

Brief report

Experimental assessment of disinfection procedures for eradication of *Aspergillus fumigatus* in food

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***Aspergillus fumigatus* spores in food may represent an infectious risk for neutropenic patients. We examined the efficiency of disinfection procedures applicable to foods for eradication of *A fumigatus*. Boiling and microwave treatment fully decontaminated an experimental spore suspension and naturally contaminated liquid foods (reconsti-**

tuted dried food, herbal tea). Full decontamination of experimentally contaminated surfaces was only obtained with 70% ethanol or heating at 220°C for 15 minutes. Pepper was decontaminated when heated for 15 minutes at 220°C but not by microwaving. Fruit skin was partially decontaminated by 70% ethanol. We conclude that *A fumiga-*

***tus* spores can be eradicated from food by heating to a temperature of at least 100°C. When foods cannot be exposed to high temperature or microwaving, ethanol only partially reduces the level of surface contamination. (Blood. 2004; 104:2000-2002)**

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Introduction

Invasive aspergillosis (IA) is a major opportunistic infection among patients with hematologic malignancies.¹ Spore inhalation is the usual route of infection, but other routes may exist.² Anaissie et al³ recently reported the presence of pathogenic molds in hospital water distribution systems, leading to aerosolization of fungal spores and potential exposure to patients. Several foods can also be colonized by *Aspergillus* and other molds and can lead to primary gastrointestinal colonization and subsequent systemic infection in animals.⁴ Massive *Aspergillus* contamination of foods was first reported in pepper,⁵⁻⁸ and subsequently in regular and herbal tea, corn, coconut, cashew nuts, coffee, beans, soy, cheese, and smoked meat.⁹⁻¹² Regarding meals served in hematology wards, we recently confirmed that pepper and tea were contaminated by *Aspergillus* and non-*Aspergillus* molds, as were also downy-skinned fruits (apricots, kiwis, and peaches), smooth-skinned fruits (apples, bananas, lemons, and oranges), freeze-dried soups, and even individual food wrappings.¹³ It has been previously recommended that immunocompromised patients should avoid such contaminated foods, and granulocytopenic patients should receive sterile diets or diets low in microbial content.¹⁴ However, such restrictions may affect the well-being of these patients.

One alternative is to disinfect potentially contaminated foods. However, little is known of the efficacy of food-disinfection procedures, especially with regard to *Aspergillus fumigatus*. This study examined the efficacy of several physical and chemical procedures that can be applied to foods and wrappings to eradicate *A fumigatus*.

Study design

Disinfection procedures

Spore suspensions and contaminated Petri dishes. Spores were collected from a culture of *A fumigatus* that was allowed to grow for 8 days on Sabouraud-chloramphenicol agar (Bio-Rad Laboratories, Marnes-la-Coquette, France), by rinsing the culture surface with 10 mL sterile water containing 0.05% Tween 80.

Then, 10 mL of the spore suspension adjusted to 3×10^8 spores/mL was exposed to the following physical disinfectant procedures: oven heating, microwave (MW) irradiation, freezing at -20°C , and heating to 60°C and 100°C (Table 1). Sterile empty Petri dishes that had been seeded with 3×10^8 spores of *A fumigatus* then air-dried were submitted to the same conditions and also to 70% ethanol solution.

Naturally and experimentally contaminated foods. Food samples were then subjected to the disinfection procedures that proved effective on spore suspensions and contaminated Petri dishes. Naturally contaminated foods consisted of commercial black-pepper sachets and tea bags. Black-pepper sachets were directly exposed to MW irradiation or oven heating (8 bags for each treatment). Tea bags were boiled in 250 mL sterile water, and the infusions were stored at 4°C for analysis. Tea from bags of the same batches was cultured on Sabouraud-chloramphenicol agar for 5 days, and infusions of bags from batches that were culture positive for *A fumigatus* were treated by oven heating or microwaving. Freeze-dried soup and fruits were experimentally contaminated; 10 mL freeze-dried soup reconstituted with boiling sterile water was seeded with *A fumigatus* (3×10^8 spores/mL), then exposed to MW irradiation or oven heating. The skins of oranges and apples were contaminated by immersion in a spore suspension of *A fumigatus* ($3 \times 10^8/\text{mL}$). The efficacy of washing the contaminated fruits with water, soap, and ethanol, alone and in combination, was then examined.

