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UTILIZATION BY LOW ENERGY PROCESSING OF PLANT ORIGIN MATERIALS CONTAMINATED WITH MYCOTOXINS

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Key words: cereals silage, lactic acid fermentation, ochratoxin A, zearalenone.

During cereals silage process 80-90% decomposition of zearalenone and ochratoxin A was observed. During lactic fermentation decomposition of ochratoxin A was in range 9-99% and was depended on activity of bacteria. The highest activity exhibited inoculum of lactic acid bacteria stored up to one week at +4°C.

INTRODUCTION

Since about 1960 with the first finding of aflatoxin B_1 in many countries started investigations on mycotoxins in food and feeds products.

On the base of our results [2, 3, 9, 19] it can be stated that percentage of samples naturally contaminated with mycotoxins was changeable and different depending on year of crops. Meanly 8% grain samples were contaminated with ochratoxin A, 0,5% with cytrynin, penicillic acid and zearalenone. Mixed feeds were contaminated with ochratoxin A in 1,5% of samples.

Additionally it was observed that every shird isolate of native microflora present in cereals was toxicogenic. Storage of cereal grains as mixed feeds in unproper conditions (high moisture) induces mycotoxins formation, losses in animal production and human health hazard.

Ensilaging of grain and green forage is well known fermentation process used for feed conservation, however zearalenone was found in ensilaged corn [8] and in our screening analyses presence of ochratoxin A in corn silage was exhibited (unpublished data). This aim of this paper was to elucidate fate of mycotoxins (zearalenone and ochratoxin A) during fermentation processes (silage and lactic fermentation).

MATERIALS AND METHODS

DECOMPOSITION OF ZEARALENONE AND OCHRATOXIN A DURING SILAGE

Ground corn grain contaminated with two levels of zearalenone 7.6 and 43.2 mg/kg and ground wheat grain contaminated with two levels of ochratoxin A 1.4 and 16.6 mg/kg were used in silage experiment. Humidity of material was adjusted to 40% and then 1% volume 24 hours old inoculum of lactic acid bacteria was added and mixed carefully. Silage process was performed in hermetic containers during 10 and 5 weeks period for corn and wheat respectively. Control experiment with mycotoxins free material was performed as well.

Zearalenone residue and pH were analysed according to method described earlier [1], and ochratoxin A residue was analysed by Nesheim method [7].

DECOMPOSITION OF OCHRATOXIN A DURING LACTIC FERMENTATION

Lactic fermentation was performed according to method continously used by Poznańskie Zakłady Przemysłu Spirytusowego in Leszno. Used in these experiments isolate of lactic acid bacteria (*Lactobacillus delbrückii*) — were stored 1 to 5 weeks at +4°C. During bacteria storage, inoculum preparing and fermentation process the following medium was used: 20% sucrose, 6% calcium carbonate and 2% rye germs. To the basic medium amounts ($\mu g/cm^3$) of ochratoxin A were added: 13,5, 1,4 in series I 1,7 and 0.17 in series II, 2.5 in series III, then autoclaved 15 min. at 121°C and inoculated with 10% volume of 24 hours old bacteria inoculum. Inoculum was prepared as follows: to the basic medium (ochratoxin A free) 10% volume of lactic acid bacteria suspension was added and incubated 24 h at 50°C. Some of media in series III were inoculated with inoculum double — incubated 24 h at 50°C. The control groups bacterial inoculum free as well as ochratoxin A free in all series were prepared.

Fermentation was performed 9 days at 50°C. Process was controlled according to routine methods (4, 5, 6): bound lactic acid according to versenate method, free lactic acid — titration method, not fermented sugars according to Nizowkin and Jemielianowa method. Ochratoxin A was analyzed as described by Nesheim [7].

