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Effects of *Lactiplantibacillus plantarum* from Homemade Pickles on Mixed Maize–Soybean Silage Quality

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Abstract: This study was conducted to determine the influence of *Lactiplantibacillus plantarum* (LpP) isolated from homemade pickles on chemical, physical, and microbiological properties, in vitro digestibility and feed value, and aerobic stability in pure maize and mixture silages of maize and soybean. The treatment groups were inoculated maize silage, inoculated soybean silage, inoculated 75% Maize + 25% Soybean silage, inoculated 50% Maize + 50% Soybean silage, inoculated 25% Maize + 75% Soybean silage, and their respective uninoculated control silages. By inoculating maize and soybean combination silages with LpP, these silages' organic matter content increased, and their neutral and acid detergent fiber contents decreased. In these silages, lactic acid bacteria content and relative feed value increased, while yeast levels decreased, compared to the control silage. Inoculation improved silage quality and aerobic stability by reducing CO_2 production, especially in pure soybean silage, and reduced the in vitro digestible organic matter and net energy lactation value of pure maize and pure soybean silages but did not affect maize-soybean mixed silages. The results showed that LpP isolated from homemade pickles can contribute to increase silage quality with respect to aerobic stability in low-structural carbohydrate contained silage materials, and maize itself for these materials. It can be suggested that LpP isolated from homemade pickles can be safely used as a bacterial inoculant for ensiling crops such as soya crops, which are difficult and risky to ensilage, alone or in mixtures with maize.

Keywords: maize and soybean silage; microbial inoculation; nutrient composition; aerobic stability; in vitro digestibility; feed value

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1. Introduction

Silage is one of the roughages commonly used in ruminant animals' nutrition. Maize is one of the most preferred silage plants around the world because of its high-energy content, deliciousness, sufficient water-soluble carbohydrates, easy ensiling, and, most importantly, cheapness [1]. However, its low crude protein content limits its use. Pure maize silage has low protein content, varying between 5.50% and 10.22% in dry matter (DM) and depending on the plant type, harvest time, soil structure, and climate characteristics, and cannot meet the protein needs of livestock alone [2]. To eliminate this weak feature of maize silage, it has become a common practice to ensile it in mixtures with other plants, such as forage soybeans.

Forage soybean, featured by rich protein and vitamins, is a quality and promising green forage source for livestock. However, as with other legumes, it has poor ensiling ability due to insufficient water-soluble carbohydrates, high-buffering capacity, and low-epiphytic lactic acid bacteria (LAB) content. Therefore, in silages containing high amounts of legumes, the pH does not decrease easily, and the amount of butyric acid increases in the medium [3]. The high-butyric acid production also creates unpleasant odors in the silage. Such silages are not consumed with appetite by animals. It is necessary to implement some strategies to prevent the problems in making silage from forage soybean, legumes, and to increase the silage's quality. These strategies include LAB inoculation, enzyme addition, molasses, nitrate, and organic and inorganic acid addition [4]. The ensiling of legume and wheat forage crops together also provides a special meaning to these strategies [5].

LABs are microorganisms widely used to improve and maintain the quality of ensiled feed [6]. These bacteria ferment easily soluble carbohydrates into organic acids, mainly lactic acid, under anaerobic conditions. Its most important species belong to *Enterococcus*, *Lactiplantibacillus* (*Lp.*, *formerly Lactobacillus*), *Pediococcus*, *Lactococcus*, and *Bacillus* [7].

Lactiplantibacillus plantarum (LpP) is a frequently preferred bacterial species in the fermented food industry because it improves the taste, texture, and shelf life of fermented foods and increases their nutritional value [7,8]. Due to these properties, it is frequently used in silage production [9]. This bacterial strain effectively produces lactic acid in silage and rapidly decreases pH. This prevents the growth and development of harmful microorganisms in the environment. As a natural consequence, they contribute to the improvement of fermentation quality and feed value in the silages to which they are added [10].

In this study, it was hypothesized that LpP isolated from homemade pickles could be inoculated into maize–soybean mixed silages to improve feed value, chemical, physical, microbiological properties, aerobic stability (improving silage storage life), in vitro digestibility, and energy value of silages. The fact that no such study has been encountered before also added original value to the study. Therefore, the aim of this study was to determine the contributions of the LpP strain isolated from homemade pickles to chemical, physical, and microbiological properties, aerobic stability, in vitro digestibility, and energy value in maize–soybean mixed silages.

2. Materials and Methods

2.1. Chemical, Physical, and Microbiological Properties

Silage maize was harvested during the milk maturity stage, and silage soybean was harvested at the 1/2–3/4 stage of seed filling. Materials were withered in the laboratory for 24 h to remove excess water and chopped into 3–5 cm pieces with a laboratory-type chopper. Initially, the fresh plant materials of experimental silages were analyzed chemically according to the procedures as provided below, and the results of these analyses are provided in Table 1.

Table 1. Nutrient composition of fresh plant materials before ensiling.

