

COMPARISON OF THE EFFECT OF BENZOIC ACID ADDITION ON THE FERMENTATION PROCESS QUALITY WITH UNTREATED SILAGES

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Abstract

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The influence of benzoic acid and formic acid (positive control) of ensilaged maize and pressed sugar beet pulp on quality fermentation processes was studied in a laboratory experiment. The effect of additive on the quality of fermentation process during maize ensiling was studied in a first model experiment. Preservatives such as formic acid and benzoic acid were added to ensiled maize at the concentration of 1L/t and 1 kg/t, respectively. When benzoic acid was used as a preservative, the pH and the N-NH₃/N total ratio decreased statistically ($P<0.05$) significantly while the titration acidity increased ($P<0.05$) in comparison with the control values. The addition of HCOOH to the maize silage reduced the level of acetic acid in dry matter by 11.3% while the overall level of acids decreased by 1.8%. The minimum loss of dry matter (2%) was found in the silage treated with formic acid in comparison with that of the control (11.0%).

Sugar beet pulp silages with benzoic acid or formic acid after 32 days of storage had a better sensuous evaluation than the control silage. The most intensive decrease of pH value was observed after formic acid addition as compared with control silage. The statistically significantly ($P<0.05$) highest lactic acid content (49.64 ± 0.28) as well as the highest ratio of LA/VFA were found in the sugar beet pulp silage with benzoic acid. Lactic acid constituted the highest percentage ($P<0.05$) of all fermentation acids in the silage with benzoic acid additive (65.12 ± 0.80). Undesirable butyric acid (BA) was not found in any variant of silages. The positive correlation between the titration acidity and acids sum in dry matter of silage conserved with formic acid was found. The additive of organic acids reduced significantly TA and fermentation acids content. Between the pH value and lactic acid content, no correlation was found.

maize silage, sugar beet pulp silage, benzoic acid, silage quality, fermentation process

Ensiling is a complex biological process based on the conversion of plant carbohydrates into individual fermentation products. The principles of ensiling technology are generally known and the respective ensiling techniques are very well elaborated. However, the quality of silage is still rather problematic.

Reduced quality of silage is often encountered in the case of legumes and grass. Low level of dry matter (DM), insufficient wilting, failure to observe techno-

logical procedures and underestimation of the effect of preservatives are the main causes of the low quality of silage. The studies conducted worldwide focus not only on the effect of bacterial inoculants but also on the protective effect of organic acids (particularly formic acid) or their salts which are currently used to preserve legumes and grasses. Benzoic acid, especially sodium benzoate, is one of the intensively studied preservatives. Benzoic acid, according to the

dissociation constant, is a weak organic acid and it does not require for its preservative effectivity such acid environment as formic acid does. It is, therefore, in the environment even less acidic and more effective than formic acid itself. Benzoic acid acts inhibiting on microorganisms in several ways. According to KYZLINK (1988), it inhibits functions of cytoplasmic membranes, of redox enzymatic activity and, simultaneously, it reacts with amino acid spectrum of microorganisms. In the environment with the pH value of ca. 4.0, it depresses effectively also yeast and fungi. To a safe inhibition of fungi, which are especially susceptible to benzoic acid, sufficient is the concentration of 0.08%, while for yeast, recommended is the benzoic acid concentration of 0.10-0.15% (KALAČ, 1975). Similarly to the other organic acids, only its undissociated form is effectively preservative. Preservation of ensiled feed using benzoic acid or sodium benzoate has been thoroughly studied. It has been demonstrated that benzoic acid additive to feeds preserved causes, among others, a significant loss decrease in dry matter and nutrients (JEPIFAKOV et al., 1983). LÄTTEMÄE et al. (1999) revealed a beneficial effect of additives containing sodium benzoate on the quality and storage stability of whole crop silage. The influence of different additives including sodium benzoate combined with lactic acid bacteria (LAB) on the quality of bale silage was studied by LINGVALL et al. (1999). The authors found that the addition of sodium benzoate and Lactisil to the silage improved the quality and storage stability of silage and reduced the number of spores which dropped below the detection limit.