RESULTS

DECOMPOSITION OF ZEARALENONE AND OCHRATOXIN A DURING ENSILAGING

The dynamics of zearalenone decomposition during ensilaging is presented on Fig. 1. After 10 weeks of fermentation still 10% and 18% of toxin residue was found in the beginning levels of contamination 2.6 and 43,2 mg/kg respectively. The highest rate of decomposition was observed during first three weeks of process. It can be pointed out that highly contaminated with zearalenone corn

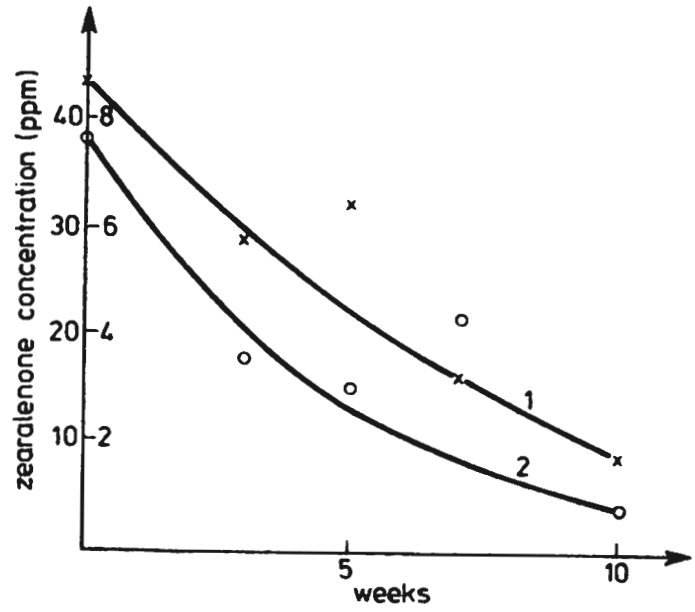

Fig. 1. Decomposition of zearalenone during ensilaging of corn grain; initial level: 1 — 43.2 ppm of zearalenone, 2 — 7.7 ppm of zearalenone

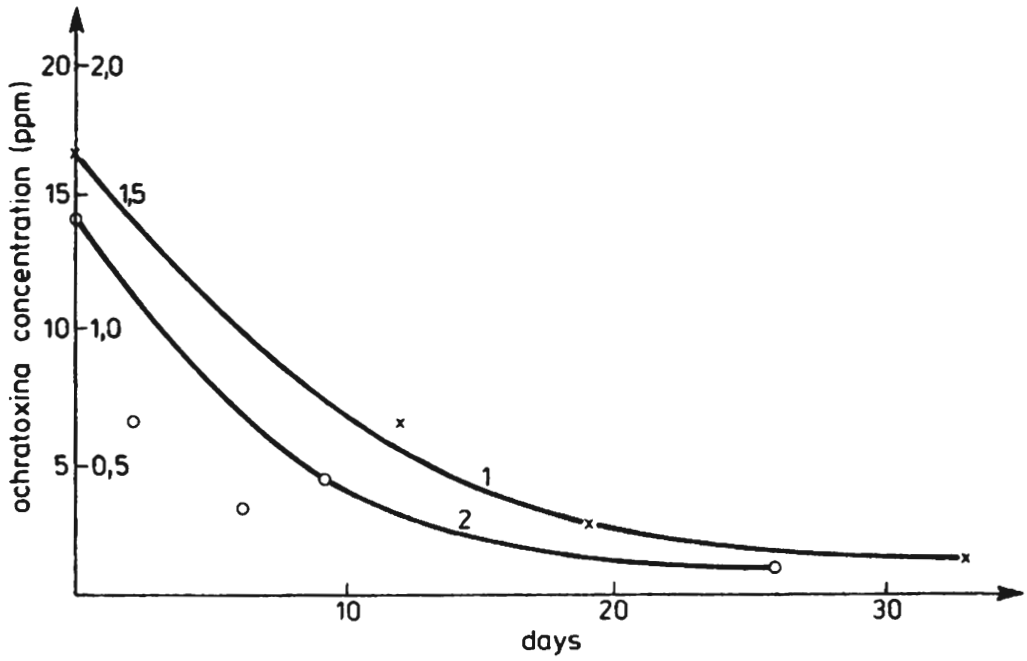

Fig. 2. Decomposition of ochratoxin A during ensilaging of wheat grain; initial level: 1 — 16.6 ppm of ochratoxin A, 2 — 1.4 ppm of ochratoxin A

