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THE EFFICACY OF COMBINED APPLICATION OF EDIBLE COATINGS AND ESSENTIAL OIL IN MANGO PRESERVATION

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Abstract. The aim of this study was to examine the effect of lemongrass oil *(Cymbopogon citratus)*, citronella oil *(Cymbopogon winterianus)* and cajeput oil *(Melaleuca leucadendron)* against *Aspergillus niger* by the agar diffusion method. The selected oil was combined with the edible film (chitosan 1 % w/v or alginate 1 % w/v) and applied to preserve Cat Chu mango (infected by *A. niger* at 10^5 spores/ml) in 14 days at 30 ^oC. The result showed that the essential oils inhibited against *Aspergillus niger* significantly among which lemongrass oil was the most effective inhibitor with a minimum inhibitory concentration (MIC) of 10 µl/mL while those of citronella oil and cajeput oil were of 20 µl/ml. The result of the mango preservation showed that all of the control samples were completely rotten after 10 days of storage, whereas the shelf life of the coated samples was longer than 10 days. The essential oils at high concentrations (10-20 µl/ml) have a negative impact on mango preservation, as they made the fruits bruised on the surface and rapidly decay. The results also indicated that alginate (1 % w/v) combines with lemongrass oil (5 µl/ml) maintained the antifungal effect after 14 days of preservation.

Keywords: *Aspergillus niger,* antifungal activity, edible coatings, essential oil, mango.

Classification numbers: 1.2.1; 1.4.6.

1. INTRODUCTION

Postharvest losses are one of the major causes of the loss of fresh vegetables during the supply chain [1]. This loss could threaten a world food security. Typically, in 1995 the amount of food lost could meet the minimum nutritional requirement for 200 million people (equivalent to the US or Indonesian population) [2]. In Vietnam, the postharvest losses of grain, vegetable

and fruit are about 10 %, 10÷20 %, and 15÷30 %, respectively [2]. In addition, the extensive use of synthetic fungicides for preserving agricultural products has significant limitations such as increased costs, handling of hazards, concern about pesticide residues and a threat to health and the environment [3]. In recent years, increased interests in the use of natural substances to reduce consequences and these problems have encouraged more detailed research on plant resources. In particular, attention has been focused on the potential application of essential oils. Many previous studies have demonstrated the antifungal effect of essential oils [4, 5, 6, 7]. In the case of *A. niger*, its growth was completely inhibited when a concentration of 0.94 % of *Citrus lemon L.*, *Citrus reticulata L.*, *Citrus paradisi L.* and *Citrus sinensis L.* was used [7]. According to Nikos et al. [5], *citronella lemongrass (Cymbopogon citratus L.)* with 25 ppm concentration inhibited up to 70 % of mycorrhizal fungi by the method of contact in the liquid phase. Essential oils can be considered as suitable substitutes for chemical additives for use in the food industry [7] due to their antimicrobial properties and the tendency to replace synthetic antifungal agents with more natural substances. The application of these natural compounds in the food industry may be a potential option, but application costs and other issues, such as strong aroma, limit their use in food preservation [8]. The edible coating is thin layers of material, created from biodegradable components that can be consumed as part of a food product and like respiratory barrier [9]. Another great advantage of this coating is the eco-friendly status because biopolymers do not cause environmental problems as packaging materials derived from non-renewable energy sources do [9]. According to Muzzarelli et al. [10], edible coatings such as chitosan are non-toxic, biodegradable and become a gel at low pH. However, edible coatings do not have an antifungal effect [9]. Therefore, combining edible coating and essential oils receives a lot of interest today. This combination offers double benefits; first, the coatings help to limit the respiration process, reduce a weight loss during storage [9]; second, this method helps to reduce the amount of essential oil used while their antifungal effects are maintained [8], limit a sensory impact caused by essential oils.

