Original Paper

Structural Parameters and Quality of Maize Silage

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Article Details: Received: 2025-06-10 | Accepted: 2025-08-26 | Available online: 2025-12-31

https://doi.org/10.15414/afz.2025.28.04.295-303

The objective of this study was to evaluate the nutritional, fermentative, and structural quality of maize silage treated with a microbial inoculant and to assess how varying particle sizes influence the content of basic nutrients, fiber fractions, and fermentation parameters. Maize silage was prepared using a two-line hybrid (FAO 480) and ensiled with an inoculant containing *Lactobacillus buchneri*, *Pediococcus pentosaceus*, and *Lactobacillus plantarum*. After two months of storage, average samples of silage were collected, mixed, and subsampled for particle size distribution and chemical analysis. The particle size of maize silage was assessed using the Penn State Particle Separator with sieves: >19 mm, 8.1–19 mm, 4.1–8 mm, and <4 mm. Maize silage (n = 3) and each particle fraction (n = 3) underwent chemical analysis. The results revealed that 73.33% of particles were retained on the first two sieves (8.1–19.0 mm and >19.0 mm), considered optimal for dairy cow feeding. Particle size had impact on silage composition. Finer particles (<4 mm) contained significantly higher levels of dry matter, crude fat, starch, organic matter, non-structural carbohydrates, and nitrogen-free extract, and lower contents of crude ash, crude protein, crude fiber, and fiber fractions. Similarly, fermentation quality was affected by particle size: finer fractions had significantly lower levels of lactic acid, acetic acid, formic acid, ethanol, fermentation products, acidity of water extract, pH, and proteolysis. Based on neutral detergent fiber and starch content, the silage met the criteria for first quality class, while its higher acetic acid concentration-linked to the use of *L. buchneri* – classified it as third quality class according to fermentation criteria. The results of this study confirmed that particle size distribution significantly affects the nutritional profile and fermentation dynamics of maize silage.

Keywords: particle size, nutritional indicators, fermentation indicators, structure of the fermented forage

1 Introduction

Maize (*Zea mays* L.) in the form of fermented feed represents a key source of energy in the diets of ruminants (Bíro et al., 2020). The optimal inclusion rate of maize silage in the diet of dairy cows typically ranges from 25% to 75% of the total forage component (Laroche, 2025). An increased proportion of maize silage in TMR enhances dry matter intake (Wang et al., 2023). Whole-plant maize silages show significant differences in the content of structural and non-structural carbohydrates, which influences the overall digestibility of organic matter and the amount of fermentable organic matter available for proteosynthesis (Doležal et al., 2012; Bíro et al., 2020; Mitrík, 2021). From the perspective of dairy cow nutrition, the nutrient content, energy value, fermentation quality, hygienic status, structural quality of silage (particle size and kernel processing), and feed management are all critical factors that significantly influence intake, rumen fermentation processes, general health, milk production, and milk quality (Worku et al., 2021; Havrdová et al., 2023; Mičiaková et al., 2025). Jančík et al. (2022) confirmed a significant effect of corn stover and kernel processing by the Shredlage technology on increasing the *in vivo* digestibility of dry matter, starch, crude fiber, organic matter, and NDF, leading to higher NEL values and thus a higher nutritional value for ruminants with the potential for improved production. Shredlage technology can significantly improve starch digestibility through the specialized treatment of spiral-groove rollers. Grain disruption is the most important factor affecting starch digestibility in corn silages (Saylor et al., 2021). Effective disruption of corn grains using Shredlage

