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Effects of harvest date and additives on maize silage quality under boreal conditions

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There is increasing interest in Finland to cultivate maize for silage, although the climatic conditions are in the borderline for maize due to the short and cool growing season. This may result in an immature crop that differs from typical maize for ensiling by having low dry matter (DM) and starch concentrations. We evaluated the preservation characteristics of forage maize during 2019 and 2020 harvested in Helsinki, Finland, at two stages of maturity. The DM concentration of the crops ranged from 230 to 360 g kg^{-1}, and starch concentration from 179 to 283 g kg^{-1} DM. The crops were ensiled in laboratory scale using four different chemical organic acid based additives or a heterofermentative lactic acid bacteria inoculant. A control silage without additive treatment was also included. All silages were well fermented with low pH (average 3.75) and proportion of ammonia N in total N (average 47 g kg^{-1}). Formic acid based additives restricted silage fermentation and most chemical additives improved the aerobic stability of maize silages compared to the control and inoculant treated silages that, under the conditions of the current study, did not differ from each other.

Key words: aerobic stability, formic acid, maturity, silage additive, *Zea mays* L.

Introduction

Maize (*Zea mays* L.) is the single most important plant species used as whole-crop cereal silage for ruminant livestock feeding (Wilkinson and Rinne 2018). Although maize is originally a tropical plant, its cultivation has continuously extended towards northern latitudes. The research interest towards maize production in Finland has a long history (Virtanen 1938, Pulli et al. 1979, Setälä et al. 1979), but the field area used for maize silage production has remained low. Finland does not publish official statistics about maize area, but based on collected data the area was only 741 ha in 2023 having decreased from 1385 ha in 2019 (Anneli Partala, Natural Resources Institute Finland, Helsinki, Finland, personal communication). However, in the neighbouring countries, maize cultivation is already more common. According to official statistics from Estonia (Statistics Estonia 2023), the area used for maize cultivation has linearly increased from 1 700 ha in 2010 to 19 000 ha in 2023. In Sweden, the maize area increased more moderately from 16 300 ha in 2010 to 22 200 ha in 2022 (Swedish Board of Agriculture 2023).

The benefits of maize, i.e., high dry matter (DM) yield per hectare at single harvest and good milk production response, have tempted farmers even in Northern Finland to sow maize despite the short growing season and frost hazards both at the beginning and the end of the growing season. Liimatainen et al. (2022) demonstrated year and location effects on the maturity of maize achieved within Finland, as the starch content was 232 g kg^{-1} DM in Helsinki (60° N) during 2019 but only 31 g kg^{-1} DM in Kuopio (63° N) in 2020. Similar variable results have been obtained in other maize experiments in Finland (Pulli et al. 1979, Rinne et al. 2014, Partti 2019, Lehtilä et al. 2023). The variable results, particularly in the main milk production areas in central Finland, have probably contributed to the low maize area currently in Finland, but the conditions for maize cultivation are likely to improve in future due to increased temperature caused by climate change (Elsgaard et al. 2012).

The feed value of maize is highly dependent on the maturity stage that has been achieved. Under Finnish conditions, milk production has either remained stable (Sairanen and Kajava 2020, Kokkonen et al. 2024) or improved (Kokkonen et al. 2022), when grass silage has been replaced with maize silage. At the same time, the nitrogen use efficiency has improved due to the markedly lower CP concentration of maize vs grass silage (87 vs 145 – 160 g kg^{-1} DM for maize and grass silage, respectively, according to Finnish Feed Tables [Luke 2024]). However, if the maize is severely immature at harvest, milk production responses are poor (Auvo Sairanen, Natural Resources Institute Finland, Kuopio, Finland, personal communication). Here, as always, comparisons between different plant species must be interpreted with care, as the quality of both maize and grass silages vary widely also within species.

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A prerequisite for successful feed use of maize is a good preservation quality of the silage. Maize is considered a material easier to ensile than grasses or legumes due to the inherently high DM concentration, low buffering capacity and high availability of fermentable substrates (McDonald et al. 1991). Under boreal conditions, DM concentration may however remain low, even under 200 g kg^{-1}, which may cause suboptimal quality of maize to be ensiled depending on the annually varying growing conditions (Partti 2019, Liimatainen et al. 2022, Lehtilä et al. 2023). The objective of the current experiment was to evaluate the fermentation quality of maize silage under Northern European growing conditions. To give some indication of the annual variation, the experiment was repeated over two growing seasons. Two growth stages were used to simulate the effect of a varying length of the growing season affecting plant maturity at harvest. In addition, chemical additives commonly used for grass silage in the region were evaluated regarding their potential to ensure quality of maize silage of variable quality prior to ensiling as chemical additives are globally rarely used in maize silage production. A heterofermentative lactic acid bacteria inoculant was added in the second year as it is globally the most common type of additive used for maize silage preservation.

Materials and methods

Experimental silage preparation

The maize (variety Pioneer P7326, FAO value 180, Pioneer Hi-Bred International, Johnston, IA, USA; the most common forage maize variety used in Finland) was grown at Viikki research farm of University of Helsinki (60° N, 25° E, 8 meters above sea level), Finland, during growing seasons 2019 and 2020. The crops were sown 5 cm deep using a seed density of 90 000 seeds ha^{-1} on 9 May 2019 and 29 May 2020 using a plastic mulch film (Oxo-Biodegradable Clear Mulch Film, Samco Agricultural Manufacturing, Limerick, Ireland). The field was fertilized prior to sowing with 150 kg N per hectare in the form of a mineral fertilizer. The following herbicide treatments were applied: when seeding, pendimethalin (Stomp, 5 l ha^{-1}, active ingredient (a.i.) 400 g l^{-1}; BASF, Ludwigshafen, Germany), and approximately 3 weeks after seeding, thifensulfuron-methyl (Harmony 50SX, 10–15 g ha^{-1}, a.i. 500 g kg^{-1}; DuPont, Wilmington, NC, USA) and rimsulfuron (Titus WSB, 30–50 g ha^{-1}, a.i. 250 g kg^{-1}; DuPont, Wilmington, NC, USA).

