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Fermentation Quality and Chemical Composition of Industrial Hemp (*Cannabis sativa* L.) Silage Inoculated with Bacterial Starter Cultures—A Pilot Study

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Abstract: Industrial hemp (*Cannabis sativa* L.) is a plant species cultivated as a raw material for fiber extraction. Alternatively, hemp biomass can be used for feeding or energy purposes. This study was conducted to investigate the effect of inoculation with a lactic acid bacteria starter culture on the fermentation and chemical compositions of hemp silages. Hemp shoots (HS) and hemp flowers (HF) were ensiled in mini laboratory silos without or with the inoculation of the commercial starter culture Lactosil Biogaz (*Lentilactobacillus buchnerii* KKP 907 p; *L. buchneri* A KKP 2047 p; *Pediococcus acidilactici* KKP 2065 p). After 7 and 42 days of ensiling, the fermentation quality and chemical compositions of the silages were assessed. The use of Lactosil Biogas for hemp resulted in a decrease in pH, increase in lactic acid (LA), and reduction in fungal abundance in the HS silage. In the case of the HF silage, the bacterial inoculation was less effective; however, an increase in LA and a decrease in butyric acid (BA) were observed. As a result of the ensilage process, decreases in crude fiber and hemicellulose were observed in the HS and HF silages. Thus, hemp ensiling with biological additives is an effective pre-treatment of hemp plants for subsequent biofuel production that can preserve the biomass and provide the year-round availability of feedstock.

Keywords: lactic acid; crude protein; fiber; hemp; *Lentilactobacillus buchnerii*; *Pediococcus acidilactici*; silage

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1. Introduction

Industrial hemp (*Cannabis sativa* L.) is a plant native to Asia and a member of the hemp family (Cannabaceae Endl). It has been cultivated worldwide for nearly 8500 years. It is an easy-to-grow, drought-tolerant plant with low soil requirements, and it grows well in neutral soil pH conditions [1]. Currently, hemp cultivation in the EU is increasing, spreading over progressively larger areas. In 2015, it was 19,970 hectares, and for 2019, the estimated area was 34,960 hectares, which accounts for an increase of about 75%. At the same time, there has also been an increase in hemp production, which rose by more than 62% over the period—from 94,120 tons in 2015 to 152,820 tons in 2019. The leaders in European hemp production are France, with more than 70% of the EU production, followed by the Netherlands with 10%, and Austria with 4%. Estimations from the European

Industrial Hemp Association (EIHA) show that the area under hemp cultivation in Europe is still growing, reaching 55,000 hectares in 2021 [2].

Cannabis has been widely cultivated due to its industrial [3], ornamental [4], nutritional [5], medicinal, and recreational potential [6]. From regulatory and application perspectives, cannabis plants are categorized based on the level of Δ9-tetrahydrocannabinol (THC), one of the most important phytocannabinoids [7]. Plants are generally classified and regulated as industrial hemp if they contain less than 0.3% THC in the dried flower (this level varies by country) or drug type with more than this threshold [8]. Industrial hemp is a multipurpose plant, the seeds of which are used for oil extraction, the stalks for a fiber source, the flowers and leaves for drug acquisition, and the residues after the separation of fibers (shive) for the furniture, automotive, and construction industries [9–12]. Moreover, industrial hemp byproducts can be used in dietary formulations, for instance, as unconventional feed sources for dairy cattle; however, the purpose of such application needs to be properly considered [13,14]. Moreover, industrial hemp cultivation also benefits the soil through the phytoremediation of contaminated soils [15]. Alternatively, hemp biomass can be used for feeding or energy purposes [16–18]. The results of studies [19] have indicated that the biomass of hemp can be used for biogas production. Importantly, hemp has a relatively low conversion efficiency for the production of biogas but produces higher-quality products in comparison to other crops, such as maize and sugar beets [20]. Hemp plants are characterized by a high content of crude fiber, which affects their energy value (important in the combustion process); however, the fiber is degraded to a limited extent during methane fermentation. For this reason, substrates with a high content of cellulose, hemicelluloses, lignin, and other structural carbohydrates should undergo preliminary hydrolysis before methane fermentation (e.g., during ensiling) [21]. The ensiling of biomass positively affects biomethanation with higher biogas yields and methane contents in comparison to fresh matter. The ensiled crop, compared to fresh substrate, has a lower crude fiber content and a higher content of metabolites formed by the anaerobic fermentation of carbohydrates. During the ensiling process, lactic acid bacteria (LAB), in addition to LA, also produces volatile fatty acids (propionic, formic, caproic, valerian) and alcohols (ethanol, methanol, propanol), which are then used by other microorganisms to synthesize acetic acid (AA) and carbon dioxide during a phase of methane fermentation called acetogenesis [22].

Plant substrates intended for methane production should be characterized by high concentrations of AA and LA and low concentrations of ammonia-N. AA is particularly important, being a direct precursor of methane. About 70% of methane is formed from AA during methane fermentation, and only 30% from hydrogen and carbon dioxide. LA lowers the pH of silage, which negatively affects the activity of methanogens; however, it is essential for preserving the shelf life of silage by inhibiting the growth of spoilage-causing microorganisms (yeast, molds, *Clostridium* sp.) [23]. Moreover, methane bacteria have a limited ability to use LA to produce methane. Therefore, the high concentrations of ethanol and BA following clostridial and heterofermentative LA fermentations have resulted in numerically higher specific methane yields [24,25]. Therefore, in the production of silage for biogas purposes, an effort should be made to reduce the formation of LA and increase the amount of AA, propionic acid, and other volatile fatty acids [26]. In this regard, microbial inoculants that contain heterofermentative strains of LAB can be helpful. In addition to LA, heterofermentative LAB synthesize a number of other metabolites (organic acids, alcohols), which are then utilized by the appropriate microorganisms in further stages of methane fermentation, which can contribute to an increase in the biogas yields from ensiled crops [27,28].

