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Ability of Different Treatments of *Saccharomyces cerevisiae* to Surface Bind Aflatoxin M_1 in Yoghurt

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ABSTRACT

Microbial detoxification is considered as one of the most common methods used for the elimination of aflatoxins. Reports indicate that *S. cerevisiae* can be effective in removing aflatoxins through the adsorption of aflatoxins to their cell wall. In the current research, the ability of *S. cerevisiae* (viable, acid-, heat- and ultrasound-treated yeasts) to bind aflatoxin M_1 was assessed in yoghurt. To this end, firstly, recombinant milk containing 12% solids, non-fat skimmed milk powder was prepared. Next, the samples were spiked by aflatoxin M_1 using different concentrations (100, 500 and 750 pg mL^{-1}). When the starter bacteria were added to the milk, the treated yeasts were added as well. The concentration of aflatoxin M_1 residue in the supernatant of the yoghurt samples after different storage times (1, 7, 14 and 21 days) was measured using the ELISA method. The results showed that all treatments containing viable, acid-, heat-, ultrasound-treated yeast and starter bacteria were able to adsorb aflatoxin M_1, and the ability of the treated yeast was significant as compared with the control ($P< 0.05$). Among the treated yeasts, the ability of the acid-treated yeasts was higher in toxin binding. Overall, it can be concluded that using *S. cerevisiae* for the biological adsorption of aflatoxin M_1 is effective in fermented dairy products.

Keywords: Biological adsorption, Cell wall, Fermentation, Mannan, Yeast.

INTRODUCTION

Some fungi produce toxic secondary metabolites which can cause acute toxic, mutagenic, teratogenic and carcinogenic effects. Aflatoxins are dangerous mycotoxins that are present in feed and food and are produced by species of Aspergillus, but *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* are of most concern (Kusumaningtyas *et al.,* 2006; Mohamadi and Alizadeh, 2010).

Aflatoxins may directly enter the human body by swallowing contaminated products or indirectly by consuming derived foods from primary contaminated materials, such as milk and dairy products from contaminated livestock. Aflatoxin B_1 is the most toxic mycotoxin, having harmful hepatotoxic, mutagenic, and carcinogenic effects on livestock. When aflatoxin B_1 in contaminated feed is ingested by livestock, it can be bio-transformed in the liver into aflatoxin M_1, a hydroxylated metabolite which is excreted in milk, tissues, and biological fluids of animals (Masoero *et al.,* 2009).

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Taking into account the health risks associated with human and livestock exposure to dietary aflatoxin levels, multiple efforts have been made for the complete removal or reduction of aflatoxin content in food products. Different methods have been used for reducing the amount of aflatoxin; for example, physical, chemical, and microbiological methods. According to the researches, microbiological method is an attractive alternative to control or reduce aflatoxin in foodstuffs (Alberts, *et al.,* 2009). *S. cerevisiae* is the most effective microorganism for binding aflatoxin B_1 in Phosphate Buffer Saline (PBS) (Phillips *et al.,* 1995; Sarimehmetoglu and Kuplulu, 2004; Shetty and Jespersen, 2006) and aflatoxins (B1, B2, G1 and G2) in PBS and cereals extracts (Hegazy *et al*., 2011), although Corassin *et al.* (2013) reported the capability of *S. cerevisiae* to bind aflatoxin M_1 in UHT skim milk.

The cell wall of *S. cerevisiae* represents about 30% (w/w) of total weight of the cell and is a bi-layered structure, the structural part of which is made up of *β*-1,3-glucan and *β*-1,6-glucan. The majority of the cell wall proteins (mannoproteins) are covalently linked to *β*-1,3-glucans through *β*-1,6-glucan chains. In addition, the cell wall is a highly dynamic structure responding quickly to changes in the environmental stresses. Based on chemical composition and physical nature of the *S. cerevisiae* cell wall, it is reasonable to think that the cell surface presents innumerable sites for the physical adsorption of molecules. Yeast cells can adsorb different molecules as complexes on their cell wall surface, such as toxins and metal ions. According to certain research, it is confirmed that removal of mycotoxins by cell wall binding is more relevant to covalent binding. Moreover, non-alive cells do not lose their ability to attract (Shetty and Jespersen, 2006). The mannan components of cell wall play a major role in aflatoxin linkage to *S. cerevisiae* (Devegowda *et al*., 1996).

