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FULL PAPER

Aloe vera gel coating incorporated with citric acid preserves the chemical and the microbiological qualities of fresh-cut melon

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Abstract

Fresh-cut fruit is categorized as minimally processed food that is susceptible to quality deterioration. In this study, Aloe vera gel coating was incorporated with 0.1% citric acid and 1 or 2% of glycerol (Gly). The effect of coating on the physicochemical properties and microbial quality of fresh-cut honeydew was studied. Fresh-cut honeydew samples were coated with different gel formulations (80% AV 1% Gly, 80% AV 2% Gly, 100% AV 1% Gly, 100% AV 2% Gly) and stored at 4°C in polyethylene bag. Physicochemical properties (weight loss, pH) and microbial quality of the honeydew samples were evaluated on storage days two, four and six of storage. The sample treated with 100% AV 2% Gly had the lowest weight loss and aerobic plate count. Further investigation was done on the titratable acidity, ascorbic acid, total polyphenol content, total yeast and mould count between coated sample (100% AV 2% Gly) and the uncoated sample. The coated sample (100% AV 2% Gly) was found to have significantly higher titratable acidity, ascorbic acid content and total polyphenol content compared to the uncoated sample ($p<0.05$). The coating of 100% AV 2% Gly significantly reduced total yeast and mould count on day six. Aloe vera-citric acid gel coating serves as a potential alternative to maintaining the quality of the melon.

1. Introduction

In recent years, fresh-cut fruits have gained popularity in the population due to the consumers' demand for healthy convenience food. The need for preparation and the relatively large size of the fruits is inconvenient for the consumer and that is how fresh-cut produce fits into their lifestyle. In general, fresh-cut produce is a highly perishable food. Fresh-cut produce including fresh-cut fruits is categorized under minimally processed food. All these minimally processed products usually involve operations including washing, cutting, peeling and shredding before packaging and storage (Rahman, 2007). Size reduction has greatly affected the quality and caused nutritional loss of produce. For example, the level of antioxidant, bioactive compounds, ascorbic acid and carotenoid present in some of the fresh -cut fruits will decrease after cutting.

Some of the major concerns associated with fresh-cut produce are physiological and biochemical changes that reduce the shelf life of the product. This is because the wounded tissue in fresh-cut produce will increase the respiration rate, oxidative browning and ethylene production in fresh-cut produce (Hodges and Toivonen, 2008). Fruit cutting increases the surface area exposed to the environment. Fresh-cut produce experiences deterioration and surface dehydration rapidly. Loss of cellular integrity might contribute to microbial invasion and growth.

Many processing techniques have been applied to improve the shelf life of fresh-cut produce. In addition to low-temperature storage, modified atmosphere packaging (MAP) can also be used to minimize quality changes of fresh-cut produce (Wilson *et al.*, 2019). Alternatively, an edible coating can be used to inhibit the produce deterioration (Horison *et al.*, 2019). In this technique, a thin layer of edible substance is usually coated on food surfaces for preserving its quality. It should be able to create a barrier that retards moisture loss and controls gas exchange to slow down respiration and deterioration (Lin and Zhao, 2007).

Melon fruits with high water activity and near-neutral pH are highly perishable especially when they were minimally processed or cut. Therefore, there is a need of applying edible coatings to preserve commodities for longer storage time. Citric acid is the

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major organic acid that occurs naturally in citrus fruits (Wu *et al.*, 2021). Citric acid provides a favourable sour taste and possesses antimicrobial properties (Mosqueda-Melgar *et al.*, 2008). It has been applied for inhibiting browning reactions in vegetables and fruit (Pace *et al.*, 2020). Glycerol serves as a plasticizer that is commonly used to increase film strength and flexibility of coatings. The addition of glycerol in Aloe vera gel coating is to improve its coating adhesion and durability during storage. However, it may affect the functionality of materials such as water vapour and gas permeability (Hassan *et al.*, 2018). Thus, the concentration of plasticizers used must be optimized.

