NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 50, 2017 (1): 55–67
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ISSN 1337-9984

VOLATILE ORGANIC COMPOUNDS IN SILAGES – EFFECTS OF MANAGEMENT FACTORS ON THEIR FORMATION: A REVIEW

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ABSTRACT

Based on empirical observations from commercial farms that well preserved, but odd-smelling maize silages may cause problems regarding feed intake and milk yield by dairy cows, volatile organic compounds (VOC) were analyzed in recent studies. The aim of this paper was to summarize the results concerning the occurrence of VOCs in silages and the effects of silage additives on their formation. Elevated levels of ethanol, ethyl acetate (EA) and ethyl lactate (EL) as well as propanol and propyl acetate (PA) occurred in maize and whole crop silages, grass/ grass-legume-mixtures silages, furthermore in sugar cane silages. Ester and ethanol levels were highest in silages stored under strict anaerobic conditions. In conclusion it can be stated that the ester concentrations were strongly correlated with the ethanol concentration and the silage pH. Results from ensiling experiments on the effects of silage additives on ester formation in different ensiling materials clearly indicated that chemical products containing active ingredients with specific antifungal effects can significantly reduce ethanol and ester concentration. Salts of sorbic, benzoic or propionic acids or mixtures are effective treatment for reducing VOC production. Buffered formic acid-containing products stimulated it due to an increase in ethanol content. A survey was carried out to investigate the incidence of VOCs in maize silages from German dairy farms and to monitor the concentrations of ethanol, n-propanol and the corresponding esters ethyl acetate, ethyl lactate and propyl acetate. With increasing compaction the contents of VOCs increased and their concentration depends on the sampling site in the silo.

Key words: volatile organic compounds; management factors; silages

INTRODUCTION

Based on empirical observations from commercial farms that well preserved, but odd-smelling maize silages may cause problems regarding feed intake and milk yield by dairy cows, volatile organic compounds (VOC) were analyzed in recent studies (Weiss *et al*., 2009a, b). Ethyl and propyl esters of lactate and acetate have been found in farm silages (Weiss, 2009a, Weiss *et al*. 2015a). Researchers have correlated feed intake negatively with concentrations of some of the VOCs (Kriszan *et al*., 2007, Raun and Kristensen, 2010, Gerlach *et al*., 2013). The knowledge on the effects of specific VOCs on feed intake by ruminants is still very limited. In addition, those substances have been discussed in relation to climate-damaging ozone formation, and it was reported that silages on dairy farms may be a significant source of VOC emission (Mitloehner *et al*., 2009).

Correlations were found among maize silages between ensiling conditions, type of silage additive as well as ethanol content and the concentrations of the ethyl esters – ethyl acetate (EA) and ethyl lactate (EL) (Weiss *et al*., 2016). Ester and ethanol levels were highest in silages stored under strict anaerobic conditions. Elevated levels of ethanol and the corresponding esters EA and EL were not only detected in maize silages, but also in silages from grass, grass-legume-mixtures, legumes, whole-crop cereals and sorghum (Weiss and Auerbach, 2012a,b; Weiss and Kalzendorf, 2016). Regardless of silage type, silage additive and ensiling conditions, in the most cases a strong correlation was found between ethanol and ester concentrations, highlighting

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Received: November 8, 2016
Accepted: January 13, 2017

the prominent role of alcohol in ester formation. Therefore, any measure that reduces ethanol will restrict ester content.

OCCURRENCE OF VOLATILE ORGANIC COMPOUNDS IN SILAGES

VOCs in maize silages

On the basis of the results from investigations in farm silages with well preserved, but odd-smelling maize silages the first laboratory scale ensiling experiments were carried out with maize (Table 1). Elevated levels of ethanol, EA and EL were detected in maize silages, but also in silages from whole-crop wheat and high-moisture corn (Table 2). Ester and ethanol levels were the highest in silages stored under strict anaerobic conditions. It was also shown that esters remain detectable in silages for a few days after opening of the silos under aerobic conditions (Weiss *et al*., 2011). Data from farm silages presented in Table 2 (whole-crop maize 6 and 7) also showed high ethanol and ester concentrations. These silages were well fermented and highly compacted. In 7 out of 14 silages biological additives were used.

Results of ensiling experiments concerning effect of storage period on fermentation pattern indicates that concentration of ethanol strongly effected formation of esters during fermentation process. Weiss *et al*. (2009b) found increasing contents of ethanol and especially lactic acid over 90 days, whereas the corresponding ethyl esters increased during the first 30 days. These findings are in accordance with results from Gerlach *et al.* (2015) who investigated the effect of storage length of different maize silage varieties.

Table 1: Characterization of the data set of maize silages (ensiling experiments, n = 439)

Type of Silage	DM $g.kg^{-1}$	n	Storage length (days)	Silage additives
Laboratory scale ensiling experiments				
Whole-crop maize 1	310	60	60, 90	biological, chemical (Weiss *et al.*, 2009a)
Whole-crop maize 2	316	30	2,14, 28, 49, 90	biological (Weiss *et al*., 2009b)
Whole-crop maize 3	349	180	2,14, 28, 49, 90	biological, chemical*)
Whole-crop maize 4	332	12	90	chemical (Weiss and Auerbach, 2012b)
Whole-crop maize 5	315 – 513	79	112 anaerobic, 0 – 8 aerobic	without (Gerlach *et al*., 2013)
Whole-crop wheat	276	34	60, 90	biological*)
High-moisture corn	635	30	97	biological, chemical (Auerbach and Weiss, 2011)
Commercial farm silages				
Whole-crop maize 6	254 – 322	3	approx. 90	without (Weiss *et al*., 2009a)
Whole-crop maize 7	299 – 403	11	approx. 90 – 180	biological , Weiss *et al*., 2016)

*(Weiss and Auerbach, unpublished)

Table 2: Contents of volatile organic compounds (VOCs), especially esters and their correlation, in different maize silages (Weiss and Auerbach, 2012a)

