



The relationship of silage temperature with the microbiological status of the face of corn silage bunkers

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ABSTRACT

A method is proposed to quantify aerobic deterioration of corn silage forage quality as related to the temperature of silage mass in the bunker. Aerobic deterioration, apart from causing nutritional value losses, affects the hygienic quality of silages through the accumulation of pathogenic organisms and their toxins. A survey was carried out in northern Italy that involved a detailed examination of silage bunker from each of 54 dairy farms. Samples from the core, the peripheral areas within 1 m from the silo walls, and the molded spots, when present, were collected. The sample and silage temperatures across the working face were measured at depths of 200 mm at 11 locations and at 7 elevations. The temperature of the central zone of the silo was defined as the reference temperature (T_{ref40}). The difference between the temperature of the silage sample and the T_{ref40} was used as a heating index associated with aerobic deterioration (dT_{ref40}). The working face area with visible molds was measured. The samples were analyzed for DM content; pH; water activity; nitrates; lactic, acetic, and butyric acids; and microbiological count of yeasts, molds, and clostridia spores. The core samples always showed a pH below 4.0 and a dT_{ref40} below 2°C, whereas the silages from the peripheral areas were split into 2 groups, one that had a pH lower than 4 and a dT_{ref40} lower than 3.5°C (53%) and one that had a pH higher than 4 and a dT_{ref40} higher than 5°C (47%). Most of the silages from the peripheral areas (94%) and all the silages from the moldy spots that have a dT_{ref40} above 5°C had a pH higher than 4.5. Furthermore, a positive dT_{ref40} higher than 5°C corresponds to a higher yeast count than 5 log cfu/g in most of the silages from the peripheral areas (93% of samples) and in almost all the silages from the molded spots. The evaluation of the extension of the visible molded areas combined with temperature measurement at 200 mm behind the feed-out face could offer a good indication of the health status of silage during consumption. Furthermore, this

method could be useful to detect early stages of the aerobic deterioration process and to improve silage management.

Key words: aerobic deterioration, silage temperature, yeast count, mold count

INTRODUCTION

Silage produced from corn is the major source of forage for lactating dairy cows in Europe and North America. Most of the silage stored in horizontal silos is exposed to air penetration, especially in the upper parts near the walls, which are difficult to pack and seal properly (Ashbell and Lisker, 1988). Much greater losses can occur, however, when a silo is opened for feeding caused by aerobic microorganisms that use the DM as a source of energy (Rees, 1982). Oxygen enables various aerobic spoilage microorganisms to become active and to multiply, causing aerobic deterioration (Woolford, 1990). Aerobic deterioration usually results in high DM loss (Bolsen et al., 1993) and the loss of important nutritional components (Kung et al., 1998). Aerobic deterioration of silage not only causes nutritional value losses (Whitlock et al., 2000), it also negatively affects the hygienic quality of silages because of the increased risk of proliferation of potentially pathogenic or otherwise undesirable microorganisms, such as molds, bacilli, and *Listeria monocytogenes* (Lindgren et al., 2002). Such activities result in the accumulation of pathogenic organisms and their toxins in the silage, with effects on animal and human health (Ivanek et al., 2006; Vissers et al., 2007), the accumulation of degradation products, and the transmission of organisms that negatively affect food quality (Wilkinson, 1999; Tabacco et al., 2009). Lactic acid, acetic acid, and water-soluble carbohydrates are the main sources of energy for the microorganisms involved in the first phase of silage deterioration (McDonald et al., 1991). The oxidation of these nutrients results in the production of carbon dioxide and water, with the evolution of heat and an increase in silage pH (McDonald et al., 1991). On the farm, this deterioration is usually manifested by an increase in temperature and by the appearance of molds in the peripheral areas of the silo (Ashbell and

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Weinberg, 1992). Silages that spoil and heat in the bunker or in the feed bunk may dramatically affect daily DMI because cows are reluctant to consume unstable silages, especially during the warm season when DMI is already compromised (Mahanna and Chase, 2003).

Because the evaluation of microbiological and chemical quality of the working face of a silage during the feed-out phase would require many samples, expensive labor and equipment, qualified personnel, and time-consuming laboratory analyses, a simple method is necessary to accurately and quickly assess silage quality. The aim of the study was to find a correlation between the microbial and chemical composition of silage during the feed-out phase and some easily measurable parameters to enable technicians and farmers to quantify the extent of aerobic deterioration at the farm level. A commercial farm survey was conducted to 1) compare the temperature at the feed-out face to silage pH, yeast and mold activity, and lactic acid concentration, and 2) to quantify the extent of aerobic deterioration through silage thermography and surface covered by visible molds.

MATERIALS AND METHODS

A survey was carried out in the western Po Plain (Italy) on 54 dairy farms with Italian Friesian cows. Farms performing well, moderately well, and poorly were selected to ensure that not only high-quality silages were surveyed. Different farm enterprises and a range of ensiling practices were examined. One corn silage bunker that had been open for at least 20 d was examined in detail on each farm. The silages were ensiled in bunker silos and were covered with at least 1 polyethylene sheet. The width of the sampled silages ranged from 5.3 to 11.5 m, the height from 1.5 to 3.3 m, and silage densities in the core from 535 to 713 kg/m³. The daily feed-out rates ranged from 7 to 25 cm in the winter period and from 8 to 33 cm in summer. Samples from the silage core, from the peripheral areas, and, where present, from visible molded spots were taken from the feed-out face of each silo to determine the fermentation characteristics, pH, DM content, yeast and mold count, and clostridia spore concentration. Before coring, the temperatures were measured within the stored silages during feed-out at a depth of 200 mm into the working face, in the same place where the samples were taken, using a digital thermometer (Testo 925, Hotek Technologies Inc., Tacoma, WA) equipped with a 600- or 1,200-mm fast-action thermocouple probe (5 mm diameter). The core and the peripheral area samples were taken using a steel corer (45 mm diameter) to a depth of about 200 mm. Two core samples were taken in the middle of the silo (108 samples in total) and 2

from the peripheral areas with no visible mold (within 1 m from the silo walls and 0.40 m from the top; 108 samples in total). A grab sample of about 0.5 kg was taken from up to 3 visibly molded spots, when present (153 samples in total).

