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ORIGINAL ARTICLE

Effect of Bread Making Process on Aflatoxin Level Changes

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KEYWORDS

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ABSTRACT: Wheat flour is a commodity with a high risk of aflatoxins (AFs) contamination. During the bread making there are many processes that can affect the AFs stability. The effect of bread making process using different yeast types on AFs levels was investigated. For this purpose, standards of AFs including B and G were added to flour and then bread loaves were prepared. Three types of commercially available yeast including active dry yeast, instant dry yeast and compressed yeast were used for dough preparation. AFs levels in flour, dough, and bread were analyzed by high performance liquid chromatography (HPLC) with fluorescence detector. The results showed that maximum reduction in aflatoxin levels observed during first proof while the least decline was seen for the baking stage. The order of AFs reduction in bread making process was $AFB_1 > AFB_2 > AFG_1$. Furthermore, the results indicated that the most effective yeast for AFs reduction was instant dry yeast.

INTRODUCTION

AFs are secondary metabolites of *Aspergillus flavus*, *A. parasiticus* and *A. nominus*. They are carcinogenic and mutagenic in humans and animals [1]. These mycotoxins can enter in human food chain by direct consumption of contaminated grain or processed food such as meat or other animal products fed with contaminated food. Although there are ways to prevent fungal growth and aflatoxin formation, so far no way has been achieved to control mycotoxins in food [2]. AFs have 18 isomers including B_1, B_2, G_1, G_2, M_1, and M_2. Among them aflatoxin B_1 is considered the strongest hepatocarcinogen agent [3].

Bread is a major nutritional component and bread making is one of the oldest food processes, known and practiced for thousands of years, all over the world [4].

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The main microbial challenges associated with bread are fungal infections and subsequent contamination with their derivatives such as aflatoxins including B_1, B_2, G_1 and G_2 (AFB_1, AFB_2, AFG_1 and AFG_2). The economic problems arising from fungi and microbial toxin have recently triggered extensive researches [5, 6]. Although the temperature used in preparing bread probably did not influence reducing aflatoxins, but activating enzymes used during the fermentation alone or in combination with heat can result in reducing aflatoxins. In other words, despite of the heat resistance of AFs, these fermentation sensitive toxins are eliminated during the baking [7].

The effects of various cooking treatments on reducing of aflatoxin toxicity were studied via simultaneous analysis of AFB_1, AFB_2, AFG_1 and AFG_2 using high performance liquid chromatography (HPLC) with a fluorescence detector [3]. It was found that combination of the moisture content and citric acid concentration, significantly affected the aflatoxin in the extruded sorghum [8]. Moreover, the recovered aflatoxin decreased with a rise in moisture content and acid concentration. AFB_1 contaminations were investigated in 373 maize samples collected during three years and the aflatoxin in maize samples varied across the years [9]. Contaminations of agricultural and food products by mycotoxin have been extensively studied in Iran [10-14], however, there is still lack of data showing the effect of process on aflatoxin level in the bread produced from contaminated flour.

This study was designed to evaluate the impact of different stages of bread preparation on AFB_1, AFB_2 and AFG_1 in dough and bread the effect of used yeast type including active dry, instant dry and compressed yeast on destruction of these aflatoxins.

MATERIALS AND METHODS

HPLC conditions

Chromatographic separation and detection was carried out using (Knauver Technology, Germany) and a fluorescence detector adjusted at excitation wavelength of 265 nm and emission wavelength of 425 nm. The separation was achieved on ODS2 C18 (250×4.6 mm, 10µm) column from Merck (Darmstadt, Germany).

Contaminated flour

A vial containing 1 mg of aflatoxin B and G was dissolved in 10 ml chloroform; this solution was added to 20 g wheat flour. This mixture was stirred so that the solvent evaporated [15].

Bread Baking

To prepare dough, 100 g flour, 2 g baker's yeast, 1 g sodium chloride and 63 ml water were mixed for five min, then, the dough fermented at 35 °C for 1.5 h to be developed. After dividing the dough, the fermentation was allowed to continue for 10 min at 25 °C. Then it was baked for 30 min at 200±5 °C.

Aflatoxins extraction from flour

To extract aflatoxins, 25.0 g of contaminated flour was mixed with 100 ml methanol (HPLC grade). The mixture was agitated on shaker at 1100 rpm for 30 min. Then, the slurry mixture was centrifuged at 6000 rpm for 30 min. (Hermale Z 200A, Germany). The supernatant, consisting of extracted aflatoxins was collected and located in rotary evaporator (RV 10B S99, IKA) under vacuum at 45 °C for 20-30 min. The solid residue was dissolved in 3.0 ml methanol (HPLC grade), filtered by a 0.22 micro-liter filter and stored in a -20 °C freezer [16].

