



# Effects of Golden Apple Snail (*Pomacea Canaliculata Lamarck*) in the Shelf Life of Tomato (*Solanum Lycopersicum*)

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## ABSTRACT

The use of different preservation techniques and methods to fruits and vegetables has been widely used to prolong their shelf life these days. This study aims to determine the effect of Golden Apple Snail (*Pomacea canaliculata Lamarck*) Chitosan on prolonging the shelf life of tomato (*Solanum lycopersicum*). The Golden Apple Snail (*Pomacea canaliculata Lamarck*) was dried and pound using mortar and pestle. The making of the coating was made through the different raw materials such as; distilled water, 2M Sodium hydroxide (2M NaOH), 2M Hydrochloric acid (2M HCl) and 50% Sodium hydroxide (50% NaOH). This study used a completely randomized design set-up consisting of three treatments T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>0</sub> as the control. The gathered data were analyzed using Analysis of Variance (ANOVA) Single Factor at 5% significant level. The treatments vary with the different amount of Golden Apple Snail (GAS) shell powder with 1g, 2g, and 3g of GAS powder for treatment T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub> respectively. With the results gathered, study on weight loss revealed no significant effect of all the treatments. On the change of color of tomato samples, a four- day delayed in color change was visibly observed on all the treated replications as compared to T<sub>0</sub>. On the overall freshness of tomato, T<sub>1</sub> presented the best result having excellent rating until the end of the study.

**Keywords :** Tomato, Chitosan, Golden Apple Snail, Demineralization, N- deacytelation, Deproteinization

## 1 INTRODUCTION

Philippines, a geographically archipelagic and mainly an Agricultural country wherein it covers a land area of 30 million hectares and about 47% is covered by Agricultural land. Because of its archipelagic nature, farmers and traders are having problem in prolonging and extending the shelf life of their crops particularly those perishable crops to be sold in either large or small market before it appears to be unappealing to the consumers. Transporting harvested crops would be a challenge for the farmers in maintaining a good quality for their produce particularly fruits and vegetables where they are inherently perishable. Furthermore, subtropical countries like Philippines acquire a warm and humid climate that could be an agent in adding more stress and decay acceleration of tropical crop produced (Philippine Statistics Authority, 2012).

Philippine Agricultural Statistics found that about 50% of loss was recorded during post- harvest from initial harvesting, grading, packaging, and transportation from field to storage and distribution to the consumers of different crops. Prior to that problem, post-harvest technologies were developed to lessen crop spoilage and deterioration to attain higher profitability. Factors like lack of knowledge and inability of the small farmers to provide proper post- harvest handling which includes storage facilities, infrastructure, cooling chains are also a contributing factor in large amount of loss produce (Lotis, 2016).

Due to the aim of resolving those post- harvest problems of crops, many technologies and methods developed were mostly Genetically Modified (GM) Product Tomatoes. But this did not resolve the problem. Instead, it resulted to be a health risk for the consumers

(Smith, 2017).

On the other side, consumers tend to patronize organic labeled products where the application of non-toxic and health friendly remedies in post-harvest methods to maintain and prolong the quality of vegetables and fruit crops are being developed.

Studies regarding this biochemical component are those that perform as a wrapping film around the fruit to slow down the respiration and transpiration process. These films could be incorporated with nutrients and preservatives and are said to be functional in various ways compared to using biodegradable plastic trays and wrapping materials that could create an extra burden of waste disposal and damages to the environment as can be observed today in our modern supermarket. This makes the idea wholesome for the consumer's perspective.

Tomato, scientifically known as *Solanum Lycopersicum*, is a well-known household commodity crop which can be grown anytime of the year. During June-October, its peak season, keeping its quality is the main challenge for farmers knowing that tomatoes are highly perishable crop due to its moisture content which has a short shelf life of about 48 hours under tropical condition. This results for the price to depreciate and with little cold storage available, much of it is wasted. To economically develop a simple and worthwhile method that is feasible for the small farm in extending the shelf life of tomato, Chitosan lysosome was developed (Park et al, 2014).

Chitosan are extracted from crustaceans like crab shells and prawn or shrimp shells. It was studied to help reduce the growth of decay-causing fungi and food borne pathogens. Chitosan coating forms a semipermeable film on the fruits' surface delaying its rate of respiration and maintaining its quality in totality (Ramanazzi et al., 2016).

