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Effect of low-dose microwave radiation on *Aspergillus parasiticus*

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ABSTRACT

The effect of low-dose microwave radiation (LDMR; 2.45 GHz, 1.5 W/g) on biochemical characteristics and mortality of *Aspergillus parasiticus* was investigated and compared to the effects of conventional heating treatment (water bath), in order to provide a theoretical basis for microwave control of mildew in rice and other food products. The effects of LT50 (52 ± 2 °C) and LT100 (72 ± 2 °C) with microwave treatment on *A. parasiticus* were both lower than those (62 ± 2 °C and 92 ± 2 °C, respectively) with conductive heating. LDMR and conventional heating treatment both caused increased cell membrane permeability, and thus an increase in electrolyte, Ca^{2+}, protein and DNA leakage, and the surface of mycelia appeared rough and swollen. LDMR was more effective in disrupting the cell membrane and causing DNA damage than conductional heating. The severity of DNA injury increased with the rise in temperature. The mechanism causing death of the mold evidently differed between LDMR and conventional heating treatment. LDMR led to the death of *A. parasiticus* mainly through the increased Ca^{2+} permeability and DNA degradation, and conventional heating treatment induced death mainly by augmenting electrolyte permeability and DNA concentration.

1. Introduction

Mold contamination of grain influences grain storage quality and is the main determinant of grain mold infection (Tsai & Yu, 1999). About 25% of grain worldwide is infected by mold and its mycotoxin each year, of which 2% loses its nutritional and economic value owing to serious infection (FAO, 2007). Although chemical fumigation (Bond, 1973; White & Loschiavo, 1985), low temperature (Luo et al., 2007), and modified-atmosphere packaging and storage (Jayas & Jeyamkondan, 2002) can control mildew, such techniques have many shortcomings, such as food safety, environmental pollution, cost, or ineffectiveness.

Aspergillus parasiticus is one of the most common molds infecting grain during storage (Reddy, Reddy, & Muralidharan, 2009; Samapundo et al., 2007). It can contaminate agricultural commodities before and during harvest, or during storage. Microwave technology has been widely used in the food industry and offers several advantages such as security, high efficiency, and environmental protection, but often affects food quality (Lewandowicz et a1., 2000; Pomerai et a1., 2003; Takagi & Yoshida, 1999). Low-dose microwave radiation (LDMR; 2.45 GHz, 1.5 w/g) (Andrea, Paola, Andrea, Francesco, & Fabio, 2004; Vadivambal, Jayas, & White, 2007) not only has certain lethal effects on molds and pests (Wang, Wig, Tang, & Hallberg, 2003; Zhao, Shao, Xing, & Qiu, 2004, 2007a), but also maintains the quality of rice grains (Zhao, Shao, Xing, Qiu, & Xu, 2007b). Although the effectiveness of microwave irradiation for sterilization has been well established by numerous studies in recent decades (Goldblith, 1967; Latimer & Matsen, 1977; Lu, Zhou, Xiong, & Zhao, 2010; Sanborn, Wan, & Bulard, 1982; Sawai, Matsumoto, Saito, Isomura, & Wada, 2009), the exact nature of the sterilization effect and whether it is due solely to thermal effects or to the 'microwave effect' is still controversial. Presently, the efficiency of LDMR treatment against *A. parasiticus* during storage is still relatively low. To optimize treatment protocols, there is a clear need to understand the effect of microwave radiation on organisms. LDMR causes a variety of biophysical, biochemical and functional changes. Microwaves can affect the mortality rate, cell structure, cell inclusion and DNA of microorganisms (Celandroni, Longo, & Tosoratti, 2004; Ovchinnikova, 1996; Sawai et al., 2009; Welt, Tong, Rossen, & Lund, 1994).

In this study, *A. parasiticus* was exposed to LDMR, which has little effect on the quality of rice. The effect of LDMR on biochemical characteristics and mortality of *A. parasiticus* was studied with conventional heating (water bath) as a control, in order to provide a theoretical basis for microwave control of mildew for rice and other food products.

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2. Materials and methods

2.1. Materials

Rice grains were obtained from Lushiyuan Food Co., Wuhan, China. All reagents used were of analytical grade. *Aspergillus parasiticus* were isolated from rice. It was identified and preserved in the laboratory.

2.2. Test methods

2.2.1. Cultivation of A. parasiticus

The *A. parasiticus* were subcultured on agar slants from the stock culture and washed with sterile water. The spores were resuspended in sterile water at a spores concentration of 106 CFU/ml. *Aspergillus parasiticus* aqueous suspensions (106 colony-forming units [CFU]/ml) were inoculated in a conical flask containing a medium composed of bean sprout liquid to produce an inoculum concentration of 10%. The bean sprout liquid consisted of 100 g soybean sprouts,50 g glucose, and 1 L distilled water (pH not adjusted). The inoculated medium was incubated at 28 ± 2 °C and shaken on a rotary incubator at 180 r/min for 48 h to prepare the spore suspension. Spores were harvested by centrifugation and washed three times with a sterile 0.9% NaCl solution. The spores were stored in a 0.9% NaCl solution, placed into vials and stored at a low temperature (4 °C) until use.

