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Inclusion of Dietary Zeolite Reduces Aflatoxin B_1 Levels in Household Bread Waste Used as Cattle Feed

M. Yavarmanesh[1,*], M. Sohrabi Balssini[1], M.R. Edalatian Dovom[1], F. Ghiamati Yazdi[2] and J. Barouei[3]

[1]*Department of Food Science & Technology, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran;* [2]*Faculty of Food Science, Gorgan University of Agricultural Sciences and Natural Resources, Iran;* [3]*Laboratory of Microbiology, School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW 2308, Australia*

Abstract: This study aimed to examine the efficacy of zeolite addition in the reduction of aflatoxin B_1 (AFB_1) levels in household bread waste used as animal feed. Three levels of zeolite (1, 3 and 5%) were added to two dry bread waste samples naturally contaminated by AFB_1 at 11.48 and 4.8 ppm levels. Samples were then analyzed before zeolite treatment (day 0) and after 7 and 14 days of storage for chemical (pH, moisture content and ash) and microbial (mesophilic bacteria and molds) changes as well as aflatoxin levels relative to the control. Data analysis showed that aflatoxin levels significantly decreased as zeolite and storage time increased, so that the lowest level of aflatoxin was found in sample treated with 5% zeolite after 14 days of storage compared with the control ($p<0.05$). Such a trend was also observed in bacterial and mold counts. However, storage time had less effect than zeolite. Chemical analyses of bread samples exhibited significant increases in pH values and corresponding decreases in moisture and ash contents in all storage time points compared with the control. These findings suggest zeolite addition as a viable method of reduction of AFB_1 levels in household bread waste used as animal feed.

Keywords: Zeolite, aflatoxin, reduction, household bread waste, cattle feed.

INTRODUCTION

Aflatoxins (AFs) are a group of structurally similar polysubstituted coumarins that are produced by common molds. One of the most important mold genera producing aflatoxins is *Aspergillus* [1]. There are three structurally similar AF compounds including AFB_2, G_1 and G_2 as well as a biotransformation compound, AFM_1 derived from AFB_1 [2]. Aflatoxins are responsible for a variety of adverse biological and health effects such as carcinogenicity, immunosuppression, mutagenicity and teratogenicity. Carry-over of aflatoxins such as AFB_1 and AFM_1 into animal tissues and fluids such as meat, egg and milk has previously been reported [3, 4].

Bread remains an important item in the Iranian diet. Overall, bread provides 47% of daily total energy intake [5]. Iranian consumers tend to eat fresh bread, not to use of stale bread and to buy more than they need. This consumer behavior has made bread as the most regularly wasted food in the Iranian homes with an estimate of 600 thousand tons per year being thrown away. There is a big traditional business that collects stale bread waste from households mainly for using as animal feed. Inappropriate storage conditions of bread waste make it as an excellent environment for growing aflatoxin-producing molds [6]. Various physical, chemical and biological methods have been developed to reduce aflatoxin levels in animal feed. Previous laboratory-scale studies have demonstrated usage of clays and other materials such as activated charcoal, bentonite, zeolite, hydrated sodium calcium aluminosilicate, sepiolite and kaolinite as potential means of reduction of aflatoxins levels in animal feed [7, 8]. However, these mainly *in vitro* results need to be confirmed by performing *in vivo* studies. Therefore these materials have not been yet approved to be used in commercial products [9]. Among these materials, natural zeolite, a crystalline and three-dimensional cage-like structure, has showed a high efficacy in binding mycotoxins [10, 11]. Due to lack of data examining the efficacy of zeolite inclusion in the reduction of AFB_1 in household bread waste used as animal feed, the current study was designed to determine chemical and microbial influences, and AFB_1 reduction effectiveness of zeolite in bread waste.

