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Ensiling whole-crop wheat and corn in large containers with *Lactobacillus plantarum* and *Lactobacillus buchneri*†

ZG Weinberg[1], G Ashbell[1], Y Hen[1], A Azrieli[1], G Szakacs[2] and I Filya[3]

[1]*Forage Preservation and By-Products Research Unit, Department of Stored Products, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel;* [2]*Department of Agricultural Chemical Technology, Technical University of Budapest, Budapest, Hungary;* [3]*Department of Animal Science, Agricultural Faculty of Uludag University, Bursa, Turkey*

The effect of applying *Lactobacillus buchneri*, alone or in combination with *Lactobacillus plantarum*, at ensiling, on the aerobic stability of wheat and corn silages was studied in 50-l plastic containers. Treatments comprised control (no additives), *L. plantarum*, *L. buchneri* and a combination of *L. plantarum*+*L. buchneri*. After 3 months of storage, the wheat silages treated with *L. buchneri* had higher acetic acid contents than the control or *L. plantarum*-treated silages, and were free of mold, whereas the top layers of the control or *L. plantarum*-treated silages were moldy. In an aerobic stability test the *L. buchneri*-treated silages were stable, whereas those treated with *L. plantarum* deteriorated. In the corn silages the effects of *L. buchneri* were not as clear and the top layer was moldy in all silages. However, *L. buchneri* also improved the aerobic stability of the corn silage, as indicated by lower yeast numbers, less CO_2 production and stable pH. It is concluded that *L. buchneri* has a potential as a silage additive that protects the silage upon aerobic exposure. The 50-l plastic containers can serve as an appropriate model to test silage additives before conducting full-scale farm experiments.
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Introduction

Silage making is a method of moist forage preservation that is based on natural fermentation whereby lactic acid bacteria (LAB) convert water-soluble carbohydrates (WSCs) to organic acids, mainly lactic, under anaerobic conditions. As a result, the pH decreases, inhibiting detrimental anaerobes, and so the moist forage is preserved. Oxygen is detrimental to silage quality because it enables such aerobic spoilage microorganisms as yeasts and molds to become active [13]. Silage may be exposed to air during storage and unloading for feeding, and is susceptible to spoilage, especially in warm climates [2]. Therefore, under warm conditions, additives that protect the silage upon exposure to air might be very useful.

Inoculants, comprising homofermentative LAB, are often used to control the ensiling fermentation by rapid production of lactic acid and the consequent decrease in pH. However, such inoculants enhance the aerobic spoilage of wheat, corn and sorghum silages [10] because, in such fermentation, not enough volatile fatty acids (VFAs) are produced to protect the silage against aerobic yeasts and molds [8]. *Lactobacillus buchneri* is a heterofermentative LAB that produces high levels of acetic acid in silage; experiments in mini-silos indicated that its application upon ensilage improved the aerobic stability of the silages [5,7,12]. Conditions in laboratory mini-silos are different from those prevailing in farm silos, with regard to mixing the additives, temperature exchange with the environment, and airtightness. Therefore, it is important to test silage additives, and inoculants in particular, on a larger scale.

The purpose of the present study was to test *L. buchneri*, alone or in combination with *Lactobacillus plantarum*, a homofermentative LAB, in whole-crop wheat and corn in 50-l containers, in order to simulate farm silos better.

Materials and methods

Experimental

Wheat at the milk stage of maturity was harvested and chopped in a commercial operation and ensiled in 1.5-l anaerobic glass jars and 50-l plastic containers. The glass jars (Weck, Wher-Oftlingen, Germany) were equipped with a lid that enabled gas release only. Each jar was filled with about 650 g (wet weight) of chopped wheat without a headspace. Hence, the packing density obtained was 433 g l^{-1}, which is about 60% of the packing density of commercial bunker silos (Israeli Extension Service information). There were three jars per treatment and they were stored at ambient temperature (25–27°C) for 3 months. At the end of the experiment, the silages were subjected to an aerobic stability test lasting 5 days, in a "bottle" system described in Ref. [1]. In this system, CO_2 production, change in pH, numbers of yeast and molds, and visual appearance serve as spoilage indicators.

