Research Paper

Effect of Different Ethylene-scavenging Packaging on Extending Shelf Life and Maintaining Fruit Quality of Guava (*Psidium guajava* L.)

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Abstract

One of the major problems with guava fruit is the huge postharvest losses due to quality issues, accelerated by its perishable nature. The current study was conducted to investigate the effect of active packaging sheets produced by partially burned rice husk charcoal powder (T_1), sepiolite clay powder (T_2), and composites prepared by rice husk charcoal and sepiolite clay (T_3), on the quality and shelf life of climacteric guava fruit. The low-cost, natural, and non-toxic ethylene absorber packaging aids were produced as lining sheets to use in guava packaging boxes. Besides, empty material sheets (T_4) and potassium permanganate powder sheets (T_5) were used as negative and positive controls, respectively. T_2 guava samples exhibited an acceptable shelf-life level (>50%) until 20 days (62.5%), while others represented a lower-than-acceptable level after 20 days of storage at 12±3 °C and 85–90% RH. All the samples maintained an acceptable shelf life for 16 days, however, reduced rapidly after 16 days, except T_2. Sepiolite clay treatments showed the lowest peel color change, the highest ascorbic acid content, and the highest firmness during the storage period. In addition, low disease incidence, disease severity, and higher marketability were also observed in T_2 compared to others. The weight loss and total soluble solids (TSS) reduction were not controlled by T_2 due to the hygroscopic nature of the material. The composite treatment, negative control, and positive control samples maintained higher TSS and lower weight loss values than both T_1 and T_2. In conclusion, the active packaging sheet produced by sepiolite clay powder is a promising packing aid as an ethylene absorber to produce active packaging for maintaining quality and extending the shelf life of guava at 12±3 °C and 85–90% RH conditions.

Keywords: Active packaging, Ethylene absorber, Guava, Quality, Shelf life

Introduction

Guava is an economically important fruit crop grown in several agro ecological zones in Sri Lanka. It is a highly demanding fruit for the fresh dessert and processed food industries due to its tastiness and nutrition profile, such as ascorbic acid, dietary fiber (Pedapati and Tiwari, 2014), vitamin A, omega-3, omega-6, unsaturated fatty acids, proteins, and minerals (Leite *et al*., 2006). Guava is greatly perishable, and a large quantity of fruits (46%) are discarded annually due to quality losses and spoilage.

Maintaining the postharvest quality of guava is still a challenge in the supply chain due to its short post-harvest life (6–8 days) attributed to its rapid respiratory and ethylene production behaviors (Forato *et al*., 2015). The climacteric nature of guava exhibits elevated respiration and an ethylene peak shortly after ripening start stage. These attributes trigger rapid quality degradation associated with high metabolic activities and respiration and transpiration rates (Kanwal *et al*. 2016). Ethylene plays an important role in increasing post-harvest losses in guava. Previous studies have reported the use of numerous technologies for extending the shelf life of guava through slowing down or inhibiting ethylene action, such as cold storage, controlled and modified atmospheres, and smart packaging. However, these technologies are more sophisticated and costly. Therefore, developing a simple and low-cost technique is essential to extending the postharvest life of guava and reducing huge postharvest losses.

The use of ethylene scavenging materials and ethylene absorbers is one of the promising technologies considered for preserving fruits and vegetables (Magaraphan and Srithammaraj, 2012). On the other hand, the use of ethylene absorbers is broadly recommended because it has been shown to enhance the shelf life of various fruits by slowing down the respiration rate and water losses (Martinez-Romero *et al*., 2006). Potassium permanganate is an accepted ethylene absorbent that oxidizes ethylene to acetaldehyde and then converts it to acetic acid. Further oxidation of acetic acid releases water and carbon dioxide (Gaikwad *et al*., 2020). At present, montmorillonite, cloister, zeolite, clay, etc. are used in the fruit and vegetable industry as natural porous ethylene scavenging materials (Bodbodak and Rafiee, 2016).

