

Combination of alginate based edible coating-betel essential oil in extending the shelf life of rose apple cv. Dalhari (*Syzygium samarangense*)

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Data Availability Statement:

All relevant data are within the paper and its Supporting Information files.

Competing Interests:

The authors declare no competing interests.

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Abstract: Rose apple cv. Dalhari is a local fruit grown in Berbah District, Special Region of Yogyakarta. This fruit perishable, easily loses its water content and is attacked by microbes. This research aimed to determine the best combination between alginate and betel essential oil to inhibit the growth of microbes and maintain quality of rose apple cv. Dalhari. Alginate and betel essential oil treatments were used at three concentration levels, alone and in combinations, respectively of 2%, 2.5%, 3% and 0%, 0.1%, 0.2%. The results showed that combination treatment of 2.5% alginate and 0.1% betel essential oil was the most effective to maintain the fruit quality. The sole addition of betel essential oil was not able to inhibit microbial growth. Furthermore, combination of alginate based edible coating and betel essential oil was able to maintain the quality of rose apple cv. Dalhari up to nine days.

1. Introduction

Rose apple cv. Dalhari is locally cultivated at Berbah District, Yogyakarta. Rose apple cv. Dalhari is one of the most famous fruits due to its large size, slightly fresh sour taste, and high water content. While, rose apple has thin skin makes low ability to maintain its water content and also is easily growth by microbes when it is kept at room temperature (Pertiwi *et al.*, 2012). Edible coating is an alternative technology to overcome the problem and its widely known as the ability to maintain the atmosphere condition around the fruit, such as control gas exchange and control water loss, maintain the fruit's texture, and also reduce the risk of microbial attack (Campos *et al.*, 2011).

Edible coating formulation can be derived from polysaccharides, lipids, proteins as long as the material are tasteless, odourless, and transparent. Polysaccharides include alginate that is widely used as primary ingredient for edible coating due to its ability in holding high oxygen and in resulting good quality of edible coating (Quirós-Sauceda *et al.*, 2014). Alginate is obtained from marine brown algae (*Phaeophyceae*) extraction. Recently, the use of edible coating in fruit preservation is combined with another

active ingredient, such as antibrowning agent, nutrients, and antimicrobial agent to decrease microbial growth (Campos *et al.*, 2011; Guerreiro *et al.*, 2015; Atarés and Chiralt, 2016; Hamed *et al.*, 2017).

Some essential oils have shown their effectiveness as food preservatives due to their antimicrobial activity and antioxidant content. Betel essential oil contains chavicol, eugenol, acetyl eugenol as the main compounds to prevent pathogen growth in different edible commodities (Prakash *et al.*, 2010; Basak and Guha, 2015). However there are still limited information available regarding the use of edible coating to maintain the freshness of rose apple cv. Dalhari. The only report from previous authors refers to effect of storage temperature of rose apple cv. Dalhari (Widoyo, 2013), and none mentioned the use of edible coatings. This study aimed to determine the effect of betel essential oil combined with alginate-based edible coatings to prevent pathogen growth, and extend the shelf life of rose apple cv. Dalhari.

2. Materials and Methods

Rose apple Fruit cv. Dalhari was harvested in Berbah District, Special Region of Yogyakarta, Indonesia in the middle of April, 60 days after flowering. Harvested fruit has characteristic red colour skin and average weight 125 g/fruit from 243 fruits. The fruit was immediately brought to Postharvest Laboratory at the Universitas Muhammadiyah Yogyakarta. The fruits were selected based on defect and size, then stored in a refrigerator (14°C) until treatment. Food grade Sodium Alginate (AL) (IIS, Indonesia) was used for coating treatment, glycerol as a plasticizer, and betel essential oil (BE).

Preparation and application of edible coating

Edible coating was prepared with diluted alginate into aquadest solution and heated in 85°C. Solution was added with 1.5% glycerol and betel essential oil in different concentration. Alginate-betel essential oil solution were formulated into nine treatments: AL 2% (w/v) (A1S0); AL 2% (w/v)-BE 0.1% (v/v) (A1S1); AL 2% (w/v)-BE 0.2% (v/v) (A1S2); AL 2.5% (w/v) (A2S0); AL 2.5% (w/v)-BE 0.1% (v/v) (A2S1); AL 2.5% (w/v)-BE 0.2% (v/v) (A2S2); AL 3% (w/v) (A3S0); AL 3% (w/v)-BE 0.1% (v/v) (A3S1); AL 3% (w/v)-BE 0.2% (v/v) (A3S2).

