INTRODUCTION

In recent decades, food contamination with natural or synthetic toxic chemical compounds has become a serious concern worldwide. The health-threatening risk of mycotoxins has drawn particular attention internationally. The Food and Agriculture Organization (FAO) has reported mycotoxin contamination in agricultural products as one of the most important food safety hazards notably in tropical and...
subtropical regions (Rahimi, Sadeghi, Bohlouli, & Karami, 2018). The most important classes of mycotoxins are aflatoxins (AFs), ochratoxins A (OTA), deoxynivalenol (DON), and zearalenone (ZEN) considering their toxicity and economic waste. Among AFs, aflatoxins B$_1$ (AFB$_1$), B$_2$ (AFB$_2$), G$_1$ (AFG$_1$), G$_2$ (AFG$_2$), M$_1$ (AFM$_1$), and M$_2$ (AFM$_2$) are the most prominent and have been observed in a variety of foodstuffs including cereals, nuts, dry fruits, milk, and dairy products (Ismail et al., 2018; Ketney, Santini, & Oancea, 2017). Aflatoxin B$_1$ (AFB$_1$) is produced as a secondary metabolite of fungal species such as Aspergillus flavus, which grow on grains during inappropriate storage (Bol, Araujo, Veras, & Welke, 2016). In addition, wheat flour and wheat flour-derived products are favored food for fungal growth and mycotoxin production (Generotti et al., 2017).

According to the Annual Agricultural Statistics of Iran, wheat is one of the mostly used cereals in Iran (Sadeghi et al., 2013). Further, wheat-derived products such as bread, biscuit, cake, and cookies are the main constituents of the human diet not only in Iran but also in many other countries (Bol et al., 2016). Traditional date cookies are one of the most popular and favorite snacks in many provinces of south Iran, especially in Khuzestan Province. Khuzestan is a tropical province with climatic conditions suitable for fungal growth and mycotoxin accumulation. Date cookie is made of wheat flour, traditional date, ginger, cinnamon, and cumin. Owing to the ingredients of the cookie and the climate conditions, a high level of AFB$_1$ contamination is probable.

AFB$_1$ is a well-characterized carcinogenic compound which severely contaminates food supplies. The International Agency for Research on Cancer has classified AFB$_1$ as the group 1 health-threatening carcinogen. Its consumption has also been proven to cause various health problems such as hepatocellular carcinoma (HCC) (Rushing & Selim, 2018). Due to the toxic effects of AFB$_1$ on human health, the European Commission has specified a maximum permitted level of 5 $\mu$g/kg in foodstuffs and 2 $\mu$g/kg in cereals and breakfast cereals (European Commission (EC), 2006). Moreover, the maximum tolerated level (MTL) of AFB$_1$ and total AFs for foodstuff in Iran are 5 and 10 $\mu$g/kg, respectively (Institute of Standards and Industrial Research of Iran (ISIRI), 2002). Therefore, the World Health Organization (WHO) has a great demand for optimizing the detoxification strategies of AFB$_1$ reduction in food products and feeds not only have a direct impact on human and animal health, but also can increase the safety of dairy products via decreasing AFM$_1$, as the main hydroxylated compound derived from AFB$_1$ contained in lactating animals feed (Corassin, Bovo, Rosim, & Oliveira, 2013; Gonçalves et al., 2017).

Recent advances in food processing, including physical, chemical, and biological manipulations have been developed to guarantee the final level of AFB$_1$ under standard limitations in food products (Cusato et al., 2013). Although aflatoxins are great heat-stable (Jager, Tedesco, Souto, & Oliveira, 2013), food processing can cause the destruction of varying degrees of aflatoxins in food products. Generally, the efficiency of thermal treatments for mycotoxins detoxification in food is highly related to factors like heating temperature, time of exposure, fermentation, pH, and also concentration and type of toxin (Ismail et al., 2018; Milani & Maleki, 2014).

