Effect of CaCl₂ and Alginate-Essential Oil Edible Coating in Maintaining Quality and Antioxidant Content in Rose Apple cv. Dalhari

A M Anjani*, C K Setiawan, N A Utama
Department of Agrotechnology, Faculty of Agriculture, Universitas Muhammadiyah Yogyakarta, Bantul 55183, Special Region of Yogyakarta-Indonesia

*E-mail: muathiaalfi@yahoo.co.id

Abstract. Information on the treatment of edible coatings alginate and various concentrations of essential oils has not been known to antioxidant and anthocyanin changes in rose apple during storage. This study was aimed to determine the effect of CaCl₂ and edible coating towards the changes in antioxidants, anthocyanins, and chlorophyll during storage. It was also to determine the concentration of the best combination of immersion of CaCl₂ and essential oils to the antioxidant, anthocyanin, and chlorophyll content found in rose apple var. Dalhari. The research was conducted by experimental CRD (Completely Randomized Design) factorial design. The first factor was the concentration of CaCl₂ consist of two levels namely 0% and 2%. The second factor was the type and concentration of essential oil consist of 3 levels, namely 1% vanilla, 0.7% cinnamon and 0% essential oil. The results of the combination of CaCl₂ and essential oils has no significant effect on antioxidant, anthocyanin, and chlorophyll content.

1. Introduction
Along with the current trend of people living on healthy lifestyles, many of them switch their diet habit from consuming fast food to healthy foods such as fruits and vegetables, which contain a lot of vitamins and minerals. Another important content is the antioxidant. Antioxidants are bioactive substances that are able to prevent cell damage in our body from the free radical exposure.

Dalhari rose apple (*Syzygium samarangense*) is an indigenous Indonesian plant that belongs to the Myrtaceae family. This plant can be found almost in all area of Indonesia because it can easily adapt to all types of soil as long as it is fertile, friable, and moist [1], but specifically for Dalhari variety, it is mostly developed in Berbah, Sleman. Rose apple has a plus point on its bell-shaped form that it can reach 150 g each with a shiny red rind. It has the water content of 90.3 g per 100 g with soft, thick, and sweet-tasted pulp (12-15 0 Brix) [2]. It also has a maximum respiration rate of 25 mg CO₂/kg when it is stored unpackaged, while for packaged rose apple possesses the maximum rate of respiration by 20 mg CO₂/kg [3].

Respiration will reduce commodity’s quality when stored. In addition, concurrently with respiration, transpiration does the same. Both occurrences damage the rose apple by losing weight and reducing its nutrient content, especially antioxidant. Efforts to lower the quality degradation during storage could be done using edible coating. It prevents damage due to mechanical handling, helps to maintain structural integrity, prevents the loss of volatile compounds, and carries additives such as
antimicrobial as well as antioxidants agents [4]. One of polysaccharide-based edible coating is alginate. The use of combination of 2% alginate edible coatings and malic acid can maintain the shelf life of fresh-cut melons for up to 10 days compared with no treatment that is only 4 days [5]. Alginate coating utilized to reduce the decline in fruit quality has not been optimal due to the unstoppable microbial growth.

The Dalhari rose apple’s rind is a focal factor during the storage process. A thin layer on Dalhari affect the growth of microbes, their presence will accelerate the decrease and worsening fruit’s quality. One way to prevent microbial growth is by applying antimicrobial agents such as vanilla and cinnamon essential oils. Vanilla essential oil contains vanilin, a phenolic compound having antioxidant and antimicrobial properties against yeast, fungi, and bacteria and it is one of the most interesting aromatic compounds. Its concentration of 3000 ppm is effective to diminish yeast growth in purées [6]. According to Ekaprasada [7], the extract of cinnamon bark (Cinnamomum burmannii Nees ex Blume) with a high content of transsinaldehyde (68.65%) is one of antioxidant sources, which is able to block free radicals or be radical scavengers.

The damage caused by the microbes lead to the declining of cell-wall’s turgidity. Kramer et al. [8] stated that the application of CaCl2 could form crosslinks between Ca2+ and pectic acid as well as other polysaccharides, thus limiting the affectivity of softening and respiration enzymes such as polygalacturonase. Thereby, it reduces respiration rates and minimize ascorbic acid degradation. S. Mola et al. [9] underlined that the combination of sodium chloride 200 mg/l, calcium chloride 20 g/l or 2%, and calcium ascorbate on fresh-cut rose apples reduced phenolic content and prevented browning and increased fruit hardness.

