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Effect of Forage Processor Roll Gap Width and Storage Length on Fermentation Profile, Nutrient Composition, Kernel Processing Score, and Starch Disappearance of Whole-Plant Maize Silage Harvested at Three Different Maturities

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Abstract: Our objective was to assess the effect of forage processor roll gap width and storage length on fermentation, nutrient composition, kernel processing score (KPS), and ruminal in situ starch disappearance (isSD) of whole-plant maize silage harvested at different maturities. Samples from a single maize silage hybrid at three harvest maturities (1/4, 1/2, and 3/4 kernel milk line (early, intermediate, and late, respectively)) processed with two roll gap widths (1 and 3 mm) were collected and stored in quadruplicate vacuum pouches for 0, 30, 120, or 240 d. Lactic acid concentrations were greater, and pH was reduced in early and intermediate maturity silage compared to late maturity silage. Ruminal isSD was greatest for early maturity silage, intermediate for the intermediate maturity silage, and lowest for the late maturity silage, but differences in isSD due to maturity were diminished after prolonged storage. Kernel processing score was greatest in late maturity silage processed through a 1 mm roll gap and lowest in late maturity silage processed through the 3 mm roll gap. For early and intermediate maturity silages, no differences in KPS were observed between the two roll gap widths. Minimal effects of maturity and roll gap width on fatty acids (FA) and amino acids (AA) were observed. Concentrations of total AA decreased as storage length progressed. Results support the premise that the silo is a dynamic system that undergoes numerous chemical changes throughout the storage period.

Keywords: maize silage; maturity; roll gap; storage length; kernel processing; fatty acids; amino acids

1. Introduction

Whole-plant maize silage (WPMS) is a vital forage source for the dairy industry in the United States, with approximately 125 million Mg of maize harvested for silage in 2020 [1]. Its reduced harvesting costs, elevated yield per area, and the flexibility to harvest maize for forage or grain have contributed to the popularity of WPMS among dairy producers. In many high-producing dairy herds, up to half of the total mixed ration (TMR) DM can be comprised of WPMS. Given its high inclusion in dairy diets, having a thorough understanding of the nutrients that WPMS supplies, as well as the factors that affect the concentrations and availability of those nutrients, is imperative.

Whole-plant maize silage is nutritionally valuable in that it is simultaneously a source of physically effective fiber (provided by the stover fraction) and energy (primarily from

starch in the kernel fraction) for the dairy cow. Starch digestibility of WPMS can be influenced by several factors, with kernel breakage being the most important [2,3]. The endosperm in maize kernels is protected by the pericarp, which is highly resistant to microbial and enzymatic degradation [4]. Therefore, breaking the pericarp is necessary to improve starch digestibility. Many self-propelled forage harvesters are fitted with processing rolls intended to break maize kernels, thereby exposing starch in the endosperm to microbial degradation in the rumen or enzymatic digestion in the small intestine. The gap width between processing rolls can be adjusted to increase or decrease the aggressiveness by which kernels are processed. Reducing the roll gap width has been shown in a meta-analytical review of the literature to increase total tract starch digestibility [5]. Ferreira and Mertens [6] developed a maize silage fragmentation index (kernel processing score; KPS) that can be used to determine the effectiveness of processing rolls at harvest. Even when the pericarp is successfully broken, however, ruminal starch degradation can be inhibited by the hydrophobic zein protein matrix that surrounds starch granules [7,8]. Increasing the length of storage of WPMS beyond 30 and up to 240 d has been shown to facilitate the breakdown of this protein matrix and increase 7 h ruminal in vitro or in situ starch degradability [9]. Plant maturity at harvest, however, has been shown to affect both the concentration of zein proteins in maize kernels and the ability of kernel processing to improve starch digestibility [3,5,10]. Plant maturity is also known to affect starch concentrations and fermentation of WPMS [9].

In addition to fiber and starch, WPMS is a significant source of other nutrients, many of which are often overlooked in silage research. Maize silage is an important source of long-chain, unsaturated fatty acids (FA), particularly oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids [11]. Although total FA concentrations in WPMS are relatively low, its high inclusion rate in dairy cow diets makes WPMS a substantial contributor to total unsaturated FA intake [12]. Similarly, concentrations of CP in WPMS are known to be relatively low, averaging around 8% of DM [13]. However, WPMS becomes a significant source of amino acids (AA) when it is included at high rates in the diet. Unfortunately, studies evaluating the factors that influence FA and AA profiles in WPMS are limited, especially over multiple storage lengths. Given the profound effect that plant and management factors can have on the quality and digestibility of WPMS, the objective of this study was to evaluate the effect of forage harvester roll gap width and length of storage on the fermentation profile, nutrient composition, KPS, and in situ starch disappearance of whole-plant maize silage harvested at three different maturities. We hypothesized that plant maturity and roll gap width would affect KPS and starch disappearance but that these effects would be reduced after prolonged ensiling.

2. Materials and Methods

2.1. Silage Production and Treatments

Whole-plant maize of a single maize silage hybrid (1024VIP, AgraTech Seeds, Inc., Atlanta, GA, USA) was harvested at three maturities, 1/4 (early), 1/2 (intermediate), and 3/4 (late) kernel milk line, from the University of Florida Plant Science Research and Education Unit (Citra, FL, USA). The three harvests occurred on 27 June, 5 July, and 12 July 2018 for the early, intermediate, and late maturity silages, respectively. Four whole-plant maize samples were collected at the time of harvest from four random locations within the same field to correspond with four replicates per treatment. Maize plants were left standing within each of these four locations to ensure all three maturities had samples collected from the same place. Whole-plant maize samples were processed with a self-propelled forage harvester (Claas of America LLC, Columbus, IN, USA) set with a theoretical length of cut (TLOC) of 22 mm and equipped with an onboard kernel processor (MCC Cracker, Claas of America LLC, Columbus, IN, USA) with a gap width of either 1 or 3 mm. Two subsamples from each roll gap width for each maturity were collected. One was immediately frozen for nutrient characterization. The second was used immediately for an evaluation of the physical characteristics of the unfermented whole-plant maize forage. These subsamples

will be referred to as unfermented samples throughout the manuscript. Maize forage samples from each roll gap width (800 g in size) were assigned to one of four storage lengths. Storage lengths were 0, 30, 120, and 240 d. Each roll gap width and storage length combination had four replicates corresponding to the four harvest locations within the field. Thus, the overall experiment consisted of 24 treatments (three maturities × two roll gap widths × four storage lengths) and 96 mini silos (four replications per treatment). All samples were vacuum-sealed in nylon-polyethylene standard barrier vacuum pouches (0.09 mm thickness, 25.4 × 35.6 cm; Doug Care Equipment Inc., Springfield, CA, USA) using an external clamp vacuum machine (Bestvac; distributed by Doug Care Equipment Inc., Springfield, CA). Mini silos with a designated storage length of 0 d were sealed and immediately frozen. All other mini silos were stored at room temperature (~20 °C) in the dark until reaching the targeted storage length.

2.2. Fermentation Profile, Physical Characteristics, Nutrients, and Digestibility Analysis

Unfermented samples were dried at 60 °C for 48 h in a forced-air oven (Heratherm OMS180; Thermo Fisher Scientific, Waltham, MA, USA) and ground to pass through a 1 mm screen in a Wiley mill (A. H. Thomas Scientific, Philadelphia, PA, USA). Dried, ground samples were then analyzed in duplicate for absolute DM, ash, NDF, CP, starch, and ether extract. Absolute DM (method 2.2.2.5) was determined by oven-drying at 105 °C for 3 h [14]. Ash (method 942.05) was determined in a furnace held at 600 °C for 8 h [15]. Concentrations of NDF (aNDFom; method 2002.04) were determined using heat-stable α-amylase, sodium sulfite, and exclusive of residual ash [16] in an Ankom 200 Fiber Analyzer (Ankom Technologies, Macedon, NY, USA). Total N (method 968.06) was analyzed by the Dumas dry combustion method [15] using a CHNS analyzer (Vario Micro Cube; Elementar, Hanau, Germany) with CP concentration calculated as N × 6.25. Starch concentrations were analyzed by a colorimetric and enzymatic method with thermostable α-amylase (Ankom Technology, Macedon, NY, USA) and amyloglucosidase (Megazyme E-AMGDF, Bray, Co. Wicklow, Ireland) enzymes as described by Hall [17]. Ether extract (standard procedure Am 5-04) was analyzed with an ANKOM XT15 Extractor (Ankom Technologies, Macedon, NY, USA) according to AOCS [18]. Unfermented whole-plant maize forage was also analyzed for KPS as described by Ferreira and Mertens [6]. Particle size distribution of unfermented whole-plant maize forage was determined using undried and unground samples as described by Kononoff et al. [19] utilizing the Penn State Particle Separator.

After the designated length of storage was reached, vacuum pouches were opened. The material was homogenized, and one-half of each sample was immediately frozen at −20 °C to stop fermentation and stored until it could be processed for further analysis. Additionally, duplicate 50-g (as-fed) silage samples were dried at 60 °C for 48 h in a forced-air oven (Heratherm OMS180; Thermo Fisher Scientific, Waltham, MA, USA) to determine the non-volatile DM concentration and ground to pass through a 1 mm screen in a Wiley mill (A. H. Thomas Scientific, Philadelphia, PA, USA) for additional analysis.

Ensiled samples were analyzed for fermentation profile, microbial counts, N fractions, water-soluble carbohydrates (WSC), starch, KPS, ruminal in situ starch disappearance (isSD), as well as FA and AA profiles. At the time of silo opening, 20-g (as-fed) silage samples were mixed with 200 mL of 0.1% peptone water (Oxoid CM0090) in a stomacher machine (Lab-Blender 400, Tekmar Company; Cincinnati, OH, USA) for 1 min. The pH of the resulting silage extract was measured using a pH meter (Maizeing model 12, Maizeing Scientific Instruments, Medfield, MA, USA). Approximately 80 mL of silage extract was filtered through two layers of cheesecloth into two 50-mL plastic centrifuge tubes. Silage extract in the first centrifuge tube was acidified with 0.4 mL of 50% sulfuric acid and centrifuged at 7000 rpm for 15 min at 4 °C. The supernatant was used to quantify ammonia-N. Additionally, a 2-mL subsample of the supernatant was collected and centrifuged again at 10,000 rpm for 10 min at 4 °C. The resulting supernatant was then filtered with a 0.22-μm syringe filter and used for quantification of lactic, acetic, propionic, and butyric acids using high-performance liquid chromatography (Merck Hitachi Elite La-Chrome;

Tokyo, Japan) with a UV detector (Merck Hitachi L-2400) set at a wavelength of 210 nm and an ion exclusion column (300 × 7.8-mm I.D.; Bio-Rad Aminex HPX-87H; Bio-Rad Laboratories, Hercules, CA, USA), with a 0.015 M sulfuric acid mobile phase and a flow rate of 0.7 mL/min at 45 °C. Concentrations of alcohols were not measured. Silage extract in the second centrifuge tube was used for yeast and mold enumeration using the method described by Schmidt and Kung (2010) [20]. Briefly, a pour plating method in a 10-fold serial dilution on malt extract agar (Difco 211220; Difco Laboratories Ltd., Detroit, MI, United States) acidified with 85% lactic acid was used for colony determination. Agar plates were incubated at 32 °C for 48 h to determine yeast counts and an additional 72 h for molds. Microbial count data were log-transformed before statistical analysis.