Each treatment was performed in several steps as

follow: the fruit was washed using sodium hypochlorite 200 µl L⁻¹ and dried at room temperature followed by dipping in edible coating solution for 2 min. The excess of edible coating solution was dripped off for 30 s before being dipped in CaCl 2% solution for 1 min. Three treated fruits were packaged with polypropylene trays with perforated cover and stored at 14°C until analysis. Analysis was performed on days 0, 3, 6, 9, 12, and 15.

Fruit quality assessment

Twenty seven groups of rose apple (9 treatments x 3 replicates) were weighed during the storage time. The weight loss (WL) of rose apple was determined using the equation:

$$WL (\%) = [(W_0 - W_t) / W_0] \times 100$$

where W₀ is the initial weight and W_t is the sample weight at time t. Fruit firmness was measured using a Fruit Hardness Tester FHT200 (Extech Instruments, USA). Each fruit was tested in three different sides with a 6 mm diameter probe on equatorial position and recorded in N/mm². Total Acidity (TA) determination was performed with methods according to ISO 750-1998. Five grams of rose apple slices were homogenised and diluted to 100 mL with distilled water. The mixture was filtered and was added 3-5 drops of phenolphthalein (1% in 95% ethanol) to 10 mL solution. The solution was then stirred and titrated with 0.1 N NaOH. TA was determined as a percent of citric acid. Total Soluble Solid (TSS) was determined using a hand-refractometer (Atago, Japan). The tools determines the juice TSS content after being homogenised the flesh and it was expressed as a brix (%). Reducing Sugar (RS) were determined according to Nelson-Somogyi Technique (Nelson, 1944) and the result showed in percentage. The analysis was performed in 540 nm abs using Spectrophotometer UVmini-1240 (Shimadzu, Japan).

Antibacteria and antiyeast activity

Bacteria and yeast were isolated from decayed rose apple using paper discs and plate count. Suspension was obtained from diluting 1 gr samples on distilled water in seven-fold dilution series. A petri dish was prepared with poured 10 ml nutrient agar medium and allowed to solidify. The 0.1% inoculum suspension was poured and flattened on the medium. Sterilized filter paper disc dipped with 0.1% betel essential oil for 15 min and then placed on the surface of a medium. The petri incubated at room temperature for 48 h. After incubation time, measurement the inhibition zones were done with a

ruler. In the other hand, 0.1% of betel essential oil was poured in medium before inoculated with 0.1 ml inoculum. After 48 h incubation at room temperature, the visible microbe was counted and the result was presented in log Colony Forming Unit (CFU).

After treatment was done, all samples were analyzed with microbial count for bacterial and yeasts based on Mola *et al.* (2017). One gram of homogenized sample was diluted using 9 ml sterilized water. Sample then diluted in seven-fold dilution series before plating. A 0.1 ml solution was poured on Plate Count Agar (PCA) surface and Potato Dextrose Agar (PDA) for enumerating total bacteria and molds. Microbiological analysis was performed in three replication and the results were expressed as CFU (Colony Forming Units) per grams fresh weight.

Sensory analysis

The sensory was analysed by 15 semi-trained panellists on the based of 5-point hedonic scales (1=very dislike; 2=dislike; 3=neither like or dislike; 4=like; 5= like very much) for the parameters of texture and appearance according to Guerreiro *et al.* (2015) with some modification. The panellists were recruited from staff and students of the department. They were trained in the initial test to be familiar with the fruit. All samples were analyzed on 0, 3, 6, 9, 12, 15 days after treatment.

Statistical analysis

The experiment was carried out in randomised experimental design. The experiment data were analysed using SAS statistical software package 9.4 for windows and Duncan multiple-range test ($P < 0.05$) was performed for mean comparisons.

