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ADSORPTION EFFECTS OF MINERAL ADSORBER ON THE CLINOPTILOLITE BASIS PART II: ADSORPTION BEHAVIOUR IN THE PRESENCE OF AMINOACIDS AND VITAMINS

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The results presented in this paper were obtained by studying the adsorption effects of a mineral adsorber based on clinoptilolite, in contact with some aminoacids (tryptophan and phenylalanine) and vitamins (A, D and E). An index of chemisorption ($C\alpha$) was developed as a measure of adsorption intensity. $C\alpha$ showed the ratio between adsorbed and total ligand concentration. The results obtained with aminoacids and vitamins were compared with previous results obtained with aflatoxins (B1, B2 and G2), zearalenone, ochratoxin and T2 toxin under. Identical conditions (pH, temperature, electrolyte). It was found that neither aminoacids nor vitamins were adsorbed by this mineral adsorber. ($C\alpha \approx 0$). The index of chemisorption was different for different toxins. The highest values were for aflatoxins - 0.80-0.90, for zearalenone $C\alpha = 0.50$, T2 toxin $C\alpha = 0.40$ and ochratoxin $C\alpha = 0.35$.

Key words: Mineral adsorber, chemisorption, aminoacid, vitamin, mycotoxin

INTRODUCTION

The effects of adsorption of mycotoxins, especially aflatoxins to different types of aluminosilicates have been approved by many authors (Harvey et al. 1989, 1992, 1993, 1994, Kubena et al. 1992, 1993, Phillips et al. 1995, Tomašević-Čanović et al. 1994, 1995). The effects are different for toxins with different functional groups. The best results were obtained with aflatoxins of the B, G and M series. It is supposed that chemisorption takes place but the mechanism of adsorption still is not clearly defined. This mode of mycotoxicosis prevention seems to be the best and in the last few years it has been widely applied (Harvey et al. 1989, 1992, 1993, 1994; Kubena et al. 1992, 1993; Rajić et al. 1995; Stankov et al. 1992).

Investigations also show that a concentration of 5 g/l of clinoptilolite based mineral adsorber in colostrum leads to significantly higher assorption of colostral immunoglobulin G in newborn calves (Stojić et al. 1995).

The adsorption of aflatoxins B1 and G2 in vitro by a mineral adsorber based on clinoptilolite were shown in Part I (Tomašević-Čanović et al. 1994).

It is very important to know what happens when aminoacids and vitamins are present together with mineral adsorber in the electrolyte. If adsorption takes place it is clear that the nutritional value of the feed would be reduced and disease may occure. For this reason, further investigation of the potential interaction of mineral adsorbers with important nutrients and feed additives is warranted. Chung and Baker (1990) reported that chemically modified phyllosilicate (HSCAS) did not impair phytate or inorganic phosphorus utilization. In other studies (Chung et al. 1990) the utilization of essential nutrients was investigated using Zn and Mn as model trace elements and vitamin A and riboflovin as model vitamins. Addition of 1% HSCAS to basal diets did not impair the utilization of riboflovin, vitamin A or Mn: however, there was a slight reduction in Zn utilization.

The adsorption of aminoacids on clinoptilolite tuff is rather weak (Petrović et al. 1995).

In this paper, the behaviour of mineral adsorber in the presence of certain aminoacids (tryptophan, phenylalanine) and vitamins (A,D and E) are shown.

MATERIALS AND METHODS

The mineral adsorber applied was a modified natural clinoptilolite obtained from Zlatokop deposit. The basic characteristics of the adsorber were presented in Part I of this investigations (Tomašević-Čanović et al. 1994).

Tryptophan and phenylalanine were produced by BDH Chemical Ltd, and were used as 1mM/dm^3 solutions in electrolyte (16.5 mg phenylalanine/100 ml and 20.6 mg tryptophan/100ml).

