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Original Paper

1,2-propanediol in maize silages with nutrients measurement using nirs technology

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2-propanediol (propanediol) represents an important glucoplastic substance for the nutritional requirements of highly productive cows, and its natural synthesis during silage fermentation is of great importance. The aim of this paper was to monitor the fermentation activity of two combinations of homo- and hetero-fermentative lactic acid bacteria (LAB) in two preparations (preparation 1: *Lentilactobacillus buchneri*, *Lacticaseibacillus rhamnosus*, *Lactiplantibacillus plantarum*; preparation 2: *Lentilactobacillus buchneri*, *Lacticaseibacillus rhamnosus*, *Lentilactobacillus diolivorans*). Propanediol production was monitored in maize silages at the following levels: cutting technology, hybrid, vegetation development, type of silage fermentation and length of fermentation time. From the point of view of silage alternatives, preparation 1 fulfilled the declared properties and had a demonstrably positive influence on the production of propanediol in maize silages from whole plants (average content of 4.9 g.kg^{-1}). Propanediol production in the silage alternative with preparation 2 (average content of 1.3 g.kg^{-1}) was higher when compared to the negative control (average content of 0.1 g.kg^{-1}). The production of propanediol decreased with increasing dry matter content, which is directly related to the advancing vegetative development of plants. The type of silage fermentation and vegetation development had a statistically demonstrable effect on the level of propanediol production in maize silages. A statistically significant difference at the hybrid level was only identified with one hybrid. No statistically significant differences were found at the level of cutting technology and length of fermentation time. The fermentation activity of the *Lentilactobacillus buchneri* strain used depended on its combination with other homofermentative and/or heterofermentative LAB strains.

Keywords: *Zea mays*, hybrids, harvesting technology, vegetation stages, dry matter content, silage, 1,2-propanediol, *Lentilactobacillus buchneri*

1 Introduction

The development and course of silage fermentation is fundamentally influenced by the fermentation's microflora. It is primarily formed by epiphytic bacteria. Targeted inoculation and guidance of the course of silage fermentation is achieved by adding various additives, in which different types and strains of lactic acid bacteria (hereinafter LAB) are most often used. These are isolated from nature and their selection depends on their unique fermentation characteristics, and their potential to interact with one another.

The aerobic stability of silage is one of the primary goals of successful silage fermentation. The dominant factor for increasing this is acetic acid (Danner et al., 2003), which is produced to varying degrees and in different ranges by LAB – lactic acid bacteria (Mitrík, 2021). Some strains of heterofermentative LAB have the ability to produce acetic acid primarily from water-soluble sugars, and secondarily through the fermentation of lactic acid (Oude Elferink et al., 2001), which creates the basis for the synergistic action of homofermentative and heterofermentative LAB. During the transformation of lactic acid to acetic acid, 1,2-propanediol (hereinafter propanediol) and ethanol are also formed (Oude Elferink et al., 2001, Danner et

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al., 2003). Propanediol is also used in the food industry to positively influence the palatability of food (Patent EP2822399A1) and it is assumed that it also improves the palatability of silages (Mitrík, 2021). It has no direct effect on increasing aerobic stability (Danner et al., 2003), but is a potential precursor of 1-propanol, which is effective against yeasts (Nishino and Touno, 2005) and is a precursor for the subsequent formation of propionic acid (Krooneman et al., 2002), which is effective against fungi. From a nutritional point of view, propanediol is an important glucoplastic substance in the nutrition of high-production cows (Wilkinson and Rinne, 2017; Lau et al., 2018), which is purposefully added to feed rations to compensate for the negative energy balance of cows, especially in the postpartum period.

The aim of this work was to monitor and evaluate the dynamics of the production of propanediol and other fermentation metabolites in silages of whole plants of different maize hybrids (*Zea mays*) using two combinations of homofermentative and heterofermentative LAB (Table 1). We monitored changes in the composition of the fermentation profile at the following levels:

- 7 different silage maize hybrids,
- harvesting and cutting technologies,
- points of vegetation development in over a time interval of 34 days,
- lengths of the fermentation process.

2 Materials and methods

Seven (7) different silage maize hybrids (FAO 200–530) from KWS SEMENA s. r. o. were sown on 28/04/2021 in four repetitions on the plot in Bátka: altitude 182 m above sea level – 48° 21' 45.9" N 20° 11' 55.7" E. Sampling was carried out at an interval of 34 days on four dates (12/08/2021 – 224th calendar day; 19/08/2021 – 231st calendar day; 2/9/2021 – 244th calendar day; 13/09/2021 – 258th calendar day).

A Class Jaguar 980 cutter with SHREDLAGE technology cylinders was used to harvest two samples with a weight of approx. 2,500 kg, i.e. half of the sown strip of each hybrid cut at each collection date. CLASSIC: theoretical cut length: 5 mm with a roller spacing of 3 mm; SHREDLAGE: theoretical cut length: 22 mm with a cylinder spacing of 1 mm (Table 7).

