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ORIGINAL RESEARCH

Chemical composition, aerobic stability and fermentation pattern of tomato pomace and pumpkin waste silage using fibrolytic enzymes and lactic acid bacteria

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Abstract

Purpose This study aimed to evaluate the effect of different additives on chemical composition, fermentation characteristics, and gas production parameters of tomato pomace and pumpkin waste silages.

Method Treatments were: tomato pomace silage, pumpkin waste silage, tomato pomace and pumpkin waste silage mix (50:50), tomato pomace and pumpkin waste silage mix treated with the *fibrinolytic* enzyme (E), tomato pomace and pumpkin waste silage mix treated with LAB made inoculants (LMI), and tomato pomace and pumpkin waste silage mix treated with E+ LMI. Representatives of samples were packed manually into laboratory silos and allowed to ensile for 1, 3, 7, 21, 45, and 90 days.

Results The results showed a significant difference between the experimental treatments in chemical composition ($p<0.05$). The treatment of pumpkin waste showed the lowest amount of dry matter (DM), insoluble fibers in neutral detergent (NDF), and insoluble fibers in acidic detergent (ADF). The value of crude protein (CP) showed a decreasing trend with increasing time after ensiling. The treatment with bacterial and enzymatic additives had a faster drop in pH and a lower final pH compared to other treatments.

Conclusion Compared with the tomato pomace and pumpkin waste silage, treatments E and E + LMI had lower acetic and butyric acid contents. During aerobic exposure, tomato pomace and pumpkin waste had the lowest pH changes in silage. Generally, applying a combination of E and LAB inoculants improved both fermentation quality and aerobic stability of silage.

Keywords Tomato pomace, Pumpkin waste, Fibrinolytic enzyme, LAB, Fermentation

Introduction

Meeting the nutritional requirements of livestock due to poor quality and quantity of pastures and lack of adequate forage and rising production costs is one of the main challenges for animal science specialists and producers. In this regard, paying attention to wastes and by-products of agricultural products due to the wastes' volume and scope of their production in animal nutrition not only cause reducing in environmental pollution and the need for costly waste management programs, but also in meeting part of the food needs for livestock as well as reducing the dependence of livestock on cereals for human consumption (Adesogan et al. 2002; Ajila et al. 2012). Among the agricultural wastes and by-products, tomato pomace and pumpkin waste are used in animal nutrition due to their nutritional values, mass production and acceptable results of other research (Ajila et al. 2012). The global production of processed tomatoes and pumpkin increased to 37.38 million and 27.6 million tons, respectively (World Processing Tomato Council 2019). According to the report (Costa and Heuvelink 2018), after potatoes, the second important vegetable product in the world was tomato (*Solanum Lycopersicum* L.). The amount of tomato as a strategic crop depends on the intensity of light, temperature, cultivation method, *etc*. (Kubota et al. 2018). The tomato

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residue and pulp containing tomato seeds and skin were obtained after extracting the tomato juice.

Tomato pomace is a by-product obtained from the tomato process for concentrating dough, extract, puree, paste, and tomato sauce. Tomato pulp contains skin, tomato seeds, some fiber, and sticky paste. The skin, seeds, and fruit of tomato pomace contain lycopene (from the skin), other carotenoids (β-carotene), and phenolic compounds. Due to the organic matter in tomato pomace, these wastes cause many problems through the growth of sewage, creek, and river plants. Tomato waste is equivalent to one-fifth of fresh tomatoes, which, with its energy, protein, and low cost of preparation, can be included in the diet of livestock and poultry or used as a substitute for some foods in the diet (Fondevila et al. 1994). Tomato pomace is relatively rich in protein (17-22 % DM) and fat (10-15 % dry matter). The fat content can exceed up to 20 % if the tomato seed ratio is high (Battaglini and Costantini 1978). The amounts of insoluble fibers in neutral detergent (NDF) and ADF were ranged from 50-72 and 39-60 % of DM, respectively (Feedipedia 2011). Its lignin content was 20-30 % of DM. However, some tomato pomace has been described as containing less than 7 % of lignin (Gasa et al. 1989; Fondevila et al. 1994). About 12,660 tons of tomato pulp is obtained in Golestan annually (Out of 81,000 tons).

Pumpkin is also one of the widely cultivated agricultural products in Golestan province for seed harvesting. Pumpkin fruit production is about 40 to 70 tons per hectare. The remained material after the seed harvesting is called pumpkin waste, which contains more than 90 % of fruit weight and includes the skin and the inner fibers. The residues have a high nutritional value and can be used to feed ruminants. Researchers report that pumpkin fruit is a source of carbohydrates, vitamins (A, C, E), lycopene, dietary fiber, and minerals (Elinge et al. 2012).

Tomato pomace and pumpkin wastes are favorable environment for the growth and reproduction of insects and pathogens. The higher water content of tomato pomace (usually more than 75 %) is the limitation for its further use in animal nutrition (Caluya 2000). Therefore, pumpkin waste cannot be stored as fresh organic material for more than 24 hours. However, due to the area under cultivation and the limited consumption by livestock in a short time, it is not possible to use all the residues at the time of extraction. Therefore, for the optimal use of tomato pomace in animal feed, its nutritional value should be calculated in various ways, including drying and siloing (Belibasakis and Tsirgogianni 1995).