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crushing rollers increases starch digestibility throughout the digestive tract (Bal et al., 2000). Ebling and Kung (2004) also found higher starch digestibility in dairy cows fed better-processed corn silages.The inclusion of maize Shredlage in TMR for dairy cows, compared to conventionally processed maize silage, resulted in a significant increase in dry matter intake and tended to increase milk fat yield and 4% fat-corrected milk (Joch et al., 2023). Fine chopping of forage is associated with alterations in rumen fermentation parameters. Feeding short corn silage in TMR increases yields of milk, protein, and lactose, but also leads to a decrease in rumen pH, indicating a higher risk of sub-acute ruminal acidosis (SARA). Additionally, cows consuming finely chopped forages exhibited changes in feed preference, selecting longer particles during SARA (Kmicikewycz and Heinrichs, 2015). While reducing forage particle size improves dry matter intake and milk yield, excessively long particles can have the opposite effect, resulting in reduced intake and performance (Nasrollahi et al., 2015; Manzocchi et al., 2020). The rumen functions as a continuous fermentation chamber, where the stabilization of hydrogen ion concentrations relies on effective salivary buffering. This buffering capacity is largely maintained through the intake of sufficient levels of physically effective neutral detergent fiber and appropriate rumination time (Hossain, 2021). Long dietary fiber particles stimulate chewing activity and salivary secretion, contributing to the maintenance of reticulorumen buffering capacity (Natnael et al., 2020; Kahyani et al., 2022). The effects of forage particle size are influenced by multiple factors, including forage type, the forage-to-concentrate ratio, and the fermentability characteristics of organic matter within the diet. For practical dairy ration formulation, an optimal forage particle length generally ranges from 8 to 19 mm, as measured using the Penn State Particle Separator, regardless of forage type (Hossain, 2021). The aim of this study was to determine the nutritional, fermentative, and structural quality of maize silage with the addition of inoculant, and subsequently assess the impact of different physical structures on the content of basic nutrients, fiber complex fractions, and fermentation parameters of maize silage.

2 Material and Methods

2.1 Ensiling, Separation, Sample Collection

The experiment was conducted in cooperation with the Agricultural Cooperative Kozárovce situated in West part of Slovakia. In this farm, a two-line maize hybrid with FAO number 480 characterized by a stay-green maturity, and grain type dent was ensiled in mid-August 2024. Maize planting parameters were as follows: sowing date on 29.04.2024 on an area of 49.93 ha, seeding density 75,000 plants.ha^{-1}, row spacing 75 cm, plant spacing in the row 17.80–18.00 cm, resulting in approximately 7.5–8.0 plants.m^{-2}. Maize was harvested at the 2/3 milk line stage using a self-propelled forage harvester KRONE BIG X with processing rollers. The material was chopped to a theoretical particle length of 15–20 mm, and an inoculant was applied at 1 g per tonne using nozzles and an applicator. The inoculant contained obligately heterofermentative LAB *L. buchneri*, facultatively heterofermentative LAB *P. pentosaceus*, and *L. plantarum*, at a minimum concentration of 3.0×10^{11} CFU.g^{-1}. The silage was stored in a recessed, non-drive-through, and roofless silo pit, covered with a thin underlay film and a multilayer cover film (milk-white from the outside), weighted with tires. After two months, partial samples were taken from the top layer of the silage mass from several spots. After mixing and splitting, final samples were taken for particle separation and chemical analysis. The particle size structure of maize silage from Agricultural Cooperative Kozárovce was evaluated using the Penn State Particle Separator according to Heinrichs and Jones (2022), with sieves corresponding to: first sieve: >19 mm, second sieve: 8.1–19 mm, third sieve: 4.1–8 mm, bottom pan: <4 mm. The maize silage samples (n = 3) and samples from each particle fraction (n = 3) were subjected to chemical analysis.

2.2 Analyzed Parameters

Sample pre-drying and determination of organic and inorganic nutrients were conducted according to Commission Regulation No. 152/2009.

Nutrient parameters:

- DM (dry matter): drying at 103 ±2 °C.
- CP (crude protein): Kjeldahl method.
- CF (crude fat): Soxhlet-Henkel extraction.
- CFI (crude fiber): Hennenberg-Stohmann method
- CA (crude ash): combustion at 530 ±20 °C in a muffle furnace.
- ST (starch): polarimetric method.
- NSC (non-structural carbohydrates): calculated as DM – (CP + CF + CA + NDF).
- NFE (nitrogen-free extract): calculated as DM – (CP + CF + CA + CFI).
- OM (organic matter): calculated as DM – CA.
- ADF (acid detergent fiber): hydrolysis in an acid detergent solution.
- NDF (neutral detergent fiber): hydrolysis in a neutral detergent solution.
- ADL (acid detergent lignin): post-hydrolysis of ADF with 72% H_2SO_4 for 3 hours.
- CEL (cellulose): calculated as NDF – ADF.
- HEMI (hemicellulose): calculated as ADF – ADL.

- All nutrient parameters are expresed in g.kg^{-1} DM, only DM content is in g.kg^{-1} original matter.
- peNDF >8 (physically effective NDF in %): calculated as (NDF content × proportion retained on the first and second sieve)/100.

