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# Ability of selected microorganisms for removing aflatoxins *in vitro* and fate of aflatoxins in contaminated wheat during baladi bread baking

Rafaat M. Elsanhoty [a,*], Mohamed Fawzy Ramadan [b,c], Sahar S. El-Gohery [d], M.F. Abol-Ela [e], M.A. Azeke [f]

[a] *Institute of Genetic Engineering and Biotechnology, Department of Industrial Biotechnology, Branch of Food and Dairy Biotechnology, Menoufia University, Egypt*
[b] *Biochemistry Department, Faculty of Agriculture, Zagazig University, Zagazig 44519, Egypt*
[c] *Institute of Scientific Research and Revival of Islamic Heritage, Umm Al-Qura University, Makkah, Saudi Arabia*
[d] *Department of Home Economic, College of Education, Dammam University, Hafr Elbatain, Saudi Arabia*
[e] *Regional Center for Food and Feed, Agric. Res. Center, Giza, Egypt*
[f] *Department of Biochemistry, Ambrose Alli University, P.M.B. 14, Ekpoma, Edo State, Nigeria*

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ABSTRACT

Aflatoxins (AFs), the secondary metabolites produced by species of *Aspergillus*, have harmful effects on humans, animals, and crops that result in illnesses and economic losses. This investigation was designed to assess the potential of four strains of lactic acid bacteria (LAB) for removing AFs *in vitro*. The stability of AFs complexes formed with LAB in viable and non-viable (heat treated) forms was assessed. The strain with the highest ability to bind AFs was selected to study its impact on removing AFs from contaminated wheat flour during baladi bread baking process. Three treatments of baladi bread produced from contaminated wheat flour were formulated including treatment (A) as control fermented by bakery yeast, treatment (B) fermented by *Lactobacillus rhamnosus* TISTR 541 and treatment (C) fermented by the mixture of bakery yeast and *L. rhamnosus*. The samples were collected during different steps of bread baking process. The results indicated that there were significant differences between the amounts of AFB and AFG detected in each sample and there were significant differences between the strains in their ability to bind AFB and AFG in the viable and heated stage. *L. rhamnosus* was the highest strain able to bind the $AFB_1$ and treatment C was the highest in removing of AFs from contaminated wheat flour during baking process.



## 1. Introduction

Aflatoxins (AFs) $B_1$, $B_2$, $G_1$ and $G_2$ are produced as secondary metabolites of fungal strains (*Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*) that grow on a variety of food and feed commodities during growth, harvest, storage and transportation (Jiang et al., 2005; Peltonen, El-Nezami, Haskard, Ahokas, & Salminen, 2001; Somorin, Bertuzzi, Battilani, & Pietri, 2012). AFs, a group of mycotoxins with mutagenic, carcinogenic, teratogenic, hepatotoxic and immunosuppressive properties, are of particular importance because of their adverse effects on animal and human health (Lewis et al., 2005; Somorin et al., 2012). The primary classes of mycotoxins are AFs, zearalenone, trichothecenes, fumonisins (FBs), ochratoxin A and ergot alkaloids (Var & Kabak, 2009). Aflatoxin $B_1$ ($AFB_1$), the most toxic AF, is of particular interest because it is a frequent contaminant of many food products and one of the most naturally occurring mutagens and carcinogens (Teniola et al., 2005).

AFs and FBs are known to be hazardous to the health of humans, in some cases directly causing illness and even death. AFs and FBs occur worldwide in maize, either alone or together (Kpodo, Thrane, & Hald, 2000). AFs are also implicated in liver cancer (JECFA, 1998; Wild & Hall, 2000). AFs have been reported to impair childhood growth in children from Benin and Togo (Gong et al., 2002). FBs were reported to be associated with oesophageal cancer in rural areas in South Africa (Rheeder et al., 1992) and liver cancer in China (Chu & Li, 1994; Ueno et al., 1997). Consumption of foods containing high levels of AFs could even lead to death as it was experienced in Kenya (Probst, Njapau, & Cotty, 2007).

