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Application of Chitosan-*Aloe vera* Gel Based Coating on Postharvest Quality and Storability of Red Chili (*Capsicum annuum* L.)

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ABSTRACT
Red chili pepper (*Capsicum annuum* L.) is a high-demand horticultural commodity in Indonesia but is vulnerable to quality deterioration. Postharvest treatment is needed to maintain the quality and extend the shelf life of red chilies. One of the promising preservation technologies to prolong the shelf-life of perishable agricultural products is edible coating application. This present work was intended to evaluate the effect of chitosan and *Aloe vera-based* coating formulations on quality attributes of red chilies after 15 days of storage at room temperature. This study used a completely randomized design (CRD) with two factors, including the concentration of chitosan (1, 1.5, and 2%) and *A. vera* (5 and 10%) that were conducted in triplicate for each treatment. The obtained results showed that the application of chitosan and *A. vera* gel as an edible coating on preserving red chilies could inhibit the deterioration of postharvest red chilies. The best formulation, a combination of 2% chitosan and 10% *A. vera*, exhibited the most minimum decay percentage (43%) of red chilies. This coating formulation also has a great antifungal activity that could effectively inhibit the mycelium growth of *Colletotrichum capsici* up to 52%.

Keywords: *Aloe vera, Chitosan, Edible coating, Red chilies, Quality change*

1. INTRODUCTION

Red chili pepper (*Capsicum annuum* L.) is a high-demand horticultural commodity in Indonesia but is vulnerable to quality deterioration. Postharvest losses of red chilies are strongly related to the metabolic activities and high-water content of harvested chilies. After harvesting, red chilies undergo both respiration and withering processes that cause the shelf life of chilies to be relatively short [1]. Besides, red chilies are vulnerable to spoilage by fungi and bacteria that cause postharvest shrinkage during storage. *Colletotrichum capsici* is a fungus commonly found in chilies [2]. For those reasons, postharvest treatments are needed to preserve and prolong the shelf life of red chilies.

One of the promising preservation techniques to prolong the shelf-life of perishable agricultural products is edible coating application [3]. Compared to cold storage technology, the edible coating requires a zero-energy cooling system [4]. The edible coating applied on fruit surface serve as a semipermeable barrier to minimize water loss and respiration and prevent microbial and fungus infection [5]. According to Hameed *et al.* [6], chilies are sensitive to chilling injuries due to low-temperature storage (< 7°C), application of edible coating can be a safe and affordable preserving alternative to maintain the quality attributes of red chilies.

The sources of edible coating material vary, including polysaccharides, lipids, proteins, or resins [7]. The well-known edible coating source is chitosan, which a type of polysaccharides. Chitosan has excellent properties, mainly has high antimicrobial activity and biocompatibility, and is non-toxic [8]. Besides, the chitosan-based edible coating forms a semipermeable film that limits water losses and the transpiration

process, extending fruits storability [9]. Some recent studies reported the application of chitosan on blueberries [10], strawberries [11], and chilies [12,13]. Another coating material with exceptional barrier properties is *Aloe vera* gel, which is biologically safe, has antimicrobial action, and delays the deterioration of fruits and vegetables [14]. In this last decade, some applications of *A. vera* gel-based coating were on fresh-cut kiwi fruit [15], strawberry [11], fresh-cut papaya [16].

The combination of more than one edible coating source gained more interest to improve edible coatings' effectiveness and stability. Poverenov *et al.* [17] observed that a coating from the combination of chitosan and gelatin on red bell peppers could significantly reduce the microbial decay incidence compared to gelatin-based coating treatment, enhance the fruit firmness and improve the shelf-life during storage. Vieira *et al.* [10] reported that edible coating from chitosan-*A. vera* liquid fraction could inhibit the microbial growth up to 50% and extend the life span of blueberries for approximately five days. These previous studies imply that combining two or more coating sources could improve the physicochemical properties of fruits better than a single source. To our knowledge, the application of *A. vera* gel and chitosan combination as an edible coating on red chilies has not been studied before. Therefore, this present work was intended to evaluate the effect of chitosan and *A. vera* based coating formulations on quality attributes of red chilies after 15 days of storage at room temperature and choose the recommended chitosan-*A. vera* formulation to preserve the quality of red chilies.

2. METHODOLOGY

At the mature state, red chili peppers were harvested from a farm field in Sukabumi, West Java. The peppers were directly transported to Bogor and prepared for the experiment the following day. Red chili peppers with defects (e.g., broken and dull outer skin) were discarded while uniform size and appearance were selected. Chitosan flakes produced from crab shells were obtained from the previous research and then ground into powder using a blender (Philips). *Aloe vera* leaves were bought from the fresh market.