From each cut sample, a roughly 30 kg coarse sample was taken from at least 10 places. The coarse samples were transported to the laboratory immediately after collection, where each of them was again thoroughly mixed and laboratory samples were taken from this material for nutrient analysis, and at the same time three silage alternatives (Table 2) were ensiled in the laboratory, each in two replications for three different periods of silage fermentation (90, 150 and 240 days). After the prescribed fermentation time, the samples were opened.

Solutions of inoculation preparations (Table 1) were prepared just before their application and distilled water was used to dilute them. The relevant solutions were applied to the sample in a plastic container using a regular plastic spray applicator in the prescribed dose (Table 1) based on the weight of the sample, and then the mass was thoroughly mixed.

The control without preservative was ensiled first. After each silage alternative, thorough disinfection with 80% ethanol was performed to avoid cross-contamination. We used standard food grade "MVAC vacuum bag 80" PE vacuum bags (manufacturer: MVAC, Canada). The weight of the samples ranged from 800 to 1,000 g of silage. The vacuum-packed samples were stored in dark conditions in a room with a temperature of 20–24 °C and the fermentation period lasted 90, 150 and 240 days.

After opening the samples, the silage was thoroughly mixed. Preparation of aqueous solution: 100 g of silage in 2,000 ml of distilled water by mixing (30,000 revolutions.1 min^{-1}) for 1 minute and subsequent filtering through a paper filter. The pH value was immediately measured on a Seven Compact S220 instrument (Mettler Toledo). Leachate samples were prepared by standard

Table 1 Silage alternative and characteristics of preparations

Preparation	0	1	2
Lentilactobacillus buchneri^{1k2075}		+	+
Lentilactobacillus diolivorans^{1k20752}			+
Lactiplantibacillus plantarum^{1k2079}		+	
Lacticaseibacillus rhamnosus^{1k20711}		+	+
CFU.1 g^{-1}		min. 3.0×10^{11}	min. 2.5×10^{11}
Dosing		1 g.1 t^{-1}	1 g.1 t^{-1}

* g.kg^{-1}

Table 2 Calibration and validation parameters of models for NIRS

Nutrient	g.kg^{-1} of dry matter	RMSEC	R^2	RMSEP	R^2
Dry matter		5.64	0.995	5.54	0.994
Crude protein		3.29	0.953	3.07	0.947
Essential extract		3.84	0.991	4.87	0.992
Ash		5.97	0.971	5.06	0.962
NDF		19.10	0.933	19.70	0.928
ADF		12.00	0.938	10.80	0.932
starch		20.30	0.983	23.10	0.974

RMSEC – root mean square error of calibration; RMSEP – root mean square error of prediction

purification (clarification, dilution, centrifugation and ultra-filtration) prior to UHPLC measurement.

Fermentation characteristics were measured on a UHPLC Dionex UltiMate 3000 Series with an AGILENT Hi-Plex H 300 × 7.7 mm column. Mobile phase 0.01M H_2SO_4 with a flow rate of 0.7 ml.min^{-1} and with a sample injection of 20 µl. Lactic acid and volatile fatty acids were measured on a UV-VIS 210 nm detector at a temperature of 40 °C, and alcohols, 1,2-propanediol (propanediol) and monosaccharides on an RI detector at a temperature of 55 °C.

Weights of 500–750 g were dried in MEMMERT UFE 500 and UFE 700 dryers at a temperature of 60 °C for 16–24 hours. The dried samples were ground on SM-100 mills (RETCH) with a 2 mm sieve and then ground on a TWISTER (RETCH) with a 1 mm sieve. The dry matter content and selected nutrients in laboratory dried samples were determined using an NIRS Antaris II FT-NIR Analyzer (Thermo Fisher Scientific) using our own validated calibration models (Table 2). The total dry matter content was evaluated on the basis of the laboratory dry matter and on the basis of the dry matter content measured by the NIRS method in the dried samples. The dry matter content of silages was corrected based on the content of individual fermentation products (Kacerovský et al., 1990). We expressed water-soluble sugars (WSC) as the sum of glucose, fructose and maltose, which were measured on UHPLC.

We performed statistical evaluations with the NCSS 12 (64 bit) program, version 12.0.18, from NCSS LLC using the following methods: ANOVA, linear correlation (Pearson), linear regression, non-linear quadratic and polynomial regression.

3 Results and discussion

3.1 Nutrient composition

We found statistically significant differences (P <0.01) in dry matter and nutrient composition between individual hybrids (Table 3). These findings are in accordance with the different level of early ripening of individual hybrids, as indicated by their inclusion in FAO maturity groups, and also large differences in the date of reaching 30% dry matter (221st to 250th calendar day). Even at the level of individual samples, we found statistically significant differences (P <0.01) in dry matter and nutrient parameters (Table 5). The average daily increase in dry matter over the course of 34 days reached 4.87 (g.kg^{-1}). day^{-1}.

