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Free radical scavenging activity of mature red tomato (*Lycopersicon esculentum* Mill.) fruit coated with hagimit (*Ficus minahassae* Miq.) extract

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Abstract: The study was conducted to determine the effect of storage time on the free radical scavenging activity (FRSA) of mature red tomatoes with hagimit extracts, to investigate the effect of timing of application of hagimit extract on the FRSA of the mature red tomatoes, and to evaluate the maximum/optimum FRSA of the mature red tomatoes coated with hagimit extracts. Coating was done on the 4th, 6th and 8th day after harvest of the tomato fruits and analyzed for FRSA. Results show that free radical scavenging activity of untreated tomato fruit drastically reduced after 6 days of storage. However, when the tomato fruits were coated with polar hagimit extracts, the free radical scavenging activity was sustained until 12 days of storage with only gradual decrease until the end of its shelf-life. Application of hagimit extracts 4, 6, and 8 days after harvest of the tomato fruits showed similar preservation of the fruits' free radical scavenging activity although fruits coated 4 days after harvest were of shorter shelf-life. Moreover, the optimum free radical scavenging activities of tomato fruits coated with aqueous, ethanolic, acetic acid extracts were 340.54, 349.01, 348.88 μmolTE/100g, respectively.

Keywords: Hagimit Extract, Tomato Shelf-Life, Free Radical Scavenging Activity, Effect of Timing of Application

1. Introduction

The tomato fruit is more important than we care to realize. It is one of the most important vegetable crops in the world which ranks second to eggplant in terms of production. Its total world production is 152.9 million ton with a value of $74.1 billion (Rakha *et al.*, 2011). It is even more important in Asia and Africa which account for more than 65% of the global tomato output (AVRDC, 2010). A tomato fruit, moreover, is a great source of antioxidants and free radical scavengers (Salas *et al.*, 2013). This is because of the presence of vitamin C and other compounds especially lycopene which is present in large amounts in the tomato pulp (Agarwal and Rao, 1998).

The common problem however, with tomato production, aside from pests, is the storage of the tomato fruits after harvest (Giovanelli *et al*, 2002). The postharvest loss of tomato due to storage worldwide is estimated at 35% of the total production (Bautista & Esguerra, 2007). A common tomato lasts at most 7 days at normal room temperature and conditions (http://www.eatbydate.com/fruits/fresh/tomatoes-shelf-life-expiration-date/). A lot of tomato fruits become dried and degraded, decreasing its antioxidant properties (Lavelli *et al.,* 1999).

Fortunately, it was found out that certain plant extracts can extend shelf life of tomato fruits (Patanao, 2013). One of these extracts is the hagimit (*Ficus minahassae* Miq.) extract (Salas *et al*., 2013). This research aimed to determine the effect of storage time on the free radical scavenging activity (FRSA) of mature red tomatoes coated with hagimit extracts; to investigate the effect of timing of application of hagimit extract on the free radical scavenging activity of mature red tomatoes; and to evaluate the maximum/optimum free radical scavenging activity of mature red tomato fruits coated with hagimit extracts stored under ambient condition.

2. Methodology

2.1. Sample Acquisition

The first step in the methodology is the acquisition of samples. Hagimit samples were gathered around the VSU area near the Mt. Pangasugan, Baybay City, Leyte. The hagimit berries were picked, air-dried and stored until the extract preparation. Approximately 40 kg of high quality mature red tomatoes, variety Diamante Max, were harvested on July 12, 2013 from the O-K Range Project at Cabintan, Ormoc City from an organic farm to avoid pesticide contamination.

2.2. Preparation of the Hagimit Extracts

The availability in the market to ordinary consumers, specifically the farmers, and the non-toxicity of the solvents were taken into consideration in choosing the extraction solvents. Three solvents were used namely vinegar, ethanol and water. The dried hagimit berries were crushed and homogenized, then soaked in ethanol, distilled water and acetic acid as extractants at room temperature for 24 hours. The sample:solvent ratio was 1:10 (w/v). The extracts were filtered using a Whatman 42 filter paper. The filtrate was transferred into a 1 liter Erlenmeyer flask and covered with black carbon paper to avoid possible pigment degradation, and stored in a refrigerator.