A number of silage inoculants with the presence of heterofermentative *Lentilactobacillus buchneri* have been developed. These bacteria produce 1,2-propanediol through the phosphogluconate pathway with the release of carbon dioxide [29–32]. Recently, another uncommon new strain, *Pediococcus acidilactici*, was isolated from ensiled maize grain and characterized by the ability to grow in medium with 1,2-propanediol as the only carbon source, revealing the ability to metabolize it. Moreover, several studies have reported

that *P. acidilactici* can inhibit the growth of pathogens during the fermentation process and food storage [33,34]. It was found that the use of inoculants containing *L. buchneri* and *P. acidilactici* resulted in an approximately 10–30% increase in the biogas yield, including methane from this raw material [35]. In terms of their use in a biogas plant, the combination of bacterial strains used in the inoculant had a positive effect on the quality parameters of the silages. Therefore, this direction of research is worth continuing [36].

This study aimed to evaluate the effect of a commercial bacterial preparation containing strains of *L. buchnerii* and *P. acidilactici* on the quality of hemp silage for biogas production.

2. Materials and Methods

2.1. Experiment Design and Treatments

Field and laboratory experiments were conducted at the Institute of Technology and Life Sciences—National Research Institute in Falenty, Poland. Industrial hemp (*Cannabis sativa* L.) was cultivated at Falenty, close to Warsaw, Poland (52°08′37.8″ N/20°55′51.92″ E 52.143833/20.931090). The hemp was sown on 4 May 2022, with an amount of 60 kg of seed per 1 ha.

Two silage experiments were carried out under laboratory conditions: with ensilaging hemp shoots (Experiment I) and hemp flowers (Experiment II). Both two-factor experiments in a split-plot design with four replications were established. The first factor was inoculation with the bacterial inoculant, and the storage length was the second factor (Figure 1).

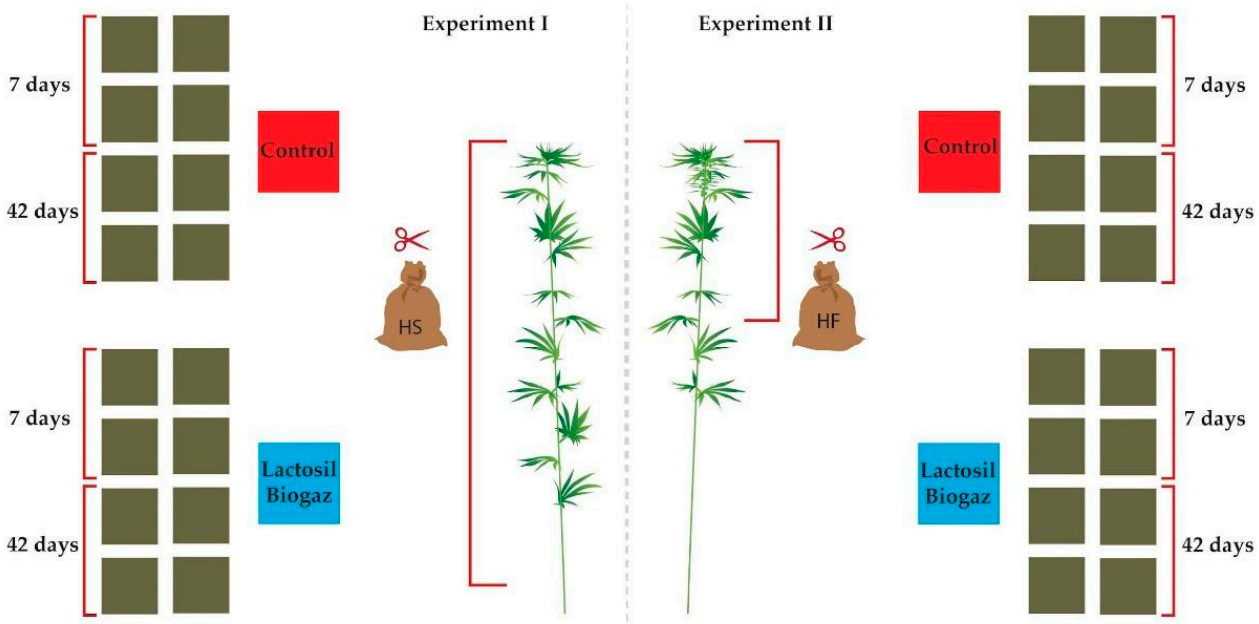


Figure 1. Scheme of silage experiments: Experiment I with ensilaging hemp shoots (HS), and Experiment II with ensilaging hemp flowers (HF).

Hemp shoots (HS) were cut a few centimeters above ground on 19 August 2022, and hemp flowers (HF) were cut on 5 September 2022. After delivery to the laboratory, the HS and HF were chopped with scissors into 2–3 cm particles. In four replications, 300 g subsamples were ensiled in mini laboratory silos (plastic beaches 30 × 40 cm) without or with the inoculation of the commercial inoculant Lactosil Biogaz *(L. buchneri* KKP/907/p; *L. buchneri* A KKP/2047/p; *Pediococcus acidilactici* KKP 2065 p.). The samples were sealed with a vacuum sealer (SilverCrest, Vancouver, BC, Canada). Experimental silos were stored in a laboratory at a stable temperature (c.a. 23 ± 2 °C) for 7 and 42 days. The dry matter contents and chemical compositions of the ensilaged material are shown in Table 1.

Table 1. Dry matter contents and chemical compositions of ensilaged material.

Parameter	HS		HF	
	Mean	**SD**	**Mean**	**SD**
DM, g kg^{-1}	283.5	6.6	310.4	72.1
CP, g kg^{-1} DM	36.2	14.0	263.2	8.4
CF, g kg^{-1} DM	495.9	12.1	355.5	4.8
Ash, g kg^{-1} DM	81.6	2.5	164.9	4.9
NDF, g kg^{-1} DM	681.6	28.7	437.2	8.5
ADF, g kg^{-1} DM	519.6	19.9	309.3	6.2
ADL, g kg^{-1} DM	79.1	4.4	78.5	0.7
OMD, %	111.6	61.4	606.1	19.9
DMD, %	149.3	67.6	664.3	22.0
WSC, g kg^{-1} DM	31.0	8.9	119.2	4.0
WSC/CP	1.0	0.3	0.5	0.0

DM—dry matter; CP—crude protein; CF—crude fiber; NDF—neutral detergent fiber; ADF—acid detergent fiber; ADL—acid detergent lignin; OMD—organic matter digestibility; DMD—dry matter digestibility; WSC—water-soluble carbohydrates; HS—hemp shoots; HF—hemp flowers.