During the last two decades, the use of chemical ethylene absorbers became popular. However, it was rejected with novel research findings of harmful effects on human health. Researchers paid attention to discovering natural ethylene absorbers. Many studies have reported that natural materials such as sepiolite clay, and active carbon have a higher potential to absorb ethylene gas. These natural GRAS (generally recognized as safe) materials are highly accepted by society because there are no hazards for humans, animals, or the environment. (Alvarez Hernández *et al*. 2018). Partially-burned rice husk charcoal has a higher efficiency to adsorb ethylene, which could be due to its micro-porous structure. It can be suggested that partially burned rice husk charcoal is an effective ethylene adsorbing material that could be effectively used as a natural and non-toxic ethylene scavenger. (Kambiz Sadeghi, Younsuk Lee & Jongchul Seo 2019). As well, sepiolite clay has also shown great potential for effective ethylene removal. (Bodbodak and Rafiee, 2016). Thus, the current study was directed toward developing ethylene-scavenging packaging sheets for guava using partially burned rice husk charcoal and sepiolite clay. Further the influence of these novel packaging sheets on the quality and shelf life of guava was evaluated.

Materials and methods

Experimental sites and fruit martial

The experiment was carried out at the Food Research Unit, Department of Agriculture, in Gannoruwa, Kandy. Green guava (*Psidium guajava* L.), variety 'apple', which was harvested at commercial maturity stage was purchased from J.M. Dassanayake Farms (Private) Limited, Dambulla. The guava samples were immediately transported to the laboratory of the Food Research Unit, Gannoruwa.

Preparation of ethylene scavenging packaging sheets

To remove impurities and other foreign materials and dust, a 400 g rice husk sample was cleaned and rinsed several times with distilled water. The cleaned rice husk sample was oven dried at 100 °C for two hours, and partially burned using a muffle furnace at 300 °C for 10 minutes. Then grind with home scale grinder and laboratory sieve (40 microns) was used to obtain fine partially-burned rice husk powder.

Sepiolite clay (2.7 kg) was dissolved in 3 L of water and filtered through a 0.025 mm mesh to remove impurities and large particles. The clay suspension was kept for 24 hours to allow sedimentation of the fine clay particles, and then the suspension was removed. Separated, the clay slurry was dried at room temperature and then oven-dried at 120 °C for 3 hours. Finally, dried clay particles were ground using a home grinder and sieved at 40 micron mesh to obtain a fine clay powder.

Prepared rice husk charcoal powder (6-7g for one sheet) and sepiolite clay powder (15-16g for one sheet) were tightly pasted on the paper sheets (470 × 240 mm) to use as a packing sheet between two guava layers in the guava boxes. Thereafter, the fabricated paper sheets were oven-dried at 60 °C for 6 hours. Fabricated packaging sheets made from rice husk powder and sepiolite clay powder were named T_1 (RHCP) and T_2 (SCP), respectively. The third group of packing sheets was prepared by using RHCP and SCP at a ratio of 1:1 (14-15g for one sheet), and it was labeled as T_3 (RHCP+SCP). The papered sheets without any ethylene scavenging materials (T_4) and potassium permanganate ($KMnO_4$) (7-8g for one sheet) (T_5) were used as negative and positive controls, respectively. The rice husk charcoal powder, sepiolite clay powder, and potassium permanganate were used to fabricate different packaging sheets.

Preparation and storage of guava samples

Green guava fruits at commercial maturity stage, uniform in shape, color, size, and free from pests and diseases were selected for the experiment. The selected guava fruits were cleaned with 0.6% chlorinated water for 10 minutes, and air-dried for 2 hours before storing. The guava fruits were randomly divided into 15 groups, and each sample (15 fruits) was stored in fifteen corrugated boxes (47 × 24 × 15 cm). Different ethylene scavenging sheets were placed in the corrugated boxes. All samples were labeled on the types of ethylene scavenging sheets (T_1, T_2 and T_3) and the controls (T_4 and T_5). The packaging sheets were kept on the bottom and in between two guava layers in the box. The guava boxes were stored in a cool room at 12±3 °C with 85–90% relative humidity (Mahajan *et al*., 2009). The parameters were measured at four-day intervals until the end of the 20-day storage period. All treatments were tested in triplicate. (n=3)

Determination of physico-chemical properties of guava

Weight loss: The percentage weight loss was calculated (75 Guava Fruits) using the method previously described by Bassetto *et al*. (2005).

$$\text{Weight loss (\%)} = \frac{\text{Initial weight of fruit (g)} - \text{Final weight of fruit (g)}}{\text{Initial weight of fruit (g)}} \times 100$$

Fruit firmness: The firmness of fruits was measured using a handheld penetrometer (model FT-327, 3-27 Lbs.) with a 5 mm diameter probe (Rangana, 2001). The measurements were taken at three different places (two polar and equator of the fruit) of one fruit, three fruits were used from one replicate (box) a total of 9 fruits were tested.