MATERIALS AND METHODS

Experimental Design

Two samples of stale bread (A and B, each 500gr) were collected from the main collection center of bread waste in Mashhad, Iran. These samples were analyzed primarily for chemical and microbial properties along with AFB_1 levels (Table **1**). Samples were then milled to a particle size of 1-2 mm (Restsch GmbH., Haan, Germany). Each sample was then divided into three portions and treated with 1, 3 or 5% w/w zeolite powder (Clinoptilolite, Afrazand Co, Iran, 1-2 mm particle size) and mixed thoroughly. Treated samples were incubated at 25°C and 20% relative humidity using a Memmert incubator (Memmert GmbH & Co.KG, Schwabach, Germany) for 14 days. After 7 and 14 days of storage,

*Address correspondence to this author at the Department of Food Science & Technology, Faculty of Agriculture, Ferdowsi University of Mashhad (FUM); Tel: +98 511 8795620; Fax: +98 511 8787430; E-mail: yavarmanesh@um.ac.ir

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Table 1. Chemical and microbial properties and AFB_1 levels of control (non-zeolite treated) bread waste samples during storage.

Characteristics		pH	Moisture%	Total count (cfu/gr)	Mold (cfu/gr)	Aflatoxin B_1 (µg/kg-ppb)	Ash (gr/100gr)
Storage time(day)	Dry bread						
0	Sample A	6.35 ± 0.2	12.6 ± 0.15	99000 ± 2500	94000 ± 1500	4.8 ± 0.1	0.94 ± 0.07
	Sample B	6.28 ± 0.1	14.9 ± 0.2	180000 ± 4000	110000 ± 2200	11.48 ± 0.2	0.98 ± 0.09
7	Sample A	6.32 ± 0.1	12.4 ± 0.2	98000 ± 3100	94000 ± 1750	4.8 ± 0.1	0.95 ± 0.06
	Sample B	6.27 ± 0.2	14.5 ± 0.25	188000 ± 4300	120000 ± 2850	11.4 ± 0.25	1.02 ± 0.08
14	Sample A	6.30 ± 0.1	12.6 ± 0.2	100000 ± 4000	96000 ± 1550	4.7 ± 0.15	0.93 ± 0.05
	Sample B	6.25 ± 0.2	14.4 ± 0.15	192000 ± 5200	115000 ± 2800	11.55 ± 0.2	0.99 ± 0.07

dry bread particles were separated from zeolite powder based on their different densities (dry bread, 0.66-0.67 gr/cm^3 and zeolite, 1-1.2 gr/cm^3) using a cyclone separator (MO119-Micro technologies, Haryana, India). Samples were then analyzed for chemical and microbial changes, and AFB_1 levels.

Chemical Analysis

In order to evaluate influences of zeolite addition on chemical properties of bread waste samples, pH values and, moisture and ash contents were analyzed using AOAC official methods 943.02, 925.10 and 930.22, respectively [12-14].

Microbial Analysis

Total count of mesophilic bacteria and molds were determined using standard plating methods [15]. After incubation, colonies were counted and microbial quality of samples was then calculated using following equation:

$N = \Sigma C / (n_1 + 0.1 n_2)\, d$

ΣC= Enumeration of colonies at two successive dilutions

n_1= Enumeration of microbial plates at the first dilution

n_2= Enumeration of microbial plates at the second dilution

d= Dilution coefficient at the first dilution

Measurement of AFB_1 Amounts

AFB_1 levels of samples were measured using high performance liquid chromatography (WATERS, Alliance system, USA) equipped with an immunoaffinity column as instructed by AOAC official method 999.07 [16]. Briefly, a linear calibration curve was obtained by injecting 500 µl of AFB_1 standards (Faroogh natural sciences laboratory) to the instrument. Then 50 µl of purified spiked sample and blank sample were injected and AFB_1 recovery was calculated. If the recovery was within the acceptable range, then unknown samples were injected to determine AFB_1 levels.

Statistical Design

A Completely randomized factorial design was used for data analysis. Statistical analysis was conducted using Minitab Version 13.20 (Minitab, Inc., State College, PA, USA) and Slide Write Version 1.0 (Advanced Graphics Software Inc., Encinitas, CA, USA). Analysis of variance and LSD test were utilized where applicable to determine statistically significant differences at $p < 0.05$.

RESULTS

Chemical and Microbial Properties of Control Samples

Chemical and microbial properties, and AFB_1 levels of control bread waste samples (with no added zeolite) in all storage time points (day 0, 7 and 14) are shown in (Table **1**). Initial concentrations of AFB_1 in bread waste samples A and B were found to be at high (11.48 µg/kg) and low (4.8 µg/kg) levels respectively.

