Degradation of fumonisin B$_1$ by cinnamon essential oil

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**A B S T R A C T**

Fumonisins are group of mycotoxins produced mainly by *Fusarium verticillioides* and *Fusarium proliferatum*. They frequently contaminate corn and corn based products, and cause several diseases in humans and animals. Fumonisin B$_1$ (FB$_1$) is the most prevalent fumonisin and is highly toxic to human and animal. The essential oils from plants offer a hope in the prevention and detoxification of these mycotoxins. The present study investigates the degradation effect of cinnamon, citral, *Litsea cubeba*, clove, eucalyptus, anise, spearmint and camphor oils on FB$_1$. The degradation level of FB$_1$ was determined by ELISA. Cinnamon oil proved to be effective essential oil in reducing FB$_1$, followed by citral, eugenol oil, eucalyptus oil, anise oil and camphor oil. The effects of incubation time, and temperature with respect to the concentration of cinnamon oil on their degradation effect on FB$_1$ by cinnamon oil were investigated. Results showed that at $120$ h time with the $280$ mg/ml concentration of cinnamon oil, under $30\, ^\circ\text{C}$ is optimal for FB$_1$ reduction. Under optimal condition, FB$_1$ was reduced from $15.03$ to $0.89$ mg/ml (94.06%); Cinnamon oil could be a promising candidate in the detoxification and control of FB$_1$ in corn based products.

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1. Introduction

Fumonisins (FBs) are mycotoxins produced mainly by *Fusarium verticillioides* and *Fusarium proliferatum* (Sydenham et al., 1990; Yoshizawa, Yamashita, & Luo, 1994). They frequently contaminate the agricultural commodities, especially maize and maize-based products (Doko & Visconti, 1994; de Nijs, Sizoo, Vermunt, Notermans, & van Egmond, 1998; Petersen & Thorup, 2001) and cause several diseases in both humans (Gelderblom et al., 1988; Marasas, Jaskiewicz, et al., 1998; Lemmer et al., 1999) and animals (Harrison, Colvin, Greene, Newman, & Cole, 1990; Haschek, Gumprecht, Smith, Tumbleson, & Constable, 2001; Smith et al., 2000).

At least 28 FBs have been isolated and characterized (Huffman, Gerber, & Du, 2010). Fumonisin B$_1$ (FB$_1$) is the most common toxic fumonisin which affects humans and animals. FB$_1$ is teratogenic (Marasas et al., 2004) and carcinogenic (Gelderblom et al., 1988, 2001; Lemmer et al., 1999). Epidemiological studies have shown that human exposure to FB$_1$ causes esophageal cancer (Marasas, Jaskiewicz, et al., 1988; Voss et al., 2002) and neural tube defects in new born infants (Hendricks, Simpson, & Larsen, 1999; Moore et al., 1997; Ncayiyana, 1986; Sydenham et al., 1990). In animals, FB$_1$ is associated with several diseases, such as leukoencephalomalacia in horses (Marasas, Kellerman, et al., 1988), pulmonary edema in swine (Harrison et al., 1990; Haschek et al., 2001; Smith et al., 2000), and liver cancer in rats (Gelderblom, Kriek, Marasas, & Thiel, 1991; Lemmer et al., 1999).

One method of inhibiting the fungal growth and/or degrading FB$_1$ involves the use of plant essential oils. Several studies have reported the inhibitory effects of cinnamon, citral, *Litsea cubeba*, clove, eucalyptus, anise, spearmint and camphor oils on the growth of different microbial species (Patil et al., 2009; Velluti, Sanchis, Ramos, Egoio, & Marin, 2003). Cinnamon and clove oils inhibited the growth of *Aspergillus flavus* and the production of aflatoxin. Furthermore, cinnamon and clove oils were effective against aflatoxin production by *A. flavus* in contaminated maize grains (Bullerman, Lieu, & Seire, 1977; Montes-Belmont & Carvajal, 1998; Sinha, Sinha, & Prasad, 1993). Velluti et al. (2003) reported that cinnamon, clove, lemongrass, oregano, and palmarose essential oils have inhibitory effect on the growth and FB$_1$ production by *F. proliferatum* in maize grains. The objective of our study was to assess the effect of cinnamon, citral, *Litsea cubeba*, clove, eucalyptus, anise, spearmint and camphor oils on the degradation of FB$_1$. 