Fermentation of food has been used as a method of preservation for centuries, and Lactic Acid Bacteria (LAB) and yeast, especially *S. cerevisiae,* are reported to reduce mold growth and aflatoxin production (Mokoena *et al.,* 2006). LAB and *S. cerevisiae*, due to their GRAS status and use as probiotics, are of particular interest for reducing the bioavailability of aflatoxin M_1 in fermented dairy products. Also, there is no previous report on the use of *S. cerevisiae* for decontamination of yoghurt containing aflatoxin M_1. Therefore, this study was carried out to evaluate the potential of *S. cerevisiae* (viable, acid-, heat- and ultrasound-treated) to remove aflatoxin M_1 from yoghurt, when added with the yoghurt starters to yoghurt prepared from milk spiked with aflatoxin M_1, and the degradation of aflatoxin M_1 in yoghurt during storage time.

MATERIALS AND METHODS

Preparation of Aflatoxin M_1 Solutions

Aflatoxin M_1 was suspended in benzene-acetonitrile (97:3, V/V) to obtain an aflatoxin M_1 stock solution concentration of 1 μg mL^{-1}. The true concentration of this stock solution was calculated using a Beer-Lambert curve and an absorption measurement at 348 nm. The stock solution was solubilized at appropriate amounts of methanol after benzene-acetonitrile evaporation by heating in a water bath (70°C for five to 10 minutes) in order to obtain aflatoxin M_1 solutions with appropriate concentrations (Zinedine *et al.,* 2005).

Activation and Preparation of the Yeast Suspension

S. cerevisiae, (PTCC 5177), was obtained from the Iranian Research Organization for Science and Technology (IROST), the Persian-type culture collection. The strain was grown on a Yeast Mould Broth (YMB; Difco) and incubated for 24 hours at 26°C. The cells were harvested by centrifugation at

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3,400×g for 10 minutes, washed twice with phosphate-buffered saline (PBS, pH 6), and the spinning of the cells at 3,400×g for 10 minutes each time. The turbidity of suspension must be standardized to match that of a 7 McFarland standard (corresponds to approximately 2.1×10^9 CFU mL^{-1}) (Peltonen *et al.,* 2001; Shetty *et al.,* 2007; Rahaie *et al.,* 2010). The 7 McFarland standard solutions were prepared by mixing 93 mL sulfuric acid 1% and 7 mL barium chloride 1.175%. (Martin and Palomino, 2009).

Yeast Treatment with Ultrasound, Heat and Acid

The activated yeast were re-suspended in 10 mL PBS and autoclaved at 121°C for 15 minutes (Shahin, 2007), or incubated at 37ºC in 2M HCl solution for one hour with mild shaking (Peltonen *et al.,* 2001; Rahaie *et al.,* 2010), or sonicate in ultrasonic bath for 15 minutes (50°C, 25 MHZ and 50% power) (Limaye and Coakley, 1998). After treatment, cells were centrifuged at 3400×g for 10 minutes and washed twice with PBS.

Contamination of Reconstituted Milk with Aflatoxin M_1 and Yoghurt Production

Reconstituted milk containing 12% total solids (non-fat) was prepared from skimmed milk powder. Some portions were contaminated with standard working solutions of aflatoxin M_1 at three different concentrations (100, 500, and 750 pg mL^{-1}) and a portion was noted as the control samples. After milk contamination, the samples were pasteurized at 90°C for 5 minutes and then cooled to 42°C. The samples were inoculated with starter cultures and the treated yeast cells were added. After mixing, homogenous samples were poured into sterile plates and incubated. After reaching pH 4.5, the samples were transferred to 4°C and stored for three weeks (Sarimehmetoglu and Kuplulu, 2004).The yoghurt samples were centrifuged to evaluate the residual aflatoxin in supernatant after 1, 7, 14, and 21 days.

Analysis of Aflatoxin M_1 by ELISA

The quantitative analysis of aflatoxin M_1 in the yoghurt samples was performed by competitive enzyme immunoassay using the RIDASCREEN Aflatoxin M_1 30/15 (Art. No. R1111, R-Bio pharm, Darmstadt, Germany) test kit.

One-hundred microliters of standard solutions and the prepared samples were added into separate microliter wells and incubated for 60 minutes at room temperature (22-25°C) in the dark. The liquid was then poured out and the wells were washed with a washing buffer (250 µL) twice. In the next stage, 100 µL of the diluted enzyme conjugate was added to the wells, mixed gently by shaking the plate manually, and incubated for 15 minutes at room temperature in the dark. The wells were again washed twice with a washing buffer. Afterwards, 100 µL of substrate/chromogen was added, mixed gently, and incubated in the dark at room temperature for 15 minutes. Finally, 100 µL of the stop reagent (1N H_2SO_4) was added into the wells and the absorbance was measured at k= 450 nm in an ELISA plate reader (ELx800, Bio-Tek Instruments, USA) (El-kest *et al*., 2015).

