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Acetic Acid Increases Stability of Silage under Aerobic Conditions

H. Danner,* M. Holzer, E. Mayrhuber, and R. Braun

Department of Environmental Biotechnology, Institute for Agrobiotechnology (IFA-Tulln), A-3430 Tulln, Austria

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The effects of various compounds on the aerobic stability of silages were evaluated. It has been observed that inoculation of whole-crop maize with homofermentative lactic acid bacteria leads to silages which have low stability against aerobic deterioration, while inoculation with heterofermentative lactic acid bacteria, such as *Lactobacillus brevis* or *Lactobacillus buchneri*, increases stability. Acetic acid has been proven to be the sole substance responsible for the increased aerobic stability, and this acid acts as an inhibitor of spoilage organisms. Therefore, stability increases exponentially with acetic acid concentration. Only butyric acid has a similar effect. Other compounds, like lactic acid, 1,2-propanediol, and 1-propanol, have been shown to have no effect, while fructose and mannitol reduce stability.

Ensilage of green forage is a traditional way to conserve animal feed, and this process is gaining importance and is replacing hay production and direct feeding of green forage. The technology is simple and includes compression of the forage, followed by airtight sealing. Subsequently the autochthonous lactic acid bacteria convert the free sugars into lactic acid. Nowadays, the use of starter cultures is increasing in order to produce high-quality feed and to ensure that there is an immediate decrease in the pH to prevent growth of undesirable microorganisms, such as clostridia. Clostridia not only form butyric acid but are also responsible for the degradation of proteins to ammonia. An increase in pH and large decreases in feeding value are the results. Furthermore, clostridia form spores, which is an important criterion of hygienic quality of silages with respect to production of milk suitable for cheese processing, as the spores cause late blowing in hard cheeses.

After silos are opened for feeding, air penetrates the silages and promotes the growth of aerobic, acid-tolerant microorganisms and the oxidation of fermentation products present in the silages. This so-called aerobic deterioration can cause spoilage of silages and may lead to potentially toxic substances or undesirable microorganisms.

The stability of silages against aerobic deterioration (aerobic stability) can vary dramatically. However, the mechanisms that prevent aerobic spoilage are not well understood. Weinberg et al. (20) investigated several homofermentative lactic acid bacteria to determine their effects on aerobic stability when they were used as silage inocula. The inoculated silages tended to spoil even faster than the control silage. O'Kiely and Muck (15) stated that aerobic stability is not related to the silage dry matter content, pH, yeast number, or glucose addition at the time of ensiling, while the effects of organic acids, such as acetic acid, lactic acid, and propionic acid, have been described to some extent (1, 13). Other authors have described the positive aspect of the formation of acetic acid by heterofermentative lactic acid bacteria, which inhibits spoilage organisms (5, 18, 19).

In silage experiments conducted at our institute, it was observed that inoculation of silages with *Lactobacillus buchneri* results in silages which are very stable against aerobic deterioration (7). Therefore, the objective of the present study was to determine the major factors that have an impact on the aerobic stability of silages.

MATERIALS AND METHODS

Cultivation and maintenance of lactic acid bacteria. All lactic acid bacterial strains were isolated from different grass silages which were provided by farmers from lower and upper Austria. An aqueous extract of silage material was made, and this extract was then diluted, plated on MRS agar (Oxoid), and incubated at 37°C for 48 h. Single colonies were subcultured on MRS agar and in MRS broth (Oxoid). Then 15% (vol/vol) glycerol was added to the microbial solutions, and the solutions were incubated for 30 min. Each mixture was dispensed into cryovials and frozen at −80°C. Single isolates were identified by using API 50CH strips (bioMerieux, Marcy l'Etoile, France) and molecular biological methods, such as sodium dodecyl sulfate-polyacrylamide gel electrophoresis of cell proteins and randomly amplified polymorphic PCR (10). Subcultures of bacteria were grown in MRS broth under anaerobic conditions with a nitrogen atmosphere. Flasks were closed tightly and gently shaken while they were incubated at 37°C.

