Cassava Peel Starch Edible Coating Enriched with Limonene EOs for Preservation of Grapes: A Sustainable Method

Putri Sayyida Ashfiya, Hayat Sholihin, Ali Kusrijadi, Fitri Khoerunnisa*

Chemistry Program, Universitas Pendidikan Indonesia, Setiabudi 229 Bandung 40154, Indonesia
*Corresponding author: Fitri Khoerunnisa, Email: fitri@upi.edu

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ABSTRACT

Grapes are perishable fruits susceptible to damage during transportation and storage post-harvesting, due to moisture loss, microbial contamination, oxidative damage, and physical degradation. These problems lead to weight loss, reduced shelf life, and deterioration of appearance, which can be overcome by using edible coating for preserving post-harvest fruits due to simplicity, non-toxicity, and cost-effectiveness. Starch is an excellent material for edible coating, with cassava peel serving as a suitable source because of good film-forming ability, biodegradability, and non-toxicity. Cassava peel starch also has the capacity to form a semi-permeable barrier that can reduce moisture and gas exchange for edible coating. However, starch is less resistant to pathogenic microbes, showing the need to incorporate limonene essential oil (EO) extracted from sweet orange peel with good antibacterial properties. Therefore, this study aimed to determine the most effective formulation of cassava peel starch and sweet orange EO in edible coating. Analysis was also carried out to evaluate the effect of coating on the quality of grapes based on weight loss, pH, antibacterial properties, and fruit organoleptic. The experiment was conducted by coating grapes using dip method and analyzed during 7 days of storage. Starch was successfully extracted from cassava peel with a facile method to obtain yield of 52.02%. The results showed that the most effective formulation of starch and EO in edible coating was 5% and 0.1%, respectively, indicating potential to significantly maintain the quality of grapes. The addition of orange peel-based EO also inhibited microbial growth during storage. At this formulation, grapes coated with EO-starch film showed an average weight loss of 5.94% and pH 4.21, with lower total microbes of 1.39×10^3 CFU/gram. This indicated that edible coating enriched limonene EO was effective in preserving grape quality and prolonging shelf life.

Keywords: Cassava peel starch, Edible coating, Sweet orange peel EO, Grapes

INTRODUCTION

Grapes (*Vitis vinifera*) are rich in vitamins C and K, antioxidants, and dietary fibers, supporting heart health and digestion (Sabra et al., 2021). These fruits spoil quickly because environmental conditions accelerate moisture loss, nutrient decline, and microbial degradation, which reduces marketability (Valenzuela, 2023; Shao et al., 2021) The quality of grapes is also related to glucose content (uncoated (6.86 g/100 g) and coated (7.77 g/100g)), decay/weight loss (uncoated (31.41%) and coated (24.91%)) (de Souza et al., 2021). To extend shelf life of grapes, edible coating has been identified as an effective strategy by forming a thin and protective layer on the surface to reduce moisture loss, microbial growth, and oxidative processes (Vaishali et al., 2019). Furthermore, it can maintain the freshness and quality during post-harvest handling and storage,

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minimizing exposure to environmental factors and preventing mechanical damage and chemical reactions (Ju et al., 2019). Among various materials, starch is widely used in food preservation due to biodegradable and film-forming properties (Priya et al., 2023)

One major source of starch is cassava (*Manihot esculenta*) peel (Weligama Thuppahige et al., 2023), containing 41.45% (Sivamania et al., 2018), higher than 30% (Azkiya et al., 2023), 23.20% to 30.17% reported in other studies (Fronza et al., 2023). The use of cassava peel starch promotes waste reduction and offers a cost-effective alternative to commercial sources. However, starch-based edible film has limited antimicrobial properties due to hydrophilic nature because it facilitates microbial growth in high-moisture environments (Muñoz-Gimena et al., 2023).

