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The use of neem oil and chitosan during pre-harvest and in the post-harvest quality of the 'Paluma' guava[1]

Uso de óleo de nim e quitosana na pré-colheita e qualidade pós-colheita de goiabas 'Paluma'

Regivânia Saraiva da Silva[2]*, Laesio Pereira Martins[3], Raunira da Costa Araujo[4], Solange de Sousa[3] and Adriana Ferreira dos Santos[5]

ABSTRACT - Guava is a fruit that is susceptible to attack by pests and diseases both pre- and post-harvest, making it important to employ techniques which maintain its quality, such as the use of neem-based products and chitosan together with cold storage. The aim of the present study was to evaluate the use of neem oil and chitosan during pre-harvest and in the post-harvest quality of the 'Paluma' guava. The experiment was conducted on a property in the district of Mamanguape, Paraíba. Eighteen guava trees were selected, and the following treatments were applied: a control; neem oil 0.5%; neem oil 1%; chitosan 0.5%; chitosan 1%; neem oil 0.5% + chitosan 0.5%; neem oil 0.5% + chitosan 1%; neem oil 1% + chitosan 0.5% and neem oil 1% + chitosan 1%. When at the commercially mature stage, the fruit was harvested, packed in a harvesting crate and transported to the Post-Harvest Physiology Laboratory (CCHSA/UFPB). The fruit was selected, stored at 24 ± 1 °C and evaluated for 10 days, and then stored at 10 ± 1 °C and evaluated for 20 days, followed by physical, chemical and enzymatic analysis. Coating with neem oil (0.5%) + chitosan (1%) during pre-harvest proved to be effective in preserving and prolonging the quality of the 'Paluma' guava during storage for 8 days at 24 ± 1 °C, and 16 days at 10 ± 1 °C.

Key words: Storage. Vegetable oil. Quality.

RESUMO - A goiaba é um fruto susceptível ao ataque de pragas e doenças na pré e na pós-colheita, tornando-se importante a utilização de técnicas para manutenção da sua qualidade, como o uso de produtos à base de nim e a utilização de quitosana, associados ao armazenamento refrigerado. Objetivou-se no presente estudo avaliar o uso de óleo de nim e quitosana na pré-colheita e qualidade pós-colheita de goiaba 'Paluma'. O experimento foi conduzido em propriedade localizada no município de Mamanguape – PB. Selecionaram-se 18 goiabeiras para aplicação dos tratamentos: controle; óleo de nim 0,5%; óleo de nim 1%; quitosana 0,5%; quitosana 1%; óleo de nim 0,5% + quitosana 0,5%; óleo de nim 0,5% + quitosana 1%; óleo de nim 1% + quitosana 0,5% e óleo de nim 1% + quitosana 1%. Os frutos foram colhidos quando apresentavam estádio de maturação comercial, acondicionados em caixa de colheita e transportados para o Laboratório de Fisiologia Pós-Colheita (CCHSA/UFPB). Realizou-se a seleção, seguida de armazenamento sob temperatura de 24 ± 1 °C, avaliado durante 10 dias, e de 10 ± 1 °C, avaliado durante 20 dias, sendo os frutos submetidos às análises físicas, químicas e enzimáticas. O uso dos revestimentos com óleo de nim (0,5%) + quitosana (1%) na pré-colheita demonstrou ser eficaz em prolongar a preservação e qualidade dos frutos de goiaba 'Paluma', durante 8 dias e 16 dias de armazenamento à temperatura de 24 ± 1 °C e 10 ± 1 °C, respectivamente.

Palavras-chave: Armazenamento. Óleo vegetal. Qualidade.