Silage inoculants were prepared in MRS broth in 1-liter bottles under anaerobic conditions. After 48 h of incubation, each suspension was concentrated by centrifugation at 16,000 × *g* for 20 min. The concentration of the cell suspension was adjusted to an optical density at 600 nm of 150 to 250. The cell suspension was frozen at −80°C and freeze-dried. The dry powder was ground and stored under dry conditions (plastic tubes) at 4°C until it was used. CFU were investigated by using the plate count method.

Silage preparation. Chopped whole-crop maize provided by a local farmer was used for all ensilage experiments. The silages described below were prepared in 6.5-liter plastic laboratory silos. Strains used for inoculation were dissolved in water and applied with a commercial hand sprayer (1 liter). Plastic bags were placed into the small basket silos to achieve optimal sealing. Each silage was compressed with a pneumatic press which was adjusted to a pressure of 8×10^5 Pa. The plastic bags were closed with cable ties after they were properly filled and compressed three times. The baskets were sealed with lids and were fermented at a constant temperature of 20°C. For each experiment one untreated lot of fresh mass was ensiled as a control. All samples were prepared in duplicate.

Aerobic stability of silages was determined by using the System Völkenrode (8). This method is based on monitoring temperature increases due to microbial activity of samples exposed to air. An apparatus that included KTY 1000 resistant thermometers (RS Components Handels GmbH, Gmünd, Austria) and on-line data acquisition for measuring up to 100 samples at the same time was constructed at our institute and used to determine aerobic stability. Silage was

* Corresponding author. Mailing address: Department of Environmental Biotechnology, Institute for Agrobiotechnology (IFA-Tulln), Konrad Lorenz Straße 20, A-3430 Tulln, Austria. Phone: 43-2272-66280-558. Fax: 43-2272-66280-503. E-mail: danner@ifa-tulln.ac.at.

TABLE 1. Silages used for blending experiments

Silage	Inoculant	Concn (g kg [dry wt]$^{-1}$) of:						% Dry matter	pH	Aerobic stability (h)	Test substances
		Glucose	Fructose	Mannitol	Lactic acid	Acetic acid	1,2-Propanediol				
EKX-1		2.9	4.7	30.2	52.1	12.8	1.4	31.0	3.74	35	Acetic acid, 1,2-propanediol
EKX-2		0.7	6.6	20.8	41.6	17.7	2.4	34.9	3.74	65	Butyric acid, acetic acid, 1-propanol
EKX-3	*L. buchneri*	0.1	3.4	0.5	3.9	58.5	27.2	37.0	4.31	181	Fructose, mannitol
EKX-4	*L. plantarum*	0.7	5.3	3.9	44.2	9.5	0.0	39.3	3.85	20	Fructose, mannitol

deemed to be stable until the internal temperature was more than 2°C above the ambient temperature.

Blending of silages. Different whole-crop maize silages were used for blending experiments and had the characteristics shown in Table 1. Silages EKX-1 and EKX-2 were provided by local farmers, and silages EKX-3 and EKX-4 were prepared by the method described above. While EKX-1 and EKX-2 were not inoculated, EKX-3 was inoculated with *L. buchneri* and EKX-4 was inoculated with *Lactobacillus plantarum*. Test substances were sprayed on the silage with a conventional hand sprayer at concentrations which have been observed in natural silages. After proper mixing, aerobic stability was determined as described above. A control was prepared by adding the same amount of water. All samples were prepared with two replicates.

Inhibitor test system. Two yeast strains (*Candida krusei* ATCC 62403 and *Pichia subpelliculosa* ATCC 16766) were used as indicator organisms for silage spoilage. The yeasts were grown on nutrient broth no. 2 (obtained from Oxoid) for 48 h; 15% (vol/vol) glycerol was added to the microbial suspensions, which were incubated for 30 min before they were frozen at −80°C until they were used. Strains were defrosted and grown on nutrient broth under aerobic conditions at 30°C for 24 h. Subcultures were grown in MRS broth at pH 4.0 to adapt the cells to the medium. After 24 h each suspension was centrifuged (3,000 rpm, 15 min; Beckman GS-6) and resuspended in fresh MRS broth at a final concentration of 10^5 cells per ml by using a Thomakammer (Brand GmbH, Wertheim, Germany).