Items	Maize	Soya Bean
Dry matter, g/kg fresh plant	319.4	279.8
Organic matter, g/kg DM	928.6	881.2
Ash, g/kg DM	71.38	118.8
Crude protein, g/kg DM	88.1	145.1
Ether extract, g/kg DM	33.9	58.5
Starch, g/kg DM	281.1	176.5
Crude fiber, g/kg DM	335.1	240.2
Acid detergent fibers, g/kg DM	163.7	322.0
Neutral detergent fibers, g/kg DM	584.2	504.2
Hemicellulose, g/kg DM	153.1	182.3

Chopped maize and soya forages were combined in different proportions based on fresh material to form the treatment groups. These chopped forage materials were packed into two layers of polyethylene bags (their manufacturing traits are mentioned in the instruction as 250 mm × 200 mm sized, 1 kg capacity, oxygen permeability 1.13 cc/m^2/day), each containing 500 g of forage. Then they were vacuum sealed using a vacuum device (Packtech PT-VKM-CPRO, Nurcivan Industrial Packacing Sytems, Kocaeli, Türkiye) and stored at room temperature 22–25 °C for 60 days. This study was planned according to a 2 × 5 factorial experimental design. Therefore, 10 silage groups were formed, half of which were inoculated with LpP, while the other half was not inoculated. Six silage samples were created from each silage group for a total of 60 silages. Four of these were used in the analyses, while the other two were kept as reserves. The silage groups were pure maize, pure soybean, 75% Maize + 25% Soybean, 50% Maize + 50% Soybean, and 25% Maize + 75% Soybean.

The LpP used in inoculation was isolated from homemade pickles according to the method detailed by Erdem et al. [11]. For this purpose, 10 mL of pickle juice were centrifuged at 3500 rpm for 10 min. The resulting precipitate was inoculated onto MRS (de Man, Rogosa, and Sharpe) agar. A biochemical fermentation kit was used to identify the bacterial strain. The isolates obtained were stored in a deep freezer at −80 °C in sterile Eppendorf tubes containing 15% glycerol. Then, the DNA of the isolates was extracted, and molecular identification was performed.

The stock culture created for the identified bacterial strain was stored at −80 °C in MRS containing 35% glycerol (*v*/*v*). Before use, the stock culture was thawed at 4 °C. And then they were incubated at 37 °C for 3 days. In the counts made, the concentration of LpP was determined as 1.0×10^6 cfu/mL. The LpP was inoculated into silages by spraying 1 mL into 1 kg of feed with a sterile syringe. The uninoculated control group was treated with the same amount of distilled water.

Silages were opened on the 60th day of fermentation, and chemical, physical, and microbial analyses, as well as aerobic stability and in vitro digestibility tests, were performed. The DM, ash, and total protein content (Kjeldahl procedure) of silages were analyzed according to AOAC [12]. Ether extract (EE) content was determined by the ANKOM XT15 ES instrument according to AOCS [13]. Crude fiber (CF), neutral detergent fiber (NDF), and acid detergent fiber (ADF) content of the silages were analyzed using ANKOM 200 Fiber Analyzer, following the method outlined by Van Soest et al. [14]. Organic matter (OM) contents of silages were obtained by subtracting ash content from dry matter (DM) content. Hemicellulose (Hcel) was calculated as the difference between the NDF content and the ADF content. Starch analysis was conducted using the polarimetric method [15].

The pH values were measured using a standard digital pH meter, while total soluble solids (TSS) content was analyzed with a digital refractometer (HI 96801, Hanna Instruments) with a sensitivity of 0.2% Brix. For this, a few droplets of juice from silage were pressed onto the digital refractometer’s surface, and the value appearing on the digital display was recorded as percentage brix (% Bx) [16]. The Flieg score (FS) was determined by the following equation [17]:

$$FS = 220 + (2 * DM\% - 15) - 40 * pH \tag{1}$$

The digestible dry matter (DDM) content of the silages and dry matter intake (DMI) amounts were determined by the following equations provided in the National Research Council, NRC [18]. The relative feed value (RFV) of the silages was calculated using the following equation suggested by Rohweder et al. [19].

$$DDM,\ \% = 88.9 - [0.779\ *\ ADF\%] \tag{2}$$

$$DMI,\ \%\ of\ Body\ Weight = 120/[NDF\%] \tag{3}$$

$$RFV = [DDM * DMI]/1.29 \tag{4}$$

The L^*, a^*, and b^* color parameters of the silages were determined using the Konica Minolta CR-410 Color Meter. The range of color values L^* brightness (100: white, 0: black), a^* from red to green ($-a^*$: green, $+a^*$: red), and b^* from yellow to blue ($-b^*$: blue, $+b^*$: yellow). Chroma (C^*), known as the saturation index, and hue angle (h^o) values were calculated with the formula [20].

$$(C)_{ab} = [(a)^2\ +\ (b)^2]^{1/2} \tag{5}$$

$$(h)_{ab} = \text{arctangent}(b/a) \tag{6}$$

The microorganism content of the silages was determined by the plate count method [21]. The determined values were converted to logarithmic colony-forming units ($\log_{10}$ cfu/g).

2.2. Aerobic Stability

The amount of CO_2, used as one of the criteria in the evaluation of the aerobic stability of silages, was determined as explained by Ashbell et al. [22]. For this purpose, a setup consisting of a 2 L polyethylene water bottle and a 600 mL glass beaker was used. Two holes, each one cm in diameter, were drilled into the top and bottom of the plastic bottle to allow air to enter and exit. The pet bottle was placed on the glass beaker, and 200 g of silage sample was placed inside. A hundred milliliters of 20% potassium hydroxide solution were placed in the beaker at the bottom of the device. The pet bottle was placed in a beaker containing potassium hydroxide solution, with the lid part down. The hole at the top of the system was left open to allow gas inflow and outflow. In this condition, the system was kept in the laboratory for 5 days (120 h). During this period, the amount of CO_2 accumulated in the potassium hydroxide solution was determined by titrating with 1 N hydrochloric acid.

