

## **EFFECTS OF HOMOFERMENTATIVE LACTIC ACID BACTERIAL INOCULANT ON THE FERMENTATION AND AEROBIC STABILITY OF SECOND CROP MAIZE SILAGE**

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### **ABSTRACT**

This study was carried out to determine the effects of homofermentative lactic acid bacterial inoculant on the fermentation and aerobic stability of second crop maize silages.

Maize was harvested at the milk stage. Inoculant -1174 (Pioneer®, USA) was used as homofermentative lactic acid bacterial inoculant. Inoculant was applied  $6.00 \log_{10} \text{cfu/g}$  silage levels. Silages with no additive served as controls. After treatment, the chopped maize was ensiled in the PVC type laboratory silos. Three silos for each group were sampled for chemical and microbiological analysis on days 2, 4, 7, 14, 21, 28 and 56 after ensiling. At the end of the ensiling period, all silages were subjected to an aerobic stability test for 14 days.

Neither inoculant improved the fermentation parameters of second crop maize silages. At the end of the ensiling period, inoculant increased lactobacilli and decreased yeast and mold numbers of silages. Inoculant treatment did not affect aerobic stability of silages.

Key words: aerobic stability, maize, inoculant, silage

### **SAŽETAK**

Svrha rada je bila utvrditi djelovanje bakterijskog cjepiva homofermentacijske mliječne kiseline na fermentaciju i aerobnu stabilnost kukuruzne silaže naknadne sjetve

Kukuruz je bran u mliječnom stadiju. Upotrijebljeno je cjepivo 1174 (Pioneer®, SAD) kao homofermentacijsko cjepivo mliječne kiseline. Cjepivo je primijenjeno  $6.00 \log_{10} \text{ cfu/g}$  razina silaže. Silaže bez aditiva poslužile su kao kontrolne. Nakon tretmana isjeckan kukuruz je siliran u laboratorijskom silosu tipa PVC. Izabrana su tri silosa za svaku skupina za kemijsku i mikrobiološku analizu 2., 4., 7., 14., 21., 28. i 56. dana nakon siliranja. Na kraju razdoblja siliranja sve su silaže podvrgnute 14 dana testiranju aerobne stabilnosti.

Niti jedno cjepivo nije poboljšalo parametre fermentacije kukuruzne silaže. Na kraju siliranja cjepivo je povećalo laktobacile i smanjilo broj kvasaca i plijesni u silažama. Tretiranje cjepivom nije djelovalo na aerobnu stabilnost silaže.

Ključne riječi: aerobna stabilnost, kukuruz, cjepivo, silaža

## INTRODUCTION

Ensiling is a preservation technology for moist whole plant forage crops which is based on lactic acid fermentation under anaerobic conditions, whereby lactic acid bacteria (LAB) convert soluble carbohydrates (WSC) into organic acids, mainly lactic acid. As a result, pH decrease and thus forage is preserved for a long time (Filya, 2000). The application of silage additives has become the conventional implement to control the ensiling process. Although the main objective in using silage additives is to ensure the fermentation process to produce well preserved silages, attention is also paid to methods of reducing ensiling losses and improving aerobic stability of silages during the feed-out period (McDonald, 1991). In order to improve the ensiling process various chemical and biological additives have been developed. Biological additives are advantageous because they are safe and easy to use, are non corrosive to machinery, do not pollute the environment, and are natural products (Sucu and Filya, 2006). Bacterial inoculants generally increase lactic acid and reduce pH, acetic acid, and butyric acid and ammonia-nitrogen levels in silage (Sheperd et.al., 1995; Aksu et.al., 2004). Inoculation of forage crops with homofermentative LAB can improve silage fermentation if sufficient fermentable substrate (WSC) is available.

Whole crop maize (*Zea mays*) is the most popular cereal crop conserved as silage in many parts of the world, and is regarded as an ideal crop for silage making because of its high yields, low buffering capacity and high WSC content (McDonald, 1981).

