Post-harvest quality assessment of *Carica papaya* L. 'Red Lady' papaya coated with rice bran wax (RBW)

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Abstract

Carica papaya L. fruits are delicate and perishable due to their high water content and would benefit from preservation methods. Waxing is a preservation method that serves as a barrier equivalent to a laminate film on the peel. Rice bran wax (RBW) as a coating has been noted to have delayed the produce ripening process. This study aimed to determine the quality of papaya coated in 15%(T1), 20%(T2), and 25%(T3) RBW emulsions against a control over a time period by assessing %weight loss, pH, fruit color, and decay incidence. Results showed that T1 had the highest weight loss percentage, pH level, and lowest decay incidence out of all treatment groups. T2 had the highest decay incidence, whereas the T3 showed the lowest %weight loss. Results had statistically insignificant differences between all treatments for all parameters. Thus, RBW is not recommended for *Carica papaya* L. fruit preservation.

Introduction. - *Carica papaya* L. fruit is produced in about 60 countries, especially in developing countries such as the Philippines which mainly grows in home gardens or as an intercrop [1, 2]. It is delicate and perishable due to its high water content and therefore lags far behind bananas and pineapples in world trade [3]. Papaya shelf-life is crucial as the papayas are stored for 1 to 2 days before being distributed to wholesaler retailers where they must last at least 3 days at the supermarket or retail shop [4]. Current preservation methods that are used in the country are the usage of modified atmosphere packaging (MAP) [5, 6] and the use of 1-methylcyclopropene (1-MCP) [7]. Waxing is another method of prolonging a fruit's shelf life by coating it in an artificial waxing material. One such material used for waxing is rice bran wax (RBW) [8, 9, 10]. This method does not harm the environment and is a cheaper alternative to other preservation practices [11].

RBW has been noted to inhibit ripening while being utilized as a wax coating for produce with it being found to be an effective way to extend tomato shelf-life of up to 27 days [8, 9]. Similar results can also be seen with limes, sweet peppers, and cherry tomatoes [8, 9]. The common material for waxing is Carnauba wax which must be imported into the country as its source plant *Copernicia prunifera* can only be found in northeastern Brazil [10]. RBW, on the other hand, can be made and procured locally in the Philippines as a by-product of the biowaste of rice milling.

The physical and chemical properties of a fruit can be analyzed to determine or approximate its maturity, namely %weight loss, pH, color, and decay incidence.

Weight loss percentage is an indicator of the fruit dehydration process due to transpiration. This parameter serves as a representation and a means to assess the efficiency of coatings in preserving fruit quality. Papayas comprise 80 to 85% water making them highly perishable as a high water content makes them highly susceptible to microbial growth [13]. They are also climacteric fruits that continue to ripen and transpire even after harvesting wherein there would be a rapid rise in the rate of oxygen and carbon dioxide exchange [14]. Papayas couldn't replace water that has been lost as water vapor once they are taken off from the main plant thus contributing to the weight loss products will experience. When moisture loss is too high, produce can experience commodity deterioration which includes symptoms like shriveling [15]. Wax coatings like RBW act as a second layer or barrier to retain moisture that could potentially lead to weight loss due to moisture loss [13].

Ripening and subsequently pH control in fruits are very important to prevent color and texture loss [17]. An increase in total acidity observed during ripening is believed to be associated with an increase in free galacturonic acids [18]. One of the important intrinsic factors influencing spoilage characteristics of fruits is pH. Fruits with a pH range of 4.5 or below do not allow the growth of most of the bacteria with exempted examples being molds, yeasts, and aciduric bacteria [19].

Color is one of the first quality attributes a customer can detect in food products [16]. Color change in papayas can be seen as their smooth thin

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green skin turns yellow or red as it ripens, and their succulent flesh ranges from yellow to orange to red [20]. Therefore, waxing can slow down the ripening process which can be reflected by the color change of the fruits.

Decay incidence is the occurrence of decay symptoms. Such symptoms can be decay and mold, sunken areas on the skin, discoloration, overripeness, softness, scarring of the skin, bruising of flesh, brown spots on the skin, and shriveled appearances [21]. In a study investigating consumers' preference for fresh table-ripe papaya [22], the top attribute that consumers deemed important in deciding their purchase is the absence of decay/damage. The high moisture content and soft texture of fruit make it susceptible to mechanical injury throughout its production and retail life [14]. Aside from being a protective barrier, wax coatings can also create a non-water-compatible surface that is not conducive to pathogen growth while sealing small cracks and dents in the exocarp to establish a barrier against pathogen entry [23].

