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Improvement of Quality of *Carica papaya L.* with Clove Oil as Preservative in Edible Coating Technology

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Abstract

We have studied utilization of essential clove oil, extracted from clove buds by hydrodistillation, as preservative in edible packaging technology. Preservative of essential clove oil was applied on chopped papaya fruits by using two methods, namely spray and brush. The effects of concentration of clove oil from 0.05 to 0.20% on the preservation of papaya fruits (*Carica papaya L.*) at room temperature (25 °C) were also evaluated. Physicochemical and in vitro microbiological activities on the papaya fruits that were stored at 25 °C and 85-90% relative humidity were investigated in details. The results indicate that the clove oil at concentration $\geq 0.10\%$ suppressed the decay time, 10% weight loss, 0.03 g citric acid/100 g in acidity titration test, and 20% pH value from those of control sample of papaya fruits kept in a storage. The population of fungi and bacteria were efficiently reduced by 90% when the clove oil at concentration $\geq 0.10\%$ was applied as preservative on papaya fruits. This finding suggested that the extracted essential clove oil acted as effective antifungal and antibacterial agents. Preservative by essential clove oil improved the quality of fruits to extend the product shelf life and to reduce the risk of microbial growth on fruits surface.

Abstrak

Peningkatan Kualitas *Carica papaya L.* dengan Minyak Cengkeh sebagai Pengawet dalam Teknologi Lapisan yang Dapat Dimakan. Peneliti telah mempelajari pemanfaatan minyak atsiri cengkeh, yang merupakan ekstrak dari tunas cengkeh dari proses hidrodistilasi, sebagai pengawet dalam teknologi kemasan yang dapat dimakan. Pengawet atsiri cengkeh diberikan pada buah pepaya cincang dengan menggunakan dua metode, yaitu menggunakan alat penyemprot dan kuas. Efek dari konsentrasi minyak cengkeh 0,05-0,20% pada penyimpanan buah pepaya (*Carica papaya L.*) pada suhu kamar (25 °C) juga dievaluasi. Kegiatan fisiokimia dan mikrobiologi in vitro pada buah pepaya yang disimpan pada 25 °C dan kelembaban relatif 85-90% diselidiki secara rinci. Hasil menunjukkan bahwa minyak cengkeh pada konsentrasi $\geq 0,10\%$ menekan waktu peluruhan, penurunan berat badan 10%, 0,03 g asam sitrat/100 g dalam tes keasaman titrasi, dan 20% nilai pH dari sampel kontrol buah pepaya di dalam tempat penyimpanan. Populasi jamur dan bakteri yang turun 90% ketika minyak cengkeh lebih dari 0,10% digunakan sebagai pengawet pada buah pepaya. Temuan ini menunjukkan bahwa ekstrak minyak atsiri cengkeh merupakan agen anti-jamur dan anti-bakteri yang efektif. Pengawetan dengan minyak atsiri cengkeh meningkatkan kualitas buah-buahan, memperpanjang umur simpan produk, dan mengurangi risiko pertumbuhan mikroba pada permukaan buah.

Keywords: antifungal, antibacterial, clove oil, edible coating, preservative

1. Introduction

Indonesia is an agricultural and tropical country which can produce huge varieties of fruits. One of important horticultural crops or cultivated plants is papaya fruit (*Carica papaya L.*). Papaya is one of the leading horticultural crops in Indonesia, and its production has

been in the top ten chart of fruit productions in Indonesia during the last five years. This is mainly because papaya is available throughout the year. Nevertheless, papaya fruit is a commodity that can damage easily if there is no treatment or further quality control.

High quality papayas preferred by consumers in general have good flavor quality, attractive in appearance, and fresh even after taken out of storage. Based on the form of packaging, papaya can be divided into primary, secondary, and tertiary categories. Primary packaging refers to direct wrapping the papaya fruits using, for example, plastics. Secondary packaging is defined as a package which covers a number of primary packaging fruits to protect them from physical damages. Tertiary or quaternary packaging refers to packaging after primary and secondary ones, and it serves as a protection during distribution of fruits [1]. Nowadays, in Indonesia, papaya fruits are packed with papers, polymers such as plastic wrap, and cardboards to prevent damages to the surface of the fruits. However, maturation process that occurs in the fruit until decomposition is not effectively suppressed [2].

