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Dual sensor measurement shows that temperature outperforms pH as an early sign of aerobic deterioration in maize silage

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High quality silage containing abundant lactic acid is a critical component of ruminant diets in many parts of the world. Silage deterioration, a result of aerobic metabolism (including utilization of lactic acid) during storage and feed-out, reduces the nutritional quality of the silage, and its acceptance by animals. In this study, we introduce a novel non-disruptive dual-sensor method that provides near real-time information on silage aerobic stability, and demonstrates for the first time that in situ silage temperature (T_{si}) and pH are both associated with preservation of lactic acid. Aerobic deterioration was evaluated using two sources of maize silage, one treated with a biological additive, at incubation temperatures of 23 and 33 °C. Results showed a time delay between the rise of T_{si} and that of pH following aerobic exposure at both incubation temperatures. A 11 to 25% loss of lactic acid occurred when T_{si} reached 2 °C above ambient. In contrast, by the time the silage pH had exceeded its initial value by 0.5 units, over 60% of the lactic acid had been metabolized. Although pH is often used as a primary indicator of aerobic deterioration of maize silage, it is clear that T_{si} was a more sensitive early indicator. However, the extent of the pH increase was an effective indicator of advanced spoilage and loss of lactic acid due to aerobic metabolism for maize silage.

Aerobic deterioration of silage is unavoidable, particularly during feed-out^{1–3} because, once a silo is opened, air freely accesses the exposed silo face. Undesirable microorganisms, mainly yeast and acid-tolerant bacteria, proliferate under these newly aerobic conditions, metabolizing residual sugars, lactic and other organic acids to CO₂, H₂O and heat^{4,5}. Consequently, silage temperature (T_{si}) increases and the silage mass becomes aerobically unstable. To evaluate this process with a quantitative threshold, the aerobic stability of silage has variously been defined as the time after opening for T_{si} to reach 1.7 °C⁶ above ambient temperature, 2 °C^{7–10}, 2.5 °C¹¹ or 3 °C^{2,12,13}, in order to evaluate effects of silage additives^{7–9,13}, assess roles of microorganisms^{2,12}, determine spatial patterns of T_{si} ¹⁰, assess impacts of site-specific bulk density in silage masses⁶, as well as various environmental effects¹⁰.

Since utilization of acids by aerobic microorganisms leads to a rise of silage pH, the pH of a silage mass reflects the extent of aerobic deterioration¹⁴. Previous studies of aerobic responses of silage pH used laboratory mini-silos^{7,15–18} and farm silos^{5,10,19–23}, both with invasive sub-sampling followed by liquid extraction of silage samples. Such ex situ pH determination may interfere with silage conditions, including the anaerobic environment, and is typically completed at daily (or longer) time intervals, which obscures important short term dynamic changes. A recent on-farm study using several silos suggested that pH may be a less sensitive indicator of spoilage progression in maize silage than T_{si} ²¹.

A recent review²⁴ suggested that an early indicator of aerobically unstable silage likely to substantially spoil within 24 h is when lactate-assimilating yeast counts are > 10⁵ cfu/g silage and, once they reach ~ 10⁷ cfu/g, silage pH and temperature begin to increase^{24,25}. Since samples sent to a forage laboratory for plate-culture counts create assessment delays of several days, an early indicator of aerobic deterioration that can be instantly measured in situ is needed to allow decisions to be made on commercial silage masses in real time.

The objectives of the study were to (1) introduce a novel method using an advanced pH sensor combined with a temperature sensor for continuous and simultaneous measurements of maize silage; (2) characterize

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	Control	Treated
Dry matter (g/kg WW)	421 ± 2.6	370 ± 4.1
pH	3.78 ± 0.07	3.62 ± 0.05
Buffering capacity (meq/100 g DM)	45.7 ± 0.56	48.4 ± 0.42
Lactic acid (g/kg DM)	51.5 ± 1.23	57.6 ± 0.79
Acetic acid (g/kg DM)	14.2 ± 0.43	16.0 ± 0.39
Butyric acid (g/kg DM)	<0.3	<0.3
Ethanol (g/kg DM)	6.24 ± 0.26	7.18 ± 0.25
Water soluble carbohydrate (g/kg DM)	21.4 ± 0.62	24.4 ± 0.55
Yeasts (log ₁₀ cfu/g WW)	5.21 ± 0.13	3.22 ± 0.15
Molds (log ₁₀ cfu/g WW)	<2	<2
Lactic acid bacteria (log ₁₀ cfu/g WW)	6.86 ± 0.23	7.11 ± 0.28
Total bacteria count (log ₁₀ cfu/g WW)	7.08 ± 0.23	7.27 ± 0.35

Table 1. Chemical and microbial analyses of the two silages at sampling, not treated or treated with a biological additive. Data are means ± SE of n = 3 replicates; WW, wet weight; DM, dry matter; cfu, colony-forming units.

