TRADE-OFF BETWEEN FERMENTATION QUALITY AND AEROBIC STABILITY OF ALFALFA SILAGE

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ABSTRACT

Alfalfa (*Medicago sativa* L.) is difficult to ensile due to high crude protein content (192 g/kg DM) and associated buffering capacity. A 4x2 factorial experiment (51-day continuous temperature monitoring) tested four preservation treatments (untreated control, enzymatic inoculant with hydrolytic enzymes, control inoculant without enzymes, formic acid) at 20 degrees C and 30 degrees C in three replicates. The enzymatic inoculant produced the most pronounced thermal response (+0.76 degrees C at 30 degrees C; Cohen's d = 0.240) and the optimal fermentation profile (LA:VFA ratio 3.46 at 30 degrees C; VFA 2.50% DM), but exhibited the lowest aerobic stability at elevated temperature (31 h vs. 93 h for formic acid). Formic acid demonstrated minimal and temperature-independent dry matter losses (0.32-0.54%) with excellent aerobic stability across both temperature regimes. Significant treatment x temperature interactions were identified for VFA ($F = 794.32$; $p < 0.001$) and DM losses ($F = 50.57$; $p < 0.001$). The study reveals a fundamental trade-off between fermentation quality and aerobic stability with practical implications for seasonal optimization of alfalfa silage conservation.

Keywords: alfalfa, silage, enzymatic inoculant, formic acid

INTRODUCTION

Ensiling of alfalfa (*Medicago sativa* L.) represents an important forage preservation technology, yet the combination of high buffering capacity (high crude protein) and low water-soluble carbohydrates makes it one of the most challenging forages to ensile. Biological additives containing lactic acid bacteria (LAB) and hydrolytic enzymes are widely used to optimize fermentation, but their temperature-dependent performance has not been systematically studied. Continuous temperature monitoring provides real-time information on silage metabolic activity and offers a novel methodological approach for comparative additive evaluation. The objective of this study was to evaluate the effects of biological and chemical preservation strategies on fermentation quality, DM losses, and aerobic stability of alfalfa silage at two contrasting storage temperatures.

MATERIALS AND METHODS

Chopped alfalfa (*M. sativa* L.) harvested at the vegetative stage (DM 359.5 g/kg FM; CP 191.9; ADF 317.2; NDF 399; ash 113 g/kg DM) was ensiled in vacuum LDPE bags (~500 g FM). Four treatments were applied: untreated control (CON) with distilled water; enzymatic inoculant (ENZ) containing *Pediococcus pentosaceus* + *Lactiplantibacillus plantarum* with beta-glucanase and xylanase (1 g/t); control inoculant (INO) with *L. plantarum* + *Levilactobacillus brevis* without enzymes (1 g/t); and formic acid (FOR, 85%, 4 L/t FM). A completely randomized 4×2 factorial design was used with two storage temperatures (20 +/– 1.5 degrees C and 30 +/– 2.1 degrees C for 4 weeks, then reduced to 20 degrees C) in three replicates (n = 24 units). Temperature was recorded by DS1922L sensors every 3 h for 51 days (13 May – 2 July 2025). After fermentation, aerobic stability was assessed over 7 days at 1-hour intervals following Honig's method (time to +2 degrees C above ambient). Fermentation quality (lactic acid, VFA) was determined by isotachophoresis (IONOSEP 2001). Data were analyzed by two-way ANOVA with Tukey HSD post-hoc test (Statistica v.13; alpha = 0.05).

RESULTS AND DISCUSSION

Two-way ANOVA (Table 1) revealed significant main effects and interactions for all fermentation parameters ($p < 0.05$). The treatment x temperature interaction was particularly strong for VFA ($F = 794.32$) and DM losses ($F = 50.57$), confirming that additive performance is highly temperature-dependent.

Fermentation profile, aerobic stability and DM losses are summarised in Table 2. All treatments produced well-fermented silage with pH 4.15–4.52 and no detectable butyric acid, confirming effective clostridial inhibition across all preservation methods and temperatures. Lactic acid content was not significantly different between treatments ($F = 26.29$; interaction $p = 0.17$), indicating that all strategies supported adequate lactic fermentation.

The enzymatic inoculant achieved the highest LA:VFA ratio (3.46) at 30 degrees C, indicating optimal homofermentative dominance with minimum undesirable by-products in the liquid phase. Paradoxically, ENZ 30 degrees C also showed the worst aerobic stability (31 h) and the highest DM losses (1.68%), compared with 93 h and 0.54% for formic acid at 30 degrees C. This fundamental trade-off arises because the high lactic acid concentration produced by efficient homofermentation provides an ideal substrate for lactate-assimilating yeasts upon aerobic exposure; aerobic stability was negatively correlated with lactic acid content ($r = -0.67$; $p < 0.01$).