The 50-l containers, lined with polyethylene bags, were filled with about 15 kg of chopped wheat with moderate compaction. The packing density obtained was only 300 g l^{-1}. Plastic tubing (3 mm i.d.) for sampling gas from the inner atmosphere were inserted through the bags to the centers of the containers. The tubes were attached to the bags by adhesive tape. The open ends of the polyethylene bags were folded and sealed with adhesive tape. There

Correspondence: Dr ZG Weinberg, Forage Preservation and By-Products Research Unit, Department of Stored Products, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel
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Table 1 Analysis of the wheat silages[1]

Treatment[2]	pH	WSC[3] (g kg^{-1} of dry matter)	Lactic acid (g kg^{-1} of dry matter)	Acetic acid (g kg^{-1} of dry matter)	Ethanol (g kg^{-1} of dry matter)	% Weight loss
Fresh wheat[4]	6.2	83±26				
1.5-l glass jars						
Control	3.9±0.1^{b}	67±4^{a}	35±3^{a,b}	10±1^{b}	2±1	0.7±0.1^{b}
L. plantarum (LP)	3.8±0.0^{c}	64±0^{a}	40±3^{a}	6±1^{c}	2±0	0.6±0.0^{b}
L. buchneri (LB)	4.0±0.0^{a}	11±1^{b}	27±2^{b}	19±1^{a}	2±0	1.2±0.0^{a}
LP+LB	3.9±0.0^{b}	14±2^{b}	33±3^{a,b}	18±2^{a}	2±0	1.1±0.0^{a}
50-l plastic containers						
Control	4.0±0.1^{b}	41±4^{a}	36±3^{a}	10±4^{b}	11±0^{b}	2.3±0.1^{a}
L. plantarum (LP)	3.9±0.0^{b}	35±2^{a}	45±6^{a}	6±1^{b}	16±0^{a}	2.5±0.0^{a}
L. buchneri (LB)	4.2±0.0^{a}	22±2^{b}	10±1^{b}	33±3^{a}	2±1^{c}	1.6±0.0^{b}
LP+LB	4.0±0.1^{b}	16±2^{b}	26±7^{a,b}	29±9^{a}	1±0^{c}	1.5±0.0^{b}

[1]Numbers following the "±" are the standard error of the mean. Superscript letters within a column and silo size indicate that a value followed by the same letter did not differ significantly ($P<0.05$) in Duncan's multiple range test from other values with the same letter.
[2]The small jars in the LB treatment, and the large containers in the LB and LP+LB treatments, also contained 3 g kg^{-1} of dry matter of butyric acid.
[3]WSC=water-soluble carbohydrate.
[4]The fresh wheat contained 433 g kg^{-1} of dry matter.

were two containers per treatment, which were stored for the same period and under the same conditions as the glass jars. At the end of the experiment the silages were also subjected to the aerobic stability test in the "bottle" system [1].

Treatments: (1) Control (no additives); (2) *L. plantarum*, ATCC 8014 (American Type Culture Collection, Manassas, VA); (3) *L. buchneri*, NRRL B-1837 (USDA Northern Regional Research Center, Peoria, IL, donated by Dr LK Nakamura); (4) *L. plantarum*+*L. buchneri*.

Microorganisms were stored as freeze-dried cultures. Before use the lactobacilli were suspended in sterile MRS broth (Difco, Detroit, MI) at 30°C. The number of lactobacilli in the suspensions was 10^{8} ml^{-1}. The treatments were applied by spraying 150 ml of the suspensions over 35 kg of chopped wheat, spread over a 1×4-m area, and mixing the wheat thoroughly. Thus, about 0.5×10^{6} colony-forming units (cfu) per gram of forage were applied. The combined treatment (*L. plantarum*+*L. buchneri*) comprised 0.5×10^{6} cfu each of *L. plantarum* and *L. buchneri* per gram of forage.

A similar experiment was performed with whole-crop corn. The differences from the wheat experiments were that no gas samples or temperature measurements were taken.

Analytical procedures

Gas samples were taken from the large wheat silages with a 50-ml syringe on day 33 and at the end of the experiment, and injected into sealed glass tubes, which had been flushed by withdrawing 300 ml of gas before taking the sample for analysis. Gas analysis included the determination of carbon dioxide, oxygen and nitrogen; it was performed with an SRI 8610 gas chromatograph (SRI Instruments, Torrance, CA) equipped with Porpak Q (for CO_2) and molecular sieve 5A (for N_2 and O_2) columns, according to Ref. [9].

Dry matter was determined by oven drying for 48 h at 60°C. WSCs were determined by the phenol sulfuric acid method [6]. Lactic acid was determined by a spectrophotometry method [3]. Ethanol and VFAs were determined in aqueous extracts by means of a gas chromatograph with a semicapillary FFAP column (Hewlett Packard, Waldbronn, Germany) over a temperature range of 45–230°C. Gas losses were evaluated according to weight loss.

