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Research article

The effects of alginate-based edible coating enriched with green grass jelly and vanilla essential oils for controlling bacterial growth and shelf life of water apples

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Abstract: This research aimed to determine the addition effect of green grass jelly and vanilla essential oils in alginate-based edible coating to extend the shelf life of water apples (*Syzygium samarangense* cv. Citra). The water apples were coated with five treatments consisting of different essential oils concentrations. Measurements of physical (weight loss), chemical (total soluble solids and titratable acidity), sensory and antibacterial (bacterial growth inhibition) properties were conducted. Each treatment was replicated three times and the results were analyzed using Completely Randomized Design (CRD). The results showed that the application of an edible coating combined with green grass jelly and vanilla essential oils produced a significantly different effect from treatments that did not use a coating. Samples treated with an edible coating showed slower biochemical changes towards ripening, better sensory qualities, and antibacterial properties. The addition of a green grass jelly and the vanilla essential oils into alginate-based edible coating were able to maintain quality and extend the shelf life of water apple fruit.
Keywords: green grass jelly; vanilla, essential oils; shelf life; water apple; edible coating

1. Introduction

The water apple (*Syzygium samarangense* cv. Citra), a local fruit found in East Java also known as wax apple or Java apple, is rich in biochemicals that are beneficial for human health. A study found that water apples have antioxidant compounds (e.g. 7-hydroxyflavone, myricetin and vitamin C), antidiabetic (e.g. 4-Hydroxybenzaldehyde, phloretin, myrigalon-G), and anticancer (2' 4'-dihydroxy-6'-methoxy-3' 5'-dimethylchalcone) [1,2]. Additionally, compounds such as flavonoid, phenolic, and tannin that have antimicrobial activities were also found in water apples [1,3]. However, similar to other fruits, the water apple has a relatively short shelf life and is more susceptible to microbial spoilage when damaged. During room temperature storage (23 ± 1 ℃, bacterial and fungal spoilage were responsible for losses of water apple fruit qualities. The spoilage bacteria *Klebsiella penumoniae* has the highest prevalence of 47.78%, while the *Penicillium purpurogenum* showed the highest prevalence among other spoilage fungi with a prevalence of 38.46% [4].

The most commonly used post-harvest treatment of water apple fruit is rinsing and air-drying by the farmer. But there were no methods added to prolong the shelf-life. Previous studies had been conducted to investigate various preservation methods of water apples. A study by Anandarista et al. (2019) reported significant differences in moisture content, total titratable acidity, texture, vitamin C content, and sensory analysis of water apple fruit that was treated with CO_2 gas packaging [5]. However, no significant difference was observed in weight loss and ash content. Another preservation method that can be applied in water apple fruit is the application of an edible coating. Sumanti et al. (2020), investigated the application of basil leaf oleoresin potential as an edible coating on extending the shelf life of water apple. Regardless, no significant difference was reported between treated and untreated samples [6].

Edible coatings based on alginate have been attracting interest in extending the shelf life of fruits. Fruits that were treated with an alginate-based edible coating showed a decreased respiration rate, as alginate has a selective permeability mechanism towards O_2 and CO_2 gases. Moreover, alginate layers have a swelling ratio and water solubility that improve the functionality of an edible coating for high-moisture surfaces of fresh-cut fruits [7]. Edible coating films of alginate can be improved by adding other components such as antioxidants and antimicrobial agents to prolong a fruit’s storage life which can prevent outgrowth of microorganism spoilage [8].

An example of antimicrobial agents added to the alginate-based edible coating is vanilla essential oils. A study investigated the effect of an edible coating consisting of alginate-based films with added vanilla essential oils on fresh-cut red pitaya; it reported that the fruit with the highest concentration of the edible coating layer exhibited the highest inhibition level of fungal spoilage [9]. Another example of compounds with antimicrobial activity that can be potentially used is green grass jelly essential oils [10]. The hydrocolloid of green grass jelly is used to delay the ripening of banana [11].