The growth inhibition test itself was performed in 96-well microtiter plates (Greiner bio-one GmbH, Kremsmünster, Austria). For yeast experiments, in each well 150 μl of test substance or an NaCl solution (positive control well) was added to a 50-μl indicator organism suspension, resulting in a final concentration of 25,000 cells per well. In the blank well (total volume, 200 μl) an NaCl solution was added instead of the yeast solution. Three replicates were prepared for each test substance. The test plates were examined with an enzyme-linked immunosorbent assay reader (Spectrafluor plus; Tecan, Grödig, Austria) to determine the optical density at 650 nm at zero time (to obtain a background value for the calculations) and then incubated at 30°C while they were gently shaken in a humidified atmosphere. Optical density measurements were taken each 24 h (up to 72 h). At the end of the experiment the percentage of growth inhibition was calculated. The growth was related to the growth in the wells containing 150 μl of an NaCl solution plus 50 μl of a yeast solution, which was defined as the 100% growth value.

Aspergillus niger IMI 096215 was grown on YGC agar (Merck) at 30°C for 4 to 10 days until sporulation occurred. Spores were harvested with a sterile 0.005% (vol/vol) aqueous solution of Triton X-100 before they were frozen at −80°C. The growth inhibition test itself was performed in 96-well microtiter plates. In each well 100 μl of test substance or of an NaCl solution (control well) was added to 100 μl of MRS bouillon (adjusted to pH 4.0) and 50 μl of a fungal spore suspension. Measurements were obtained as described above for yeasts. The growth was related to the growth in the wells containing 100 μl of an NaCl solution and 100 μl of MRS bouillon plus 50 μl of a spore suspension, which was defined as the 100% growth value.

Chemical analysis. Silage was extracted by using a laboratory homogenizer (IUL Instruments Masticator Silver no. 0420/0520) and distilled water at a ratio of water to silage material (fresh weight) of 1:5. After 4 min of extraction at 8 strokes per second, the liquid was decanted into centrifugation tubes and centrifuged at 16,000 × *g* for 20 min. The supernatant was filtered (0.2-μm-pore-size membrane filter; Pall Gellman Sciences) and analyzed with a high-performance liquid chromatograph (Hewlett-Packard HP1100) equipped with a refractive index detector (HP 1047A). A Polyspher OA KC RT300-700 column (Merck) was used at 42°C. The flow rate of the mobile phase (0.01 N H_2SO_4) was 0.4 ml min^{-1}.

Dry weight was determined by heating material at 105°C for 24 h. The pH of silage was measured by using 20-g samples homogenized with 80 ml of water after 30 min.

RESULTS

Effects of different homo- and heterofermentative lactic acid bacteria on whole-crop maize. Whole-crop maize with 9.5 × 10^6 CFU of epiphytic lactic acid bacteria per g (fresh weight) was inoculated with different homo- and heterofermentative lactic acid bacteria (7.0 × 10^8 CFU g [fresh weight]$^{-1}$) and compared to a control to which bacteria were not added. After 74 days of fermentation at room temperature, the silos were opened, and the metabolites (organic acids and alcohols), pH, sugars, and aerobic stability were analyzed.

The results are shown in Table 2. In all batches glucose was degraded after 74 days (data not shown), while minor amounts of fructose were still detectable. The major fermentation products were lactic acid, acetic acid, and mannitol. Butyric acid was formed only in the uninoculated control silage, while sig-

TABLE 2. Sugar and bacterial metabolite contents of whole-crop maize silage treated with different homo- and heterofermentative lactic acid bacteria after 74 days of fermentation

Inoculant	pH	Concn (g kg [dry wt]$^{-1}$) of:						Aerobic stability (h)
		Fructose	Mannitol	Lactic acid	Acetic acid	Butyric acid	1,2-Propanediol	
Whole-crop maize	5.80	44.0	ND[a]	1.0	0.9	ND	0.2	ND
Control	3.81	4.4	30.1	42.9	16.5	2.8	0.1	40
L. rhamnosus 147	3.84	3.0	15.2	34.3	9.6	ND	0.2	31
P. pentosaceus 159	3.88	3.3	9.9	31.9	8.4	ND	0.6	31
L. plantarum 268	3.83	3.7	3.0	34.8	8.1	ND	0.3	26
L. plantarum 322	3.86	5.3	4.9	35.4	9.0	ND	0.1	29
L. buchneri 218	4.11	2.4	0.3	1.4	55.3	ND	11.6	274
L. brevis 312	3.86	0.7	4.9	26.1	28.6	ND	ND	72

[a] ND, not detectable (below the detection limit).