Fermentation parameters:
- LA (lactic acid), AA: (acetic acid), BA (butyric acid), FA (formic acid): isotachophoresis.
- AWE (acidity of water extract): alkalimetric titration to pH 8.5.
- pH: electrometrically using a pH meter.
- ALC (alcohols): microdiffusion using Conway dishes.
- FP (fermentation products): calculated as volatile fatty acids+ LA + ALC.
- NH_3 (ammonia): microdiffusion using Conway dishes.
- DP (degree of proteolysis): NH_3-N / total Kjeldahl N × 100.
- All fermentation parameters are expresed in g.kg^{-1} DM, only DP is in %, and AWE is in mg KOH.100 g^{-1} silage.

2.3 Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics version 26.0. Descriptive statistics were obtained via One-way ANOVA, and differences between groups were evaluated using independent samples *T*-test.

3 Results and Discussion

According to Natnael et al. (2020), the physical effectiveness of fiber is primarily assessed based on the fiber's physical properties to stimulate rumination and salivary buffering. Therefore, the concept of physically effective fiber is important and can be expressed as the extent to which the fiber's physical structure stimulates chewing and saliva production necessary for buffering, while also contributing to the formation of the floating mat of large particles in the rumen. The optimal balance between physically effective fiber and easily degradable carbohydrates in the ration is critical not only for maintaining proper rumen function but also for stable health and increased milk production. By observing structural indicators (Table 1), it was found that the structure of maize silage (MS) differed slightly from the recommendations by Heinrichs and Jones (2022), although the desired proportion on the first two sieves 48–73% was only slightly exceeded in the analyzed MS, with a value of 73.33%.

According to Bíro et al. (2020), the optimal dry matter (DM) content range for maize silage is 300.00–350.00 g.kg^{-1} of original matter. Doležal et al. (2012) recommend a slightly broader range of 280.00–350.00 g.kg^{-1} of original matter, while Mitrík (2021) considers 300.00 g.kg^{-1} of original matter as the minimum acceptable threshold. The analyzed maize silage from silo pit had a DM content of 330.10 g.kg^{-1} of original matter (Table 2), which was consistent with the previous recommendations. The dry matter content increased linearly (P <0.05) as particle size decreased. According to Beauchemin (2018), higher dry matter content in finer particles may contribute to better nutrient utilization during animal feeding, as such material typically has greater energy value and improved digestibility. This indicates that optimal silage quality requires consideration of proper particle size during its production and storage. Maize silage typically contains low levels of crude protein (CP) (Khan et al., 2015), which may reduce the activity of fermentative bacteria if an insufficient amount is present, thereby affecting lowering digestibility. The crude protein value is frequently used as a quality indicator, but not for silages of carbohydrate (energy) nature. Seasonal and maturity stage factors influence crude protein concentration; in colder months, forages tend to have higher crude protein concentrations compared to those harvested in warmer periods. The crude protein content in maize silage decreased linearly with decreasing particle size. The <4.0 mm fraction had the lowest CP content, which was statistically significant (P <0.05) compared to the other fractions. The crude fat (CF) in maize silage refers to the total lipid content, including triglycerides and other fat-soluble substances. It is a key parameter for assessing the energy value of silage, as fat includes nutritionally important components. The crude fat in maize silage is typically measured as the rest from ether extract, which includes fat-soluble substances such as triglycerides, phospholipids, and fat-soluble vitamins (Khan et al., 2015). According to Ferreira and Mertens (2005), CF content in maize silage can vary depending on harvest maturity, maize variety, and environmental

Table 1 Physical structure of maize silage from silo pit and comparison with the recommendation according to Henrichs and Jones (2022)

n = 3	Particle size			
	>19.0 mm	8.1–19.0 mm	4.1–8.0 mm	<4.0 mm
Maize silage	2.22 ±0.32%	71.11 ±2.03%	13.18 ±1.11%	13.49 ±1.14%
Henrichs and Jones (2022)	3–8%	45–65%	20–30%	<10%