Type of Silage	Lactic acid	Acetic acid	Ethanol	Ethyl acetate (EA)	Ethyl lactate (EL)	Regression EA+EL(y), Ethanol (x)	
	$g.kg\ DM^{-1}$	$g.kg\ DM^{-1}$	$g.kg\ DM^{-1}$	$mg.kg\ DM^{-1}$	$mg.kg\ DM^{-1}$	y = ax + b	R^2
Whole-crop maize 1	6.9 – 74.8	5.8 – 79.4	0.9 – 51.7	12 – 284	16 – 379	12.50x + 91.2	0.70
Whole-crop maize 2	32.5 – 119.8	8.6 – 25.8	3.2 – 28.3	55 – 343	30 – 683	26.47x + 121.5	0.65
Whole-crop maize 3	13.7 – 67.4	0.5 – 26.7	3.3 – 20.1	38 – 639	0 – 224	18.10x + 91.7	0.20
Whole-crop maize 4	73.8 – 124.6	5.3 – 29.2	6.2 – 50.8	116 – 262	156 – 661	11.55x + 266.0	0.93
Whole-crop maize 5	0 – 75.5	0 – 36.6	0 – 36.9	0 – 1109	0 – 986	52.51x + 0.2	0.88
Whole-crop wheat	20.7 – 99.9	9.1 – 42.4	21.9 – 121.8	84 – 951	309 – 1277	6.76x + 684.0	0.24
High-moisture corn	6.1 – 20.7	1.0 – 14.5	0.2 – 7.6	0 – 107	0 – 47	17.62x + 0.3	0.78
Whole-crop maize 6	11.3 – 70.8	25.8 – 48.7	21.0 – 64.0	357 – 789	118 – 1263		
Whole-crop maize 7	37.2 – 86.9	10.4 – 28.3	1.1 – 24.1	12 – 64	47 – 1305		

VOCs in grass silages

Extensive literature search yielded one study only by Krizsan *et al*. (2007), who detected variable concentrations of esters in grass silage, but the mean content never exceeded 30 mg.kg DM^{-1}. Therefore, the aim of investigations with grass silages (Table 3) was to determine the incidence of VOCs in grass silages, particularly ethanol and the ethyl esters of lactic and acetic acids.

Grass silages contained high ethanol and ester concentrations, particularly in those from trials 1 and 2 (Table 4). This may be attributed to the lower storage temperature, which promotes ester formation. Weiss *et al.* (2009a) observed that maize silages stored at 20 °C had higher ester contents than were detected at 35 °C. The correlation coefficients presented in Table 4 varied widely between 0.35 and 0.85, depending on the trial.

Table 3: Characterization of the data set from grass silages (laboratory scale ensiling experiments, n = 620)

Trial	Silage DM ($g.kg^{-1}$)	n	Storage length (days)	Silage additive type used in experiment
1	211 - 438	213	252 - 266	biological, chemical, molasses (Lengyel *et al*., 2012)
2	191 - 464	209	252 - 266	biological, chemical, molasses (Lengyel, unpublished data)
3	230 - 318	49	81	biological, chemical (Nadeau, unpublished data)
4	318 - 383	12	91	biological (Nadeau, unpublished data)
5	223 - 299	45	90	biological, chemical (Kalzendorf, unpublished data)
6	274 - 357	17	142	biological (Nadeau, unpublished data)
7	283 - 373	12	270	chemical (Nadeau, unpublished data)
8	202 - 219	21	131	biological, chemical (Kalzendorf, unpublished data)
9	223 - 240	21	121	biological, chemical (Kalzendorf, unpublished data)
10	243 - 268	21	139	biological, chemical (Kalzendorf, unpublished data)

Table 4: Fermentation products, pH and ester concentrations in grass silages (n = 620) (Weiss and Auerbach, 2013)

Trial	pH	Lactic acid (g.kg DM^{-1})	Acetic acid (g.kg DM^{-1})	Ethanol (g.kg DM^{-1})	Total esters* (mg.kg DM^{-1})	Correlation** r_s	*P* value
1	3.7 - 6.7	0 - 99.5	1.5 - 62.8	0.7 - 39.6	0 - 3540	0.35	< 0.001
2	3.6 - 5.8	0 - 89.8	2.0 - 46.7	0 - 35.3	0 - 3995	0.37	< 0.001
3	4.0 - 4.5	60.6 - 117.5	11.1 - 36.5	2.2 - 18.7	0 - 359	0.91	< 0.001
4	3.8 - 4.2	42.7 - 81.8	13.2 - 35.4	6.7 - 12.0	216 - 455	0.52	ns
5	3.8 - 4.5	32.3 - 89.2	14.2 - 76.7	1.6 - 13.1	73 - 378	0.64	< 0.001
6	4.2 - 4.9	30.0 - 116.7	19.7 - 49.3	2.4 - 7.8	0		-
7	4.3 - 4.7	36.6 - 86.5	7.5 - 13.3	2.1 - 19.9	0 - 161	0.65	< 0.05
8	3.8 - 4.2	42.6 - 105.1	8.4 - 19.9	0.9 - 15.1	0 - 378	0.84	< 0.001
9	3.9 - 4.3	49.9 - 110.6	1.6 - 13.9	1.0 - 14.1	0 - 189	0.85	< 0.001
10	4.0 - 4.7	24.0 - 76.2	14.0 - 31.5	3.9 - 12.3	62 - 272	0.85	< 0.001

*sum of ethyl acetate and ethyl lactate, **correlation between ethanol and total ester concentrations
r_s - Spearman rank correlation coefficient, ns - not significant

The pH of the silages had a pronounced effect on ester levels (Table 5). Strong relationships ($r_s > 0.50$) were mostly observed when the pH of the silages did not exceed the value of 4.25. This is in line with observations by Hangx *et al*. (2001) who found ester reactions be stimulated by low pH in the environment.

