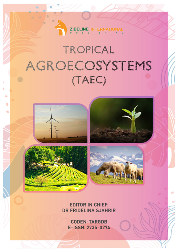
Tropical Agroecosystems (TAEC)

DOI: http://doi.org/10.26480/taec.02.2020.88.91

ZIBELINE INTERNATIONAL PUBLISHING

ISSN: 2735-0274 (Online)
CODEN: TARGDB



CrossMark

RESEARCH ARTICLE

EFFECT OF POST HARVEST TREATMENTS ON SHELF LIFE AND QUALITY OF SWEET ORANGE (*Citrus sinensis)* IN GAASC, GOKULESHWOR, BAITADIS

Rinicha Bhandari*, Puja Acharya, Prabha Sapkota, Manasa Acharya, Pramisha Adhikari, Ramesh Upreti

Gokuleshwor agriculture and animal science college, Tribhuvan University, Baitadi, Nepal.
****Corresponding authors email: rinichabhandari@gmail.com***

ARTICLE DETAILS

Article History:

Received 13 June 2020
Accepted 14 July 2020
Available Online 29 July 2020

ABSTRACT

The experiment was carried out at horticulture laboratory, Gokuleshwor, Baitadi during 28th December 2018 to 19th January 2019, to study the effect of post harvest treatments on quality and shelf life of sweet orange (*Citrus sinensis*) cultivar Local. It was carried out in Completely Randomized Design (CRD) with five treatments of different types of post harvest treatments viz. T_1 = control (distilled water), T_2 = bavistin (0.1%), T_3 = calcium chloride (1%), T_4 = Jeevatu (5%) and T_5 = cinnamon oil (2%) replicated four times. Among these post harvest treatments, T_1 showed highest percentage of weight loss (15.83%), lowest firmness (2.22 kg/cm^2) and highest TSS (10.70˚ Brix), lowest TA (0.395%) at final day of storage as compared to other treatments. Bavistin was found as the most effective in reducing the physiological loss in weight (10.80%), retained maximum firmness (3.13 kg/cm^2), highest tritrable acidity (0.76%), highest pH (5.08). The minimum total soluble solid (8.75˚Brix) was retained by cinnamon oil. This study revealed that sweet orange treated with bavistin recorded lowest physiological loss in weight (31.77%) and retains more firmness (24.73kg/cm^2) than that of control .Thus, present findings indicate that sweet oranges treated with bavistin increase the shelf life where as cinnamon oil also found to be promising treatment for retaining the quality of the sweet oranges stored up to 28th days under laboratory condition.

KEYWORDS

Bavistin, postharvest treatments, cinnamomum oil, jeevatu, tritrable acidity.

1. INTRODUCTION

Citrus sinensis, sweet orange is a small tree in the Rutaceae (citrus family) that originated in southern China, where it has been cultivated for millennia. Sweet oranges are now grown commercially worldwide in tropical, semi-tropical, and a few warm temperate regions and became the foremost widely planted angiospermous tree within the world. Sweet oranges are the world's most popular fruit and are eaten fresh and used for juice. The sweet orange tree is a small, spiny tree, typically growing to 7.5 m, but occasionally reaching the heights up to 15 m, generally with a compact crown. The fruits ripen to orange or yellow. The fruit skin (rind or peel) contains numerous small oil glands. The flesh or pulp of the fruit is typically juicy and sweet, divided into 10 to 14 segments (although there are seedless varieties) and ranges in color from yellow to orange-red. In Nepal, an area covered by sweet orange is 3443ha and production is 33558 ton with the productivity of 9.7 ton/ha. While in the Baitadi district area covered by sweet orange is 21 ha, production 200 ton with the productivity of 9.5 ton/ha (MoAD, 2018).

Postharvest loss of fruits is a global challenge. Since mandarin may be non-climacteric and perishable fruit, it can't be kept for an extended time during transportation and storage. Mandarin fruits are often kept hardly for 1 - 2 weeks depending upon temperature and humidity. The large volume of the losses starts right from harvesting and loss increases many folds during the postharvest steps. Worldwide postharvest loss in fruits and vegetables is as high as 30% - 40% and even much higher in developing countries like Nepal (Rokaya et al., 2016). Post-harvest loss is mainly due to the lack of cold storage facility, transport problem, lack of sound marketing facility, post-harvest disease, insect, and pest. Proper time and method of harvesting along with transportation, storage, and proper packaging materials also determines the post-harvest losses of sweet orange (Arun and Ghimire, 2019). Physical weight loss and quality deterioration are the main causes of post-harvest losses. Proper methods of preserving sweet oranges are not used to date. Although the mid-hill region of Nepal has huge potentiality for sweet orange and have the potentiality to export, we are dependent on import from different countries to full fill the demands of the Nepalese consumers (Bhattarai, 2018).

