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Effect of propionic and formic acid mixtures on the fermentation, fungi development and aerobic stability of maize silage

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Abstract: The aim of the performed investigations was to determine the impact of preparations containing mixtures of propionic and formic acids (KP, KPM, KM) on yeast and mould fungi cell counts, chemical composition and aerobic stability of maize (*Zea mays* L.) FAO 250 (PIONEER) silages with 33% dry matter content in the first year and 35% dry matter content in the second and third years of experiments. Analyses were carried out at two dates: first, after opening the foil sleeve and then following a 7-day aerobic stability test. The performed chemical analyses included: determination of dry matter (DM), water soluble carbohydrates (WSC), crude protein (CP), lactic acid (LA), acetic acid (AA), butyric acid (BA), pH and deoxynivalenol (DON). The applied preservatives reduced significantly ($P<0.01$) the development of yeasts from log cfu 5.81 in the control (CCS) to log cfu 5.10 in the KM combination and also of moulds from log cfu 3.85 in the control to log cfu 3.11 in the KP sample. In addition, they also exerted a significant effect ($P<0.01$) on the DM increase from 320.5 g kg^{-1} in the control to 340 g kg^{-1} in the KM sample as well as on a significant ($P<0.01$) increase in WSC concentrations from 41 g kg^{-1} DM in the control to 48.5 g kg^{-1} DM in the KM sample. The employed chemical preparations also increased significantly ($P<0.01$) concentrations of crude protein, and reduced significantly ($P<0.01$) levels of lactic, acetic, and butyric acids and pH in comparison with the control. Last but not least, they reduced DON content in silages from maize and improved aerobic stability of silages subjected to 7-day oxygen exposure.

key words: silage, mould fungi, chemical preservatives, aerobic stability.

INTRODUCTION

Maize (*Zea mays* L.), due to its high carbohydrate concentration and low protein content and hence, low buffer capacity, is usually considered as an easily ensiled plant. The quality of the obtained silage depends, to a large extent, on the quality of the ensiled material, the applied ensiling technology as well as the way of its feeding. Maize silage constitutes environment very rich in nutrients and, therefore, provides a good substrate for the development of mould fungi (*Fusarium, Aspergillus*, *Penicillium, Mucor* etc.) and yeasts which may cause considerable nutrient losses once the silos are open during the feed-out phase. In addition, the above fungi can produce mycotoxins. The most common mycotoxins comprise: aflatoxins (AFLA), ochratoxin A (OTA), trichothecene (T2 toxin, HT–2 toxin) deoxynivalenol (DON), diacetoxyscirpenol (DAS) and zearalenone (ZON). Another dangerous development is the possibility of transfer or carry-over of mycotoxins to products of animal origin, especially milk and its products posing a serious hazard to consumers (Grajewski et al., 2007). That is why all measures should be undertaken to protect silages against their excessive fungal attack. Among increasingly popular and frequently applied silage additives are: formic and propionic acids, their mixtures and salts which can inhibit the development of undesirable microorganisms in silages exposed to aerobic conditions (Selwet et al., 2008).

The objective of the experiments was to assess the impact of preparations containing mixtures of formic and propionic acids (inhibitors) on changes in the cell counts of mould fungi and yeast, chemical composition and on aerobic stability of maize silages exposed to air during the process of feeding to animals.

MATERIAL AND METHODS

Silages were prepared from whole maize (*Zea mays* L.) FAO 250 (Pioneer) plants of 33% DM content in the first year and 35% DM content in the 2nd and 3rd years of investigations and harvested at the dough stage (cut height – 30 cm). The chopped plant material was ensiled in foil sleeves of AG BAG Company. Samples for analyses were

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taken after 120 days from ensiling after opening the foil sleeve prior to stability test and after 7-day aerobic test.

The experimental treatments comprised: CCS – control treatment (maize without additives), KP – maize supplemented with 85% propionic acid and 15% formic acid, KPM – maize + 50% propionic acid and 50% formic acid, KM – maize + 25% propionic acid and 75% formic acid. The doze of experimental preparations was 2 1 t^{-1}.