Mangoes are commercially cultivated in more than 103 countries worldwide and production is increasing each year due to increasing consumer [11]. However, commercial mangoes are limited by their perishable nature which easily attacked by molds. According to Prakash O et al. [12], *Aspergillus niger* is one of the major causes of black rot of mango. Therefore, control of *A. niger* during the preservation is very necessary. Previous studies showed that essential oils combined with chitosan and alginate have been shown effective in increasing shelf life [8, 13, 14, 15, 16]. However, there are few studies comparing the preservation efficacy of these two coatings published. Therefore, in this study, lemongrass oil *(Cymbopogon citratus)*, citronella oil *(Cymbopogon winterianus)* and cajeput oil *(Melaleuca leucadendron)* original from Vietnam were evaluated for antifungal activity against *A. niger* with an agar diffusion method. The essential oil having the best antifungal activity was combined with edible films (chitosan and alginate) and applied in preserving Cat Chu mango (infected with *A. niger* 5 log(CFU/g)) in 14 days at 30 °C.

2. MATERIALS AND METHODS

2.1. Materials

The essential oils used in this study were lemongrass oil *(Cymbopogon citratus*), citronella oil (*Cymbopogon winterianus*) and cajeput oil *(Melaleuca leucadendron)* from Vietnam.

Essential oils were diluted in xanthan gum (Himedia) 0.3 % w/v at different concentrations (5; 10; 20; 50; 75 and 100 µl/ml) and homogenized until the emulsion. The diluted essential oils are tested for antifungal efficacy.

Aspergillus niger M1 was isolated from mangoes in Cao Lanh district, Dong Thap province and was subsequently sequenced and identified by Nam Khoa Company. Cat Chu Mango was harvested hard-green stage in the garden, My Xuong Commune, Cao Lanh District, Dong Thap Province.

2.2. Methods

2.2.1. Determination of minimum inhibitory concentration (MIC) by agar diffusion method

The experiment based on the study of Lieu et al. [6] with slight modifications. Briefly, the test strains suspensions were spread over the surface of PDA plates (at a final concentration of 5 log CFU/ml approximately) and allowed to dry in 5 min. The essential oils in xanthan gum (0.3 % w/v) at different concentrations were spotted on these media agar (10 µl) and the emulsifying agents were used at controls. The plates were incubated in 24 h at 30 ^{o}C. After 24 h incubated, Petri dishes were examined by inhibition zone. The MIC values were determined as the lowest concentration of oil preventing the visible growth of microorganisms.

The essential oil, which has the best antifungal effect was directly analyzed by gas chromatography coupled to mass spectrometry (Agilent GC 7890B GC System, 7010 GC/MS Triple Quad). The column used was an HP-5MS (30 m long, 0.25 mm and 0.25 µm film thickness). The operating conditions were as follows: Helium was used as a carrier gas with a back pressure of 0.8 atm; flow rate of 1.0 ml/min; split 1:20 and injection volume 0.2 µl. The injector temperature was 250 ^{o}C and the oven temperature program started at 60 ^{o}C for 5 min and then increased at a rate of 5 ^{o}C/min up to 150 ^{o}C, and increased from 150 ^{o}C to 280 ^{o}C at 10 ^{o}C/min. The constituents in the essential oils were identified by computer matching of their mass spectral fragmentation patterns with those of compounds in the data bank NIST 98 and Wiley 275 library.

2.2.2. Evaluation of mango preservation by edible coatings and essential oil

Mangoes were washed with saline water in 2 minutes and were dried in air at ambient temperature. Then the mangoes were treated in different ways including spraying by essential oil at different concentration (E sample); coating by chitosan 1 % w/v (C sample); coating by Ca-alginate 1 % w/v (A sample); coating by chitosan (1 % w/v) combined essential oil at different concentration (CE) and coating by Ca-alginate (1% w/v) combined essential oil at different concentration (AE). Coatings samples were processed as described by Azarakhsha et al. [13]: the mangoes were dipped in the film-forming solutions (chitosan 1 % w/v or alginate 1 % w/v) for two minutes then the mangoes were dried out in the case of chitosan film or were dipped with