In the case of tomato, the application of crab or prawn shell based on Chitosan is not economically reasonable. Crabs or prawn shells are much expensive and is rarely available as to be used as one component of the biofilm in treating a whole year available crop like tomato.

The Golden Apple Snail (*Pomacea canaliculata* Lamark), the farmer's enemy in the rice field are much numerous during rice planting season also contains Chitosan.

With this, the researcher came up with the idea of the application of Golden Apple Snail Chitosan in prolonging the shelf life of tomato which will be a cheaper source of Chitosan.

## 2 METHODOLOGY

### 2.1 EXTRACTION OF CHITOSAN FROM GOLDEN APPLE SNAIL SHELLS

Figure 1 presents the flow of the process of extraction of Chitosan. The activities involved were gathering of materials, washing snails with water, desiccating at room temperature, boiling for meat separation of shellfish, sun drying, baking in hot air oven, powderization, demineralization, deproteinization, decolorization, N-deacetylation, and analysis of data.

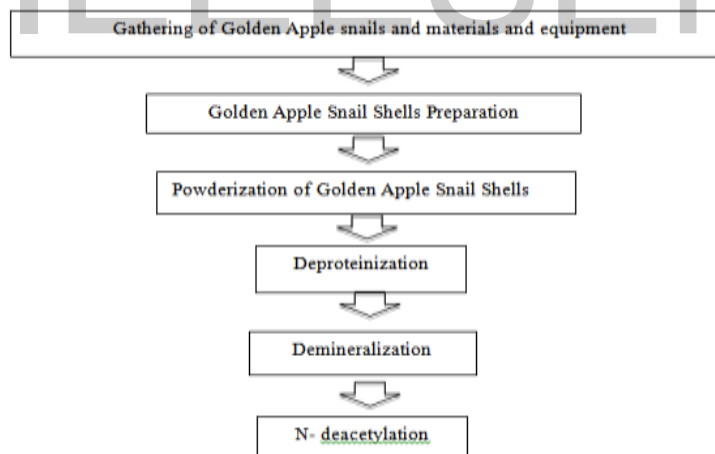


Figure 1. Flow chart of the method of extraction of Chitosan

#### 2.1.1 Gathering of Golden Apple Snails and materials and equipment

In this research, the Golden Apple snail was obtained at Pamucutan Rice fields, Zamboanga City. The raw materials used in extraction of Golden Apple Snail Chitosan are distilled water, 2M Sodium hydroxide (NaOH), 50% Sodium hydroxide (NaOH), and 2M Hydrochloric acid (2 M HCl) obtained at the College of Agriculture Science Laboratory, Western Mindanao State University.

The equipment used was cooking pot, hot air oven, desiccator, mortar and pestle, blender, analytical balance, 20 ml beaker, mechanical stirrer, and pH meter.

### 2.1.2 Golden Apple Snail Shells Preparation

The Golden Apple Snail (GAS) shells were washed with water until mud was removed. The GAS was boiled using a cooking pot to easily separate the meat from its shell. After cooking, the meat was removed and separated from the shells. The remaining shells were oven dried for 1 hour at 100°C to sterilize and for easy powderization of the shells. After 1 hour, the oven dried shells were desiccated using a desiccator located in the Science Laboratory of the WMSU – College of Agriculture at room temperature.

### 2.1.3 Powderization of Golden Apple Snail Shells

The Golden Apple Snail Shells were crushed with mortar and pestle and further powderized using a blender to create Golden Apple Snail shells powder.

### 2.1.4 Deproteinization

Deproteinisation of powdered Golden Apple Snail shells was done using a 2M Sodium hydroxide (2M NaOH) and powderized Golden Apple Snail (GAS) with a ratio of 20 ml: 1 g (w/v) respectively at a room temperature. The treatment was mixed using a mechanical stirrer for duration of 2 hours. Afterwards, the treated GAS was washed with water and water was filtered until it has a neutral pH.

