Efficacy of Local Propolis as Edible Coating of Tangerine cultivar Garut (*Citrus reticulata* Blanco)

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**Abstract.** The tangerine var. Garut (*Citrus reticulata Blanco*) is one of the promising local Indonesia fruit commodity. In order to penetrate to new markets, extended shelf life is a necessity. Concern on the energy cost and health made application of natural products as coating material as the best option for post-harvest protection. This research focused on examining the effectiveness of local propolis as the coating material for tangerine var. Garut. The propolis used in this study originated from Trigona laeviceps farm in Maribaya, West Java. Propolis was extracted with propylene glycol solvent. Propolis extract then diluted in propylene glycol to produce extracts with concentration 5%, 10%, and 15% and applied as biocoat of the tangerine by dip method for a minute. Fruits washed by ethanol 70% in a minute were designated as positive control and fruits without any treatment as the negative control. All fruits were stored in room temperature for 34 days. Measurement of weight and diameter, the firmness of mesocarp and endocarp, the temperature of the rind and the flesh, and content of sucrose, total soluble solids, and vitamin C conducted every 3 days. At the same time, digital pictures were taken to observe the changing of color pigment on the fruit skin. Observation of physical and chemical characteristics of fruit showed that propolis coating maintained endocarp firmness, diameter, and vitamin C level for also delayed the decaying process 20% longer than the control with the best concentration for application was 10%.

**1. Introduction**

Tangerine is one of important local fruit with export potency and huge local market. There are several varieties of tangerine such as Garut, Tawangmangu, Soe NTT, and Batu. This fruit is considered as favorite choice for local people due to their rich sweet taste (the sucrose content of mature about 48-50% of total juice) and high antioxidant content (in form of vitamin C, flavonoid, and limonoid). Local buyers also consume this fruit due to their healthy properties as this fruit also rich polysaccharide, vitamin, lipid, and mineral such as cellulose, pektin, citric and malat acid, lipid, carotenoid, vitamin C, vitamin A, vitamin K, thiamin, riboflavin, niacin, inositol, biotin, pyridoxine, pantothenate and paminobensoat acid, kolin, folic acid, potassium, and nitrogen [1][2].

Tangerine is consumed as mature fruit as the characteristic of mature fruits completely different to immature ones [2]. Tangerine is a non-climacteric fruit that produces the relatively low amount of ethylene, reduce their respiration rate after harvest, and slow their ripening process when detached from the plant [3][4]. During postharvest period fruit, physical and chemical parameters in fruit change with time [5][6]. In the long term storage, losses in physiological and biochemical along with
phytopathological agents resulting major deterioration of fruit [4]. Some of the symptoms of the
deterioration are increasing sucrose content, decreasing organic acid content, ripening of peel,
chlorophyll breakdown, and carotenoid accumulation, softening of exocarp and pulp, and weight loss
[7][8][9][10][11]. Furthermore, fungal decay also limits the storage life of orange [12] and reduce the
market penetration of local tangerine to another area in Indonesia as not all area in Indonesia suitable
for tangerine production. Application of chemical fungicide could negatively affect human health and
environment while it could not act as protection agent against physical loss.

Another approach to improve the shelf life of fruit is by applying natural material as coating agent.
The application of coating technique is common in post harvest protection of orange and citrus
[13][14][15][16]. One of the natural materials which is considered safe for human while has
antibacterial and antifungal properties is propolis [17][18][19][20][21][22]. Wax inside propolis
provides hydrophobic properties which allowed propolis to be stick on many types of surface, reduce
direct contact of fruit to air, and control respiration and transpiration rate of fruit [23]. Because of these
properties, propolis have been applying as coating material for various agricultural products
[24][25][26][27]. In this study, we tested the benefit of application propolis coating as postharvest
technique for local tangerine cultivar Garut (Citrus reticulata Blanco).

2. Methods
The study was conducted at Toxicology Laboratory of School of Life Sciences and Technology,
Institut Teknologi Bandung. About 180 tangerines picked in the same time, originated from the farm at
Garut, West Java, were used.

2.1. Propolis extraction
Raw propolis was extracted by ethanolic extraction to obtain the highest amount of phenolic
compound [28]. Prior to use, propolis extracts were stored inside the freezer with average temperature
-100 C. Propolis extract then diluted with propylene glycol, stirred with stir plate for 10 hours, to
obtain 5%, 10%, and 15% solution. Unlike ethanol, propylene glycol dissolved beeswax inside the
propolis and produced a gummy oily solution [29] which easier to be applied as the coating material.