TABLE 3. Aerobic stabilities of whole-crop maize silages EKX-1 and EKX-2 blended with various substances at different concentrations

Silage	Additive	Amt added (g kg [dry wt]$^{-1}$)	Concn determined (g kg [dry wt]$^{-1}$)	Aerobic stability (h)
EKX-1	Acetic acid (buffered)	22.2	35	57
EKX-1	Acetic acid (buffered)	44.4	57.2	92
EKX-1	Acetic acid (buffered)	66.7	79.5	150
EKX-1	Acetic acid (pure)	22.2	35	79
EKX-1	Acetic acid (pure)	44.4	57.2	106
EKX-1	Acetic acid (pure)	66.7	79.5	339
EKX-1	1,2-Propanediol	11.1	12.5	35
EKX-1	1,2-Propanediol	22.2	23.6	35
EKX-1	1,2-Propanediol	44.4	45.8	39
EKX-2	Butyric acid	5	5	81
EKX-2	Butyric acid	10	10	184
EKX-2	Butyric acid	20	20	188
EKX-2	Acetic acid (pure)	20	37.7	139
EKX-2	Acetic acid (pure)	40	57.7	186
EKX-2	1-Propanol	3	4	65
EKX-2	1-Propanol	5	6	58
EKX-2	1-Propanol	10	11	65
EKX-2	1-Propanol	20	21	115

nificant amounts of 1,2-propanediol were detected in the silage inoculated with *L. buchneri*. Silages inoculated with homofermentative lactic acid bacteria (*Lactobacillus rhamnosus*, *Pediococcus pentosaceus*, *L. plantarum*) had pH values around 3.8 and lactic acid concentrations between 31.9 and 35.4 g kg (dry weight)$^{-1}$. In the same silages the acetic acid concentrations were significantly lower than the concentration in the control batch, which was not inoculated, and the aerobic stabilities ranged from 26 to 31 h, which were lower than the aerobic stability in the control (40 h).

In contrast, inoculation with heterofermentative lactic acid bacteria (*L. buchneri*, *Lactobacillus brevis*) led to silages which were much more stable against aerobic deterioration. High concentrations of acetic acid were observed, especially in silages inoculated with *L. buchneri* (55.3 g kg^{-1}) and with *L. brevis* (28.6 g kg^{-1}). The final pH of the silage inoculated with *L. buchneri* was 4.11, which was the highest value observed. In the same silage, almost no lactic acid was found, but in addition to acetic acid there were significant amounts of 1,2-propanediol, indicating that aerobic stability is not determined by the concentration of lactic acid or the final pH.

Evaluation of the effect of blending with different substances on preservation of silage. During silage fermentation several compounds are produced. Therefore, it is difficult to evaluate the potential of a single substance to inhibit spoilage microorganisms in silage experiments. In this work we attempted to do this by adding single substances at concentrations which have been observed in natural silages, before the aerobic stability was measured. A comparison with the control was used to quantify the effects of each substance.

Whole-crop maize silage produced without addition of starter cultures was used for this set of experiments to determine the effects of single compounds on the aerobic stability of silages. Different substances, such as acetic acid, butyric acid, 1,2-propanediol, 1-propanol, mannitol, and fructose, were added to the silage at concentrations which can be observed in natural silages before the aerobic stability was measured.