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[2]Programa de Pós-Graduação em Tecnologia Agroalimentar, Departamento de Gestão e Tecnologia Agroindustrial, Centro de Ciências Humanas Sociais e Agrárias, Universidade Federal da Paraíba/UFPB, Bananeira/PB, Brasil, regivania.saraiva@gmail.com (ORCID ID 0000-0002-2935-3964)

[3]Departamento de Gestão e Tecnologia Agroindustrial, Centro de Ciências Humanas Sociais e Agrárias, Universidade Federal da Paraíba/UFPB, Bananeiras-PB, Brasil, laesiopm@gmail.com (ORCID ID 0000-0002-3252-0807), solange_ufpb@yahoo.com.br (ORCID ID 0000-0002-8587-4828)

[4]Departamento de Agricultura, Centro de Ciências Humanas Sociais e Agrárias, Universidade Federal da Paraíba/UFPB, Bananeiras-PB, Brasil, arinuar@hotmail.com (ORCID ID 0000-0002-8917-8360)

5Unidade Acadêmica de Tecnologia de Alimentos, Centro de Ciências e Tecnologia Agroalimentar, Universidade Federal de Campina Grande/UFCG, Pombal-PB, Brasil, adrefesantos@ccta.ufcg.edu.br (ORCID ID 0000-0001-6442-7255)

INTRODUCTION

The guava (*Psidium guajava* L.) is a plant native to South America, occupying a prominent place among Brazilian tropical fruits due to its pleasant aroma, distinctive flavour and high nutritional value. IBGE data (2018) show that guava production in Brazil was 578,608 thousand tons, where the largest producers were the states of Pernambuco and São Paulo, with 200,042 and 195,406 tons respectively, contributing significantly to domestic production. Paraíba is in 16th place, with a production of 2,326 thousand tons. As a fruit, the guava can be consumed *in natura*, as well as used in the processing industry to obtain by-products such as juices, nectars, pulps, ice cream, jellies and jams. It is a source of vitamin C, with a higher content than other citrus fruits; it contains high levels of sugars, vitamin A, and group B vitamins, such as thiamine and niacin, in addition to significant levels of phosphorus, potassium, iron and calcium, and is rich in fibre (NASCIMENTO, 2010).

The guava is a fruit that is susceptible to attack by pests and diseases both pre- and post-harvest, resulting in changes in appearance, flavour, texture and colour, which are reflected in the visual and nutritional quality of the product '*in natura*'. It is therefore important to use appropriate techniques to maintain the quality of the fruit, avoiding water loss, slowing the metabolism, and maintaining the quality of the product during long periods of storage (CANUTO *et al*., 2010).

The products extracted from the seeds of *Azadirachta indica* A. Juss., known as neem, are used in the control of pests and agricultural diseases, where they mainly act to inhibit the development of insects and mites. They contain insecticidal compounds, belonging to the group of limonoids known as meliacins or tetranortriterpenoids, the principal representatives of the class of terpenoids that show insecticidal action (SCHLESENER *et al*., 2013).

Chitosan is a polysaccharide derived from the shell of crustaceans, and can be found naturally in some fungi; it is the product of the deacetylation reaction of chitin. It is a non-toxic, biodegradable, biocompatible polymer, and is produced from renewable natural sources. The applicability and insertion of such polymers is widespread, and as they are considered a fungicide and bactericide, they are used as film for covering fruit. The use of natural polymers based on chitin and chitosan has seen important advances in biotechnology, where their advantages include being easily available, biocompatible and biodegradable (DIAS *et al*., 2013).

A combination of methods to promote better fruit quality has been employed in recent years. The use of an edible coating, together with refrigerated storage, helps to minimise post-harvest loss, extends the period of marketing and maintains fruit quality, enabling a modified atmosphere to form around the fruit (CHITARRA; CHITARRA, 2005). Evaluating quality indices in the 'Pedro Sato' guava, Fonseca *et al*. (2016), found that edible coatings based on starch and alginate were efficient in delaying ripening in guava when stored for four days under refrigeration (10 °C).

As such, the aim of this study was to evaluate the use of neem oil and chitosan during pre-harvest and in the post-harvest quality of the 'Paluma' guava.