The main objective of this study is to investigate the effect of Aloe vera gel combined with citric acid and glycerol as a composite edible coating on fresh-cut honeydew. The physicochemical and microbial quality of coated fresh-cut honeydew were conducted to study the functions and effects of Aloe vera gel coating with different concentrations (80% AV 1% Gly, 80% AV 2% Gly, 100% AV 1% Gly and 100% AV 2% Gly).

2. Materials and methods

2.1 Extraction of Aloe vera gel

Aloe vera (*Aloe barbadensis* Miller) leaves and honeydew melons (*Cucumis melo var. inodorus*) were purchased from the market located in Kuala Lumpur. The Aloe vera leaves were washed using distilled water before the Aloe vera matrix was cut out from the outer cortex of the leaves by using a sterilized knife. Distilled water was used to rinse off the poisonous yellow colour Aloe latex on the Aloe vera matrix. The transparent matrix was then crushed using a blender and the mucilaginous gel was filtered using a cheesecloth to remove the fibrous material. The Aloe vera gel was then kept in screw cap bottles before application of Aloe vera gel on fresh-cut honeydew.

2.2 Preparation of coatings

The four Aloe vera gel coatings of different formulations were prepared by mixing Aloe vera gel, distilled water and glycerol according to the formulations shown in Table 1. The citric acid (0.1% v/v Aloe vera gel coating) was added to each coating solution mixture and homogenized using a homogenizer with a speed of 7000 rpm for three minutes.

2.3 Preparation of fresh-cut honeydew

The surface of honeydew melon was washed with water to remove the dirt that may cause contamination to the fruit flesh. The knife and containers were sterilized using hot boiled water. The honeydew melon was cut into small cubes (3×3×3 cm). All the fresh-cut honeydew cubes were distributed randomly between five different treatments (control and four different Aloe vera gel coatings).

Table 1. Formulations of Aloe vera gel coating

	Aloe vera gel solution		Glycerol	Citric acid
Formulation	Aloe vera gel (%)	Distilled water (%)	(v/v Aloe vera gel solution) (%)	(v/v Aloe vera gel solution) (%)
A	80	20	1	0.1
B	80	20	2	0.1
C	100	-	1	0.1
D	100	-	2	0.1

2.4 Preparation of Aloe vera gel coatings

The respective honeydew cubes were immersed into the Aloe vera gel coating solutions of different concentrations for 3 mins. To control fresh-cut honeydew samples, the honeydew cubes were immersed in distilled water for three minutes. Then, a sieve was used to drip off the excess coating material on the surface of honeydew cubes for 2 mins. The weight of three coated honeydew cube samples from each treatment was measured and recorded for weight loss analysis.

2.5 Evaluation of the efficiency of different aloe vera gel coatings

All the control and coated honeydew cube samples were sealed separately in a polystyrene bag and kept at 4±1°C. The experiments were divided into two stages. In the first stage, different coating formulations were compared. pH, weight loss, and total plate count were conducted on two, four and six days of storage. In the second stage of the experiment, Aloe vera gel coating with the best performance in weight loss and total plate count was chosen for further investigation. Further analysis was conducted between coated and uncoated samples including titratable acidity, ascorbic acid content, total polyphenol content, total yeast and mould count.

2.6 pH and weight loss

Three honeydew cubes from each treatment were crushed to obtain the juice. The pH was measured using a pH meter (Mettler Toledo). For weight loss, the weight of honeydew was measured on the day of treatment for initial weight and the weight of the respective samples was measured after two, four and six days of storage for final weight. The weight measurement of honeydew from each treatment was triplicated (n = 3). The weight loss percentage of honeydew was calculated as the

percentage loss of initial weight of coated sample and control sample.

2.7 Total plate count and total yeast and mould count

A total of 10 g of sample was measured and mixed with 90 mL of sterile peptone water. The mixture was homogenized and a serial dilution was conducted. For total plate count, the spread plate method was performed in triplicate on the plate count agar (PCA). Total aerobic plate count was enumerated after incubation at 37°C for two days. Total yeasts and mould count were enumerated by using potato dextrose agar (PDA) after incubation for five days at 30°C. The results obtained were expressed as colony-forming units (CFU) per g.