2.2.2. Sample pretreatment

For the LDMR treatment, samples were irradiated using a QW-15HM microwave oven (Guangzhou Kewei Microwave Oven Energy, China) with a 900 W capacity and 2.45 GHz frequency. The effective length, width and height of the oven were 508 mm, 420 mm and 305 mm, respectively. The temperature of the spore suspensions was measured immediately after irradiation with a JDDA80 point thermometer (Wuhan, Jingda Instrument Factory Co., China) at different times. Five groups of 100 mL spore suspensions each were placed in conical flasks. The suspensions were exposed to microwave irradiation 1min, 2min, 3min, 4min, and 5min at rates of 1.5 W/g, so as to produce a range of final temperatures according to a predetermined standard temperature–time curve. The spore suspensions were exposed to microwave irradiation at a fixed output power (1.5 W/g) for different exposure times to obtain a number of suspension temperatures (30, 50, 60 and 70 °C). To determine the effect of conductive heat treatment, the spore suspensions subjected to a constant temperature of 30, 50, 60 and 70 °C in water bath.

2.2.3. Determination of A. parasiticus mortality

Immediately after LDMR or conductive heating treatment, the conical flask containing 100 mL mold suspension was placed in an ice bath for 10 min. The colonies were determined as described elsewhere (Yao & Mainelis, 2006). The mortality of *A. parasiticus* was quantified with the following equation:

$$M(\%) = \left(1 - \frac{N_i}{N_e}\right) \times 100 \quad (1)$$

where M is the mortality, N_i is the CFU number for the treated microorganisms, and N_c is the CFU number for the control microorganisms.

After LDMR and conventional heating, the mold suspension was incubated at 28 ± 2 °C and shaken at 180 r/min for 10, 20, 30, 40, 50, 60, 70, or 80 h. The mold culture liquid was vacuum-filtrated for 5 min, and then washed three times with sterile water to collect mycelia. The mycelia were dried to a constant weight at 105 °C.

2.2.4. Determination of protein and DNA leakage

The mold suspension before and after treatment was filtered and the absorbance of DNA and protein in the supernatant was measured with a UV-2600 spectrophotometer (Younikang Co., Shanghai, China) at 280 nm and 260 nm, respectively. The blank was composed of sterile physiological (0.85%) saltwater (Woo, Rhee, & Park, 2000).

2.2.5. Determination of electrolyte leakage

Electrolyte leakage (EL) from cells treated with LDMR and conventional heating was measured as described previously (Soro, née, Djè, ., & Thonart, 2010; Campanha et al., 2007). The treated mold suspension was left to stand for 10 min, then stirred gently with a glass rod at 25 °C. The initial electrical conductivity (E_i) and total electrical conductivity (E_t) of the treated mold suspension was measured using an FE30 conductivity meter (Mettler-Toledo Instruments Co., China). The mold suspension was then placed into a 100 °C boiling water bath for 15 min to induce electrolyte leakage, which was calculated as follows:

$$\mathrm{EL}\ (\%) = [1 - (1 - \mathrm{Ei}/\mathrm{Et})/(1 - \mathrm{Ci}/\mathrm{Ct})] \times 100 \quad (2)$$

where C_i is the initial electrical conductivity of the control, and C_t is the total electrical conductivity of the control.

2.2.6. Determination of Ca^{2+} permeability

After microwave or conventional heating treatment, the mold suspension was washed three times with sterile distilled water, then vacuum-filtrated, and finally dried at 105 °C for 4 h, after which the weight (M) was measured. The quantity of Ca^{2+} released after microwave or conventional heating was determined by a colorimetric system (Campanha et al., 2007). The reaction involved the binding of Ca^{2+} to purple-colored phthalein in an alkaline medium. The system consisted of: Reagent 1 contained 920 mmol/L buffer, pH 12, and 19 mmol/L sodium azide; Reagent 2 contained 320 mmol/L o-cresolphthalein complexone, 13 mmol/L hydroxyquinoline and 130 mmol/L hydrochloric acid; and the standard solution contained 10 μg/mL Ca^{2+} and 0.1% formol. The absorbance was determined at 570 nm with a 722S spectrophotometer (Precision Scientific Instruments Co., Shanghai, China). The Ca^{2+} permeability was expressed by the following equation:

$$\mathrm{Ca^{2+}permeability}(\%) = \frac{\mathrm{A_{test}}}{\mathrm{M} \times \mathrm{A_{standard}}} \times 100 \quad (3)$$

2.2.7. Mycelium morphology

Mycelium morphology was examined with a JSM-6390LV scanning electron microscope (NTC, Japan) at an accelerating voltage of 20 kV. The dried cell materials were fixed to copper stubs using double-adhesive tape and coated with gold (Marcin, Sławomir, Agnieszka, Jarosław, & Marcin, 2007).

2.2.8. Determination of DNA damage

DNA damage was measured using the cetyl-trimethylammonium bromide (CTAB) method (Fang, Hammar, & Grumet, 1992; Kieser, Bibb, Buttner, & et al, 2000; Taylor & Powell, 1982), using phenol-chloroform extraction. Mycelia were placed in a small, clean glass mortar; 0.9 mL extraction buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris–HCl [pH 8], 0.2% polyvinyl-polypyrrolidone [PVPP], 0.01% sodium dodecyl sulfate [SDS], and 0.2% α-mercaptoethanol) was added to the mortar and the mycelia ground to a paste, which was transferred to a 2 mL centrifuge tube with the tip cut off and replaced with a 0.5-cm suction head. After extraction at room temperature for 45 min, samples were washed twice with 0.6 mL chloroform-isoamyl alcohol (24:1, v/v) followed