The crop was harvested at two dates with 3-week intervals to obtain early (E) and late (L) maturity in order to evaluate the effect of plant developmental stage on the results. The rationale to harvest the maize early was to simulate a situation where a short growing season under boreal conditions limits maize development. The harvest dates were 17 September and 8 October in 2019, and 22 September and 14 October in 2020, resulting in growing season lengths of 131 and 152 days in 2019, and 116 and 138 days in 2020 for E and L, respectively. The ear development stages determined with R-scale were R4 (dough maturity) in 2019 and R5 (dent maturity) in 2020 at the early harvest, while for the late harvest, R5 stage was reached both in 2019 and 2020.

The crop was harvested with a farm-scale JF FH 1300 chopper (Kongskilde Agriculture, Albertslund, Denmark) leaving a ca. 15 cm stubble and with a chop length of 0.5–1 cm. No cracker was used at harvest. The chopped maize was immediately ensiled into pilot scale silos. A control treatment (CON) without additive application was used for all four maize batches. In addition, four different chemical additives were applied (Table 1). The compounds in the additives were formic acid, sodium formate, propionic acid, sorbic acid, potassium sorbate and sodium benzoate in various combinations and application levels. The treatments were coded based on the effective compounds so that F = formic acid, P = propionic acid, S = sorbic acid and B = benzoate, and compounds were either in acid or salt form. The number after the letter describes the proportion of the compound (%), resulting in additive treatments of F64P20S3, F83, P45B14 and B22S12. The dose of additives was 4 l ton^{-1} fresh matter except for B22S12, which was applied at a rate of 2 l ton^{-1}, following the commercial application recommendations of the manufacturers. During 2020, a lactic acid bacteria inoculant (LAB) which contained both homofermentative and heterofermentative strains was also used at a rate of 2 × 10^{5} colony forming units g^{-1} fresh feed, and the solution was produced using 1 g of product per 10 litres of tap water.

Four replicate silos were prepared for each treatment. The additives, and tap water for CON, were manually sprayed on 10 kg batches of chopped maize forage for each individual replicate, and carefully mixed. After that, the forage was placed into the cylindrical silos (internal diameter 14 cm, volume 12 litres) in ca. 500 g batches and an 8-kg lead plummet was let to drop freely 10 times on the top of the forage to compact it. Densities obtained were 926 and 797 kg m^{-3} in 2019, and 809 and 689 kg m^{-3} in 2020 for E and L, respectively. After sealing the silos with a plastic cover, plastic lid, an 8 kg lead plummet, and a water bag, they were stored at room temperature (ca. 20 °C), protected from light and opened after a storage time of 127, 127, 121 and 120 days for E2019, L2019, E2020 and L2020, respectively. The silos were equipped with a drain for effluent release, but none of the silos produced effluent.

Table 1. Additives used for experimental maize silage preservation

Treatment abbreviation	Commercial name (manufacturer)	Application rate	Active ingredients
CON		10 l t^{-1}	None (tap water)
F64P20S3	AIV Ässä Na (Eastman, Oulu, Finland)	4 l t^{-1} (+ 6 l t^{-1} tap water)	Formic acid, propionic acid, potassium sorbate, sodium formate
F83	AIV 2000 Plus Na (Eastman, Oulu, Finland)	4 l t^{-1} (+ 6 l t^{-1} tap water)	Formic acid, sodium formate
P45B14	GrasAAT (Addcon, Bitterfeld-Wolfen, Germany)	4 l t^{-1} (+ 6 l t^{-1} tap water)	Propionic acid, sodium propionate, sodium benzoate
B22S12	Kofasil Stabil (Addcon, Bitterfeld-Wolfen, Germany)	2 l t^{-1} (+ 8 l t^{-1} tap water)	Sodium benzoate, potassium sorbate
LAB [1)]	Feedtech Silage F600 (DeLaval, Tumba, Sweden)	1 g t^{-1} (2 ×10^5 cfu g^{-1}) mixed with 10 l t^{-1} tap water	*Lentilactobacillus buchneri* (DSM 13573, 1k20733; min. 1×10^{11} cfu g^{-1}), *Lactiplantibacillus plantarum* (DSM 3676, 1k20731; min. 0.5×10^{11} cfu g^{-1}), *Lactiplantibacillus plantarum* (DSM 3677, 1k20732; min. 0.5×10^{11} cfu g^{-1})

[1)] LAB was only included during year 2020

Chemical and statistical analyses

Fresh and ensiled maize samples were analysed for chemical composition and fermentation quality by routine methods as described by Seppälä et al. (2016). The oven DM determined at 105 °C overnight was corrected for loss of volatile compounds according to Huida et al. (1986). Starch was analysed from fresh maize samples, and from silages in 2019 according to Salo and Salmi (1968) using Shimadzu double-beam UV-VIS spectrophotometer UV-1800 (Shimadzu Co., Kyoto, Japan). Formic acid was determined only from those samples that had been treated with additives containing it. *In vitro* organic matter digestibility was determined only for the maize materials prior to ensiling and it was calculated from cellulase solubility utilizing the conversion equation for whole-crop silages (Huhtanen et al. 2006). The microbial analyses were conducted immediately after silo opening as described by Franco and Rinne (2023). When opening the silos, the visually spoiled silage was evaluated using a mould score from 0 to 3 (0 = no mould, 1 = slight mouldiness, 2 = moderate mouldiness, 3 = severe mouldiness). For aerobic stability measurements, silage was placed into polystyrene boxes with internal dimensions of 13.3 cm × 13.3 cm × 10.3 cm. Temperature was automatically recorded at 10-minute intervals and aerobic stability was defined as the time taken to increase the temperature of the sample for 2 °C above the ambient temperature.