2.2. Chemical Composition of Raw Material and Silages

After 7 and 42 days of ensiling, silos were opened and intended for chemical and microbiological analyses. An amount of 30 g of silage was taken for the pH and fermentation product determination. The pH was determined using the potentiometric method immediately after the sample preparation. The pH of the test solution was measured with a calibrated pH meter (Si Analytics, Suite, WA, USA) in duplicate.

The extract of macerated silage was prepared with distilled water and filtered through two layers of cheesecloth. An amount of 20 g of plant material was transferred to a beaker with 300 mL of distilled water and thoroughly mixed, and it was then sealed tightly with foil and left in the refrigerator at +6 °C for 24 h. On the next day, the extract was filtered through cheesecloth into glass flasks. To remove macromolecular compounds (proteins and polysaccharides), 5 mL of Carrez I and Carrez II reagents was added to each test sample, filtered into clean plastic bottles, and frozen at −20 °C for further analysis.

Determination of the L- and D-lactic acid and acetic acid contents was conducted using enzymatic methods by measuring the increase in the NADPH concentration at 340 nm, according to the manufacturer's protocols (Megazyme International Ireland, Bray, Wicklow, Irlandia). The 3-hydroxybutyric acid concentration was measured spectrophotometrically at 492 nm using a Megazyme D-3-hydroxybutyric acid kit. All experiments were carried out in triplicate, on the basis of which, according to the manufacturer's protocol, the average value was calculated.

The DM content of the ensilaged material and silages was determined according to ISO standards [37]. Three subsamples (200 g) of each ensiled sample were dried in 70 °C to constant moisture to determine the chemical composition of the silage, including the crude protein (CP), crude fiber (CF), crude ash (inorganic matter; CA), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent fiber (ADL), organic matter digestibility (OMD), dry matter digestibility (DMD), and water-soluble carbohydrates (WSC). Near-infrared reflectance spectroscopy (NIRS) technology (NIRFlex N-500, Büchi, Flawil, Switzerland), using a global calibration equation, was used to estimate the nutritive quality of the silage. The cellulose content was calculated as ADF-ADL, and the hemicellulose as NDF-ADF. The same procedure was used for the chemical composition evaluation of the silage samples.

2.3. Microbial Analyses

The total mesophilic bacteria (TMB), yeast, and molds (CFU g^{-1}) were determined by the serial dilution method. Under sterile conditions, 10 g of plant material was introduced into 100 mL of sterile 0.85% NaCl solution, and a tenfold dilution of samples was made for the analysis by the pouring method on nutrient agar (Merck) for the determination of

the total mesophilic bacteria counts. Samples were incubated at 28 ± 2 °C for 72 ± 2 h. Yeast and molds were cultured on a yeast peptone dextrose agar medium (YPDA) (peptone: 20 g L^{-1}; glucose: 20 g L^{-1}; yeast extract: 10 g L^{-1}; agar: 20 g L^{-1}) with chloramphenicol (0.1 g L^{-1}) at 30 °C for 48 ± 2 h. The yeast and mold count determinations were followed by a macroscopic evaluation.

2.4. Statistical Analysis

The results of the microbial analyses were expressed as logarithmic values. Statistical analyses, including the analysis of variance (ANOVA) and Tukey HSD post hoc test, were conducted separately for the HS and HF silages, according to the experimental data models designed as a split-plot experiment. All the calculations were performed using Statistica V. 6. (Statsoft) and MS Excel. The statistical significance was set at a p-value < 0.05.

3. Results

3.1. Fermentation Quality of Industrial Hemp Silage

3.1.1. Experiment I—HS Silage

The pH value of the HS silage ranged from 5.6 (HS silage with bacterial inoculant after 42 days) to 6.1 (HS control silage after 7 days) (Figure 2a). The pH of the HS silage was influenced by both the application of the bacterial inoculant and the length of the ensiling period. The pH of the HS silage prepared with the bacterial starter culture was significantly ($p < 0.05$) lower (5.84) than that of the control silage (6.16), regardless of the length of the ensiling period. Regardless of the bacterial additive used, the pH of the HS silage averaged 6.27 on day 7 of ensiling, and it dropped significantly ($p < 0.05$) to 5.73 after 42 days of ensiling (Table 2).

Table 2. Average values of fermentation quality parameters of HS silage for treatments and storage length.

Parameters	Treatment		Storage Length	
	Control (n = 8)	Bacterial Inoculant (n = 8)	7 Days (n = 8)	42 Days (n = 8)
pH	6.16 a	5.84 b	6.27 a	5.73 b
LA, g kg^{-1} DM	0.52 b	3.24 a	3.41 a	0.36 b
AA, g kg^{-1} DM	24.68 a	26.93 a	41.46 a	10.14 b
BA, g kg^{-1} DM	0.19 a	0.19 a	0.20 a	0.18 a
SA, g kg^{-1} DM	27.70 a	28.12 a	45.07 a	10.74 b
TMB, log CFU g^{-1}	7.60 a	7.74 a	7.96 a	7.38 b
Molds, log CFU g^{-1}	1.39 a	0.67 b	1.17 a	0.89 a

LA—lactic acid; AA—acetic acid; BA—butyric acid; SA—sum of acids; TMB—total mesophilic bacteria count. Means with the same letter do not differ significantly at $p < 0.05$ in Tukey's HSD test.