The effects of different concentrations of acetic acid and 1,2-propanediol on the aerobic stability of whole-crop maize silage EKX-1 are shown in Table 3. Untreated silage containing acetic acid at a concentration of 12.8 g kg^{-1} and 1,2-propanediol at a concentration of 1.4 g kg^{-1} was stable for 35 h. Addition of buffered acetic acid solutions resulted in clear increases in stability up to 150 h at a concentration of 79.5 g kg^{-1}, while addition of pure acetic acid at the same concentration dramatically increased the stability to 339 h. Similar effects of acetic acid were obtained with whole-crop maize silage EKX-2 (Table 3). The control containing acetic acid at a level of 18 g kg^{-1} exhibited an aerobic stability of 65 h, while addition of 20 g kg^{-1} and addition of 40 g kg^{-1} increased the stability to 74 and 121 h, respectively. 1,2-Propanediol had no effect at concentrations up to 23.6 g kg^{-1}; at a concentration of 45.8 g kg^{-1} the stability was comparable to that of the untreated silage. However, such high concentrations of 1,2-propanediol have never been found in silages.

Addition of 5 g of butyric acid per kg (dry weight) of silage improved the aerobic stability to 16 h, while 10 g kg^{-1} resulted in an aerobic stability of more than 180 h. This proves that butyric acid is even more effective for stabilizing silages against aerobic deterioration. However, butyric acid in silages is an indicator of contamination with clostridia, which is not desirable in silages at all.

Another substance investigated was 1-propanol. The increase in temperature above the 2°C limit was more or less equal to the increase in the control silage up to a 1-propanol concentration of 10 g kg^{-1}. Addition of more than 20 g of 1-propanol per kg (dry weight) of silage increased the stability from 65 to 115 h. However, such a high level of 1-propanol has never been found in natural silages.

Addition of mannitol or fructose to silages reduced the aerobic stability. This conclusion was reached by blending two completely different silages with mannitol or fructose. Silage EKX-3 was a silage which had been fermented with *L. buchneri*, while EKX-4 was a silage obtained by fermentation with *L. plantarum*. It was observed that addition of 10 g of mannitol kg^{-1} to silage EKX-3 containing a high level of acetic acid (58.5 g kg^{-1}) with an aerobic stability of more than 180 h reduced the stability to 80 h (Table 4). The same amount of

TABLE 4. Effects of addition of mannitol and fructose on the aerobic stabilities of silages obtained by fermentation with *L. buchneri* (EKX-3) and *L. plantarum* (EKX-4)

Silage[a]	Additive		Aerobic stability (h)
	Compound	Concn (g kg [dry wt]$^{-1}$)	
EKX-3			181
EKX-3	Fructose	10	103
EKX-3	Fructose	20	103
EKX-3	Mannitol	10	80
EKX-3	Mannitol	20	82
EKX-4			20
EKX-4	Fructose	10	15
EKX-4	Fructose	20	16
EKX-4	Mannitol	10	15
EKX-4	Mannitol	20	15

[a] Silage EKX-3 contained 58.5 g of acetic acid kg (dry weight)$^{-1}$, 0.5 g of fructose kg (dry weight)$^{-1}$, and 3.4 g of mannitol kg (dry weight)$^{-1}$. Silage EKX-4 contained 9.5 g of acetic acid kg (dry weight)$^{-1}$, 3.9 g of fructose kg (dry weight)$^{-1}$, and 5.3 g of mannitol kg (dry weight)$^{-1}$.

mannitol had a similar effect in an already unstable silage (EKX-4) with a low acetic acid concentration (9.5 g kg^{-1}) and reduced the stability from 20 to 15 h. With the same two silages, addition of 10 g of fructose kg^{-1} decreased the stability from 180 to 103 h and from 20 to 15 h, respectively (Table 4). Therefore, we concluded that both mannitol and fructose are suitable carbon sources for spoilage microorganisms like yeasts or fungi. However, a higher concentration of mannitol or fructose (20 g kg^{-1}) gave the same stabilities that were observed with 10 g kg^{-1}. This indicates that 10 g of mannitol kg^{-1} or 10 g of fructose kg^{-1} is adequate to overcome substrate limitation for the spoilage organisms and that the growth rate is determined only by the inhibitors present in silage.