will be not detoxified completly this way even after long (3 months) process. Ochratoxin A was decomposed more quickly and the rate of decompositions did not much depend on contamination level. The highest decomposition rate was observed during first 10 days (Fig. 2) and after 4 weeks only 10% of the beginning mycotoxin concentration was observed in both groups with level of contamination 1.4 and 16.6 mg/kg. Final pH value was 3.7 and ochratoxin A did not influenced ensilage process.

DECOMPOSITION OF OCHRATOXIN A DURING LACTIC FERMENTATION PROCESS

Results are presented in Tables 1-3. In control groups (bacterial inoculum free) decomposition of ochratoxin A during 9 days process at 50°C was not

observed which suggests that lactic acid bacteria probably due to hydrolitic peptydases are able to hydrolyze ochratoxin A.

The significant influence of bacteria age on process efficiency was observed and the highest decomposition rate was stated when one week old lactic acid bacteria inoculum was used (Tab. 2). Older bacteria were less active and 5 week old bacteria after 9 days of fermentation decomposed only 10% of the initial ochratoxin A content (Tab. 1). Efficiency of older then 1 week bacteria can be increased through their double — passage (Tab. 3).

Presence of ochratoxin A in fermentation medium did not influence efficiency of lactic acid fermentation mentioned process.

New compound with blue fluorescence and lower R_f value than ochratoxin A

Table 1. Decomposition of ochratoxin A during lactic fermentation using inoculum of 5 weeks old lactic acid culture

Variant of fermentation	Time of incubation (days)	Content or acidity				
		ochratoxin A ($\mu g/cm^3$)	sugar %	bound %	free %	total %
Control fermentation	2	0	6.2	3.9	0.1	4.0
	7	0	—	—	—	—
	9	0	4.2	5.7	0.2	5.9
Variant I a) without bacteria	2	12.3	7.5	1.8	0.05	1.85
	7	11.8	—	—	—	—
	9	12.0	7.3	1.2	0.05	1.25
b) with bacteria	2	13.5	6.4	2.8	0.1	2.9
	7	11.7	—	—	—	—
	9	13.0	4.3	4.1	0.1	4.2
Variant II a) without bacteria	2	1.5	7.1	1.9	0.04	1.94
	7	1.7	—	—	—	—
	9	1.6	7.0	1.4	0.05	1.45
b) with bacteria	2	1.4	6.6	3.8	0.1	3.9
	7	1.7	—	—	—	—
	9	1.5	4.0	5.6	0.2	5.8
Variant III a) without bacteria	2	0.11	6.9	1.9	0.05	1.95
	7	0.12	—	—	—	—
	9	0.11	7.0	1.5	0.07	1.57
b) with bacteria	2	0.15	6.7	3.9	0.1	4.0
	7	0.14	—	—	—	—
	9	0.12	4.4	5.9	0.2	6.1

Variant I — ochratoxin A concetration level 13,5 $\mu g/cm^3$
Variant II — ochratoxin A concentrarion level 1,4 $\mu g/cm^3$
Variant III — ochratoxin A concentration level 0,15 $\mu g/cm^3$

Table 2. Decomposition of ochratoxin A during lactic fermentation using inoculum of 1 week old lactic acid bacteria

