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Thermostability of Ochratoxin A in Wheat under Two Moisture Conditions

HAMID BOUDRA,* PIERRETTE LE BARS, and JOSEPH LE BARS

Laboratoire de Pharmacologie-Toxicologie, Institut National de la Recherche Agronomique, 31931 Toulouse Cedex, France

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The decomposition of ochratoxin A (OTA) was examined, under different temperature and moisture conditions. The calculated half-lives, corresponding to 50% values, were 707, 201, 12, and 6 min, respectively, at 100, 150, 200, and 250°C for dry wheat and 145, 60, and 19 min, respectively, at 100, 150, and 200°C for wheat heated under wet conditions. The presence of water (50%) increased the decomposition of OTA at 100 and 150°C; the opposite was observed at 200°C. Complete destruction of OTA within the limits of this study (100 to 250°C) was not obtained.

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Several *Penicillium* and *Aspergillus* species produce ochratoxin A (OTA), which has clearly been shown to be a toxic substance, with nephrotoxic, immunosuppressive, teratogenic, and carcinogenic effects in many species (3, 6, 9). Some findings indicate that OTA may be implicated in the etiology of Balkan endemic nephropathy (13). OTA can also occur in tropical areas. Recently, the natural occurrence of OTA in food (17) and in human blood serum in North Africa (1, 7) has been reported.

In spite of the great importance of wheat as the principal food in the daily diet of many people in North Africa, relatively few studies have been made on the stability of OTA. After harvest, wheat is subjected to many technological processes in which temperature is the main parameter. Various temperature and moisture conditions are used in food processing and cooking. Therefore, the purpose of the present study was to determine the effects of different thermal treatments under two moisture conditions (dry and wet) on the stability of OTA in wheat by submitting a culture of a toxigenic strain of *Aspergillus ochraceus* on wheat to different time-temperature combinations.

OTA-contaminated wheat. Two 500-ml Erlenmeyer flasks were filled with 100 g of dry wheat added to 100 ml of water and autoclaved at 120°C for 20 min. The flasks were inoculated with spore suspensions from a 15-day-old malt agar culture of a toxinogenic strain of *A. ochraceus*. They were incubated at 28°C for 2 weeks. The flask contents were washed several times to eliminate spores and mycelia. The contaminated whole wheat was dehydrated in a vacuum oven for 48 h at 45°C; the oven contained a water-absorbent silica gel which was periodically changed. Finally, the dried wheat cultures were ground (Waring blender) and sieved (0.5-mm diameter). The OTA concentration in this contaminated wheat was considered the initial concentration (C_0).

Heating experiments. The experimental procedure was designed by taking into consideration three principal factors, i.e., temperature, duration of treatment, and moisture. After preliminary experiments, the following plan was chosen. One gram of contaminated wheat was put into stoppered test tubes (20 ml; 12 by 12 mm). To simulate wheat paste (wet conditions), 1 ml of water was added to a second series of tubes containing contaminated wheat. These samples were held for different times at four different temperatures before extraction of the toxin (Table 1). Such a small quantity would allow the rapid and precise determination of the effective temperature and exposure time within the sample. The samples were immersed in an oil bath in a thermoregulated oven in order to minimize the delay in obtaining an even temperature of the substrate. The heating conditions we used are typical for wheat cooking. Every treatment was performed in triplicate.