Crude protein was analyzed as previously described. Soluble CP (SCP) was determined by weighing 0.25 g of dry ground sample into a 50-mL plastic centrifuge tube with 25 mL of warm (39 °C) McDougall's buffer. Centrifuge tubes were capped, placed on their side, and agitated in an incubator shaker (Serial # 000134322; C24 Incubator Shaker; New Brunswick Scientific Co., Edison, NJ, USA) set at 39 °C for 3 h. The mixture was then filtered through a 125-mm circular Whatman Grade 541 hardened ash-less filter paper. Filter paper and the residue retained on it were dried in a forced-air oven set at 60 °C for 12 h. The residue was then collected from the filter paper and analyzed for CP as previously described. Soluble CP was calculated by subtracting 100 minus the concentration of insoluble CP. Ammonia-N in the silage extract was quantified using a Technicon AutoAnalyzer (RFA-300, Alpkem Corporation, Clackamas, OR, USA) adapted from the Noel and Hambleton method for colorimetric ammonia quantification [21].

Concentrations of WSC were determined by the anthrone reaction assay [22]. Starch concentrations were determined as previously described. Kernel processing score was determined using dried, unground silage samples [6]. To determine isSD, a ruminal in situ incubation was conducted at the University of Florida Dairy Unit (Gainesville, FL, USA) under a protocol approved by the University of Florida, Institute of Food and Agricultural Sciences, Animal Care Research Committee. Dacron polyester bags (R1020, 10 × 20 cm and 50 ± 10 μm porosity; Ankom Technology, Macedon NY, USA) containing 15 g of undried and unground samples were incubated in two lactating Holstein cows. The bags were incubated for 7 h and placed in mesh laundry bags with a rubber weight to ensure their submersion in the ventral rumen. Once removed, the bags were submerged in ice water for 15 min to inhibit ruminal microbial activity and rinsed with room temperature tap water to wash off any large particles adhered to the bags. The bags were then placed in clean laundry bags for further rinsing in a washing machine (Roper RTW4516F*, Whirlpool Corp., Benton Harbor, MI, USA) set on the rinse and spin cycle with room temperature water for 30 min. The individual in situ bags were then dried in a forced-air oven set at 60 °C for 48 h and weighed. Residues from the same silo incubated in different cows were combined and ground to pass through a 1-mm sieve using a Cyclone sample mill (UDY Corporation, Fort Collins, CO, USA). Ground residues were analyzed for starch as previously described.

The fatty acid profile, the concentration of free FA, and the AA profile were determined by Cumberland Valley Analytical Services (Waynesboro, PA, USA). For the determination of the FA profile, samples were prepared using a direct methylation procedure based on methods described by Sukhija and Palmquist [23]. Fatty acid analysis was performed using gas chromatography with an RTX-2330 column (30 m × 0.32 mm i.d. and 0.2 μm film thickness; Restek Corp., Bellefonte, PA, USA) installed in a Clarus 580 gas chromatograph with a flame ionization detector and split capillary injection (PerkinElmer Instruments, Shelton). Concentrations of free FA were determined by extracting fat from samples using a Foss Soxtec 8000 extraction unit (Foss North America, Eden Prairie, MN, USA). The extracted fat was titrated with 0.01 M sodium hydroxide dropwise until a light pink color persisted for 30 sec or longer as described by AOCS Official Method Ca 5a-40 [24]. Final free FA concentrations were based on the total volume of sodium hydroxide used during the titration step. Amino acid profile was determined with near-infrared reflectance

spectroscopy (NIRS) using a Foss 5000 (Foss North America Inc., Eden Prairie, MN, USA) on dried, ground samples. Calibration equations for NIRS analysis of AA were based on a modified version of the procedure described by Gehrke et al. [25], in which a 21-h hydrochloric acid hydrolysis step precedes the analysis of AA via high-performance liquid chromatography using a Shimadzu HPLC (Shimadzu Scientific Instruments, Columbia, MD, USA) fitted with a photodiode array detector followed by post-column derivatization with a Pinnacle PCX (Pickering Laboratories, Mountain View, CA, USA). Analysis of sulfur amino acids (cysteine and methionine) required a 16.5-h performic acid peroxidation step prior to the 21-h acid hydrolysis based on a modification to the procedures described by Mason et al. [26] and Elkin and Griffith [27].

2.3. Kernel Sample Collection and Analysis

At the time of each harvest, eight random ears were collected, husked, and immediately frozen for the characterization of the maize kernels. All eight ears were hand-shelled while frozen and composited into one sample. Twenty random kernels were selected from each sample for analysis of vitreousness by manual dissection [28]. The remaining kernels were dried at 60 °C for 48 h in a forced-air oven and ground to pass through a 6-mm screen in a Wiley mill. A portion of the ground sample was dry-sieved using a Tyler Ro-Tap Shaker (model RX-29; W.S. Tyler, Mentor, OH, USA) using a set of nine sieves (W.S. Tyler) with nominal square apertures of 4.75, 3.35, 2.36, 1.70, 1.18, 0.60, 0.30, and 0.15 mm and pan [29] to determine particle-size distribution. Geometric mean particle size (μm) and surface area (cm^2/g) were calculated using a log-normal distribution [30].

The additional ground sample was used in a ruminal in situ incubation to determine the kinetics of starch digestion. The in situ incubation was conducted at the University of Florida Dairy Research Unit (Gainesville, FL, USA) under a protocol approved by the University of Florida, Institute of Food and Agricultural Sciences, Animal Care Research Committee. Dacron polyester bags (R1020, 10 × 20 cm and 50 ± 10 μm porosity; Ankom Technology, Macedon NY, USA) containing 5 g of DM of dried, ground (6 mm) sample were incubated in two lactating Holstein cows. Bags were incubated in reverse chronological order at 120, 48, 6, and 0 h to ensure bags were removed and washed at the same time. Each maturity (early, intermediate, and late) and incubation time point combination were duplicated within each cow. Following incubation, bags were removed and washed as previously described. Duplicate residues from each cow were ground to pass through a 1-mm screen using a Cyclone sample mill (UDY Corporation, Fort Collins, CO, USA) and analyzed for starch as previously described.

The remaining kernel samples were ground to pass through a 1-mm screen in a Wiley mill and sent to Dairyland Laboratories, Inc. (Arcadia, WI, USA) for nutrient analysis. Crude protein concentration (method 990.03) was determined via analysis for N [16]. Neutral detergent fiber concentration (method 2002.04) was determined using an amylase-treated method corrected for residual ash [16]. The starch concentration was determined according to Vidal et al. [31]. Ether extract concentrations (method 920.39) were determined with the use of diethyl ether on a Foss Soxtec 2047 (Foss North America, Eden Prairie, MN, USA) [16]. Ash concentration was determined using the AOAC method 942.05 [16].

2.4. Statistical Analysis

Data were analyzed as a split-split-plot design using PROC MIXED of SAS (SAS Institute Inc., Cary, NC, USA) with maturity as the main plot, roll gap width within maturity as the subplot, and storage length within roll gap width and maturity as the sub-subplot. The model included the fixed effects of maturity, roll gap, storage length, and their interactions. Repetition, repetition × maturity and repetition × roll gap (maturity) were included as random effects. Repetition × maturity and repetition × roll gap (maturity) were defined as error terms to test the effects of the main and subplot, respectively. Residual error was used to test the effects of the sub-subplot. Mini silo was the experimental unit. Means were determined using the LSMEANS statement and compared using the

Bonferroni *t*-test option after a significant overall treatment *F* test. Orthogonal contrasts were used to evaluate ensiling effects (0 d vs. 30 d) as well as linear and quadratic effects of storage length (from 30 to 240 d). Unequal spacing between measured storage lengths was accounted for in the analysis using PROC IML. Interactions were partitioned using the SLICE option to study the effects of maturity and roll gap within each day of storage. Organic acids and pH data were analyzed without d 0 using the model above, with the exception that orthogonal contrasts to evaluate ensiling effects (0 d vs. 30 d) were not performed. Statistical significance was declared at $p \leq 0.05$.

3. Results and Discussion

3.1. Chemical and Physical Characteristics of Unfermented Samples

Nutrient composition, KPS, and particle size distribution of unfermented WPMS are in Table 1. Concentrations of DM, aNDFom, and starch ranged from 29.5% to 36.9%, 43.3% to 51.2%, and 20.1% to 29.8%, respectively. Overall, concentrations of DM and starch increased with increasing maturity. During maize plant maturation, sugars in the maize kernels are converted to starch, resulting in increasing concentrations of DM and starch [32]. Concentrations of CP and aNDFom were more variable, however, and did not follow a similar pattern. In many cases, greater starch concentrations in mature maize plants will correspond to reduced concentrations of CP and NDF [3].

Table 1. Nutrient composition and particle size distribution of unfermented whole-plant maize silage (*n* = 2 for each maturity by processing combination) [1].

