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)^{A}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
	30	13684	156	434.27	3.53	0.99
	60	23698	213	506.77	3.63	0.99
ME	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
	30	23010	304	632.17	3.23	0.99
	60	12554	150	369.00	3.23	0.99
FA	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
	30	15362	238	495.57	3.83	0.99
	60	16224	182	399.94	3.93	0.99
FB	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
	30	23388	184	420.78	2.01	0.99
	60	16160	199	463.02	3.61	0.99
AA	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
	30	29438	267	617.13	3.20	0.99
	60	18603	243	494.16	3.69	0.99
AB	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 %)

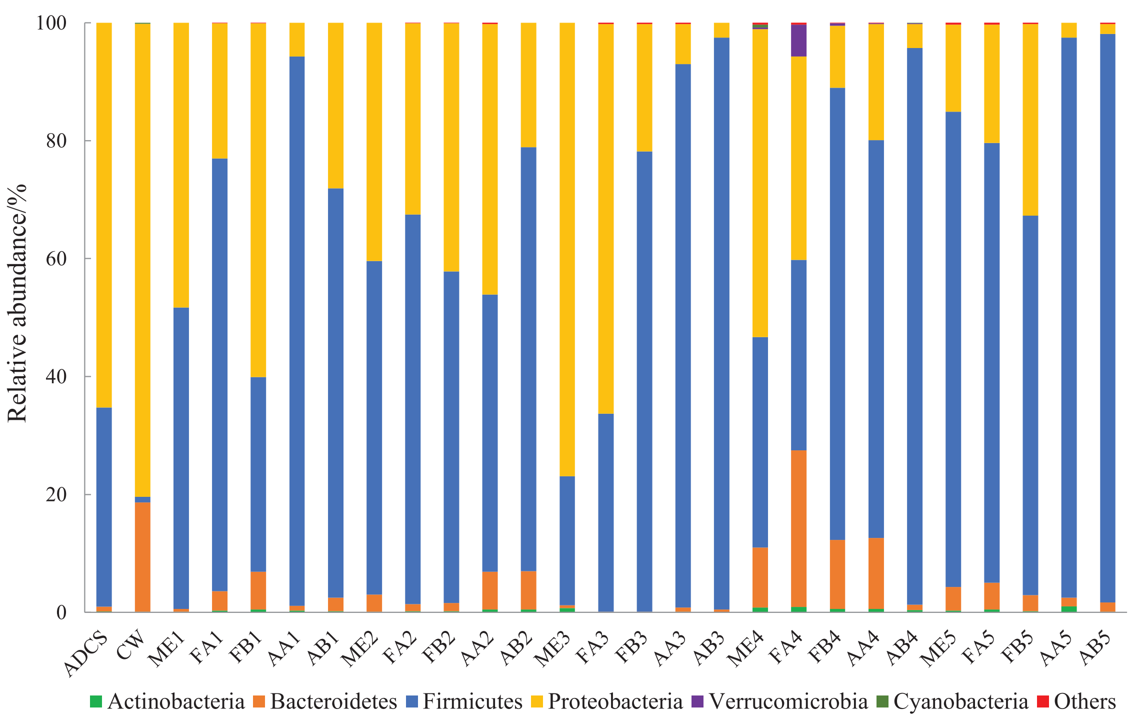

Fig. 2. Relative abundance of bacterial community at the phylum level. ADCS=air-dried corn stover, CW=cabbage waste, ME=mixed silage of ADCS and CW without any additives (negative control group), FA=mixed silage with low dose of formic acid (0.3 %), FB=mixed silage with high dose of formic acid (0.6 %), 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