3.2 Silage alternative

The dry matter content in all three silage alternatives (Table 4) did not show statistically significant differences. Each of the groups consisted of an extensive set of 168 silage samples (Table 4), which testifies to a broad and standard starting base for each group. The addition of silage additives affects the presence of *L. buchneri* in maize silages (Mitrík et al., 2019; Kalúzová et al., 2022). We found very significant statistical differences (P <0.01) in the content of propanediol between the silage alternatives. Preparation 1 reached significantly the highest content of 4.9 g.kg^{-1}, which is also in accordance with the results of other authors (Weiss et al., 2005; Kleinschmit and Kung, 2006; Arriola et al., 2021; Huang et al., 2021), indicating very good performance of the *L. buchneri* strain in combination with two homofermentative strains. Despite using the same strain of *L. buchneri* in preparation 2, the production of propanediol was almost 3.8 times lower. The control contained only trace amounts of propanediol, which did not exceed 1.00 g.kg^{-1} even in the maxima, which is also in accordance with the results of Kleinschmit and Kung (2006). The results show that the use of a particular strain/species and its combination with other LAB affects the success of propanediol production. At the level of all silage alternatives, we found a statistically significant correlation (P <0.01) between propanediol and lactic acid (r -0.356), acetic acid (r 0.415) and ethanol (r -0.381). The fermentation parameters and their ratios when

Table 3 Hybrids – nutrient composition of fresh material before ensiling and dry matter content and fermentation parameters** in the silage alternative: Preparation 1

Hybrid	1	2	3	4	5	6	7	Total/average
n (fresh samples – all)	72	72	72	72	72	72	72	504
FAO group	200	240	280	340	420	480	530	356
30% DM content ***	221	212	232	237	245	243	250	235
Dry matter (g.kg^{-1})	402.1 ±52.1^{34567}	407.9 ±59.5^{34567}	341.7 ±66.8^{12567}	338.7 ±87.8^{12567}	307.2 ±70.1^{12347}	307.8 ±67.2^{12347}	267.7 ±53.7^{123456}	339.0 ±81.4
WSC (g.kg^{-1} DM)	55.8 ±13.9^{567}	48.3 ±13.9^{3567}	59.1 ±27.3^{2567}	55.0 ±23.0^{567}	67.5 ±21.6^{12347}	69.6 ±22.5^{12347}	83.9 ±34.9^{123456}	62.8 ±28.6
NDF (g.kg^{-1} DM)	389.6 ±32.5^{567}	387.6 ±28.4^{567}	400.6 ±39.0^{7}	389.3 ±29.9^{567}	410.4 ±32.4^{1247}	405.3 ±35.9^{1247}	426.9 ±54.4^{123456}	401.4 ±39.2
ADF (g.kg^{-1} DM)	208.5 ±20.8^{47}	207.1 ±8.2^{47}	210.2 ±6.2^{47}	195.1 ±16.6^{123567}	211.2 ±17.0^{47}	210.5 ±23.6^{47}	226.2 ±32.5^{123456}	209.8 ±22.1
STARCH (g.kg^{-1} DM)	276.4 ±33.1^{2567}	299.0 ±19.9^{13567}	264.6 ±55.3^{247}	297.6 ±50.5^{3567}	246.5 ±46.6^{1247}	249.9 ±65.0^{1247}	201.0 ±78.8^{123456}	262.2 ±61.6
EE (g.kg^{-1} DM)	26.3 ±2.6^{234567}	24.7 ±1.9^{1567}	24.2 ±3.4^{1567}	23.6 ±3.9^{1567}	21.6 ±2.5^{12347}	22.3 ±3.4^{12347}	20.3 ±3.4^{123456}	23.3 ±3.6
ASH (g.kg^{-1} DM)	48.4 ±3.9^{234567}	45.2 ±1.1^{1456}	44.0 ±3.2^{147}	41.9 ±2.5^{123567}	43.8 ±2.7^{1247}	43.6 ±2.3^{1247}	45.2 ±3.4^{13456}	44.6 ±3.4
n (silage – preparation 1)	24	24	24	24	24	24	24	168
Dry matter*	398.2 ±51.2^{34567}	404.9 ±60.2^{34567}	337.9 ±69.8^{127}	333.9 ±92.3^{127}	302.4 ±72.5^{12}	302.9 ±69.1^{12}	264.5 ±54.0^{1234}	335.0 ±54.0
pH	3.96 ±0.1^{567}	3.95 ±0.1^{57}	3.99 ±0.1^{567}	3.96 ±0.1^{567}	3.88 ±0.1^{1234}	3.88 ±0.1^{134}	3.86 ±0.1^{1234}	3.92 ±0.1
Lactic acid*	18.9 ±5.4^{3457}	19.4 ±5.7^{34567}	12.8 ±6.0^{12}	14.4 ±5.3^{12}	14.6 ±6.4^{12}	15.2 ±4.5^{2}	13.6 ±5.3^{12}	15.6 ±5.3
Acetic acid*	11.8 ±7.0	9.4 ±5.1^{3567}	16.4 ±7.9^{2}	14.7 ±9.6	17.6 ±10.9^{2}	16.4 ±8.3^{2}	16.2 ±7.7^{2}	14.7 ±7.7
Butyric acid*	0.0 ±0.0	0.0 ±0.2	0.0 ±0.0	0.0 ±0.1	0.1 ±0.3	0.0 ±0.1	0.0 ±0.0	0.0 ±0.0
Propionic acid*	0.0 ±0.0	0.0 ±0.0	0.2 ±0.5	0.2 ±0.4	0.1 ±0.4	0.0 ±0.2	0.1 ±0.3	0.1 ±0.3
Formic acid*	0.6 ±0.2^{37}	0.6 ±0.3^{37}	0.4 ±0.2^{12}	0.5 ±0.2	0.5 ±0.2	0.5 ±0.2	0.4 ±0.2^{12}	0.5 ±0.2
Ethanol*	5.9 ±3.3^{24567}	2.8 ±3.3^{1}	4.2 ±2.7^{456}	2.2 ±1.4^{13}	2.3 ±1.5^{13}	1.6 ±0.9^{13}	3.2 ±2.3^{1}	3.2 ±2.3
1,2-propanediol*	3.9 ±4.1^{6}	2.8 ±3.4^{6}	4.7 ±4.4	4.8 ±3.4	5.6 ±5.4	7.5 ±4.7^{12}	5.2 ±4.2	4.9 ±4.2
1-propanol*	0.5 ±1.5	0.3 ±1.1	0.8 ±1.7	1.1 ±1.8	0.9 ±1.8	0.9 ±1.7	1.3 ±2.0	0.8 ±2.0
1-butanol*	0.1 ±0.6	0.0 ±0.1	0.0 ±0.1	0.2 ±1.0	0.0 ±0.1	0.0 ±0.1	0.0 ±0.1	0.1 ±0.1