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Table 1. Experimental assessment of disinfection procedures

Procedures	Spore suspension, log ₁₀ spores/mL	Inoculated Petri dishes, log ₁₀ spores/mL
Controls	5.75 ± 0.49	5.80 ± 0.75
Freezing at -20°C, 24 h	5.15 ± 0.34	5.70 ± 0.63
MW irradiation		
800 W, 1 min	3.45 ± 1.40	5.70 ± 0.82
800 W, 2 min	0*	5.40 ± 0.70
Oven heating		
150°C, 5 min	0.05 ± 0.16*	2.75 ± 0.72*
150°C, 10 min	0*	1.55 ± 0.50*
150°C, 15 min	0*	2.50 ± 0.91*
220°C, 5 min	0.10 ± 0.32*	0.45 ± 0.76*
220°C, 10 min	0*	0.10 ± 0.32*
220°C, 15 min	0*	0*
Hot water		
60°C	5.75 ± 0.54	5.60 ± 0.57
100°C	5.15 ± 0.63	4.95 ± 0.76
70% ethanol	—	0*

Results are shown for 10-mL suspensions containing 3×10^8 spores of *A fumigatus*/mL and Petri dishes contaminated with 3×10^8 spores of *A fumigatus* (mean log value ± SD of 10 determinations).

— indicates not done.

*Significantly different from the untreated control group at $P < .05$.

Assessment of the disinfection procedures

After treatment, 100- μ L samples of each spore suspension and reconstituted freeze-dried soup and tea infusion were cultured in Sabouraud-chloramphenicol broth (Bio-Rad Laboratories) for 72 hours at 32°C, then titrated by a limiting dilution method (10-fold dilutions) in 96-well plates.

Moist swabs were applied to the entire inner surface of contaminated Petri dishes and fruit skins, then agitated in 1 mL sterile distilled water; spores were counted in 100- μ L samples, as described for spore suspensions.

Spore titers were expressed as log₁₀ spores/mL. The mean spore titer of 8 to 10 replicate experiments was calculated for each treatment and control.

The contents of black-pepper sachets were suspended in 1 mL sterile distilled water and seeded on Sabouraud-chloramphenicol agar. The rate of culture positivity after treatment was expressed as a percentage of untreated controls.

Analysis of variance analysis was used to compare results between treated samples and untreated controls. A P value less than .05 was considered significant.

Table 2. Efficacy of disinfection procedures on food

Disinfection procedures	Oranges, log ₁₀ spores/mL	Apples, log ₁₀ spores/mL	Black, pepper, log ₁₀ spores/mL	Freeze-dried soup, log ₁₀ spores/mL
Control	4.19 ± 0.37	4.44 ± 0.32	7.00 ± 0.82	6.63 ± 0.25
MW, 800 W, 2 min	—	—	1.13 ± 1.13*	0*
Oven heating				
220°C, 5 min	—	—	0.31 ± 0.53*	—
220°C, 15 min	—	—	0*	0*
70% ethanol	0.56 ± 0.50	0.75 ± 0.89	—	—
Washing				
Water washing, 1 min	2.69 ± 0.53*	4.00 ± 0.53	—	—
Water + soap washing, 1 min	2.38 ± 0.58*	3.63 ± 1.27*	—	—
Water + soap + 70% ethanol washing, 1 min	0.75 ± 0.53*	1.38 ± 0.52*	—	—

Results show the fungal load after decontamination of pepper naturally contaminated by *A fumigatus* and on foods (oranges, apples, and freeze-dried soups) experimentally contaminated with a suspension containing 3×10^8 spores of *A fumigatus*/mL (mean log value ± SD of 8 determinations).

