

The logo for AgroSym 2022 is an oval with a dark red background and a gold border. Inside the oval, the word "AgroSym" is written in a gold, serif font, with a small stalk of wheat above the 'y'. Below "AgroSym", the year "2022" is written in a smaller, gold, sans-serif font.

AgroSym
2022

A large, oval-shaped photograph of a sunflower field under a blue sky with light clouds. The sunflowers are in various stages of bloom, with bright yellow petals and dark brown centers. The leaves are green and large.

BOOK OF PROCEEDINGS

*XIII International Scientific Agriculture Symposium
"AGROSYM 2022"
October 6-9, 2022*

The logo for AgroSym 2022 features a green leaf icon above the text "AGRO 2022" in a bold, sans-serif font. Below "AGRO 2022", the word "sym" is written in a smaller, blue, sans-serif font.

AGRO 2022
sym

BOOK OF PROCEEDINGS

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“AGROSYM 2022”**



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Jahorina, October 06-09, 2022, Bosnia and Herzegovina**

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PREFACE

Dear colleagues,

The Faculty of Agriculture of the University of East Sarajevo (Bosnia and Herzegovina), the Faculty of Agriculture of the University of Belgrade (Serbia), and CIHEAM - Mediterranean Agronomic Institute of Bari (CIHEAM Bari, Italy) organized from 6 to 9th October 2022 on Jahorina mountain (East Sarajevo, Bosnia and Herzegovina) the 13th International Scientific Agriculture Symposium “AGROSYM 2022”. The symposium was organized for the third time in a hybrid format, with in-person participation (around 300 participants) and online participation (400 participants), because of the prescribed restrictions caused by the COVID-19 pandemic and the world’s political situation.

AGROSYM 2022 made an important contribution to agriculture science and practice in different topics: plant production, plant protection, animal husbandry, environmental protection, organic farming, agroforestry, agroecology, and rural development. The Symposium topics cover all branches of agriculture as well as forestry and agroforestry. The scientific committee received around 700 papers and after review, it accepted 668 papers, 159 for oral presentations and 509 for poster presentations, which represents over 1500 authors from more than 80 countries worldwide.


During AGROSYM 2022, we had the opportunity to share the results of the current research at the international level and new information relating, inter alia, to biotechnology, world markets and agricultural knowledge and innovation systems in the European Union, especially in plenary papers. Based on many investigations, we can see that practices based on the concept of sustainable agriculture are gaining more and more attention. The goal for sustainable agriculture must be to meet society’s needs, not only the production of goods such as food and fiber but also the maintenance or restoration of ecosystem services such as watershed protection, healthy soil and the biodiversity on which humanity depends.

Big thanks to all members of the scientific committee and the staff from the symposium secretariat for their continued efforts and hard work that made this symposium possible and successful. I should like to thank my colleagues from the organizing committee, particularly the Dean of the Faculty of Agriculture of the University of East Sarajevo, professor Vesna Milic, as a host and chairperson. Special thanks to His Excellence, Prof. Sinisa Berjan, for all he has done to bring this event together; it has been a considerable logistical exercise.

Finally, I would like to thank all the authors, reviewers, session moderators and colleagues for their help in preparing and editing these e-Proceedings. Special thanks go to the organizers, partners and sponsors for their unselfish collaboration and comprehensive support.

Editor in Chief

East Sarajevo, 25 October 2022



Academician Dusan Kovacevic,
Academy of Engineering Sciences of Serbia

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EFFECTS OF BACTERIA AND ENZYME MIXTURE INOCULANTS ON QUALITY OF HIGH-MOISTURE MAIZE GRAIN SILAGE

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Abstract

The objective of this study was to evaluate the effect of applying lactic acid bacteria (LAB) and enzymes mixture inoculants (Sil-All and Silaprilis) on the chemical composition and fermentation of high-moisture grain silage of two maize hybrids Zenit and ZP 735. Maize hybrids were harvested at 68-72% of dry matter. Commercial inoculants were prepared and sprayed following the manufacturer's specifications. Silages were stored in glass jars with a special valve filled with water in the middle of the lid. Significant differences between hybrids were found for ash, crude protein, pH, and acetic acid. The hybrid Zenit had significantly higher ash (14.9 g kg⁻¹ dry matter (DM)), pH (4.03), and acetic acid (6.3 g kg⁻¹ DM), and significantly lower crude protein (89.0 g kg⁻¹ DM) than hybrid ZP 735 (12.5 g kg⁻¹ DM, 3.98, 5.1 g kg⁻¹ DM and 101.2 g kg⁻¹ DM, respectively). Compared to control, LAB+enzymes mixture inoculants stimulated ensiling of high-moisture maize grain. Inoculants decreased the contents of ammonia nitrogen and acetic acid, and pH value, and increased the contents of dry matter, ash, crude protein, crude fat, and lactic acid during silage fermentation than control. Accordingly, the application of LAB+enzymes mixture inoculants is justified and they can be recommended for high-quality silage production in feeding livestock.