WSC (glucose + fructose + mannose); NDF – neutral detergent fibre; ADF – acid detergent fibre; EE – crude fat (ether extract); * g.kg^{-1}; ** average of 90, 150 and 240 days of fermentation; *** calendar day of eaching 30% dry matter content; indexes statistically significant differences in the row (P <0.01)

Acta fytotechn zootechn, 26, 2023(2): 138–146
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Table 4 Silage alternative – fermentation parameters**

Preparation	0	1	2
n	168	168	168
Dry matter*	346.2 ±80.2	335.0 ±82.8	335.9 ±80.8
pH	3.87 ±0.10^{12}	3.92 ±0.10^{02}	3.81 ±0.11^{01}
Lactic acid*	20.9 ±4.8^{1}	15.6 ±6.0^{02}	20.1 ±4.5^{1}
Acetic acid*	5.9 ±2.8^{12}	14.7 ±8.6^{02}	12.1 ±6.2^{01}
Butyric acid*	0.0 ±0.1	0.0 ±0.1	0.0 ±0.1
Propionic acid*	0.0 ±0.0^{1}	0.1 ±0.3^{02}	0.0 ±0.1^{1}
Formic acid*	0.6 ±0.2^{12}	0.5 ±0.2^{02}	0.6 ±0.2^{01}
Ethanol*	4.9 ±3.3^{12}	3.2 ±2.7^{0}	2.9 ±2.6^{0}
1,2-propanediol*	0.1 ±0.5^{12}	4.9 ±4.4^{02}	1.3 ±1.8^{01}
1-propanol*	0.1 ±1.1^{12}	0.8 ±1.7^{02}	1.4 ±1.7^{01}
1-butanol*	0.1 ±0.7		0.1 ±0.5

* g.kg^{-1}; ** average of 90, 150 and 240 days of fermentation; indexes statistically significant differences in the row (P <0.01)

Table 5 Vegetation development – nutrient composition and fermentation parameters**