* Corresponding author. Present address: Faculty of Agriculture and Veterinary Medicine, Department of Food Science and Human Nutrition, Qassum University, Kingdom of Saudi Arabia. Tel.: +966 55 8996968; fax: +966 63801360.
*E-mail addresses:* rafaatmhamed@yahoo.com, hassanienmohamed@yahoo.com (R.M. Elsanhoty).

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Mycotoxins cause an undesirable effect (mycotoxicosis) when animals and human are exposed. Biological effects include liver and kidney toxicity, nervous system effects and estrogenic effects. Effects of processing on mycotoxin contamination in food products are being investigated and this strategy was showing great promise for mycotoxin reduction. The use of physical methods, including cleaning, separation of screenings, washing, aqueous extraction, dehulling and milling, has been shown to be effective, to a certain extent, in reducing mycotoxins in cereals (Charmley & Prelusky, 1995; Shetty & Bhat, 1999; Voss, Poling, Meredith, Bacon, & Saunders, 2001). AFs and FBs levels in tortillas were found to be significantly reduced due to alkaline cooking (Voss et al., 2001).

Due to the increasing number of studies on the toxic nature of AFs, there is a need to control AFs levels in food. Methods of screening can be classified in two categories: (1) prevention of mold contamination and growth, and (2) detoxification of contaminated products, but those methods till now not accepted (Mishra & Das, 2003). Fermentation of food has been used as a method of preservation for centuries, wherein lactic acid bacteria (LAB) are reported to reduce mold growth and AFs production (Elsanhoty, 2008; Mokoena, Chelule, & Gqaleni, 2006). LAB and bifidobacteria, due in large part to their Generally Recognize as Safe (GRAS) status and use as probiotics, are of particular interest for reducing the bioavailability of AFs. Several studies have screened these microorganisms for the ability to bind AFs and have reported a wide range of species and strains with specific binding capacities (Gratz, Mykkanen, & El-Nezami, 2005; Haskard, El-Nezami, Kankaanpaa, Salminen, & Ahokas, 2001; Peltonen et al., 2001; Shahin, 2007; Zinedine, Faid, & Benlemlih, 2005).

This work was planned to screen four LAB and bifidobacteria strains for their ability to remove AFs. The screening was focused on the ability to remove the substances that can be used as a biodetoxification. The best performing strains were further investigated for their ability to bind AFB and AFG from contaminated wheat flour during bread baking as a fate study for AFs during the baking process and using the strain as a starter in dough.

## 2. Materials and methods

### 2.1. Bacterial strains and preparation of cultured bacteria and bacterial cell

Four strains of LAB and probiotic were obtained from different international culture collections (Table 1). These strains were selected based either on their common use in food industry or on available information regarding their effects on food mutagens. Strains were cultured on deMan, Rogosa and Sharpe (MRS, Difico Laboratories) agar plates. Anaerobic strains were kept in an anaerobic jar (Anaerogen, Oxoid). Fully grown colonies were stored on plates at 4 °C until needed with sub culturing on a monthly basis. For long-term conservation of strains, spore or cell suspensions were kept in cryovials at −80 °C with 90% glycerol as cryoprotectant.

**Table 1**
Origin of bacterial strains investigated for its ability of binding of AFs.

| Strain | Origin[a] | Oxygen requirement |
|---|---|---|
| *Lactobacillus acidophilus* ATCC 20552 | 1 | Anaerobic |
| *Lactobacillus rhamnosus* TISTR 541 | 2 | Aerobic |
| *Lactobacillus sanfranciscensis* DSM20451 | 3 | Aerobic |
| *Bifidobacterium angulatum* DSMZ 20098 | 3 | Anaerobic |

[a] (1) Egyptian Microbial culture collection (EMCC) at Cairo Microbiological Resources Centre (Cairo MIRCEN), Faculty of agriculture, Ain Shams University (Egypt). (2) Institute of Scientific and Technological Research, Bangkok (Thailand). (3) Centre for culture collection (DSMZ-Germany).

