Effect of High Temperature with *Litsea cubeba* Pers. to Control Mold Growth on Bamboo Food Packaging and Its Possible Modes of Action

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This study examined the effect of high temperature and *Litsea cubeba* oil with its main components against molds (*Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp., *Penicillium cyclopium*, *Rhizopus* sp., *Fusarium* sp., and *Cladosporium* sp.) on bamboo food packaging. Response surface methodology (RSM) with X_1 (concentration of *L. cubeba* oil at 100, 300, and 500 mg g^{-1}), X_2 (temperature at 60, 80, and 100 °C), and X_3 (time at 12, 14, and 16 h) was used to find the inhibitory periods of natural mold on packaging plates. The physical properties and the change of chemical components on the bamboo packaging plate before and after temperature treatment were determined to find the mode of action using gas chromatography–mass spectrometry (GC-MS). High temperature (at 100 °C) was a good inhibitor of all mold growth with the MIC (minimum inhibitory concentration) of 300 mg g^{-1}; without heat treatment, no MIC was found. In addition, it was found that by using 300 mg g^{-1} of *L. cubeba* oil at 100 °C with an exposure time of 12 h, spore germination on the bamboo surfaces was completely inhibited for at least 290 days. After using high temperature, citral was detected on the surface of the packaging plates. Therefore, these components could be the key factors for inhibiting molds.

Keywords: Litsea cubeba oil; Mold; Bamboo; Packaging plate; Heat

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INTRODUCTION

Petroleum-based food packaging types such as plastic and foam are having a major long-term impact on the environment because they are non-biodegradable. These kinds of packaging are hard to degrade naturally, and they may take 100 years or more to decompose (Satyanarayana *et al*. 2009). As an alternative to solve this problem, there is presently great interest in developing renewable raw materials (Cao *et al.* 2013). Cellulose has been identified as a suitable packaging material (Shahroze *et al.* 2018).

Bamboo is a lignocellulose material that is an abundant natural resource in Asia (Zakikhani *et al*. 2016). Generally, bamboo takes a short time to grow and has high productivity (Hu *et al.* 2016). The bamboo plate is one of the newest packaging innovations due to its light weight, high strength, and ability to form many shapes (Kumar *et al.* 2017). Bamboo fiber has a higher Young's modulus over density than wood and steel. Paper and foam made from bamboo are available in the worldwide market (Win *et al.* 2012; Liu and Wang 2013). However, due to its degradation properties, bamboo packaging plates are easily destroyed by natural mold when they are kept in wet conditions or high relative humidity, such as tropical rainforest areas (Matan *et al*. 2011; Theapparat *et al*. 2015;

Sequeira *et al*. 2017). Normally, this packaging must be stored in dry conditions within a large container to provide moisture control, and such a system uses more energy. In this research, the application of an essential oil (*Litsea cubeba*) to protect against molds on bamboo packaging plates was selected.

Litsea cubeba is found in southern China, Japan, and Southeast Asian countries (Yang *et al*. 2014). Citral is the main component (78.7 to 87.4%) of *L. cubeba* oil (Si *et al*. 2012), which is similar to citrus fruit (Lante and Tinello 2015). *L. cubeba* oil has shown good antifungal and antimicrobial activity (Liu and Yang 2012; Suhem *et al*. 2015) in both *in vitro* (Gogoi *et al*. 1997) and *in vivo* tests (Luo *et al*. 2004). However, *L. cubeba* has its own distinct flavor and aroma that could have a sensory effect if it is directly added into foods. Within this research, to reduce such an effect, *L. cubeba* was instead added into the packaging rather than being adding directly into the foods. Therefore, the main objective of this study was to assess mold growth on bamboo packaging plates using high temperature.

EXPERIMENTAL

Chemicals Used in the Study

L. cubeba oil, derived by steam distillation from the fruits, was purchased from the Thai China Flavors & Fragrance Industry Company of Thailand (Nonthaburi, Thailand). Citral, citronellal, 4-hydroxybenzaldehyde, and linalool oxide were purchased from Sigma-Aldrich of Singapore (Nucleos, Singapore).

