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# Utilization of Anthocyanin in Parijoto Extract as Additives in Edible Coating to Extend Shelf-Life of Food Stuffs

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**Abstract.** The exploration of Parijoto (Medinilla speciosa Blume) continues to be increased because the color and content of anthocyanin compounds in Parijoto are interesting to study. Foodstuffs, especially fruits, which have a high level of consumption in society, need attention in their packaging and storage. The purpose of this study was to determine the profile of the food ingredient, in this case cherry tomatoes were used which had been coated with an edible ingredient added with Parijoto extract. The profile in question includes physical and organoleptic appearance, moisture content and mass of food ingredients. This study used UV-Vis and HPLC spectrophotometry to determine the anthocyanin content, both quantitatively and qualitatively. Testing the profile of each food ingredient using standard laboratory methods. The anthocyanin contents identified in Parijoto were Delfinidin-3-glycosides and Cyanidin-3-glycosides with a total content of 0.05065%w/w or 0.5065 mg/g. Foodstuffs packaged with edible coatings show a longer shelf life and minimal quality loss when compared to foodstuffs that are not coated. In addition, the coating made with the addition of Parijoto extract also showed a longer shelf life and minimal quality loss than tomatoes coated with an edible coating solution without Parijoto extract.

**Keywords** : Parijoto, edible coating, shelf life, food stuffs

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## Introduction

Indonesia is very rich in tropical fruit diversity. Increasing consumer awareness in the health sector has spurred the development of packaging technology, transportation, and marketing systems. Tropical fruits typical of Indonesia contain bioactive components that are beneficial for health, thus positioning the fruit as functional food. Compounds such as phenolics, carotenoids, organic acids, vitamins, and fiber function to increase immunity, antioxidants, antimicrobials, facilitate digestion, overcome obesity, anticancer and anti-inflammatory [1]. Some anthocyanin compounds that are very abundant are pelargonidin, peonidin, cyanidin, malvidin, petunidin, and delphinidin [2].

The physical appearance of the fruit, especially the skin, generally determines the level of consumer preference. Fruit that has physical damage to the skin is susceptible to physiological and pathological changes, so that it ultimately has a shorter shelf life. Even the slightest physical damage to the fruit skin can make it susceptible to dehydration, ethylene gas production, degradation of sensory components (color, odor, taste), production of unwanted metabolites and enzymatic reactions, stimulate ripening, aging and decreased fruit integrity, to microbial growth that results in toxic properties during storage and transportation [3]. The use of edible coating is one way that is very helpful in maintaining the quality and extending the shelf life of fruit products. Edible coating is a protective layer or thin packaging in the form of edible material (can be consumed) which is applied to environmentally friendly and biodegradable food ingredients. Edible coating functions as a protector of food ingredients against chemical, physical and biological changes and as a carrier of additives that can increase shelf life or certain purposes for handling fruit until ready to be consumed. The application of edible coating can also improve appearance (the color of food ingredients becomes bright and glowing), maintain moisture, prevent weight loss of food ingredients and act as an antimicrobial.

Various polysaccharide mixtures that can function as carrier substances for other materials can be used as the core matrix of edible coatings, including antimicrobial solution materials so that they have great potential to be collaborated into a single material that can improve the quality and shelf life of fresh materials [4]. The bioactive components of edible coatings depend on the characteristics of the product and the type of matrix polymer. Active compounds in the form of antioxidants, antimicrobials, nutrients, vitamins, antibrowning, enzymes and probiotics can be applied to the coating matrix to help maintain the quality of the coated product. The components of edible coatings consist of hydrocolloids (polysaccharides, proteins, alginates), lipids (fatty acids, aryl glycerides, waxes), and composites (proteins, polysaccharides-proteins, fats-polysaccharides). This material is formulated with surfactants and plasticizers. These three components can provide maximum protection when combined. The application methods for coating on fresh cut fruit are dipping, foaming, spraying, casting, and controlled drops. The advantage of using edible coating is that some active ingredients can be incorporated into the polymer matrix and consumed with food so that it can maintain its nutritional and sensory attributes.