The purpose of this study was to focus on the effects of homofermentative LAB inoculants on the fermentation and aerobic stability characteristics of second crop maize silages.

## MATERIALS AND METHODS

Materials and silage preparation maize (*Zea mays* L.) was harvested at the milk stage of maturity ( $23.7 \pm 0.65\%$  DM). Whole plants were chopped about 2.0 cm and ensiled in PVC types silos with three replications. Three jars from each group were sampled for chemical and microbiological analysis on days 2, 4, 7, 14, 28 and 56 after ensiling. At the end of the ensiling period, the silages were subjected to an aerobic stability test for 14 days.

The following treatments were used in the experiment:

Control (no additive).

Inoculant (I): Inoculant-1174 (Pioneer®,USA) containing *Lactobacillus plantarum* and *Enterococcus faecium*. Final application rate of  $6.00 \log_{10}$  cfu/g of fresh maize.

The application rate determined by the manufacturers stated the level of LAB in the products. On the day of the experiment, inoculants were suspended in 20 ml of tap water and the whole suspension was sprayed over 10 kg (wet weight) of the chopped forage spread over a 1 x 4m area. All inoculants were applied to the forages in a uniform manner with constant mixing.

## Analytical procedures

Chemical analyses were performed in triplicate. The DM content of the fresh materials was determined by drying at 60 °C for 48 h in a fan-assisted oven (Akyıldız, 1984). pH in fresh and material and silage samples was measured according to British standard method (Anonymous, 1986). Buffering

capacity (Bc) in fresh material was estimated as described by Playne and McDonald (1966). The ammonia nitrogen (NH<sub>3</sub>-N) content of silages was determined, according to Anonymous (1986). The WSC content of silages was determined by spectro-photometer (Shimadzu UV-1201, Kyoto, Japan) after reaction with an antron reagent Anonymous (1986).

Crude protein (CP), and crude fiber (CF) were determined following the procedure of Association of Official Analytical Chemists (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to the method of Goering and Van Soest (1983).

Microbiological evaluation included enumeration of lactobacilli on pour-plate Rogosa agar (Oxoid CM627, Oxoid, Basingstoke, UK), and yeast and moulds on spread-plate malt extract agar (Difco, Detroit, MI, USA) acidified with lactic acid to pH 4.0. Plates were incubated for 3 days at 30 °C (Seale et. al., 1986). All microbiological data were transformed to log<sub>10</sub>.

The statistical analysis of the results included one-way analysis of variance and Duncan multiple range tests, which were applied to the results using the Statistical Analysis System (1988).

### **Aerobic stability test**

The silages stored for 56 days in experiment were used. After emptying PVC half of the initial contents were again put into the bottle without compaction. The top was left uncovered, and a thermometer was placed in the centre of the silage. The PVC was kept in a room maintained at 18-20°C, and daily changes in the temperature were recorded for 7 days. Aerobic deterioration was considered to have started when the difference between the silage and surrounding air reached 2°C.

## **RESULTS**

The chemical composition of the fresh and ensiled second crop maize silage is given Table 1. All silages were well preserved. In the experiment neither LAB inoculant improved the fermentation parameters of second crop maize silages. The pH of all silages decreased faster and to a greater extent. During

fermentation, no significant difference was shown between the pH values of control and inoculated silages ( $P>0.05$ ; Fig.1 ). In the experiment the WSCs in all silages decreased with decreased in pH. Inoculant treatments did not affect the concentration of WSC and  $\text{NH}_3\text{-N}$  of the silages. After 4 days of ensiling, the silages inoculated silages had higher lactic acid and lower acetic acid levels than control silages ( $P<0.05$ ). The same trends were showed at 14, 21, 28 and 56 days of ensiling. During fermentation no butyric acid was present in the silages.

The microbial composition of the second crop maize silages is given Table 2. Lactobacilli numbers of increased and yeast numbers decreased of second crop maize silages compared with the control silages.