conditions. Silage produced from maize harvested at an earlier maturity stage typically has higher CF content, as younger kernels contain more lipids. Although fat contributes only partially to the total energy value of silage, it plays a key role in providing essential fatty acids and fat-soluble vitamins. The <4.0 mm particle fraction had the highest CF content, which was statistically significant (*P* <0.05) compared to the other fractions. Grant (2019) states that the proportion of different plant parts in the silage determines the amount of crude fiber, starch, and crude protein. Maize silage typically contains low CP and provides fermentable starch (energy), along with varying levels of physically effective fiber depending on particle size. Crude fiber (CFI) negatively affects nutritional value due to its lower digestibility compared to starch. The <4.0 mm particle fraction had the lowest CFI content, which was statistically significant (*P* <0.05). The crude ash (CA) includes all inorganic matter (minerals) in the feed, as well as potential contaminants such as soil or sand. This indicator is important for assessing the purity and quality of the feed, as high CA content may indicate contamination or a lower-quality of organic fraction (Grant, 2019). The CA content decreased linearly (*P* <0.05) as particle size decreased in the maize silage. Non-structural carbohydrates (NSC) are an important energy component of maize (Wu et al., 2019). Analysis of maize silage fractions showed that NSC content increased linearly (*P* <0.05) with decreasing particle size. The <4.0 mm fraction had more than double the NSC content compared to the >19 mm fraction. The nitrogen-free extract (NFE) represents the proportion and content of soluble carbohydrates in the feed. Nitrogen-free extract is considered important because it consists of readily available carbohydrates, mainly sugars and starch, which provide energy to the animal. It is a crucial factor in assessing the energy value of feed and silage. The NFE is especially useful for estimating digestible energy in ruminant nutrition, where fermentable carbohydrates in the rumen can be used by microorganisms as an energy source (Grant et al., 2020). The <4.0 mm particle fraction had the highest NFE content, which was statistically significant (*P* <0.05). Organic matter (OM) includes carbohydrates (such as starch and fiber), proteins, fats, and other biologically active compounds present in the maize plant. Organic matter in maize silage provides the main nutrients and energy required by ruminants, particularly dairy cows, to maintain production and health. Organic matter plays an important role in supplying energy through carbohydrate content – especially starch, which is a key energy source for ruminants. The higher the OM content, the more energy and nutrients are available to the animals. The quality of OM based on its digestibility and fermentability affects the overall efficiency of silage in ruminant rations (Grant et al., 2022). Organic matter content increased linearly (*P* <0.05) as particle size decreased in the maize silage. According to Haselmann et al. (2019), maize kernels primarily contain starch (ST), which makes up approximately 75% of the dry matter of the kernel and serves as the primary energy source. The nutritional value of maize silage largely depends on the content and degree of starch degradation. The maize endosperm contains more than 85% starch, which is a significant substrate for rumen fermentation, leading to the production of propionic acid-important energy source. In whole-plant maize silage, typical ST content ranges from 25 to 30% of DM. Increasing ST content and its digestibility enhances milk production in dairy cows fed predominantly maize silage. Starch digestibility is strongly influenced by maturity stage, kernel processing, and storage duration, and correlates with nitrogen content. The average ST content of the maize silage

Table 2 The effect of different physical structures of maize silage on the content of basic nutrient

n = 3	Particle size				MS from silo pit
	>19.0 mm	8.1–9.0 mm	4.1–8.0 mm	<4.0 mm	
DM*	284.10 ±0.07[a]	316.53 ±0.02[b]	342.40 ±0.42[c]	408.13 ±0.02[d]	330.10 ±0.42
CP**	70.48 ±1.08[a]	67.18 ±0.25[a]	66.39 ±0.33[a]	63.85 ±0.05[b]	69.31 ±0.75
CF**	19.09 ±0.34[a]	18.98 ±0.06[a]	24.64 ±0.30[b]	26.83 ±0.02[c]	21.40 ±0.16
CFI**	194.81 ±1.92[a]	209.73 ±4.33[a]	166.67 ±1.96[b]	72.47 ±0.86[c]	154.51 ±3.18
CA**	48.87 ±0.22[a]	46.19 ±0.27[b]	42.31 ±0.02[c]	33.43 ±0.19[d]	42.37 ±0.78
NSC**	119.13 ±0.93[a]	132.95 ±0.18[b]	180.82 ±0.31[c]	282.67 ±0.11[d]	173.69 ±0.64
NFE**	666.76 ±3.56[a]	657.94 ±4.29[a]	700.01 ±1.97[b]	803.46 ±0.62[c]	712.42 ±1.82
OM**	951.14 ±0.22[a]	953.81 ±0.27[b]	957.70 ±0.02[c]	966.58 ±0.19[d]	957.63 ±0.78
ST**	141.61 ±0.84[a]	172.49 ±0.16[b]	145.02 ±0.81[c]	446.73 ±1.44[d]	281.40 ±2.69