Analytical procedure. The extraction method of Fröhlich et al. (5) was modified as follows. Contaminated wheat (1 g) was extracted by overnight soaking in 10 ml of chloroform acidified with 800 μl of *o*-phosphoric acid followed by mechanical agitation for 1 min. The mixture was filtered through separator phase paper (Whatman SP) and evaporated to dryness under nitrogen at 45°C. The residue was redissolved in chloroform-acetic acid (9.9:0.1). Separation of OTA was performed by bidirectional thin-layer chromatography. Silica gel on an aluminum sheet (20 by 10; Merck 5553) was activated before use for 1 h at 100°C. Five microliters of the extract was spotted on a line 6 cm from the bottom of the plates. Prechromatographic cleanup was carried out with anhydrous diethyl ether developed over the whole plate. The portion of the plate (1.5 cm above OTA spots) which contained impurities was cut off. For final separation, the plate was developed in the opposite direction with toluene-ethyl acetate-formic acid (6:3:1). OTA was quantified by fluorimetric detection at 333 nm (model CS 930 scanning densitometer; Shimadzu Corp., Kyoto, Japan).

The percentage of OTA decomposition as measured by temperature-versus-time exposures is reported in Table 1. The decomposition of OTA depends on moisture conditions and treatment duration.

Ground dry wheat heated at 100 and 150°C for various time periods decomposed moderately. To obtain a significant reduction (ca. 20%) of the percentage of recovered OTA, 80 and 32 min at 100 and 150°C were required, respectively (Table 1). At 200 and 250°C, 93.5 and 88.3% of the initial OTA were destroyed within 48 and 16 min, respectively. OTA decomposition was greatly increased even after the shortest heating period used (2 to 6 min). Moreover, the heated product showed a new fluorescent spot similar to that of the original OTA (Table 1). When these extracts were used in five different solvent systems, partial separation was obtained in toluene-acetic acid (99:1).

Heating of wheat under wet conditions resulted in high percentages (>60%) of OTA decomposition (Table 1). The presence of water appeared to increase the decomposition of OTA at 100 and 150°C. In contrast, at 200 and 250°C, this

* Corresponding author.

TABLE 1. Effect of heat treatment on decomposition of OTA in dried and moistened ground wheat

Heating time (min)	$(C_0 - C)/C_0$ (%)[a]						
	100°C		150°C		200°C		250°C (dry)
	Dry	Wet	Dry	Wet	Dry	Wet	
2							30 ± 2
4							35 ± 3
6					25 ± 7	12 ± 10	
8							47 ± 1
12					44 ± 3	24 ± 14	
16			14 ± 2				88 ± 3[b]
20	2 ± 2						
24					80 ± 1[b]	61 ± 3	
32			20 ± 3	35 ± 1			
40	17 ± 4						
48					94 ± 4[b]		
60		18 ± 1					
64			32 ± 7	56 ± 2			
80	21 ± 2						
120		51 ± 7					
128			39 ± 2	73 ± 13			
160	21 ± 0.2						
240		66 ± 3					

[a] Results are the means ± standard deviations of three determinations. C_0, initial OTA concentration.
[b] Conditions under which a new spot appeared on thin-layer chromatography plates.

phenomenon was reversed. For each temperature, the ln of the ratio of resulting OTA to initial content (ln C/C_0) is linearly correlated to exposure time (Fig. 1a and b). The calculated half-lives corresponding to the 50% value were 707, 201, 12, and 6 min, respectively, at 100, 150, 200, and 250°C for dry wheat and 145, 60, and 19 min, respectively, at 100, 150, and 200°C for wheat heated under wet conditions.

The decrease in the amount of recovered OTA after heat treatments followed first-order kinetics, as is often the case for thermal decomposition of organic compounds: ln $C/C_0 = -kt$