Culture Preparation

Seven strains of molds (*Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp., *Penicillium cyclopium*, *Rhizopus* sp., *Fusarium* sp., and *Cladosporium* sp.), isolated from the bamboo packaging plate, were obtained from the Innovation of Essential Oil for Food Safety and Packaging laboratory of Walailak University in Nakhon Si Thammarat, Thailand. All molds were grown on malt extract agar (MEA; Merck Ltd., Songkhla, Thailand) at 25 °C for 7 days. Suspensions were collected by flooding the surface of the MEA tube with 9 mL of sterile water. Viable molds were counted using MEA (7 $\log_{10}$ CFU ml^{-1}).

Effect of High Temperature on Mold Growth on the Bamboo Packaging Plate

The bamboo packaging composites was first prepared by mixing 5.0 g of bamboo fiber, 1.5 g of corn starch (Thai Holdings, Bangkok, Thailand), and 1.5 g of tapioca starch (Thai Wai Food Products, Bangkok, Thailand) into 20 g of distilled water and then it was boiled for 10 min. The bamboo solution was sterilized at 121 °C for 15 min in an autoclave (Suhem *et al*. 2017). The solution was held for approximately 5 min or until the temperature dropped to 50 °C, and *L. cubeba* oil, citral, citronellal, 4-hydroxy-benzaldehyde and linalool oxide were added at concentrations from 50 to 500 mg g^{-1} into the paste. Next, the paste was poured into the round stainless plates, with a diameter of 5 cm. The bamboo plates were dried in an electric oven (FD23, Binder, Tuttlingen, Germany) at 100 °C for 14 h. Controls were carried out using the same procedure but drying with low temperature (30 °C). All packaging was kept at 27 ± 2 °C with 65 ± 5% relative humidity (RH) in an environmental chamber until the moisture content fell below 12%.

One mL of each mold strain suspension was inoculated on a surface of treated

bamboo packaging and kept at 25 °C and 100% RH for 7 days. Next, 25 g of each specimen (n = 3) was used for visible cell counts. Compared to the control, the ones with the lowest essential oil concentration showing no visible mycelium growth were regarded as the minimum inhibitory concentration. Three replicates were prepared for each treatment.

Optimization of Drying Process Parameters for Control (Natural Mold) Using Response Surface Methodology

Experimental design

A central composite face-centered design (CCF) with three factors, X_1 (concentrations of *L. cubeba* oil at 100, 300, and 500 mg g^{-1}), X_2 (temperatures at 60, 80, and 100 °C), and X_3 (times at 12, 14, and 16 h) was employed for this study. The second-order polynomial equation for a 3-factor system is shown in Eq. 1,

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j \quad (1)$$

where Y is the predicted response, β_o is the interception coefficient, β_i are the linear terms, β_{ii} are the quadratic terms, β_{ij} are the interaction terms, and x_i and x_j are the coded levels of the independent variables. Models of the two responses were expressed in terms of coded variables without considering the statistically insignificant terms. The quality of the fit was checked with the coefficient of determination R^2 and its statistical significance was found by the F-test. Statistical analysis was performed by using Statistica software (StatSoft, Oklahoma, USA).

Mold test on the bamboo packaging plates

Bamboo paste containing *L. cubeba* oil at 100, 300, and 500 mg g^{-1} was prepared according to the process described above. After preparation, the paste was poured into a molding and dried at different temperatures (60, 80, and 100 °C) for 12, 14, and 16 h. The average moisture content of the packaging specimens after conditioning at 20 °C and 65% RH for 48 h was 12 ± 1% (n = 10).

To evaluate the mold growth on the bamboo packaging plates, all plates were kept at 25 °C and 100% RH. Each plate (n = 5) was individually rated for mold growth (ASTM D4445 1998). When evaluated with the naked eye, the inhibitory period in days was determined by visualization using a microscope and then recorded. The time periods needed for the initiation of mold growth on bamboo packaging plates were recorded.

Properties of the Packaging Plate and Mold Morphology

A bamboo packaging plate containing 300 mg g^{-1} of *L. cubeba* oil after heat treatment at 100 °C for 12 h was selected for this study. The control (a plate containing 300 mg g^{-1} of *L. cubeba* oil and 300 mg g^{-1} of vegetable oil /or from Morakot Industries, Bangkok, Thailand) was carried out using the same method but without heat treatment (drying at 30 °C for 16 h). The plate properties and gas chromatography-mass spectrometry (GC-MS) were then measured.