2.3. Phytochemical Coating and Storing of Tomatoes

The tomato samples were placed in net bags and soaked in 1% of the different hagimit extracts for 5 minutes. After coating, the tomato fruits were air-dried for 30-60 minutes. The tomato fruits were stored in paper boxes placed in trays. They were kept inside a room at ambient temperature throughout the analysis. Moreover, the placement of the different treatments and replicates was randomized. Coating of tomato was done on the 4th, 6th and 8th day after harvest to evaluate the effect of the timing of application on its free radical scavenging activity.

2.4. Preparation of Tomato Extracts

The tomato from each treatment and replication were chosen randomly and weighed. Each tomato was chopped and mixed with a corresponding amount of ethanol, given a 1:10 (w/v) ratio for the tomato and ethanol. The mixture was further homogenized using a blender. The solution was allowed to stand for 1 hour to maximize extraction. Afterwards, it was filtered using a Whatman 42 filter paper. The extracts were then placed in 25 mL screw capped vials covered with carbon paper to minimize degradation due to light reaction. The extracts were stored in a refrigerator prior to analysis.

2.5. Determination of Free Radical Scavenging Activity

Lastly, free radical scavenging activities of the tomatoes were determined. DPPH (1,1-diphenyl-1-picrylhydrazyl) assays were utilized. Free radical scavenging activity determination was done by making antioxidant assays with DPPH. The DPPH solutions were prepared by dissolving 2.25 mg of the DPPH in 75 mL of ethanol and then diluted to 100 mL with distilled water. DPPH standard solutions were stored in the refrigerator wrapped with carbon paper.

An external calibration was first done to provide a basis of the free radical scavenging activity of the extracts. Laboratory grade trolox was used as a standard for the antioxidant. Different concentrations namely, 0.00001 M, 0.0001 M, 0.0002 M, 0.0003 M, 0.0004 M and 0.0005 M of trolox standard were prepared.

Aliquots of 0.1 mL of the standard trolox solutions were allowed to react with 3.9 mL of the standard DPPH solution. Similarly, 0.1 mL of the tomato extract was reacted with 3.9 mL of the DPPH solution.

The mixtures were allowed to react for 1 hour with shaking. Afterwards, the absorbance of the resulting solutions were read in a Shimadzu UV-Vis spectrophotometer using ethanol as the reference. Free radical scavenging activity test was done whenever possible throughout the average shelf-life of the tomato fruits.

The experiment Trolox was used as the standard free radical scavenger. The DPPH assay yields a scavenging value equivalent to that of a certain amount of the standard Trolox. The absorbance of the assays are correlated with the standards using a UV-Visible Spectrometer.

The experiment has a total of 13 treatments namely:

T_0 = Untreated tomato (Control);

T_1 = Tomato coated with acetic acid (Solvent Control);

T_2 = Tomato coated with ethanol (Solvent Control);

T_3 = Tomato coated with water (Solvent Control);

T_4 = Tomato coated with alum solution (Positive Control);

T_5= Tomato coated with acetic acid hagimit extract (4 days after harvest);

T_6= Tomato coated with ethanolic hagimit extract (4 days after harvest);

T_7 = Tomato coated with aqueous hagimit extract (4 days after harvest);

T_8= Tomato coated with acetic acid hagimit extract (6 days after harvest);

T_9= Tomato coated with ethanolic hagimit extract (6 days after harvest);

T_{10} = Tomato coated with aqueous hagimit extract (6 days after harvest);

T_{11}= Tomato coated with acetic acid hagimit extract (8 days after harvest);

T_{12}= Tomato coated with ethanolic hagimit extract (8 days after harvest);

T_{13}= Tomato coated with aqueous hagimit extract (8 days after harvest).

3. Results and Discussion

The average shelf-life of uncoated tomatoes lasted until the 15th day after harvest. The tomatoes coated on the 6th day after harvest with acetic acid hagimit extract meanwhile lasted up to 49 days. Table 3 shows the average free radical scavenging activity (FRSA) of mature red tomatoes coated with polar hagimit extracts on the 4th day after harvest (4DAH). The free radical scavenging activity of uncoated mature red tomato increased until the 6th day of storage. From the 6th day onwards however, a continuous decrease in the FRSA was observed until the 12th day after which its average shelf-life ended.