The allocation of the grass silages to different ethanol classes was done as described by Weiss and Auerbach (2012a) and showed clear effects of ethanol content on the relationship between pH and total ester concentration (Table 6). Within each ethanol class, a great variation in ester concentration was observed. The detected ester levels in grass silages were extremely high compared with those reported by Weiss and Auerbach (2012b) for maize silages.

Table 5: Relationship between ethanol and ester contents in grass silages (n = 620) as affected by pH (Weiss and Auerbach, 2013)

pH class	n	Total esters* (mg.kg DM-1)	Ethanol (g.kg DM-1)	Correlation**	
				r_s	*P* value
> 3.50 - 3.75	19	482 - 3995	0 - 35	0.60	< 0.01
> 3.75 - 4.00	126	0 -1856	1 - 40	0.72	< 0.001
> 4.00 - 4.25	176	0 - 920	1 - 25	0.55	< 0.001
> 4.25 - 4.50	131	0 - 762	1 - 18	0.21	< 0.05
> 4.50 - 4.75	81	0 - 550	1 - 24	0.26	< 0.05
> 4.75 - 5.00	42	0 - 384	0 - 38	0.49	< 0.001
> 5.00 - 5.25	26	0 - 255	1 - 37	0.49	< 0.05
> 5.25 - 5.50	10	63 - 211	4 - 28	-0.35	ns
> 5.50	9	0 - 171	3 - 24	0.25	ns

*sum of ethyl acetate and ethyl lactate, **correlation between ethanol and total ester concentrations
r_s - Spearman rank correlation coefficient, ns - not significant

Table 6: Relationship between pH and ester contents in grass silages (n = 620) as affected by ethanol (Weiss and Auerbach, 2013)

Ethanol class (g.kg DM-1)	n	Total esters* (mg.kg DM-1)	pH range	Correlation**	
				r_s	*P* value
≤ 5	257	0 - 1180	3.7 - 5.8	-0.12	ns
> 5 - 10	181	0 - 1856	3.8 - 6.7	-0.46	< 0.001
> 10 - 15	100	0 - 1147	3.7 - 5.7	-0.66	< 0.001
> 15 - 20	39	87 - 3116	3.7 - 5.7	-0.88	< 0.001
> 20 - 25	21	0 - 3540	3.6 - 6.1	-0.93	< 0.001
> 25 - 30	12	63 - 3589	3.7 - 5.3	-0.83	< 0.001
> 30 - 35	5	274 - 2054	3.8 - 4.8	-0.60	ns
> 35 - 40	5	182 - 3995	3.7 - 5.2	-0.90	< 0.05

*sum of ethyl acetate and ethyl lactate, **correlation between ph and total ester concentrations
r_s - Spearman rank correlation coefficient, ns - not significant

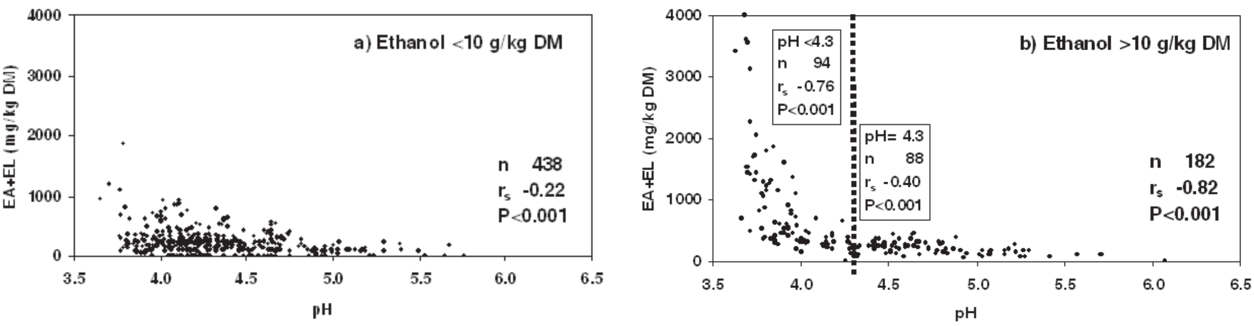

Fig. 1: Total ester concentrations as affected by ethanol class a) ≤ 10 g.kg DM^{-1}, b) >10 g.kg DM^{-1} (Weiss and Auerbach, 2013)

As shown in figure 1a, the correlation between total ester content and pH in grass silages was very weak (r_S = -0.22; $P < 0.001$) up to an ethanol content of 10 g.kg DM^{-1}, whereas a very strong negative relationship was found (r_S = -0.82; $P < 0.001$) at higher ethanol levels (Figure 1b). The least correlation existed if silage pH exceeded the threshold value of pH 4.3.

In summary it can be stated that grass silages may also contain ethyl esters. However, the relationship between ethanol and ethyl esters in grass silages seems to be not as close as that for maize silages. This can be explained by the fact that the intensity of ester reactions is affected by the pH of the silage and grass silages often have pH values above 4.0. As a consequence,

Table 7: Concentrations of fermentation products in sugarcane silages (n = 33), Daniel *et al.* (2013a)

Common name	Mean	SD[a]	Min.	Max.
	g.kg^{-1}			
DM oven[b]	28.3	4.0	22.2	34.9
DM corr[c]	31.1	3.1	26.7	36.5
	g.kg DM^{-1}			
Ethanol	54.2	48.1	5.0	154.5
Acetic acid	32.8	11.5	14.3	53.5
Lactic acid	26.0	20.9	6.5	60.4
	mg.kg DM^{-1} [d]			
Propane-1,2-diol	1532	2348	< 100	12186
Ethyl lactate	697	799	132	2401
Acetone	573	527	< 5	2072
Butane-2,3-diol	358	250	< 100	905
Propionic acid	284	350	< 100	1107
n-Butyric acid	273	369	< 100	1383
Ethyl acetate	167	174	< 5	597
2-Butanol	135	194	< 5	538
Methanol	133	359	< 100	1555
Propanol	123	81	< 5	290
iso-Butyric acid	< 100	55	< 100	274

[a]Standard deviation.
[b]Dry matter determined by oven drying (predrying at 55 °C for 72 h followed by drying at 105 °C for 12 h).
[c]Dry matter corrected for volatile compounds (Weissbach, 2009).
[d]iso-valeric acid, n-valeric acid, and caproic acid were below the limit of detection of 100 mg.kg DM^{-1};
1-butanol was below the limit of detection of 5 mg.kg DM^{-1}.

the correlation coefficients decrease with increasing pH. In conclusion it can be stated that the ester concentrations are strongly correlated with the ethanol concentration and the silage pH.