Development of edible packaging technology is currently of concern in the industry, dealing particularly with fresh foods, in order to extend the shelf life and to maintain the food products. The packaging technology for food products has evolved rapidly. Packaging technology with edible coating film is usually utilized to maintain the freshness of fruits, to minimize the process of skin shrinkage caused by evaporation of most of the water contained in the fruits, and to increase a physical barrier to pathogens (bacteria, viruses, and other microorganisms) that can cause diseases [2]. The major edible coating is made from calcium ascorbate (Nature Seal™), but this anti-browning agent is not effective for anchoring process of decay in papaya [3].

Edible coating films have also been developed to which an active substance is added to improve main components or matrix of package performance. A number of studies have been devoted on essential oils as active substance for packaging [4]. These oils are biodegradable, non-toxic, and have good bioavailability properties for fresh products. Some essential oils have been known to have preserving effect because they have antioxidant, antimicrobial, and insecticide activities [4].

In this study, we explore the use of clove oil as an additional element in packaging, expecting that the clove oil would increase protective packaging on papaya from biological damage. Compared with essential oils from other plants, clove oil contains high eugenol, which can act as antimicrobial, antioxidant, and insecticidal activities [5]. The use of clove oil as an additive in fruit preservative, however, has not been widely studied. The clove oil used in this study is a clear yellow oil of lipid in fluid phase obtained from extraction of the clove plant, such as leaves, woods, and clove buds.

We note that application of preservative coatings is generally done by soaking method though this method

has some drawbacks, including wasteful of solution preservatives, high levels of contamination, and low time efficiency [6]. Therefore, the use of brush and spray techniques to coat the surfaces of fruits in the preservative coating processes in this study were expected to improve the quality of fruits, to extend the product shelf life, and to reduce the risk of microbial growth on fruits surface.

2. Experiment

Materials. Dried clove buds (*Syzygium aromaticum*) were obtained from traditional market in Jakarta, Indonesia. Tween 80 obtained from Brataco. Papaya fruits (*C. papaya* L.) were harvested at color index 3rd from commercial farm in Depok, Indonesia. They were visually inspected defects and blemishes.

Extraction of clove oil. Clove oil was extracted by hydrodistillation method with ratio of solid and solvent being 1:2. Dried clove buds were grinded for 1 h to obtain fine powder with a uniform particle size. Distilled water was used as solvent [7]. The mixture of distilled water and the powder of clove buds was heated for 1 h in the distillation flask as starter (warm-up), and then distillation was continued for 4 h at 110 °C [8]. During the distillation, the tube was streamed with cold water from cooling bath which was filled by ice-water.

Application of clove oil solution as preservative. Preservation properties of clove oil were evaluated by following procedure. First, we mixed extracted clove oil (0.05, 0.10, 0.15, and 0.20% v/v, respectively) and Tween 80 at a concentration of 0.20% v/v in 100 mL distilled water for 1 h at room temperature. A solution containing 0% clove oil was used as a control solution. Tween 80 was used as an emulsifier to dissolve the clove oil in distilled water. To ensure the solvation, the mixture was further stirred and heated at 40 °C for 1 h. A solution containing 0% clove oil was used as a control solution.

Preservative of clove oil were applied on chopped papaya fruits using two methods, namely spray and brush. With these two methods, the mixture was either sprayed or brushed on the papaya fruits using spray bottle or brush, respectively.

Physicochemical properties. Papaya fruit samples were weighed and blended using a kitchen blender for 1 min. Two independent samples were prepared for each concentration of clove oil as well as for a control solution. Acidity test was analyzed using 942.15 method and expressed as citric acid per 100 g fruit. pH was measured by pH-meter.