Silos were weighed after filling and immediately before opening to calculate ensiling losses, and the observed weight loss was multiplied by a factor of 1.44 to account for the H_2O formation remaining in the silo while CO_2 is lost, according to Knický and Spörndly (2015). The fermentability coefficient (FC) was calculated according to DLG (2020) as:

FC = (DM (g kg^{-1}) + 8 water soluble carbohydrates (WSC; g kg^{-1} DM) / buffering capacity (g lactic acid 100 g^{-1} DM))/10.

Data was analysed with maturity stage and additive treatment as fixed effects and replicate as a random effect using the MIXED procedure (SAS Inc. 2002-2012, Release 9.4; SAS Inst. Inc., Cary, NC, USA) of SAS Statistical package. The different years were analysed separately because an additional additive treatment (LAB) was included in 2020. All data excluding LAB were also analysed together to evaluate the effect of the year, and results are discussed in text, when relevant. Contrasts were used to evaluate the effect of harvest. The pairwise comparisons of the treatment means were performed using Tukey's test at a probability level of $p<0.05$.

Results

Characterisation of maize before ensiling is presented in Table 2. The DM concentrations of maize before ensiling were higher in 2019 than in 2020 (320 vs 247 g kg^{-1}, respectively) and the higher starch content in 2019 than in 2020 (276 vs 213 g kg^{-1} DM, respectively) was in line with the higher DM concentration. Postponing the harvest by 3 weeks increased the DM concentration in both years, and starch concentration in 2020 but not in 2019. The WSC concentration reflected the starch concentration so that it was high when starch was low (E2020) and vice versa. The changes in ash and CP concentration and buffering capacity in relation to postponed harvest were minor, but neutral detergent fibre concentration increased.

Table 2. Description of maize batches before ensiling

Year	2019		2020	
Maturity	Early	Late	Early	Late
Harvest date	17 Sep	8 Oct	22 Sep	14 Oct
Temperature sum[1] since sowing, degree days	1473	1519	1334	1504
Dry matter (DM), g kg^{-1}	281	360	230	264
Buffering capacity, g lactic acid 100 g^{-1} DM	1.56	1.70	1.82	1.36
Fermentation coefficient	55	56	92	60
In DM, g kg^{-1}				
Ash	41	46	54	57
Crude protein	75	79	66	61
Starch	283	268	179	246
Water soluble carbohydrates	52	43	157	57
Neutral detergent fibre	395	434	451	478
Soluble N, g kg^{-1} N	370	510	610	410
In vitro organic matter digestibility	0.731	0.718	0.694	0.685
Microbial counts, cfu g^{-1}				
Total bacteria	3.5×10^7	3.1×10^8	6.2×10^7	6.9×10^7
Yeasts	2.5×10^6	2.4×10^7	1.7×10^6	5.1×10^6
Moulds	5.0×10^6	2.3×10^6	6.3×10^5	6.1×10^5

[1] Sum of effective temperature (mean daily temperature in °C minus 5) obtained from the closest station of the Finnish Meteorological Institute.

Silage characteristics are presented in Tables 3 and 4 for years 2019 and 2020, respectively. Progressing growing time decreased silage CP concentrations ($p<0.01$) during both years (77 vs 72 g kg^{-1} DM in E vs L, respectively), while ash decreased in 2019 ($p<0.01$) but increased in 2020 ($p<0.01$) although numerical differences were small and probably of minor importance considering feed use. The starch concentration in E2019 was higher in fresh material compared to the mean of ensiled samples (283 vs 240 g kg^{-1} DM) while in L2019 the values were much closer (268 vs 259 g kg^{-1} DM).

Based on the fermentation coefficient, all materials were classified as easy to ensile (fermentation coefficient > 45; Table 2). In line with this, the fermentation quality of the control silages was in general good (pH on average 3.81, proportion of ammonia-N in total N 65 g kg^{-1}, no excessive amounts of acetic or butyric acids) as indicated in Tables 3 and 4. The differences in DM concentration of maize before ensiling were reflected in silage fermentation quality so that the pH was higher in 2019 than in 2020 (3.79 vs. 3.70, $p<0.01$) while acetic acid concentration was lower (14.8 vs 20.7 g kg^{-1} DM, $p<0.01$). When both years were analysed together, postponed harvest increased pH (3.71 vs 3.78, $p<0.01$) and amino acid degradation (42 vs 52 g ammonia-N kg^{-1} total N, $p<0.01$) similarly in both years as the interaction effect was not significant.

All chemical additives had some positive features on the fermentation quality of the silages. The formic acid containing additives F64P20S3 and F83 had rather consistent effects on reducing pH, ammonia-N proportion and production of fermentation acids compared to CON ($p<0.05$). Additive P45B14 decreased pH in L2019, ammonia-N in both maize batches in 2019, and acetic acid in E2019 and L2020 compared to CON ($p<0.05$). Additive B22S12 decreased pH in L2019 and ammonia-N proportion in E2019 but did not affect fermentation quality in 2020. Ethanol contents were elevated in silages treated with formic acid as the main effective ingredient ($p<0.05$ for most pair-wise comparisons between F64P20S3 and F83 vs CON, P45B14 and B22S12). The only significant effect of LAB compared to CON (evaluated only in 2020) was a reduced ethanol concentration.

