FTB Food Technology & Biotechnology

original scientific paper
ISSN 1330-9862
doi: 10.17113/ftb.56.01.18.5455

Effects of Formic or Acetic Acid on the Storage Quality of Mixed Air-Dried Corn Stover and Cabbage Waste, and Microbial Community Analysis

Haiwei Ren[1,2]*, Cong Wang[1], Wenguang Fan[1], Bingyun Zhang[1], Zhizhong Li[1] and Dong Li[3]

[1]School of Life Science and Engineering, Lanzhou University of Technology, 287 Langongping Road, 730050 Lanzhou, PR China
[2]Key Laboratory of Complementary Energy System of Biomass and Solar Energy, Gansu Province, 287 Langongping Road, 730050 Lanzhou, PR China
[3]Key Laboratory of Environmental and Applied Microbiology, Environmental Microbiology Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Science, No. 9, Section 4, Renmin South Road, 610041 Chengdu, PR China

Received: July 30, 2017
Accepted: February 1, 2018

***Corresponding author:**
Phone: +869312976385;
Fax: +869312973367;
E-mail: rhw52571119@163.com

ORCID IDs: 0000-0001-6745-8727 (Ren), 0000-0001-9408-7845 (Wang), 0000-0002-4200-606X (Fan), 0000-0002-2905-362X (Zhang), 0000-0001-8157-4783 (Li, Z), 0000-0002-8581-8658 (Li, D)

Paper was presented at the 7th International Forum on Industrial Bioprocessing - IFIBiop 2017, May 21-24, 2017, Wuxi, PR China

SUMMARY

A mixture of air-dried corn stover and cabbage waste was ensiled to preserve lignocellulosic biomass for use as biofuel. Furthermore, the effects of different fresh mass fractions (0.3 and 0.6 %) of formic or acetic acid on the mixed silage quality were evaluated to guarantee its quality. The application of formic or acetic acid prior to mixing the silage led to higher water-soluble carbohydrate fractions than the negative control, indicating that both acids contributed to preservation of water-soluble carbohydrates during storage for 170 days. The dry matter content was also increased after storage from 90 to 170 days. It was found that the content of neutral and acid detergent fibre, cellulose and holocellulose (the sum of cellulose and hemicellulose) in mixed silage treated with formic or acetic acid was significantly lower than that obtained in the negative control. The pH and the ratio of ammoniacal nitrogen to total nitrogen in mixed silage treated with acetic acid also significantly decreased. Furthermore, the addition of formic or acetic acid significantly weakened the fermentation intensity of lactic acid, depending on the ratio of lactic to acetic acid, as well as the ratio of lactic acid to total organic acids. The number of bacterial species and their relative abundance shifted during silage mixing, wherein microbial communities at phylum level mainly consisted of Proteobacteria and Firmicutes. The dominant bacteria were also observed to shift from *Lactobacillus* and *Enterobacter* in presilage biomass to *Lactobacillus* and *Paralactobacillus*. Specifically, *Enterobacter* disappeared after 130 days of storage. In conclusion, the addition of a low dose of acetic acid to fresh mass (0.3 %) could effectively improve the fermentation quality and is conducive to the preservation of the organic components.

Key words: air-dried corn stover, cabbage waste, mixed ensiling, formic and acetic acids, fermentation quality, microbial community diversity

INTRODUCTION

Growing energy consumption and diminishing fossil fuel supplies have led to increased research on renewable bioenergy, such as biogas and bioethanol (*1*). Lignocellulosic biomass, such as energy crops (*e.g.* prairie grasses), agricultural residues (*e.g.* corn stover, wheat straw), forestry products (*e.g.* poplar) and food processing byproducts (*e.g.* sugar cane bagasse), represents the most optimal candidate as a sustainable future energy or fuel supply (*2*). However, it is not realistic to produce bioenergy from fresh green biomass without a storage facility due to their seasonal dependence and discontinuous availability. Thus, fresh biomass needs to be preserved and stored to keep it available throughout the year for continuous utilization and minimization of losses whilst maintaining good final quality and high energy conversion value. Dry and wet storage are two common approaches to preserving lignocellulosic biomass. Generally, the dry storage method includes sun drying, hot air circulation drying and mechanical ventilation (*3,4*). Compared with dry storage, wet storage is a promising alternative, providing several advantages including lower energy costs, lower dry matter losses and fire risk, and high harvest efficiency, which is conducive to the improvement of the biological digestibility for biorefinery utilization after storage (*5-9*). Silage, a representative wet

storage, can preserve valuable moisture and cellular biomass components such as carbohydrates, which makes the substrate available for bioenergy production all year long, independent of harvest time (*10*).

China is an agricultural country with abundant crop residues, with up to 320 million metric tonnes of corn (*Zea mays* L.) produced in 2016, which is expected to climb further in the following years (*11*). Corn stover represents a substantial source of lignocellulosic biomass and is the prevailing material producing bioenergy because of abundant cellulose and hemicellulose (*12*). In the majority of cases, corn stover collected after harvest is available once a year, which requires preservation and storage to ensure a year-round continuous supply. Thus, to maintain a sustainable supply of corn stover with consistent quality, silage presents an attractive option (*3*). Microbiologically, silage represents a wet preservation method for maintaining nutrient components of moist crops through the activity of lactic acid bacteria (LAB), which convert water-soluble carbohydrates into organic acids, resulting in low pH range of 4.0-4.5, effectively inhibiting the growth of undesirable bacteria (*e.g. Clostridia* and *Enterobacteria*) (*13*). Generally, corn stover is wilted and dried to yellow stalks and collected after corn harvest in the autumn. As a result, the free sugars in air-dried corn stover are not readily available, as the moisture is almost entirely evaporated during wilting and field air drying, which limits silage due to low water-soluble carbohydrate and high lignocellulosic contents (*14*). To overcome this limitation, addition of vegetable wastes as a source of water-soluble carbohydrates and moisture to initiate fermentation has been used to complement bulk air-dried corn stover on the basis of physical structure, nutrient and moisture contents (*11*). Nevertheless, the lignified nature and associated recalcitrance of air-dried corn stover, in combination with both insufficient numbers of epiphytic microflora and low moisture or water-soluble carbohydrate content, could result in a failure of the start-up of silage fermentation, or at least a delay in the initial pH drop. Thus, the prospect of application of silage additives to the mixture of vegetable wastes and air-dried corn stover represents a potential approach to ensure a quick decrease in pH, while potentially improving silage conservation with minimal fermentation losses.

Furthermore, a variety of compounds (*e.g.* biological, nutrient, acid, feed ingredients and byproducts) have been used as silage additives, as has been reported in detail in the literature. Extensive research over recent years has explored the potential of using organic acids (*e.g.* formic, acetic, malic, citric and succinic acids) as options to improve silage performance. Ke *et al*. (*15*) found that including malic or citric acid to alfalfa silage effectively improved fermentation characteristics, while limiting proteolysis. Aksu *et al*. (*16*) found that application of formic acid, molasses and inoculant additives can increase the lactic acid content in ensiled material. Interestingly, acetic acid has been proven by Danner *et al*. (*17*) to be the sole substance responsible for increased aerobic stability, and that acetic acid acts as an inhibitor of spoilage microorganisms. The findings of Schmidt and Kung Jr (*18*) showed that addition of acetic acid could also effectively reduce silage pH and prevent its spoilage. Moreover, Li *et al*. (*19*) found that formic acid yielded higher quality of alfalfa silage if ensiled before killing frost.

Currently, very little information is reported on ensiling of air-dried corn stover mixed with cabbage wastes, which would improve trans-seasonal preservation of this feedstock for biorefinery activities. Furthermore, little information is available regarding the application of formic or acetic acid in mixed air-dried corn stover and cabbage waste silages, and whether these organic acids have positive effects on promoting the fermentation quality is not clear. In view of this, and the fact that the preliminary experiments (*11*) were undertaken in laboratory-scale tank of 1.5 L, the objective of the present study is to dynamically investigate the effects of different mass fractions of formic or acetic acid on the fermentation characteristics of mixed silages for 170 days at a moderate scale of 50 L, taking into account the chemical composition, fermentation quality and microbial community structures to evaluate the efficacy of additives.

MATERIALS AND METHODS

Feedstock

Air-dried corn stover was collected post-harvest from Yuzhong county (Lanzhou, PR China). The moisture content of air-dried corn stover chopped to lengths of 15-20 mm on fresh mass basis was 10.23 %. The cabbage waste was collected from the Qilihe vegetable market (Lanzhou, PR China).

Mixed air-dried corn stover and cabbage waste silage

A mass of 3.23 kg of air-dried corn stover and 9.87 kg of cabbage waste were prepared and mixed in 50-litre laboratory-scale tank, then sealed in soft plastic container (Minguan New Energy Technology Co. Ltd, Chendu, PR China), respectively. The mixed silage treatments were applied as follows: (*i*) without any additives (negative control group), (*ii*) low dose of formic acid (0.3 %), (*iii*) high dose of formic acid (0.6 %), (*iv*) low dose of acetic acid (0.3 %), and (*v*) high dose of acetic acid (0.6 %). The negative control was prepared by adding only water, corresponding to a moisture content on fresh mass basis of 73 % consistent in all five groups, and all treatments were stored at (18±1) °C for 170 days. There were three silage replicates per treatment and 105 silage bags were used. Bags were opened 30, 60, 90, 130 and 170 days after the start of the experiment for analysis of the chemical composition, fermentation quality and microbial community diversity.

Chemical analysis

Representative samples of silage after varied intervals were oven-dried and ground through a 1-mm screen prior to chemical analysis. Dry matter content was determined by oven drying the material at 105 °C (Keweiyongxing Instrument Co. Ltd, Beijing, PR China) to constant mass, and subsequently ground in a cutting mill (Kanghe Machinery Manufacturing Co. Ltd, Jiangyin, PR China) for further analysis.

Water-soluble carbohydrates were determined by the anthrone-sulfuric acid method (*20*). The neutral and acid detergent fibre and acid detergent lignin content were determined according to the Van Soest method (*21*). Hemicellulose content was calculated as the difference between neutral and acid detergent fibre, while cellulose content was determined as the difference between acid detergent fibre and acid detergent lignin. Total nitrogen content was determined by the Kjeldahl method according to Tangka (*22*). The crude protein content was calculated as nitrogen mass fractions multiplied by conversion constant (6.25) (*11*).