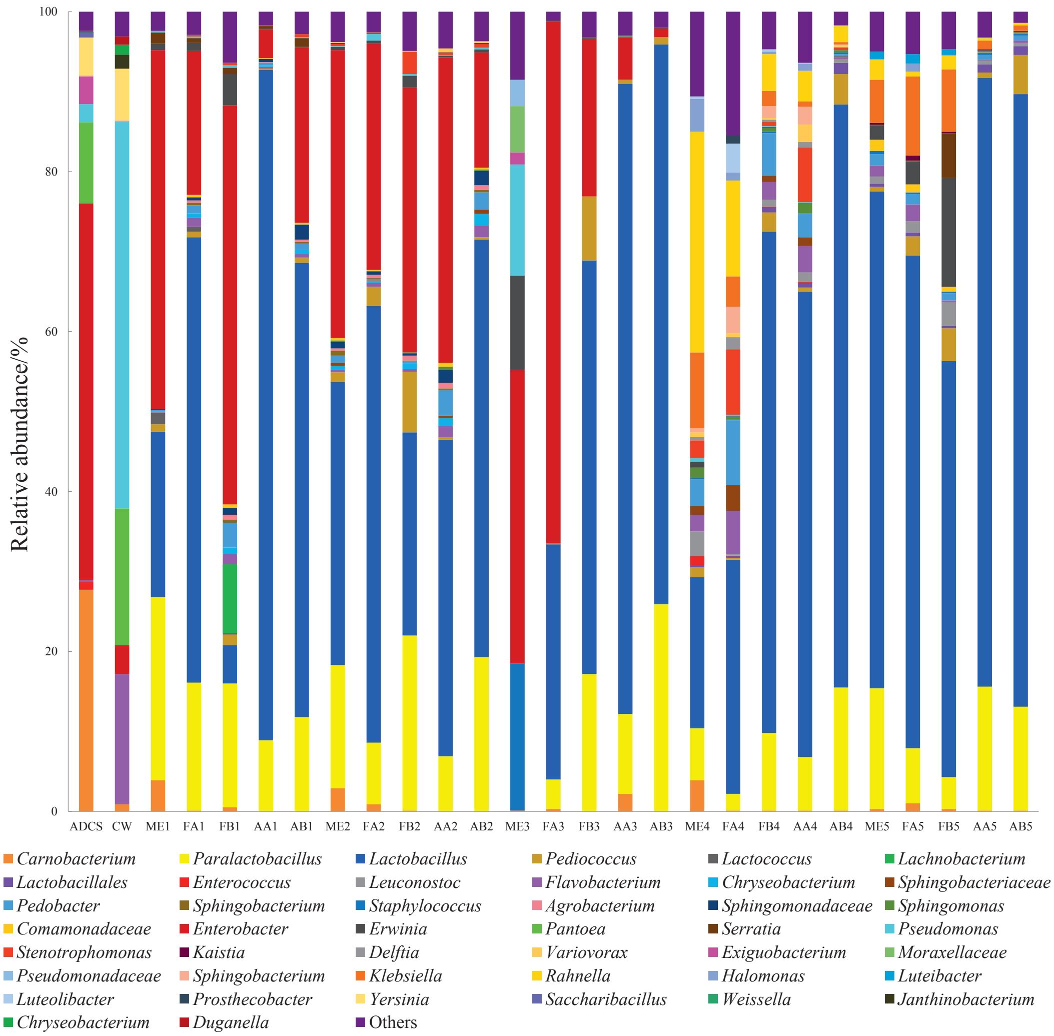

Fig. 3. Relative abundance of bacterial community at the genus level. ADCS=air-dried corn stover, CW=cabbage waste, ME=mixed silage of ADCS and CW without any additives (negative control group), FA=mixed silage with low dose of formic acid (0.3 %), FB=mixed silage with high dose of formic acid (0.6 %), 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.

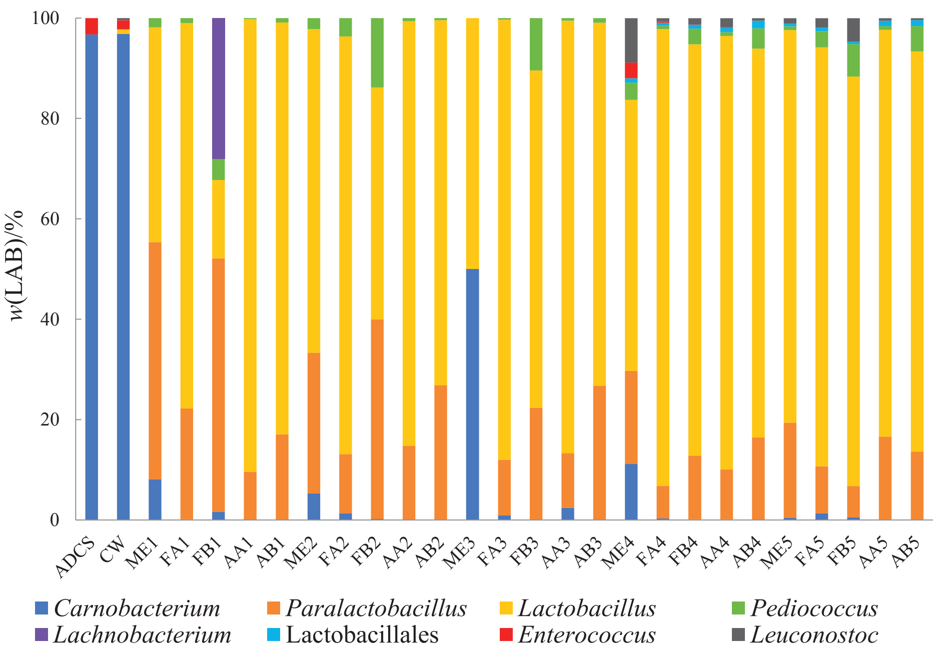

Fig. 4. Relative fraction of lactic acid bacterial (LAB) during 170 days of storage. ADCS=air-dried corn stover, CW=cabbage waste, ME=mixed silage of ADCS and CW without any additives (negative control group), FA=mixed silage with low dose of formic acid (0.3 %), FB=mixed silage with high dose of formic 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).

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