Statistical Analysis

The data were analyzed using a completely randomized design. The three factors were: aflatoxin concentration at three levels (100, 500, and 750 pg mL^{-1}); yeast type at four levels (viable, acid-, heat- and ultrasound-treated); and storage time at four levels (1, 7, 14, and 21 days). Statistical analyses were performed by a DUNCAN test and ANOVA with repeated measures, using the SPSS software package program.

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P values of < 0.05 were considered to be significant.

RESULTS AND DISCUSSION

Table 1 shows that the effect of treatment type, initial concentration of toxin, and storage time on the percentage of aflatoxin M_1 bound in yoghurt are significant (P< 0.05). Statistically significant differences were found between treatment and the control samples, although there was no significant difference between treatments (P< 0.05). The highest amounts of aflatoxin adsorption were related to acid- (76.46%), heat- (76.39%), ultrasound- (75.99%) treatments and viable yeast (74.20%), respectively. Similar results were obtained by Rahaie *et al*. (2010) who showed that the cell treatment under acid condition had the highest adsorption ability of the aflatoxin among the three yeast treatment types (viable yeast, acid, and heat treatment). The acidic conditions could affect polysaccharides by releasing monomers and further fragmentation into aldehydes after the breaking down of the glycosidic linkages (Bejaoui *et al*., 2004). According to the previous research (Hasakard *et al*., 2001), it is feasible that in acidic conditions some linkages are intracellular. Moreover, heat-treated yeast reduces toxins more compared to viable cells. Heating may cause the denaturation of proteins or the formation of Millard reaction products in the cell wall, and may also increase the permeability of the outer layer of the cell wall due to the dissolution of some of the mannoprotein from the cell surface (Zelotik *et al*., 1984) leading to the changed accessibility of the differently hidden binding sites (Rahaie *et al.,* 2010, Shetty *et al.,* 2007).

Comparing viable and unviable yeast cells (treated with heat, acid, and ultrasound), it was shown that unviable cells had a greater capacity for aflatoxin binding and, consequently, toxin reduction. Our results were not in agreement with Hegazy *et al*. (2011) who found that non-viable yeast had

Table 1. The mean (±SD) of aflatoxin M_1 bound (%) by *S. cerevisiae* (viable and treated with ultrasound, heat or acid) in yoghurt contaminated with aflatoxin M_1 during storage times.[a]

Treatments	Initial concentration			Time				*P* Value					
	100	500	750	1	7	14	21	T	C	Ti	T×C	T×Ti	C×Ti	T×C×Ti
Control	$^{A}90.3\pm0.4^{a}$	$^{C}29.5\pm5.2^{b}$	$^{B}49.5\pm8.3^{b}$	$^{D}52.3\pm29.9^{b}$	$^{C}54.8\pm28.6^{b}$	$^{B}56.4\pm29.7^{b}$	$^{A}62.0\pm23.6^{b}$	0.0001	0.0001	0.042	0.0001	0.0001	0.103	0.0001
Viable	$^{C}47.9\pm8.0^{b}$	$^{B}84.9\pm0.2^{a}$	$^{A}89.8\pm0.1^{a}$	$^{A}76.5\pm17.5^{a}$	$^{A}71.0\pm24.7^{a}$	$^{A}74.4\pm20.5^{a}$	$^{A}74.5\pm20.2^{a}$							
Acid	$^{B}54.3\pm11.7^{b}$	$^{A}85.1\pm0.1^{a}$	$^{A}90.0\pm0.3^{a}$	$^{AB}77.9\pm16.9^{a}$	$^{A}80.6\pm11.3^{a}$	$^{B}72.7\pm23.1^{a}$	$^{AB}74.7\pm19.9^{a}$							
Heat	$^{B}54.2\pm15.8^{b}$	$^{A}85.1\pm0.6^{a}$	$^{A}89.9\pm0.6^{a}$	$^{B}72.5\pm23.2^{a}$	$^{B}75.3\pm19.2^{a}$	$^{B}74.1\pm20.6^{a}$	$^{A}83.7\pm11.5^{a}$							
Ultrasound	$^{C}53.3\pm6.8^{b}$	$^{B}84.9\pm0.1^{a}$	$^{A}89.9\pm0.1^{a}$	$^{A}78.9\pm13.5^{a}$	$^{AB}76.4\pm17.2^{a}$	$^{B}74.9\pm19.5^{a}$	$^{B}73.8\pm21.2^{a}$							

[a] Different superscript with capital letter (A-D) or lowercase letter (a-b) within a row or column, respectively significances at $P< 0.05$.

