# IMPACT OF ENSILING TIMING AND INOCULANT USE ON FERMENTATION DYNAMICS AND AEROBIC STABILITY IN WHOLE-PLANT MAIZE SILAGE

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## ABSTRACT

Atypical ensiling conditions, such as delayed ensiling and inadequate sealing, frequently occur in practice and may impair silage quality. This study evaluated the effects of a 24 h delay before ensiling, sealing conditions during the delay, and inoculation with *Lentilactobacillus buchneri* and *Lactococcus lactis* on maize silage quality. Whole-plant maize (*Zea mays* L.; 36% dry matter) was ensiled for 60 days in 3.0 L laboratory containers with or without a commercial inoculant. Forage was ensiled immediately or after a 24 h delay, during which it was either covered or uncovered. Treatments were replicated five times, Delayed ensiling significantly impaired fermentation, increased dry matter losses, and reduced aerobic stability and hygienic quality ($p < 0.01$). Inoculation reduced temperature rise during the delay (by 8.1–8.2 °C; $p < 0.01$), lowered silage pH, decreased ammonia-N, alcohols, and butyric acid, and increased lactic and acetic acid concentrations ($p < 0.01$). Inoculated silages also showed lower dry matter losses during fermentation and aerobic exposure and reduced yeast and mold counts ($p < 0.01$). Minimizing aerobic exposure and using LAB-based inoculants can mitigate the negative effects of delayed ensiling.

Keywords: aerobic exposure, ensiling challenge, *Lentilactobacillus buchneri, Lactococcus lactis,* maize silage

## INTRODUCTION

During practical silage production, forage may remain exposed to oxygen for extended periods due to long transportation distances from the field or delays in filling and sealing the silo. Such exposure prolongs plant respiration, reduces the availability of soluble carbohydrates, and may negatively affect subsequent fermentation processes (Bruning *et al*., 2018; Michel *et al*., 2017). The presence of oxygen in the ensiled mass promotes the activity of aerobic microorganisms, leading to aerobic deterioration, nutrient losses, and reduced silage quality. During this phase, undesirable microorganisms metabolize plant substrates, which delays the development of lactic acid bacteria (LAB), increases pH, and favors the growth of yeasts and molds. As a result, the nutritional value of silage declines and the risk of spoilage increases, particularly during feed-out (Weiss *et al*., 2022). The use of silage additives, particularly LAB-based inoculants, has been widely recommended to enhance fermentation, suppress undesirable microorganisms, and improve aerobic stability (Puntillo *et al*., 2020). Therefore, the objective of this study was to evaluate the effects of overnight interrupted ensiling and the application of a LAB-based inoculant on fermentation characteristics, dry matter recovery, and aerobic stability of whole-plant maize silage.

## MATERIAL AND METHODS

After harvesting, maize forage was transported to the laboratory for silage preparation. The chopped forage was divided into two equal portions (200 kg each), thoroughly mixed, and sampled to determine chemical composition and yeast and mold counts. One portion remained untreated, while the other was treated with an inoculant containing lactic acid bacteria (LAB) strains in a 50:50 ratio of *Lentilactobacillus buchneri* DSM 22501 and *Lactococcus lactis* DSM 11037 (Novonesis, Horsholm, Denmark). The inoculant was applied at $1.5 \times 10^5$ colony-forming units (cfu) per gram of fresh crop. A portion of each treatment was immediately ensiled into 3 L mini-silos, while another portion (30 kg per treatment) was placed in 80 L plastic boxes (70 × 40 × 28 cm). The forage was compressed to fill 2/3 (60 L) of each box, achieving a density of 174 ± 3 kg/m$^3$, and left for 24 h either uncovered or covered to simulate farm conditions where ensiling may be delayed overnight. After 24 h, the forages were sampled for chemical composition and yeast and mold counts before being placed into mini-silos. The experiment followed a completely randomized design with six treatments and five replicates: T0C-non-inoculated, promptly ensiled; T1C-non-inoculated, 24 h delay, uncovered; T2C-non-inoculated, 24 h delay, covered; T3I-inoculated, promptly ensiled; T4I-inoculated, 24 h delay, uncovered; and T5I-inoculated, 24 h delay, covered. For mini-silo preparation, the forage was weighed to achieve a density of 200 kg DM/m$^3$ and packed into 3 L glass jars. After 60 days fermentation, mini-silos were opened, weighed to determine fresh weight and DM losses, and sampled for chemical and microbiological analyses. Aerobic stability was evaluated during a 7 and 17-day exposure period by monitoring temperature, yeast and mold counts, pH, chemical changes, and fresh weight loss. and data were analyzed using SAS MIXED procedures. Significance was declared at $p < 0.01$.

