Philippine Fruits as Free Radical Scavengers Enhancing the Shelf-Life of Green Chili Pepper (*Capsicum annuum* L.)

Bianca Mariel DP. Juacalla, Jasmin F. Velasco, Arlyn Q. Ariola

Abstract—Fruits and vegetables such as green chili peppers (GCP) were considered as perishable agricultural commodities that undergo high postharvest losses. The preservation process is inevitable in the agricultural industry and thus, safe alternative methods should be developed. The study determined the potential of aqueous phytochemical extracts on extending the shelf-life and influencing the free radical scavenging activity (FRSA) and weight loss of the harvested GCP fruits. The extracts were derived from starfruit (*Averrhoa carambola*), duhat (*Syzygium cumini*) and guyabano (*Annona muricata*) as these fruits were known to have natural antioxidants and could play a role in preventing oxidative damage. Weight loss (%) and shelf-life (days) were observed and FRSA was determined spectrophotometrically according to 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The results showed enhanced shelf-life and free-radical scavenging activity of harvested GCP fruits. Chilies that were treated with starfruit and guyabano extracts exhibited significantly higher free radical scavenging activity (75.35 ± 0.15% and 74.25 ± 0.07% DPPH inhibition at 100 µg GAE, respectively) and extended shelf-life (11-15.33 days and 11-14.67 days, respectively). These findings suggested the potential application of phytochemical extracts to prolong the shelf-life of vegetables, particularly green chili peppers.

Index Terms— *Annona muricata*, *Averrhoa carambola*, *Capsicum annuum*, oxidative damage, phytochemical coatings, free radical scavenging activity, shelf-life, *Syzygium cumini*

1 Introduction

FRUITS and vegetables were one of the important crops in farming industry and considered a means of improving the livelihoods of smallholder farmers around the world as it was considered as sources of income of many people and considered to belong in staple foods for humankind. The perishable nature of vegetables demand comprehensive planning for movement, storage, processing and distribution.

Capsicum annuum (green chili peppers, GCP) undergo high postharvest losses due to poor postharvest handling during transportation and storage [1]. GCPs are characterized by high moisture content and active metabolism and as a consequence, significant losses resulting in senescence, desiccation, physiological disorders, mechanical injuries and microbial spoilages occur at any point from harvest through utilization [2].

Vegetables and fruits are considered the best source for antioxidants. Antioxidants are key factors used for the enhancement of the life of any species. However, there are chemicals that promote the shelf-life of harvested fruits and vegetables that are toxic and are harmful to human health as they can cause degenerative diseases so it is beneficial to the people and to the food industry to discover different ways of preserving fruits and vegetables with the assessment of toxicity to ensure the safety of foods to human health and wellness.

There is enough food in the world for everyone, but one-third of all food was wasted globally according to the Food and Agriculture Organization. Reducing food loss was the most sustainable alternative to increasing food production [3].

Food loss in developing countries like the Philippines occurs even before consumption as this were already accounted as early as reproduction, postharvest stages and storage of agricultural produce. Studies of post-harvest losses of vegetables identified losses in the range of 20-40% [4].

As an answer to this problem, the researcher made a contribution to the prevailing issues related to food loss particularly in fruits and vegetables. Shelf-life enhancement of fruit vegetables particularly green chili peppers was conducted for the prevention of decay that will help the farmers, retailers and wholesalers to gain good earnings and other people in buying and consuming this agricultural produce with high standard and quality.

Leaves of starfruit (*Averrhoa carambola*), duhat (*Syzygium cumini*), and guyabano (*Annona muricata*) were used as they possess phytochemicals and other phytoconstituents which scavenge free radicals from the organisms' body and contain the bioactive compounds that could play a role in preventing oxidative damage which was one of the main causes of aging and shelf-life reduction [5], [6], [7].

This study can help in crop preservation particularly in fruits and vegetables which are highly perishable and are considered short duration crops. It can also become an essential principle in post harvesting system as it can

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contribute to the development of protocols in assessing the shelf-life enhancement of green chili. Therefore, this study can diminish much concern regarding the postharvest losses as it will provide good quality product and contributes to a good market standard.

2 Objectives of the Study

The study aimed to determine the potential of phytochemical extracts of starfruit, duhat, and guyabano on assessing the free radical scavenging activity before and after coating the GCPs; improving the shelf-life of harvested GCPs; and decreasing the weight loss during storage. Specifically, the study seeked to determine the: (1) potential of phytochemical extracts of different fruits to extend the shelf-life of harvested GCP fruits; (2) significant differences in the shelf-life of chili coated with different phytochemical extracts; (3) free radical scavenging activity after coating the GCPs; (4) significant differences in the free radical scavenging activity of the green chili before and after coating of different phytochemical extracts; (5) average weight loss of the green chili pepper fruits coated with phytochemical extracts under ambient condition; and (6) significant differences in the average weight loss of the green chili pepper coated with different phytochemical extracts.