Total ascorbic acid content: A guava juice sample was prepared from a blended and homogenized whole guava fruit. The iodine titration method was used to determine the total ascorbic acid content of juice samples (Suntornsuk *et al*., 2002). For the titration procedure: the guava sample 1ml was extracted from 9 ml of metaphosphoric acid, and titrated against the prepared dye solution. The titration was completed with a pink color in the expected time (15 seconds) according to preliminary studies. The ascorbic acid content of the sample was calculated using the formula below (Rangana, 2001)

$$\text{Ascorbic Acid (mg/100 g)} = \frac{\text{Titre} \times \text{Dye factor} \times \text{Volume made up}}{\text{Aliquot extract taken} \times \text{Weight of sample taken}} \times 100$$

Total soluble solids: The total soluble solids of guava were determined using a digital refractometer (Atago Co., Ltd., Japan). Guava homogenate was prepared by crushing and blending the guava fruit, and two drops of the pulp were then carefully applied on the refractometer using a plastic dropper. A reading was obtained directly as a percentage of soluble solids concentration.

Determination of disease development

Disease incidence: Disease incidence (DI) (% of diseased fruits) was calculated following the method described by Akhtar and Alam (2002). To calculate the DI, eight guava fruits were used for each sample in triplicate. DI was measured over four-day-intervals of the storage period using the following equation.

$$\text{DI (\%)} = \frac{\text{Number of diseased fruits in the sample}}{\text{Number of total fruits in the sample}} \times 100$$

Disease severity index: The disease severity index (DSI) was calculated using the assessment provided by Akhtar and Alam (2002). Eight guava fruits were used for each sample, and each sample was prepared in triplicate. DSI was calculated using the percentage of diseased area on the guava peel after 20 days. A 5-point hedonic scale (20%, 21-40%, 41-60%, 61-80%, and > 81%) to determine the diseased area on the fruit's peel. For each scale, the scores were offered as 1, 2, 3, 4, and 5, respectively. DSI was calculated using the following equation:

$$\text{Disease Severity Index (\%)} = \frac{\sum (a + b + c\ldots)}{N + Z} \times 100\%$$

Where, Σ (a+ b+ c...) = summation, score of diseased fruit, N = total number of fruit in sample, Z = the score of the highest diseased sample.

Shelf life study: The shelf life of the guava samples was determined by an assessment previously described by Beverly *et al*., (1993). The marketability was calculated in order to assess the shelf life of guava samples in 26 days. The surface appearance features were used to determine the marketing quality attributes. A fruit was deemed unmarketable if it displayed one or more of the following traits: ripening, shriveling, dehydration, apparent mold growth, rotting, or black spots. As a result, if any fruit sample's marketability drops by more than 50%, it is considered to have reached the end of its shelf life. (Ayele *et al*., 2012). The percentage of marketability was calculated by using the following equation:

$$\text{Marketability (\%)} = \frac{\text{Number of marketable fruits}}{\text{Total number of fruits}} \times 100\%$$

Data analysis

Completely Randomized Design (CRD) was used as the experimental design for the study. All the tests described above were performed in three replicated unless otherwise specified. Statistical analysis, two-way ANOVA was performed using the Minitab version 17 to compare the mean differences between groups. Turkey post hoc tests was used to find the least significant difference between the compared groups ($P < 0.05$).

Results and discussion

Analysis of physico-chemical parameters

Weight loss can be occured due to various factors such as transpiration, respiration, and evaporation of moisture from the fruit surface. According to the test results, weight loss was not significantly different among treatments ($P \geq 0.05$). Different ethylene scavenging materials did not affect the weight loss of guava, but storage times showed a significant difference in the weight loss of guava ($P \geq 0.05$). The changes in weight loss of guava fruit during storage were shown in Figure 1. Weight losses in all treatments slowly increased, with a similar trend for the whole 20-day storage period.