Preparation 1	Collection (calendry day)			
	1 (224)	2 (231)	3 (244)	4 (258)
n	126	126	126	126
Dry matter (g.kg^{-1})	270.9 ±47.3^{234}	301.1 ±50.3^{134}	347.7 ±62.2^{124}	436.4 ±47.2^{123}
Increase***		4.3	3.6	6.3
WSC* (g.kg^{-1} DM)	86.0 ±23.9^{234}	64.8 ±13.7^{134}	44.2 ±12.3^{124}	56.0 ±6.9^{123}
NDF (g.kg^{-1} DM)	441.2 ±43.5^{234}	397.9 ±24.9^{14}	392.9 ±15.4^{14}	373.5 ±31.0^{123}
ADF (g.kg^{-1} DM)	231.7 ±25.4^{234}	208.1 ±11.8^{134}	200.9 ±8.6^{12}	198.6 ±20.2^{12}
Starch (g.kg^{-1} DM)	202.1 ±66.0^{234}	252.8 ±49.7^{134}	286.0 ±22.4^{124}	307.7 ±39.1^{123}
EE (g.kg^{-1} DM)	19.1 ±3.2^{234}	22.9 ±1.8^{134}	26.5 ±2.7^{124}	^{24}.6 ±1.2^{123}
ASH (g.kg^{-1} DM)	45.1 ±1.1^{234}	46.5 ±2.1^{134}	43.2 ±4.2^{12}	43.6 ±4.1^{12}
n	84	84	84	84
Dry matter*	265.6 ±48.6^{234}	294.7 ±50.6^{134}	^{34}5.2 ±62.8^{124}	4^{34}.3 ±47.1^{123}
pH	3.90 ±0.10^{2}	3.96 ±0.10^{1}	3.93 ±0.09	3.93 ±0.1^{2}
Lactic acid*	13.5 ±7.6^{34}	12.3 ±3.^{234}	16.5 ±4.5^{124}	20.0 ±4.5^{123}
Acetic acid*	18.3 ±8.^{134}	20.3 ±8.7^{34}	12.6 ±6.6^{124}	7.5 ±3.7^{123}
Butyric acid*	0.0 ±0.1	0.0 ±0.2	0.0 ±0.2	0.0 ±0.0
Propionic acid*	0.1 ±0.4	0.2 ±0.5^{34}	0.0 ±0.2^{2}	0.0 ±0.1^{2}
Formic acid*	0.5 ±0.3	0.5 ±0.2	0.5 ±0.2	0.5 ±0.2
Ethanol*	3.4 ±2.6^{24}	1.9 ±1.2^{14}	2.5 ±1.5^{4}	4.9 ±3.7^{123}
1,2-propanediol*	7.4 ±4.8^{34}	7.3 ±3.9^{34}	4.1 ±3.3^{124}	0.9 ±1.0^{123}
1-propanol*	0.7 ±1.22	1.8 ±2.4^{134}	0.7 ±1.6^{2}	0.0 ±0.1^{2}
1-butanol*	0.0 ±0.1	0.1 ±0.5	0.0 ±0.0	0.1 ±0.8

* g.kg^{-1}; WSC* (glucose + fructose + mannose); ** average of 90, 150 and 240 days of fermentation; *** increase in dry matter content (g.kg^{-1}).deň$^{-1}$; indexes statistically significant differences in the row (P <0.01)

using preparation 1 point to the successful course of the secondary production of acetic acid from lactic acid, which is also confirmed by:

- the lowest lactic acid content,
- the highest acetic acid content,
- higher ethanol content than preparation 2,

which is in agreement with the description of fermentation pathways characteristic of *L. buchneri* (Oude Elferink et al., 2001; Krooneman et al., 2002; Rooke and Hatfield, 2003). Homofermentation supported by the inclusion of *L. plantarum* in preparation 1 most likely supported higher lactic acid formation in the first stages of fermentation, thus creating the basis for its secondary fermentation by the *L. buchneri* strain.

3.3 Influence of other factors

In accordance with the goals of this work and also based on the evaluation of the results at the level of silage alternatives, we made further evaluations at the level of preparation 1.

3.3.1 Vegetative development

Changes in the nutrient composition during vegetation development document the average nutrient contents, which differed statistically significantly in individual phases of development (Table 5). The contents of propanediol, like dry matter content and nutrients, differed statistically significantly in the course of vegetation development. With dry matter of up to 300 g.kg^{-1}, the production of propanediol reached a level of around 7 g.kg^{-1}. In the range of dry matter from 300 g.kg^{-1} to 400 g.kg^{-1}, the production of propanediol dropped to approximately half, and then at dry matter above 400 g.kg^{-1} it dropped to 0.9 g.kg^{-1}, while the lactic acid content was the highest (Table 8). This indicates that at a higher to high dry matter content (above 350 g.kg^{-1}) the conditions for the secondary conversion of lactic acid to acetic acid are not suitable, and these findings are also in accordance with the results of other authors (da Silva et al., 2022).

3.3.2 Length of fermentation time

The length of the fermentation period did not have a statistically significant effect on the production of propanediol. However, a slight decrease in propanediol content with increasing fermentation time is evident (Table 6).

The lower level of propanediol and the simultaneous statistically demonstrable increase in the content of propionic acid and 1-propanol on the 240th day of fermentation indicate that epiphytic strains of *L. diolivorans* could also have been used during in this phase of fermentation (Krooneman et al., 2002).

3.3.3 Cutting technology

When examining the technology used for cutting the green matter and its influence on silage fermentation when inoculated with preparation 1, including in each alternative all 7 hybrids, 4 vegetation stages and 3 lengths of fermentation time (Table 7) we only found significant differences for lactic acid, formic acid and ethanol.