The edible coating formulations were arranged by using a Completely Randomized Design (CRD) factorial with two factors, where A = the concentration of chitosan (1, 1.5, and 2% w/v) and B = the concentration of *A. vera* gel (5 and 10% w/v). Each treatment was conducted in triplicate. Non-coated peppers were used as a control treatment. The quality attributes of samples were evaluated after 15 days of storage.

Chitosan solutions (1, 1.5, and 2 %) were prepared according to Muthmainnah *et al.* [12] by dissolving chitosan powder (1, 1.5, and 2 g) to 1% acetic acid (100 mL). The solutions were stirred at 400 rpm and heated at 50 °C for 60 minutes. Finally, chitosan solutions were filtered using filter paper with a vacuum pump to remove impurities. *Aloe vera* gel was prepared by peeling the cortex of *A. vera* leaves, cutting the flesh into small pieces, and then blending. The pulp and gel mixtures were filtered using a sieve to produce clear gel and then was pasteurized at 75 °C for 15 minutes. The gel was cooled at room temperature, and the pH was adjusted to 4 before further use. Edible coating solutions were prepared by mixing chitosan solution with *A. vera* gel in the predetermined ratio and mixed for 5 minutes using a magnetic stirrer.

During coating application, 50 g of red chili peppers were dipped in the coating solution for 5 seconds and dried at the shady and open place for 30-60 minutes until thoroughly dried. As a control, peppers were submerged only in distilled water. The coated samples were packed in transparent plastic boxes and stored at room temperature for 15 days. Attributes studied in this research include decay incidence (%), weight loss (%), pH (%), and soluble solid content (SSC).

2.1. Decay incidence

Red chili with the fungal mycelia growth on its surface was considered decay. The decay incidence was evaluated by using the following formula [17]:

$$decay\ incidence\ (\%) = \frac{number\ of\ decayed\ chilies}{total\ number\ of\ chilies} x\ 100 \quad (1)$$

2.2. Weight loss

Samples were weighed every three days using a two-decimal digital scale. The following Equation (2) calculated the weight loss percentage of red chilies [6], where W_1 is the weight of the sample before storage, and W_2 is the weight after storage for 15 days

$$weight\ loss\ (\%) = \frac{W_1 - W_2}{W_1} x\ 100 \quad (2)$$

2.3. pH

The pH of red chilies from each treatment was measured before and after 15 days of storage. A 10 g of red chilies from each treatment were cut into small pieces and ground using a blender with 100 mL of

distilled water. The pH measurement was done by using a pH meter (Satorius) [11].

2.4. Soluble solid content (SSC)

The soluble solid content of extracted red chili juice was measured using a digital refractometer, and the value was expressed in percentage [11].

2.5. Antifungal activity assay

The poisoned food technique was conducted according to Satish *et al.* [18] to determine the percent of mycelial inhibition of *Colletotrichum capsici*. This fungus was isolated from rotten chili seeds. First, the rotten chili seeds were dipped in sterile water and dried using sterile tissue. These seeds were dipped again in Alcohol (70%) for 30 s continued the dipping process in sterile water three times. Chili seed was inoculated to potato dextrose agar (PDA) and incubated at room temperature (25-28°C) for one week. The *C. capsici* was identified using a microscope by observing of the shape of the hyphae and conidia. The pure culture of *C. capsici* isolate was used for the antifungal activity assay.

A total of 9 mL of PDA media was poured into a petri plate, and 1 mL of chitosan and *A. vera* mixture solution was added with a predetermined concentration variation, continuously shaking until homogeneous. A mixture of media with distilled water was used as a negative control. Once the PDA media solidified, aseptically inoculated *C. capsici* isolates (1 cm diameter). These petri plates were then incubated at 37°C for seven days. The diameter of the fungal pathogen colonies contained in the media was measured. The percent (%) mycelium inhibition of each treatment was determined using the following Equation (3), where C is the radius of fungal mycelium growth in negative control media (cm). T is the radius of fungal mycelium growth in treated media.

$$\text{mycelium inhibition (\%)} = \frac{C-T}{C} x\ 100 \quad (3)$$

2.6. Statistical analysis

The data were analyzed using IBM SPSS software 16.0 and subjected to a two-way analysis of variance (ANOVA) ($p < 0.05$). Tukey's HSD (Honestly Significant Difference) test examined the significant differences between treatments.