### 2.1.5 Demineralization

The residue from deproteinisation was weighed with analytical balance. Demineralization was done using diluted Hydrochloric acid (2 M HCl) solution. The sample was treated with 20 ml Hydrochloric acid (2 M HCl) and 1g from the residue of deproteinisation at a room temperature. The treatment was done for duration of 2 hours using a mechanical stirrer rotating at all times. After the two- hour duration, it was washed with water and the water was filtered until it has a neutral pH.

### 2.1.6 N- deacetylation

The residue from demineralisation was weighed with analytical balance. Then, it underwent deacetylation with 50 % Sodium Hydroxide (NaOH). The ratio was 20 ml: 1g (w/v) of the NaOH and the residue from the demineralization respectively at temperature of 14°+10°C. The treatment was done for duration of 2 hours using a mechanical stirrer rotating at all times. The mixture was washed with water and water was filtered until it has a neutral pH. It was boiled in boiling water bath for another 4 hours and filtered to dry in an oven at 100+10°C for 1 hour.

## 2.2 Application of Edible Coating for Tomato Preservation

Figure 2 shows the flow of the preparation of the application of Golden Apple Snail Chitosan to the sampled tomatoes.

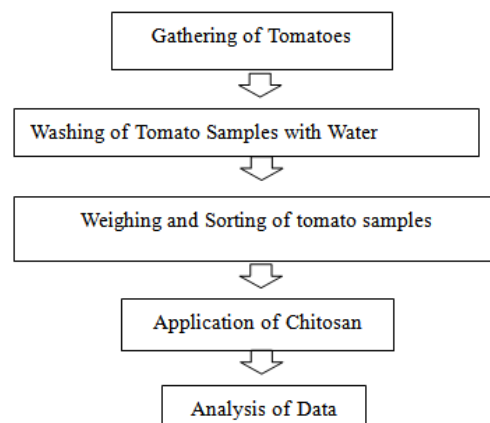


Figure 2. Flow chart of the method of application of Golden Apple Snail Chitosan to the tomato samples

### 2.2.1 Gathering of Tomatoes

In this research, the tomatoes were obtained at Butalian, Zamboanga Sibugay. The raw materials gathered were tomatoes and Golden Apple Snail Chitosan.

The equipment and materials used were analytical balance, 500 ml plastic container, newspaper, digital camera, pen and notebook.

### 2.2.2 Washing of Tomato Samples with Water

Tomato samples were washed with flowing water to remove possible dust, dirt and other unwanted organisms present on the sam-

ples. The washed tomato samples were placed on a clean newspaper to dry-up.

### 2.2.3 Weighing and Sorting of Tomato Samples

The tomato samples with the same harvest period, free from blemishes and fungal infections, were weighed using an analytical balance and sorted base on its weight, and size basis.

### 2.2.4 Preparation of set up

The weighed and sorted tomatoes were placed inside a 500 ml disposable plastic cup, 5 pieces each of the container.

The set- up of the treated tomatoes was covered with mosquito net to prevent from attack of rodents and insects.

### 2.2.5 Application of Chitosan

The weighed and sorted tomatoes were characterized based on its size, and weight. Samples with almost the same size and weight were combined. Different chitosan concentrations were labeled  $T_1$ ,  $T_2$ ,  $T_3$  and  $T_0$ ,  $T_0$  with no chitosan concentration. Table 1 shows the amount of Golden Apple Snail (GAS) powder per treatment. Each concentration had 3 replications having 5 tomato samples per replication. Tomato samples in each replication except  $T_0$  were dipped in their designated chitosan concentration until all tomatoes were totally coated. Tomatoes were drained for 1 minute to remove excess liquid before it was placed in a 500 ml plastic container.

The weight of GAS chitosan were identified after the extraction of Chitosan from Golden Apple Snail shells had been tested base from their respective chitosan percentage.