Wettability measurement

A contact angle goniometer (Kyowa Interface Science, Niiza, Japan) was used to measure the spreading of the distilled water on the bamboo packaging plate. Drops of distilled water were made by a micro-syringe. Five seconds after dropping, five images were captured for every second of contact angle analysis. Each sample was subjected to 8

4-angle position measurements: vertical left, vertical right, horizontal left, and horizontal right. Finally, the images of the distilled water drops were analyzed with a computer contact angle software (FAMAS, Niiza, Japan).

Water absorption

The water absorption was done according to the work of Alamri and Low (2013). The samples were prepared at dimensions of 10 mm × 10 mm × 3.5 mm. The water absorption test was carried out by immersing the specimens in the deionized water for 10 min. The packaging weight was measured after 10 s. Then, to remove the excess water from their surfaces, each sample was dried for 30 min at 100 °C before being weighed. The water absorption (%) was calculated according to Eq. 2,

$$\text{Absorption (\%)} = (W_2\text{-}W_1)/W_1 \times 100 \quad (2)$$

where W_1 was the initial weight of the bamboo tray and W_2 was the weight of the bamboo tray.

Mold morphology

Mold morphology on selected packaging plates was examined after incubation by using a Leica DVM6 digital microscope (Leica Microsystems AG, Heerbrugg, Switzerland).

GC-MS Analysis

The bamboo packaging plates with 300 mg g^{-1} of *L. cubeba* oil that were heated at 100 °C for 12 h and then dried at 30 °C for 16 h were subjected to GC-MS. Using a method adapted from Friedman *et al*. (2000) the essential oil components were extracted from 10 g of the bamboo packaging plates having five replicate specimens. First, the pieces of the packaging plates were added to a glass tube with 4 mL of ethyl acetate. The tube was sealed with a Teflon-lined cap and mixed by gentle shaking. Next, the specimen was held for 30 min before being extracted with ethyl acetate twice more. The combined ethyl acetate extracts were reduced to dryness under a stream of nitrogen at room temperature. Finally, the residue of each was dissolved in 1 mL of ethyl acetate in 1 µL aliquots.

Analyses of *L. cubeba* oil and the extract solutions (1 µL) were carried out on a gas chromatograph (Hewlett Packard Model 7890A, California, USA) equipped with a DB-5 (J&W Scientific, California, USA) column (30 cm × 0.25 mm ID) and a film thickness of 0.25 µm. The average helium carrier gas flow rate was 1 mL min^{-1}. The split ratio of the column oven was 50:1, and the injector and detector temperatures were set at 250 °C and 260 °C, respectively, with the temperature held at 60 °C for 30 s, increased to 150 °C at 40 °C min^{-1}, and then maxed to 260 °C at 2 °C min^{-1}. The solution was manually injected (1.0 µL). The identification of the constituents was based on a comparison with authentic samples, their Kovats indices, and computer matching with the NIST 0.8 L (Database/ ChemStation data system).

Statistical Analysis

All results were expressed as mean ± standard deviation (n = 3). Data were statistically treated by one-way ANOVA and Duncan's post hoc test, with $P < 0.05$ being considered to be statistically significant. Statistical analysis was performed using Statistica software (StatSoft, Oklahoma, USA).

RESULTS AND DISCUSSION

Effect of High Temperature on Mold Growth

The antifungal activity of *L. cubeba* oil and its main components (citral, citronellal, 4-hydroxybenzaldehyde, and linalool oxide) against mold on bamboo packaging plates is summarized in Table 1. The results showed that citral had the highest antifungal activity. The MICs for citral ranged from 100 to 140 mg g^{-1} when the bamboo plates were dried at 100 °C for 14 h, while the MICs ranged from 300 to 350 mg g^{-1} for *L. cubeba* oil. On the other hand, when the plates were dried at 30 °C, the MIC values of the plates containing citral increased to 300 to 400 mg g^{-1}. Furthermore, no MICs were found when *L. cubeba* oil and vegetable oil (control) were incorporated into the tray. Although high temperature could reduce the antifungal activity of essential oil, results from this study confirm that it could enhance antifungal activity of *L. cubeba* oil. From this study, using high temperature for drying plates with citral (main component of *L. cubeba* oil) showed greater inhibition of all mold with lower MIC values. Therefore, citral might be the key factor for inhibiting mold growth after heat drying.