3. RESULTS

3.1. Microbial decay incidence

The incidence of microbial decay on red chilies with various coating treatments ranged from 43-100%. A two-way ANOVA analysis revealed a significant interaction between the effect of chitosan and *A. vera* concentration on incidence decay of red chilies after storage for 15 days at room temperature. Coating red chilies with a mixture of 2% chitosan and 10% *A. vera* resulted in the significantly lowest percentage of decay, which was 43% (Figure 1). In contrast, the coating combination of *A. vera* concentrations with 1% chitosan solution exhibited a maximum decay (100%), and with 1.5% of chitosan solution resulted in a slight decrease of decay incidence but not significant (94.14-95.56%).

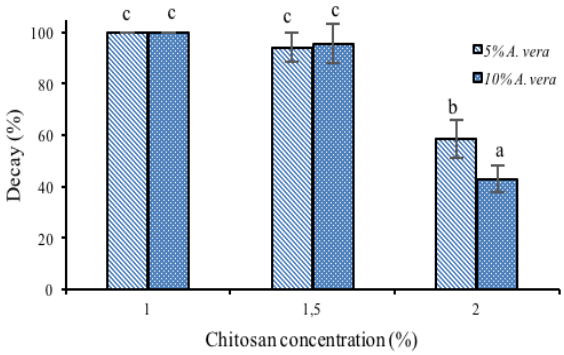

Figure 1. Effect of edible coating on decay incidence of red chilies after 15 days storage. The bars indicate the standard error of each treatment (n = 3). The significance value is indicated by the difference letter on top of each bar, $p < 0.05$.

These results showed that the chitosan concentration affects the microbial decay incidence on red chilies. The *A. vera* concentration also affects the inhibition of microbial decay mainly when combined with 2% of chitosan solution.

Chitosan has been known for its antimicrobial activity. Chitosan could restrict the microbial growth and coat the preserved product so that there is minimal interaction between the product and its environment. El Ghaouth *et al.*[19] tated that chitosan acts on host-pathogen, causing severe cellular damage and inhibiting the enzyme secretion. Edirisinghe *et al.* [20] explained that chitosan performs its antifungal activity by activating the defense enzymes of plants or fruit. A bibliometric review from Salgado-Cruz *et al.* [21] summarized several fungi on postharvest fruit effective to inhibited through the coating application from pure chitosan or incorporated with other coating compounds, including *Colletotrichum gloeosporioides* on Mango [22], papaya [23] and guava [24]; *Botrytis cinerea* on strawberry [25], blueberry [26], and cherry tomato [27]; B*urkholderia seminalis* on apricot fruit [28].

Table 1. Main effects of chitosan and *A. vera* treatments as edible coatings on weight loss of red chilies during storage.

	Weight loss (%)				
	3 days	6 days	9 days	12 days	15 days
Chitosan concentration					
1%	0.88±0.52 ^{b}	1.72±0.18 ^{b}	2.46±0.21 ^{b}	3.21±0.21 ^{b}	3.97±0.19 ^{b}
1.50%	0.66±0.85 ^{a}	1.55±0.27 ^{ab}	2.35±0.43 ^{a}	3.30±0.46 ^{b}	3.86±0.25 ^{b}
2%	0.67±0.09 ^{a}	1.34±0.29 ^{a}	1.93±0.26 ^{a}	2.58±0.17 ^{a}	3.41±0.28 ^{a}
A. vera concentration					
5%	0.77±0.09 ^{x}	1.61±0.25 ^{x}	2.36±0.35 ^{x}	3.11±0.44 ^{x}	3.80±0.33 ^{x}
10%	0.70±0.15 ^{x}	1.46±0.31 ^{x}	2.13±0.38 ^{x}	2.95±0.45 ^{x}	3.70±0.36 ^{x}

* letters that follow the number showing significant or not significant differences, $p < 0.05$.

On the other hand, *A. vera* also greatly influences on the inhibition of microbial incidence. *A. vera* gel contains many bioactive compounds responsible for antifungal and antibacterial activity against numerous microorganisms, such as *Penicillium digitatum, Rhizopus stolonifera*, and *Otritys cinerea* [29]. Vieira *et al.* [10] observed that both liquid and pulp fractions of *A. vera* could restrict the mycellium growth of *Aspergillus niger, Botytris cinerea and Penicillium expansum* with the best inhibition occurred to *B. cinerea*. Barragan-Menendez *et al.* [30] reported that the coating formulation of 100% of *A. vera* and 10 $g.L^{-1}$ glycerol could restrict the growth of *C. gloeosporioides*. *Aloe vera* gel coating also showed good results in inhibiting the growth of destructive microorganism on strawberry [31] and kiwi [15].