Influences of Zeolite on pH Values

As indicated in (Fig. **1**), increases in zeolite percentage and storage time were associated with significant increases in pH values of samples compared with the control.

Influences of Zeolite on Moisture Content

A significant decrease in moisture content was observed in samples treated with 5% zeolite compared with the control. Furthermore, a synergistic effect of increased storage time with 5% zeolite on moisture content on moisture content was observed.

Influences of Zeolite on Ash Levels

As shown in (Fig. **1**), ash levels decreased as zeolite percentage increased. However, this decrease reached significant statistical difference in samples treated with 5% zeolite after 14 days of storage relative to 1% zeolite.

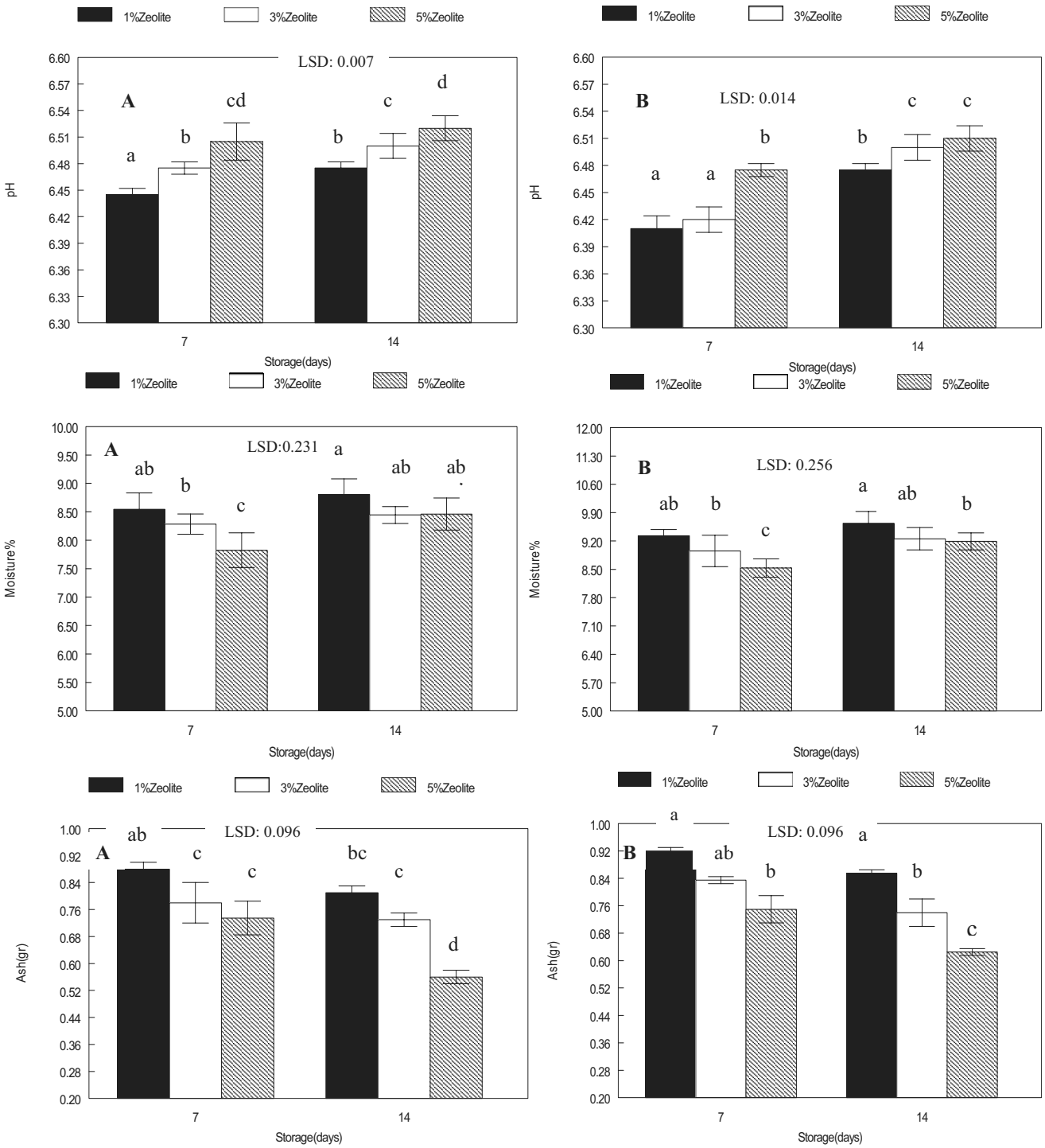

Fig. (1). Interaction between zeolite percentage and storage time on pH, moisture content and ash levels of bread waste samples (A: sample A; B: sample B). Bars having a different letter indicate significant differences ($p \leq 0.05$).