Samples for analyses weighing 1 kg were collected from AG BAG foil sleeves. Analytical solutions were prepared by adding 90 cm^3 of physiologic salt solution (NaCl) to 10 g of silage sample and homogenized for 10 min. Mould fungi and yeast cell counts were assayed using the plate method from consecutive solution dilutions on the oxytetracycline–glucose–yeast–extract agar (Oxoid) substrate. Incubation lasted 5 days and the incubation temperature was 25°C.

Basic composition of feeds was determined according to AOAC (1990), WSC – in accordance with the methodology given by McDonald and Henderson (1964), while ADF and NDF – according to Van Soest et al. (1991). pH values were determined with the assistance of the pH meter of Hann Instruments in suspensions prepared from 20 g silage and 180 m^3 demineralised water homogenized for 10 minutes.

Concentrations of fatty acids and ethanol were assessed using a gas chromatographer equipped with a FID detector, glass Supelco 80/100 Chromosorb WAW column 2 m long, I.G 2 mm filled with GP 10% SP-1200/1% H_3PO_4 and Varian 8200 CX autosampler. The gas carrier was hydrogen (flow – 30 cm^3 min^{-1}), furnace temperature – 120°C, injection temperature – 250°C and detector temperature – 300°C. Fluka Company acids were used as standards.

Deoxynivalenol (DON) was determined in accordance with the methodology given by Wiśniewska-Dmytrow and Kozak (2006). Vomitoxin was extracted from the examined material with the aid of water in the presence of polyethylene glycol. The extract was purified on the VICAM immunological affinity column (DON test™ HPLC) which contained antibodies specific for this mycotoxin. The deoxynivalenol (vomitoxin) was eluted from the column with the assistance of methyl alcohol. After thickening, the eluent was determined qualitatively and quantitatively with the liquid chromatography (LC) method using a UV-VIS detector.

The aerobic stability test was performed on 500 g samples placed in PCV containers with holes 5 mm in diameter. Samples were placed in a room where the temperature was 20°C ± 2. Changes in pH were measured every 24 h.

The effect of factors differentiating chemical composition and fungal cell counts in the examined silages was subjected to statistical analysis. Calculations were carried out employing the GLM procedure of the SAS program (1999) using the Tukey test.

RESULTS

Table 1 presents mean counts of mould fungi and yeast cells found in the experimental silages.

The applied chemical preparations reduced significantly ($P<0.01$) yeast cell counts (log cfu) in comparison with the control in which the counts were found the highest. The employed preparations reduced yeast cell counts by: KP – 6.5%, KPM – 5.7%, KM – 12.0%.

The highest ($P<0.01$) mould fungi cell counts (log cfu) were determined in the control sample and the inclusion in the silage of silage additives reduced mould cell counts in comparison with the control by: KP – 20.3%, KPM – 7.8%, KM – 7.2%.

Table 1. Microbiological analyses of corn silages before the aerobic exposure (log cfu).

Item	Year	Treatments				Mean
		CCS	KP	KPM	KM	
Yeasts	I	5.81	5.59	5.62	5.10	**5.53A**
	II	6.22	6.10	6.09	5.51	**5.98B**
	III	5.96	5.10	5.23	5.20	**5.37A**
Mean		**5.99a**	**5.60b**	**5.65b**	**5.27c**	
Mould	I	3.85	3.11	3.64	3.59	**3.55A**
	II	3.22	2.45	3.11	3.10	**2.97B**
	III	2.98	2.45	2.51	2.63	**2.64B**
Mean		**3.35a**	**2.67b**	**3.09c**	**3.11c**	

A, B – means in columns designated with the same letters do not differ significantly at the level of $P<0.01$.
a, b, c – means in rows designated with the same letters do not differ significantly at the level of $P<0.01$.
CCS – corn control silage, **KP –** corn + 85% propionic acid and 15% formic acid, **KPM** – corn + 50% propionic acid and 50% formic acid, **KM** – corn + 25% propionic acid and 75% formic acid.

Table 2 shows the chemical composition of the examined silage directly after opening the foil sleeve.