Table 1. MICs of *L. cubeba* and its Main Components

Molds	Drying at 30 °C				
	Essential Oil (mg g^{-1})	Main Components of Essential Oil (mg g^{-1})			
	L. cubeba	Citral	Citronellal	Hydroxybenzaldehyde	Linalool Oxide
Aspergillus niger	> 500	300	> 500	> 500	> 500
Aspergillus flavus	> 500	300	> 500	> 500	> 500
Penicilium sp.	> 500	350	> 500	> 500	> 500
Rhizopus sp.	> 500	300	450	> 500	> 500
Penicillium cyclopium	> 500	400	> 500	> 500	> 500
Fusarium sp.	> 500	300	> 500	> 500	> 500
Cladosporium sp.	> 500	300	> 500	> 500	> 500
	Drying at 100 °C				
	Essential Oil (mg g^{-1})	Main Components of Essential Oil (mg g^{-1})			
	L. cubeba	Citral	Citronellal	Hydroxybenzaldehyde	Linalool Oxide
Aspergillus niger	300	100	450	> 500	> 500
Aspergillus flavus	300	100	> 500	> 500	> 500
Penicilium sp.	300	100	450	> 500	> 500
Rhizopus sp.	300	100	450	> 500	> 500
Penicillium cyclopium	250	140	> 500	> 500	> 500
Fusarium sp.	300	100	450	> 500	> 500
Cladosporium sp.	300	100	450	> 500	> 500

L. cubeba oil has been used to address issues of medical concern such as inflammation and headaches; it has also been used for food flavoring (Wang *et al*. 2009). For the purpose of food safety, Suhem *et al*. (2015) showed that *L. cubeba* vapor could prevent the growth of *Aspergillus flavus* on snack bars and also could strongly inhibit the accumulation of aflatoxin B1 in licorice after being inoculated and incubated with *A. flavus* for 20 days (Li *et al*. 2016). The results from this experiment have confirmed that *L. cubeba* in bamboo trays could prevent the growth of *A. flavus* and other mold strains but only after drying at a high temperature.

Table 2. Qualitative GC-MS Analysis of Chemical Compositions in *L. cubeba* Oil Treated Bamboo Fiber Packaging Before and After Heat Drying at 100 °C for 10 min

Compounds	Retention Time	Kovats Index (KI)*	Relative Contents (%)	
			Before Heat Curing	After Heat Curing
Limonene	5.22	1030	4.12 ± 0.13	3.39 ± 0.21
Carvone	7.61	1248	0.87 ± 0.12	NF
Citronellal	8.12	1157	3.15 ± 0.12	3.42 ± 0.10
Menthol	9.21	2103	0.83 ± 0.21	NF
E-Citral	10.99	1255	54.47 ± 0.91	52.70 ± 3.12
Z-Citral	11.13	1240	31.09 ± 1.23	29.97 ± 1.09
Geraniol	11.78	1266	3.33 ± 0.32	2.45 ± 0.62
Hydroxybenzaldehyde	13.63	960	NF	4.40 ± 0.34
Linalool Oxide	14.95	1172	NF	3.46 ± 0.54
Total Identified			96.89	96.37
*KI: Kovats index on DB-5 column. NF: not found				

The possible biosynthetic pathway of citral during heat treatment is shown in Fig. 3. High temperature treatment could cause citral to degrade into various compounds. Two components, *i.e.* hydroxybenzaldehyde (Wang *et al.* 2004; Weerawatanakorn *et al*. 2015) and linalool oxide (King and Dickinson 2000; Serra *et al*. 2017), were detected after the heat treatment at 100 °C for 10 min. However, citral alone or a combination of it with other essential oil components may be recommended as a natural preservative according to its highly antifungal activity against a food-infesting mold such as *A. flavus* and aflatoxin secretions (Miron *et al*. 2014). Citral has a high boiling point of around 229 °C (Lide 2005); this is nearly the same as *L. cubeba* oil, which is around 232 °C (ChemicalBook, 2017).. Therefore, setting the temperature of bamboo plate drying at 100 °C might change the form of this main component rather than destroying mold. From the GC-MS results (Table 2), higher peak areas of both E-citral and Z-citral after heat drying at 100 °C were detected. In addition, citronellal peak areas were greater after using high temperature and were more effective against molds with MIC values from 450 mg g^{-1}, except for *A. flavus* and *P. cyclopium* (MIC > 500 mg g^{-1}). Although, hydroxybenzaldehyde and linalool oxide were found after using high temperature, no MIC values were found for either component. Moreover, limonene (Negro *et al.* 2016) and geraniol (Miron *et al*. 2014) showed good properties against microorganisms and using low temperature was recommended (Negro *et al*. 2016).