$CaCl_2$ for five minutes and dried in the case of alginate. The samples which covered by the coatings combined essential oil was processed as described by Rojas et al. [15]. Briefly, the essential oil in xanthan gum was mixed in the film-forming solutions, then the mangoes were dipped in the mixture in five minutes and dried out. After that, all samples were sprayed with *A. niger* spores at a concentration of 10^5 spores/ml. The mangoes sprayed with *A. niger* without any treatment were used as the control. All the samples were allowed to dry naturally at room temperature. To avoid changing the air around the fruit, six holes with 7 mm diameter were cut on nylon bags, then mangoes were stored in these bags at 30 ^oC. The data were collected after treatment (day 0) and every 2 days during the 14 days of storage. Each treatment was carried on 20 fruits. When they had a bruise, which had 2 mm diameter or more, they were considered to be mold damage.

2.2.3. Statistical analysis

The data were subjected to analysis of variance (ANOVA) using Statraphics 15 followed by Student-Newman-Keuls t-test to compare means, with a significance level of 5 % when the significant difference between treatments was noted. All tests were performed in triplicate and the data expressed as means ± standard deviation

3. RESULTS AND DISCUSSION

3.1. Antifungal activity of essential oils in agar diffusion method

The antifungal activity of lemongrass oil, citronella oil, and cajeput oil was shown in Figure 1. The antifungal zone depends on the concentration and type of oil. The diameter of the antifungal zone of lemongrass oil, citronella oil, and cajeput oil was 6÷34 mm; 8÷28 mm and 4÷17 mm, respectively (Figure 1). Lemongrass oil showed the best antifungal activity ($p < 0.05$), its MIC value was 10 µl/ml, while an antifungal efficacy of cajeput oil at all of the concentrations was lowest (Figure 1). The MIC of citronella oil was 20 µl/ml and there was no difference ($p > 0.05$) to that of cajeput oil.

The major components of lemongrass oil were confirmed and listed in Table 1. β-citral was identified as the main compound with the highest peak area percentage (41.2 %). α-citral (39.80 %) was the second major compound detected in the lemongrass oil, followed by Neryl acetate (8.1 %); Caryophyllene (1.5 %), Linalool (1.5 %), Caryophyllene oxide (1.1 %). Other compounds such as Verbenol, Carveol, Eucalyptol, etc. were found to be at the trace level.

Table 1. Major components of lemongrass oil.

Components	**%***	**Components**	**%***
β-Citral	41.2	Citronellal	0.3
α -Citral	39.8	Verbenol	0.6
Camphene	0.3	Carveol	1.0
5-Hepten-2-one, 6-methyl-	1,5	Neryl acetate	8.1
Limonene	0.2	Caryophyllene	1.5
Eucalyptol	0.5	Caryophyllene oxide	1.1
Linalool	1.5		

* Percent of the peak area of the evaporated organic compound.

In previous studies, the antifungal activity of lemongrass oil was more effective than commercial bifonazole [4]. Similarly, the growth of *A. niger* in the liquid phase was inhibited (91 %) when there was 1000 ppm citronella lemongrass [17]. Research of Viuda-Martos et al. [7] showed that *A. niger*'s growth was affected by citrus oils such as *Citrus lemon L., Citrus sinensis L., citrus (Citrus reticulata L.)* and *grapefruit (Citrus paradisi L.)* at 0.94 % (w/v). The chemical composition of essential oils was analyzed in the previous study, in the essential oil of *C. winterianus*, 23 compounds were identified (96.50 % of total oil). The main components were citronellal (27.00 %) and α-citral (22.78 %) [4], in *C. citratus*, α-citral was highest at 40.79 %, followed by β-citral at 31.85 % [5]. The variety in the amount and components of essential oil leading to the antimicrobial activity of essential oil is not due to a single mechanism, but different mechanisms at the cellular level. In general, essential oils can degrade the cell wall, disturb the phospholipid bilayer of the cytoplasmic membrane, and damage the membrane proteins leading to increased permeability of the cell membrane and loss of cellular constituents [18]. In the present study showed that the antifungal efficacy depended on the type of the essential oil in which lemongrass oil exhibited the best result ($p < 0.05$) (Figure 1). The result in Table 1 showed that α-citral and β-citral of lemongrass oil from Vietnam were 39.8% and 41.2 %, respectively. This suggests that α-citral and β-citral, which are the main ingredients in lemongrass oil, had the best ability to inhibit *A. niger*. The lemongrass oil, which could inhibit *A. niger* at low concentration was selected for mango preservation alone or combined with edible films in the next step.