When analyzing means obtained from three years of experiments, in comparison with the control, samples supplemented with experimental chemical preparations revealed a significant ($P<0.01$) increase of dry matter, WSC and crude protein. The content of dry matter increased by: KP – 5.9%, KPM – 7.1%, KM – 7.7%, that of WSC by: KP – 13.0%, KPM – 15.7%, KM – 13,7% and of crude protein by: KP – 8.3%, KPM – 9.4%, KM – 8.9%, respectively.

Following the application of the experimental differentiating factors, concentrations of lactic, acetic and butyric acids were found significantly ($P<0.01$) lower in comparison with the control. The concentration of lactic acid was by 7.8% lower in the KP sample, by 8.4% in KPM and by 8.6% in KM. The content of acetic acid decreased by: 27.9% in the KP sample and by 25.8% in KPM and KM samples, while that of butyric acid, by 73.8% in the KP sample and by 75.4% in KPM and KM.

Table 2. Chemical analyses of corn silages before the aerobic exposure (g kg^{-1} DM).

Item	Year	Treatments				Mean
		CCS	KP	KPM	KM	
DM	I	320.5	337.1	339.2	340.1	**334.2A**
(g kg^{-1})	II	323.2	351.1	349.9	352.2	**344.1B**
	III	319.2	331.1	342.6	345.1	**334.5A**
Mean		**321.0a**	**339.8b**	**343.9c**	**345.8c**	
WSC	I	41.01	47.12	49.01	48.51	**46.41A**
	II	42.03	46.45	46.87	46.27	**45.40A**
	III	40.01	45.43	46.52	45.11	**44.27B**
Mean		**41.01a**	**46.33b**	**47.45b**	**46.63b**	
CP	I	76.45	81.14	83.05	82.21	**80.71A**
	II	73.89	80.32	81.21	80.45	**78.97B**
	III	71.12	80.01	80.21	80.34	**77.92B**
Mean		**73.82a**	**80.49b**	**81.49b**	**81.00b**	
LA	I	80.00	72.15	74.21	71.98	**74.58A**
	II	82.47	76.14	75.38	76.45	**77.61B**
	III	82.01	77.02	74.32	75.01	**77.09B**
Mean		**81.49a**	**75.10b**	**74.64b**	**74.48b**	
AA	I	12.56	10.03	9.94	10.01	**10.63A**
	II	13.12	9.12	9.75	9.09	**10.27A**
	III	13.54	9.12	9.41	10.01	**10.52A**
Mean		**13.07a**	**9.42b**	**9.70b**	**9.70b**	
BA	I	0.51	0.25	0.20	0.24	**0.30A**
	II	0.67	0.12	0.14	0.12	**0.26B**
	III	0.66	0.11	0.11	0.10	**0.24B**
Mean		**0.61a**	**0.16b**	**0.15b**	**0.15b**	
DON	I	0.70	-	-	-	**0.175**
(µl l^{-1})	II	0.71	-	-	-	**0.177**
	III	0.80	-	-	-	**0.200**
Mean		**0.74**				

DM – dry matter, WSC – water soluble carbohydrates, CP – crude protein, LA – lactic acid, AA – acetic acid, BA – butyric acid, DON – Deoksynivalenol.
Other explanations see Table 1.

Table 3. Microbiological analyses of corn silages after 7 days of the aerobic exposure (log cfu)

Item	Year	Treatments				Mean
		CCS	KP	KPM	KM	
Yeasts	I	6.89	5.61	5.70	5.27	**5.87A**
	II	6.99	6.17	6.20	5.99	**6.34B**
	III	6.97	5.21	5.31	5.51	**5.75A**
Mean		**6.95a**	**5.66b**	**5.74b**	**5.59b**	
Mould	I	4.17	3.23	3.76	3.63	**3.70A**
	II	4.09	2.65	3.23	3.18	**3.29B**
	III	4.34	2.68	2.68	2.71	**3.10B**
Mean		**4.20a**	**2.85b**	**3.22c**	**3.17c**	

Explanations see Table 1.