	Early		Intermediate		Late	
Item	**1 mm**	**3 mm**	**1 mm**	**3 mm**	**1 mm**	**3 mm**
DM, % as-fed	31.2 ± 0.1	29.5 ± 0.4	34.3 ± 0.1	33.8 ± 0.1	36.9 ± 0.3	36.5 ± 0.4
CP, % of DM	8.4 ± 0.3	6.8 ± 0.3	7.8 ± 0.3	7.1 ± 0.3	5.8 ± 0.3	6.7 ± 0.3
aNDFom, % of DM	48.5 ± 1.4	48.8 ± 0.2	45.1 ± 0.2	44.1 ± 0.3	51.2 ± 1.3	43.3 ± 1.0
Starch, % of DM	22.0 ± 1.1	20.1 ± 0.1	28.5 ± 0.7	28.1 ± 0.3	26.3 ± 1.1	29.8 ± 0.5
EE, % of DM	3.9 ± 0.6	3.8 ± 0.2	4.3 ± 0.4	3.5 ± 0.2	2.9 ± 0.1	4.0 ± 0.2
Ash, % of DM	4.4 ± 0.2	4.3 ± 0.1	4.2 ± 0.1	3.9 ± 0.1	3.6 ± 0.2	3.7 ± 0.2
KPS [2], % of starch	63.9 ± 5.0	54.5 ± 4.4	65.7 ± 6.0	60.3 ± 3.4	74.2 ± 2.2	55.5 ± 1.5
Particle size sieves [3], % as-fed retained						
19 mm	44.6 ± 1.7	34.0 ± 3.7	19.5 ± 3.0	37.6 ± 9.6	30.1 ± 2.4	29.2 ± 3.0
8 mm	38.3 ± 0.1	51.4 ± 2.4	52.9 ± 4.3	42.0 ± 9.4	49.9 ± 2.2	53.4 ± 2.3
4 mm	8.3 ± 1.2	6.7 ± 0.8	11.4 ± 0.3	9.7 ± 0.1	8.7 ± 0.8	8.0 ± 0.8
Bottom pan	8.8 ± 0.5	7.9 ± 0.5	16.2 ± 0.9	10.8 ± 1.3	11.4 ± 0.7	9.4 ± 0.6

[1] Treatments were whole-plant maize silage harvested at 3 maturities, 1/4 (Early), 1/2 (Intermediate), and 3/4 (Late) kernel milk line, processed through 2 roll gap widths, 1 or 3 mm. [2] KPS = kernel processing score; measured as % starch passing through a 4.75 mm sieve as described by Ferreira and Mertens [6]. [3] Particle size was measured using the Penn State Particle Separator as described by Kononoff et al. (2003).

Roll gap width had minor effects on the nutrient composition of the unfermented WPMS. Overall, there was considerable variability in nutrient composition among unfermented WPMS harvested at the three different maturities and processed through the two roll gap widths. As a physically and chemically heterogenous feedstuff, a certain degree of variability in the nutrient composition is to be expected. In this case, concentrations of nutrients in the unfermented WPMS were well within the range of those commonly reported in WPMS studies [13].

In unfermented WPMS, KPS ranged from 54.5% to 74.2%. Across all maturities, a roll gap width of 1 mm improved KPS compared to a roll gap width of 3 mm. The improvement in KPS achieved by reducing the roll gap width from 3 to 1 mm ranged from 5.4%-units to 18.7%-units. The particle size distribution of the unfermented WPMS was also highly variable. Maturity and roll gap width had a minor influence on particle size distribution. At equal TLOC settings, kernel processing has been shown to reduce the percentage of

particles greater than 18 mm in size by 20% [3]. No such pattern was observed in this study, however.

3.2. Unfermented Maize Kernels

Chemical composition, particle size, and in situ starch disappearance of unfermented maize kernels at the maturity stage are in Table 2. Concentrations of DM, CP, and starch ranged from 58.5% to 70.9%, 10.4% to 10.8%, and 67.3% to 68.6%, respectively. As expected, values of DM concentration and kernel vitreousness increased with increasing maturity. The vitreousness range in the present study (38.9% to 66.6% of endosperm) was similar to that reported by Correa et al. [33]. Vitreousness is negatively related to ruminal in vitro starch digestibility [33]. Both kernel DM and vitreousness have been reported to increase with increasing maturity [3,33]. Concentrations of starch and CP did not follow a similar pattern. The geometric mean particle size of kernels ground to pass through a 6 mm screen increased from 1619.6 to 1996.9 µm with increasing maturity. Correspondingly, the particle surface area decreased from 24.6 to 21.8 cm^2/g with increasing maturity. Increasing kernel vitreousness with increasing maturity may have challenged kernel particle size reduction, resulting in larger particles overall.

Table 2. The chemical composition, particle size, and in situ starch disappearance of unfermented maize kernels (n = 2 for each maturity) [1].

Item	Early	Intermediate	Late
Nutrient			
DM	58.5 ± 0.1	66.8 ± 0.1	70.9 ± 0.1
CP	10.8 ± 0.1	10.4 ± 0.1	10.7 ± 0.1
aNDF	7.3 ± 0.6	6.5 ± 0.6	7.4 ± 0.1
Starch	67.3 ± 0.1	68.6 ± 0.1	68.0 ± 0.1
EE	4.2 ± 0.2	4.4 ± 0.1	5.1 ± 0.1
Ash	1.6 ± 0.1	1.4 ± 0.1	1.4 ± 0.1
Endosperm			
Vitreousness, % of endosperm	38.9 ± 5.5	45.4 ± 1.5	66.6 ± 7.4
Geometric mean particle size [2], µm	1619.6 ± 73.6	1857.2 ± 85.1	1996.9 ± 92.0
Particle surface area [2], cm^2/g	24.6 ± 0.5	22.5 ± 0.5	21.8 ± 0.5
Ruminal in situ starch disappearance			
0 h, % starch	19.4 ± 0.1	19.4 ± 0.1	16.0 ± 0.1
6 h, % starch	51.5 ± 3.6	45.2 ± 0.1	42.2 ± 2.7
48 h, % starch	99.9 ± 0.1	99.0 ± 1.2	99.7 ± 0.3
120 h, % starch	100.0 ± 0.1	100.0 ± 0.1	100.0 ± 0.1

[1] Treatments were maize kernels collected at 3 maturities, 1/4 (Early), 1/2 (Intermediate), and 3/4 (Late) kernel milk line. [2] Analyzed on samples dried and ground to pass through a 6 mm screen.

Ruminal in situ starch disappearance at 0 h was greater in early and intermediate maturity kernels compared to late maturity kernels. Starch disappearance after 6 h of ruminal incubation decreased from 51.5% to 42.2% as kernel maturity increased. Starch disappearance after 48 and 120 h was greater than 99% for all three kernel maturities. These results suggest that soluble starch was reduced in the late maturity kernels and that kernel vitreousness likely explains the reduction in 6 h starch disappearance observed with increasing kernel maturity.

3.3. Silage Fermentation Profile and Nutrient Composition

P-values and standard errors for effects of maturity, roll gap width, storage length, and their interactions on fermentation profile and nutrient composition are in Table 3. Main effects are discussed only if the interaction effects were not significant ($p > 0.05$). The effect of maturity, roll gap width, and storage length on pH is in Figure 1A. An interaction between maturity and storage length was observed for pH ($p < 0.001$). The pH of late maturity silage was greater than that of the other two maturities at d 30, 120, and 240. Additionally, pH decreased from d 30 to 120 across all maturities. However, pH of early

and intermediate maturity silages stabilized between d 120 to 240 of storage, while that of late maturity silage increased from d 120 to 240. These results are supported by our lactic acid data (Figure 1B), which suggest a more robust fermentation in early and intermediate maturity silages compared to late maturity silage. Silage pH was unaffected by roll gap width ($p = 0.45$).

An interaction between maturity, roll gap width, and storage length was observed for concentrations of lactic acid ($p = 0.04$; Figure 1B). Lactic acid concentrations were similar among all maturity and roll gap combinations at d 30. At d 120, concentrations of lactic acid were greatest in early maturity silage processed through a 1- and 3-mm roll gap, and in intermediate maturity silage processed through a 1 mm roll gap. Concentrations of lactic acid were lowest in late maturity silage processed through a 3 mm roll gap. After 240 d of storage, lactic acid concentrations were greatest in intermediate maturity silage processed through a 3 mm roll gap and lowest in late maturity silage processed through a 1- and 3-mm roll gap. Reduced concentrations of organic acids are frequently observed in more mature maize silages [9]. Metabolic water available for the growth of bacteria in the silo becomes limiting as maize plants mature and DM concentrations increase [34].

An interaction between maturity, roll gap width, and storage length was also observed for concentrations of acetic acid ($p = 0.02$; Figure 1C). After 30 d of storage, concentrations of acetic acid were similar among all maturity and roll gap width combinations. At 120 d, concentrations of acetic acid were greatest in late maturity silage processed through a 1 mm roll gap and lowest in late maturity silage processed through a 3 mm roll gap. After 240 d, acetic acid was greatest in the intermediate maturity silages and lowest in late maturity silage processed through a 3 mm roll gap. Acetic acid concentrations typically decrease as maize silage maturity increases and moisture decreases [9], but results can vary. Windle et al. [35] reported similar concentrations of acetic acid in WPMS ensiled at 31% and 40% DM. Similarly, Ferraretto et al. [36] found that concentrations of total acids were not different between early and late maturity maize silages. Concentrations of propionic and butyric acid were measured but not detected in this experiment. Therefore, these data are not presented in any tables or figures.

Table 3. Statistical analysis (*p*-values) for the effect of maturity [1] (M), roll gap width [2] (R), storage length [3] (S), and their interactions on the fermentation profile and nutrient composition of whole-plant maize silage (total *n* = 96).