Although each component has a preservation effect, the combination of alginate-based with the addition of antimicrobial agents such as green grass jelly and vanilla essential oils as edible coating, in particular, to delay bacterial growth has not been investigated yet. Therefore, this study aimed on investigating the effect of alginate-based edible coating enriched with green grass jelly and vanilla essential oils as antimicrobial agents in controlling bacterial growth and preservation methods to extend the shelf life of water apples.

2. Research methods

A preliminary investigation was conducted to determine the concentration of essential oils that would be used in this study. To investigate the edible coating effect on the shelf life of water apples, microbiological (bacterial growth), physical, chemical, and organoleptic analyses were conducted.

2.1. Determination of vanilla essential oils concentration

Before the addition of essential oil in the edible coating mixture, the adequate concentration was determined. The concentration should be kept at the least amount for economic purposes, but also showed maximum inhibition of bacterial growth. The concentration of green grass jelly essential oils used in this study had been determined by a previous study [12]. In this study, the adequate concentration of vanilla the maximum inhibition was determined using the paper diffusion method and expressed by the minimum inhibitory concentration.

The determination was started by isolating bacteria from spoiled water apple fruit. The spoiled water apples that rot during its time in the tree were chosen according to the weight criteria of ±100 grams. The chosen fruit was cut and mashed using a mortar. One gram of mashed fruit was inserted in an Erlenmeyer flask filled with 99 mL of water. The flask was stirred until homogenous and 1 mL of the solution was added to get a dilution series of 10^{-4}. The diluting steps were repeated until a dilution series of 10^{-10} was obtained. Each suspension with dilution series of 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10} was taken about 0.1 mL and transferred into Petri dishes PCA (Merck, Darmstadt, Germany). The Petri dishes were incubated for 48 hours at room temperature and then observed for any bacterial growth. The bacteria that grew in the plate were inoculated and cultured into PCA media [13].

The paper disk method as a preliminary test was conducted according to a study by Mith et al. (2014) with some modifications [14]. The suspension of bacteria was poured into the surface of a petri dish filled with PCA agar. A paper disk with 1 cm diameters treated with four different concentrations of vanilla essential oil was put on top of the media. The concentrations used were 0%, 0.1%, 0.3%, and 0.6%. The petri dish was incubated at room temperature for 48 hours. The inhibition zone observed was measured using calipers and the results were expressed in cm.

2.2. Sample preparation

A sample of 205 water apples of similar size and age (two months) was chosen. The selected water apple had ±100 grams of weight. The fruits were washed with sodium bisulfate (0.2 gram/L) and dried before being stored at a temperature of 14 ℃ until they were tested.

2.3. Production and application of the edible coating

The production of the edible coating was started by the making of green grass jelly powder using the method by Nurmalasari et al. (2017) [15]. Green grass jelly leaves were washed with water and dried in an oven at 50 ℃ for 18 hours. The dried leaves were ground and sieved using a 0.5 mm diameter sieve, producing a green grass jelly powder. In accordance to the method established by Olivas et al. (2007), the edible coating was produced by diluting the powdered green grass jelly in 1 L of water and stirred at 50 ℃ for 30 minutes About 20 gram/L of alginate was added and the solution was stirred again at 75 ℃ for 30 minutes until homogenous. Lastly, glycerol (0.5 gram/L) was added and stirred at 85 ℃ for 10 minutes and vanilla essential oils (Lansida, Yogyakarta, Indonesia) were added. For the application of the edible coating, dried fruits were dipped into solutions of edible coating for 3 minutes and then submerged in a solution of 2% $CaCl_2$ for ±15 minutes until the layer was formed [16]. Five different treatments were applied, as follows:

A: 0.2% green grass jelly + 0.3% vanilla essential oils

B: 0.2% green grass jelly + 0.6% vanilla essential oils
C: 0.4% green grass jelly + 0.3% vanilla essential oils
D: 0.4% green grass jelly + 0.6% vanilla essential oils
E: No edible coating applied

After the application of the edible coating, the water apples were dried, packed, and stored at 14 ℃ during observation days.