Inhibition of spoilage microorganisms by selected compounds. Different groups of microbes are known to act as spoilage organisms, especially after a silo is opened and the silage is exposed to air. Important strains of spoilage organisms belong to the yeast and mold groups (11). An experiment was designed to evaluate the potential of substances produced by lactic acid bacteria (lactic acid and acetic acid, as well as 1,2-propanediol) to inhibit known silage spoilage organisms. A growth inhibition test was performed with certain test organisms which are known to influence the aerobic stability of silages. Selected strains, including two yeasts (*C. krusei* ATCC 62403 and *P. subpelliculosa* ATCC 16766) and one mold (*A. niger* IMI 096215), were purchased from strain collections and tested for inhibition by different substances as described above.

Figure 1 shows the results of the growth inhibition test with yeasts performed at pH 4. In some cases at low substance concentrations the growth of the indicator organisms was more than 100%. This might have been due to the ability of the indicator organisms to degrade the test substance. Acetic acid was the substance which had the greatest inhibitory effect on yeast growth. An acetic acid concentration of 20 g liter^{-1} in the test mixture was enough to completely inhibit the growth of the selected yeasts at pH 4. Also, some inhibitory effects of lactic acid were observed. However, only growth of *P. subpelliculosa* was totally inhibited at concentrations around 50 g liter^{-1}. Growth of *C. krusei* was even improved by lactic acid, which indicates that this yeast is able to degrade lactate at pH 4.0. No negative effect of 1,2-propanediol on the growth of the yeasts investigated was observed.

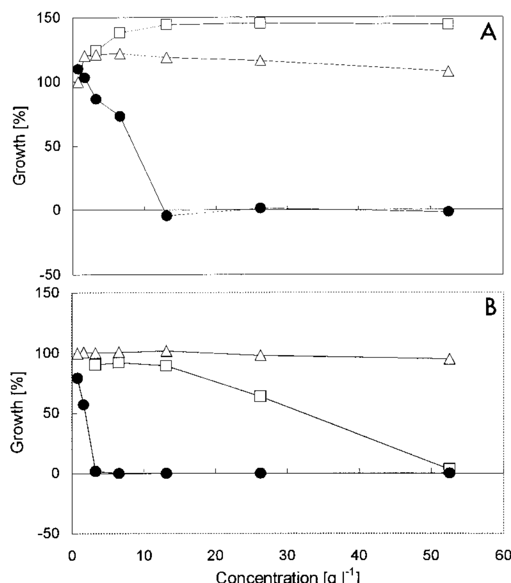

FIG. 1. Effects of single substances on the yeasts *C. krusei* ATCC 62403 (A) and *P. subpelliculosa* ATCC 16766 (B) at pH 4. Symbols: □, lactic acid; △, 1,2-propanediol; ●, acetic acid.

The results obtained in the experiments with *A. niger* were quite similar (Fig. 2). Again, acetic acid had the greatest negative effect on the growth of the microbes. Concentrations less than 3 g liter^{-1} resulted in complete inhibition of the mold. The influence of lactic acid at concentrations up to 27 g l^{-1} was negligible. *A. niger* proved to be tolerant to 1,2-propanediol at concentrations up to 27 g liter^{-1} (the highest concentration investigated).

DISCUSSION

Effects of homo- and heterofermentative lactic acid bacterial inoculants on the stability of whole-crop maize silage. In the present study, we demonstrated that inoculation of whole-crop maize with different lactic acid bacteria results in different metabolites and different aerobic stabilities. The most obvious difference between the bacteria used in this study was the variation in the amount of lactic acid produced during silage

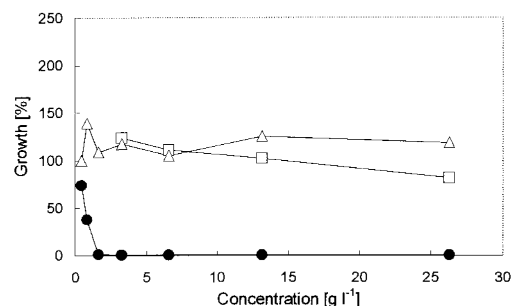

FIG. 2. Effects of single substances on *A. niger* IMI 096215 at pH 4. Symbols: □, lactic acid; △, 1,2-propanediol; ●, acetic acid.