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low binding effect. Aflatoxin binding appears to be a physical phenomenon with non-viable and physically altered cells binding significantly higher levels of toxin than their viable counterparts. Similar results were reported by Rahaie *et al.* (2010) and Shetty *et al.* (2007). In addition, there will be innumerable physico-chemical changes taking place in the cell wall during the heat treatment resulting in exposing more binding sites. The nature of cell wall components involved in mycotoxin binding is still not clear and carbohydrate rich mannoproteins or glucans may be the likely candidates involved in the binding. Raju and Devegowda (2000) attributed the aflatoxin binding by yeast cell walls to mannan oligosaccharides. However, systematic studies with the intact cells and isolated cell walls are still needed to understand the chemistry of binding.

The results revealed that the initial concentration of aflatoxin M_1 had a significant effect ($P< 0.05$) on the amount of aflatoxin M_1 bounded by the control, viable, acid-, heat-, and ultrasound-treated yeasts, while there were no statistically differences between treatments at each initial concentration of toxin ($P< 0.05$) (Table 1). The highest percentage of toxin removal was related to acid-treated yeast and the control samples at 750 and 100 pg mL^{-1} of initial concentration of toxin, respectively. The results of this study are in agreement with El-Nezami *et al*. (1998), Elsanhoty *et al*. (2014) and Peltonen *et al.* (2001) studies who reported that the relative amounts of aflatoxin removed by viable as well as heat- and acid-treated bacteria depend on initial concentrations of toxin. These results were similar to those obtained by Shetty *et al.* (2007). They reported that the absolute amounts of the bounded aflatoxin B_1 increased steadily with increasing aflatoxin B_1 concentrations and the initial concentration of aflatoxin B_1 had a remarkable influence on the binding capacity.

The results indicated that the effect of the storage time on aflatoxin M_1 bound in different treatments was significant ($P< 0.05$), although the storage time did not show any significant effect on the binding of the aflatoxin M_1 by a viable yeast ($P> 0.05$).

Figure 1 shows the interaction effect of initial concentration of toxin and the storage time on aflatoxin M_1 bound in different treatments in yoghurt. The results indicated different trends in all treatments during different storage time.

It is clear from the Figure 1a that with an increase in the storage time, the percentage of aflatoxin M_1 bound increased in the control samples, while the increase in the initial concentration of the toxin reduced its removal. The results revealed that the toxin was bounded at about 90% by yoghurt starter bacteria and then remained constant at the lowest initial concentration of the toxin (100 pg mL^{-1}) for the first day of storage (Figure 1-a). However, with an increasing concentration, the binding amount decreased considerably, as it declined to 29.45% and 49.53% at 500 and 750 pg mL^{-1}, respectively. Moreover, differences between the initial toxin concentrations were significant ($P<0.05$). Similar results were obtained by El-Khoury *et al.* (2011), El-Nezami *et al.* (1998), Hassanin (1994), Lee *et al.* (2003), Pranoto *et al.* (2007), and Sarimehmetoglu and Kuplulu (2004). Mycotoxin binding was dependent on its solution concentration and was always linear at low level of aflatoxin B_1 and showed the transition to a plateau with higher toxin concentrations. The amount of toxin removed increased with increasing aflatoxin B_1 concentration, but the percentage removed decreased with increasing toxin concentration, because the saturation started (Lee *et al*., 2003).

According to Figure 1 (b-e), a similar rising trend of removal toxin is observed by an increase in initial concentration of the toxin from 100 to 750 pg mL^{-1}, in viable, acid-, heat- and ultrasound-treated yeast, while there is no regular trend during storage times of yoghurt.