Fermentation quality analysis

The silage extract was prepared by mixing 25 g of various silage samples with 225 mL of distilled water in a homogenizer (JJ-2; Guohua Instruments Co. Ltd, Suzhou, PR China). The slurry mixture was then filtered through four layers of cheesecloth, and the filtrate was used for determination of pH, ammoniacal nitrogen, lactic acid and volatile fatty acid contents. The pH was measured immediately using a pH meter (UB-10; Denver Instruments Co. Ltd, Beijing, PR China). Part of the volume was centrifuged (H2500R; Xiangyi Instrument Co. Ltd, Changsha, PR China) at 3000×*g* for 20 min at 4 °C and the supernatant was filtered through a 0.22-μm syringe filter (MET Laboratories Inc., Baltimore, MD, USA) and used to quantify lactic acid and volatile fatty acids. Lactic acid was determined using a biosensor (SBA--40X; Bosheng Biotechnology, Jinan, PR China), and 0.4 μL of samples were analyzed to determine ethanol and volatile fatty acid contents using gas chromatograph (GC-2014C; Shimadzu, Kyoto, Japan), which was equipped with a pillar KB-Wax and flame ionization detector. The temperatures of sample injector, detector and column were set at 250, 250 and 130 °C, respectively, and nitrogen gas was used as the carrier gas. All of the chemical analyses were conducted in triplicate, and the results were expressed on dry mass basis, except for dry mass content (expressed as % on fresh mass basis) and ammoniacal nitrogen (expressed as % of total nitrogen) (*11*).

Microbial community analysis

The silages were randomly subsampled from various layers and then mixed to generate a homogeneous sample for microbial community analysis. High-throughput 16S rDNA sequencing technology was adopted to analyze the changes of population structure during ensiling. Total DNA was extracted using Water DNA isolation kit (FuJi Biotechnology Co. Ltd, Chengdu, PR China) according to the manufacturer's instructions. For sequencing, the 16S rRNA genes were amplified with primers 338F (5-ACTCCTACGGGAGGCAGCA-3) and 806R (5-GGACTACHVGGGTW TCTAAT-3) targeting the bacterial and archaeal V3-V4 region in the 16S rRNA gene with a 10-mer barcode at the 50 end of primer 338F. To minimize PCR bias, two PCR reactions were set up for each sample, and the PCR products in the replicate reactions were pooled (*13*). The amplicons from each sample were pooled with an equimolar concentration and sequenced with the Illumina MiSeq platform. The details of PCR and related procedures were described previously by Li *et al.* (*13*). Sequencing reads with primer sequences were removed.

Statistical analysis

Data analysis from each ensiling period was performed using SPSS v. 20 software (*23*), and one-way analysis of variance (ANOVA) with Tukey's multiple comparison test at a 0.05 % significant level was used to determine the effects of additives.

RESULTS AND DISCUSSION

Analysis of air-dried corn stover and cabbage waste

The chemical compositions of air-dried corn stover, cabbage waste and their mixture before storage are shown (**Table 1**). The content of dry matter in air-dried corn stover was found to be 89.8 %, while neutral detergent fibre, acid detergent fibre and acid detergent lignin contents in air-dried corn stover were 76.5, 46.5 and 7.6 %, respectively. The air-dried corn stover used in this study contained approx. 39.0 % cellulose and 30.0 % hemicellulose, which represent biodegradable polysaccharides that can be converted into bioenergy through biological conversion. It is noteworthy that the moisture content in air-dried corn stover was merely 10.2 %, which is relatively low. Furthermore, the content of water-soluble carbohydrates was 8.1 %, which was slightly less than the typical values (*i.e.* 8-10 %), due to free sugar consumption and water evaporation during wilting and field drying (*24*). The moisture and water-soluble carbohydrate content in cabbage waste were 91.4 and 14.6 %, respectively, which were both significantly higher than those of air-dried corn stover. Therefore, the trans-seasonal mixed storage of air-dried corn stover and cabbage waste was feasible on the basis of strong complementarity in terms of nutrient constituents and moisture content.

Table 1. Chemical compositions of pre-ensiled air-dried corn stover and cabbage waste

Material	*w*(compound)/%							
	DM	WSC	NDF	ADF	ADL	CL	HC	HoC
ADCS	89.8	8.1	76.5	46.5	7.6	39.0	30.0	68.9
CW	8.6	14.6	36.3	33.8	16.2	17.6	2.4	20.0
ADCS+CW	28.8	9.5	67.7	44.0	10.0	34.1	23.7	27.7

ADCS=air-dried corn stover, CW=cabbage waste, DM=dry matter, WSC=water-soluble carbohydrates, NDF=neutral detergent fibre, ADF=acid detergent fibre, ADL=acid detergent lignin, CL=cellulose, HC=hemicellulose, HoC=holocellulose. DM was determined on fresh and all other components on dry mass basis

Fig. 1 shows that the relative abundance of undesirable microorganisms or non-lactic acid bacteria in cabbage waste was about 99.1 %, mainly including *Pseudomonas* (48.4 %), *Pantoea* (17.1 %), *Flavobacterium* (16.3 %), *Yersinia* (6.5 %), *Enterobacter* (3.6 %) and *Exiguobacterium* (0.04 %), which indicated that LAB were scarcely present in pre-ensiled cabbage waste. Once discarded and exposed to outdoor weathering, cabbage waste is perishable and causes heavy odour and leachate, and thus utilization of this material is essential for environmental protection. With respect to the relative abundance of LAB in air-dried corn stover, nearly 29.8 % of the community was comprised mainly of *Carnobacterium*, *Enterococcus* and *Lactococcus*. Thus, while cabbage waste brings nutrients and moisture, air-dried corn stover contributes LAB, underscoring the complementarity of both pre-ensiled materials. These results are supported by Li and Nishino (*25*), who reported that *Pseudomonas*, *Lactobacillus* and *Rahnella* are often found in pre-ensiled wilted Italian ryegrass crops. Heron *et al*. (*26*) also reported that *Erwinia* and *Rahnella* often dominate in fresh grass, but these genera were not discovered in air-dried corn stover and cabbage waste, indicating that bacterial populations may vary according to pre-ensiled material variety, geographic area, climate or growing stage.

Dynamic analysis of the content of organic chemical composition during 170 days of storage

As can be seen in **Table 2**, the dry matter content in negative control group, and treatments with formic (both high and low dosage) or acetic (high dosage) acid showed a decreasing trend. However, with the addition of low dosage of acetic acid the dry matter was observed to increase first and then decrease. Smaller dry matter reduction than in the control indicated that the addition of formic or acetic acid was beneficial for preserving dry matter, especially during 90-170 days of storage. Furthermore, it is well-known that high water-soluble carbohydrate content is desirable for the silage process, as it provides readily accessible substrates for bacteria to produce lactic acid and other organic acids, which can reduce the pH of silage and facilitate the preservation of feedstock (*27*). When the period of ensiling was extended, the water-soluble carbohydrate content in all five groups was found to significantly ($p<0.05$) decrease, compared to their content before storage. However, the water-soluble carbohydrate content increased significantly ($p<0.05$) after addition of formic or acetic acid compared with the negative control group during storage (except the silage treated with low dose of formic acid on day 170), which is in agreement with the results found by Li *et al.* (*19*), who indicated that application of formic or acetic acid results in increased water-soluble carbohydrate and crude protein contents. On the other hand, substantial changes in the amounts of structural carbohydrates occurred during ensiling. The contents of dry matter and structural carbohydrates were found to be increased to some extent, which was congruent with the results reported by Van Ranst *et al*. (*28*). In previous work it was concluded that the content of dry matter and structural carbohydrates tended to decrease with storage duration (*6*); however, this trend was inconsistent among various silage types.

The air-dried corn stover before and after ensiling showed distinct differences in their chemical compositions. With time, the holocellulose content increased in negative control group and treatments with formic acid (both high and low dosage), but it first increased and then decreased in silages treated with acetic acid (both high and low dosage). Holocellulose content during ensiling did not increase in silages treated with acetic or formic acid. Instead, the holocellulose content in the majority of treatments decreased to different extents. A similar trend was found for the cellulose content in silages treated with acetic or formic acid. However, the holocellulose content was well preserved in silage treated with acetic acid compared with negative control group during storage for 170 days. The acid detergent lignin content in silages treated with acetic or formic acid was shown to have an increasing tendency compared with the negative control. The addition of formic acid likely contributed to the decrease of neutral detergent fibre and the addition of acetic acid was conducive to the decrease of acid detergent fibre. Pakarinen *et al*. (*9*) studied the chemical composition of whole crop corn before and after 4- and 8-month ensiling, and

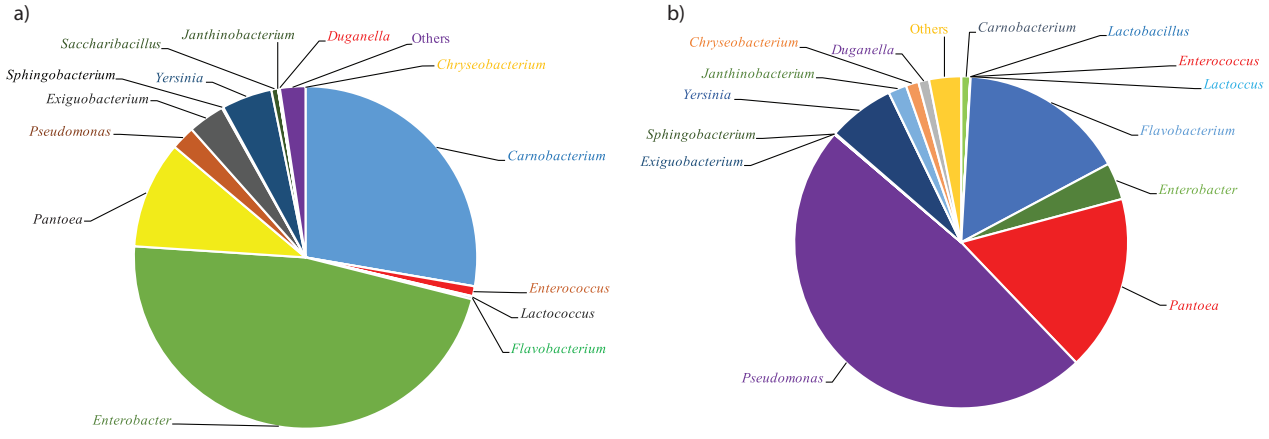

Fig. 1. Relative abundance of microbial community of pre-ensiled: a) air-dried corn stover and b) cabbage waste at the genus level

