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Application of Lemongrass Oil in Chitosan as Antimicrobial During Storage of Crystal Guava Fruit

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ABSTRACT

Guava crystal is a fruit that has a high selling value. One of the obstacles to marketing crystal guava fruit is the fast decay process. Many factors cause fruit to rot quickly, including microbial contamination post-harvesting. This study evaluates the effectiveness of adding lemongrass oil in chitosan coatings to protect crystal guava from microbiological damage during storage. The fruit is coated with 1% chitosan and added to lemongrass oil according to the treatment. The study used a completely randomized design with three replications. The treatment tested included 0.0, 0.1, 0.3, and 0.5% lemongrass oil. The content of lemongrass oil compounds was analyzed using gas chromatography-mass spectroscopy (GC-MS). The antibacterial test was carried out using the disc method. Thge parameters observed were the total plate count and total fungal count of crystal guava fruit on days 0, 3, 6, 9, 12, 15, and 18 after storage. GC-MS analysis showed that the main content of lemongrass oil is two isomers of citral, i.e., neral (38.54%) and geranial (39.26%). The results showed that the total fungal and plate count increased during storage. Chitosan coating with lemongrass oil more effectively inhibits *Staphylococcus aureus* **than** *Escherichia coli***. Adding 0.3% and 0.5% lemongrass oil in chitosan could restrain the rate of microbes in crystal guava until the 18th day after storage. Chitosan treatment with 0,3% lemongrass oil is recommended to be the best treatment for applying on crystal guava.**

Keywords: citral, GC-MS, *Psidium guajava* **L., total plate count, total fungal count**

INTRODUCTION

'Crystal' guava (*Psidium guajava* L.) is one of the guava cultivars with high economic value. This guava has a sweet taste, a crispy texture, and a small number of seeds, so consumers prefer it. Farmers are also happy to cultivate crystal guava because it is not difficult to maintain, bears fruit all year round, and has a higher selling value than other varieties of guava (Rustani and Susanto 2019). Guava is a climacteric fruit because it still undergoes ripening even though it has been harvested from the tree (Nair *et al.* 2018). Metabolic activity after the fruit is harvested causes a rapid decline in the quality of climacteric fruit. In tropical countries such as Indonesia, the storage of crystal guava fruit at room temperature only lasts about 8 to 9 days (Widodo and Maretha 2013). In addition to metabolic processes, microbial contamination in the post-harvest process contributes to the deterioration of guava quality (Botelho *et al.* (2016).

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The decline in this crystal guava's quality negatively impacts its selling value, causing losses for traders (Nagaraju and Banik 2019).

Nowadays, various types of plastics made from polyethylene (PE), polypropylene, and polyethylene terephthalate (PET) are used in the fruit storage process (Petkoska *et al.* 2021). Crystal guava is generally coated with plastic wrap to reduce microbial contamination during postharvest (Rana *et al.* 2015). However, plastic packaging is no longer recommended due to the impact of waste on the environment and the health hazards caused by toxic components entering the fruit (Sharma *et al.* 2024). Using *edible coating* with antimicrobial properties is one of the alternative technologies to protect fruits from damage caused by contaminants during the post-harvest process (Guerra *et al.* 2015). Edible coatings are thin layers that can be applied directly to food products such as fruits (Yashaswini and Iyer 2019). Edible coatings with natural ingredient formulations with antimicrobial and *biodegradable properties* are considered an environmentally friendly technology that increases the shelf life of crystal guava. One of the materials that has received much attention in manufacturing edible coatings is chitosan.

Chitosan is a polysaccharide-based biopolymer that is more naturally abundant than cellulose (Feyzioglu and

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Tornuk 2016). This biopolymer is one of the promising materials to be applied as a food preservative because it is safe to consume, has antimicrobial properties, is biocompatible with coated surfaces, is easily decomposable, has low oxygen permeability, and is commercially available at a low price (Aider 2010; Keawchaoon and Yoksan 2011; dos Santos *et al.* 2012; Barikani *et al.* 2014; Wang *et al.* 2014). The characteristics of chitosan can be improved by adding sodium tripolyphosphate (NaTPP) as a crosslinking agent. According to Abraham *et al.* (2018), crosslinking can improve chitosans' mechanical strength, solubility, permeability, and stability. In some previous studies, chitosan can be added with essential oils to enhance its antimicrobial properties. In addition to being antimicrobial, essential oils act as preservatives (Ribeiro-Santos *et al.* 2017). Research by Basaglia *et al.* (2021) said that adding 0.5% cinnamon oil to chitosan protected pineapples from microbial contamination during storage. Similar results were also obtained by Hasheminejad and Khodaiyan (2019), who found that adding 0.15% clove essential oil to chitosan inhibits the microbial rate of pomegranates during storage.