Influences of Zeolite on Microbial Loads

Microbial loads of samples were strongly affected by zeolite percentage. Combined effect of zeolite inclusion and storage time on microbial changes is shown in (Fig. **2**). A significant interaction effect was observed between zeolite level and storage time was observed in the reduction of microbial loads. While total mesophilic bacterial count significantly decreased in samples treated with 5% zeolite, mold counts significantly dropped in 3 and 5% zeolite compared with 1% zeolite.

Interaction Effect Between Zeolite Levels and Storage Time on AFB_1 Levels

A significant interaction effect was observed between zeolite level and the storage time in the reduction of AFB_1 (Fig. **2**). Moreover, it appears that the influence of the storage time was stronger than zeolite level.

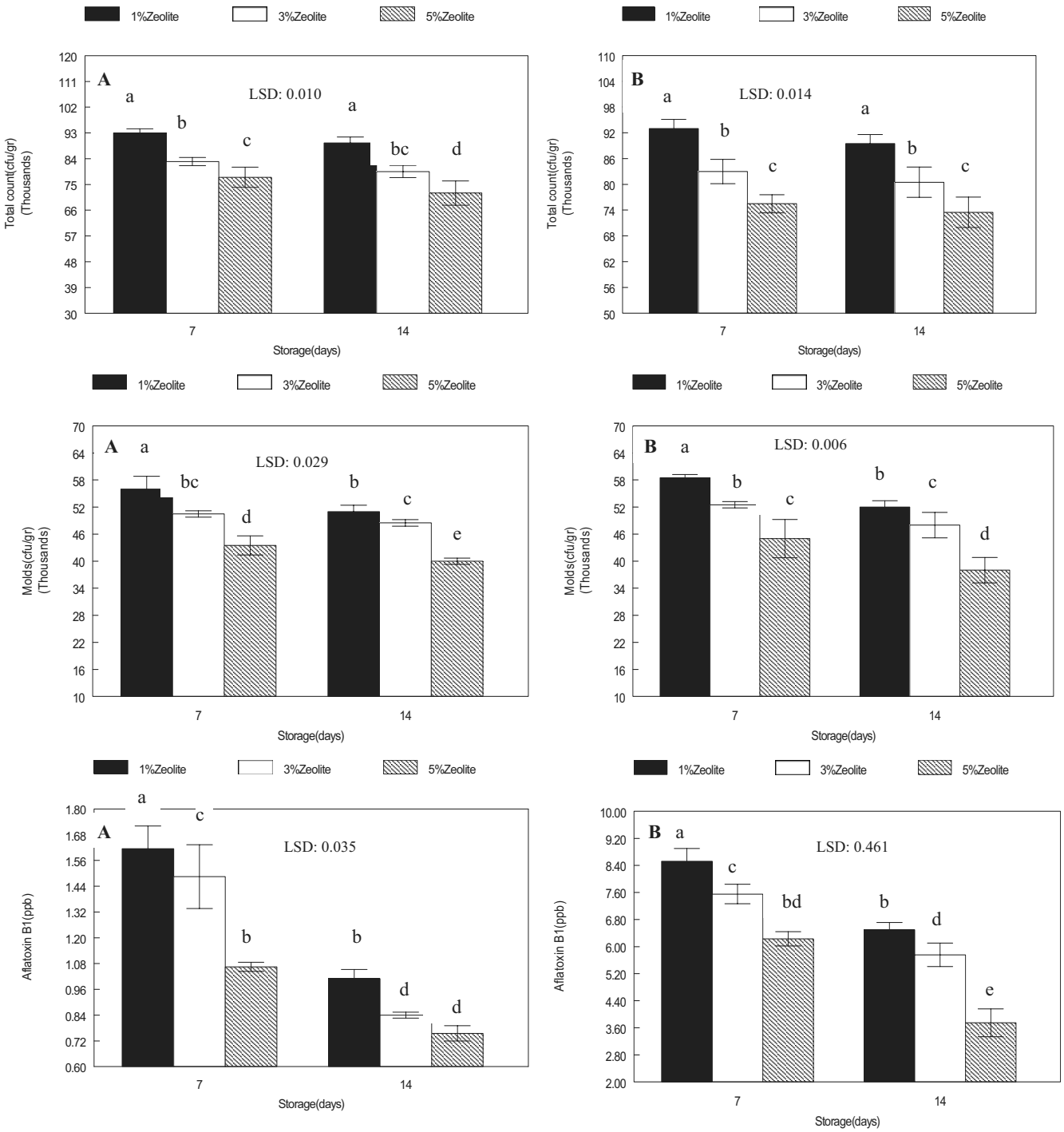

Fig. (2). Interaction between zeolite percentage and storage time on the total mesophilic bacterial counts, mold counts and AFB_1 levels in bread waste samples (A: sample A; B: sample B). Bars having a different letter indicate significant differences ($p \leq 0.05$).

DISCUSSION

Zeolite compounds have been used for years as anti-caking agents and aflatoxin adsorbents in animal feeds [17]. Because of their negative surface charge, natural zeolites (clinoptilolite) have been considered to be effective in absorbing polar mycotoxins such as aflatoxins [11]. However, there have been no previous studies examining the effect of zeolite inclusion on the reduction of aflatoxin contamination, and chemical and microbial qualities of household bread waste used as animal feed. In the present study, significant increases in pH values and corresponding decreases in moisture content and ash levels were observed in all time points during the storage as zeolite increased. This might show that addition of zeolite to the bread waste provides optimal pH conditions for molds proliferation and as a result aflatoxin production (AFB_1). Previous studies have shown that inorganic compounds such as zeolite can absorb organic acids using surface functional groups, thus results in increased pH values [18]. Specifically such an effect has been reported