The core depth (about 200 mm) and weight were measured to determine the silage density (Muck and Holmes, 2000). The temperatures were measured in the same silos inside the stored silages during feed-out at a depth of 200 mm from the working face. Measurements were taken at 11 locations across the working face (0.25, 0.5, 1.0, 2.0, and 3.0 m from both the right and left side walls and in the middle of the silo face) and at 7 elevations (0.1, 0.2, 0.3, 0.6, 1.0, and 2.0 below the top surface and 0.2 m above the base), as reported by Borreani and Tabacco (2009). Ambient temperature was recorded at the same time. The working face area with visible molds was also determined and measured. The central silo zone temperature ($h/2$ and $x/2$, where h = height of the silo and x = width of the silo) was measured at depths of 200, 400, 600, and 1,200 mm from the working face and defined as the reference temperature (T_{ref}). The difference between the silage sample temperature and the T_{ref} was used as an index of heating associated with aerobic deterioration (dT_{ref}). The climatic patterns of the western Po Plain (latitude from 45°40'N to 44°40'N; 1977–2008 period) were characterized by mean daily temperature increases of 0.6°C in January to 22.1°C in July [average minimum January temperature = -3.8°C (lowest = -17.8°C) and average maximum July temperature = 28.5°C (highest = 38.0°C)].

Sample Preparation and Analyses

The silage samples were split into 2 subsamples. One subsample was oven-dried at 60°C for 72 h to determine the DM content, air equilibrated, weighed, and ground in a Cyclotec mill (Tecator, Herndon, VA) to pass a 1-mm screen. The dried silage samples were analyzed for total nitrogen, according to the Dumas method (Schindler and Knighton 1999), using a nitrogen analyzer Micro-N (Elementar, Hanau, Germany). The second subsample was stored as a wet sample at -30°C. Wet samples were extracted using a stomacher blender (Seward Ltd., Worthing, UK) for 4 min in distilled water at a ratio of water to sample material (fresh weight) of 9:1 or in H₂SO₄ (0.05 mol) at a ratio of acid to sample material (fresh weight) of 5:1. The nitrate (NO₃) contents were determined in the water extract through semiquantitative analysis using Merckoquant test strips (detection limit = 100 mg of NO₃/kg of fresh matter; Merck, Darmstadt, Germany). The ammonia nitrogen (NH₃-N) content, determined using a specific

electrode, was quantified in the water extract. The lactic and monocarboxylic acids (acetic, propionic, and butyric acids) were determined by HPLC in the acid extract (Canale et al., 1984). Duplicate analyses were performed for all the determined parameters. The duplicates were averaged and the means were considered as observations in the statistical analysis. The water activity (a_w) of the silage was measured at 25°C using an AquaLab Series 3TE (Decagon Devices Inc., Pullman, WA) that used the chilled-mirror dew point technique on a fresh sample.

To conduct the microbial counts, 30 g of sample was transferred into sterile homogenization bags, suspended 1:10 (wt/vol) in a peptone physiological salt solution (1 g of neutralized bacteriological peptone and 9 g of sodium chloride/L), and homogenized for 4 min in a laboratory stomacher blender. The dilution was also prepared at a 1:4 sample to peptone physiological salt solution ratio for the clostridial spore count of the core silages. The serial dilutions were prepared and mold and yeast numbers were determined using the pour plate technique with 40.0 g/L of yeast extract glucose chloramphenicol agar (Difco, West Molesey, Surrey, UK) after incubation at 25°C for 3 and 5 d for yeast and mold, respectively. The mold and yeast colony forming units were enumerated separately, according to their macromorphological features. The mean count of the duplicate subsamples was recorded for the total yeasts and molds on plates that yielded 10 to 100 cfu/Petri dish.

The clostridia spore concentration was determined using the most probable number (MPN) procedure. A serial 10-fold dilution was prepared in a ringer solution (Oxoid Ltd., Hampshire, UK). Tubes from each dilution step, containing 9 mL of sterilized reinforced *Clostridium* medium (Merck, Darmstadt, Germany) supplemented with sodium lactate 60% (wt/wt) syrup (25 mL/L), agar (15 g/L), and 0.2 g/L of D-cycloserine (added through 0.2 μ m-sterile filter; Jonsson, 1990), were each inoculated with 1 mL. The tubes were heated in a water bath for 10 min at 80°C to inactivate the vegetative cells and to trigger the spore germination. The tubes were sealed with paraffin and incubated for 7 d at 37°C. A tube scored positive if it exhibited abundant gas formation after incubation.

Statistical Analysis

All the microbial counts were \log_{10} transformed to obtain log-normal distributed data. To calculate the averages, the values below the detection level (detection levels: 15 clostridia spores/g, 10 yeast/g, and 10 mold/g) were assigned a value corresponding to half

of the detection level (i.e., 7.5 clostridia spores/g, 5 yeast/g, and 5 mold/g; Tabacco et al., 2009).

To evaluate statistical significance between the various locations of the silo face, the data were analyzed using the ANOVA procedure and significant differences among means were determined by Tukey multiple range test. Pearson correlation coefficients of corn silage chemical and microbiological composition, difference between silage temperature and reference temperature of the core of the silage measured at 400 mm behind the silage working face (dT_{ref40}), and temperature of the silage sample and their level of significance were determined. Data of pH, yeast count, mold count, and lactic acid content were regressed on dT_{ref40} , visibly molded spot (**MOLD_SPO**; 1 = silage visibly molded, 0 = no visible mold), and $dT_{ref40} \times$ MOLD-SPO as independent variables. Multiple regression analysis was performed using the stepwise selection procedure of SPSS (SPSS Inc., Chicago, IL) to select the best regression model at $P < 0.05$. The best equation for each parameter was selected using the coefficient of determination and root mean square error. All the reported determination coefficients (R^2) were adjusted for degrees of freedom. Unless stated, all regression coefficients were significant at $P < 0.05$. All the above statistical analyses were performed using SPSS (version 16 for Windows, SPSS Inc.).