Siloing is a common method that preserves and stores moist forage in an anaerobic and acidic environment (McDonald et al. 1991). In this method, due to the activity of lactic acid-produced by bacteria under anaerobic conditions, water-soluble carbohydrates in forage are converted to organic acids, mainly lactic acid, which reduces the pH and thus protects the forage from microbial spoilage (Filya 2003). The main biochemical processes involved in silo fermentation, such as acidic conditions (fermentation of carbohydrates by microorganisms) and enzymatic hydrolysis of structural carbohydrates by plant and bacterial enzymes, affect the overall nutritional value of silage. Silo additives have been used as a management tool to improve the nutritional value of ensiled materials. In practice, these additives are used to stimulate fermentation, reduce nutrient loss, increase aerobic stability, and ultimately improve and increase livestock production (Yitbarek and Tamir 2013) through various chemical and biological additives. Biological additives have advantages in comparison to chemical additives due to their safety, ease of use, non-corrosion of agricultural machinery, and non-contamination. Among biological additives, lactic acid bacteria with homogeneous or heterogeneous fermentation (homofermentative or heterofermentative) has been used as silage additive (Oude Elferink et al. 2001).

Enzymes are another class of biological additives especially those with *cellulolytic*, *hemicellulolytic*, and *amylolytic* activity, which have been used to break down fiber into water-soluble carbohydrates (WSC) to ferment lactic acid bacteria (Kung and Ranjit 2001) in silage. The primary function is to break down cell wall compounds and grain starch in crops to improve silage fermentation (Muck and Bolsen 1991). The enzyme increases lactic acid production (Rauramaa et al. 1987; Jaakkola et al. 1991; Kung et al. 1991), decreased silage pH (Rauramaa et al. 1987; Kung et al. 1991; Stokes 1992), decreased acetic acid concentrations (Jaakkola et al. 1991; Stokes and Chen 1994) as well as increasing (Dean et al. 2005), decreasing (Jaakkola et al. 1991) or being ineffective (Stokes and Chen 1994) on aerobic stability. Due to the nutritional values of tomato pomace and pumpkin wastes, in terms of the amount of crude protein and soluble carbohydrates (as an energy source), the combination of mentioned material in the

preparation of silage may be able to support a better fermentation process in the silo. On the other hand, lactic acid produced by bacteria does not have much ability to reduce plant cell walls; it was hypothesized that the use of an enzyme additive could help lactic acid bacteria to break down the cell wall for energy supply.

Therefore, this study aimed to investigate the effect of LAB inoculant and fibrolytic enzymes on chemical composition, aerobic stability, and fermentation properties of tomato pomace and pumpkin waste silage.

Materials and methods

Silage preparation

Tomato pomace and pumpkin waste were supplied from local paste factories and local fields (Golestan, Iran). Fresh pumpkin pomace was provided from the field around Gonbad Kavous, chopped under farm condition to the length of 2-3 cm, and used aeration drying. Treatments were: 1) tomato pomace silage, 2) pumpkin pomace silage, 3) mix of 50 % tomato pomace and 50 %pumpkin silage (w/w), 4) treatment 3+ Enzyme (1g in DM), 5) treatment 3+ LAB-made inoculants (LMI) and 6) treatment 3+ Enzyme + LMI inoculant. The LAB inoculant was applied at a level of 1×10^8 cfu/g of fresh weight. Presentative forage samples (3kg) were packed manually, in triplicate, into a mini silo of small plastic pouche. Air was withdrawn from the plastic pouches by means of a vacuum cleaner. The filled silos were completely closed and stored in a compartment of the laboratory at ambient temperature (20–25 °C) and allowed to ensile for 1, 3, 7, 21, 45 and 90 days. Four replicate pouches were prepared for each ensiling time and experimental forage.

Preparation of bacterial inoculants

The LAB inoculant was a combination of L. acidophilus PTCC (Persian Type Collection Culture) 1643, *L. casei* PTCC 1608, *L. Plantarum* PTCC 1058, *Enterococcus faecium* PTCC 1238, and *Pediococcus pentosaseous* PTCC 1426. These strains were purchased from Iran Scientific and Industrial Organization for making LAB-made inoculants (LMI). A vial of freeze-dried LAB (*L. casei*, *L. Plantarum* and *P. pentosaseous*) and *E. faecium* were individually inoculated into 10 mL MRS (de Man, Rogosa, Sharpe) broth (Merck, KGaA Germany) and Brain Heart Infusion (BHI), respectively and incubated at 37 °C for 24 h under anaerobic conditions. By pour plating serial 10fold dilutions (in sterile ringer's solution) on-demand, Rogosa, sharp agar, and SLB agar plates were incubated anaerobically at 37 °C for 48 h. 109 CFU LAB/ml of culture was produced after 24 h of culture.

Silage sampling

After designated ensiling times, silos were opened and the top of silage was disposed of from each mini silo due to spoilage, then samples were taken from the upper, middle, and lower levels of each silo.

Chemical analysis

Dry matter (DM) was determined by drying. The dry matter (DM) contents of ensiled forage were determined by drying the samples to a constant weight at 60 °C for 3 days. The dried samples were ground to pass a 1 mm screen for later analysis. Nitrogen (N) content was analyzed by the Kjeldahl method (AOAC 2000). The CP was calculated as N × 6.25. Neutral detergent fiber (NDF) and acid detergent fiber (ADF; without amylase) were determined (Van Soest et al. 2000).