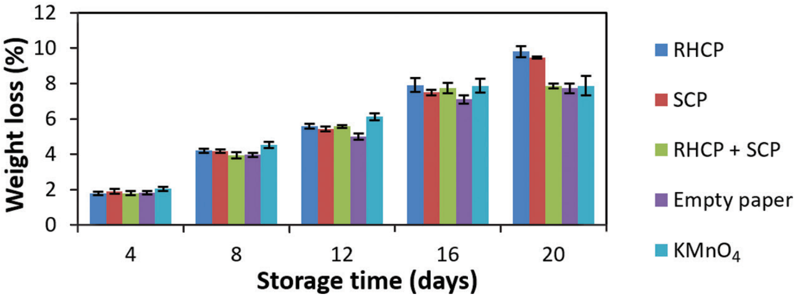

Figure 1. Effect of ethylene scavenging materials and storage days on weight loss (%) of guava during storage at 12± 3 °C for 20-days storage period. Vertical bars indicate the standard error of the mean for three replicates. RHCP= rice husk charcoal paper, and SCP= sepiolite clay paper.

After 20 days storage, the minimum cumulative weight loss that was observed with the negative control (T_4) was 7.73% while the maximum cumulative weight loss that was observed with the rice husk charcoal treatment was 9.81%. Further all treatments and positive control exhibited higher weight loss values in progressing the storage due to the hygroscopic ability of sepiolite clay, charcoal, and KMnO4. These results were in agreement with the similar finding reported by Garcı´a-Garcı´a *et al*. (2013). They observed that sepiolite clay and sepiolite + permanganate - coated paperboard absorbed more water. Based on the channel structure, the highly specific surface, and the material's ability to absorb water, this fact is attributed to the water affinity of sepiolite. However, many studies reported that the weight loss increased with storage progress (Bassetto *et al*., 2005; García García *et al*., 2013), and similar results were observed in the current study.

During storage, the total soluble solids (TSS) content of guava can be changed due to various factors such as respiration, transpiration, and enzymatic activity. In the current study, no significant ($P\geq$ 0.05) differences were shown among the treatments and storage period. The TSS content of all samples increased slowly with a similar trend during the 20-day storage period (Figure 2). The initial TSS content of all guava samples was recorded as less than 7.5 ºBrix and started to slowly increase thereafter until day 16 with ripening, and thereafter the TSS content of all treated and control samples started to reduce slowly with senescence (Figure 2). Similar findings were reported for the remarkable increase in total sugars after the climacteric ethylene peak in guava. It was found that storing guava samples in an ethylene-scavenging package with potassium permanganate and sepiolite clay delayed the increase in TSS, which ultimately increased the time the fruit could be stored in a cold storage. When an ethylene scavenger based on KMnO4 is added to the banana packages, the changes in TA and TSS are also controlled (Tzeng *et al*., 2019). According to the studies conducted by Bashir and Abu-Goukh (2003) and Sadeghi (2019), the increment of TSS was slightly controlled by the ethylene scavenger materials compared to the negative control. The results of the current study were also the same as in previous reports.

The ascorbic acid content of all samples slightly increased until day 12 with ripening (Figure 3), and it is general for all fruits. After day 12, the ascorbic acid content of all samples started to reduce with senescence. However, these results are contradictory to the results of Katumbi *et al*., (2021), who estimated the ascorbic acid concentration to grow consistently and peak at day 7 of storage. Ascorbic acid content usually increases during the ripening of guava fruit and then decreases during senescence (Taylor 1993). However, in this study the rate of loss of ascorbic acid in guava was faster in the negative control sample as compared to other treatments during the 20-days storage period. Storage days affected the ascorbic acid content significantly, which slightly increased at first and then decreased rapidly irrespective of the treatments as the storage period progressed. The results are similar to the findings of Kumar (2000), who described that the amount of ascorbic acid in Kinnow fruit (*Citrus reticulata* cv. Kinnow Blanco) decreased with storage time. Due to its antioxidant properties, ascorbic acid helps shield the plant from oxidative damage. However, due to its solubility in water, ascorbic acid undergoes rapid degradation due to oxidation during post-harvest storage. Sepiolite clay treatment maintained the highest ascorbic acid content significantly more than all other samples, and the positive control sample also maintained its ascorbic content in a similar range with sepiolite clay treatment.