However, from a quantitative point of view, these differences are not significant. The average production of

Table 6 Length of fermentation time – fermentation parameters**

Preparation 1	Number of days		
	1 (90)	2 (150)	3 (240)
n	56	56	56
Dry matter*	339.3 ±81.7	334.4 ±83.1	331.1 ±84.2
pH	3.86 ±0.08^{23}	3.94 ±0.10^{1}	3.97 ±0.10^{1}
Lactic acid*	17.8 ±5.4^{23}	14.8 ±5.6^{1}	14.2 ±6.3^{1}
Acetic acid*	11.2 ±7.0^{3}	12.9 ±6.7^{3}	19.8 ±9.4^{12}
Butyric acid*	0.0 ±0.0	0.0 ±0.0	0.1 ±0.2
Propionic acid*	0.0 ±0.0^{3}	0.0 ±0.2^{3}	0.2 ±0.5^{12}
Formic acid*	0.6 ±0.3^{23}	0.4 ±0.2^{1}	0.5 ±0.2^{1}
Ethanol*	2.9 ±2.7	3.4 ±2.8	3.3 ±2.6
1,2-propanediol*	5.2 ±5.1	5.6 ±4.5	4.0 ±3.3
1-propanol*	0.0 ±0.1^{3}	0.3 ±0.8^{3}	2.1 ±2.4^{12}
1-butanol*	0.1 ±0.4	0.1 ±0.7	0.0 ±0.0

* g.kg^{-1}; ** average of 90, 150 and 240 days of fermentation; indexes statistically significant differences in the row (P <0.01)

Table 7 Cutting technology – fermentation parameters**

Preparation 1	Technology	
	classic (1)	shredlage (2)
Theoretical cut length (mm)	5	22
Cylinder spacing (mm)	3	1
GMPS (mm)	2.3 ±0.3	4.3 ±0.4
>4.75 mm (%)	32.0 ±3.0	48.0 ±4.0
n	168	168
Dry matter*	330.6 ±84.5	339.3 ±81.1
pH	3.92 ±0.10	3.92 ±0.1[1]
Lactic acid*	14.3 ±5.8[2]	16.9 ±5.9[1]
Acetic acid*	14.7 ±7.9	14.6 ±9.3
Butyric acid*	0.0 ±0.2	0.0 ±0.1
Propionic acid*	0.1 ±0.3	0.1 ±0.3
Formic acid*	0.4 ±0.2[2]	0.5 ±0.2[1]
Ethanol*	3.6 ±3.2[2]	2.7 ±2.1[1]
1,2-propanediol*	4.6 ±4.4	5.2 ±4.5
1-propanol*	1.0 ±1.9	0.6 ±1.5
1-butanol*	0.0 ±0.1	0.1 ±0.6

* g.kg^{-1}; ** average of 90, 150 and 240 days of fermentation; GMPS – geometric mean particle size; indexes statistically significant differences in the row (P <0.01)

propanediol when using both technologies is at the level of approximately 5 g.kg^{-1} and does not show statistically significant differences.

3.3.4 Maize silage hybrid

In the production of propanediol, we found statistically significant differences between a pair of hybrids (1 and 2) in comparison with hybrid 6 (Table 3). The highest average propanediol production of 7.5 g.kg^{-1} was achieved by hybrid 6 (FAO 480; dry matter content 300 g.kg^{-1} reached on the 243rd calendar day) and the lowest 2.8 g.kg^{-1} by hybrid 2 (FAO 240; dry matter content of 300 g.kg^{-1} reached on the 212th calendar day). At the level of all hybrids and when using the silage alternative with preparation 1, we also found statistically significant (P <0.01) correlations between dry matter content and the following fermentation products:

- propanediol (r -0.590),
- lactic acid (r 0.629),
- acetic acid (r -0.627).

Based on these relationships, we proceeded to create 8 groups (Table 8) with a dry matter range of 200 to 550 g.kg^{-1}, with intervals of 50 g.kg^{-1}. We also found statistically significant differences (P <0.01) in propanediol, lactic acid and acetic acid between individual groups. Propanediol peaked (8.58 g.kg^{-1}) at a dry matter content of 250 g.kg^{-1} (Figure 1) and dropped at a dry matter content of 300 to 350 g.kg^{-1} to a level of approximately 5.0 g.kg^{-1}. At a dry matter content of 400 g.kg^{-1}, propanediol production dropped to 2.21 g.kg^{-1} and continued to drop below

Table 8 Dry matter content (group) – fermentation parameters** in the silage alternative with Preparation 1

Group	1	2	3	4	5	6	7	8	Average
DM content*	200	250	300	350	400	450	500	550	
Replication (*n*)	14	94	61	65	33	51	17	1	
LA *	12.18^{567}	10.95^{34567}	14.02^{2567}	16.89^{26}	19.59^{123}	20.87^{1234}	20.24^{123}	21.44	15.57
AA*	18.84^{567}	21.56^{34567}	17.08^{24567}	11.94^{23}	8.21^{123}	8.49^{123}	6.10^{123}	6.47	14.65
ETH*	5.13^{2}	2.06^{167}	2.76^{6}	2.73^{6}	2.95^{6}	5.43^{2345}	4.38^{2}	5.45	3.17
PPD *	4.07^{2}	8.58^{134567}	5.84^{2567}	4.77^{267}	2.21^{23}	0.83^{234}	0.41^{234}	0.30	4.92

* g.kg^{-1}; ** average of 90, 150 and 240 days of fermentation; indexes statistically significant differences in the row (P <0.01); DM – dry matter; LA – lactic acid; AA – acetic acid; ETH – ethanol; PPD – 1,2-propanediol