Microbiological test. The growth of bacterial and fungi in each sample were analyzed by microbiological test

using potato-dextrose agar (PDA) and Nutrient Agar(NA) medium, respectively, for every single concentration of clove oil as preservative. In this microbiological test, papaya fruits were coated by different clove oil concentrations from 0.05 to 0.20% v/v, and they were kept for 3 days. The fruits was then extracted, and the papaya juice was diluted by 10^{-5} and subjected for microbiological activity through total plate count (TPC) technique. The cultures were incubated for 24 h at room temperature 25 °C.

3. Results and Discussion

GC-MS characterization of clove oil. Chemical compounds in extracted clove oil were obtained from GC-MS. As listed in Table 1, clove oil extracted from dried clove buds using water as solvent had 86.39% eugenol; 8.72% 1-caryphyllene; 1.44% humulene; 0.27% of the allyl-phenol; 0,44% delta-cardinene; 0.42% 4-terpineol; and 2.32% other minor elements. Eugenol, the highest content in the clove oil, is volatile and bioactive. The latter properties might be related to its preservative activity to extend the shelf life of papaya fruit.

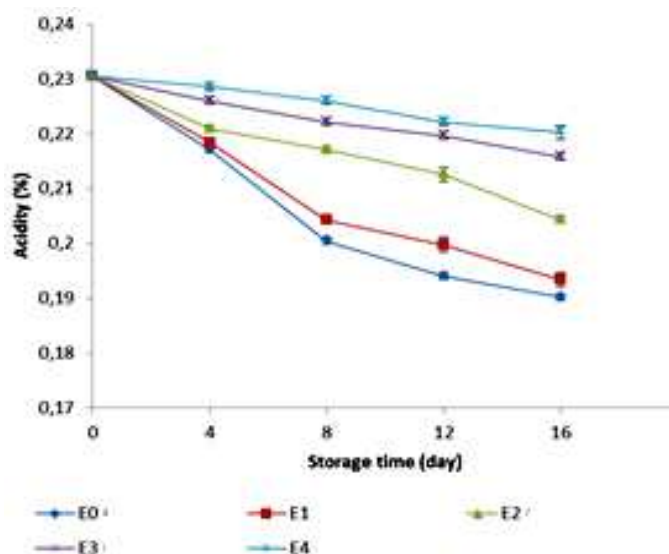
Physicochemical properties. Effects of clove oil on physicochemical properties of papaya fruits were evaluated by measuring weight loss during storage. By varying the concentration of the clove oil, we found that at $\geq 0.10\%$ v/v the total amount of weight loss was 5.84 and 5.97 g for papaya fruits coated by brush and spray method, respectively. The weight loss was about 10% higher compared with that the control sample. This indicates that the rate of weight loss is reduced when the fruits are coated with clove oil, attributed to the additional protection against diffusion through stomata and excretion caused by bacteria and fungi metabolisms.

Acidity measurement. In Figure 1 (a), acidity is 23% for fresh sample, and it tended to decrease with storage time. Interestingly, we found that the decrease in the acidity could be suppressed by addition of clove oil concentration. In other words, the decreasing rate of the acidity was lower for higher concentration of coating clove oil. We also found that the tendency of acidity decrease using spray and brush method was similar to each other (Figure 1(a) and (b)), indicating the same concentration of clove oil had the same effect on the quality of papaya fruits during storage, irrespective of the coating method.

pH measurements. Papaya fruits showed pH in the range from 5.7 to 7.1. As the acidity changed with storage time, it was not surprising that the pH of the papaya fruits changed with storage time. Figure 3 shows the pH at different storage times for both brush and spray methods. The two methods again show a similar tendency (Figure 2(a) and (b)), supporting the acidity data.