To enhance effectiveness, natural antimicrobials such as essential oil (EO) are often added to starch coating. Limonene-rich oil from sweet orange and other citrus peels shows strong antimicrobial activity (Patricia et al., 2019). Particularly, sweet orange provides EO with over 90% limonene, making it abundant and cost-efficient (Anmol et al., 2021; Kačániová et al., 2024; Yunilawati et al., 2021). Adding this EO to starch-based films can suppress microbial growth and extend fruits shelf life (Ghareaghaji et al., 2021). In previous studies, cassava peel starch coating, often combined with chitosan or plasticizers, has reduced weight loss by 8.12% after 20 days of storage and maintained quality in fruits such as oranges and pineapples (Apriyani et al., 2020; Mudaffar, 2020; Handayani et al., 2018). Orange EO in pectin coatings also preserved apples by preventing shrinkage, weight loss, and microbial growth (Sumonsiri et al., 2020), and chitosan coatings supported grape quality during storage (Hilma et al., 2018). However, the combined application of cassava peel starch and EO on grapes remains unexplored, potentially offering improved biodegradable and antimicrobial preservation.

Based on the description above, this study aims to investigate the effect of incorporating sweet oranges EO into cassava peel starch-based edible coating on the shelf life of grapes. The fabricated starch is characterized using Fourier Transform Infrared Spectroscopy (FTIR). After coating, grapes are stored for 15 days and observed daily based on parameters such as percentage weight loss, pH changes, and organoleptic properties, including freshness and color appearance. Additionally, microbial total analysis is conducted to monitor microbial growth during storage. By exploring the combination of cassava peel starch and EO, this study introduces an innovative and sustainable method to post-harvest preservation, distinguishing from previous reports and contributing to the development of eco-friendly food packaging solutions.

METHODS

Materials

The main materials of this study were the cassava peel obtained from the local market. Grapes (*Vitis vinifera*) were sorted based on similarity in size obtained from the local market (the age before harvesting was 120 days). Additionally, other materials were aquadest, sorbitol as a plasticizer, CMC (Carboxy Methyl Cellulose), and sweet orange peel EO with purity 60% commercially available (Lansida, Indonesia). The chemicals used for analysis included Luff school solution, NaCl (Merck, Germany), and nutrient agar (Merck, Germany).

Starch extraction of cassava

The outer part of the cassava peel was removed, followed by rinse using water, and then cut into small pieces to enhance its surface contact. Cassava peel was soaked for 24 hours and then rinsed. After that, cassava peel was dried for 15 minutes and crushed using a blender with water in a 1:3 weight ratio. The starch slurry was filtered using a cloth filter to separate the filtrate. The filtrate was left for 24 hours to precipitate. The precipitate was separated and dried in an oven at 60° C for 8 hours. The dried starch was sieved using an 80-mesh and evaluated for starch content using the Luff-Schoorl method was adapted from Ifmaily (2018). A 0.1 g sample of cassava peel starch was hydrolyzed with 5 mL of 25% HCl in 50 mL of distilled water at 100°C for 2 hours, neutralized with 25% NaOH (pH 7) and filtered. A total of 25 mL of aliquot of the filtrate was reacted with 25 mL of Luff-Schoorl reagent, heated under reflux for 10 minutes, and cooled. After adding 15 mL of 20% KI and 25 mL of 25% H_2SO_4, the solution was titrated with 0.1 N sodium thiosulfate using 0.5% starch indicator. A blank was prepared using distilled water, and starch content was calculated based on the titration difference between the blank and the sample, with reducing sugar content determined from the difference between inverted and non-inverted sugars, multiplied by 0.9. Additionally, cassava peel starch was characterized with FTIR spectrophotometer to confirm that the extract was starch. Cassava peel starch was characterized using FTIR spectrophotometer "Prestige 21 Shimadzu FTIR Spectrometer" at 1400–400 cm^{-1}, resolution of 2 cm^{-1}, 100 scans with KBr pellet method.