3 Methodology

The experimental design of research was utilized to determine the ability of the phytochemical extracts of starfruit, duhat and guyabano leaves in extending the shelf-life, influencing free radical scavenging activity, and decreasing the weight loss of harvested GCPs. The study was composed of two experiments. The first experiment included the analysis of the ability of the extracts from the leaves of four fruits in enhancing the shelf-life of GCPs with the assessment of its weight loss and visual quality rating. Leaves of starfruit, duhat and guyabano were ground followed by extracting its bioactive compounds. Extraction method used was percolation to get most of the antioxidants from the leaves. On the second experiment, two extracts were selected among the three phytochemical extracts due to its effective results manifested on the chillies' weight loss and shelf-life on the first experiment. A separate set of chillies were coated using only these two extracts, stored at ambient condition for few days and were tested for its free radical scavenging activity.

GCPs locally known as 'siling haba' were harvested in a farm located at Brgy. Concepcion, Lumban, Laguna, Philippines. They were sorted according to their sizes and weight and cleansed with tap water before being coated by the leaves extract. They were coated by immersing the green chilies in the aqueous phytochemical extracts for five minutes followed by surface drying. Fruits were weighed and initial analyses were done accordingly.

The coated fruits of green chilies were placed on trays in triplicates in an ambient temperature. Weight loss [8] was recorded every three interval days. A replicate or a treatment is terminated once the vegetable fruits attained a 50% weight loss. Weight loss was expressed in percent (%). The formula used was:

$$\text{weight loss (\%)} = [(\text{initial wt} - \text{final wt})/\text{initial wt}] \times 100$$

Phenolic extracts of chillies uncoated and coated with the selected phytochemical extracts were obtained. The antioxidant activity was measured in terms of free radical scavenging ability using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) [9] with some modifications wherein the working solution of the radical was prepared by diluting the DPPH stock solution with methanol. The DPPH is a stable free radical with purple color (absorbed at 517nm). If free radicals have been scavenged by the phenolic compounds in the extracts, DPPH will change its color to yellow. Briefly, different concentrations (20, 40, 60, 80 and 100μg GAE) of chili phenolics were added to 5mL of 0.1mM DPPH in methanol. The mixtures were stirred and stand for 15 minutes. The absorbance was read at 517nm using water to zero the instrument. The DPPH reagent and methanol served as control and DPPH inhibition was calculated using the formula:

$$\%\ \text{DPPH Inhibition} = [(A_{\text{Control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

Analysis of variance (ANOVA) was used to determine whether there are any statistically significant differences between the means of all the treatments and the differences were assessed using Tukey's Honesty Significant Difference (HSD) at 5% probability.

4 Literature Review

Food preservation for commodities such as fruits and vegetables can be measured through prolonged shelf-life and enhanced free-radical scavenging activity. These were the two factors that were tested in the study.

Shelf-life is the time it takes a food product to deteriorate to an unacceptable degree under specific storage, processing and packaging conditions. The shelf-life of food depends on how it is processed, stored and packaged. Measurable food quality factors include nutrient content, color & appearance, moisture content, physical shape or size, mechanical properties, flavor panel score, and toxicant level (chemical risk) [10].

Free radical scavenging activity is performed mainly by substances called antioxidants. Antioxidants are molecules that delay, prevent or remove oxidative damage to a target molecule [11]. Oxidation reactions produce free radicals that can start multiple chain reactions that eventually cause damage or death to the cell. Antioxidants remove these free radical intermediates by being oxidized themselves, and inhibit other oxidation reactions, thus stopping the harmful chain reactions [12]. Molecules of antioxidant with radical scavenging ability are thought to wield possible protective outcome against the free-radical damage [13].

Plants are affluent source of free radical scavenging molecules, such as vitamins, terpenoids, phenolic compounds, lignins, tannins, flavanoids, alkaloids, coumarins, and other metabolites, which are rich in antioxidant activity. Therefore, much attention has been focused on the use of antioxidants,

especially natural antioxidants, to inhibit lipid peroxidation, or to protect against the damage of free radicals [14].

However, many researchers reported the adverse effects of synthetic antioxidants such as toxicity and carcinogenicity. Natural antioxidants are in high demand for application as nutraceuticals, bio-pharmaceuticals, as well as food additive because of consumer preference [15]. The recent study opted to use natural antioxidants from plants such as starfruit (*Averrhoa carambola*), duhat (*Syzygium cumini*), and guyabano (*Annona muricata*). Studies showed that starfruit, duhat, and guyabano were important sources of bioactive compounds that could play a role in preventing oxidative damage to cells [5], [16], [17], [18].

