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# Effects of different temperatures and durations of heating on the reduction of Ochratoxin a in bread samples

**Masoud Hashemi-Karouei[1], Issa Gholampour-Azizi[2], Samaneh Rouhi[2], Mahdi Tashayyo[1]**

1 Department of Microbiology, Islamic Azad University, Tonekabon Branch, Tonekabon, Iran
2 Department of Microbiology, Islamic Azad University, Babol Branch, Babol, Iran

**Original Article**

## Abstract

Ochratoxin A (OTA) is a mycotoxin produced in corn, rice, and flour. It is a major concern for animal and human health. The purpose of this study was the evaluation of OTA contamination in bread samples gathered from bakeries, in different temperatures and durations of heating. In this study, 32 samples (4 samples of flour and 28 samples of bread) were randomly collected from different bakeries in Babol city, Mazandaran province, Iran, in fall 2013. The OTA content of the samples was measured in different temperatures and durations of heating using competitive direct enzyme-linked immunosorbent assay (CD-ELISA) method. Nonparametric Kruskal-Wallis was applied for data analysis. Results proved that reduction in the amount of OTA in samples during the heating process was significant and longer duration of heating was more effective, than raising the temperature, on OTA reduction. The highest percentage of OTA reduction occurred in constant temperature and when 2 minutes were added to the original time of heating. The lowest reduction rate of this toxin was observed in constant temperature and when 4 minutes was deducted from the original time in each bakery. Our study showed that bread and flour samples do in fact contain OTA, but this toxin is being reduced through heating. Since bread is the most consumed food in the world and also Iran, determination, management, and reduction of OTA in bread should be considered seriously.

**KEYWORDS:** Bread, Ochratoxin A Reduction, Temperatures, Heating

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## Introduction

Mycotoxins are secondary metabolites that are produced by fungi. Aflatoxins, trichothecenes, zearalenone, fumonisins, and ochratoxins are important mycotoxins found in cereals.[1] Ochratoxin has three types of A, B, and C which have some chemical differences. This toxin has a colorless and crystallized compound. Ochratoxin A (OTA) is a common type of Ochratoxin.[2] The chemical structure of OTA is 7-L-β-phenylalanylcarbonyl-5-chloro-8-hydroxy-3, 4-dihydro-3-R-methyl-isocoumarin.[3] OTA is produced by Aspergillus ochraceus, Aspergillus carbonarius, and Aspergillus niger in tropical regions, and by Penicillium verrucosum in temperate regions.[4] This toxin has been found in different foods such as corn, rice, soybean, coffee, cocoa, bean, and pea and also corn derivatives such as flour, bread, and pasta.[5] OTA has nephrotoxic, immunotoxic, carcinogenic, teratogenic, and genotoxic effects and it has been associated with human and animal kidney diseases.[6] The International Agency for Research on Cancer (IARC) has given a group 2B classification to OTA.[3] The Iranian maximum tolerated level for OTA is 5 ng/g in human

**Corresponding Author:**
*Mahdi Tashayyo*
*Email: microbiol_sci@yahoo.com*

food.[7] European Commission (EC) has determined the maximum tolerated OTA level to be 5 ng/g for cereals and 3 ng/g for their derived products. According to EC, the tolerable weekly intake (TWI) of OTA is 120 ng/kg bw/week for humans.[6] Several studies have shown the production of mycotoxins in food products, and the reduction and change in concentration of different mycotoxins based on heating in human food. Meca et al., in Canada, detected Fusarium mycotoxin [Beauvericin (BEA) (5 mg/kg)] decomposition in barley and wheat flour during beer and bread making. During the bread making process, BEA reduction ranged from 75 to 95%.[8] van der Stegen et al., in the Netherlands, studied green coffee contaminated by OTA and showed that during the roasting time which lasted from 2.5 to 10 minutes, OTA was reduced 69%.[9] Kristensen et al., in Denmark, using 64 °C for 10.5 minutes, reduced 99% of the yeast and 98% of the filamentous fungi in rye bread and confirmed that drum drying did not destroy OTA.[10] In another study in Iran, Gholampour Azizi reported that from 100 grape juice and raisin samples, 32 grape juice and 4 raisin samples had contamination rates higher than that of the EC limit (10 µg/kg).[11] The climatic conditions of Mazandaran province (northern Iran) provide a good environment for fungi growth and mycotoxins occurrence such as OTA (because in northern regions of Iran, the weather is humid and mild). Bread is the most consumed food in the world and Iran. Therefore, the purpose of this study was to evaluate OTA in several bread samples, based on different temperatures and durations of heating, using the competitive direct enzyme-linked immunosorbent assay (CD-ELISA) method in Babol city (Mazandaran province, Northern Iran).