3.2. Weight loss

Based on a two-way ANOVA analysis, there was no significant interaction between the effect of chitosan and *A. vera* concentrations on weight loss of red chilies. However, the main effect analysis showed significant weight losses on chitosan treatments from 3 to 15 days after storage (Table 1). The lowest weight loss occurred in 2% chitosan treatments, where after 15 days of storage, the weight loss of red chilies was only 3.41%. Meanwhile, the *A. vera* treatments did not significantly affect the weight loss of red chilies during 15 days of storage. The overall coated red chilies' weight loss was considerably lower than untreated red chili (6.43%).

Table 2. Effect of edible coating on pH and SSC of red chilies.

	pH		SSC	
	0 day	15 days	0 day	15 days
Chitosan concentration				
1%	4.61±0.17 ^{a}	4.35±0.12 ^{a}	1.23±0.16 ^{a}	0.77±0.08 ^{a}
1.50%	4.71±0.11 ^{a}	4.5±0.09 ^{a}	1.08±0.08 ^{a}	0.73±0.08 ^{a}
2%	4.68±0.01 ^{a}	4.47±0.11 ^{a}	0.97±0.08 ^{a}	0.70±0.06 ^{a}
A. vera concentration				
5%	4.69±0.13 ^{a}	4.45±0.12 ^{a}	1.12±0.20 ^{a}	0.74±0.09 ^{a}
10%	4.63±0.13 ^{a}	4.44±0.13 ^{a}	1.07±0.1 ^{a}	0.72±0.07 ^{a}

* letters that follow the number showing significant or not significant differences, $p < 0.05$

These results suggested that low concentrations of chitosan and *A. vera* edible coatings (1% and 5%, respectively) could be used to reduce the weight loss of red chili at least up to 2.5% compared to uncoating chilies.

As a crucial parameter, weight loss determines the quality and life span of fruits. Fruit weight loss during postharvest is primarily correlated with moisture evaporation and respiration through the fruit surface [9]. The edible coating has been proven to maintain the fruit quality by creating a physical barrier limiting the respiration rate and water evaporation during storage. Some previous studies have reported the significance of chitosan-based edible coating in reducing the weight loss of *Capsicum* sp. The optimum chitosan concentration varied depending on the types of chilies/peppers, the storage condition, and the combination with other coating materials. Adetunji *et al.* [13] noted that a significant weight loss reduction of green bell peppers was observed on the coating treatment with 1.5% of chitosan stored on the evaporative cooling system. Poverenov *et al.* [17] stated that the mixture coating made from 2% chitosan and 1% gelatin on red bell peppers could prolong the cold storage period up to 21 days. Muthmainnah *et al.* [12] reported that the application of 1.5% of chitosan combined with 10% gum Arabic could significantly inhibit the respiration rate of chilies (*Capsicum frustecens* L.).

A. vera gel coating also could control the weight loss of some fruits such as strawberry [11,31], sliced kiwi fruit [15], and papaya [32]. *A. vera* gel mainly contains polysaccharides, so it has a good characteristic as a barrier on fruit surface [14]. In this study, the effects of *A. vera* treatments were not significant due to the improper storage conditions and *A. vera* concentration. A study from Suriati *et al.* [33] revealed that the *A. vera* gel-based coating is relatively stable when stored at cool temperatures compared to room temperature. Besides, higher concentrations of *A. vera* gel might give better results in reducing weight loss. A study from Qamar *et al.* [11] observed that *A. vera* gel coating (100%) showed the lowest weight loss (13%) of refrigerated strawberry fruits compared to uncoated fruits (27%). Ul Hasan *et al.* [34] reported that *A. vera* coating (50%) could significantly reduce the weight loss of green chilies (7.4%) compared to control (12.8%) after 28 days of cold storage (10°C).

3.3. pH

The pH of coated red chilies was not significantly different in any coating formulations at 15 days of storage (Table 2). Overall, the pH changes of coated red chilies during 15 days storage were around 4-7 %, which is relatively small compared to uncoated red chilies, 18 %. This means the coatings could maintain the pH value of red chilies with the slightest pH change compared to uncoated ones.

Although, the concentration of chitosan and *A. vera* and the interaction of these two factors did not significantly affect the pH change among the coated red chilies ($p > 0.05$). The result obtained is similar to several publications related to coating fruits. Qamar *et al.* [11] reported that *A. vera* gel-coated strawberries had the least pH change after 12 days of storage. Vieira *et al.* [10] found that chitosan-*A.vera* gel-coated blueberries preserved their pH better than uncoated blueberries. The pH value of fruits and vegetables is related to the organic acids. An increase in the pH value indicates a decrease in acidity caused by acid formation decline and the losses of some organic acid contents during storage [35].

