საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 18, №2, 2024
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 18, no. 2, 2024

Biotechnology

Aflatoxins Adsorption by Lignin Treated with Sodium Hydrocarbonate Using Technogenic Raw Materials

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This study examines aflatoxins adsorption by lignin treated with sodium hydrocarbonate. Food safety and security issues are of great relevance in the modern world. Food safety is secured only when healthy and full-value products are in sufficient quantity and accessible for everyone. Numerous organizations worldwide along with researchers work to solve this problem and find ways to ensure food safety. Mycotoxins are one of the major challenges for food safety and security, since they contaminate products during storage and transportation. Aflatoxins, which belong to the class of mycotoxins, are acute carcinogenic, mutagenic and teratogenic toxins. They are the products of *Aspergillus* fungi metabolism, which grow mainly on cereal crops and nut crops. Aflatoxins are also found in milk and meat products. Toxins penetrate animal organism from contaminated cereals. This study examines aflatoxins adsorption by lignin treated with sodium hydro-carbonate. As an adsorbent a lignin treated with sodium hydrocarbonate was used. We have infected pieces of bread with *Aspergillus parasiticus* and *Aspergillus flavus* microorganisms, which generate different groups of toxins. A sample was placed along with lignin treated with sodium hydrocarbonate, and adsorption properties were studied. Using thin-layer chromatography, aflatoxins concentration and type have been identified in the specimen under investigation and in lignin. The method employed by us, is ecologically clean, safe, technologically simple and accessible. We have studied the adsorption properties of lignin treated with sodium bicarbonate with the purpose of aflatoxin deactivation. This method is in full compliance with food safety requirements. *© 2024 Bull. Georg. Natl. Acad. Sci.*

aflatoxin, mycotoxin, adsorption, lignin

Food harmlessness and safety is of great relevance in the modern world. Food safety is provided only when healthy and full-value products are in sufficient quantity and accessible to everyone. Solution of this problem is looked for by many global organizations along with scientific teams, which set a goal of finding ways of food safety provision [1, 2].

Mycotoxins, which contaminate products during their storage and transportation, are one of the main of the problems of food zero harm and safety. Aflatoxins, which belong to mycotoxin class are the acute carcinogenic, mutagenic and teratogenic toxins. These are the products of metabolism *Aspergillus fungi*, which mainly grow on cereal

crops and nut trees. Aflatoxin is also detected in dairy and meat products. Toxins penetrate animal organisms from contaminated cereals [3].

Fungi appearance in cereal crops and nut trees is caused by hot and humid environment, as well as by the use of fungicides and pesticides, which increases formation of different mycotoxins 100 times.

Several groups of aflatoxins: B_1, B_2, G_1, G_2 are singled out.Among this group of secondary metabolites, the most common are toxins of B_1, T_2 group, which according to International Agency for Research on Cancer (IARC) studies conducted in 1993 are qualified as carcinogens [4].

Metabolite of aflatoxin B_1 is classified as a potent carcinogen, that is why its permissible content in food products is brought under regulation in Europe in America, in particular, it equals to 0.05 mkg/kg in Europe and 0.5 mkg/kg in America.

Penetration of mycotoxins in low doses into organism leads to the development of chronic aflatoxicosis, which is featured by immune system suppression, DNA damage and oncogenes activation. The presence of furan and coumarin groups in the main structure of toxin is probably linked with its carcinogenesis [5].

Aflatoxins are stable in the neutral environment. It has been established that *Aspergillus parasiticus* and AFT grow well at 33°-38°C, and pH=5. *Aspergillus flavus* produces the largest amount of aflatoxins at temperatures from 24° to 28°C, under conditions of 80% humidity [6].

Aflatoxins detoxication is possible via mechanical, physical and chemical ways. The application of different types of adsorbents is an effective method for vegetables and cereals protection from aflatoxins. This method is accessible and effective when properly used.

Aflatoxin adsorbents are divided into several groups: mineral, carbon-containing, polysaccharides and mixed adsorbents.

Our work sets a goal of product protection from aflatoxin formation via adsorption using cheap, simple and safe adsorbents.

The application of lignin treated with $NaHCO_3$ as adsorbent is safe, technologically simple and accessible [7].

100 g of sawdust were treated with 4% $NaHCO_3$ solution. We took 50 g of obtained specimen and placed it along with mouldy bread into exicator. We made 7-week observation over sawdust mass. Every week results of weighing are given in Table, Figure.

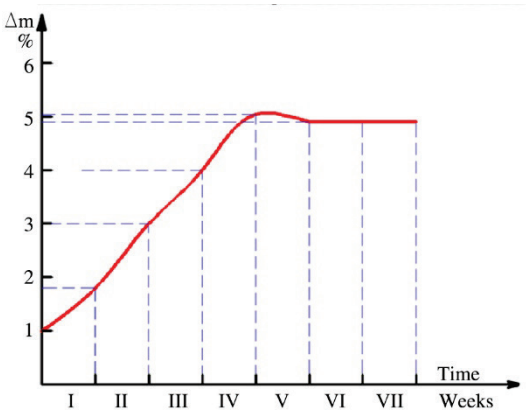

Fig. Diagram of time dependence of sawdust mass change.

Based on these data, one may conclude that lignin treated with $NaHCO_3$ has adsorption properties: considerable increase of mass during the first 4 weeks confirms the enhancement of adsorption properties. After slight decrease registered on the fifth week, this parameter remained constant for next three weeks [8].