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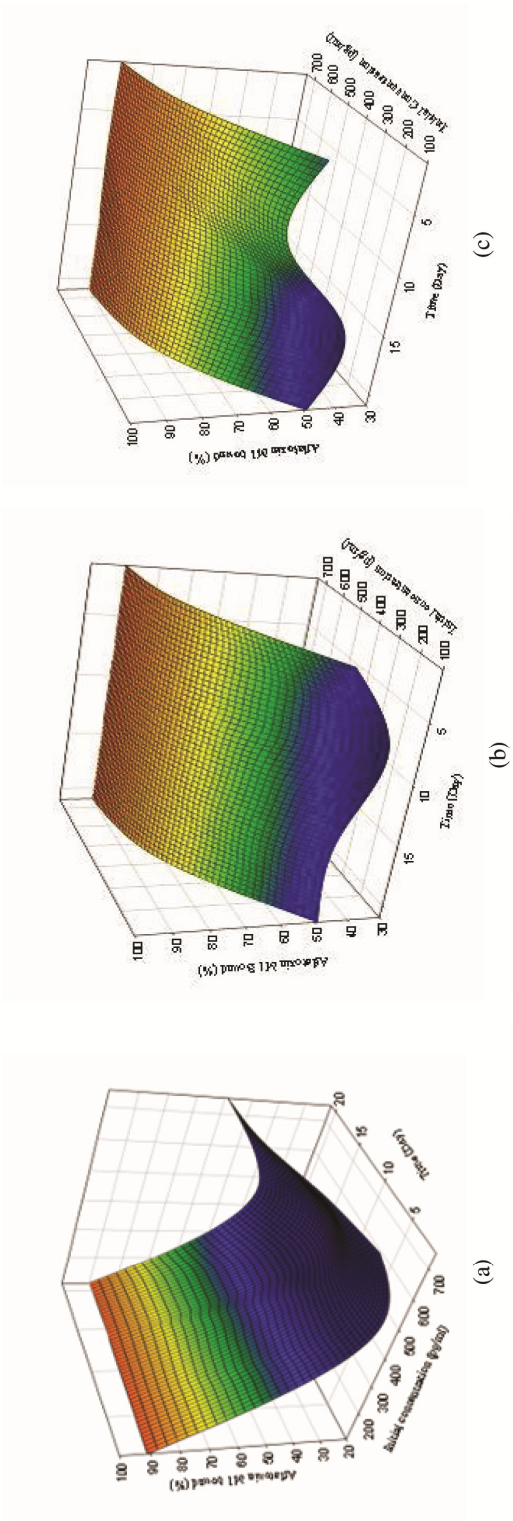

Figure 1. The interaction effect of initial concentration of toxin and storage time on aflatoxin M_1 bound. (a: Control; b: Viable; c: Acid; d: Heat, and e: Ultrasound) at three replicates.

The results indicate that although removal of the toxin at 500 and 750 pg mL^{-1} increased, this increment was not significant for viable yeast ($P< 0.05$). A significant reduction in aflatoxin M_1 bound was observed at an initial concentration of 100 pg mL^{-1} at seven day of storage compared to the previous and succeeding days of storage ($P> 0.05$). In total, this toxin concentration had significant differences compared to the other concentrations during storage ($P< 0.05$) (Figure 1-b). *S. cerevisiae*, in its viable and untreated form, could bind more than 89% of aflatoxin M_1 at its initial concentration of 750 pg mL^{-1}. However, the toxin binding was around 84% for the contaminated samples, containing 500 pg mL^{-1} and, with a reduction of the initial concentration to 100 pg mL^{-1}, the aflatoxin binding decreased to about 47%. These results were similar to those obtained by Kusumaningtyas *et al.* (2006) and Shetty *et al.* (2007). They concluded that the absolute amounts of aflatoxin B_1 that were bound increased steadily with increasing aflatoxin B_1 concentrations.

As Figure 1-c shows the highest amount of aflatoxin was removed from the supernatant of acid-treated yeast after seven days of storage and removal of the toxin was 66.36, 85.11, and 90.27% at 100, 500, and 750 pg mL^{-1} toxin concentrations, respectively. However, these differences during storage time were significant only at the lowest toxin concentration ($P< 0.05$). The highest removal percentage was obtained after seven days storage of the yoghurt samples containing acid-treated yeasts. The reason could be related to a chemical change in the bacteria cell wall structure due to an increase in acidity during storage. Pranoto *et al.* (2007) reported that at low pH (≤ 5), amount of bound aflatoxin by bacteria was higher than at pH 6 and 7. Besides affecting aflatoxin, pH also affected lactic acid bacteria itself. Haskard *et al.* (2001) reported that acid treatment could affect components of cell wall like polysaccharide and peptidoglycan. Acid could destroy cell wall of bacteria, thereby causing aflatoxin B_1 to be easily bound by constituents of sitoplasmic membrane. Furthermore, it makes aflatoxin B_1 to be bound rapidly at lower pH.

As shown in Figure 1d, the highest and lowest removal percentages are related to 750 and 100 pg mL^{-1} concentrations for the heat-treated yeast, respectively; however, no significant differences were observed between the 500 and 750 pg mL^{-1} concentrations ($P> 0.05$). According to the results obtained, the toxin binding by the heat-treated yeast increased after 21 days of yoghurt storage, although no significant increase was observed until 14 days ($P> 0.05$) (Figure 1-d). Bejaoui *et al.* (2004), Corassin *et al.* (2012), El-Nezami *et al.* (1998), Rahaie *et al.* (2010), Sahebghalam *et al.* (2013), Shetty *et al.* (2006), and Shetty *et al.* (2007) found that the amounts of toxin removed by heat-treated yeast depend on initial toxin concentrations.