Aflatoxins extraction from dough

To extract aflatoxins from dough, 10.0 g dough was diluted with 25 ml distilled water and stirred by a mixer. The mixture centrifuged at 6000 rpm for 10 min. Other extraction procedures were similar to those of aflatoxin extraction from flour.

Aflatoxins extraction from bread

The baked bread samples were completely dried after one day and then crushed by a mill (Sunny, No. SBG - 420) for five min to create fine or medium-size powder 25.0 g of the powder was mixed with 100 ml methanol. It was placed on shaker at 1100 rpm for 30 min. Other extraction procedures were similar to what previously explained [16, 17].

Aflatoxins analysis by HPLC

Determination of AFB_1, AFB_2 and AFG_1 levels in control and samples were carried out by HPLC using a fluorescence detector (excitation at 365 nm, emission at 425 nm) and an ODS2 C18 column (250×4.6 mm, 10 µm). The mobile phase was mixture of deionized water, acetonitril, methanol, and acid acetic (59:29:29:1 v/v respectively). The flow rate was 1 ml/min [16].

STATISTICAL ANALYSIS

Duncan's method, together with one-way analysis of variance (ANOVA), was performed to evaluate changes in the levels of aflatoxin. A *P*-value of less than 0.05 ($P< 0.05$) was considered to show statistical significance. All analyses were performed in SPSS software program version 16 (SPSS Inc., Chicago, IL, USA) and the graphics were done in Excel.

RESULTS

Effect of bread making process on AFB_1

The effect of bread making process on AFB_1 is shown in Figure 1. There was a significant difference among the flour, primary dough, final dough, and bread prepared by active dry, instant dry, and compressed yeast ($P<0.05$).The most difference was observed after the first proofing for all three types yeasts. In other words, baking stage had the least effect on reduction of AFB_1.

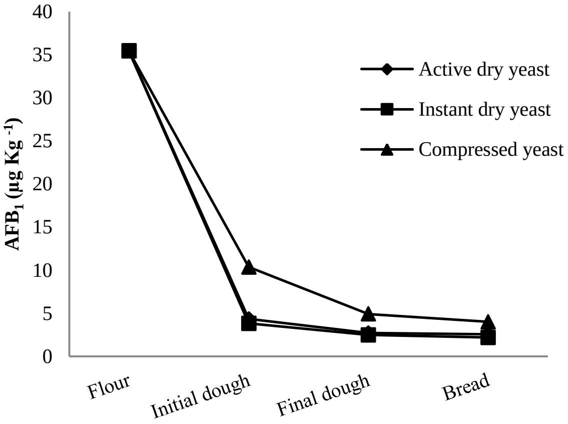

Figure 1. The effect of bread making process on AFB_1 using three types of yeast

Effect of bread making process on AFB_2

Figure 2 shows the effect of bread making process using different yeasts types on AFB_2.

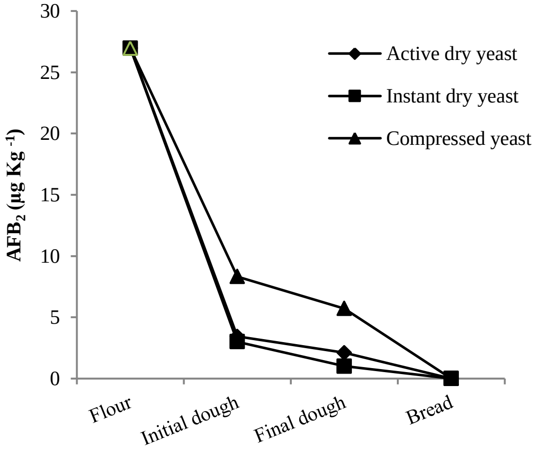

Figure 2. The effect of bread making process on AFB_2 using three types of yeast

There was a significant difference between the flour, primary dough, final dough, and bread containing active dry, instant dry, and compressed yeast ($P<0.05$). The most reduction was observed after the first proofing for all three types yeasts. Furthermore, it should be mentioned that, AFB_2 was not detected in the bread.

Effect of bread making process on AFG_1

Figure 3 shows the effect of bread making process using different yeast types on AFG_1. As can be seen, there is no considerable differences between the active dry, instant dry, and compressed yeast, however, a significant difference in AFG_1 levels was observed between the flour, primary dough, final dough, and bread ($P<0.05$). No AFG_1 was detectable in bread.