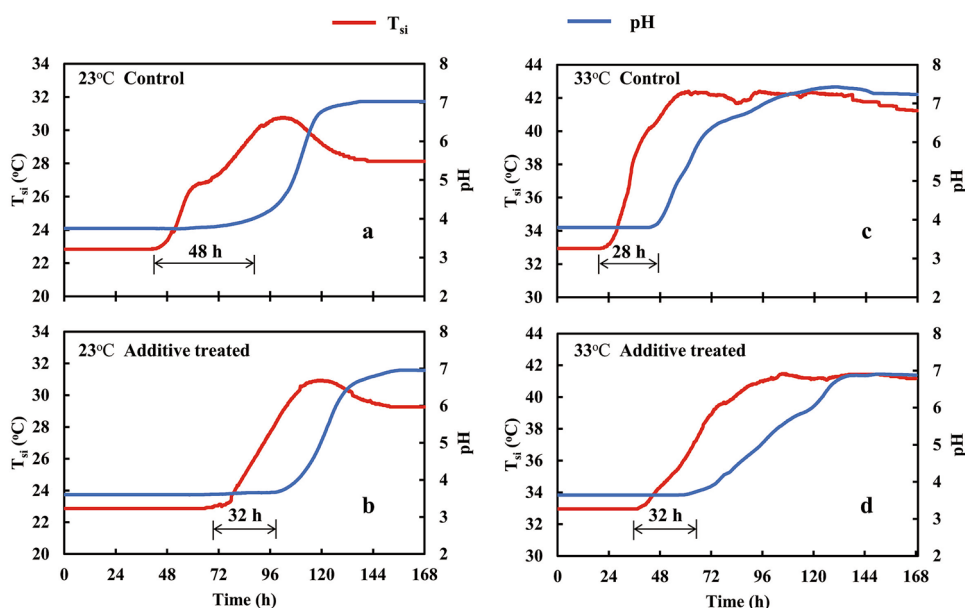


Figure 1. Silage temperature (T_{si}) and pH patterns in control (a, c) or biological additive-treated (b, d) maize silage samples incubated at 23 °C (a, b) or 33 °C (c, d) for 168 h of incubation (T_i).

relationships between pH and T_{si} determined in situ following aerobic exposure of maize silage; and (3) evaluate aerobic loss of lactic acid associated with the temporal courses of T_{si} and pH change. Silages treated or untreated with a microbial inoculant, in three replicated bunker silos each, were used to create a robust data set to better examine patterns of T_{si} and pH change after exposure to air, the dual-sensor, and relationships with organic acid content. Comparison of the silage treatments per se was not the study purpose.

Results

Initial information on the two silages. According to a quality classification for maize silage⁵, initial counts of yeast and mold suggest excellent aerobic stability of the additive-treated silage, but reduced aerobic stability in the untreated silage (Table 1). In addition, the amount of acetic acid, and the ratio between lactic and acetic, in the additive-treated silage were not consistent with a maize silage that underwent heterolactic fermentation¹.

Aerobic responses of T_{si} and pH. The time courses of pH and T_{si} (each course represents the mean of three replicates) from the silages incubated at 23 °C (T_i) (Fig. 1a,b) and 33 °C (Fig. 1c,d) were similar. Increases of T_{si} began earlier for 33 °C silage following exposure to air. Assuming that:

	Control				Treated			
	23 °C	33 °C	SEM	P	23 °C	33 °C	SEM	P
Dry matter (g/kg WW)	405	411	2.9	NS	359	358	2.3	NS
pH	7.0	7.2	0.098	NS	6.88	6.84	0.200	NS
Lactic acid (g/kg DM)	6.21	4.13	0.149	**	8.47	4.87	0.171	**
Relative loss of lactic acid (%)	87.9	92.0	0.41	**	85.3	91.6	0.31	**
Acetic acid (g/kg DM)	5.24	3.27	0.154	**	6.06	5.69	0.129	NS
Butyric acid (g/kg DM)	<0.38	<0.38	–	–	<0.38	<0.38	–	–
Water soluble carbohydrate (g/kg DM)	6.95	5.87	0.189	*	7.66	6.26	0.296	*
Ethanol (g/kg DM)	3.89	2.89	0.156	*	2.56	2.76	0.113	NS
Yeasts (log ₁₀ cfu/g WW)	5.83	7.79	0.290	**	6.52	5.52	0.360	NS
Molds (log ₁₀ cfu/g WW)	<2	<2	–	–	<2	<2	–	–
Lactic acid bacteria (log ₁₀ cfu/g WW)	7.14	7.33	0.448	NS	8.28	8.64	0.212	NS
Total bacteria count (log ₁₀ cfu/g WW)	7.74	7.42	0.449	NS	8.31	8.51	0.264	NS