2.3. In Vitro Digestibility and Feed Value

In vitro organic matter digestibility and energy value of samples were determined using the ANKOM^{RF} Gas Production System (Ankom Technologies, Macedon, NY, USA), which consists of a 250 mL glass bottle and a module connected to them [23]. The amount of gas production (GP) occurring in the system was used as the basic data in determining the metabolizable energy content (ME_{gp}) and net energy lactation (NE_l) content of the silages. When the ambient pressure inside each unit rises above 1.5 kPa, the module's valve automatically releases the gas collected at the top of the glass bottles. The internal pressure of the glass bottles was measured every 5 min. The buffer solution required for the system was prepared according to Menke and Steingass [23] and mixed with rumen fluid in a ratio of 4:1 (80 mL artificial saliva solution and 20 mL rumen fluid) to create buffered rumen fluid. The buffer solution was created from 499.3 mL of distilled water, 250 mL of macro mineral solution, 0.12 mL of micromineral solution, 250 mL of buffer solution, 1.25 mL of resazurin solution, and 24 mL of reduction solution. Rumen fluid was obtained from three male Simmental bulls, aged 24–30 months, with a live weight of 450–500 kg, slaughtered in a special integrated meat facility, and consumed a ration prepared with 65% concentrate + 35% alfalfa hay. The rumen content was brought to the laboratory in approximately 15 min in a thermos set at 39 °C containing CO_2 After temperature and pH controls (6.23–6.28) were made, it was filtered using a four-layer cheesecloth and fresh. For testing, 1 g of sample was placed into each preheated bottle and 100 mL of buffered rumen fluid was added. The bottles were incubated in water at 39 °C for 24 h. Each sample was evaluated in three replicates. The determined gas pressure was first converted to mole units (Equation (7)) and then to milliliter (mL) units (Equation (8)) as follows:

$$n = (pV)/(RT) \tag{7}$$

$$GP,\ \mathrm{mL} = n * 22.4 * 1000 \tag{8}$$

n (mol): Produced gas; p (kilopascals, kPa): Pressure; V (L): Topside volume in the bottle; T (Kelvin, K): Temperature; R (L·kPa/K/mol): Gas constant, 8.314472.

Using the amounts of gas produced, in vitro organic matter digestibility (IVOMD), ME_{gp} and NE_l of silages were calculated with the help of equations defined by Menke and Steingass [23].

$$IVOMD,\ \% = 14.88 + 0.8893 * GP + 0.448 * CP + 0.651 * ash \tag{9}$$

$$ME_{gp}, \mathrm{MJ/kgDM} = \left(2.20 + 0.136 * GP + 0.057 * CP + 0.0029 * CP^2\right) \tag{10}$$

$$NE_l,\ \mathrm{MJ/kgDM} = \left(0.54 + 0.0959 * GP + 0.038 * CP + 0.001733 * CP^2\right) \tag{11}$$

GP: Net gas production for 24 h (mL/200 mg DM), *CP:* Crude protein (%), *EE*: Ether extract (%).

2.4. Statistical Analysis

The data collected were analyzed by using general linear model (GLM) in SPSS software (Windows version of SPSS, released 25.00). Also, the means were compared by using the Multiple Duncan Range Test within the same software at $p < 0.005$ level.

3. Results and Discussion

3.1. Chemical, Physical, and Microbiological Properties

Data on the nutritional content of silages are provided in Tables 2 and 3. It was observed that both, together, ensiling of maize-soybean plants and/or inoculation with LpP were effective on the nutritional composition of the silages evaluated in the research. The dry matter content of the silages ranged from 294.4 to 332.3 g/kg. Inoculant application increased the DM content of pure maize, 75% Maize + 25% Soybean, and 50% Maize + 50% Soybean silages. The values agreed with values reported by Serbester et al. [24] for maize silages (28 and 29%) and Carpici [25] for maize + soybean mixed silages (19.66 and 32.55%). On the contrary, Erdal et al. [26] reported lower silage DM in maize and soybean mixture silages (22.18–25.23%). The LpP inoculation increased the OM and EE contents of the silages. However, a decrease was observed in the CF, ADF, NDF, and Hcel contents ($p < 0.001$). It was determined that as the amount of soybean plant in the mixture increased, the CP content of the silage also increased ($p < 0.001$). This may be because the soybean plant has a higher protein content than the maize plant (Table 1). This finding is consistent with the findings of Kızılşimşek et al. [27], who reported that the crude protein content of silage increases as the soybean content in silage increases. The LpP inoculation increased the CP content of pure maize, while it decreased it in other silages ($p < 0.001$). This result may be explained that LpP preferred the use of carbohydrates rather than that of crude protein in maize. On the contrary, the other silage materials had more CP content than that of maize. These higher CP contents were most likely degraded by plant proteases during fermentation process. Indeed, Zhang et al. [28] and Da Silva et al. [29] reported that during silage fermentation, real proteins in the silage may be degraded and non-protein nitrogenous compounds may increase in the environment. Less proteolysis may have occurred in maize silage. The EE content increased with the effect of both LpP inoculation and mixture ratios ($p < 0.001$).