3. Results

Weight loss is one of the most important freshness indicators in fruit. As shown in figure 1, the weight loss increases in all fruit treatment during 15 days storage. However, a combination of alginate 2.5% and betel essential oil 0.1% can significantly ($P < 0.05$) prevent the fruit weight loss, while the treatment of alginate 2% has shown the highest weight loss. Weight loss of rose apple has no relation to its firmness. It can be shown in figure 2. Although it shows that the value of firmness is not constant, overall the trend tends to show the similar pattern

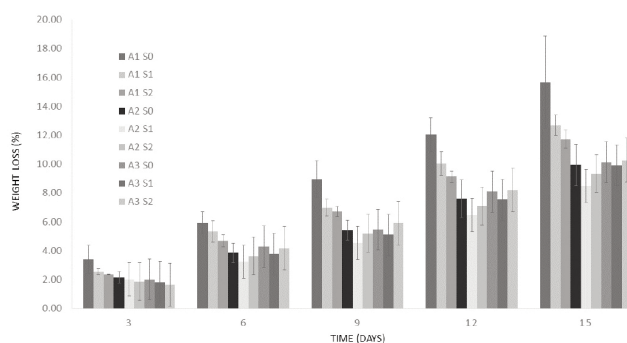


Fig. 1 - Effect of alginate based edible coating-betel essential oil on weight loss of rose-apple cv. Dalhari.

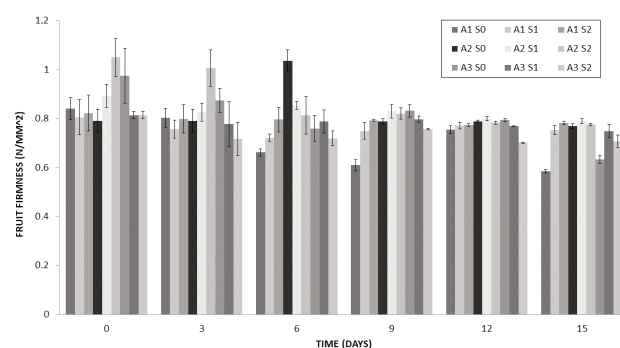


Fig. 2 - Effect of alginate based edible coating-betel essential oil on fruit firmness of rose-apple cv. Dalhari.

during 15 days of storage. Alginate can maintain the fruit firmness' decline even there is no significant effect between all treatment ($P > 0.05$). A similar trend was also shown in pineapples (Azarakhsh *et al.*, 2012), and apple (Rojas-Graü *et al.*, 2007 a) (Oms-Oliu *et al.*, 2008).

The TA, TSS and RS content show a regular decreasing during 15 days of storage time. The TA decreases after three days storage time in all treatment. However, as can be seen in figure 3A, the fruit treated with 3% alginate shows slower TA reduction. The value of TSS slowly decreases on all treatments during 15 days storage (Fig. 3B) despite no significant effect ($P > 0.05$) is observed. Rose apple fruit being non-climacteric fruit, tends to maintain similar TSS content during storage. Concerning RS content, there is no significant effect ($P > 0.05$) in all treatments. RS value decreased after three days storage as showed in figure 3C.

The effect of the edible coating containing a different concentration of betel essential oil on microbial growth is shown in figure 4A and 4B. As

