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ORIGINAL ARTICLE

Impact of Lactic Acid Bacteria Cells on the Aflatoxin B_1 in Wheat Flour During Manufacture Fino Bread

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KEYWORDS

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Fino bread

ABSTRACT: Lactic acid bacteria (LAB) play many function roles during the preparation food; one of those roles is to remove or reduce mycotoxins from contaminated food. Therefore, this study aimed to study the impact of five strains from LAB (*Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Bifidobacterium bifidum*, *Streptococcus thermophilus* and *Lactobacillus reuteri*) to reduce aflatoxin B_1 (AFB_1) during manufacturing Fino bread. Also, this study has been extended to evaluate the qualities and characteristics of the Fino bread manufactured by treated wheat flour by LAB cells. The data reflected that the percentages of reduction of AFB_1 after mixing ingredients were 9.7, 8.5, 7.04, 7.4 and 5.5% with addition *L. rhamnosus*, *L. plantarum*, *Bifidobacterium bifidum*, *Str. Thermophilus* and *L. reuteri*, respectively. Moreover, the results indicated that the addition of *L. rhamnosus* and *L. plantarum* cells given the highest percentage of removal AFB_1 after fermentation stage were 60.5 and 54.25%, respectively, while the lowest reduction of AFB_1 recorded with the addition of *L. reuteri* cells was 42.25%. AFB_1 reduction has reached 100% in blends treated with *L. rhamnosus* and *L.plantarum* cells in the final bread. The results indicated that the increase in water absorption and the dough development time as well as dough weakening. Finally, the addition of LAB cells didn't show any significant differences in taste, color, odor, and texture for the final bread.

INTRODUCTION

Aflatoxins (AFs) are secondary metabolites produced by many fungi particularly *Aspergillus* species on a large range of agricultural commodities in the field, and also during post-harvest operations and storage, AFs usually enter the body via ingestion of contaminated foods. The four major naturally produced AFs are known as AFB_1, AFB_2, AFG_1 and AFG_2. AFB_1 has been known as the most potent toxin among various AFs and all mycotoxins [1, 2]. Agency for Research on Cancer (IARC) classified AFB_1 as group 1 carcinogens. AFs have negatively affect health of livestock and poultry due to contaminated feeds. as well as, they significantly limit the development of international trade as a result of strict regulation in high-value markets [3, 4]. There are many methods used to reduce of AFs, one of these methods are to use microorganisms such lactic acid bacteria (LAB) are generally regarded as safe (GRAS) for human and animal consumption, environmentally friendly and low a cost-benefit. Numerous studies have demonstrated that many LAB strains can remove AFs. Removal efficiency ranges from small amounts to an almost complete removal [5-7]. AFB_1 reduce by LAB through many mechanisms such as chemical/enzymatic degradation, metabolic conversions, as well as adsorption processes, all these processes

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without producing any toxic products. On the other hand, in digestive system LAB decrease the amount of toxin available by complex formed between the toxin and the cell wall LAB, it will excrete in the feces [8-10]. So that the objective of this study was to determine if LAB cells could remove of AFB_1 during manufacturing Fino bread as well as evaluation qualities and characteristics of the Fino bread their affected manufactured by treated wheat flour by LAB cells.

MATERIALS AND METHODS

Materials

LBA strains

Five strains from LAB (*Lactobacillus rhamnosus, Lactobacillus plantarum, Bifidobacterium bifidum, Streptococcus thermophilus and Lactobacillus reuteri*) were used in this study, which were isolated from some local dairy products and identified using -50CHLAPI-identification system (BioMerieux) [11].

Wheat flour samples

This study used seven samples wheat flour as naturally contaminated by AFB_1 according to the results obtained from the second year of the project No. 11040205 from samples which collected from local markets from Cairo and Giza.

Dough ingredients

Active dry yeast, sodium chloride and sunflower oil were procured from local market in Giza.