Table 2. Final (*i.e.*, 168 h of aerobic incubation) chemical and microbial analyses of the two silages, not treated or treated with a biological additive. WW, wet weight; DM, dry matter; cfu, colony-forming units. *, $P < 0.05$; **, $P < 0.01$; NS, $P > 0.05$.

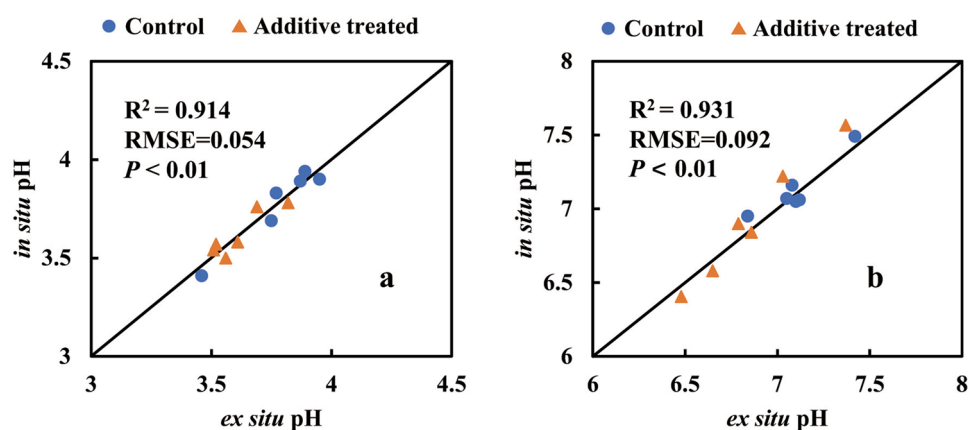


Figure 2. Piecewise comparison of in situ and ex situ pH in the 3.5 to 7.5 range (low pH group obtained before the experiment; high pH group at the end of aerobic exposure). The solid line is the line of equality. 24 paired data in the subfigures were measured from the samples in the same instrumented jar each.

$T_{si} - T_c = 2\text{ °C}$ is the threshold of instability^{7–10}, the time sequence of these samples to cross the threshold was: control (30 h, 33 °C; 54 h, 23 °C) and treated (52 h, 33 °C; 84 h, 23 °C). This resulted in lower final lactic acid contents for 33 °C incubated silage than 23 °C (Table 2). The T_{si} at 23 °C increased with a subsequent decline, whereas T_{si} at 33 °C increased to plateau. Generally, there was a lag of 28 to 48 h between the onset of a T_{si} increase and onset of a pH increase following aerobic exposure.

Validation of the in situ measurement of pH. Figure 2 ($n = 12 \times 2$) shows the piecewise relationship between silage pH resulting from water-extraction (*i.e.*, ex situ determination) and direct measurement (*i.e.*, in situ), where paired values of in situ and ex situ were measured from the same instrumented jar/pH-sensor. The in situ and ex situ measurements agreed well, and were consistent with a 1:1 relationship of (*i.e.*, $R^2 = 0.914$, $RMSE = 0.054$, $P < 0.01$) over the pH range of 3.5 to 4 (Fig. 2a, initial period) and ($R^2 = 0.931$, $RMSE = 0.092$, and $P < 0.01$) over the pH range 6.5 to 7.5 (Fig. 2b, final period).

A general comparison between the additional jars with ex situ and in situ determinations using the instrumented jars over the process of experiment is in Fig. 3 ($n = 120$). In contrast to Fig. 2, the higher R^2 of 0.985 and higher RMSE (0.187) is likely due to more data. Nevertheless, both Figs. 2 and 3 demonstrate that in situ pH measurements from 3.6 to 7.4 (Fig. 1) were accurate, and that the dual-sensor technique suited the mini-silos.

