ZEARALENONE ADSORPTION ON A NATURAL ZEOLITE MODIFIED WITH DIFFERENT SURFACTANTS

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ABSTRACT

Adsorption of zearalenone (ZEN) by a natural zeolite modified with different amounts of benzalkonium chloride (BC) and cetylpyridinum chloride (CP) at pH 3 and 7 was investigated. Organozeolites (ZCPCs and ZBCs) were prepared with surface coverages of 20, 50, and 100 mmol of CP or BC /100 g natural zeolite. Results showed that adsorption of ZEN by the natural zeolite was low and adsorption by the ZCPCs and ZBCs increases with increasing amounts of solid phase in suspension. ZEN adsorption also increased with increasing amounts of CP or BC at the zeolitic surface. At the highest level of both surfactants (10 mmol/100g), a slightly higher adsorption of ZEN was observed when the zeolitic surface was modified with CP ions.

Keywords: mycotoxins, zearalenone, adsorption, zeolite, surfactants.

INTRODUCTION

Mycotoxins are a group of structurally diverse secondary fungal metabolites that occur as contaminants of grain worldwide. Contamination of cereal grains and animal feeds with mycotoxins continues to be a serious concern throughout the world. It has been estimated that mycotoxin contamination may affect as much as 25% of the world's food crops each year. The most common mycotoxins found in animal feed are aflatoxins, ochratoxins, trichothecenes, fumonisins, zearalenone, and ergot alkaloids [1].

Zearalenone (ZEN) is a non-steroidal mycotoxin produced by certain species of the genus *Fusarium*. Although ZEN intoxication is not commonly fatal, it can induce infertility, abortion, and other breeding problems in livestock [2, 3]. The chemical structure of ZEN is presented in Fig. 1.

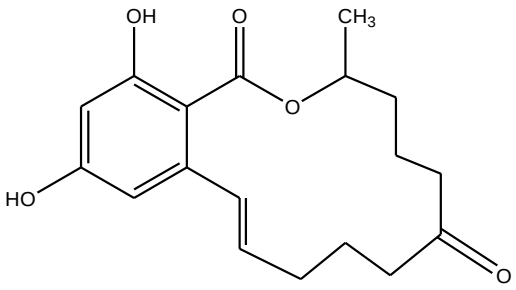

Figure 1. Chemical structure of zearalenone.

One approach to detoxify of animal feedstuffs, contaminated by mycotoxins, is the addition of nutritionally inert mineral sorbents, usually aluminosilicates (zeolite –

clinoptilolite and bentonite – montmorillonite) to the diet to decrease the bioavailability of the mycotoxins in the gastrointestinal tract of animals [4]. The natural zeolite (clinoptilolite) has the ability to bind aflatoxin B_1 under *in vitro* conditions. However, due to its hydrophilic and negative surface, the zeolite can not bind other mycotoxins, like ZEN. We previously reported that modification of the clinoptilolite with different levels of the surfactant – octadecydimethylbenzyl ammonium (ODMBA) chloride increased adsorption of ZEN. It was determined that ZEN adsorption increased with increasing amounts of the surfactant at the zeolitic surface indicating that ODMBA ions may be the active sites responsible for ZEN adsorption [5, 6].

Benzalkonium chloride (BC) and cetylpyridinum chloride (CP) are cationic surfactants widely employed as preservatives, solubilizing or wetting agents in various pharmaceutical preparations. They are considered safe for human use as preservatives for drugs, in eyewashes, spermicidal creams, and in several cleaners and disinfectants. The surface of the natural zeolite - clinoptilolite was modified with three different levels of CP and BC and the *in vitro* adsorption of ZEN at pH 3 and 7 was studied. The aim of this research was to determine if the type and amount of organic cation influences ZEN adsorption.

EXPERIMENTAL

A natural zeolite from the Zlatokop deposit (Vranje, Serbia) was used as the starting material for preparation of the organozeolites. The mineralogical composition of the natural zeolite was primarily clinoptilolite with smaller amounts of feldspar, quartz and pyrite as measured by qualitative X-ray powder diffraction analysis (XRPD). The cation exchange capacity (CEC) of the zeolitic tuff was 146 mmolM^+/100g, while the external cation exchange capacity (ECEC) was 10 mmolM^+/100g.