MATERIAL AND METHODS

Guava fruit were obtained from a property located in the district of Mamanguape, Paraíba. The guava orchard was approximately five years old. Selecting the orchard considered the time since pruning, the time of the harvest, and the lack of any type of product applied to the orchard, as well as the use of cropping treatments, and plants chosen for their good development.

The district of Mamanguape, Paraíba, is located in the North-Coastal mesoregion, at 6°35'05" S and 35°23'50" W. The local climate is tropical rainy, with dry summers and a rainy season from February to October; the average rainfall is 1,634.2 mm (SOUZA; SOUZA, 2010).

Eighteen guava trees were randomly selected and identified, with each treatment consisting of two plants. The orchard was selected immediately after the plants were pruned, and was free from herbicides to avoid any interference in the end results of the experiment. Throughout the experimental period, cropping treatments were carried out, including supplying water via micro-sprinkler irrigation, the elimination of spontaneous plants and the application of fertiliser.

A backpack sprayer with a full cone-shaped nozzle was used to apply the solutions. Three litres of solution were used per plant (Table 1), comprising the nine treatments, one of which was considered the control (distilled water), and the others containing a mixture of neem oil and chitosan in different concentrations. Tween® 80 surfactant (0.01% v/v) was added to each solution, which was applied until it ran off the fruit. Applications were made in the morning, 150 days after flowering, by which time the fruit was fully developed.

The guava were harvested when the colour of the epidermis indicated commercial maturity, approximately 30 days after application (Stage 2 - fruit with a light g4een colour), packed in harvesting crates and transported to the post-harvest physiology laboratory of the Federal University Paraíba, where the experiment was set up and conducted.

Table 1 - Pre-harvest treatments applied to the 'Paluma' guava stored at room temperature and under refrigeration

Treatment Number	Applied Treatment
1	Control
2	Neem (0.5%)
3	Neem (1%)
4	Chitosan (0.5%)
5	Chitosan (1%)
6	Neem (0.5%) + Chitosan (0.5%)
7	Neem (0.5%) + Chitosan (1%)
8	Neem (1%) + Chitosan (0.5%)
9	Neem (1%) + Chitosan (1%)

The fruit were selected based on uniform size and the absence of physical damage. Fruit for each treatment were then placed in polypropylene trays and stored at room temperature (24 ± 1 °C) for 10 days, and under refrigeration (10 ± 1 °C) in a BOD (biochemical oxygen demand) refrigerated incubator for a further 20 days at a relative humidity of 85 ± 5%.

Preparing the solutions:

- Chitosan solutions - Polymar brand commercial 85% deacetylated chitosan was used to prepare the 0.5% and 1% solutions by adding acetic acid to the solution at a concentration of 4000 ppm (0.4%), homogenising until a viscous liquid was obtained, and then correcting to pH 4.0 with 0.01 M NaOH solution.

- Neem oil solution - Neemax commercial neem oil was mixed in distilled water, adding Tween 80 (0.1% v/v) as surfactant to reduce the surface tension of the water and improve the solution adhering to the fruit.

- Mixing the solutions - the two individually prepared solutions were mixed according to the concentration of each treatment, with Tween 80 (0.1% v/v) added as surfactant.

Evaluations were made when setting up the experiment and during storage, using three replications of nine fruit for each treatment, giving a total of 972 fruit. The fruit stored at 24 ± 1 °C were evaluated at intervals of two days, and those stored at 10 ± 1 °C every four days, when the physical, chemical and enzymatic analysis was carried out.