## Materials and Methods

In this study, 32 samples from 4 bakeries (8 samples per bakery: 1 sample of flour and 7 samples of bread) in Babol were randomly collected. The constant temperature and time of heating in each bakery, which prepared normal bread, were bakery A: 320 °C and 6 minutes, B: 185 °C and 8:45 minutes, C: 280 °C and 6:30 minutes, and D: 200 °C and 8:30 minutes. Samples from each bakery included: 1 sample of wheat flour (the flour with which bread was baked and was the basis for other samples and it was not heated), 1 sample of normal bread baked for the original time and with constant temperature of heating in the bakeries, 1 sample baked for the original time and constant temperature increased by 20 °C from the original temperature of heating in the bakeries, 1 sample baked for the original time and constant temperature deducted by 20 °C, 1 sample baked for the original time and constant temperature deducted by 40 °C, 1 sample baked constantly with the original temperature and time of heating increased by 2 minutes, 1 sample baked constantly with the original temperature and time of heating deducted by 2 minutes, 1 sample baked constantly with the original temperature and time of heating deducted by 4 minutes.

In this procedure all samples were dried and ground to powder. Subsequently, 20 g of each sample was mixed with 100 cc of 70% methanol in a blender, and then, the mixture was shaken in an Erlenmeyer flask continuously for 3 minutes. After settling, the extract was filtered with Whatman filter paper No.1 (Kamyab Tabib Tajhiz, Iran).[12]

The OTA content of the samples was measured using the AgraQuant OTA assay kit (Romer Lab, Singapore) which was based on the CD-ELISA format. Then, 200 ml of conjugated enzyme was added to uncoated-antibody wells, and then, 100 ml of both standard solution and sample extract were added to it. Subsequently, 100 µl of these solutions were transferred to coated-antibody microplate wells and were incubated at room temperature (20-25 °C) for 10 minutes. OTA in samples and standards competitively bind to solid phase antibody specific to this toxin. Tetramethylbenzidine/

hydrogen peroxide was used as a substrate for color development. After the washing phase, 100 µl of enzyme substrate was added to wells and incubated at room temperature for 5 minutes. After this step, a blue color was observed in the wells. Finally, 100 µl of stopping solution was added to stop the reactions, as a result, the blue color changed to yellow. The color intensity was inversely proportional to the OTA concentration and was measured with the ELISA reader in the wavelength of 450-630 nm (according to the manufacturer's instructions).

Data were calculated by a nonparametric Kruskal-Wallis test (to check normality of data) using SPSS software (version 18, SPSS Inc., Chicago, IL, USA) ($P < 0.05$).

## Results and Discussion

### Results of OTA reduction in bakeries

In bakery A, the highest reduction percentage of OTA (16.53%) was observed when the bread sample was baked in a constant temperature (320 °C) and time was increased by 2 minutes (8 minutes) from the original time (6 minutes). The lowest reduction (9.09%) was observed with a constant temperature (320 °C) and the deduction of time by 4 minutes (2 minutes) in the same bakery (Table 1).

In bakery B, the highest percentage of OTA reduction (88.93%) was observed in bread samples baked in constant temperature (185 °C) in the same bakery and time increased by 2 minutes (10:45 minutes) from the original time (8:45 minutes). The lowest reduction (80.33 %) of this toxin in bakery B was observed in samples baked in constant temperature (185 °C) and time deducted by 4 minutes (4:45 minutes) (Table 2).

In bakery C, the highest percentage of OTA reduction (87.32%) was observed in bread sample baked in a constant temperature (280 °C) in the same bakery and time increased by 2 minutes (8:30 minutes) from the original time (6:30 minutes). The lowest reduction (74.85%) of this toxin in this bakery was observed in a constant temperature (280 °C) and when time was deducted by 4 minutes (2:30 minutes) (Table 3).