against the *Candida albicans* acid production [19]. While as a general consensus, increase in pH up to the neutral values provides optimal conditions for microbial growth [2], a significant decline was found in microbial loads (mesophilic bacteria and molds) and therefore aflatoxin production (AFB_1). The most likely explanation for decreases in microbial loads and AFB_1 levels relates to significant decreases in water activity (moisture content) and the minerals (ash) induced by zeolite incorporation. Previous research has shown that Zeolite can entrap free water and cations due to its molecular sieving and ion exchange property [20]. Moreover, zeolite can be used for cell exclusion via its ability to absorb bacterial cells with high selectivity [21]. It has also been reported that zeolite decreases substance uptake at the cell surface. This zeolite property along with factors such as its higher maintenance coefficient and substrate transport limitation are other potential underlying mechanisms in decreased microbial growth in zeolite-treated bread waste [18]. Previous research has also shown that natural zeolite (Clinoptilolite) adsorbs mycotoxins such as aflatoxins (AFB_1) more effectively than other natural or modified clays because of its hydrophilic surface [11]. Therefore, decrease in aflatoxin levels in zeolite-treated bread waste samples is more likely due to direct adsorption of aflatoxin and indirectly via growth inhibition of molds including aflatoxin-producing ones during storage. The latter effect, as mentioned above is because of alterations in the chemical properties, in particular, reduced moisture content of zeolite-treated bread waste.

CONCLUSION

As in most of courtiers, bread is the most wasted food in Iran. Bread waste is separately collected from Iranian households and is mainly used as animal feed. However, inappropriate storage of collected bread waste increases risk of growth of aflatoxin-producing molds and incidence of aflatoxin. The current study showed that addition of natural zeolite (Clinoptilolite) to the bread waste decreases aflatoxins (AFB_1) levels and inhibit bacterial and mold growth during storage. The findings therefore confirm that it is possible to utilise zeolite in the bread waste used as animal feed, in order to potentially decrease aflatoxin which is a potential threat to human and animal health. However, the effect of zeolite addition to bread waste on palatability of zeolite-treated bread for animals and on chemical composition of animal products such as meat and milk needs to be further investigated.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1] Huwiga A, Freimund S, Kappelib, H. Mycotoxin detoxication of animal feed by different adsorbents. Toxicol Lett 2001; 122: 179-88.