The LA (lactic acid) content of the HS silage varied over a very wide range: from 0.30 (control silage, day 42) to 6.07 g kg^{-1} DM (bacterial inoculant, day 7) (Figure 2b), and depended on both the LAB additive used and the length of storage (Table 2). The average LA concentration in the HS silages with the bacterial additive was 3.24 g kg^{-1} DM, which was significantly higher ($p < 0.05$) than in the control silage (0.52 g kg^{-1} DM). During the storage period, a decrease in the LA content was observed (on average, from 3.41 on day 7 to 0.36 g kg^{-1} DM on day 42). The decrease was higher in the case of the silage with the bacterial addition, in which the LA content was reduced from 6.07 g kg^{-1} DM (day 7) to 0.42 g kg^{-1} DM (day 42). In the case of the control silage, the decrease was insignificant (Figure 2b).

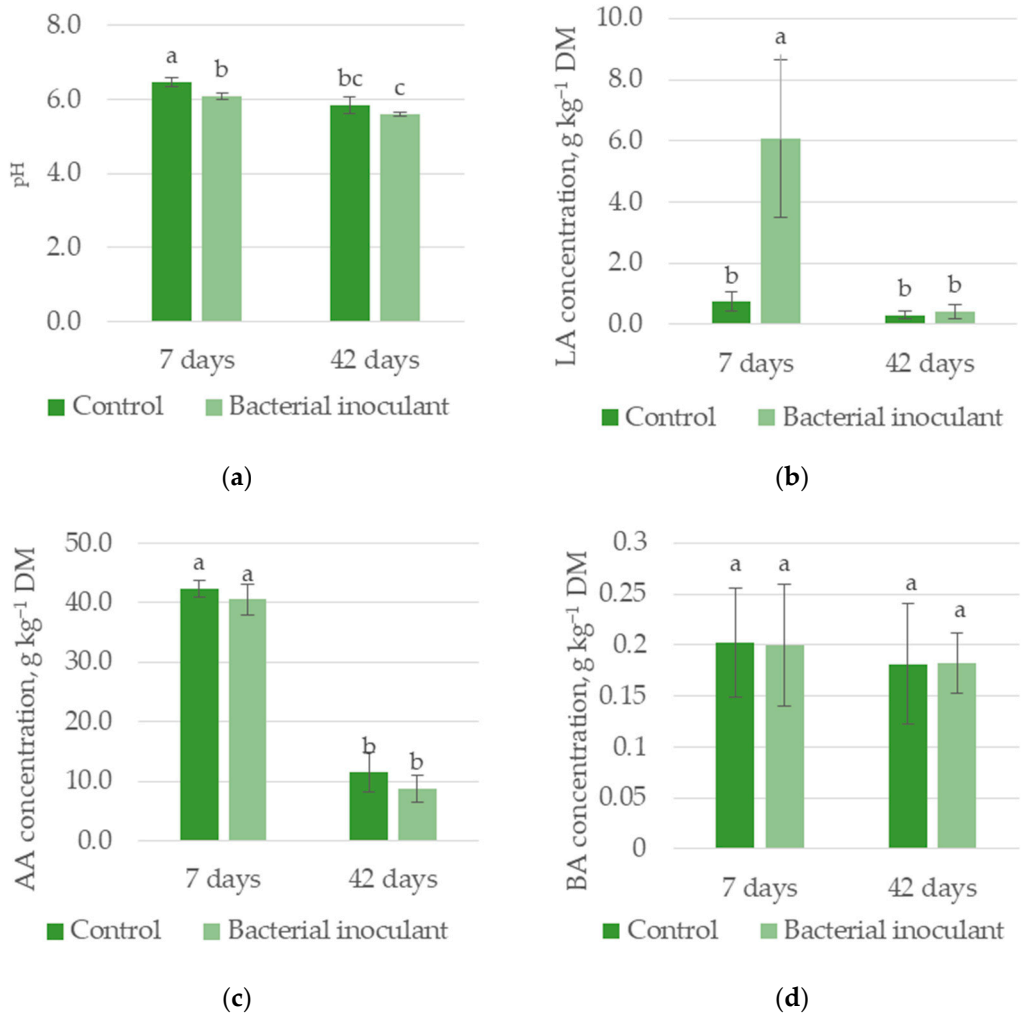
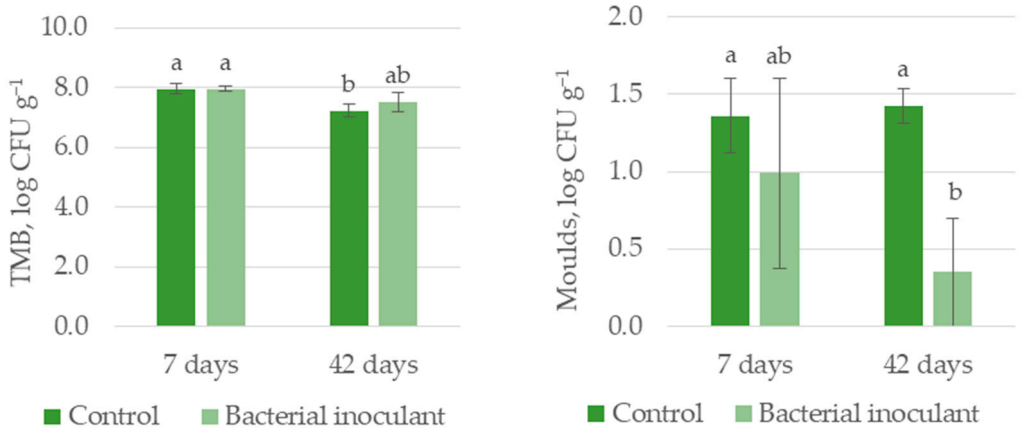


Figure 2. Parameters of fermentation quality of HS silage: (**a**) pH; (**b**) LA (lactic acid); (**c**) AA (acetic acid); (**d**) BA (butyric acid). Error bars indicate the standard deviation of the mean. Values marked with at least one similar letter are not significantly different at $p < 0.05$ in Tukey's HSD test.