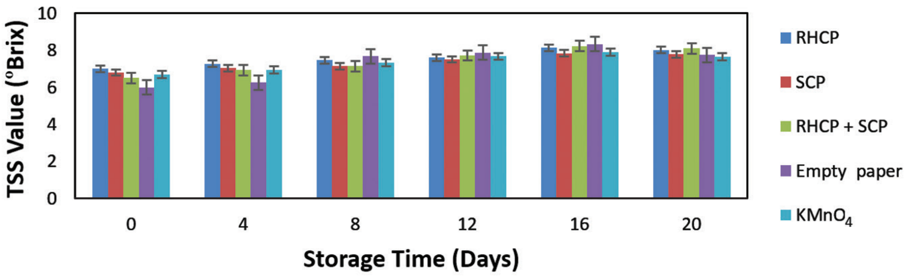

Figure 2. Effect of ethylene scavenging materials and storage days on TSS (^{0}Brix) of guava during storage at 12± 3 °C for 20-days storage period. Vertical bars indicate the standard error of the mean for three replicates. RHCP= rice husk charcoal paper, and SCP= sepiolite clay paper

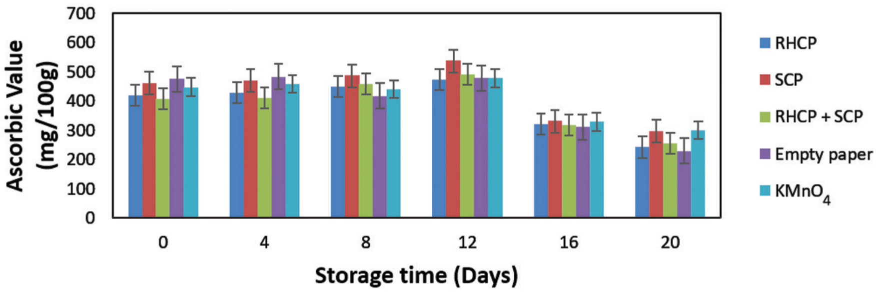

Figure 3. Effect of ethylene scavenging materials and storage days on ascorbic acid content (mg/100 g) of guava during storage at 12 ±3 °C for 20-days storage period .Vertical bars indicate the standard error of the mean for three replicates. RHCP= rice husk charcoal paper, and SCP= sepiolite clay paper.

One of the key quality characteristics that affects whether a product is accepted by the consumer is firmness. Firmness often reveals the biochemical components, water content, and cell wall makeup of the fruit. The loss of firmness after storage in fruits and vegetables is the most obvious textural alteration. (Martínez-Romero *et al*., 2006). According to the results, there were significant differences among the treatments and storage days ($P \leq 0.05$). The flesh firmness of all guava samples slightly decreased after day 4, but it was not significant. However, on day 8, the firmness values of sepiolite clay treatment, and rice husk charcoal + sepiolite clay treatment were significantly higher compared to the negative control. On days 12, the firmness values were significantly higher for all treatments when compared to the negative control. On day 16 and 20, the firmness values of sepiolite clay treatment were (9.3 N, 7.2N) significantly higher when compared to all other treatments. The results showed that the sepiolite clay-treated samples maintained the firmness significantly better than all other samples.

As guava fruit ripens, it becomes more susceptible to fungal and bacterial diseases, which can lead to fruit rot, mold, and decay. According to the Disease Incidence (DI) results, there were significantly different DI values among treatments ($P \leq 0.05$), as well as among storage days. On day 4, there was a comparatively higher DI value for the negative control (16.67 %) compared to other treatments. The negative control sample showed significantly higher DI values on days 12, 16 and 20 compared to all other treatments (Figure 4). During storage, the sepiolite clay-treated sample showed the lowest DI values, and they were significantly lower than all other samples. In comparison, the DI values of all samples increased in a similar pattern, and at the end of the storage, the negative control and positive control samples exhibited the highest DI values (87.5% and 75%). The highest DI values of KMnO4 treated samples might be due to the chemical stress generated by $KMnO_4$.