Table 2. Effect of formic or acetic acid on the chemical compositions of mixed silage of air-dried corn stover and cabbage waste before and after ensiling

Treatment	*t*/day	*w*(compound)/%							
		DM	WSC	NDF	ADF	ADL	CL	HC	HoC
ME	0	(28.83±0.01)^{Aa}	(9.53±0.20)^{Aa}	(67.67±0.39)^{Da}	(44.01±1.09)^{Ca}	(9.95±0.21)^{Ba}	(34.06±1.08)^{Da}	(23.66±0.74)^{Ca}	(57.72±0.34)^{Da}
	30	(28.60±0.01)^{Ba}	(1.94±0.05)^{BCe}	(71.34±0.14)^{Ca}	(44.54±0.43)^{BCa}	(5.88±0.26)^{Ed}	(38.66±0.69)^{Ca}	(26.79±0.56)^{Aa}	(65.45±0.14)^{Ba}
	60	(28.38±0.01)^{Cb}	(2.12±0.05)^{Bd}	(71.13±0.25)^{Cc}	(45.49±0.09)^{Ba}	(10.98±0.85)^{Aa}	(34.51±0.90)^{Dc}	(25.64±0.22)^{Bd}	(60.15±0.77)^{Cd}
	90	(26.53±0.01)^{De}	(2.06±0.16)^{BCd}	(74.52±0.15)^{Aa}	(47.40±0.36)^{Aa}	(4.65±0.07)^{Fd}	(42.75±0.33)^{Aa}	(27.12±0.48)^{Ab}	(69.88±0.22)^{Aa}
	130	(23.35±0.01)^{Ee}	(1.04±0.09)^{De}	(71.67±0.01)^{Ca}	(44.78±0.04)^{BCb}	(7.07±0.35)^{Dc}	37.71±0.39)^{Ca}	(26.89±0.04)^{Ab}	(64.60±0.36)^{Ba}
	170	(22.66±0.01)^{Fe}	(1.88±0.09)^{Cd}	(72.82±0.53)^{Bb}	(48.04±0.53)^{Aa}	(8.03±0.07)^{Cb}	(40.02±0.46)^{Ba}	(24.78±1.06)^{Bc}	(64.79±0.59)^{Ba}
FA	0	(28.83±0.01)^{Aa}	(9.53±0.20)^{Aa}	(67.67±0.39)^{Ea}	(44.01±1.09)^{Ca}	(9.95±0.21)^{Ba}	(34.06±1.08)^{Ca}	(23.66±0.74)^{Da}	(57.72±0.34)^{Ea}
	30	(28.03±0.01)^{Bc}	(6.53±0.09)^{Bab}	(69.41±0.35)^{Dc}	(42.68±0.17)^{Db}	(6.01±0.07)^{Dd}	(36.67±0.24)^{Bc}	(26.73±0.52)^{Ba}	(63.40±0.29)^{Bc}
	60	(27.42±0.01)^{Cd}	(4.08±0.14)^{Db}	(67.98±0.37)^{Ed}	(43.66±0.30)^{Cb}	(5.29±0.23)^{Ec}	(38.36±0.15)^{Aa}	(24.32±0.06)^{CDe}	(62.69±0.20)^{BCc}
	90	(26.62±0.01)^{Fd}	(3.71±0.16)^{Eab}	(72.25±0.12)^{Bd}	(44.99±0.16)^{ABd}	(10.89±0.29)^{Ab}	(34.10±0.40)^{Cd}	(27.26±0.37)^{Bb}	(61.36±0.37)^{Dc}
	130	(27.11±0.01)^{Da}	(4.43±0.14)^{Ca}	(70.57±0.37)^{Cc}	(45.82±0.25)^{Aa}	(8.51±0.39)^{Cb}	(37.31±0.47)^{Ba}	(24.75±0.50)^{Cc}	(62.06±0.74)^{CDbc}
	170	(26.67±0.01)^{Ec}	(1.32±0.16)^{Fe}	(74.08±0.89)^{Aa}	(44.92±0.16)^{Bd}	(8.17±0.15)^{Cb}	(36.75±0.01)^{Bc}	(29.17±0.73)^{Aab}	(65.92±0.74)^{Aa}
FB	0	(28.83±0.01)^{Aa}	(9.53±0.20)^{Aa}	(67.67±0.39)^{Ea}	(44.01±1.09)^{Ba}	(9.95±0.21)^{ABa}	(34.06±1.08)^{Ba}	(23.66±0.74)^{Ca}	(57.72±0.34)^{Ca}
	30	(28.29±0.01)^{Bb}	(6.62±0.43)^{Ba}	(69.90±0.31)^{Cb}	(44.36±0.22)^{Ba}	(9.02±0.19)^{BCb}	(35.34±0.19)^{Bd}	(25.54±0.09)^{Bc}	(60.88±0.27)^{Bd}
	60	(27.71±0.01)^{Cc}	(2.34±0.00)^{Dc}	(68.57±0.04)^{Dd}	(40.95±0.39)^{Cc}	(6.72±1.35)^{Db}	(34.23±1.55)^{Bc}	(27.62±0.36)^{Ac}	(61.85±1.33)^{Bc}
	90	(27.18±0.01)^{Dc}	(3.89±0.11)^{Ca}	(72.09±0.18)^{Bd}	(46.31±0.06)^{Ac}	(10.77±0.21)^{Ab}	(35.55±0.27)^{Bc}	(25.78±0.12)^{Bc}	61.32 (±0.39)^{Bc}
	130	(26.19±0.01)^{Fd}	(1.50±0.09)^{Ed}	(70.06±0.03)^{Cd}	(46.04±0.16)^{Aa}	(8.33±0.49)^{Cb}	(37.70±0.64)^{Aa}	(24.03±0.19)^{Cd}	(61.73±0.47)^{Bbc}
	170	(26.56±0.01)^{Ed}	(2.12±0.14)^{Dc}	(72.70±0.20)^{Ab}	(46.76±0.15)^{Ab}	(9.52±0.34)^{Ba}	(37.24±0.36)^{Abc}	(25.94±0.24)^{Bc}	(61.17±0.54)^{Ab}
AA	0	(28.83±0.01)^{Ba}	(9.53±0.20^{Aa}a	(67.66±0.39)^{Ea}	(44.01±1.09)^{BCa}	(9.95±0.21)^{Aa}	(34.06±1.08)^{CDa}	(23.66±0.74)^{Ea}	(57.72±0.34)^{Fa}
	30	(27.46±0.01)^{Dd}	(3.74±0.19)^{Cd}	(68.67±0.02)^{Dd}	(42.96±0.20)^{Db}	(9.44±0.17)^{ABa}	(33.52±0.48)^{De}	(25.72±0.18)^{Dbc}	(59.24±0.30)^{Ee}
	60	(31.56±0.01)^{Aa}	(4.20±0.09)^{Bb}	(72.82±0.51)^{Bb}	(41.11±0.42)^{Ec}	(4.51±0.30)^{Dc}	(36.61±0.12)^{Bb}	(31.70±0.47)^{Ab}	(68.31±0.44)^{Aa}
	90	(28.06±0.11)^{Ca}	(2.62±0.00)^{Ec}	(74.23±0.10)^{Ab}	(46.81±0.13)^{Ab}	(7.27±0.06)^{Cc}	(39.54±0.07)^{Ab}	(27.42±0.05)^{Cb}	(66.96±0.04)^{Bb}
	130	(26.44±0.01)^{Fb}	(3.02±0.19)^{Dc}	(71.21±0.09)^{Cb}	(44.77±0.15)^{Bb}	(9.80±0.50)^{Aa}	(34.97±0.35)^{Cb}	(26.44±0.06)^{Db}	(61.41±0.41)^{Dc}
	170	(26.84±0.01)^{Eb}	(3.61±0.19)^{Cb}	(72.10±0.87)^{Bb}	(43.58±0.32)^{CDe}	(9.28±0.05)^{Ba}	(34.30±0.28)^{CDd}	(28.53±0.63)^{Bab}	(62.83±0.82)^{Cb}
AB	0	(28.83±0.01)^{Aa}	(9.53±0.20)^{Aa}	(67.66±0.39)^{Ca}	(44.01±1.09)^{BCa}	(9.95±0.21)^{Ba}	(34.06±1.08)^{BCa}	(23.66±0.74)^{Ea}	(57.72±0.34)^{Fa}
	30	(25.38±0.01)^{Fe}	(5.35±0.14)^{Bc}	(71.00±0.27)^{Ba}	(44.71±0.50)^{Ba}	(7.12±0.06)^{Dc}	(37.59±0.57)^{Ab}	(26.29±0.47)^{Dab}	(63.88±0.30)^{Cb}
	60	(26.07±0.01)^{Ee}	(4.79±0.14)^{Ca}	(73.78±0.28)^{Aa}	(40.56±0.56)^{Dc}	(6.86±0.30)^{Db}	(33.70±0.86)^{Cc}	(33.23±0.59)^{Aa}	(66.92±0.45)^{Ab}
	90	(27.37±0.01)^{Bb}	(3.58±0.14)^{Eb}	(73.87±0.14)^{Ac}	(45.05±0.24)^{ABd}	(14.61±0.04)^{Aa}	(30.43±0.20)^{De}	(28.82±0.38)^{Ba}	(59.26±0.18)^{Ed}
	130	(26.24±0.01)^{Dc}	(4.08±0.23)^{Db}	(71.23±0.30)^{Bb}	(43.59±0.17)^{Cc}	(8.64±0.41)^{Cb}	(34.95±0.24)^{Bb}	(27.64±0.35)^{Ca}	(62.59±0.52)^{Db}
	170	(27.16±0.01)^{Ca}	(3.95±0.11)^{Da}	(74.11±0.07)^{Aa}	(45.82±0.34)^{Ac}	(8.28±0.71)^{Cb}	(37.54±0.37)^{Ab}	(28.29±0.26)^{BCb}	(65.84±0.64)^{Ba}