VOCs in sugar cane silages

In tropical areas ensiled sugarcane is important forage with more than 400 g.kg DM^{-1} water soluble carbohydrates which act as substrates for intensive fermentation (Daniel *et al.*, 2013a). Ethanol is the main fermentation end product in sugar cane silages (Kung and Stanley, 1982). Concerning feed intake Daniel *et al.* (2013b,c) reported no difference when fresh sugar cane silage was compared with oven-dried material resulting in the loss of volatiles, which was reconstituted with water before feeding. On the one hand ethanol has been correlated with esters and other volatile organic compounds (Weiss *et al*., 2009a) and, on the other, Kriszan *et al*. (2007) and Gerlach *et al*. (2013) observed negative correlations between some VOCs and feed intake.

Daniel *et al*. (2013a) found that the VOCs comprised up to 22 % of the sugarcane dry matter. Table 7 contains data concerning the occurrence of VOCs in sugarcane silages, without additives, with sodium benzoate, and with *Lactobacillus buchneri*. In addition to high contents of ethanol, acetic acid, and lactic acid, 1,2-propanediol, ethyl lactate, acetone, 2,3-butanediol, propionic acid, n-butyric acid, ethyl acetate, 2-butanol, methanol, propanol, and iso-butyric acid were also found (Daniel *et al*., 2013a).

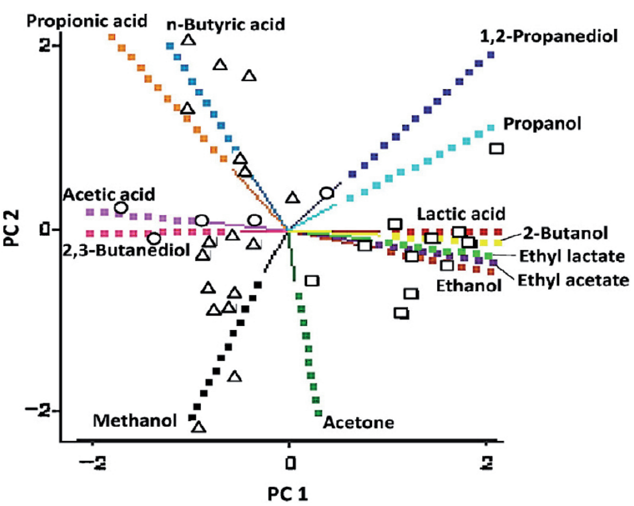

Fig. 2: Principal component analysis of volatile organic compounds in sugarcane silages. PC 1, first principal component (0.48); PC 2, second principal component (0.15). Silages were untreated (□), treated with sodium benzoate (Δ) and inoculated with *Lactobacillus buchneri* (O) (Daniel *et al.*, 2013a)

Daniel *et al*. (2013a) performed a statistical calculation with principal component analysis using PRINCOMP procedure of SAS (Figure 2). They postulated some functional relationships among the fermentation end-products in sugarcane silages. Ethanol was negatively associated with acetic acid and 2,3-butanediol, but positively correlated with lactic acid and esters.

EFFECTS OF SILAGE ADDITIVES ON FORMATION OF VOCs

Whole crop maize silage

The findings that VOCs are frequently found in silages and may detrimentally affect feed intake by dairy cattle (Weiss *et al.*, 2009a; Weiss and Auerbach, 2012a) have initiated more research with focus on the use of silage additives to reduce ethanol and ester formation. It is well known that silage additives can alter ethanol contents thereby exerting an effect on ethyl ester production. Weiss and Auerbach (2012b) tested the effects of chemical silage additives on fermentation pattern, production of VOCs and aerobic stability of maize silage. They found that treatment had significant effects on all parameters tested, except pH, which was very low in all silages (Table 8). DM losses were highest in acid treatments, whereas a significant reduction in DM loss was found by liquid mixture of sodium benzoate and potassium sorbate (SBPS). These observations can be explained by differences in ethanol concentrations, whose formation always results in CO_2 release, which escapes from the silo. The most significant reduction in ethanol was caused by the chemical additive SBPS, whereas acid additives (FAPA, FAPAP) stimulated ethanol production.

The concentration of ethyl esters in this study were also clearly affected by the concentration of ethanol and the respective organic acids. In general, lactate content was high, and SBPS increased the concentration of this fermentation acid over that of silages of all other treatments. Hafner *et al.* (2014, 2015) confirm these findings. They postulated that especially potassium sorbate is an effective additive for reducing production of ethanol and ethyl esters in corn silage. Acetic acid concentration was reduced by all used additives. As contents of lactic acid were higher than those of acetic acid, the formation of ethyl lactate was also more pronounced than that of ethyl acetate. SBPS decreased contents of ethanol, ethyl lactate (EL) and ethyl acetate (EA). FAPA and FAPAP stimulated the production of ethanol and EL, whereas no effect was found on EA. By using all experimental data from all individual silages of all treatments (n = 12), a very high correlation was found between ethanol and total ester concentrations (R^2 = 0.985). Elevated ethanol production in anaerobic conditions can be attributed to the activity of yeasts, which may have been present at

Table 8: Effects of silage additives on DM losses, fermentation pattern, VOCs and aerobic stability of whole-crop maize silage (DM 332 g.kg^{-1}); Weiss and Auerbach (2012b)