2.4. Physical parameters analysis

The analysis of physical parameters was conducted using weight loss measurement. From a total of 10 samples in each different treatment groups investigated, three water apples were chosen randomly to represent the group and measured for its weight. The weight loss was calculated every three day during three weeks of storage at 14 ℃. The weight loss was reported as a percentage and was calculated by dividing the difference between initial and final weight loss by the initial weight.

2.5. Chemical parameters analysis

2.5.1 Total soluble solids

From a box of 10 water apple fruit, three samples were chosen randomly during weight loss analysis were used in the total soluble solids' analysis. The samples were mashed using a mortar and were taken via pipette for transfer to the handheld digital refractometer (ATAGO, Tokyo, Japan). This analysis was conducted after the fruits were stored at 14 ℃ after storage days of 0, 3, 6, 9, 12, and 15. The total mass solids were reported in the percentage Brix unit.

2.5.2. Titratable acidity

An analysis of titratable acidity was conducted to investigate total organic acid in the sample. The titratable acidity measurement was conducted as previously done by Nejad et al. (2014) [17]. The mashed samples from total soluble solids were measured into 5 grams and inserted into a 100 mL volumetric flask. The flask was filled with water to the mark and stirred until homogenous. The solution was filtered, and 10 mL of the filtrate was transferred in an Erlenmeyer. Two to three drops of indicator phenolphthalein (PP) were dripped into the solution before titration with NaOH 0.1 N was conducted. The measurement was done once every three days while the samples were stored at 14 ℃.

2.6. Sensory analysis

From a box consist of 10 water fruit apples, three pieces were chosen randomly for each different edible coating treatment to be measured according to its sensory qualities. The organoleptic parameters investigated were color, texture, aroma, taste, and overall likeness. This analysis was conducted once every three days by trained panelists as the samples were stored at 14 ℃. The panelists were asked to score each parameter using a 5-point scale, with score 1 as the lowest and score 5 as the highest.

2.7. Antibacterial analysis

The analysis in this study was done by determining the inhibition of bacterial growth on different concentrations of essential oils added in the alginate-based edible coating. The analysis was conducted by the paper diffusion method as previously done by Mith et al. (2014) with some modifications [14]. The measurement was started by preparing the bacterial suspension retrieved from water apple fruit. The samples were selected by randomly choosing one piece of water apple fruit. The chosen sample were mashed using a mortar, weighed for 1 gram, and inserted into an Erlenmeyer filled with 99 mL of distilled water. The solution was mixed until homogenous to become a dilution of 10^{-2}. The filtrate was taken for 1 mL and added to a test tube filled with 9 mL of distilled water (10^{-3}). The dilution was repeated until the distillation series of 10^{-7}. From the dilution series 10^{-5}, 10^{-6}, and 10^{-7}, 0.1 mL of solution was transferred into five Petri dishes with PCA agar (Merck, Darmstadt, Germany) and spread out using a spatula. A paper disk with 1 cm diameters that had been added with different concentrations of essential oils according to the specified treatment was placed on top of the PCA agar. The plates were stored at 14 ℃ for 15 days and the inhibition zone was observed on day 0, 4, 8, 12, and 15.

2.8. Data analysis

This study was compiled based on a Completely Randomized Design (CRD) with a single factor design. All data were analyzed using Duncan's multiple range test with a significant level of ≤ 0.05.

3. Results and discussion

3.1. Determination of vanilla essential oils concentration

To determine the amount of vanilla essential oils concentration that will be used, the concentration with the highest minimum inhibitory concentrations will be chosen. According to Table 1, the concentration of 0.3% and 0.6% vanilla essential oil showed a high minimum inhibitory concentration with similar results. Therefore, two variation concentrations of vanilla essential oils added to the edible coating mixture were 0.3% and 0.6% in this study. Vanilla is one of the most potent plant sources of antimicrobial components [18]. The vanilla essential oils can significantly inhibit the growth of *S. aureus, E. coli*, and *L. innocua* [19,20]. The effect of vanilla essential oils is bactericidal and give less severe membrane damage when compared to the phenolic flavor compound, carvacrol [20].