Item	M	R	S	M × R	M × S	R × S	M × R × S	SEM
Fermentation profile [4]								
pH	<0.001	0.45	<0.001	0.87	<0.001	0.61	0.13	0.04
Lactic acid, % DM	<0.001	0.05	0.10	0.30	<0.001	0.01	0.04	0.55
Acetic acid, % DM	0.16	0.11	<0.001	0.15	<0.001	0.43	0.02	0.30
Yeast count, log cfu/g	0.11	0.65	<0.001	0.65	0.01	0.01	0.10	0.64
Mould count, log cfu/g	0.18	0.01	0.01	0.29	0.01	0.23	0.43	0.59
Nutrients								
DM, % as-fed	<0.001	0.25	0.01	0.01	0.01	0.74	0.15	0.26
WSC [5], % DM	0.01	0.36	<0.001	<0.001	<0.001	<0.001	0.02	1.50
CP, % DM	0.01	0.80	<0.001	0.01	0.22	0.06	0.11	2.32
SCP [6], % CP	0.02	0.45	<0.001	0.62	0.01	0.01	0.10	0.25
Ammonia-N, % CP	0.01	0.01	<0.001	0.09	<0.001	0.13	0.11	0.47
Starch, % DM	0.01	0.59	<0.001	0.01	0.48	0.12	0.11	3.29
isSD [7], % starch	<0.001	0.01	<0.001	0.07	0.01	0.90	0.05	2.82
KPS [8], % starch	0.08	0.01	<0.001	0.01	0.01	0.63	0.36	0.04

[1] Whole-plant maize was harvested at 3 maturities: 1/4 (Early), 1/2 (Intermediate), and 3/4 (Late) kernel milk line. [2] Maize was processed through rolls with a gap width of either 1 or 3 mm using a self-propelled forage harvester. [3] Mini-silos were stored for either 0, 30, 120, or 240 d. [4] Propionic and butyric acids were measured but not detected. [5] WSC = water-soluble carbohydrates [6] SCP = soluble crude protein [7] isSD = 7 h ruminal in situ starch disappearance of undried, unground samples [8] KPS = kernel processing score; measured as % starch passing through a 4.75 mm sieve as described by Ferreira and Mertens [6].
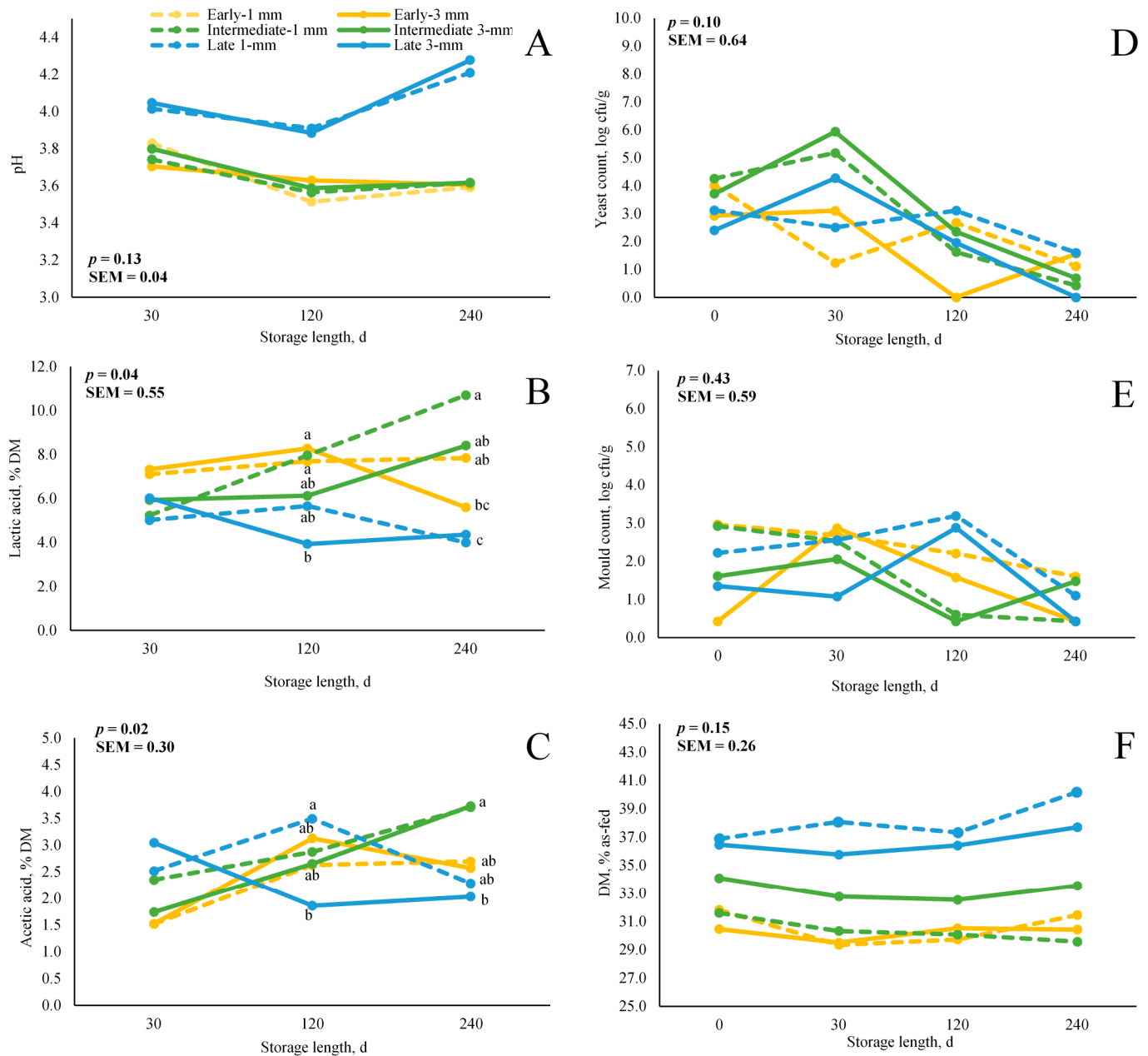
Figure 1. The effect of maturity, roll gap width, and storage length on pH (**A**), lactic (**B**) and acetic acid (**C**) concentrations, yeast counts (**D**), mould counts (**E**), and concentrations of DM (**F**) in whole-plant maize silage (*n* = 96). Means within the same day with different letters (a,b,c) differed (*p* ≤ 0.05). Whole-plant maize was harvested at 3 maturities (1/4 (Early), 1/2 (Intermediate), and 3/4 (Late) kernel milk line) and processed through rolls with a gap width of either 1 or 3 mm using a self-propelled forage harvester. Silage was stored for 0, 30, 120, or 240 d.

The effects of kernel processing on WPMS fermentation are not well-documented. Johnson et al. [37] conducted two experiments to evaluate the effects of hybrid, maturity, and mechanical processing on chemical and physical characteristics of WPMS. Researchers observed that lactic acid concentrations were greater for processed maize silage (kernel processing rolls set at 1 mm apart) compared to unprocessed maize silage in one experiment but not the other. In both experiments, pH and acetic acid concentrations were unaffected by kernel processing [37]. In a similar study, Johnson et al. [38] reported marginal effects of mechanical processing on WPMS fermentation, with reduced lactic acid and greater acetic acid concentrations in processed (kernel processing rolls set at 1 mm apart) compared to un-processed WPMS. It is important to note that the physical differences between unprocessed

and processed WPMS are likely more pronounced than those between WPMS processed through a 1- or 3-mm roll gap. The effect of kernel processing on WPMS fermentation likely requires additional investigation. The effects of storage length on fermentation of WPMS are clear. It is well-accepted that organic acids accumulate in the silo during storage, resulting in a reduction in pH [9]. Studies by Windle et al. [35] and Der Bedrosian et al. [39] reported a pH decline, as well as gradual increases in lactic and acetic acid concentrations in WPMS, as the storage length progressed up to 150 d or more.

The effects of maturity, roll gap width, and storage length on counts of yeasts and molds are in Figure 1D,E, respectively. An interaction between maturity and storage length was observed for yeast counts ($p = 0.01$). Across all maturities, yeast counts decreased as storage length increased from 0 to 240 d. At d 0, 120, and 240, yeast counts were not different between the three maturities. At d 30, however, yeasts counts were greater in the intermediate maturity silage compared to early and late maturity silages. An interaction between roll gap width and storage length was also observed for yeast counts ($p = 0.01$). Again, yeast counts decreased with prolonged storage in silage processed through a 1 mm roll gap and a 3 mm roll gap. Counts of yeasts were not different between the two roll gap widths at d 0, 120, and 240. At d 30 of storage, yeast counts were greater in silage processed through a 3 mm roll gap compared to a 1 mm roll gap. An interaction between maturity and storage length was observed ($p = 0.01$) for mold counts. Overall, mold counts decreased over the course of the storage period. At d 0, 30 and 240, mold counts were not different among the three maturities. At d 120, however, mold counts were greatest in the late maturity silage and lowest in the intermediate maturity silage. Roll gap width was also found to affect mold counts ($p = 0.01$). Interestingly, mold counts were greater in silage processed through a 1 mm roll gap compared to a 3 mm roll gap (2.1 vs. 1.4 log cfu/g, respectively). Generally, yeast and mold counts tend to decrease with storage due to the accumulation of organic acids and decline in pH in the silo [40]. Counts of molds observed in this study were relatively low for most samples, an indication that fermentation and extended storage sufficiently inhibited the growth of these organisms. Likewise, most yeast counts for early maturity were below 3 log cfu/g supporting our premise of better fermentation when WPMS is ensiled at earlier maturity.

The effect of maturity, roll gap width, and storage length on concentrations of DM is in Figure 1F. A maturity by roll gap interaction was observed for DM ($p = 0.01$). As expected, DM concentrations were greatest in the late maturity silage. Concentrations of DM were intermediate in the intermediate maturity silage processed through a 3 mm roll gap and lowest in the intermediate maturity silage processed through a 1 mm roll gap and the early maturity silages. A maturity by storage length interaction was also observed for DM ($p = 0.01$). Across all storage lengths, DM concentrations were greatest in the late maturity silage. Concentrations of DM were similar between early and intermediate maturity silages at d 0, 120 and 240. At d 30, however, DM concentrations were greater in the intermediate maturity silage compared to the early maturity silage. Although these results are representative of typical maturity effects reported in the literature, it is important to note that this study was conducted with a single maize silage hybrid harvested at one geographical location. Future research evaluating treatment effects on different hybrids harvested from a variety of locations is warranted.