The preservative effect of a mixture of organic acids containing 1% of benzoic acid during ensiling of grass-alfalfa mixed is reported by POTKANSKI et al. (1999). THYLIN et al. (2000) describe the effect of biological additives and sodium benzoate mixed with LAB on the quality of silage. Analogous results were published by RAMMER et al. (1999). The using of benzoic acid alone or its salt can be successful according to PODKOWKA and RYDZIK (1971) not only in carbohydrate feeds with rapid acidification in 0.15-0.30% ration, but also ensiling alfalfa and clover crops (NOVÁK and KALAČ, 1974). From the number of previous studies (PODKOWKA, 1972; PODKOWKA and PAULI, 1973) it is seen that the concentration of benzoic acid or sodium benzoate at ensiling clover crops should not exceed 0.40%. A beneficial effect of benzoic acid on ensiling of rot crops is reported by SLAVÍK et al. (1970), STIBIC et al. (1966), and sugar-beet pulp, by (NONN, 1983). NONN (1983) at ensiling of sugar-beet pulp with dry matter content lower than 20% found that the benzoic acid additive in the ration of 1.5 kg per 1 t fresh sugar-beet pulp pressed inhibited significantly the un-

desirable decomposition of pectin, acetic and butyric acids creation, and inhibited seriously alcohol creation. Benzoic acid added had a favourable effect on the resulting quality of fermentation process, and also affected positively the silage consistency, because it led to loss decrease in silage structures (COURTIN and SPOELSTRA, 1989).

The use of a number of the other preservatives in ensiling of sugar beet pulp was studied also by DOLEŽAL (2003), KOPŘIVA et al. (1991), LOUČKA et al. (1999) and others.

The main aim of the present paper was to evaluate the effect of benzoic acid on the fermentation of maize containing an increased level of dry matter, and sugar-beet pulp and to compare the quality of chemically treated silage with that of the control silage (untreated) and of the silage treated with formic acid.

MATERIALS AND METHODS

Experiment 1

The first model experiment on maize containing 340.2 g of dry matter per kg on average was carried out in the laboratories of the Mendel University of Agriculture and Forestry in Brno. The maize was ensiled into 50 L aluminium experimental vessels. Three separate batches of 30-35 kg of fresh maize were collected. Both control maize (untreated) and the maize supplemented with either 1.0 kg of benzoic acid/t or 1 L formic acid /t were pressed into 50 L experimental vessels. For each treatment, 30 kg of maize were mixed and pressed. The vessels were closed with a lid covered with a diluted solution of molasses and stored at 20-25 °C prior to analysis. After 6 months, 6 samples were taken from each experimental vessel and analysed for fermentation parameters.

Experiment 2

The second model experiment was based on assessments of the effect by benzoic acid applied homogeneously by hand in the ration of 1.5 g per kg sugar-beet pulp pressed with dry matter content of 122.7 g/kg. In six repeats, fresh sugar-beet pulp was ensiled in 4 L preserving jars immediately following after treatment. The fresh pulp was ensiled in 4-L preserving jars immediately following treatment. Silages with benzoic acid were compared with the control untreated silage and with the silage treated with formic acid (positive control). The level of addition of formic acid was 4 ml per kg of pressed pulp. The preserving jars were closed thoroughly and stored in the laboratory at the temperature of 27 ± 3 °C. Six of the experimental jars were opened after 32 days of storage and analysed.

Analytical methods:

Dry matter was determined at 105 °C (drying till constant weight) according to Czech National Stand-

ard 467092-42. All analytical procedures including the preparation of aqueous extracts were described previously (DOLEŽAL, 2002). The samples of silage were analysed for the pH value, volatile acids and lactic acid content. The ethanol, volatile fatty acid and lactic acid contents were determined as described previously (HARTMAN, 1974, 1980). Individual fermentation parameters of model silages were worked up statistically using variation analysis.

RESULTS AND DISCUSSION

The average content of dry matter in ensiled maize was 340 g/kg. Ensiled maize contained 86.7 g of CP, 22.4 g of lipids, 564.4 g of N free extractives, 244.9 g of crude fibre, and 9.95 MJ of metabolisable energy per kg of dry matter. The concentration of WSC (water soluble saccharide) was 226.1 g/kg. The average values of fermentation parameters of model maize silage are provided in Table I. It follows from the results in Table I that both acids (formic acid and benzoic acid) added to the maize silage at the given concentration did not significantly reduce the levels of lactic acid and acetic acid and did not affect the overall production of acids. The level of lactic acid in the maize silage treated with benzoic acid was only insignificantly increased compared with the control. Similarly, the presence of formic acid did not reduce the production of lactic acid and the level of acetic acid did not decrease significantly in comparison with that of other experimental silages. This indicates that formic acid might inhibit heterofermentative bacteria. However, the concentration used in the experiment (1 L/t) did not lead to significant inhibition of these bacteria. Our findings are in good agreement with the results reported by THYLIN et al. (2000). Silage extracts differed significantly ($P < 0.05$) by their pH values. Of all silages, the silage treated with benzoic acid showed the lowest pH (3.83 ± 0.01). The critical pH value, which is in agreement with our previous findings, was not exceeded. Unlike formic acid, benzoic acid is a weak acid, as can be concluded from its dissociation constant, and therefore requires less acidic environment to function as an efficient preservative. Furthermore, the above mentioned pH determined