The consistency and stability of edible coating will be optimal if additives are added. The type and concentration of additives determine the consistency and stability of edible coating. The ability of edible coating as a matrix or carrier of additives is greatly influenced by the molecular structure, molecular size, and chemical content. Several things that need to be considered in the application of edible coating are surface coverage, application time and method, storage conditions, and composition and thickness of the layer. The problem of applying edible coating to fresh cut fruit is the difficulty of the material's adhesion to the surface of the fruit slices which are hydrophilic. The ability of adhesion is largely determined by the surface tension which will affect the thickness of the layer and the structure of the edible coating particles on the surface of the fruit. Different concentrations and soaking times also produce different layer thicknesses [5]. Packaging treatment with edible coating can increase the shelf life of cut fruit (up to 2 days) compared to the control [6].

This study aims to determine the type and amount of total anthocyanin content of Parijoto extract and to determine the effect of edible coating with the addition of Parijoto extract on fruit packaging.

## Experimental

**The Simplisia preparation.** The tools used include HPLC, UV Visible spectrophotometer, rotary evaporator, water bath, kitchen equipment, scales, and standard laboratory glassware, polystyrene box. This study used fresh Parijoto fruit, ethanol p.a., methanol p.a., Magnesium pieces, Na alginate, distilled water, 1.5% glycerol, cherry tomatoes (in uniform size), 200 µg/L chlorine solution, 2% CaCl2 solution. Fresh Parijoto is washed, then sliced and dried by airing it. After drying, it is ground again and weighed.

**Parijoto extraction.** Dry simplicia was macerated with ethanol solvent with a ratio of 1:10. The maceration process was carried out for a total of 5 days; including 3 days of maceration and 2 days of remaceration. The stirring process was carried out twice a day. On the 3rd day of maceration, the maceration results were filtered using flannel cloth. Remaceration was carried out again on the dregs with a new solvent with the same treatment. The extract obtained was evaporated using a rotary evaporator at a temperature of 600C. The thick extract remaining after solvent evaporation was then weighed and the percentage of yield was calculated.

**Qualitative Analysis of Flavonoids in Extract.** A total of 0.5 mL of the sample was dropped on to a glass slide. Then 3 drops of methanol were added and stirred until homogeneous. After that, small pieces of Magnesium were added, then 3 drops of concentrated HCl were added. The formation of a yellow-orange color indicates the presence of flavonoid compounds [7].

**Quantitative Analysis of Flavonoids in Extract.** The method using quercetin standards, that is 90 ppm quercetin in methanol, then made several concentrations of 80, 90, 100, 110, and 120 ppm. From each concentration of the quercetin standard solution, 3 mL of methanol, 0.2 mL of 10% AlCl3, 0.2 mL of 1 M potassium acetate were added, and add aquadest up to 10 mL, incubated for 30 minutes at room temperature. The absorbance was measured on UV-Vis spectrophotometry with a wavelength of 415 nm [8] [9].

**Analysis of Anthocyanin Content in Parijoto Extract.** Identification of Anthocyanin types was carried out using Vertex column type HPLC, Eurosphere 100-5 C18, 150 x 4.6 mm (SN:GJ96) with a mobile phase of 1% Formic acid:acetonitrile (85:15).

**Antioxidant Analysis in Parijoto Extract.** In this research, we studied the identification of antioxidants using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method radical scavenging activity. A total of 24 mg of DPPH were dissolved in 100 mL of methanol for making the stock solution. Filtration of DPPH stock solution using methanol yielded a usable mixture with an absorbance of around 0.973 at 517 nm. In a test tube, 3 mL DPPH workable solutions were combined with 100 µL of extract. Three milliliters of solution containing DPPH in 100 µL of methanol is often given as a standard. After that, the tubes were kept in complete darkness for 30 min. The absorbance was therefore determined at 517 nm with three replications [10]. The percentage of antioxidants or Radical Scavenging Activity (RSA) was calculated using Eq. (1).

$$\% \text{ of antioxidant activity} = [(Ac - As) \div Ac] \times 100 \quad (1)$$

where: Ac= Control reaction absorbance; As= Testing specimen absorbance.