The average AA (acetic acid) values in the control HS silage and in the silage with the bacterial inoculant were similar: 24.68 g kg^{-1} DM in the control silage and 26.93 g kg^{-1} DM in the experimental silage (Table 2). The storage time was a significant factor. On day 7, the AA content was significantly ($p < 0.05$) the highest (an average of 41.46 g kg^{-1} DM for both silages). A fourfold decrease in the AA content to 10.14 g kg^{-1} DM was observed in both silages during the storage period (Table 2). The butyric acid content was low and did not depend on the factors studied (Table 2).

The average TMB counts in the control HS silage and in the silage with the bacterial inoculant were similar (7.60 log CFU g^{-1} and 7.74 log CFU g^{-1}, respectively) (Table 2). The storage time was an important factor. On day 7, the TMB counts averaged 7.96 log CFU g^{-1}, and they decreased significantly ($p < 0.05$) to 7.38 log CFU g^{-1} during ensiling, regardless of the treatment (Table 2).

The applied bacterial inoculant significantly ($p < 0.05$) reduced the fungal abundance—from 1.39 log CFU g^{-1} in the control silage to 0.67 log CFU g^{-1} (Table 2). The control silage at both day 7 and day 42 was characterized by a higher mold number than the silage with the bacterial starter culture (Figure 3b).



(**a**) (**b**)

Figure 3. Microbial quality of HS silage: (**a**) TMB counts; (**b**) mold counts. Error bars indicate the standard deviation of the mean. Values marked with at least one similar letter are not significantly different at $p < 0.05$ in Tukey's HSD test.

3.1.2. Experiment II—HF Silage

The average pH values of the HF silage on day 7 were high and ranged from 8.24 (control silage) to 8.18 (silage with the addition of the inoculant). After 42 days of the experiment, there were significant decreases in the pH values in the control and inoculated silages, to 6.18 and 6.05, respectively (Figure 4a). The above data indicated a nonsignificant effect of the inoculant on the pH of the HF silage.

The LA content of the HF silage ranged from 1.85 g kg^{-1} DM (control silage, day 7) to 3.02 g kg^{-1} DM (inoculated silage, day 42) (Figure 4b). As in the HS silage, it depended on both the bacterial additive used and the length of the ensiling time (Table 3). The application of the bacterial inoculant increased the LA content of the HF silage, on average, from 1.92 g kg^{-1} DM in the control silage to 2.49 g kg^{-1} DM in the experimental silage (Table 3). An increase in the LA content was also observed during the storage period: slightly less in the control silage and higher in the silage with the bacterial inoculant (Figure 4b).

Table 3. Average values of fermentation quality parameters of HF silage for treatments and storage length.

Parameters	Treatment		Storage Length	
	Control (n = 8)	Bacterial Inoculant (n = 8)	7 Days (n = 8)	42 Days (n = 8)
pH	7.21 a	7.12 a	8.21 a	6.11 b
LA, g kg^{-1} DM	1.92 b	2.49 a	1.90 b	2.51 a
AA, g kg^{-1} DM	6.32 a	6.20 a	6.36 a	6.16 a
BA, g kg^{-1} DM	0.23 a	0.11 b	0.18 a	0.16 a
SA, g kg^{-1} DM	8.42 a	8.84 a	8.44 a	8.83 a
TMB, log CFU g^{-1}	8.69 a	8.79 a	8.85 a	8.63 b
Molds, log CFU g^{-1}	2.52 a	1.96 a	2.48 a	2.00 a

LA—lactic acid; AA—acetic acid; BA—butyric acid; SA—sum of acids; TMB—total mesophilic bacteria count. Means with the same letter do not differ significantly at $p < 0.05$ in Tukey's HSD test.

The average AA content in the HF silage was lower than in the HS silage. It ranged from 5.8 g kg^{-1} DM (inoculated silage, day 42) to 6.9 g kg^{-1} DM (control silage, day 7) (Figure 4c) and did not depend on the factors tested (Table 3).