The triangulation gridding method with a linear interpolation option of Surface Mapping System (version 6.0.3; Golden Software, 1995) was used to interpolate the temperature and the dT_{ref} of the silage face values. Gridding is the process of using original data points (observations) in an XYZ data file to generate calculated data points on a regularly spaced grid. The interpolation schemes estimate the value of the surface at locations where no original data exists, on the basis of the known data values. The Surface Mapping System program was then used to generate the grid temperature contour map of the silage face. The silage surface areas with $dT_{ref} > 5^\circ\text{C}$ or $> 10^\circ\text{C}$ were estimated using the option grid volume of the Surface Mapping System program.

RESULTS

The mean chemical and microbial composition from the core, peripheral areas, and molded spots of the silages are reported in Table 1. The cores were characterized by a DM content ranging from 26.2 to 41.4%, with a mean value of 34.3%, an a_w of 0.981, and a pH of 3.64. The lactic acid ranged from 1.44 to 8.98% of DM, and the acetic acid from 0.17 to 5.48% of DM, but the butyric acid was always below the detection limit. The microbial characteristics of the core resulted in a

Table 1. Mean values (ranges in parentheses) of chemical and microbiological characteristics from the core, peripheral areas, and molded spots of corn silages observed in the farm survey (n = 369)

Item ¹	Silage core (n = 108)	Peripheral areas (n = 108)	Molded spots (n = 153)	SE	P-value
DM content (%)	34.3 (26.2–41.4)	34.1 (16.3–47.4)	33.4 (11.5–48.4)	0.313	0.284
Water activity	0.981 (0.960–0.990)	0.988 (0.963–1.00)	0.993 (0.970–1.00)	0.000761	<0.001
pH	3.64 ^c (3.45–4.03)	4.97 ^b (3.53–8.71)	6.84 ^a (4.70–8.33)	0.0860	<0.001
Yeast (log cfu/g)	2.93 ^c (<1.00–5.70)	5.48 ^b (<1.00–8.57)	6.33 ^a (3.00–8.40)	0.124	<0.001
Mold (log cfu/g)	1.76 ^c (<1.00–4.04)	3.71 ^b (<1.00–6.65)	8.00 ^a (5.70–9.40)	0.160	<0.001
Clostridia spores (log MPN/g)	1.36 ^c (<1.18–2.36)	2.75 ^b (<1.18–6.46)	5.08 ^a (1.48–7.04)	0.241	<0.001
Sample temperature (°C)	18.6 ^c (12.0–22.9)	30.6 ^b (7.6–51.8)	35.4 ^a (12.4–54.5)	0.634	<0.001
dT _{ref40} (°C)	–1.5 ^c (–5.9–0.9)	9.9 ^b (–5.7–33.5)	13.3 ^c (–6.0–33.5)	0.598	<0.001
Nitrates (mg/kg of fresh matter)	349 ^a (<100–3,367)	298 ^a (<100–4,791)	48 ^b (<100–1,523)	37.9	<0.001
Lactic acid (% DM)	5.45 ^a (1.44–8.98)	2.91 ^b (<0.001–7.09)	0.02 ^c (<0.001–0.85)	0.149	<0.001
Acetic acid (% DM)	1.67 ^a (0.17–5.68)	1.63 ^a (<0.001–6.16)	0.02 ^b (<0.001–0.72)	0.0626	<0.001
Butyric acid (% DM)	<0.001 ^b	0.03 ^a (<0.001–0.64)	0.02 ^a (<0.001–0.24)	0.00589	0.002

^{a–c}Means within a row with different superscripts differ ($P < 0.05$).

¹MPN = most probable number; dT_{ref40} = difference between silage temperature and reference temperature of the core of the silage measured at 400 mm behind the silage working face.

mean yeast count of 2.93 log cfu/g, a mold count of 1.76 log cfu/g, and a clostridial spore content of 1.36 log MPN/g. The dT_{ref40} of the core silages ranged from –5.9 to 0.9, with a mean value of around zero.

The samples from the peripheral areas with no visible molds presented chemical values that ranged from values similar to those of the core to values that are characteristic of deeply altered silage (pH as high as 8.71, absence of lactic and acetic acids, and presence of butyric acid, with values of up to 0.64% on DM). As expected, the samples from the molded spots presented deeply altered chemical and microbial profiles, with a mean pH of 6.84, an a_w of 0.993, yeast as high as 6 log cfu/g, mold of around 8 log cfu/g, and clostridial spores from 1.48 to 7.04 log MPN/g. Furthermore, the microbial activity of the deteriorated silage altered the acid contents, with an absence of lactic and acetic acids and a butyric acid content that ranged from <0.001 to 0.24% of DM. The correlations between the dT_{ref40} and chemical and microbial composition of the silage samples are reported in Table 2. The dT_{ref40} showed positive

correlation with pH, a_w , yeast count, mold count, and clostridia spores, whereas it was negatively correlated with lactic acid, acetic acid, and nitrate concentration. Other silage variables were variously intercorrelated.

The silage temperature in the core was 18.6°C on average, with values ranging from 12.0 to 22.9°C. Higher values were observed in the peripheral areas and in the molded spots of the silo, with temperatures of up to 54.5°C. The temperature of the silage core in the studied silos and the ambient temperatures are reported in Figure 1 as a function of the sampling time. The reference temperature averages measured in the central zone of the silos are reported in Figure 2 as a function of depth from the feed-out face for winter, spring, and summer consumptions. The temperatures were almost constant for all the seasons below 400 mm from the working face, with lower values observed over the spring period. The T_{ref} measured at a depth of 400 mm (T_{ref40}) was used as a reference for the method and ranged from 12.4 to 25.7°C, with a mean value of 21.5, 16.4, and 19.5°C in winter, spring, and summer, respectively.