*Lactobacilli* were cultivated in MRS broth and bifidobacteria were grown in MRS broth with supplementation with cysteine and incubated for 24 h at the suitable growth temperature (Table 1). An appropriate volume of culture was used to inoculate 150 mL cultures and incubated for 24 h at 37 °C in an anaerobic jar (Anaerogen, Oxoid). Cultured bacteria were collected using of centrifuge at 6000*g* for 15 min under cooling (10 °C) and under sterile conditions to remove the supernatant. The bacterial cells were washed five times by 5 mL sterile dionoized water to avoid the effect of bacterial concentration on $AFB_1$ removal of phosphate-buffered saline (PBS; pH 7.3, 0.01 M) prior to use for AFs binding ability.

### 2.2. Determination of AFs binding properties

Solid $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ (Sigma, St. Louis, Mo.) were dissolved in acetonitrile–benzene (3:97, v/v) to obtain AFs concentration of 4 mg/mL and keep as stock solution. The concentration was determined spectrophotometrically at 348 nm. To prepare an aqueous solution, 50 μL of methanol was added then made to volume with PBS, or PBS was added directly and the acetonitrile–benzene was evaporated by heating in a water bath at 80 °C for 10 min. The bacterial pellet was suspended in PBS (1.5 mL) containing 15 μg of $AFB_1$ per mL and incubated at 37 °C for 24 h. The bacteria were pelleted by centrifuge at 3000 × *g* for 15 min at 10 °C. The samples of supernatant fluid (200 μL) containing unbound $AFB_1$ were collected and stored at −20 °C. All assays were performed in triplicates, and both positive controls (bacteria suspend in PBS) and $AFB_1$ controls (5 μg/mL of $AFB_1$ in PBS) were included prior to analysis by high performance liquid chromatography (HPLC).

### 2.3. Determination of complex stability

To study complex stability, cultured bacterial samples ($10^8$ bacteria) were used. In the surface binding study, cultured ($10^8$ bacteria) bacterial samples were used. Bacteria were either incubated as viable (in 4 mL of PBS for 1 h) or heat treated (boiled in 4 mL of PBS for 1 h). All bacterial samples were centrifuged and the supernatant was removed prior to AFs binding assays. All incubations were carried out at 37 °C, and all centrifugations were at 2500 × *g* for 10 min (10 °C).

### 2.4. Preparation of grain samples

Wheat grain samples were scratched by shaking with sand for 1 min, disinfested by immersing in 5% sodium hypochlorite for 2 min, washed thoroughly with sterilized water and dried at 44 °C for 42 h. All samples were divided into sub-samples and transferred into 3 sterilized plastic suck (1 L).

### 2.5. Inoculation of spore suspension and production of AFs in wheat

Spore suspension was prepared from pure cultures of *A. flavus* (21 days old) grown on PDA plates (9 cm). These plates were flooded with 15 mL of sterilized distilled water and brushed thoroughly for 1–2 min. The suspension was filtered through three layers of cheesecloth to remove the mycelia residues. Number of spores/mL was counted in the collected spore suspension using a Spencer haemocytometer to about $10^6$ spores/mL. Spore suspension was inoculated to test grains and give a final density of approximately 3000–3500 spore/g of wheat grains as described by Eisa et al. (1996). Moisture content of wheat grains was adjusted wherein the required volume of water needed for each moisture level was calculated according to the following

$$S = \frac{\text{required moisture content} - \text{initial moisture content}}{100 - \text{required moisture content}} \times 100$$

$S$ is the water volume required for 100 g of wheat grains to reach 20% then stored at room temperature for 20 days. AFs production was determined in 100 g from wheat grain samples then the grains were milled to give wheat flour (82%). After the sieving screen (40 mesh), the flour samples produced from infected wheat were taken to determine $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$.