Methods

Preparation of flour used in blends

This study used natural contamination wheat flour by AFB_1 at 8.5 (µg kg^{-1}) that calculated after mixing the seven samples. On the other hand, one kilogram from wheat flour free from AFB_1 was used as a control sample.

Preparation of bacteria culture cells

LAB were grown in 100 ml MRS, MRS modified, and M17 broth (pH 6.8) for 16 h, then centrifugation of the culture at 5000 rpm for 20 min at 4°C, and filtration of the supernatant through a sterilized filter (0.2 m, Millipore), to take all free cells.

Extraction of AFB_1 from wheat flour

Mixed 25gm of flour with 100 ml methanol. The mixture was shaken at 1100 rpm for 30 min. Then, the slurry mixture was centrifuged for 30 min at 6000 rpm. The supernatant was collected, and placed in a rotary evaporator under a vacuum at 45 °C for 20-30 min. Used 3.0 ml methanol to dissolved the residue, and filtered by a 0.22 micro-liter filter to determine by HPLC [12].

Preparation of blends wheat flour

Addition of cells of LAB as (Table 1) to 1000 g of wheat flour contaminated with AFB_1 at (8.5 µg kg^{-1}) during making Fino bread as follows:

In the pilot plant at the National Research Centre (NRC) in Dokki, Egypt, different Fino bread blends were prepared using wheat flour with active dry yeast (1.5 %), NaCl (1.5%), sugar (2 %), shortening (1%), bread improver (1%), and water (an amount needed to achieve 500 Brabender Units of consistency). Fino bread was made according to Hussein and Ibrahim [13].

Table 1. The treatments of wheat flour

Blend No.	Component
T1	Control
T2	Fino bread blend+ 100mg *Lactobacillus rhamnosus* cells
T3	Fino bread blend+ 100mg *Lactobacillus plantarum* cells
T4	Fino bread blend+ 100mg *Bifidobacterium bifidum* cells
T5	Fino bread blend+ 100mg *Streptococcus thermophilus* cells
T6	Fino bread blend+ 100mg *Lactobacillus reuteri* cells

Extraction of AFB_1 from dough

Ten-gram dough was mixed with 25 ml distilled water and stirred by a mixer. For 10 min, the mixture was centrifuged at 6000 rpm. Other extraction procedures were similar to those of AFB_1 extraction from flour as previously.

Extraction of AFB_1 from Fino bread

After one day of drying, the baked bread samples were milled for 5 min to produce a fine or medium-size powder, and 25.0 g of the powder was mixed with 100 ml methanol. It was shaken for 30 min at 1100 rpm. Other extraction procedures were similar to those described previously. The finally, AFB_1 determined by HPLC [14]

Rheological properties for dough after adding LAB cells

Farinograph test

Water absorption (%), arrival time(min), dough development time (min), dough stability(min) and degree of weakening (BU) were determined using brabender farinograph (model No. 178507) [15].

Freshness of Fino bread

Alkaline water retention capability (AWRC) was used to assess the freshness of bread samples after 1, 2, and 3 days of storage at room temperature [16, 17].

Sensory evaluation

In this test, samples were made from wheat flour free from AFB_1

Fino bread samples were coded and presented to fifteen - member panel of judges who are familiar with the product for sensory evaluation. The panelists scored the taste, colour, odor, texture and overall acceptability of the bread using a five-point hedonic scale, where 5 indicates extremely like and 1 extremely dislike [18].

Statistical analysis

The statistical analysis was carried out using one-way ANOVA using SPSS, ver. 22 **(**IBM Corp. Released 2013). Data were treated as a complete randomization design according to Steel *et al*, 1997 [18]. Multiple comparisons were carried out applying Duncun test the significance level was set at probability of P value <0.05.