**Preparation of Alginate-Based Edible Coating Solution.** Edible coating solution was prepared by dissolving alginate powder into distilled water (1:10) and heated at 85ºC using a water bath for 30 minutes until the solution became clear. The solution was then added with 1.5% glycerol as a plasticizer (adhesive). After the alginate edible coating solution was formed, Parijoto thick extract was added according to the desired variation.

**Tomato Selection.** The selected tomatoes are tomatoes that have almost the same size, approximately 100 grams. Tomato storage is at a temperature of 150C before coating. Tomatoes are first washed with a chlorine solution of 200 µg/L and then aired until dry.

**Coating Process and Observation of Tomato Shelf Life.** The prepared tomatoes were then dipped into each coating solution, namely the one with Parijoto extract added and the solution containing only the coating solution without Parijoto extract. The tomatoes were then dipped back into the 2% CaCl2 solution until a layer was formed. After the layer was formed, store it at room temperature using a polystyrene box and wrapping for 15 days.

***Tomato Quality Test After Coating***

**Weight loss percentage (%).** Weight loss of tomatoes was done by the method according to [11], which weighed before treatment and after treatment, then the difference in tomato weight was calculated as weight loss using the Eq. (2).

$$Weight\ loss = (Wa-Wb)/Wa \times 100\% \quad (2)$$

Wa = initial weight before treatment; Wb = final weight after treatment.

**Organoleptic (scoring).** Testing using a scoring method with a scale of 0-4;

Where 0 = no wrinkles; 1 = a little (± 5% of the surface area); 2 = moderate (5-20% of the surface area); 3 = quite a lot (20-50% of the surface area); 4 = very much (> 50% of the surface area).

## Result and Discussion

The dry simplicia obtained from the grinding and drying of fresh Parijoto fruit is 100 grams (Figure 1). The thick Parijoto extract obtained through extraction from 100 grams of simplicia is 19.013 grams. Solvent evaporation (Figure 2) uses a temperature range that is at the ethanol vapor point so that the solvent can separate. The evaporation temperature setting is not too high so that the extracted active compounds are not degraded and remain stable so that further analysis can be properly validated. The result of solvent evaporation is a thicker extract containing the active compounds of Parijoto fruit. The thick extract is then weighed and stored in a tightly closed place and protected from light. The extraction yield at this stage is 19.013% or 19.013 grams of thick Parijoto fruit extract. The yield of the thick extract obtained is higher than the previous reference, which is 17.56% [12] with the same solvent. The storage time and humidity of Parijoto fruit until the extraction process of fresh Parijoto fruit apparently affect the extraction yield. One which can affect anthocyanin degradation was Polyphenol oxidase (PPO) as common endogenous enzymes. PPO is an enzyme that contained with copper-core, with a di-nuclear copper center. Anthocyanin was one of the substrate of PPO enzyme can react directly, although they are weak substrates for it, but mostly anthocyanin degradation involves a co-oxidation of enzymatically generated o-quinones and/or secondary oxidation products formed from quinone [13][14]. The next activity is a qualitative test of Parijoto extract which produces a yellow-orange color in the solution. This color is formed due to the presence of gamma benzopyran flavonoid (anthocyanin backbone) which reacts with the presence of Mg and Cl-. Anthocyanin is a type of flavonoid. The reaction results shown in Figure 3 and the reaction in Figure 4 have been stated in the research of [15].