2.8 Titratable acidity and ascorbic acid content

Titratable acidity was determined according to the method of AOAC (2000). For ascorbic acid, 2,6-dichloroindophenol (DCIP) titration method was used to determine the ascorbic acid content of the sample (AOAC, 1990). Measurement for each treatment was triplicated.

2.9 Total polyphenol concentration

The total polyphenol concentration was determined using the gallic acid equivalence assay (AOAC, 2017). A stock solution of gallic acid (100 ppm) and serial dilution of gallic acid solution were prepared. Folin-ciocalteau (FC) reagent was prepared by diluting with the same extraction solvent of the sample (80% ethanol) at a ratio 1:10 and kept in dark conditions.

A 10 g of sample was crushed and makeup to 100 mL of 80% ethanol in a volumetric flask. 0.5 mL of the sample extract was mixed with 2.5 mL of FC reagent and 2.0 mL of 7.5% sodium carbonate solution (after five minutes). Approximately 0.5 mL of each gallic acid standard solution will be mixed with 2.5 mL of FC reagent and 2.0 mL of 7.5% sodium carbonate solution (after five minutes) as well. The mixture for each standard solution and sample solution were incubated in dark conditions for one hour and then the absorbance of the mixture was measured at 765 nm using a spectrophotometer. The phenolic content calculated was expressed as mg GAE/ 100g.

2.10 Statistical analysis

Data of each treatment obtained from each test were analyzed with a one-way analysis of variance (ANOVA) using SPSS software (IBM SPSS Statistics Version 22). Mean values from triplicate measurements were calculated and reported as mean±standard deviation. HSD Turkey test ($p<0.05$) was used to examine the presence of significant differences between treatments.

3. Results and discussion

3.1 pH

Table 2 shows the pH of uncoated and coated samples. In general, the pH increased throughout the storage regardless of treatments. The pH value of the uncoated fresh-cut honeydew was generally greater than that of the coated fresh-cut honeydew throughout storage. The lower pH values of coated fresh-cut might be caused by the citric acid added into the Aloe vera gel coatings.

Table 2. pH value of fresh-cut honeydews with different formulations during chilled storage

Sample	pH		
	Storage Time (days)		
	2	4	6
Control	5.93±0.06^{a}	5.99±0.11^{a}	6.20±0.13^{a}
A (80% AV, 1% Gly)	5.64±0.09^{b}	5.61±0.17^{b}	6.09±0.07^{ab}
B (80% AV, 2% Gly)	5.64±0.09^{b}	5.70±0.11^{ab}	6.11±0.22^{a}
C (100% AV, 1% Gly)	5.55±0.11^{b}	5.62±0.05^{b}	5.68±0.14^{b}
D (100% AV, 2% Gly)	5.63±0.04^{b}	5.63±0.11^{b}	5.91±0.18^{ab}

Values are presented as mean±standard deviation (n = 3). Values with different superscripts within the same column are significantly different ($p<0.05$).

Generally, fruits with a lower pH do not support the growth of bacteria. However, melons have a higher pH with a high water activity which is susceptible to microbial growth (Bassett and McClure, 2008). *Listeria monocytogenes* had been implicated in a multistate outbreak in the United States in 2011 and cantaloupe was the source of contamination (McCollum *et al*., 2013). *Listeria monocytogenes* are of concern due to their ubiquitous occurrence in the environment and variety of food (Wai *et al.*, 2019).

At a storage period of four days, the pH of coated samples A, C and D were significantly lower ($p<0.05$) than that of the control sample. In this case, a lower pH value of coated honeydew samples might help to retard the microbial growth across the storage period.

3.2 Weight loss

All the treatments experienced weight loss across the storage time (Table 3). However, the weight loss of coated samples was generally lower than that of control although the differences were not significant statistically ($p>0.05$). Weight loss is a common phenomenon for fresh-cut fruits that contain high water content such as melons. Weight loss in fresh-cut fruit is mainly caused by loss of water (Koh *et al.*, 2017). It is hypothesized that the edible coating applied on fresh-cut melons could help to reduce fluid loss. The edible coating might serve as a protective layer or sacrificial layer for moisture loss

to reduce weight loss.