Table 2. Pearson correlation coefficients of corn silage chemical and microbiological composition and difference between silage temperature and reference temperature of the core of the silage measured at 400 mm behind the silage working face (dT_{ref40}) (n = 369)

Item	dT _{ref40}	DM content	Water activity	pH	Yeast	Mold	Clostridia spores	Sample T ¹	Nitrates	Lactic acid
DM content	0.163**									
Water activity	0.411**	–0.274**								
pH	0.583**	0.184**	0.514**							
Yeast	0.606**	NS	0.462**	0.439**						
Mold	0.588**	NS	0.562**	0.840**	0.515**					
Clostridia spores	0.468**	NS	0.421**	0.668**	0.382**	0.747**				
Sample T	0.985**	0.184**	0.450**	0.625**	0.589**	0.626**	0.488**			
Nitrates	–0.282**	–0.312**	–0.225*	–0.404**	NS	–0.302**	–0.372**	–0.309**		
Lactic acid	–0.645**	–0.144*	–0.576**	–0.723**	–0.442**	–0.634**	–0.510**	–0.663**	0.221**	
Acetic acid	–0.565**	–0.138*	–0.459**	–0.636**	–0.518**	–0.546**	–0.542**	–0.589**	0.335**	0.497**

¹Sample T = sample temperature.

* $P < 0.05$; ** $P < 0.01$; NS = $P > 0.05$.

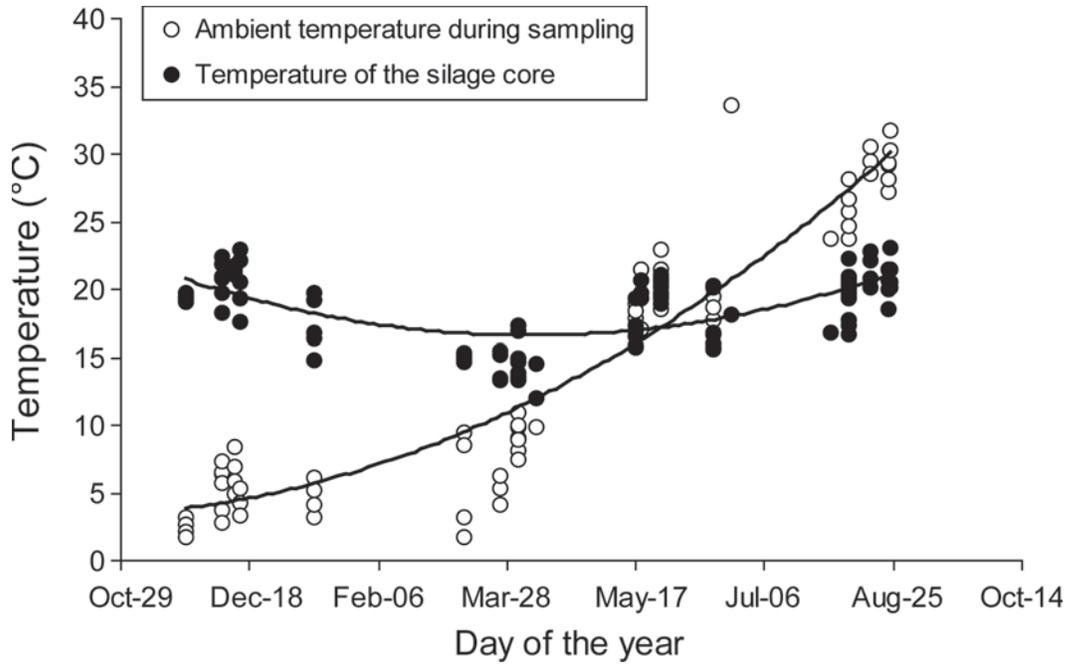


Figure 1. Temperature of the silage core and ambient temperature in the studied silos as a function of the sampling time.

As a consequence, the calculated difference between silage sample temperature and T_{ref40} (dT_{ref40}) was 9.9 and 13.3°C on average for the silage from the peripheral areas and from the molded spots, respectively, whereas it was close to 0°C in the silage core (Table 1).

The relationships between dT_{ref40} and pH, the yeast count, and the mold count are reported in Figures 3, 4, and 5, where all the data from the survey have been pooled together. The regression equations that describe the relationships between pH, yeast count, mold count,

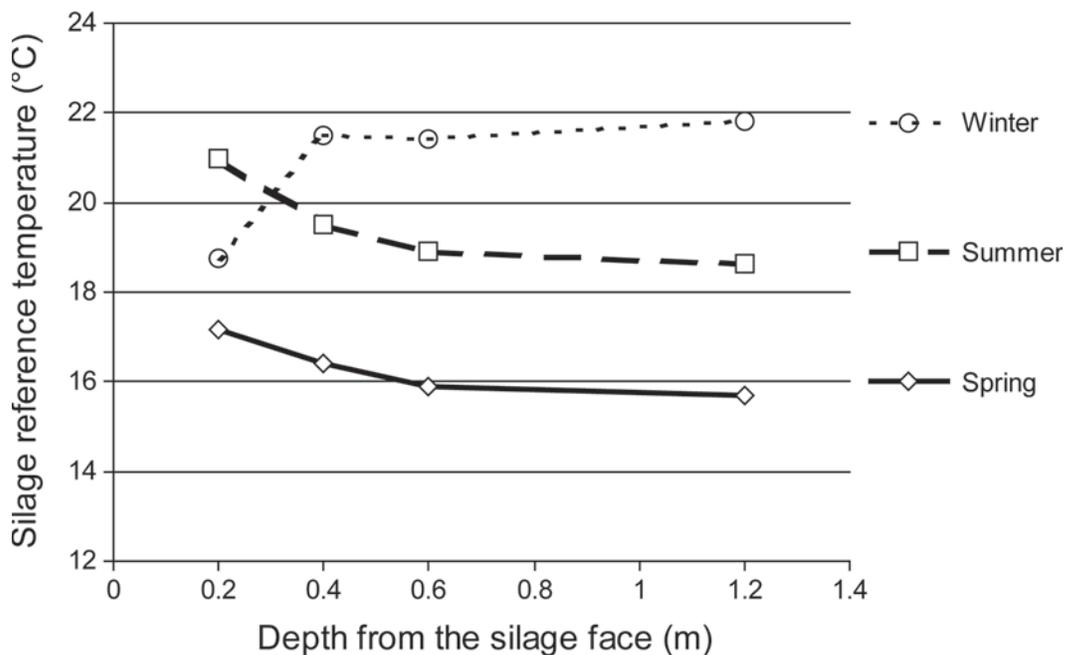


Figure 2. Average reference temperature (T_{ref}) profiles in the studied silos as a function of depth from the feed-out face in winter, spring, and summer periods.