Parameter	Treatment				SED	Significance
	CON	SBPS[5]	FAPA[6]	FAPAP[7]		
DM loss (%)	6.5^{b}	4.3^{a}	7.5^{c}	7.5^{c}	0.31	***
WSC[1,2]	13.9^{a}	17.3^{ab}	20.5^{b}	19.2^{b}	1.28	**
pH	3.65	3.53	3.63	3.63	0.05	*
NH_3-N (g.kg^{-1} total N)	108^{a}	106^{a}	90^{b}	87^{b}	3.28	***
Lactic acid[2]	86.5^{a}	118.8^{b}	84.6^{a}	78.2^{a}	7.31	**
Acetic acid[2]	22.2^{b}	13.1^{a}	5.7^{a}	5.7^{a}	2.53	***
Propionic acid[2]	0.5^{a}	0.3^{a}	1.2^{b}	1.7^{c}	0.08	***
Ethanol[2]	23.2^{b}	6.5^{a}	49.1^{c}	46.5^{c}	1.57	***
1,2-propanediol[2]	0.3^{b}	0.4^{b}	0^{a}	0^{a}	0.08	***
Ethyl lactate[3]	398^{b}	166^{a}	617^{c}	612^{c}	39.3	***
Ethyl acetate[3]	223^{b}	123^{a}	189^{b}	184^{b}	16.1	**
Total ethyl esters[3]	621^{b}	289^{a}	806^{c}	795^{c}	31.3	***
ASTA[4] (days)	5.9^{a}	12.7^{b}	14.0^{b}	14.0^{b}	1.17	***

[1]water-soluble carbohydrates; [2]g.kg DM^{-1}; [3]mg.kg DM^{-1}; [4]aerobic stability; means in columns with unlike superscripts differ significantly at $P < 0.05$ (Tukey test); [5]liquid mixture of 21.9 % sodium benzoate and 13.2 % potassium sorbate, 2 l/t; [6]liquid mixture of 35 % formic and 12 % propionic acids, 25.5 % sodium formate, 1.5 % sodium benzoate, 4 L.t^{-1}; [7]liquid mixture of 48.8 % formic acid/ formate, 18.4 % propionic acid/propionate, 6.1 % sodium, 4 L.t^{-1}.

high numbers during the initial stages of fermentation but died off during later storage.

Further investigations by Weiss *et al*. (2015b, 2016) with maize confirmed that silage additives containing sodium benzoate, calcium propionate and potassium sorbate were superior to other treatments regarding suppression of ethanol and ester formation as well as improvement of aerobic stability, with and without air ingress.

Sorghum silages

A study of Auerbach and Weiss (2012) with sorghum silages aimed at testing the effects of different silage additives on dry matter (DM) losses, fermentation pattern, VOCs production and aerobic stability of this type of silages. Sorghum was chosen as silage type because it represents an important forage source for ruminants in semi-arid regions, and its production often bears the risk of excessive ethanol fermentation so that high concentrations of VOCs are to be expected.

Lactic and acetic acids were affected by variety and treatment, and an interaction was determined between the two factors for lactic acid (Table 9). Ethanol was reduced by *Lactobacillus buchneri* (LB) at all inoculation rates, and the lowest levels were consistently found if a mixture of sodium benzoate and potassium sorbate (BS) were used. The use of *Lactobacillus plantarum* (LP) alone or in combination with LB1 did not affect ethanol production when compared with control silages. The concentrations of reaction products of ethanol and organic acids – ethyl lactate and ethyl acetate – were affected by variety and treatment. Application of BS and LB (regardless of inoculation rate) caused the lowest ester contents, and no differences between CON, LP and LP+LB1 were found.

Grass silages

However, the knowledge of the formation of VOCs in grass silages and the effects of additives thereon is also still very limited. Weiss and Auerbach (2015) carried out a laboratory ensiling experiment with fourth-cut natural grassland, wilted overnight to 26.8 % DM. Forages received the treatment with 21 commercial additives (Table 10) which were obtained from the German marketplace and used according to the instructions of the manufacturers.

Grass silages were well fermented as reflected by low pH (Table 10) and no butyric acid was found (data not given). The production of lactic acid was stimulated by some additives of the types Ho, HoHe and HoCh whereas the pure He inoculant as well as two chemicals reduced it. The treatment with homofermentative LAB, either applied alone or in combination with antimycotic chemicals, always resulted in lower acetate levels. The lowest ethanol and ethyl ester contents were detected in silages that had received

Table 9: Effects of silage additives on volatile organic compounds of sorghum silages (Auerbach and Weiss, 2012)

Treatment	Lactic acid (g.kg DM^{-1})		Acetic acid (g.kg DM^{-1})		Ethanol (g.kg DM^{-1})		Ethyl esters[1] (mg.kg DM^{-1})	
Variety	Goliath	Maya	Goliath	Maya	Goliath	Maya	Goliath	Maya
CON[3]	92.0^{bA}	40.3^{cB}	24.9^{abA}	27.3^{bcA}	31.7^{cA}	34.2^{cdA}	381^{dA}	587^{dB}
LP[4]	90.2^{bA}	38.3^{bcB}	20.2^{aA}	22.0^{aA}	34.9^{cA}	28.8^{cA}	506^{dA}	586^{cdA}
LB[5]1	24.3^{aA}	22.8^{bA}	47.4^{bcB}	37.0^{abdA}	19.9^{bA}	19.5^{bA}	251^{cA}	414^{bcB}
LB[5]2	22.3^{aA}	24.6^{bA}	51.6^{cB}	45.5^{cdA}	18.9^{bA}	18.3^{bA}	245^{bcA}	365^{abcB}
LB[5]3	19.9^{aA}	17.8^{aA}	53.5^{cA}	51.8^{dA}	17.3^{bA}	19.6^{bA}	214^{abA}	299^{aB}
LP+LB1[6]	103.6^{bA}	26.9^{abcB}	24.3^{abA}	22.8^{abA}	34.2^{cA}	39.5^{dA}	460^{dA}	559^{dB}
BS[7]	95.4^{bA}	24.4^{bB}	27.6^{abA}	27.9^{bA}	6.9^{aA}	7.7^{aA}	131^{aA}	239^{abA}
SEM	8.18	1.83	3.05	2.46	2.23	2.31	31.1	33.2
Significance level[2]								
Variety	***		*		ns		***	
Treatment	***		***		***		***	
Variety x Treatment	***		ns		ns		ns	

