

Research Article

Inhibition of *Aspergillus niger* on tea box packaging made of rubberwood treated with vapour of peppermint oil

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Abstract

Antifungal activities of rubberwood treated with vapour of peppermint oil against *Aspergillus niger* identified from rubberwood surfaces was investigated. The vapour of peppermint oil at 60°C was employed to determine the minimal inhibitory concentration (MIC using the concentration of substances between 100 and 900 $\mu\text{l ml}^{-1}$). Peppermint oil exhibited high fungal activities with the MIC of 300 $\mu\text{l ml}^{-1}$ against the test moulds. Moreover, peppermint oil at the MIC was capable of providing complete protection from mould growth on rubberwood for up to 12 weeks at storage conditions of 25°C and 100% RH. To evaluate consumer acceptability of rubberwood packaging treated with vapour of peppermint oil (300 $\mu\text{l ml}^{-1}$) in food applications, a sensory test of tea kept inside a box made of oil treated rubberwood was carried out using a 9-point hedonic scale. Results of the sensory test showed no significant (overall liking) differences ($p < 0.05$) in consumer hedonic test between the treated and the controlled tea after 1 week of storage. These findings demonstrated the good potential of essential oil treated rubberwood in food packaging applications.

Keywords: *Aspergillus niger*; Rubberwood; Essential oil; Packaging; Sensory test

Introduction

Mould growth on rubberwood is one of major problems in the wood industries. Mould grows rapidly in a humid climate, normally within seven days on the surface of wood without preservative [1]. Discolouration caused by mould on wood is objectionable to buyers. *Aspergillus niger* is one of major fungi identified from rubberwood surfaces. *Aspergillus* is a cosmopolitan fungus with air-borne infective propagules and it routinely contaminates food, feed and agricultural commodities such as wood. It has been reported that *Aspergillus niger* is a serious problem from the viewpoint of public health [2]. Currently, chemical fungicides such as methyl bromide are commonly used to control the growth of mould, but are not appropriate for many food packaging applications. Consequently, developing natural alternatives that are environmentally friendly and have low mammalian toxicity are needed. Matan *et al.* [3], utilized essential oils as alternative choices to chemical fungicides. Essential oils have been widely used in food applications and the antifungal effects of essential oils have been extensively studied [4]. The microbiocidal action of essential oil is based on various compounds such as aldehydes and alcohol. However, little is known of how to apply the oil as preservative in lumber. Therefore, this study was initiated to determine the antifungal activity of peppermint oil in the vapour phase against *Aspergillus niger* on the surface of rubberwood and its potential for use in tea box packaging, a product that traditionally uses wood.

Material and Methods

Essential oils, chemicals and rubberwood

Food-grade peppermint oil containing 61% menthol derived by steam distillation was provided by Thai China Flavours & Fragrances Industry Co., Ltd. Bangkok, Thailand. Rubberwood specimens were prepared from freshly cut rubberwood lumber obtained from the plantation site in Nakhon Si Thammarat province, Thailand. The average moisture content of the rubberwood specimens before testing was $48 \pm 2\%$ ($n=10$).

Cultures and preparation of inoculums

Aspergillus niger WU 0701 was identified from rubberwood surfaces. The code refers to strains held in the culture collection of the Wood Science and Engineering Research Unit, Walailak University, Nakhon Si Thammarat province, Thailand. Spores of test mould were obtained from mycelia grown on malt extract agar (MEA; Merck Ltd, Thailand) medium at 30°C for 14 days, and were collected by flooding the surface of the plates with ~5 ml sterile saline solution (NaCl, 8.5 g l⁻¹ water) containing Tween 80 (0.1% v/v). After counting the spores using a hemocytometer, the suspension was standardized to concentrations of 10⁷ spores ml⁻¹ by dilution with sterile water before use. The viability of all strains was checked using quantitative colony counts at 10⁷ CFU ml⁻¹.