**Table 1. Distribution and reduction of Ochratoxin A (OTA) contamination in bread samples of bakery A**

| Bakery | Time (min) | Temperature (°C) | OTA contamination (ppb) | OTA reduction (%) |
|---|---|---|---|---|
| Wheat flour | - | - | 48.4 | Basis |
| Normal bread | 6 | 320 | 41.2 | 14.88 |
| Bread sample | 6 | 340 | 41.4 | 14.46 |
| Bread sample | 6 | 300 | 42.1 | 13.02 |
| Bread sample | 6 | 280 | 43.5 | 10.12 |
| Bread sample | 8 | 320 | 40.4 | 16.53 |
| Bread sample | 4 | 320 | 43.5 | 10.12 |
| Bread sample | 2 | 320 | 44.0 | 9.09 |

Normal bread: Bread prepared in constant temperature and duration of heating in any bakery without a change in those factors; Basis: flour not affected by different temperatures and durations of heating for reduction of OTA and chosen as the basis for comparison with the bread samples; OTA: Ochratoxin A

**Table 2. Distribution and reduction of Ochratoxin A (OTA) contamination in bread samples of bakery B**

| Bakery | Time (min) | Temperature (°C) | OTA contamination (ppb) | OTA reduction (%) |
|---|---|---|---|---|
| Wheat flour | - | - | 48.8 | Basis |
| Normal bread | 8:45 | 185 | 7.1 | 85.45 |
| Bread sample | 8:45 | 205 | 6.1 | 87.50 |
| Bread sample | 8:45 | 165 | 7.5 | 84.63 |
| Bread sample | 8:45 | 145 | 7.4 | 84.84 |
| Bread sample | 10:45 | 185 | 5.4 | 88.93 |
| Bread sample | 6:45 | 185 | 9.1 | 81.35 |
| Bread sample | 4:45 | 185 | 9.6 | 80.33 |

* Normal bread: Bread prepared in constant temperature and duration of heating in any bakery without a change in those factors; Basis: flour not affected by different temperatures and durations of heating for reduction of OTA and chosen as the basis for comparison with the bread samples OTA: Ochratoxin A

**Table 3. Distribution and reduction of Ochratoxin A (OTA) contamination in bread samples of bakery C**

| Bakery | Time (min) | Temperature (°C) | OTA contamination (ppb) | OTA reduction (%) |
|---|---|---|---|---|
| Wheat flour | - | - | 48.9 | Basis |
| Normal bread | 6:30 | 280 | 7.2 | 85.28 |
| Bread sample | 6:30 | 300 | 10.1 | 79.35 |
| Bread sample | 6:30 | 260 | 7.6 | 84.46 |
| Bread sample | 6:30 | 240 | 7.8 | 84.05 |
| Bread sample | 8:30 | 280 | 6.2 | 87.32 |
| Bread sample | 4:30 | 280 | 11.0 | 77.51 |
| Bread sample | 2:30 | 280 | 12.3 | 74.85 |

Normal bread: Bread prepared in constant temperature and time in bakery without change in the temperature and time of heating; Basis: flour not affected by different temperatures and durations of heating for reduction of OTA and chosen as the basis for comparison with the bread samples; OTA: Ochratoxin A

**Table 4. Distribution and reduction of Ochratoxin A (OTA) contamination in bread samples of bakery D**

| Bakery | Time (min) | Temperature (°C) | OTA contamination (ppb) | OTA reduction (%) |
|---|---|---|---|---|
| Wheat flour | - | - | 48.8 | Basis |
| Normal bread | 8:30 | 200 | 8.4 | 82.79 |
| Bread sample | 8:30 | 220 | 7.7 | 84.22 |
| Bread sample | 8:30 | 180 | 8.4 | 82.79 |
| Bread sample | 8:30 | 160 | 9.6 | 80.33 |
| Bread sample | 10:30 | 200 | 7.6 | 84.43 |
| Bread sample | 6:30 | 200 | 8.9 | 81.76 |
| Bread sample | 4:30 | 200 | 9.8 | 79.92 |

Normal bread: Bread prepared in constant temperature and time in bakery without change in the temperature and time of heating; Basis: flour not affected by different temperatures and durations of heating for reduction of OTA and chosen as the basis for comparison with the bread samples; OTA: Ochratoxin A

In bakery D, the highest percentage of reduction in this toxin (84.43%) was observed in constant temperature of 200 °C and when time was increased by 2 minutes (10:30 minutes) from the original time (8:30 minutes). the Lowest reduction of OTA (79.92%) was observed in the constant temperature of 200 °C and when the time was deducted by 4 minutes (4:30 minutes) from the original time (8:30 minutes) (Table 4). In all bakeries, it was specified that temperature and time of heating are prominent factors for reduction of OTA in bread samples. All bread samples showed less OTA contamination than raw flour.