**Table 1**

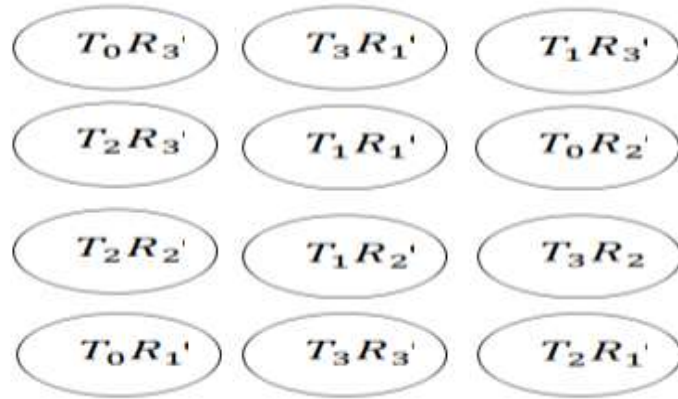
*Weight of Golden Apple Snail (GAS) Powdser after extraction for the different treatments replicated three times*

Treatment	Weight of GAS Powder (g)
$T_0$	0
$T_1$	0.3
$T_2$	2
$T_3$	4

**Table 2**

*Portion of the different ingredients of the tomato coating before application*

Treatments	Mixture	
	Weight of GAS Powder (g)	Distilled Water (ml)
control	0	0
1	0.3	60
2	2	60
3	4	60



**Figure 3.** Experimental layout set- up used during the conduct of the experiment using complete randomized method.

## Parameters gathered

### *Weight of Tomatoes*

All tomatoes were weighed during the initial application of treatments and throughout the entire duration of the study using an analytical balance.

### *Tomato Color*

The tomato samples gathered were green in color upon harvest. Tomatoes color was recorded using food color chart every other day for the entire duration of 28 days.