One of the essential oils that has the potential to be added to edible coatings is lemongrass oil (*Cymbopogon citratus*). The main component of kitchen lemongrass oil is citral. The citral content of kitchen lemongrass provides strong antimicrobial properties (Kumar *et al.* 2012). Lemongrass essential oil is safe to consume because it is included in the *Generally Recognized as Safe* (GRAS) by the *Food and Drug Administration* (FDA) (FDA 2020), so it can be added to *edible* coatings. Research conducted by Azarakhsh *et al.* (2014) showed that adding 0.3% and 0.5% lemongrass oil in alginate could resist the microbial rate in cut pineapples during storage. In addition, the addition of 0.1% lemongrass oil in arabic gum is also good at resisting the rate of decay of pomegranates (Kawhena *et al.* 2022). The use of chitosan with the addition of lemongrass essential oil to coat crystal guava fruit has never been reported, so this study needs to be conducted. It examines the effect of adding lemongrass oil to chitosan as a coating for crystal guava fruit on antimicrobial activity during the storage period.

METHODS

The research was conducted at the IPB Biopharmaceutical Laboratory and the Integrated Laboratory of the Industrial Spice Crops Research Institute, Parungkuda, Sukabumi. The study used a Complete Random Design (RAL) design with 3 replicates each. The crystal guava fruit used in this study is physiologically mature. The ingredients included chitosan with medium molecular weight (degree of deacetylation 75–85%), lemongrass essential oil, NaTPP, Tween 80, *Staphylococus aureus* (ATCC 6538), *Escherichia coli* (ATCC 25922), nutrient agar (Oxoid), chloramphenicol (Nacalai Tesque Inc.), potato dextrose agar*,* plate count agar, CH3COOH, NaCl, and NaOH obtained from Merck. Meanwhile, the equipment used was gas chromatography-mass spectrometry (GC-MS) (Agilent Technologies 5977), Ultra-Turrax homogenizer (T18, IKA), hot plate stirrer (IKA), and incubator (Memmert).

Compound Identification of Lemongrass Essential Oil Using GC-MS

Lemongrass essential oil was analyzed following Hien Tran *et al.* (2019). The column used was HP5-MS. The flow rate of the carrier gas (He) was 1 mL/min with an injection temperature of 250°C. The sample was injected using a split ratio of 1:100. The temperature program was as follows: the initial holding temperature was 50°C for 2 minutes, then increased by an increment rate of 2°C/min to 80°C. Next, it was raised by 5°C/min to 150°C, and subsequently increased to 200°C at a rate of 10°C/min. Finally, the temperature was raised to 300°C at an increment of 20°C/min, with a hold time of 5 minutes at this final temperature.

The mass spectrometer operated at an electron energy of 70 eV. Compound identification included the determination of the chemical name, structure, and molecular weight. This identification was accomplished by aligning the chromatogram peaks with the spectra of known compounds from the Wiley 9 database and corroborating the data with information from *PubChem*.

Antibacterial Activity Test of Lemongrass Essential Oil

The test of the antibacterial activity of lemongrass essential oil refers to CLSI (2018). The test cultures used were *S. aureus* and *E. coli*. The bacterial suspension was prepared by putting the bacterial culture into a 0.9% NaCl solution. Then, 0.1 mL of culture suspension was inoculated into 100 mL of NA. The medium was poured into a petri dish of 20 mL and left to stand until it hardened. The antibacterial test was carried out using the disc diffusion method. On each disc paper, 20 μL of lemongrass essential oil was inoculated with concentrations of 0.1%, 0.3%, and 0.5%. The medium was incubated for 24 hours at a temperature of 37°C. The antibiotic chloramphenicol at 1 mg/mL concentration was

used as a positive control and 1% Tween 80 as a negative control. The measurement of the inhibition zone was using a caliper.