Table 3. The effect of harvest date and silage additive treatment on maize silage conservation characteristics during 2019. For additive treatment descriptions, see Table 1.

	Early harvest					Late harvest					SEM 1)	*p*-value
	CON	F64P20S3	F83	P45B14	B22S12	CON	F64P20S3	F83	P45B14	B22S12		Harvest
Dry matter, g kg^{-1}	255^{b}	255^{b}	249^{b}	263^{b}	258^{b}	317^{a}	311^{a}	313^{a}	307^{a}	313^{a}	3.4	<0.01
Density, kg fresh matter $(m^{3})^{-1}$	904^{abc}	932^{a}	926^{ab}	920^{ab}	949^{a}	753^{d}	821^{bcd}	818^{bcd}	807^{cd}	784^{d}	22.8	<0.01
pH	3.78^{cd}	3.69^{e}	3.75^{d}	3.80^{bc}	3.79^{bcd}	3.91^{a}	3.77^{cd}	3.78^{cd}	3.84^{b}	3.84^{b}	0.010	<0.01
Ammonium-N, g kg^{-1} N	67^{a}	20^{f}	22^{f}	48^{d}	54^{cd}	66^{a}	36^{e}	37^{e}	57^{bc}	63^{ab}	1.6	<0.01
In dry matter, g kg^{-1}												
Ash	44^{abc}	47^{ab}	48^{a}	45^{abc}	46^{abc}	42^{bc}	43^{abc}	44^{abc}	42^{bc}	41^{c}	1.1	<0.01
Crude protein	80^{abc}	80^{ab}	81^{a}	77^{abcd}	80^{abc}	74^{bcd}	74^{cd}	76^{abcd}	73^{d}	72^{d}	1.3	<0.01
Water soluble carbohydrates	25^{c}	22^{c}	16^{d}	35^{ab}	25^{c}	34^{ab}	15^{d}	13^{d}	37^{a}	31^{b}	1.0	0.09
Starch	243	231	242	245	238	262	252	261	263	259	11.3	0.01
Ethanol	5.7^{de}	18.5^{ab}	22.6^{a}	5.1^{e}	3.9^{e}	6.2^{cde}	12.2^{bcd}	12.7^{bc}	3.5^{e}	4.5^{e}	1.39	<0.01
Formic acid		10.0	9.8				7.9	8.6			0.18	--
Lactic acid	70.7^{a}	43.9^{d}	45.5^{d}	65.6^{ab}	67.4^{a}	54.4^{c}	39.5^{d}	42.2^{d}	59.2^{bc}	58.5^{c}	1.37	<0.01
Acetic acid	20.2^{a}	16.6^{b}	15.0^{b}	17.2^{b}	21.0^{a}	10.8^{c}	12.7^{c}	11.9^{c}	11.2^{c}	11.3^{c}	0.47	<0.01
Propionic acid	0.21^{f}	1.53^{c}	0.86^{de}	6.49^{a}	0.31^{f}	0.11^{f}	1.03^{d}	0.50^{ef}	4.56^{b}	0.12^{f}	0.101	<0.01
Propionic acid, corr. 2)	0.21^{b}	0^{b}	0^{b}	1.49^{a}	0.31^{b}	0.11^{b}	0^{b}	0^{b}	0.26^{b}	0.12^{b}	0.092	<0.01
Butyric acid	0.01^{ab}	0.01^{ab}	0^{b}	0^{b}	0.02^{ab}	0.03^{ab}	0.03^{ab}	0.01^{ab}	0.05^{a}	0.02^{ab}	0.009	<0.01
Volatile fatty acids, total 2)	20.4^{ab}	15.2^{c}	14.4^{c}	18.7^{b}	21.4^{a}	10.9^{d}	11.4^{d}	11.2^{d}	11.5^{d}	11.5^{d}	0.52	<0.01
Fermentation acids, total 2)	91.1^{a}	59.1^{cd}	59.8^{cd}	84.3^{a}	88.8^{a}	65.3^{bc}	50.9^{e}	53.4^{de}	70.7^{b}	69.9^{b}	1.65	<0.01
Fermentation products, total 2)	96.8^{a}	77.6^{cd}	82.5^{bc}	89.4^{ab}	92.7^{a}	71.5^{def}	63.1^{f}	66.0^{ef}	74.1^{cde}	74.4^{cde}	2.08	<0.01
Lactic acid:acetic acid	3.5^{bc}	2.6^{d}	3.0^{cd}	3.8^{b}	3.2^{bcd}	5.0^{a}	3.1^{cd}	3.6^{bc}	5.3^{a}	5.2^{a}	0.14	<0.01
Aerobic stability (2 °C), h	49^{c}	310^{a}	283^{a}	77^{bc}	187^{ab}	41^{c}	265^{a}	297^{a}	86^{bc}	94^{bc}	27.4	0.17
Fermentation losses, g kg^{-1}	9.2^{b}	11.6^{ab}	13.3^{ab}	29.8^{a}	9.3^{b}	9.8^{b}	10.2^{b}	10.8^{ab}	7.7^{b}	8.4^{b}	4.02	0.04
Mould score	1.19	1.50	0.94	1.00	0.94	1.06	0.88	0.94	1.25	0.94	0.244	0.52
Total bacteria, cfu g^{-1}	8.4×10^{5b}	1.8×10^{5b}	1.3×10^{6b}	1.6×10^{6ab}	1.0×10^{6b}	9.3×10^{6a}	8.7×10^{4b}	2.5×10^{5b}	4.3×10^{6ab}	2.0×10^{6ab}	1.6×10^{6}	0.04
Yeasts, cfu g^{-1}	3.6×10^{3ab}	3.9×10^{2ab}	1.6×10^{2b}	6.6×10^{2ab}	1.5×10^{4a}	6.8×10^{3ab}	1.0×10^{2b}	5.9×10^{3ab}	1.6×10^{3ab}	5.6×10^{2ab}	3.0×10^{3}	0.63
Moulds, cfu g^{-1}	2.7×10^{3}	5.0×10^{2}	1.9×10^{3}	6.6×10^{2}	3.1×10^{3}	2.6×10^{3}	3.3×10^{3}	2.5×10^{2}	1.8×10^{4}	1.7×10^{4}	7.6×10^{3}	0.20

1) Standard error of the mean presented for the comparison of all 10 treatments; 2) Corrected by removing 80 % of the calculated amount of propionic acid added with the additives. Treatment means with the same letter within a row are not significantly different ($p \geq 0.05$) based on Tukey's test.