Preparation and application of coating solution

Coating solution was prepared by mixing starch with distilled water at varying concentrations of 3, 5, and 7%, which was heated at 80°C. Subsequently, 1% of CMC and 2% of sorbitol were added to the mixture and stirred until homogeneous to improve the solution

Table 1. Composition of edible coating

Coating Series*	Edible Coating Component			
	Starch (%)	Sorbitol (%)	CMC (%)	EO (%)
Control	-	-	-	-
P3	3	2	1	-
P5	5	2	1	-
P7	7	2	1	-
PEO	5	2	1	-
PEO1	5	2	1	0.1
PEO15	5	2	1	0.15
PEO2	5	2	1	0.2

*P3, P5, and P7 represent coatings containing 3%, 5%, and 7% starch, respectively. PEO1, PEO1.5, and PEO2 indicate coatings with starch combined with 0.1%, 0.15%, and 0.2% essential oil, respectively.

and enhance plasticity, as shown in Table 1. The solution was left at room temperature, added with EO, and homogenized for further application. Before coating process, grapes were cleaned and sorted by uniform size (about 3 cm in diameter). The selected grapes (without stems) were coated by the dip coating method and immersed twice for 15 seconds (Handayani et al., 2018). Subsequently, coated grapes were dried, put in a plastic container (Polyethylene (PE)), and stored at room temperature for 7 days, while the control fruits did not receive any treatment. The effect of EO starch-based edible coating on fruits quality was evaluated daily by measuring weight loss, pH, organoleptic, and microbial tests.

Weight loss determination

The weight of grapes was recorded from days 0 to 7. The calculation was performed as the percentage of weight loss compared to the initial weight of grapes according to a standard method of AOAC (Helrich, 1990; Khodaei et al., 2021). All measurements were repeated three times, and the percentage of weight loss was calculated using Equation (1):

$$weight\ loss = \frac{initial\ weighti - final\ weight}{initial\ weight} x100\% \quad (1)$$

pH measurement

To measure the pH of the sample, 1 gram of the sample was crushed and taken, then it was dissolved with 10 mL of water, filtered, and measured using a pH meter (Yin et al., 2012). The pH meter must first be calibrated using pH 4 and pH 7. pH measurement was repeated 3 times.

Organoleptic test

Organoleptic test was carried out based on appearance and color by 15 untrained panelists (Tanone & Prasetya, 2019). The assessment focused on key attributes including appearance, aroma, texture, and overall acceptability, using a hedonic scale to measure preferences. The panelists rated freshness and color appearance of fruits by appearance criteria ranging from 1 to 5, indicating 1: extremely rotten, 2: rotten, 3: slightly fresh, 4: fresh, and 5: very fresh. Color parameter was rated on a range of 1 to 5, indicating 1: extremely pale, 2: pale, 3: slightly bright, 4: bright, and 5: very bright.

Microbial Total test

The microbiological test was performed using the Total Plate Count method. The sample was diluted to 10^{-1}, 10^{-2}, and 10^{-3}. Four test tubes containing 9 ml of distilled water were prepared and labeled as tubes 1-3. A 1 gram of the homogenized sample was added to tube 1. Subsequently, 1 mL was pipetted and transferred to tube 2, with a similar step repeated until tube 3. The 1 mL of dilutions of tube 3 were plated into pre-sterilized petri dishes. Liquid nutrient agar (NA) at the temperature of 44-46°C was added 9 mL per petri dish containing the diluted sample. The mixture was homogenized by rotating petri dishes, which were allowed to cool and solidify. Petri dishes were incubated in an inverted position at approximately 37°C for 24-48 hours (Fujikawa & Tsubaki, 2019), and microbiological test was conducted on days 1, 3, 5, and 7.

Statistical analysis

The SPSS ver. 24 was used to analyze the collected data, and the results obtained were expressed as mean ± standard deviation (SD), examined using One-way Analysis of Variance (ANOVA) at a significant level of $p<0.05$. This was followed by the Tukey HSD test at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Cassava peel starch

Starch yield from the extraction of starch from cassava peel was found to be approximately 6.33%, as shown in Table 2. The value was higher compared to previous studies, which produced 5.97% of starch (Alfian et al., 2020).

The percentage of starch obtained was 56.02%, which was higher than the 44% previously reported (Mudaffar, 2020). Although the extraction method used was similar, the difference may be due to variations in cassava peel source, sample preparation, or specific extraction conditions. This suggested that cassava peel could be a promising starch suitable for edible coating applications. Figure 1 shows FTIR spectra of cassava peel starch.