Keywords: *grain maize silage, lactic acid bacteria+enzymes mixture inoculants, chemical composition, fermentation parameters.*

Introduction

Maize grain is an important component of livestock feed ingredients due to its availability and nutritional quality. In Serbia, the production of maize is high, with a yield of 7.9 million tons in 2020 (*Statistical Yearbook of the Republic of Serbia, 2021*). The practice is to store dry grains of maize. However, high-moisture maize grain also had a high nutritional value and energy content, is highly digestible for livestock, and doesn't require drying. The maize grain contains high starch content (60-72%) with a ratio of amylose to amylopectin from 20:80 to 30:70. The higher the content of amylopectin makes it favorable for livestock feeding because it is easy to digest (Copeland *et al.*, 2009). It is suitable for silage making and can be used as a supplement for feeding dairy cows and fattening cattle and as a basic raw material (with a proportion of 50 to 70%) for feeding pigs and poultry. Ensiling high-moisture maize grain increases ruminal and total-tract starch digestibility compared with dry maize (Ferraretto *et al.*, 2013) and may increase lactation performance by dairy cows due to the increased surface area for bacterial enzymatic degradation and the breakdown of the hydrophobic starch-protein matrix (Castro *et al.*, 2019).

However, the ensiling of wet grain carries a greater risk of fungal contamination and spoilage compared to other forages because moisture content at harvest may vary leading to a loss of nutrients (Hoffman and Ocker, 1997). For this reason, good ensiling practices are mandatory which includes the use of silage additives (bacterial inoculants, enzymes, and their combinations). The bacterial inoculants reduce pH thereby favoring the growth of aerobic microorganisms and preventing the growth of undesirable aerobic organisms (Zielińska and Fabiszewska, 2018). Also, they reduce dry matter and nutrient loss and improve the aerobic stability in fermented silage (Queiroz *et al.*, 2013). In the world market for improving the quality of maize silage fermentation, silage bacterial inoculants containing homo-fermentative *Lactobacillus plantarum*, *L. acidophilus*, *Pediococcus pentosaceus*, *P. acidilactici*, and *Enterococcus faecium* and/or hetero-fermentative bacteria like *Lactobacillus buchneri* have been developed. It has been proven that the homofermentative inoculants enhance fermentation and improve starch digestibility in feed high-moisture maize (Saylor *et al.*, 2020). *Lactobacillus plantarum* dominates in silage inoculants (Oliveira *et al.*, 2017). These bacteria in silages is being highly competitive with epiphytic lactic acid bacteria decrease pH and increase lactic acid production preventing dry matter and nutritional losses (Guan *et al.*, 2020), but usually reduces the aerobic stability of silage (Silva *et al.*, 2018). Consequently, dual-purpose inoculants were developed containing homofermentative and heterofermentative lactic acid bacteria. However, LAB from inoculants does not have a consistent effect on fiber degradation into fermentable sugars due to which enzymes are added to help bacteria produce lactic acid and render the silage more digestible during feed out (McDonald *et al.*, 1991; Sheperd and Kung, 1996). Enzyme addition to silage inoculants aims to break down forage fiber during fermentation into soluble sugars, and so help bacteria produce lactic acid, which helps to lower silage pH (Ordaz, 2017).

The study aimed to analyse the effects of lactic acid bacteria+enzymes mixture inoculants on the chemical composition and fermentation parameters of high-moisture maize grain silage.

Materials and Methods

The study was carried out at the Institute for Animal Husbandry, Zemun, Serbia in 2019. Maize hybrids Zenit and ZP 735 were harvested at 68-72% of dry matter which is the appropriate point for grain silage. Approximately 100 kg of grains were processed by a hammer mill to a 3-4 mm particle size. Three replicated piles containing approximately 10 kg of samples were prepared for each treatment (total of 24 piles). Samples were sprayed uniformly with a solution of two lactic acid bacteria+enzyme mixture inoculants according to the manufacturer's specifications. Inoculant Sil-All (Alltech, UK) contains *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Pediococcus pentosaceus* and *Propionibacteria acidipropionici* bacteria together with amylase, cellulase, β -glucanase and xylanase enzymes. Inoculant Silaprilis (Timac Agro, USA) contains *Lactobacillus plantarum*, *Pediococcus acidilactici* and *Propionibacteria acidipropionici* bacteria together with β -glucanase and xylanase. Also, control silage (non-inoculated) was included. Silages were stored in glass jars (1.5 l). The jars had a special valve filled with water in the middle of the lid. The valve was served to create an anaerobic fermentation environment. The silos were stored at 22–25°C in dark conditions for a period of 60 days. *After 60 days of ensiling, silage samples were taken for analysis.* The contents of dry matter (DM), ash, crude fat, crude protein, acid (ADF) and neutral detergent fibres (NDF) were determined according to AOAC (2000). The content of cellulose was determined by the Weende method. The content of soluble