Table 4. The effect of harvest date and silage additive treatment on maize silage conservation characteristics during 2020. For additive treatment descriptions, see Table 1.

	Early harvest						Late harvest						SEM 1)	*p*-value
	CON	F64P20S3	F83	P45B14	B22S12	LAB	CON	F64P20S3	F83	P45B14	B22S12	LAB		Harvest
Dry matter, g kg^{-1}	226^{b}	232^{b}	226^{b}	222^{b}	226^{b}	221^{b}	260^{a}	265^{a}	264^{a}	265^{a}	264^{a}	262^{a}	2.6	<0.01
Density, kg fresh matter (m^{3})$^{-1}$	805^{a}	801^{a}	819^{a}	812^{a}	812^{a}	806^{a}	697^{b}	690^{b}	697^{b}	677^{b}	696^{b}	678^{b}	19.1	<0.01
pH	3.75^{abc}	3.66^{bcd}	3.59^{d}	3.67^{bcd}	3.67^{bcd}	3.64^{bcd}	3.78^{ab}	3.62^{cd}	3.69^{bcd}	3.77^{abc}	3.77^{abc}	3.87^{a}	0.031	<0.01
Ammonium-N, g kg^{-1} N	61^{bcd}	20^{f}	19^{f}	53^{d}	58^{cd}	63^{bc}	68^{ab}	31^{e}	36^{e}	66^{ab}	62^{bc}	72^{a}	1.7	<0.01
In dry matter, g kg^{-1}														
Ash	54^{c}	54^{bc}	58^{abc}	58^{abc}	56^{abc}	57^{abc}	58^{abc}	56^{abc}	59^{a}	60^{a}	59^{a}	59^{ab}	1.0	<0.01
Crude protein	72^{abcd}	75^{abc}	74^{abc}	75^{ab}	74^{abcd}	76^{a}	72^{abcd}	71^{bcd}	70^{d}	71^{cd}	71^{cd}	71^{cd}	0.9	<0.01
Water soluble carbohydrates	34^{bc}	57^{a}	27^{bcd}	43^{ab}	41^{ab}	20^{cd}	10^{d}	16^{cd}	15^{cd}	34^{bc}	24^{bcd}	11^{d}	4.2	<0.01
Ethanol	5.1^{c}	12.2^{ab}	14.1^{a}	5.7^{c}	3.3^{c}	7.5^{bc}	13.7^{a}	12.9^{a}	15.1^{a}	6.2^{c}	6.4^{c}	7.3^{bc}	1.05	<0.01
Formic acid	-	11.1	11.2	-	-	-	-	10.5	10.6	-	-	-	0.18	-
Lactic acid	70.0^{ab}	38.5^{c}	54.4^{abc}	68.7^{ab}	71.9^{ab}	74.2^{a}	52.1^{bc}	39.1^{c}	42.6^{c}	64.4^{ab}	58.4^{abc}	53.0^{bc}	4.11	<0.01
Acetic acid	25.2^{ab}	18.0^{ab}	20.3^{ab}	21.6^{ab}	26.0^{ab}	27.5^{a}	28.4^{a}	14.2^{b}	13.8^{b}	14.1^{b}	25.5^{ab}	28.5^{a}	2.50	0.11
Propionic acid	0.21^{g}	2.45^{c}	1.25^{de}	9.13^{a}	0.22^{g}	0.26^{g}	0.28^{g}	1.57^{d}	0.89^{ef}	7.42^{b}	0.22^{g}	0.52^{fg}	0.105	<0.01
Propionic acid, corr. 2)	0.21^{cd}	0^{d}	0^{d}	3.23^{a}	0.22^{cd}	0.26^{cd}	0.28^{cd}	0^{d}	0^{d}	2.42^{b}	0.22^{cd}	0.52^{c}	0.098	0.17
Butyric acid	0.01	0.03	0.03	0	0	0	0	0.03	0	0.02	0	0	0.019	0.40
Volatile fatty acids, total 2)	25.4^{ab}	18.0^{ab}	20.4^{ab}	24.9^{ab}	26.2^{ab}	27.7^{a}	28.7^{a}	14.3^{b}	13.8^{b}	16.5^{ab}	25.7^{ab}	29.1^{a}	2.57	0.11
Fermentation acids, total 2)	95.4^{ab}	56.6^{c}	74.8^{bc}	93.6^{ab}	98.1^{a}	102.0^{a}	80.8^{ab}	53.4^{c}	56.3^{c}	80.9^{ab}	84.1^{ab}	82.1^{ab}	4.50	<0.01
Fermentation products, total 2)	100.5^{a}	68.7^{c}	88.8^{abc}	99.3^{a}	101.4^{a}	109.5^{a}	94.5^{ab}	66.2^{c}	71.4^{bc}	87.1^{abc}	90.6^{abc}	89.3^{abc}	4.93	<0.01
Lactic acid:acetic acid	2.8^{bc}	2.1^{bc}	2.7^{bc}	3.2^{b}	2.8^{bc}	2.7^{bc}	1.9^{c}	2.7^{bc}	3.1^{bc}	4.6^{a}	2.4^{bc}	2.2^{bc}	0.23	0.40
Aerobic stability (2 °C), h	50^{c}	157^{abc}	160^{abc}	107^{bc}	276^{a}	52^{c}	158^{abc}	281^{a}	264^{a}	139^{abc}	247^{ab}	164^{abc}	31.4	<0.01
Fermentation losses, g kg^{-1}	18.2	13.4	19.9	11.6	12.0	13.4	16.7	14.6	15.0	12.8	9.9	14.8	2.62	0.59
Mould score	0.88^{ab}	1.50^{ab}	1.63^{a}	1.38^{ab}	0.13^{b}	0.88^{ab}	1.81^{a}	1.44^{ab}	1.31^{ab}	1.50^{ab}	0.63^{ab}	1.44^{ab}	0.29	0.09
Yeasts, cfu g^{-1}	3.0×10^{5}	3.8×10^{4}	2.5×10^{2}	1.4×10^{3}	2.5×10^{2}	1.5×10^{5}	2.8×10^{4}	1.0×10^{2}	1.0×10^{2}	2.9×10^{2}	6.8×10^{3}	3.5×10^{3}	6.7×10^{4}	0.06
Moulds, cfu g^{-1}	9.2×10^{2}	1.3×10^{4}	3.1×10^{3}	7.8×10^{3}	1.8×10^{2}	9.3×10^{2}	5.4×10^{3}	2.5×10^{2}	3.2×10^{2}	3.5×10^{2}	4.6×10^{3}	5.2×10^{3}	4.1×10^{3}	0.49