Evaluation of the FRSA of the tomatoes showed that the phytochemical coatings were able to slow down the degradation of the free radical scavenging components of the tomatoes. FRSA of coated tomatoes were sustained between the 10th to 19th day and only gradual decreases were observed onwards until the termination of its shelf-life.

The increase in free radical scavenging activity could be attributed to the further ripening of the tomato fruits. This is because harvested fruits undergo several metabolic processes, some of which are essential to reach the desired state of maturity such as those attributed to further ripening (Bargel and Neinhuis, 2005). As the tomato ripens it produces and develops more lycopene, thus enhancing its antioxidant activity (Radzevičius et al., 2009). The rapid decrease in the FRSA afterwards could be attributed to the fast degradation of the mature red tomato fruit, being on the last stage of maturity. Most of the metabolic processes that harvested fruits undergo are degradative in nature especially after full maturity (Yang et al., 2013) which can include the degradation of antioxidant components. According to Ruiz et al. (2001), the degradation of tomato fruits could be related to the degradation of its components especially the free radical scavenging components because these components help in the preservation of the fruit. Thus, the positive effect of the polar hagimit extracts on the preservation of the tomato fruits could be attributed to the conservation of the free radical scavenging components.

The comparison between FRSA of the uncoated and those coated mature red tomatoes indicates that the phytochemical coatings have deliberately slowed down the reduction in the FRSA. It could be seen that the FRSA of the tomato fruits coated with acetic acid hagimit extract (AAHE) continuously increased until the 10th day of storage. The tomato fruits coated with aqueous hagimit extract, meanwhile showed an optimum FRSA at the 12th day of storage. This is also true with the tomato fruits coated with the ethanolic hagimit extracts, which had its peak FRSA on the 12th day, exhibiting the highest FRSA among the treatments with the value of 349.01±5.90 µmolTE/100g. After the 12th day, the above-mentioned treatments inevitably experienced a decrease in the FRSA although gradual, with the ethanolic hagimit extract still having higher FRSA than the other two treatments (AAHE & AQHE). It can then be inferred that the different polar hagimit extracts were able to sustain the FRSA of the tomato fruits until the 10th to 12th day and was able to slow down the degradation of the free radical scavenging components of the fruit.

The preservation of the FRSA of the coated tomato fruits could be attributed to the different phytochemicals found in the polar hagimit extracts. Both the acetic acid and aqueous hagimit extracts contain flavonoids, tannins and polyphenolic compounds, meanwhile, the ethanolic hagimit extract also contain the above-mentioned phytochemicals with the addition of alkaloids (Perez, 2014). Phenolics contribute largely to antioxidant activities of different plant extracts because of their ability to donate hydrogen atoms or electrons in capturing free radicals (Stoilova et al., 2008). Moreover, phenolic compounds have properties that enable them to adsorb and neutralize free radicals, to quench singlet and triplet oxygen or decomposing peroxides (Hasan et al., 2008). The enhanced performance of the ethanolic hagimit extract in extending the FRSA of the tomato fruits might be attributed to the alkaloids present in them. Alkaloids in plants are considered as byproducts of the plant metabolism as stimulators or regulators which can have pharmacological and other bioactive properties (Ikan, 1969) such as anti-ripeners or shelf-life enhancers (Salas et al., 2013).

Assessment of the effect of different timing of applications of the phytochemical coating indicated that tomatoes coated on the 6th and 8th day have longer shelf-lives than those coated on earlier days such as on the 4th day. The FRSA however, show that they had a similar trend which is shown in Figure 1. Preservation of the FRSA lasts until the 12th day regardless of the timing of application and then small incremental decrease is observed.

Lastly, although tomatoes coated on the 4th day lasted shorter than those coated later, the maximum/optimum FRSA were observed among them. The tomatoes coated with hagimit extract had an optimum FRSA of 340.54±6.27 µmolTE/100g; The tomatoes coated with ethanolic hagimit extract meanwhile, had a FRSA of 349.01±5.90 µmolTE/100g; the tomatoes coated with acetic acid hagimit extract had the maximum FRSA of 348.88±4.70 µmolTE/100g. Moreover, the FRSA of the tomato fruits prior to termination is comparable to that of the FRSA of the tomato at the start of the harvest as shown in Figure 2.