Silages treated with the experimental chemical preparations were characterized by lower pH levels than control samples (Fig. 1-3). Deoxynivalenol (DON) was found present only in the control samples (Table 2).

Table 4. Chemical analyses of corn silages after 7 days of the aerobic exposure (g kg^{-1} DM).

Item	Year	Treatments				Mean
		CCS	KP	KPM	KM	
DM	I	300.1	321.2	323.1	322.1	**316.6A**
(g kg^{-1})	II	299.8	333.1	334.2	333.7	**325.2B**
	III	288.2	329.1	332.6	325.1	**318.7A**
Mean		**296.0a**	**327.8b**	**330.0b**	**327.0b**	
WSC	I	37.41	46.01	47.10	47.15	**44.42A**
	II	35.11	40.18	41.16	41.05	**39.37B**
	III	30.01	42.41	44.53	43.10	**40.01B**
Mean		**34.18a**	**42.87b**	**44.26c**	**43.77c**	
CP	I	64.48	78.17	79.25	78.49	**75.10A**
	II	62.44	77.11	76.13	77.51	**73.30B**
	III	68.02	78.11	77.91	78.04	**75.52A**
Mean		**64.98a**	**77.80b**	**77.76b**	**78.01c**	
LA	I	62.45	70.14	69.97	69.87	**68.10A**
	II	62.11	70.26	70.84	71.18	**68.60A**
	III	52.11	70.12	70.12	70.11	**65.61B**
Mean		**58.89a**	**70.17b**	**70.31b**	**70.39b**	
AA	I	7.11	9.01	9.00	8.97	**8.52A**
	II	6.41	8.12	8.21	7.99	**7.68B**
	III	9.54	7.12	7.41	7.01	**7.77B**
Mean		**7.67a**	**8.08b**	**8.21b**	**7.99b**	
BA	I	0.32	0.19	0.18	0.20	**0.22A**
	II	0.40	0.09	0.09	0.09	**0.17B**
	III	0.31	0.10	0.11	0.11	**0.16B**
Mean		**0.34a**	**0.13b**	**0.13b**	**0.13b**	
DON	I	120.0	12.01	8.01	3.01	**35.76A**
(µl l^{-1})	II	110.1	11.03	7.10	3.12	**32.83B**
	III	111.1	11.02	6.20	3.13	**32.86B**
Mean		**113.7a**	**11.35b**	**7.10c**	**3.09d**	

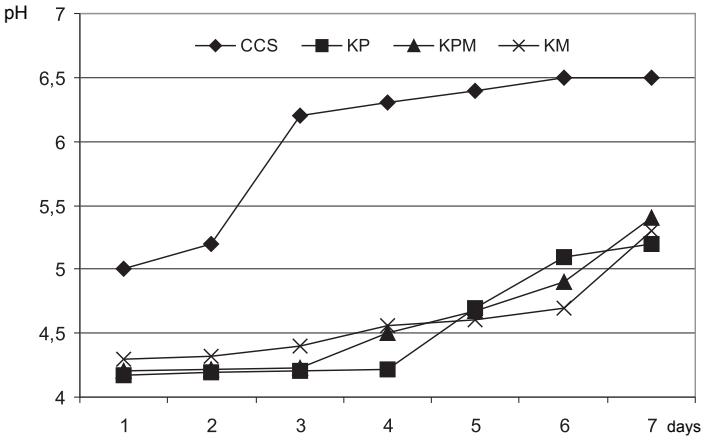
Explanations see Table 2.

The quality of experimental silages was assessed again following 7 days of exposure to air in order to check the impact of the examined preparations on biological and chemical transformations taking place during aerobic silage degradation.

Mould fungi and yeast cell counts in all samples were found higher in comparison with the results recorded before the aerobic test. The highest increases in cell counts (log cfu) were observed in the control samples, by 16.0% in the case of yeasts and by 25.4% – in mould fungi. The lowest increase in the yeast cell counts of 1.1% was recorded in the KP sample and of mould fungi cells (1.9%) – in the KM sample (Table 3).