1) Standard error of the mean, presented for the comparison of all 12 treatments. 2) Corrected by removing 80 % of the calculated amount of propionic acid added with the additives. Treatment means with the same letter within a row are not significantly different ($p \geq 0.05$) based on Tukey's test.

In general, additives had positive effects on the aerobic stability of the silages (Fig.1), but the responses varied in different silage batches. Further, the dispersion within data was large particularly in 2020 so that even rather large numerical differences did not reach significance, and the only significant difference compared to CON was the longer aerobic stability of B22S12 in E2020 ($p<0.05$). The average weight loss during fermentation was 13.2 g kg^{-1}, and the only significant difference was a higher value for P45B14 than in CON in E2019 ($p<0.05$). Total bacteria count was higher in CON L2019 than in formic acid treated silages ($p<0.05$) and yeast count was higher in B22S12 than in F83 ($p<0.05$), but no other significant differences were noted for microbial counts.

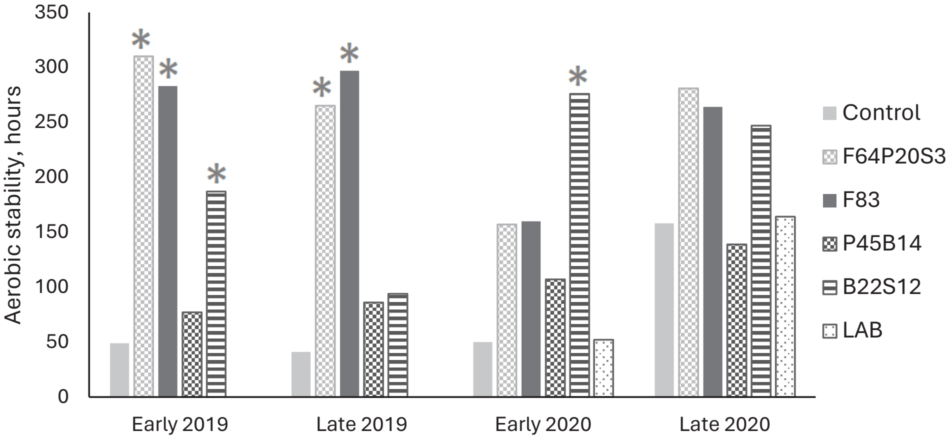

Fig. 1. Aerobic stability of maize silages harvested at early or late maturity stage in 2019 and 2020, and preserved without additive (Control) or using different combinations and amounts of organic acids (see Table 1). The bars marked with stars denote comparisons significantly ($p<0.05$) differing from Control within each silage batch.

Discussion

Maize composition prior to ensiling

The maturity of maize for silage is assessed based on the DM concentration of the crop, and target level for it is 300 – 350 g kg^{-1} (Khan et al. 2015). The range in maize maturity achieved in various Finnish data sets has been large, but this DM target has rarely been achieved. The DM levels in the present data, particularly in 2020, were clearly below this target, although in L2019 it was as high as 360 g kg^{-1}. The DM concentration has important implications for silage making as low water activity restricts fermentation and reduces the risk of malfermentation (McDonald et al. 1991), affects effluent production which typically ceases above DM concentration of 250 – 300 g kg^{-1} (Jones and Jones 1995) and has consequences for aerobic stability by affecting the porosity of silage mass in the silo.

When silage fermentation quality is considered, the starch content per se may not be very important, but rather the decrease in WSC and increase in DM concentration that change concomitantly with the increased maize maturity. The starch content however plays an important role regarding the nutritional value of the crop with a target value of ca. 300 g kg^{-1}. Such values are generally reached in main maize silage producing areas in Central Europe and Northern America as Khan et al. (2015) reported an average starch concentration of 333 g kg^{-1} DM and respective DM concentration of 338 g kg^{-1} in a data set based on 176 maize silages.