Mould test on rubberwood

Sets of five random replicate specimens were evaluated for resistance to mould fungi according to the American Society for Testing and Material D4445-91 [5], with peppermint oil at various concentrations ranging from 100 to 900 µl ml⁻¹. Vegetable oil was used as a control. Different dilutions of the oil were made with methanol. The vacuum treatment schedule included 30 min vacuum at 100mmHg. Whilst under vacuum, peppermint oil of 100 to 900 µl ml⁻¹ was poured over to cover the test specimens placed in the beaker inside a

vacuum desiccator. After vacuum release, specimens were left to soak in the peppermint oil at atmospheric pressure for 30 min. Following this, treated specimens were held in a closed container overnight at 60°C before inoculation with spores of the test mould and were incubated at 25°C with 100% RH in an environmental chamber (Contherm, Petone, New Zealand) for 12 weeks. The specimens were then individually rated for mould growth on a scale of 0 to 5, with 0 denoting clean specimens and 5 representing heavy mould growth (0=clean, 1=20%, 2=40%, 3=60%, 4=80%, 5=100% of mould growth). Percent of stain and mould (based on control) for each essential oil concentration was calculated according to

$$\text{Percent of stain and mould (based on control)} = (A/B) \times 100$$

A = Total score for mould at each concentration of peppermint oil

B = Total score for each mould at control.

Sensory evaluation

Tea boxes (100mm x100mm in cross section by 500 mm long) made of 5mm thick rubberwood lumber which was vacuum-treated with peppermint oil (300 $\mu\text{l ml}^{-1}$) and without essential oil (as a control) were prepared. Teabags (Lipton tea, Unilever Ltd., Thailand) were kept inside the boxes for 1 week and 12 weeks prior to presenting to the consumers. A 9-point hedonic scale ranging from “like extremely” to “dislike extremely” was used to determine the degree of acceptance of tea in terms of colour, odour, flavour, aftertaste and overall liking. On pre-test and post-test days, the tea was served by brewing one teabag in 160 ml of boiling water for 3 min and then 40 ml of cold water was added so that the tea was immediately at a consumable temperature. Panelists (47 people) were selected among students and staff at Walailak University. The panelists evaluated two tea products including the control. The responses were converted to numerical values ranging from 1 for “dislike extremely” to 9 for “like extremely”.

Statistical analyses

All variables were tested for normality applying the Komogorov-Smirnov test and homogeneity of variances was assessed using Levene's test. Data transformation was done, where necessary. All results were expressed as mean \pm SD. The data was statistically treated by one-way ANOVA and Duncan's post hoc test with $P < 0.05$ considered to be statistically significant.

Results and Discussion

Inhibition of Aspergillus niger on rubberwood by vapour of peppermint oil.

Antifungal activity of rubberwood treated with vapour of peppermint oil at various concentrations in comparison with controls are shown in Figure 1. Vapour of peppermint oil at a concentration about 300 $\mu\text{l ml}^{-1}$ showed complete inhibition against *A. niger* for up to 12 weeks. The finding indicates that menthol, a major volatile component of peppermint oil might play a role in preventing spore germination of *A.niger* [3]. It is also possible that a specific alcohol or a combination of components in peppermint oil could contribute to the good antifungal properties of the oil vapour and, to a certain extent, the effectiveness of peppermint oil as an antifungal agent [6-8].

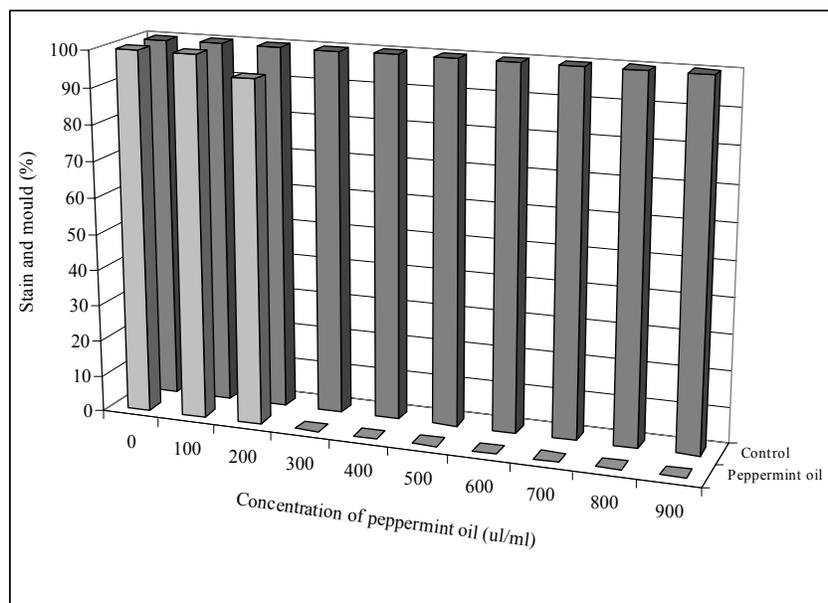
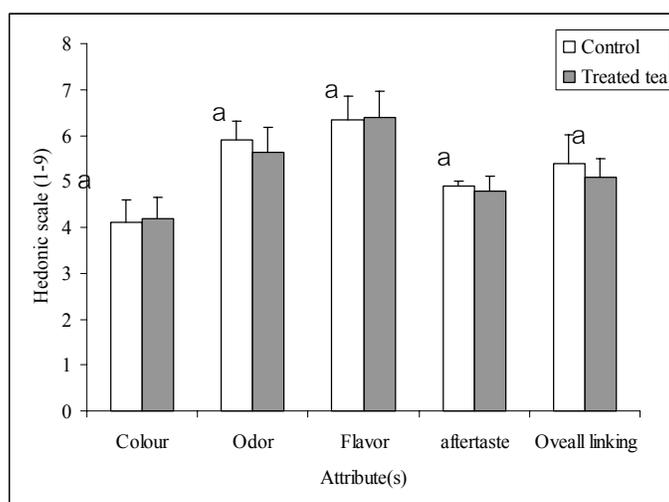