Optimization of Bamboo Tray Drying and Natural Protection

The inhibitory periods of treated bamboo trays using different concentrations of *L. cubeba oil*, temperature, and drying times are shown in Table 3. The constants and the coefficients of the regression equation obtained after ANOVA are presented in Table 3. The concentration of essential oil, time, and temperature ($P < 0.05$) were significant factors in extending the shelf-life of bamboo trays. The inhibitory period (Y) fit by the polynomial equation (Eq. 3) was fit to a polynomial expression ($R^2 = 0.83$) as follows,

$$Y = 237.65+16.39X_1 – 10.78X_2 + 9.56X_3 – 33.54X_1^2 \text{ -}11.75\ X_1X_2 – 26.75\ X_1X_3$$

(3)

where Y is the inhibitory period (days), X_1 is the concentration of essential oil, X_2 is the time, and X_3 is the temperature.

Response surface plots are also shown in Fig. 1 (a-c). The best condition against natural mold on trays for at least 290 days was *L. cubeba* oil at 100 mg g^{-1} and then drying at 300 °C for 12 h (see Tables 3 and 4). The results confirmed that using high temperature (100 °C) for drying could enhance antifungal activity of *L. cubeba* and extend the shelf-life of the bamboo trays. Lower temperatures (60 °C and 80 °C) showed lower inhibitory periods. Nevertheless, the control (trays without *L. cubeba* oil) found mold growth on the surface after 1 week of storage in 100% relative humidity at 25 °C.