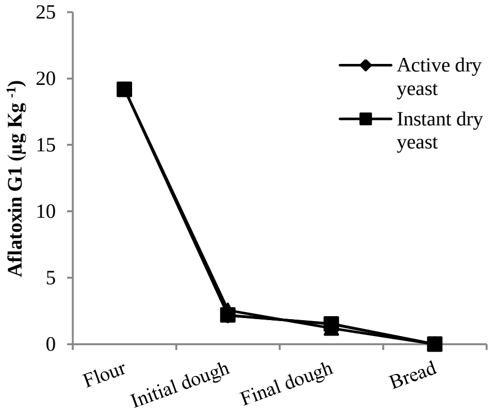

Figure 3. The effect of bread making processes on AFG_1 using three types of yeast

Effect of yeast type on aflatoxins during the first proof

According to Figure 4, the least reduction in the level of AFB_1 and AFB_2 was observed using compressed yeast. Additionally, as mentioned previously, the reduction in G_1 was not affected by the yeast type.

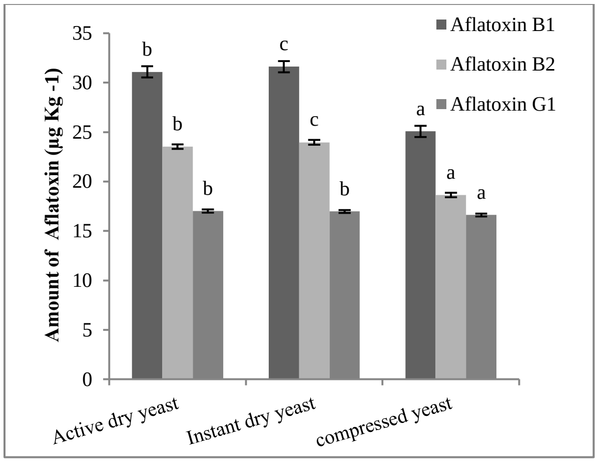

Figure 4. The aflatoxin reduction after the first proof

Effect of yeast type on aflatoxins during the final proof

According to Figure 5, the highest reduction in aflatoxins level, especially for AFB_1, was obtained in dough containing compressed yeast. It was significantly different from other yeasts ($P<0.05$). Similarly, reductions in samples containing active dry yeast and instant dry yeast were significantly different.

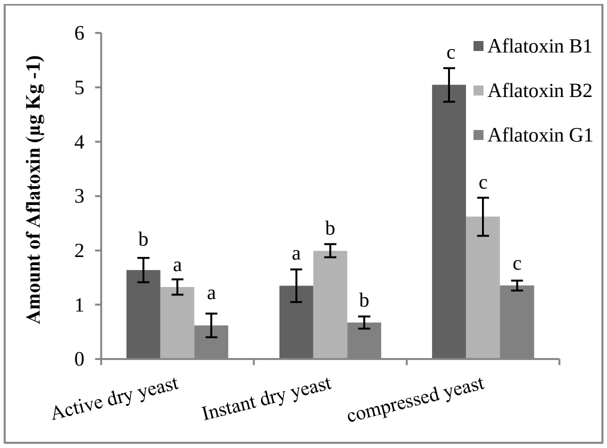

Figure 5. The aflatoxin reduction after the final proof

Effect of baking on aflatoxins

According to Figure 6, AFB_1 and AFB_2 reduction in dough containing compressed yeast was the highest. It was significantly different from other yeasts ($P<0.05$). However, for AFG_1, destruction was less than two other yeasts.