RESULTS AND DISCUSSION

Effect of addition of LAB cells to wheat flour on AFB_1

The data recorded in (Table 2) shown the content of AFB_1 in the blend used in manufacturing Fino bread treated by various LAB cells. In case wheat flour (T2) treated by *L. rhamnosus* the content of AFB_1 increased to 7.3±0.02 and 3.16±0.07 (µg kg^{-1}) after the mixing step directly and fermentation, respectively compared with control sample was 8.09±0.06 and 8.0±0.09 (µg kg^{-1}), whereas after baking, the measurement using HPLC did not show any AFB_1 in the final product, also this result was with *L. plantarum* (T3). Also, many food processes which are done on cereals such as cleaning, milling, baking, frying, roasting, and extrusion have effects destroy on AFs in final products [20]. Analysis of variance and Duncan analysis showed a significant ($p \leq 0.05$) between differences treatment on content of AFB_1. The obtained data in (Figure 1) reflected that the percentages of reduction or remove of AFB_1 after mixing ingredients were 9.7, 8.5, 7.04, 7.4 and 5.5% after addition *L. rhamnosus, L. plantarum, Bifidobacterium bifidum, Str. Thermophilus* and *L. reuteri*, respectively. Moreover, the same results in (Figure 1) indicated that addition of *L. rhamnosus* and *L. plantarum* cells given the highest percentage of removal AFB_1 after fermentation stage were 60.5 and 54.25, respectively, while the lowest reduction of AFB_1 recorded with addition of *L. reuteri* cells was 42.25%. AFB_1 reduce has reached 100% in blends treated with *L. rhamnosus* and *L.plantarum* cells. Reducing of content, the AFB_1 during Fino bread making may be due to the thermal decomposition of the toxin, and AFB_1 binding with LAB cells during fermentation process, as the presence of lactic acid bacteria cells increases the actual fermentation process of the dough and hence adsorption AFB_1 on surface of LAB cells and addition to the activity of some enzymes for Baker's yeast, or bacteria cells, it makes

AFB_1 easy to influence by baking heat [21-23]. On the other hand, adding water during mixing the dough help in opening the lactone ring in AFB_1.The results indicated the most reduction in AFB_1 level with all LAB cells was observed after baking because this stage takes place after two stages of fermentation, the first stage (The first fermentation stage) was 30 min in duration, and the second was for 45 min, as well as the effect of heat during the baking, which reaches more than 300°C for 10 to 15 min inside the oven, so AFB_1 might become negligible to be not measurable [24].

Table 2. Concentrations of AFB_1 (µg Kg^{-1}) after manufacturing stages of the Fino bread.

Steps	Blends No*					
	T1	T2	T3	T4	T5	T6
Mixing	8.09±0.06[a]	7.3±0.02[d]	7.4±0.02[c]	7.52±0.01[c]	7.49±0.014[c]	7.64±0.03[b]
Fermentation (The first fermentation stage)	8.0±0.09[a]	3.16±0.07[e]	3.66±0.12[d]	4.06±0.08[c]	4.54±0.15[b]	4.62±0.18[b]
Final bread (after baking)	5.3±0.145[a]	ND[c]	ND[c]	0.246±0.02[b]	0.340±0.02[b]	0.353±0.014[b]

*Mean values in the row with the same letter are not significant difference at 0.05 levels.