Table 3 gives the results of the aerobic exposure test second crop maize silages. Silage deterioration indicators are pH, temperature change and increase in yeast and mold numbers. The inoculated silages had higher pH, but lower enterobacteria, mould and yeasts numbers of than the control silages.

## DISCUSSION

The success of a bacterial inoculant as a silage additive depends on many factors, such as the type and properties of the crops to be ensiled, climatic conditions, epiphytic microflora, ensiling technique and the properties of the inoculant (Henderson, 1984). Until now homofermentative LAB inoculants have been added to silage in order to stimulate lactic acid fermentation, accelerating the decrease in pH and thus improving silage preservation. In this experiment, neither homofermentative LAB inoculant improved lactic acid production second crop maize silages. During fermentation inoculant increased lactic acid and decreased acetic acid production of silages. Bolsen et al. (1989) concluded that whole crop maize was fermented rapidly and that bacterial inoculants had little effect on the rate and efficiency of silage fermentation. Observations reported by other researches (Buchanan et. al., 1981; Moon, 1981) were similar, and the present finding further confirm these earlier conclusions. Seale (1986), in his review on bacterial inoculants for silages, reported that suitable fast acid producing strains in sufficient numbers might be as effective as silage additives if the DM and WSCs of the crop are high enough. In the present study, all silages had lower pH values at an earlier stage of ensiling.

Neither LAB inoculant affected concentrations of NH<sub>3</sub>-N of second crop maize silages compared with control silage (except 2 day) McDonald et al., (1991) reported that lower pH values inhibited protein degradation in silages. Therefore, concentrations of NH<sub>3</sub>-N of all second crop maize silages were low in the experiment. At the end of the ensiling period, LAB inoculants improved the microbiological composition of second crop maize silages as expected. LAB inoculant increased lactobacilli and mould, decreased yeast numbers of second crop maize silages compared with the control silages. These findings are agreement with those reported by Spoelstra (1991), Filya (2003), Sucu and Filya (2006) and Ozduven et al., (2009).

**Table1. Chemical analysis of the second crop maize silages**

Item	At time ensiling	Control	Inoculant	P
pH	5.62	4.23±0.04	4.20±0.05	NS
Bc, mEq NaOH/kg DM	107.28	-	-	
DM, % in FM	24.76	23.00±0.00	23.10±0.32	NS
NH <sub>3</sub> -N, g/kg DM	-	0.30±0.03	0.36±0.08	NS
WSC, g/kg DM	71.58	16.28±0.78	14.02±0.45	NS
Lactic acid, % FM	-	1.82±0.06	2.06±0.56	0.05
Acetic acid, %FM	-	0.66±0.44	0.64±0.13	NS
LAB, log <sub>10</sub> cfu/g FM	2.51	4.19	4.28	0.05
Yeast, log <sub>10</sub> cfu/g FM	-	NF	NF	
Moulds, log <sub>10</sub> cfu/g FM	-	NF	NF	
Crude protein, %DM	6.29	7.00±0.08	6.92±0.08	NS
ADF, %DM	30.37	35.67±0.26	34.73±1.34	NS
NDF, %DM	55.95	56.00±1.53	54.09±2.39	NS
ADL, %DM	2.45	6.99±0.55	8.01±0.81	NS
Cellulose, %DM	24.01	26.46±0.26	27.30±0.36	NS
EE, %DM	1.43	1.54±0.02	2.10±0.06	0.01
Ash, %DM	10.16	10.69±0.09	10.60±0.14	NS