nitrogen/total nitrogen was measured by the method of Licitra *et al.* (1996). The content of NH₃-N/total nitrogen was determined by the distillation method using a Kjeltec 1026 analyser. The GC-2014 gas chromatograph (Shimadzu, Kyoto, Japan) was used for the determination of the lactic, acetic, and butyric acids content. The pH data were obtained with a digital HANNA HI 83141 Portable PH Meter.

Analysis of variance (ANOVA) was used to compare datasets, using Statistical software Statistica version 10 (StatSoft, Tulsa, Oklahoma, USA). The randomized complete block analysed the trial with three replicates. Tukey's test ($P \leq 0.05$) was used to compare means.

Results and Discussion

The chemical composition of the maize grain sample before ensiling is shown in Table 1.

Table 1. Chemical composition of wet grain maize sample before ensiling

Parameter	Zenit	ZP 735
Dry matter (DM) (g kg ⁻¹)	680.5	680.9
Ash (g kg ⁻¹ DM)	15.11	13.5
Crude fat (g kg ⁻¹ DM)	54.8	57.3
Crude protein (g kg ⁻¹ DM)	100.0	105.1
Cellulose (g kg ⁻¹ DM)	41.9	41.5
Acid detergent fiber (ADF) (g kg ⁻¹ DM)	39.0	39.5
Neutral detergent fiber (NDF) (g kg ⁻¹ DM)	240.0	246.2

As shown in Tables 2 and 3, the maize hybrids differ considerably in the contents of ash, crude protein and acetic acid, and pH value. Hybrid Zenit was found to have significantly higher contents of ash (14.9 g kg⁻¹ DM) and acetic acid (6.3 g kg⁻¹ DM), pH value (4.03), and significantly lower content of crude protein (89.0 g kg⁻¹ DM) compared to hybrid ZP 735 (12.5 g kg⁻¹ DM, 5.1 g kg⁻¹ DM, 3.98, and 101.2 g kg⁻¹ DM, respectively). So, the chemical composition and fermentation characteristics of wet grain silage high may be affected by the hybrid difference.

The contents of dry matter (582.3 g kg⁻¹ DM), ash (14.9 g kg⁻¹ DM), crude protein (102.6 g kg⁻¹ DM), crude fat (41.0 g kg⁻¹ DM), and lactic acid (72.7 g kg⁻¹ DM) were significantly higher in silage treated with Silaprilis compared to control (561.6 g kg⁻¹ DM, 13.0 g kg⁻¹ DM, 81.4 g kg⁻¹ DM, 38.2 g kg⁻¹ DM, and 67.5 g kg⁻¹ DM). Contrary, the contents of ammonia nitrogen (49.0 g kg⁻¹ DM), acetic acid (5 g kg⁻¹ DM), and pH value (3.97) were significantly lower in silage treated with inoculant Silaprilis compared to control (80.4 g kg⁻¹ DM, 6.1 g kg⁻¹ DM, and 4.04, respectively). Similar conclusions have also been reported by Rafiuddin *et al.* (2021), their study found that the application of Sil-All ® 4X4 (*L. plantarum*, *Enterococcus faecium*, *Pediococcus acidilacti*, and *Lactobacillus salivarius* + α -amylase, cellulase, hemicellulose and pentosanase) could reduce dry matter loss, decrease pH and increase protein content and lactic acid in silage maize.

The silages treated with inoculants, especially with Silaprilis decreased dry matter losses. This indicated good fermentation and preservation of nutrients. Higher ash content indicates that bacteria from inoculants use soluble components and so increase ash content, similarly to Đorđević *et al.* (2020). When ensiling, it is very important to maintain a high level of protein in silages. As evident from the results, the silages treated with inoculants had a higher protein

content compared to the control. This can be related to the fact that the addition of inoculants in silages increased lactic acid fermentation and sugar utilization and reduced pH and proteolytic activity, as evidenced by the research by Abdul Rahman *et al.* (2017). Also, Xing *et al.* (2009) reported that the sharp decline in pH of inoculated silage with inoculant and enzymes was the major reason for reducing the protein degradation which contributes to higher dry matter content.

Table 2. Chemical composition of maize by wet grain silage (untreated silage and silage treated with inoculants).