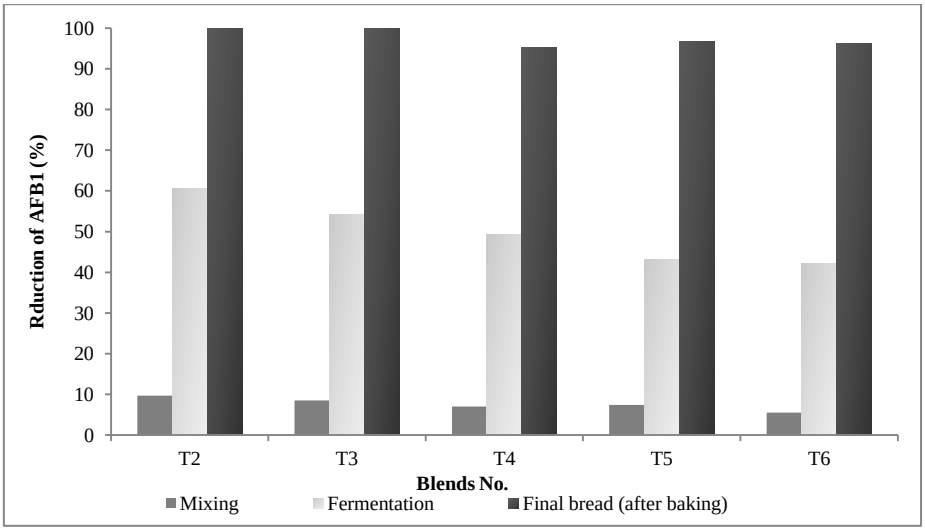

Figure 1. The percentages of reduction for AFB_1 after manufacturing stages of the Fino bread from wheat flour treated by LAB cells

Farinograph and extensograph properties for dough

Addition of LAB cells to wheat flour led to increase water-absorption and the dough development time as well as dough weakening. On the other hand, decreased stability of dough compared with control sample as in the Table 3. The results in Table 4 reflect highly enhancement in the strength characteristics of the resulted dough from flour after addition of LAB cells. The higher values of resistance and extension were 135mm and 540 B.U, after addition of *L. rhamnosus* and *L. plantarum* cells. LAB cause change in pH value which results in a change in the properties of the gluten network can result in changes in rheological properties [25].

Physical properties of Fino bread

According to the results, the addition of LAB cells has led to an increase in the volume of Fino bread as (Figure 2), as well as significant were observed in samples. On the other hand, the higher volume of bread could be due to higher gas (carbon dioxide) production during fermentation, it is due to increasing of monosaccharide (xylose) production by LAB action [26]. Increased of volume bread may due to improving gas retention capacity of gluten network due to reduction of disulfide bonds by acidic condition in presence of sourdough which results in more network flexibility [27].

Table 3. Effect of addition of LAB cells to wheat flour on farinograph parameters

Blends No	Faringraph parameters				
	Water absorption (%)	Arrival time (min)	dough development time (min)	Stability (min)	Dough weakening
T1 (control)	60.5	1.5	4.0	6.0	35
T2	61.6	1.5	4.5	5.5	40
T3	61.0	1.5	4.5	5.5	40
T4	61.5	1.5	4.5	5.5	40
T5	61.7	1.5	4.5	5.5	40
T6	61.3	1.5	4.5	5.5	40

Table 4. Effect of addition of LAB cells to wheat flour on extensograph parameters.

Blend (No.)	Extensibility (*E*) (mm)	Resistance to extension (*R*) (BU)	Ratio (*R*/*E*)
T1	120	500	4.16
T2	135	540	4.0
T3	135	540	4.0
T4	130	530	4.07
T5	130	530	4.07
T6	130	530	4.07

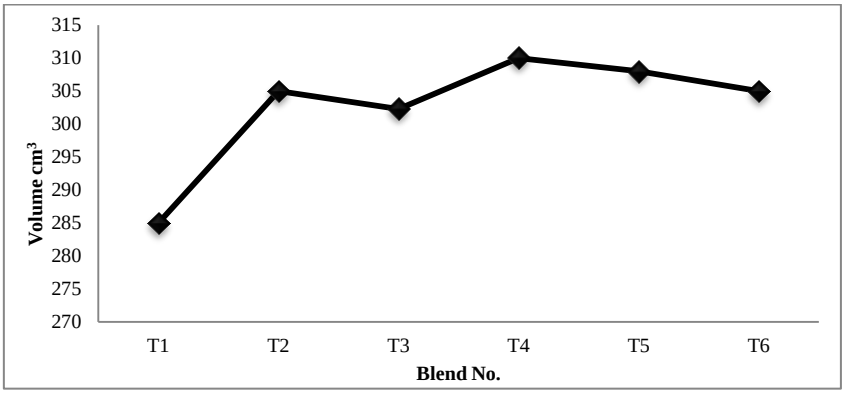

Figure 2. Volume of Fino bread manufactured from wheat flour treated by LAB cells