Table 2. Starch yield from cassava peel extraction

Cassava part	Mass (gr)
Cassava peel	2640
Starch	167

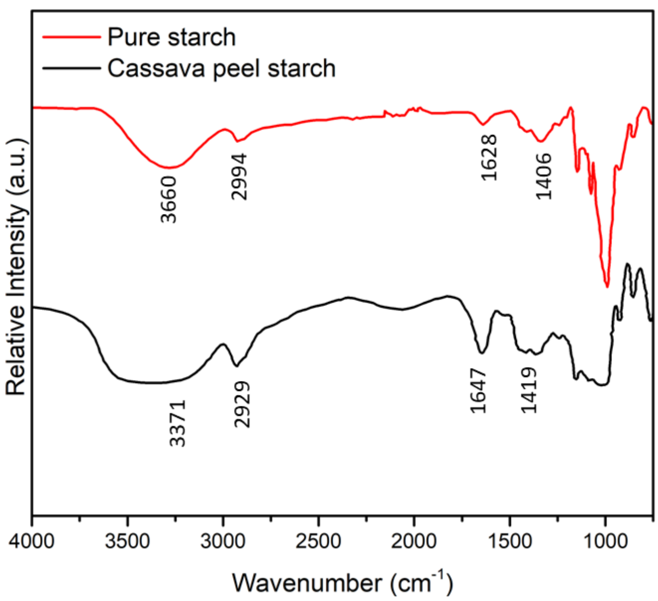

Figure 1. FTIR spectra of pure starch and cassava peel starch

FTIR spectra of pure starch showed the adsorption band at 3660, which corresponded to the stretching vibration of O–H groups, associated with hydrogen bonding in polysaccharides. The band at 2994 cm⁻¹ was related to the asymmetric stretching of the C–H bond. Additionally, the peaks at 1628 cm⁻¹ corresponded to the C=O group stretching vibration, while 1404 cm⁻¹ was associated with C-H bending vibration.

Cassava peel starch spectra showed an absorption peak with band shifts compared to pure starch. The broad band at 3371 cm⁻¹ corresponded to the stretching vibration of the O–H group, indicating the presence of O-H group. The absorption band at 2929 cm⁻¹ represented the stretching vibration of the C–H bond. The presence of two bands at 1647 cm⁻¹ suggested the occurrence of water-associated vibrations. Furthermore, the peak at 1419 cm⁻¹ corresponded to C-H bending vibrations. These results are in line with previous studies, confirming the presence of O–H, C–H, and C–O functional groups characteristic of starch (Maulida et al., 2016). The shifting peaks correspond to the impurity content in cassava peel starch.

Determination of starch composition for edible coating

Figure 2 shows the weight loss, pH, and organoleptic test during 7 days of storage of coated grapes. As presented in Figure 2a, all samples experienced weight loss during storage due to natural processes such as respiration and transpiration. These processes included the diffusion of water vapor and the breakdown of carbohydrate compounds, causing a decrease in fruit weight (Bovi et al., 2018; Rossi et al., 2022). Transpiration and respiration are physiological processes capable of causing a major loss in fresh fruit. Specifically, transpiration contributes by allowing water vapor to escape from fruits surface, while respiration consumes carbohydrates, producing CO_2, energy, and evaporated water (Lufu et al., 2019). In addition, water loss during storage also reduces quality and causes damage to the fruit (Handayani et al., 2018). It is suggested to further measure the respiration to confirm the result.

Coated grapes have a lower weight loss percentage than controlled grapes (without coating treatment). The edible coating can inhibit the transpiration and respiration processes in fruit, preventing it from shrinking. The coating acts as a barrier, reducing water vapor and oxygen transmission, thus minimizing shrinkage. This result aligned with previous research found that the cassava peel starch-based coated pineapple had a lower weight loss percentage than pineapple without edible coating treatment (Maharsih & Pusfitasari, 2021). The lowest percentage of weight loss was found at a concentration of 5%. Lower concentrations might not provide complete coverage. Whereas the high concentration tends to be more viscous, and it was easy to crack, resulting in an edible coating with a lower ability to inhibit respiration on the grape.