The AFs occurrence and the effect of the food processing on detoxification have been studied in the previous researches (Ahmadi, 2020; Hajmohammadi et al., 2020; Milani, Nazari, Saman, Bamyar, & Maleki, 2018; Rastegar et al., 2017). However, as far as we know, there are little data about AFB$_1$ incidence in wheat flour and its derived products in Iran. Furthermore, there is no study regarding the influence of its ingredients and processing agents on final AFB$_1$ content in the traditional Iranian bakery products such as date cookies. Thus, the objectives of this survey were to investigate the effect of biological (fermentation), physical (thermal process), and chemical (spices) treatments on AFB$_1$ reduction during the traditional cookie-making from the wheat flour samples of Khuzestan (southern part of Iran) and to determine an optimal production process to produce the final product with the lowest possible level of AFB$_1$.

## 2 | MATERIALS AND METHODS

### 2.1 | Chemicals and reagents

Crystalline AFB$_1$ was bought from Sigma-Aldrich (USA). For the clean-up step of samples, AFLA-test immunoaffinity column (LC Tech GmbH) was used following the manufacturer’s advice. Whatman filter paper No.1 was supplied. Methanol, acetonitrile, water, and phosphate-buffered saline (PBS) were obtained from Merck. All solvents were of HPLC grade. The water used in all experiments was generated by a water purification system (Millipore).

The AFB$_2$ stock solution was prepared by adding 1 mg AFB$_2$ to 10 ml HPLC grade methanol, which was then kept at ~20°C and was brought to room temperature before use. Then, the standard working solutions were prepared by adding adequate dilutions of the stock solution at methanol: water ratio of 40:60 v/v ranging from 0.02 to 20 ng/ml and were kept at 4°C. The working standard was renewed every two weeks. To minimize the health risks caused by AFs pollution, all laboratory glassware used was washed by sodium hypochlorite (5%) before discarding. The Iranian wheat flour samples were randomly bought from the local bakeries and Saccharomyces

### Highlights

- Fates of aflatoxin B$_1$ from wheat flour to Iranian traditional cookies were investigated.
- Biological (yeast), physical (heat), chemical (spices) methods, on AFB$_1$ reduction were surveyed.
- The cookie-making process was controlled by modifying technological factors on AFB$_1$ decrement.
- AFB$_1$ levels in all samples were analyzed by reverse-phase HPLC with a fluorescence detector.
cerevisiae was purchased from Khamir Mayeh-Khuzestan (Dez mayeh, Iran). Moreover, dates, ginger, cinnamon, cumin, and other additives were obtained from markets in Khuzestan Province, Southwest of Iran.

2.2 | Spiking process of wheat flour

The initial content of AFB1 in wheat flour was evaluated by high-performance liquid chromatography (HPLC) with a fluorescence detector (FLD). The mean AFB1 concentration detected was approximately 3.14 µg/kg in the wheat flour samples, which was probable to decline to a level below the detection limits of the methods during the production process used in this research. Further, this toxin was not found in the analyzed batches of other formulation additives. To simulate the possible highest level of AFB1 contamination, a part of blank wheat flour was contaminated by adding an AFB1 methanolic solution with a concentration of 1,000 ng/ml (Institute of Standards and Industrial Research of Iran (ISIRI), 2002). No change was observed in the flour texture using this ratio of organic solution/flour compared to the blank samples.

The flour spiking process was done in a glass bottle. Then, each part of the spiked flour was homogenized and the mycotoxin solvent was evaporated. The contaminated samples were analyzed before processing, whose levels were compatible with the recovery rate of this method (Bol et al., 2016).

2.3 | Dough fermentation process

The dough was created with contaminated flour (25 g), water (17 ml), and Saccharomyces cerevisiae (commonly known as baker’s yeast). To investigate the effects of fermentation on AFB1 contamination, the samples were prepared with two levels of baker’s yeast (1%, 2% w/w). The yeast survivability was evaluated by microbial examination as CFU per gram of dry matter. The yeast was mixed with water and the yeast cell suspension was counted by the plate counting method on the yeast extract-glucose-chloramphenicol (YGC) agar (Merck, Germany). The average number of viable yeast cells was 12 × 1010 (CFU/g) in triplicate after 5 days of incubation at 25°C (Rad & Kasaie, 2017). The dough samples (5 and 10 µg/kg) were physically mixed until they shaped an elastic and nonsticky structure. The prepared samples were fermented in the oven at 35°C for 30, 60, and 90 min.