An interaction between maturity, roll gap width, and storage length was observed for concentrations of WSC ($p = 0.02$; Figure 2A). At d 0, concentrations of WSC were greatest in the early maturity silage processed through a 3 mm roll gap and in the intermediate maturity silage processed through a 1 mm roll gap. Concentrations of WSC were lowest in the late maturity silage processed through a 3 mm roll gap. As expected, concentrations of WSC were greater in unfermented WPMS compared to WPMS stored for 30 d. Concentrations of WSC subsequently decreased throughout the storage period. At d 30, concentrations of WSC were greater in the early maturity silages and in the intermediate maturity silage processed through a 1 mm roll gap compared to the other maturity and roll gap width combinations. Interestingly, concentrations of WSC were greater in the early

maturity silage processed through a 3 mm roll gap compared to the other maturity and roll gap width combinations at d 120 and 240. Maize silage contains considerable levels of WSC [41], which favor the proliferation of anaerobic bacteria [9]. The continued use of WSC by these organisms during storage explains the gradual reduction in WSC concentrations observed in this experiment.
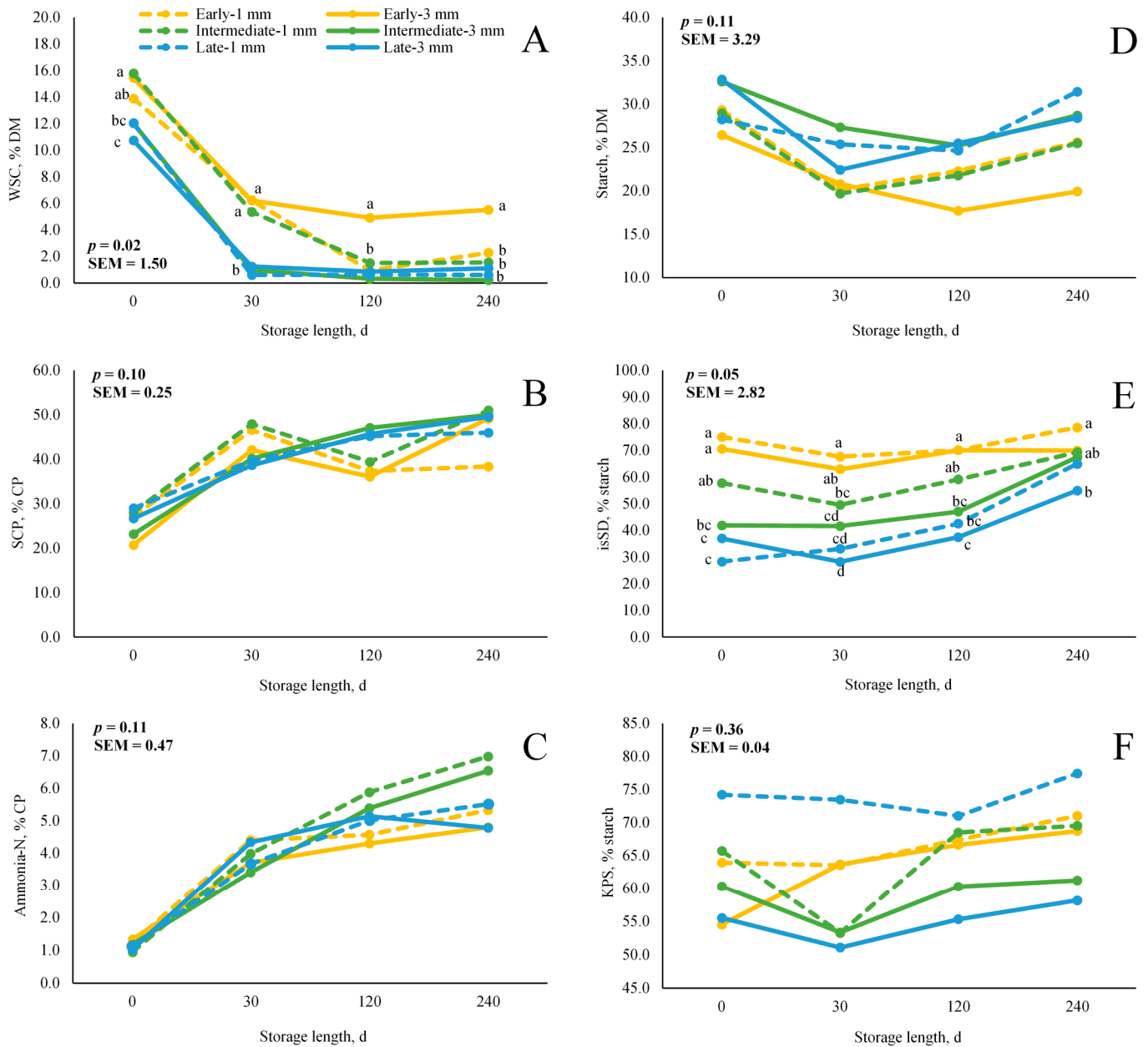

Figure 2. The effect of maturity, roll gap width, and storage length on WSC (**A**), SCP (**B**), ammonia-N (**C**), starch (**D**) concentrations, as well as isSD (**E**) and KPS (**F**) of whole-plant maize silage (*n* = 96). Means within the same day with different letters (a,b,c) differed (*p* ≤ 0.05). Whole-plant maize was harvested at 3 maturities (1/4 (Early), 1/2 (Intermediate), and 3/4 (Late) kernel milk line) and processed through rolls with a gap width of either 1 or 3 mm using a self-propelled forage harvester. Silage was stored for 0, 30, 120, or 240 d.

An interaction between maturity and roll gap width was observed for concentrations of CP (*p* = 0.01; data not shown in tables or figures). Overall, concentrations of CP were greatest in the early maturity silage processed through a 1 mm roll gap and in the intermediate maturity silage processed through a 3 mm roll gap (8.3% on average). Concentrations

of CP were lowest in the late maturity silages (7.6% on average). An effect of storage length was also observed for concentrations of CP ($p = 0.01$; data not shown in tables or figures). Concentrations of CP increased with ensiling ($p = 0.01$; 7.2% vs. 7.8% at d 0 and 30, respectively) and increased quadratically ($p = 0.01$) from 7.8% to 8.3% as storage length increased from 30 to 240 d. Although effects of ensiling and storage length were observed for concentrations of CP, differences were of minimal magnitude, which agrees with previous literature [35,39].

The effects of maturity, roll gap width, and storage length on concentrations of SCP and ammonia-N are in Figure 2B,C, respectively. An interaction between maturity and storage length was observed for SCP ($p = 0.01$). Overall, concentrations of SCP increased from 0 to 240 d of storage across all maturities. Concentrations of SCP were not different between the three maturities at d 0, 30, and 240. At d 120, however, concentrations of SCP were greater in the late maturity silage compared to the early maturity silage. An interaction between roll gap width and maturity was also observed for SCP ($p = 0.01$). Concentrations of SCP increased with storage in silages processed through a 1 mm roll gap and in silages processed through a 3 mm roll gap. At d 0 and 30, SCP concentrations were greater in silages processed through a 1 mm roll gap compared to a 3 mm roll gap. After 240 d, however, concentrations of SCP were greater in silages processed through the 3 mm roll gap. Roll gap width did not affect SCP concentrations at d 120. An interaction between maturity and storage length was observed for ammonia-N ($p < 0.001$). Overall, concentrations of ammonia-N increased from d 0 to d 240 of storage. Ammonia-N concentrations were not different between the three maturities at d 0 and 30. After 120 d, concentrations of ammonia-N were greater in the intermediate maturity silage compared to those in the early maturity silage. After 240 d of storage, concentrations of ammonia-N were greater in the intermediate maturity silage compared to the early and late maturity silages. An effect of roll gap width was also observed for ammonia-N concentrations ($p = 0.01$). Concentrations of ammonia-N were greater in silage processed through a 1 mm roll gap compared to a 3 mm roll gap (4.0% vs. 3.8% of CP, respectively).

It is well-documented that substantial proteolysis occurs in WPMS during storage, as evidenced by increasing concentrations of ammonia-N and SCP [9]. Junges et al. [42] reported that bacterial activity was the primary contributor to proteolysis in maize silage (60%), followed by plant enzymes (30%), fungi (5%), and fermentation end-products (5%). It is likely that continued proteolytic activity by microbial and plant proteases throughout the storage period resulted in the accumulation of SCP and ammonia-N in this study. Others have reported increasing concentrations of these N fractions in WPMS stored for an extended period [35,39,43]. Concentrations of SCP and ammonia-N have been shown to be correlated with starch digestibility of WPMS [9].

The effect of maturity, roll gap width, and storage length on concentrations of starch is in Figure 2D. An interaction between maturity and roll gap width was observed for starch concentrations ($p = 0.01$). Across all storage lengths, concentrations of starch were greatest in intermediate maturity silage processed through a 3 mm roll gap (28.5%) and lowest in early maturity silage processed through a 3 mm roll gap (21.2%). Starch concentrations were also affected by storage length ($p < 0.001$). Starch concentrations were decreased by ensiling ($p < 0.001$; 0 d vs. 30 d). A positive linear effect of storage length was observed for starch concentrations ($p < 0.001$; 30 d to 240 d).

An interaction between maturity, roll gap width, and maturity was observed for isSD ($p = 0.05$; Figure 2E). Across all maturity and roll gap width combinations, isSD increased with prolonged storage. At 0 d, isSD was greatest for the early maturity silage, intermediate for the intermediate maturity silage, and lowest for the late maturity silage. These differences persisted from d 0 to d 120. At 240 d of storage, differences between treatment combinations were diminished, with isSD being greater in early maturity silage processed through a 1 mm roll gap compared to late maturity silage processed through a 3 mm roll gap. Ruminal starch digestibility is often found to be greater in early maturity compared to late maturity WPMS [36,39,44]. In a meta-analysis by Ferraretto and Shaver [5],

a reduction in total-tract starch digestibility was observed for diets containing WPMS with >40% DM. The profound effect of plant maturity on starch digestibility is likely related to an increase in the proportion of vitreous endosperm in mature maize kernels [3]. Our results suggest that differences in isSD due to maturity have the potential to be diminished with prolonged storage. In contrast to the well-established literature [9], the most pronounced effects of fermentation on isSD in the present study were not at the onset of fermentation (30 d) but instead of after 120 d of storage. Although a similar lag on starch digestibility was previously reported [35], these results should be interpreted cautiously. Further research elucidating the mechanism causing this lag in certain silages is warranted. Across all lengths of storage, however, isSD was influenced to a greater extent by maturity than by roll gap width. Ferraretto and Shaver [5] reported 5.9% and 2.8%-units greater total-tract starch digestibility when WPMS was processed using 1 to 3 mm roll gap settings compared with 4 to 8 mm processed or unprocessed WPMS.

The effect of maturity, roll gap width, and storage length on KPS is in Figure 2F. An interaction between maturity and roll gap width was observed for KPS ($p = 0.01$). Across all maturity and roll gap width combinations, KPS was greatest in the late maturity silage processed through a 1 mm roll gap and lowest in the late maturity silage processed through the 3 mm roll gap. For early and intermediate maturity silage, no differences in KPS were observed between the two roll gap widths. These findings suggest that reducing the roll gap width from 3 mm to 1 mm may improve KPS of late maturity WPMS without substantial benefit to that of early and intermediate maturity WPMS. An interaction between maturity and storage length was also observed for KPS ($p = 0.01$). Overall, KPS increased marginally d 0–240 for all three maturities. At d 0, 120, and 240, KPS was not different between the three maturities. At d 30, however, KPS was greater in early maturity silage compared to the intermediate maturity silage.