Tomato Color

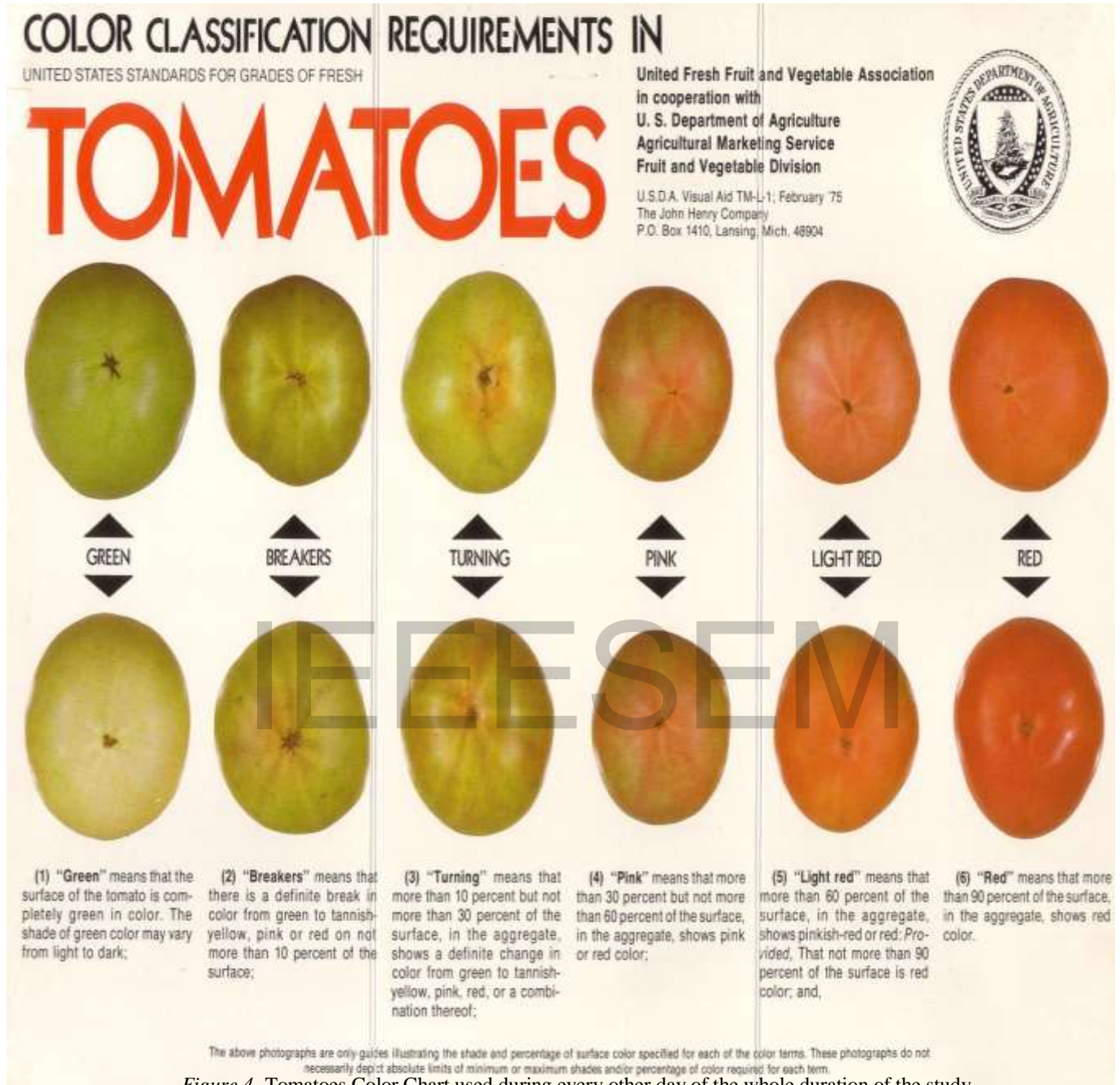


Figure 4. Tomatoes Color Chart used during every other day of the whole duration of the study

The tomatoes color category is based from the United States Department of Agriculture (2011), and the use of color coding is based from the research work of Janice Vancleave, "Browning of Apples: Data Analysis", (2010).



Table 3

*Rating codes used for measuring the color stages of tomato.*

Code	Tomato Color Stages
1	Green
2	Breaker
3	Turning
4	Pink
5	Light Red
6	Red
7	Rot

### Overall freshness of Tomatoes

The tomato samples upon harvest and after treatment were observed every five (5) days during the whole duration of the study using the rating scales for measuring the overall freshness of tomato fruits.

Table 4

*Rating scale for measuring the overall freshness of tomato in terms of pod shelf life, yellowing cracking, shriveling and rotting qualities*

Rating Descriptor	Percent	Ratings
4 Excellent	0	Overall appearance excellent; Fresh fruits
		Overall appearance good, some fruits showing little or no
3 Good	25	sign of rotting/ blackening at the tips; few defects ; acceptable firmness of fruits
		Appearance of the limit of being acceptable, fruits flaccid;
2 Acceptable	50	fruit tips brown or black; some with brown streaks; several/many defects
1 Unacceptable	75	No freshness, fruits with black streaks and brown grooves
		Musty odor, fruits turn brown, slimy decayed, many streaks
0 Poor	100	and blackness

### 2.2.6 Analysis of Data

The results were analyzed using Single Factor Completely Randomized Design (CRD). Analysis of variance (ANOVA) was used in

the analysis of data at 5% significance was used to test the significant difference in the treatment means, and a two- way table was used for data interpretation.

### 3 RESULTS AND DISCUSSION

This research tested the effect of the different amounts of Golden Apple Snail (GAS) powder with three treatments replicated three times evaluated from the parameters gathered namely, total weight loss, change in color, and overall freshness of the tomato samples.

For the total weight loss, the weight of the tomato samples was recorded after every treatment application and after 28 days of the study. The changes in the color of tomato were observed every other day of the study using a tomato color chart. The overall freshness of tomatoes in terms of pod shelf life, yellowing cracking, shriveling and rotting qualities were identified using the rating scale for the overall freshness of tomatoes and observed every other day of the study.

#### 3.1 Weight Loss

The highest weight loss was obtained from the control samples,  $T_0$ , with no GAS powder chitosan while the lowest weight loss was obtained from  $T_1$  with 0.3g of GAS powder chitosan resulting to 25 g of weight loss from the initial weight. Table 6 shows the results using ANOVA Single Factor. The table shows non-significant results between the treatments with the computed F value of 0.908271 that is greater than the tabular F value at 5%.

Based on the results gathered, similar studies from the Journal of Food Processing and Preservation (2008), fruits and vegetables with chitosan coating help restrain the weight of the commodity longer under controlled condition. The result implies that GAS chitosan is not as effective in restraining the commodity's weight under uncontrolled condition compared to store under controlled condition.