Data are expressed as mean value±standard deviation. Different capital letters in the same row show significant difference among different days in the same treatment at p=0.05 level. Different lower-case letters in the same row show significant difference among different treatments on the same days at p=0.05 level. DM=dry matter, WSC=water-soluble carbohydrates, NDF=neutral detergent fibre, ADF=acid detergent fibre, ADL=acid detergent lignin, CL=cellulose, HC=hemicellulose, HoC=holocellulose. ME=mixed silage of air-dried corn stover and cabbage waste without any additives (negative control group), FA=mixed silage with low dose of formic acid (0.3 %), FB=mixed silage with high dose of formic acid (0.6 %), AA=mixed silage with low dose of acetic acid (0.3 %), AB=mixed silage with high dose of acetic acid (0.6 %). DM was determined on fresh and all other components on dry mass basis

the results indicated that the water-soluble carbohydrate and cellulose contents increased with the addition of formic acid after 4 months, while the lignin content also increased regardless of the used additives. Weinberg and Chen (*14*) reported that the expected increase in neutral detergent fibre content occurred only with wheat silage from the milk stage, probably due to the acid hydrolysis of hemicelluloses, which kept neutral detergent fibre contents constant over time.

Dynamic analysis of the fermentation quality during 170 days of storage

The fermentation quality during 170 days of mixed ensiling, including the changes in pH, the contents of lactic, acetic, propionic and butyric acids, as well as the ratios of lactic to acetic acid and ammoniacal nitrogen to total nitrogen are shown in **Table 3**. The pH range of 3.7-4.2 is generally considered beneficial for the preservation of cereal forages (*29*). Over the extended storage period, the pH was observed to increase first and then subsequently decrease in negative control and treatments with a low dose of formic acid and high doses of formic and acetic acids, wherein the pH after treatment with acetic acid promptly dropped to less than 4.2 on day 30, and remained low until 170 days of ensiling, suggesting that the mixed silages with the addition of acetic acid were well preserved with significantly ($p<0.05$) lower pH values than that of control, resulting in growth inhibition or reduced survival of yeasts and moulds. It should be noted that no significant differences ($p>0.05$) with respect to the pH value after treatment with formic acid compared with the negative control were observed. On average, pH values were higher in mixed silages treated with formic acid than in those treated with acetic acid (4.21 *vs.* 4.01; $p<0.05$), and with the increase of formic acid mass fraction (*i.e.* from 0.3 to 0.6 %), the pH in ensiled forages increased accordingly. Earlier criteria for the effective preservation of ensiled crops included

Table 3. Effect of formic and acetic acids on the fermentation quality of mixed silage of air-dried corn stover and cabbage waste

Treatment	*t*/day	pH	*w*(compound)/%					LA/AA	LA/TOA	(AN/TN)/%
			LA	AA	PPA	BA	EA			
ME	30	$(4.15\pm0.01)^{Cc}$	$(3.78\pm0.04)^{Bb}$	$(0.77\pm0.02)^{Cc}$	$(0.09\pm0.01)^{ABa}$	$(0.25\pm0.03)^{Ba}$	$(0.71\pm0.04)^{Ac}$	4.91	0.77	$(2.1\pm0.2)^{Ab}$
	60	$(4.36\pm0.01)^{Bc}$	$(5.07\pm0.03)^{Aa}$	$(1.27\pm0.05)^{BCa}$	$(0.08\pm0.01)^{Ba}$	$(0.21\pm0.01)^{Cc}$	$(0.67\pm0.05)^{ABa}$	3.99	0.76	$(1.8\pm0.3)^{ABb}$
	90	$(4.43\pm0.01)^{Aa}$	$(3.05\pm0.02)^{Cc}$	$(1.45\pm0.08)^{Bbc}$	$(0.11\pm0.02)^{Aa}$	$(0.32\pm0.02)^{Aa}$	$(0.50\pm0.02)^{Cd}$	2.10	0.62	$(1.7\pm0.3)^{BCb}$
	130	$(3.95\pm0.01)^{Dd}$	$(2.51\pm0.02)^{Dc}$	$(1.11\pm0.06)^{BCd}$	$(0.10\pm0.00)^{ABbc}$	$(0.15\pm0.02)^{Dc}$	$(0.58\pm0.05)^{BCc}$	2.26	0.65	$(1.6\pm0.1)^{BCb}$
	170	$(3.93\pm0.01)^{Ed}$	$(3.77\pm0.01)^{Ba}$	$(2.1\pm0.6)^{Ad}$	$(0.01\pm0.00)^{Cd}$	$(0.01\pm0.00)^{Ee}$	$(0.56\pm0.09)^{Cd}$	1.83	0.64	$(1.34\pm0.09)^{Cb}$
FA	30	$(4.26\pm0.01)^{Ba}$	$(1.44\pm0.01)^{Ec}$	$(0.29\pm0.03)^{Ed}$	ND	$(0.15\pm0.03)^{Bc}$	$(0.36\pm0.04)^{Cd}$	4.97	0.77	$(2.0\pm0.3)^{Bb}$
	60	$(4.39\pm0.01)^{Ab}$	$(2.95\pm0.02)^{Bd}$	$(0.56\pm0.02)^{Dd}$	$(0.08\pm0.00)^{Ba}$	$(0.28\pm0.02)^{Ab}$	$(0.37\pm0.01)^{Cc}$	5.27	0.76	$(1.3\pm0.2)^{Cc}$
	90	$(4.06\pm0.01)^{Cc}$	$(2.20\pm0.01)^{Dd}$	$(1.04\pm0.07)^{Bd}$	$(0.08\pm0.01)^{Bb}$	$(0.13\pm0.01)^{Bc}$	$(0.60\pm0.07)^{Bc}$	2.12	0.64	$(3.0\pm0.6)^{Aa}$
	130	$(3.94\pm0.01)^{Ed}$	$(2.49\pm0.01)^{Cc}$	$(0.77\pm0.08)^{Cc}$	$(0.08\pm0.00)^{Bbc}$	$(0.12\pm0.02)^{BCcd}$	$(0.4\pm0.1)^{Cb}$	3.23	0.72	$(0.66\pm0.05)^{Dab}$
	170	$(4.00\pm0.01)^{Db}$	$(3.04\pm0.02)^{Ab}$	$(2.9\pm0.1)^{Ac}$	$(0.10\pm0.01)^{Ac}$	$(0.10\pm0.01)^{Cb}$	$(0.78\pm0.07)^{Ac}$	1.05	0.50	$(2.8\pm0.5)^{Aa}$
FB	30	$(4.21\pm0.01)^{Cb}$	$(0.48\pm0.01)^{Ed}$	$(0.21\pm0.01)^{Cd}$	ND	$(0.26\pm0.01)^{Ba}$	$(0.25\pm0.01)^{De}$	2.29	0.51	$(3.1\pm0.3)^{ABa}$
	60	$(4.58\pm0.01)^{Aa}$	$(1.62\pm0.02)^{Cd}$	$(0.76\pm0.01)^{Cc}$	$(0.08\pm0.00)^{Ca}$	$(0.33\pm0.03)^{Aa}$	$(0.5\pm0.1)^{Cb}$	2.13	0.58	$(2.1\pm0.1)^{Bab}$
	90	$(4.41\pm0.01)^{Bb}$	$(1.49\pm0.02)^{De}$	$(0.84\pm0.07)^{Ce}$	$(0.09\pm0.01)^{Cb}$	$(0.17\pm0.02)^{Cb}$	$(0.87\pm0.03)^{Bab}$	1.77	0.58	$(0.93\pm0.03)^{Dcd}$
	130	$(4.10\pm0.01)^{Eb}$	$(2.58\pm0.01)^{Bb}$	$(1.6\pm0.1)^{Bb}$	$(0.13\pm0.03)^{Ba}$	$(0.32\pm0.03)^{Aa}$	$(0.75\pm0.05)^{Ba}$	1.61	0.56	$(1.37\pm0.07)^{Cb}$
	170	$(4.13\pm0.01)^{Da}$	$(3.05\pm0.03)^{Ab}$	$(4.6\pm0.8)^{Aa}$	$(0.18\pm0.03)^{Ab}$	$(0.12\pm0.01)^{Da}$	$(1.22\pm0.08)^{Ab}$	0.66	0.38	$(2.7\pm0.4)^{Aa}$
AA	30	$(4.22\pm0.01)^{Ab}$	$(3.44\pm0.01)^{Bb}$	$(0.97\pm0.03)^{CDb}$	$(0.09\pm0.01)^{Ba}$	$(0.21\pm0.03)^{Bb}$	$(0.83\pm0.07)^{Bb}$	3.55	0.73	$(0.48\pm0.07)^{Cd}$
	60	$(4.09\pm0.01)^{Be}$	$(3.14\pm0.01)^{Cc}$	$(0.93\pm0.04)^{Db}$	$(0.08\pm0.01)^{Ba}$	$(0.20\pm0.00)^{Bc}$	$(0.32\pm0.01)^{Dc}$	3.38	0.72	$(1.3\pm0.1)^{Bc}$
	90	$(3.83\pm0.01)^{Ee}$	$(4.17\pm0.02)^{Aa}$	$(1.41\pm0.08)^{Bc}$	$(0.08\pm0.00)^{Bb}$	$(0.09\pm0.02)^{Cd}$	$(0.92\pm0.04)^{Aa}$	2.96	0.73	$(0.65\pm0.04)^{Cde}$
	130	$(3.97\pm0.01)^{Dc}$	$(2.04\pm0.03)^{Ed}$	$(1.15\pm0.01)^{Cc}$	$(0.09\pm0.00)^{Bbc}$	$(0.27\pm0.03)^{Ab}$	$(0.57\pm0.05)^{Cb}$	1.77	0.57	$(1.06\pm0.07)^{Bb}$
	170	$(4.01\pm0.01)^{Cb}$	$(3.02\pm0.01)^{Db}$	$(2.7\pm0.2)^{Abc}$	$(1.18\pm0.08)^{Aa}$	$(0.03\pm0.00)^{Dd}$	$(0.80\pm0.03)^{Bc}$	1.12	0.44	$(3.2\pm0.3)^{Aa}$
AB	30	$(3.90\pm0.01)^{Dd}$	$(3.90\pm0.02)^{Aa}$	$(1.4\pm0.1)^{Da}$	$(0.09\pm0.01)^{Ba}$	$(0.19\pm0.01)^{Bbc}$	$(1.31\pm0.04)^{Ba}$	2.87	0.70	$(1.3\pm0.1)^{Bc}$
	60	$(4.16\pm0.01)^{Ad}$	$(3.45\pm0.04)^{Bb}$	$(1.3\pm0.1)^{Da}$	$(0.09\pm0.01)^{Ba}$	$(0.27\pm0.02)^{Abc}$	$(0.72\pm0.07)^{Ca}$	2.70	0.68	$(1.2\pm0.2)^{Bc}$
	90	$(3.91\pm0.01)^{Dd}$	$(3.45\pm0.01)^{Bb}$	$(1.55\pm0.04)^{Ca}$	$(0.09\pm0.01)^{Bb}$	$(0.10\pm0.01)^{Cd}$	$(0.81\pm0.01)^{Cb}$	2.23	0.66	$(1.3\pm0.2)^{Bbc}$
	130	$(4.13\pm0.01)^{Ba}$	$(2.74\pm0.03)^{Ca}$	$(2.00\pm0.09)^{Ba}$	$(0.11\pm0.01)^{Aab}$	$(0.12\pm0.01)^{Ccd}$	$(0.74\pm0.06)^{Ca}$	1.37	0.55	$(1.12\pm0.06)^{Bb}$
	170	$(3.96\pm0.01)^{Cc}$	$(1.59\pm0.02)^{Dc}$	$(3.15\pm0.05)^{Ab}$	$(0.09\pm0.01)^{Bc}$	$(0.07\pm0.01)^{Dc}$	$(1.43\pm0.06)^{Aa}$	0.50	0.32	$(2.0\pm0.3)^{Ab}$