Edible Coating Preparation

The chitosan coating with lemongrass oil was prepared using Wu *et al.* (2016) method with some modifications. The coating solution was made by dissolving chitosan (1% (w/v)) in dilute acetic acid (1% (v/v)). Tween 80 (0.3% b/v) was added to a 1% chitosan solution and stirred for 90 minutes. After homogenization, essential oils of various concentrations (0.0%, 0.1%, 0.3%, and 0.5%) are dripped into the solution while stirring at 20000 rpm (rotor diameter 12.7 mm). The concentration of 0.0% was the coating of chitosan without lemongrass oil. The 0.2% TPP solution (b/v) was slowly incorporated while being homogenized. The volume between the chitosan and TPP solutions was 1:1. From this procedure, 4 edible coating samples were obtained to be tested, namely chitosan with zero, 0.1%, 0.3%, and 0.5% lemongrass oil. The coating emulsion that has been made was stored at a temperature of 4°C until use.

Antibacterial Test of Edible Coatings

The chitosan edible coating solution made previously with lemongrass oil of various concentrations (0.1%, 0.3%, and 0.5%) was tested for its antibacterial activity by calculating the resulting inhibition zone using a caliper. This test referred to CLSI (2018). As a positive control, 1 mg/mL chloramphenicol was used.

Crystal Guava Coating

Crystal guava fruits were harvested from plantations in the Rancabungur area, Bogor Regency. The feasibility of the fruit was sorted before being tested. Due to disease attacks or physical impacts, the fruit was chosen with a smooth surface texture and no brownish spots. Before coating, the fruit was cleaned with running water, rinsed with distilled water, and dried. The process of dyeing crystal guava fruit was following Hasheminejad and Khodaiyan (2019). Each crystal guava was dipped in a solution of chitosan edible coating in additions of 0.1%, 0.3%, and 0.5% for 2 minutes. The fruit was then dried and aired for ± 30 minutes. Crystal guava was used as a control and dipped in sterile distilled water. All fruit were stored at 25–27°C with a relative humidity of 65–70%. Testing was carried out with a time interval of every 3 days until the 18th day of storage.

Microbial Analysis

A total of 10 g of samples were dissolved with 90 mL of sterile saline solution (8.5 g of NaCl L^{-1}) and tested as follows (Rinaldi *et al.* 2013).:

1. Total plate number: One mL of sample dilution was poured on a Petri plate and then PCA media. After the

medium hardened, it was incubated at 30°C for 48 hours.

2. Mold and yeast test: One mL of dilution was put on a Petri plate. Then, the PDA medium, which was previously homogenized with 100 mg of L^{-1} chloramphenicol, was poured. The PDA media was stored at 25°C for 4–6 days.

All microbial counts obtained from both tests are reported as colony-forming unit logs per gram $(g⁻¹ CFU logs)$.

Data Analysis

The data of the research results were analyzed with ANOVA using IBM SPSS version 27. If the results show a real influence, continue with the Duncan Test with 95% confidence ($α = 0.05$).

RESULTS AND DISCUSSION

Chemical Constituents in Lemongrass Oil

The GC-MS spectra (Figure 1) shows that there are 3 main compounds in lemongrass oil, including β-myrcene, β-citral (neral), and α-citral (geranial). The peak of the βmyrcene appeared at a retention time of 10.71 minutes with an abundance of 7.74%, nerals appeared at 30.60 minutes with an abundance of 38.54%, and geranial with an abundance of 39.26% appeared at 32.83 minutes (Table 1). Based on these results, lemongrass oil contains antimicrobial compounds because there are nerals and geranials. The two compounds are 2 isomer compounds from the citral. Citral is a compound of the aldehyde monoterpene group that contributes significantly to the bioactivity of lemongrass essential oil. As an antimicrobial, citrals damage the cell membrane lining, interfering with quorum sensing and inhibiting the action of enzymes in microbial metabolism (Gutiérrez-Pacheco *et al.* 2023). Some bioactivities of citrals include antimicrobial, anti-inflammatory, antiparasitic, allelopathic, and mosquito repellent (Ganjewala *et al.* 2012).

The results of this study are slightly different from those of Bharti *et al.* (2013), who stated that there are four main compounds in lemongrass essential oil: neral, geranial, myrcene, and geraniol. The composition of these different compounds can be affected by several factors, including the part of the plant used, the geobotanical conditions of the growing environment, the age of the plant, and the harvest time (Oladeji *et al.* 2019).