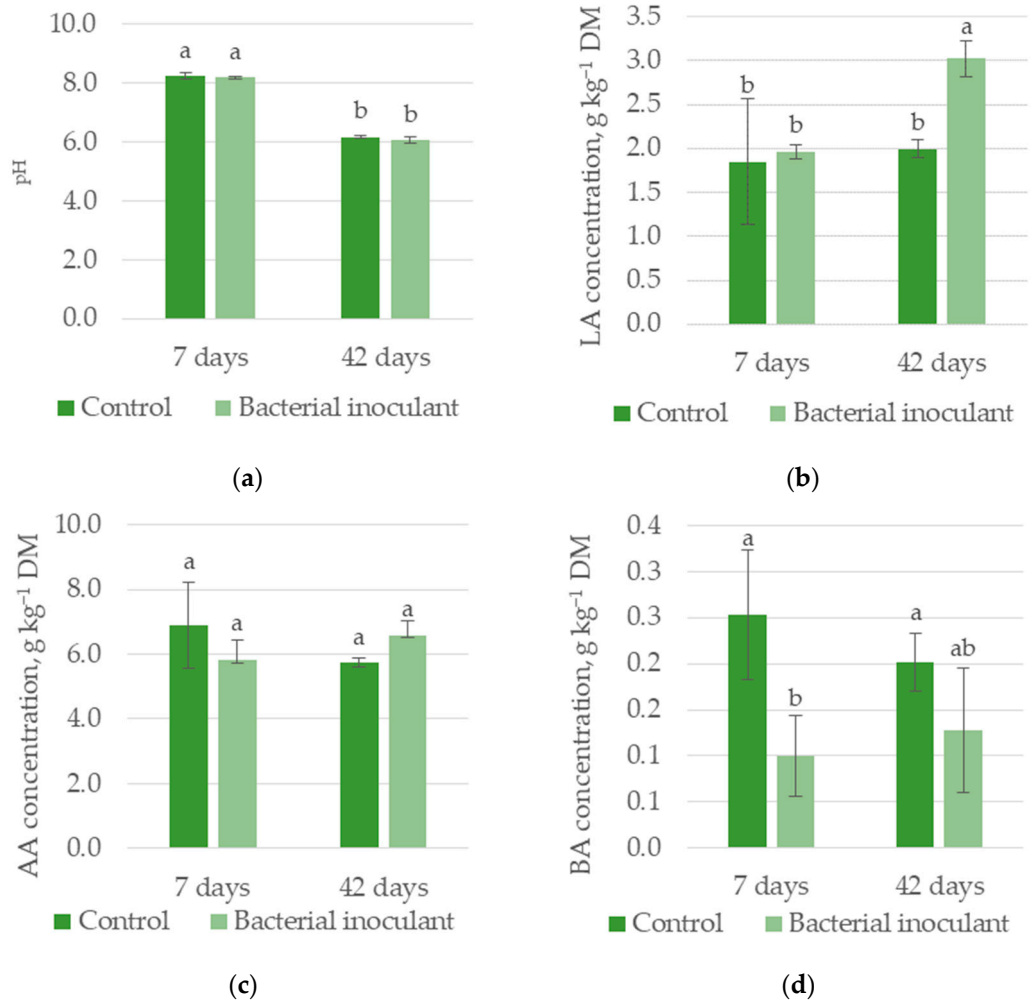
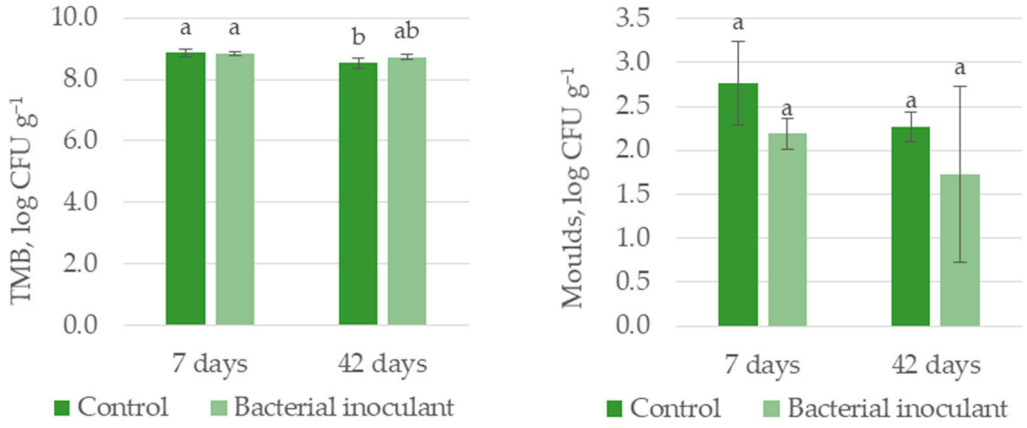


Figure 4. Parameters of fermentation quality of HF silage: (**a**) pH; (**b**) LA; (**c**) AA; (**d**) BA. Error bars indicate the standard deviation of the mean. Values marked with at least one similar letter are not significantly different at $p < 0.05$ in Tukey's HSD test.

The butyric acid content was low (Figure 4d). On average, the application of the inoculant contributed to a significant reduction in this acid concentration from 0.23 to 0.11 g kg^{-1} DM. The effect of the storage length on the BA concentration was not significant (Table 3).

The average TMB counts in the control and experimental HF silages were similar—8.69 log CFU g^{-1} and 8.79 log CFU g^{-1}, respectively (Table 3). The storage time was an important factor. On day 7, the TMB counts averaged 8.85 log CFU g^{-1}, and they decreased significantly ($p < 0.05$) to 8.63 log CFU g^{-1} during ensiling (Table 3).

The fungal number in the HF silage ranged from 1.72 log CFU g^{-1} to 2.76 log CFU g^{-1} (Figure 5b) and did not depend on the factors studied (Table 3).



(**a**) (**b**)

Figure 5. Microbial quality of HF silage: (**a**) TMB counts; (**b**) mold counts. Error bars indicate the standard deviation of the mean. Values marked with at least one similar letter are not significantly different at $p < 0.05$ in Tukey's HSD test.

3.2. Chemical Composition of Industrial Hemp Silage

The use of a bacterial inoculant did not affect the chemical composition of the HS silage (Table 4). During the ensiling process, there was a significant increase in the CP (on average, from 33.3 to 67.9 g kg^{-1} DM) and a decrease in the ADF (on average, from 522.5 g kg^{-1} DM on day 7 to 505.7 g kg^{-1} DM on day 42). Relative to ensiled material, both silages showed an increase in CP, ash, and digestibility of dry matter and organic matter, and a decrease in crude fiber and NDF fractions, including hemicellulose, was observed.

Table 4. Chemical composition of HS silage.

Parameters	Treatment		Storage Length	
	Control (n = 8)	Bacterial Inoculant (n = 8)	7 Days (n = 8)	42 Days (n = 8)
DM, g kg^{-1}	282.0 a	287.3 a	285.8 a	283.5 a
CP, g kg^{-1} DM	52.9 a	48.3 a	33.3 b	67.9 a
CF, g kg^{-1} DM	484.6 a	487.1 a	492.2 a	479.4 a
Ash, g kg^{-1} DM	101.7 a	105.4 a	103.3 a	103.8 a
NDF, g kg^{-1} DM	649.6 a	649.7 a	658.3 a	641.0 a
ADF, g kg^{-1} DM	516.0 a	512.2 a	522.5 a	505.7 b
Cellulose, g kg^{-1} DM	436.6 a	436.1 a	446.6 a	426.1 b
Hemicellulose, g kg^{-1} DM	133.6 a	137.5 a	135.9 a	135.2 a
ADL, g kg^{-1} DM	79.4 a	76.1 a	75.9 a	79.6 a
OMD, %	13.15 a	16.35 a	15.26 a	14.25 a
DMD, %	17.63 a	21.17 a	20.88 a	17.91 a

DM—dry matter; CP—crude protein; CF—crude fiber; NDF—neutral detergent fiber; ADF—acid detergent fiber; ADL—acid detergent lignin; OMD—organic matter digestibility; DMD—dry matter digestibility. Means with the same letter do not differ significantly at $p < 0.05$ in Tukey's HSD test.