3.2. Wight loss

Weight loss of harvested fruit is generally caused by moisture loss from transpiration [21]. The weight loss of the water apple samples showed an increasing trend for all different treatments throughout the observation days (Table 2). The samples with edible coating treatments did not show a significant difference in weight loss. This implies that green grass jelly and vanilla essential oils as an edible coating cannot reduce the weight loss rate of water apples. In fruit and vegetables, weight loss can happen during storage and is strongly linked with water loss [22]. When the fruit was harvested from the plants, it used its water content for the transpiration process. Weight loss is an undesirable parameter in fresh fruits as it may reduce the fruits' economic value [23]. According to Krochta et al. (1994), the hydrocolloid and lipids that formed the edible coating layer were able to maintain O_2 and CO_2 gases, but the coating layer had a weak ability to withstand moisture loss as it was hydrophilic. Thus, the application of alginate-based coating with the addition of green grass jelly and vanilla essential oils may not be able to reduce moisture loss, which consequently did not hinder the weight loss rate.

Table 1. Minimum inhibitory concentration of vanilla essential oils (cm).

Dilution	The concentration of vanilla essential oil (%)			
	0	0.1	0.3	0.6
10^{-7}	0	0.28	0.29	0.19
10^{-8}	0.14	0	0.39	0.38
10^{-9}	0	0.19	0.38	0.14
10^{-10}	0	0.14	0.39	0.49
Total	0.035	0.1525	0.3625	0.3

Table 2. Weight loss analysis result (%).

Treatments	Observation days				
	3	6	9	12	15
A	1.34[a]	2.16[a]	2.83[a]	3.64[a]	4.75[a]
B	1.61[a]	2.30[a]	2.73[a]	3.37[a]	4.20[a]
C	1.31[a]	2.09[a]	2.67[a]	3.36[a]	4.32[a]
D	1.28[a]	1.76[a]	2.48[a]	3.35[a]	4.23[a]
E	1.34[a]	1.84[a]	2.69[a]	3.21[a]	4.23[a]

Note: The results with the same superscript letter(s) and order indicate that no significant difference was found between the results ($p > 0.05$) using the Duncan Multiple Range Test.

3.3. Total soluble solids

From Figure 1, it can be seen that the total soluble solids showed fluctuating results throughout the observation days. In Table 3, the total soluble solids result observed after varied treatments showed significant differences during observation days, except on observation day 6. The total soluble solids can be used to indicate the ripening of fruit. As reported in a previous study by Rooban et al. (2016), fruits will have a low level of total soluble solids, which will increase through the ripening process. The change of soluble solids may decrease at the start due to the conversion of starch from sugar, and less sweet fruit will be produced. However, the change will gradually increase because of the re-conversion of starch into simple sugars [26].

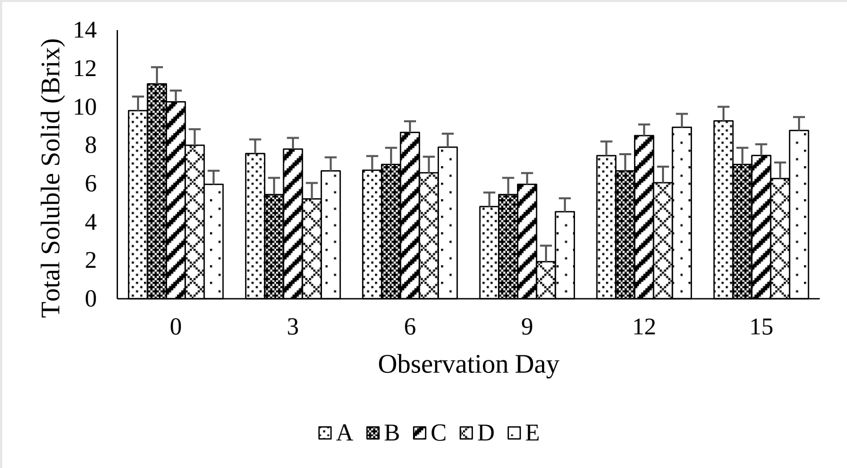

Figure 1. Total soluble solids analysis result.