Antibacterial Activity of Lemongrass Essential Oil

Some microbes that can contaminate fruit during postharvest include *Staphylococcus aureus, E. coli, Bacillus cereus, S. typhimurium, Aspergillus flavus*, and

Figure 1 GC-MS chromatrogram of lemongrass oil.

Table 1 Chemical constituents in lemongrass oil

Compound	RT (min)	Peak area (%)
β-Myrcene	10.71	7.74
β-Pinene	10.85	4.59
β - Ocimene	13.09	0.79
Linalool	16.81	3.36
β -Citral (Neral)	30.60	38.54
α-Citral (Geranial)	32.83	39.26
Geranyl acetate	37.04	3.17
α-Bergamotene	38.58	0.87
2-Norpinene	40.47	0.79
Selina-6-en-4-ol	43.34	0.88

A. parasiticus (Chawla *et al.* 2021). In this study, the microbes used were *E. coli* and *S. aureus*. Before being used as an edible coating, Lemongrass oil was tested first to see the effectiveness of the concentration used. The results showed that the diameter of the inhibitory zone of *E. coli* and *S. aureus* bacteria increased with the increase in lemongrass oil concentration. The Duncan test showed significant differences between the three concentrations (0.1%, 0.3%, and 0.5%) tested in *E. coli* and *S. aureus* bacteria (Table 2). This result is in line with previous research, which showed that lemongrass oil was able to inhibit the growth of bacteria, both grampositive bacteria (*S. aureus B. cereus,* and *B. subtilis)* and gram-negative bacteria (*E. coli* and *K. pneumoniae)* (Naik *et al.* 2010). Therefore, lemongrass oil with a concentration of 0.1% to 0.5% can be used as an antimicrobial in crystal guava storage.

Antibacterial Activity of Edible *Coatings*

Table 3 shows the diameter of the chitosan inhibition zone produced by adding lemongrass oil against the two bacteria tested. The results showed increased coating resistance and increased concentration of lemongrass oil

added. Duncan's test on the two bacteria showed significant differences in the inhibition zones produced.

The inhibition of lemongrass oil is higher when used with chitosan than when not used with chitosan. This is because chitosan itself has antimicrobial activity. Sulistijowati *et al*. (2019) reported that using chitosan as a coating has antibacterial and antifungal properties. Research on the use of essential oils with chitosan has also been conducted using clove, thyme, cinnamon, lime, and nettle oils (Sotelo-Boy ́as *et al.* 2015; Wu *et al.* 2016; Bagheri *et al.* 2021). The results show that adding essential oils to chitosan has good potential as a coating with antimicrobial activity.

The comparison between Table 2 and Table 3 shows a difference in the increase in inhibition between *E. coli* and *S. aureus*. The increase in inhibition of *S. aureus* bacteria is higher compared to *E. coli*. This occurs because *S. aureus* bacteria do not have an outer membrane like *E. coli,* making it easier for chitosanbased coatings to attach directly and accelerate the destruction of vital activity in *S. aureus* (Duan *et al.* 2019). The same results were also seen in the study of Goy *et al.* (2016), demonstrating that at a chitosan concentration

Table 2 Diameter of the inhibitory zone of lemongrass essential oil against *E.coli* and *S. aureus*

Remarks: Numbers followed by the same letter show no apparent difference based on the Duncan test at α = 0.05.

Table 3 Diameter of the inhibition zone of the chitosan–lemongrass oil coating

Remarks: Numbers followed by the same letter show no significant difference based on the Duncan test at α = 0.05.

of 1%, the resulting inhibitory power against *S. aureus* bacteria was higher than that of *E. coli.*

Microbiological Analysis

The total plate number and the number of yeasts were measured to determine the coating's ability to control the ra te of microorganisms in crystal guava fruits during storage. The results show that the large number of microorganisms that contaminate during storage can accelerate fruit decay. This is in line with Karanth *et al.* (2023), mentioning that the growth and contamination of mesophilic bacteria and fungi during fruit harvesting, processing, and packaging can accelerate fruit spoilage during storage.