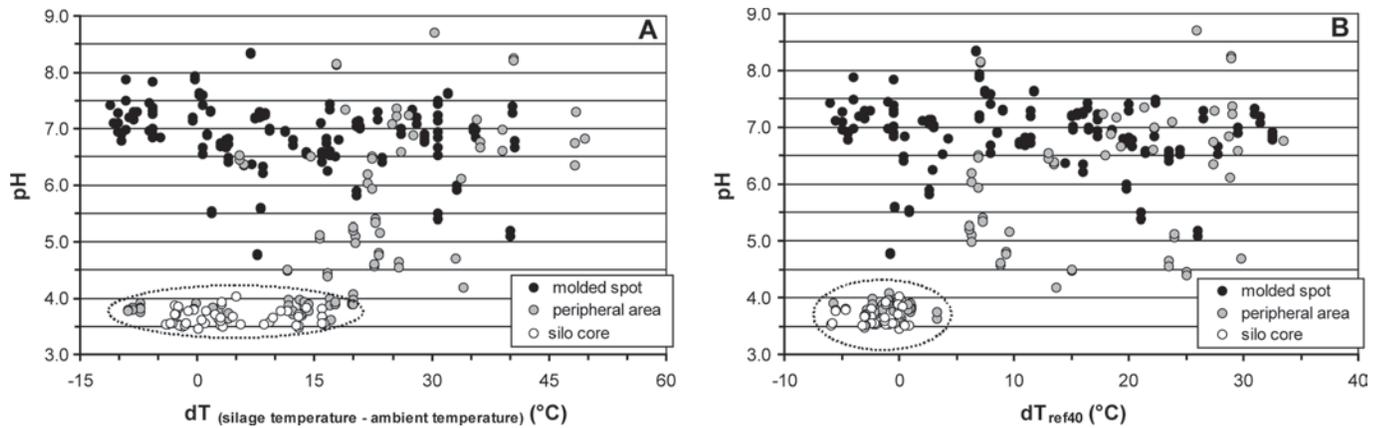


Figure 3. pH related to A) difference between silage temperature and ambient temperature (dT) and B) difference between silage temperature and reference temperature of the core of the silage measured at 400 mm behind the silage working face (dT_{ref40}) in the studied farm corn silages. The samples grouped in the dotted circle are well-conserved silages.

and lactic acid and dT_{ref40} , MOLD-SPO, and $dT_{ref40} \times$ MOLD-SPO as independent variables are presented in Table 3. The core samples always showed a pH below 4.0 and a dT_{ref40} below 2°C, whereas the silages from the peripheral areas were split into 2 groups, one that had a pH lower than 4 and a dT_{ref40} lower than 3.5°C (53%) and one that had a pH higher than 4 and a dT_{ref40} higher than 5°C (47%). A positive dT_{ref40} higher than 5°C was associated with a pH higher than 4.5 in most of the silages from the peripheral areas (48/51 samples) and in all the silage from moldy spots. Several samples from visible moldy spots (39/153) presented an altered pH with a dT_{ref40} close to zero. Furthermore, a positive dT_{ref40} higher than 5°C corresponded to a yeast count higher than 5 log cfu/g in most of the silages from the peripheral areas (51/55 samples) and from almost all the silages from the molded spots (109/113). The

yeast count tended to decrease for molded spots with dT_{ref40} lower than 5°C. The samples with visible mold had a mold count higher than 6 log cfu/g in almost all the samples. To partially explain the further pH and mold count increase with the progressive cooling of visible molded silage, the MOLD_SPO and $dT_{ref40} \times$ MOLD_SPO variables were tested in the regression analysis. The coefficient of determination of the regression equations for pH and mold count greatly increased from 0.414 and 0.263 (data not shown), when only dT_{ref40} was used, to 0.820 and 0.843, respectively.

The relationship between the pH and lactic acid concentration is reported in Figure 6 and the relative regression equation is given in Table 3, where all the data from the survey are pooled together. It is clear that when the pH is higher than 4.5, the lactic acid is below 1.0% on DM. All except for 4 of the molded

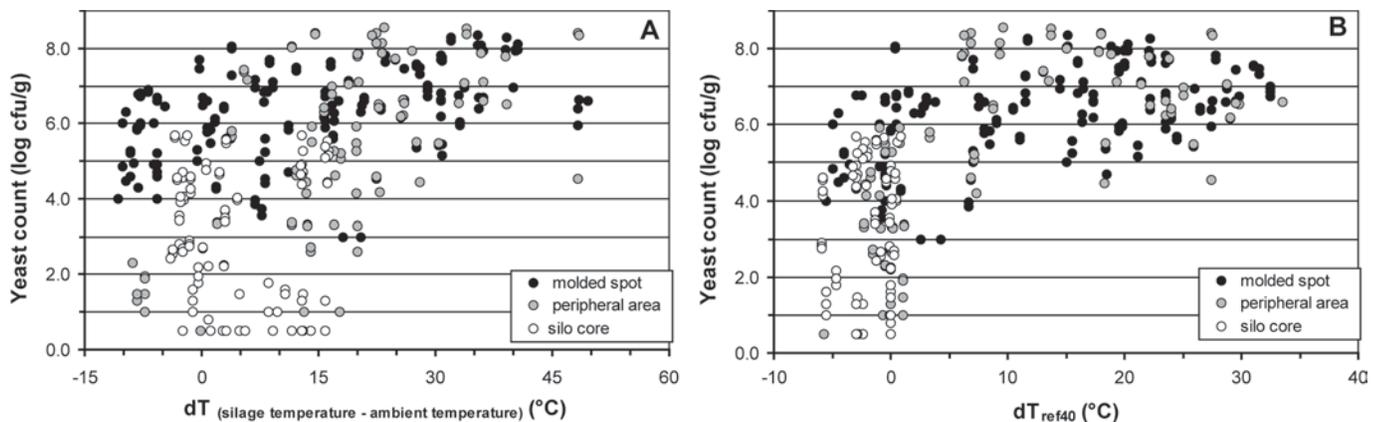


Figure 4. Yeast count related to A) difference between silage temperature and ambient temperature (dT) and B) difference between silage temperature and reference temperature of the core of the silage measured at 400 mm behind the silage working face (dT_{ref40}) in the studied farm corn silages.