Table 3. Total Soluble Solids Analysis Result (%Brix).

Treatments	Observation days					
	0	3	6	9	12	15
A	9.80[a]	7.56[ab]	6.70[a]	4.80[a]	7.46[b]	9.26[a]
B	11.20[a]	5.43[bc]	7.00[a]	5.43[a]	6.66[c]	7.00[b]
C	10.26[a]	7.80[a]	8.66[a]	5.96[a]	8.50[a]	7.46[b]
D	8.00[ab]	5.2[c]	6.56[a]	1.93[b]	6.05[d]	6.26[c]
E	5.96[b]	6.66[abc]	7.90[a]	4.53[a]	8.93[a]	8.76[a]

Note: The results with the same superscript letter(s) and order indicate that no significant difference was found between the results ($p > 0.05$) using the Duncan Multiple Range Test.

Over the last three days of observation, the samples treated with the highest edible coating concentration combination significantly showed the lowest increase rate of total soluble solids. The results indicate that the composition of the edible coating used can increase the shelf life of water apples. The change of total soluble solids is highly correlated with the respiration rate of fruit after harvested. The sugar content of fruit is used in the biochemical process, including respiration. A low respiration rate indicates a slower increase of sugar conversion to simple sugars, consequently extending the shelf life [27,28].

3.4. Titratable acidity

The titratable acidity of water apples with different treatments of edible coating is shown in Figure 2. A sudden high increase of titratable acidity was observed in the samples with no edible coating on day 9. On the last observation day, samples treated with the lowest concentration of green grass jelly and vanilla essential oils had the same result of titratable acidity (0.38%) as untreated samples. In contrast, the samples with the highest concentration of essential oil edible coating showed the lowest titratable acidity (0.26%). However, there was no significant difference between the different treatments on the last observation day as seen in Table 4.

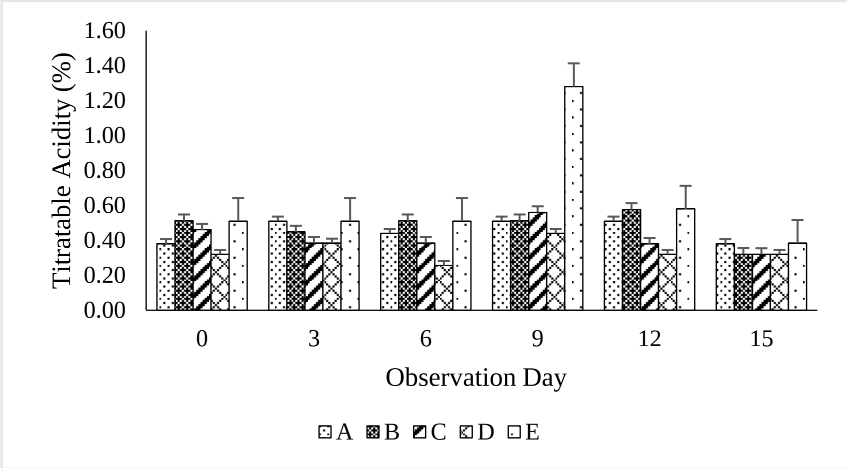

Figure 2 Titratable Acidity Analysis Result.

Table 4 Total Titratable Acid Analysis Result (%).