## RESULTS AND DISCUSSION

The data in Table 1 show that inoculated silages, whether promptly ensiled (T3I) or ensiled after a 24 h delay either uncovered (T4I) or covered (T5I), had 2.1–2.5% higher ($p < 0.01$) DMc compared with non-inoculated silages (T0C–T2C). Inoculated silages also showed 34.1–36.2% lower ($p < 0.01$) dry matter (DM) losses regardless of ensiling time or covering conditions. Non-inoculated silages ensiled after a 24 h delay (T1C and T2C) had significantly lower ($p < 0.01$) crude protein and starch contents than the other treatments. The LAB count in inoculated silages (T3I–T5I) was 1.1–1.4 $\log_{10}$ cfu g$^{-1}$ higher ($p < 0.01$) than in non-inoculated silages (T0C–T2C). At the same time, inoculated silages contained 1.0–1.2 $\log_{10}$ CFU/g fewer yeasts and 1.2–1.9 $\log_{10}$ CFU/g fewer molds ($p < 0.01$). Weiss *et al.* (2022) indicated that factors such as increased availability of WSC, air ingress and higher temperature during fermentation promote yeast growth in silage. The lowest yeast counts among inoculated silages were observed in promptly ensiled T3I, while T0C had the lowest yeast and mold counts among non-inoculated treatments. Significant differences were observed in fermentation characteristics. Inoculated silages (T3I–T5I) had lower pH values (by 0.08–0.16 units; $p < 0.01$) than non-inoculated silages. The highest pH ($p < 0.01$) occurred in the delayed, uncovered treatment (T1C). Inoculation significantly reduced ($p < 0.01$) $NH_3$-N, alcohols, and butyric acid concentrations. The lowest (by 26.8–39.6%; $p < 0.01$) ammonia-N values were observed in T3I and T5I. Ethanol production

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was highest (by 68–96 %; $p < 0.01$) in T1C silages. Inoculation also increased ($p < 0.01$) lactic acid, acetic acid, and 1,2-propanediol concentrations, with the largest increases in lactic acid and 1,2-propanediol in T3I and acetic acid in T4I. After 7 days of aerobic exposure, promptly ensiled inoculated silage (T3I) retained 8.1% more DM and 9.2% more starch ($p < 0.01$) than delayed, uncovered non-inoculated silage (T1C). Inoculated silages showed 58.7% to 2.4-fold lower DM losses and 2.6–3.0% higher metabolizable energy than non-inoculated silages ($p < 0.01$). Aerobic stability was also significantly improved, with inoculated silages remaining stable for 165.6–250.8 h and reaching peak temperatures 3.0–4.6 °C lower ($p < 0.01$). During 7 and 17 days of aerobic exposure, yeast and mold counts in inoculated silages remained significantly lower than in non-inoculated treatments.

**Tab. 1:** Chemical and microbial characteristics and aerobic stability of maize silage ensiled into mini-silo under the and fermented for 60 days (data given in g/kg DMc unless stated otherwise)