### 2.6. Baladi bread baking

Baladi bread was manufactured according to Khorshied, Emora, and Hawas (1989). The contaminated wheat flour (82% extraction) was divided into three portions (A, B and C) of 300 g each. The first portion was used to manufacture of baladi bread as a control (A) wherein bakery yeast was used for fermentation. The second portion (B) was baked baladi bread using *L. rhamnosus* TISTR 541 with 5% (incubated 24 h) from the flour basis. The third portion (C) was used to bake baladi bread fermented by mixture of 1% yeast and 2% (24 h incubation) *L. rhamnosus* TISTR 541. In all treatment salt (1% flour basis) and 210 mL of water added. All materials were mixed, the dough was covered and left for 20 min at 28–30 °C then manually divided. Dough was placed on wooden boards previously dusted with wheat bran for 15–20 min, flattened manually (proofing at 28–30 °C for 60 min, RH = 75–85%), then final flattening was done with removal of excess bran. The baking was carried out at 450–500 °C for 1–1.5 min. Finally, the bread was aerated at room temperature for 30 min at least. The samples were collected from all treatments after mixing directly, after fermentation for 1 h and 2 h and after baking and kept at −2 °C to determine AFs using HPLC.

### 2.7. Determination of AFs in residual $AFB_1$ in supernatant, wheat, dough and bread samples

Standards for $AFB_1$ were obtained from Sigma (St. Louis, MO, USA). Stock solutions and standards were prepared and assayed according to Roos, Van Der Kamp, and Marley (1997) using HPLC. The HPLC analysis was conducted using a HP gradient system, fitted with an HP1100 pump, a HP1100 fluorescence detector (detector wavelength was set at 360 nm for excitation, and at 440 nm for emission) and a D-2500 integration system. Twenty five microliter of the samples were injected into a $C_{18}$ column (25 cm × 4.6 mm ID; Merck, Darmstadt, Germany) at a flow rate of 1 mL/min. The level of AFs was measured in part per billion (ppb).

### 2.8. Statistical analysis

All assays in this study were carried out in triplicate. Statistical analysis of the data was carried out according to Gomez and Gomez (1984, pp. 139) to identify significant differences between bacterial strains and bread treatments. The results are considered to be statistically different at ($P < 0.05$).

## 3. Results and discussion

Physical and chemical detoxification methods have limitations, such as loss of product nutritional value, organoleptic qualities, undesirable health effects, and high cost (Basappa & Shantha, 1996). Beneficial microorganisms such as bacteria and fungi substantially contribute to reducing AFs in contaminated media (Farzaneh et al., 2012; Phillips, Clement, & Douglas, 1994). Bacteria have more application for the aflatoxin remediation because of some advantages such as more elimination within short time as well as producing non pigments (Laciakova, Cicoova, Mate, & Lasciak, 2008). Several studied have been published about $AFB_1$ reduction by some bacterial isolates. LAB were found to be active in removing $AFB_1$ primarily by the adhesion method (Farzaneh et al., 2012; Gratz et al., 2005; Khanafari, Soudi, Miraboulfathi, & Karamei Osboo, 2007).

### 3.1. Binding of $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ and complex stability

Several strategies including physical, chemical and biological methods have been investigated to inactivate or reduce the bioavailability of AFs. Probiotics, living microorganisms which, when ingested at sufficient numbers, exert a beneficial effect on the host organism beyond inherent general nutrition (CAST, 2007; Hernandez-Mendoza, Garcia, & Steele, 2009), hold great promise for reducing the bioavailability of AFs. LAB are most commonly consumed. The objectives of this study were to screen four strains of LAB for the ability to bind AFs and investigate the stability of the bacterial–AFs complexes.

The bacterial cell envelope appears to be the site of AFs binding, with cell wall polysaccharides and peptidoglycan thought be the molecules of greatest importance (Haskard et al., 2001; Lahtinen, Haskard, Ouwehand, Salminen, & Ahokas, 2004). The stability of the AFs–bacterial cell complex is also a key consideration when evaluating a strain ability to reduce AFs bioavailability in foods, as AFs release during gastric passage would have clear negative health implications. Binding of AFs to bacterial cells has been demonstrated to be reversible and the stability of the AFs–bacterial cell complex dependent on the strain utilized, conditions utilized

**Table 2**
Percentages of $AFB_1$ and $AFB_2$ bound to viable and heat-treated bacteria and remaining bound after up to five washes.