DM – dry matter; CP – crude protein; CF – crude fat; CFI – crude fiber; CA – crude ash; NSC – non-structural carbohydrates; NFE – nitrogen-free extract; OM – organic matter; ST – starch; *$g.kg^{-1}$ of original matter; **$g.kg^{-1}$ of dry matter; MS – maize silage; values with the different index in row means *P* <0.05

was 281.40 g per kilogram of dry matter, thus meeting the requirement for first quality class (≥275.00 g.kg^{-1} DM) according to Mitrík (2021). In maize silages, according to Heuzé et al. (2017), starch content ranges from 168.00 to 406.00 g.kg^{-1} DM (average 291.00 g). The mean starch value in the analyzed maize silage was comparable with the findings of Heuzé et al. (2017). Rajčáková et al. (2013) reported ST values of 314.30 g.kg^{-1} DM in maize silage samples from Slovakia with a DM content of 349.40 g.kg^{-1} original matter. The <4.0 mm fraction had the highest ST content, which was statistically significant (P <0.05).

According to Hristov et al. (2020), acid detergent fiber (ADF) consists mainly of cellulose, lignin, and a fraction of crude protein bound to the ADF matrix. This fiber fraction is closely associated with the indigestibility of forages and represents an important indicator when calculating the energy value of feed. The higher the ADF content, the lower the digestibility of the feed and the less metabolizable energy it contains. High ADF values negatively affect the nutritional value of the feed, reduce dry matter intake, and limit overall nutrient utilization. Therefore, monitoring ADF is especially important when formulating rations for dairy cows and fattening animals. According to Heuzé et al. (2017), the average ADF content in maize silages with dry matter content between 30–35% was 233.00 g.kg^{-1} DM, with a minimum of 186.00 g and a maximum of 332.00 g. The analyzed maize silage had a lower ADF value of 177.57 g.kg^{-1} DM (Table 3). The <4.0 mm particle fraction had the lowest ADF content, which was statistically significant (P <0.05). Chewing time is strongly influenced by the intake of neutral detergent fiber (NDF), particle size in the ration, and forage fragility (hardness). Complex interactions exist among these factors, and the correlation between chewing time and individual ration components is generally low to moderate. Chewing time is more strongly correlated with NDF intake than with the NDF content in the ration. The correlation between chewing time and forage NDF content in total mixed rations was r = 0.19 (P <0.05) (Beauchemin, 2018). The NDF content in the maize silage from the silo pit was 340.76 g.kg^{-1} DM, fulfilling the criterion for first quality class (≤380.00 g.kg^{-1} DM) according to Mitrík (2021). Based on its basic nutritional quality, the content of neutral detergent fiber and starch, the maize silage from the silo pit was classified as first quality class (Mitrík, 2021). The <4.0 mm particle fraction had a statistically significantly the lowest NDF content (P <0.05). The physically effective fiber (peNDF >8) value of the maize silage from the silo pit was 24.99%. According to Nasrollahi et al. (2015), the minimum peNDF >8 value in total mixed rations for dairy cows should be greater than 18.00%. Acid detergent lignin (ADL) is a polymeric component of plant cell walls that provides structural rigidity and support to the plant. The ADL is not digestible by animal enzymes. Its content increases with plant maturity and tends to be higher in plants grown in warmer climates. The higher the ADL content in forages, the lower the digestibility of neutral detergent fiber, which means the feed will be less digestible and provide less usable energy (Haselmann et al., 2019). The maize silage sample from the silo pit had ADL value of 14.70 g.kg^{-1} DM, which was lower than the range reported by Pastierik et al. (2014), who noted values from 16.80 to 32.10 g.kg^{-1} DM. The <4.0 mm particle fraction had the lowest ADL content, which was statistically significant (P <0.05). Nikolić et al. (2020) reported cellulose (CEL) content ranging from 178.60 to 209.20 g.kg^{-1} DM in various maize hybrids. The maize silage from the silo pit had a lower CEL content (162.87 g.kg^{-1} DM) in comparison with Nikolić et al. (2020). Cellulose content decreased linearly (P <0.05) with decreasing particle size of the maize silage. Hemicelluloses (HEMI) are a group of polysaccharides found in plant cell walls. They are heteropolysaccharides composed of various sugars, including pentoses (e.g., arabinose, xylose) and hexoses (e.g., mannose, galactose). Hemicelluloses differ from cellulose in their structure, being less ordered and having lower molecular weight (Ning et al., 2016). According to Nikolić et al. (2022), hemicelluloses are present alongside cellulose and lignin in plants and form an important fiber component