There are limited investigations conducted utilizing RBW as the material for waxing on papaya and significantly bigger fruit sizes than sizes found in current RBW studies.

This study's findings will contribute to the enhancement of papaya preservation techniques for producers, particularly in exploring alternative wax materials like RBW, thereby expanding the existing body of research in this field. The additional information on RBW provided by this study may encourage other researchers to further investigate RBW or present different methods for using it as a wax coating.

This study aimed to determine the quality of *Carica papaya* L. 'Red lady' papaya coated with RBW. The study specifically aimed to:

(i) measure the papaya fruits' percentage weight loss, pH level, fruit color, decay incidence, and mean values of the treatment groups [(T1) RBW: 15% (w/v), (T2) RBW 20% (w/v), (T3) RBW 25% (w/v)] and the control group;

(ii) compare the papaya fruits' percentage weight loss, pH level, fruit color, decay incidence, and mean values of the treatment groups and the control group; and

(iii) determine if there is a statistical difference in the mean percentage weight loss, mean pH level, mode of fruit color, and mode decay incidence of papaya between the treatment and control groups.

Methods. - This study used an experimental approach to examine the quality of Red Lady papaya. The study lasted four weeks, with one and a half weeks allotted for material procurement and authentication and setup preparation, another one and a half weeks for data collection, and the final week used for value computation and data analysis.

Each papaya was given a code and was randomly assigned to a group. There were three experimental groups and one control group, with each group having three replicates as per the standard control of the Randomized Complete Block Design (RCBD) with a total of nine papayas per group and a total of 36 papayas overall.

The experimental phase was conducted for nine days, and an assessment of the fruit color was done daily during the whole duration of the data-gathering period. The weight was measured on day 0 to get the initial weight and on day nine to get the final weight. The pH and decay were analyzed on the last day of the data-gathering period.

Sample Collection. Samples of the papayas were collected from Spring Bloom Farms, Pavia, Iloilo. They were noted to be unripe based on the color maturity index of Basulto et al. [20]. Samples were picked based on the following criteria: (i) must be Red Lady variety with certification from the Department of Agriculture Western Visayas (DA - WV); (ii) must have no decay sign; (iii) must have no pests; and (iv) must not be shriveled.

Sample Preparation. The papayas were washed with distilled water as recommended by the Food and Agriculture Organization (FAO) of the United Nations [14]. A total of 36 papayas were used, and samples were randomly assigned to different treatments using the fishbowl method. The RBW and the Polysorbate 80 were purchased from Cyleina Organics and Crafts and Cedar Alley Enterprise, respectively.

Wax Preparation. RBW coating emulsion was prepared using a method adapted from Abrihami et al. [10]. The RBW was melted using the double boiler method. This method utilizes the heat of trapped steam between two pots to melt solid wax. A small pot containing the RBW was placed inside a larger pot that was partially filled with water. Using a burner, the water was boiled into a simmer. As adapted from Abhirami et al. [10], the amounts used for the treatments are the following: 15% (T1), 20% (T2), and 25% (T3) were 600 g, 800 g, and 1,000 g of RBW respectively. The hot wax was added to a bucket with 300 mL of emulsifier Polysorbate 80 (Tween 80) and 4 L of hot distilled water, homogenized using a stand mixer for 10 minutes at 18,000 rpm. The treatments were quantified using weight per volume (w/v), which was determined by the ratio of the wax weight to the water volume.

Waxing. The papayas were divided into three blocks with 9 papayas per block. The dip coating method was used to apply the wax on the papayas wherein the whole fruit was dipped in the emulsion for 3 minutes. After waxing, the papayas were left to dry for 5 hours and transferred to their assigned tables. A mosquito net was suspended on each table to prevent insects from contaminating the samples.

%Weight loss. For the weight loss percentage determination, the samples were weighed before (day 0) and after (day 9) storage. The papayas were weighed using a calibrated digital weighing scale. They were all recorded and computed in a Google sheet.

The equation for calculating the percentage of weight loss given below was based on the study of Zhang et al. [8]; where m and m0 are the final and initial weights of each papaya fruit, respectively.

$$\%weight\ loss = (m_0 - m)\ (m_0^{-1})$$

Fruit color. The fruit color of the papayas was observed and documented every 5 pm from day 1 to day 9. The color change of the papaya was based on the maturity index of Maradol papaya in the study of Basulto et al. [20]. (See Table 1 and Figure 1).