So far, the information about alginate treatment as edible coating and various essential oil concentrations to the antioxidant and anthocyanin changes in rose apples during the storage process has not been known. The results of Setyaningrum’s study confirmed that the antiradical capacity of Salam leaf extract had a high correlation to its anthocyanin content [9]. Based on the mentioned description, it is necessary to study Dalhari rose apples because of its high anthocyanin that potential as an antioxidant agent. Therefore, knowing the changes of chemicals, especially Dalhari’s antioxidants that has been treated using edible coating and essential oils in order to maintain the Dalhari’s quality is indispensable.

2. Materials and Methods

This research used the rose apples with grade A Dalhari variety that has a size approximately 150 grams in one kilogram contained 8 pieces. In addition, the state of their rind is not defective and shaped like a perfect bell. The fruits were picked directly from the farmers in Jogotirto, Krasakan, Berbah, Sleman. Other components used include alginate, vanilla and cinnamon essential oils, CaCl2, glycerol, ethanol p.a, HCl 1%, aquadest, 80% acetone, DPPH, and Na-citrate.

The instruments used in this research were knives, blenders, jars, beaker, analytical balance, sterofoam, spray bottles, stirrer rods, micro pipettes, drop pipettes, measuring pipettes, measuring flasks, measuring cylinders, test tubes, and UV-Vis Spectrophotometers.

The experimental method is arranged in Random Factorial Design (RAL) factorial (2x3) with 2 main factors. First was CaCl2 concentration consisting of two levels 0% and 2%. The second one was the concentration of essential oils, which consisted of 3 levels, 0%, 1% of vanilla essential oil, and 0.7% of cinnamon essential oil. Hence, there were 6 treatment combinations were obtained, while for the comparison, it used the negative controls and for each combination were done on triplicates. Also, the experimental unit employed 3 samples and 6 targets. The required rose apples were 189 pieces and combination treatments were as follows:

| Combination | Description                                      |
|-------------|--------------------------------------------------|
| A0C0        | 2% alginate                                      |
| A0C2        | 2% alginate + 2% CaCl2                           |
| A1C0        | 2% alginate + 1% Vanilla                         |
| A1C2        | 2% alginate + 2% CaCl2 + 1% Vanilla              |
| A2C0        | 2% alginate + 0.7% cinnamon                      |
| A2C2        | 2% alginate + 2% CaCl2 + 0.7% cinnamon           |
Control: without any treatment

2.1. The selection of Dalhari Rose Apples
Rose apples that would be treated with alginate edible coating must posses the same stage and size. Guava water is picked directly from farmers in Jogotirto, Krasakan, Berbah, Sleman. Then, fruits were washed with the 200 μl L-1 chlorine solution, dried and cleaned from unnecessary parts.

2.2. CaCl2 Immersion Treatment
Fruits were dipped into CaCl2 in each concentration treatment, 0% and 2%. Immersion was carried out for 1 minute then dried for 30 minutes.

2.3. Making Alginate Edible Coating
The edible coating solution was prepared by dissolving the alginate powder into the aquadest and heated to 85 ºC for 30 minutes until the solution became transparent. Then, this solution was added by 2.5% glycerol as a plasticizer (adhesive). After the alginate edible coating was formed, the vanilla and cinnamon essential oils were added according to the treatment compositions.

2.4. CaCl2 Immersion
The fruits were dipped into 2% CaCl2 solution until the layer was formed. The immersion was performed for 1 minute and dried for 30 minutes.

2.5. Sample Preparation
Dalhari rose apples were cut into small pieces and mashed.

2.6. Extraction for Chlorophyll Pigment Test
Chlorophyll a and chlorophyll b were measured using the Lichtenthaler and Wellburn (1983) method with few modifications. A total of 5 grams sample were homogenized using 25 ml p.a acetone (80%). The mixture was stored at 5 °C for 30 minutes then measured its absorbance at 645 nm and 662 nm.

2.7. Anthocyanin Extraction
Anthocyanins were extracted from 5 grams of mashed rose apple with wet maceration technique using 1% HCl in 20 ml methanol. The maseration was carried out for 16 hours then filtered and the filtrate was collected. The extraction was done until all anthocyanins were perfectly extracted.

