






Assessment of Maize Silage Quality Under Different Pre-Ensiling Conditions

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Abstract: Maize silage suffers from several factors that affect the final quality and, to some extent, pre-ensiled conditions that can be potentially tuned during harvesting. After assessing new indices for silage quality under lab-scale conditions, several trials have been conducted to find associations between fresh maize characteristics and silage features. Among the first, we included field input levels, FAO class, maturity stage, use of bacterial inoculants, sealing delay and chemical traits, whereas, among the latter, we assessed density and porosity, pH, fermentative profile, dry matter loss and aerobic stability. The trials were conducted using vacuum bags or mini silo buckets. More than 1500 maize samples harvested in Northeast Italy were analysed during the 2016–2022 period. Moreover, to evaluate silage aerobic stability, the fermentative profile and temperature were measured 14 days after the opening of the silo. The association between silage quality and aerobic stability was assessed, and a prognostic risk score was used to calculate the probability of aerobic instability. The dataset could provide baseline information to promote the continuous improvement of maize silage management from different botanical and crop fields, thus improving agronomic and animal farm resource allocation from a precision agriculture perspective.



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Keywords: corn silage; heterofermentative inoculation; homofermentative inoculation; aerobic stability; survival analysis; fermentation quality; growing condition; maturity class; fermentative quality index; lab-scale silages

1. Summary

Maize (*Zea mays* L.) silage is a primary source of fiber and energy in dairy and beef cattle diets [1] and is frequently used as the main roughage ingredient in total mixed rations (TMR) [2]. According to the Italian National Institute of Statistics (ISTAT), in 2020, farms located in Northern Italy produced more than 81% of the entire Italian milk yield (1.33×10^9 kg) [3], showing an increase of 125% between 2006 and 2019; in the same period, the arable land sown for maize silage production, starting from 299,663 ha in 2006, increased by 146% [3–6]. Due to its importance in dairy and beef cattle rations, maize silage quality has been studied extensively with regard to its chemical, microbial and organoleptic traits and dry matter (DM) and quality loss [1,7]. Furthermore, the content of volatile fatty acids (VFA), alcohols and pH are of paramount importance to discriminate between excellent, average or poor fermentation during the ensiling process [8]. Pre-ensiled fresh harvested

maize (FHM) composition and miscellaneous management factors, such as the rapidity of packing, pack density, additives used, chop length and silo covering procedures, are known to affect silage quality [1,7,9,10]. Overall, the use of inoculants favours a rapid decrease in substrate pH during ensiling [9,11]. An in-depth insight into the most suitable dosage of additives is still debated since it was pointed out that aerobic stability is dose-dependent, and many research trials were carried out applying more than the maximum recommended dose suggested by the selling companies [12].

A large amount of yield and nutrient characteristic data from specific agronomic locations at different harvesting times has now been made available through precision farming technologies. Farmers and maize growers can use this information to make more informed decisions on harvesting and transforming the whole plant into silage [10]. Pre-ensiled traits of ears, grains or whole plants, together with harvest conditions, could be used to estimate the risk of aerobic instability and the likelihood of attaining good silage quality [13,14]. For this purpose, low-cost, eco-friendly, automated and rapid sensor devices, coupled with accurate chemometric modelling, could be used for chemical maize characterisation to help farmers enhance silage quality under field conditions. From 2016 to 2022, a research group from the Department of Animal Medicine, Production and Health at Padova University carried out several trials with the following aims:

1. Evaluate the effectiveness of a set of fermentative quality indexes to estimate the quality of fermentation of whole-plant maize silage in a lab-scale ensiling system [15].
2. Verify the influence of the FAO class of maize hybrids harvested at different maturity stages and grown in agronomic areas with different yield potentials on their ensilability capacity. The latter was assessed using the fermentative profile and fermentation quality indexes tested in a previous trial [16].
3. Evaluate the effectiveness of a multivariate approach and multiple linear regression in predicting the potential of freshly harvested maize (FHM) to ensure silage fermentation quality based on the chemical composition of the harvested whole-plant maize [17].
4. Assess the effect of hetero- and homo-fermentative inoculants on maize silage fermentative quality and aerobic stability under different conditions—progressive delay in silo sealing, different maturity at harvest and different exposure times to air after the opening of the silo—and propose a predictive model of maize silage aerobic stability potential based on the FHM composition using a nomogram ranking the risk of silage degradation [13,14].

