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Influence of chemical inhibitors on fermentation process and hygienic quality of high moisture corn

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ABSTRACT

The aim of work was to find influence of chemical inhibitors on fermentation process and hygienic quality of high moisture corn. In experiment was used high moisture maize corn with content of dry matter from 602.0 to 613.3 g. After harvest was corn directly mechanically processed on crusher. Three different variants of high moisture corn were examined: K – untreated control, A – treated with a chemical inhibitor containing propionic acid and formic acid in application dose 3.5 kg per ton of maize corn, B – treated with a chemical inhibitor containing propionic acid, formic acid, benzoic acid and calcium formiate in application dose 3.4 kg per ton of maize corn. The maize corn was filled into plastic bins and placed in laboratory of conservation at 18-22^{o}C. After four months were plastic bins opened and the average samples were determined for content of nutrients, parameters of fermentation process and content of microscopic fungi. For determination of colony-forming units (cfu) 20 g samples of ground corn were soaked in 180 ml sterile tap-water containing 0.02 % Tween 80 and then 30 min. homogenized. Dilutions (from 10-1 to 10-4) in sterile tap-water with 0,02 % Tween 80 were prepared and 1 ml aliquots were incubated on three plates of Malt agar and Czapek-Dox agar with streptomycin (to inhibit the bacterial growth) and Petri dishes were inoculated using the spread-plate technique and incubated at 25 °C. Total cfu.g^{-1} counts of samples were determined after 5-7 days of incubation. For isolation and identification of genera of fungi Czapek-Dox agar and malt agar tender the same incubation conditions as cfu were used. Taxonomic identification of all colonies of isolated fungi was made according to Klich (2002), Samson et al. (2002), Hoog et al. (2000).

Moulds and mycotoxins are common contaminants of forage crops and silages made from them in many areas of the world. They pose a potential health hazard to domestic livestock. Changes in environmental conditions from pre-ensiling through fermentation result in the establishment of a characteristic mycoflora, mainly represented by Penicillium, Aspergillus and Monascus species. In addition to field-derived mycotoxins, the proliferation of these filamentous fungi upon subsequent exposure to air during feedout can result in further increase in the mycotoxin load of silages. Good management of the growing crop, the ensiling process and the unloading phase must be employed to minimize moulds and mycotoxins.

In our experiment addition of chemical inhibitors in conservation of high moisture corn did not affect occurrence of species and genus, but inhibited number of the microscopic fungi. From results follows that the highest value of pH had maize corn with addition of inhibitor containing organic acids and salts of acids in the cause the lowest content of lactic acid. High moisture corn with chemical additives had lower total content of fermentation acids. Addition of chemical inhibitors decreased content of lactic acid, acetic acid and did not affect content of butyric acid. After application of chemical additive consisting of organic acids had high moisture corn the lowest value of pH. Treatment of maize corn with chemical inhibitor composed of propionic and formic acid influenced hygienic quality and fermentation process expressively.

Keywords: high moisture corn, fermentation, microscopic fungi, chemical inhibitors

INTRODUCTION

Preservation systems of high moisture maize corn have influenced favourably cattle performance (Buchannan et al., 2003). To ensure good health status and high performance of cattle it is essential to produce silages with high nutrition value and meeting hygienic criteria at once. In addition to eventual contamination by pathogenic microorganisms e.g. Clostridium botulinum, Listeria monocytogenes and Escherichia coli O157 Fenlon et al., 2000; Thylin, 2000) the occurrence of filamentous fungi and their secondary toxic metabolites (mycotoxins)

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are the possible source of low animal performance and health disorders of farm animals (Bauer, 2002). The presence of various species of microscopic fungi and their metabolites affect sensoric feed properties as well as chemical and nutrition changes, feed intake and storage looses negatively (Sauer, 1988). One way to eliminate these undesirable effects is application of preserving agents. From the viewpoint of preventive treatment and inhibition of unfavourable microorganisms' development, warming-up and degradation of ensiled feeds there were studied different kinds of chemical substances (Jones, 1974). The propionic acid is most used preserving agent that is used separately or in combination with other acids (Bíro et al., 2003; Petersson, 1989). It is characterized by strong antifungal effect (Herting, et al., 1974). Similar inhibition effect has formic acid and acetic acid (Peterson et al., 1989). The application rate of propionic acid for preservation of moisture corn depends on moisture content. In recommended moisture content of corn about 35-40 % the application rate of propionic acid range from 14 to 17.5 g.kg-1 (Jones, et al., 1974). New approaches to ensiling additives development lead to the combined application of several groups of preparations in order to reach high efficiency and large utilization range. Combined applications of homofermentative lactic bacteria with benzoic acid and sorbic acid as well as mixture of homofermentative and heterofermentative lactic bacteria affect aerobic stability positively and so are able to reduce risks for growth of fungi and mycotoxins production in silages (Auerbach et al., 2000; Driehuis, et al., 2001; Owen, 2002).