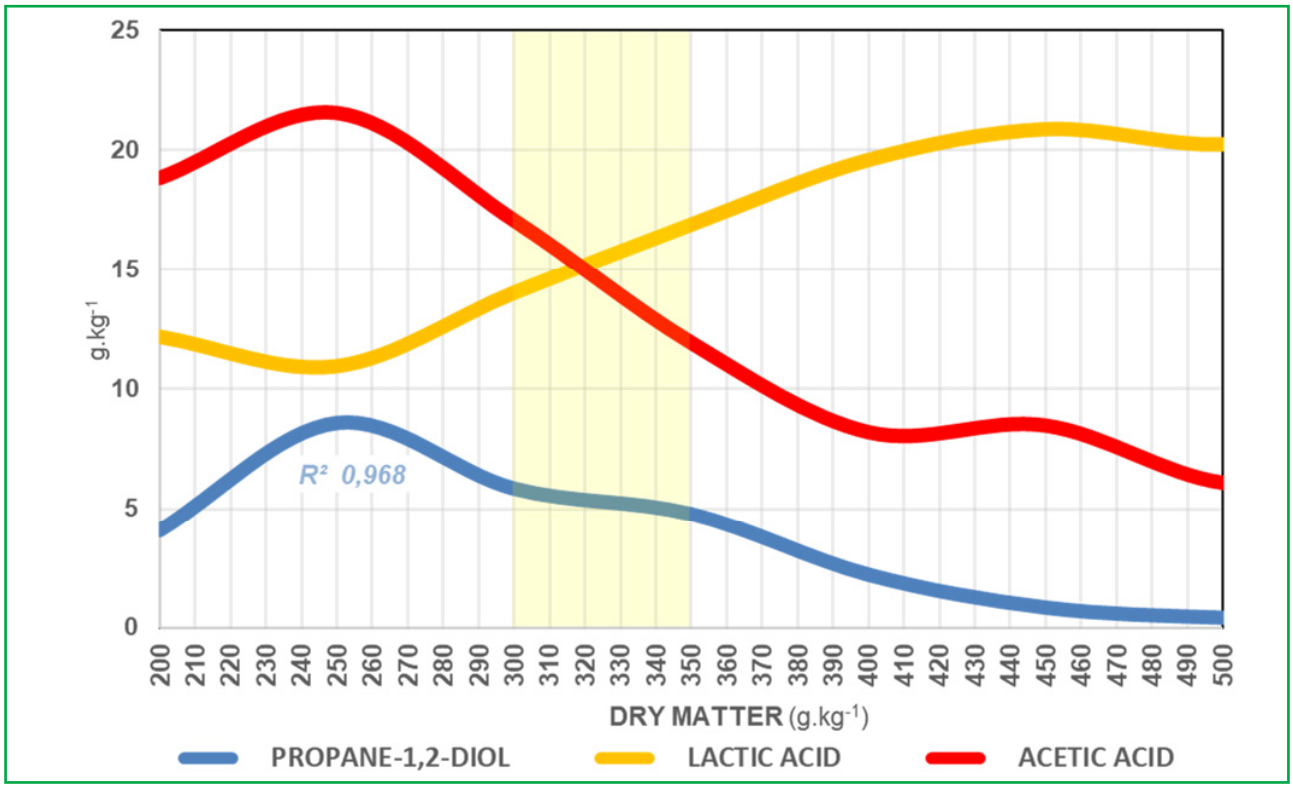

Figure 1 Propanediol and dry matter content

1.00 g.kg^{-1} thereafter. A significant decrease in the level of propanediol at a dry matter content above 300 g.kg^{-1} was also recorded by Da Silva et al. (2022). These results again indicate that the intensity of the secondary fermentation of lactic acid to acetic acid and propanediol decreases with increasing dry matter content.

4 Conclusion

The same *L. buchneri* strain was able to increase propanediol production almost 3.8-fold under the same conditions if it was inoculated in combination with two homofermentative LAB strains. The combination of two heterofermentative LAB strains (*L. buchneri* and *L. diolivorans*) with one homofermentative strain (L. rhamnosus) did not produce increased amounts of acetic acid. The results achieved and the differences between the preparations indicate that the performance of the same *L. buchneri* strain in the production of propanediol depends, with great probability, on its action in combination with other Lactobacillus species.

Preparation 1 had a demonstrably positive influence on the production of propanediol in maize silages (average content 4.9 g.kg^{-1}), fulfilled the declared properties and is strongly assumed to positively influence the health of highly productive cows. The production of propanediol in the silage alternative with preparation 2 (average content 1.3 g.kg^{-1}) was higher than in the negative control (average content 0.1 g.kg^{-1}), but only approximately at a third of the level compared to preparation 1. Propanediol production culminated on the 150th day of fermentation. Propanediol production decreased as vegetation development advanced (increasing dry matter content).

The results of this work significantly indicate that the nutrient characteristics of silage hybrids in individual vegetation stages can create different fermentation starting points for the course of the silage process, and this issue will require further monitoring.