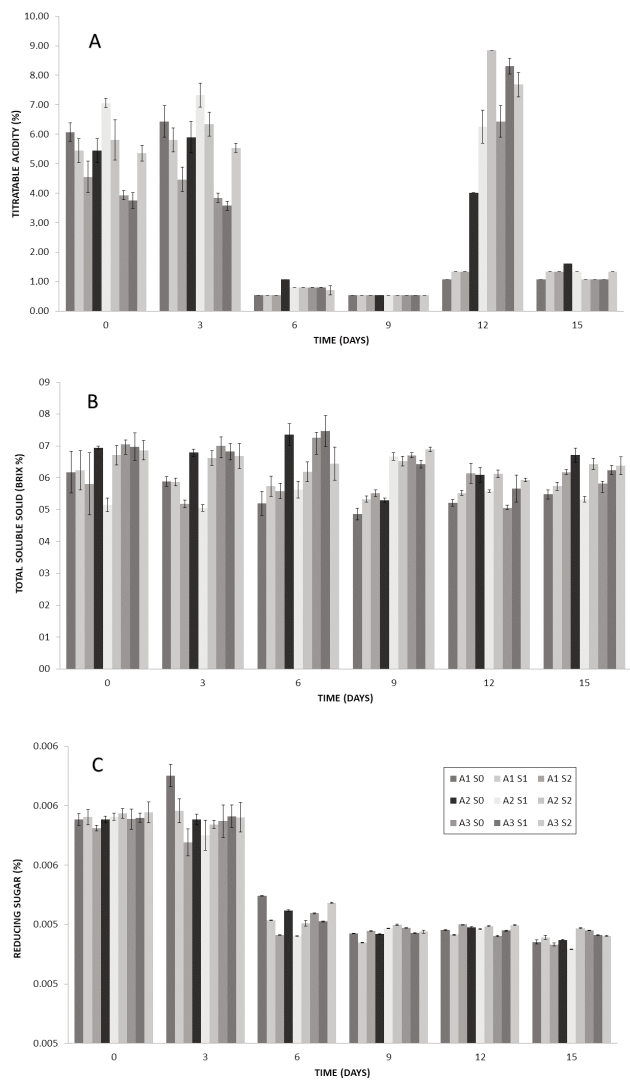


Fig. 3 - Effect of alginate based edible coating-betel essential oil on TA (A), TSS (B) and RS (C) of rose-apple cv. Dalhari.

shown in the figure, edible coating can inhibit both bacteria and yeast in rose apple cv. Dalhari. The increase in the population of both bacteria and yeast is observed during 15 days of storage time. The addition of betel essential oil can inhibit the yeast growth until nine days, while bacteria can be shown in no essential oil addition treatment after six days storage (Fig. 4A).

The sensory quality evaluation is not significantly different ($P>0.05$) during 15 days of storage time based on both treatments. The result indicates that different changes happen in the treatment during storage time although panellists did not apprehend those differences. The result also showed that rose apple is not suitable for consumption at 9 d after storage since most panellists give a score under minimum acceptable value in overall score (Fig. 5).

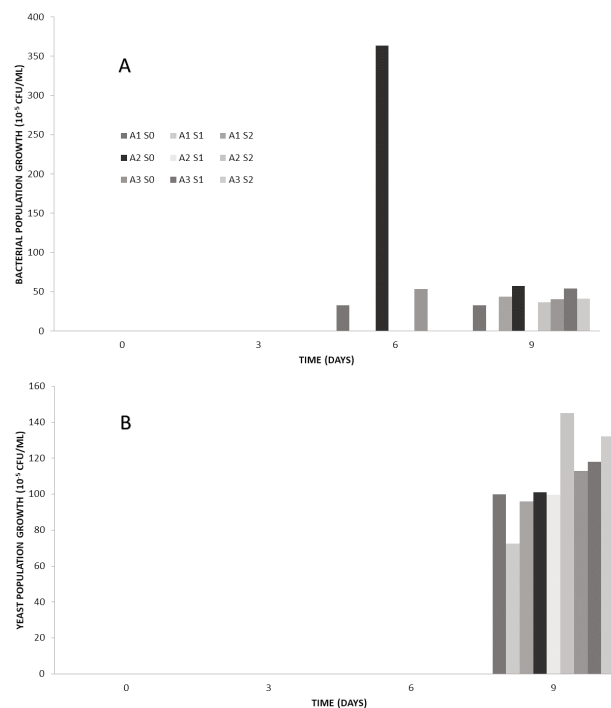


Fig. 4 - Effect of alginate based edible coating-betel essential oil on bacteria (A) and yeast (B) population of rose-apple.

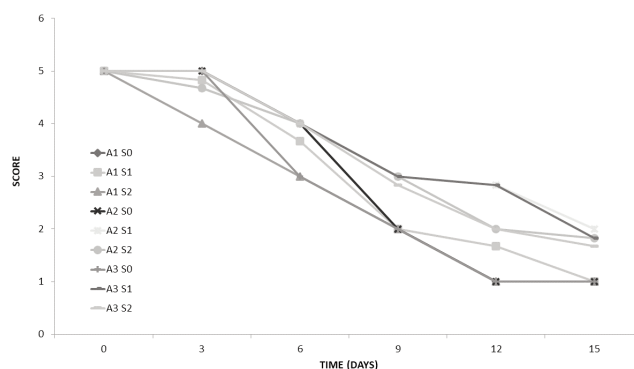


Fig. 5 - Effect of alginate based edible coating-betel essential oil on panelist preferences of rose-apple cv. Dalhari.