Nowadays the demand for the fruit crop is increased day by day. This shows the scope of the fruit crop world widely. The climate, soil, topography, and altitude of mid-hills are suitable for citrus cultivation but due to the lack of knowledge, technology, scope, and economic value people are not aware of this. As we know the economic value of the fruit crop is much higher than vegetables and cereals, the farmers of mid-hills can increase their socio-economic conditions by cultivating citrus fruits such as Sweet orange, mandarin, lime, lemon, etc. Thus, this study is very important to enhance the sweet orange shelf life and quality. Postharvest treatments play a big role in extending the time period of the fruits

Quick Response Code	Access this article online	
	Website: www.taec.com.my	**DOI:** 10.26480/taec.02.2020.88.91

Cite The Article: Rinicha Bhandari, Puja Acharya, Prabha Sapkota, Manasa Acharya, Pramisha Adhikari, Ramesh Upreti(2020).Effect Of Post Harvest Treatments On Shelf Life And Quality Of Sweet Orange (*Citrus Sinensis*) In Gaasc, Gokuleshwor, Baitadis. *Tropical Agroecosystems, 1(2): 88-91.*

(Gotame et al., 2015). Nepal faces big losses of mandarin annually due to not adopting proper postharvest handling practices during harvesting, transportation, and storage (Rokaya et al., 2016).

Hence, to minimize these postharvest losses and to maximize the quantitative and qualitative parameters along with prolongation of storage capacity, postharvest treatments with wax and other safe fungicides are urgent for effective marketing of mandarin within the country. The present findings indicate that mandarin is often stored up to four weeks when treated with the wax also as wax with bavistin within the condition with 14 °C – 18 °C and 45% - 73% relative humidity (Rokaya et al., 2016). Reduction of PLW and shriveling and increase in TSS and overall acceptability of sweet orange can be enhanced with proper use of post-harvest chemicals. This study was conducted to evaluate the effect of post harvest treatments on shelf life and quality of sweet orange. Among five treatments T_1= control (distilled water) T_2= bavistin, T_3= calcium chloride, T_4= jeevatu, and T_5= cinnamon oil.

2. METHODOLOGY

2.1 Experiment location detail

The study was conducted at Horticulture Laboratory of Gokuleshwor Agriculture and Animal Science College (GAASC), Gokuleshwor, Baitadi. It lies in the Longitude 80º50' East and Latitude 24º75' North and elevation of 700 masl.

2.2 Preparation of experiment samples

Fresh sweet oranges were harvested from sweet orange tree with the help of hooked stick located within local community of GAASC, Baitadi. Defect free light yellow stages of sweet oranges of local variety were selected. The sweet oranges were harvested on january 8, (2019) and were brought to the lab in jute sack. Each treatment per replication consist 15 sweet oranges of uniform sizes which were kept in open plastic tray. Destructive and non-destructive samples were prepared for each treatment. Out of 15 sweet oranges within plastic tray, 5 of them were tagged as non-destructive sample and used for weight loss observation at every two day's interval whereas remaining sweet oranges taken as destructive sample and used for observation of TA, TSS, pH and firmness at every 7 day's interval after post-harvest treatments.

2.3 Experimental design and treatment details

The experimental set up was done in Completely Randomized Design (CRD) with five treatments of different plastic tray, each replicated four times. The five different treatments used for the study as suggested by (Rokaya et al., 2016); T_1: Control (Distilled water), T_2: Bavistin (0.1%), T_3: Calcium chloride (1%), T_4: Jeevatu (5%), and T_5: Cinnamon oil (0.2%).

2.4 Observations taken

2.4.1 Storage conditions

Temperature and RH during storage were monitored using temperature-RH measuring device. A digital recording device (digital hygrometer thermometer) was used for this purpose. Maximum, minimum and average RH was recorded by digital thermometer. Similarly, maximum, minimum and optimum temperature was also recorded during those days. Every day the recording was done at 5:00 pm.