Temporal variation of pH versus lactic and acetic acids. Profiles of pH versus lactic and acetic acids over time in silages incubated at 23 °C and 33 °C are present in Fig. 4. For those incubated at 23 °C, the lactic acid content began to decline at 40 h (control) or 70 h (treated silages) and continued to decline to very low levels. In contrast, lactic acids of these silages incubated at 33 °C decreased earlier and faster, reflecting the influence of the incubation temperature on microbial metabolic activity. The lactic acid contents in all samples were initially

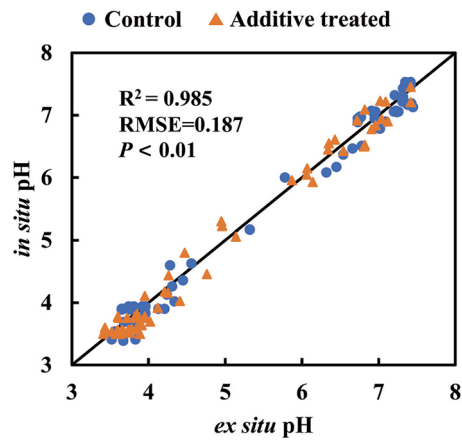


Figure 3. Comparison of in situ data from the 12 instrumented jars and ex situ data from the additional 120 jars. The solid line is the line of equality.

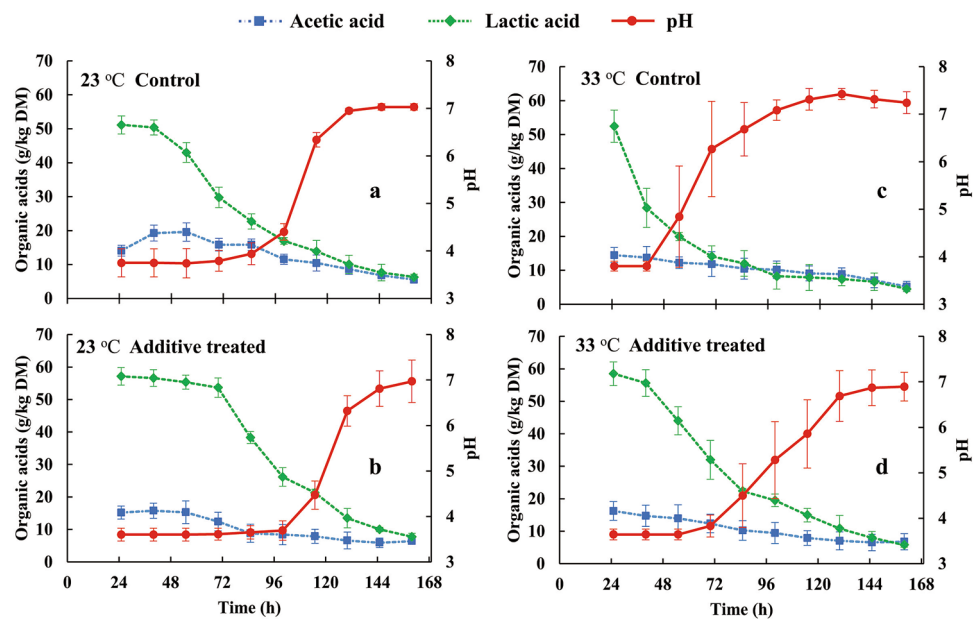


Figure 4. Acetic acid, lactic acid and pH patterns in control (a, c) and biological additive-treated samples (b, d) at 23 °C (a, b) or at 33 °C (c, d) for 168 h of incubation.

higher than those of acetic acid which, in the silages incubated at 23 °C, increased between 24 and 48 h, then declined to low levels. In general, the patterns in Fig. 4 show that most lactic acid in each sample was metabolized before the pH began to increase.

Estimating relative loss of lactic acid (RLLA). Figures 1 and 4 suggest that the relative loss of lactic acid in these maize silages can be estimated from the combined information in the time courses of T_{si} and pH. In the initial 2 to 3 days, lactic acid declined but remained the primary organic acid (Fig. 4), although it is likely that buffering capacity of the silages mitigated a pH change despite the decline of lactic acid. This allowed RLLA to be estimated as: $\Delta T_{si} = T_{si} - T_c$ as an index of its loss (Fig. 5). The relationships of RLLA (0–60%) and ΔT_{si} (0 to 6–10 °C), on the left side of Fig. 5, were linear ($R^2 \geq 0.901$, $P < 0.01$). After T_{si} stabilized (Fig. 1), RLLA could be estimated from the quadratic increase in pH ($R^2 \geq 0.781$, $P < 0.01$; right side of Fig. 5). The non-linear relationship between pH and RLLA was likely due to changes in the acetic acid content. In the final stage, contents of lactic and acetic acids were similar (Fig. 4). Thus, for these maize silages over the range of 0 to 60% loss of lactic acid, ΔT_{si} was a useful predictive tool. However, as RLLA became $\geq 60\%$, estimation based on pH became more appropriate, albeit increasingly uncertain due to the non-linear relationship between pH and RLLA (right side of Fig. 5).