[2] Jay JM, Loessner MJ, Golden DA. Modern Food Microbiology, 7th ed. Springer Science: New York 2005.

[3] Veldman A, Meijes AC, Borggreve GJ, *et al.* Carry-over of aflatoxin from cow's food to milk. Anim Product 1992; 55: 163-8.

[4] Yiannikouris A, Jouany JP. Mycotoxins in feeds and their fate in animals: A review. Anim Res 2002; 51: 81-99.

[5] Shahnoushi N, Saghaian S, Reed M, Firoozzare A, alerajabi, M. Investigation of factors affecting consumers' bread wastage. J Agri Econ Dev 2013; 2: 246-54.

[6] Mortazavi SA, Rahimi Yazdi S, Ghiafe Davoodi M. Proceedings of National Conference of Food Science & Technology 1993. Evaluation of Aflatoxigenic Molds and Measurement of Their Aflatoxins in Dry Breads. PP. 133-147.

[7] Phillips TD, Kubena LF, Harvey RB, *et al.* Hydrated sodium calcium aluminosilicate: A high affinity sorbent for aflatoxin. Poult Sci 1988; 67: 243-7.

[8] Ramos AJ, Fink-Gremmels J, Hernandez E. Prevention of toxic effects of mycotoxins by means of nonnutritive aadsorbent compounds. J Food Prot 1996; 59: 631-41.

[9] Doll S, Danicke S, Valenta H, *et al. In vitro* Studies on the evaluation of mycotoxin detoxifying agents for their efficacy on deoxynivalenol and zearalenone. Arch Anim Nutr 2004; 58: 311-24.

[10] Papaioannou D, Katsoulos PD, Panousis N, *et al.* The role of natural and synthetic zeolites as feed additives on the prevention and/or the treatment of certain farm animal diseases: A review. Micropor Mesoporous Mat 2005; 84: 161-70.

[11] Phillips TD, Sarr AB, Grant PG. Selective chemisorptions and detoxification of aflatoxins by phyllosilicate clay. Nat Toxins 1995; 3: 204-13.

[12] AOAC 943.02. pH of Flour, Potentiometric Method. Official Methods of Analysis of AOAC International, 18th ed. Gaithersburg, Maryland, USA 2005.

[13] AOAC 925.10. Moisture in Bread. Official Methods of Analysis of AOAC International, 18th ed. Gaithersburg, Maryland, USA 2005.

[14] AOAC 930.22. Ash of Bread. Official Methods of Analysis of AOAC International, 18th ed. Gaithersburg, Maryland, USA 2005.

[15] Pouch Downes F, Ito K. Compendium of Methods for the Microbiological Examination of Foods, 4th ed. American Public Health Association, Washington, DC 2001.

[16] AOAC 999.07. Aflatoxin B_1 & Total Aflatoxins in Peanut Butter, Pistachio Paste, Fig Paste & Paprika Powder. Immunoaffinity Column Liquid Chromatography with Post-Column Derivatization. Official Methods of Analysis of AOAC International, 18th ed. (Chapter 49, Natural Toxins). Gaithersburg, Maryland, USA 2005.

[17] Jaynes WF, Zartman RE, Hundnall WH. Aflatoxin B1 adsorption by clays from water and corn meal. Appl Clay Sci 2006; 36: 197-205.

[18] Punyapalakul P, Soonglerdsongpha S, Kanlayaprasit C, *et al.* Effects of crystalline structures and surface functional groups on the adsorption of haloacetic acids by inorganic materials. J. Hazard. Mater 2009; 171: 491-9.

[19] Nikawa H, Yamamoto T, Hamada T, *et al*. Antifungal effect of zeolite-incorporated tissue conditioner against *Candida albicans* growth and/or acid production. J Oral Rehabil 1997; 24: 350-7.

[20] Loosdrechet MCM, Lyklema J, Norde W, *et al*. Influence of interfaces on microbial activity. Microbiol Rev 1990; 54: 75-87.

[21] Kubota M, Nakabayashi T, Matsumoto Y, *et al.* Selective adsorption of bacterial cells onto zeolites. Colloid Surf 2008; 64: 88-97.

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