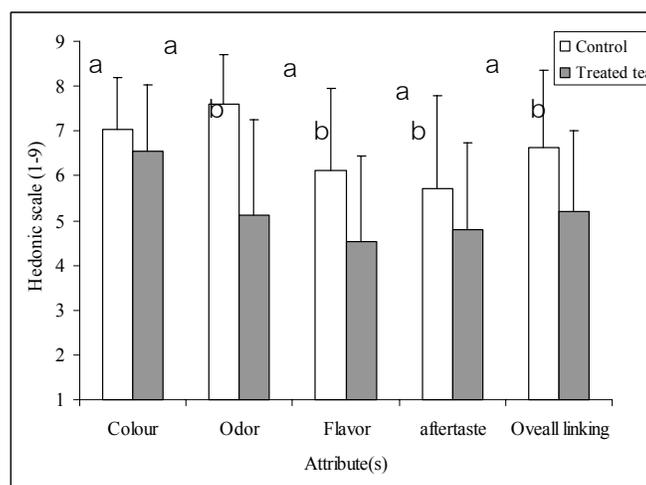
Figure 1. Percentages of stain and mould growth on rubberwood treated with vapour of peppermint oil at 100-900 $\mu\text{l ml}^{-1}$ after 12 weeks.

Sensory evaluation of tea kept inside oil-treated rubberwood box

Concentration of peppermint oil used in the sensory evaluation test was chosen as the lowest level possible (i.e. at the MICs; $300 \mu\text{l ml}^{-1}$) in order to minimize the effect this might have on the quality of the product (tea in this case). The results of sensory evaluation attributes of tea kept inside the box made of oil-treated rubberwood for 1 week and 12 weeks are summarised in Figure 2. The data were subjected to the ANOVA test. No significant differences of colour, odour, flavour, aftertaste and overall liking ($p < 0.05$) in consumer hedonic test were found between tea kept in packaging made of oil-treated rubberwood and the controls after 1 week. It can be noticed, however, that peppermint oils at the MICs slightly deteriorated odour, flavour and aftertaste but did not at all affect the colour of tea. Taste and aftertaste of tea kept inside rubberwood boxes treated with vapour of peppermint oil deteriorated after 12 weeks of storage in comparison with the control.



(A)



(B)

Figure 2. Means of hedonic scores of affective attributes for tea samples kept in boxes made of rubberwood treated with vapour of peppermint oil at $300 \mu\text{l ml}^{-1}$ for (A) 1 week and (B) 12 weeks.

^{a-b} Mean value by the same letter are not significantly ($P < 0.05$) different according to Duncan test following ANOVA

Conclusions

Peppermint oil exhibited antifungal activities against *Aspergillus niger*, identified from rubberwood with the MIC values of $300 \mu\text{l ml}^{-1}$. Peppermint oil at the MIC was capable of providing total protection from mould growth on rubberwood for at least 12 weeks under storage conditions of 25°C and 100% RH. Results of sensory evaluation tests revealed that no significant differences of colour, odour, flavour, aftertaste and overall liking ($p < 0.05$) in consumer hedonic test were found between tea kept in packaging made of oil-treated rubberwood and the controls after 1 week. There were differences of odour, flavour, aftertaste and overall liking between the treatment and the control after 12 weeks of storage.

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