Table 1. Description of each maturity stage of Maradol Papaya from Basulto et al. [20]

Maturity Stage	Description
Green fruit	Green skin without yellow stripe, pulp very hard and white in color; seeds well-formed but white or slightly darker in color.
1	Green skin with a light yellow stripe; pulp exhibits some areas with orange color, is very hard, and contains large amounts of latex.
2	Green skin with a well-defined yellow stripe; the pulp is orange in color near the seed cavity and light green near the skin, although still hard and with large amounts of latex.
3	One or more orange-colored stripes in the skin; pulp almost completely orange in color, except near skin, still hard but contains less latex.
4	Skin clearly orange in color with some light green areas; pulp completely orange, except near peduncle, softer than in stage 3, but still too hard for consumption, low latex content.
5	Skin displays orange characteristic of Maradol variety, pulp firmness appropriate for consumption, latex no longer present.
6	Conditions similar to stage 5, but with a more intense orange color in the skin and softer pulp still adequate for consumption

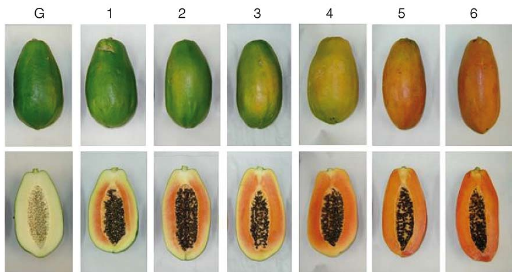

Figure 1. Maturity index of Maradol papaya (Basulto et al. 2009)

The assessments and pictures of the color change of the papayas from day 1 to day 9 were plotted on the Google Docs application.

pH determination. The pH of the control and treated samples was measured using a benchtop pH meter. The pH of each group was determined by taking a sample of their mesocarp or the fleshly middle layer of the fruit's pericarp and then feeding it into a juicer to obtain the liquid solution. The liquid solution was then transferred to a small plastic cup, and its pH value was measured using the pH meter. The pH level of the papayas in each treatment was done on the last day (day 9) of the data gathering period.

Decay Incidence. In this study, papaya is considered to be experiencing decay when the visual signs of quality loss can be seen on the exocarp or the outer layer skin of the fruit; such defects can be decay and mold, sunken areas on the skin, discoloration, overripe, soft, scarring of the skin, bruising of flesh, brown spot on the skin, and shriveled appearances [19]. One of the symptoms of plant disease is fruit spots, definite and localized areas of softening discoloration, and disintegration of tissue [21]. These spots can merge to form rot. Any fruit with visible microorganism growth is also considered decayed.

Decay incidence was based on the visual observation of decay spots recorded using the method of Cao et al. [24]. Their four-grade scale is as described: 0 = no decay; 1 = slight decay, covering up to 25% of the fruit surface; 2 = moderate decay, covering >25% but <50% of the fruit surface; 3 = severe decay, covering >50% of the fruit surface. Results will be expressed as a decay index after using the following formula as used by Cao et al. [24]:

$$DI = \frac{(1 \times N_1 + 2 \times N_2 + 3 \times N_3) \times 100}{(3 \times N)}$$

where DI is the decay index, N is the total number of fruits, and N_1, N_2, and N_3 is the number of fruits showing slight decay, moderate decay, and severe decay, respectively.

Data Analysis. Data were plotted using Google Docs and Google Sheets before being processed with the Statistical Package for the Social Sciences (SPSS) ver.28 program. To determine whether the data are statistically significant, one-way ANOVA was utilized at a 95% confidence interval for the parametric data of weight and pH. At the same time, the Kruskal-Wallis H test was used for the nonparametric data of color and decay incidence.

Safety Procedure. Polysorbate 80 poses physical hazards and potential health effects to researchers. It may cause skin and eye irritation. Moreover, RBW may also cause mild eye irritation to the researchers when not used properly during waxing. The heated emulsion of RBW, Polysorbate 80, and distilled water may cause burns to the hand of the researcher during the waxing process. To avoid these, proper and cautious handling of the substance was always observed. The researchers also wore protective clothing such as gloves and laboratory gowns.

For the disposal of the RBW, as it is wax that can be reused, the remaining treatments were stored away in a cool place in the researchers' homes.

To avoid the spread of the COVID-19 virus, researchers strictly adhered to quarantine protocols and wore face masks. They also avoided touching the eyes, nose, and mouth and washed their hands thoroughly with a sanitizer or alcohol.