The increased vitreousness and kernel hardness associated with increasing maturity may presumably affect the reduction in kernel particle size associated with harvesting and processing. Effects of maturity on KPS have been variable in the literature, however. When processed through a conventional kernel processor (2 mm roll gap), Ferraretto et al. [36] reported numerically greater processing scores in *bm3* gene mutation and dual-purpose maize silage hybrids harvested at a late maturity compared to the same hybrids harvested at early maturity. Bueno et al. [44] observed greater kernel processing scores in unprocessed WPMS harvested at 30% DM compared to 40% DM.

In this study, the improvement in KPS observed with prolonged storage suggests that the degradation of the protein matrix surrounding starch granules after 240 d may have been detrimental to the structural integrity of the kernels, making them more susceptible to a reduction in particle size and increasing KPS. Studies evaluating the influence of storage length on KPS are scarce, and reports have been variable. Ferraretto et al. [45] conducted two experiments to determine the influence of ensiling on KPS. In the first experiment, WPMS ensiled for 30 d had a 10%-unit increase in KPS compared to unfermented samples (60.1% vs. 50.2%, respectively). In the second experiment, WPMS ensiled for 120 d tended to have a greater KPS than WPMS stored for 0 d (67.2% vs. 60.3%, respectively), but not 30 d (63.6%). Agarussi et al. [46], however, found that the KPS of WPMS ensiled for 120 d was not different from that of fresh WPMS. However, the authors suggested that the very low KPS at ensiling (28.8%) may have contributed to the absence of an ensiling effect.

3.4. Silage FA Composition

P-values and standard errors for the effect of maturity, roll gap width, storage length, and their interactions on silage FA composition are in Table 4. The main effects are presented and discussed only if the interaction effects were not significant ($p > 0.05$). Concentrations of all FA in early, intermediate, and late WPMS can be found in Supplementary Tables S1–S3, respectively. The majority of the FA found in WPMS were C18:2 and C18:1, followed by C16:0 and C18:3. Baldin et al. [12] found that C18:2 constituted more than 45% of the total FA in WPMS.

Table 4. Statistical analysis (*p*-values) for the effect of maturity [1] (M), roll gap width [2] (R), storage length [3] (S), and their interactions on the fatty acid profile of whole-plant maize silage. (total *n* = 96).

Item, % DM [4]	M	R	S	M × R	M × S	R × S	M × R × S	SEM
C12:0	<0.001	0.13	0.30	0.06	0.03	0.37	0.65	0.0004
C14:0	0.01	0.01	0.11	0.01	<0.001	0.75	0.12	0.0005
C15:0	<0.001	0.07	0.01	0.17	0.01	0.51	0.02	0.0001
C16:0	0.01	0.31	0.27	<0.001	0.51	0.02	0.61	0.0174
C16:1	0.06	0.29	<0.001	0.16	0.02	0.01	0.87	0.0008
C17:0	0.14	<0.001	0.01	0.01	0.07	0.64	0.16	0.0002
C18:0	<0.001	0.01	0.42	<0.001	0.48	0.14	0.42	0.0027
C18:1 *cis*-9	<0.001	0.07	0.02	<0.001	0.72	0.13	0.30	0.0402
C18:1 *trans*-9	0.55	0.06	<0.001	0.01	0.62	0.61	0.59	0.0002
C18:1n-7	<0.001	<0.001	<0.001	0.32	0.63	0.04	0.04	0.0042
C18:2n-6	<0.001	0.05	<0.001	<0.001	0.54	0.02	0.36	0.0540
C18:3n-3	<0.001	0.40	<0.001	0.09	0.76	0.44	0.32	0.0128
C20:0	<0.001	0.16	0.85	<0.001	0.49	0.12	0.45	0.0007
C20:1	0.01	0.01	0.13	0.01	0.16	0.81	0.52	0.0005
C20:2n-6	0.04	0.48	0.49	0.03	0.81	0.17	0.18	0.0002
C20:5	0.31	0.66	0.25	0.76	0.65	0.06	0.41	0.0003
C22:0	0.39	0.01	<0.001	0.01	0.55	0.52	0.32	0.0006
C22:1	0.01	0.94	0.32	0.01	0.19	0.82	0.37	0.0004
C24:0	0.10	0.49	<0.001	0.05	0.50	0.82	0.34	0.0006
C24:1	0.72	0.06	0.45	<0.001	0.09	0.21	0.02	0.0013
Sum of C12:0 to C22:6 FA	<0.001	0.49	0.04	<0.001	0.61	0.04	0.41	0.1169
Free FA	0.05	0.76	<0.001	<0.001	<0.001	0.24	<0.001	0.0960

[1] Whole-plant maize was harvested at 3 maturities: 1/4 (Early), 1/2 (Intermediate), and 3/4 (Late) kernel milk line. [2] Maize was processed through rolls with a gap width of either 1 or 3 mm using a self-propelled forage harvester. [3] Mini-silos were stored for either 0, 30, 120, or 240 d. [4] FA analyzed but not detected were C22:5n-3 and C22:6n-3.

The effect of maturity, roll gap width, and storage length on concentrations of C18:0 is in Figure 3A. An interaction between maturity and roll gap width was observed for C18:0 (*p* < 0.001). Concentrations of C18:0 were greatest in the late maturity silage processed through a 1 mm roll gap, followed by intermediate maturity silage processed through a 3 mm roll gap, intermediate maturity silage processed through a 1 mm roll gap, and early maturity silage processed through a 1 mm roll gap. Concentrations of C18:0 were lowest in early maturity silage processed through a 3 mm roll gap. Storage length had no effect (*p* = 0.42) on C18:0 concentrations.

The effect of maturity, roll gap width, and storage length on concentrations of C18:1 *cis*-9 is in Figure 3B. An interaction between maturity and roll gap width was observed for C18:1 *cis*-9 (*p* < 0.001). Concentrations of C18:1 *cis*-9 were greatest in the late maturity silages and the intermediate maturity silage processed through a 3 mm roll gap, intermediate in intermediate and early maturity silages processed through a 1 mm roll gap, and lowest in early maturity silage processed through a 3 mm roll gap. An effect of storage length was also observed for concentrations of C18:1 *cis*-9 (*p* = 0.02). Concentrations of C18:1 *cis*-9 decreased quadratically (*p* = 0.03) from 30 to 240 d of storage.

The effect of maturity, roll gap width, and storage length on concentrations of C18:2n-6 is in Figure 3C. An interaction between maturity and roll gap was observed for C18:2n-6 (*p* < 0.001). Similar to what was observed for C18:1 *cis*-9, concentrations of C18:2n-6 were greatest in the late maturity silages and the intermediate maturity silage processed through a 3 mm roll gap, intermediate in intermediate and early maturity silages processed through a 1 mm roll gap, and lowest in early maturity silage processed through a 3 mm roll gap. An interaction between roll gap width and storage length was also observed for C18:2n-6 (*p* = 0.02). Concentrations of C18:2n-6 were greater in WPMS processed through a 3 mm roll gap compared to a 1 mm roll gap at d 0. However, no differences in C18:2n-6 concentrations between the two roll gap widths were observed from d 30 to 240.
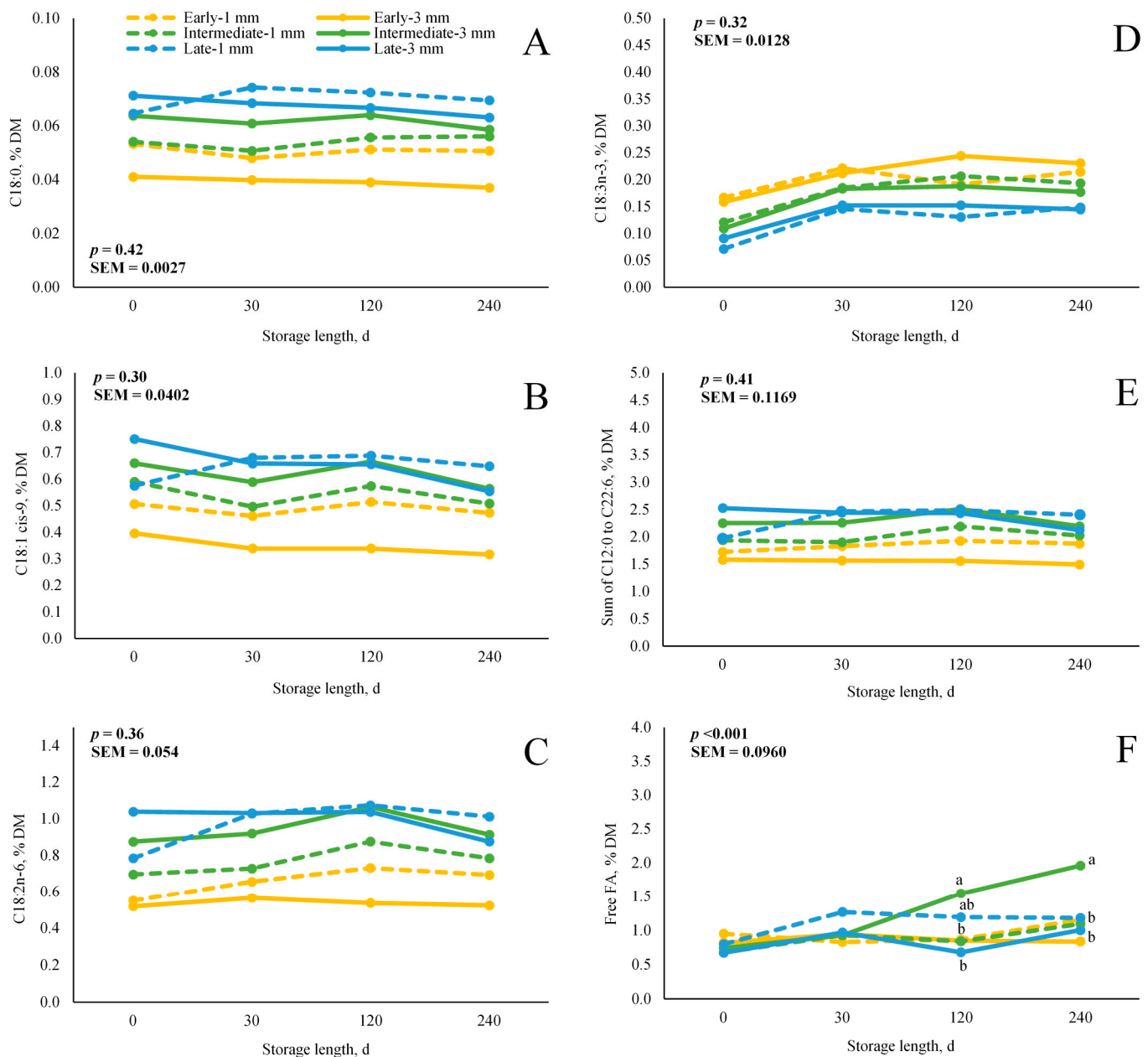
Figure 3. The effect of maturity, roll gap width, and storage length on C18:0 (**A**), C18:1 *cis*-9 (**B**), C18:2n-6 (**C**), C18:3n-3 (**D**), sum of C12:0 to C22:6 (**E**), and free FA concentrations (**F**) in whole-plant maize silage (*n* = 96). Means within the same day with different letters (a,b) differed (*p* ≤ 0.05). Whole-plant maize was harvested at 3 maturities (1/4 (Early), 1/2 (Intermediate), and 3/4 (Late) kernel milk line) and processed through rolls with a gap width of either 1 or 3 mm using a self-propelled forage harvester. Silage was stored for 0, 30, 120, or 240 d.