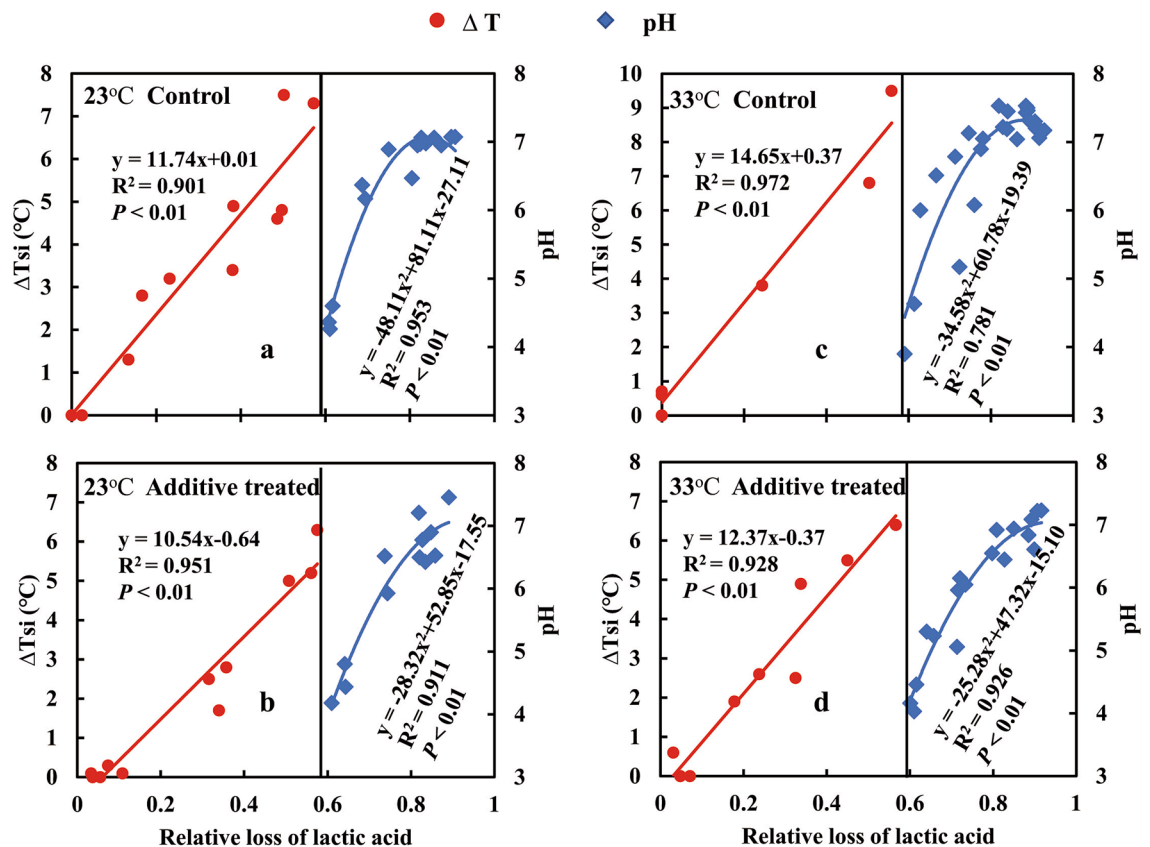


Figure 5. Estimates of the relative loss of lactic acid (RLLA) in the control silage (a, c) and biological additive-treated silage (b, d) at 23 °C (a, b) and at 33 °C (c, d) using T_{si} and pH.

Discussion

The slower changes in pH versus T_{si} over time of incubation after air exposure may reflect buffering capacity of the silage^{4,10}. Since buffering is a normal property of plant material²⁶, a time lag is inherent but likely situationally specific. During the lactic acid generating fermentation phase, a delayed pH decline has been observed^{27–29}. However, effects of buffering on this lag time relative to the pH increase after silo opening have not been adequately addressed, although a potential role of buffering in aerobic stability was previously suggested based upon limited experimental support^{1,30}. Our results, measured in situ, provide the first characterization of the time lag between T_{si} and pH in response to aerobic exposure, and demonstrate that a delayed pH increase during feed-out (*i.e.*, de-acidification) is affected by the concentration of lactic acid and buffering capacity of the silage mass.