Table 2. Dry matter, organic matter, ash, crude protein, and ether extract contents of the silages.

	Silages	Dry Matter (g/kg)	Organic Matter (g/kg DM)	Ash (g/kg DM)	Crude Protein (g/kg DM)	Ether Extract (g/kg DM)
Uninoculated	Maize	303.4 ^{b}	952.9 ^{b}	47.1 ^{g}	65.0 ^{h}	48.2 ^{i}
	Soybean	296.7 ^{b}	901.9 ^{h}	98.1 ^{a}	159.6 ^{a}	93.1 ^{b}
	75% Maize + 25% Soybean	296.8 ^{b}	925.7 ^{d}	74.3 ^{e}	99.4 ^{de}	74.3 ^{f}
	50% Maize + 50% Soybean	298.2 ^{b}	926.9 ^{d}	73.1 ^{e}	102.0 ^{d}	60.4 ^{g}
	25% Maize + 75% Soybean	303.2 ^{b}	905.2 ^{f}	94.8 ^{b}	117.1 ^{c}	83.0 ^{c}
	SEM	1.947	6.107	6.107	10.219	5.321
Inoculated	Maize	332.3 ^{a}	968.2 ^{a}	31.8 ^{h}	68.2 ^{g}	56.6 ^{h}
	Soybean	294.4 ^{b}	918.9 ^{e}	81.1 ^{d}	124.7 ^{b}	95.5 ^{a}
	75% Maize + 25% Soybean	328.0 ^{a}	942.3 ^{c}	57.7 ^{f}	97.9 ^{e}	76.2 ^{e}
	50% Maize + 50% Soybean	306.9 ^{b}	953.8 ^{b}	46.3 ^{g}	88.4 ^{f}	61.7 ^{g}
	25% Maize + 75% Soybean	303.5 ^{b}	914.6 ^{e}	85.4 ^{c}	117.3 ^{c}	79.6 ^{d}
	SEM	3.088	6.806	6.806	6.767	4.600
	Pooled SEM	2.529	4.860	4.860	6.059	3.432
p-values	Silage	0.000	<0.001	<0.001	<0.001	<0.001
	Inoculant	<0.001	<0.001	<0.001	<0.001	<0.001
	Silage × Inoculant	<0.001	<0.001	<0.001	<0.001	<0.001

SEM, standard error of means. $^{a–h}$ Means with different superscripts in the same column differ significantly ($p < 0.05$).

Table 3. Crude fiber, starch, acid detergent fiber, neutral detergent fiber, and hemicellulose contents of the silages.

	Silages	Crude Fiber (g/kg DM)	Starch (g/kg DM)	ADF (g/kg DM)	NDF (g/kg DM)	Hcel (g/kg DM)
Uninoculated	Maize	184.3 ^{e}	409.6 ^{b}	223.1 ^{d}	406.7 ^{a}	183.6 ^{a}
	Soybean	157.6 ^{f}	166.5 ^{g}	290.2 ^{a}	364.5 ^{b}	150.7 ^{b}
	75% Maize + 25% Soybean	184.6 ^{e}	247.8 ^{e}	219.0 ^{d}	340.4 ^{bc}	113.5 ^{d}
	50% Maize + 50% Soybean	213.1 ^{b}	220.3 ^{f}	266.9 ^{c}	407.5 ^{a}	140.6 ^{bc}
	25% Maize + 75% Soybean	217.6 ^{a}	106.3 ^{h}	290.4 ^{a}	398.4 ^{a}	99.6 ^{d}
	SEM	7.302	33.99	10.45	9.06	9.98
Inoculated	Maize	146.9 ^{g}	415.9 ^{a}	166.1 ^{g}	305.3 ^{d}	131.6 ^{c}
	Soybean	200.0 ^{c}	62.3 ^{j}	277.2 ^{b}	349.4 ^{bc}	66.9 ^{e}
	75% Maize + 25% Soybean	160.0 ^{f}	299.0 ^{c}	180. 7 ^{f}	284.0 ^{e}	96.8 ^{d}
	50% Maize + 50% Soybean	161.4 ^{f}	255.6 ^{d}	189.0 ^{e}	300.6 ^{de}	105.0 ^{d}
	25% Maize + 75% Soybean	191.3 ^{d}	80.9 ^{i}	70.8 ^{bc}	309.3 ^{d}	35.8 ^{f}
	SEM	6.760	44.740	15.822	7.461	11.119
	Pooled SEM	5.336	27.358	10.366	10.212	9.287
p-values	Silage	<0.001	<0.001	<0.001	<0.001	<0.001
	Inoculant	<0.001	<0.001	<0.001	<0.001	<0.001
	Silage × Inoculant	<0.001	<0.001	<0.001	<0.001	<0.001

ADF, acid detergent fiber; NDF, neutral detergent fiber; Hcel, hemicellulose; SEM, standard error of means. $^{a–h}$ Means with different superscripts in the same column differ significantly ($p < 0.05$).