Under Finnish conditions, the DM and starch concentrations have typically been much lower. The maize crops produced simultaneously with the current material but in Kuopio had starch concentrations of 111 and 31 g kg^{-1} DM in 2019 and 2020, respectively (Liimatainen et al. 2022). Lehtilä et al. (2023) evaluated the effect of plastic mulch film at seeding on maize development and noted increases in both DM and starch concentrations. Again, in their data, clear differences were noted between geographical locations within Finland as averaged over 2019 and 2020, starch was 158 and 122 g kg^{-1} DM with and without the mulch in Helsinki (60° N) and 61 and 19 g kg^{-1} DM in Kuopio (63° N). Mussadiq et al. (2012) documented a similar clear geographical effect on maize development at different locations in Sweden (56 – 60 °N). Rinne et al. (2014) reported a starch concentration of 94 g kg^{-1} DM for nine different maize varieties with DM concentration as low as 162 g kg^{-1}, while Seleiman et al. (2017) harvested maize at 120 and 150 days after sowing, which resulted in starch concentrations of 111 and 214 g kg^{-1} DM. All silages in the current experiment reached starch levels that are at the high end compared to previous Finnish observations, even at the earlier maturity stage. This can be explained by the location in the South of

Finland, favourable weather conditions during the experimental years, use of plastic mulch at seeding, and the early-maturing maize variety used.

The smaller quality difference caused by 3-week difference in growing time of maize in 2019 compared to 2020 may partly be explained by the higher level of maturity achieved already at the earlier harvest time in 2019, which was also supported by the clearly earlier sowing date in 2019 compared to 2020. In addition, the weather conditions for the 3-week additional growth period in the autumn varied markedly between the years. Accumulation of the effective temperature was only 46 degree days in 2019 compared to 170 degree days in 2020.

The information on maize silage composition under practical conditions is starting to accumulate under boreal conditions (Laura Vaarnas, Valio Ltd., Seinäjoki, Finland, personal communication). The average Finnish farm sample DM concentration of maize silages during years 2014 – 2023 was 266 g kg^{-1} (n = 666) and starch concentration 156 g kg^{-1} DM (n = 329). The values in Estonia in 2023 were 331 g DM kg^{-1} and 315 g starch kg^{-1} DM (Andres Olt, Estonian Agricultural University, Tartu, Estonia, personal communication).

Maize silage fermentation quality

Maize silage is in general easier to ensile than grass or legume crops due to the lower buffering capacity and higher DM concentration and amount of fermentable substrates (McDonald et al. 1991). From silage preservation point of view, starch concentration is of minor importance as the lactic acid fermentation uses WSC as the primary substrate (McDonald et al. 1991). Lehtilä et al. (2023) observed a drastic decline in WSC from 245 to 78 g kg^{-1} DM with progressing growth, while simultaneously the starch concentration increased from 21 to 201 g kg^{-1} DM. Similar trends were reported by Mussadig et al. (2012) and Seleiman et al. (2017) as WSC are converted to starch with progressing physiological maturity of the maize plants. Although WSC concentrations were lowish in the current maize material prior to ensiling, it did not seem to restrict fermentation as low pH values in response to high fermentation acid production were achieved. The clearly higher sum of fermentation end products + residual WSC in both silage batches from 2019 as well as from L2020 than the WSC concentration of the pre-ensiled crop (103 vs 51 g kg^{-1}, respectively) indicates that there must have been some hydrolysis of starch or fibre fractions during the preservation phase. There was a clear decline of 43 g kg^{-1} DM in starch concentration in E2019 while in L2019 only 9 g kg^{-1} DM) when comparing the fresh and ensiled materials showing that at least in L2019, starch contributed only in a very limited extent as a substrate to fermentation. The greater loss in E2019 may be related to the earlier maturity of the seeds, or potentially overestimation of starch in fresh material due to uneven sampling.

Buffering capacity is another factor affecting silage preservation, but none of the studies reviewed earlier reported changes of buffering capacity in relation to maize plant maturity. The current maize crops showed no clear trends in buffering capacity in relation to maturity, and based on the fermentation coefficient, they were all classified as easy to ensile (DLG 2020). This supports the common use of lactic acid bacteria inoculants as additives in maize silage preservation, but the effect of chemical additives may be more consistent, as they are not as dependent on the complicated biological interactions in the silage fermentation process, and restriction of fermentation has potential to decrease DM losses during fermentation (da Silva and Kung 2022, Davies 2023).

There is a wide range of different chemicals that are being used as silage additives, and commercial products are often blends with several compounds with various activities (Muck et al. 2018). Further, the amounts of active ingredients vary in different commercial products. Formic acid based additives have shown consistent improvements in silage quality (Setälä et al. 1979, Seppälä et al. 2016, Franco and Rinne 2023) even in very challenging conditions such as extremely low DM grass and clover materials (Rinne et al. 2023). In line with this, F64P20S3 and F83 had positive effects on maize silage quality by restricting lactic and acetic acid production and degradation of N compounds. The effects of the other two chemical additives P45B14 and B22S12 on silage fermentation characteristics were not as clear when compared with CON, as the mode of action of their active ingredients propionic acid, benzoic acid and sorbic acid is more directed to controlling aerobic stability (Kung et al. 1998, Muck et al. 2018).

The responses to lactic acid bacteria inoculants are variable and depend at least on the quality and quantity of the epiphytic flora as well as the added strains, the availability of substrates and temperature, that affect the viability of the inoculants. Some experiments have reported significant improvements in maize silage fermentation quality in response to lactic acid bacteria inoculation (Hu et al. 2009, Jatkauskas et al. 2018), while Partti (2019) reported even decreased fermentation quality, and in the current experiment, no effects were observed. The responses to lactic acid bacteria have been variable also for grass silages under Finnish conditions as in some cases improvements were noticed (Franco et al. 2022a, Franco and Rinne 2023) while in other cases not (Franco et al. 2022b, Rinne et al. 2023).