2.2. Coating mechanism
In this study, 180 tangerines were used as the sample. Tangerines were divided into 5 groups of
application. One group was dedicated to control which did not receive any treatment (NC), one group
as control positive in which tangerines were dipped into 70% ethanol (PC), others were treatment
group in which tangerines were dipped into 5% (P5), 10% (P10), and 15% (P15) propolis solution,
respectively.

2.3. Fruit weight and diameter
Fruit weight was measured by analytic weigher while diameter by digital caliper. Both data were
collected from 15 fruits every 3 days for 34 days.

2.4. Fruit hardness and skin elasticity
Fruit hardness and skin elasticity measured by Hardness Tester Penetrometer FHM-1. Data were
collected from 15 fruits every 3 days for 34 days.

2.5. Total soluble solid and sucrose content
Total soluble solid was measured by refractometer Atago PAL-J while sucrose by refractometer
Milwaukee MA871. Measurement method based on Abbe principle and determined by degree Brix
(%Brix) [30]. Data were collected from 15 fruits every 3 days for 34 days.
2.6. **Titratable acidity**
The level of Titratable Acidity (TA) was determined by titration of tangerine juice with NaOH 0.01 mol/L, using 1% phenolphthalein as an indicator. The value was expressed as the percentage of citric acid, the dominant acid in orange fruit [4]. The measurement of TA was conducted every 3 days since observation day 22nd to day 34th.

2.7. **Vitamin C content**
Vitamin C content was measured by iodometric titration method [31]. In this method, about 1/3 part of endocarp was squished to obtained the fruit juice and added with aquadest until 100 mL then mixed thoroughly. About 25 mL of solution mixed with 5 mL of sulphic acid 10% and 1 mL of 1% amylum. Resulted solution then titrated with iodine 0.01 N until the colour of solution change into dark colour, amount of iodine required for this process was measured to calculate Vitamin C content by formulae

2.8. **Data analysis**
All observed data were analyzed by Two Way ANOVA with the confidence level of 0.05. Post-Hoc Tukey Test was applied for the significant result. All analysis were conducted by SPSS 19.

3. **Results and discussion**

3.1. **Morphological changes of fruits**
A significant change in morphology was observed on the peel color. In the beginning, all fruits were green yellowish. At second and third week significant change in color was observed. Fungal started attacked the control groups and P5 at the fourth week (50 and 60%, respectively). In the end of observation, group P10 received less fungal attack than other groups (35%), followed by P15 (40%) (Figure 1).

![Figure 1](image)

**Figure 1** Morphological change in fruit after 34 days storage at room temperature. A: Negative Control (NC), B: Positive Control (PC), C: Coated with propolis 5% (P5), D: Coated with propolis 10% (P10), E: Coated with propolis 15%

Change in peel color mostly caused by chlorophyll breakdown and carotenoid accumulation [11]. When the fruit rotten, pigment production stop due to reducing respiration and metabolism also cell death [32][33]. In this study, tangerine can be stored in good condition at room temperature for 2-3 weeks which agree with Chen et al. [34]. The inability of propolis coating to improve the shelf life of tangerine probably related with extraction method of propolis. Although the extraction method intended to obtain the highest amount of antioxidant of propolis, it reduces the wax content [35]. Wax could act as additional protection layer which maintains the water content which important for fruit metabolism. However, the result indicated the content of propolis could act as antifungal that reduces fungal attack on tangerine. These findings are in agreement with various reports on the effectiveness of ethanolic extract propolis to prevent the fungal attack on citrus and other fruits [36][37][38][39][40].
3.2. Fruit weight and diameter

Significant weight loss was experienced by tangerine of group NC, PC, and P5 after 34 days storage period showed inability of propolis coating to maintain the weight of the fruits (ANOVA, \( F = 2.128, P = 0.081 \)) (Figure 2). The significant loss was started since day 16\(^{th} \) (ANOVA, \( F = 4.710, P<0.05 \)) and level weight loss reached more than 10% of total weight since day 28\(^{th} \). However, the final weight of tangerine of group P10 and P15 still in the range of standard size of local tangerine which is 100-210 gram [41]. The coating of fruit surface has been widely used in post harvest treatment of fruit to reduce dehydration and water loss. The finding of this study confirmed some previous reports on the ability of propolis coating to reduces weight loss rate of fruit [4][31][40][42][43].

On the other hand, it seems that fungal, fruit flies, and loss of moisture as the main factors caused the loss in other groups. Flavonoid and phenol of propolis may deter the infestation of tangerine by microbes, fungi, and fruit flies which explain the result [44][45].