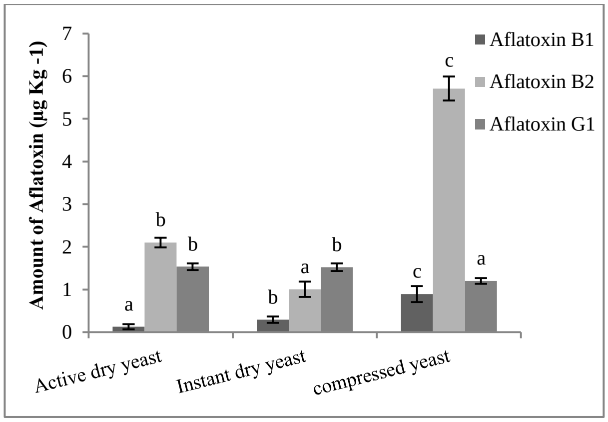

Figure 6. The aflatoxin reduction after baking

DISCUSSION

Results of this study showed that processing of wheat flour into bread could significantly reduce the aflatoxins level. The largest decline in all stages of the process could be observed between flour and primary dough (first proof) because it was longer than final proof and performed at temperature closer to optimum for *S. cerevisiae* i.e. 37°C. Among all AFs, the most destruction in the first proof was seen for AFB_1, but AFG_1 was stable at this conditions. The least decline was seen for the baking stage. The aflatoxins have high decomposition temperatures ranging from 237 °C to 306 °C. Solid AFB_1 is quite stable to dry heating at temperatures below its thermal decomposition temperature of 267 °C [18]. The moisture content is a critical factor on aflatoxins inactivation especially for $AFB_{1.}$ The presence of water helps in opening the lactone ring in AFB_1 [19].The baking had the most destructive effect on AFB_2 on bread prepared using compressed yeast.

The order of stages effectiveness on AFs destruction was as follows: first proofing>second proofing>baking. Probably because of heat resistance of aflatoxins, baking under the certain temperature had little effect on AFs destruction.

The most decrease in AFs during bread making process was for AFB_1, since AFB_1 had lower molecular weight and smaller molecular size and probably was more affected by destructive factors. The least decreasing AF was seen for AFG_1. This can be elucidated the smaller lactones rings in AFG_1 because AFs destruction mainly occurred on lactones ring rupture.

There are some reports on the fate of mycotoxins during the fermentation process. Earlier work showed that OTA, AFB_1 and AFB_2 at 0.19, 0.95 and 0.95 µg/m1 respectively were degraded in 87–91% by three strains of *S. cerevisiae* during fermentation of wort at 25 °C for 8 days [20]. Reduction of pH during fermentation, due to organic acids produced by the yeasts is likely resulted in decrease or degradation of AFs [21].

Many food-processing operations (such as roasting, cooking, frying, and baking) include heat treatment. Therefore, thermal inactivating of mycotoxins has been thoroughly studied. Mycotoxins differ in their stability under heat treatments. Aflatoxins are resistant to thermal inactivation and are destroyed only at temperatures of around 25 ºC [22]. However, fermentation can sensitize them due to enzymes, ethanol and Co_2 production. The effect of various treatments such as washing, heating and steaming on reduction of aflatoxin toxicity was investigated. It was concluded that concentration of AFB_1 is more reduced by heating than washing treatment [3].

Reduction of aflatoxin during heat processing depends on the moisture content of the product. In cottonseed meal with 30% moisture content, an 85% reduction in AFs was recorded following heat treatment of 100 ºC for 2 h but with the same temperature and time combination, only 50% of the AFs were destroyed when the meal moisture content was 6.6% [23]. In the light of the data accumulated from several studies of the effect of moisture content on aflatoxin degradation, it can be concluded that degradation is enhanced at higher moisture content [22].

Study on effect of fermentation period has shown that there is a significant falling in aflatoxin content in Idli up to 22 hr of fermentation [24]. These results agree with those obtained in this study.

In investigation of the effect of yeast type on AFs, it was found that the compressed yeast had the least effect on all AFs during the first proof. The higher moisture content of this type of yeast than two others, leads to less alive yeast cell in the same weight, consequently less destructive activity on AFs. Difference in ability of yeasts for aflatoxin reduction during the first proof is because of difference in its manufacture and preparation methods. The most destructive effect among three types of yeast was related to instant dry yeast. This is due to maintenance of enzymes activity during fabricating this kind of yeast. Moreover, this yeast unlike active dry yeast needs less amount of water for activation, so available water is enough for instant dry yeast activity.

In the final proof, the most effective yeast was compressed yeast. Probably higher Co_2 producing ability and resulted lower pH is the reason of high destructive capability at the final proof. The difference between active dry yeast and instant dry yeast in this stage because of their resemblance was not considerable.

During the baking, the most decrease was seen for AFB_1 and AFB_2 using compressed yeast. AFG_1 destruction through the baking was a little. It seems that AFG_1 has been sensitized to heat during fermentation less than two others. During the baking with compressed yeast, higher moisture content of yeast and/or its lower pH may enhance the effect of temperature on AFs destruction. The moisture is an important factor to reduce AFs by heating because the moisture is required to hydrolyze the lactone ring of the AFs during the baking [25].

Previous researches showed that rise in moisture content significantly influenced the reduction of aflatoxin, which confirms our findings [8].

CONCLUSIONS

The effect of bread making with various kinds of yeasts on aflatoxins indicated significant depletion during the first proof due to longer time and the baking stage had the least effect on aflatoxins. Overall, the compressed yeast during the bread making process according to the fermentation and baking was the most effective in reducing levels of aflatoxins.

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