Based on the observation from day 0 to the last day of the experiment, there was an increase in the total plate number. The total plate numbers of control and chitosan treatments were statistically different on the day of processing, and on day 3, storage did not show any significant difference (Table 4). The new difference was seen on the sixth day of storage. Based on the Duncan test, since the day of processing, the control treatment and chitosan have shown significant differences with the chitosan treatment given lemongrass oil concentrations of 0.1%, 0.3%, and 0.5%. The same pattern of results was obtained until the 15th day of storage. On that day, the treatment on the control fruit could not be tested because the crystal guava fruit rotted on the 12th day of storage, while the fruit with chitosan treatment could not be tested on day 18 because the fruit had also rotted on the 15th day. These results showed that chitosan with the addition of lemongrass oil concentrations of 0.1%, 0.3%, and 0.5% could reduce the rate of bacterial contamination in crystal guava fruits during the storage period. The effect of adding essential oils to chitosan in carrots, pomegranates, and pineapples in previous

studies also had a good effect (Martínez-Hernández *et al.* 2017; Hasheminejad and Khodaiyan 2019; Basaglia *et al.* 2021).

The data on the total plate number of chitosan treatment with the addition of 0.3 and 0.5% of lemongrass oil was similar from the beginning to the end of the study. Based on the results, the two treatments are equally good in inhibiting bacterial contamination in crystal guava fruits. This inhibition occurs because edible coatings can form a membrane that inhibits the entry of nutrients into the cell, thereby disrupting the metabolism of bacteria (Rochima *et al.* 2018). In line with the results of the total plate number, the number of yeasts also increased in all treatments given during the fruit's storage period. The control treatment and chitosan have shown significant differences on the 6th day of storage (Table 5).

Chitosan treatment with the addition of 0.3% and 0.5% lemongrass oil indicated the same ability to inhibit the rate of yeast during the shelf life of crystal guava fruit. However, the chitosan treatment with 0.1% lemongrass oil did not show the same pattern. On the 3rd, 15th, and 18th-day observations, the yeast yield from 0.1% lemongrass oil was significantly different from adding 0.3 and 0.5%, while the observations on other days were not significantly different. It means that the application of 0.1% lemongrass oil was not as good as the treatment of 0.3 and 0.5% in inhibiting the rate of yeast in crystal guava fruit.

The success in inhibiting the rate of yeast in chitosan fed with lemongrass oil is suspected because lemongrass oil has an antifungal function. The ability of lemongrass oil to inhibit the growth of fungi has also been demonstrated (Tzortzakis and Economakis 2007), which found that lemongrass oil can inhibit the growth of

Remarks: NA (*Not Applicable*), A number followed by the same letter shows no significant difference based on the Duncan test at $\alpha = 0.05$.

Table 5 Changes in the number of crystal guava fruit mold during storage

Remarks: NA (Not Applicable), A number followed by the same letter shows no significant difference based on the Duncan test at $α = 0.05$.

Colletotrichum coccodes, Cladosporium herbarum, Rhizopus stolonifera, and *Aspergillus niger*.

These findings show that adding lemongrass oil to chitosan can increase the antimicrobial ability of crystal guava coatings. This is proven by its ability to inhibit the rat e of increase in the number of total plates and yeast. The concentration of 0.3% and 0.5% lemongrass oil looks equally good in inhibiting microbes in crystal guava fruit during storage. Both concentrations of lemongrass oil added with chitosan can resist fruit spoilage until the 18th day after storage. Fruit spoilage can be affected by the growth of microorganisms, bacteria, and fungi. According to Jahun *et al.* (2021), microorganisms present in fruit will release their enzymes into food and absorb nutrients, causing fruit to rot quickly. A concentration of 0.3% lemongrass oil is recommended compared to 0.5% for efficiency in the use of materials and costs. Hence, adding 0.3% lemongrass oil to the chitosan coating is the best treatment because it can prevent spoilage and extend the shelf life of crystal guava.

CONCLUSION

Lemongrass oil contains citral chemicals that can be used to reduce antibacterial activity. Using chitosan

edible coating with lemongrass oil can withstand yeast growth and increase the total plate number of crystal guava fruit during storage. The inhibitory zone caused by chitosan coating created by adding lemongrass oil to S. aureus is greater than that of E. coli. Adding 0.3% and 0.5% lemongrass oil can resist the microbiological rate in crystal guava fruits until the 18th day, preventing the fruit from rotting and increasing the fruit's shelf life. In this investigation, the treatment of chitosan with 0.3% lemongrass oil was indicated as the optimum treatment for crystal guava storage.

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