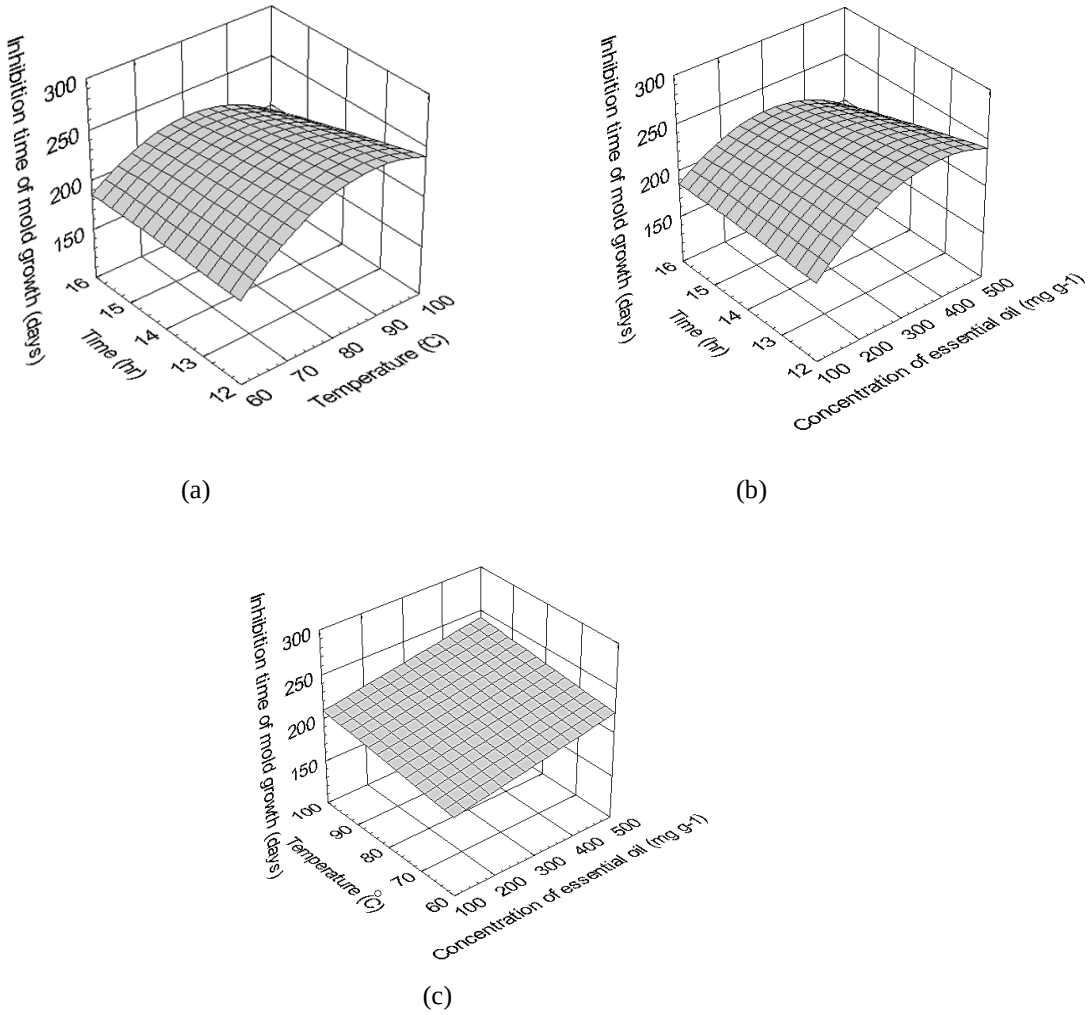


Fig. 1. Response surface plots showing the effect of drying time (h) and temperature (°C) (a), time (h) and concentration (mg g^{-1}) (b), and temperature (°C) and time (h) (C) on the growth on the mold of bamboo packaging

Table 3. Central Composite Face-centered Design (X_1 = Concentrations; mg g^{-1}, X_2= Temperatures; °C; X_3= Times; hours) and Responses for the Growth of Natural Mold (RSM) on Surface of Bamboo Fiber Packaging with *L. cubeba* oil

Experiment	Factor (Coded)			Factors (Uncoded)			Observed Inhibition Time of Mold Growth (Days) Y_0	Predicted Inhibition Time of Mold Growth (Days) Y_i	$Y_0 - Y_i$
	X_1[a]	X_2[b]	X_3[c]	X_1	X_2	X_3			
1	-1	-1	-1	100	12	60	122	150	-28
2	-1	0	-1	100	14	60	157	15	6
3	-1	1	-1	100	16	60	175	150	25
4	-1	-1	0	100	12	80	206	187	19
5	-1	0	0	100	14	80	194	188	6
6	-1	1	0	100	16	80	181	189	-8
7	-1	-1	1	100	12	100	217	223	-6
8	-1	0	1	100	14	100	205	224	-19
9	-1	1	1	100	16	100	210	225	-15
10	0	-1	-1	300	12	60	230	239	-9
11	0	0	-1	300	14	60	230	228	2
12	0	1	-1	300	16	60	190	184	6
13	0	-1	0	300	12	80	225	248	-23
14	0	0	0	300	14	80	235	238	-23
15	0	1	0	300	16	80	230	152	78
16	0	-1	1	300	12	100	290	258	32
17	0	0	1	300	14	100	267	247	20
18	0	1	1	300	16	100	230	236	-6
19	1	-1	-1	500	12	60	270	260	10
20	1	0	-1	500	14	60	230	238	-8
21	1	1	-1	500	16	60	217	215	2
22	1	-1	0	500	12	80	227	243	-16
23	1	0	0	500	14	80	227	220	7
24	1	1	0	500	16	80	217	218	-1
25	1	-1	1	500	12	100	222	226	-4
26	1	0	1	500	14	100	187	203	-16
27	1	1	1	500	16	100	165	181	-16
28	0	0	0	300	14	80	235	248	-13
29	0	0	0	300	14	80	235	248	-13
30	0	0	0	300	14	80	235	248	-13
31	0	0	0	300	14	80	235	248	2

Table 4. Analysis of Variance (ANOVA) for the Response Surface Quadratic Model for the Inhibition Time of the Mold Growth Value of Bamboo Fiber Packaging with *L. cubeba* Oil

	P-value
X_1	0.0004*
X_2	0.0124*
X_3	0.0244*
X_1X_1	0.0000*
X_1X_2	0.0239*
X_2X_2	0.8703
X_3X_1	0.0000*
X_3X_2	0.1616
X_3X_3	0.6719
*(P < 0.05)	