While LpP inoculation increased the starch content of pure maize silage, it decreased it in pure soybean silage ($p < 0.001$, Table 3). In this study, the starch content of maize silage was determined as 409.6 in uninoculated maize silage and 415.9 g/kg DM in inoculated maize silage. Khan et al. [30] reported that the starch content of maize silage could increase up to 380–400 g/kg DM, depending on the harvest period of maize. Heinzen Jr et al. [31] emphasized that the starch content in silage may be related to the vegetation period of the plant, initial crude protein, and starch density. In this study, the LpP inoculation to both pure and mixed silages of maize and soybean plants decreased the ADF, NDF, and Hcel contents of the silages ($p < 0.001$). Both mixture proportion and LpP inoculation were effective in this decrease. Khan et al. [30] reported that the nutritional differences of maize silages may be caused by many factors, but the most important factor among these factors is the maturity degree of the plant. Marbun et al. [32], who reported that LpP application increased the CP content and decreased the NDF content in maize silage, also obtained similar results. Sarubbi et al. [33] emphasized that DMI is critical for animal performance and that decreasing ADF and NDF content of forages can significantly increase feed intake. Indeed, in this study, it was observed that the decrease in ADF and NDF content of silages increased the calculated DMI values (Table 4). On the other hand, the reason for the decrease in the cell wall components of silages may be the consumption of nutrients such as TSS, CP, and other nutrients by LAB. This may have increased the bacteria's affinity for cell wall components. These findings agree with those of Ni et al. [34], who determined that LpP application decreases the cell wall content of silages.

Table 4. The relative feed value of the silages and the values of the relevant parameters.

	Silages	Digestible Dry Matter (%)	Dry Matter Intake (% of Body Weight)	Relative Feed Value
Uninoculated	Maize	71.5 ^{d}	2.95 ^{e}	164 ^{ef}
	Soybean	66.3 ^{g}	3.30 ^{d}	169 ^{de}
	75% Maize + 25% Soybean	71.8 ^{d}	3.53 ^{c}	196 ^{c}
	50% Maize + 50% Soybean	68.1 ^{e}	2.95 ^{e}	156 ^{f}
	25% Maize + 75% Soybean	66.3 ^{g}	3.02 ^{e}	155 ^{f}
	SEM	0.813	0.077	5.58
Inoculated	Maize	76.0 ^{a}	3.94 ^{b}	232 ^{b}
	Soybean	67.3 ^{f}	3.44 ^{cd}	179 ^{d}
	75% Maize + 25% Soybean	74.2 ^{c}	4.23 ^{a}	245 ^{a}
	50% Maize + 50% Soybean	67.8 ^{ef}	4.00 ^{b}	230 ^{b}
	25% Maize + 75% Soybean	74.8 ^{b}	3.8 8 ^{b}	204 ^{c}
	SEM	1.233	0.090	8.00
	Pooled SEM	0.808	0.104	7.19
p-values	Silage	<0.001	<0.001	<0.001
	Inoculant	<0.001	<0.001	<0.001
	Inoculant x Silage	<0.001	<0.001	<0.001

SEM, standard error of means. $^{a–g}$ Means with different superscripts in the same column differ significantly ($p < 0.05$). I, >151, the best quality; 125–151, prime quality; 103–124, II. Quality; 87–102, III. Quality; 75–86, IV. Quality; < 75, V. quality or poor quality.

The RFV of the silages and the values of the relevant parameters are presented in Table 4. The DDM and DMI values were found between 66.3 and 76.0% and 2.95 and 4.23%, respectively. The RFV values ranged from 155 to 245. The LpP inoculation of the mixed silages of maize and soybean increased the DDM value of the silages. The highest DDM was obtained for inoculated pure maize (76.0), while the lowest DDM was obtained for uninoculated 25% Maize + 75% Soybean (66.3). The LpP inoculation increased RFV in experimental silages compared with control silages, except for pure soybean silage ($p < 0.001$). RFV is an important index value that reveals feed quality and has been used by scientists and other industry representatives for many years. The NDF and ADF are used to determine this index. Higher RFV values indicate higher forage quality [19]. The highest RFV value was obtained in inoculated 75% Maize + 25% Soybean (245.45), while the lowest value was obtained in 25% Maize + 75% Soybean silage (154.79). According to the RFV scale, all silages showed first-quality class value (>151).

The color parameter values of silages at the opening time are provided in Table 5. The colorimetric evaluation method was used to evaluate silage in terms of color [35]. This method offers a more objective assessment compared to the sensory scoring method. The color of quality silages varies from light olive green to brownish olive, depending on the plant variety. Dark black-colored silages are undesirable silages. While mixing silage materials affected the L^* values (lightness) of silages, LpP application did not affect them. The L^* values obtained in the study were determined between 31.2 and 33.6. It is thought that animals prefer silages with high L^* values [36]. The mixing of silage materials did not affect the a^* value of the silages ($p > 0.05$) but significantly affected the b^* and the C^* values ($p = 0.014$). Similarly, LpP treatment did not affect the a^* value of silages ($p > 0.05$) but significantly affected the b and C values ($p = 0.001$). There was no difference between silages in terms of the h° value ($p > 0.05$). When the color parameters were evaluated as a whole, the LpP inoculation to maize and soybean mixed did not cause any negative effects on the color quality of the silages. This can be explained by the obtained silage fermentation quality by LpP treatment for all experimental silages. Increasing lactic acid bacteria density is reflected in the pH of the silage, causing the pH to decrease. As a result, the development of undesirable microorganisms in the silage is prevented, the fermentation process is improved, and the silage color remains in its natural state.

Table 5. The color parameter values at the opening time of silages.