[1]sum of ethyl acetate and ethyl lactate; [2]means in columns with unlike superscripts and means within rows bearing unlike capital superscripts differ significantly at $P \le 0.05$ (Tukey test); [3]Control; [4]*L. plantarum* , 1 x 10^5 cfu.g^{-1} forage; [5]*L.buchneri* (1 x 10^5 cfu.g^{-1} forage; 2 (2.5 x 10^5 cfu.g^{-1} forage), (5 x 10^5 cfu.g^{-1} forage); [6]*L. plantarum* + *L. buchneri* (2 x 10^5 cfu.g^{-1} forage); [7]500 g.t^{-1} sodium benzoate + 300 g.t^{-1} potassium sorbate (applied in 2 L.t^{-1} aqueous solution)

Table 10: Effects of additives on fermentation pattern, volatile organic compounds and aerobic stability of grass silage stored for 72 days (Weiss and Auerbach, 2015)

Treatment	pH	Lactic acid[1]	Acetic acid[1]	Ethanol[1]	EE[2,3]	Propanol[3]	Acetone[3]	Methanol[3]	2-Butanol[3]	AS[4]
Con[5]	4.0	82.0	14.1	10.2	344	236	0	697	205	7.4
Ho[6]	3.9*	85.1	7.9*	8.7	301	0*	119*	789	224	3.3*
Ho[6]	3.9*	96.0*	11.0§	9.0	316	23*	131*	844§	222	7.0
Ho[6]	3.8*	87.2	7.9*	7.5$^{\#}$	258	0*	109*	796	212	2.3*
Ho[6]	3.8*	90.9§	8.5*	8.2§	284	0*	108*	845§	128§	1.8*
Ho[6]	3.8*	91.2§	7.6*	7.5*	309	0*	99*	686	152	2.7*
Ho[6]	3.8*	88.6	8.9*	7.7$^{\#}$	261	0*	89*	694	160	4.3§
He[7]	4.1*	61.1*	22.8*	13.4*	353	1080*	100*	817	195	8.8
HoHe[8]	3.9$^{\#}$	85.2	14.0	10.5	384	44*	126*	828	208	6.3
HoHe[8]	3.9*	79.6	11.3	8.7	342	100§	76*	816	197	7.2
HoHe[8]	3.9*	86.8	11.3	8.8	411	72$^{\#}$	111*	878$^{\#}$	130§	6.4
HoHe[8]	3.9*	96.2*	10.2§	7.8$^{\#}$	329	0*	108*	853§	214	5.4
HoHe[8]	3.9*	89.0	12.3	8.8	327	78$^{\#}$	99*	786	196	6.7
HoCh[9]	3.9*	93.2§	10.3§	9.8	300	0*	18	641	155	7.4
HoCh[9]	3.9*	81.7	10.2§	8.9	292	0*	24	660	187	8.1
HoCh[9]	3.9*	85.0	9.5$^{\#}$	8.6	272	0*	69*	692	179	6.8
HoCh[9]	3.9*	87.0	7.7*	7.5*	208	0*	51*	598	202	7.3
HoCh[9]	3.9*	84.2	8.6*	7.5*	265	0*	97*	729	241	10.9§
Ch[10]	4.0$^{\#}$	72.4§	16.5	2.3*	80*	499*	0	809	161	15.0*
Ch[11]	4.0	77.9	15.6	4.2*	143*	165*	0	611	153	15.0*
Ch[12]	4.0	61.0*	11.6	4.5*	105*	0*	0	583	164	14.1*
Ch[12]	3.9*	78.1	11.5	3.2*	61*	0*	0	630	209	15.0*

Means of each additive treatment in columns bearing unlike superscripts differ compared with untreated; $^{*}P < 0.001$, $^{\#}P < 0.01$, $^{\S}P < 0.05$; [1]g.kg DM^{-1}; [2]ethyl lactate + ethyl acetate; [3]mg.kg DM^{-1}; [4]aerobic stability, days; [5]untreated; [6]homofermentative LAB; [7]heterofermentative LAB; [8]combination of homo- and heterofermentative LAB; [9]combination of homofermentative LAB and antimycotic chemical(s); [10]nitrite, hexamine, sorbate; [11]nitrite, benzoate, sorbate; [12]buffered formic and propionic acid blends.

chemical additives. There was a strong positive linear correlation between these two parameters (R^2 = 0.72, P < 0.001). The production of 1-propanol was the highest in silages treated with the heterofermentative inoculant.

Legume silages

Investigations concerning the effect of wilting and silage additives on silage quality of lucerne, red clover and grass mixtures (Weiss and Kalzendorf, 2016) demonstrated the occurrence of VOCs in legume silages. The DM content and silage additives affect the concentrations of alcohols, acids and esters. However, yeast counts were high and increased during wilting period. In accordance to the fact that under anaerobic conditions yeasts are responsible for ethanol formation, the ethanol content in silages without any additives was between 4.8 and 10.9 g.kg DM^{-1} with a strong negative correlation to DM content (R^2 = 0.81) and positive correlation to ester content (R^2 = 0.65). Therefore elevated levels of alcohols and esters occur in silages with low DM. The total esters ranged between 124 und 197 mg.kg DM^{-1} in untreated silages and consisted of only ethyl lactate. These ester contents are comparable with contents in grass silage (Weiß and Auerbach, 2013) considering the pH level between 4.0 and 6.3. Silage additives with LAB did not primarily affect the contents of ethanol, the same applies for the contents of esters. The additive salts containing benzoate, nitrite and hexamine strongly reduced the ethanol and ester contents. According to Woolford (1975) these substances are able to inhibit yeasts and possibly heterofermentative LAB which also produce ethanol.