Microbiological analysis was performed on pooled samples except for replicate samples, which differed considerably in their appearance. Microbiological evaluation included enumeration of lactobacilli on pour-plate Rogosa agar (Oxoid CM627, Oxoid, Basingstoke, UK), and of yeast and molds on spread-plate malt extract agar (Difco) acidified with lactic acid to pH 4.0. Plates were incubated for 3 days at 30°C.

Table 2 Microbiological data for the wheat silages

Container	Treatment	LAB (log cfu g^{-1} of dry matter)	Yeasts (log cfu g^{-1} of dry matter)	Molds (log cfu g^{-1} of dry matter)
Fresh wheat		4.9	5.6	3.9
1.5-l glass jars	Control	6.6	3.7	<2.0
	L. plantarum (LP)	5.5	4.9	<2.0
	L. buchneri (LB)	4.9	<2.0	<2.0
	LP+LB	4.8	<2.0	<2.0
50-l Plastic Container	Control	7.7	4.6	3.8
	L. plantarum	7.0	4.3	3.2
	L. buchneri	4.9	<2.0	<2.0
	LP+LB	4.5	<2.0	<2.0

Table 3 Results of the 5-day aerobic stability test of the wheat silages

Treatment[1]	pH[2]	CO_2[2] (g kg^{-1} of dry matter)	Yeasts (log cfu g^{-1} of dry matter)	Molds (log cfu g^{-1} of dry matter)
1.5-l glass jars				
Control	3.9±0.0^{b}	0	5.7	2.0
L. plantarum (LP)	3.8±0.0^{b}	1.8±1.8	<2.0	<2.0
L. buchneri (LB)	4.0±0.0^{a}	0	<2.0	<2.0
LP+LB	3.9±0.0^{b}	0	<2.0	<2.0
50-l plastic containers				
Control	4.1±0.1	4.8±0.6^{b}	7.3	7.3
L. plantarum (LP)	4.8±0.5	32.2±2.3^{a}	8.5	7.6
L. buchneri (LB)	4.1±0.0	0^{c}	4.3	3.5
LP+LB	4.0±0.1	1.7±0.4^{b,c}	4.3	<2.0

[1]All samples were clean with a pleasant odor.
[2]Means of pH and CO_2 are followed by the standard error of the mean. Superscript letters within a column and silo size indicate that a value followed by the same letter did not differ significantly ($P<0.05$) in Duncan's multiple range test.

Statistical analyses included one-way analysis of variance and Duncan's multiple range test, which were applied to the results using Statistical Analysis System (SAS, Cary, NC).

Results

Wheat

Gas analysis within the large containers revealed that the CO_2 contents on day 33 were 21.5±2.7% in the control and *L. plantarum*, and 9.6±0.3% in the *L. buchneri* and *L. plantarum*+*L. buchneri* treatments; at the end of the experiment the CO_2 percentage had decreased to 10.0±1.5 and 6.2±0.5, respectively. The O_2 percentages ranged between 5.0 and 8.1 on day 33 and between 6.0 and 10.3 at the end of the experiment with no marked differences between treatments. In all cases the rest of the gas was nitrogen. The higher levels of CO_2 in the control and *L. plantarum* silages are explained by the fact that in these treatments there developed yeasts and molds that produced CO_2, whereas the *L. buchneri* inhibited these microorganisms, therefore, less CO_2 was detected. These results are supported by the higher yeast and mold counts (Table 2) and the higher ethanol levels (Table 1) obtained in the control and *L. plantarum* silages from the large containers.

After 3 months of ensiling, the top layers of the large control and *L. plantarum* silages were completely moldy and the amount discarded was 3.0–3.5% of the original mass; the top layers of the *L. buchneri* and *L. plantarum*+*L. buchneri* silages were clean and free of mold.

Table 1 gives the results of the chemical analysis of the small and large wheat silages. The control and the *L. plantarum*-treated silages contained more residual WSCs than the silages treated with *L. buchneri* or *L. plantarum*+*L. buchneri*. The latter had significantly higher levels of acetic acid. Silages treated with *L. plantarum* had a significantly higher content of lactic acid than silages treated with *L. buchneri*.

Table 2 gives the microbiological counts of the wheat silages; no yeasts or molds were detected in silages treated with *L. buchneri* and *L. plantarum*+*L. buchneri*, whereas appreciable numbers of these microorganisms were detected in the control and the *L. plantarum* silages.

Table 3 gives the results of the aerobic stability test of the small and large wheat silages. All of the small wheat silages remained stable, therefore, the advantage of *L. buchneri* was not apparent.