Table 3 The effect of different physical structures of maize silage on fiber fraction

n = 3	Particle size				MS from silo pit
	>19.0 mm	8.1–19.0 mm	4.1–8.0 mm	<4.0 mm	
ADF*	225.43 ±0.71^{a}	225.89 ±3.01^{a}	182.23 ±1.13^{b}	94.59 ±1.82^{c}	177.57 ±2.44
NDF*	442.18 ±1.75^{a}	447.61 ±0.53^{a}	338.57 ±1.55^{b}	183.28 ±0.04^{c}	340.76 ±2.60
ADL*	14.94 ±0.12^{a}	20.29 ±2.07^{a}	17.72 ±0.31^{b}	10.16 ±0.46^{c}	14.70 ±0.42
CEL*	210.49 ±0.58^{a}	205.60 ±0.30^{b}	164.51 ±0.82^{c}	84.43 ±2.28^{d}	162.87 ±2.84
HEMI*	216.75 ±2.46^{a}	221.72 ±2.48^{a}	156.34 ±2.68^{b}	88.70 ±1.79^{c}	163.20 ±0.16
peNDF >8 of MS from silo pit (%)					24.99

ADF – acid detergent fiber; NDF – neutral detergent fiber; ADL – acid detergent lignin; CEL – cellulose; HEMI – hemicellulose; peNDF >8 – physically effective NDF; *g.kg^{-1} of dry matter; MS – maize silage; values with the different index in row means P <0.05

(a fraction of NDF) in animal feeds. After acid detergent treatment, HEMI are solubilized, while cellulose and lignin remain in the residue as the acid detergent fiber content. Hemicelluloses also play an important role in digestion, as they are partially degraded in the rumen, allowing for the production of fermentation products such as acetate and butyrate, which serve as energy sources for ruminants. The <4.0 mm particle fraction had the lowest HEMI content, which was statistically significant (*P* <0.05).