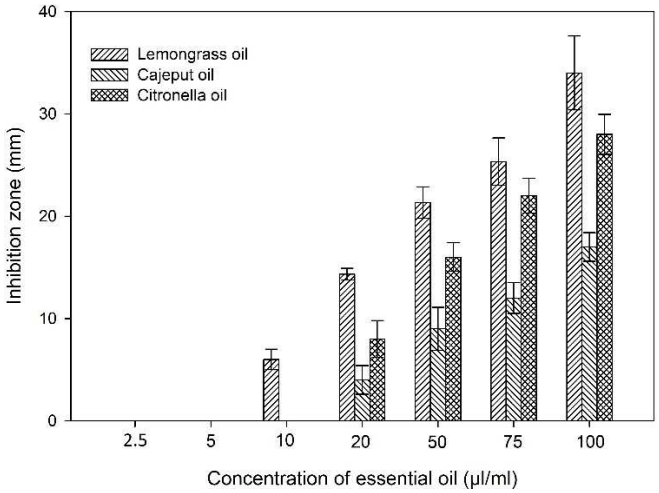

Figure 1. The antifungal activity of essential oil on *A. niger.*

3.2. The effect of essential oil and edible film on mango preservation

The influence of essential oil and edible film on mango preservation were shown in Figures 2, 3, 4. In the control samples, the mangoes were not rotten after 4 days of storage. However, mango damage began after 4 days of storage with 11 % of damage and a sharp increase of 50 % after 6 days of storage and total damage after 10 days of storage (Figure 2). In E samples, the concentration of essential oil showed significant influence on mango preservation in which the concentration of lemongrass oil over the MIC not only did not increase the storage efficiency but also caused damage to the sample. The samples sprayed with 20 µl/ml essential oil were rotten

of 26 % on the 2nd preservation day and completely rotten after 6 days of storage (Figure 2). Samples sprayed at MIC concentrations were rotted after 6 days (10 %) and were completely damaged after 14 days of storage. This result was better ($p < 0.05$) than the control, but it was not as good as the result of samples sprayed with 2.5 and 5 µl/ml essential oil. The samples, which were sprayed with 2.5 or 5 µl/ml essential oil, had higher efficiency. They weren't damaged after 6 days and samples sprayed with 2.5 µl/ml were damaged 10 %, samples sprayed with 5 µl/ml were damaged of 6 % after 8 days. However, samples of 2.5 and 5 µl/ml were damaged 59 % and 42 %, respectively at day 14 of storage (Figure 2). In the A and C samples, the results showed that mangoes treated with coatings had a better storage efficiency ($p < 0.05$) than the control samples (Figure 3). The samples treated with chitosan or Ca-alginate were not damaged after 8 days of storage and damaged less than 10 % after 10 days of storage. The edible with Ca-alginate as coater showed protected effect ($p < 0.05$) better than chitosan. In the AE and CE samples which were treated by a combination of lemongrass oil and the edible film showed the best result ($p < 0.05$) at low concentration of essential oil (5 µl/ml), namely they were not rotten after 14 days of storage in case of AE samples and 10 days of storage in case of CE samples. The result also indicated that the mangoes were rotten quickly at high concentration of essential oil (20 µl/ml) which were rotten completely after 6 days of storage (Figure 4). The antifungal activity of the essential oil has been demonstrated in many previous studies [4, 5, 7, 17]. However, the application of essential oil in preserving agricultural products is complex due to the characteristics of the essential oil such as the volatile and surfactant characteristics (burns). The present study showed that the lemongrass oil at 2.5 and 5 µl/ml concentration did not show an antifungal activity in *in vitro* test (Figure 1). However, in preservation test, the lemongrass oil at 2.5 and 5 µl/ml concentration had a preservative efficiency ($p < 0.05$) higher than 10 µl/ml (the MIC of lemongrass in the *in vitro* test) (Figure 2). The essential oil at the low concentration (MIC or lower) significantly reduced the antifungal activity, whereas the mangoes were rotten at the high concentration of the essential oil (higher than the MIC value). This happens due to the essential oil at high concentration could damage the fruit surface so the mold would damage and destroy the fruits quickly [15].