| Microorganism | Bound $AFB_1$ and $AFB_2$ (±SD) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Viable | | | | Heat-treated | | | |
| | Initial | | Final | | Initial | | Final | |
| | $AFB_1$ | $AFB_2$ | $AFB_1$ | $AFB_2$ | $AFB_1$ | $AFB_2$ | $AFB_1$ | $AFB_2$ |
| *Lactobacillus acidophilus* ATCC 20552 | 53.7 ± 1.9 | 51.9 ± 2.1 | 24.3 ± 2.3 | 19.8 ± 4.1 | 73.8 ± 0.9 | 69.9 ± 2.3 | 30.9 ± 2.7 | 35.6 ± 3.6 |
| *Lactobacillus rhamnosus* TISTR 541 | 79.4 ± 3.4 | 69.6 ± 3.1 | 35.8 ± 3.7 | 34.1 ± 1.3 | 84.7 ± 2.3 | 80.1 ± 1.8 | 72.7 ± 2.9 | 68.99 ± 1.9 |
| *Lactobacillus sanfranciscensis* DSM20451 | 22.6 ± 1.7 | 21.6 ± 4.1 | 1.9 ± 3.6 | 2.7 ± 2.4 | 44.5 ± 2.1 | 35.4 ± 1.9 | 16.6 ± 2.3 | 11.5 ± 1.4 |
| *Bifidobacterium angulatum* DSMZ 20098 | 49.8 ± 1.8 | 50.8 ± 2.2 | 16.8 ± 2.9 | 18.9 ± 2.1 | 69.5 ± 1.7 | 62.9 ± 2.8 | 33.1 ± 1.7 | 36.4 ± 3.1 |

Bacteria were incubated in PBS (4 mL) at 37 °C for 1 h (viable), boiled in PBS (4 mL) for 1 h (heat-treated).
Initial, percentage of $AFB_1$ removed after $10^8$ bacteria were incubated with AFs.
Final, percentage of AFs remaining bound to the bacteria after five washes (1.5 mL each) with deionized sterile water. When the amount of AFs released was below the HPLC limit of detection prior to the fifth wash, AFs remaining bound after the first, second, third, or fourth wash was used, as indicated in parentheses.
Results are the average ± SD for triplicate samples.

**Table 3**
Percentages of $AFG_1$ and $AFG_2$ bound to viable and heat-treated bacteria and remaining bound after up to five washes.

| Microorganism | Bound $AFB_1$ and $AFB_2$ (±SD) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Viable | | | | Heat treated | | | |
| | Initial | | Final | | Initial | | Final | |
| | $AFG_1$ | $AFG_2$ | $AFG_1$ | $AFG_2$ | $AFG_1$ | $AFG_1$ | $ABG_1$ | $ABG_2$ |
| *Lactobacillus acidophilus* ATCC 20552 | 50.7 ± 2.1 | 53.5 ± 2.3 | 21.4 ± 2.2 | 186 ± 3.7 | 71.7 ± 1.3 | 67.9 ± 21 | 31.9 ± 2.2 | 34.6 ± 3.3 |
| *Lactobacillus rhamnosus* TISTR 541 | 69.4 ± 2.3 | 65.7 ± 3.1 | 34.9 ± 3.5 | 36.1 ± 1.3 | 86.2 ± 2.1 | 81.4 ± 1.6 | 74.7 ± 2.6 | 65.18 ± 2.3 |
| *Lactobacillus sanfranciscensis* DSM20451 | 23.6 ± 1.9 | 21.8 ± 2.6 | 2.7 ± 3.3 | 4.7 ± 2.1 | 41.4 ± 1.9 | 33.4 ± 2.1 | 17.6 ± 1.9 | 18.4 ± 1.8 |
| *Bifidobacterium angulatum* DSMZ 20098 | 44.8.±2.5 | 52.8 ± 2.4 | 16.8 ± 2.9 | 15.9 ± 3.1 | 66.8 ± 1.9 | 61.7 ± 2.3 | 35.4 ± 1.8 | 32.2 ± 2.1 |

Results are the average ± SD for triplicate samples.

during complex formation and the treatment used to assess stability (Haskard et al., 2001).