2.4.2 Physiological loss in weight (PLW)

It was calculated as the percentage weight loss of the initial weight. Initial weight of each sample per replication was taken. The weight of the sample was taken on two day's interval after setting of the experiment. Weight loss was taken with the help of digital balance having capacity to weight from 1 mg to 5 kg. The formula used for the calculation was (Aborisade and Ajibade, 2010):

$$PLW\% = \frac{initial\ wt. - final\ wt.}{initial\ wt.} X\ 100\%$$

2.4.3 Firmness (kg/cm2)

Firmness of the samples was measured with the help of penetrometer (analog) at 7 day's interval after the initiation of the experiment. Peel was removed from the sample and penetration operation was done. From each sample, 3 data were taken which helps in precision.

2.4.4 Total soluble solid (TSS ºBrix)

The Total Soluble Solid content of the fruits was analyzed at the post-harvest laboratory of GAASC, Baitadi. The TSS was determined by hand held refractometer at 7 day's interval after the initiation of the experiment. A drop of juice was squeezed from the fruit sample on the prism of the refractometer and TSS content was recorded. TSS was expressed as °Brix. From each sample, 3 data were taken which helps in precision.

2.4.5 Titrable acidity (TA)

The Titrable Acidity (TA) content of the fruit was analyzed at the postharvest laboratory of GAASC lab. It was determined from 2 ml fruit juice diluted in 10 ml distilled water, titrated with 0.1 N NaOH using phenolphthalein indicators (2-3 drops), and calculated as percent citric acid. Percent titrable acidity was calculated by using the following formula as suggested.

$$TA\% = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 0.064 \times 100}{\text{Volume of juice titrated}}$$

•Acid milliequivalents (mEq) factor for citric acid

2.4.6 pH of fruit juice

pH of the fruit juice was measured by using digital pH meter. pH meter was placed on juice for 2 minutes and reading was noted.

2.5 Statistical analysis

Experimental data were analysed using Gen-stat software of 15th edition and treatment means were separated using Duncan,s Multiple Range Test (DMRT) at 5% level of significance as stated cited (Gomez and Gomez, 1984; Rokaya et al., 2016). Tables were constructed using MS-Excel 2013 and MS-word 2013.

3. RESULTS AND DISCUSSION

3.1 Physiological loss in weight

Table 1: Effect of postharvest treatments on physiological loss in weight (%) of sweet orange (*Citrus sinensis)* at different days during 2018-2019 at GAASC

Physiological loss in weight (%)				
Treatment	7th days	14 days	21 days	28 days
Control	2.580	6.70^{a}	11.06^{a}	15.83^{a}
Bavistin powder	2.423	4.51^{b}	8.13^{b}	10.80^{c}
Calcium chloride	2.348	5.36^{b}	9.09^{ab}	11.70^{bc}
Jeevatu	2.578	6.62^{a}	11.03^{a}	14.73^{a}
Cinnamon oil	2.104	5.02^{b}	9.36^{ab}	12.69^{b}
F-test	NS	**	*	**
SEm (±)	0.1845	0.335	0.694	0.560
LSD (0.05)	0.5561	1.070	2.093	1.689
CV (%)	15.3%	12.6%	14.3%	8.5%
Grand mean	2.407	5.64	9.73	13.15

Means with same letter (letters) within column do not differ significantly at p=0.05 by DMRT, SEM = Standard error of means, LSD=Least Significant Difference, CV=Coefficient of variance, NS, *, ** indicate non-significant and significant at P<0.05 and significant at P<0.01 respectively.

Physiological loss in weight (PLW) was significantly increased in all the treatments with the advancement of the storage period. The increasing trends in the PLW was found maximum in the fruits with untreated as a control in each days up to 28th days of storage period and the losses ranged from 2.580% to 15.83% from 7th to 28th days of storage (Table 1). Minimum percentage of PLW was observed in the fruits treated with bavistin powder in all the days during storage and the losses ranged from 2.243% to 10.80% followed by calcium chloride which losses ranged from 2.34% to 11.70% from 7th to 28th days of storage period. The maximum weight loss in control is due to the rot of fruits (Jafarpour, 2012; Rokaya et al., 2016; Deka et al., 2006). Cinnamon oil coating could reduce the loss in weight than that of control (Win et al., 2007).