![Figure 2](image)

**Figure 2** Change in fruit weight and diameter of tangerine for 34 days storage at room temperature

Group P10 and P15 also experienced the least diameter reduction showed that propolis coating did not significantly maintain the diameter of the fruits (ANOVA, \( F = 2.858, P = 0.027 \)). Significant diameter loss started from day 25\(^{th} \) (ANOVA, \( F = 3.735, P<0.05 \)) (Figure 2). Until the end of observation period, most of the diameter of fruit still comply with level 2 of Indonesia Standard (SNI) (61-70 mm) while some of the fruits of group NC, PC, and P5 reduced their quality to level 3 of SNI (50-60 mm) [46].

3.3. Exocarp elasticity and firmness of mesocarp and endocarp

Good quality tangerine has high exocarp elasticity. The result showed that all groups experienced significant loss on the elasticity of exocarp after 10 days. The loss of elasticity significantly occurred since day 7\(^{th} \) (ANOVA, \( F = 68.801, P<0.05 \)) although propolis application did not maintain the elasticity of exocarp (ANOVA, \( F = 1.334, P = 0.261 \)) (Figure 3). The finding agreed with the study of Singh and Reddy [47] about the loss of citrus peel elasticity during the storage period. Possible loss of moisture could responsible to this finding.
Figure 3 Change in elasticity of exocarp of tangerine for 34 days storage at room temperature

Observation on the hardness of mesocarp and endocarp showed that level of degradation of mesocarp was higher than endocarp, especially for NC and PC group. Although propolis coating did not prevent degradation of exocarp, it maintained the hardness of mesocarp (ANOVA, $F = 3.582$, $P = 0.009$) and endocarp (ANOVA, $F = 8.332$, $P < 0.05$). Significant loss of mesocarp hardness recorded after 10$^{th}$ day while the loss of endocarp hardness recorded after 28$^{th}$ days and higher loss recorded on both control groups (Figure 4). Good hardness mesocarp for local tangerine is more than 3 N (Suswono and Syarif, 2014). The hardness level was maintained until 13$^{th}$ day.

Figure 4 Change in mesocarp and endocarp hardness of tangerine for 34 days storage at room temperature

The higher level of loss of mesocarp and endocarp firmness in control group could be caused by higher respiration rate and attack by microbe and fungi also infestation by fruit flies as showed by the result of morphological changes [49][50].

3.4. Total Soluble Solute (TSS) and sucrose content
Total soluble solute (TSS) and sucrose content were increasing during storage period for all groups. Significantly increased value of TSS observed at day 13$^{th}$ while sucrose at day 16$^{th}$ (ANOVA, $F = 9.484$, $P < 0.05$). However, there was no effect of propolis application to preservation of TSS (ANOVA, $F = 0.86$, $P = 0.49$) (Figure 5).
Figure 5 Change in Total Soluble Solute and Sucrose Content for 34 days storage at room temperature.

According to SNI, good tangerine has TSS level of 8% Brix with Sucrose level between 4.8 to 5.0 (Pangestu, 2009). Based on this result, propolis coating did not affect to the level of TSS and sucrose content. It seems ripening process of tangerine as non climacteric fruit relatively independent from respiration rate and water loss which altered by application of coating material on the fruit surface.

On the other hand, level of citric acid was decreasing with time especially after day 31st. However, the citric acid level of all fruit coated with propolis significantly higher (ANOVA, P<0.05) (Figure 6). However, during observation, level of citric acid was between 6-9 g/L which is considered as good citric acid level of fruit from citrus group [51].

Figure 6 Change in Citric Acid Content.

Decreasing citric acid level during ripening period is common for citrus [52]. As a non climacteric fruit, during ripening period, activity of citric synthase and aconitase which use to citric acid production is reduced [53]. Citric acid and other organic acids inside the fruit also use as the energy source for fruit respiration [54].

3.5. Vitamin C content
Level of vitamin C significantly reduced with storage period and started from day 4th (ANOVA, F=118.128, P<0.05). Among groups, P10 and P15 showed less vitamin C loss compared with others (ANOVA, F=7.715, P<0.05) (Figure 7). According to SNI, good vitamin C content inside citrus juice is 31 mg/ 100 gram [46] which was recorded until 1-week observation.
Gambar 7 Change in Vitamin C of tangerine for 34 days storage at room temperature

Higher vitamin C loss in all control groups and the P5 group could be related to the infestation of fruit by microbes and fruit flies due to the destruction of enzymes for vitamin C production by both microbes and insect larvae activities [55]. Longer storage period also increased the contact with air and induced the oxidative reaction of vitamin C [56] which may be explained the lower vitamin C loss in fruit coated with propolis.

4. Conclusion
Application of propolis as the coating for local tangerine maintained some parameter related to the quality of tangerine during long-term storage. Observation of physical and chemical characteristics of fruit showed that propolis coating maintained endocarp firmness, diameter, and vitamin C level for also delayed the decaying process 20% longer than the control with the best concentration for application was 10%.

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