The AFs binding properties of four LAB and bifidobacteria strains under study are given in Tables 2 and 3. Data showed that strains under investigation have bound $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ efficiently. *L. rhamnosus* TISTR 541 had higher ability (in percentages) to bound $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ than other tested microorganisms (*Lactobacillus acidophilus* ATCC 20552, *Lactobacillus sanfranciscensis* DSM20451, *Bifidobacterium angulatum* DSMZ 20098) in viable and heat-treated stage. Results obtained by HPLC from cultured and heat treatments indicated that the majority of bound $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ are attached to the bacterial surface. AFs were detected on the cultured pellets and at the end of washing of strains. There were significant differences between the amounts of $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ detected in each sample type ($P < 0.05$) and there were significant differences between the strains in their ability to bind AFs in the viable and heat stage treatments. There is considerable variation in the percentage of $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ bound initially and after washing. *L. rhamnosus* TISTR 541 strains was the most effective in initial binding and also retaining $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$, suggesting that the complexes formed with these strain was the most stable. Treatment of bacteria with heat may affect the $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ binding mechanism. Heat treatments also significantly enhanced the ability of bacteria to remove $AFB_1$, $AFB_2$, AFG1 and $AFG_2$. Similar results were obtained by Haskard et al. (2001) and Peltonen et al. (2001) who shown that the relative amounts of $AFB_1$ removed by viable and heat-treated bacteria depend on initial AFs concentrations. The obtained results were in agreement with the results obtained by Hwang, Lee, Kim, Lee, and Jun (2005), Shahin (2007) and Zinedine et al. (2005) who reported that there were a wide range of genus, species and strains with specific binding capacities. LAB and bifidobacteria, have reduced the bioavailability of AFs when these studies have screened dairy strains of LAB to bind a common food carcinogen ($AFB_1$). Heat-killed bacteria have also been reported to bind $AFB_1$ in a strain specific manner (Oatley, Rarick, Ji, & Linz, 2000) where $AFB_1$ was bound to the surface components of LAB (Haskard et al., 2001). El-Nezami, Ploychronki, Salminen, and Mykkanen (2002) demonstrated that strains of *L. rhamnosus* GG and *L. rhamnosus* Lc-705 have the ability to remove zearalenone and its derivative α-zearalenol (55%) with a rapid reaction instantly after mixing with the bacteria. Turbic, Ahokkas, and Haskard (2002) showed that $AFB_1$ (77–99%) and ochratoxin A (36–76%) were removed by *L. rhamnosus* strain in high and moderate amounts. In addition, only minimal amounts of other aromatic dietary substances such as caffeine, vitamin B12 and folic acid were also removed (9–28%). The destruction of specific components of the bacterial cell wall, e.g., carbohydrates and proteins, resulted in reduction in $AFB_1$ binding. The differences in $AFB_1$ binding by the strains are probably due to different bacterial cell wall and cell envelope structures (Haskard et al., 2001). From the obtained results we could conclude that LAB and bifidobacteria under investigation had ability to bind $AFB_1$, wherein *L. rhamnosus* TISTR 541 strain was the highest active strain and therefore it was chosen to remove or reduce of $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ from bread produced from contaminated wheat.

### 3.2. Effect of LAB on AFs levels in dough and baladi bread

Wheat is susceptible to these fungi infections through its growth, harvest, transport, and storage (Giray, Girgin, Basak Engin, Aydın, & Sahin, 2007). Bread is an important staple food in many countries. Since the consumption of bread and bakery products shows an increasing trend in most developing countries, so, bread safety and quality is of major concern. The ability of LAB to inhibit