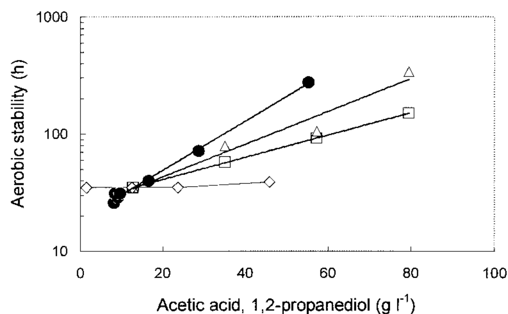

FIG. 3. Correlation of aerobic stability with the acetic acid contents of silages inoculated with different homo- and heterofermentative lactic acid bacteria after 74 days of fermentation and of silage EKX-1 after treatment with different levels of acetic acid and 1,2-propanediol. Symbols: ●, experiment with different inocula (R^2 = 0.9958); □, blending with buffered acetate (R^2 = 1.0000); △, blending with unbuffered acetate (R^2 = 0.9526); ◇, blending with 1,2-propanediol.

fermentation. This can be expected in general from homo- and heterofermentative lactic acid bacteria. While inoculation with homofermentative lactic acid bacteria led to silages with high lactic acid contents, inoculation with heterofermentative lactic acid bacteria resulted in high levels of acetic acid. Several authors have described similar effects in silages (2, 4, 18). However, inoculation with *L. buchneri* 218 not only resulted in the formation of acetic acid but also resulted in a reduction in the lactic acid level after 74 days of fermentation and in the production of 1,2-propanediol. Similar observations with *L. buchneri* were reported by Oude Elferink et al. (16), who proposed a novel pathway for ATP generation by anaerobic lactic acid degradation to acetic acid and 1,2-propanediol.

The aerobic stabilities of the strains investigated showed a high dependence on the acetic acid content. Homofermentative lactic acid bacterial additives even reduced the stability compared to the stability of the control silage, as acetic acid was also formed to a lesser extent. This confirms the results reported by Inglis et al. (9), who found that silage starters consisting exclusively of homofermentative lactic acid bacteria reduced aerobic stability. *L. brevis* 312 influenced a silage positively and resulted in a satisfying aerobic stability (72 h after 74 days of fermentation). However, the greatest stability was observed with *L. buchneri* 218.

The correlation between acetic acid content and aerobic stability is shown in Fig. 3. Bearing in mind that aerobic stability is a parameter that represents nothing but the growth of spoilage microorganisms, it is not surprising that the effect of acetic acid on aerobic stability is an exponential function with an actual high correlation value of almost 1.0 observed. Therefore, acetic acid acts as an inhibitor of spoilage organisms by decreasing the maximum growth rate of these organisms (17). The high correlation value also indicates that acetic acid is the sole parameter that influences the aerobic stability of silages under comparable conditions.

Effect of pH on the stability of silages. In general, it might be expected that spoilage microorganisms are inhibited more effectively at lower pH values than at higher pH values, which are closer to the optimum pH range of the spoilage organisms. However, no such correlation between the pH of a silage and its aerobic stability was observed. Of the silages shown in Table 1, for instance, EKX-3 showed the highest stability, 181 h at pH 4.31, while all other silages had lower pH values (around pH 3.8) and dramatically lower stabilities which ranged from 20 to 65 h. Similar data are presented in Table 2. Again, the silage with the highest pH (pH 4.11) had the greatest stability (274 h) compared to the stabilities at pH values around 3.8 to 3.9 (between 26 and 72 h). These observations were validated by addition of buffered and pure acetic acid solutions to whole-crop maize silage (Table 3). Buffered acetic acid did not influence the pH in the silage but increased the aerobic stability. Addition of pure acetic acid lowered the pH and resulted in a clear increase in aerobic stability compared to the stability observed with buffered acetic acid. This proves that aerobic stability at a constant pH depends only on the type and concentration of organic acids.

Effects of different substances on the stability of silages. Several experiments were carried out in this study to determine the specific effects of single compounds on the aerobic stability of silages. Again, acetic acid was found to be one of the most effective substances for inhibition of spoilage microorganisms. The amount of acetic acid in silages necessary to successfully preserve the silages after the silos are opened can be quite high. The silage used in this experiment required a concentration of acetic acid of more than 50 g kg^{-1} for a stability of 100 h (Fig. 3). However, according to the model proposed above, this value can change dramatically, mainly depending on the initial occurrence and composition of spoilage organisms, which are inhibited by acetic acid at various concentrations. This also explains the variation in the steepness of the curves shown in Fig. 3 but does not change the major conclusion, namely, that acetic acid is responsible for aerobic stability of silages.