2.8. Determination of Total Anthocyanin Content by Different pH Method
The appropriate dilution factor for samples should be determined first by dissolving them with KCl buffer pH 1.0 to obtain an absorbance of less than 1.2 at 510 nm. Next, the absorbance of aquadest was measured at the reference wavelength (510 and 700 nm) to find the zero point. The 510 nm is the maximum wavelength for cyanidin-3-glucoside while the 700 nm was used to anticipate the precipitate that still present in the sample. If the sample is completely transparent then the absorbance at 700 nm must be zero. Two solutions with the sample were prepared; first sample used KCl buffer pH 1.0 and for the second one used Na-citrate buffer pH 4.5. Each sample was dissolved with mentioned buffer solution based on predetermined DF (dilution factor). Samples dissolved using buffer pH 1.0 were left for 15 minutes before measurement, while for samples dissolved in 4.5 pH buffer were readily measured after being allowed to mix for 5 minutes. Absorbances were observed at 520 and 700 nm.

2.9. The Antioxidant Activity Test by DPPH Method
The 11.8 mg of DPPH was dissolved in 150 ml methanol (200 μM). One gr sample was suspended in 10 mL methanol. One mL of stock solution was put into the test tube. Then, 1 ml DPPH (200 μM) was added and incubated in a dark room for 30 minutes. Afterwards, the mixture was diluted to 5 ml using methanol. The blank solution used was 1 ml DPPH dissolved in 4 ml methanol and the absorbance was measured at 517 nm.
2.10. The Observed Parameters
The storage of alginate-coated rose apple was conducted for 15 days and observed on the 0th, 3rd, 6th, 9th, 12th, and 15th day.

2.10.1. Chlorophyll Content
Calculating chlorophyll a and b using this formula:
Chlorophyll a (μg/kg) = \[11.75 \times \text{Abs 662} \times 2.35 \times \text{Abs 645}\] ......
Chlorophyll b (μg/kg) = \[18.61 \times \text{Abs 645} \times 3.96 \times \text{Abs 662}\] ......

2.10.2. Anthocyanin Content
The absorbance of diluted sample (A) was obtained using equation 3.
\[A = (A_{510} - A_{700}) \times \text{pH 1,0} - (A_{510} - A_{700}) \times \text{pH 4,5} \] ......
The anthocyanin content was calculated with equation 4.
Total anthocyanin (mg/L) = \[(A \times BM \times DF \times 1000)/(g \times b)\] ......

Note:
BM = molecular mass of cyanidine-3-glucoside = 449.2 g/mol
DF = dilution factor
g = molar absorptivity of cyanidine-3-glucoside = 26.900 L/(mol.cm)
b = weight of sample

2.10.3. Antioxidant Content
The calculation of DPPH's and solution tests’ anti free radical absorbance was performed with:
Antioxidant activity (%) = \[(\text{OD Blank} - \text{OD sample})/\text{OD Blank}] \times 100\%

2.11. Statistical Analysis
Observed data would be analysed using one-way ANOVA with variance in 5% level of error. If it is found the obvious difference among treatment effects, it would be performed further analysis Duncan Multiple Range Test with 5% level and SAS Contrast to compare all treatments with controls.

3. Result and Discussion
3.1. Chlorophyll Content
The color of the fruit is caused by the pigment contained in the fruit. Plants have some chlorophyll types, but the most are chlorophyll a and chlorophyll b. During the fruit ripening process, the color changes from green to yellow, orange, red, blue, or other colors. The color change as a result of chlorophyll is breakdown and there are syntheses of other pigment [11]. During ripening, the rind changes from green to a different bright color. The most obvious change is the degradation of chlorophyll content while accompanied by other pigment synthesis, which is usually anthocyanin or carotenoid [12].