After the baking process, sample preparation for AFB1 analysis was done according to the manufacturer’s immunoaffinity columns (IAC) instructions (LCTech GmbH, Germany). One gram NaCl was added to 20 g of the thoroughly homogenized powered samples. Then, 100 ml methanol: water (8:2) mixture was added to perform extraction by a laboratory blender at high speed. After 3 min stirring, the sample was filtrated twice, and 14 ml of the filtered sample was diluted with 86 ml PBS solution. The IAC was equilibrated by passing 10 ml PBS solution at a flow rate of 1–2 drops per second. The diluted extract was then passed through that. The column was eluted with 10 ml distilled water. Afterward, AFB1 was washed with 2 ml methanol. The gathered solvent was evaporated with nitrogen gas at room temperature, reconstituted by 1 ml methanol: water (1:1, v/v), and saved at −18°C until analysis. Eventually, 100 µl elution was injected into the HPLC (Imperato, Campone, Piccinelli, Veneziano, & Rastrelli, 2011).

The measurement of AFB1 levels was carried out in triplicate by an HPLC system Knauer (AZURA) equipped with a fluorescence detector (RF-20A) with photochemical postcolumn derivatization of UV system (LCTech). The wavelengths of the fluorescence detector were set at 329 and 460 nm for excitation and emission, respectively. The HPLC column was Knauer C18 analytical column (250 4.6 mm I.D., 5 mm) with a precolumn, and the temperature of the column was held at 40°C. The mobile phase used was acetonitrile/water (90:10) at a flow rate of 1.5 ml/min.
2.7 Validation of method

The HPLC quality control parameters like the calibration curve linearity, the limit of detection (LOD), the limit of quantification (LOQ), and repeatability of AFB1-contaminated samples were obtained to validate the method developed in this study. Moreover, the recovery percentage of AFB1 in samples was measured to confirm the accuracy of the method. The linear calibration curve was acquired by analyzing the linear least square regression of the peak area against the concentration of AFB1. The calibration plot was linear for AFB1 in the concentration range of 0.02-20 μg/kg, which showed a correlation $R^2$ of 0.996. The equation calibration curve was obtained by the following equation: $y = 39.359x + 14.592$. LOD and LOQ were 0.005 μg/kg and 0.015 μg/kg, respectively. Due to the precision of the method applied, flour, dough, and cookie were experimentally spiked with 5 and 10 μg/kg concentrations of AFB1. The recovery rate and standard deviation (SD) were calculated ($n=3$) afterward (Table 1). The mean recovery rate of the chromatographic analysis of AFB1 for two concentrations indicated the method applied was highly reliable for AFB1 detection in these samples.

2.8 Statistical analysis

All statistical analyses were done by SPSS (version 16.0) software. Results are presented as mean ± standard deviation. Evaluation of the AFB1 levels was performed by one-way analysis of variance (ANOVA) together with Duncan’s test. Pearson’s correlation coefficient was used to determine the correlation between AFB1 level and pH. Independent samples t test was used to compare the means between the two groups. $p < .05$ was considered significant for all experimental data.

3 RESULTS AND DISCUSSION

3.1 Effect of fermentation on AFB1

Results of AFB1 changes during dough fermentation, as one of the main steps of cookie-making, are shown in Table 2. AFB1 concentrations in the mixed dough were approximately similar to those of the flour. Based on our results (Table 2), the fermented dough samples showed a significant decrement in AFB1 levels compared with the control dough sample with an increase in the yeast content from 1% to 2% w/w ($p < .05$). Moreover, the longer the time of fermentation (from 30 to 90 min), the more the reduction of AFB1. Furthermore, the results showed that fermentation had a more efficient effect on reducing the higher concentration of AFB1 (10 μg/kg) compared to the lower concentration (5 μg/kg). The lowest amount of AFB1 (7.63 μg/kg) was found in the sample with 2% yeast and 90 min fermentation when the initial concentration of toxin was high.