The enzymatic inoculant produced the most pronounced thermal signature (+0.76 degrees C above CON at 30 degrees C; Cohen's d = 0.240), reflecting hydrolytic enzyme activity liberating fermentable substrates from cell walls, which fuels increased microbial metabolic heat. At 20 degrees C the thermal effect was smaller (+0.18 degrees C; Cohen's d = 0.136), demonstrating the temperature-dependence of enzymatic kinetics. Formic acid showed minimal temperature deviation from control (non-significant at 30 degrees C; Cohen's d = 0.032), consistent with its non-biological acidification mechanism.

Tab. 1: Two-way ANOVA: F-values for key fermentation parameters

Source of variation	df	pH	VFA	DM losses
Treatment (T)	3	1245.4*	2285.6*	109.7*
Temperature (°C)	1	58.33*	81.00*	38.45*
T × °C interaction	3	102.6*	794.3*	50.57*

* $p < 0.05$; NS = not significant; VFA = volatile fatty acids; DM = dry matter.

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Tab. 2: Fermentation profile, DM losses and aerobic stability of alfalfa silage

Treatment	pH	LA (% DM)	VFA (% DM)	LA/VFA	DM losses (%)	Aerobic stability (h)
CON 20 °C	4.150^{a}	9.40^{a}	3.42^{c}	2.75^{b}	0.43^{c}	57
CON 30 °C	4.160^{a}	9.10^{a}	3.33^{b}	2.73^{b}	1.62^{d}	76
ENZ 20 °C	4.190^{b}	8.83^{a}	2.94^{e}	3.00^{bc}	0.61^{c}	>168
ENZ 30 °C	4.245^{c}	8.66^{a}	2.50^{g}	3.46^{a}	1.68^{d}	31
INO 20 °C	4.375^{d}	7.61^{a}	3.00^{f}	2.54^{b}	0.20^{a}	>168
INO 30 °C	4.310^{e}	7.86^{a}	3.53^{a}	2.22^{c}	0.88^{c}	40
FOR 20g°C	4.515^{f}	6.95^{a}	2.56^{h}	2.71^{b}	0.32^{ab}	93
FOR 30 °C	4.400^{d}	7.12^{a}	2.82^{d}	2.52^{bc}	0.54^{bc}	76
Pooled SEM	*0.003*	*0.024*	*0.010*	*0.002*	*0.090*	—

Means with the same superscript letter within columns do not differ (p > 0.05, Tukey HSD). Shaded rows highlight the key trade-off. CON = control; ENZ = enzymatic inoculant; INO = inoculant without enzymes; FOR = formic acid; LA = lactic acid; VFA = volatile fatty acids (acetic + propionic); SEM = standard error of mean; aerobic stability = time to +2 degrees C above ambient

Formic acid demonstrated the smallest temperature sensitivity among all treatments: DM losses increased by only 71% from 20 to 30 degrees C (0.32–0.54%), compared with 337% for INO and 174% for ENZ. Aerobic stability for formic acid was 76–93 h (classified as excellent by Honig's system) at both temperatures, outperforming all biological treatments and supporting its reconsideration as a reliable preservative for warm climates or slow feed-out systems.

The control inoculant (INO) achieved the lowest DM losses at 20 degrees C (0.20%) with perfect aerobic stability (>168 h), demonstrating that heterofermentative LAB without enzymatic supplementation can be highly efficient in temperate storage conditions. At 30 degrees C, INO showed the highest total VFA (3.53% DM) and acetic acid (3.21% DM), likely reflecting greater heterofermentative activity under warmer conditions.

CONCLUSIONS

Continuous temperature monitoring identified characteristic thermal signatures of individual preservation strategies, with the enzymatic inoculant showing the highest metabolic response (+0.76 degrees C at 30 degrees C). A fundamental trade-off was identified between fermentation quality and aerobic stability: enzymatic inoculant achieved the best fermentation profile (LA:VFA 3.46) but the poorest aerobic stability (31 h) at 30 degrees C, while formic acid provided excellent stability (76–93 h) and minimal, temperature-independent DM losses (0.32–0.54%). For immediate consumption in moderate climates (below 25 degrees C), enzymatic inoculants with rapid feed-out are recommended.

For long-term storage or warm climates (above 25 degrees C), formic acid offers the most economically reliable performance. Preservation strategy must be selected in combination with anticipated storage temperature and feed-out management.

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