The objective of the study was to evaluate effect of chemical inhibitors on fermentation process and hygienic quality (microscopic fungi occurrence) of high moisture corn.

MATERIALS AND METHODS

Biological material for experiment was obtained from Slovak Agriculture University, Experimental Farm in Kolíňany. The maize corn harvested with higher moisture content (dry matter content from 602,0 to 613,3 g) was mechanically processed by means of moisture corn crusher immediately after harvest. Ensilage matter was filled into PVC bins of 50 dm3 volume and was hermetically sealed, subsequently. In K variant (control) we ensiled moisture corn without preservative agents; in variants A and B we used various preservative agents. In variant A was applied chemical inhibitor composed of propionic acid and formic acid in dose of 3.5 $kg.t^{-1}$. In variant B was applied chemical inhibitor composed of propionic acid, formic acid, benzoic acid and calcium formiate in dose of 3.4 $kg.t^{-1}$. Each of variants was ensilaged in three repetitions and stored at temperature 18 – 22 °C for four months in Laboratory of Feed Preservation at Department of Animal Nutrition, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra. After termination of fermentation process the vessels were opened and we took average samples for analysis of nutrient content and basic fermentation characteristics according to the Regulation of Ministry of Agriculture of the Slovak Republic no 1497/4/1997-100 [16]. The occurrence of microscopic fungi was identified in average samples consequently. For assessment of colony-forming units (cfu) in 1 g of substrate we used the plate dilution method. Twenty g of sample was added to the 180 ml sterile water that contents 0.02 % of Tween 80. This way prepared dilution (10-1) was homogenized at horizontal shaker for 30 minutes. For inoculation on the agar growth media we used 1 ml of dilution 10-1 to 10-4 in three repetitions. The number of microscopic fungi was quantified after 5 day dark incubation in 25 ± 1 °C. Subsequently the microscopic fungi were identified either directly or after inoculation and cultivation (5-10 days) at identification nutritious media.

In-process of identification of fungi genus and species the following characteristics were studied:

a) cultivation traits: colony growth rate, colony shape, edging of colony, colony surface, colony colour, production and secretion of pigment to the environment, production of exudates at the colony surface,
b) morphological traits: presence of asexual spores, their shape, size, the way of their production and structurization, type of vegetative fructification structure, its shape and structurization, presence of special formations (rhizoids, sclerotia, chlamydospores, stolones etc.), presence of sexual fructification structures and spores.

Morphological characters were specified in slide cultures and preparations with lacto-phenol. The classification of single fungi genus and microscopic fungi species was done according to the following authors Klich (2002), Samson et al. (2002), Hoog et al. (2000).

For cultivation of microscopic fungi we used two cultivation media to perceive greater genus and specie spectrum of fungi, which is malt agar and Czapek – Dox agar with Bengali red and tetracycline. Bengali red has antibacterial effect and limit growth of microscopic fungi with rapid mycelia growth (e.g. Rhizopus, Mucor, Absidia and others). Antibiotic tetracycline is used for inhibition of bacteria growth.

RESULTS AND DISCUSSION

a) Fermentation process

Dry matter content in particular variants vary from 598.1 to 603.8 g, whereas the differences were not significant statistically (tab. 1). Buchannan et al. [4] indicate the optimum moisture of preservation grain in the range from 200 to 350 g.kg-1. Maize grain with addition of inhibitors at the base of organic acids and their salts (B) has the highest pH because of the lowest content of lactic acid. In variant A we recorded the lowest pH. The differences compared to the control variant K were statistically significant. The content of lactic acid in preserved grain was higher than 10 g per kg of original mass only in control variant K. Chemical additives inhibited lactic-fermentation bacteria growth as we found out that the content of lactic acid per kg of origin mass was only 8.8 g (A) and 4.1 g (B). Sebastian et al. (1996) had recorded the same results after application of propionic acid. In comparison to variant without treatment we identified that the content of fermentation acids were lower in variant A and B. The application of propionic acid, which was component part of both additives, expressed significantly on decrease of acetic acid content. Decrease in acetic acid content consequently after propionic acid

Table 1: Result of fermentation process

Variant n = 3		Dry matter g	pH	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Formic acid
						g.kg^{-1} of dry matter		
K	$\bar{x}$	603.8	3.75	24.3	3.7	0.2	0.6	0.2
	s	0.609	0.006	0.678	0.264	0.009	0.065	0.034
	v	1.008	0.154	2.794	7.076	4.605	11.271	20.24
A	$\bar{x}$	600.0	3.74	14.7	1.3	7.2	0.7	0.9
	s	0.275	0.006	0.538	0.059	0.435	0.311	0.196
	v	0.459	0.155	3.673	4.726	6.056	46.236	20.858
B	$\bar{x}$	598.1	3.89	6.8	1.3	1.4	0.5	3.1
	s	0.285	0.031	0.554	0.274	0.326	0.056	0.848
	v	0.477	0.786	8.142	21.651	23.170	12.167	27.737
d K – A		-	+	+++	+++	+++	-	++
d K – B		-	++	+++	+++	++	-	++
d A – B		-	++	+++	-	+++	-	+