Table 3. Regression models of pH, yeast count, mold count, and lactic acid on dT_{ref40} ¹ and MOLD_SPO² as independent variables (n = 369)

Equation no.	Equation model	Adjusted R ²	RMSE ³
1	$\text{pH} = +2.999 \times \text{MOLD_SPO} + 0.105 \times dT_{\text{ref40}} - 0.111 \times \text{MOLD_SPO} \times dT_{\text{ref40}} + 3.937^4$	0.820	0.685
2	$\text{Yeast count (log cfu/g)} = +0.232 \times dT_{\text{ref40}} - 0.00510 \times dT_{\text{ref40}}^2 + 0.997 \times \text{MOLD_SPO} + 3.728$	0.505	1.62
3	$\text{Mold count} = +6.120 \times \text{MOLD_SPO} + 0.104 \times dT_{\text{ref40}} - 0.133 \times \text{MOLD_SPO} \times dT_{\text{ref40}} + 2.270$	0.843	1.19
4	$\text{Lactic acid (\% DM)} = +0.254 \times dT_{\text{ref40}} + 0.00607 \times dT_{\text{ref40}}^2 - 1.748 \times \text{MOLD_SPO} + 4.092$	0.524	1.71

¹ dT_{ref40} = difference between silage temperature and reference temperature of the core of the silage measured at 400 mm behind the silage working face.

²MOLD_SPO: 1 = silage visibly molded, 0 = no visible mold.

³RMSE = root mean square error.

⁴All coefficients are significantly different from zero ($P < 0.05$).

samples presented a lactic acid content below the detection limit.

The thermography and the dT_{ref40} contour maps of 2 silage faces of 2 surveyed bunker silos are reported in Figure 7 as an example of the application of silage temperature measurement on farms. The bunker in part A of the figure did not show any visibly molded spots and only a small area with a dT_{ref40} higher than 5°C, which involved 3.7% of the total silage surface, whereas the bunker in part B had 23.2% of its surface aerobically deteriorated, with dT_{ref40} higher than 5°C or spots with visible mold.

DISCUSSION

It has long been recognized that the presence of air produces deleterious effects on silage (Beck and Gross, 1964; Woolford, 1990) and oxygen enables various aerobic spoilage microorganisms, which survive in the anaerobic phase of ensiling, to become active and to multiply, causing aerobic deterioration (Woolford, 1990).

A method to evaluate the incoming of aerobic deterioration in laboratory-scale experiments was first proposed in the 1970s by German researchers (Pahlow and Muck, 2009) and was developed in the subsequent years until it became a standardized methodology (Ranjit and Kung, 2000) that is today adopted in all experiments set up to establish the aerobic stability of silages under different conditions (i.e., inoculum, additives, and crop characteristics). In short, this method, which is always performed under well-controlled laboratory conditions, involves continuous temperature measurements of samples placed inside insulated polystyrene boxes and measurement of the ambient temperature as the reference value. Aerobic stability is defined as the number of hours that silage is exposed to air before a 2°C increase in temperature above the ambient temperature. It is well known that, during aerobic stability tests on corn silages, when the temperature starts to increase it rapidly reaches a peak in less than 24 h because lactate-assimilating yeasts, which are considered the primary initiators of aerobic spoilage, are capable of rapid growth in the presence of oxygen (Lowe et al., 2000; Tabacco

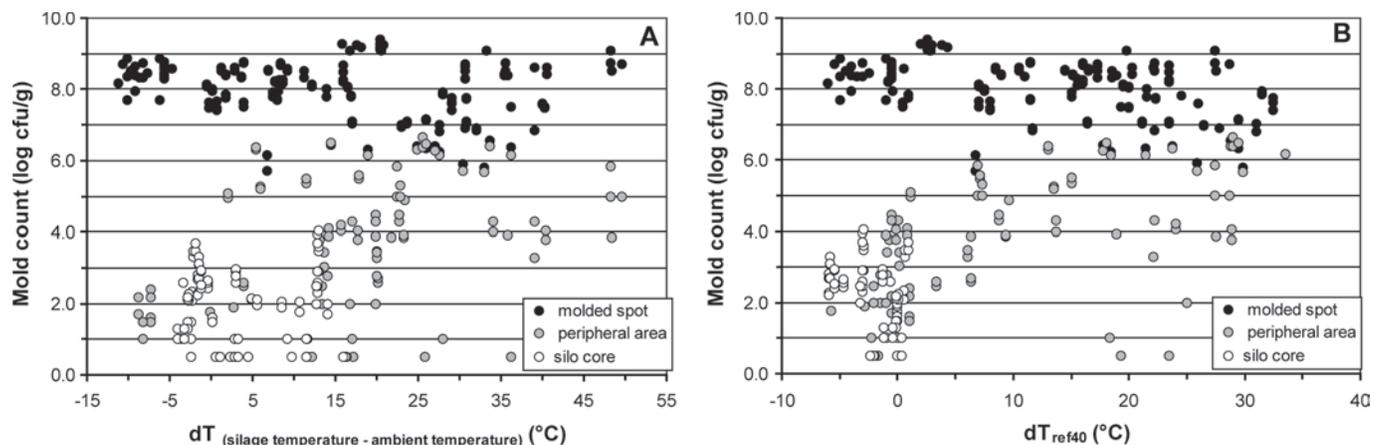


Figure 5. Mold count related to A) difference between silage temperature and ambient temperature (dT) and B) difference between silage temperature and reference temperature of the core of the silage measured at 400 mm behind the silage working face (dT_{ref40}) in the studied farm corn silages.