Table 1. *Average free radical scavenging activity (µmolTE/100g) of mature red tomato fruits coated with polar hagimit extracts as affected by storage time*

Storage Time (days)	Free Radical Scavenging Activity (µmolTE/100g)			
	Uncoated	AAHE at 4DAH	ETHE at 4DAH	AQHE at 4DAH
0	270.61±10.99[a]			
6	341.98±11.07[c]	330.69±4.76[b]	335.76±13.94[b]	330.74±17.33[b]
8	338.81±10.47[c]	339.78±2.76[b]	345.50±1.87[b]	330.89±18.35[b]
10	332.81±24.45[c]	339.57±13.03[b]	347.26±25.51[b]	331.19±5.28[b]
12	266.36±3.46[b]	348.88±4.70[b]	349.01±5.90[b]	340.54±6.27[b]
19		280.73±6.90[a]	289.60±2.90[a]	280.78±3.69[a]

*AAHE=Acetic Acid Hagimit Extract ETHE= Ethanolic Hagimit Extract AQHE = Aqueous Hagimit Extract

*Values with different letters differ significantly at α=0.05 using Tukey's HSD.

The data obtained provide valuable data on the trend of the free radical scavenging activity of the tomato fruits throughout its average shelf life. It provides an insight on the development of the FRSA of the tomato fruits at different days after harvest. By evaluating the FRSA of the tomatoes, the analysis on the efficiency of the different extracts will not be limited to the physical degradation of the tomato but could also be extended to the quality and health benefit inside the fruit, a feature that other information, such as visual quality rating, could not indicate.