in the silage extract may have some beneficial effect on yeast and other fungi. The lowest pH value in the experimental silage is in good agreement with the overall titration acidity (1741.82 ± 11.31 mg KOH), which is significantly increased ($P < 0.05$) in the silage treated with benzoic acid. The silage treated with formic acid showed the lowest titration acidity, which was lower ($P < 0.05$) than that of the control (untreated silage). Low titration acidity of this silage may be a consequence of a decreasing production of lactic acid and acetic acid. No statistically significant differences between individual model silages were found in the production of fermentation acids. However, in the case of silage preserved with formic acid (11.5%) the level of acetic acid was lower compared with that of the control. In the case of silage treated with benzoic acid the trend of increasing amount of acids was observed. This fact may result from the application of a low level of benzoic acid (1 kg/t). It is known that aromatic acids including benzoic acid have remarkable preservative and antifungal properties when present at certain concentrations (1.5-2.0 kg/t carbohydrate-containing crops). Benzoic acid can also be used as a suitable additive to preserve protein crops that are difficult to ensile. The amount of benzoic acid recommended for this purpose is 3-4 kg/t. Our results differ from the findings of LINGVALL et al. (1999) who found that the levels of both fermentation products and spores of *Clostridium* sp. in the grass preserved with LAB and sodium benzoate (400 g/t) decreased. A significant decrease of fermentation acids in ensiled feeds treated with sodium benzoate or benzoic acid was also reported by NONN (1987), THYLIN et al. (2000), POTKANSKI et al. (1999) and other authors. Our experiment revealed significant differences in the value of $N-NH_3/N$ total that reflects the level of protein decomposition. The statistically lowest value of $N-NH_3/N$ total ($P < 0.05$) was found in the case of silage preserved with benzoic acid (4.47 ± 0.17) while the control silage and the silage treated with formic acid showed a higher value of $N-NH_3/N$ total. It was found that the level of $N-NH_3/N$ total in silage is indirectly proportional to both titration acidity and the pH.

I: Chemical composition of maize silages

Silage	DM (g/kg)	pH	TA (mg KOH/100 g)	LA	AA	Sum of acids	N-NH ₃ N total	LA/AA
				g/kg DM				
Control untreated	330.97 ± 6.92	^b 3.90 ± 0.01	^b 1642.30 ± 8.05	62.30 ± 2.32	17.37 ± 2.19	79.67 ± 3.65	^b 5.56 ± 0.23	3.64 ± 0.49
Formic acid (1 ml/1 kg)	342.98 ± 5.11	^b 3.95 ± 0.01	^a 1512.12 ± 8.92	62.80 ± 11.61	15.40 ± 1.22	78.20 ± 11.26	^b 6.72 ± 1.13	4.12 ± 0.95
Benzoic acid (1 g/1 kg)	335.10 ± 4.31	^a 3.83 ± 0.01	^c 1741.82 ± 11.31	65.13 ± 1.66	17.40 ± 1.61	82.53 ± 2.21	^a 4.43 ± 0.17	3.77 ± 0.39

LA ... lactic acid, AA ... acetic acid, TA ... titrating acidity

Differences between values marked with different letters in the same row are significant (P<0.05).