Figure 1e shows the interaction effect of the initial toxin concentration and yoghurt storage time on the amount of bounded aflatoxin M_1 for ultrasound-treated yeast. Although according to the results, an increase in the removal of toxin from the supernatant was observed at 500 and 750 pg mL^{-1} initial concentrations; this increment was not significant ($P> 0.05$). At the lowest toxin concentration, a significant difference was observed during storage time. The highest percentage of toxin removal for the ultrasound-treated yeast was related to the highest level of aflatoxin, which was 750 pg mL^{-1}. Furthermore, the results illustrated that an increase in the initial concentration of the toxin from 100 to 750 pg mL^{-1} caused a significant increase in toxin binding for the ultrasound-treated yeast ($P< 0.05$). For these yeasts, the highest toxin binding percentage was obtained in the first days of storage, and a significant decrease was observed for the bounded aflatoxin M_1 after 14 days of yoghurt storage ($P< 0.05$).

CONCLUSIONS

There are no previous studies to evaluate the effect of *S. cerevisiae* on removal of aflatoxin M_1 in yoghurt. Although low levels

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of aflatoxin M_1 in yoghurt can be achieved by prevention through controlling contamination levels of aflatoxin B_1 in feed, our results indicate that viable and nonviable cells of *S. cerevisiae* may be useful for significantly removing aflatoxin M_1 from yoghurt containing up to 750 pg mL^{-1}. Viable, acid-, heat- and ultrasound-treated *S. cerevisiae* cells have a high efficiency to bind aflatoxin M_1 in yoghurt. Therefore, the methods of aflatoxin removal employing *S. cerevisiae*, mainly those strains that are already currently used in food products, have a potential application for reducing the levels of aflatoxin M_1 in yoghurt and other fermented foods at the household and industrial level. However, aiming the commercial application in the dairy industry, further studies are needed to investigate the mechanisms involved in the removal process of the toxin by *S. cerevisiae* and the factors that affect the stability of the toxin sequestration such as the concentration of yeast, strains of yeast, acidity, and type of starter culture.

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REFERENCES

1. Alberts, J. F., Gelderblomb, W. C. A., Botha, A. and Van Zyl, W. H. 2009. Degradation of Aflatoxin B_1 by Fungal Laccase Enzymes. *Int. J. Food Microbiol.,* **135:** 47-52
2. Bejaoui, H., Mathieu, F., Taillandier, P. and Lebrihi, A. 2004. Ochratoxin A Removal in Synthetic and Natural Grape Juices by Selected Oenological *Saccharomyces* Strains. *J. Appl. Microbiol.,* **97(5):**1038–1044.
3. Bilek, S. E. and Turantas, F. 2013. Decontamination Efficiency of High Power Ultrasound in the Fruit and Vegetable Industry: A Review. *Int. J. Food Microbiol*., **166:** 155–162.
4. Brackett R. E. and Marth, E. H. 1982. Association of Aflatoxin M_1 with Casein. Z. *Lebensm Unters Forsch*, **174:** 439-441.
5. Corassin, C., Bovo, F., Rosim, H. and Oliveira, R. E. 2013. Efficiency of *Saccharomyces cerevisiae* and Lactic Acid Bacteria Strains to Bind Aflatoxin M_1 in UHT Skim Milk. *Food Control.,* **31:** 80-83
6. Devegowda, G., Arvind, B. R. and Morton, M. G. 1996. *Saccharomyces cerevisiae* and Mannanoligosacharides to Counteract Aflatoxicosis in Broilers. *Proc. Aust. Poul. Sci. Symp. Sydney,* **8:** 103–106.
7. El-kest, M. M., El-Hariri, M., Khafaga, N. I. and Refai, M. K. 2015. Studies on Contamination of Dairy Products by Aflatoxin M_1 and Its Control by Probiotics. *J. Glob. Biosci.,* **4(1):** 1294-312.
8. El-Khoury, A., Atoui, A. and Yaghi, J. 2011. Analysis of Aflatoxin M_1 in Milk and Yogurt and Aflatoxin M_1 Reduction by Lactic Acid Bacteria Used in Lebanese Industry. *Food Control*., **22:** 1695-1699.
9. El-Nezami, H., Kankaanpaa, P., Salminen, S. and Ahokas, J. 1998. Ability of Dairy Strains of Lactic Acid Bacteria to Bind a Common Food Carcinogen, Aflatoxin B_1. *Food Chem. Toxicol*., **36:** 321-326.
10. Elsanhoty, R. M., Salam, S. A., Ramadan, M. F. and Badr, F. H. 2014. Detoxification of Aflatoxin M_1 in Yoghurt Using Probiotics and Lactic Acid Bacteria. *Food Control.,* **43:** 129-34.
11. Haskard, C. A., El-Nezami, H. S, Kankaanpaa, P. E., Salminen, S. and Ahokas, J. T. 2001. Surface Binding of Aflatoxin B1 by Lactic Acid Bacteria. *Appl. Environ. Microbiol*., **67:** 308-316.
12. Hassanin, N. 1994. Stability of Aflatoxin M1 during Manufacture and Storage of Yoghurt, Yoghurt-cheese and Acidified Milk, *J. Sci. Food Agri*., **65:** 31-34.
13. Hegazy, E. M., Sadek, Z. I., El-Shafei, K. and Abd El-Khalek, A. B. 2011. Aflatoxins Binding by *Saccharomyces cerevisiae* and *S. boulardii* in Functional Cereal Based Ice-cream. *Life Sci. J.,* **8(4):** 75-81.
14. Kusumaningtyas, E. Widiastuti, R. and Maryam, R. 2006. Reduction of Aflatoxin B1 in Chicken Feed by Using *Saccharomyces cerevisiae*, *Rhizopus oligosporus* and Their Combination. *Mycopathologia*, **162:** 307–311.
15. Lee, Y. K., El-Nezami, H., Haskard, C. A., Gratz, S., Puong, K. Y., Salminen, S. and Mykkanen, H. 2003. Kinetics of Adsorbtion