ANOVA analysis results showed a significant effect of starch concentration treatment on the percentage of weight loss during storage, with a significance of 0.017 ($p<0,05$). Additionally, respiration affects the content of organic acid in fruits, because of conversion into sugar (Ma et al., 2022). Based on the results in Figure 2b, grapes tended to increase pH during storage due to a decrease in organic acids during the respiration process. This suggests that edible coating preserves pH level of fruits, as also reported in previous studies (Breceda-Hernandez et al., 2020). Coated grapes with a 5% concentration have the lowest pH. ANOVA analysis showed that there was no significant effect of starch concentration treatment on pH during storage, with a significance ($p>0,05$).

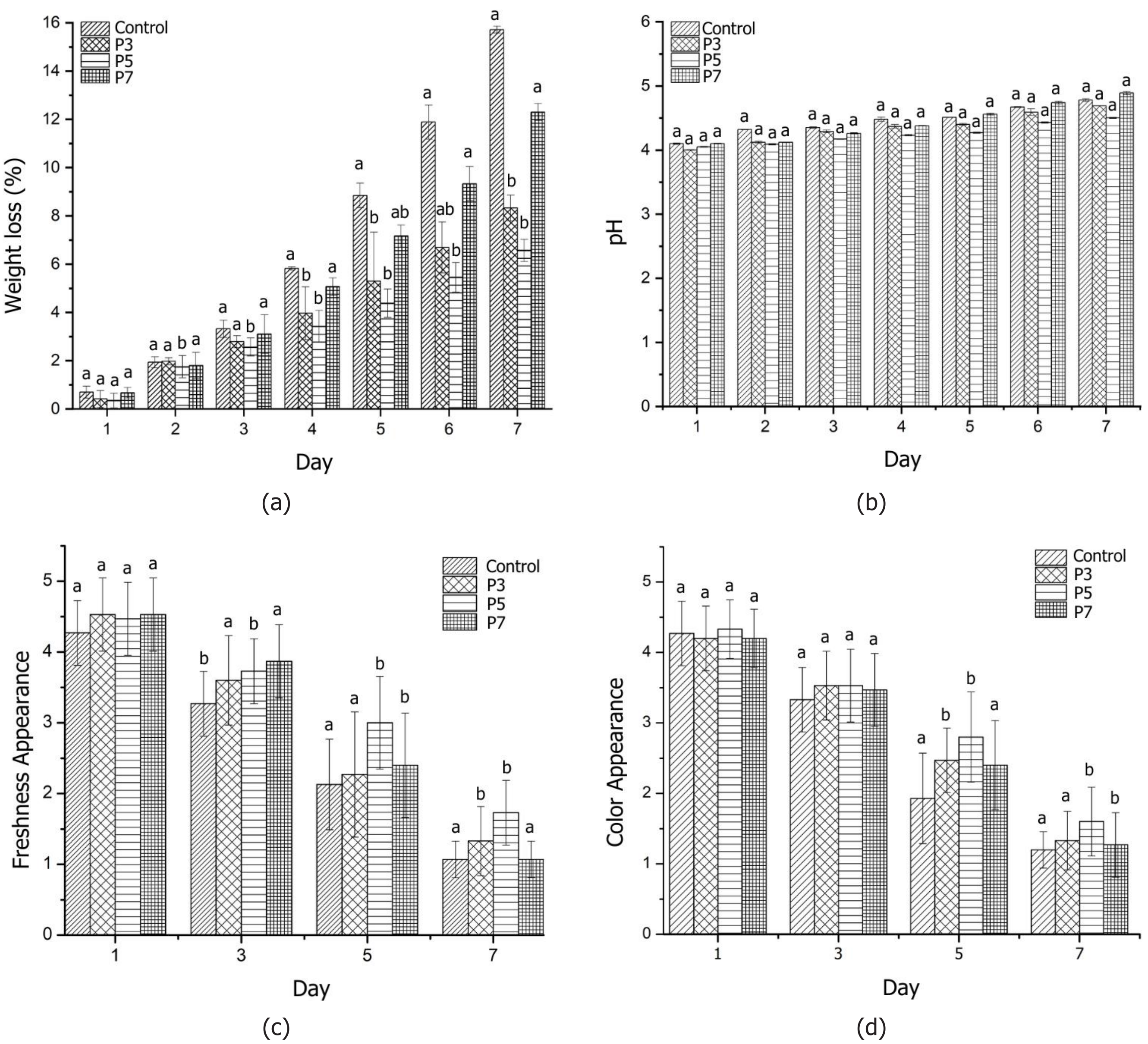

Figure 2. Weight loss (a), pH (b); freshness (c); and color of grapes during storage (d) (N= 3, mean values with error bars that show the standard error of the mean). Different letters show a significant difference ($p < 0.05$) for each sample (starch variation) in the same storage day

