/kg of DM, respectively. A different amount of digestibility (841 g/kg DM after 48h) were reported by Lashkari and Taghizadeh (2015) for OP. Chemical compositions of the feedstuff can be the source of the differences. As it is obvious, in our study the inclusion of OP in the treatments lead them to a more extent of digestion.

4.7. Fermentation parameters

Confirmed by several researchers that no lactate production occurs during pectin, as the main source of energy in the SBP, fermentation [45–47]. recent findings have reported that the extent of pectin fermentation of decreases when the pH value is reduced. The optimum amount of SBP was estimated by Münnich et al. (2017) as > 200 g/kg of the dietary DM when dry matter intake of the animal is ≤ 3.5% of body weight. In a study conducted by Steyn et al. (2017) they found no significant changes in the rumen pH while working on the effect of dried AP when replaced by maize. In the present study, orange pulp showed the lowest amount of NH_3-N within the treatments; the most probable reason can be the capacity of the polyphenols in the OP to bind the protein and therefore to decrease the fermentation extent of it [49], however the CP level of the OP as the only source of the protein in the digestion culture was less than the others. Reduced level of the ammonia nitrogen can result in an improved level of the nitrogen utilization and it can decrease the nitrogen excretion as a contaminator to the environment [9]. No significant differences in the amount of VFA, particularly acetate, propionate and butyrate, were reported when the SBP were used in the ruminant's ration [19, 50], also the similar results were reported by Steyn et al. (2017) for AP.

5. Conclusion

According to the results we obtained from the present study orange pulp and sugar beet pulp had higher amount of methane emission and cumulative biogas production. Images of FESEM revealed that more broken edges were for orange pulp while sleek surface was for apple pomace. Here in this study we conclude that beside reducing environmental contamination by the agro-industrial by-products, a positive effect of AIBPs on degradation and biogas kinetics, methane emission and in vitro fermentation parameters were found that describes they can be used as a good source of non-fiber energy sources.

Declarations

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Rusinamhodzi, L., *Challenges in maximizing benefits from ecosystem services and transforming food systems*, in *The Role of Ecosystem Services in Sustainable Food Systems*. 2020, Elsevier. p. 263-274.
2. Greening, C., et al., *Diverse hydrogen production and consumption pathways influence methane production in ruminants.* The ISME Journal, 2019: p. 1.
3. Thauer, R.K., *The Wolfe cycle comes full circle.* Proceedings of the National Academy of Sciences, 2012. **109**(38): p. 15084-15085.
4. Chun, H., et al., *Maternal exposure to air pollution and risk of autism in children: A systematic review and meta-analysis.* Environmental Pollution, 2020. **256**: p. 113307.
5. Arku, R.E., et al., *Long-term exposure to outdoor and household air pollution and blood pressure in the Prospective Urban and Rural Epidemiological (PURE) study.* Environmental Pollution, 2020: p. 114197.
6. Anderson, J.O., J.G. Thundiyil, and A. Stolbach, *Clearing the air: a review of the effects of particulate matter air pollution on human health.* Journal of Medical Toxicology, 2012. **8**(2): p. 166-175.
7. Liu, J., et al., *Air pollution exposure and adverse sleep health across the life course: A systematic review.* Environmental Pollution, 2020: p. 114263.
8. Vermeulen, S.J., B.M. Campbell, and J.S. Ingram, *Climate change and food systems.* Annual review of environment and resources, 2012. **37**.
9. Correddu, F., et al., *Can Agro-Industrial By-Products Rich in Polyphenols be Advantageously Used in the Feeding and Nutrition of Dairy Small Ruminants?* Animals, 2020. **10**(1): p. 131.
10. Ribeiro, G.O., et al., *Repeated inoculation of cattle rumen with bison rumen contents alters the rumen microbiome and improves nitrogen digestibility in cattle.* Scientific Reports, 2017. **7**(1): p. 1-16.
11. Lan, W. and C. Yang, *Ruminal methane production: Associated microorganisms and the potential of applying hydrogen-utilizing bacteria for mitigation.* Science of The Total Environment, 2019. **654**: p. 1270-1283.
12. Henderson, G., et al., *Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range.* Scientific reports, 2015. **5**: p. 14567.
13. Palangi, V. and M. Macit, *Indictable Mitigation of Methane Emission Using Some Organic Acids as Additives Towards a Cleaner Ecosystem.* Waste and Biomass Valorization, 2021: p. 1-10.
14. Leahy, S.C., et al., *The genome sequence of the rumen methanogen Methanobrevibacter ruminantium reveals new possibilities for controlling ruminant methane emissions.* PloS one, 2010. **5**(1): p. e8926.
15. Fontenot, J., et al., *Supplementation of apple pomace with nonprotein nitrogen for gestating beef cows. I. Feed intake and performance.* Journal of animal science, 1977. **45**(3): p. 513-522.