Maize crop DM increases with progressing maturity, which has similar effects as grass silage wilting on silage quality. Reduced water activity restricts fermentation and risks for clostridial activity and butyric acid formation decrease (McDonald et al. 1991). Lynch et al. (2012) noted that the increasing DM with postponed maize harvest decreased fermentation and directed it to be more homolactic. The harvest time effect was similar in the current material, and some significant maturity × additive interactions were observed indicating that the differences between additive treatments were smaller in late rather than early harvest time. This can probably be attributed to the higher DM content of them similarly as noted for grass and clover materials (Seppälä et al. 2016, Rinne et al. 2023).

It makes sense to target high DM content of maize silage as it prevents effluent production (Jones and Jones 1995) and ensures high nutritional quality of maize linked with higher starch concentration (Khan et al. 2015). The fermentation quality of low DM maize silage (DM 190 g kg^{-1}) was poor (very high lactic and acetic acid concentrations) as reported by Partti (2019). The data from Finnish farm samples indicate that the average fermentation quality was rather good (pH 3.89, volatile fatty acids 16 g kg^{-1} DM and ammonia-N 25 g kg^{-1} N; n = 666 during 2014–2023; personal communication, Laura Vaarnas, Valio Ltd., Seinäjoki, Finland).

Under Finnish conditions, maize is typically harvested in October, when the temperature starts to fall, which may restrict in-silo fermentation. In the current experiments, the silages were fermented inside at room temperature so that the influence of cool temperature could not be evaluated. The average outdoor temperatures in Finland for October, November, December, January, February, March and April are 2.9. –2.1, –5.6, –8.3, –8.5, –4.4 and 1.4 °C (average for years 1990–2020, Finnish Meteorological Institute, Helsinki, Finland), respectively, so that it might be needed to evaluate the effects of low temperatures on maize silage fermentation. The low outside temperatures may also decrease silage heating at feed-out during winter.

Maize silage aerobic stability

Aerobic stability is an important practical factor for successful use of silage and in a worst-case scenario, the silage is only appropriate for composting. According to a farm survey conducted in Sweden (Nadeau et al. 2010), heating of maize silos, particularly during summer, is a common problem. In order to control silage heating, it is crucial to prevent the growth of yeasts, that typically initiate the aerobic deterioration (Wilkinson and Davies 2013). This requires good silo management to prevent air ingress into the silo such as tight compaction, not too high DM content of the crop and fast removal of feed. Although there were slight differences in the density of the different maize batches of the current study, they were unlikely to affect the aerobic stability although it is considered an important factor at farm scale. The lack of density effect under experimental conditions on aerobic stability was demonstrated by Franco et al. (2022a) and can be related to the laboratory silos being more airtight than farm scale silos, and the aerobic stability test being commenced immediately after silo opening so that air-ingress into the silage mass during the storage and feed-out phases is not mimicked.

Different types of silage additives are used to improve the aerobic stability (Muck et al. 2018). There are two main approaches in additive use to control heating that rely on different mechanisms. The use of heterolactic lactic acid bacteria, particularly *L. buchneri*, produces acetic acid with strong antifungal properties (Wilkinson and Davies 2013). The drawbacks of this method are the unpredictable production of acetic acid, and high DM losses linked with secondary fermentation of lactic acid to acetic acid (Davies 2023). The use of chemical additives is a more direct method, where antifungal substances are directly applied into the biomass, and results tend to be more consistent (da Silva and Kung 2022, Davies 2023). On the other hand, limitations of chemical additives include higher costs and corrosiveness of acids.

Chemical additives showed relatively good efficacy in improving aerobic stability in the current data (Fig. 1), although numerical improvements of P45B14 did not reach statistical significance in any of the 4 silage batches evaluated. The experimental procedure of mixing P45B14 with water may have precipitated benzoic acid, which could have reduced the efficacy of the product. The heterolactic inoculant was used only in 2020, but it did not modify the fermentation, i.e., acetic acid concentration was not increased, and subsequently, no differences in aerobic stability could be detected compared to CON. Formic and propionic acid based additives have shown good results in improving aerobic stability in grass silages under Finnish conditions (Seppälä et al. 2016, Franco et al. 2022a,b, Rinne et al. 2023).

The ability of lactic acid bacteria to improve aerobic stability, particularly inoculants that contain heterolactic strains such as *L. buchneri*, depends on how well they are able to modify the silage fermentation and increase the acetic acid concentration. This was not the case in the current experiment, but has been shown in other cases when heterolactic lactic acid bacteria have been applied (Hu et al. 2009, Tabacco et al. 2011, Queiroz et al. 2013, Jatkauskas et al. 2018). In the data of Queiroz et al. (2013) including nine different additives, the only inoculant containing *L. buchneri* and a chemical additive containing benzoate were effective in improving the aerobic stability against control, while other inoculants and chemical additives were not, highlighting that more work is needed to consistently improve the aerobic stability.

Conclusions

Maize silage of good fermentation quality could be obtained under the conditions of the current experiment (DM above 230 g kg^{-1}) even without the use of additives. However, application of chemical additives further improved several parameters of fermentation quality as well as aerobic stability of at least some batches of the maize silages. The inoculation with a heterofermentative lactic acid bacteria inoculant was not effective, but it was only applied during the second year of the study. Based on the current experiment and other experimental and practical experiences, good preservation of maize silage can be successfully achieved under boreal conditions. However, materials compromised by exceptionally short growing season resulting in very low DM concentration or severe frost damages were not included in the current data set. With maize silages, proper silage making techniques including the choice of an appropriate additive should be followed to prevent quality and quantity losses, similarly as with any other biomasses.

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