**Figure 1.** Dry simplicia (ready for extraction)

**Figure 2.** Evaporation of solvents using a rotary evaporator

**Figure 3.** Qualitative test of Parijoto extract

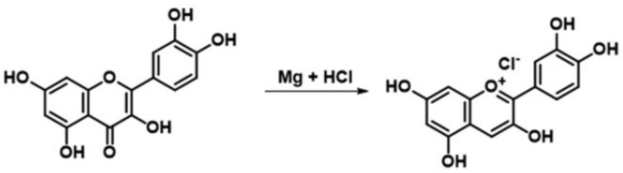


**Figure 4.** Flavonoid reactions with Mg and HCl [15]

The results of the determination of total flavonoid levels using UV Vis spectrophotometers showed that the total flavonoid levels in Parijoto extract were 80.276 ppm. Quercetin was used as a standard solution because quercetin is a flavonoid of the flavonol group which has a hydroxyl group at the C-3 or C-5 atom, a keto group at C-4 and has neighboring flavones and flavonols that flavonols which they can form complexes with $AlCl_3$ [16]. The reaction is shown in Figure 5. The results of quantitative analysis was showed in Table 1.

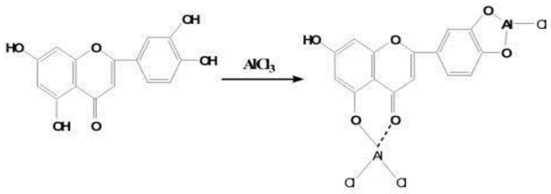

**Figure 5.** Complexes formation between Flavonol with $AlCl_3$ [17]

**Table 1.** Content of Total Flavonoids in Parijoto Extract

| No | Replication | Concentration | Absorbance |
|---|---|---|---|
| 1 | 1 | 80.282 | 0.478 |
| 2 | 2 | 80.209 | 0.477 |
| 3 | 3 | 80.337 | 0.478 |

The next identification is the type of Anthocyanin after it is confirmed that there is a flavonoid content. Identification of the type of anthocyanin was carried out using High Performance Liquid Chromatography (HPLC). The resulting chromatogram is as shown in Figure 6. The anthocyanin compounds that were successfully identified in the Parijoto extract were Delfinidin-3-glycoside and Cyanidin-3-glycoside. Identification by HPLC showed that the types of Anthocyanins in Parijoto extract were Delfinidin-3-glycoside and Cyanidin-3-glycoside. The total Anthocyanin content was 0.05065% w/w or 0.5065 mg/g. The content of each type of Anthocyanin is stated in the Table 2 and the detection is in Table 3. In this study showed that the highest content type of Anthocyanin in the sample is Delfinidin-3-glycoside. It is reached 0.328 mg/g content in extract

The presence of Anthocyanins in Parijoto extract indicates that the eligibility requirements for an antioxidant/antibacterial additive in edible coating are met. The presence of Anthocyanin in Parijoto extract indicates that the minimum requirements for an addition in edible coating are met and can be used as an additional antioxidant/ antibacterial material in edible coating.

Antioxidant properties of Parijoto extract was done by DPPH method because the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a popular, quick, easy, and affordable approach for the measurement of antioxidant characteristics which includes the use of the free radicals used for assessing the potential of substances to serve as hydrogen providers or free -radical scavengers (FRS)[10]. Measurement of the DPPH test results using a spectrophotometer and the % inhibition value of each concentration (Inhibition concentration/ IC50) was obtained. The result of antioxidant activity of extract was stated in IC50 19.706 µg/mL.

**Table 2.** Content Type of Anthocyanin in Parijoto

| Sample | Delfinidin-3-glycoside (mg/g) | Cyanidin-3-glycoside (mg/g) | Pelargonidin-3-glycoside (mg/g) |
|---|---|---|---|
| Parijoto extract | 0.328 | 0.045 | - |