Equation 1. Formula used for the average weight loss of the tomatoes

$$\text{Eq. 1. Average Weight Loss} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

**Table 5**

*Average weight loss for the different treatments with varying GAS powder chitosan*

Treatment	Weight of GAS Powder Chitosan(g)	Average Weight Loss(g)
Control( $T_0$ )	0	45
<b>T1</b>	0.3	25
<b>T2</b>	2	28
<b>T3</b>	4	29

**Table 6**

*ANOVA result on weight loss of tomato samples with 3 treatments and the controlled sample replicated 3 times*

Source of Variation	df	Sum of Square	Mean Square	F value	Tabular F (5%)
<b>Treatment</b>	3	755	251.6667	0.908271 ns	4.07
<b>Error</b>	8	2216.667	277.0833		
<b>Total</b>	11	2971.667			

cv= 52.29

ns= non-significant



### 3.2 Change in color of tomato samples

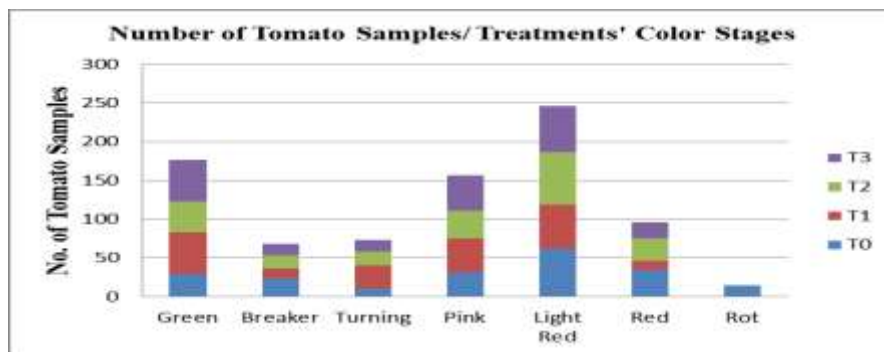
The result in the change of color of tomatoes every treatment were analyzed using a categorical data Two- Way Table presented in table 7. The result shows during the entire 28 days of data gathering- 210 under T0, 29(21.23%) green, 23 (8.20%) undergo breaker, 12 (8.81%) undergo turning, 33 (18.82%) undergo pink, 63 (29.67%) undergo light red, 36 (11.58%) undergo red, and 14 (1.69%) become rot.; 210 under T1, 54(21.23%) green, 13 (8.20%) undergo breaker, 30 (8.81%) undergo turning, 45 (18.82%) undergo pink, 56 (29.67%) undergo light red, 12 (11.58%) undergo red, and 0 (1.69%) become rot. 210 under T2, 42 (21.23%) green, 17 (8.20%) undergo breaker, 18 (8.81%) undergo turning, 36 (18.82%) undergo pink, 66 (29.67%) undergo light red, 31 (11.58%) undergo red, and 0 (1.69%) become rot. 210 under T3, 54(21.23%) green, 15 (8.20%) undergo breaker, 15 (8.81%) undergo turning, 45 (18.82%) undergo pink, 60 (29.67%) undergo light red, 21 (11.58%) undergo red, and 0 (1.69%) become rot. As the result interpreted using a stacked column graph, T0 has the lowest number of tomato samples remained green from the gathering of data until the end of the study while T1 has the most number of samples remained green followed by T3 and T2. At the red stage of the tomato, T0 has the most number of samples while T1 has the least result followed by T3 and T2. Furthermore, only T0 resulted to have rotten tomato samples.

Similar research from an article, Food Processing and Technology, chitosan coating has certain preventive effect against microbes and decay which support the result in this study that treatments with GAS chitosan is also an effective coating in delaying the ripening process of the tomatoes.

**Table 7**

*Result in change of color of tomato gathered every second day of the entire duration of the study*

Treatment	Green	Breaker	Turning	Pink	Light		Rot	Total
					Red	Red		
<b>T0</b>	29	23	12	33	63	36	14	210
<b>T1</b>	54	13	30	45	56	12	0	210
<b>T2</b>	42	17	18	36	66	31	0	210
<b>T3</b>	54	15	15	45	60	21	0	210
<b>Total</b>	179	68	75	159	245	100	14	840



**Figure 5. Result of change in color for every treatment during the entire 28 days duration of the study**

### 3.3 Overall freshness of tomato

The result of the overall freshness of the tomato samples of each treatment replicated 3 times was gathered every other day of the whole duration of the study. Table 8 shows the number of tomato sample for every replication categorized in terms of same percentage ratings. Treatment 1 presented the highest number of tomatoes in terms of excellent overall freshness while treatment 3 has the lowest number of tomatoes in terms of overall freshness. Treatment 3 presented the highest number of tomatoes with good overall freshness while T0 has the lowest. On the acceptable category, T0 has the highest number of tomatoes and T3 has the lowest number.