Figures 2c and 2d show test results for freshness and color, where grapes coated with 5% concentration have higher scores than others. This suggests that using edible coating with an optimized composition helps sustain the natural brightness of fruits, as grapes contain an antioxidant compound giving color to fruits rind. The stability of anthocyanins is determined by several factors, e.g., pH, oxygen, temperature, and light (Khoo et al., 2017). In case of an interaction between fruits and oxygen from the surrounding environment, there is damage to anthocyanins that affects color. ANOVA analysis showed that there was a significant effect of starch concentration treatment on the appearance and color of grapes during storage, with a significance p<0,05.

Based on the observations, the 5% starch concentration was found to be the best result for coating grapes, effectively maintaining quality during storage. This result corresponded to previous studies, where a 5% concentration of starch maintained the quality of avocado during storage period (Sivamania et al., 2018).

Determination of EO concentration

Figure 3 shows the weight loss, pH, and organoleptic test (freshness and color) of grapes coated with EO incorporated edible film. As presented in Figure

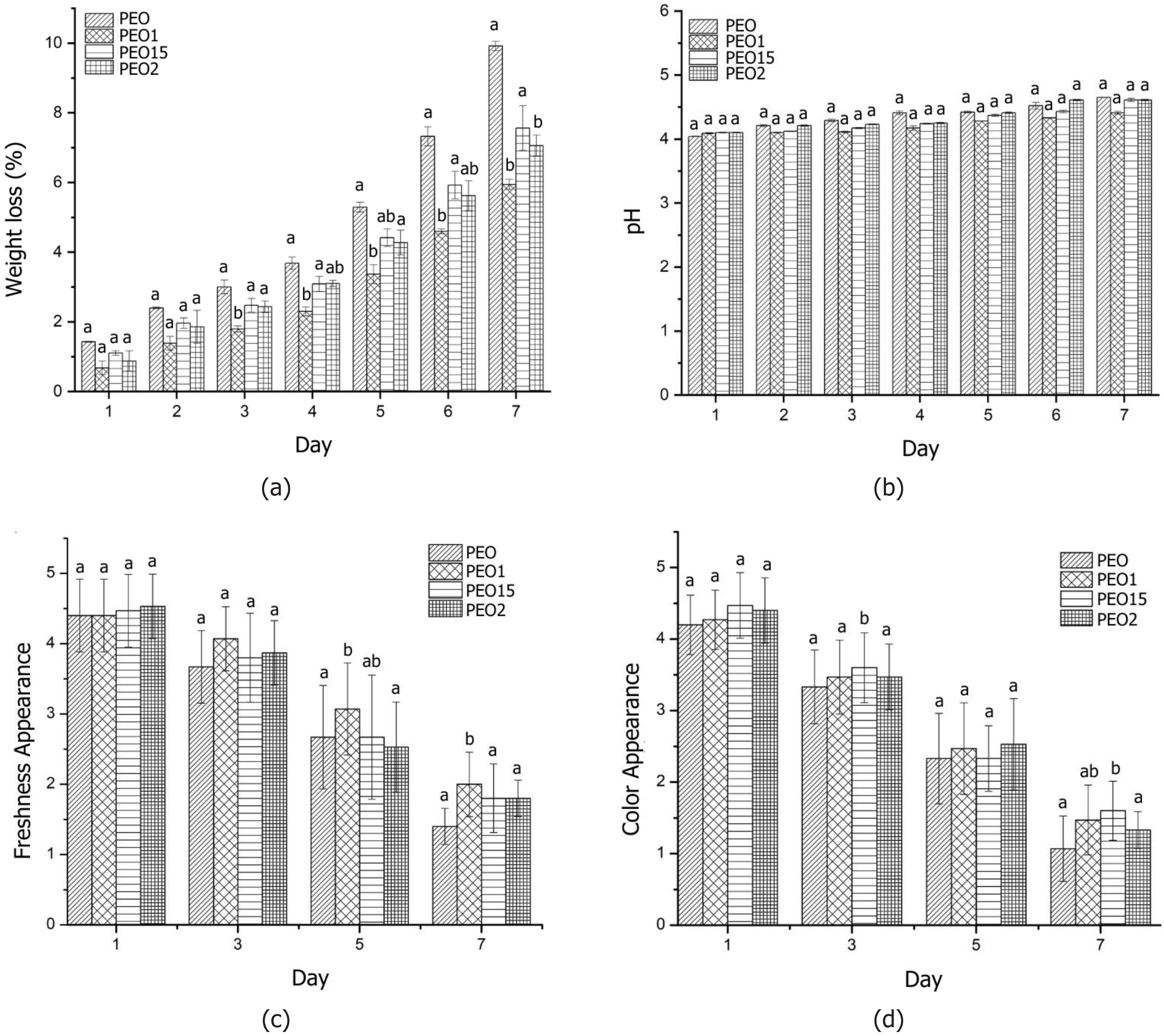

Figure 3. Weight loss (a), pH (b); freshness (c) and color of coated grapes during storage (d) (N=3, mean values with error bars that show the standard error of the mean). Different letters show a significant difference ($p < 0.05$) for each sample (EO variation) in the same storage day

3a, coated grapes with the addition of EO have a lower weight loss percentage compared to those without EO. This suggests that EO serves as hydrophobic component in coating emulsion and barriers against moisture loss, thereby reducing the diffusion of water to maintain the mass of fruits (Yousuf et al., 2021). The hydrophilic and hydrophobic ratio is essential because concentration influences water vapor permeability. Specifically, weight loss is attributed to moisture loss from the tissue. Water vapor loss occurs mostly through the hydrophilic component of coating, which explains the reduction in weight from fruit tissue when coated with EO. The lowest weight loss recorded was 5.94%, with pH gradually increasing during storage (Figure 3b). ANOVA results showed that EO concentration significantly influenced weight loss ($p < 0.05$) but did not affect pH ($p > 0.05$). The 0.1% EO treatment had slightly lower final pH of 4.21, indicating minimal moderation of pH by EO.