2. Data Description

The trials are summarised and classified as follows. Each section contains information common to all the trials enumerated in the subsections, while the subsections add details specific to each trial. However, the trials described in the subsections may not include all experimental theses, replicates, chemical or physical characteristics, or methodological methods announced in the relevant section.

2.1. Trial #1: 2016 to 2019, Testing Hybrids

Data were collected over four consecutive years (y2016, y2017, y2018 and y2019) from maize cultivated in the Veneto region (Northeast Italy) using 37 maize hybrids of early (EA; FAO class 200, $n = 19$) and late (LA; FAO class 600–700, $n = 18$) ripening classes. The average yield per hectare was 23.5, 22.3 and 24.0 tonnes of DM of FHM biomass for y2016, y2017 and y2018, respectively (y2019 unknown). Each hybrid was harvested in up to three plots corresponding to three areas (level input field) defined by different pedoclimatic characteristics. Each growing area was characterised by an input field (IF) level, which refers to soil fertility defined as “low” (IFL, medium-heavy soil with an FHM biomass production of 49.6 tonnes ha^{-1}), “medium” (IFM, medium-light soil with an FHM biomass production of 54.3 tonnes ha^{-1}) and “high” (IFH, clay soil with an FHM biomass production of 68.6 tonnes ha^{-1} ; IFH). In y2017, y2018 and y2019, the trial was performed

only on IFM and IFH productivity plots. For each plot, every hybrid was harvested twice in two different subplots. Each hybrid was sown in both subplots in 4 rows \times 10 plants. In total, 40 plants were grown per hybrid and subplot. In the main plot field, some external rows of generic seeds were sown and excluded from the trial. At the core of the field, EA and LA hybrids were sown at precise densities to maximise production, corresponding to 95,000 and 70,000 plants per ha, respectively. Whole-plant maize (WPM) was manually harvested at a stubble height of approximately 20 cm and chopped at a theoretical length-of-cut of 20.0 mm using a self-propelled forage harvester. For each plot and subplot, WPM was sampled at three phenological maturity stages (MSe): early (EH; 1/3 milk line phase), medium (MH; at 2/3 milk line phase) and late harvest (LH; 5 d after the 2/3 milk line phase). For each plot, subplot and maturity phase, about five plants were harvested, chopped and mixed to obtain one sample. Each sample was split into two subsamples. Processing and near-infrared (NIR) spectroscopy analyses were carried out with two scans on the subsamples, but averages of scans and subsamples were performed before statistical analysis. To avoid any changes caused by respiration activity, the FHM subsamples were promptly placed in a large vessel (approximately 1.8 ± 0.2 kg) and scanned twice using a portable NIR system (poliSPEC^{NIR}, ITPhotonics, Breganze, Italy). Each scan was performed for 10 sec, with an integration time of about 10 msec. Thus, most of the within-sample variability was mostly acquired. Subsamples were quickly ensiled in vacuum-packed bags (Orved 2633040, Orved SpA, Musile di Piave, VE, Italy), as described in [15], and stored in a dark room at 23 °C for 60 days [16]. Silage subsamples were scanned twice using a FOSS NIRSystem 5000 scanning monochromator (FossNIR-System, Hillerød, Denmark) and predicted using the calibration curve, as previously reported by the authors [15,16,18].

2.2. Trial #2: 2018 to 2022, Testing Silage Quality and Dry Matter Loss

Trial #2 refers to Trial #2.a (in buckets or bags, testing different doses of inoculants in the input field did not estimate fertility (IFN) and LA hybrids) and Trial #2.b (in buckets, monitoring an operative scenario). Additionally, Trial #2.a was arranged in Trial #2.a.1.a (in buckets, simulating the feed-out rate), Trial #2.a.1.b (in buckets, without simulating the feed-out rate) and Trial #2.a.2 (in bags). Trials #2.a.1.b, #2.a.2, and #2.b have not yet been used in previous publications, and therefore no article is associated with them into the spreadsheets of the stored "datasilage.xlsx" file.