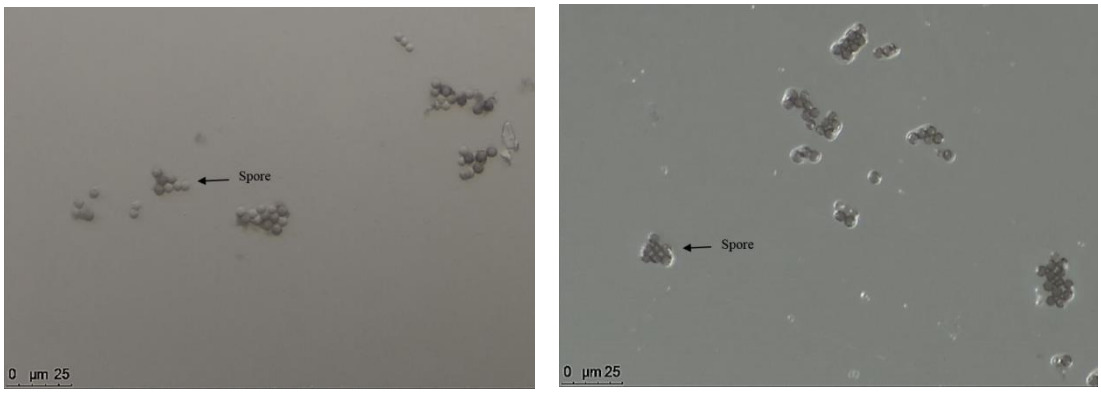
This study agreed with scientific reports about the effect of temperature (mild heat to high) on controlling microorganisms. Haberbeck *et al*. (2012) reported that using oregano oil with temperatures at 90 °C to 100 °C inhibits *Bacillus coagulans* spores. Using lime oil with heat curing at 70 °C enhances the inhibition of *A. niger* on sedge *(Lepironia articulata)* after 18 weeks of storage time (Matan *et al*. 2013). Using similar techniques, Matan *et al.* (2012) showed that garlic oil with heat curing at 100 °C for 24 h inhibits the growth of *A. niger* on rubberwood for at least 40 weeks. Therefore, high temperature asserts positive influences on the antifungal activity of *L. cubeba*. High temperature does not decompose citral but the formation of cintronellal could be observed. These compounds might protect packaging from molds for long periods of storage in accelerated conditions.

Possible Modes of Action

The results from the wettability test suggested that there were significant changes in the contact angle between the treated trays (100 °C) with *L. cubeba* oil at 300 mg g^{-1} (109 °C ± 1°C), the trays with *L. cubeba* oil at 300 mg g^{-1} (93 °C ± 2 °C) dried at lower temperatures (30 °C) and the control trays without essential oil (68 °C ± 2 °C). The water absorption of treated bamboo trays showed a significant decrease (P<0.05) of 75% from the control (without essential oil). Hydrophobicity is an important characteristic of bamboo trays, and the results from this study demonstrated that water could not be easily adsorbed into the treated specimens. Therefore, after drying at 100 °C *L. cubeba* oil had a hydrophobic effect on the trays. Furthermore, the effect of *L. cubeba* with high temperature drying for inhibiting mold spores on bamboo trays was observed in this study. Figure 2a shows *A. niger* spores on bamboo trays were covered by essential oil components and could not be germinated for at least 290 days of storage. This result agreed with the work of Tzortzakis and Economakis (2007), which found that fungal spore production inhibited up to 70% at 25 ppm of lemongrass oil concentration when compared with equivalent plates stored in ambient air. Furthermore, with the highest oil concentration (500 ppm) employed, fungal sporulation was completely retarded. In contrast, without essential oil and high temperature, no spore covering was found on the packaging surfaces (Fig. 2b). After drying, citral might be the main active component against all mold.

Table 5. Physical Properties of Bamboo Fiber Packaging With and Without *L. cubeba* Oil

Physical Properties	Bamboo Fiber Packaging	
	Without *L. cubeba* oil	With *L. cubeba* oil
Water Contact Angle (°)	68.00 ±1.54[b]	108.86 ± 0.92[a]
Water Absorption (%)	198.09 ± 5.65[a]	122.80 ± 4.34[b]
Total Color Difference (ΔE)	0.67 ± 0.34[a]	0.59 ± 0.21[b]
[a-b] Mean in each row with different superscript letters are significantly different ($p < 0.05$)		



(a) (b)

Fig. 2. Spore of *Aspergillus niger* on the control packaging for 1 day (a) and on the packaging with *L. cubeba* oil at concentration 300 mg g^{-1} for 10 days (b) after incubation at 25 °C with 100% RH