Table 4 Analysis of the corn silages[1]

Treatment	PH	WSC[2] (g kg^{-1} dry matter)	Lactic acid (g kg^{-1} dry matter)	Acetic acid (g kg^{-1} dry matter)	Ethanol (g kg^{-1} dry matter)	% Weight loss
Fresh corn[3]	5.7	56±6				
1.5-l glass jars						
Control	3.6±0.0^{b}	18±5	43±3^{a}	10±1^{b,c}	3±0^{a,b}	0.4±0.0^{b}
L. plantarum (LP)	3.6±0.0^{c}	14±2	33±1^{a,b}	9±1^{c}	2±0^{b}	0.3±0.0^{b}
L. buchneri (LB)	3.9±0.1^{a}	11±1	21±6^{b,c}	17±1^{a,b}	3±0^{a,b}	0.8±0.1^{a}
LP+LB	3.9±0.1^{b}	13±3	19±5^{c}	22±4^{a}	4±1^{a}	0.9±0.2^{a}
50-l plastic containers						
Control	3.8±0.1^{b}	8±0	25±2^{a}	10±1^{b}	2±0^{c}	1.7±0.2
L. plantarum (LP)	3.8±0.1^{b}	12±3	26±3^{a}	9±1^{b}	4±0^{a}	1.5±0.0
L. buchneri (LB)	4.1±0.0^{a}	13±1	9±1^{b}	23±2^{a}	4±1^{a}	1.7±0.0
LP+LB	4.0±0.0^{b}	11±1	16±2^{a,b}	20±1^{a}	3±1^{b}	1.6±0.0

[1]Numbers following the "±" are the standard error of the mean. Superscript letters within a column and silo size indicate that a value followed by the same letter did not differ significantly ($P<0.05$) in Duncan's multiple range test from other values with the same letter.
[2]WSC=water-soluble carbohydrates.
[3]The fresh corn contained 351 g kg^{-1} dry matter.

Table 5 Microbiological data for the corn silages

Container	Treatment	LAB (log cfu g^{-1} of dry matter)	Yeasts (log cfu g^{-1} of dry matter)	Molds (log cfu g^{-1} of dry matter)
Fresh corn		5.3	5.9	3.0
1.5-l glass jars	Control	7.4	3.1	<2.0
	L. plantarum (LP)	8.5	3.3	2.2
	L. buchneri (LB)	7.2	<2.0	3.2
	LP+LB	7.3	<2.0	2.2
50-l plastic containers	Control	7.9	2.7	3.8
	L. plantarum	8.1	3.2	3.9
	L. buchneri	7.8	3.9	2.7
	LP+LB	6.5	<2.0	3.2

Samples from the control, and more so from the *L. plantarum*-treated large wheat silages spoiled upon aerobic exposure, whereas those treated with *L. buchneri* or *L. plantarum*+*L. buchneri* remained stable. This was indicated by lower yeast and mold counts and lower CO_2 production (although CO_2 production from the *L. plantarum*+*L. buchneri* silages was not significantly different from the control silages).

Corn

After 3 months of ensiling, the top layers of all the large corn silages were moldy, and the amount discarded ranged from 11% in the control to 6–7% in the other silages. The silages under the moldy layer were clean and of good quality.

Table 4 gives the results of chemical analyses of the small and large corn silages. The control and the *L. plantarum*-treated large silages contained more lactic acid and less acetic acid than silages treated with *L. buchneri* or *L. plantarum*+*L. buchneri*. In the small silos the trend was similar, although not always statistically significant. Yeast and mold populations were generally low, with no marked differences among treatments (Table 5).

Table 6 gives the results of the aerobic stability test of the small and large corn silages. These results were not as clear-cut as those of the tests of the wheat silages, and in some cases replicates did not agree. After aerobic exposure, all replicates of the small control and *L. plantarum* silages became moldy, whereas among the small *L. buchneri* and *L. plantarum*+*L. buchneri* silages only one sample out of three was moldy. In the small control and *L. plantarum* silages the pH increased more than in the *L. buchneri* and *L. plantarum*+*L. buchneri* silages. Less CO_2 was produced in the L. plantarum+*L. buchneri* silages, but the microbiological counts were not consistent. The aerobic stability test of samples from the large silages gave better results with regard to the ability of *L. buchneri* to protect the silage. This was apparent from visual appraisal, the lower pH values, the smaller amounts of CO_2 and the generally lower fungal counts.