The surfactants, cetylpyridinum chloride (CP) and benzalkonium chloride (BC), were purchased from Sigma-Aldrich Co. The organozeolites were prepared by treating the zeolite with either CP or BC equivalent to 20, 50 and 100% of its ECEC (2, 5 and 10 mmol/100g). The natural zeolite (20 g) was mixed with 100 mL of each surfactant solution in a mixer at 5,000 rpm for 10 min at 50°C. The organozeolites were rinsed with distilled water and dried at 60°C. The CP organozeolites were denoted as ZCP-2, ZCP-5, ZCP-10 and the BC samples were denoted as ZBC-2, ZBC-5 and ZBC-10.

In vitro ZEN adsorption by the organozeolites was performed using the following procedure: duplicate aliquots of 0.1 M phosphate buffer (adjusted to pH 3 or 7) containing 2 ppm ZEN in solution (10 mL) were added to 15 mL Falcon polypropylene tubes to which had been added 20, 10, 5 or 2 mg of each adsorbent. In order to eliminate exogenous peaks, controls were prepared by adding 10 mL of 0.1 M phosphate buffer (pH 3 or 7) plus 20 mg adsorbent to Falcon tubes. The Falcon tubes were placed on a rotator shaker for 30 min at room temperature. The suspensions were centrifuged at 13,000 rpm for 5 min and 2 mL of the aqueous supernatant was removed for ZEN analysis. An aliquot of the original buffered ZEN test solution was used as the HPLC standard. The initial and final concentrations of ZEN were determined by HPLC and the percent of ZEN bound was calculated from the difference between the initial and final concentration in the aqueous supernatant after equilibrium.

RESULTS AND DISCUSSION

The organozeolites ZCPs and ZBCs were obtained by the ion exchange of inorganic cations at the zeolitic surface with surfactants. The highest levels of CP or BC in the organozeolites were equal to the ECEC of the natural zeolite.

Zearalenone is hydrophobic and is only slightly soluble in water. From chemical structure (Fig. 1), ZEN is a diphenolic compound with an estimated pK_{a1} = 7.62 [2],

suggesting that at pH 3, it is mainly in the neutral form, while at pH 7, the phenolate anion is present in solution.

Preliminary ZEN adsorption results ($C_{0\ ZEN}$ = 2 ppm; C_{susp} = 4 g/L) showed that the adsorption index on the natural zeolite was 7% at pH 3 and 7. Results for ZEN adsorption by the natural zeolite modified with different levels of CP (ZCPs) and BC (ZBCs) ,with different amounts of solid phase in suspension, and at pH 3 and 7 are presented at Figs. 2 and 3.

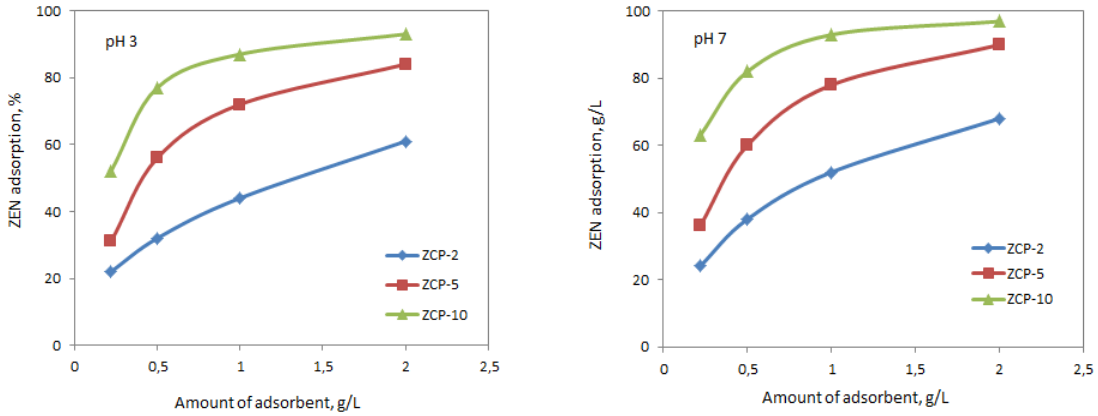

Figure 2. ZEN adsorption by organozeolites: ZCP-2, ZCP-5 and ZCP-10; pH 3 and 7.