The effect of benzoic acid added to sugar-beet pulp pressed (Experiment 2) is evident from Tab. II and Fig. 1 - 4. The results presented in the table indicate that after 32 days of storage, there are differences in values of fermentation products between individual groups. The lowest pH value (3.69 ± 0.012) was found in the with formic acid additive, while the highest one (3.77 ± 0.008) was in the silage with benzoic acid. These trends correspond to the nature of the acid used, as they were described previously by KALÁČ (1975) and KYZLINK (1988). The highest titration acidity (559.93 ± 6.046) was determined in the control silage, and it corresponds to the violent fermentation with a higher total creation of fermentation acids. In contrast to that, the experimental silages, especially with formic acid, indicated a significant decrease of titration acidity caused by statistically significant (P<0.05) reduction of lactic acid creation (11.76 ± 0.442) compared with the control untreated silage (57.80 ± 0.876), partly also with that with benzoic acid (49.64 ± 0.282). The formic acid additive in the ration of 4 ml per kg fresh sugar beet pulp caused a more remarkable inhibition of fermentation process than 1.5 g of benzoic acid per 1 kg of sugar beet pulp. Higher acetic acid and total acid contents in the control silage signalise the heterofermentative type in fermentation, supported likely also by decomposition of pectin substances. The effect of additive of formic and benzoic acid on the regression relation in comparison with control silage is evident from Fig. 2 - 4. A higher production of acetic acid in the control silage was caused not only by the higher temperature in the storage first phase, low saccharide content, but also evidently by the metabolization of pectin substances and hemicellulose. It is evident that the control silage, regards the lactic acid and acetic acid contents, had a worse up to unsuccessful quality than the experimental silages. Our results confirm the previous results by NONN (1983), KUBADINOV et al. (1984) and

by others that appropriate chemical preservatives inhibit decomposition processes in the sugar beet pulp silage. A significant inhibiting effect was registered in the experimental silages in ethanol creation, compared with control silage (6.91 ± 2.010). In the experimental silages, alcohol was not diagnosed. This finding is in agreement with the knowledge of KALÁČ (1987) that, at pectin substance fermentation, simultaneously an increased creation of methanol and total alcohol occurs. The alcohol higher production in silages is also associated with activity of undesirable microorganisms, which have the optimum conditions at this temperature range for their destructive activities (FLÁM, 1986).

SUMMARY

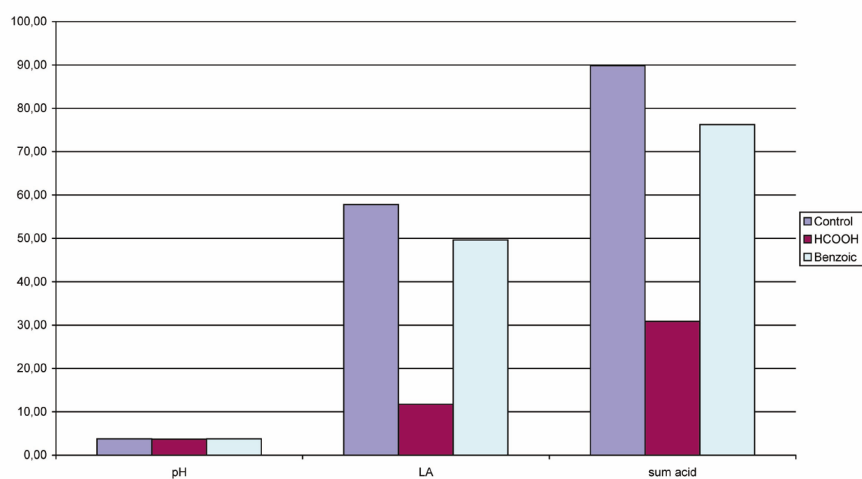
Chemical preservatives were shown to be useful in ensiling carbohydrate-containing feed such as maize. The use of chemical preservatives leads to the decrease of the pH while the titration acidity increases. Furthermore, chemical preservatives inhibit the growth of undesirable bacteria so that the level of volatile fatty acids (VFA) is low. Another advantage of the preservatives is that they reduce protein decomposition (CP) and the loss of dry matter. Chemical preservatives, which control the fermentation process, are necessary to maintain residual carbohydrates in silage. The main advantage of aromatic acids is that they show not only antibacterial effects but also antifungal effects. Suitable application dosage is a prerequisite. It was shown that the concentration of formic acid (1 L/t) and benzoic acid (1 kg/t) used in our experiment was not sufficient to maintain high quality of maize silage in all respects. The amount of both benzoic acid and formic acid to preserve carbohydrate-containing feeds should therefore be increased to 1.5-2.0 kg/t.