Variant of fermentation	Time of incubation (days)	Content or acidity				
		ochratoxin A $\mu g/cm^3$	sugar %	bound %	free %	total %
Control fermentation	0	0	7.5	1.8	0.05	1.85
	2	0	—	—	—	—
	9	0	3.8	4.5	0.17	4.67
Variant I a) without bacteria	0	2.1	7.3	1.8	0.05	1.85
	2	2.2	—	—	—	—
	9	1.9	7.5	1.3	0.04	1.34
b) with bacteria	0	1.7	7.7	1.8	0.05	1.85
	2	0.2	—	—	—	—
	9	traces	3.4	4.5	0.15	4.65
Variant II a) without bacteria	0	0.17	6.9	1.8	0.05	1.85
	2	0.20	—	—	—	—
	9	0.23	7.1	1.4	0.04	1.44
b) with bacteria	0	0.17	7.3	1.8	0.05	1.85
	2	traces	—	—	—	—
	9	traces	3.4	4.4	0.14	4.54

Variant I — ochratoxin A concentration level 1,7 $\mu g/cm^3$
Variant II — ochratoxin A concentration level 0,17 $\mu g/cm^3$

Table 3. Decomposition of ochratoxin A during lactic fermentation using inoculum of 3 week old lactic acid bacteria

Variant of fermentation	Time of incubation (days)	Content or acidity				
		ochratoxin A $\mu g/cm^3$	sugar %	bound %	free %	total %
Control without bacteria	0	2.7	6.6	0.5	0,04	0.54
	2	3.2	—	—	—	—
	9	3.3	6.7	0.4	0,02	0.42
Variant I a) without ochratoxin A	0	0	7.1	1.3	0.05	1.35
	0	2	—	—	—	—
	9	0	3.6	8.4	0.22	8.62
b) with ochratoxin A	0	2.4	7.0	1.2	0.05	1.25
	2	2.8	—	—	—	—
	9	2.0	4.2	6.0	0.15	6.15
Variant II a) without ochratoxin A	0	0	7.3	1.1	0.05	1.15
	2	0	—	—	—	—
	9	0	2.4	9.2	0.29	9.49
b) with ochratoxin	0	2.6	7.4	1.0	0.05	1.05
	2	1.5	—	—	—	—
	9	0.2	3.4	6.8	0.18	6.98

Variant I — inoculum once passaged
Variant II — inoculum double passaged

appeared on thin layer chromatogram of fermentation medium extract. The properties of ochratoxin A decomposition product are under the study.

CONCLUSION

According to the performed investigations the following conclusions can be suggested:

1. Ochratoxin A and zearalenone in ensilaging process and ochratoxin A in lactic fermentation are decomposed with efficiency up to 90% of initial level of mycotoxin concentration.
2. New compound-product of ochratoxin A decomposition was observed on TL chromatograms.
3. Efficiency of ochratoxin A decomposition in lactic acid fermentation process depended on age of bacteria the highest being for one week old lactic culture.
4. Presence of mycotoxins did not influence fermentation process.

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ZASTOSOWANIE NISKOENERGETYCZNEGO PROCESU OBRÓBKI SUROWCÓW ROŚLINNYCH (ZIARN ZBÓŻ) SKAŻONYCH MYKOTOKSYNAMI

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Streszczenie

Badano zachowanie się ochratoksyny A oraz zearalenonu podczas procesów fermentacyjnych (fermentacja mlekowa i silosowanie): 80-90% rozkładu wymienionych toksyn zaobserwowano w ziarnie zakażonej kukurydzy podczas 10-tygodniowego silosowania oraz podczas 5-tygodniowego silosowania w ziarnie zakażonej pszenicy. Obecność mykotoksyn nie wpłynęła na przebieg procesów fermentacji.

Podczas fermentacji mlekowej obecna w kulturach bakteryjnych ochratoksyna A rozkładała się do nowego związku wykazującego niebieską fluorescencję. Najwyższy (90%) stopień rozkładu obserwowano przy zastosowaniu jednotygodniowej kultury bakteryjnej przetrzymywanej w temp. 4°C. Starsze kultury bakteryjne (trzytygodniowe) wykazywały podobną aktywność przy podwójnym ich pasażowaniu. Wiek kultury bakteryjnej, sposób przygotowania inoculum oraz obecność ochratoksyny A nie wpływały na ilość produkowanego kwasu mlekowego.