Table 1. Chemical Compounds in Clove Oil

Composition	Percentage (%)
Eugenol	86.39
1-Caryphyllene	8.72
Humulene	1.44
Para-allylphenol	0.27
4-Terpineol	0.42
Delta-Cadinene	0.44
Para-allylphenol	0.27
Iseugenol acetate	0.20
Anethol	0.18
Methylenedioxybenze	0.16
Others	1.51



(a) Brush Method

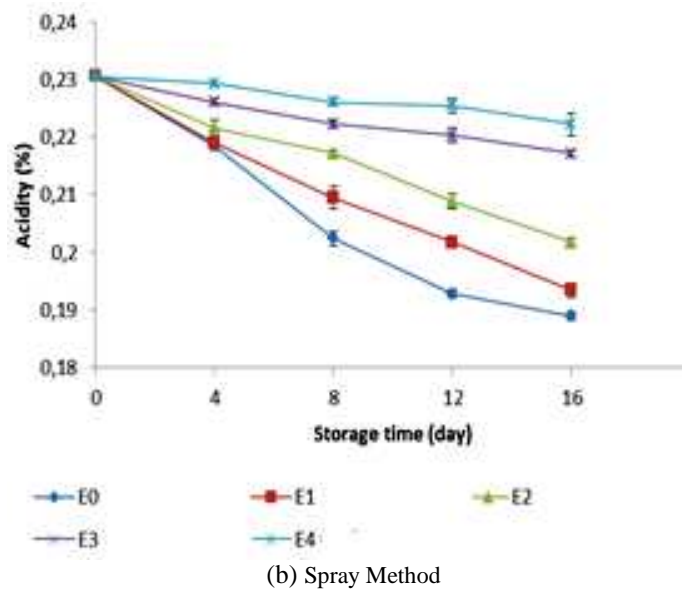


Figure 1. The Effect Concentration of Clove Oil at Titratable Acidity Test Using Brush Method (a) and Spray Method (b), where E0 = Control, E1 = 0.05wt%, E2 = 0.1 wt%, E3 = 0.15wt% and E4 = 0.2wt%

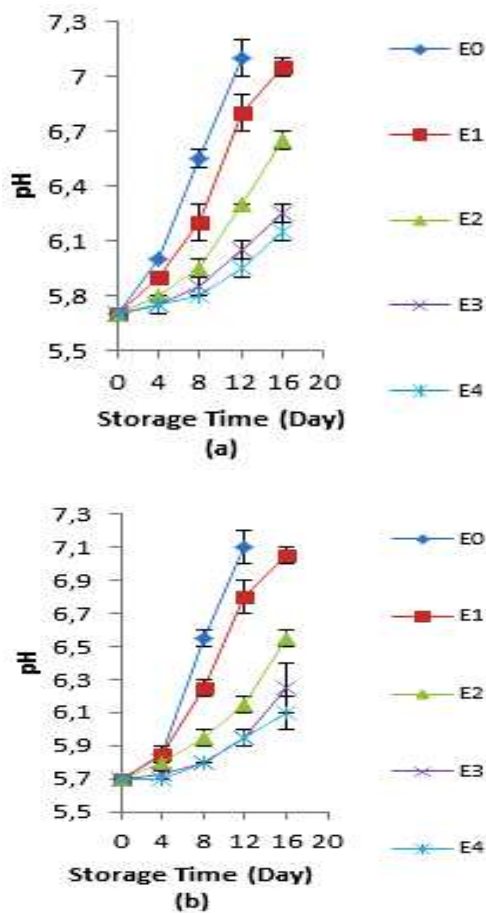


Figure 2. The Effect of Clove Oil Concentrations at pH in the Range 5.7–7.1 with (a) Brush and (b) Spray Method, where E0 = Control, E1 = 0.05wt%, E2 = 0.1 wt%, E3 = 0.15wt% and E4 = 0.2wt%

The increase in pH or the decrease in the acidity of the papaya fruits with the storage time can be caused by many factors.