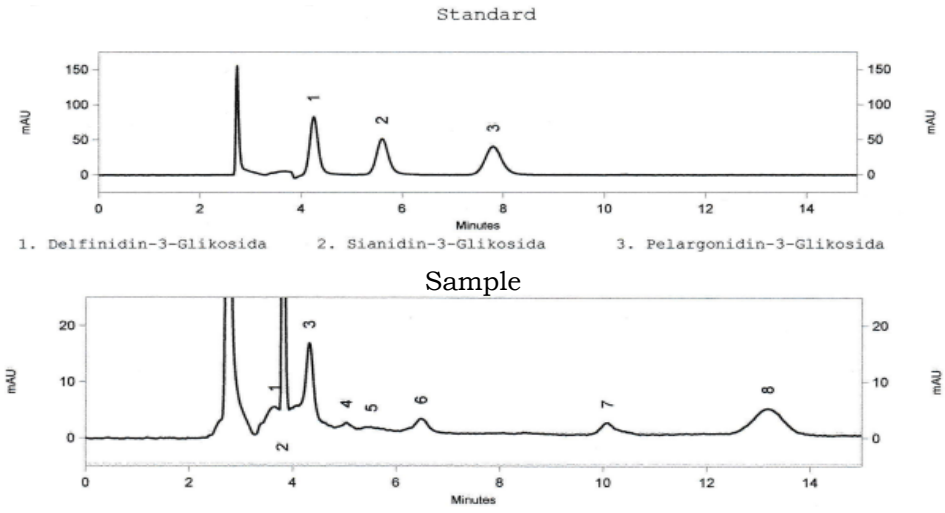


**Figure 6.** Chromatogram of Sample and Standard

The next step is to make an edible coating solution with tomato preparation for coating. In this edible coating solution, an alginate base is used because of its several advantages, namely it is rigid, edible and renewable, making it suitable for the purpose of food packaging sustainability. The making of an edible coating solution is accompanied by the preparation of tomatoes used for coating. Tomatoes are first washed and pretreated with chlorine and stored at a certain temperature to sterilize tomatoes from dirt and microorganisms that stick to them. Alginate base is used in this study because it has several advantages, namely it is rigid, edible and renewable, making it suitable for the purpose of food packaging sustainability. Edible coating made from an alginate base can produce good antioxidant activity, especially if alginate is combined with Parijoto extract containing anthocyanin as an active substance. According to [18] if anthocyanin interacts with alginate, a bond can occur between alginate and anthocyanin, so that if anthocyanin is bound by alginate, the benefits of anthocyanin in an edible coating can be felt more by the coated food.

The results of the treatment of food packaging, namely tomatoes using an edible coating solution showed differences in shelf-life parameters and water content. Packaging using edible packaging materials (can be eaten directly) has special advantages, namely it is practical and easy to use and apply. Packaging with a solution is done by simply dipping the food to be packaged into the edible coating solution. In terms of appearance, packaging using this edible material is quite attractive, namely it still displays the original color and texture of the packaged food, unlike plastic, canned or other packaging so that the original form of the food can be monitored directly by consumers.

In addition, the shelf life of food packaged with edible coating shows longer durability than uncoated food. Coating carried out with the addition of Parijoto extract also shows longer shelf life results than tomatoes coated without Parijoto extract. The results of each treatment are shown in Figures 5, 6 and 7 (after 8 days of storage). Details of water content and shelf life after treatment are listed in Table 1.

The surface of tomatoes coated without Parijoto extract and those without coating treatment began to grow mold. Meanwhile, tomatoes coated with Parijoto extract have not grown mold. From the results of the three treatments of tomatoes, weight loss of materials and water content in food materials, it can be observed that coating with the addition of Parijoto extract has a positive effect on the shelf life of food materials, there are longer shelf life, less weight loss and less water content loss compared to others.