**Table 4**
AFs levels in contaminated flour, dough and different samples collected during baladi bread baking.

| Samples | Aflatoxins ppb | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Treatment A[a] | | | | Treatment B[b] | | | | Treatment C[c] | | | |
| | $AFB_1$ | $AFB_2$ | $AFG_1$ | $AFG_2$ | $AFB_1$ | $AFB_2$ | $AFG_1$ | $AFG_2$ | $AFB_1$ | $AFB_2$ | $AFG_1$ | $AFG_2$ |
| Contaminated Flour | 29.7 ± 0.9 | 4.28 ± 1.2 | 40.30 ± 1.6 | 5.23 ± 0.9 | 29.7 ± 0.9 | 4.28 ± 1.2 | 40.3 ± 1.6 | 5.23 ± 0.9 | 29.7 ± 0.9 | 4.28 ± 1.2 | 40.3 ± 1.6 | 5.23 ± 0.9 |
| Dough after mixing directly | 23.4 ± 1.8 | 3.54 ± 0.8 | 28.36 ± 2.2 | 3.90 ± 1.02 | 20.5 ± 2.4 | 2.96 ± 0.4 | 25.9 ± 1.6 | 3.63 ± 1.2 | 18.3 ± 1.4 | 2.58 ± 1.1 | 19.8 ± 2.4 | 3.49 ± 0.9 |
| Dough after fermentation for 1 h. | 19.9 ± 1.4 | 2.70 ± 1.6 | 18.60 ± 2.4 | 2.88 ± 1.1 | 14.1 ± 2.9 | 1.99 ± 0.3 | 16.9 ± 2.1 | 2.35 ± 0.6 | 12.6 ± 2.2 | 1.88 ± 0.7 | 14.2 ± 2.3 | 2.11 ± 1.1 |
| Dough after fermentation for 2 h. | 16.2 ± 2.1 | 1.89 ± 2.1 | 15.19 ± 1.7 | 1.98 ± 0.6 | 12.4 ± 3.2 | 1.12 ± 0.2 | 12.6 ± 1.3 | 1.54 ± 0.4 | 10.0 ± 2.1 | 1.32 ± 0.2 | 10.9 ± 2.1 | 1.76 ± 0.3 |
| Bread after baking and aeration | 13.0 ± 1.2 | 1.36 ± 1.3 | 12.79 ± 2.4 | 1.54 ± 0.6 | 10.0 ± 2.6 | 1.09 ± 0.3 | 10.1 ± 2.2 | 1.31 ± 0.2 | 9.12 ± 2.1 | 0.96 ± 0.2 | 8.96 ± 1.2 | 1.07 ± 0.2 |

[a] Treatment (A): Baladi bread as control fermented using yeast.
[b] Treatment (B): fermented by *Lactobacillus rhamnosus* TISTR 541.
[c] Treatment (C): fermented by the mixture of yeast and *Lactobacillus rhamnosus* TISTR 541.

*Aspergillus*, *Fusarium*, and *Penicillium*, the main contaminants in bread, was recently evaluated (Gerez, Torino, Rollán, & Font de Valdez, 2009). It was also reported that sourdough LAB strains are able to inhibit mold growth in bakery products (Corsetti, Gobbetti, Rossi, & Damiani, 1998; Lavermicocca et al., 2000).

The results in Table 4 show the effect of LAB on the AFs levels in dough and different samples collected during baladi bread baking. The results indicate that there were decreasing in AFs levels in all treatments during mixing and fermentation process (after 1 h and 2 h), and after baking and aeration. There were significant differences ($P < 0.05$) between the different treatments in the reduction of $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$. The treatment (C) that fermented by mixture of bakery yeast and *L. rhamnosus* TISTR 541 was higher in the decreasing of AFs than other treatments. This may be due to the ability of *L. rhamnosus* to bind the AFs from the contaminated flour. Similar results were obtained by Haskard et al. (2001), Peltonen et al. (2001) and Shahin (2007) who shown that the relative amounts of $AFB_1$ removed by viable and heat-treated bacteria depend on initial AFs concentrations.

## 4. Conclusion

The present study demonstrates the ability of some strains of LAB and bifidobacteria to reduce the initial concentration of $AFB_1$ in MRS broth in a viable stage and killed stage. The results presented support the conclusions of previous researchers that the ability to bind AFs and the stability of the bacterial cell–AFs complex are bacterial strain dependent traits. On the other hand, these strains have ability to remove AFs from contaminated wheat flour during baladi bread baking. Depending on these results the application of theses strains for removal of mycotoxins from contaminated food and feed is recommended to improve food and feed safety. The future trends are to identify the genetic characteristics that gave the LAB and bacteria the ability to remove or reduce of AFs from food or feed products.

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