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2. Materials and methods

2.1. Chemicals

High performance liquid chromatography (HPLC) grade methanol and acetonitrile were purchased from Thermo Fisher scientific (Fisher Chemicals HPLC, USA). FB1 standard was purchased from Sigma–Aldrich Chemicals (USA). All other reagents used in the analysis were of analytical grade. FB1 was dissolved in acetonitrile/water (1:1, v/v) at a concentration of 0.2 mg/ml as a standard stock solution and stored at -20 °C. Working standard solutions were prepared by diluting the FB1 standard stock solution in acetonitrile/water (1:1, v/v) and stored at 4 °C.

2.2. Essential oils

Seven essential oils were used in the experiments: cinnamon (85% Cinnamic aldehyde), citral (96% Citral), eugenol (99% Eugenol), eucalyptus (80% Cineole), anise (92% Anethole), peppermint (50% Methol), and camphor (55% Borneol) oils (Jiangxi Xue Song Natural Medicinal Oil Co., Ltd, China).

2.3. Solution model preparation

FB1 was added to screw-caped tubes containing 50 μl of acetonitrile/water (1:1, v/v) resulting in a 20 μg/ml concentration of FB1. Ten microliters of the seven essential oils was added to the tubes (the concentration of cinnamon, citral, eugenol, eucalyptus, anise, peppermint and camphor oils was 140, 160, 160, 130, 150, 85 and 90 μg/ml, respectively), which were subsequently tightly closed and shaken at 200 rpm and 25 °C. Aliquots were drawn at 72 h and detected by using ELISA kit, according to the manufactures protocol.

2.4. Effect of incubation time, different concentration and temperature of cinnamon oil on FB1 degradation

A full factorial design was used. The factors were incubation time, concentration, and temperature. The responses were the concentration and the degradation rate of FB1. With 140 μg/ml concentration of cinnamon oil at 25 °C, the effect of incubation time was analyzed (24, 48, 72, 96, 120 h). Then, with the temperature at 25 °C and the time at 120 h, the effect of the concentration of cinnamon oil was analyzed (0, 70, 140, 210, 280 μg/ml). With the incubation time at 120 h and 280 μg/ml concentration of cinnamon oil, the effect of the temperature was analyzed (20, 25, 30, 35 °C).

2.5. Statistical analyses of the data

All the experiments were done in triplicates. The data were expressed as percentage and mean ± RSD, were calculated by using SAS 9.2 (SAS Institute, Cary, NC, USA). Statistical significance was judged at the 5% level.

3. Results

3.1. Effect of the seven essential oils on the degradation rate of FB1

Of the seven essential oils tested, peppermint oil had no effect on the degradation of FB1 (Fig. 1). On the other hand, cinnamon, citral, eugenol, eucalyptus, anise, and camphor oils reduced the level of FB1. Cinnamon oil proved to be the effective essential oil with the highest rate of FB1 degradation (66.65%). It was followed by citral (53.19%), eugenol oil (24.91%), and eucalyptus oil (21.33%). Cinnamon oil could degrade aflatoxin B1, B2, G1, and G2 in maize (data not shown). Thus, cinnamon oil can be exploited for reducing FBs and aflatoxins which are often seen together in contaminated maize.

3.2. Effect of incubation time on the degradation of FB1 by cinnamon oil

Incubation time had a significant effect on the degradation of FB1 by cinnamon oil (Fig. 2). The degradation rate of FB1 depends on the incubation time. The degradation rates of FB1 increased gradually with the extension of incubation time. At 120 h of incubation, cinnamon oil showed the highest rate of FB1 degradation (72.92%).
3.3. Effect of the concentration of cinnamon oil on the degradation of FB1

The concentration of cinnamon oil had a significant effect on the degradation of FB1 (Fig. 3). The degradation rate of FB1 also depends on the concentration of cinnamon oil, and the degradation rate of FB1 increased gradually with increasing cinnamon oil concentration. When the concentration of cinnamon oil was at 280 μg/ml, the degradation rate of FB1 (93.35%) was at peak. The second highest rate of FB1 degradation (88.00%) was achieved with the concentration of cinnamon oil at 210 μg/ml.

3.4. Effect of temperature on the degradation of FB1 by cinnamon oil

Temperature had a significant effect on the degradation of FB1 (Fig. 4) and the degradation rate of FB1 gradually increased with increasing temperature. The highest degradation rate of FB1 (94.06%) was achieved at 30 °C. No significant differences in the degradation of FB1 at 25, 30, or 35 °C were observed. Therefore, the degradation of FB1 by cinnamon oil can be performed at room temperature (25 °C).