In the case of the HF silage, the use of a bacterial inoculant resulted in a decrease in crude ash and an increase in the ADL fraction (Table 5). During the ensiling process, there was a significant increase in the protein, crude ash, ADL fiber fraction, and DMD digestibility, with a significant decrease in the hemicellulose, cellulose, and lignins included in the NDF fraction (Table 5). As a result of these changes, after 42 days of ensiling, the control silage contained the following: 261.1 g kg^{-1} DM CP; 349.2 g kg^{-1} DM CF; 192.5 g kg^{-1} DM crude ash; 400.1 g kg^{-1} DM NDF; 333.9 g kg^{-1} DM ADF; and 87.6 g kg^{-1}

DM ADL. The silage prepared with the Lactosil Biogaz inoculant contained the following: 259.8 g kg^{-1} DM CP; 337.0 g kg^{-1} DM CF; 187.4 g kg^{-1} DM crude ash; 373.7 g kg^{-1} DM NDF; 328.0 g kg^{-1} DM ADF; and 89.9 g kg^{-1} DM ADL. In relation to the ensiled material, both silages (control and experimental) showed increases in ash, ADL, and organic matter digestibility, and decreases in protein, crude fiber, and NDF, including hemicellulose and dry matter digestibility.

Table 5. Chemical composition of HF silage.

Parameters	Treatment		Storage Length	
	Control (n = 8)	Bacterial Inoculant (n = 8)	7 Days (n = 8)	42 Days (n = 8)
DM, g kg^{-1}	335.2 a	330.5 a	328.7 a	337.0 a
CP, g kg^{-1} DM	244.3 a	245.5 a	229.4 b	260.5 a
CF, g kg^{-1} DM	341.9 a	346.0 a	344.8 a	343.1 a
Ash, g kg^{-1} DM	189.8 a	180.9 b	180.8 b	189.9 a
NDF, g kg^{-1} DM	405.6 a	407.6 a	426.3 a	386.9 b
ADF, g kg^{-1} DM	331.0 a	337.1 a	337.2 a	331.0 a
Cellulose, g kg^{-1} DM	246.6 a	250.2 a	254.5 a	242.3 b
Hemicellulose, g kg^{-1} DM	74.6 a	70.5 a	89.1 a	55.9 b
ADL, g kg^{-1} DM	84.4 b	86.9 a	82.7 b	88.7 a
OMD, %	54.94 a	52.48 a	52.91 a	54.52 a
DMD, %	61.12 a	59.22 a	58.03 b	62.30 a

DM—dry matter; CP—crude protein; CF—crude fiber; NDF—neutral detergent fiber; ADF—acid detergent fiber; ADL—acid detergent lignin; OMD—organic matter digestibility; DMD—dry matter digestibility. Means with the same letter do not differ significantly at $p < 0.05$ in Tukey's HSD test.

4. Discussion

Ensiling proved to be a suitable method to store the biomass, allowing its use for energy sources throughout the year. Hemp ensiling is a novel approach; hence, studies on it are still limited. Different biological and chemical additives designed for feed preservation have been tested for the preservation of hemp plants in model trials and at a farming scale. The preservation results were evaluated for biological inoculants and acidic or alkaline chemical additives (e.g., caustic soda). However, the majority of trials have led to the conclusion that control samples ensiled without any additives lead to similar biochemical effects and good hemp preservation [22,38,39]. The results obtained by [40] showed that it was possible to produce microbiologically stable material from chopped hemp by ensiling it in film tubes (240 µm thickness) of a volume of 61–75 m^3 and at a pressing density of 540–695 kg m^{-3}, without the addition of an ensiling agent. Other studies showed that *Cannabis* sp. was a poor substrate for ensiling, and that its intake and fiber digestibility were lower than in conventional silage. Nevertheless, the authors concluded that ensiling seemed to be an excellent method for the biopreservation of *Cannabis* sp. [41].

The quality of the ensiled plant mass depends on many factors, including the moisture content, buffering capacity, sugar content, and types of organisms that dominate the process. Management factors, such as the speed of packing, pack density, type of additive used, chop length, covering management, and silo management during feed-out, can also affect silage fermentation and its subsequent quality [42]. In an ideal fermentation, homolactic acid bacteria use water-soluble carbohydrates for growth and produce only lactic acid. However, the fermentation of forage crops is very complex and involves many types of microorganisms, resulting in a variety of different end products [43].

The main parameter enabling the assessment of silage fermentation is the pH and the concentrations of organic acids and alcohols [42]. The final pH of silage is affected by many factors, but it is mostly related to the concentration of LA, because it is about 10–12 times stronger than any of the other major acids found in silages and exceeds the

buffering capacity of the crop. The pH of the HS silage at the beginning of the experiment was about 6.0, and during the ensiling process, it dropped to 5.8. A significantly higher ($p < 0.05$) initial pH was observed in the HF silage (value over 8.0), which also significantly dropped ($p < 0.05$) during the ensiling process. The high initial pH value may indicate the high buffering capacity of this plant material due to its high protein (263.2 g kg^{-1} DM) and ash (164.9 g kg^{-1} DM) contents, similar to legume crops [44]. Comparable pH values (5.5–5.8) were stated by [22] and [39]. A relatively low pH value of the hemp silage—namely, 4.5—was observed in an experiment with ensilaging crops for biogas production by [45].

The concentrations of LA in the control hemp silages after 42 days of ensilage were rather low: 0.30 and 1.99 g kg^{-1} DM in the HS and HF silages, respectively. Typically, the concentrations of LA in commonly fed silages range from 20 to 40 g kg^{-1} DM, but they can be considerably higher in silages with low concentrations of DM (<30%) [42]. According to other studies, the LA concentration in hemp silage fluctuated from 14.0 g kg^{-1} DM [45] to 17.0 g kg^{-1} DM [40]. In the hemp silage evaluated by [46], LA was not detected. Similarly, in a study conducted by [39], LA was not detected after 12 months of hemp storage.