Vitamins A, D and E were of pharmaceutical grade, and were used in the following amounts:

1.5 mg/100 ml electrolyte - Vitamin A

10 mg/100 ml electrolyte - Vitamin E

12.5 mg/100 ml electrolyte - Vitamin D

The electrolyte was prepared using the predominant inorganic components present in the gastric juice of animals. It contained 0.1 M HCl/dm^3 and 0.05M NaCl/dm^3. The pH was adjusted to 7.0 with 0.1N NaOH.

The test method for determination: Aliquots (0.4 cm^3) were taken from 100 ml of electrolyte containing defined amounts of the examined components (16.5mg Phe; 20.6 mg Try; 1.5 mg Vit A; 10mg Vit E; 12.5mg Vit D), for determination of the total amount present in solution (C_t). To the electrolyte 1g mineral adsorber was added and the suspension was placed in a water bath (37^{0}C), and slightly shaken.

For determination of non-adsorbed additives, after 2h an aliquot was taken for analysis. The mineral product was separated by centrifuging. The concentrations of non adsorbed (C_f) aminoacids or vitamins in supernatant solution were determined (C_f) by HPLC (with UV-VIS detector).

RESULTS AND DISCUSSION

In contrast to the reaction with aflatoxins B1 and G2, which were adsorbed on the mineral adsorber (Part I), the investigated ligands (tryptophan, phenylalanine, vitamins A, D and E) were not adsorbed.

In Figure 1 the spectra of tryptophan in clear electrolyte (Figure 1-1) and in the presence of mineral adsorber are presented (Figure 1-2). It is clear that there was no difference in the intensity and that all tryptophan was present in the electrolyte after contact with the adsorber for two hours.

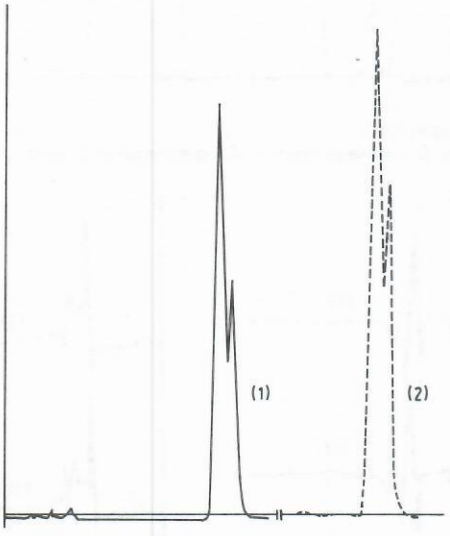
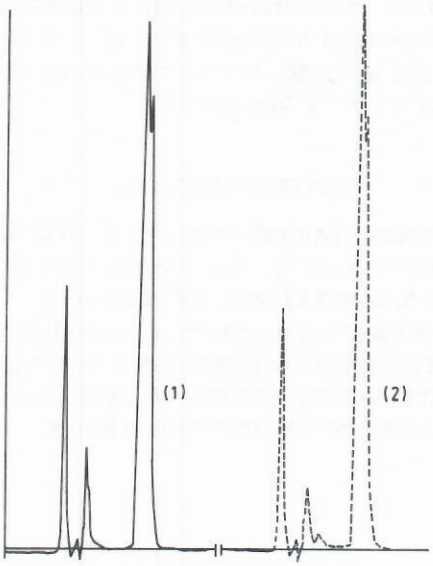


Figure 1 Tryptophan spectra
1 - clear system; 2 - in the presence of 1 g mineral adsorber

In Figure 2 the spectra for phenylalanine are presented, showing the same behaviour, i.e. no adsorption. No difference in the intensity in the clean solution and in the supernatant after two hours adsorber/phenylalanine contact was noted.

Similar results were obtained with vitamins A, D and E. In Figure 3 curves obtained for vitamin A in electrolyte - curve (1) and in supernatant after two hours adsorber/vitamin A contact - curve (2) are given. The same intensity confirms that the process of adsorption did not occur.