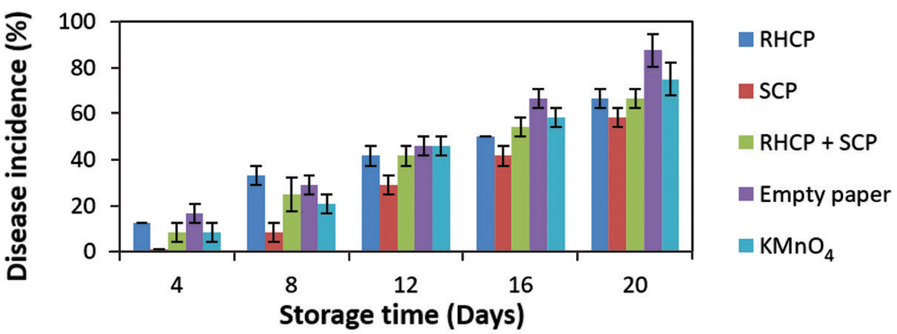

Figure 4. Effect of ethylene scavenging materials and storage days on disease incidence (%) of guava during storage at 12 ±3 °C for 20-days storage period. Vertical bars indicate the standard error of the mean for three replicates. RHCP= rice husk charcoal paper, and SCP= sepiolite clay paper.

The Disease Severity Index (DSI) is a quantitative measurement of disease development that takes into account both the incidence (proportion of infected fruit) and severity (degree of disease symptoms) of the disease. Results in Figure 5 indicate that the DSI was significantly ($P \leq 0.05$) affected by the treatments. The DSI of guava samples was measured on day 20 (the end of the storage) by measuring the disease-affected surface area of the guava. Significantly higher DSI values were observed with the positive and negative controls in comparison to the other samples. The highest DSI (78.88%) was observed in the negative control, while the lowest value (58.71%) was observed in the sepiolite clay treatment. The DSI of all treatments was significantly lower than that of both positive and negative controls. As the results show, sepiolite clay-treated samples exhibited lower DSI as well as higher firmness values. There was a relationship between the DSI and fruit firmness due to fungi, bacteria, and other microorganisms degrading the cell wall polysaccharides by releasing cell wall-depredating enzymes, which reduced the fruit firmness (Juge, 2006). The results of the present study showed the rapid loss of fruit firmness in negative control samples (Figure 6), which coincided with the highest disease severity index.

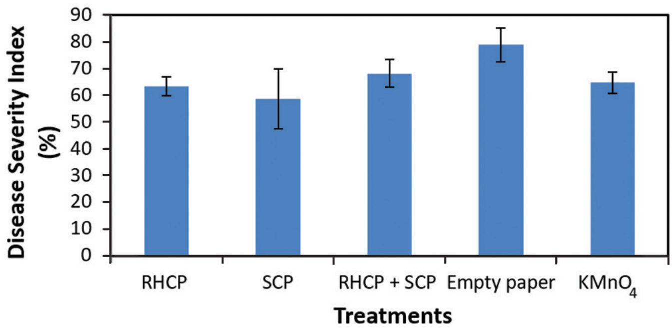

Figure 5. Effects of ethylene scavenging materials on disease severity index (%) of guava during storage at 12±3 °C after 20-days storage period. Vertical bars indicate the standard error of the mean for three replicates. RHCP= rice husk charcoal paper, and SCP= sepiolite clay paper.

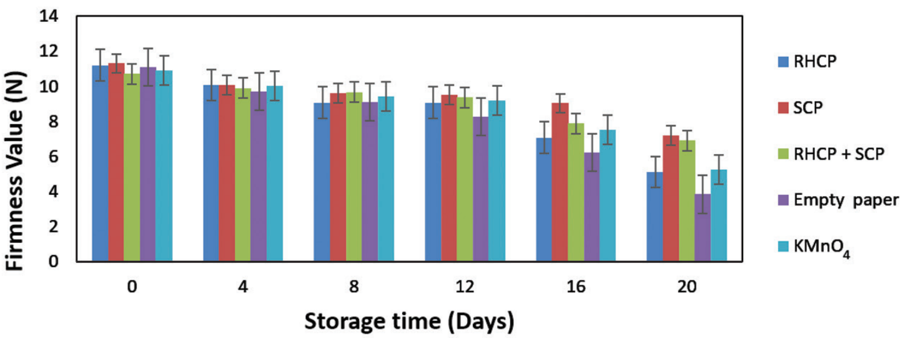

Figure 6. Effect of ethylene scavenging materials and storage days on fruit firmness (N) of guava during storage at 12 ±3 °C for 20-days storage period .vertical bars indicate the standard error of the mean for three replicates. RHCP= rice husk charcoal paper, and SCP= sepiolite clay paper.