16. Toyokawa, K., et al., *Studies on the utilization of rice straw, 7: The effect of rice straw on making silage of apple pomace as addition and its rearing effect for lambs as roughage.* Bulletin of the Faculty of Agriculture Hirosaki University, 1977.
17. Toyokawa, K., et al., *The effects of apple pomace silage on the milk production and the reduction of the feed cost for lactating cows.* Bulletin of the Faculty of Agriculture-Hirosaki University (Japan), 1984.
18. Ghoreishi, S.F., R. Pirmohammadi, and A.T. Yansari, *Effects of ensiled apple pomace on milk yield, milk composition and DM intake of Holstein dairy cows.* Journal of Animal and Veterinary Advances, 2007. **6**(9): p. 1074-1078.
19. Münnich, M., et al., *A meta-analysis of feeding sugar beet pulp in dairy cows: effects on feed intake, ruminal fermentation, performance, and net food production.* Animal Feed Science and Technology, 2017. **224**: p. 78-89.
20. Zema, D., et al., *Valorisation of citrus processing waste: A review.* Waste Management, 2018. **80**: p. 252-273.
21. Kara, K., et al., *Investigation in terms of digestive values, silages quality and nutrient content of the using pomegranate pomace in the ensiling of apple pomace with high moisture contents.* Journal of applied animal research, 2018. **46**(1): p. 1233-1241.
22. Paya, H., et al., *Evaluation of rumen fermentation kinetics of some by-products using in situ and in vitro gas production technique.* Slovak Journal of Animal Science, 2012. **45**(4): p. 127-133.
23. Lashkari, S. and A. Taghizadeh. *Digestion kinetics of carbohydrate fractions of citrus by-products*. in *Veterinary Research Forum*. 2015. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
24. Muela, C.R., et al., *Concentration of acid detergent fiber, neutral detergent fiber and lignin during solid state fermentation of apple (Malus domestica) derivates.* Cuban Journal of Agricultural Science, 2017. **51**(1).
25. García-Rodríguez, J., et al., *Chemical Composition, In Vitro Digestibility and Rumen Fermentation Kinetics of Agro-Industrial By-Products.* Animals, 2019. **9**(11): p. 861.
26. AOAC, *Official methods of analysis of AOAC*. 2005, Washington: AOAC.
27. Van Soest, P.v., J. Robertson, and B. Lewis, *Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition.* Journal of dairy science, 1991. **74**(10): p. 3583-3597.
28. Care, I.C.o.A., *Guide to the care and use of experimental animals*. 1995, Isfahan University of Technology Isfahan, Iran.
29. Shirmohammadi, S., et al., *Ruminal and post-ruminal barley grain digestion and starch granule morphology under three heat methods.* Annals of Applied Biology, 2020.
30. Parakhia, M.V., *Application of Scanning Electron Microscope in Agriculture.* 2017.
31. McDougall, E., *Studies on ruminant saliva. 1. The composition and output of sheep's saliva.* Biochemical journal, 1948. **43**(1): p. 99.
32. Fedorah, P.M. and S.E. Hrudey, *A simple apparatus for measuring gas production by methanogenic cultures in serum bottles.* Environmental Technology, 1983. **4**(10): p. 425-432.
33. Adejoro, F.A., et al., *Replacing urea with nitrate as a non-protein nitrogen source increases lambs' growth and reduces methane production, whereas acacia tannin has no effect.* Animal Feed Science and Technology, 2020. **259**: p. 114360.
34. Khajehdizaj, F.P., A. Taghizadeh, and B.B. Nobari, *Effect of feeding microwave irradiated sorghum grain on nutrient utilization, rumen fermentation and serum metabolites in sheep.* Livestock Science, 2014. **167**: p. 161-170.
35. Broderick, G. and J. Kang, *Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media1.* Journal of dairy science, 1980. **63**(1): p. 64-75.
36. Larsen, M., et al., *Evaluation of the ororuminal FLORA sampling device for rumen fluid sampling in intact cattle.* Journal of dairy science, 2020. **103**(1): p. 447-450.
37. Getachew, G., H. Makkar, and K. Becker, *Tropical browses: contents of phenolic compounds, in vitro gas production and stoichiometric relationship between short chain fatty acid and in vitro gas production.* The Journal of Agricultural Science, 2002. **139**(3): p. 341-352.
38. Menke, K., et al., *The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro.* The Journal of Agricultural Science, 1979. **93**(1): p. 217-222.
39. Mirzaei-Aghsaghali, A. and N. Maheri-Sis, *Nutritive value of some agro-industrial by-products for ruminants-A review.* World J. Zool, 2008. **3**(2): p. 40-46.
40. Sun, K., et al., *Manganese oxide nanorods supported on orange peel-based carbon nanosheets for high performance supercapacitors.* Int. J. Electrochem. Sci, 2015. **10**: p. 2000-2013.