| Item | T0C | T1C | T2C | T3I | T4I | T5I | SE | p-Value |
|---|---|---|---|---|---|---|---|---|
| DMc, g/kg | 348.8$^{bc}$ | 345.8 $^{c}$ | 347.4$^{c}$ | 356.2$^{a}$ | 354.5$^{ab}$ | 355.2$^{a}$ | 3.085 | <0.01 |
| Crude protein | 92.7$^{ab}$ | 88.4 $^{c}$ | 89.9$^{bc}$ | 93.8$^{a}$ | 90.6$^{abc}$ | 91.6$^{abc}$ | 1.147 | 0.01 |
| WSC | 13.6 | 11.9 | 11.5 | 13.8 | 16.2 | 12.7 | 2.189 | 0.335 |
| Lactic acid | 46.59$^{bc}$ | 38.36$^{d}$ | 39.59$^{cd}$ | 60.54$^{a}$ | 49.78$^{b}$ | 51.6$^{b}$ | 2.320 | <0.01 |
| Acetic acid | 16.6$^{c}$ | 18.38$^{c}$ | 17.42$^{c}$ | 20.63$^{b}$ | 24.70$^{a}$ | 22.60$^{b}$ | 0.642 | <0.01 |
| Ethanol | 26.09$^{b}$ | 30.87$^{a}$ | 27.50$^{b}$ | 15.52$^{c}$ | 17.93$^{c}$ | 17.60$^{c}$ | 0.952 | <0.01 |
| 1,2-propanediol | 1.93$^{d}$ | 2.35$^{d}$ | 2.11$^{d}$ | 8.89$^{a}$ | 4.37$^{c}$ | 6.84$^{b}$ | 0.209 | <0.01 |
| Ammonia-N, g/ kg N | 52.68$^{b}$ | 63.01$^{a}$ | 55.37$^{ab}$ | 38.03$^{c}$ | 40.35$^{bc}$ | 38.56$^{c}$ | 3.208 | <0.01 |
| pH | 3.88$^{bc}$ | 3.98$^{a}$ | 3.90$^{b}$ | 3.80$^{d}$ | 3.82$^{cd}$ | 3.80$^{d}$ | 0.020 | <0.01 |
| DM loss | 61.63$^{a}$ | 69.61$^{a}$ | 64.54$^{a}$ | 40.04$^{b}$ | 44.39$^{b}$ | 42.54$^{b}$ | 2.863 | <0.01 |
| Yeast, $log_{10}$ CFU/g | 2.38$^{b}$ | 3.30$^{a}$ | 3.10$^{a}$ | 1.40$^{c}$ | 2.07$^{b}$ | 1.99$^{b}$ | 0.143 | <0.01 |
| Mold, $log_{10}$ CFU/g | 2.23$^{b}$ | 3.18$^{a}$ | 3.03$^{a}$ | 1.00$^{c}$ | 1.28$^{c}$ | 1.16$^{c}$ | 0.096 | <0.01 |
| **Aerobic stability test** | | | | | | | | |
| Aerobic stability, h | 144.0$^{c}$ | 146.4$^{c}$ | 171.6$^{c}$ | 394.8$^{a}$ | 312.0$^{b}$ | 363.6$^{ab}$ | 26.020 | <0.01 |
| Highest temp., °C | 27.72$^{a}$ | 28.14$^{a}$ | 27.34$^{a}$ | 23.12$^{c}$ | 25.12$^{b}$ | 23.44$^{bc}$ | 0.634 | <0.01 |
| Yeast, $log_{10}$ CFU/g | 8.21$^{a}$ | 9.00$^{a}$ | 8.22$^{a}$ | 2.64$^{b}$ | 2.88$^{b}$ | 2.78$^{b}$ | 0.357 | <0.01 |
| Mold, $log_{10}$ CFU/g | 6.47$^{a}$ | 6.18$^{a}$ | 6.42$^{a}$ | 1.95$^{b}$ | 2.11$^{b}$ | 2.03$^{b}$ | 0.306 | <0.01 |
| pH | 5.22$^{a}$ | 5.36$^{a}$ | 5.28$^{a}$ | 3.90$^{c}$ | 4.14$^{b}$ | 4.10$^{b}$ | 0.052 | <0.01 |
| DM loss | 148.93$^{a}$ | 153.22$^{a}$ | 133.58$^{a}$ | 62.94$^{c}$ | 89.09$^{b}$ | 78.92$^{b}$ | 10.575 | <0.01 |

T0C-non-inoculated promptly ensiled; T1C-non-inoculated with ensiling pause for 24 h uncovered; T2C-non-inoculated with ensiling pause for 24 h covered-up; T3I-inoculated and promptly ensiled; T4I-inoculated with ensiling pause for 24 h uncovered; T5I-inoculated with ensiling pause for 24 h covered-up; DMc-dry matter corrected for the loss of volatiles; WSC-water soluble carbohydrates; letters a,b,c in rows descri$^{b}$e significant differences between treatments at $p < 0.01$.

## CONCLUSION

The use of a microbial inoculant significantly improved maize silage fermentation, nutrient preservation, and aerobic stability despite delayed ensiling. Inoculation increased LAB populations enhanced lactic and acetic acid production, and reduced pH, ammonia-N, alcohols, and undesirable microorganisms. As a result, inoculated silages exhibited lower dry matter losses, higher metabolizable energy, and improved stability during aerobic exposure, effectively mitigating the negative effects of delayed ensiling

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