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References

Arriola, K.G., Oliveira A.S., Jiang Y., Kim D., Silva H.M., Kim S.C., Amaro F.X., Ogunade I.M., Sultana H., Pech Cervantes A.A., Ferraretto L.F., Vyas D., & Adesogan A.T. (2021). Meta-analysis of effects of inoculation with *Lactobacillus buchneri*,

with or without other bacteria, on silage fermentation, aerobic stability, and performance of dairy cows. *J. Dairy Sci.,* 104, 7653–7670. https://doi.org/10.3168/jds.2020-19647

da Silva, E. B., Liu, X., Mellinger, C., Gressley, T.F., Stypinski, J.D., Moyer, N. A., & Kung, Jr. L. (2022). Effect of dry matter content on the microbial community and on the effectiveness of a microbial inoculant to improve the aerobic stability of corn silage; *J. Dairy Sci.,* 105, 5024–5043. https://doi.org/10.3168/jds.2021-21515

Danner, H., Holzer, M., Weidenholzer, E., & Braun, R. (2003). Acetic Acid Increases Stability of Silage under Aerobic Conditions. *Applied and Environmental Microbiology,* 69(1), 562–567.

Huang, Z., Wang, M., Ke, W., & Guo, X. (2021). Screening of High 1,2-propanediol Production by *Lactobacillus buchneri* Strains and Their Effects on Fermentation Characteristics and Aerobic Stability of Whole-Plant Corn Silage. *Agriculture*, 11(7), 590. https://doi.org/10.3390/agriculture11070590

Kacerovský, O., Babička, L., Bíro, D., Heger, J., Jedlička, Z., Lohniský, J., Mudřík, Z., Roubal, P., Svobodová, M., Vencl, P., Vrátny, P., & Zelenka, J. (1990). *Testing and judging feeds* (Zkoušení a posuzování krmiv). Státni zemědelské nakladatelství Praha.

Kalúzová, M., Kačániová, M., Bíro, D., Šimko, M., Gálik, B., Rolinec, M., Hanušovský, O., Felšöciová, S., & Juráček, M. (2022). The Change in Microbial Diversity and Mycotoxins Concentration in Corn Silage after Addition of Silage Additives. *Diversity*, 14, 592. https://doi.org/10.3390/d14080592

Kleinschmit, D. H., & Kung, L. Jr. (2006). The Effects of *Lactobacillus buchneri* 40788 and *Pediococcus pentosaceus* R1094 on the fermentation of Corn Silage; *J. Dairy Sci.,* 89, 3999–4004.

Krooneman, J., Faber, F., Alderkamp, A.C., Qude Elferink, S.J.H.W., Driehuis, F., & Cleenwerck, I. (2002). *Lactobacillus diolivorans* sp. nov. a 1,2-propanediol-degrading bacterium isolated from aerobically stable maize silage. *International Journal of Systematic and Evolutionary Microbiology*, 52, 639–46.

Lau, N., Kramer, E., & Hummel, J. (2018). Impact of grass silage with high levels of propylene glycol on ketosis prophylaxis during transition phase and early lactation. In *Proceedings of the 18th International Silage Conference*, Bonn, Germany, 24–26 July 2018 (pp. 308–309).

Mitrík, T. (2021). *Ensilage – illustrated manual for expert a begginer alike*. Wageningen Academic Publishers. https://doi.org/10.3920/978-90-8686-911-4

Mitrík, T., Kořínek, D., & Mitrík, A. (2019). Heterofermentative LAB and 1,2-propanediol production in maize silages with lower dry matter content. *18th international symposium forage conservation*, Brno.

Nishino, N., & Touno, E. (2005). Ensiling characteristics and aerobic stability of direct-cut and wilted grass silages inoculated with *Lactobacillus casei* or *Lactobacillus buchneri*. *J Sci Food Agric,* 85, 1882–1888. https://doi.org/10.1002/jsfa.2189

Oude Elferink, S. J. W. H., Krooneman, J., Gottschal, J.C., Spoelestra, S. F., Faber, F., & Driehuis, F. (2001). Anaerobic Conversion of Lactic Acid to Acetic Acid and 1,2-Propanediol by *Lactobacillus buchneri. Applied and environmental microbiology*, 2001, 125–132.

Patent EP2822399A1. *Food and beverage products containing 1,3-propanediol and methods of modifying flavor release using 1,3-propanediol*. https://patents.google.com/patent/EP2822399A1/en

Rooke, J.A., & Hatfield, R.D. (2003). Biochemistry of ensiling. In Buxton, D.R., Muck, R.E., & Harrison, J.H. (eds.) *Silage science and technology*. Wiley, Hoboken, NJ, USA.

Weiss, K., Kroschlewski, B., & Auerbach, H. (2005). Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage. *J. Dairy Sci.,* 99, 8053–8069.

Wilkinson, J.M., & Rinne, M. (2017). Highlights of progress in silage conservation and future perspectives. *Grass Forage Sci.,* 00, 1–13. https://doi.org/10.1111/ gfs.12327