	Silages	Lightness (L^*)	Redness (a^*)	Yellowness (b^*)	Chroma (C^*)	Hue Angle (h°)
Uninoculated	Maize	29.7^{bcd}	1.84	8.88^{c}	9.08^{c}	78.3^{ab}
	Soybean	26.0^{cd}	2.23	9.02^{c}	9.30^{c}	76.2^{abc}
	75% Maize + 25% Soybean	28.8^{bcd}	2.68	9.84^{bc}	10.2^{c}	74.8^{abc}
	50% Maize + 50% Soybean	30.3^{abc}	3.60	10.4^{bc}	11.0^{bc}	70.9^{c}
	25% Maize + 75% Soybean	25.3^{d}	1.69	8.53^{c}	8.71^{c}	78.7^{a}
	SEM	0.702	0.255	0.235	0.282	1.187
Inoculated	Maize	33.6^{a}	3.68	14.6^{a}	15.3^{a}	72.3^{bc}
	Soybean	26.1^{cd}	1.81	9.26^{c}	9.44^{c}	78.8^{a}
	75% Maize + 25% Soybean	31.2^{ab}	2.25	12.3^{ab}	13.3^{ab}	78.1^{ab}
	50% Maize + 50% Soybean	29.8^{abcd}	2.43	10.5^{bc}	10.8^{bc}	76.9^{abc}
	25% Maize + 75% Soybean	28.1b^{cd}	2.63	10.2^{bc}	10.5^{bc}	75.6^{abc}
	SEM	0.682	0.167	0.444	0.495	0.702
	Pooled SEM	0.529	0.139	0.364	0.406	0.610
p-values	Silage	0.001	0.091	0.021	0.014	0.339
	Inoculant	0.061	0.537	0.001	0.001	0.639
	Silage × Inoculant	0.489	0.004	0.016	0.010	0.024

SEM, standard error of means; ns = not significant. $^{\text{a–d}}$ Means with different superscripts in the same column differ significantly ($p < 0.05$).

The TSS, pH, and Flieg score values of silages are provided in Table 6. The TSS is an important source of energy used by lactic acid bacteria. It may vary according to the maturity level of the plants and grain-binding rate. Higher TSS indicates a more total soluble sugar presence [16]. This contributes to improving silage quality. In our study, the TSS content of the silages was determined to be between 18.5–19.5% Bx, and mixing maize and soybean or inoculating with LpP did not affect the TSS Bx value of the silages ($p > 0.05$). These data were higher than the values reported by Singh and Choudhary [16] for maize (8.51 to 11.22% Bx). These differences can be attributed to the differences in the maturity of the plant material, grain-binding rate, and environmental conditions. The pH value is a critical indicator for the fermentation profile and fermentation quality of silages. In this study, pH values were measured between 4.67 and 5.42. The LpP inoculation decreased the pH value of pure soybean and 50% Maize + 50% Soybean silages ($p < 0.001$) but did not affect other silages. In this study, the pH values are similar to the pH values obtained in earlier studies with maize and soybean-based silages. Serbester et al. [24] reported pH values of 4.0–5.5 in silages consisting of pure maize, pure soybean, and their mixture. The similar results (3.74-5.5) were reported in some studies [25,26,37,38] as well. The FS is used as an indicator of silage fermentation quality and provides important clues about silage quality. The LpP inoculation of maize and soybean-based silages increased the FS values of pure soybean and 50% Maize + 50% Soybean silages ($p < 0.001$) but was not effective in the other silages. The inoculation application promoted the good-quality maize silage to the excellent class and the medium-quality pure soybean silage to the medium-quality class.

Table 6. Total soluble solids, pH, and Flieg score properties of silages.

	Silages	Total Soluble Solids (% Bx)	pH$_0$	Flieg Score [1]	Silage Quality
Uninoculated	Maize	18.5	4.84 ^{cd}	72.1 ^{ab}	Good
	Soybean	19.5	5.42 ^{a}	47.5 ^{d}	Medium
	75% Maize + 25% Soybean	18.5	4.83 ^{cd}	71.2 ^{ab}	Good
	50% Maize + 50% Soybean	18.5	5.03 ^{bc}	63.6 ^{bc}	Good
	25% Maize + 75% Soybean	19.5	5.38 ^{a}	50.5 ^{d}	Medium
	SEM	0.233	0.087	3.467	-
Inoculated	Maize	19.3	4.67 ^{d}	80.4 ^{a}	Excellent
	Soybean	19.2	5.06 ^{bc}	61.6 ^{bc}	Good
	75% Maize + 25% Soybean	19.3	4.87 ^{cd}	75.9 ^{a}	Good
	50% Maize + 50% Soybean	18.7	4.75 ^{d}	76.6 ^{a}	Good
	25% Maize + 75% Soybean	18.5	5.21 ^{ab}	57.3 ^{cd}	Medium
	SEM	0.136	0.044	2.077	-
	Pooled SEM	0.116	0.041	1.866	-
p-values	Silage	0.494	<0.001	<0.001	-
	Inoculant	0.700	0.001	<0.001	-
	Silage × Inoculant	0.140	0.048	0.620	-

pH$_0$; pH value at opening time; ns = not significant, SEM, standard error of means, $^{a–d}$ Means with different superscripts in the same column differ significantly ($p < 0.05$). [1] 0–20, poor; 21–40, low-quality; 41–61, medium; 61–80, good; >80, excellent.