41. Lashkari, S. and A. Taghizadeh, *Nutrient digestibility and evaluation of protein and carbohydrate fractionation of citrus by-products.* Journal of animal physiology and animal nutrition, 2013. **97**(4): p. 701-709.
42. Taghizadeh, A., et al., *In vitro gas production profiles in some concentrate ingredients.* J Anim Vet Adv, 2008. **7**(2): p. 137-139.
43. Hynes, D.N., et al., *Effects of concentrate crude protein content on nutrient digestibility, energy utilization, and methane emissions in lactating dairy cows fed fresh-cut perennial grass.* Journal of dairy science, 2016. **99**(11): p. 8858-8866.
44. Pirmohammadi, R., et al., *Chemical composition, digestibility and in situ degradability of dried and ensiled apple pomace and maize silage.* Small ruminant research, 2006. **66**(1-3): p. 150-155.
45. Mojtahedi, M. and M.D. Mesgaran, *Effects of the inclusion of dried molassed sugar beet pulp in a low-forage diet on the digestive process and blood biochemical parameters of Holstein steers.* Livestock Science, 2011. **141**(2-3): p. 95-103.
46. Shahmoradi, A., et al., *Effects of partial replacement of barley grain with beet pulp on performance, ruminal fermentation and plasma concentration of metabolites in transition dairy cows.* Journal of animal physiology and animal nutrition, 2016. **100**(1): p. 178-188.
47. Strobel, H.J. and J.B. Russell, *Effect of pH and energy spilling on bacterial protein synthesis by carbohydrate-limited cultures of mixed rumen bacteria.* Journal of Dairy Science, 1986. **69**(11): p. 2941-2947.
48. Steyn, L., R. Meeske, and C. Cruywagen, *The effect of dried apple pomace as a replacer for maize in the concentrate for Jersey cows grazing ryegrass pasture on production and rumen metabolism.* Animal Feed Science and Technology, 2017. **234**: p. 264-273.
49. Toral, P.G., et al., *Tannins as feed additives to modulate ruminal biohydrogenation: Effects on animal performance, milk fatty acid composition and ruminal fermentation in dairy ewes fed a diet containing sunflower oil.* Animal Feed Science and Technology, 2011. **164**(3-4): p. 199-206.
50. Mahmoud, A.E., et al., *Impact of Inclusion Dried Sugar Beet Pulp in Ruminant's Ration on Rumen Parameters in vitro.* Pakistan Journal of Zoology, 2019. **51**(6): p. 2281.

Figures

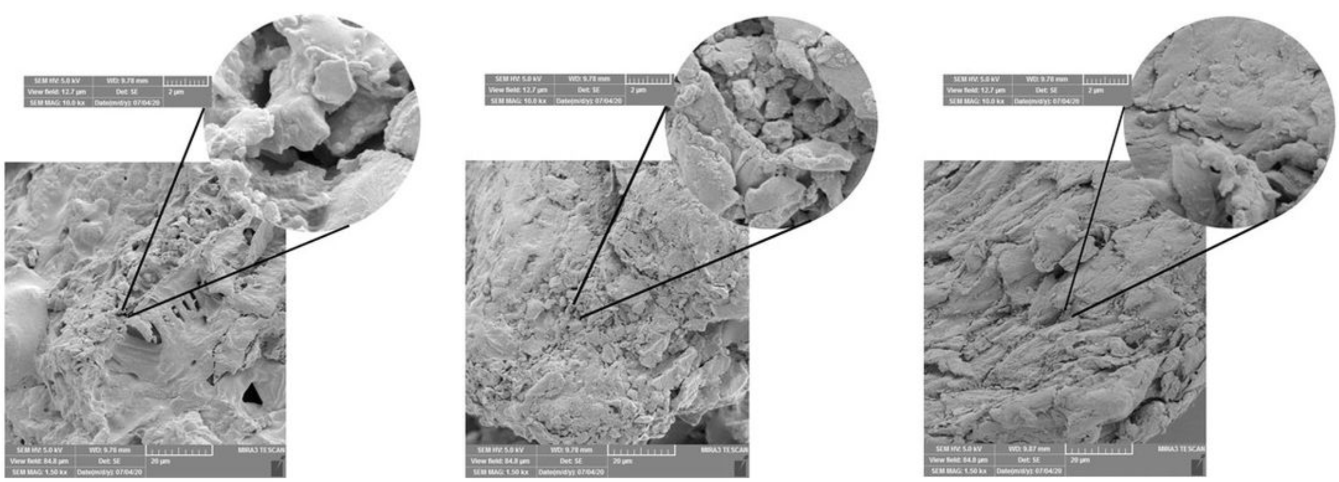

Figure 1

FESEM (MIRA3TESCAN) image (HV= 5.00 KV, MAG= 1.5 KX, Bar=20 μm & MAG= 10 KX, Bar=2 μm). apple pomace (AP), Orange pulp (OP) and Beet pulp sugar (BPS) from left to right, respectively.

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