+P<0,05 ++P<0,01 +++ P<0,001; $\bar{x}$ – average; s – standard deviation; v – coefficient of variance; d – difference between variants;
P – signifficant diffrences

Table 2: Occurrence of species of microscopic fungi isolated from preserved high moisture corn

Variant n = 3	Species of microscopic fungi	on Czapek-Dox agar	on malt agar
		cfu . g^{-1}	
K	*Acremonium sp., Penicillium sp., Rhizopus stolonifer*	0 6.104 76.103	3 3.104 74.103
A	*Acremonium sp., Fusarium sp., Mucor circinelloides, Mucor racemosus Paecilomyces variotii, Penicillium sp., Scopulariopsis sp.*	6.102 11.10 0	7.104 1.102 1.104
B	*Fusarium sp., Paecilomyces variotii, Penicillium sp., Rhizopus stolonifer*	6.104 0 3	5.104 3.102 2.103

application was recorded by Kung et al. (2004). In corn grain was stated occurrence of butyric acid; the highest content was in variant A.

b) Hygienic quality

The number of microscopic fungi in preservation moisture corn on Czapek – Dox agar was from 0 to 76.103 cfu per gram of dry mass (tab. 2). The number of microscopic fungi was influenced by treatment methods in particular variants. The greater mycological contamination was recorded in variant without additives (K). In maize grains treated by chemical additive (A) we stated the lowest microscopic fungi content, on the contrary. Sebastian et al. (1996) determined lower content of microscopic fungi after propionic acid application too, in comparison to control variant. The abundance of microscopic fungi on malt agar was in range from 3 to 74.103 cfu per g of dry matter. Also the samples of control variant K had the highest number of microscopic fungi on Czapek – Dox agar, whereas the lowest content was recorded in maize grain in variant B. Organic acids as propionic acid, butyric acid, benzoic acid and sorbic acid, as well as their salts are strong inhibitors of microscopic fungi (Fellner, 2001; Danner, 2003).

From analyzed samples we isolated and identified 7 representatives of mycromycetes genus (tab. 2), in variant A we recorded occurrence 6 genus, in variant B representatives of 4 genus and in variant without treatment (K) the occurrence of 3 genus was stated. The genus Acremonium, Fusarium, Paecilomyces, Penicillium a Rhizopus participated in contamination of maize grain, mostly. The genus Penicillium, Fusarium, Claviceps and Aspercillus are important producers of mycotoxins (Diekman, 1992). Penicillum sp. was the only species, which was isolated in all experimental variants. Sorbic and benzoic acids are particularly effective in growth inhibition of toxinogenic microscopic fungi Penicillium roqueforti, which occurs in silages generally. Current studies revealed that aerobic degradation of fermented crops caused by P. roqueforti fungi could be prevented by benzoic acid application, what stopped mycotoxins biosynthesis at the same time (Auerbach et al., 2000). Influence of used chemical inhibitors had not significant effect on genus or species representation of microscopic fungi in analysed samples of maize grain. Čonková et al. (1993) testing effect of five organic acids determined that propionic acid in concentration of 20 $ml.l^{-1}$ inhibited growth of five micromycetes species, while formic acid with the same concentration inhibited growth of two micromycetes species, only.

CONCLUSSIONS

Microscopic fungi and mycotoxins are the common contaminants of feeds and silages, which are produced from them. They present serious risks for farm animals. The environmental changes before ensilage and during the whole fermentation process resulted in development of characteristic microflora represented by species of Penicillium genus. Beside field-derived mycotoxins the proliferation of these filamentous fungi during silage handling and subsequent oxidation could result into further mycotoxin load of silages. To minimise contamination of silages by microscopic fungi and mycotoxins must by implemented suitable crop management, ensiling process and handling of preserved feeds.

From the observation of chemical inhibitors effects on fermentation process and hygienic quality of moisture corn grain results that:

1. Chemical additives decreased content of lactic acid, acetic acid as well as total content of fermentation acids, and never affect butyric acid content.
2. After application of chemical inhibitor at organic acids base (propionic acid, formic acid) the preservation corn grain has the lowest statistically significant pH.
3. Application of chemical inhibitors never effect genus or species representation of microscopic fungi.
4. Chemical preparations inhibited number of microscopic fungi.
5. The fermentation process and hygienic quality of moisture corn was mostly influenced by chemical inhibitor composed by propionic acid and formic acid.

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