Data are expressed as mean value±standard deviation. Different capital letters in the same row show significant difference among different days in the same treatment at p=0.05 level. Different lower-case letters in the same row show significant difference among different treatments on the same days at p=0.05 level. AN/TN=the ratio of ammoniacal nitrogen to total nitrogen, LA=lactic acid, AA=acetic acid, PPA=propionic acid, BA=butyric acid, EA=ethanol, TOA=total organic acid, ME=mixed silage of air-dried corn stover and cabbage waste without any additives (negative control group), FA=mixed silage with low dose of formic acid (0.3 %), FB=mixed silage with high dose of formic acid (0.6 %), AA=mixed silage with low dose of acetic acid (0.3 %), AB=mixed silage with high dose of acetic acid (0.6 %), ND=not detected. All measurements were made on dry mass basis

a high content of lactic acid and a pH below 4.5 after the fermentation phase. The five types of mixed silages all had a lower pH in the range of 3.83-4.58, and were all below 4.5 except for the pH of the silage treated with high dose of formic acid (4.58). In well-preserved silage, LAB dominated the fermentation, rapidly producing the low pH conditions that help to preserve the silage. Lactic acid is the major organic acid responsible for decreasing silage pH because it has a lower dissociation constant (pK_a=3.86) (*30*). The changes in pH values were the result of changes in organic acid content. Considerable lactic acid content was obtained in the five types of mixed silages, wherein the lactic acid content in silage treated with acetic acid tended to be greater than that of negative control and silage treated with formic acid after 30, 90 and 130 days of storage, respectively, suggesting that the addition of acetic acid could ensure rapid and vigorous fermentation by promoting the production of lactic acid. Statistical analysis showed that the pH and lactic acid content were significantly affected by the addition of acetic acid, but this was not the case with formic acid. The lactic acid content of the silages was influenced significantly by the dose of additives throughout the storage. Moreover, the lactic acid content of all mixed silages was higher than that (2.0 %) of good-quality silages (except for silages treated with a low dose of formic acid on day 30, high dose of formic acid on days 30-90, and high dose of acetic acid on day 170), which were all well preserved at low pH (*24*). With prolonged storage period, a trend of increased acetic acid content in the five types of mixed silages was observed.

Acetic acid is a short-chain fatty acid with substantial antifungal activity capable of reducing yeast and mould growth, and this is enhanced with decreasing pH value. The acetic acid content in silage treated with acetic acid was significantly greater ($p<0.05$) than that of negative control and silage treated with formic acid during storage for 30-130 days. Rapid decrease in the pH of the silage treated with acetic acid over time was partly due to the accumulation of acetic acid, especially in the silage treated with high dose of acetic acid. The propionic acid content in all silages after 170 days of storage was less than 10 %, which is in agreement with the findings of Levital *et al.* (*31*). Butyric acid is usually indicative of low-quality silage, and is produced by undesirable clostridial fermentations (*32*). Content of butyric acid in silage reported by Nkosi *et al.* (*33*) was lower than 1 %, representing well-preserved silage. In the work presented here, the butyric acid contents of five types of silages were all lower than 0.5 % during 170 days of storage, indicating good preservation. It has been reported that propionic acid is

produced from lactic acid, and the presence of butyric acid in silage is a sign of fermentation by undesirable microorganisms, which should be avoided at all costs (*34*). Furthermore, the ratio of lactic to acetic acid and the ratio of lactic to total organic acids in all five silages decreased with the prolonged storage, and the three silages (negative control and those treated with low dose of formic or acetic acid) had higher ratio of lactic to acetic acid than those treated with high dose of formic and acetic acids. The ratio of lactic to acetic acid in negative control and treatments with low dose of formic or acetic acid was more than 3:1 when stored for 30 to 60 days (**Table 3**). Kung Jr and Ranjit (*35*), as well as Stokes and Chen (*36*) reported that the ratio of lactic to acetic acid of more than 3:1 was an indication of a homofermentative lactic bacteria dominant fermentation. Thus, considering this, the results found here suggest that homofermentative lactic acid bacteria dominated in negative control and silages treated with low dose of formic or acetic acid between 30 and 60 days. The ratio of lactic to acetic acid decreased after 90 days due to fermentation of pentose sugars released from hemicellulose by acid hydrolysis (*36*). In the present study, the lower pH, the ratio of lactic to total organic acids, and lactic acid contents in silages treated with formic or acetic acid indicate that the addition of formic or acetic acid during ensiling could alter or suppress fermentation.

Ammoniacal nitrogen, expressed as percentage of total nitrogen, gradually showed an increasing trend over time, indicating that protein degradation and deamination occurred during the later period of storage. The ratio of ammoniacal nitrogen to total nitrogen in silage treated with acetic acid was significantly lower ($p<0.05$) than that of negative control over 30-90 days, suggesting that proteolysis was reduced by lowering plant enzyme activity or inhibiting undesirable microorganisms with the addition of acetic acid, potentially reducing the number of yeasts that cause spoilage in the presence of air. Some authors have described the benefits of the inhibition of spoilage organisms (*37*), demonstrating that lowered pH value in ensiled forage can effectively inhibit proteolysis because plant enzymes are quickly inactivated under these conditions (*15*). The ratio of ammoniacal nitrogen to total nitrogen in silage treated with formic acid, on average, was significantly higher ($p<0.05$) than that of negative control when stored between 90 and 170 days due to the higher pH values and weakened inhibitory effects, but ammonium content in all five silages was lower than the recommended value of 10 % of total nitrogen, indicating good silage, which demonstrated that the total nitrogen or crude protein in silages were well preserved (*38*). However, a greater reduction of the ratio of ammoniacal nitrogen to total nitrogen in silages treated with acetic acid was observed than in those treated with formic acid, which helps to explain the lower pH values achieved with the acetic acid treatment. In brief, organic acids, such as formic or acetic acid, represent feasible methods for promoting a rapid decline of silage pH and improving the fermentation quality of ensiled forage, with the application of acetic acid being especially efficacious.

Effects of additives on microbial community of mixed silage of air-dried corn stover and cabbage waste

The microbiota profile is another indicator of silage quality. Importantly, good quality silage should not contain any pathogenic and spoilage bacteria. The recovered reads of all five silages summed up to 593 875, and these reads were clustered into a total of 5669 operational taxonomic units (OTU) of genus at a 3 % dissimilarity level (**Table 4**). Silages treated with formic or acetic acid had higher OTU numbers than negative control on day 30, the silage treated with high dose of acetic acid had a higher OTU number on day 60, silages treated with formic or acetic acid had lower OTU numbers on days 90 and 170, and the silage treated with high dose of formic acid had a lower OTU number on day 130. Another bacterial community richness estimator, Chao, was utilized to estimate the number of OTUs (*39*). The Chao index showed a similar trend to OTUs. These two indices showed that with respect to the bacterial community richness, the higher OTUs indicated a richer bacterial community. The diversity index of microbial population, as represented by the Shannon index (*39*), varied within the range of 3.27-4.58 for negative control, 1.96-5.15 for treatment with low dose of formic acid, 3.26-3.93 for treatment with high dose of formic acid, 2.01-4.23 for treatment with low dose of acetic acid and 2.43-3.69 for treatment with high dose of acetic acid. The coverage values were around 0.99, suggesting that most of bacterial species were detected. Results from the Shannon index, Chao index and reads of observed species (**Table 4**) indicated that most samples had a high bacterial biodiversity.

There were seven bacterial phyla, namely, Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Verrucomicro, Cyanobacteria and others (**Fig. 2**). Proteobacteria (65.3 %) and Firmicutes (33.8 %) were dominant in air-dried corn stover, while the relative abundance of Bacteroidetes (0.8 %) and Actinobacteria (0.2 %) was low. Proteobacteria (80.2 %) and Bacteroidetes (18.6 %) were dominant in cabbage waste, and the relative abundance of Firmicutes (0.9 %), Cyanobacteria (0.2 %) and Actinobacteria (0.1 %) was low.