The results of this survey showed that the amount of yeast and the fermentation time were effective parameters for the AFB1 reduction during fermentation. Therefore, these results are in accordance with the results reported by El-Banna and Scott (1983). They reported that, on average, 19% of the added AFB1 was destroyed after the fermentation process. A similar order was reported by Uma Reddy, Gulla, and Nagalakshmi (2010). They found that a significant fall in AFB1 content in a higher fermentation time. These results are in agreement with those obtained in this study. In another study, researchers reported that the fermentation process under 30°C for 50 min using 1% yeast reduced the AFB1 level by 6% (Bol et al., 2016), which is not as effective as that of the present study. It could be due to the lower yeast concentration and shorter fermentation time. Moreover, other studies (Mozaffary, Milani, & Heshmati, 2019; Valle-Algarra et al., 2009) on mycotoxin reduction during wheat bread fermentation showed increased in fermentation time and yeast concentration could have more effect on reducing mycotoxins, that was in good agreement with our results.

Scientists believe that the biological methods can not only decrease the toxic component but also maintain the nutritional values of foods and therefore are considered a safe way for aflatoxin detoxification in the food industry (Gonçalves et al., 2020; Peng et al., 2018). S. cerevisiae is one of the most effective microorganisms to remove or degrade AFB1 in the food products. Furthermore, it has shown the potential ability to reduce AFM1 in the dairy industry (Gonçalves et al., 2017, 2020).

S. cerevisiae has capable of reducing mycotoxins under soft conditions without using any chemical substances, by binding mycotoxins to its cell wall (Karazhyan, 2017). Many researchers (Aazami, Nasri, Mojtahedi, & Bataccone, 2019) have previously reported that S. cerevisiae is able to change the chemical bonds between AFs and food proteins by binding toxins to its own cell wall polysaccharides (such as mannose and glucan) and to transform AFs into less toxic substances by releasing degrading enzymes, lactic acid, ethanol, and CO2. This supposition was validated by Shetty, Hald, and Jespersen (2007). They observed that even inactivated S. cerevisiae cells could effectively reduce the amount of AFB1 via treatment at 120°C. These results suggest that the heat-resistant enzymes of S. cerevisiae are probably important in the destruction of aflatoxin. In another study, the effect of fermentation process on detoxification in food products was investigated and results indicated that AFB1 could be absorbed by the baker’s yeast cell wall (Joannis-Cassan, Tozlovanu, Hadjeba-Medjdoub, Ballet, & Pfohl-Leszkowicz, 2011). Based on the published results, the yeast cell wall is able to absorb AFB1 up to 29%. The results of

| Spiked level (μg/kg) | Wheat flour | Dough | Cookie |
|---------------------|-------------|-------|--------|
| Low                 | 98.4 ± 0.09 | 92.4 ± 0.11 | 97.4 ± 0.09 |
| High                | 98.6 ± 0.13 | 92.2 ± 0.12 | 99.1 ± 0.09 |
samples with a higher initial concentration of AFB1. The present study indicated more decrement in the amount of toxin in the fermentation; however, its mechanism is still unknown (Rastegar et al., 2017; Uma Reddy et al., 2010).

Researchers have reported since the binding of AF to mannanoprotein (mannan), one of the main cell wall ingredients of S. cerevisiae, is physical (hydrogen, and van der Waals), it increases over time and at higher toxin concentrations (Shetty et al., 2007). Increasing the concentration of toxins, including their charge distribution, polarity, solubility, size, and shape dissociation, affect the adsorption process during fermentation; however, its mechanism is still unknown (Rastegar et al., 2017; Uma Reddy et al., 2010).