Based on the organoleptic assessment (Figures 3c and 3d), the freshness and color appearance of the coated grapes with the addition of 0.1% EO show the highest score. The organoleptic scores of the coated grapes with the addition of EO are higher compared to the coated grapes without the addition of EO. It can happen due to the EO's role as an antimicrobial that inhibits fruit spoilage and maintains the fruit's physiological appearance, both in freshness and color appearance. Generally, microbial contamination is a major cause of quality deterioration in post-harvest fruits and vegetables due to the influence of fungi and bacteria. According to Khodaei et al. (2021), these putrefactive microorganisms significantly influence the overall quality of fruits. Similar to the appearance test, color evaluation of grapes coated with EO also produced a higher score, as shown in Table 3.

Table 3. Appearance of grapes during 7 days

Days	Control	Coated	Coated with EO
1			
3			
5			
7			

As presented in Table 3, the three fruit treatments showed no effects until day 3. However, on day 5, uncoated fruits started showing signs of decay, including the presence of rot and bacteria. Coated samples appeared fresher and brighter than the uncoated, as previously presented in Figures 2c and 2d. Fruits coated with the addition of EO had the freshest appearance and the best-preserved color compared to others, appearing fresh on day 7. This discovery was also consistent with the panelists results presented in Figures 3c and 3d. Grapes treated with coating have higher scores, which show the potential to maintain appearance. Several factors can contribute to fruit rotting, including fruit transpiration and bacteria that cause decay and change appearance. A high water loss can also lead to withering and wrinkles on fruits, causing loss of freshness (Novita et al., 2012). Applying the coating on fruits can maintain freshness and inhibit the spoilage process by inhibiting transpiration process and ultimately preventing softening. The higher organoleptic scores for coated grapes with EO can be attributed to the reduced fruit spoilage, which is supported by the lower weight loss observed in these samples. The coating likely helped maintain fruit integrity by reducing moisture loss and delaying deterioration, contributing to better appearance and overall acceptability