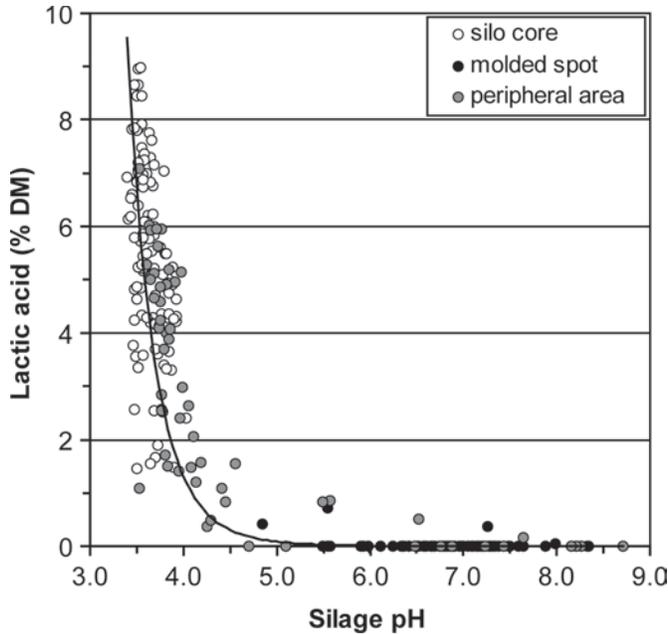


Figure 6. Relationship between lactic acid and silage pH in the studied farm corn silages. Regression equation: lactic acid (% DM) = $3.018 \times 10^7 \times \text{pH}^{-12.23}$; $R^2 = 0.895$ and root mean square error = 1.34.

et al., 2009). Honig and Woolford (1980) stated that because the oxidation process is accompanied by the evolution of heat, an increase in temperature is a convenient indicator of the extent and intensity of aerobic

deterioration in both experimental and practical conditions. A first attempt to establish which area of the silo is subject to aerobic deterioration at a farm level by the mean of temperature measurement was made by Ruppel et al. (1995). These authors measured temperatures inside storage silage during feed-out at depths of 200 mm at various locations across the working face and the ambient temperatures at the same time. They then used silage temperature above ambient temperature as an index of heating associated with aerobic deterioration (dT). The main problem of this method is that the ambient temperature is not always a suitable reference, especially in temperate environments where it is subject to seasonal and daily fluctuations (i.e., up to 25°C in spring and fall in northern Italy). Our results indicate that when the ambient temperature is used as the reference value to calculate the dT , it gives biased indications of the healthy status of the silage, with more than 35% of the core samples having dT higher than 5°C without showing any evident alteration in terms of pH (Figure 3A).

In our survey, the silage temperature of the core samples taken 200 mm behind the silage face ranged from 12.0 to 22.9°C, with measured ambient temperatures ranging from 2.2 to 33.6°C. The highest temperatures of the silage core were observed close to the harvesting season, in fall and winter, whereas the coldest temperatures were measured at the beginning of spring and early summer. This is because of the progressive

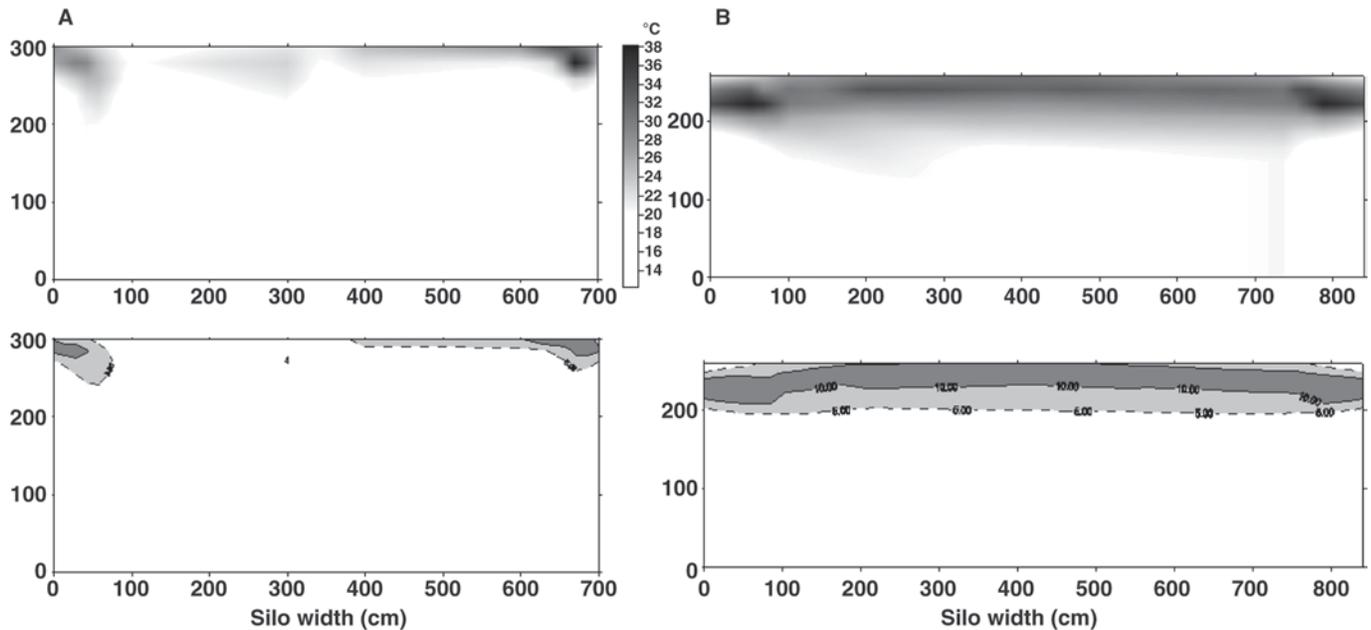


Figure 7. Examples of thermal images (upper panel) and calculated isothermal contour map (lower panel) of $dT_{\text{ref40}} > 5^\circ\text{C}$ (light gray) and $dT_{\text{ref40}} > 10^\circ\text{C}$ (dark gray) of the working face of 2 of the surveyed bunker silos. A) Bunker with no visible spoiled areas. Total silos surface 21 m^2 (3.0 m \times 7.0 m), 0.78 m^2 aerobic spoiled surface with $dT_{\text{ref40}} > 5^\circ\text{C}$, and 0.17 m^2 with $dT_{\text{ref40}} > 10^\circ\text{C}$. B) Bunker with visible molded spots. Total silos surface 21.8 m^2 (2.6 m \times 8.4 m), 5.06 m^2 aerobic spoiled surface with $dT_{\text{ref40}} > 5^\circ\text{C}$, and 2.46 m^2 with $dT_{\text{ref40}} > 10^\circ\text{C}$.