Factor		DM g kg ⁻¹	Ash g kg ⁻¹ DM	Crude protein g kg ⁻¹ DM	Crude fat g kg ⁻¹ DM	Cellu- lose g kg ⁻¹ DM	NDF g kg ⁻¹ DM	ADF g kg ⁻¹ DM
Hybrid (A)	Zenit	575.5	14.9 ^a	89.0 ^b	39.2	34.1	190.8	33.9
	ZP 735	564.2	12.5 ^b	101.2 ^a	40.7	34.2	198.6	33.3
Inocu- lation (B)	Control	561.6 ^b	13.0 ^b	81.4 ^b	38.2 ^b	34.6	177.1	31.6
	Sil-All	565.7 ^{ab}	13.2 ^{ab}	101.3 ^a	40.6 ^{ab}	33.9	203.1	34.3
	Silaprilis	582.3 ^a	14.9 ^a	102.6 ^a	41.0 ^a	34.0	203.9	34.9
F test	A	ns	**	*	ns	ns	ns	ns
	B	*	**	*	*	ns	ns	ns
	A×B	ns	**	ns	*	*	ns	ns

DM – dry matter; NDF – Neutral detergent fiber; ADF – Acid detergent fiber; Distinct letters in the row indicate significant differences according to Tukey's test ($P \leq 0.05$); ns – non significant; * – significant at 5 % level of probability; ** – significant at 1% level of probability.

Table 3. Fermentation characteristics of maize by wet grain silage (untreated silage and silage treated with inoculants).

Factor		NH ₃ -N g kg ⁻¹ TN	pH	Lactic acid g kg ⁻¹	Acetic acid g kg ⁻¹	Butyric acid g kg ⁻¹
Hybrid (A)	Zenit	69.7	4.03 ^a	69.6	6.3 ^a	0.10
	ZP 735	54.8	3.98 ^b	71.2	5.1 ^b	0.17
Inoculation (B)	Control	80.4 ^a	4.04 ^a	67.5 ^b	6.1 ^a	0.17
	Sil-All	57.4 ^{ab}	4.00 ^b	70.9 ^{ab}	5.0 ^b	0.11
	Silaprilis	49.0 ^b	3.97 ^c	72.7 ^a	5.0 ^b	0.12
F test	A	ns	**	ns	**	ns
	B	*	**	*	*	ns
	A×B	ns	*	ns	*	ns

DM – dry matter; TN – total nitrogen; Distinct letters in the row indicate significant differences according to Tukey's test ($P \leq 0.05$); ns – non significant; * – significant at 5 % level of probability; ** – significant at 1% level of probability.

Crude fat is an important source of energy for animals. Our data showed greater crude fat content in silages treated with inoculants than in control. However, a significant difference was detected between the Silaprilis treatment and control. There were no significant differences among the inoculant treatments in terms of cellulose, NDF, ADF, and butyric acid. Similarly, Ranjit and Kung (2000) found that the silage inoculants have no effect on the ADF and NDF content of maize silage. The content of NH₃-N/total nitrogen ranged from 49.0 g kg⁻¹ (Silaprilis treatment)

to 80.4 g kg⁻¹ DM (control) and was less than 100 g kg⁻¹ which suggests that harmful bacteria were effectively inhibited. The pH of the silage varied from 3.97 to 4.04, indicating that the silages were adequately fermented and achieved good fermentation. Acceptable silages generally contain > 65% lactic acid, <4% acetic acid, and <0.5% butyric acid of dry matter (Shaver, 2003). Based on the results obtained for acids, all silages met good fermentation quality. The greater lactic acid production in the high moisture silage of maize treated with Silaprilis is most likely related to the greater concentration of enzymes in him. A lower concentration of butyric acid showed that the silages has not undergone clostridial fermentation. It is understandable because the spores of *Clostridium* spp do not grow if the pH of silage is 4 or less (Cai *et al.*, 1998).

We observed an interaction between hybrid, and inoculant for ash, crude fat, cellulose, acetic acid, and pH value. The interaction between hybrid and inoculant resulted in a significant increase in ash, crude fat, and cellulose and in a significant decrease in acetic acid, and pH value.

Conclusions

The choice of maize hybrids can affect the quality of high-moisture grain silage. Our results showed that the hybrid ZP 735 had a higher content of crude protein and lower contents of ash and acetic acid, and pH compared to hybrid Zenit. Compared to control, bacteria+enzyme mixture inoculants stimulated ensiling. Inoculants decreased the ammonia nitrogen and acetic acid contents, and pH value, and increased dry matter, ash, crude protein, crude fat, and lactic acid contents, especially Silaprilis. Accordingly, the application is justified and they can be recommended for high-quality silage production.

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