Treatments	Observation Days					
	0	3	6	9	12	15
A	0.38^{ab}	0.51^{a}	0.44^{a}	0.51^{b}	0.51^{a}	0.38^{a}
B	0.51^{a}	0.44^{a}	0.51^{a}	0.51^{b}	0.57^{a}	0.32^{a}
C	0.46^{ab}	0.38^{a}	0.38^{a}	0.57^{b}	0.38^{ab}	0.32^{a}
D	0.32^{b}	0.38^{a}	0.25^{a}	0.44^{b}	0.32^{a}	0.26^{a}
E	0.51^{a}	0.51^{a}	0.51^{a}	1.28^{a}	0.57^{a}	0.38^{a}

Note: The results with the same superscript letter(s) and order indicate that no significant difference was found between the results ($p > 0.05$) using the Duncan Multiple Range Test.

The level of titratable acidity explained the changes in organic acid during ripening and storing in fruit. In relation to shelf-life, titratable acidity will decrease with the increase of storage time [29]. The decrease of titratable acidity was highly related to high respiration and ripening rate as the organic acid was used as one of the main substrates on respiration [30]. Thus, the higher rate of titratable acidity loss indicates a shorter shelf-life.

Figure 2 shows that the samples that were not treated with an edible coating had a great increase followed by a fast decrease of titratable acidity. This could happen due to the samples with no edible coating had reached its peak ripening state. A similar result was reported by Rooban et al. (2016), which explained that titratable acidity will increase significantly and then decrease during the ripening period of both climacteric and non-climacteric fruit [25]. As the fruit undergoes ripening, the acidity level will decline due to the decarboxylation of carboxylates and gluconeogenesis. Organic acid acts as the main respiratory substrate and is converted to a greater length of other substances, thus it will decrease during ripening [31,32]. Samples that were treated with an alginate-based edible coating and essential oils did not go through changes as the untreated samples did, indicating that the treated samples had not reached its peak of ripe state. The trend of titratable acidity in samples with the highest concentration of essential oils was the most stable throughout the observation days. This implies that the analyzed edible coating can extend the shelf life of water apples.

3.5. *Sensory analysis*

Based on the sensory analysis (Figure 3), samples coated with the highest concentration of essential oils had the best score on color and taste parameters. The samples with treatment A, which were applied with the alginate-based edible coating with the addition of 0.2% green grass jelly and 0.3% vanilla essential oils, showed the highest texture and overall score. On the other hand, the samples with no edible coating treatment had the lowest score on all parameters compared to samples coated with an alginate-based and essential oils coating. The edible coating causes water apples to have slower respiration and transpiration rates, which will delay certain biochemical changes, including decreased organoleptic parameters due to ripening. These results were also shown in previous studies, which reported the highest sensory measurement level in fruits that were treated with an edible coating [33–36].

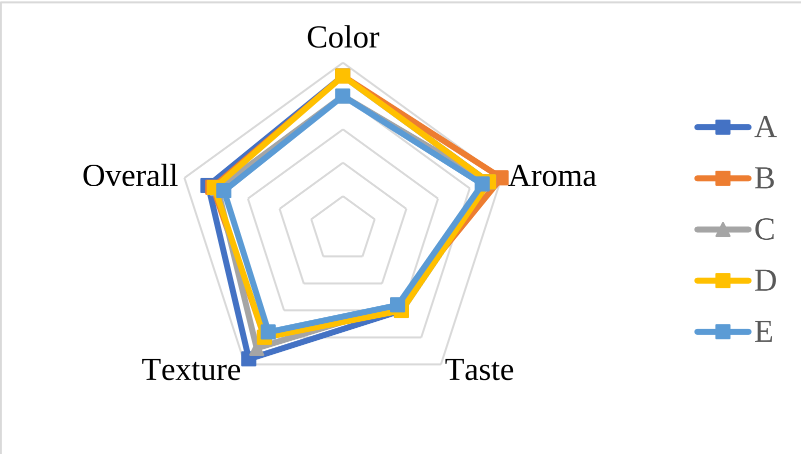

Figure 3. Sensory analysis result.