Results and Discussion. - The study aimed to determine the quality of *Carica papaya L.* coated with different concentrations of RBW by assessing their weight loss, pH level, fruit color, and decay incidence.

Weight loss analysis. All samples had weight loss. Among the treatment and control groups, treatment 3 yielded the least mean percentage weight loss at 18.54%, followed by control (18.68%), T2 (21.11%), and T1 (21.58%). It could be inferred that T3 allowed for the lowest transpiration rate and water content loss. This may be due to the concentration of the RBW or material component of the film which affects the water vapor permeability properties of the wax [25]. Similarly, the study of Jutamongkon et al. [9] reported that higher RBW concentrations resulted in less weight loss in tomatoes. Zhang et al. [8] also found that RBW-coated cherry tomatoes had a significantly lower weight loss compared to the control group. Percentage weight loss is an indicator of the fruit dehydration process due to transpiration. It can be used as a representation of a way to determine coating efficiency in the preservation of fruit quality [10].

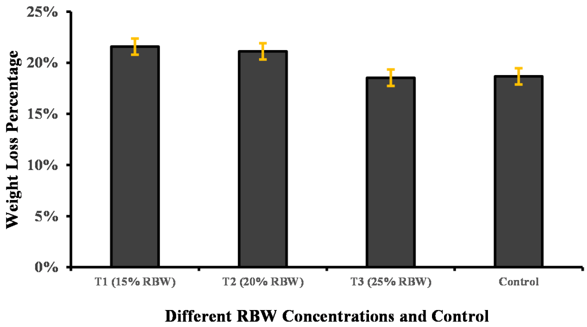

Figure 2. Mean percentage weight loss of the groups

However, one-way ANOVA results revealed that there was no statistically significant difference between the treatment and control groups ($F(3,32)=[0.757]$, $p=0.527$). The weight loss of the coated fruits was not significantly different from the control group. Furthermore, after the means of Day 1 and Day 9 of each treatment and the control group underwent a paired t-test, it was found that RBW neither slowed down nor sped up the weight loss rate. This indicates that there is not enough evidence to prove that a relationship between RBW coatings at different concentrations and weight loss of fruits exists, which may be attributed to the small sampling size. This indicates that there is not enough evidence to prove that a relationship between RBW coatings at different concentrations and weight loss of fruits exists, which may be attributed to the small sampling size. It is to note as well that there are only two data points observed on day 0 and day 9 which could not be represented entirely and thus inconclusive.

pH analysis. It is observed that all groups have yielded a pH value that is between 5 and 6. The mean pH values of the T1 papayas fall under the pulp's standard pH, which is between 5.5 and 5.9 [26]. The rest of the treatments and the control group fall a little lower than the range stated.

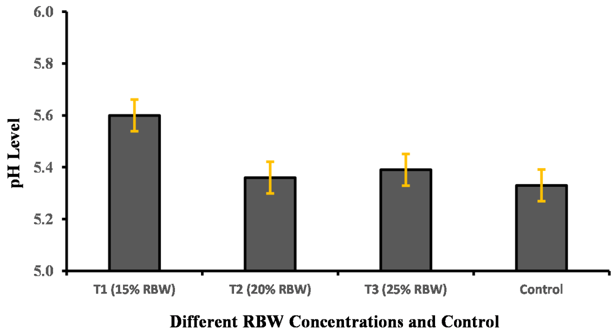

Figure 3. Mean pH levels of the treatments and the control group

One-way ANOVA results revealed that there was no statistically significant difference between the treatment and control groups ($F(3,32)=[0.987]$, $p=0.411$). This suggests that with or without wax, the pH level of the papayas relatively stayed within the normal range. The results of this analysis denote that RBW did not significantly change the pH of the papaya.

Batista-Silva et al. [27] claimed that the organic acid content of fruits decreases with maturation due to the breathing process or sugar conversion. This results in a rise in pH as the acidity of the fruit decreases. In a study conducted by Gomez et al. [28], it was noted that papayas in the mature green stage were more acidic. However, papayas at the intermediate and ripe stages had less acidity and were found to be sweeter than green papayas. The acidity is low because the papayas in the different treatment groups and the control group have a pH level below but near neutral. This signifies that the papaya fruits are marketable because of their taste in relation to their pH levels.

Fruit color analysis. A higher ranking in the color scale means there is a higher number of papayas observed that had higher valued scores on the papaya maturity index. A higher-valued score based on the papaya maturity index corresponds to how far along the papaya is in its ripening based on the visual evidence of its pericarp color.