The hydrophilic nature of the coating material leads to higher water vapour permeability of the coating material (Rahman, 2007). Aloe vera gel consisted mainly of hydrophilic polysaccharides. This nature may limit their efficiency to act as a barrier to water transfer and retard moisture loss. It might explain the insignificant result observed among the treatments.

Table 3. Weight loss percentage of fresh-cut honeydew with different formulations during chilled storage.

Sample	Weight loss (%)		
	Storage Time (days)		
	2	4	6
Control	1.20±0.60	1.33±0.72	2.36±1.80
A (80% AV, 1% Gly)	0.94±0.54	1.22±0.52	1.32±1.25
B (80% AV, 2% Gly)	1.10±0.96	1.16±0.59	2.26±0.76
C (100% AV, 1% Gly)	0.89±0.55	1.01±0.20	1.31±0.89
D (100% AV, 2% Gly)	0.82±0.20	1.08±0.35	1.17±0.54

Values are presented as mean±standard deviation (n = 3). There was no significant difference among samples (p>0.05).

3.3 Total plate count

As shown in Figure 1, the total plate count of the coated samples and control samples increased slowly during the experimental period. In the first four days of storage, all the coated fresh-cut had a lower count compared to control except for sample A on Day 4, although the differences were not significant statistically (p>0.05). It indicates that the gel coatings might have inhibitory effects on the growth of aerobics mesophilic bacteria at the initial stage of storage. Similarly, Aloe vera gel was reported to reduce the microbial population in strawberry fruit (Sogvar *et al.*, 2016). In the present study, the observed microbial growth inhibition is possibly due to the combined effect of Aloe vera and citric acid. Aloin is the possible component that contributes to its antimicrobial activity in Aloe vera (Zapata *et al.*, 2013). A fresh-cut fruit is a wounded tissue, leakage of fruit juice provides nutrients for bacterial growth. Microbial growth inhibition help extend the shelf life of fresh-cut fruits.

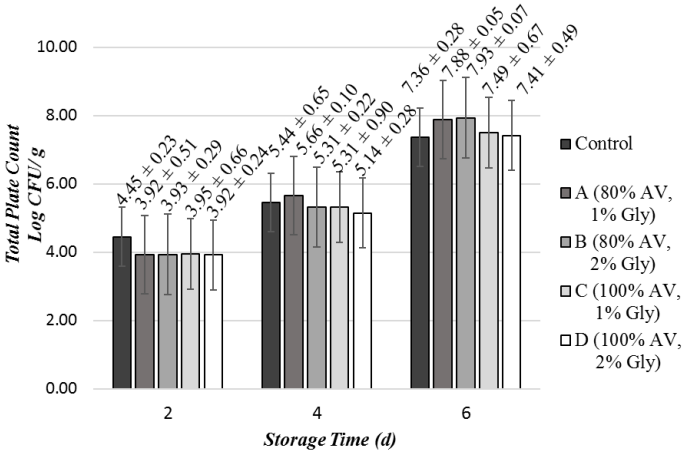

Figure 1. Total plate count of fresh-cut honeydew with different formulations during chilled storage. Values are presented as mean±standard deviation (n = 3). There was no significant difference among treatments (p>0.05).

3.4 Titratable acidity

By comparing the pH, weight loss, and total plate count of different Aloe vera gel coatings, Aloe vera gel coating D (100% AV, 2% Gly) had the lowest weight loss significantly (0.82±0.20%) on day two. For total plate count, the lowest log CFU/g was recorded in Aloe vera gel coating D after 2 days and 4 days of storage. Therefore, formulation D (100% AV, 2% Gly) was chosen for further evaluations including titratable acidity, total polyphenol, ascorbic acid, yeast and mould count.

Total titratable acidity can serve as a quality indicator for fruit. Generally, the total titratable acid of the coated sample was higher than those of the uncoated sample during storage (Table 4). After four days and six days of storage, titratable acidity levels decreased in all fruit samples. A possible reason for the decline of total titratable acid during storage is the acids being metabolized in the respiratory process (Wan and Lam, 1984).