Butyric acid proved to be another effective inhibitor of aerobic deterioration. Frequently, uninoculated silages have very high contents of this substance, which is usually produced by clostridia resulting from contamination with soil or slurry. Very low levels of this organic acid can increase the stability of silages under aerobic conditions. Addition of 10 g of butyric acid per kg (dry weight) resulted in an aerobic stability that was 119 h longer (Table 3). Similar results were reported by Beck et al. (3), who used silages containing 10^5 CFU of yeasts per g (dry weight) and 10 g of butyric acid kg^{-1}, which proved to be much more stable than comparable silages with lower yeast and butyric acid contents. Ohyama et al. (14) observed no aerobic deterioration with silages containing more than 5 g kg (dry weight)$^{-1}$. Butyric acid is usually not desired in silages and therefore is not a suitable tool to increase aerobic stability, but the results explain the occasionally higher aerobic stabilities of uninoculated silages than of inoculated silages.

Also, 1-propanol, a substance that is found in small quantities in silages together with butyric acid or is formed as a fermentation product from 1,2-propanediol by anaerobic bacteria that are distinct from *L. buchneri* (16), was added to silage before the aerobic stability was tested. However, the influence of 1-propanol on silage stability turned out to be negligible at low concentrations. Addition of 20 g kg (dry weight)$^{-1}$ resulted in a slight improvement in the aerobic stability. However, such a high concentration of 1-propanol could never be found in natural silages. Only Henderson (6) described silages that had very low dry matter contents (about 133 g kg [fresh weight]$^{-1}$) and higher contents of this substance (about 20 g kg [dry

weight]$^{-1}$). In this special case it has to be considered that the active concentration related to the fresh matter was still quite low as the water content was relatively high.

Under aerobic conditions the oxidation of mannitol to fructose can be completed easily when oxygen is present as an electron acceptor. This equilibrium is probably the reason for the similar aerobic stability results when fructose or mannitol is added to silage. The effect on reducing the aerobic stability was even stronger with mannitol. Polyols have also been described to be a reserve carbon source for germinating spores of *Aspergillus* sp. (21). However, the preserving effects of acetic acid are much stronger than the preserving effects of sugars or mannitol.

Effects of single substances on the growth of spoilage organisms. Three substances, lactic acid, acetic acid, and 1,2-propanediol, were evaluated to determine their inhibitory effects on spoilage organisms. It can be assumed that the antimicrobial action of lactate or acetate is caused by the lipophilic, undissociated acid molecules which penetrate the bacterial plasma membrane. The antimicrobial effect of an organic acid, therefore, depends upon its pK_a and the pH of the medium. Lactic acid has a pK_a of 3.86 and is therefore a stronger acid than acetic acid, which has a pK_a of 4.75. This results in the higher antimicrobial activity of acetic acid in surroundings where the pH values are low (around pH 4), since a greater proportion of the acetate is not dissociated. Lactic acid exhibited clearly lower inhibition of the yeasts and mold tested. Similar results were reported by Moon (12), who observed that for the same degree of inhibition, lactate concentrations had to be about two times higher than the concentrations of acetate. No inhibitory effect of 1,2-propanediol on the test organisms was detected.

Conclusions. We demonstrated that under constant conditions (dry matter content, similar concentrations and compositions of spoilage organisms) the aerobic stability of silages is determined only by the concentration of acetic acid. This was concluded from the results obtained with silages inoculated with different homo- and heterofermentative lactic acid bacteria, as well as from the results of blending experiments and studies of inhibitory test systems with spoilage organisms. Acetic acid obviously acts as an inhibitor of the growth of spoilage organisms. Therefore, acetic acid increases the aerobic stability exponentially. Only butyric acid was found to have a comparable effect, while lactic acid, 1,2-propanediol, and 1-propanol have no effect and fructose or mannose even decreases aerobic stability.

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