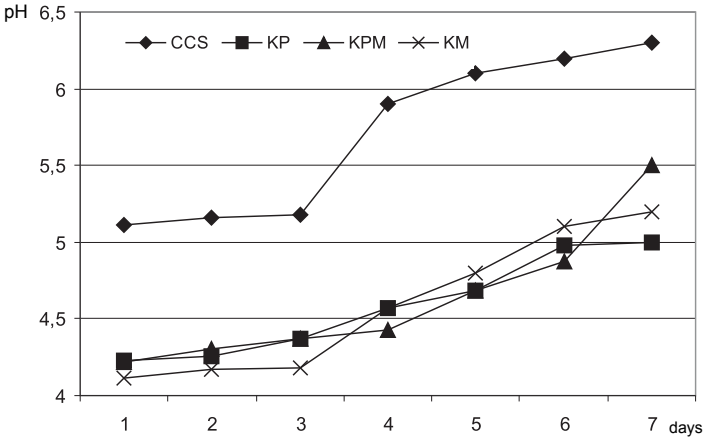
The chemical composition of experimental silages following 7-day aerobic test is presented in Table 4.

The seven-day aeration of silages resulted in losses of: dry matter, WSC, protein, lactic, acetic and butyric acids in all silage samples. The highest losses were observed in the control samples and they amounted to: 7.8% in the case of dry matter, 16.7% for WSC, 12% for crude protein, 27.7%



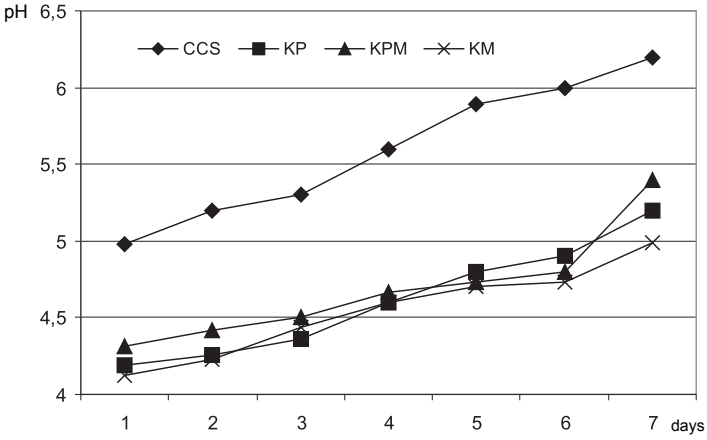
CCS – corn control silage
KP – corn + 85% propionic acid and 15% formic acid
KPM – corn + 50% propionic acid and 50% formic acid
KM – corn + 25% propionic acid and 75% formic acid

Fig. 1. Value pH in the silages after 7 days of the aerobic exposure – 1st year.



Explanations see Fig. 1.

Fig. 2. Value pH in the silages after 7 days of the aerobic exposure – 2nd year.



Explanations see Fig. 1.

Fig. 3. Value pH in the silages after 7 days of the aerobic exposure – 3rd year.

for lactic acid, 41.3% for acetic acid and 44.3% for butyric acid. All the employed preservatives were found to reduce losses of the selected chemical parameters in experimental silages. The smallest losses were determined in dry matter – by 3.5% – in the KP sample, WSC – by 6.1% – in KM, protein – by 3.3% – in KP, lactic acid – by 5.5% – in KM, acetic acid – by 14.2% – in KP and butyric acid – by 13.3% – in KPM and KM.

Values of pH increased in all silages after 7 days of the aerobic test with the highest increases recorded in the control silages (Fig. 1-3). Levels of pH in aerated silages were measured every 24 hours throughout the duration of the test. The obtained results show slowing down of pH levels in silage treated with the experimental silage additives. In control silages, stronger pH value increases were recorded between days 2 and 3 of the aerobic test (Fig. 1-3).

Aeration was also found to increase deoxynivalenol (DON) concentrations in silages with the highest increase of the mycotoxin of 99.3% determined in the control. Silages treated with experimental chemical additives contained significantly ($P<0.01$) lower deoxynivalenol levels.