Figure 1 and 2 showed that chlorophyll a and chlorophyll b in both essential oil and CaCl\(_2\) treatments have an upward trend during observation. When the ripening process, chlorophyll content will be degraded. In general, there are 3 reactions of chlorophyll degradation, namely peophytins reaction, chlorophyllid formation, and oxidation. Peophytins reaction is a reaction that form peophytin, a form of chlorophyll losing its Mg\(^{2+}\) ion resulting its color is not green but brownish green. The second reaction is a reaction that forms chlorophyllid. Chlorophyllid is formed from the hydrolysis reaction in both acid and base conditions. The activity of chlorophyllase enzyme will result in the formation of chlorophyllid. This enzyme will hydrolyze the phytol chain in chlorophyll thus chlorophyllide and fitol will be formed. The last reaction is oxidation. Oxidation can occur either enzymatically or non-enzymatically. The enzymatic oxidation reaction involves the enzyme lipoxigenase (linoleic oxidoreductase). This enzyme catalyzes the oxidation in the presence of oxygen and lipid. Non-enzymatic oxidation reactions occur spontaneously by the atmospheric oxygen during meskipin storage in dark conditions [13].
Figure 1. Comparison on the level of chlorophyll a (top) and chlorophyll b (bottom) of Dalhari rose apple treated with various essential oils.

On 0th day, the chlorophyll content was low due to chlorophyll degradation. According to Nuri and Fitri [13], factors that affect this degradation are heat and light exposure, oxidizer, and environmental pH. In agreement with Kyzlink [14], peophytins reaction will run faster when exposed to the heat. The heat accelerates the peophytins because it denatures the protein. In addition to heat, chlorophyll degradation is also caused by oxidation. The use of alginate and CaCl₂ will inhibit oxidation, hence chlorophyll degradation process will be reduced.

3.2. Total Anthocyanin

The pigments that responsible for the formation of red, blue, and purple in fruits, vegetables, and ornamental plants are anthocyanins. Recently, research on anthocyanin pigments has been intensified recently because of their promising health benefits as food antioxidants [15]. Fig. 3 showed the increase of anthocyanin levels from 0th to 9th day and then decreased on 12th and 15th day. The content of anthocyanin is lower than chlorophyll content because chlorophyll is the main pigment present in all plants, so even though the fruit is red, it does not mean that the fruit has dominant antocyanin pigments, but chlorophyll does [16].

According to Winarno [17], the pigment anthocyanin (flavonoid) consists of three functional group, namely the basic ring consisting of aglyonous group (without sugar), aglycons or sugar groups, and native organic acids (e.g. coumarat, coufeit or pherulic). The anthocyanin molecule is composed of an esterified aglycone (anthocyanidin) with one or more sugars (glycon). Timberlake and Bridle [18] stated that the sugars making up the anthocyanin are monosaccharides (usually glucose, galactose, rhamnosa, and arabinose), disaccharides (two monosaccharides with a combination of four monosaccharides above the xylose, like rutinose), trisaccharides (three monosaccharides containing a combination of the above sugar in linear or branch position).

The presence of sugar clusters which include monosaccharides, disaccharides, and trisaccharides will affect the stability of anthocyanins. When the sugar group leaves, anthocyanins become unstable. When heating it in concentrated acids, anthocyanins rupture into anthocyanidins and sugars. This
anthocyanin is a polar dye and dissolves well in a polar solvent. Factors that influence the stability of anthocyanins are the structure of anthocyanins and other components contained in the foodstuff. Factors that affect non-enzymatic anthocyanin stability are pH, temperature, and light [19].

Figure 2. Comparison on the level of chlorophyll a (top) and chlorophyll b (bottom) of Dalhari rose apple treated with CaCl₂.

Figure 3. Comparison on the total anthocyanin of Dalhari rose apple treated with essential oils (top) and CaCl₂ (bottom).
3.3. Antioxidant Activity
The presence of antioxidant activity resulted in the color change of the sample in the DPPH solution from purple to yellow. The same result was confirmed by Andayani et al. [20] that the antioxidant activity causing discoloration of DPPH solution in ethanol which was originally a deep purple to pale yellow.

Histogram in Figure 4 displayed a descending trend of antioxidants. However, the treatment of 1% vanilla essential oil has a relatively constant trend. The presence of essential oils supposed to increases the content of Dalhari’s antioxidants. Vanillin is a phenolic compound that exhibits antioxidant properties. According to Ekaprasada [7], cinnamon bark extract (Cinnamomum burmannii Nees ex Blume) with a high content of trans-sinamaldehyde content (68.65%) to be a source of antioxidant compound. The histogram in Figure 3 showed the stagnant activity of the 0% and 2% CaCl2 treatments, no increase or decrease from the observation on the 0th, 3rd, 6th, 9th, 12th, and 15th day.