Phenylalanine spectra
Figure 2 1 - clear system; 2 - in the presence of 1 g mineral adsorber

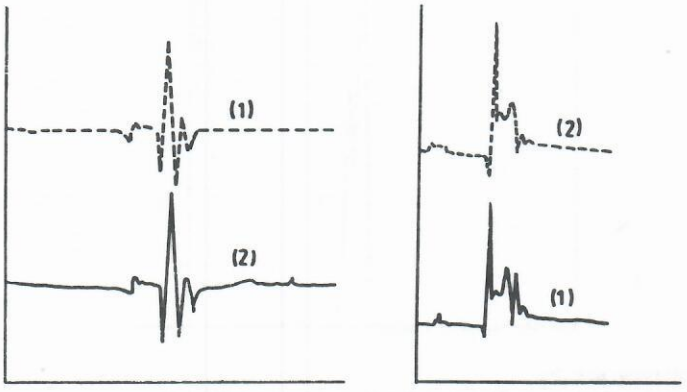


Figure 3. Vitamin A
1 - clear system; 2 - in the presence of mineral adsorber
Figure 4 Vitamin D
. 1 - clear system; 2 - in the presence of mineral adsorber

The results obtained for vitamin D (Figure 4) were similar. Namely, this mineral adsorber could be used for adsorption of aflatoxins without impairing the utilization of this vitamin.

The results for vitamin E (Figurte 5), also show that this mineral adsorber had no afinity to adsorb it.

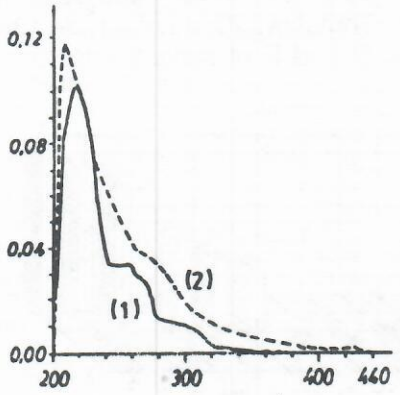


Figure 5. Vitamin E
1 - clear system; 2 - in the presence of mineral adsorber

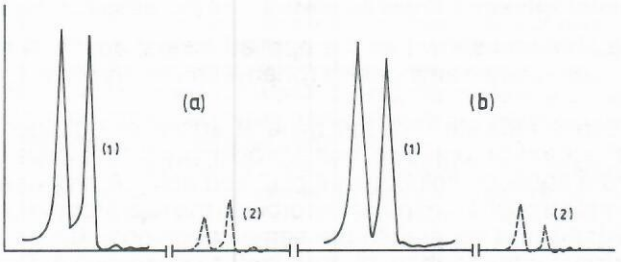


Figure 6. Aflatoxin B1 (a) and G2 (b) spectra
1 - clear system; 2 - in the presence of mineral adsorber

The same results were obtained at pH2 and pH7. In order to provide a comparison, the spectra of aflatoxin B1 (Figure 6a) and aflatoxin G2 (Figure 6b) are presented, showing differences between the curves with and without mineral adsorber.

The chemisorption index ($C\alpha$) was developed, allowing direct comparison of the ability of the examined mineral adsorber to adsorb different ligands (mycotoxins, aminoacids, vitamins).

The chemisorption index was determined by the expression:

$$C\alpha = \frac{C_t - C_f}{C_t}$$

where $C\alpha$ is the concentration of free ligand in the supernatant after separation of the mineral adsorber, C_t the total concentration of ligand to be determined. $C\alpha$ indicates the strength of binding of different ligands to the mineral adsorber.

rezultatima za aflatoksine (B1, B2 i G2), zearalenon, ohratoksin i T2 toksin. Za ispitivanja je korišćen isti mineralni adsorber, a i uslovi ispitivanja (pH, temperatura, elektrolit) su bili identični. Dobijeni rezultati su pokazali da ispitivani mineralni adsorber ne adsorbuje aminokiseline (triptofan i fenilalanin) kao ni vitamine (A, D. i E). Indeksi hemisorpcije različiti su za različite toksine. Najveći je za aflatoksine i kreće se od 0.80-0.90, dok je za zearalenon 0.50, T2 toksin -0.40 i ohratoksin 0.35.