cooling of the silage mass in winter, as also observed by Green et al. (2009) on corn silage in Denmark and by Williams et al. (1997) on grass silage in the United Kingdom. Muck and Huhnke (1995) found similar results in Wisconsin, studying air movement in bunker silos and subsequent aerobic deterioration at the silage face. These authors observed that the temperature of a winter corn silage in the core increased from 12.5 to 20°C with increasing depth from the face, whereas in a summer silo temperature decreased with increasing depth from 25 to 20°C. It is interesting to observe from these data that the temperature of the silage core remained almost constant from a depth of 400 to 1,000 mm, with temperatures around 20°C both for winter and summer silos. For these reasons, steam could be released from the core of the silo in temperate environments during the winter season because of the difference between the retained heat (produced by respiration during silo filling and to a lesser extent by fermentation processes) and the ambient temperature. Therefore, the presence of steam from the core of the silo does not always mean that silage is deteriorating (Borreani and Tabacco, 2007). From our data, it is evident that ambient temperature influences the temperature of the core of the silage only to a depth of 200 mm from the feed-out face, whereas months are necessary to detect temperature variations of aerobically stable silage below 400 mm from the silo surface (seasonal variations; Figure 2). For these reasons, we suggest that the T_{ref} measured at a depth of 400 mm (T_{ref40}) could be assumed as a robust reference value to calculate the dT_{ref40} relative to different locations of the feed-out face of the silages. The temperatures of the peripheral areas and of the molded spots of the surveyed silages were very high (>30°C) in more than 55% of the samples. This is in agreement with data reported by Ashbell and Lisker (1988) and by Ruppel et al. (1995), who found higher temperatures in the upper part of the silages near the walls, which are difficult to seal properly. The method proposed here allows a dT_{ref40} to be calculated for each silage sample, which can indicate the status of the silage and any silage zone of the working face without visible mold to be classified as aerobically stable or with ongoing aerobic deterioration.

Lactic acid and water-soluble carbohydrates are the main sources of energy for the microorganisms involved in aerobic deterioration. The oxidation of these nutrients results in the production of carbon dioxide and water, with the evolution of heat (McDonald et al., 1991). The release of water during the oxidation process also increases the a_w of the silages that, together with lactic acid depletion, leads to a less inhibiting environment for microbial activity. When well-fermented silages with low values of residual sugars are considered (such

as corn silage; Weinberg et al., 2001), the main oxidized product is likely to be lactic acid. With the complete oxidation of lactic acid (15.16 kJ/g), the temperature could increase in the silage mass to 70°C, assuming a specific heat capacity of 1.89 kJ/kg/°C for the silage DM and no loss of heat to the atmosphere (McDonald and Whittenbury, 1973). In most practical situations, a part of the heat produced through oxidation will be dissipated into the atmosphere, although temperatures of up to 54.5°C have been recorded in the peripheral areas and in the molded spots of the studied silages in our experiment. These data are in agreement with previous studies on corn silages (Bolsen et al., 1993; Ruppel et al., 1995). When the calculated dT_{ref40} of the peripheral areas and of the molded spots was higher than 5°C, the yeast count was higher than 5 log cfu/g and the pH was higher than 4.5, whereas when dT_{ref40} was higher than 10°C, the mold count also showed values higher than 4 log cfu/g. Furthermore, when the absolute temperature increased to over 35°C, the dT_{ref40} was higher than 10°C, and nitrate concentration was lower than 200 mg/kg of fresh matter, it was possible to find a high spore clostridia count, as reported by Borreani and Tabacco (2008).

Because the microbial population increased during aerobic deterioration in an exponential manner, the silage from the peripheral areas and from the molded spots had the potential of contaminating feed-out silage to a great extent, even when it was included in very small amounts, whereas the fermentative profile could be unaffected or slightly modified. From a practical perspective, the strategy of diluting contaminated silage with other mold-free feeds or silage is not a healthy solution, especially for milking dairy cows or young cows. General recommendations suggest that silages with 10^4 cfu/g are generally safe to feed to ruminants, whereas levels in excess of 5×10^6 to 1×10^7 cfu/g should be discarded (Mahanna and Chase, 2003; Chen and Weinberg, 2009). The calculated dT_{ref40} of a silage face should be used to create a map of the whole working face with the aid of mapping system computer programs (e.g., Figure 7) to obtain management indications to help prevent aerobic spoilage and to estimate the quality of the silage fed to animals. Furthermore, if those areas are related to the DM densities of the silages, it is also possible to calculate the amount of deteriorated silage that should be discarded and the economic value of the DM lost.

The data of this research show that increases in temperature at the silo face of farm bunkers are clearly linked to aerobic microbial activity (especially mold growth), and changes of silage pH and the measurement of the temperature could have applications in alerting farmers to the onset of aerobic deterioration

and could be useful as an index to identify the invisible altered mass of silage. The evaluation of the extension of the visible molded areas combined with temperature measurements at 200 mm behind the feed-out face could be used to obtain a good indication of the health status of the silage during consumption. Furthermore, the application of this method could be useful to detect the aerobic deterioration process in its early stages and to quantify the extent of the spoiled areas to correctly discard the spoiled silage and to improve silage management.

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