Determination of silage pH, N-NH3 and VFA in silage samples

A sub-sample of silage (50 g) was homogenized in 450ml of distilled water (w/v) to produce a dilution of 1:10, and homogenized for 5 min at room temperature and then filtered through double-layered cheesecloth. The pH of water extracts was measured immediately by pH meter (Metrohm, 691 models).

For determination of NH_3-N, a portion of the extract (5 ml) was filtered and added to 5 ml of 0.2 N HCl (v/v) and frozen immediately at -20 °C. Ammonia-N in silages extract was determined by the phenol–hypochlorite reaction (Broderick and Kang 1980).

To determine concentrations of acetic, propionic, butyric, iso-butyric and iso-valeric acids in extraction liquid of silage samples, the liquid was decanted into centrifuge tubes and centrifuged at 26,000 g for 30 min (Tjardes et al. 2000). The supernatant was filtered and analyzed with a high-performance liquid chromatography device (Agilent 1100 HPLC, Germany) equipped with a refractive index detector (HP 1047A). An Aminex Hpx 87H column (Germany) (300 × 7.8 mm

column) was used. The flow rate of the mobile phase (0.005 M H_2SO_4) was 0.6 ml/min at 41 °C (Canale et al. 1984).

Aerobic stability

After ensiling for 90 days, the residual silages of each silo were placed loosely in a 2 kg plastic bucket at room temperature (25 °C) without sealing to test their aerobic stability for 7 days. No physical packing of the silage took place. Each bucket was covered with a double layer of cheesecloth to avoid contamination and to allow air penetration. A thermometer was placed in the geometric center of each silage mass and temperatures were recorded every 2 h. When the temperature of the silage increased by 2 °C above the ambient temperature, the silage was recognized as undergoing aerobic deterioration (Pitt et al. 1991).

In vitro gas production measurement

In vitro cumulative gas production was determined using 120 ml serum bottles (Theodorou et al. 1994). A buffered mineral solution (Menke et al. 1979) was prepared and placed in a water bath at 39 °C under continuous flushing with CO2. Rumen fluid was collected before morning feeding from three ruminal fistulated sheep (45±2 Kg) The sheep were housed in individual cages, fed a 40:60 concentrate: forage (approximately 1.5 kg) at the maintenance level with free access to drinking water. *In vitro* gas production was measured in triplicate on composite samples from the same treatment silos. For each replicate, samples of 200 mg DM silages obtained from the days 90 mini silos were used. The bottles were then filled with 30 ml of incubation medium that consisted of 10 ml of rumen fluid plus 20 ml of buffer solution and placed in a water bath at 39 °C. Gas production was recorded at 2, 4, 8, 16, 24, 48, and 72 h. Total gas values were corrected for blank incubation and gas values were expressed in ml g-1 of DM. A pressure transducer and LED digital read-out voltmeter were used to measure the headspace gas pressure in the culture bottles. Volumes of gas at the top of the culture bottles were transferred into a syringe by the withdrawal of the syringe plunger until the pressure became zero. Gas volume and headspace pressure were recorded. Following the procedure (Theodorou et al. 1994), linear regression analyses were determined for headspace gas pressure versus gas volume. Data for gas production at the different time treatments were fitted with the nonlinear equation of P= b (1-e-ct) (Orskov and Mcdonald 1979), where P is gas production in time t, b is potential gas production and c is the rate of gas production of the insoluble fraction. The organic matter digestibility (OMD) and metabolizable energy (ME) (Menk et al. 1979) and SCFAs (Makkar 2005) were estimated using equations on Menke et al. (1979) as:

OMD, % = 14.88+ 0.889 GP + 0.45 CP_1 + 0.065 A

ME, (MJ/kg DM) = 2.20+0.136 GP + 0.0574 CP_2

SCFA, (mmoL) = -0.00425 + 0.0222 GP

Where, GP: 24 h net gas production (ml/200 mg DM), CP_1: Crude protein (%), A: Ash content (%), CP_2: g/kg DM.

Statistical analysis

Statistical analyses were performed using the GLM procedure of SAS (Version 9.1, SAS Institute) according to a completely randomized design. The least significant difference (LSD) test was used to compare the means.

Results and discussion

Chemical composition and fermentation characteristics

The effect of enzymatic and bacterial additives on the mixed composition of pumpkin waste and tomato pomace residues silage is shown in Table 1. The results showed that the dry matter of combination of pumpkin waste and tomato pomace silage was higher than pumpkin waste silage and lower than tomato pomace silage. However, there was no significant difference between the treatments in terms of dry matter content (P> 0.05). As the ensiling time increased, the dry matter content of the silages did not follow a specific trend. The amount of crude protein in silages was not affected by the treatments until 7 days after ensiling. However, no significant differences were observed between experimental treatments on days 21, 45, and 90. The amount of crude protein had a decreasing trend and the greatest decrease in the amount of crude protein was shown in the silage of pumpkin waste and the mixed silage of tomato pomace and pumpkin waste (2.4 % and 15.4 %, respectively). In this investigation, there was no significant difference in NDF and ADF between experimental treatments (except pumpkin waste silage) at all times

after ensiling. Although in all silages the concentration of ammonia nitrogen increased with increasing ensiling time, from the 21st day onwards, there was no significant difference between the experimental treatments. There was a significant difference in ammonia nitrogen concentration between experimental treatments up to 7 days ($P<0.05$). With increasing ensiling time, the concentration of ammonia nitrogen had an increasing trend, the amount of increase in these treatments was determined to be 2.1, 1/2, 1.76, 1.96, 1.23, and 1.76 mg/dl, respectively. The pH of different silages was affected by experimental treatments during the ensiling time. The lowest pH (3.83) was observed on day 90 for bacteria-treated silage added bacterial additive silage. With increasing silage time, the pH value decreased. In this regard, the greatest decrease in pH during the times after ensiling was observed in silage with bacterial additive (1.14). The rate of decrease in pH for treatments up to the 7th day after ensiling was 0.44, 0.59, 0.47, 0.63, 0.83 and 0.71, respectively.