3.2 Total soluble solid (TSS)

Table 2: Effect of postharvest treatments on TSS of sweet orange (*Citrus sinensis*) at different days during 2018-2019 at GAASC.

Total soluble solid (^{0}Brix)				
Treatment	7 days	14 days	21 days	28 days
Control	8.55	9.15	11.80	10.70
Bavistin powder	8.22	8.50	10.10	9.25
Calcium chloride	7.45	7.78	10.12	10.00
Jeevatu	7.82	8.32	10.35	9.25
Cinnamom oil	8.10	8.22	8.45	8.75
F-test	NS	NS	NS	NS
SEm (±)	0.368	0.491	0.497	0.507
LSD (0.05)	1.110	1.479	1.498	1.528
CV (%)	9.0%	11.8%	9.5%	10.6%
Grand mean	8.15	8.32	10.43	9.59

Means with same letter (letters) within column do not differ significantly at p=0.05 by DMRT, SEM = Standard error of means, LSD=Least Significant Difference, CV=Coefficient of variance, NS, *, ** indicate non significant and significant at P<0.05 and significant at P<0.01 respectively.

As shown in Table 2, TSS increased with the increasing period of storage in all the treatments for 21st days and the increasing trend was higher in control (distilled water) than the treated fruits. Control fruits showed the maximum TSS content during the storage and ranged from the 8.55 ˚Brix (7 days) to the 11.80˚ Brix (21 days). At the final day of storage i.e. at 28th days, TSS slightly decreased than that of 21st day, highest TSS was observed in control (10.70 ^{0} Brix) and the lowest was observed in cinnamon oil (8.75 ^{0} Brix) but they are not statistically different with each other. All the treatments are statistically at par at every 7th, 14th, 21st and 28th day of storage. The trend showed that fruits treated with cinnamon oil showed gradual increment in the TSS change whereas in control, it was increased at faster pace. The faster TSS increment in the untreated fruits might be due to faster metabolic activities through respiration and transpiration than in treated fruits with different post harvest treatments (Rokaya et al., 2016). The gradual increment in TSS of fruits treated with coating material may be justified by the twin role of coating material, acting as a physical barrier for transpiration losses and creating a modified atmosphere resulting in building of internal CO_2 and depletion of O_2 (Jholgiker and Reddy, 2007).

3.3 Titratable acidity (TA)

Table 3: Effect of post harvest treatments on TA (%) of sweet orange (*Citrus sinensis*) at different days during 2018 at GAASC

Titratable acidity (%)				
Treatments	7th days	14th days	21st days	28th day
Control	1.275	0.780	0.688	0.395^c
Bavistin powder	1.490	1.136	0.814	0.760^a
Calcium chloride	1.424	1.083	0.776	0.752^a
Jeevatu	1.488	0.886	0.675	0.579^b
Cinnamon oil	1.582	0.887	0.702	0.505^{bc}
F-test	NS	NS	NS	**
SEm (±)	0.0814	0.0916	0.0484	0.516
LSD (0.05)	0.2453	0.2761	0.1459	0.1555
CV (%)	11.2%	19.2%	13.2%	17.2%
Grand mean	1.452	0.954	0.731	0.598

Means with same letter (letters) within column do not differ significantly at p=0.05 by DMRT, SEM = Standard error of means, LSD=Least Significant Difference, CV=Coefficient of variance, NS, *, ** indicate non significant and significant at P<0.05 and significant at P<0.01 respectively.

The data presented in Table 3. revealed that TA significantly decreased with the advancement of the storage period. Among the different treatments in 7th day cinnamon oil shows significantly higher TA whereas lowest TA was shown by control (distilled water) which is statistically non-significant. Similarly, at 14th and 21st days no significant difference of TA was seen. The TA was recorded maximum in the fruits treated with bavistin 0.1% (0.76%) which is statistically at par with calcium chloride 1% (0.752%) as against control (0.395%) at the end of the storage. The higher acidity in the bavistin treated fruits might be due to the lesser utilization of the acids in the respiration process during the storage whereas untreated had minimum acids which might be due to faster utilization of the acids in the respiration process during storage (Rokaya et al., 2016; Sonkar et al., 2009; Deka et al., 2006).