In a study where maize silage was exposed to air³¹, the authors noted that yeast counts increased from 3 to 6.5 (\log_{10} cfu/g) while pH remained < 4 in the initial 130 h. Thereafter, pH increased rapidly to 6.3 by 20 h. The authors³¹ attributed this outcome to buffering capacity, and presented numerous models associated temporal counts of yeast and mold to predict buffering capacity²⁶. Tables 1 and 2, show that silage yeast counts increased at both incubation temperatures, and the only slight increase of acetic acid at 23 °C between 24 to 48 h (Fig. 4a) could be related to the microbial profile of yeasts over time.

pH has been regarded as an indicator/inhibitor of microbial activity/growth in silage^{1,22}, with a lower pH reflecting stronger suppression to microbial activity^{30,32}. However, simultaneous measurements of T_{si} and pH (Fig. 1) during the early unstable period of silage after exposure to air demonstrate the immediate onset of aerobic activity based on the T_{si} increase. That pH remained low for an additional 1 to 2 days is likely due to the buffering capacity of the maize silage. Overall, this suggests that pH may be an inadequate measure of microbial activity when silage is in the early aerobically unstable phase after silo opening³¹.

The pH time courses were dominated by variations in lactic acid during the initial period (Fig. 4) since its contents were higher than those of acetic acid. However, contents of these acids later converged (Fig. 4). Even with similar concentrations, it was likely lactic acid which was the primary pH determinants because the pKa of lactic acid (3.86), its acid dissociation constant, coincided with the lowest pH value (3.6, Fig. 1), whereas the pKa value of acetic acid is higher (4.75) reflecting it being a ten times weaker acid than lactic acid^{30,33,34}. Thus, as the weaker acid, acetic had more undissociated molecules in silage water when the pH was < 4.75³⁴. In addition, as acetic acid is more volatile than lactic^{2,29,30}, and thus more acetic acid would have been lost by volatilization. Thus, pH is a useful indicator of aerobic loss of lactic acid (right sides of Fig. 5), but only after buffering capacity is exceeded.

Using this novel dual-measurement technique we found that pH is not as effective T_{si} as an earlier aerobic marker of maize silage spoilage, but is effective at longer times of air exposure. Indeed, both T_{si} and pH have been suggested as indicators of oxidative degradation^{5,10,13,21,35–38}. The ($\Delta T_{si} = +2$ °C) as the threshold of aerobic stability

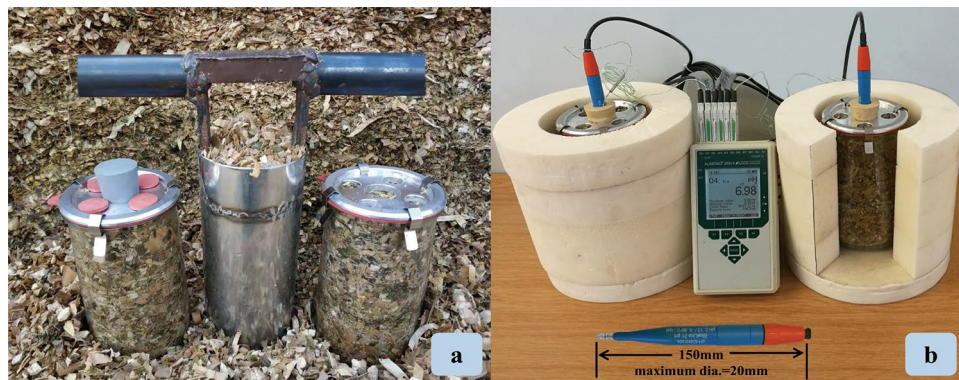


Figure 6. Silage probed using the core sampling device and immediately packed into jars in the on-farm silo (a), and the instrumented jars with insulating cover ready for laboratory incubation (b).

has been widely accepted^{1,7,8}, while other studies have suggested a pH-based threshold of aerobic deterioration when pH exceeds the initial value by 0.5 units^{17,39–41}. Our case study suggests that: $\Delta T_{si} = +2\text{ }^{\circ}\text{C}$, represents a 11 to 25% loss of lactic acid from these maize silages whereas: $\Delta T_{si} = +3\text{ }^{\circ}\text{C}$, also a commonly accepted threshold of aerobic deterioration, represents 18 to 35% loss. In contrast, at $\Delta\text{pH} = 0.5$ unit, over 60% of lactic acid had been lost, thereby further supporting the suggestion²¹ that pH is a less sensitive indicator of aerobic deterioration than is T_{si} for maize silage. However, for farm silos where ambient temperature fluctuates diurnally^{5,10,21}, this may interfere with determining the threshold of aerobic stability. As the chemical definition of pH is independent of ambient temperature, this may be an additional advantage for outdoor investigation.