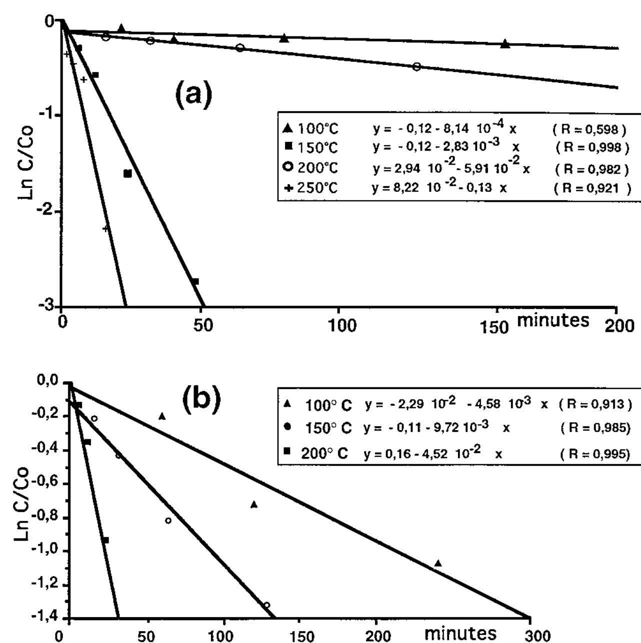

FIG. 1. Kinetics of the decrease of OTA concentration in wheat culture of *A. ochraceus* subjected to different heat treatments: (a) dry conditions; (b) wet conditions.

(2). The 50% half-lives calculated from this equation highlight the fact that OTA is a relatively thermostable mycotoxin. Such an evaluation of the decay suitably points out the effects of the main thermal treatments used in technological processes.

The destruction of OTA after dry wheat was heated at 100°C for 20 min averaged only 2% of the toxin. Our results are in agreement with partial data reported by previous workers: El-Banna and Scott (4) observed that only 6% of the OTA added was destroyed during cooking of polished wheat at 100°C for 30 min. Under conditions simulating the roasting process (ca. 20 min at 200 to 250°C), Levi et al. (10) have shown that 80% of OTA added to green coffee was destroyed, and Tomova (18) noted considerable reduction of OTA in white flour heated at 250°C for 40 min. Similarly, we found that experimental roasting, prior to the preparation of wheat flour as used for an Algerian meal (Bsissa), caused noticeable losses of OTA (80 to 88%).

The thermal decomposition depends on moisture conditions. The presence of water (50%) increased the decomposition of OTA at 100 and 150°C. At 100°C, there was no change in 20% of OTA destruction after 40 to 160 min of dry heating, whereas upon wet heating at the same temperature, more than 50% of the OTA was destroyed after 120 min (Table 1). This physical property, enhanced with the effect of humidity, was encountered in other mycotoxins: for example, Mann et al. (12) observed that increasing moisture content of oilseed meals resulted in increased rates of aflatoxin degradation. Forty percent of fumonisin B_1 and fumonisin B_2 was recovered in cornmeal after heating at 190°C, but only 20 to 30% was recovered in moist cornmeal after heating at 190°C (15). However, Trenk et al. (19), studying the effect of autoclaving, observed the opposite result; i.e., OTA was more readily destroyed in dry cereal than when water was present. Such a discrepancy might be due to different technical conditions, mainly the presence of steam during autoclaving (15 lb/in^2 at 121°C), and artificial spiking of pure OTA by these authors.

Two fluorescent products have been observed only after heating OTA in dry wheat at higher temperatures (200 and 250°C). Unidentified similar fluorescent materials of unknown

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toxicity have been reported by Tomova (18), Trenk et al. (19), and Trivedi et al. (20). Since the products of OTA decomposition have not been identified, there is no evidence that the observed thermal instability results in detoxification. Heated products have a toxicity in HeLa cells (8, 20) and in pigs (11) similar to that of the original toxin. In contrast, an extract of heated cornmeal showed an almost complete loss of fusarin C mutagenicity (16). Similarly, the decomposition products of zearalenone in bread lacked estrogenic activity (14).

It is concluded that a complete thermal destruction of OTA during the cooking process of wheat within the limits of this study (100 to 250°C) was not achieved. Because of the possibility of finding OTA in end products after thermal treatments, an efficient decontamination procedure is required, but the best protection would be to prevent toxin formation. This study provided information concerning the thermostability of OTA in dry ground wheat and ground wheat paste. Nevertheless, for further investigations, it will be interesting to evaluate the toxic potential of heated materials.

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