In general, the differing amounts of AFB1 in dough preparation stages, including kneading and fermentation, are probably explained by the variation of initial yeast content, the time and temperature of fermentation, and the effect of enzymes like a-amylase, glucose-oxidase, xylanase, cellulase, and protease (Vidal, Sanchis, Ramos, & Marin, 2016). The pH reduction and acidic condition by hydration products (Adebo, Njobeh, Gbashi, Nwinyi, & Mavumengwana, 2017; Aiko, Edamana, & Mehta, 2016). Based on the results of these studies, the pH drop of dough during fermentation, due to the production of organic acids (succinic acid and carbon dioxide) by the yeasts, resulted in the decomposition or inactivating of AFs (Milani & Heidari, 2017). The pH reduction and acidic condition by hydration of AFB1 at the terminal furan ring could decompose this toxin to less toxic products (Adebo, Njobeh, Gbashi, Nwinyi, & Mavumengwana, 2017; Aiko, Edamana, & Mehta, 2016). Based on the results of these studies, the organic acids and other compounds produced by the yeast can influence AFB1, which is an effective way for detoxification of AFs in comparison with other chemical methods (Milani & Heidari, 2017).

| Yeast content (%) | Spiked concentration (µg/kg) | Fermentation time (min) | AFB1 (µg/kg) | Reduction of AFB1 (%) | AFB1 (µg/kg) | Reduction of AFB1 (%) | AFB1 (µg/kg) | Reduction of AFB1 (%) |
|------------------|-----------------------------|-------------------------|--------------|-----------------------|--------------|-----------------------|--------------|-----------------------|
|                  |                             | 30 min                  |              |                       | 60 min       |                        | 90 min       |                       |
| 0%               | 5                           | 4.62 ± 0.026<sup>Aa</sup> | 7.6%         | 4.6 ± 0.026<sup>Aa</sup> | %8.0         | 4.59 ± 0.026<sup>Aa</sup> | %8.2         |                       |
|                  | 10                          | 9.22 ± 0.026<sup>Aa</sup> | 7.8%         | 9.2 ± 0.026<sup>Aa</sup> | %8.0         | 9.19 ± 0.01<sup>Aa</sup> | %8.1         |                       |
| 1%               | 5                           | 4.53 ± 0.036<sup>Bb</sup> | 9.4%         | 4.44 ± 0.043<sup>Bb</sup> | %11.2        | 4.35 ± 0.036<sup>Bb</sup> | %13.0        |                       |
|                  | 10                          | 8.94 ± 0.036<sup>Ba</sup> | 10.6%        | 8.75 ± 0.036<sup>Bb</sup> | %12.5        | 8.54 ± 0.036<sup>Bc</sup> | %14.6        |                       |
| 2%               | 5                           | 4.26 ± 0.045<sup>Ba</sup> | 14.8%        | 4.12 ± 0.026<sup>Cb</sup> | %17.6        | 3.98 ± 0.036<sup>Cc</sup> | %20.4        |                       |
|                  | 10                          | 8.29 ± 0.026<sup>Cb</sup> | 17.1%        | 7.95 ± 0.052<sup>Cb</sup> | %20.5        | 7.63 ± 0.036<sup>Cc</sup> | %23.7        |                       |

| Content         | Mean ± SD                   | Reduction (%)          |
|-----------------|-----------------------------|------------------------|
| Yeast content   |                             |                        |
| 0%              |                             |                        |
| 1%              |                             |                        |
| 2%              |                             |                        |

Note: Means ± standard deviations of triplicate independent experiments are shown. Different superscript capital letters within a column indicate statistically significant differences in AFB1 changes at different yeast levels (in the same concentration). Different superscript small letters within a row indicate statistically significant differences in AFB1 changes at different times; if they have a common letter, they are not significantly different.

**FIGURE 1** The correlation between concentration changes of AFB1 and pH during dough fermentation.
aromatic herbs contain a lot of phenolic compounds, flavonoids, alkaloids, steroids, terpenoids, saponins, and tannin, agents that offer antioxidant and antibacterial properties to them and make them capable of removing food toxins and biological contaminations (Lv et al., 2012; Noroozi et al., 2019). The effect of spices on the AFB1 content of cookies after thermal processing is exhibited in Figure 2.