3.4 Firmness

Table 4: Effect of postharvest treatments on Firmness of sweet orange (*Citrus sinensis*) at different days at GAASC

Firmness (kg/cm^2)				
Treatment	7th days	14th days	21st days	28th days
Control	5.15	4.20^b	3.10	2.11
Bavistin powder	6.53	5.58^a	4.17	3.13
Calcium chloride	6.85	4.40^b	3.55	2.93
Jeevatu	5.05	4.21^b	3.62	3.08
Cinnamon oil	5.08	4.32^b	3.38	2.55
F-test	NS	*	NS	NS
SEM	0.423	0.330	0.386	0.457
LSD	1.274	0.995	1.163	1.377
CV (%)	15.3%	14.7%	21.6%	33.1%
Grand mean	5.53	4.50	3.57	2.76

Means with same letter (letters) within column do not differ significantly at p=0.05 by DMRT, SEM = Standard error of means, LSD=Least Significant Difference, CV=Coefficient of variance, NS, *, ** indicate non-significant and significant at P<0.05 and significant at P<0.01 respectively.

The fruit firmness was decreased with the advancement of the storage period in all the treatments. As shown in Table 4. the decreasing trend was started from the first week to the end of storage in all the treatments. During 7th days, calcium chloride treated fruits resulted in more intact (6.85 kg/cm^2) and lowest was observed in fruits treated with Jeevatu (5.05 kg/cm^2). At 14th days highest firmness was recorded in bavistin (5.58 kg/cm^2) treated fruits whereas lowest was seen in control (4.20 kg/cm^2). At 21st and 28thdays no significant changes were recorded in different treatments. However, at the end of the storage, the firmness was observed maximum in the fruits treated with bavistin powder (3.13 kg/cm^2) and lowest was observed in control (2.11 kg/cm^2). The decline in the firmness might be due to moisture loss from the fruits cells. The fruits treated with bavistin showed the more intact (firmness) than other treatments which might be due to fungicides acts as moisture and microbial inhibitor that reduces the respiration and transpiration of the fruits (Rokaya et al., 2016).

3.5 pH

Table 5: Effect of post harvest treatments on pH of sweet orange (*Citrus sinensis*) at GAASC

pH				
Treatment	7th days	14th days	21st days	28th days
Control	4.55	4.47	4.35	4.33
Bavistin powder	5.03	4.92	4.85	4.72
Calcium chloride	4.58	4.47	4.35	4.28
Jeevatu	4.58	4.45\	4.47	4.42
Cinnamon oil	5.08	4.85	4.83	4.65
F-test	NS	NS	NS	NS
SEm (±)	0.377	0.372	0.358	0.349
LSD (0.05)	0.803	0.792	0.763	0.744
CV (%)	11.20%	11.30%	11.10%	11.00%
Grand mean	4.76	4.63	4.57	4.48

Means with same letter (letters) within column do not differ significantly at p=0.05 by DMRT, SEM = Standard error of means, LSD=Least Significant Difference, CV=Coefficient of variance, NS, *, ** indicate non significant and significant at P<0.05 and significant at P<0.01 respectively.

The above table 5. shows that in different post harvest treatments the pH value decreases slightly from starting to end of storage period. In control from 7th to 28th days pH decrease slightly from 4.5 to 4.33. Similarly, other treatments showed same pattern of pH value, slightly decreasing from 7th to 28th days of storage. At initial stage pH of sweet orange is highest in cinnamon oil and lowest was seen in control. Although the results are statistically non significant, from 14th, 21st, 28th days of storage highest pH was observed in Bavistin where as lowest was observed in Jeevetu at 14th days and at 21st, 28th days calcium chloride showed lowest pH. The decrease in pH indicates the increased acidity of the fruit and this might be due to the formation of acidic compounds due to degradation of reducing sugars. This study shows the effect of calcium on pH of fruit juice and it was higher (4.33) in control than that of calcium treated fruits (4.28). Since calcium chloride is acidic in nature it might have lowered the pH of the treated fruits which is in lined with the findings of (Martin-Diana et al., 2007).