Effect of addition LAB cells on alkaline water retention capacity (AWRC) of Fino bread

Alkaline water retention capacity (AWRC) is a simple and quick test to follow staling of bread. Higher values of AWRC mean higher freshness. The results presented in Figure 3 shown that the AWRC for Fino bread with zero time were 282 in control samples, while the highest value was 289 with wheat flour treated by *Streptococcus thermophilus* as well as all treatment increased the values of AWRC compared with control sample. At 1st to 3rd day results indicated that addition of LAB cells to wheat flour improves the AWRC. Finally, these results indicated that the addition of LAB cells has the ability to improving the AWRC at a different rate, but the all addition was better than the control sample. This improvement may be due to increased break down starch to glucose units with production of higher levels of reducing sugars as a result of the activity of LAB, a factor that would raise the water absorption and increase bread shelf-life [28-30].

Sensory evaluation of bread

Note: this test was performed on samples not contaminated with toxin (AFB_1)

The obtained results are shown in (Table 5) from this results could be noticed that addition of LAB cells didn't show any significant differences on taste, color, odor, and texture for bread**.** The addition of LAB cells to flour used in the manufacture of bread give bread has sensory acceptance as well as LAB could be incorporated into

bread to provide its beneficial health effects, especially, reduce of AFB_1 from flour-contaminated.

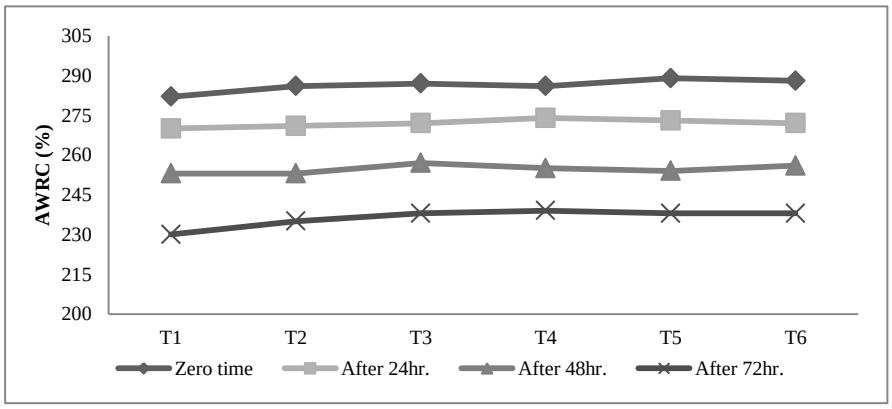

Figure 3. Alkaline water retention capacity (%) of Fino bread prepared by wheat flour treated by LAB cells.

Table 5. Scores for sensory attributes of Fino bread samples

Blend NO.	Sensory attributes*				
	Taste (5)	Color (5)	Odor (5)	Texture (5)	Overall acceptability (5)
T1	4.45±0.11[a]	4.11±0.20[a]	4.65±0.22[a]	4.65±0.25[a]	4.77±0.34[a]
T2	4.41±0.32[a]	4.15±0.22[a]	4.59±0.25[a]	4.62±0.24[a]	4.72±0.33[a]
T3	4.44±0.25[a]	4.22±0.27[a]	4.61±0.28[a]	4.71±0.22[a]	4.62±0.38[a]
T4	4.55±0.31[a]	4.09±0.24[a]	4.52±0.31[a]	4.75±0.21[a]	4.66±0.28[a]
T5	4.22±0.20[a]	4.21±0.23[a]	4.44±0.24[a]	4.69±0.27[a]	4.70±0.24[a]
T6	4.40±0.25[a]	4.19±0.19[a]	4.51±0.2[a]	4.70±0.25[a]	4.7±0.30[a]

*Mean±SE

Availability of data and materials

The manuscript contains the evidence that backs up the results of this study.

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Conflict of interests

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

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