Sugar cane silages

The study of Cardoso *et al*. (2016) to evaluate the chemical composition, fermentation pattern and microorganisms of sugar cane without and with chemical additives and inoculants (Figure 3) confirmed that a correlation between ethanol and ethyl esters is strong. In sugarcane silages with CaO this chemical additive inhibited ethanol and ester formation.

White lupin-wheat silages

Laboratory ensiling trials with white lupin-wheat silages (König *et al*., 2015) demonstrated the occurrence of volatile compounds, also esters, in this special ensiling material. The authors found that increased proportion of lupin increased the concentration of VOCs and confirmed the safe effect of chemical additives due to their influence on fermentation pattern.

Summary

Results from ensiling experiments on the effects of silage additives on ester formation in different ensiling materials clearly indicated that chemical products containing active ingredients with specific antifungal effects can significantly reduce ester concentration. Salts of sorbic, benzoic or propionic acids or mixtures are effective treatment for reducing VOCs production.

Buffered formic acid-containing products, which were always applied at 4 L.t^{-1} stimulated it due to an increase in ethanol content (Weiss and Auerbach, 2012b; Auerbach and Weiss, 2012).

VOCs IN MAIZE SILAGES IN GERMAN DAIRY FARMS

A survey has been carried out to investigate the incidence of VOCS in maize silages from German dairy farms and to monitor the concentrations of ethanol, n-propanol and the corresponding esters ethyl acetate, ethyl lactate and propyl acetate, depending on the sampling site in the silo and the compaction of silages (Weiss *et al*., 2015a).

The survey included a detailed examination of silages stored in bunker silos on 52 dairy farms. Most silages were produced without silage additives (n = 43), whereas 9 farms had used biological additives. The highest contents of fermentation acids (acetic, lactic and propionic acids) and alcohols (methanol, ethanol, n-propanol) in maize silages were found in the bottom, highly compacted core and to some extent in middle core samples taken from bunker silos (Table 11), which supports empirical observations by Weiss *et al*. (2009a). Ethanol was detected at up to 17.8 g.kg DM^{-1} and the highest n-propanol level was 20.2 g.kg DM^{-1} (Figure 4a). In agreement with data by Weiss *et al*. (2009a), ethyl lactate (EL) concentrations in maize silages were higher than the levels of ethyl acetate (EA) and propyl acetate (PA) (Figure 4b). The contents of total esters (up to 925 mg.kg DM^{-1}) were higher than in silages from laboratory ensiling trials (Weiss *et al*., 2009a). With increasing compaction, the concentrations of n-propanol and ethanol as well as those of the ethyl esters EA and EL (Figure 4) and aerobic stability (R^2 = 0.920, P < 0.001) increased (data not shown). This may be explained by the usually lower pH in the bottom, more compacted and less air-affected zones in farm silos. Esterification processes were shown to be stimulated by low pH (Weiss and Auerbach, 2013).

Table 11: Fermentation characteristics of maize silages on 52 German dairy farms in different sections of bunker silos (mean ± SEM, g.kg DM^{-1} unless otherwise stated) (Weiss *et al*., 2015a)

Parameter	BC[4]		MC[5]		TE[6]		*P*-Value
DM (%)	34.1	± 0.5	33.4	± 0.4	34.0	± 0.5	0.950
pH	3.85[a,b]	± 0.18	3.83[a]	± 0.02	3.89[b]	± 0.03	0.036
Lactic acid	49.3[b]	± 2.6	51.4[b]	± 1.9	41.8[a]	± 2.1	0.001
Acetic acid	23.0[b]	± 1.2	19.5[a]	± 0.9	19.6[a]	± 1.0	0.009
Prop. acid[1]	0.8[b]	± 0.2	0.4[a]	± 0.1	0.6[a,b]	± 0.1	0.028
Methanol	0.3[b]	± 0.0	0.2[a]	± 0.0	0.3[b]	± 0.0	0.008
Ethanol	6.9[b]	± 0.5	5.9[a,b]	± 0.4	5.1[a]	± 0.4	0.001
2-Butanol	0.2[b]	± 0.1	0.2[a,b]	± 0.1	0.1[a]	± 0.0	0.015
n-Propanol	4.4[b]	± 0.7	2.7[a]	± 0.5	2.1[a]	± 0.4	0.001
Ethyl acetate[2]	51[a,b]	± 4	40a	± 3	59[b]	± 5	0.007
Ethyl lactate[2]	210[b]	± 17	176[a,b]	± 15	150[a]	± 14	0.003
Propyl acetate[2]	44	± 17	30	± 7	46	± 16	0.626
Total esters[2]	305	± 24	246	± 18	255	± 24	0.080
Ammonia	1.3[b]	± 0.0	1.1[a]	± 0	1.1[a]	± 0.0	< 0.001
WSC[3]	8.2[a]	± 0.7	10.5[b]	± 1.0	9.9[a]	± 0.7	0.001
AS (d)	7.2	± 4.8	6.6	± 4.1	6.3	± 4.2	0.2613
Yeasts (log cfu.g FM^{-1})	4.7[a]	± 4.6	6.2[b]	± 5.9	6.1[b]	± 5.8	< 0.001
Compaction (kg.m^{-3})	256	± 5.6	226	5.8	217	± 5.9	< 0.001

[1]Propionic acid; [2]mg.kg DM^{-1}, [3]water-soluble carbohydrates; [4]Bottom core; [5]Middle core; [6]Top edge; means in rows with unlike superscripts differ at $P < 0.05$ (Tukey's test).