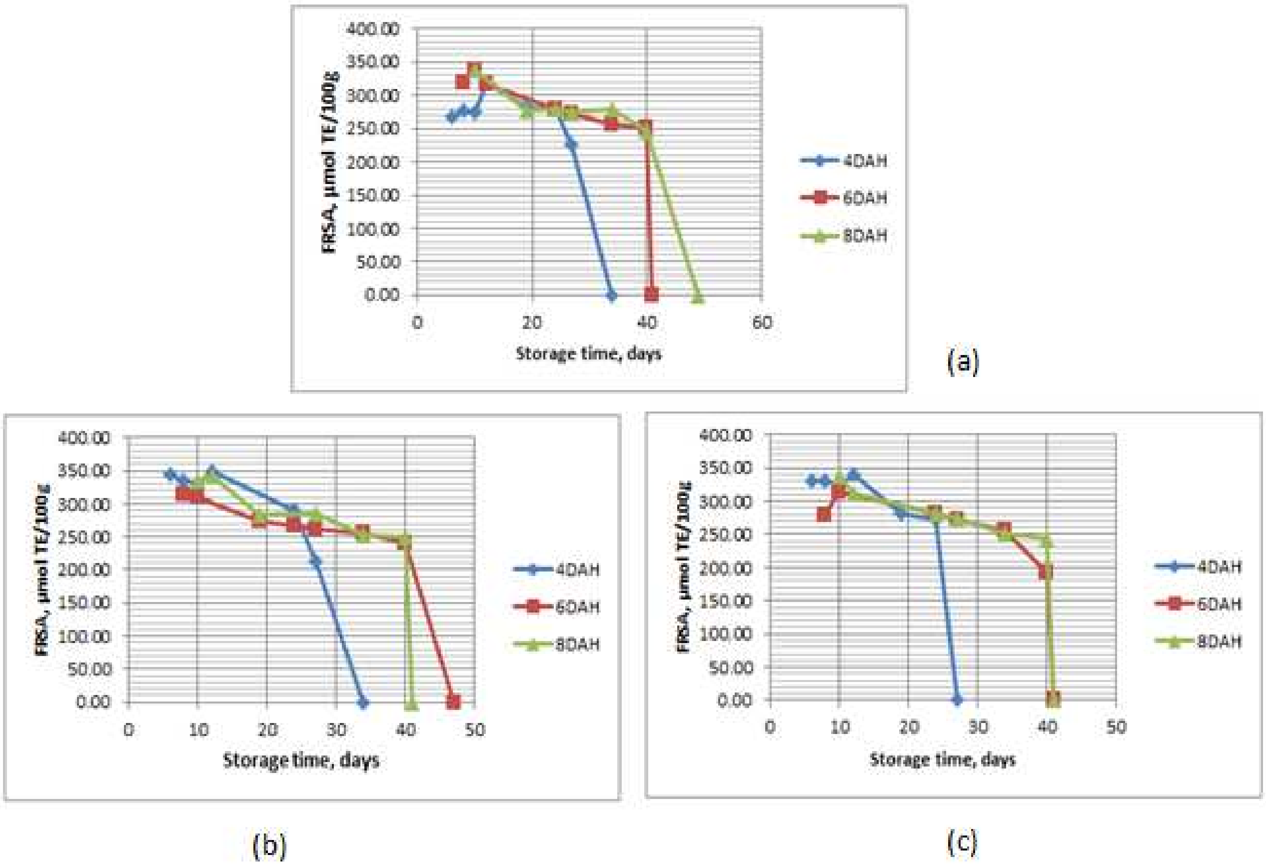

***Figure 1.** FRSA of mature tomato fruits coated with (a) acetic acid, (b) ethanolic and (c) aqueous hagimit extracts as affected by timing of application.*

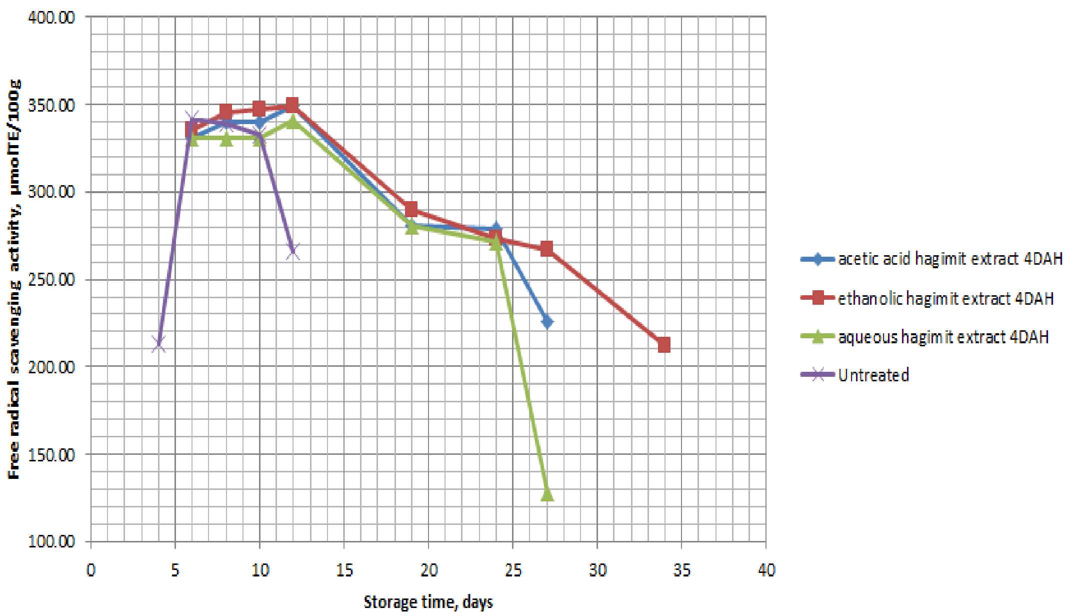

***Figure 2.** FRSA of mature tomato fruits coated with hagimit extracts at 4 days after harvest.*

The extension of the FRSA of the tomatoes means that the different polar hagimit extracts not only extend the average shelf-life of the tomato fruits but they also preserve the quality and the health benefits of the fruits. This fact could have vast effect in agricultural development particularly in solving the problems on postharvest losses and availability of food products in the market. Post-harvest loss due to storage comprises 35% (Bautista & Esguerra, 2007). The different polar hagimit extracts have the potential to further lessen the postharvest loss of tomato fruits as experienced by the farmers. This in turn will bring in more income and improve the state of the agriculture industry of the country.