The effect of maturity, roll gap width, and storage length on concentrations of C18:3n-3 is in Figure 3D. There tended to be an interaction between maturity and roll gap width for C18:3n-3 concentrations (*p* = 0.09). An effect of maturity was observed for concentrations of C18:3n-3 (*p* < 0.001), with concentrations of the FA decreasing from 0.20% to 0.13% as maturity increased from early to late. An effect of storage length was also observed for C18:3n-3 (*p* < 0.001). Ensiling (d 0 vs. 30) increased concentrations of C18:3n-3 from 0.12% to 0.18% (*p* < 0.001). No differences in C18:3n-3 concentrations were observed from d 30 to 240.

Baldin et al. [12] reported a negative correlation between C18:3 and C18:2 in WPMS, but not between C18:3 and C18:1. Changes in WPMS FA composition with increasing maturity are not well-documented. Baldin et al. [12] observed that total FA and C18:2

concentrations were positively correlated with starch and negatively correlated with NDF in fresh whole-plant chopped maize and maize silage samples from multiple hybrids. However, this was thought to reflect the FA composition of kernels and the proportion of grain in maize silage, not plant maturity. More than 80% of the total FA in WPMS were in the kernels, 11.8% in the leaves, 5.1% in the stalk, 1.7% in the cob, and 1.0% in the husk and shank [12]. Alves et al. [11] observed that ensiling decreased concentrations of C18:2n-6 and C18:3n-3. In that study, total FA content was not affected by ensiling [11]. In a study by Agarussi et al. [46], no effects of ensiling (0 vs. 120 d) were observed on the profile of major LCFA found in maize silage (C16:0, C18:0, C18:1, C18:2, C18:3). In the current study, observed changes in concentrations of individual FA were very small, even in the most abundant FA present in WPMS (C18:1 *cis*-9, C18:2n-6, and C18:3n-3). These changes, even at high inclusion rates of maize silage in the diet, would be unlikely to influence FA metabolism or animal performance.

The effect of maturity, roll gap width, and storage length on the sum of C12:0 to C22:6 FA is in Figure 3E. An interaction between maturity and roll gap width was observed for the sum of C12:0 to C22:6 FA ($p < 0.001$). Similar to what was observed for concentrations of C18:1 *cis*-9 and C18:2n-6, the sum of C12:0 to C22:6 FA was greatest in the late maturity silages and the intermediate maturity silage processed through a 3 mm roll gap, intermediate in intermediate and early maturity silages processed through a 1 mm roll gap, and lowest in early maturity silage processed through a 3 mm roll gap. Similar to C18:2n-6, an interaction between roll gap width and storage length was observed for the sum of C12:0 to C22:6 FA ($p = 0.04$), with more FA in WPMS processed through a 3 mm roll gap compared to a 1 mm roll gap at d 0, but not at d 30 to 240. An interaction between maturity, roll gap width, and storage length was observed for concentrations of free FA ($p < 0.001$; Figure 3F). Concentrations of free FA increased slightly from d 0 to d 30 and were not different between the maturity and roll gap width combinations at these time points. From d 30 to d 240, concentrations of free FA continued to increase for intermediate maturity silage processed through a 3 mm roll gap. Free FA concentrations were greater with this treatment combination than the others at both 120 and 240 d. Minimal changes in free FA concentrations occurred with the other maturity and roll gap width combinations from d 30 to 240. Few studies have investigated the effect of WPMS processing and storage on concentrations of free FA. Huang et al. [47] suggested that the fast release of FA and starch may increase the production of biohydrogenation intermediates linked to milk fat depression. In that study, soluble FA in maize silage was 25 ± 14% of total FA and was found to be positively correlated with soluble starch and soluble DM in maize silage. Baldin et al. [12] suggested that highly digestible starch and polyunsaturated fatty acids (PUFA) supplied by maize silage may put cows at risk of developing diet-induced milk fat depression. Our results suggest that differences in maturity, kernel processing, and storage length are unlikely to affect PUFA supplied by WPMS.

3.5. Silage AA Composition

P-values and standard errors for the effect of maturity, roll gap width, storage length, and their interactions on silage AA composition are in Table 5. The main effects are presented and discussed only if the interaction effects were not significant ($p > 0.05$). Concentrations of all AA in early, intermediate, and late WPMS can be found in Supplementary Tables S4–S6, respectively.

Table 5. Statistical analysis (*p*-values) for the effect of maturity [1] (M), roll gap width [2] (R), storage length [3] (S), and their interactions on the amino acid profile of whole-plant maize silage. (Total *n* = 96).

Item, % DM	M	R	S	M × R	M × S	R × S	M × R × S	SEM
EAA [4]								
Arginine	<0.001	0.42	<0.001	0.83	<0.001	0.01	0.57	0.006
Histidine	<0.001	0.18	<0.001	0.01	0.37	0.03	0.78	0.006
Isoleucine	0.47	0.04	0.01	0.02	0.96	0.03	0.17	0.007
Leucine	<0.001	0.53	0.01	0.01	0.39	0.01	0.47	0.021
Lysine	<0.001	0.61	<0.001	0.03	<0.001	0.02	0.01	0.003
Methionine	0.44	0.12	0.02	0.01	0.61	0.01	0.16	0.004
Phenylalanine	0.02	0.16	0.01	0.01	0.45	0.11	0.26	0.014
Threonine	0.01	0.85	<0.001	0.92	0.47	<0.001	0.61	0.006
Valine	0.01	0.17	0.01	0.69	0.88	0.03	0.43	0.008
Sum of EAA	0.14	0.40	<0.001	0.06	0.79	0.01	0.38	0.065
NEAA [5]								
Alanine	0.01	0.07	<0.001	0.96	<0.001	0.44	0.26	0.022
Aspartic acid	<0.001	0.02	<0.001	0.46	0.01	0.01	0.02	0.014
Cysteine	0.01	0.53	<0.001	<0.001	<0.001	<0.001	0.72	0.003
Glutamic acid	0.01	0.98	<0.001	0.01	0.11	0.02	0.83	0.031
Glycine	0.05	0.36	<0.001	0.93	0.89	0.01	0.39	0.008
Proline	0.01	0.12	0.24	<0.001	0.91	0.02	0.27	0.019
Serine	<0.001	0.17	<0.001	0.72	0.01	0.01	0.25	0.012
Tyrosine	<0.001	0.11	<0.001	0.11	0.03	0.04	0.17	0.006
Sum of NEAA	0.40	0.17	<0.001	0.05	0.73	0.01	0.39	0.094
Sum of total AA	0.25	0.25	<0.001	0.06	0.77	0.01	0.39	0.159
AA Protein, % CP	<0.001	0.70	<0.001	0.75	<0.001	0.25	0.29	0.510

[1] Whole-plant maize was harvested at 3 maturities: 1/4 (Early), 1/2 (Intermediate), and 3/4 (Late) kernel milk line. [2] Maize was processed through rolls with a gap width of either 1 or 3 mm using a self-propelled forage harvester. [3] Mini-silos were stored for either 0, 30, 120, or 240 d. [4] EAA = essential AA. [5] NEAA = non-essential AA.

An interaction between maturity, roll gap width, and storage length was observed for lysine concentrations (*p* = 0.01; Figure 4A). Overall, concentrations of lysine decreased from d 0 to d 240. At d 0, concentrations of lysine were greatest in early maturity silage processed through a 3 mm roll gap and lowest in late maturity silage processed through a 1 mm roll gap. At d 30, lysine concentrations were greatest in the early maturity silage processed through a 1 mm roll gap, intermediate in early maturity silage processed through a 3 mm roll gap, and lowest in the other maturity and roll gap width combinations. At d 120, concentrations of lysine were greatest in the early maturity silage processed through the 3 mm roll gap and lowest in the intermediate maturity silages. After 240 d of storage, lysine concentrations were greatest in the early maturity silage processed through a 3 mm roll gap, intermediate in early maturity silage processed through a 1 mm roll gap and the late maturity silages, and lowest in the intermediate maturity silages. Differences between the various maturity and roll gap width combinations within a given d of storage are likely of minor biological significance.