**Figure 4.** Comparison on antioxidant activity of Dalhari rose apple treated with essential oils (top) and CaCl2 (bottom).

4. Conclusion
The combination of CaCl2 and essential oil in alginate edible coating not affected weight loss, texture, titrated acids, total dissolved solids, chlorophyll content, anthocyanin levels, and antioxidant activity of Dalhari rose apple. The combination of CaCl2 and essential oil in alginate edible coating not affected weight loss, texture, titrated acids, total dissolved solids, chlorophyll content, anthocyanin levels, and antioxidant activity of Dalhari rose apple.

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References
[1] Hariyanto B 1992 Jambu Air Jenis, Perbanyakan, dan Perawatan Penebar Swadaya: Jakarta
[2] Amalia S 2013 Perubahan Total Antioksidan Buah Jambu Air (Syzygium samarangense) Varietas Dalhari Selama Pengemasan Dan Penyimpanan Suhu 50°C Skripsi Jurusan Teknologi Pangan Dan Hasil Pertanian Fakultas Teknologi Pertanian Universitas Gadjah Mada Yogyakarta
[3] Patria G D 2013 Perubahan Sifat Fisik dan Kimia Jambu Air (Syzygium samarangense) varietas Dalhari selama Penyimpanan pada Suhu 5°C Fakultas Teknologi Pertanian UGM Yogyakarta.
[4] Kester J, Fennema O R 1988 Food Technology 42:47-59.
[5] Raybaudi-Massilia R M, Mosqueda-Melgar J, Martin-Bellos O 2008 International Journal of Food Microbiology 121 313–327.
[6] Cerrutti P, Alzamora S M, Vidales S L 1997 Journal of Food Science 62(3) 608-610.
[7] Ekaprasada M T 2009 Isolasi Senyawa Antioksidan Kulit Batang Kayu Manis (Cinnamomum burmanii Nees ex Blume) Skripsi: Universitas Indonesia
[8] Kramer G F, Wang C Y, Conway W S 1989 Journal of American Society of Horticultural Sciences 114 942-946.
[9] S Mola, Uthairatanakij A, Sriloang V, Aiamla-or S, Jitareerat P 2016 Agriculture and Natural Resource 50 331-337.
[10] Setyaningrum, A 2010 Kapasitas Antiradikal Ekstrak Antosianin Buah Salam (Syzygium polyanthum) Segar dengan Variasi Proporsi Pendarat dan Teknologi Pangan UNS Solo.
[11] Mercubuana 2014 Kematangan dan Indeks Kematangan. http://ebook.repo.mercubuanayogya.ac.id/Kuliah/materi_20142_doc/7_Pematangan%20Buah.pdf.
[12] Khandaker M M, Alebidi A I, Hossain A S, Mat N, Boyce A N 2015 Journal of Sustainability Science and Management 10(1) 66-75.
[13] Andarwulan N, Faradilla R H F 2012 Pewarna Alami untuk Pangan http://seafast.ipb.ac.id/tpc-project/wp-content/uploads/2013/03/09-hijau-klorofil.pdf.
[14] Kyzlink V 1990 Principles of Food Preservation Tokyo: Elsevier.
[15] Ronald E W 2001 The Possible Health Benefits of Anthocyanin Pigments and Polyphenolics Department of Food Science and Technology, Oregon State University, Corvallis.
[16] Maulid R R, Laily A N 2015 Kadar Total Pigmen Klorofil dan Senyawa Antosianin Ekstrak Kastuba (Euphorbia pulcherrima) Berdasarkan Umur Daun In: Prosiding Seminar Nasional Konservasi dan Pemanfaatan Sumber Daya Alam 225-230.
[17] Winarno 2004 Kimia Pangan Dan Gizi PT Gramedia Pustaka Utama: Jakarta.
[18] Timberlake C F, Bridle P 1980 Anthocyanins Development In: Food Colours (Ed) Walford J Applied Science Published Ltd: New York.
[19] Salisbury F B, Ross W C 1991 Fisiologi Tumbuhan ITB Press: Bandung
[20] Andayani R, Lisawati Y, Maimunah 2008 Jurnal Sains dan Teknologi Farmasi 13(1) 1-9.