- Weight loss (%) - Calculated from the difference between the initial weight of the fruit and the weight at the time of each analysis, using a semi-analytical balance (BEL 503, Piracicaba, São Paulo);

- Titratable acidity (% citric acid) - Determined by titration with 0.1 N NaOH solution added to 10 g of pulp diluted in 50 ml of distilled water until a light pink colour was obtained. The results were expressed as a percentage of citric acid, as per the Instituto Adolfo Lutz - IAL (2008);

- Ascorbic acid (mg.100 g^{-1}) - determined by spectrophotometry, according to Pearson, 1976. Approximately 0.6 g of the sample were weighed in a beaker. Ten ml of 0.4% oxalic acid were added and stirred for 5 minutes. The sample was transferred to a 10 ml volumetric flask, and the volume topped up with oxalic acid. The solution was then filtered. The spectrophotometer was zeroed with distilled water at a wavelength of 520 nm. One ml of 0.4% oxalic acid was transferred to a test tube, 9 ml of the DFI dye solution (2,6-dichlorophenolindophenol) were added and the L_1 reading was taken. Ascorbic acid crystals were then added to the test tube to decolourise the solution, and the L_{1A} reading was taken. One ml of the filtrate was transferred to each of two test tubes and 9 ml distilled water were added to one of the test tubes. The device was reset to zero using this solution. Nine mL of DFI were added to the other test tube, and the L_2 reading was taken. Some ascorbic acid crystals were added to this test tube and the L_{2A} reading was taken.

- Total extractable polyphenols (mg.100 g^{-1}) - Carried out as described by Larrauri, Rupérez and Saura-Calixto (1997). Four ml of 50% methanol were added to 1 g of crushed pulp and left to extract for 1 hour. The mixture was then centrifuged at 15,000 rpm for 15 minutes. The supernatant was transferred to a 15 -mL Falcon tube and 4 mL of 70% acetone were added to the sediment and allowed to extract for 1 hour. This suspension was again centrifuged, the supernatant was added to the supernatant from the first centrifugation and the volume (11 ml) was topped up with distilled water. An aliquot of 0.20 ml of the extract was added to a test tube containing 0.80 ml distilled water, 1 ml Folin-Ciocalteu reagent, 2.0 ml 20% sodium carbonate and 2.0 mL distilled water. The test tube was shaken for 5 seconds and allowed to rest for 30 minutes; the reading was taken by spectrophotometer, at a wavelength at 700 nm, with the results expressed in mg.100 g^{-1};

- Total antioxidant activity (g sample/g DPPH) - Antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent method (BRAND-WILLIAMS; CUVELIER; BERSET, 1995). Using the extract obtained for total extractable polyphenols, three different dilutions (100, 50 and 10 μg mL^{-1}) were prepared in triplicate. In a dark environment, a 0.1 ml aliquot of each dilution was transferred to test tubes with 3.9 ml of the DPPH radical (0.06 mM) and homogenised in a test-

tube shaker. Then, 0.1 mL of the control solution (methyl alcohol, acetone and water) were mixed with 3.9 mL of the DPPH radical and homogenised. Methyl alcohol was used as the blank to calibrate the spectrophotometer (RUFINO *et al*., 2007). After 40 minutes incubation (determined following the kinetics) at room temperature and protected from light, reduction of the free DPPH radical was measured by reading the absorbance at 515 nm against the respective blank of each dilution.

- Peroxidase activity (Unit of Enzymatic Activity - UEA/min/g) - extraction was carried out as per the method described by Wissemann and Lee (1980). The activity was measured as recommended by Matsuno and Uritani (1972). The results were expressed in $UEA.g^{-1}$ fresh $matter.min^{-1}$;

Experimental design and statistical analysis

The experimental design was completely randomised (CRD) for the guava kept at 24 and 10 °C. A 9 x 6 factorial scheme was used, with three replications of nine fruit, where the first factor corresponded to the treatments and the second to the storage times. The statistical analysis was carried out separately for each temperature.

The data were subjected to analysis of variance and the mean values were compared by Tukey's test at 5% probability with the aid of the SAS® v9.1 statistical software (2013), licensed to the Centre for Human, Social and Agrarian Sciences at the Federal University of Paraíba.