## Results of statistical analysis

The results indicate that in all bakeries, OTA level is higher than recommended standard values. Depending on the OTA amount in wheat flour, different temperatures and times of heating caused significant reductions in OTA level in bread ($P < 0.05$), which ranged from 9.09-16.53%, 80.33-88.93%, 77.51-87.32%, and 79.92-84.43% in bakeries A, B, C, and D, respectively.

## More information about this toxin and comparison with other studies

OTA is a type of mycotoxin that occurs frequently in cereals and can be found in starch-rich foods such as grains, cereal, and bread.[13,14] Valle-Algarra et al.,[13] in Spain, showed that OTA reduced by 32.9% during the baking process. Our study showed that the highest reduction of OTA was 88.93% (10:45 minute, 185 °C) in bakery B when bread samples were baked in a constant temperature in the same bakery and time of heating was increased by 2 minutes. A higher rate of OTA reduction was achieved in our study compared to that in the study by Valle-Algarra et al. Different baking conditions, such as dough fermentation or yeasts selected in industrial fermentations, are effective on the levels and reduction of mycotoxins in flour that are used to bake bread.[13] Scudamore et al., in Britain, showed

that OTA decreased only a small amount during the bread making process.[15] We showed that the highest rates of reduction in bakeries A, B, C, and D were 16.53% (8 minutes, 320 °C), 88.93% (10:45 minutes, 185 °C), 87.32% (8:30 minutes, 280 °C), and 84.43% (10:30 minutes, 200 °C), respectively. Different researches reported that food processing (cleaning, milling, baking, and fractionating), hygienic conditions, and workers' health in the bakeries can be effective on the levels of mycotoxin contamination of flour and bread.[14-16] Pacin et al., in Argentina, after studying several wheat flour samples, reported that French bread and Vienna bread were contaminated with deoxynivalenol (DON) (35.5 µg/kg and 22 µg/kg, respectively). OTA was not detected in the flour in their study. A 33 and 58.5% reduction in DON after the bread making process was observed in French bread and Vienna bread, respectively.[17] In our study, 32 samples were contaminated by this toxin. Common processes in manufacturing and physical treatment of grain, such as scouring and cleaning the grain prior to milling, can reduce the initial contamination by mycotoxins in the raw material used for bakery and bread making.[17,18] Osborne et al. showed that when OTA was added to flour samples which were subsequently baked into bread, it was recovered after the baking processes without becoming decomposed.[19] Furthermore, in our study, toxins, in some cases, were destroyed in the samples. For example, in bakery A samples, the level of OTA was higher than the other samples. This toxin has a moderately stable molecule and is able to survive most food processes; thus, it is only partly destroyed during cooking and bread making processes. Baking and roasting bread can reduce the existing OTA content up to 20%.[20,21] Castells et al., in Spain, reported that the cooking temperature of 160 °C applied for 70 seconds reduces OTA up to 86% in barley bread.[22] In our research, the highest reduction (88.93%) of OTA was observed when the bread sample was baked in a constant temperature (185 °C) and 2 minutes was added to the original time (10:45) (bakery B). Different factors that influence the OTA content and quantity in food products are: different ecological niches of the ochratoxigenic mycobiota, such as Aspergillus spp. and Penicillium verrucosum, and accurate agricultural practices, such as better harvest procedures and storage conditions, the type of grains, nature and extent of technological advancements in agricultural equipments.[12,23] Since most wheat flour is processed into various foods such as bread and consumption of a food that is contaminated by OTA causes different diseases in humans, greater attention should be paid to OTA in wheat and wheat products. Results of our study are effective in the management, detection, and reduction of fungi growth and OTA production in food.

## Conclusion

In this study, OTA concentration in several samples was measured after employing different temperatures and durations of heating using ELISA. Therefore, we can conclude that there was a significant relationship between OTA reduction in the samples, and temperature and durations of heating ($P < 0.05$). The highest rate of reduction in OTA was observed when a constant temperature was maintained in each bakery and when the time of heating was increased by 2 minutes from the original time.

## Conflict of Interests

Authors have no conflict of interests.

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