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and Desorbtion of Aflatoxin B_1 by Viable and Non Viable Bacteria. *J. Food Protec.*, **66(3):** 426-430.

16. Limaye, M. S. and Coakley, W. T. 1998. Clarification of Small Volume Microbial Suspensions in an Ultrasonic Standing Wave. *J. Appl. Microbiol*. **84**:1035–1042.
17. Martin, A. and Palomino, J. C. 2009. *Procedure Manual, Nitrate Reductase Assay: Drug susceptibility testing for Mycobacterium tuberclusis*. Procedure Manual, Version 2, Mycobacteriology Unit, Institute of Tropical Medicine, Antwerp, Belgium.
18. Masoero, F., Gallo, A., Diaz, D., Piva, G. and Moschini, M. 2009. Effects of the Procedure of Inclusion of a Sequestering Agent in the Total Mixed Ration on Proportional Aflatoxin M_1 Excretion into Milk of Lactating Dairy Cows. Anim. Feed Sci. Technol., **150:** 34–45.
19. Mohamadi, H. and Alizadeh, M. 2010. A Study of the Occurrence of Aflatoxin M1 in Dairy Products Marketed in Urmia, Iran. *J. Agri. Sci. Technol.,* **12:** 579-583
20. Mokoena, M. P., Chelule, P. K., and Ggaleni N. 2006. The Toxicity and Decreased Concentration of Aflatoxin B1 in Natural Lactic Acid Fermented Maize Meal. *J. Appl. Microbiol.* **100**:773–777.
21. Peltonen, K., EL-Nezami, H., Haskard, C., Ahokas, J. and Salminent, S. 2001. Aflatoxin B_1 Binding by Dairy Strains of Lactic Acid Bacteria and Bifidobacteria. *J. Dairy Sci.,* **84:** 2152–2156.
22. Phillips, T. D., Bashir Sarr, A. and Grant, P. G. 1995. Selective Chemisorption and Detoxification of Aflatoxins by Phyllosilicate Clay. *Natur. Toxin.* **3:** 204–213.
23. Pranoto, Y., Amanah, H., Utami, T. and Rahayu, E. 2007. Study on Factors Affecting Aflatoxin B1 Binding by Lactobacillus Acidophilus SNP-2. *International Agricultural Engineering Conference,* Bangkok, Thailand.
24. Rahaie, S., Razavi, S. H. and Emam Jomeh, Z. 2010. The Ability of *Saccharomyces Cerevisiae* Strain in Aflatoxin Reduction in Pistachio Nuts. *Iran. J. Food Sci. Technol., (JFST),* **7(1):** 81-88.
25. Raju, M. V. L. N. and Devegowda, G. 2000. Influence of Modified Glucomannan on Performance and Organ Morphology, Serum Biochemistry and Hematology in Broilers Exposed to Individual and Combined Mycotoxicosis (Aflatoxin, Ochratoxin and T-2 toxin). *Br. Poult. Sci*. **41**:640-650.
26. Rasic, J. L., Skrinjar, M. and Markov, S. 1991. Decrease of Aflatoxin B1 in Yoghurt and Acidified Milks. *Mycopathologia*, **113:** 117-119.
27. Sahebghalam, H., Mohammadi Sani, A. and Mehraban, M. 2013. Assessing the Ability of *Saccharomyces cerevisiae* to Bind Aflatoxin B1 from Contaminated Medium. *Nutr. Food Sci.,* **43(4):** 392-397.
28. Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. *Molecular Cloning: A Laboratory Manual*. 2nd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, USA.
29. Sarimehmetoglu, B. and Kuplulu, O. 2004. Binding Ability of Aflatoxin M_1 to Yoghurt Bacteria. *Ankara Univ. Vet. Fat Derg*. 51:195–198.
30. Shahin, A. A. M. 2007. Removal of Aflatoxin B_1 from Contaminated Liquid Media by Dairy Lactic Acid Bacteria. Int. J. Agri. Biol., **9(1):** 71-75.
31. Shetty, P. and Jespersen, L. 2006. *Saccharomyces cerevisiae* and Lactic Acid Bacteria as Potential Mycotoxin Decontaminating Agents. *Trend. Food Sci. Technol.,* **17:** 48–55.
32. Shetty, P. H., Hald, H. and Jespersen, L. 2007. Surface Binding of Aflatoxin B_1 by *Saccharomyces cerevisiae* Strains with Potential Decontaminating Abilities in Indigenous Fermented Foods. *Int. J. Food Microbiol.,* **113:** 41–46.
33. Suttajit, M. 1989. Prevention and Control of Mycotoxins. In: "*Mycotoxin Prevention and Control in Food Grains*", (Eds.): Semple, R. L., Firo, A. S., Hicks, P. A. and Lozare, J. V. Vialedelle Termedi Caracalla, FAO, Rome, Italy.
34. Tabata, S., Kamimura, A., Ibe, H., Hasimoto, H., Ida, M., Tamura, Y. and Nishima, T. 1993. Aflatoxin Contamination in Foods and Foodstuffs in Tokyo: 1986-1990. *J. AOAC Int.,* 76:32-35.
35. Zlotnik, H., Fernandez, M. P., Bowers, B. and Cabib, E. 1984. Saccharomyces Cerevisiae Mannoproteins form an External Cell Wall Layer That Determines Wall Porosity. *J Bacteriol*. **159**:1018–1026.
36. Zinedine, A., Faid, M. and Belemlih, M. 2005. *In vitro* Reduction of Aflatoxin B_1 by Strains of Lactic Acid Bacteria Isolated from Moroccan Sourdough Bread. *Int. J. Agri. Biol.,* **7:** 67-70.