Overall, Firmicutes and Proteobacteria were still dominant in all five silages after 170 days. An increasing trend of Actinobacteria and Firmicutes in silages treated with formic or acetic acid was observed in comparison with negative control. With prolonged storage period, relative abundance of Actinobacteria first increased, followed by a decrease in negative control and silages treated with formic and high dose of acetic acid, while in the silage treated with low dose of formic acid it reached a maximum (0.9 %) on day 130. Similarly, Actinobacteria in silage treated with low dose of acetic acid was observed to decrease first and then increase, with the relative abundance reaching a maximum (1.0 %) on day 170. However, with prolonged storage period, Firmicutes was observed to decrease and then increase in negative control and silages treated with low dose of formic or acetic acid, while it increased first and then decreased in silages treated with high dose of formic or acetic acid. Firmicutes in silages treated with high dose of formic acid (78.1 %) and silages treated with high dose of acetic

Table 4. Diversity statistics of bacterial community during ensiling

Treatment	*t*/day	Read	OTU	Chao*	Shannon*	Coverage
ME	30	13684	156	434.27	3.53	0.99
	60	23698	213	506.77	3.63	0.99
	90	30110	155	223.00	4.58	0.99
	130	20379	283	564.80	4.57	0.99
	170	27917	320	716.51	3.27	0.99
FA	30	23010	304	632.17	3.23	0.99
	60	12554	150	369.00	3.23	0.99
	90	28430	103	101.00	1.96	0.99
	130	34983	318	583.05	5.15	0.99
	170	22164	305	611.03	3.63	0.99
FB	30	15362	238	495.57	3.83	0.99
	60	16224	182	399.94	3.93	0.99
	90	29162	122	142.00	3.26	0.99
	130	21260	265	534.22	3.85	0.99
	170	24903	275	574.83	3.56	0.99
AA	30	23388	184	420.78	2.01	0.99
	60	16160	199	463.02	3.61	0.99
	90	26271	124	121.00	2.22	0.99
	130	25088	292	610.76	4.23	0.99
	170	22363	264	575.90	2.91	0.99
AB	30	29438	267	617.13	3.20	0.99
	60	18603	243	494.16	3.69	0.99
	90	27308	147	151.00	2.43	0.99
	130	32644	292	536.45	3.45	0.99
	170	28772	268	512.43	2.65	0.99

*Chao and Shannon indices (*39*), OTU=operational taxonomic units, ME=mixed silage of air-dried corn stover and cabbage waste without any additives (negative control group), FA=mixed silage with low dose of formic acid (0.3 %), FB=mixed silage with high dose of formic acid (0.6 %), AA=mixed silage with low dose of acetic acid (0.3 %), AB=mixed silage with high dose of acetic acid (0.6 %)

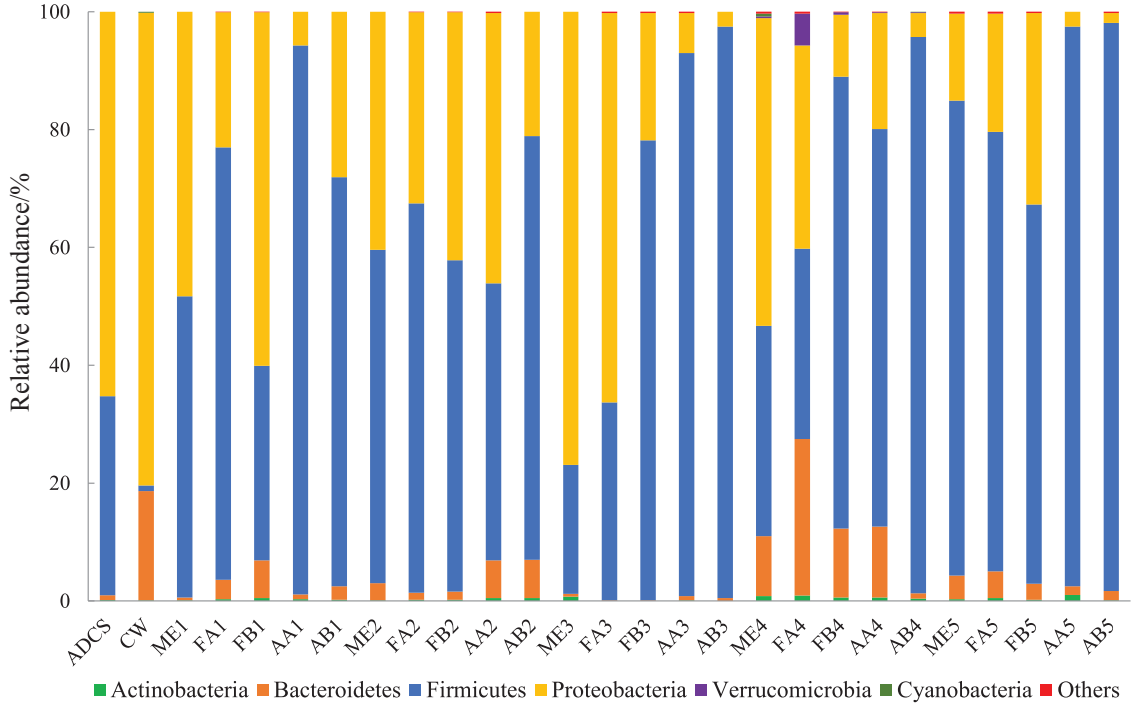

Fig. 2. Relative abundance of bacterial community at the phylum level. ADCS=air-dried corn stover, CW=cabbage waste, ME=mixed silage of ADCS and CW without any additives (negative control group), FA=mixed silage with low dose of formic acid (0.3 %), FB=mixed silage with high dose of formic acid (0.6 %), AA=mixed silage with low dose of acetic acid (0.3 %), AB=mixed silage with high dose of acetic acid (0.6 %), 1, 2, 3, 4, 5=storage period of 30, 60, 90, 130 and 170 days, respectively

acid (97.0 %) reached maximum values on day 90. The most abundant bacteria on the phylum level in the mixed silages, with or without additives, were Firmicutes.

The dominant genus in air-dried corn stover included *Enterobacter* (47.1 %), *Carnobacterium* (27.7 %), *Pantoea* (10.1 %), *Yersinia* (4.8 %), *Exiguobacterium* (3.5 %), *Pseudomonas* (2.3 %) and so on (**Fig. 3**), in cabbage waste it included *Pseudomonas* (48.4 %), *Pantoea* (17.1 %), *Flavobacterium* (16.3 %), *Yersinia* (6.5 %), *Enterobacter* (3.6 %) and *Carnobacterium* (0.9 %). The relative abundance of non-LAB in cabbage waste was 99.1 % (minimal LAB presence). *Enterobacteria* are non-spore forming, facultative anaerobes that can ferment lactic acid to acetic acid and other products, thus causing a loss of nutritional value in silage (*40*). After being discarded, cabbage waste is easily decomposed and mixed with air-dried corn stover, and is complementary to lactic acid bacteria.

Relative abundance of LAB was higher than of non-LAB in silages with added formic or acetic acid than in the negative control, except for the silage with high dose of formic acid on day 30, with low dose of acetic acid on day 60 and with low dose of formic acid on day 130. With prolonged storage, the amount of LAB species increased, and the relative abundance of LAB in silage with high dose of acetic acid increased first, from 69.2 to 96.8 %, and then remained in the range of 96.1-96.8 %. The relative abundance of some harmful bacteria such as *Flavobacterium* and *Pseudomonas* in all five silages decreased significantly in comparison with air-dried corn stover and cabbage waste, and the presence of harmful bacteria such as *Enterobacter*, *Pantoea*, *Yersinia* and *Exiguobacterium* was not observed. Parvin *et al.* (*41*) observed a shift in bacterial community structure from *Enterobacter* to *Lactobacillus*, *Pediococcus* and *Lactococcus* after 30 days of silage by comparing bacterial communities over time. Li and Nishino (*42*) also reported that *Lactobacillus*, *Pediococcus*, *Weissella* and *Klebsiella* were found in both the pre-ensiled crop and the silage by the analysis of evolution in bacterial communities in whole corn silage. In fact, great variations were found among the relative abundances of detected bacteria before and after fermentation of silages treated with