Discussion

In a warm climate such as that of Israel, whole-crop cereal silages are susceptible to aerobic deterioration. This is because aerobic yeasts are most active at 20–30°C [2]. Therefore, efforts are being made to find suitable additives that would inhibit fungi and protect the silage upon aerobic exposure. Bacterial inoculants are preferred over chemical additives because they are safe (nonhazardous), easy-to-use, noncorrosive to farm machinery, do not pollute the environment, and are regarded as natural products. Most commercial inoculants for silage include homofermentative LAB such as *L. plantarum*, *Enterococcus faecium* and *Pediococcus* spp., because they produce large amounts of lactic acid in the silage in a short time and so stabilize it with minimal losses. However, such strains enhance

Table 6 Results of the 5-day aerobic stability test of the corn silages

Treatment	Visual appraisal	pH[1]	CO_2[1] (g kg^{-1} dry matter)	Yeasts[2] (Log cfu g^{-1} of dry matter)	Molds[2] (Log cfu g^{-1} of dry matter)
1.5-l glass jars					
Control	Moldy	7.6 ± 0.1^{a}	37.7 ± 1.8	6.5	7.6
L. plantarum (LP)	Moldy	$7.1\pm0.1^{a,b}$	49.8 ± 3.3	<2.0	7.4
L. buchneri (LB)	2 Clean, 1 moldy	$5.3\pm1.1^{b,c}$	37.8 ± 22.6	<2.0	4.6, 7.4
LP+LB	2 Clean, 1 moldy	4.7 ± 0.0^{c}	18.3 ± 13.9	2.8	3.1, 7.6
50-l plastic containers					
Control	Moldy	6.6 ± 0.6	$36.5\pm0.5^{a,b}$	8.1	7.4
L. plantarum (LP)	Moldy	6.6 ± 0.3	50.8 ± 9.8^{a}	<2.0	7.2
L. buchneri (LB)	Clean	4.7 ± 0.3	9.8 ± 5.4^{b}	3.4, 7.4	3.3, 5.4
LP+LB	1 Clean, 1 moldy	5.2 ± 1.0	$20.1\pm10.8^{a,b}$	3.7	4.5

[1]Means of pH and CO_2 are followed by the standard error of the mean. Superscript letters within a column and silo size indicate that a value followed by the same letter did not differ significantly ($P<0.05$) in Duncan's multiple range test.
[2]In cases in which microbiological counts did not agree, values of both replicates are given.

aerobic deterioration of whole-crop cereal silages, probably because not enough VAFs are produced to inhibit fungi [10]. Such microorganisms are now being used in our experiments to enhance aerobic spoilage and to challenge other microbial strains to overcome such spoilage. Other bacteria that were tested for their ability to stabilize whole-crop cereal silages upon aerobic exposure included a propionic acid bacterium, which was tried with the hope that the propionic acid produced in the silage would suppress yeasts and molds that spoil silages under aerobic conditions. However, this microorganism had only a marginal effect on the aerobic stability of whole-crop cereal silages, because it could not withstand silage conditions [11]. *L. buchneri* is a heterofermentative LAB, which was isolated from corn silage [5] and which produces high levels of acetic acid in silage. Results with this microorganism in mini-silos were promising with regard to aerobic stability [5,7,12]. However, conditions prevailing in farm silos might be different in many respects from those in mini-silos and, therefore, it is important to test any new additive under farm conditions. Since treating whole bunker silos is expensive and complicated (because of the need for a parallel control silage), it was decided to test *L. buchneri* in 50-l plastic containers before treating whole silos.

Results from the 1.5-l jars and the 50-l containers agreed in general, but some differences were noticed that could have partially evolved from the different packing density of the two silo types: fermentation losses were larger in the large containers, and in the experiment with wheat, *L. buchneri*-treated silages from the large containers contained higher levels of acetic acid than those from the small jars. When some air is present in the silage during storage, there is a shift in the metabolism of the homofermentative LAB and they also produce acetic acid [4]. The control and the *L. plantarum*-treated silages from the large containers deteriorated upon aerobic exposure, whereas those from the small jars remained stable. Therefore, we think that such large containers, that are not hermetically sealed, may serve as a model that simulates farm silos better than the tightly sealed glass jars.

In the experiment with wheat *L. buchneri* was very effective in preserving the silage upon aerobic exposure and in the experiment with corn *L. buchneri* improved the aerobic stability of the silage, although not completely, maybe because the corn was more sensitive to air than the wheat.

In conclusion, the results indicate the *L. buchneri* can be included in silage inoculants in order to stabilize silages upon aerobic exposure.

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