The level of heat and humidity in the environment around the food material greatly affects the evaporation process of the water in the material. Each food material has a relative humidity balance, namely the humidity at a temperature when the food material will not lose water to the air or will not take water vapor from the atmosphere [19]. When the relative humidity of the air is higher than the balance, the food material will actually draw water vapor from the air. This is what happened when testing tomato storage in a closed container. Even though it is in a closed place, tomatoes still release water into their environment, but at the same time the humidity in the closed container is also very high, as a result, in tomatoes without coating treatment, the growth of mold is very progressive because the surface of the food material is

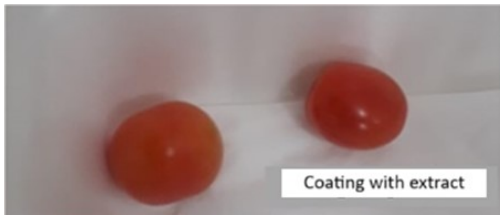


**Figure 7.** Tomatoes coated with additional Parijoto extract

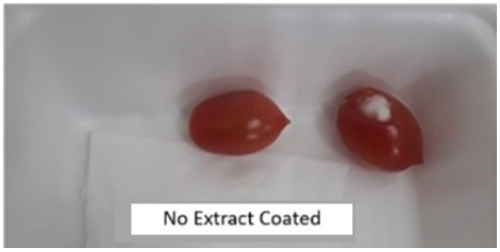


**Figure 8.** Tomatoes coated without Parijoto extract


**Figure 9.** Non-coated tomatoes

**Table 3.** Tomato Weight Loss Comparison

| Sample | Weight (mg) | | | | | Average weight loss percentage (%) |
|---|---|---|---|---|---|---|
| | Day 4 | Day 6 | Day 8 | Day 10 | Day 12 | |
| Coated with additional extract | 6234,6 | 6168,1 | 6132,7 | 6032,4 | 5997,5 | 1,129515 |
| | 7473,1 | 7408,1 | - | - | - | |
| | 12413,7 | 12270 | - | - | - | |
| | 9653,3 | 9487,9 | - | - | - | |
| | 8272,2 | 8202,7 | 8165,6 | 8062 | 8037,3 | |
| Coated without extract | 9925 | 9769,2 | 9658,5 | 9249,3 | 9080 | 1,454893 |
| | 8120,2 | 7991,4 | - | - | - | |
| | 14762,1 | 14552,5 | - | - | - | |
| | 8766,1 | 8669,3 | 8607,5 | 8439,5 | 8383,3 | |
| | 10574,4 | 10405,8 | - | - | - | |
| Non coated | 10181,2 | 10042,5 | 9907,9 | 9555,2 | 9520,6 | 1,797152 |
| | 12418,6 | 12282,4 | - | - | - | |
| | 12209,4 | 12029,2 | - | - | - | |
| | 12024 | 11822 | - | - | - | |
| | 9953,9 | 9821,6 | 9752,6 | 9234,5 | 9040,5 | |
| | 8395 | 8223,6 | 8130,7 | 6827 | 6607,1 | |

in direct contact with the humid air in the container so that the results are overgrown with mold. The results shown in this study are in line with the existing theory that low water content can extend the shelf life of a material. This is because low water content can limit the occurrence of chemical reactions that cause fruit ripening or decay and microbial growth [20].

## Conclusion

From the research that has been done, it can be concluded that from the extraction and analysis of Parijoto content, it is known that the type of Anthocyanin compound in Parijoto extract, namely Delfinidin-3-glycoside and Cyanidin -3-glycoside with a total content of 0.05065% w/w or 0.5065 mg/g. Packaging of food ingredients (tomatoes) with edible coating made by adding Parijoto extract has a positive effect when compared to edible coating without Parijoto extract and food ingredients without coating. The positive effect is that it can better maintain the quality of tomatoes (weight loss and minimal water content loss) and extend the shelf life of tomatoes. This study still has limitations, namely the weight and harvest age of the food ingredients being compared are not exactly the same, but as much as possible in this study using food ingredients (tomatoes) that have the same level of ripeness and size.

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## Author Contributions

I.D.M concept the project, funding acquisition, investigation, writing – original draft, R. L. V investigate and coordinate project administration and analysis, R. R checking data curation, resources, A. F. resources, validation, M. U. checking data, visualization, M. R. R. investigation, visualization.

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