**Table 8**

*Result of the overall freshness of tomato gathered every 5 days of the study*

	Excellent	Good	Acceptable	Unacceptable	Poor	Total
<b>T0</b>	8	1	6	0	0	15
<b>T1</b>	15	0	0	0	0	15
<b>T2</b>	14	1	0	0	0	15
<b>T3</b>	6	5	4	0	0	15
<b>Total</b>	43	7	10	0	0	60

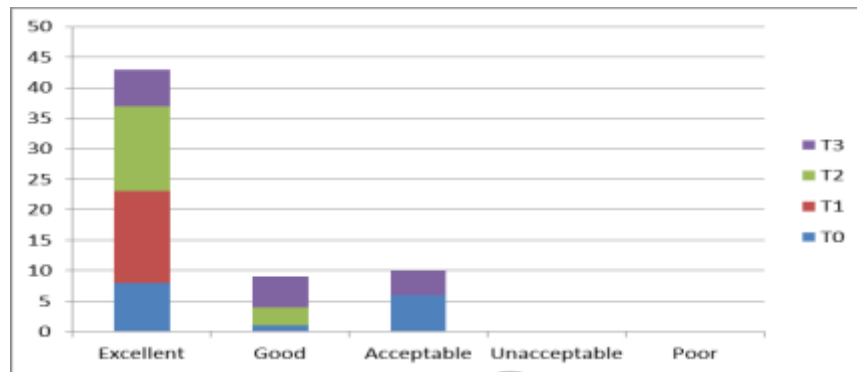


Figure 6. Graphical interpretation of the overall freshness of the tomato samples

#### 4 CONCLUSION

The study conducted aimed to know the most effective edible coating of tomato with different concentration of Golden Apple Snail (GAS) shell powder by determining the weight loss, change in color and overall freshness of tomato samples. Based on the three parameters gathered, weight loss in all treatments have no significance at all technically, GAS chitosan is not sufficient in restraining the tomatoes weight under uncontrolled storage condition however, the change in color and the overall freshness of tomatoes resulted to have prolonged and restrained their metabolic process for a longer days compared to the samples without treatment. Among all treatments, treatment with the least GAS chitosan have resulted the best result. T1, having the least amount of GAS chitosan resulted best result in terms in the change in color and overall freshness of tomatoes. T2, having 2 gram of GAS chitosan resulted to have better result on the overall freshness compared to T3 with the higher amount of GAS chitosan that have shriveled over time. In conclusion, GAS chitosan coatings could be a good alternative in extending the tomatoes shelf life and preserving the good quality of the product for a longer time.

#### 5 RECOMMENDATION

The researcher recommends extending the number of days of study until the controlled samples will deteriorate in order to know and better distinguish the extent of the shelf life of the treated tomato samples based from the controlled samples also, the researcher recommends testing for the total soluble solids and firmness of tomato samples on the effect of the different amount of GAS chitosan coating for the tomatoes.