Besides that, color is one of the main factors considered by consumers when selecting food. A nutritious food with a good taste and texture will not be selected if it does not have an appealing color (Dey & Nagababu, 2022). Therefore, the color of the food becomes the main attractive aspect of eating it. Grape contains an antioxidant compound that gives color to the fruit's rind. The stability of anthocyanins is determined by several factors, including pH, oxygen, temperature, and light (Khoo et al., 2017). In case of an interaction between the fruit and oxygen from its surrounding environment, it suggests the damage of anthocyanins and thereby ruins the fruit's color. ANOVA analysis showed that there was a significant effect of EO concentration on the freshness appearance of grapes during storage, with a significance p<0,05. However,

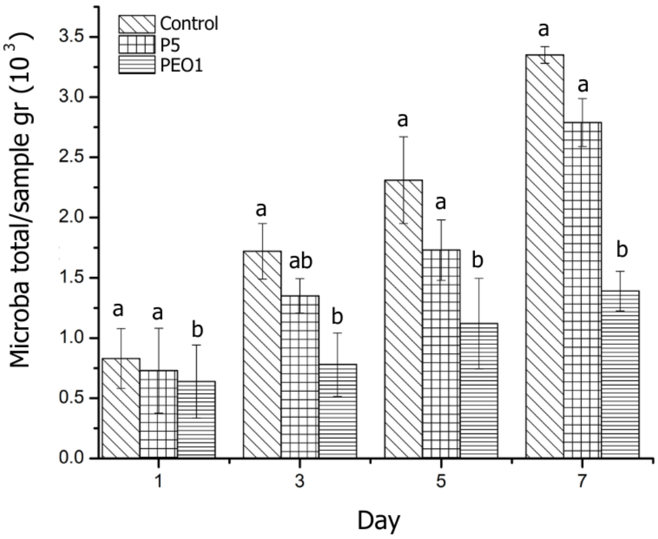

Figure 4. Microbial total of grapes during the storage period (N= 3, mean values with error bars that indicate the standard error of the mean). Different letters represent a significant difference ($p < 0.05$) for each sample (EO variation) on the same storage day

the addition of EO did not substantially influence color appearance ($p>0.05$).

Based on the weight loss percentage, pH, and grape organoleptic assessment, the optimum condition of EO added to cassava peel starch was obtained at a concentration of 0.1%. Although pH levels did not show a significant effect, the result indicated that EO with a concentration of 0.1% had the lowest pH level. The concentration of 0.1% was also selected as the best concentration due to the requirement for minimal materials with good result. Therefore, the optimum concentration of edible coating was found to be 5% of starch with the addition of 0.1% EO.

Microbial total test

Figure 4 shows the increase in total microbes in grapes during the storage period, with the controlled grapes having a higher number compared to coated. This shows that edible coating treatment on grapes can reduce microbial growth.

Grapes coated with the addition of EO have a smaller number of microbes during the storage period compared to the control. Specifically, microbial total count of coated grapes was 1.39×10^3 CFU/gram, while uncoated samples had 3.35×10^3. The variation in results is attributed to the presence of limonene on sweet orange EO, which is the main component of citrus essential (Golmohammadi et al., 2018). Limonene can cause damage to the cell wall structure and the bacteria DNA structure, thereby interfering with the growth (Patricia et al., 2019). This result suggests that adding EO significantly improves antimicrobial activity of edible coating, preventing grapes from bacteria growth.

CONCLUSION

In conclusion, this study successfully extracts starch from cassava peel with a facile method, which produces a yield of 52.02%. The results show that the best composition of starch and EO in edible coating was 5% and 0.1, respectively, suggesting the potential to significantly maintain the quality of grapes. The addition of orange peel-based EO can inhibit microbial growth on grapes during storage. At this optimum concentration, grapes coated with EO-starch film show a mean weight loss of 5.94%, pH 4.21, and lower total microbes of 1.39×10^3 CFU/gram. This indicates that edible coating improves the marketability and shelf life of grapes, thereby supporting environmental sustainability by reducing food waste and promoting the use of natural and biodegradable sources.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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