Green chili (*Capsicum annuum*) is one of the most important vegetable crops in the world. Due to the perishable nature, it is susceptible to fast quality changes and spoilage (through wilting, shriveling, pathogenic disorders, water loss, etc.) after harvest under improper post-harvest management like prolonged storage, improper handling, transportation, microbial infection, etc. [19], [20].

The main factors for the quality degradation of pepper during prolonged storage include poor external appearance, injury, decay development and shriveling associated with weight loss [21]. The weight loss of the fruit was principally due to the loss of water in transpiration and to a lesser extent to the loss of carbon in the respiration process [22].

One potential for preserving fruit quality is by using natural alternative edible coatings from other plants. The edible coatings provide an additional protective coating and reduce oxidation in fruit and vegetables that can influence various changes in color, firmness, sensory quality, inhibition of microbial growth, and production of ethylene in fruits and thus can extend the shelf-life [23], [24], [25], [26].

5 Discussion

5.1 Shelf-life and Weight Loss Evaluation

Table 1 shows the shelf-life (measured in average number of days) and average weight loss of GCPs coated with aqueous phytochemical extracts. Data revealed that there was a significant difference between the treatments as influenced by the aqueous phytochemical extracts in terms of shelf-life. T1 which was the untreated green chili fruits that served as the control exhibited an average shelf life of 11 days and got the lowest shelf-life, while T5 treated with starfruit extract got the highest number of days pertaining to its shelf-life with 15.33 days. The aqueous extract of guyabano and duhat extended the shelf-life of green chili pepper up to 14.67 and 12.67 days, respectively. The results may be attributed to the presence of bioactive components in the leaves extracts [27], [28], [29], [30].

On the 3rd day of storage, green chilies that were untreated exhibited the highest weight loss with 8.72% while fruits with application of guyabano extract showed the lowest weight loss with 5.54% followed by the ones treated by starfruit, duhat, and distilled water which showed weight loss of 7.07%, 7.08% and 7.28%, respectively. On this day, the fruits treated with phytochemical extracts and distilled water as well as the untreated ones, were not significantly different. This could be attributed to the rate of physiological processes such as the respiration rate and the delayed peak of ethylene production in the peppers [31].

TABLE 1
Shelf-Life (average number of days) and Average Weight Loss of the GCPs Coated with Aqueous Phytochemicals Stored Under Ambient Conditions

Treat-ments*	Shelf-life (Days)	Average Weight Loss (%)				
		Days of Storage				
		3	6	9	12	15
T1	11	8.72	26.05	34.15	52.82	62.31
T2	11.33	7.28	24.41	31.13	51.64	60.82
T3	12.67	7.08	15.28	26.67	47.59	58.87
T4	14.67	5.54	11.9	20.31	34.36	49.44
T5	15.33	7.07	16.87	22.56	28.84	48.67
P values	0.000	0.189	0.002	0.001	0.000	0.000
				0.000		

*Treatments:
T1 - Control/Untreated
T2 - Distilled Water
T3 - Duhat extract
T4 - Guyabano Extract
T5 - Starfruit extract

On the 6th day of storage, green chilies that were untreated and treated with distilled water exhibited the highest weight loss of 26.05% and 24.41%, respectively. Fruits coated with guyabano extract showed the lowest weight loss with 11.9% followed by the extracts of duhat and starfruit with 15.28% and 16.87%, respectively.

On the 9th day of storage, green chilies that were untreated and treated with distilled water still exhibited the highest weight loss of 34.15% and 31.13%, respectively. Fruits coated with guyabano extract showed the lowest weight loss with 20.31% followed by the extracts of starfruit and duhat with 22.56% and 26.67%, respectively.

On the 12th day of storage, coated green chilies of starfruit and guyabano extract significantly exhibited lower weight loss compared to the control group and the ones treated with distilled water. Green chilies that were untreated and treated with distilled water exhibited the highest weight loss of 52.82% and 51.64%, respectively. Fruits coated with starfruit extract showed the lowest weight loss with 28.84% followed by the extracts of guyabano and duhat with 34.36% and 47.59%, respectively.

On the 15th day of storage, coated green chilies of starfruit and guyabano extracts significantly exhibited lower weight loss compared to the control group and the ones treated with distilled water. Green chilies that were untreated and treated with distilled water exhibited the highest weight loss of 62.31% and 60.82%, respectively. Fruits coated with starfruit extract showed the lowest weight loss with 48.67% followed by the guyabano and duhat extracts with 49.44% and 58.87 %, respectively.