2.2.1. Trial #2.a: Testing Different Doses of Inoculants in IFN Fertility and LA Hybrids

Samples (y2018, $n = 8$; y2019, $n = 240$; y2020, $n = 135$; y2021, $n = 48$; y2022, $n = 36$) were collected during the summer season in Lonigo (Northeast Italy; 45° 23' lat. N, 11° 23' long.; IFN) from KWS Kelindos, a single maize hybrid of late-ripening (LA) class (FAO class 600–130 d). The maize was sown in a single trial field and harvested with a mono-row self-propelled maize harvester equipped with a kernel unit and a theoretical length cut of 12.7 mm [13,19]. Maize in a single-trial field (plot) was harvested in three contiguous subplots (replicates). Plots consisted of plants of the same hybrid, with an inter-row spacing of 0.75 m, at the standard density recommended by the Italian Ministry of Agriculture for the different FAO classes, corresponding to 70,000 plants per ha. Plants from the central rows of the sub-areas were harvested and included in the trial, whereas plants at the ends of the rows were excluded [16]. The FHM was mixed at best from each sub-area and then divided for further use. As reported in Serva et al. [13], FHM plants were ensiled by testing three mixtures of obligate heterofermentative (He) *Lentilactobacillus buchneri* (Lb) CCM 1819 (KWS Lactostability, AGRAVIS Raiffeisen AG, Munster, Germany) at three different concentrations (CFU g^{-1} of FHM) and a mixture of homo-fermentative (Ho) lactic acid bacteria (*Lactiplantibacillus plantarum* NCIMB 30083–1k207736, *L. plantarum* NCIMB 30084–1k207737 *Peditococcus pentosaceus* DSM 23688–1k1010, *Peditococcus pentosaceus* DSM 23689–1k1019 and *Enterococcus faecium* 22502–1k20602) at a standard dose (SD) of 3×10^5 CFU g^{-1} of FHM. The doses used for the inoculation with He were as follows: standard dose (SD), 2.02×10^5 ; half dose (HD), 1.01×10^5 ; and double dose (DD), 4.04×10^5 . The inoculation

was performed in a large sterile container, allowing for adequate mixing. For each tested mixture of inoculants (MXe), silages were prepared with delays of 0, 6 and 20 h from the harvest time (D0, D6 and D20, respectively) to evaluate the delay effect (DLe). In y2019 and y2020, every combination of MXe per DLe was repeated within each MSe harvesting thesis (EH, MH, LH and very late [VLH = 5 days after LH] in y2019 only). The freshly harvested maize (FHM) and silage samples were analysed using NIR spectroscopy, as described in Trial #1.

Trial #2.a.1.a: Testing Different Doses of Inoculants in Buckets, in IFN Fertility and LA Hybrids to Simulate the Feed-Out Rate

Maize samples from trials conducted in y2018, y2019 and y2020 were ensiled in a 20 L circular truncated conical plastic bucket and pressed with the use of a 1-tonne hydraulic press (141 kg cm^{-2}) with the ideal purpose of reaching a density of 225 kg DM m^{-3} of ground FHM [20]. The buckets were shielded using a $150 \text{ }\mu\text{m}$ SealPlus Film permeable to oxygen at a daily rate of $48 \text{ cm}^3 \text{ m}^{-2}$ at $23 \text{ }^\circ\text{C}$ and 65% RH (SealPlus by Gamma Srl, Mondovi, Italy) and siled with robust tape. The sealed buckets were stored in a dark room for 60 days at a stable temperature of $23 \pm 1 \text{ }^\circ\text{C}$, ensuring better anaerobic ensiling conditions. To simulate the feed-out rate in a farm-scale silo-bunker after opening the buckets (opening day 1, OT1), a 5 cm top layer was unloaded to discharge possible undesirable spoiled silage (spoiled top layer, Spl). Approximately 1.0 kg of sample was taken from the bucket's upper end and immediately analysed using NIR instruments for proximate composition and fermentative profile, as described in previous studies [15,16]. The buckets with the remaining ensiled fraction were left in a dark, temperature-stable room, leaving the silage surface exposed to air. After 48 h (opening day 3, OT3), a further 1.0 kg of sample was taken and analysed for OT1. The same procedure was repeated 96 h after day 1 (day 5, OT5). The maize silage was sampled three times in each bucket and analysed for proximate composition and fermentative profile. A data logger was repositioned 7.0 cm under the silage surface of OT1, -3 and -5, recording the temperature every 15 min with a precision of $0.1 \text{ }^\circ\text{C}$ (Elitech USB Temperature Datalogger RC-5, London, UK). The data logger was repositioned 7 cm below the silage surface at each sampling time.