RESULT AND DISCUSSION

There was a significant difference ($p \leq 0.05$) between storage times for weight loss in the 'Paluma' guava stored at a temperature of 24 ± 1 °C (Table 2). The fruit from the treatment with neem oil (0.5%) + chitosan (1.0%) showed a smaller loss, around 12.81% on the 8 th day of storage, while the fruit from the control treatment showed greater weight loss (15.57%), affecting the quality and external appearance of the fruit, characterised by the presence of spots and rot.

Regardless of the applied treatment, there was a significant loss ($p \leq 0.05$) in fresh weight during storage for the 'Paluma' guava stored at 10 ± 1 °C (Table 2). The application of neem oil (0.5%) + chitosan (1%) showed a weight reduction (10.41%) on the 16 th day of storage compared to the other treatments. As such, the efficiency of neem oil (0.5%) + chitosan (1%) is obvious, providing a barrier that prevented the loss of moisture, and that can be a viable alternative for retaining fresh weight in the 'Paluma' guava. However, according to the results, there was no difference to the other treatments.

Weight loss depends on such factors as variety, the characteristics of the product surface, the stage of development and the surface to volume ratio, all of which result in a loss of turgor. The difference in vapour pressure between the fruit and the environment, influenced by the temperature and relative humidity, should also be considered (CHITARRA; CHITARRA, 2005).

The coating based on neem oil (0.5%) + chitosan (1.0%) resulted in a longer storage time for the 'Paluma' guava, possibly by forming a barrier that minimised the rate of respiration and reduced water loss (which did not differ from the other treatments). However, the different temperatures were also a factor influencing storage, as it was found that when fruit treated with neem oil (0.5%) + chitosan (1%) were exposed to a temperature of 10 ± 1 °C, they showed a smaller loss, equal to 10.41% on the 16 th day, compared to those submitted to the treatment with neem oil (0.5%) + chitosan (1%) and exposed to a temperature of 24 ± 1 °C, which showed losses of 12.81% on the 8 th day of storage.

There was a significant difference ($p \leq 0.05$) in the levels of citric acid in the 'Paluma' guava stored at both 24 ± 1 °C and 10 ± 1 °C, showing an increase during storage for all treatments (Table 3). The fruit treated with neem oil (0.5%) + chitosan (1.0%) and kept at 24 ± 1 °C showed the highest value (0.85%) compared to fruit from the control treatment and the treatment with chitosan (0.5%), which showed the lowest values (0.62%) on the 8th day of storage.

The increase in total acidity may have been due to the concentration of acids for later conversion into sugars, such as the increase in polygalacturonic acid and the production of intermediate acid compounds during the Krebs cycle; on the other hand, in most fruit, the concentration of organic acids tends to decrease at maturation, a result of the respiratory process (CHITARRA; CHITARRA, 2005).

For the levels of titratable acidity in the 'Paluma' guava kept at 10 ± 1 °C, it was found that, during storage, each treatment showed an increase in citric acid content, while guava treated with neem oil (0.5%) + chitosan (1%) and neem (0.5%) presented the highest values (0.96% citric acid) on the 16th day of storage (which did not differ from the other treatments), whereas for the same storage time, the lowest value for acidity (0.75% citric acid) was found in the fruit treated with neem (1%).

The initial values for titratable acidity, both in fruit stored at 10 ± 1 °C and fruit stored at 24 ± 1 °C, were higher than those found by Sitorus, Karo and

Table 2 - The effect of different concentrations of neem oil and chitosan on weight loss (%) in the 'Paluma' guava stored at temperatures of 24 ± 1 °C and 10 ± 1 °C, RH 85 ± 5%