The dry matter content of tomato pomace and pumpkin waste on the first day after ensiling was 25.39 % and 14.98 %, respectively, which reached 20.84 % by mixing the two. The dry matter content of good quality silage should be in the range of 20-35 % (Ergül 1988), which the combination of these two materials has produced silage with a dry matter of about the desired range.

In previous studies, it was reported that dry matter content of pumpkin waste silage (Ulger et al. 2018) was 9.33 % and for tomato pomace silage 26.9 % (Gallo et al. 2017). However, the use of bacterial and enzymatic additives did not affect the dry matter content of tomato pomace silage and pumpkin waste silage ($P>0.05$). The use of bacterial and enzymatic additives in forage silage did not affect the amount of dry matter. The results of this study suggested that a mixture of tomato pomace silage and pumpkin waste silage can increase the dry matter content of pumpkin waste (Adesogan et al. 2004).

The highest and lowest amounts of raw ash were related to pumpkin waste and tomato pomace silage, respectively. The amount of raw ash of tomato pomace during silage was in the range of 13-16 %, which was higher than the reported values (Ulger et al. 2018).

The crude protein content of tomato pomace and pumpkin waste silages for the first day after ensiling was 16.2 % and 15.15 %, respectively. Our findings are in agreement with those of Barroso (2002) who reported that the crude protein of pumpkin waste silage was 16.5 %. The amount of crude protein was significantly affected by the treatments with increasing time after ensiling and had a decreasing trend. In many studies, the use of bacterial and enzymatic additives reduced (Xing et al. 2008; Gallo et al. 2001) or was ineffective (McAllister et al. 1995; Aksu et al. 2006; Kizilsimsek et al. 2007) on crude protein content of silages.

In terms of ammonia nitrogen concentration, there was a significant difference among treatments up to day 7 after ensiling ($P <0.05$), which was consistent with other researchers (Hasnat et al. 2017). With increasing days after ensiling, the concentration of ammonia nitrogen increased, which was in the line with the decreasing trend of crude protein content. In this regard, the lowest amount of increase in ammonia nitrogen concentration was observed in the enzyme-treated treatment. Ammonia nitrogen is an indicator of the decomposition of peptides and amino acids and is a criterion for the quality of silage. In agreement with the results of the present study, a meta-analytic study of the findings of 46 studies has shown that bacterial additives do not affect ammonia nitrogen concentrations (Keedy and Murphy 1994). It was observed that up to day 4 after ensiling, soluble crude protein and non-protein nitrogen increased in all processed silages due to high and rapid degradation of protein in the initial phase of silage (Hasanat et al. 2007). There was an increase that was in the line with the decreasing trend in the amount of crude protein. In this regard, the lowest amount of increase in ammonia nitrogen concentration was observed in the treatment with the enzyme-treated silage.

Silage pH is an important indicator in the evaluation of silages, which can be measured to determine the production of lactic acid and the quality of the fermentation process. In many studies, no effect on pH in corn silage resulting from the use of enzymes with bacterial additives (Higginbotham et al. 1996; Stokes and Chen 1994) has been reported. In this study, the rate of decrease in pH up to day 7 after ensiling was higher in silages containing enzyme and enzyme- + bacterial inoculant. Also, the final pH on day 90 after ensiling was lower in enzyme and enzyme + bacterial treated silages, compared to other treatments. This is probably due to the addition of enzymes, in which the enzymes increase the activity of lactic acid-producing bacteria by breaking down the cell wall to release water-soluble carbohydrates, and finally decreases the pH. A faster decrease in silage pH, especially during the fermentation phase, can be achieved by inactivating plant proteases and

Table 1 Effect of additives on chemical composition (DM basis) of Tomato pomace and Pumpkin waste silage at several days after ensiling