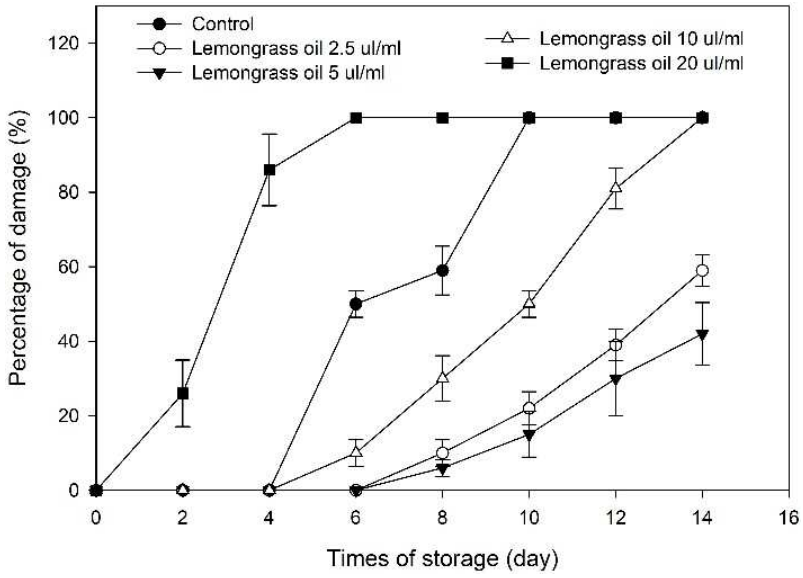

Figure 2. The influence of essential oil on mango preservation.

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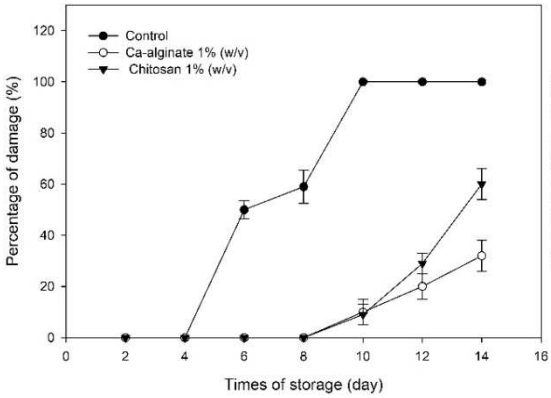

Figure 3. The influence of edible film on mango preservation.

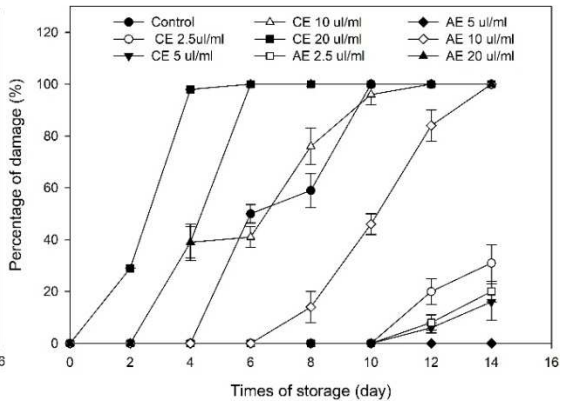

Figure 4. The influence of the combination of lemongrass oil and edible film on mango preservation. (AE: Ca-alginate – lemongrass oil; CE: Chitosan-lemongrass oil).