AA is the second most abundant acid found in silage, usually ranging from 10 to 30 g kg^{-1} DM and inversely related to the DM content. After 42 days of ensilage, the concentrations of AA in the control hemp silages were 11.5 and 5.7 g kg^{-1} DM in the HS and HF silages, respectively. A similar concentration of AA (7.7 g kg^{-1} DM) was recorded in the hemp silage prepared by [45]. A very low AA concentration (1.0 g kg^{-1} DM) was noticed by [40]. An extremely high AA concentration was observed by [39]: after 6 months of storage, it was 17.4 g kg^{-1} DM, and after 12 months, it increased to 108.4 g kg^{-1} DM.

The BA concentration in the hemp silages was low. The presence of this acid indicates the metabolic activity of bacteria of the genus *Clostridium*, which leads to large losses of DM and the poor recovery of energy [47]. In well-fermented silages, BA should not be detectable. The lack of butyric acid in hemp silage ensiled without any additives in the tube was also found by [40]. In the hemp silage evaluated by [39], after one year of storage, the n-butyric acid concentration was 64.5 g kg^{-1} DM, and the i-butyric acid amounted to 6.3 g kg^{-1} DM.

The ensiling process may occur either naturally, with epiphytic microorganisms present on the plant material, or with the addition of microbial inoculants to improve the process, resulting in better silage quality. Microbial inoculants are commercially available for use in silage, and LAB are the main microorganisms used for this purpose [48]. In general, studies with LAB inoculants have shown that inoculation before ensiling enhances the fermentation quality of the ensiled forage [28,49]. In addition, the inoculation of LAB can help shorten the fermentation process and ensiling time, as well as directly maintain the silage quality by increasing the lactic acid content and reducing butyric acid [50]. However, the determining factors for the successful application of microbial inoculants in silage are the forage type [51] and the purpose of the silage (for feed or biogas production).

The microbial formulation used in the experiments was evaluated for the ensilage of biomass intended for the production of biogas. The bacterial preparation Lactosil Biogaz contains *P. acidilactici* and two heterofermentative bacteria of *L. buchneri* strains. The research results showed that *P. acidilactici* is a homofermentative LAB species, active within the pH range of 5.0–6.5 and capable of dominating the early stages of the fermentation phase [44]. The rapid growth of *Pediococcus* at a high pH probably produces a greater lactate concentration relative to other individual bacteria. Two remaining LAB strains are obligatorily heterofermentative bacteria, with the extraordinary ability to biotransform LA into AA, 1,2-propanediol, ethanol, and CO_2. According to [52], 1,2-propanediol is metabolized by other microorganisms to 1-propanol and propionic acid.

As expected, the use of the commercial bacterial inoculant Lactosil Biogaz for hemp ensilage resulted in a decrease in pH, an increase in LA, and a reduction in fungal abundance in the HS experimental silage in comparison to the control. In the case of the HF silage, the

bacterial inoculation was less effective, but an increase in LA and a decrease in BA were also observed.

Energy crops intended for biogas production should have the same content and digestibility of components as biomass for ruminant feed [53]. The reason is the process of methane fermentation, which resembles the process that occurs in the digestive tracts of ruminants. The results obtained for the chemical composition of the hemp silage are comparable to those published by other authors [22,39,40]. As stated earlier by [54], the chemical composition and digestibility of hemp biomass vary depending on the plant part tested. In our study, the whole hemp plant was characterized by lower CP and WSC and a more favorable CP/WSC ratio (0.95 vs. 0.45) compared with the flowers. The whole-plant samples also had higher fiber concentrations with low digestibility.

Cellulose and hemicellulose are sources of easily fermentable simple sugars. The most energetic cellulose is surrounded by both hemicellulose and lignin fragments, making it difficult for methane fermentation bacteria to access [55]. The appropriate pre-treatment of the lignocellulosic complex can increase the biodegradation rate of these structures, thereby increasing the biogas production efficiency and reducing the fermentation time [56]. Ensiling is a pre-treatment method that is usually utilized to store wet feedstock before processing [57]. Moreover, industrial hemp is a source of phenolic compounds, which can inhibit biogas production during anaerobic digestion. A promising method of pre-treatment might be the enzyme-catalyzed approach. Using a laccase treatment, the authors of [58] decreased the phenolic compound concentration in hemp straw and significantly lowered ($p < 0.05$) the inhibition levels for biogas production. A new, interesting direction of studies on microbial inoculants for the preservation of green biomass is the selection of microorganisms with specific enzyme activities [59].

As confirmed by the results of our study, hemp ensiling with bacterial inoculants may function as a beneficial pre-treatment for lignocellulosic materials before further processing to methane [60]. A decrease in the crude fiber and hemicellulose fraction was observed in the HS silage. Similarly, in the HF silage, as a result of the ensiling process, a drop in the crude fiber and hemicellulose was observed. This corresponds to the results reported by [39], who observed a marked decrease in the hemicellulose content over a storage period of 12 months. Still, contradictory results were obtained by [40], where, after 36 weeks, the crude fiber remained nearly unchanged in comparison to the ensilaged material and dropped slightly in the silage with additives. Our study was limited to assessing the feasibility of making hemp silage for energy use. There is a need for further research on hemp ensiling for its use as a feed, and with the use of other biological formulations that facilitate the ensiling process.

5. Conclusions

Ensiling can be an effective method for pre-treating hemp plants intended for subsequent biogas production, which can preserve the harvested biomass and ensure the year-round availability of the feedstock. The use of bacterial inoculants containing homo- and heterofermentative strains of LAB for hemp ensiling is recommended to improve the fermentation quality, and to increase the degradation of hemicellulose. The results can be used in hemp biomass management to improve the ensiling process aimed at increasing the degradation of hemicellulose and enhancing the efficiency of biogas production. Because the number of studies on hemp ensilage is still limited, there is a need for further experiments to expand the understanding of the ensiling of hemp biomass for energy and feed purposes.

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