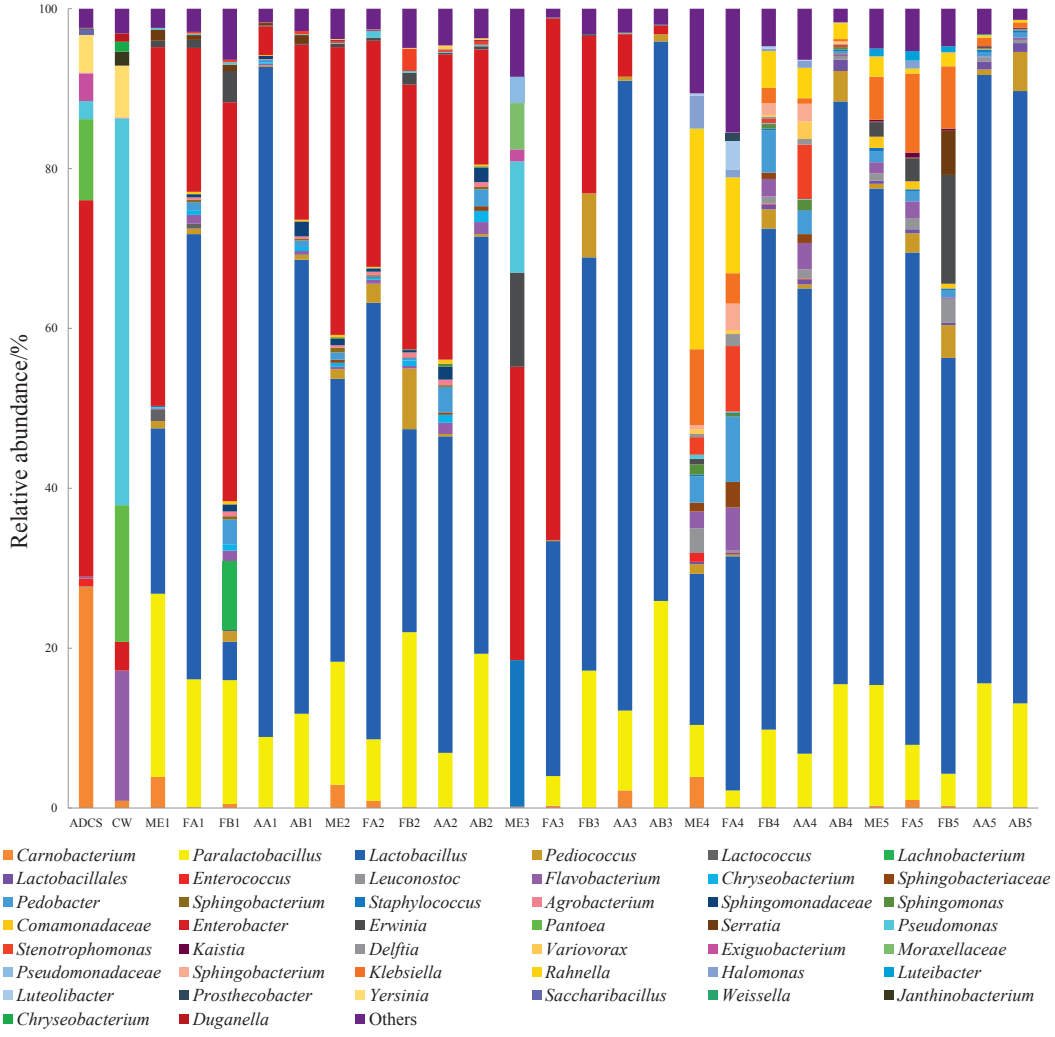

Fig. 3. Relative abundance of bacterial community at the genus level. ADCS=air-dried corn stover, CW=cabbage waste, ME=mixed silage of ADCS and CW without any additives (negative control group), FA=mixed silage with low dose of formic acid (0.3 %), FB=mixed silage with high dose of formic acid (0.6 %), AA=mixed silage with low dose of acetic acid (0.3 %), AB=mixed silage with high dose of acetic acid (0.6 %), 1, 2, 3, 4, 5=storage period of 30, 60, 90, 130 and 170 days, respectively

formic or acetic acid in different ensiling phases. Before fermentation, *Carnobacterium* was the most abundant species in the silage samples. After fermentation, *Lactobacillus* (83.8 %) and *Enterobacter* (49.9 %) appeared to be the dominant bacteria in the silages (**Fig. 3**).

This shows that the addition of formic or acetic acid can help to establish LAB as dominant bacteria during storage, and effectively inhibit or reduce the growth of harmful bacteria. It was found that Firmicutes was the dominant phylum during this process, while *Lactobacillus* was the dominant observed genus, which is in agreement with the findings of Ennahar *et al.* (*43*).

The main LAB in air-dried corn stover were *Carnobacterium* and *Enterococcus*, with respective fractions of 96.7 and 3.3 % (**Fig. 4**). In cabbage waste, the majority of LAB were *Carnobacterium* and *Enterococcus*, with relative fractions of 96.9 and 1.8 %, respectively, while also containing a small amount of *Lactobacillus* (0.9 %) and *Leuconostoc* (0.5 %). *Paralactobacillus* and *Pediococcus* also appeared in all five silages after day 170, while *Lachnobacterium* appeared earlier in silage treated with high dose of formic acid on day 30. Furthermore, Lactobacillales appeared in all treatments after day 130. *Lactobacillus* was the dominant LAB genus throughout the whole storage period, and the proportion of LAB increased from 0.9 % in cabbage waste on day 0 to 91.0 % in silage treated with low dose of formic acid on day 130. Overall, LAB populations as a whole reached a maximum in silage treated with high dose of formic acid on day 90. Tohno *et al.* (*44*) reported that lactic acid bacteria typically associated with silage belong to the genera *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Leuconostoc*, *Enterococcus* and *Weissella*. LAB are characterized by their acid tolerance and final pH values reached 3.8 at the end of the corn silage fermentation stage. To conclude, the addition of formic or acetic acid, especially acetic acid, has mostly positive effects on fermentation during mixed silage of air-dried corn stover and cabbage waste. Apart from regulating the pH and the content of microorganism metabolites (volatile fatty acids and ammoniacal nitrogen), the formic or acetic acid additives also modulate the bacterial composition and community structure in these fermented silages.

CONCLUSIONS

The mixed silage with addition of formic or acetic acid preserved effectively the dry matter and water-soluble carbohydrate content, and the content of neutral and acid detergent fibre decreased compared with the negative control. The addition of formic or acetic acid was shown to increase the relative abundance of the dominant bacterium at the phylum and genus levels, while decreasing and even suppressing harmful bacteria, such as *Enterobacter*, *Pantoea*, *Yersinia* and *Exiguobacterium*. In general, formic or acetic acid both contributed to producing the high quality fermented silage, but the application of acetic acid was superior to formic acid. Therefore, the addition of 0.3 % acetic acid represents a cost-effective approach for the preservation of air-dried corn stover by ensiling. Dynamic changes in organic components, intermediate fermentation products and microbial communities were determined, deepening the understanding of the improved quality of trans-seasonal preservation of air-dried corn stover. However, the bacterial microbiota profiles described in this paper are restricted to the genus level, and studies on bacterial profiles at the species level will be imperative in future work.

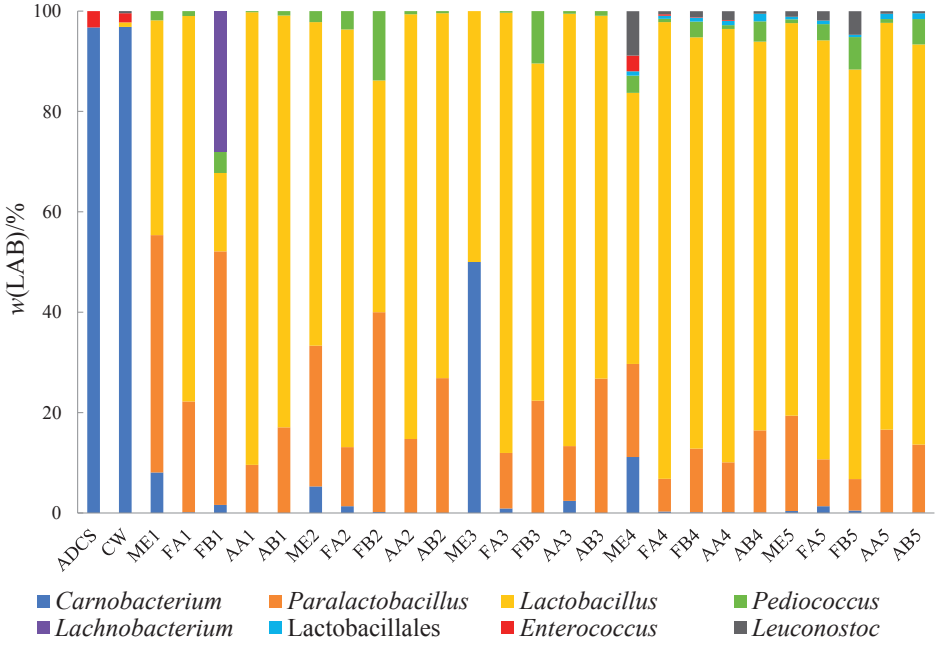

Fig. 4. Relative fraction of lactic acid bacterial (LAB) during 170 days of storage. ADCS=air-dried corn stover, CW=cabbage waste, ME=mixed silage of ADCS and CW without any additives (negative control group), FA=mixed silage with low dose of formic acid (0.3 %), FB=mixed silage with high dose of formic acid (0.6 %), AA=mixed silage with low dose of acetic acid (0.3 %), AB=mixed silage with high dose of acetic acid (0.6 %), 1, 2, 3, 4, 5=storage period of 30, 60, 90, 130 and 170 days, respectively

ACKNOWLEDGEMENTS

This work was financially supported by the National Natural Science Foundation of China (51666010, 51366009), Natural Science Foundation of Gansu (1606RJZA206, 1606RJYA287, 17JR5RA117), Key Laboratory of Environmental and Applied Microbiology, Chengdu Institute of Biology, Chinese Academy of Sciences (KLCAS-2016-10), Hong Liu Excellent Young Teachers Cultivation Project of Lanzhou University of Technology (Q201207).