Most of them is the absence of protective layer; therefore, the fruits had access to air exchange and large environmental contacts, allowing metabolic processes by microbial contaminants. It is suggested that the essential oil containing phenolic components, such as eugenol in clove oil, interacted with membrane cell [9]. Thus, these components affect the metabolic cycle and senescence. However, fungi and bacteria involved in the metabolic processes of papaya fruits remained unknown. Nevertheless, we found that susceptibility of bacteria to essential oils increased at low pH value. This has been attributed to an increase in hydrophobicity of the fruits [10], hence the lipids in the membrane cells of the target bacteria can be dissolved.

Antimicrobial activities. We found that the clove oil was able to reduce and diminish the growth of fungi and bacteria. The populations of fungi and bacteria significantly decreased in the presence of clove oil. More interestingly, the population of fungi and bacteria were efficiently reduced by 90% when the clove oil was more than 0.10% (Figure 3). This finding evidenced that clove oil can inhibit the growth of both fungi in PDA medium and bacteria in NA medium. Inhibition of growth of the microorganisms should be related to the high content of eugenol in the clove oil which acts an antimicrobial agent. Eugenol has abroad-spectrum antimicrobial effect against *Enterobacter* [11,12], and it is also active against gram-positive bacteria [13]. This further indicates that essential oils, such as clove oil

containing eugenol and other phenolic components, are potential for strong antibacterial activities against pathogens in food spoilage, as reported by Lambert et al. [14].

The phenolic components are considered to be able to destroy the outer membrane of gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP. The presence of eugenol which leads to a decrease in the function of the cell wall and cell lysis occurs in a high level. Essential oils can provoke depolarization of mitochondrial membrane by reducing the membrane potential in a prokaryotic cell [9], and they contribute in synthesis of bacterial toxins. Therefore, we proposed the mechanism of inhibition of growth of the microorganisms by clove oil as follows: (i) interaction of eugenol with their cell wall, (ii) reaction with the cell membrane, resulting in an increase in permeability and damage in the cell, and (iii) inactivation of enzymes that are responsible for growth and synthesis, leading to inhibition, destruction, or damage on genetic material [15].

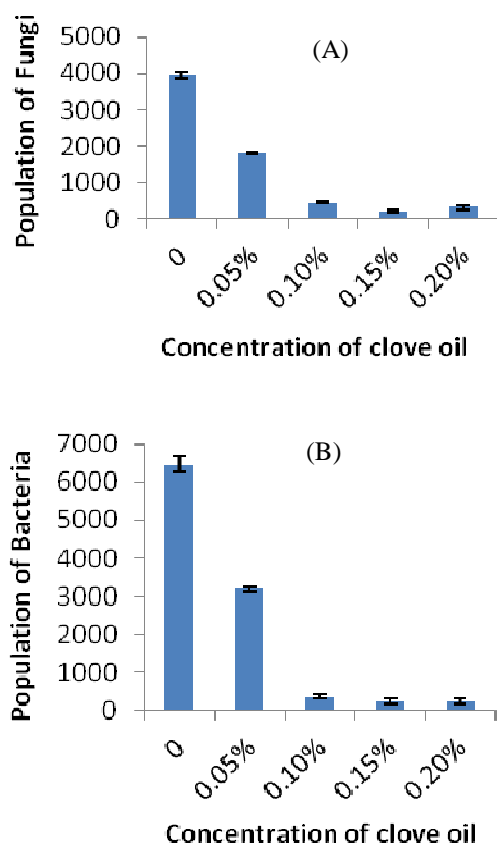


Figure 3. Effect Concentration of Clove Oil at Number of Microbial Colonies with:(A) Brush Method and (B) Spray Method

4. Conclusions

Physicochemical and antimicrobial activities of extracted clove oil as preservation in papaya fruits were investigated. We demonstrated that the clove oil shows fascinating antifungal and antibacterial activities, thus it can be a useful active preservative for edible coating of papaya fruits. We also show that both spray and brush methods could be generalized to deliver the extracted clove oil in packaging technology, as the two methods exhibit the similar effects on the pH, weight loss, and acidity of the papaya fruits.

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