A higher concentration of lactic acid ensures better fermentation progress and higher silage quality (Karnatam et al., 2023). The maize silage from the silo pit had a lactic acid (LA) content of 15.25 g.kg^{-1} of original matter. The lactic acid content decreased with decreasing particle size. The <4.0 mm fraction had a statistically significantly lower LA content (*P* <0.05) compared to the 8.1–19.0 mm and >19.0 mm fractions (Table 4). In dry matter, the LA content in the maize silage from the silo pit was 46.18 g, and it decreased linearly (*P* <0.05) with decreasing particle size. Acetic acid (AA) also plays an important role in silage stabilization, particularly at the beginning of fermentation or when the silage is exposed to air (increases aerobic stability) (Karnatam et al., 2023). However, high concentrations of AA may indicate silage quality issues, such as excessively high moisture content or insufficient compaction during ensiling. While AA is important for silage stability, its excessive concentration can have a negative effect on dry matter intake. Silages with high AA content may lead to reduced dry matter intake because acetic acid can cause undesirable taste or irritate the animal's digestive tract. The AA content in the maize silage from the silo pit was 41.93 g.kg^{-1} DM, which was the result of the additive applied, containing *Lactobacillus buchneri*. *L. buchneri*, as an obligately heterofermentative lactic acid bacterium, has been shown to significantly increase acetic acid concentrations, as confirmed by the study of Juráček et al. (2024). According to Mitrík (2021), the threshold for AA content is ≤30.00 g.kg^{-1} DM for the first quality class, 30.01–40.00 g for the second, and 40.01–50.00 g for the third quality class. The <4.0 mm fraction had a statistically significantly the lowest acetic acid content (*P* <0.05). The lactic acid to acetic acid ratio in the maize silage from the silo pit was 1.10 : 1. Among the individual particle size fractions, the ratio remained relatively balanced, ranging from 1.08 : 1 to 1.13 : 1. According to Kitaw et al. (2024), silages containing butyric acid (BA) typically exhibit a characteristic rancid butter odor, often have an olive-green coloration, and possess poor palatability for animals. This results in reduced feed intake, increased DM losses, and elevated ammonia content. Although butyric acid by itself may not drastically reduce intake, its presence is associated with protein degradation and the formation of compounds such as ammonia, biogenic amines, and amides, which can negatively affect animal health and productivity. Butyric acid was not detected in the analyzed silage samples. According to Mitrík (2021), the threshold for the first quality class for butyric acid is ≤3.00 g.kg^{-1} DM. Formic acid (FA) may contribute to silage pH regulation and stabilization of the fermentation process, particularly in silages with high sugar content or those prone to excessive gas production. Formic acid may inhibit the growth of undesirable microorganisms such as Clostridia, which produce butyric acid. Although FA has a positive impact on the fermentation process, its high concentrations can negatively affect the palatability of the silage and animal health (Serva, 2024). The FA content in the <4.0 mm fraction was statistically significantly the lowest (*P* <0.05). The maize silage from the silo pit was classified as acidic, with an average acidity of water extract (AWE) value of 1952.50 mg KOH.100 g^{-1} silage. According to Doležal et al. (2012), the reference value for AWE in maize silages ranges from 1,200.00 to 1,600.00 mg KOH.100 g^{-1} silage. The AWE values of the individual fractions exceeded 1600.00 mg KOH.100 g^{-1} silage, and they decreased linearly (*P* <0.05) with decreasing particle size. According to Smith (2019), the pH value of maize silage is one of the most important indicators of fermentation quality. Optimal pH values for well-fermented maize silage range between 3.7 and 4.2. Such values indicate successful fermentation. If the pH exceeds 4.2, this may suggest the presence of undesirable microorganisms, such as Clostridia, which degrade proteins and produce BA and ammonia, thereby reducing silage stability, nutritional value, and intake. Conversely, very low pH may result from excessive production of organic acids, especially in silages with high sugar content. Maintaining pH within the optimal range is essential for silage stability, palatability, and nutritional value (Karnatam et al., 2023). The pH value in the maize silage from the silo pit was 3.84, fulfilling the requirement for the first quality class (≤4.55) at the given dry matter content according to Mitrík (2021). The <4.0 mm particle fraction had the lowest pH value, which was statistically significant (*P* <0.05). According to Bíro et al. (2020), alcohol in silage (ALC) is a product of the activity of heterofermentative *lactic acid bacteria*, but it is also a byproduct of sugar fermentation by yeasts, which may be present during silage ensiling or storage. The most common alcohol is ethanol, which is the dominant volatile compound in maize silage. The presence of alcohol is not necessarily problematic, but high ethanol concentrations can reduce silage intake in animals and negatively affect milk flavor and aroma (Zurak et al., 2018). The maize silage from the silo pit had ALC content of 7.68 g per kilogram of dry matter. In the finest particle fraction (<4.0 mm), the alcohol

Table 4 The effect of different physical structures of maize silage on fermentation parameters

n = 3	Particle size				MS from silo pit
	>19.0 mm	8.1-19.0 mm	4.1-8.0 mm	<4.0 mm	
LA*	17.49 ±0.18[a]	17.06 ±0.28[ab]	16.54 ±0.11[bc]	15.17 ±0.07[c]	15.25 ±0.18
LA**	61.56 ±0.64[a]	53.90 ±0.89[b]	48.29 ±0.37[c]	37.17 ±0.17[d]	46.18 ±0.59
AA**	54.59 ±1.68[a]	49.72 ±1.27[b]	44.04 ±1.19[c]	33.34 ±0.61[d]	41.93 ±2.11
LA/AA	1.13 : 1	1.08 : 1	1.10 : 1	1.11 : 1	1.10 : 1
BA**	ND	ND	ND	ND	ND
FA**	4.65 ±0.01[a]	4.55 ±0.04[a]	3.83 ±0.5[b]	2.99 ±0.01[c]	3.97 ±0.05
AWE***	2187.00 ±14.14[a]	2107.00 ±4.24[b]	2054.25 ±0.21[c]	1865.50 ±3.54[d]	1952.50 ±7.78
pH	3.84 ±0.01[ab]	3.84 ±0.01[a]	3.79 ±0.01[b]	3.81 ±0.01[c]	3.84 ±0.01
ALC**	5.27 ±0.51[a]	6.37 ±0.25[a]	2.00 ±0.04[b]	1.98 ±0.70[bc]	7.68 ±1.00
FP**	126.03 ±2.90[a]	114.54 ±2.48[b]	97.51 ±1.72[c]	75.48 ±1.24[d]	99.76 ±3.75
DP (%)	4.57 ±0.07[a]	4.70 ±0.02[a]	3.37 ±0.01[b]	3.18 ±0.01[c]	3.92 ±0.04