3.6. Antibacterial analysis

The inhibition activity towards bacterial growth of an alginate-based edible coating with different concentrations of essential oils was measured using the disk diffusion method. After each observation day, the inhibition zone on the top of the filter paper disk indicated the antibacterial properties of the edible coating [37]. The inhibition zone of different edible coating treatments of water apples is shown in Figure 4. Samples treated with the highest concentration of an alginate-based coating with added green grass jelly and vanilla essential oils had an inhibition zone towards bacterial growth on all observation days.

Similar studies reported the antibacterial activity of green grass jelly and vanilla essential oils. According to Kusmardiyani, Insanu, and Asyhar (2014), green grass jelly contains the flavonoid compound of the 3-*O*-glycosidic flavonol. A study by [38] investigated green grass jelly antimicrobial properties in *Salmonella typhi* biofilm formation. Cava-Roda et al. (2012) reported the antimicrobial activity of vanilla essential oils against *L. monocytogenes* and *E. coli* O157: H7 in whole and skim milk. The study also stated that the inhibitory action of vanilla was bacteriostatic, not bactericidal. According to Fitzgerald et al. (2005), aldehyde groups such as 3-anisaldehyde or guaiacol in vanilla are compounds with antimicrobial activity. At a low pH such as in fruits, the antimicrobial activity will be enhanced due to better binding between antimicrobial molecules and the hydrophobic zone of the membrane, thus improving its antimicrobial activity [41].

On observation days 8 and 12, samples with no edible coating produced an inhibitory zone. This can be caused by the compounds originated from a water apple fruit that had antibacterial activity. The water apple fruit is naturally containing polyphenolic compounds such as ethanol and methanol, which have antimicrobial properties [42].

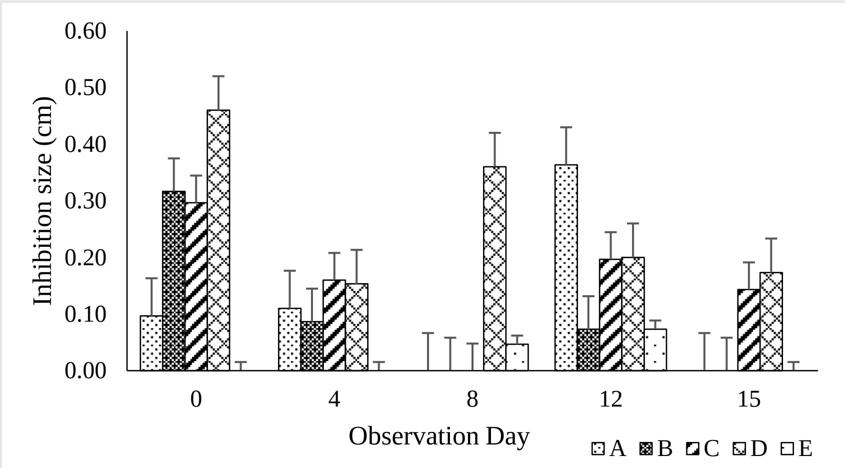

Figure 4. Minimum inhibitory zone analysis result.

4. Conclusions

This study reported that there were no significant differences in weight loss and titratable acidity for different concentrations of green grass jelly and vanilla essential oil in an alginate-based edible coating of water apple fruit. The samples treated with 0.4% green grass jelly and 0.6% vanilla essential oils addition exhibit the significantly lowest total soluble solids and widest inhibitory zone towards bacterial growth. From the sensory analysis, the addition of 0.2% green grass jelly and 0.3% vanilla essential oils showed the highest overall score. These results indicate that the addition of green grass jelly and vanilla essential on the application of alginate-based edible coating can be conducted to hinder bacterial growth and prolong the shelf-life of water apple fruit.

Conflict of interest

All authors declare no conflicts of interest in this paper.

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