Items	Ensiling days	Treatments						SEM	P-value		
		1	2	3	4	5	6		T	D	T×D
DM	1	25.39^{a}	14.98^{c}	20.84^{b}	21.11^{b}	20.89^{b}	20.53^{b}	0.502	0.0001	0.0001	0.1119
	3	26.60^{a}	15.89^{c}	21.72^{b}	20.67^{b}	22.52^{ab}	21.11^{b}				
	7	25.40^{a}	15.48^{c}	20.96^{b}	20.41^{b}	22.57^{b}	20.76^{b}				
	21	25.93^{a}	15.8^{c}	21.76^{b}	20.93^{b}	22.12^{b}	21.59^{b}				
	45	26.61^{a}	16.05^{d}	22.05^{bc}	23.43^{ab}	22.51^{bc}	21.54^{c}				
	90	28.53^{a}	16.50^{c}	21.72^{b}	21.81^{b}	22.06^{b}	23.10^{b}				
CP	1	17.60	15.15	16.20	16.00	15.50	16.10	0.481	0.0001	0.0001	0.2670
	3	16.70	14.20	15.50	15.90	15.55	16.35				
	7	16.40	14.10	15.40	14.75	14.65	16.15				
	21	16.25^{b}	13.05^{c}	13.75^{bc}	14.15^{b}	14.25^{b}	15.75^{a}				
	45	16.30^{a}	12.75^{b}	13.90^{ab}	13.8^{ab}	13.85^{ab}	14.45^{a}				
	90	15.55^{ab}	11.95^{c}	12.45^{bc}	13.65^{ab}	13.90^{a}	13.80^{ab}				
NDF	1	58.30^{a}	45.20^{b}	54.70^{a}	57.10^{a}	56.20^{a}	56.40^{a}	10.42	0.0001	0.0645	0.1223
	3	57.81^{a}	44.70^{b}	54.15^{a}	56.40^{a}	54.60^{a}	55.30^{a}				
	7	57.32^{a}	44.20^{b}	53.60^{a}	55.4^{a}	53.05^{a}	54.20^{a}				
	21	56.85^{a}	43.20^{b}	52.80^{a}	52.60^{ab}	51.90^{ab}	53.15^{ab}				
	45	56.71^{a}	43.56^{b}	52.95^{a}	51.50^{a}	51.35^{a}	52.72^{a}				
	90	56.50^{a}	43.35^{b}	53.20^{a}	52.00^{a}	50.20^{ab}	51.50^{a}				
ADF	1	45.10^{a}	34.15^{b}	45.70^{a}	44.50^{a}	44.40^{a}	45.10^{a}	6.18	0.0001	0.030	0.125
	3	44.59^{a}	32.63^{b}	45.13^{a}	43.58^{a}	43.10^{a}	44.95^{a}				
	7	44.11^{a}	33.15^{b}	44.61^{a}	42.73^{a}	41.80^{a}	43.85^{a}				
	21	44.20^{a}	31.15^{b}	44.08^{a}	41.75^{a}	39.41^{ab}	43.76^{a}				
	45	43.51^{a}	32.50^{b}	43.83^{a}	41.48^{a}	49.22^{a}	41.42^{a}				
	90	43.31^{a}	32.37^{b}	43.58^{a}	40.98^{a}	39.11^{a}	41.50^{a}				
Ash	1	4.78^{c}	16.10^{a}	8.78^{bc}	9.01^{b}	8.83^{bc}	9.38^{b}	0.132	0.0001	0.0001	0.0001
	3	4.39^{c}	13.66^{a}	9.44^{b}	10.00^{b}	8.61^{bc}	9.45^{b}				
	7	4.40^{d}	14.24^{a}	9.41^{bc}	9.59^{b}	9.34^{c}	9.30^{c}				
	21	4.30^{d}	14.59^{a}	8.88^{c}	9.14^{b}	8.94^{bc}	9.06^{bc}				
	45	4.29^{e}	13.06^{a}	8.84^{c}	8.53^{d}	9.06^{bc}	9.28^{b}				
	90	4.32^{c}	13.46 ^{a}	8.98^{b}	8.79^{b}	8.93^{b}	8.78^{b}				
EE	1	6.05^{a}	1.80^{b}	5.35^{a}	5.15^{a}	5.50^{a}	5.05^{a}	0.475	0.0001	0.0001	0.0001
	3	6.35^{a}	1.45^{c}	4.75^{b}	3.75^{b}	3.85^{b}	6.30^{a}				
	7	5.70^{a}	1.40^{c}	4.50^{b}	4.95^{ab}	5.60^{ab}	5.80^{a}				
	21	6.45^{ab}	3.70^{c}	7.35^{ab}	7.05^{ab}	8.05^{a}	5.30^{bc}				
	45	4.85^{bc}	3.75^{c}	6.30^{a}	5.15^{ab}	5.30^{ab}	6.25^{a}				
	90	6.20^{bc}	2.55^{d}	4.15^{dc}	7.2^{ab}	8.70^{a}	6.25^{bc}				
$N\text{-}NH_3$	1	0.24 ^{b}	0.25^{b}	0.45^{a}	0.20^{b}	0.23^{b}	0.17^{b}	0.057	0.038	0.0001	0.383
	3	0.44^{ab}	0.29^{ab}	0.5^{a}	0.30^{ab}	0.31^{ab}	0.25^{b}				
	7	0.57^{ab}	0.70^{a}	0.65^{a}	0.69^{a}	0.38^{b}	0.39^{b}				
	21	1.08^{a}	1.40^{a}	1.17^{a}	1.34^{a}	1.06^{a}	1.68^{a}				
	45	1.37^{a}	1.66^{a}	1.41^{a}	1.49^{a}	1.22^{a}	1.66^{a}				
	90	2.34^{a}	2.35^{a}	2.21^{a}	2.16^{a}	1.46^{a}	1.93^{a}				
pH	1	4.83^{d}	5.12^{a}	4.94^{bc}	4.98^{b}	4.97^{b}	4.91^{c}	0.0009	0.0001	0.0001	0.0001
	3	4.58^{e}	4.87^{a}	4.78^{b}	4.62^{d}	4.66^{c}	4.64^{cd}				
	7	4.39^{c}	4.53^{a}	4.47^{b}	4.35^{c}	4.14^{e}	4.20^{d}				
	21	4.25^{b}	4.43^{a}	4.37^{a}	4.19^{b}	4.05^{c}	4.10^{c}				
	45	4.15^{b}	4.28^{a}	4.27^{a}	4.07^{c}	3.93^{d}	3.98^{d}				
	90	4.06^{bc}	4.15^{ab}	4.21^{a}	3.95^{cd}	3.83^{d}	3.90^{d}				