Treatment	Storage Time (24 ± 1 °C)					
	0	2	4	6	8	10
Control	0.00 eA	5.15 dA	8.44 cA	12.18 bA	15.57 aA	18.48 aA
Neem (0.5%)	0.00 dA	4.76 cA	7.45 cA	10.83 bA	14.47 aA	17.57 aA
Neem (1%)	0.00 dA	4.60 cA	7.51 cA	11.19 bA	14.61 aA	17.35 aA
Chitosan (0.5%)	0.00 dA	4.47 cA	7.18 cA	10.47 bA	13.63 abA	16.25 aA
Chitosan (1%)	0.00 eA	4.26 dA	7.89 cA	10.97 bcA	13.83 abA	16.23 aA
N (0.5%) + C (0.5%)	0.00 dA	4.45 cA	7.58 cA	11.31 bA	15.28 aA	16.96 aA
N (0.5%) + C (1%)	0.00 eA	4.23 dA	6.81 cdA	9.89 bcA	12.81 abA	15.25 aA
N (1%) + C (0.5%)	0.00 dA	4.31 cA	6.92 cA	10.33 bA	13.54 aA	16.29 aA
N (1%) + C (1%)	0.00 dA	4.73 cA	7.57 cA	10.96 bA	14.10 abA	16.96 aA
Treatment	Storage Time (10 ± 1 °C)					
	0	4	8	12	16	20
Control	0.00 eA	4.62 dA	7.99 cAB	11.22 bAB	16.26 aA	18.33 aAB
Neem (0.5%)	0.00 eA	4.06 dA	7.98 cAB	11.39 bAB	13.98 abAB	16.53 aABC
Neem (1%)	0.00 eA	4.56 dA	7.77 cAB	10.88 bAB	13.01 abBC	15.32 aBCDE
Chitosan (0.5%)	0.00 eA	2.93 deA	5.88 cdB	8.70 bcB	10.85 abBC	13.01 aDE
Chitosan (1%)	0.00 dA	4.12 cA	6.96 cAB	10.14 bB	12.23 abBC	14.56 aCDE
N (0.5%) + C (0.5%)	0.00 eA	4.54 dA	8.08 cAB	11.09 bAB	13.35 abABC	15.57 aABCD
N (0.5%) + C (1%)	0.00 eA	3.60 dA	6.24 cdB	8.73 bcB	10.41 abC	12.16 aE
N (1%) + C (0.5%)	0.00 eA	3.54 dA	6.18 cdB	8.82 bcB	10.70 abC	12.59 aDE
N (1%) + C (1%)	0.00 eA	5.89 dA	9.95 cA	13.58 bA	13.48 bABC	18.78 aA

*Mean values followed by the same uppercase letters in a column and lowercase letters on a line do not differ statistically by Tukey's test at 5% probability. **N = neem; Q = chitosan

Table 3 - The effect of different concentrations of neem oil and chitosan on titratable acidity (% citric acid) in the 'Paluma' guava stored at temperatures of 24 ± 1 °C and 10 ± 1 °C, RH 85 ± 5%

Treatment	Storage Time (24 ± 1 °C)					
	0	2	0	2	0	2
Control	0.37 dCD	0.54 bAB	0.44 cF	0.59aDE	0.62 aE	0.52 bCD
Neem (0.5%)	0.43 dAB	0.51 cB	0.46 dF	0.71aA	0.73 aC	0.65 bB
Neem (1%)	0.39 eBCD	0.52 cAB	0.60 bA	0.69aAB	0.69 aD	0.46 dE
Chitosan (0.5%)	0.40 eBCD	0.55 dA	0.59 cAB	0.69aAB	0.62 bcE	0.64 bB
Chitosan (1%)	0.41 eABC	0.43 eC	0.50 dE	0.65bBC	0.72 aCD	0.56 cC
N (0.5%) + C (0.5%)	0.40 eBCD	0.45 dC	0.55 cCD	0.57cE	0.81 aB	0.70 bA
N (0.5%) + C (1%)	0.37 eD	0.43 dC	0.51 cDE	0.66bBC	0.85 aA	0.54 cCD
N (1%) + C (0.5%)	0.38 dCD	0.54 cAB	0.56 cBC	0.68bAB	0.73 aC	0.69 bA
N (1%) + C (1%)	0.44 eA	0.50 dB	0.57 cABC	0.62bCD	0.74 aC	0.51 dD