Toxin concentration has been determined in lignin and bread specimen: we took sawdust treated

Table. The change in sawdust mass within 7 weeks

Weeks	I	II	III	IV	V	VI	VII
Lignin mass according to weeks	50.9	51.5	52.0	52.52	52.45	52.45	52.45
percentage content	1.8%	3%	4%	5.04%	4.9%	4.9%	4.9%

with 52 g $NaHCO_3$ and placed it into 500 ml flask, then added 150 ml acetone and water (85:15), shook them up for 45 min and then filtered. Obtained filtrate was moved to separating funnel, and 100 ml of 4% NaCl solution with 50 ml of hexane were added. This mass was divided into layers. Lower layer was moved to separating funnel with addition of 25 ml of chloroform. Layers were divided again and this procedure was repeated three times. Then the obtained fraction was filtered by Na_2SO_4. A filtrate was reduced to 5 ml by evaporation [9].

Using thin-layer chromatography we determined aflatoxin concentration and type in the researched specimen. Using capillary tube we applied 20 mkl of solution and 5 mkl aflatoxin-containing working solution to silufol paper. We placed a plate into methanol:acetin (1:99) solution, then took it from solution, dried for 5 minutes and developed it under ultraviolet light emission. Based on surveyed specimen spots spread on thin-layer chromatography plate and its color we confirmed that aflatoxin B_1 run height (Rf) in our surveyed specimen coincides with a standard, light emission color transforms from dark-blue to yellow that evidences aflatoxin presence in the specimen [10].

Aflatoxin concentration was determined by the following formula:

$$C = \frac{V_1}{V_2} \cdot \frac{h}{h_{st}} \cdot \frac{m_{st}}{M},$$

where: V_1 is 200 ml of chloroform extract; V_2 – 20 mkl, volume of extract, applied by us to silufol; h – Rf, mm; h_{st} – standard Rf, mm; m_{st} – aflatoxin mass; M – surveyed specimen mass; $C \approx 0.4 \cdot m$ mkg/kg.

Our study was targeted at the improvement of storage conditions of agricultural products, in order to protect them from further contamination. We have studied adsorption properties of lignin treated with hydro-carbonate, with the purpose of aflatoxin deactivation. Aflatoxin's concentration and type were identified in the specimen and in lignin using thin-layer chromatography.

The method used by us, is ecologically clean, safe, technologically simple and accessible, so it completely meets the food safety requirements.

ბიოტექნოლოგია

ტექნოგენური ნედლეულის გამოყენებით აფლატოქსინების ადსორბცია ნატრიუმის ჰიდროკარბონატით დამუშავებული ლიგნინით

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ნაშრომში წარმოდგენილია აფლატოქსინების ადსორბცია ნატრიუმის ჰიდროკარბონატით დამუშავებული ლიგნინით. დღევანდელ მსოფლიოში მეტად აქტუალურია სურსათის უვნებლობისა და უსაფრთხოების პრობლემა. სასურსათო უსაფრთხოება მხოლოდ მაშინაა დაცული, როდესაც ჯანსაღი და სრულფასოვანი პროდუქტი ყველასათვის საკმარისი და ხელმისაწვდომია. ამ პრობლემის გადასაჭრელად მუშაობს მსოფლიოს მრავალი ორგანიზაცია მეცნიერთა ჯგუფებთან ერთად, რომელთა მიზანია მოძებნონ გზები სურსათის უსაფრთხოების უზრუნველსაყოფად. სურსათის უვნებლობისა და უსაფრთხოების ერთ-ერთი ძირითადი პრობლემაა მიკოტოქსინები, რომლებიც შენახვისა და ტრანსპორტირების დროს აბინძურებს პროდუქტს. აფლატოქსინები, რომლებიც მიკოტოქსინების კლასს მიეკუთვნება, წარმოადგენს ძლიერ კანცეროგენულ, მუტაგენურ და ტერატოგენურ ტოქსინებს. ისინი ასპერგილუსის სოკოების მეტაბოლიზმის პროდუქტს წარმოადგენს, რომლებიც ძირითადად მარცვლეულ კულტურებსა და თხილეულზე იზრდება. აფლატოქსინები ასევე აღმოჩენილია რძესა და ხორცპროდუქტებში. ცხოველის ორგანიზმში ტოქსინები დაბინძურებული მარცვლეულიდან აღწევს. ჩვენ მიერ მოხდა ნატრიუმის ჰიდროკარბონატით დამუშავებული ლიგნინის გამოყენება ადსორბენტად. დავასნებოვნეთ პურის ნაჭრები *Aspergillus parasiticus* და *Aspergillus flavus* მიკროორგანიზმებით, რომლებიც წარმოქმნიან სხვადასხვა ჯგუფის ტოქსინებს. ნიმუში მოვათავსეთ ნატრიუმის ჰიდროკარბონატით დამუშავებულ ლიგნინთან და შევისწავლეთ ლიგნინის ადსორბციული თვისებები. თხელფენოვანი ქრომატოგრაფიით განისაზღვრა აფლატოქსინის კონცენტრაცია და ტიპი საკვლევ ნიმუშსა და ლიგნინში. მეთოდი, რომელიც გამოვიყენეთ არის ეკოლოგიურად სუფთა, უსაფრთხო, ტექნოლოგიურად მარტივი და ხელმისაწვდომი. შევისწავლეთ ლიგნინის ადსორბციული თვისებები აფლატოქსინის გასაუვნებელყოფად. მეთოდი სრულად აკმაყოფილებს სურსათის უსაფრთხოების პირობებს.

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Received November, 2023