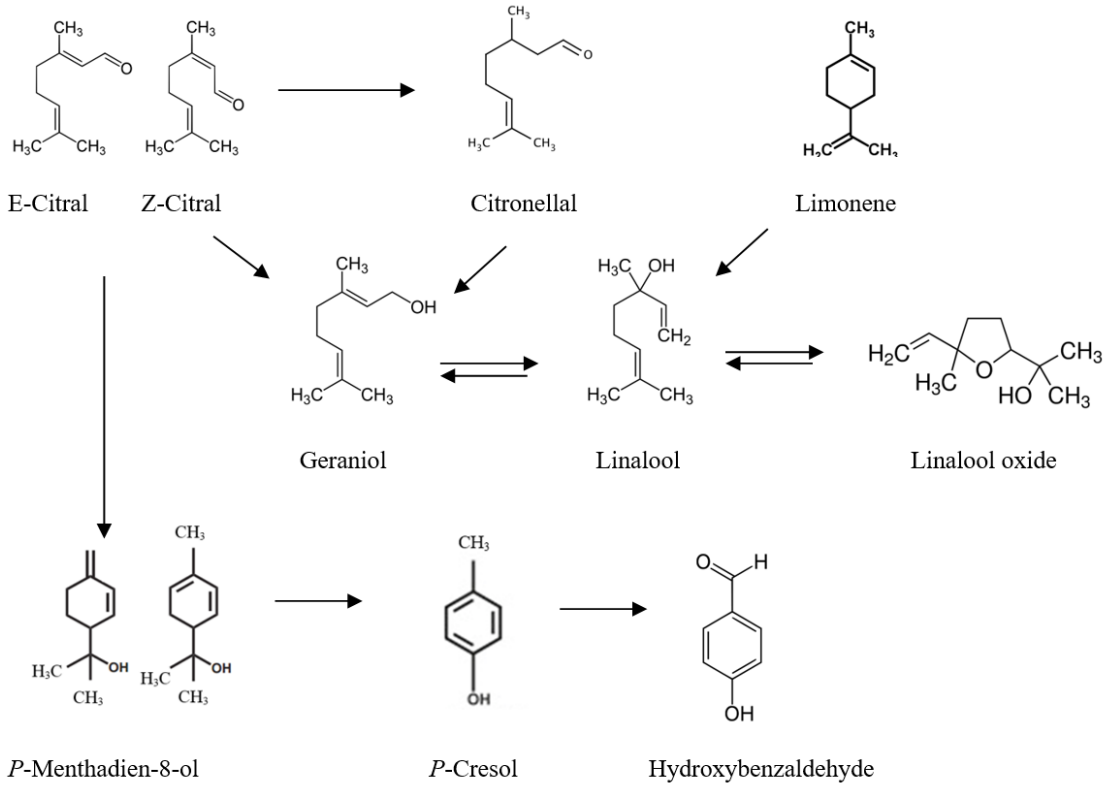


Fig. 3. Possible pathway of citral during heat treatment (King and Dickinson 2000; Wang *et al.* 2004; Vilella *et al*. 2005; Weerawatanakorn *et al.* 2015; Serra *et al*. 2017)

Li *et al*. (2014) found that citral at the concentration of 400 µg mL^{-1} completely inhibits spore germination of *Magnaporthe grisea* on the potato dextrose agar (PDA) after 24 h. The antifungal activity of citral has also been explained by Tao *et al.* (2014) who stated that citral disrupts the cell membrane integrity and membrane permeability of *P. italicum*. When combined with another technique such as high hydrostatic pressure, citral has a greater effect on mold spore inactivation (Palhano *et al*. 2004). Thus, a combination of citral with a thermal treatment at 95 °C can inhibit the germination or outgrowth of *Alicyclobacillus acidoterrestris* spores (Huertas *et al.* 2014). The results from this study are in agreement with previous results.

CONCLUSIONS

1. Overall, drying a bamboo tray containing *L. cubeba* at 300 mg g^{-1} at a high temperature (100 °C) for 12 h can enhance the antifungal activity of *Litsea cubeba* on a bamboo packaging tray. *Litsea cubeba* components after heat treatment can be released and coated on the surface of a bamboo tray and prevent mold spore germination.
2. After drying at a high temperature with *Litsea cubeba*, the shelf-life of a bamboo tray increased from 7 days to at least 290 days.
3. Lower water adsorption and lower wettability properties of a bamboo tray were detected after heat treatment with *Litsea cubeba* oil.
4. The use of high temperature for drying bamboo packaging trays is normally used in the packaging industry. Also, the application of *Litsea cubeba* for food flavor and medicine is increasing. Presently, the combined use of essential oil and high temperature drying could limit fungal infections on bamboo trays during long-term storage. This process is interesting and easy to apply in large-scale production of the trays.

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