Continued Table 3

Treatment	Storage Time (10 ± 1 °C)					
	0	4	8	12	16	20
Control	0.37 fC	0.49 eD	0.64 dC	0.74 cC	0.86 bC	0.98 aA
Neem (0.5%)	0.43 eAB	0.69 dA	0.76 cAB	0.81 bB	0.96 aA	0.71 dDE
Neem (1%)	0.39 eBC	0.53 dC	0.57 dD	0.68 cD	0.75 bD	0.96 aA
Chitosan (0.5%)	0.40 eBC	0.56 dC	0.63 cC	0.75 bC	0.93 aAB	0.74 bCD
Chitosan (1%)	0.41 fABC	0.61 eB	0.74 dAB	0.88 bA	0.95 aA	0.78 cC
N (0.5%) + C (0.5%)	0.40 fBC	0.61 eB	0.78 dA	0.87 cA	0.92 bAB	0.97 aA
N (0.5%) + C (1%)	0.37 dC	0.66 cA	0.64 cC	0.76 bC	0.96 aA	1.00 aA
N (1%) + C (0.5%)	0.38 eC	0.49 dD	0.56 cD	0.69 bD	0.93 aAB	0.68 bE
N (1%) + C (1%)	0.44 eA	0.55 dC	0.73 cB	0.81 bB	0.91 aB	0.89 aB

*Mean values followed by the same uppercase letters in a column and lowercase letters on a line do not differ statistically by Tukey's test at 5% probability. **N = neem; Q = chitosan

Lubis (2014) of 0.20, 0.18, 0.16 and 0.12 g citric acid/100 g of fresh matter at concentrations of 1, 2, 3 and 4% chitosan respectively. Cerqueira *et al*. (2011), determining the effect of protein and chitosan coatings on conserving the 'Kumagai' guava, saw an increase in titratable acidity as maturation advanced.

The levels of ascorbic acid in the guava increased during each evaluation period in some treatments, showing a significant interaction between storage time and treatment (Table 4). It was found that on the 8th day of storage, fruit stored at 24 ± 1 ºC and treated with neem (0.5%) + chitosan (1%) showed the highest values (43.06 mg.100 g^{-1}) for ascorbic acid compared to the other treatments.

According to Chitarra and Chitarra (2005), ascorbic acid is an antioxidant compound synthesised by fruit and vegetables; its content varies according to species, cultivar, environmental factors and the degree of maturation, and tends to decrease with ripening and senescence.

The fruit treated with neem (0.5%) + chitosan (1%), and stored at 10 ± 1 °C, had the highest levels of ascorbic acid (46.32 mg.100 g^{-1}) on the 16 th day of storage compared to the other fruit (not differing from other treatments). This behaviour is similar to that seen by Hong *et al*. (2012), who found that a coating based on chitosan at a concentration of 1 and 2% resulted in a smaller reduction in the levels of ascorbic acid in guava stored at 11 ºC for 12 days.

Table 5 shows the levels of total extractable polyphenols in the guava. Fruit treated with chitosan (0.5%) and stored at 24 ± 1 °C showed the highest levels of polyphenols (61.63 mg.100 g^{-1}) compared to the other treatments. There was a significant difference between treatments and storage times.

The lowest values were found in fruit treated with neem (0.5%) + chitosan (1%), of 41.06 mg.100 g^{-1}. It was found that combining neem and chitosan at the respective concentrations afforded a reduction in total extractable polyphenols in the guava pulp.

Fruit treated with neem (0.5%) + chitosan (0.5%) and stored at 10 ± 1 °C had the highest levels of polyphenols (60.63 mg.100 g^{-1}) compared to the treatment with neem (0.5%) + chitosan (1%), which had a polyphenol content of 54.37 mg.100 g^{-1}. Melo *et al*. (2008), explained that variations in the level of polyphenols were possibly due to the maturity of the fruit and to the temperature.