As well from the second model experiment of assessments on the effect of the formic and benzoic additives

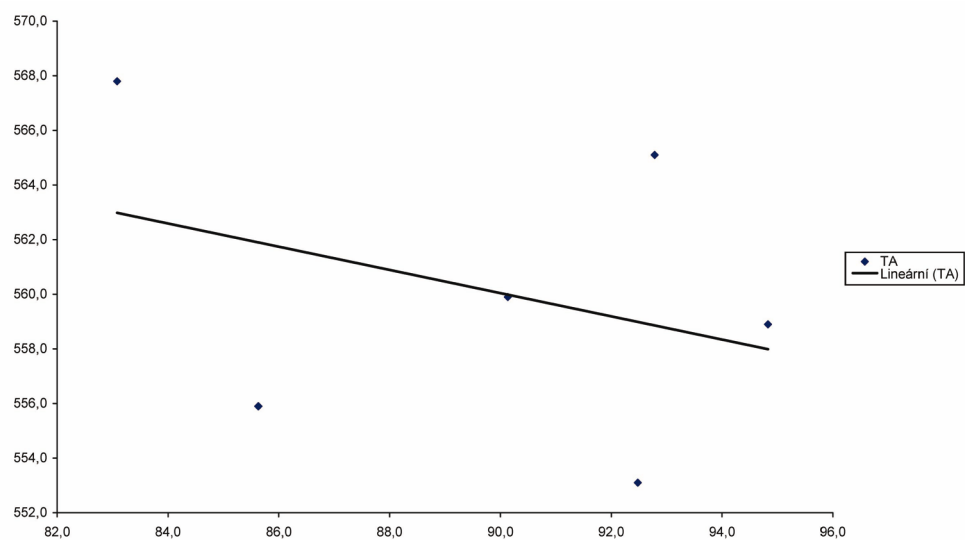
II: The average of fermentation parameters of sugar beet pulp silage after 32 days of storage

Silage	DM g/kg	pH	TA mg/100 g	NH ₃ mg/kg	LA g/kg DM	AA g/kg DM	Σ acids in DM	LA/VFA	Alcohol g/kg	%LA/Σ acids in DM
Untreated control (0)	^b 127,60 ± 2,610	^b 3,73 ± 0,005	^a 559,93 ± 6,046	55,30 ± 0,008	^a 57,80 ± 0,876	^a 32,02 ± 3,347	^a 89,82 ± 4,1634	^a 1,82 ± 0,196	^a 6,91 ± 2,010	^a 64,35 ± 1,983
Formic acid (4 ml/kg)	^a 137,10 ± 0,573	^c 3,69 ± 0,012	^c 392,27 ± 3,697	55,10 ± 0,007	^c 11,76 ± 0,442	^b 19,11 ± 0,285	^c 30,87 ± 0,494	^b 0,62 ± 0,024	0	^b 38,09 ± 0,997
Benzoic acid (1,5 g/1 kg)	^a 136,10 ± 1,661	^a 3,77 ± 0,008	^b 439,33 ± 3,653	55,20 ± 0,007	^b 49,64 ± 0,282	^{ab} 26,59 ± 0,944	^b 76,23 ± 1,003	^a 1,87 ± 0,065	0	^a 65,12 ± 0,801

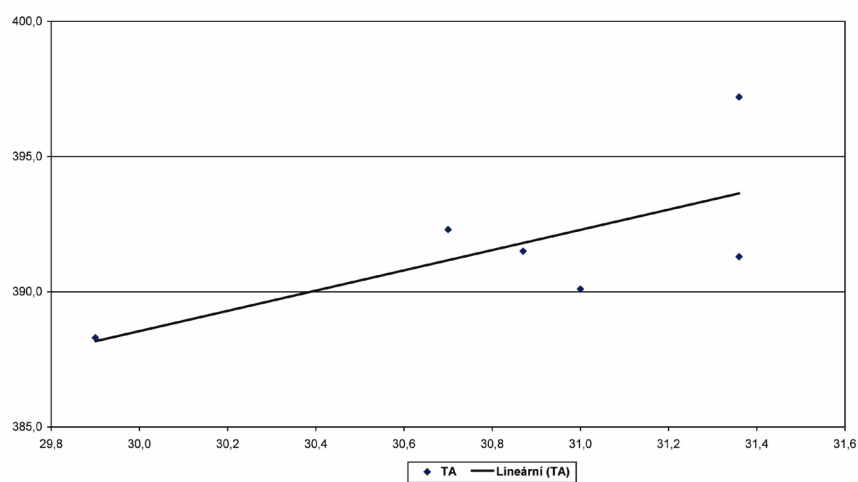
DM ... dry matter, TA ... titrating acidity, LA ... lactic acid, AA ... acetic acid, VFA ... volatile fatty acids, Σ ... sum of acids
Differences between values marked with different letters the same row are significant (P<0.05).