1) Tomato pomace silage, 2) Pumpkin waste silage, 3) Tomato pomace and Pumpkin waste silage mix (50:50), 4) Tomato pomace and Pumpkin waste silage mix treated with Enzyme, 5)Tomato pomace and Pumpkin waste silage mix treated with lab made inoculants (LMI) and 6) Tomato pomace and Pumpkin waste silage mix treated with Enzyme + LMI, DM: Dry matter, CP: Crude protein, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, EE: Ether extract, D: Effect of ensiling days; T: Effect of treatments; T×D: Effect of treatment and ensiling days interactions; SEM: Standard error of the mean. The means within a row without common letter differ ($p<0.05$).

preventing the growth of undesirable microorganisms such as yeast (Kung and Shaver 2001). The bacteria used to prepare the bacterial additive in this study were heterofermentative, which showed that these bacteria increased the rate of silo acidification, reduced the final pH and proteolysis in the silo, and reduced the risk of Clostridium fermentation in the silage (Keady et al. 1994; Ohyama et al. 1975). In general, this is because the decomposition process is strongly influenced by the availability of degradable carbohydrates and domination of favorable bacteria during the silage process.

Pumpkin waste silage had the lowest amounts of insoluble fibers in neutral detergent and insoluble fibers in acidic detergent. The effect of time after ensiling on the amount of insoluble fibers in acidic detergent which had a decreasing trend was significant. In this study, the use of enzymatic and bacterial additives did not have a significant effect on cell wall concentration, which was in contrast with the results of (Xing et al. 2008; Tang et al. 2000). Studies using fibrolytic enzymes as additives have reported reductions in ADF and NDF levels (McAllister 2001; Xing et al. 2008; Tang et al. 2000; Zobell et al. 2000; Aksu et al. 2006). The cellulose and xylanase enzymes can break down lignocellulose bonds, making them more exposed to digestion by microorganisms. In contrast, in line with the present results, bacterial additives did not affect the cell wall concentration of silage (Islam et al. 2001).

Fermentation quality

The results of the concentrations of volatile fatty acids in different silages (Table 2), showed that there was a significant difference between treatments ($P \leq 0.0001$). The concentration of acetate and propionate in tomato pomace silage was higher than other silages (14.09 and 5.07 mmol/L, respectively). The lowest amount of acetate was observed in pumpkin waste silage (4.40 mmol/L) and propionate in pumpkin waste and tomato pulp silage with the enzyme (2.54 mmol/L). Acetate concentration in the pumpkin and tomato pomace silage was 11.21 mmol/L. Among the additive treatments, the use of bacterial additive did not affect acetate concentration (11.44 mmol/L); however, the use of enzyme additives and bacterial-enzyme composition significantly reduced the amount of acetate (10.39 and 9.36 mmol/L, respectively). Bacterial and enzyme-bacterial combination increased the amount of propionate in pumpkin and tomato pomace silage (3.58 and 4.25 mmol/L, respectively). The concentration of butyrate in tomato pomace and pumpkin silage was the same as tomato pomace (1.08 mmol/L). The addition of enzymes, bacteria, and their combination reduced the concentration of mentioned fatty acid (1.04 mmol/L). The most remarkable amount of butyrate was observed in pumpkin silage (1.01 mmol/L).

The concentration of isobutyrate in pumpkin silage was higher than other treatments (0.049 mmol/L), which was not significantly different from silages prepared from a mixture of pumpkin and tomato pomace with the enzyme (0.04 mmol/L). The concentration of this fatty acid in silage prepared from pumpkin waste with tomato pomace was 0.04 mmol/L. The use of bacterial additive and combination of bacteria with enzyme reduced isobutyrate (0.03 and 0.02 mmol/L, respectively). The amount of valerate and isovalerate in tomato pomace silage and pumpkin silage (0.08 and 0.02 mmol/L, respectively) was less than the silage prepared from their combination (0.11 and 0.10 mmol/L, respectively). The use of enzymes, bacteria, and their combinations reduced the amount of valerate fatty acid in silage prepared from a mixture of pumpkin and tomato pulp (0.02, 0.03 mmol/L, respectively). Also, the combination of bacteria and enzyme reduced the amount of isovalerate in this silage (0.02 mmol/L).

The highest amounts of acetic acid and butyric acid were observed in tomato pomace silage. Treatments with enzymatic-bacterial and enzymatic additives had significantly lower acetic acid content compared to mixed silage of tomato pulp and pumpkin waste. In many studies, the use of enzymatic additives (Jaackola et al. 1991; Stokes and Chen 1994; Rodrigues et al. 2001; Lynch et al. 2014; Kung et al. 2004) and bacterial additive (Whiter and Kung 2001; Sadeghi et al. 2012; Jatkauskas and Vrotniakiene 2004) reduced the amount of acetic acid. The lack of effect of bacterial additive on the amount of acetic acid in this study was in line with the results of (Filya et al. 2006; Koc et al. 2008; Adesogan et al. 2004; Gordon et al. 1999). The optimal amount of butyric acid in the treatments with additives showed a significant decrease compared to the silage of a mixture of tomato pulp and pumpkin nuts. A decrease in the amount of butyric acid can be considered as optimal fermentation in silages with additives. Perhaps the reason can be attributed to the faster decrease in pH in these treatments, which resulted in reduced protein degradation, because proteolytic *Clostridia* mainly ferments amino acids into products such as acetic acid, butyric acid, and amines.