Extrapolation of our outcomes with maize silage to grass and/or legume silages should be done with care as maize silage has a relatively low acid buffer capacities^{42–44} for a silage. Consequently, silage with higher acid buffer capacity may exhibit different pH time delays relative to T_{si} in response to air exposure and subsequent aerobic deterioration.

Conclusions

Time courses of T_{si} and pH simultaneously measured in maize silages in situ using a novel dual-sensor documented lags of pH change relative to those in T_{si} at two incubation temperatures. These lags, likely due to ongoing losses of lactic acid and silage buffering capacity that delayed the pH rise following aerobic exposure, demonstrate that pH is a less sensitive indicator than T_{si} of short time aerobic deterioration of maize silage. However, pH is an effective indicator of advanced spoilage and loss of lactic acid due to aerobic metabolism.

Our results refer to a case observation using the dual-sensor tool that is different from ex situ method. We presented the comparison/discussion of the in situ and ex situ data and outcomes recommend this simpler and faster in situ method for mini-silo studies. As all maize samples measured were extracted non-destructively from farm silos, this technique is promising for farm level use, although a protective shield for the glass electrode of pH sensor is necessary. The stronger conclusion that the temperature outperforms pH as an early sign of aerobic loss of various silage depends on extending experiments, associated with multiple affecting factors in future study.

Methods

Experimental materials. A total of 132 glass jars (2 (two farms that provided different silage samples) \times 3 (three silos of each farm for replicates) \times 2 (incubated at 23 $^{\circ}\text{C}$ and 33 $^{\circ}\text{C}$) \times 11 (1 in situ + 10 ex situ measurements)) were filled with whole crop maize (*Zea mays* L.) silage. Control samples (66 jars, sampling date June 17, 2019) were collected from three bunker silos (40 \times 6 \times 3.5 m^3) located at the Frankenforst Research Farm (University of Bonn, Bonn, Germany). Additive treated samples (66 jars, sampling date June 29, 2019) were collected from three bunker silos (30 \times 5 \times 2.5 m^3) at a nearby private farm where these silos were inoculated at harvest with a biological additive containing *Lactobacillus buchneri*, *Lactobacillus plantarum*, and *Lactobacillus rhamnosus* (BONSILAGE FIT M (liquid), 170713, homo- and hetero-fermentative, H. Wilhelm Schaumann GmbH, Germany) at 1 g/t wet weight). Both farms cultivated cv. Susann S260 (Saaten Union, Germany) with similar seeding (late April, 2018) and the same harvest (August 28, 2018) dates, and had similar unloading rates (0.5 m/d).

To facilitate sampling from the silos, while minimizing air entry, a metallic coring device (inner diam. 9.5 cm, length 25 cm; Fig. 6a) was constructed with the same inner diameter as the glass jars (1.5 L, inner diam. 10 cm, depth 20 cm; Fig. 6a) into which the samples were placed for deterioration assessment. The sampling area (1.8 m \times 1.4 m) was located in the center of each silo. Prior to sampling from the bunker, 20 cm of the surface of the exposed face was removed. Sampled silage was immediately packed to a density of 220 kg/m^3 dry matter (DM) in glass jars which were tightly sealed with aluminum lids (diam. 10 cm, thickness 1 cm), rubber O-rings and four elastic clamps (Fig. 6a).

In situ silage pH sensor. The study required validation that the pH sensor used is suitable for liquids and maize silage with typically high water content (> 600 g/kg) under high compaction/density (500 to 600 kg/

m³ wet weight). Since maize silage is a H₂O-rich porous material⁴⁵, its dense compaction enables good contact between the pH-electrode and the water phase in the silage. The lids of the glass jars were perforated with five holes (Fig. 6b), with the center hole (diam. 3 cm) allowing insertion of a glass pH electrode (2–13 pH/±0.01 pH, BlueLine 21, SI Analytic GmbH, Mainz, Germany; Fig. 6b) and a thermocouple (NiCr-Ni, diam. 1 mm, −40–160 °C/±0.1 °C, Alborn Mess-und Regelungstechnik GmbH, Germany). The other holes (diam. 2 cm) were air inlets for the aerobic-exposure measures when rubber stoppers were removed. Ex situ determination of silage pH followed a standard protocol of extraction of 25 g of maize silage with 0.225 L deionized water for 30 min^{4,14,23}.