Studies about the preservation of edible coating have also been reported in previous studies [9, 14, 15]. Savage [19] reported that apples treated with chitosan reduced mold rates for 12 weeks at 5°C. Similarly, chitosan coating showed up to effectively inhibit the growth of microorganisms [14]. The storage efficiency of the coatings come from the ability to reduce the respiratory rate of fresh fruit, slowing the dehydration process on the fruit surface, reducing the moisture absorption and oxidation, avoiding the loss of incense and preventing intrusion of bacteria [9], leading to extending the shelf life of fruit. In the present study, chitosan and alginate enhanced significantly ($p < 0.05$) the shelf life of mangoes compared to control samples in which A sample had the best result (Figure 3). The coating at high concentration (over 1 % w/v) would affect the sensory properties while using at low concentrations (less than 1 %), a preservation efficiency of the coating reduced (Data not shown). However, in the present study, chitosan film although prolonged mango preservation, mango rotting was still happened (Figure 3). This happens revealed that the chitosan film is not effective for fungal resistance.

Edible coating of the three groups: polysaccharides, proteins, and lipids have great advantages [9] and when they combined with essential oils, this method effectively slows down the rate of evaporation of the antifungal agent, so active compounds are retained at high concentration on the fruit surface (where is the main way to infect of microbial) for a long time. This makes the method more effective in reducing the level of microorganisms than direct applying to the surface of the product by the spray method [8]. Edible coatings combined with essential oils showed an effectiveness in previous studies. The combination of cinnamon oil and Ca-alginate film extended the shelf life of melon slices over 21 days of storage at 5 °C [16]. Similarly, the edible coating formed by alginate combined with lemongrass 0.3 % w/v has the potential to extend the shelf-life and maintain the quality of fresh pineapple [13]. In this study, lemongrass oil combined with chitosan or alginate showed a significantly extended shelf life compared to control samples in which alginate showed a better storage efficacy than chitosan (Figure 3). However, when the concentration of lemongrass oil was 10 µl/ml or more than 10 µl/ml (MIC value from *in vitro* experiment), the rate of damage in mangoes was higher than the low concentration. Rojas-Graü et al. [15] reported that citronella oils at 1.0 and 1.5 % (w/w) concentration combined with alginate had an effect on the texture of fresh apples which leading to bruised and rotten rapidly, because the high concentrations of essential oils have a negative impact on the skin of the fruit shells [8], making mangoes damaging as soon as treated with

coatings. Then they are waterlogged and rotten rapidly. The present study showed that lemongrass oil at 2.5 µl/ml or 5 µl/ml did not show the *A. niger* resistance by agar diffusion method, but these concentrations had better ($p < 0.05$) mango preservation efficiency when combined with the edible coatings than the minimum inhibitory concentration (10 µl/ml) and 5 µl/ml of essential oil combined with alginate gave the best results (Figure 4). Therefore, the combination of Ca-alginate film and lemongrass oil (5 µl/ml), eventually leading to have dual efficiency: First, the low concentrations of essential oils ensure no damage to the fruit surface during storage; Second, the Ca-alginate film helps to maintain the antifungal agent of the essential oil during storage.

4. CONCLUSIONS

The results show that all three kinds of lemongrass oil, citronella oil, and cajeput oil have antifungal effect against *A. niger*. Lemongrass oil, which β-citral (41.2 %) and α-citral (39.80 %) were the main compounds showing the best antifungal activity whose MIC value was 10 µl/ml, compared to 20 µl/ml in the cases of citronella oil and cajeput oil. In mango preservation experiments (infected by *A. niger* 10^5 spores/ml), the mangoes were rotten after 10 days of storage in the control samples. The concentration of lemongrass oil showed significant influence on mango preservation in which the concentration of lemongrass oil over the MIC not only did not increase the storage efficiency but also caused damage to the mangoes. The samples sprayed with 20 µl/ml of essential oil were completely rotten after 6 days of storage, whereas the samples of 2.5 and 5 µl/ml were damaged of 59 % and 42 %, respectively at day 14 of storage. The combination of edible coatings and essential oils significantly improved mangoes preservation. The combination of lemongrass oil at 5 µl/ml (1/2 MIC value) and Ca-alginate 1 % (w/v) showed the best results, the samples were not rotten after 14 days. The edible film can help to reduce the amount of used essential oil that still ensures effective antibacterial.

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