Various studies have approved the detoxification ability of herbal compounds, spices, and other ingredients used in food production, some of which are mentioned below. A study indicated the essential oils of orange and lemon lead to 90% reduction in AF production (Hasan, 2000). In agreement with these results, other researchers reported that ajwain extract (carom), used as a spice was able to destroy AFs, by modifications of the lactone ring in the aflatoxin structure (Velazhahan et al., 2010). Several studies (Panda & Mehta, 2013; Vijayanandraj et al., 2014) have shown that extracts of medicinal plants such as Ocimum tenuiflorum and aqueous extracts of vasaka leaves (Adhatoda vasica) detoxify AF even at room temperature.

Spices are used as a natural way to reduce food contamination since these compounds are environmentally friendly and they can be safely used against microbial, fungal, and toxic contaminations (Ismail et al., 2018; Velazhahan et al., 2010). Our results showed that the spices, such as cumin, ginger, and cinnamon, used in the formulation of cookies did not significantly reduce AFB1 content (p > .05). This result could be attributed to the use of plant powder, which was less effective than its essential oil and extract. This issue, in particular, requires further research.

### 3.4 | The effect of baking process on AFB1 levels

To investigate the effect of different baking protocols as the final step of cookie-making, the optimized conditions for dough fermentation (2% yeast with 90 min fermentation) were applied and the samples containing 7.63 and 3.98 μg/kg AFB1 were then subjected to various baking conditions (temperature/time combination) in the oven. Next, the samples with or without fermentation were compared. According to the data (Table 3), all three combinations of the baking process could efficiently decrease the initial contamination level, but the maximum reduction in AFB1 concentration was expected to occur at 280°C because its baking temperatures are higher than those of others.

The baking process is an important physical procedure for the destruction of AFB1, a toxin with low molecular weight and small molecular size that mainly opens the lactone rings and makes AFs sensitive to other reactions altering the binding properties of terminal furan ring. Such structural changes could ultimately accelerate the degradation reactions of AFs. Moreover, heat treatment increases the toxin adsorption ability of these cells by creating active adsorption sites in the cell wall of saccharomyces (Milani et al., 2018).

Different experimental researches (Bullerman & Bianchini, 2007; Generotti et al., 2017; Rahimi et al., 2018) have revealed that an optimal combination of time/temperature processing, especially at higher baking temperatures, can considerably reduce the AFB1 contamination. Based on our results, the major effect of the baking time/temperature was reported for the fermented samples with higher initial AFB1 levels whose baking process was done at 280°C for 15 min. Thermal destruction products and mycotoxin-conjugated forms were not detected by the analytical procedure applied in the present study.

Based on the results obtained, the highest reduction (%) of AFB1 in samples with 5 and 10 μg/kg AFB1 after fermentation were 74.2% and 75.9% and the highest reduction (%) in samples without yeast were 57.4% and 53.9% respectively. Detailed results are presented in Table 2. The amount of AFB1 reduction was greater than those reported in most studies. Thus, the higher reduction in our study was due to the use of more effective parameters during product preparation and processing (samples with 2% yeast, 90 min fermentation

![Figure 2](image-url)
time, and baking process at 280°C for 15 min). Moreover, due to the size and shape of the cookies, the heat penetration may be higher. Other data obtained from studies on bread, biscuit, and cake regarding the impact of baking time/temperature on the removal of mycotoxins were in accordance with our results (Generotti et al., 2017; Valle-Algarra et al., 2009).