When the silages were opened, LAB, yeast, and mold contents were determined as in Table 7. The LAB and yeast count of the silages after opening were found to be between 6.17–7.00 and 4.63–7.03 log$_{10}$ cfu/g, respectively. Mixturing maize and soybean, and LpP inoculation affected the LAB and yeast levels of the silages ($p < 0.001$). The LpP inoculation in pure soybean silage and 75% Maize + 25% Soybean silage increased the LAB content of the silage compared to their control ($p < 0.001$) but did not affect it in the other silages. This can be explained that they consume more nutrients, especially protein, for the developing LAB. Zeng et al. [2] and Ni et al. [34] also reported similar results for pure soybean silage. The LpP inoculation did not affect the yeast count of silages in pure maize and soybean ($p > 0.05$) but decreased it in all mixed silages ($p < 0.001$). This result might be attributed to the help of LpP in sustaining the lactic acid concentration of silages. The increase in the number of LAB and the decrease in the number of yeasts indicate that LpP added to the silage works effectively and lactic acid is produced, which prevents the growth of harmful bacteria by rapidly lowering the pH. Yeasts cause aerobic spoilage and, therefore, worsen the quality and feed value of silage. These types of silage pose a serious risk to animal health and animal products. In this study, no mold was observed in any of the silages at the time of opening. These results are consistent with previous findings [1,34], which reported the decreased yeast count of inoculation application in soybean silages.

Table 7. Lactic acid bacteria, yeast, and mold content at the opening time.

	Silages	**Lactic Acid Bacteria (log$_{10}$ cfu/g)**	**Yeast (log$_{10}$ cfu/g)**	**Mold (log$_{10}$ cfu/g)**
Uninoculated	Maize	6.40^{de}	6.23^{b}	nd
	Soybean	6.73^{bc}	7.03^{a}	nd
	75% Maize + 25% Soybean	6.17^{f}	6.13^{b}	nd
	50% Maize + 50% Soybean	6.83^{ab}	6.23^{b}	nd
	25% Maize + 75% Soybean	6.53^{cd}	6.17^{b}	nd
	SEM	0.071	0.098	
Inoculated	Maize	6.30^{ef}	6.13^{b}	nd
	Soybean	7.00^{a}	6.93^{a}	nd
	75% Maize + 25% Soybean	6.70^{bc}	4.63^{d}	nd
	50% Maize + 50% Soybean	6.90^{ab}	5.37^{c}	nd
	25% Maize + 75% Soybean	6.70^{bc}	4.70^{d}	nd
	SEM	0.067	0.237	-
	Pooled SEM	0.051	0.146	-
p-values	Silage	<0.001	<0.001	-
	Inoculant	<0.001	<0.001	-
	Silage × Inoculant	0.003	<0.001	-

nd, not detected; SEM, standard error of means. $^{\text{a–f}}$ Means with different superscripts in the same column differ significantly ($p < 0.05$).

3.2. Aerobic Stability

The pH$_{5d}$, carbon dioxide, mold, and yeast contents determined after aerobic stability testing of silages are provided in Table 8. Aerobic stability is generally a measure of the chemical and microbiological changes that occur during the exposure of silages to oxygen [3]. In this study, pH, CO_2 production, and the growth of yeast and mold on the fifth day after opening the silages were used as indicators to assess aerobic deterioration. The pH$_{5d}$ values of silages were determined between 4.35 and 6.97. While the type of silage production significantly affected the pH$_{5d}$ value ($p = 0.009$), inoculation application had no effect ($p > 0.05$). The CO_2 production values were found to be between 1.70 and 5.91 g/kg DM in this study. The higher CO_2 and pH values in silages are considered an indication of further growth of spoilage-causing microorganisms [22,39]. These types of silages negatively affect animal performance. The LpP application did not affect the

CO_2 production value of pure maize silage. However, it significantly reduced the CO_2 production values of pure soybean, 75% Maize + 25% Soybean, and 50% Maize + 50% Soybean silages; although it did not statistically affect the CO_2 production values of 25% Maize + 75% Soybean silage, it decreased numerically. Meanwhile, the CO_2 production values obtained in this study were lower than the values reported for soybean (27.9–23.6 g CO_2/kg DM) by Nkosi et al. [40]. These differences may be due to the difference in additives used in silage and the effectiveness of lactic acid bacteria during fermentation. Mixing maize and soybean plants affected yeast growth, whereas LpP inoculation did not. This result is consistent with the findings of Lee et al. [39]), who reported that LpP inoculation had no impact on the aerobic stability of maize silage. Similarly, Wang et al. [41] reported in their study that lactic acid bacteria were not effective in preventing yeast and mold growth in wheat, sorghum, and corn silages. Neither the silage mixing ratios nor the inoculation application had a significant effect on mold growth.

Table 8. The pH_{5d}, carbon dioxide, mold, and yeast contents determined after aerobic stability testing of silages.