In addition, the results could be very beneficial in terms of the proper timing of application of the phytochemical coatings in its future use in agriculture. With these information the forthcoming users of the coatings can properly time the application of the hagimit extracts to suit their preferences. If enhancement of the antioxidant property is the priority, an earlier application could be employed. On the other hand, if preservation of the tomato fruits is of importance later application could be used.

Lastly, these figures are very beneficial in pinpointing the period wherein the coated tomato exhibits their optimum free radical scavenging activity. By identifying these periods, one can determine the interval wherein the tomato fruits are healthiest to consume. Moreover, this could benefit future researchers wherein there is a need to extract large amounts of free radical scavenging components of the tomato fruits. The use of different free radical scavenging components on treatments of diseases is widely applied in medicine (Delanty and Dichter, 2000). An example of these is the use of the antioxidants in amending many disorders such as multistage human carcinogenesis (Tsao *et al.*, 2004). This property is responsible for the significant protective effects of antioxidants in human carcinogenesis (Greenwald, 2002). With these results, one can determine the most appropriate time to consume or use the tomato fruits for maximum/optimum free radical scavenging components. By doing these, the body can have a larger intake of antioxidants that will help in the amelioration of the said diseases.

4. Summary, Conclusions and Recommendations

The study was intended to evaluate the effect of storage time on tomatoes coated with polar hagimit extracts, namely acetic acid, ethanolic and aqueous extracts; investigate the effect of different timing of applications on the free radical scavenging activity of the coated tomato and lastly, to evaluate the maximum free radical scavenging activity of the coated tomatoes stored under ambient condition.

The average shelf-life of uncoated tomatoes lasted until the 15th day after harvest. The tomatoes coated on the 6th day after harvest with acetic acid hagimit extract meanwhile lasted up to 49 days. Evaluation of the FRSA of the tomatoes showed that the phytochemical coatings were able to slow down the degradation of the free radical scavenging components of the tomatoes. Free radical scavenging activity of coated tomatoes were sustained between the 10th to 19th day and only gradual decrease were observed onwards until the termination of its shelf-life.

Assessment of the effect of different timing of applications of the phytochemical coating indicated that tomatoes coated on the 6th and 8th day have longer shelf-lives than those coated on earlier days such as on the 4th day. The FRSA however, show that they had a similar trend. Sustenance of the FRSA lasts until the 12th day regardless of the timing of application and then small incremental decrease is observed.

Lastly, although tomatoes coated on the 4th day lasted shorter than those coated later, the maximum/optimum FRSA were observed among them. The tomatoes coated with aqueous hagimit extract had an optimum FRSA of 340.54±6.27 µmolTE/100g; The tomatoes coated with ethanolic hagimit extract meanwhile, had a FRSA of 349.01±5.90 µmolTE/100g; the tomatoes coated with acetic acid hagimit extract had the maximum FRSA of 348.88±4.70 µmolTE/100g.

The polar *Ficus minahassae* Miq. extracts were able to delay the free radical scavenging of the tomatoes; with the acetic acid showing the most promising results by extending shelf-life up to 49 days; application of phytochemical coating at later days such as the 6th and 8th day after harvest extend the shelf-life of the tomatoes longer; the general trend of FRSA however, is similar regardless of the timing of application; and the maximum/optimum FRSA of the coated tomatoes with aqueous, ethanolic and acetic acid were 340.54±6.27, 349.01±5.90, 348.88±4.70 µmolTE/100g, respectively.

The following recommendations are proposed for further studies on the potential of *Ficus minahassae* Miq. extracts: other non-toxic extracting solvents ought to be explored; application of the plant extract on other agricultural products; use of different antioxidant assays to evaluate FRSA of the tomatoes; evaluate the mechanism as to how the extracts preserve FRSA of tomato fruit; toxicity of the plant extract be determined; evaluation of the stability of the plant extracts; evaluation of antimicrobial property of the plant extracts; application of polar hagimit extracts on the 12th day after harvest and beyond; field evaluation of the polar hagimit extracts on truckloads of tomato fruits; acceptability test of coated tomato fruits; and adoption of phytochemical coating technology by farmers and entrepreneurs.

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