4. Discussion

In China, FB1 is widespread in grains, especially in maize and maize-based products. FB1 has been implicated in several human and animal diseases. Currently, there are no effective control measures for this mycotoxin. FB1 is highly stable molecule. Physical methods like boiling treatment (Alberts et al., 1990), autoclaving treatment (Murphy, Hopmans, Miller, & Hendrich, 1995), ethanol fermentation (Bothast, Bennet, Vancauwenberge, & Richard, 1992), physically removing the “fines” or sieving from maize of bulk shipments (Sydenham, Van der Westhuizen, Stockenstr, Shephard, & Thiel, 1994), or the addition of adsorbents (Huwig, Freimund, Kappeli, & Dutler, 2001) are not significantly effective in reducing and/or degrading FB1. Chemical methods such as ammonia treatment (Park, Rua, Mirocha, Abd-Alla, & Weng, 1992), alkalis treatment (Sydenham, Shephard, Thiel, Marasas, & Stockenstr, 1991) or bleach treatments (Murphy et al., 1995) are also not significantly effective. Even though FB1 is rapidly degraded by ozone gas (O3) to 3-keto FB1 and possibly other products, treating of FB1 with O3 is ineffective in diminishing its toxicity (Mckenzie et al., 1997).

Since FBs are resistant to most of physical and chemical treatment, researchers have focused on microbes that can degrade FBs. In 1999, Exophiala spinifera, a black yeast fungus was identified as one that can do oxidative deamination of hydrolyzed FB1 (Blackwell, Gilliam, Savard, DavidMiller, & Duvick, 1999). In 2000, three Bacillus spp. strains and a yeast strain were able to partially degrade FBs were isolated from corn and silage microflora (Camilo, Ono, Ueno, & Hirooka, 2000). In 2005, a Sphingomonas spp. strain MTA144 with strong FBs degrading activity was isolated (Täubel, Ono, Ueno, & Hirooka, 2000). In 2006, a bacterial strain NCB 1492, supposed to be related to the Delftia/comamonas group that was able to hydrolyze and deaminate FB1, was isolated from soil samples using an enrichment culture procedure (Benedetti, Nazzi, Locci, & Firrao, 2006). Microbial degradation is one of the effective methods for the detoxification of FB1. However, higher cost limits the use of microbes for degrading FBs.

FB1 is resistant to many degradation methods. Therefore, more effective methods to degrade FB1 are required. In 2003, a group of researchers assessed the effect of seven naturally occurring phenolic compounds from plants on FB1 degradation. They reported that chlorophorin, irokom, maakianin, vanillic acid, and caffeic acid were effective in the degradation of FB1 (Beekrum, Govinden, Padayachee, & Odlav, 2003). In this study, the effect of cinnamon, citral, L. cubeba, clove, eucalyptus, anise, spearmint, and camphor oils on the degradation of FB1 was evaluated. The results revealed that cinnamon oil was the most effective compound in degrading of FB1, followed by citral, eugenol, eucalyptus, anise, and camphor oil. Under optimum conditions, FB1 concentration was reduced from 15.03 to 0.89 μg/ml (i.e., a degradation rate of 94.06%).

In an attempt to investigate natural fungicides, Chao and Young examined the inhibitory effects of 45 oils on different microbe strains (i.e., one yeast, one phage, eight bacterial, and two fungal strains). The results revealed that cinnamon oil had a strong inhibitory effect against all tested organisms and phage (Chao, Young, & Oberg, 2000). The inhibitory effect of cinnamon oil on growth and aflatoxin production by A. flavus has been reported (Bullerman et al., 2006).
In maize grain, cinnamon oil had high inhibitory effect on aflatoxin production by A. flavus after 10 days even under favorable conditions for mycotoxin production (Sinha et al., 1993). The inhibitory effect of cinnamon oil on growth and FB1 production by F. verticillioides and F. proliferatum has been assessed (Velluti et al., 2003). The results of their study indicated that cinnamon oil had a strong inhibitory effect on the growth of F. verticillioides under both temperatures (20 and 30 °C) and water activity (a_w) (0.950 and 0.995). A significant inhibitory effect on FB1 production by F. verticillioides was obtained at 30 °C and 0.995 a_w. The results in the present study suggest that cinnamon oil could be effective in controlling growth and FB1 production by F. verticillioides in maize grains, and could be effective in the reduction of FB1. Thus, cinnamon oil could be used for controlling and reducing of FB1 in maize grains.

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