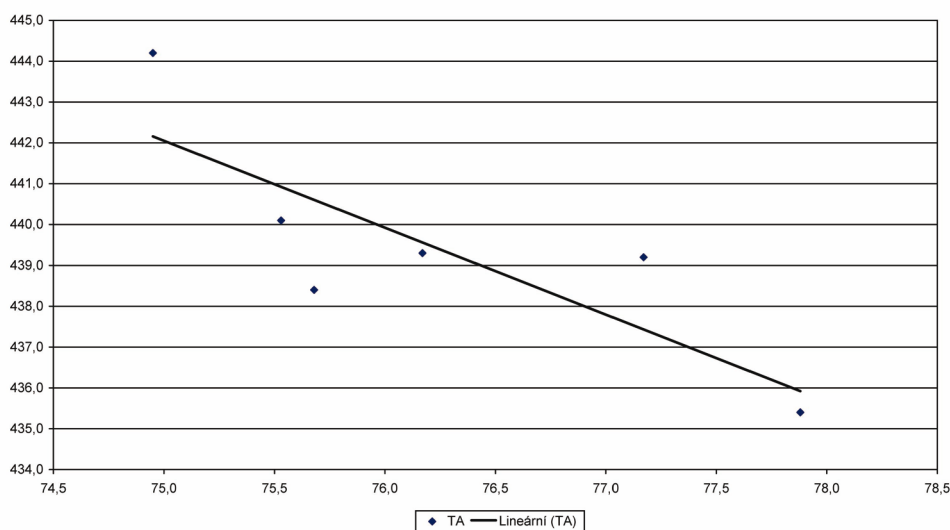
1: Influence of trial on parameters of silages acids



2: The Relation between the parameters of TA and acids sum in control silage



3: Influence of formic acid on regresion parameters between TA and sum acids



4: Influence of benzoic acid on regression parameter between TA and sum acids

in ensiling of sugar beet pulp pressed (DM 122.7 g/kg) on fermentation quality, the knowledge has resulted in that the formic or benzoic acid additives lead to the significant ($P < 0.05$) inhibition in creation of all fermentation acids, but especially of acetic acid. This reduction of acids is supported by a lower titration acidity. The

pH value was significantly lower ($P < 0.05$) in the sugar-beet pulp silage with formic acid. In the experimental silages, the better structure and significant inhibition of dry matter losses than in the control silage were found. The experimental silages compared with control silage contained no alcohol.

SOUHRN

Vliv přidavku kyseliny benzoové na kvalitu fermentačního procesu v porovnání s neošetřenými silážemi

Potvrdilo se, že i při silážování sacharidových krmiv, jako např. silážní kukuřice, lze použít chemické konzervační prostředky, které způsobí nejen přímou redukci pH, zvýší titrační kyselost siláže a současně potlačí nežádoucí skupiny bakterií a tím i tvorbu těkavých mastných kyselin. Předností aplikace těchto prostředků při konzervaci krmiv je také snížení rozkladu dusíkatých látek, snížení ztrát sušiny a celková redukce fermentace. Významnou předností je také větší uchování zbytkových sacharidů v silážích. Technologickou výhodou aromatických kyselin je vedle baktericidních účinků také vysoký protiplísňový efekt. Ukázalo se, že kyselina mravenčí v dávce 1 l/t nebo dávka kyseliny benzoové 1 kg/t silážní kukuřice není zcela dostačující ve prospěch všech kvalitativních ukazatelů fermentace kukuřičné siláže.

Také z druhého pokusu, ve kterém byl ověřován vliv kyseliny mravenčí a benzoové při silážování lisovaných cukrovarských řízů s obsahem sušiny 122,7 g/kg, vyplynul poznatek, že přidavek kyseliny mravenčí nebo benzoové způsobuje významnou ($P < 0,05$) inhibici tvorby všech kvasných kyselin, zejména však kyseliny octové. U pokusných siláží byla zjištěna lepší struktura a významné omezení ztrát sušiny než u kontrolní siláže. Pokusné siláže ve srovnání s kontrolní siláží neobsahovaly žádný alkohol.

kukuřičná siláž, siláž z cukrovarských řízů, kyselina benzoová, kvalita siláží, fermentační proces

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