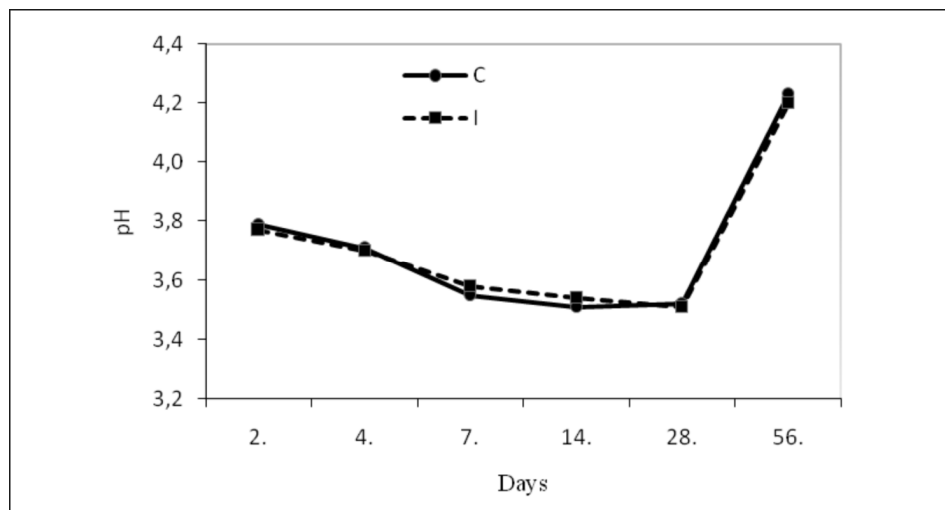
Bc: Buffering capacity, DM: Dry matter; NH<sub>3</sub>-N: Ammonia nitrogen; WSC: Water soluble carbohydrate; LAB: lactic acid bacteria; ADF: Acid detergent fiber; NDF: Neutral detergent fiber; ADL: Acid detergent lignin; EE: Ether extract; log cfu, logarithm colony forming unit; FM: Fresh Matter; NF: Not Found; NS: Not Significant.

**Table 2. Results of the aerobic stability test of the second crop maize silages.**

Items	Control	Inoculant	P
pH	7.29±0.21	7.47±0.13	NS
DM,% in FM	24.40±1.40	24.96±0.78	NS
WSC, g/kg KM	-	-	
Yeast, log <sub>10</sub> cfu/g FM	6.35	5.41	0.05
Moulds, log <sub>10</sub> cfu/g FM	3.90	4.35	0.05

DM: Dry matter; FM: Fresh Matter; WSC: Water soluble carbohydrate; LAB: Lactic acid bacteria; NF: Not Found; NS: Not Significant

**Figure 1. pH change in second crop maize silages**



Based on temperature changes, LAB inoculated silage was considered to have deteriorated after exposure to air (Figure 6). The silage temperature peaked after 5-6 days at 2°C above the ambient and cooled quickly thereafter.

Filya *et al.* (2000) hypothesized that homofermentative LAB inoculants produced mainly lactic acid, which could serve as a substrate for lactate-assimilating yeasts upon aerobic exposure. Thus, only small amounts of