## REFERENCES

- [1] Bourtoom, T. (2008). Edible films and coatings: characteristics and properties. Retrieved from: [http://www.google.com/url?q=http://www.ifr.upm.edu.my/15%2520\(3\)%25202008/01.%2520Bourtoom,%2520T.pdf&sa=U&ved=2ahUKEwjWxMWRvvXdAhWDp48KHSxXAJ8QFjABegQIChAB&usg=AOvVawoqShSPU6\\_k3gtomSvNEPrK](http://www.google.com/url?q=http://www.ifr.upm.edu.my/15%2520(3)%25202008/01.%2520Bourtoom,%2520T.pdf&sa=U&ved=2ahUKEwjWxMWRvvXdAhWDp48KHSxXAJ8QFjABegQIChAB&usg=AOvVawoqShSPU6_k3gtomSvNEPrK).
- [2] Department of Agriculture (2000). Management options for the Golden Apple Snail. Retrieved from: <http://www.applesnail.net>
- [3] Department of Agriculture (2001). Management option for golden apple snail. Retrieved from: <http://www.google.com/url?q=http://www.pinoyrice.com/wp-content/uploads/management-option-for-the-golden-apple-snail.pdf&ved=2ahUKEwjfx-vDwvPdAhVKpo8KHceVBisQFjAAegQIAxAB&usg=AOvVaw2TerZPOwfecPxIJorQQ5L8>
- [4] Gianfranco Romanazzi, Erica Feliziani, Silvia Bautista Baños & Dharini Sivakumar (2015): Shelf Life Extension of Fresh Fruit and Vegetables by Chitosan Treatment, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2014.900474. Retrieved from: <http://dx.doi.org/10.1080/10408398.2014.900474>
- [5] Isaac Koho Arah, Gerald K. Ahorbo, Etornam Kosi Aku, Ernest Kodzo Kumah, and Harrison Amaglo (2016). Postharvest handling practices and treatment methods for tomato handlers in developing countries: a mine review. Retrieved from: <https://www.hindawi.com/journals/aag/2016/6436945>.
- [6] Lotis E. Mopera (2016). Food Loss in the Food Value Chain: The Philippine Agriculture Scenario. Retrieved from the Journal of Developments in Sustainable Agriculture.
- [7] Jeffrey Smith (2017). Throwing biotech lies at tomatoes- part 1: killer tomatoes. Retrieved from: <https://m.huffpost.com/us/entry/803139>
- [8] Philippine Statistics Authority (2012). Selected Statistics Agriculture. Retrieved from: <http://www.google.com/url?q=https://psa.gov.ph/sites/default/files/Selected%2520Statistics%25202012.pdf&sa=U&ved=2ahUKEwiSnYC91fxdAhUGTo8KHYZwCdoQFjADegQIB&usg=AOvVaw1TE5EyhL-BeTFC7A4jxyK5>.
- [9] Rice Research Bank (n.d.). Golden apple snail. Retrieved from: <http://www.knowledgebank.irri.org/step-by-step-production/growth/pests-and-diseases/golden-apple-snails>
- [10] Soon Ju Park, Ke Jiang, Lior Tal, Yoav, Oron Gar, Dani Zamir, Yuval Eshed, and Zachary B. Lippman (2014). Optimization of Crop Productivity in Tomato Using Induced Mutations in the Florigen Pathway. Retrieved from: <https://www.ncbi.nlm.nih.gov/labs/pubmed/25362485-optimization-of-crop-productivity-in-tomato-using-induced-mutations-in-the-florigen-pathway/>.
- [11] Thillai natarajan S., Kalyanasundaram N., and Ravi S. (2017). Extraction and characterization of chitin and chitosan from achatinodes. Retrieved from: <https://www.omicsonline.org/open-access/extraction-and-characterization-of-chitin-and-chitosan-from-achatinodes.php?aid=93097>
- [12] United States Food Drug Administration (2013). Optimization and Characterization of Chitosan Films for Transdermal Delivery of Ondansetron. Retrieved from: [http://www.google.com/url?q=https://www.researchgate.net/publication/236691844\\_Optimization\\_and\\_Characterization\\_of\\_Chitosan\\_Films\\_for\\_Transdermal\\_Delivery\\_of\\_Ondansetron&sa=U&ved=2ahUKEwjB6ZNY\\_XdahWHuo8KSYiCpoQFjACegQIBhAB&usg=AOvVaw2b78mfvWFdLibeEb53Nba](http://www.google.com/url?q=https://www.researchgate.net/publication/236691844_Optimization_and_Characterization_of_Chitosan_Films_for_Transdermal_Delivery_of_Ondansetron&sa=U&ved=2ahUKEwjB6ZNY_XdahWHuo8KSYiCpoQFjACegQIBhAB&usg=AOvVaw2b78mfvWFdLibeEb53Nba).
- [13] Yi Wu and Marilyn Schnepf (2002). Development and Application of Multicomponent Edible coatings and Films: A Review. Retrieved from: [https://doi.org/10.1016/SI043-4526\(02\)44007-7](https://doi.org/10.1016/SI043-4526(02)44007-7).