A result similar to our study was reported by Banna et al. They found that 55% of the AFB₁ content in flour was reduced in the Egyptian bread after fermentation and the subsequent baking (El-Banna & Scott, 1983). Furthermore, Bol et al., (2016) investigated the processing effect on AFB₁ levels in bakery products and they reported 36, 40, and 70% mycotoxin reduction in bread, biscuit, and cake during processing, respectively. Similarly, Hwang et al. surveyed the effects of different cooking procedures on AF levels in flour, dough combination containing microorganisms and enzymes, fermentation rate, and baking temperature (Schaarschmidt & Faulh-Hassek, 2018). Based on the results of this study, the baking process was not capable of entirely eliminating AFB₁ from the investigated samples. However, the AFB₁ level was significantly lower in the baked cookies than in dough and flour (p < .05).

### 4 | CONCLUSION

The current study investigated the effect of the modifications of technological factors and other ingredients in the recipe of traditional date cookie on the final AFB₁ content. Based on the data obtained, decrement of pH value after fermentation under different conditions decreased AFB₁ during cookie manufacturing, especially when the initial content of toxin was high in the samples. The results indicated that the baker's yeast content, time, and pH values involved in fermentation play a basic role in AFB₁ reduction. The formulation of the recipe, despite its phenolic content, and antioxidant properties did not show a significant effect on AFB₁ decrement. Enhancement of baking temperature/time at an admissible technological range especially in products fermented prior to the baking process effectively reduced AFB₁ content. In general, it was found that AFB₁ decreased during different treatments (various fermentation times, yeast levels, and time/temperature combinations of the baking process) applied in this study as a detoxification process. The present study provides an optimized preparation protocol for traditional cookies and demonstrates how exact control of the traditional process may decrease AFB₁ impact as a serious health-threatening toxin in the final product. Such studies are crucial to adopt suitable management strategies to ensure the safety of the local food supply in the country.

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### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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**TABLE 3** The effect of fermentation and baking process (three main protocols) on the changes of AFB₁ concentration

| Baking stage | Spiked concentration (µg/kg) | Treatment A<sup>a</sup> Concentration of AFB₁ (µg/kg) | Reduction of AFB₁ (%) | Treatment B<sup>b</sup> Concentration of AFB₁ (µg/kg) | Reduction of AFB₁ (%) |
|--------------|-----------------------------|---------------------------------|-------------------------|---------------------------------|-------------------------|
| D (0 min)    | 5                           | 4.62 ± 0.026<sup>a</sup>        | 7.6%                    | 3.98 ± 0.036<sup>a</sup>        | 20.4%                   |
|              | 10                          | 9.22 ± 0.026<sup>a</sup>        | 7.8%                    | 7.63 ± 0.036<sup>a</sup>        | 23.7%                   |
| C (160°C–45 min) | 5                           | 2.60 ± 0.036<sup>b</sup>        | 48%                     | 1.69 ± 0.026<sup>b</sup>        | 66.2%                   |
|              | 10                          | 5.30 ± 0.036<sup>b</sup>        | 47%                     | 3.08 ± 0.036<sup>b</sup>        | 69.2%                   |
| C (220°C–25 min) | 5                           | 2.45 ± 0.04<sup>c</sup>         | 51%                     | 1.52 ± 0.03<sup>c</sup>         | 69.6%                   |
|              | 10                          | 5.11 ± 0.026<sup>c</sup>        | 48.9%                   | 2.94 ± 0.043<sup>c</sup>        | 70.6%                   |
| C (280°C–15 min) | 5                           | 2.13 ± 0.036<sup>d</sup>        | 57.4%                   | 1.29 ± 0.02<sup>d</sup>         | 74.2%                   |
|              | 10                          | 4.61 ± 0.034<sup>d</sup>        | 53.9%                   | 2.41 ± 0.045<sup>d</sup>        | 75.9%                   |

Note: The treatments were nominated by D (Dough before baking process) or C (cookie after the baking process).<sup>a</sup> Treatment (A): samples without yeast, <sup>b</sup> treatment (B): samples with 2% yeast and optimal fermentation conditions. Means ± standard deviations of triplicate independent experiments are shown. Different superscript capital letters in the same concentration within a column indicate statistically significant differences (p < .05) among values; if they have a common letter, they are not significantly different.
ETHICAL APPROVAL
This article does not involve any human or animal experiments.

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