Table 2 Effect of additives on fermentation acids (mmol/L) of Tomato pomace and Pumpkin waste silage at day 90 after ensiling

Items	Treatments						SEM	P-value
	1	2	3	4	5	6		
Fermentation								
Acetic acid	14.09^{a}	4.40^{e}	11.21^{b}	11.44^{b}	10.39^{c}	9.36^{d}	0.021	0.0001
Propionic acid	5.07^{a}	ND	2.97^{d}	3.58^{c}	2.54^{e}	4.25^{bc}	0.013	<0.0001
Butyric acid	1.08^{a}	1.01^{c}	1.08^{a}	1.04^{b}	1.04^{b}	1.04^{b}	0.002	<0.0001
Isobutyric acid	0.04^{bc}	0.049^{a}	0.04^{bc}	0.04^{ab}	0.03^{cd}	0.02^{e}	0.102	<0.0001
Isovaleric acid	0.02^{b}	0.02^{b}	0.1^{a}	ND	ND	0.02^{e}	0.008	<0.0001
Valeric acid	0.08^{b}	0.08^{b}	0.11^{a}	0.02^{c}	0.03^{c}	0.03^{c}	0.002	<0.0001
Gas production								
a+b (ml/gDM)	276.1^{c}	342.2^{a}	284.5^{c}	282.9^{c}	283.2^{c}	310.4^{b}	7.64	0.045
C (ml/h)	0.0307	0.0282	0.0325	0.0320	0.0298	0.269	0.002	0.032
OMD (%)	45.83^{c}	53.61^{a}	49.61^{abc}	47.61^{bc}	50.72^{ab}	50.72^{ab}	3.29	0.036
ME (MJ/kg DM)	6.93^{c}	8.12^{a}	7.51^{abc}	7.20^{bc}	7.07^{bc}	7.68^{ab}	0.077	0.036
SCFA (mmoL)	0.76^{c}	0.96^{a}	0.86^{abc}	0.81^{bc}	0.78^{bc}	0.89^{ab}	0.002	0.031

Treatments: 1) Tomato pomace silage, 2) Pumpkin waste silage, 3) Tomato pomace and Pumpkin waste silage mix (50:50), 4) Tomato pomace and Pumpkin waste silage mix treated with Enzyme, 5) Tomato pomace and Pumpkin waste silage mix treated with LAB-made inoculants (LMI) and 6) Tomato pomace and Pumpkin waste silage mix treated with Enzyme + LMI. SEM: Standard error of the mean. The means within a row without common letter differ ($p<0.05$)

Gas production parameters

The effect of using bacterial and enzymatic additives on the parameters of gas production of tomato pomace and pumpkin waste silage is shown in Table 2. The results showed that tomato pomace and pumpkin waste silages had the lowest and the highest gas production potential, respectively. However, combination of pumpkin waste and tomato pomace silage (284.5 ml/g DM) had higher and lower gas production potential than tomato pomace silage (276.1 ml/g DM) and the pumpkin waste silage (342.2 ml/g DM), respectively. The gas production potential of silages containing bacterial and enzymatic additives was not significantly different from tomato pomace and pumpkin waste silage. In contrast, the use of enzymatic and bacterial additives combination caused a significant increase in gas production potential ($P<0.05$). Dry matter digestibility, metabolizable energy, and concentration of short-chain fatty acids in the treatment with enzymatic-bacterial additive were higher compared to tomato pomace and pumpkin waste silages.

In this study, a mixture of tomato pomace and pumpkin waste improved gas production potential and organic matter digestibility. However, the use of enzymatic and bacterial additives alone in the silage of a mixture of tomato pomace and pumpkin waste did not improve gas production parameters, which is consistent with the other results (Radigius et al. 2001). However, many studies have reported an increase in gas production as a result of the use of enzymes (Colombatto et al. 2004) and bacterial additives (Haghparvar et al. 2012). However, the combined use of enzymes and bacterial additives significantly improved the gas production potential and gas fermentation parameters compared to other mixed silages of tomato pulp and pumpkin waste.

Theoretically, cellulase or hemicellulase enzymes can break down lignocellulosic bonds and thus provide more of these substances to lactic acid-producing bacteria (Tang et al. 2000; Colombatto 2000). Lactic acid bacteria cannot use complex carbohydrates as an energy source to make lactic acid as they do not have the enzymes needed to hydrolyze the cell wall (Eun and Beauchemin 2007). In silo conditions, enzymes are also released through cell disruption during forage chopping, thereby breaking down complex carbohydrates to provide soluble sugars to lactic acid bacteria (Rotz and Muck 1994). Bacterial additives also aim to create

a dominant population in the population of silage microorganisms. Perhaps the failure of using enzymatic and bacterial additives alone and a significant increase in gas production potential in treatments with both additives can be attributed to the synergistic effect of the two. Some researchers have reported the interaction between forage and enzymes, the explanation of which has not been explained by its biological mechanism (Mendoza et al. 2014).

In treatments with additives, the rate of drop in pH was higher than in other treatments. Therefore, it can be said that hydrolytic enzymes in mini-silos have less opportunity to hydrolyze the fiber, so more structural carbohydrates remain intact, which can affect the amount of gas production. Inoculation of fibrolytic enzymes and lactic acid bacteria destroys the cell wall but does not always improve silage digestibility *in vitro*. It has been explained that the addition of fibrolytic enzymes may additionally reduce the digestible portions of structural polysaccharides (Nadeau et al. 2000; Dehghani et al. 2012).