The shelf life of guava and other fruits is the length of time that the fruit remains fresh and marketable during storage. The percentage marketability of guava obtained with the duration of storage in different ethylene scavenging materials did not show any significant differences among the treatments and days ($P \geq 0.05$). The marketability percentage of all guava samples decreased in a similar trend during storage. Rice husk charcoal-treated samples and sepiolite clay-treated samples showed the highest marketable condition (95.83 %, 100 %) at 4 days after storage. Control samples could maintain marketability for up to 8 days of storage. The highest reduction in percentage of marketable fruits was noticed at 26 days in the negative control sample (8.33%) and the treatment, rice husk charcoal+sepiolite clay, was in a second-lowest marketable (12.5%) condition (Figure 7). Slightly better results were obtained with rice husk charcoal, sepiolite clay treatments (62.5% and 70.83%). After day 16, or 20 the percentage marketability of all samples was reduced to lower than 50%, except for sepiolite clay treated samples and RHCP treated sample. The sepiolite clay treated sample extended the marketable storage life until 20 days for guava.

At the end of the 26-days storage duration, all treatments were not up to marketable conditions. However, on 20 days of storage, only sepiolite clay-treated samples exhibited 62.5% of marketable condition. This shows guavas could be stored with sepiolite clay materials to preserve their marketability for up to 20 days. Sepiolite clay treatment was significantly better with higher fruit performances, such as higher fruit firmness, ascorbic acid, and lower DI and DSI, which also directly affect the shelf life of guava. Magaraphan, R. and Srithammaraj (2012) reported similar results with storage studies of tomatoes using sepiolite clay treatment. However, there is limited research on the ethylene scavenging capacity of sepiolite clay and rice husk charcoal. Similarly, rice husks containing active packaging material can provide an alternative ethylene scavenging material in place of chemical and polymer-based materials to prolong the 16-day postharvest life of tomatoes (Haider *et al*., 2020).

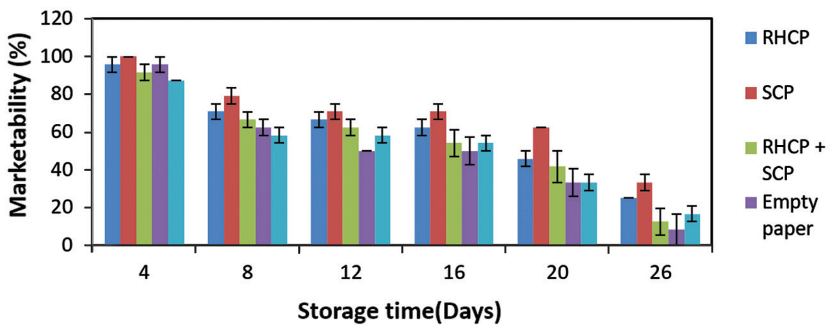

Figure 7. Effects of ethylene scavenging materials and storage days on marketability (%) of guava fruits during storage at 12±3 °C for 26 days storage period. Vertical bars indicate the standard error of the mean for three replicates. RHCP= rice husk charcoal paper, and SCP= sepiolite clay paper.

Conclusions

The current study focused on preparing a low-cost, natural ethylene scavenging packaging aid to use in active packaging to extend the shelf life and quality of harvested guava. The TSS and weight loss of guava were not controlled by any type of tested material. Among the tested materials, sepiolite clay maintained comparatively higher levels of shelf life, fruit firmness, ascorbic acid, and low disease incidence and low disease severity index. Therefore, the packaging sheets produced by sepiolite clay have a good potential to extend shelf life while maintaining quality.

Packaging material produced by sepiolite clay, can be used as an alternative to ethylene scavenging to replace chemical and polymer-based materials to prolong the postharvest life of guava. It was also observed that an increase in the rice husk charcoal and potassium permanganate samples slightly enhanced ethylene adsorption capacity.

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