DISCUSSION

Investigations on the effect of chemical preservatives on changes in cell counts of various microorganisms, chemical composition and aerobic stability of silages have been conducted by different researchers and vary significantly. It is also a fact that different preparations which can facilitate preservation of forages are widely used by farmers. The results of this research project showed that the inclusion in maize silages of preparations containing mixtures of formic and propionic acids reduced yeast and mould fungi cell counts in them. These results are corroborated by experiments carried out by Kung et al. (2004a), Selwet (2005) and Guerre et al. (2000). However, there are other papers which do not confirm this dependence and in which researchers reported intensive growth of fungi and increased mycotoxin production following the treatment of plant material with preservatives. This may be attributed to the response of moulds to environmental stress, especially during the full access of oxygen following the opening of the silo (Selwet, 2004).

According to some researchers, chemical additives containing short-chain organic acids and

their mixtures can have a positive influence on changes in silage chemical composition (Kostulak-Zielińska et al., 2002).

Silages treated with experimental chemical preparations were characterized by higher dry matter concentrations which could have been associated with the limitation of development of certain groups of microorganisms and, consequently, with smaller losses of nutrients. Increased dry matter concentrations in maize silages supplemented with chemical additives were also reported by Driehuis and Oude-Elfering (2000) as well as Raczkowska-Werwinska et al. (2008). The performed investigations also revealed increased WSC concentrations in comparison with control silages which could have been caused by restricted sugar fermentation by yeast cells and different groups of bacteria such as *Enterobacteriaceae* and *Clostridium*. These results are confirmed by investigation carried out by Kung et al. (2004b) and Selwet (2005). Silages treated by organic acids were also characterised by higher protein concentrations probably due to limited growth of microorganisms leading to reduced intensity of protein proteolysis (Selwet, 2008). However, different results were reported by Kleinschmit et al. (2005). In their experiments, the addition of organic acids increased lactic acid concentrations. These results were confirmed by experiments carried out by Steidlova and Kalač (2002) but not by trials conducted by Selwet (2008). It is possible that organic acids could have reduced counts of useful lactic bacteria and, consequently, decreased lactate concentrations. Concentrations of acetic acid were also decreased. Similar results were reported by Haigh (1998) and Selwet (2008), although experiments carried out by Kung et al. (2004a) fail to corroborate them. However, it should be stressed that silages treated by organic acids contained higher quantities of acetic acid following the stability test in comparison with the control samples. On the one hand, this is a favourable phenomenon because acetic acid may act as an inhibitor of yeast development, but on the other hand, its high concentration may limit feed intake by animals. The experimental differentiating factors reduced butyric acid concentrations in silages whose quantities in total organic acids should be as small as possible and decreased pH of silages in comparison with the control. These results were corroborated by studies conducted by Nadeau et al. (2000) but not by Kleinschmit et al. (2005). The highest concentrations of mycotoxins (DON) in the examined silages were determined in the control samples. Literature data confirm the effect of chemical additives on reduced concentrations of mycotoxins in silages (Selwet et al., 2008).

Recapitulating the results obtained in the course of 3 years of experiments, certain conclusions can be drawn. It appears sensible to use chemical preservatives containing mixtures of propionic and formic acids since they improve the hygiene value of silages. In addition, also the development of mould fungi was limited, including those which are responsible for the production of toxins that can cause diseases both in animals and in humans. Silage aerobic stability was also improved as a result of appropriate concentrations of lactic, acetic and butyric acids in the feed. In comparison with the control samples, production of mycotoxins in silages treated with the experimental chemical additives declined.

CONCLUSIONS

1. Preparations containing mixtures of propionic and formic acids can be recommended during the ensiling process of whole maize plants as inhibitors of yeast and mould growth as well as preservatives which can reduce nutrient losses.
2. The application of propionic and formic acid mixtures used at different proportions appears to be a very effective method for improving silage aerobic stability during aeration.
3. The observed synergistic influence of propionic and formic acids effectively reduced deoxynivalenol (DON) concentrations in maize silages during their aerobic decomposition.

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