Trial #2.a.1.b: Testing Different Doses of Inoculants in Buckets in IFN Fertility and LA Hybrids without Simulating the Feed-Out Rate

In contrast to the previously described Trial #2.a.1, in y2021 and y2022, after 60 days of ensiling, the 20 L buckets were opened, and a 15 cm thick layer of silage was removed to discharge the eventually spoiled silage, which was not further considered for quality analysis, and 1.0 kg of maize silage was promptly submitted to NIR analysis. The remaining portion was loosened in a 20 L open and square polystyrene pan, $495 \times 295 \times 140 \text{ mm}$ in dimension. A data logger was positioned 7 cm below the silage surface of the 20 L polystyrene pan, recording the temperature every 30 min. Therefore, no OT1, OT3 and OT5 were performed. Moreover, the maize used for y2022 was sown in June and harvested in September–October. In 2021, two pre-ensiled densities (DEN) were tested to reach the final low point (DENl of approximately 140 kg DM m^{-3}) and high point (DENh of approximately 200 kg DM m^{-3}).

Trial #2.a.2: Ensiling in Bags

In 2019, vacuum-packed silage bags were prepared at the same time as the buckets, but only for delay 0 (DL0). In total, 60 samples were collected in 5 MXe (3 He + 1 Ho + C) \times 1 DLe \times 4 MSe \times 3 replicates. Each experimental combination was repeated twice ($n = 120$), and only the average values were reported. The FHM was immediately packed in the bags, as described in Trial #1 and by Andrighetto et al. [15]. Finally, all replicates were stored at $23 \pm 1 \text{ }^\circ\text{C}$ and were opened for analysis of the maize silage after 60 days of conservation [16]. The analyses of the FHM and silage were performed using an NIR instrument, as reported in Trial #1.

2.2.2. Trial #2.b: Ensiling in Buckets to Monitor an Operative Scenario

With the single aim of recording DM loss, FHM samples were collected in the Veneto region (Northeast Italy) during the summer season of y2018 ($n = 16$) and y2019 ($n = 71$). The samples were kept on commercial farms and ensiled using the 20 L buckets, as reported in Trial #2.a.1.a and Trial #2.a.1.b, regardless of the use of inoculants, sealing delay (always considered at zero hours), maturity stage at harvest and replicate. After 60 days of ensiling, the buckets were opened, and the samples were kept at OT1, -3 and -5, as described in Trial #2.a.1.a.

2.3. Dataset Description

The data are stored in the “datasilage.xlsx” file, which consists of four sheets of reporting data, as detailed below:

1. Sheet 1, “dictionary keys”, contains details of the variables described in Sheets 2, 3 and 4. Column A is the variable name, Column B is the variable description—including the unit of measurement and the possible values for categorical variables—and Column C reports some details or explanations.
2. Sheet 2, “elab.all”, reports the trial number (#) and reference of the published articles (Columns A and B), the sample id (Columns C and D), the tested theses (Columns E–O), the type of data as singular or arithmetic mean (Trial #2.a.1.a and Trial #2.b) from different silage layer values (Columns P), the FHM composition (Columns Q–Y), an empty Column (Z), the silage composition (Columns AA–AS), the DM loss (DMloss, %) and DM recovery (DMr, %) (Columns AT and AU), FQI (Column AV), porosity and density (Columns AW–AX), and reference methods used for FHM and silage sample analyses (Columns AY and AZ).
3. In Sheet 3, “elab.temp”, Columns A to P report the corresponding columns (A–P) of sheet “elab.all” for a proper sample link. Columns Q to S report the indicator (event = 1) for aerobic instability, the time to event for aerobic instability, and the cumulative temperatures (TCUM) of aerobically unstable samples, respectively.
4. Sheet 4, “elab.buckets”, reports data only for Trial #2.a.1.a and Trial #2.b. Columns A to P report the corresponding columns (A–P) of sheet “elab.all” for a proper sample link. The FHM (Columns Q–Y), silage Spl (Columns AA–AS), silage OT1 (Columns AU–BM), silage OT3 (Columns BO–CG) and silage OT5 (Columns CI–DA) chemical traits are reported. DMloss, DMr, density and porosity are reported in Columns DC–DF. The reference methods used for FHM and silage sample analyses are reported in Columns DH and DI.

In general, the same sample can be linked among Sheets 2, 3 and 4 and can be easily recognised by the “fresh.id”, which is the ID key for a sample search.