Figure 2. Water soluble carbohydrate (WSC) change in second crop maize silages

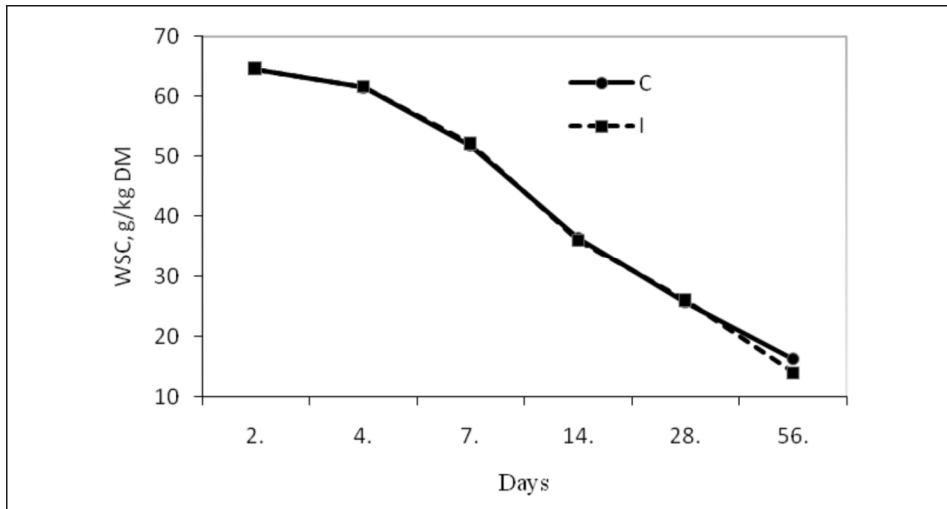


Figure 3.  $\text{NH}_3\text{-N}$  change in second crop maize silages

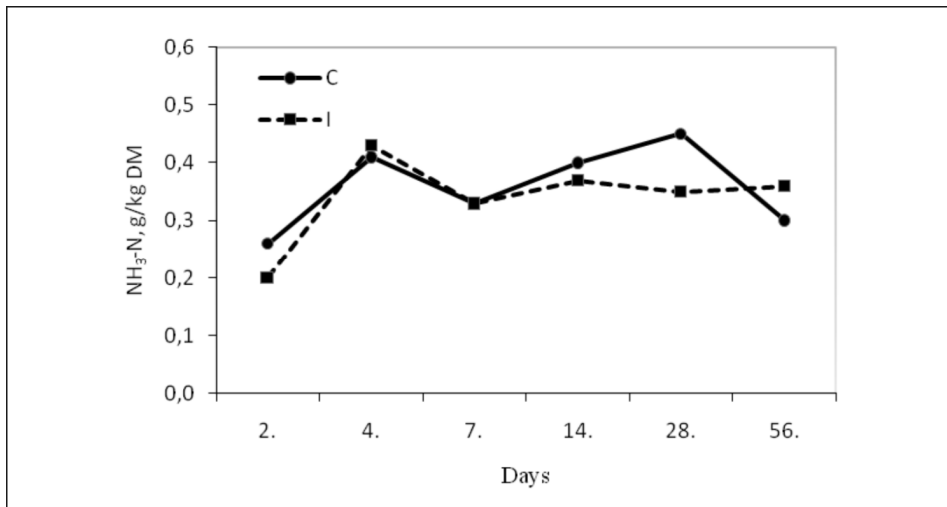




Figure 4. Lactic acid concentration change in second crop maize silages

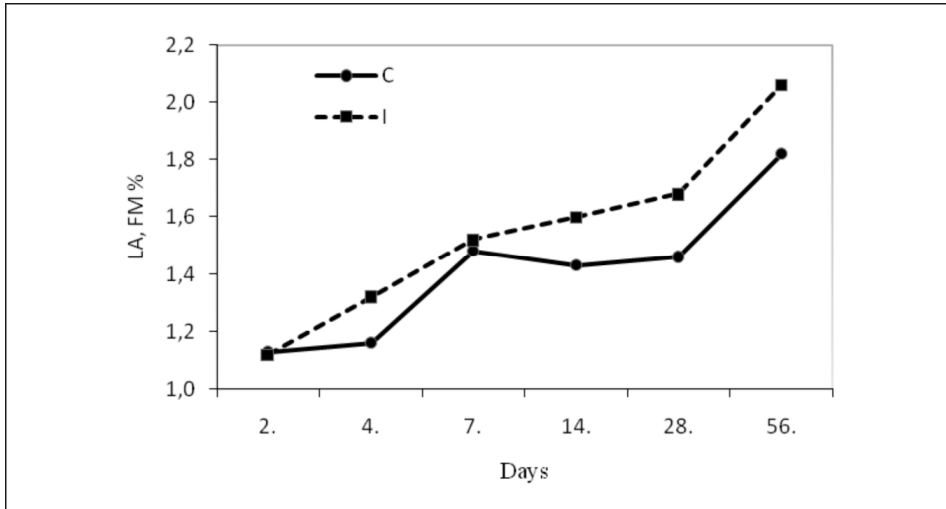
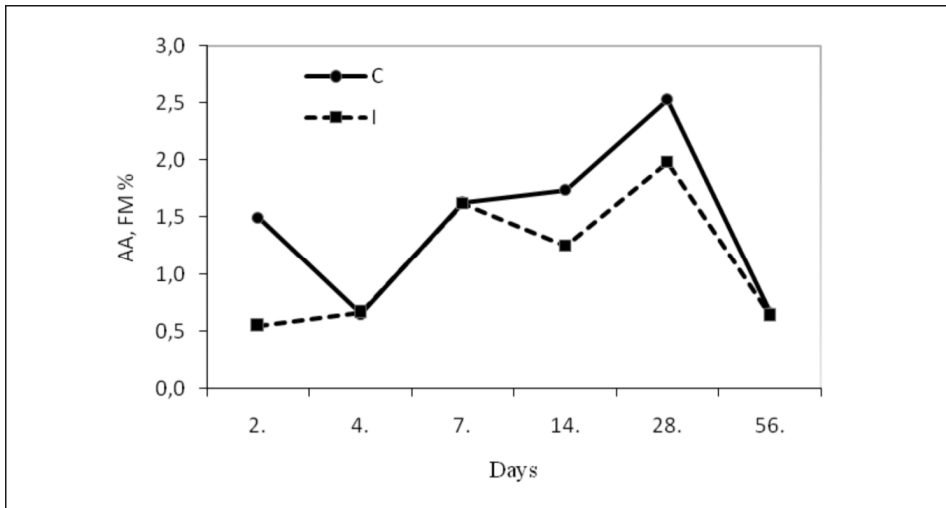
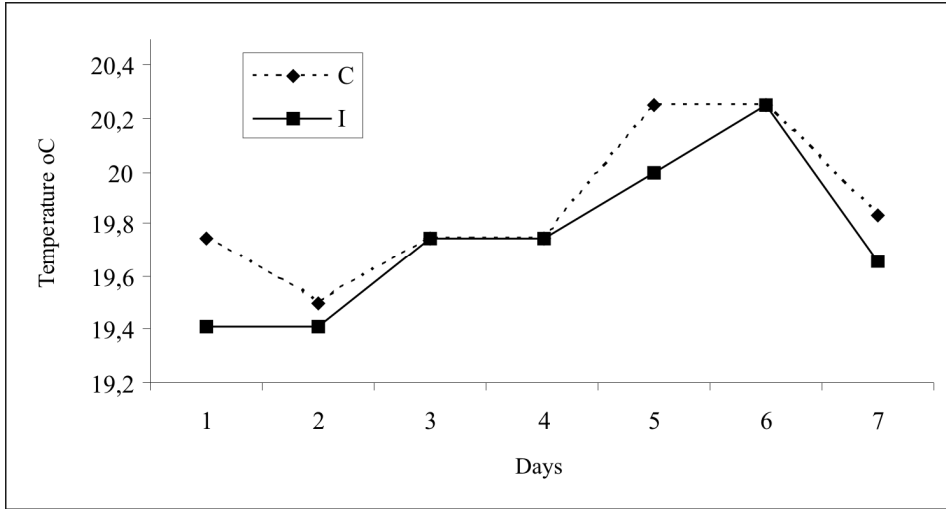


Figure 5. Acetic acid concentration change in second crop maize silages



**Figure 6. Changes in temperatures after aerobic stability exposure of second crop maize silages**



shortchain volatile fatty acids (VFAs) such as acetic, propionic and butyric acids are produced. These VFAs can inhibit yeasts and molds, making silages treated with homofermentative LAB inoculants deteriorate faster upon exposure to air. This difference between our results and those published by Ohyama *et al.* (1975) and Pahlow (1982) is probably due to the fact that these researchers infiltrated air into the silage during the ensiling period from the beginning.

## CONCLUSION

The results of the present study showed that homofermentative LAB inoculant did not improve the fermentation parameters or aerobic stability of second crop maize silage.

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