4. Discussion and Conclusions

Reducing effect from essential oil to weight loss can be described with conjunction of betel essential oil with polysaccharides based edible coating. This relation make the edible coating more stable and permeable. Similar result was described in the experiment using lemongrass (Azarakhsh *et al.*, 2014), cinnamon and palmarosa (Raybaudi-Massilia *et al.*, 2008), oregano and vanillin (Rojas-Graü *et al.*, 2007 a). The use of alginate as edible coating is expected can decrease the weight loss. In the present study, the results show that increasing

alginate concentration determine an increase in weight loss. In a previous study, this phenomenon has been reported and weight loss was explained as coming from edible coating itself (Guerreiro *et al.*, 2015).

Addition of betel essential oil does not affect the firmness (Fig. 3). In a previous study, the use of lemongrass essential oil on alginate based edible coating decreases the fruit firmness due to its lower pH that triggers pectic acid hydrolysis (Rojas-Graü *et al.*, 2007 a). Fresh cut pineapple which are treated with alginate-lemongrass essential oil edible coating shows a decrease in firmness value during storage (Azarakhsh *et al.*, 2014).

In TA observation, the result show that high concentration of alginate can decrease oxygen permeability which led reduction on the respiratory process (Campos *et al.*, 2011). TA changes are related to organic acid production and an acidity reduction maybe represent as a metabolic changes result in fruit or caused by the use of organic acid as respiratory substance (Selcuk and Erkan, 2015). TSS content is a critical attribute which has contribution to quality and consumer acceptability. Our result demonstrate that alginate based edible coating shows ability to maintain the TSS content of rose apple. This findings are in line with the previous study in sweet cherry (Díaz-Mula *et al.*, 2012), guava (Nair *et al.*, 2018), cantaloupe (Zhang *et al.*, 2015), blueberry (Mannozi *et al.*, 2017) and Ber fruit (Ramana Rao *et al.*, 2016).

RS value is an essential indicator for determining respiratory process in the fruit. Betel essential oil addition does not affect the RS value. However, many studies showed alginate can maintain RS value which is reported in carambola (Gol *et al.*, 2015), cherry (Díaz-Mula *et al.*, 2012)

Fresh fruit has high nutrient and high water content which allow microbial to multiply (Rojas-Graü *et al.*, 2007 a). Betel essential oil can inhibit the microbiological growth in food preservation (Prakash *et al.*, 2010). In a previous study, the result showed that there is no fungi that can grow in media which have been added with betel essential oil more than 0.6% (v/v) (Basak and Guha, 2015). However, our previous study in preferences (unpublished data) on alginate-betel essential oil edible coating showed that most panellists accepted to consume rose apple with the addition of essential oil less than 0.2%.

Addition betel essential oil shows proper appreciation up to 9 d in appearance value. This result is related to low microbial spoilage compared

to no addition of essential oil. Similarly, changes are shown in rose apple cv. Dalhari texture preference. The panellist show acceptable score up to 9 d. This overall result is consistent with in previous study where it is no differences between fruit with low concentration essential oil addition with control in sensory test (Raybaudi-Massilia *et al.*, 2008; Azarakhsh *et al.*, 2014; Bustos *et al.*, 2016; Guerreiro *et al.*, 2016).

In conclusion, edible coating treatment with alginate 2.5% and betel essential oil 0.1 % maintained the quality of rose apple cv. Dalhari by reducing weight loss, fruit firmness, microbial growth and appearance. In a study of microbial growth inhibition using paper disc test, betel essential oil has shown to inhibit both yeast and bacteria. However, in this experiment, a combination of alginate-betel essential oil has not shown the different effect to microbial growth on rose apple cv. Dalhari. The main limitation of this study is the lack of information in the fruit. Future research needs to be done for more findings and better understanding.

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