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جذب سطحی آفلاتوکسین M1 توسط مخمر ساکارومیسس سرویزیه در ماست

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چکیده

توکسین‌زدایی میکروبی یکی از روش‌های حذف آفلاتوکسین‌ها از جمله آفلاتوکسین M1محسوب می‌شود. گزارشات نشان دهنده آن است که مخمر ساکارومیسس سرویزیه از طریق جذب سطحی آفلاتوکسین‌ها به دیواره سلولی خود، می‌تواند در حذف آن مؤثر باشند. در این تحقیق توانایی مخمر ساکارومیسس به شکل‌های زنده، تیمارشده با اسید، اولتراسوند و حرارت، در میزان جذب آفلاتوکسین M1 در ماست بررسی گردید. بدین منظور شیر بازسازی‌شده حاوی ۱۲ درصد ماده جامد بدون چربی از پودر شیر پس‌چرخ تهیه شد. سپس نمونه‌ها با غلظت‌های ۱۰۰، ۵۰۰ و ۷۵۰ پیکوگرم در میلی‌لیتر آفلاتوکسین M1 آلوده شدند. در زمان افزودن باکتری‌های آغازگر به شیر، مخمرهای تیمارشده نیز اضافه گردید. غلظت آفلاتوکسین باقیمانده در سوپرناتانت نمونه‌های ماست در روزهای اول، هفتم، چهاردهم و بیست و یکم پس از تولید ماست، توسط روش الایزای رقابتی تعیین شد .یافته‌های حاصل از این تحقیق نشان داد هرچند، همه‌ی تیمارهای مورد بررسی شامل مخمر زنده، مخمر تیمار شده با اسید، تیمار شده با اولتراسوند، تیمار شده با حرارت و فاقد مخمر، قادر به جذب آفلاتوکسین M1 می‌باشند، اما توانایی مخمر زنده، مخمر تیمار شده با اسید، اولتراسوند و حرارت در جذب مقادیر مختلف توکسین در مقایسه با آغازگرهای ماست به لحاظ آماری معنی‌دار می‌باشد (P<۰/۰۵). در میان مخمرهای تیمارشده نیز توانایی مخمر تیمارشده با اسید در جذب توکسین بیشتر از سایر تیمارها به دست آمد. در مجموع می‌توان نتیجه گرفت استفاده از مخمر ساکارومیسس سرویزیه (زنده، تیمار شده با اسید ، اولتراسوند و حرارت) در جذب بیولوژیکی آفلاتوکسین M1 از فرآورده‌های لبنی تخمیری بسیار موثر خواهد بود.

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