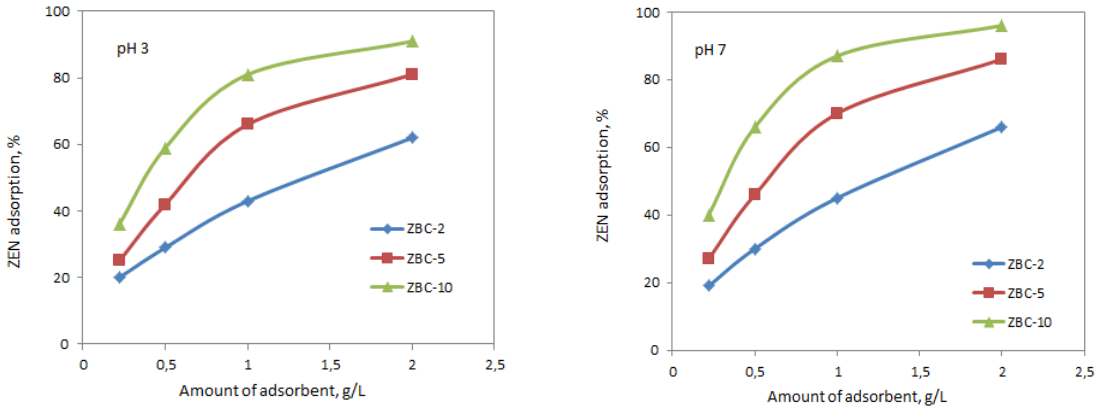

Figure 3. ZEN adsorption by organozeolites: ZBC-2, ZBC-5 and ZBC-10; pH 3 and 7.

As can be seen from Figs. 2 and 3, for both the ZCPs and ZBCs at both pHs, adsorption of ZEN increased with increasing amounts of solid phase in suspension. Also, at the same amount of solid phase in suspension, adsorption of ZEN increased with increasing amounts of both organic cations at the zeolitic surface. Thus, the highest adsorption of ZEN was achieved when the zeolitic surface was totally covered with either CP or BC (ZCP-10 and ZBC-10). At the highest level of solid phase in suspension (2 g/L), similar ZEN adsorption indexes were obtained for ZCPs and ZBCs. At pH 3, ZEN adsorption by ZCPs was: 61% for ZCP-2, 84% for ZCP-5 and 93% for ZCP-10, while for ZBCs, adsorption of ZEN was 62%, 81% and 91% for ZBC-2, ZBC-5 and ZBC-10, respectively. At pH 7, the following ZEN adsorption indexes were obtained: 68% for ZCP-2, 90% for ZCP-5, 97% for ZCP-10, 66% for ZBC-2, 86% for ZBC-5 and 96% for ZBC-10. At the lowest level of solid phase in suspension (0.2 g/L) at pH 3 and 7, the differences in ZEN adsorption by ZCPs and ZBCs were more visible and slightly higher ZEN adsorption indexes were observed for the ZCPs. For example, the organozeolite with the highest amount of surfactant ZCP-10, ZEN adsorption was 52% at pH 3 and 63% at

pH 7, while for ZBC-10, ZEN adsorption was 36 and 40% at pH 3 and 7, respectively. Both organozeolites had a slightly higher adsorption of ZEN at pH 7, where ZEN exists in solution in the neutral and partly in the anionic form. These results were similar to previous results of ZEN adsorption by the natural zeolite modified with different levels of ODMBA [5] where the ZEN adsorption increased with increasing levels of CP or BC in the organozeolites. The results confirmed that the degree of hydrophobicity of the zeolitic surface plays an important role in ZEN adsorption and that long chain organic cations (surfactants) are the active sites responsible for its adsorption.

CONCLUSION

Organozeolites were prepared by treatment of the natural zeolite with different levels of benzalkonium chloride (BC) and cetylpyridinum chloride (CP), surfactants commonly used in the pharmaceutical industry. *In vitro* adsorption of ZEN by these organozeolites was studied at pH 3 and 7. Increased adsorption of ZEN with increasing amounts of organic cations at the zeolitic surface confirmed that either CP or BC at the zeolitic surface are responsible for ZEN adsorption. However, to better understand the ZEN adsorption mechanism, more detailed experiments on ZEN adsorption by organozeolites will be the subject of future research.

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