Table 4. Titratable acidity of coated and uncoated fresh-cut honeydews during chilled storage

Sample	Titratable Acidity (g of citric acid/ 100g)		
	Storage Time (days)		
	2	4	6
Coated (100% AV, 2% Gly)	0.09±0.01[a]	0.09±0.01[a]	0.07±0.01[a]
Uncoated	0.09±0.01[a]	0.06±0.00[b]	0.05±0.01[b]

Values are presented as mean±standard deviation (n = 3). Values with different superscripts within the same column are significantly different (p<0.05).

A significant difference was observed between the coated and uncoated samples after four days and six days of storage. The Aloe vera coating might help in better retention of titratable acidity. This result is similar to the study by Hassanpour (2014) associated with raspberry fruit. Wounding tissue may induce ethylene production (Saltveit, 1999). In this study, the protective coating may prevent the wounded tissue to contact the environment. Low oxygen reduces respiration and inhibits ethylene action and hence slowing the rate of organic acid degradation.

3.5 Ascorbic acid content

Figure 2 presents the ascorbic acid concentration of uncoated and coated fresh-cut honeydew stored at 4±1°

C. Based on the figure, the trend of the ascorbic acid concentration of both samples is decreasing along with storage time. The coated fresh-cut honeydew had a significantly higher level of ascorbic acid than uncoated samples throughout the storage period ($p<0.05$). It shows that the gel coating had effectively retained the ascorbic acid in fresh-cut honeydew.

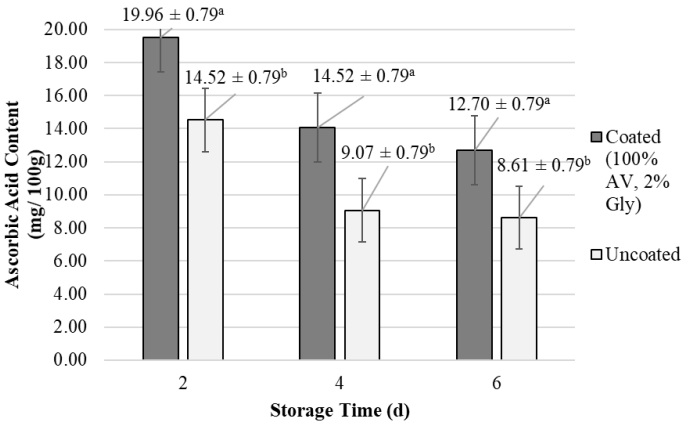

Figure 2. The ascorbic acid content of coated and uncoated fresh-cut honeydews during storage. Values are presented as mean±standard deviation (n = 3). The different superscript indicates a significant difference between treatments ($p<0.05$).

The presence of oxygen is an important contributor to ascorbic acid oxidation (Amaro *et al.*, 2015). The retention of ascorbic acid was possibly attributed to the modification of the atmosphere with gel coating. Hence, the autoxidation of ascorbic acid was greatly reduced. Besides, the ascorbic acid concentration of coated fresh-cut honeydew might be contributed by the Aloe vera gel which contains antioxidants such as α-tocopherol and ascorbic acid as well (Radha *et al.*, 2015).

3.6 Total polyphenol content

Figure 3 displays the total polyphenol content in coated and uncoated fresh-cut honeydew samples during storage at 4±1°C. The coated fresh-cut honeydew retained most of the phenolic content throughout the storage compared to the uncoated fresh-cut honeydew. The polyphenol content of the uncoated sample decreased from 39.24±0.29 (day two) to 29.37±0.57 mg GAE/ 100 g of sample (day six). The polyphenol content of coated sample decreased from 45.50±0.67 (day two) to 40.48±0.13mg GAE/ 100 g of sample (day six).

The uncoated fresh-cut honeydew sample had a significantly lower ($p<0.05$) polyphenol content than a coated fresh-cut honeydew sample. About 100 g of honeydew melon contains only 59.33 mg of total phenolic compound (Amaro *et al.*, 2015). The reduction of total polyphenol content in uncoated fresh-cut honeydew samples might be attributed to the damage to cell structure that leads to the degradation of phenolic compounds. In this case, the Aloe vera gel coating was effective to reduce the rate of biochemical changes and degradation of phenolic compounds by acting as a protective barrier on the fruit surface.