Experimental procedure. The experiment used temperatures of 23 and 33 °C (T_c) in an incubator (KBF-S 720, control range: 0 to 70 °C, accuracy: ±0.3 °C, vol. 0.97×0.58×1.25 m, BINDER GmbH, Tuttlingen, Germany). According to a suggestion³⁶, all mini-silos/jars were covered with insulated sleeves throughout (Fig. 6b). Prior to and after the experiment, all pH sensors of the instrumented jars were calibrated with standard calibration liquids: HI 70,004 (pH=4.01) and HI 70,007 (pH=7.01), (Hanna Instruments, Inc. Woonsocket, USA). The pH tip and a thermocouple (1 mm diameter, T-type) were inserted together into the sample at the same depths (8 cm) in each jar. Both sensors are connected to a data-logger (ALMEMO-2890–9, AHLBORN GmbH, Germany) with data recorded at 10 min intervals over the 168 h experimental cycles. Each instrumented jar, and 10 additional jars (1.5 L) as a group all containing the same maize silage and packed to the same density. These additional jars served as sampling points during aerobic deterioration measures. During each sampling (15 h interval beginning 24 h after ensilage), a subsample of 150 g was removed from 8 cm behind the face in these additional jars, to same depth as the in situ pH and thermocouple sensors. A 100 g subsample was sealed with a vacuumizer (Boss Mini-Max, Helmut Boss Verpackungsmaschinen KG, Bad Homburg, Germany) to remove air, frozen and shipped on ice to a commercial laboratory for chemical and microbial analyses within 24 h. The remaining 50 g subsample was divided equally, each with 225 g deionized water, to determine pH ex situ, which used for evaluation of in situ the measurements from the instrumented jars.

Statistical analysis. Experimental data were analyzed using IBM SPSS v25.0 (IBM Co., Armonk, NY, USA). Linear regression, curve fitting and fitting errors were evaluated using coefficient of determination (R²), significance (P) and root mean square error (RMSE). A T-test was conducted to determine effect of T_c (i.e., incubated at 23 or 33 °C) on chemical and microbial composition at the end of the experiment for final-data processing.

Chemical analysis. Dry matter was measured by drying at 60 °C for 48 h in a forced-air oven³⁹. Buffering capacity (BC) was determined by the method of the literature⁴⁶. Acids (i.e., lactic, acetic, butyric) and ethanol were determined using high-performance liquid chromatography (LC-2010AHT, Shimadzu Corp., Kyoto, Japan), with an integrated UV-detector. Water soluble carbohydrate (WSC) content was determined enzymatically⁴⁷.

Microbial analysis. According to the method of the literature³, 30 g of silage was suspended in 270 ml of ¼-strength ringer solution (2.25 g/l NaCl, 0.105 g/l KCl, 0.06 g/l CaCl₂, 0.05 g/l NaHCO₃) (Merck, Darmstadt, Germany) and homogenized in a mixer for one minute. From this suspension, total bacterial counts were analyzed on plate-count agar (5.0 g/l enzymatic digest of casein, 2.5 g/l yeast extract, 1.0 g/l glucose, 15 g/l agar, pH=7.0) (Merck, Darmstadt, Germany) after aerobic incubation at 30 °C for 2 days. Lactic acid bacteria were quantified on MRS agar (Merck, Darmstadt, Germany) after anaerobic cultivation for 3 days at 30 °C. Yeasts and molds were detected using yeast extract glucose chloramphenicol (YGC)-agar (5.0 g/l yeast extract, 20.0 g/l glucose, 0.1 g/l chloramphenicol, 14.9 g/l agar, pH = 6.6) (Merck, Darmstadt, Germany) after incubation at 25 °C for 3 days.

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Author contributions

For this research article, G.S. Y.S. W.B. and C.M. conceived and designed the experiments; G.S. Y.S. W.B. C.M. A.L. I.A. M.T. F.K. and Y.W. performed the experiments; G.S. Y.S. W.B. and C.M. analyzed the data; Y.S. W.B. C.M. A.L. I.A. M.T. F.K. Y.W. and D.A.G. contributed to the reagents/materials/analysis tools; Y.S. D.A.G. and G.S. wrote the paper.

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Competing interests

The authors declare no competing interests.

Additional information

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