The effect of maturity, roll gap width, and storage length on concentrations of methionine is in Figure 4B. An interaction between maturity and roll gap width was observed for methionine concentrations (*p* = 0.01). Concentrations of methionine were greatest in the early maturity silage processed through the 1 mm roll gap and lowest in the early maturity silage processed through the 3 mm roll gap. Concentrations of methionine were intermediate with the other treatment combinations and were not different from one another. An interaction between roll gap and storage length was also observed for methionine (*p* = 0.01). Concentrations of methionine were not different between the two roll gap widths at d 0, 30, and 120. At d 240, however, methionine concentrations were greater in silage processed through the 1 mm roll gap compared to the 3 mm roll gap.
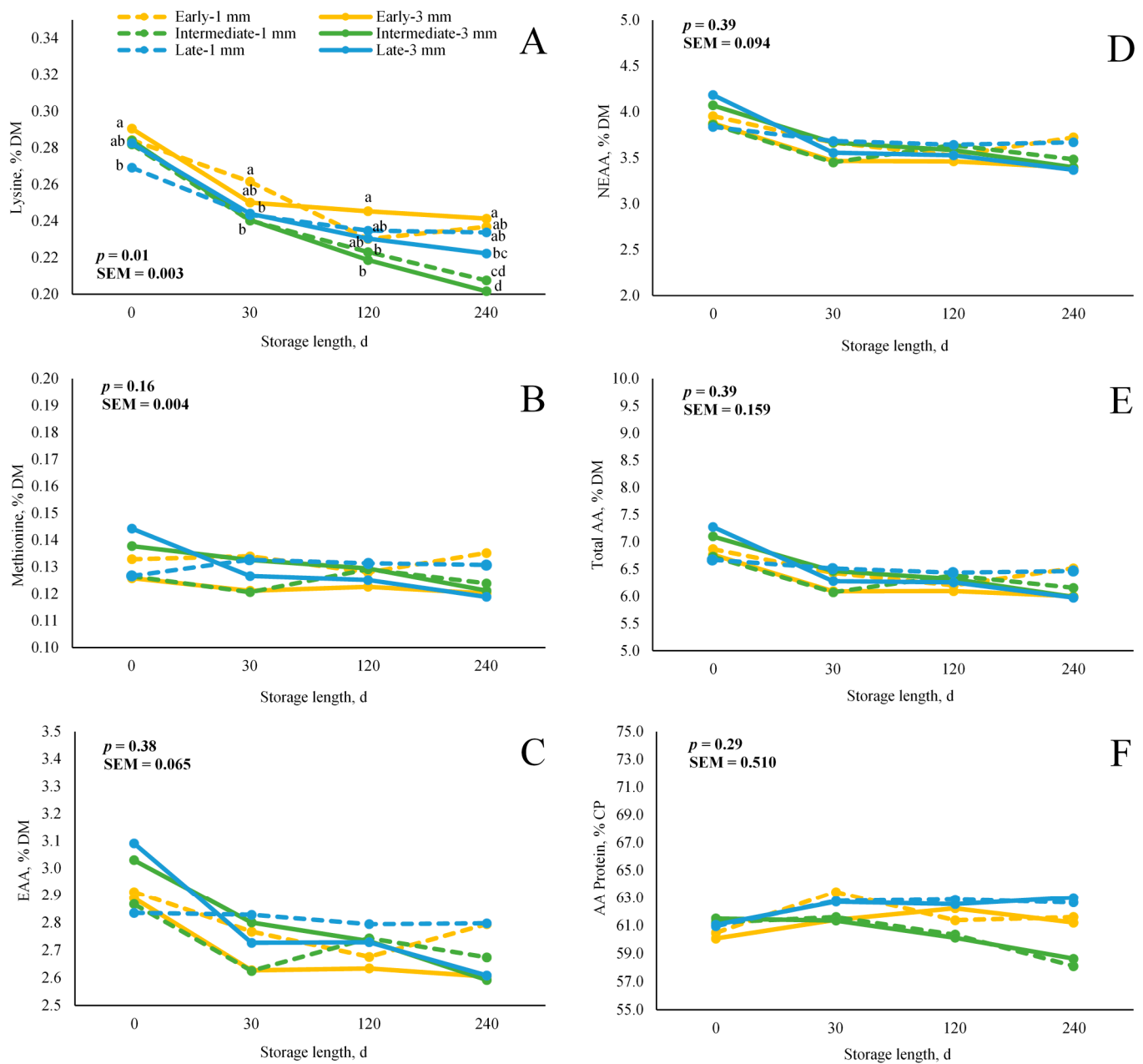
Figure 4. The effect of maturity, roll gap width, and storage length on lysine (**A**), methionine (**B**), EAA (**C**), NEAA (**D**), total AA (**E**), and AA protein (% CP) (**F**) concentrations in whole-plant maize silage (*n* = 96). Means within the same day with different letters (a,b,c,d) differed (*p* ≤ 0.05). Whole-plant maize was harvested at 3 maturities (1/4 (Early), 1/2 (Intermediate), and 3/4 (Late) kernel milk line) and processed through rolls with a gap width of either 1 or 3 mm using a self-propelled forage harvester. Silage was stored for 0, 30, 120, or 240 d.

It has been suggested that the AA profile of WPMS is related to the relative proportions of maize kernels and stover material in the silage [48]. The four main components of a maize kernel are the endosperm, germ, bran, and tip cap. Each component contains protein of varying types and amounts. The endosperm, the largest part of the kernel, contains 60% zein, 26% glutelin, and about 6% each of albumin and globulin proteins [49]. Zein protein is rich in glutamic acid (20%), leucine (19%), proline (10%), and alanine (12%) [50]. As the grain fraction increases, concentrations of AA in WPMS likely increase. In a study of 1243 maize silage samples collected across two years (2018 and 2019), Huang et al. [48] observed that leucine concentrations were positively correlated with starch concentrations, supporting this hypothesis. Concentrations of leucine [51] are also greater in maize grain

relative to WPMS (11.2% vs. 8.6% of DM), suggesting that the kernel fraction, rather than the stover fraction, is the primary source of leucine in WPMS.

The effect of maturity, roll gap width, and storage length on concentrations of essential AA (EAA) is in Figure 4C. An interaction between roll gap width and storage length was observed for EAA ($p = 0.01$). Concentrations of EAA, which decreased from d 0 to d 120, were not different between WPMS processed through the 1 mm roll gap and that processed through the 3 mm roll gap. After 240 d of storage, concentrations of EAA were lower in silage processed through the 3 mm roll gap compared to the 1 mm roll gap. Maturity did not affect concentrations of EAA ($p = 0.14$).

The effect of maturity, roll gap width, and storage length on concentrations of non-essential AA (NEAA) is in Figure 4D. An interaction between maturity and roll gap width was observed for NEAA ($p = 0.05$). Concentrations of NEAA were greatest in early maturity silage processed through a 1 mm roll gap, intermediate silage processed through a 3 mm roll gap, and late maturity silage processed through a 1 mm roll gap, intermediate in intermediate silage processed through a 1 mm roll gap and in late maturity silage processed through a 3 mm roll gap, and lowest in early maturity silage processed through a 3 mm roll gap. These differences in NEAA concentrations are likely of minor biological significance. An interaction between roll gap width and storage length was also observed for NEAA concentrations. Similar to what was observed with EAA, concentrations of NEAA decreased from d 0 to d 120 in WPMS processed through the 1 mm roll gap and through the 3 mm roll gap. After 240 d of storage, concentrations of NEAA were lower in silage processed through the 3 mm roll gap compared to the 1 mm roll gap.

The effect of maturity, roll gap width, and storage length on concentrations of total AA is in Figure 4E. The interaction between maturity and roll gap width tended to have an effect on total AA ($p = 0.06$). An interaction between roll gap width and storage length was also observed for total AA concentrations ($p = 0.01$). Similar to what was observed with EAA and NEAA, concentrations of the total AA decreased from d 0 to d 120 in WPMS processed through the 1 mm roll gap and through the 3 mm roll gap. After 240 d of storage, concentrations of total AA were lower in silage processed through the 3 mm roll gap compared to the 1 mm roll gap.

The effect of maturity, roll gap width, and storage length on concentrations of AA protein (% CP) is in Figure 4F. An interaction between maturity and storage length was observed ($p < 0.001$) for AA protein. Concentrations of AA protein increased from d 0 to d 30 across all maturities. From d 30 to d 240, concentrations of AA protein decreased for both the early and intermediate maturity silages. Concentrations of AA protein were relatively unchanged from d 30 to d 240 for late maturity silage. At d 0 and d 30, AA (% CP) concentrations were not different between the three maturities. At d 120 and 240, however, concentrations of AA protein were lower in intermediate maturity silage compared to the early and late maturity silages. These data fit well with our ammonia-N results, which suggest increased deamination of AA in intermediate maturity silage at d 120 and 240.

The effects of mechanical processing and storage on the AA profile of WPMS are often overlooked in silage research. Der Bedrosian and Kung [52] observed that ensiling had no effect on the concentrations of total NEAA and total AA. However, ensiling caused a slight decrease in total EAA [52]. Changes in concentrations of the total AA have been reported when forages are ensiled [53]. Ensiling has also been shown to increase concentrations of free AA [54]. Both plant and microbial proteases in the silo are capable of degrading plant proteins to peptides and free amino acids [55]. Amino acids may subsequently be deaminated by silage microbes [56]. Huang et al. [48] observed that lysine and arginine were negatively associated with ammonia-N concentrations, suggesting changes in N fractions (AA, soluble CP, and ammonia-N) can be attributed to the combination of proteolysis and deamination during storage. Decreases in concentrations of arginine with ensiling have been reported by others as well [53,57].

4. Conclusions

Overall, this experiment demonstrated the influence that plant maturity, processing roll gap width, storage length, and their interactions could have on maize silage fermentation profile, nutrient composition, and starch disappearance. Lactic acid concentrations were greater, and pH was reduced in early and intermediate maturity silage compared to late maturity silage, indicating a less-thorough fermentation with delayed harvest. Although isSD was greatest for early maturity silage, intermediate for the intermediate maturity silage, and lowest for the late maturity silage from d 0 to d 120, our results suggest that differences in isSD due to maturity were diminished when WPMS was stored for 240 d. Furthermore, our results also suggest that reducing roll gap width from 3 mm to 1 mm may improve KPS of late maturity WPMS without substantial benefit to that of early and intermediate maturity WPMS. Together, these findings emphasize the fermentation and digestibility advantages that accompany proper harvest maturity and indicate that aggressive kernel processing and prolonged storage have the potential to improve starch availability of late maturity WPMS. When combined with our SCP and ammonia-N data, this study highlights the chemical and physical effects of proteolysis on maize kernels over the course of the storage period. Concentrations of EAA, NEAA, total AA, and AA protein decreased as the storage length progressed. Therefore, when WPMS is included at high rates in the diet, it may be beneficial to consider how the supply of AA coming from WPMS may change throughout the feed-out phase. Fatty acids were relatively unaffected by the storage length, however, suggesting that WPMS stored for a prolonged period is unlikely to put cows at a greater risk of developing diet-induced milk fat depression. Future research evaluating the effects of these treatment combinations on different maize silage hybrids harvested from multiple geographical locations is warranted.

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