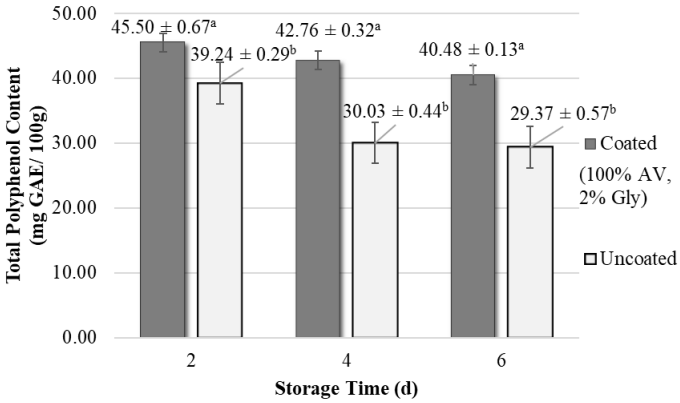

Figure 3. The total polyphenol content of coated and uncoated fresh-cut honeydews during storage. Values are presented as mean±standard deviation (n = 3). The different superscript indicates a significant difference between treatments ($p<0.05$).

3.7 Total yeast and mould count

Total yeast and mould count of fruit in both treatments increased during storage, but it was higher in uncoated honeydew (Figure 4). In the first two days, the results were not significantly different ($p>0.05$). After four and six days of storage, Aloe vera coating demonstrates the antifungal effect in the present study. The gel coating (100% AV 2% Gly) showed a significant effect in reducing the total yeast and mould count ($p<0.05$). 0.25 log reduction and 0.72 log reduction of yeast and mould count were recorded by coated fresh-cut honeydew on day four and day six respectively as compared to control. Total yeast and mould count are some of the factors that will affect the shelf life of fresh-cut. The high yeast and mould count of fresh-cut honeydew indicate spoilage potential.

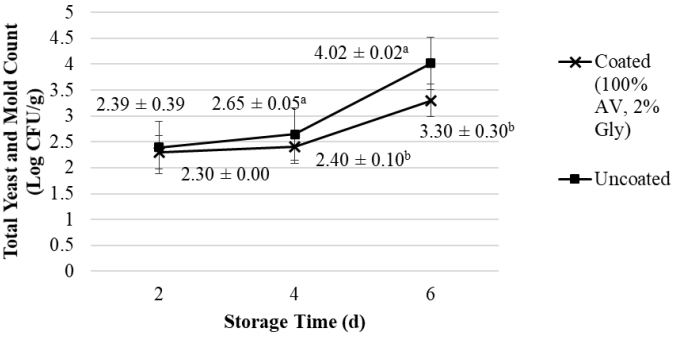

Figure 4. Total yeast and mould count of coated and uncoated fresh-cut honeydews during storage. Values are presented as mean±standard deviation (n = 3). Different superscript indicates significant difference between treatments ($p<0.05$).

Aloe vera gel is reported for its antifungal activity against common fruit pathogens. The gel was found to inhibit mycelial growth against *A. alternate* (Saks and Barkai-Golan, 1995). Fungi were more tolerant to colder

conditions compared to bacteria (Pietikäinen *et al*., 2005). Low-temperature storage does not prevent spoilage, fungi still can grow in this condition (Baert *et al.*, 2007). Therefore, the application of Aloe vera gel coating could help to retard the growth of fungi.

4. Conclusion

The application of Aloe vera-citric acid gel coatings (80% AV 1% Gly, 80% AV 2% Gly, 100% AV 1% Gly and 100% AV 2% Gly) reduced the pH of fresh-cut honeydew significantly but their effects on weight loss and total plate count of fresh-cut honeydew were not significant. Aloe vera-citric acid gel coating (100% AV and 2% Gly) was found to have a significant effect in preserving the titratable acid, ascorbic acid and total polyphenol content of fresh-cut honeydew compared to the uncoated sample. Also, this gel coating exhibited a significant log reduction in the total yeast and mould count of honeydew. In conclusion, Aloe vera-citric acid gel coating provides a potential alternative to help preserve the quality of fruit.

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