Cumulative weight loss differed significantly ($P \leq$

0.05) over the storage period. This could be due to the physiological processes like transpiration, ethylene production and respiration and is mainly attributed to the continuous moisture and other nutrient loss as the green chilli fruits are alive [32] and storing chilies under ambient condition shows significantly higher weight loss as they were exposed to high temperature and lower relative humidity and resulted to heat damage thus all the physiological and metabolic activities might have increasing [33].

5.2 Assessment of the Phenolic Content of the Green Chili Peppers with Aqueous Phytochemical Coatings

Table 2 shows the total phenolic content of the chilies untreated, treated with distilled water and with the selected two phytochemical extracts, guyabano and starfruit extracts. The chili samples treated with starfruit and guyabano extracts showed significantly higher total phenolic contents than that treated with distilled water and the untreated sample. Such increase may be attributed to the added phenolics and other chemical components such as flavonoids, saponins, steroids, alkaloids and triterpenes [34], [35] from starfruit and guyabano extracts.

TABLE 2
TOTAL PHENOLIC CONTENT OF UNTREATED AND TREATED CHILI SAMPLES (DRY BASIS)

Sample Treatment	Total phenolic content (mg GAE/g)
Untreated	5.75 ± 0.02
Distilled water	6.35 ± 0.02
Starfruit extract	6.75 ± 0.03
Guyabano extract	6.79 ± 0.02

5.3 Evaluation of Free-radical Scavenging Activity

Table 3 shows the free radical scavenging activity of the green chili pepper fruits uncoated and coated with the selected two aqueous phytochemical extracts and distilled water. The FRSA was done in five phenolic concentrations (20, 40, 60 80, and 100 μg GAE) of chili. At the phenolic concentration of 20 μg GAE, chilies that were treated with starfruit and guyabano extract exhibited significantly higher free radical scavenging activity than that treated with distilled water and the untreated samples which may indicate that such treatment improved the antioxidant capacity of chili. However, such trend was not consistently observed in 40-80 μg GAE phenolic concentrations. At 100 μg GAE phenolic concentration only starfruit extract showed a significant effect while guyabano extract did not. This may be attributed to the high bioactive components of the starfruit extract such as alkaloids, glycosides, phenol, tannins, flavonoids, protein and diterpenes that made its significant result in the free radical scavenging activity as compared with other treatments [36].

TABLE 3
ANTIOXIDANT ACTIVITY BASED ON FREE RADICAL SCAVENGING ACTIVITY (FRSA) OF GREEN CHILI SAMPLES

Phenolic concentration (ug GAE)	Free radical scavenging activity (% DPPH inhibition)			
	Untreated	Treated with distilled water	Treated with starfruit extract	Treated with guyabano extract
20	22.56 ± 0.11	22.23 ± 0.07	23.77 ±0.29	23.99 ± 0.15
40	37.60 ± 0.40	37.89 ± 0.18	38.74 ± 0.22	37.05 ± 0.07
60	52.27 ± 0.18	51.31 ± 1.14	52.49 ± 0.18	50.04 ± 0.95
80	62.77 ± 0.04	61.96 ± 0.70	62.91 ± 0.04	62.23 ± 0.11
100	74.10 ± 0.07	74.03 ± 0.07	75.35 ± 0.15	74.25 ± 0.07

6 CONCLUSION

The study focused on the free radical scavenging activity, weight loss and shelf-life of green chili pepper (GCP) fruits with phytochemical coatings stored under ambient condition. On the first experiment, GCPs were coated with the aqueous extract from the guyabano, duhat, and starfruit. Initial data were gathered such as the weight of the fruits and then GCPs were stored at ambient temperature. Results showed that cumulative weight loss differed significantly over the storage period.

The most effective phytochemical extract that prolonged the shelf-life of chili on the first experiment was the starfruit extract followed by the extract of the guyabano. These two leave extracts were used in the second experiment for comparative assessment of the free radical scavenging activity (FRSA) of the chilies using DPPH free-radical scavenging method. This was tested to determine if guyabano and starfruit can influence shelf-life and quality of chilies. Phenolic contents from chilies were also extracted and the results showed that chilies coated with the extracts of starfruit and guyabano exhibit significantly higher FRSA than positive and negative control groups.

In conclusion, the phytochemical extracts derived from starfruit and guyabano were potential shelf-life enhancer of harvested GCPs. The extracts also had a significant effect on GCPs in terms of reducing the cumulative weight loss and enhancing the FRSA.

7 RECOMMENDATIONS

Conducting further studies on the capability of starfruit and guyabano leaves' ethanolic and acetic acid extract in prolonging the shelf-life of the GCPs and assessment of taste of the fruit and vegetables coated with phytochemical extracts are recommended. In addition, application of these phytochemical extracts to other fruits and vegetables under different storage condition was suggested. Commercial products that can aid in post-harvest management may also be developed in the future.

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