3. Methods

An NIR portable poliSPEC^{NIR} (ITPhotonics srl, Breganze, Italy) with robust calibration curves was used to analyse DM, ash, crude protein (CP), ether extract (EE), α -amylase neutral detergent fiber (aNDF), acid detergent fiber (ADF), sulphuric acid detergent lignin (lignin), water-soluble carbohydrates (WSC) and starch in the FHM samples [13,16]. A benchtop FOSS NIRSystem 5000 scanning monochromator (FOSS, Hillerød, Denmark), with the calibration described by Andrighetto et al. [15]—built with the use of a large dataset—was used to analyse silage subsamples. The reference methods used to calibrate the NIR instrument for proximate composition were detailed and described in previous studies [15,16,18] and reported on further, along with those for the fermentative profile. The DM and ash were determined according to #934.01 and #942.05 [21]. The AOAC methods #2001.11 [22], #2003.05 [21] and #996.11 [23] were used for CP, EE and starch, respectively. The aNDF and ADF fiber fractions were determined using an AnkomFiber Analyser (Ankom Technology Corporation, Fairport, NY, USA). The aNDF was performed with sodium sulphite, heat-stable α -amylase and F57 bags with 25 μ m pore size and included residual ash [24,25]; non-sequential ADF was evaluated according to Vogel et al.,

1999 [26,27] and lignin in sulphuric acid [28]. Lactic, acetic, propionic, butyric acid and ethanol were extracted in an acid solution (sulphuric acid 0.6 N) and analysed using high-performance liquid chromatography (HPLC); ammonia was determined using a Megazyme assay kit, and pH was determined by a standardised procedure [29].

The DM content of maize silage considered the presence of volatile carbon compounds, which might be lost in oven dissection [30,31]; thus, a correction was applied as $DM_{corrected} = 2.22 + 0.96 \times DM_{uncorrected}$ [30].

The DM mass (DM_{mass} , kg) was calculated as the mass weighed (kg) corrected by its DM content (%). The DM density (DM_d , kg $DM_{mass} m^{-3}$) was calculated as the amount of DM_{mass} per $1 m^3$ (kg m^{-3}) [14]. The holder volume was 20 L for the buckets (fixed value), whereas the volumes of the bags were measured by submerging them into a graduated vessel containing water and observing the increment in the water level. The DM_r was calculated as the ratio of the DM_{mass} of the silage to the DM_{mass} of the corresponding FHM sample, while DM_{loss} is reported in Equation (1) as follows:

$$DM_{loss} = [(FHM \ DM_{mass} - silage \ DM_{mass}) / FHM \ DM_{mass}] \times 100 \quad (1)$$

For Trials #2.a.1.a and Trials #2.b, the silage DM_{mass} was calculated as the sum of DM_{mass} from layers Spl, OT1, OT3 and OT5. The porosity was calculated according to the formula proposed by Richard et al. [1,32] and reported in Equation (2) as follows:

$$\Phi = 1 - \rho_{wb} \times \{[(1 - DM) / \rho_w] + [(DM \times OM) / \rho_{om}] + [(DM \times (1 - OM)) / \rho_{ash}]\} \quad (2)$$

where ρ_{wb} is bulk density on the wet basis ($g \ cm^{-3}$); ρ_w is water density ($1 \ g \ cm^{-3}$); OM is organic matter; ρ_{OM} is organic matter density ($1.6 \ g \ cm^{-3}$); and ρ_{ash} is ash density ($2.5 \ g \ cm^{-3}$).

The silage analysis results were used to calculate a fermentation quality index (FQI) according to the method reported by Andrighetto et al. for quality index I1 [15]. Aerobic silage stability was defined as the time required to exceed room temperature of $2 \ ^\circ C$ [21–23]. Aerobic instability in maize silages was defined as when their temperature exceeded $2 \ ^\circ C$ of the room temperature [14,21,23]. The cumulative temperature ($TCUM$, $^\circ C$) was defined as the sum of the values exceeding the threshold “room temperature plus $2 \ ^\circ C$ ” [33,34].

4. Conclusions

This paper presented a large dataset of FHM and silage features of maize collected from different experimental trials covering a 7-year period. Due to its high use in dairy and beef farming, the characterisation of physico-chemical traits and nutritive value of maize silage was a priority in this research to ensure a highly productive performance and to prevent the onset of feed-related diseases. However, the lack of shared datasets, which typically have regional relevance, makes multivariate modelling approaches, such as machine or deep learning, less reliable in estimating maize silage fermentative quality. Large datasets can instead be appropriately merged and data mined conveniently to achieve better predictive accuracy. Our data are freely available to researchers who might combine them with their observations to produce a more robust interpretive model or to enhance a comprehensive understanding of the ensiling process and fermented preservation. Sharing data can be used for comparative assessment with other international silage production systems and may represent a first step in encouraging central database development.

5. Patent

These data were partially used to deposit patent number EP3586646A1/WO2019243615A1 and further extensions.

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