LA – lactic acid; AA – acetic acid; BA – butyric acid; FA – formic acid; AWE – acidity of water extract; ALC – alcohols; FP – fermentation products; DP – degree of proteolysis; *g.kg^{-1} of original matter; **g.kg^{-1} of dry matter; ***mg KOH.100 g^{-1} silage; MS – maize silage; ND – not detected, values with the different index in row means P <0.05

content was significantly lower (P <0.05) compared to the 8.1–19.0 mm and >19.0 mm fractions. The content of fermentation products (FP) decreased linearly (P <0.05) with decreasing particle size. The analysis of maize silage fractions confirmed that increasing dry matter content was associated with decreasing concentrations of FP. The degree of proteolysis (DP) in maize silage is generally lower than in alfalfa silages. This is due to maize's lower buffering capacity, lower initial nitrogen content, and higher levels of easily fermentable sugars, which promote rapid pH decline and improve fermentation stability. Rapid acidification helps to inhibit plant protease and microbial activity, thereby limiting protein breakdown (Kitaw et al., 2024). The degree of proteolysis in the maize silage from the silo pit was 3.92%. The <4.0 mm particle fraction had the lowest value, which was statistically significant (P <0.05). According to the fermentation indicators defined by Mitrík (2021) namely, the contents of butyric and acetic acid and the pH value – the maize silage from the silo pit was classified in the third quality class, due to the elevated concentration of acetic acid.

Conclusions

In terms of nutrient parameters, the maize silage with the addition of an inoculant was classified as first quality class based on its content of neutral detergent fiber and starch. According to fermentation indicators, the maize silage exhibited a higher concentration of acetic acid, thus meeting the criteria for the third quality class. The elevated acetic acid concentration was related to the composition of the applied silage additive, which contained *Lactobacillus buchneri*. Analysis of the physical structure of the maize silage confirmed that particles larger than 19 mm accounted for 2.22%, particles sized 8.1 to 19 mm accounted for 71.11%, particles sized 4.1 to 8 mm represented 13.18%, and particles ≤4 mm made up 13.49%. The proportion of particles retained on the first two sieves was 73.33%, which can be considered optimal for the utilization of maize silage in dairy cow nutrition. The findings of this study confirm that particle size distribution exerts a statistically significant influence on both the nutritional profile and fermentation parameters of maize silage. Finer particles (<4 mm) exhibited elevated concentrations of dry matter, starch, crude fat, non-structural carbohydrates, nitrogen-free extract, and organic matter, alongside reduced crude protein, crude ash crude fiber and its fractions. These compositional shifts underscore the relationship between particle size reduction and the accumulation of energy-dense components, which may enhance the silage's energy value but simultaneously compromise its role as a source of physically effective fiber. Fermentation parameters displayed similar particle-dependent trends, with finer fraction contain significantly lower concentrations of lactic, acetic, formic acid, pH, fermentation products, and alcohols, alongside reduced proteolysis, and acidity of water extract.

Acknowledgement

Thanks for the cooperation to the Agricultural Cooperative Kozárovce.

The article was addressed within the project VEGA no. 1/0321/23: Effective management of the ruminant nutrition using a modern monitoring of the internal environment.

Conflict of Interest

The authors declare that there is no conflict of interest.

Authors Contribution

Miroslav Juráček – results and discussion, Daniel Bíro – methodic supervisor, Petr Doležal – manuscript conceptualization, Milan Šimko – final manuscript preparation, Branislav Gálik – technical support, Michal Rolinec – data processing, Ondrej Hanušovský – statistical evaluation, Matúš Džima – sample preparation, Mária Kapusniaková – laboratory analysis, Michal Radošovský – data collection

AI and AI-Assisted Technologies Use Declaration

No generative AI tools/AI-assisted technologies were used during the preparation of the manuscript.

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