— indicates not done.

*Significantly different from the untreated control group at $P < .05$.

Results and discussion

Spore suspensions and contaminated Petri dishes

Full decontamination of spore suspensions was obtained after at least 5 minutes at 150°C in a conventional oven and 2 minutes of exposure to 800-watt MW irradiation ($P < .05$; Table 1). Freezing at -20°C for 24 hours, and addition of hot water (60°C and 100°C) had no significant effect on spore titers. MW irradiation and hot water had no significant effect on Petri dish surface contamination. Heating at 150°C for 5 to 15 minutes resulted in significant but incomplete decontamination. Fungal load was reduced by 90% after 5 to 10 minutes at 220°C. Complete eradication was obtained only after heating at 220°C for 15 minutes or with 70% ethanol ($P < .05$).

Naturally and experimentally contaminated foods

A fumigatus was cultured from all untreated pepper sachets. Complete decontamination was obtained only when bags were heated for 15 minutes at 220°C in a conventional oven (Table 2). Tea from 30 bags was culture positive in 24 cases, and 8 (33%) of the corresponding infusions were also culture positive. Complete decontamination of tea infusions was obtained by exposure to MW for 2 minutes (which resulted in boiling). Oven heating at 220°C for 2 minutes was partially effective (data not shown). All 8 samples of reconstituted freeze-dried soup were decontaminated by boiling.

Washing with water or soap was ineffective on experimentally contaminated apples but had a small significant effect on oranges. Exposure to 70% ethanol, used alone or preceded by washing with soap, significantly reduced the fungal load. These results are shown in Table 2.

Fungal decontamination of food served to at-risk patients mainly relies on thorough cooking. However, no consensus approach has been agreed on,^{15,16} and little is known about the susceptibility of filamentous fungi to biocides. Most disinfectants with fungicidal activity are designed for soil and surface treatment (not food decontamination), and their activity has mainly been

tested against *Aspergillus niger*. We chose to study *A fumigatus*, one of the most prevalent and life-threatening pathogenic molds, that has been isolated from several types of food.⁵⁻¹³

After experimental contamination, we found that prolonged exposure to temperatures of at least 100°C is necessary to eradicate *A fumigatus* spores in liquid suspension. Freezing was ineffective, as previously reported.¹⁷ Experimentally contaminated dry surfaces (Petri dishes) were partly decontaminated by lengthy heating in an oven, but not by MW irradiation. We confirmed that 70% ethanol is highly effective for decontaminating dry surfaces¹⁸⁻²¹ and also found that washing with soap and water before ethanol treatment has no added benefit.

These results were confirmed on naturally and experimentally contaminated foods. Oven heating of reconstituted dried foods at the boiling point was the only effective method. Exposure of black pepper to high temperatures was effective but resulted in a marked loss of flavor. With regard to surfaces that can be naturally contaminated, such as fruits and individual food wrappings, washing with water followed by 70% ethanol treatment was the most effective procedure, although complete eradication was never obtained.

Various non-*Aspergillus* molds have emerged as significant

pathogens in immunosuppressed patients.²² Our findings can probably be extrapolated to other potentially pathogenic yeasts and molds, because *A fumigatus* spores are highly resistant to physical and chemical disinfectants.^{18-21,23} Few data are available on non-*Aspergillus* molds. *Fusarium* species are sensitive to alcoholic solution²⁴ and to high water temperature,²⁵ and MW heating to the boiling point is a rapid and convenient method for eradicating *Candida albicans* from milk.²⁶ These are also the conditions that we found most effective on *A fumigatus* spores.

Our results provide a basis for guidelines on *A fumigatus* decontamination of foods and supplement existing recommendations for the prevention of fungal contamination during food storage and meal preparation. These recommendations should probably be extended to other naturopathic products and herbal medicines that are frequently contaminated by various *Aspergillus* species and can also contain *Aspergillus*-derived mycotoxins.²⁷⁻³⁰

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