T1 (15% RBW), T2 (20% RBW), and the control group showed similar progression in color wherein they consistently had a color score mode of 2 for the first five days and it increased to 3 on the sixth up until the seventh day. The fruit color on days 8 and 9 was not recorded anymore due to the presence of decay obscuring the color of the papayas.

There was no statistically significant difference between the change in fruit color of the treatment groups and the control group as determined by the Kruskal-Wallis H test in the SPSS software. However, the fruit color of treatment 3 was not recorded due to it being obscured by the wax.

Fruit quality is influenced by chlorophyll loss, carotenoid synthesis, and unmasking of pigments such as carotenoids during development [17]. This affects color intensity and uniformity. The results show that the different concentrations of wax did not greatly affect the change in color of the treatments in comparison to the control group. It can be inferred that the RBW did not alter the respiration rate to the extent of significance.

Decay incidence analysis. T1 showed the least decay percentage while the higher RBW concentration tied for the highest decay percentage. This ranking is also reflected in the modes of the decay scores of each treatment (Figure 5).

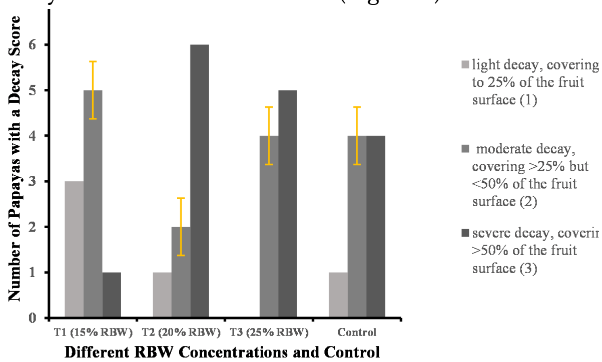

Figure 4. Decay index score modes of the treatments and control group.

Table 3. Decay index percentages and description of the treatments and the control group

Treatment	Decay Index Percentage	Description
Control	56.70%	severe decay
(T1) RBW: 15% (w/v)	43.20%	moderate decay
(T2) RBW 20% (w/v)	62.10%	severe decay
(T3) RBW 25% (w/v)	62.10%	severe decay

The Kruskal-Wallis H test resulted in no significant differences (h = 7.179, p = .066, df = 3) between the 3 treatments and control for decay incidence. This indicates that coating *Carica papaya* L. 'Red Lady' Papaya with RBW has no significant effect on inhibiting microbial growth or decay symptoms. Waxing creates a hydrophobic film barrier on the surface of the fruit [29]. which blocks out pores in the cuticle of the exocarp for less water vapor loss [30]. This could mean that RBW is also sealed in possible moisture which is conducive to microbial growth as water is essential for the normal body functioning of organisms [23]. Although not part of the study's parameters, it has been observed that treated papayas' mesocarp can be considered unripe inside for having firm flesh that would have been softening due to ripening [31]. The flesh is also lacking in the bright orange color that ripened papayas have [19].

Papayas may also be more susceptible to fungal invasion during ripening as their normal range of pH, which the papayas of the study have, is lower than that of the pH range of 4.5 or below which helps in inhibiting the growth of most of the bacteria in fruits [32]. It is also suggested that as the pH of the tissue increases, skin layers soften, soluble carbohydrates build up, and defense barriers weaken which could explain the eventual microbial growth, discoloration, and sunken areas observed on the papayas [31].

Limitations. It is to note that temperature was excluded as a variable in the experiment as of the time the study was conducted, there was not any available equipment for it, and was deemed an uncontrolled variable. It could be that heat from the wax, despite being let cool, influenced the progression of the papaya quality.

Conclusion. - The results obtained from each parameter showed that there was no significant difference in weight loss, pH, fruit color, and decay incidence between the treatment groups and the control group.

Recommendations. - It is recommended to use an advanced tool or software such as ImageJ to measure the total area of decay and the fruit color of the papaya. The use of a homogenizer is also advised to homogenize the papaya flesh in measuring the pH level. Furthermore, it is advised to replace the Brand X used by the study with a self-produced RBW as well as the characterization of said RBW. The addition of parameters to be analyzed such as firmness, ethylene production, titratable acidity, flesh characterization, microbial infestation, and soluble solids content is also recommended to examine further the ripening of the fruit physically and chemically. The cosmetic attributes that wax coatings can provide for papayas can also be investigated for consumer preferences.

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