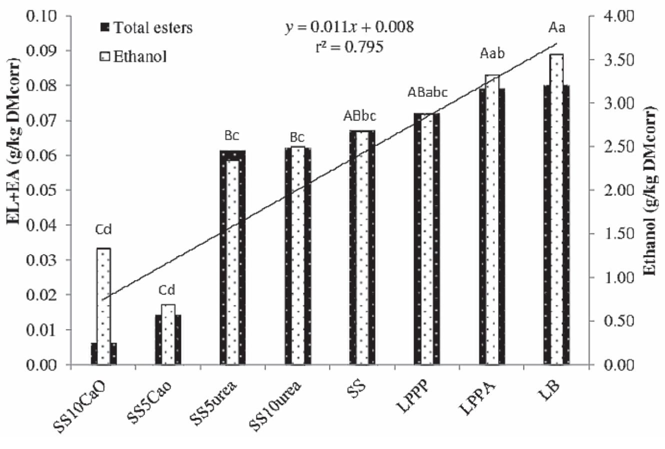

Fig. 3: Correlation between ethyl acetate and ethyl lactate (EL + EA) and ethanol contents in silage, averages in g.kg^{-1} of DMcorr. Sugarcane silage without inoculant (SS), SS with *Lactobacillus buchneri* (LB), SS with *Lactobacillus plantarum* and *Pediococcus pentosaceus* (LPPP), SS with *Lactobacillus plantarum* and *Propionibacterium acidipropionici* (LPPA), SS with 5 g.kg^{-1} CaO (SS5CaO), SS with 10 g.kg^{-1} CaO (SS10CaO), SS with 5 g.kg^{-1} urea (SS5urea), and SS with 10 g.kg^{-1} urea (SS10urea) (Cardoso *et al*., 2016)

ESTIMATION OF ESTER CONTENT

Based on a total of 1148 data sets from grass silages (Weiss and Auerbach, 2013) as well as from silages from whole-crop maize, whole-crop wheat, sorghum, high-moisture corn (Weiss and Auerbach, 2012a), a regression model was used to describe the relationship between total ester and ethanol concentrations, which is valid for all silage types. As shown in figure 5, each incremental increase in ethanol content by 5 g.kg DM^{-1} resulted in increased total ester concentration by 114 mg.kg DM^{-1} ($R^2 = 0.76$). Therefore, the following equation can be applied to calculate ester concentration in silages based on their ethanol content: predicted total ester concentration [mg.kg DM^{-1}] = ethanol concentration [g.kg DM^{-1}] x 114/5. The use of this predictive model offers the possibility to avoid laborious and expensive chemical ester analyses.

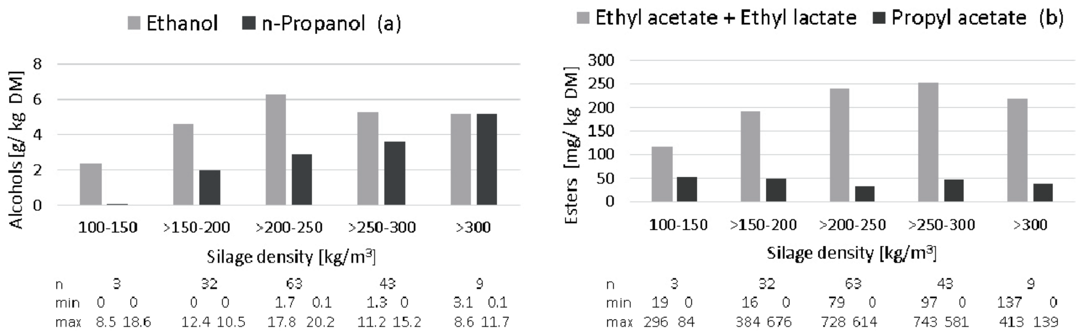

Fig. 4: Average concentrations of ethanol and n-propanol (a) and the esters ethyl acetate + ethyl lactate and propyl acetate (b) as affected by silage density (Weiss *et al.*, 2015a).

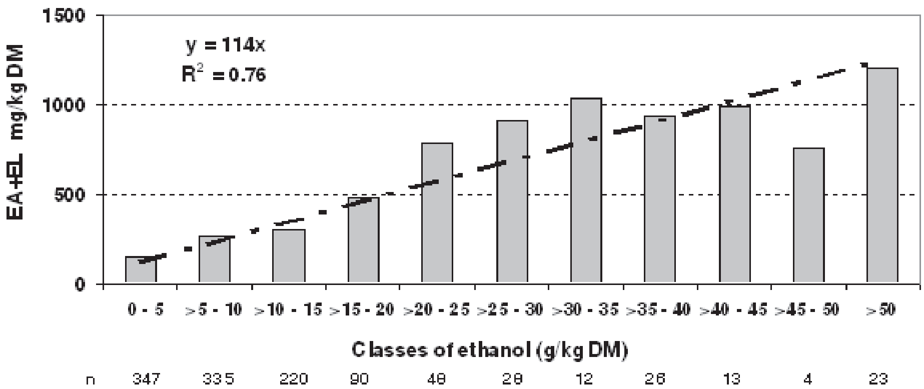

Fig. 5: Average total content of esters (ethyl acetate and ethyl lactate) in classes of ethanol in silages from whole-crop maize, whole-crop wheat, sorghum, high-moisture corn and grass (n = 1148) (Weiss and Auerbach, 2013)

CONCLUSIONS

With regard to the current body of evidence on VOCs formation in silages and their potential negative impact on feed intake in dairy cows and goat it can be stated that the reduction in ethanol production may lead to lower levels of ethyl esters. This is substantiated by data from ensiling experiments on the effects of different silage additives on ester formation in maize, grass, legume and sorghum silages. Only chemical products containing active ingredients with specific antifungal properties (sodium benzoate, potassium sorbate) consistently and significantly reduced ethyl ester concentrations.

ACKNOWLEDMENTS

I deeply acknowledge the expert technical support by the staff of the Common Laboratory of Analysis, Humboldt Universität zu Berlin, especially by Gabriele Sommer and by Manuela Alt. I would like to thank Horst Auerbach, Christine Kalzendorf, Elisabet Nadeau, Luis Gustavo Nussio, Johannes Thaysen and ADDCON GmbH, Bonn, Germany, for partial funding of the studies.

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