REFERENCES

1. Triolo JM, Sommer SG, Møller HB, Weisbjerg MR, Jiang XY. A new algorithm to characterize biodegradability of biomass during anaerobic digestion: Influence of lignin concentration on methane production potential. Bioresour Technol. 2011;102:9395-402. https://doi.org/10.1016/j.biortech.2011.07.026
2. Chandra R, Takeuchi H, Hasegawa T. Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production. Renew Sust Energ Rev. 2012;16:1462-76. https://doi.org/10.1016/j.rser.2011.11.035
3. Richard TL. Challenges in scaling up biofuels infrastructure. Science. 2010;329:793-6. https://doi.org/10.1126/science.1189139
4. Chen Y, Sharma-Shivappa RR, Chen C. Ensiling agricultural residues for bioethanol production. Appl Biochem Biotechnol. 2007;143(1):80-92. https://doi.org/10.1007/s12010-007-0030-7
5. Pakarinen O, Lehtomäki A, Rissanen S, Rintala J. Storing energy crops for methane production: Effects of solids content and biological additive. Bioresour Technol. 2008;99(15):7074-82. https://doi.org/10.1016/j.biortech.2008.01.007
6. Herrmann C, Heiermann M, Idler C. Effects of ensiling, silage additives and storage period on methane formation of biogas crops. Bioresour Technol. 2011;102:5153-61. https://doi.org/10.1016/j.biortech.2011.01.012
7. Herrmann C, Heiermann M, Idler C, Prochnow A. Particle size reduction during harvesting of crop feedstock for biogas production I: Effects on ensiling process and methane yields. Bioenerg Res. 2012;5(4):926–36. https://doi.org/10.1007/s12155-012-9206-2
8. Vervaeren H, Hostyn K, Ghekiere G, Willems B. Biological ensilage additives as pretreatment for maize to increase the biogas production. Renew Energ. 2010;35:2089-93. https://doi.org/10.1016/j.renene.2010.02.010
9. Pakarinen A, Maijala P, Jaakkola S, Stoddard FL, Kymäläinen M, Viikari L. Evaluation of preservation methods for improving biogas production and enzymatic conversion yields of annual crops. Biotechnol Biofuels. 2011;4:20. https://doi.org/10.1186/1754-6834-4-20
10. Cui Z, Shi J, Wan C, Li Y. Comparison of alkaline- and fungi-assisted wet-storage of corn stover. Bioresour Technol. 2012;109(4):98-104. https://doi.org/10.1016/j.biortech.2012.01.037
11. Ren H, Xu N, Li J, Li Z, Zhao T, Pei F, et al. Effects of different mixed ratio of maize straw and cabbage wastes on silage quality. J Biobased Mater Bioenergy. 2015;9(1):88-94. https://doi.org/10.1166/jbmb.2015.1494
12. Hess JR, Kenney KL, Wright CT, Perlack R, Turhollow A. Corn stover availability for biomass conversion: Situation analysis. Cellulose. 2009;16(4):599-619. https://doi.org/10.1007/s10570-009-9323-z
13. Li L, Sun Y, Yuan Z, Kong X, Wao Y, Yang L, et al. Effect of microalgae supplementation on the silage quality and anaerobic digestion performance of Manyflower silvergrass. Bioresour Technol. 2015;189:334-40. https://doi.org/10.1016/j.biortech.2015.04.029
14. Weinberg ZG, Chen Y. Effects of storage period on the composition of whole crop wheat and corn silages. Anim Feed Sci Technol. 2013;185:196-200. https://doi.org/10.1016/j.anifeedsci.2013.08.009
15. Ke WC, Ding WR, Xu DM, Ding LM, Zhang P, Li FD, Guo XS. Effects of addition of malic or citric acids on fermentation quality and chemical characteristics of alfalfa silage. J Dairy Sci. 2017;100:8958–66. https://doi.org/10.3168/jds.2017-12875
16. Aksu T, Baytok E, Karslı AM, Muruz H. Effects of formic acid, molasses and inoculant additives on corn silage composition, organic matter digestibility and microbial protein synthesis in sheep. Small Ruminant Res. 2006;61(1):29-33. https://doi.org/10.1016/j.smallrumres.2004.12.013
17. Danner H, Holzer M, Mayrhuber E, Braun R. Acetic acid increases stability of silage under aerobic conditions. Appl Environ Microbiol. 2003;69(1):562-7. https://doi.org/10.1128/AEM.69.1.562-567.2003
18. Schmidt RJ, Kung Jr L. The effects of Lactobacillus buchneri with or without a homolactic bacterium on the fermentation and aerobic stability of corn silages made at different locations. J Dairy Sci. 2010;93(4):1616-24. https://doi.org/10.3168/jds.2009-2555
19. Li P, Ji S, Hou C, Tang H, Wang Q, Shen Y. Effects of chemical additives on the fermentation quality and N distribution of alfalfa silage in south of China. Animal Sci J. 2016;87(12):1472–9. https://doi.org/10.1111/asj.12600
20. Leyva A, Quintana A, Sánchez M, Rodríguez EN, Cremata J, Sánchez JC. Rapid and sensitive anthrone–sulfuric acid assay in microplate format to quantify carbohydrate in biopharmaceutical products: Method development and validation. Biologicals. 2008;36(2):134-41. https://doi.org/10.1016/j.biologicals.2007.09.001
21. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci. 1991;74(10):3583-97. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

22. Tangka JK. Analysis of the thermal energy requirements for the extraction of leaf protein concentrate from some green plants. Biosyst Eng. 2003;86(4):473-9. https://doi.org/10.1016/j.biosystemseng.2003.08.014
23. IBM SPSS Statistics for Windows, v. 20, Armonk, USA: IBM Corp; 2011. Available from: https://www-01.ibm.com/software/analytics/spss/products/statistics/.
24. Van Vuuren AM, Bergsma K, Frol-Kramer F, Van Beers JAC. Effects of addition of cell wall degrading enzymes on the chemical composition and the in sacco degradation of grass silage. Grass Forage Sci. 1989;44(2):223–30. https://doi.org/10.1111/j.1365-2494.1989.tb01930.x
25. Li Y, Nishino N. Bacterial and fungal communities of wilted Italian ryegrass silage inoculated with and without Lactobacillus rhamnosus or Lactobacillus buchneri. Lett Appl Microbiol. 2011;52(4):314-21. https://doi.org/10.1111/j.1472-765X.2010.03000.x
26. Heron SJE, Wilkinson JF, Duffus CM. Enterobacteria associated with grass and silages. J Appl Microbiol. 2008;75(1):13-7. https://doi.org/10.1111/j.1365-2672.1993.tb03401.x
27. Weiland P. Biogas production: Current state and perspectives. Appl Microbiol Biotechnol. 2010;85(4):849-60. https://doi.org/10.1007/s00253-009-2246-7
28. Van Ranst G, Fievez V, De Riek J, Van Bockstaele E. Influence of ensiling forages at different dry matters and silage additives on lipid metabolism and fatty acid composition. Anim Feed Sci Technol. 2009;150(1-2):62-74. https://doi.org/10.1016/j.anifeedsci.2008.08.004
29. Kung L, Shaver R. Interpretation and use of silage fermentation analysis reports. Focus on Forage. 2001;3(13):1-5.
30. Jian W, Lei C, Yuan XJ, Guo G, Li JF, Bai YF, Shao T. Effects of molasses on the fermentation characteristics of mixed silage prepared with rice straw, local vegetable by-products and alfalfa in Southeast China. J Integr Agric. 2017;16(3):664-70. https://doi.org/10.1016/S2095-3119(16)61473-9
31. Levital T, Mustafa AF, Seguin P, Lefebvre G. Effects of a propionic acid-based additive on short-term ensiling characteristics of whole plant maize and on dairy cow performance. Anim Feed Sci Technol. 2009;152(1-2):21-32. https://doi.org/10.1016/j.anifeedsci.2009.03.010
32. Pahlow G, Muck RE, Driehuis F, Oude Elferink SJWH. Microbiology of ensiling. In: Buxton DR, Muck RE, Harrison JH, editors. Silage Science and Technology. Agron Monog. 42. Madison, WI, USA: ASA, CSSA, SSSA; 2003.
33. Nkosi BD, Meeske R, van der Merwe HJ, Groenewald IB. Effects of homofermentative and heterofermentative bacterial silage inoculants on potato hash silage fermentation and digestibility in rams. Anim Feed Sci Technol. 2010;157(3–4):195-200. https://doi.org/10.1016/j.anifeedsci.2010.03.008
34. Dunière L, Gleizal A, Chaucheyras-Durand F, Chevallier I, Thévenot-Sergentet D. Fate of Escherichia coli O26 in corn silage experimentally contaminated at ensiling, at silo opening, or after aerobic exposure, and protective effect of various bacterial inoculants. Appl Environ Microbiol. 2011;77(24):8696-704. https://doi.org/10.1128/AEM.06320-11
35. Kung Jr L, Ranjit NK. The effect of Lactobacillus buchneri and other additives on the fermentation and aerobic stability of barley silage. J Dairy Sci. 2001;84(5):1149-55. https://doi.org/10.3168/jds.S0022-0302(01)74575-4
36. Stokes MR, Chen J. Effects of an enzyme-inoculant mixture on the course of fermentation of corn silage. J Dairy Sci. 1994;77(11):3401-9. https://doi.org/10.3168/jds.S0022-0302(94)77282-9
37. Acosta Aragón Y, Jatkauskas J, Vrotniakiene V. The effect of a silage inoculant on silage quality, aerobic stability and milk production. ISRN Vet Sci. 2014;2012:ArticleID 345927. https://doi.org/10.5402/2012/345927
38. Ni K, Wang F, Zhu B, Yang J, Zhou G, Pan Y, et al. Effects of lactic acid bacteria and molasses additives on the microbial community and fermentation quality of soybean silage. Bioresour Technol. 2017;238:706-15. https://doi.org/10.1016/j.biortech.2017.04.055
39. Zhao Y, Yu J, Liu J, Yang H, Gao L, Yuan X, et al. Material and microbial changes during corn stalk silage and their effects on methane fermentation. Bioresour Technol. 2016;222(12):89-99. https://doi.org/10.1016/j.biortech.2016.09.113
40. McEniry J, Allen E, Murphy JD, O'Kiely P. Grass for biogas production: The impact of silage fermentation characteristics on methane yield in two contrasting biomethane potential test systems. Renew Energy. 2014;63(1):524-30. https://doi.org/10.1016/j.renene.2013.09.052
41. Parvin S, Wang C, Li Y, Nishino N. Effects of inoculation with lactic acid bacteria on the bacterial communities of Italian ryegrass, whole crop maize, guinea grass and rhodes grass silages. Anim Feed Sci Technol. 2010;160:160-6. https://doi.org/10.1016/j.anifeedsci.2010.07.010
42. Li Y, Nishino N. Effects of inoculation of Lactobacillus rhamnosus and Lactobacillus buchneri on fermentation, aerobic stability and microbial communities in whole crop corn silage. Grassl Sci. 2011;57(4):184-91. https://doi.org/10.1111/j.1744-697X.2011.00226.x
43. Ennahar S, Cai Y, Fujita Y. Phylogenefic diversity of lactic acid bacteria associated with paddy rice silage as determined by 16S vibosomal DNA analysis. Appl Environm Microbiol. 2003;69(1):444-51. https://doi.org/10.1128/AEM.69.1.444-451.2003
44. Tohno M, Kitahara M, Irisawa T, Masuda T, Uegaki R, Ohkuma M, Tajima K. Lactobacillus silagei sp. nov. isolated from orchardgrass silage. Int J Syst Evol Microbiol. 2013;63(Pt 12):4613-8. https://doi.org/10.1099/ijs.0.053124-0