4. Conclusion

From this study it was observed that sweet orange treated with post harvest treatments had an extended life than those left untreated (control). So it is better to treat sweet oranges with any post harvest treatments than leaving untreated. Among the different post harvest treatments, control showed highest percentage of weight loss (15.83%), lowest firmness (2.22 kg/cm^2) and highest TSS (10.70˚ Brix), lowest TA (0.395%) and lowest pH (4.33) at final day of storage as compared to other treatments. At the end of the study bavistin 0.1% shows lowest physiological loss in weight (10.80%), highest Titratable acidity (0.76%), highest firmness (3.15 kg/cm^2) and highest pH (4.72). Lowest increasing trend of TSS was observed in cinnamon oil (8.75 ^{0}Brix).

Thus, this study revealed that sweet orange treated with bavistin recorded lowest physiological loss in weight (31.77%) and retains more firmness (24.73%) than that of control. Sweet oranges treated with cinnamon oil shows low increasing trend of TSS. Therefore, sweet oranges treated with bavistin showed long shelf life and quality when stored for 28 days where as cinnamon oil is also seen promising in maintaining the quality of sweet oranges. Furthermore, the study on posthavrset life is still lacking and no any appropriate recommendations for improving fruit quality has been reported in sweet orange, thus, introduction to these topics should be prime concern.

Acknowledgements

This paper is a part of Undergraduate Practicum assessment of the authors and R. Upreti is the academic advisor. The research was partially funded by Gokuleshwor Agriculture and Animal Science college, Nepal. The authors would like to acknowledge Sandesh Thapa, Rita Bhandari, Archana Acharya and Anusha khanal for their help and support throughout the research period.

Data availability statement

All the data associated with this research has been presented in the manuscript.

Conflict of interests

Authors do not have any type of competing interests.

References

Aborisade, A.T., Ajibade, A.A., 2010. Effect of prestorage curing on storage life, internal and external qualities of sweet orange (Citrus sinensis). Rev. Bras. Frutic., 32 (3), Pp. 910–915.

Arun, G.C., Ghimire, K., 2019. Estimating post-harvest loss at the farm level to enhance Food Security: A Case of Nepal. Int. J. Agric. Environ. Food Sci., 3, Pp. 127–136.

Bhattarai, D.R., 2018. Postharvest horticulture in Nepal. Hortic. Int. J., 2 (6), Pp. 458–460.

Deka, B., Sharma, S., Borah, S.C., 2006. Post-harvest management practices for shelf-life extension of Khasi mandarin. Indian Journal of Horticulture, 63 (3), Pp. 251-255.

Gomez, A.A., Gomez, K.A., 1984. Statistical Procedures For Agricultural Research, 6. newyork: John Wiley & Sons Ltd.

Gotame, T.P., Subedi, G.D., Dhakal, M., Khatiwada, P.P., 2015. Postharvest Handling of Asian Pear in Nepal. NARC Publication Serial No. 00211-025 (2015/16).

Jafarpour, M., Fatemi, S., 2012. Post harvest treatments on shelf life of sweet orange 'Valencia. Journal of medicinal plant research, 6 (11), Pp. 2117-2124.

Jholgiker, P., Reddy, B.S., 2007. Effect of different surface coating material on post-harvest physiology of Annona squamosa L. fruits under ambient and zero energy cool chamber storage. Indian Journal of Horticulture, 64 (1), Pp. 41-44.

Martín-Diana, A.B., Rico, D., Frías, J.M., Barat, J.M., Henehan, G.T.M., Barry-Ryan, C., 2007. Calcium for extending the shelf life of fresh whole and minimally processed fruits and vegetables: a review. Trends in Food Science & Technology, 18 (4), Pp. 210-218.

MOAD, 2018. Statistical Information In Nepalese Agriculture.

Rokaya, P.R., Baral, D.R., Gautam, D.M., Shrestha, A.K., Paudyal, K.P., 2016. Effect of Postharvest Treatments on Quality and Shelf Life of Mandarin (Citrus reticulata Blanco). Am. J. Plant Sci., 7 (7), Pp. 1098–1105.

Sonkar, R., Sarnaik, D., Dikshit, S., 2009. Individually stretch cling film wrapped kinnow mandarin under ambient storage. Indian J. Hortic., 66 (1), Pp. 22–27.

Win, N.K.K., Jitareerat, P., Kanlayanarat, S., Sangchote, S., 2007. Effects of cinnamon extract, chitosan coating, hot water treatment and their combinations on crown rot disease and quality of banana fruit. Postharvest Biology and Technology, 45 (3), Pp. 333-340.