	Silages	pH_{5d}	CO_2 (g/kg DM)	Yeast (log_{10} cfu/g)	Mold (log_{10} cfu/g)
Uninoculated	Maize	4.65 ^{c}	5.91 ^{a}	6.22 ^{a}	1.17
	Soybean	6.97 ^{a}	5.35 ^{a}	5.49 ^{a}	0.00
	75% Maize + 25% Soybean	5.11 ^{bc}	3.15 ^{b}	1.52 ^{cd}	0.00
	50% Maize + 50% Soybean	4.35 ^{c}	3.34 ^{b}	0.00 ^{d}	1.84
	25% Maize + 75% Soybean	5.28 ^{bc}	2.08 ^{cd}	0.00 ^{d}	0.00
	SEM	0.343	0.482	0.764	0.422
Inoculated	Maize	5.33 ^{bc}	5.38 ^{a}	5.32 ^{ab}	1.17
	Soybean	6.37 ^{ab}	2.92 ^{bc}	4.78 ^{ab}	0.00
	75% Maize + 25% Soybean	5.11 ^{bc}	1.76 ^{d}	2.78 ^{bc}	1.84
	50% Maize + 50% Soybean	4.65 ^{c}	1.83 ^{d}	1.52 ^{cd}	0.00
	25% Maize + 75% Soybean	5.52 ^{abc}	1.70 ^{d}	0.00 ^{d}	1.52
	SEM	0.240	0.483	0.639	0.494
	Pooled SEM	0.204	0.362	0.490	0.321
p-values	Silage	0.009	0.001	0.001	0.849
	Inoculant	0.677	0.001	0.661	0.663
	Silage × Inoculant	0.722	0.030	0.479	0.477

pH_{5d}, the fifth day pH value; SEM, standard error of means; ns = not significant. ^{a,b,c,d} Means with different superscripts in the same column differ significantly ($p < 0.05$).

3.3. In Vitro Digestibility and Feed Value

The in vitro gas production technique, which is an important technique for estimating the digestibility and feed value of feeds, is widely used, especially due to its similarity to in vivo evaluations and the ability to evaluate a larger number of feeds in a shorter time. In vitro organic matter digestibility and energetic values of the silages that are the subject of this study, determined by the in vitro method, are provided in Table 9. The LpP inoculation reduced the IVOMD and NE_l value of pure maize and pure soybean silages but did not affect it in maize–soybean mixed silages. The highest IVOMD was obtained in uninoculated pure maize (68.6%), and the lowest value was obtained in inoculated soybean silage (58.3%). The inoculation application reduced the ME_{gp} value of pure maize but did not affect other silages. The highest ME value was obtained in the uninoculated pure maize silage (9.62), while the lowest values were obtained in the uninoculated 75% Maize + 25%

Soybean silage (6.95). The highest NE_l value was obtained in the pure maize silage without inoculant (6.07), while the lowest values were obtained in the inoculated 75% Maize + 25% Soybean silage (4.53). In contrast to this study, Marbun et al. [32] and Kansagara et al. [42] reported that in vitro GP and IVOMD were not affected by the application in maize silage inoculated with LpP. The differences between the studies in terms of GP value and IVOMD are thought to be due to the differences in the chemical composition of the maize and soybean plants used.

Table 9. The digestibility and energetic values of silages.

	Silages	**IVOMD (%)**	**MEgp, (MJ/kg DM)**	**NEl, (MJ/kg DM)**
Uninoculated	Maize	68.6^{a}	9.62^{a}	6.07^{a}
	Soybean	59.7^{d}	7.48^{d}	5.25^{bc}
	75% Maize + 25% Soybean	58.5^{e}	6.95^{e}	4.53^{d}
	50% Maize + 50% Soybean	65.1^{b}	8.48^{b}	5.48^{b}
	25% Maize + 75% Soybean	63.0^{c}	8.21^{bc}	5.31^{bc}
	SEM	1.221	0.308	1.672
Inoculated	Maize	64.8^{b}	8.44^{bc}	5.25^{bc}
	Soybean	58.3^{e}	7.14^{de}	4.72^{d}
	75% Maize + 25% Soybean	59.0^{de}	7.00^{e}	4.57^{d}
	50% Maize + 50% Soybean	64.2^{b}	8.28^{bc}	5.32^{bc}
	25% Maize + 75% Soybean	62.0^{c}	7.99^{c}	5.06^{c}
	SEM	0.891	0.202	0.103
	Pooled SEM	0.751	0.184	0.103
p-values	Silage	<0.001	<0.001	<0.001
	Inoculant	<0.001	0.001	<0.001
	Silage × Inoculant	0.002	0.009	0.008

IVOMD, in vitro organic matter digestibility; MEgp, metabolizable energy based on gas production; NE_l, net energy lactation; SEM, standard error of means; ns = not significant. ^{a,b,c,d,e} Means with different superscripts in the same column differ significantly ($p < 0.05$).

4. Conclusions

In maize and soybean-mixed silages, LpP isolated from homemade pickles showed positive effects on chemical, physical, and microbiological properties, aerobic stability, in vitro digestibility, and energy value in maize–soybean mixed silages. Especially, (a) LpP mainly worked to degrade the crude fiber content of silage materials, except soyabean, (b) LpP increased the organic matter of silages but did not prevent the crude protein contents of silage materials (except maize), (c) the LpP inoculation to maize and soybean mixed did not cause any negative effects on the color quality of the silages, (d) LpP improved aerobic stability mainly in soyabean and soyabean dominant silages, and (e) LpP increased feed value especially on maize and maize dominant silages. Therefore, due to the difficulties and risks involved in ensiling soybean crops, mixing them half and half (50% Maize + 50% Soybean) with easily ensiled crops such as maize and inoculating them with LpP can contribute positively to obtaining quality and reliable silages. In future, more comprehensive research is needed on a single bacterial species, bacterial combinations, or bacterial–enzyme combinations to obtain better-quality silages from legume–cereal mixed green fodders, which have become widespread in recent years.

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