3.4. Soluble solid contents (SSC)

Soluble solid contents decreased for all uncoated and coated red chilies over 15 days (Table 2). However, the SSC reductions in coated red chilies (22-43%) were considerably smaller than uncoated ones (67%), indicating that coatings might preserve the SSC of chilies. Statistical results showed no significant difference among treatments, which the SSC values of all treatments were 0.7-0.8 %. These results trends were similar to [11], where SSC of coated and uncoated strawberries decreased over storage, but uncoated samples had the lowest SSC. Some studies reported that edible coating applications tend to maintain the SSC of blueberry [10] and mangoes [36]. During the postharvest period, the soluble solid content of vegetables and fruits tends to rise, caused by ripening process [11].

3.5. Antifungal activity

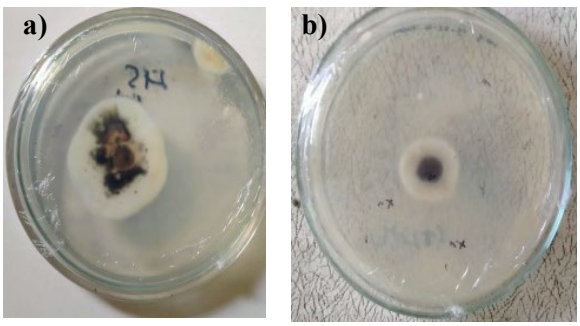

Figure 2. Mycelium growth of *Colletotricum capsici* on PDA medium. **a)** control; **b)** with the addition of 2% chitosan and 10% *A. vera* gel at day 7.

The mycelial growth of *C. capsici* is presented in Figure 2a. Identification of macroscopic results showed that *C. capsici* mycelium has different colors of colonies in which the upper part of the petri dish had white-grey

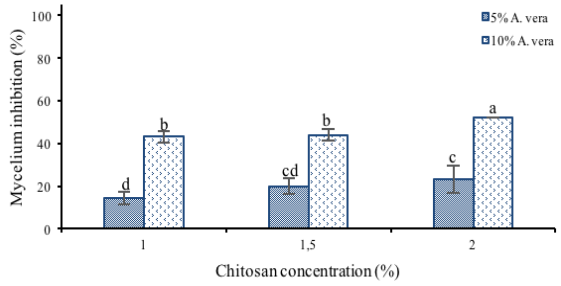

Figure 3. Mycelium inhibition of *Colletotricum capsici* on coating formulation modified PDA medium. The bars indicate the standard error of each treatment (n = 3). The significance value is indicated by the difference letter on top of each bar, $p < 0.05$.

colonies. In contrast, the bottom part of the petri had blackish brown colonies. The mycelium growth of this fungus was relatively slow in the first 24 hours, so the mycelium growth was observed after 7 days. Mycelial growth inhibition of *C. capsici* on agar medium with different coating formulations ranged between 14.4-52%. The effect of chitosan and *A. vera* concentrations on the mycelium inhibition of *C. capsici* showed a statistically significant interaction (Figure 3). The highest mycelium inhibition, 52%, was obtained from adding 2% chitosan solution and 10% of *A. vera* gel to PDA agar (Figure 2b). Overall, higher significant inhibitions were observed from 10% of *A. vera* gel treatments than 5% of *A. vera* gel treatments (19.2%). These results follow Nidiry *et al.*[37] in which the extract of *A. vera* gel showed high antifungal activity on *C. gloeosporioides* and *C. capsici*.

Chitosan and *A. vera* gel-based coatings could be used as a postharvest strategy alternative to improve the quality of red chilies during storage. The best formulation, a combination of 2% chitosan and 10% *A. vera,* exhibited the most minimum decay percentage (43%) of red chilies. This coating formulation also has a great antifungal activity that could effectively inhibit the mycelium growth of *Colletotrichum capsici* up to 52%.

An edible coating made of from the mixture of *A. vera* and chitosan is generally safe. It may provide a low-cost coating compared to solely chitosan coating or the use of pure *A. vera* gel (100%). Some improvements in technical coating application and formulation are worth exploring to increase coating performance effectively.

AUTHORS' CONTRIBUTIONS

All authors have contributed to finishing this manuscript. Yora Faramitha: conception, experimental and analysis design, data analysis, interpretation, discussion, drafting, and revisions. Fitria Febriyanti: performing the experiments, collecting data, and data analysis; Tiana Fitrilia, Firda Dimawarnita, and Siswanto: discussions and revisions.

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