Aerobic stability

In the Fig. 1, silages treated with enzymatic additive and enzymatic and bacterial mixtures significantly increased aerobic stability compared to other treatments. The lowest value of aerobic stability was observed in the treatment of pumpkin waste silage. Bacterial additive silage did not differ significantly from tomato pomace silage and tomato pomace and pumpkin waste silage. In terms of pH changes during exposure to air, the lowest increase in pH was observed in the silage of a mixture of tomato pulp and pumpkin waste.

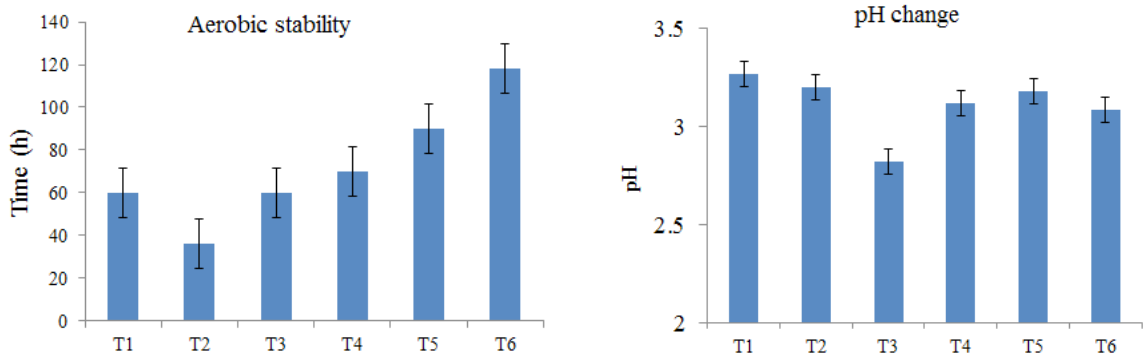

Fig. 1 Effect of additives on Aerobic stability of Tomato pomace and Pumpkin waste silage at several days after ensiling

Treatments: 1) Tomato pomace silage control (untreated); 2) Pumpkin waste silage, 3) Tomato pomace and Pumpkin waste silage mix (50:50), 4) Tomato pomace and Pumpkin waste silage mix treated with Enzyme, 5) Tomato pomace and Pumpkin waste silage mix treated with lab-made inoculants (LMI) and 6) Tomato pomace and Pumpkin waste silage mix treated with Enzyme + LMI.

When these silages are exposed to the air, fungi and molds use lactic acid as an energy source and break down lactic acid, causing it to grow. Fungi and molds also grow as lactic acid breaks down. As the amplitude of decomposition increases, the temperature of the silo also increases, and a favorable environment is provided for the growth of *Clostridia* (Valizadeh et al. 2007). Butyric acid and acetic acid have an antimycotoxin role and prevent the growth of adverse microorganisms (Aksu et al. 2006). Bacterial additives increase the amount of lactic acid and decrease the amount of acetic acid in silage (Hassanat et al. 2007). It has been suggested that due to the use of bacterial additives, after opening the doors of the silos, the acidity increases, and fungi and molds start to grow and produce carbon dioxide. As a result, the temperature of the silo increases. Bacterial additives produce lactic acid only under anaerobic conditions. While under normal fermentation conditions, various volatile fatty acids such as propionic acid, acetic acid, and butyric acid are also produced. These acids have antimycotoxin properties and prevent the growth of fungi and molds under aerobic conditions. Researchers showed that the use of bacterial additives increases the concentration of lactic acid and decreases acetic and butyric acid (Kung et al. 2004).

However, in this study, bacterial additive silage did not have a significant effect on aerobic stability. In some studies (Kung and Ranjit 2001), the use of bac-

terial additives may reduce aerobic stability during the exit of silage from the silo. The application of bacterial additives has been shown to increase waste on the dry matter in corn silage (Kung et al. 2004). The pervious study showed that the application of homofermentative bacterial additives increases the population of yeasts and fungi in corn and sorghum silages (Filya 2003). It has been suggested that silages with higher lactic acid concentrations decompose faster than silages with low lactic acid concentrations. Homofermentative bacterial additives increase the rate of lactic fermentation. While heterogeneous fermentation additives cause more aerobic stability of silage (Kung and Ranjit 2001). It is hypothesized that high levels of soluble carbohydrates combined with lactic acid, as well as unprotected volatile fatty acids in bacterial additive-treated silages, are directly associated with increased spoilage and decreased aerobic stability. Because both soluble carbohydrates and lactic acid are initial materials for the growth of fungi and molds (Adesogan et al. 2004). The results showed that butyric acid increases the aerobic stability of silage. Butyric acid has stronger antimycotoxin properties than acetic acid. The presence of these two acids in silage increases aerobic stability (Adesogan et al. 2004).

Conclusion

The results of this study showed that the use of different additives (fibrolytic enzyme and bacterial additive produced in the laboratory) separately and together improved some properties of silage mixture of tomato pomace and pumpkin waste silage. In treatments with additives, the rate of decrease in pH was significant compared to other treatments. In terms of gas production parameters, the combined use of both additives caused the highest amount of gas production potential. The use of bacterial and enzymatic additives alone and in combination caused a significant improvement in the aerobic stability of a mixture of tomato pomace and pumpkin silage. However, it seems that although the use of these additives has been able to improve some of the properties of silage, performance trials are needed to determine the effect of using these additives *in vivo* conditions.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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