The results found by Vieira *et al*. (2011), when evaluating total phenolic content and antioxidant activity *in vitro* using the methods of free-radical capture: DPPH (1,1-diphenyl-2-picrylhydrazyl radical) and ABTS (2,2'azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) radical, found intermediate values for total phenolic compounds of around 104.76 ± 4.39 in aqueous extract, higher values than those found in the present work, which varied from 41 to 73 mg.100 g^{-1} for guava stored at 24 ± 1 °C, and 37 to 73 mg.100 g^{-1} for guava stored at 10 ± 1 °C.

A significant effect was found between treatments and storage times (Table 6). At a temperature of 24 ± 1 °C, the greatest antioxidant activity was found in guava treated with neem (0.5%) + chitosan (1%), and neem (0.5%) + chitosan (0.5%), of 6.81 and 7.22 g sample/g of DPPH at

the start respectively, followed by a reduction at the end of storage.

Guava treated with neem (1%) + chitosan (1%) showed little variation in antioxidant activity during

Table 4 - The effect of different concentrations of neem oil and chitosan on the levels of ascorbic acid (mg.100g^{-1}) in the 'Paluma' guava stored at temperatures of 24 ± 1 °C and 10 ± 1 °C, RH 85 ± 5%

Treatment	Storage Time (24 ± 1 °C)					
	0	2	4	6	8	10
Control	27.10 cCD	27.46 cEF	50.93 aAB	45.21 abAB	42.11 abA	37.67 bcAB
Neem (0.5%)	30.56 aCD	23.64 abF	20.41 abD	20.25 abE	15.99 bC	11.30 bE
Neem (1%)	53.35 aA	52.29 aABC	49.53 aAB	27.72 bDE	16.15 bC	19.89 bCDE
Chitosan (0.5%)	21.46 bcD	33.66 abDEF	39.62 aBC	31.48 abCDE	21.26 bcBC	16.28 cDE
Chitosan (1%)	35.59 aBC	43.65 aBCD	44.40 aABC	40.19 aABCD	37.11 aA	32.45 aABC
N (0.5%) + C (0.5%)	34.36 abBCD	39.80 aCDE	35.48 abC	34.02 abBCD	32.72 abAB	25.32 bBCD
N (0.5%) + C (1%)	44.47 bAB	58.36 aA	53.75 abA	52.49 abA	43.06 bA	42.64 bA
N (1%) + C (0.5%)	32.42 bBCD	51.86 aABC	52.19 aA	50.08 aA	32.16 bAB	24.69 bBCDE
N (1%) + C (1%)	23.16 cCD	54.66 aAB	51.38 aAB	44.26 abABC	42.24 abA	36.33 bAB
Treatment	Storage Time (10 ± 1 °C)					
	0	4	8	12	16	20
Control	27.10 aC	31.51 aC	34.51 aB	37.51 aB	40.10 aAB	34.93 aA
Neem (0.5%)	30.56 bBC	47.84 aAB	43.17 abAB	38.92 abB	37.24 abAB	38.57 abA
Neem (1%)	53.35 aA	43.01 abABC	37.25 bB	32.54 bB	34.34 bAB	37.28 bA
Chitosan (0.5%)	21.46 bC	35.22 abBC	37.30 aB	35.32 abB	32.53 abAB	30.01 abA
Chitosan (1%)	35.59 aBC	45.37 aABC	37.88 aB	35.87 aB	36.26 aAB	34.12 aA
N (0.5%) + C (0.5%)	34.36 aBC	44.67 aABC	42.20 aAB	42.39 aAB	37.84 aAB	32.64 aA
N (0.5%) + C (1%)	44.47 abAB	54.38 abA	54.95 abA	56.00 aA	46.32 abA	41.24 bA
N (1%) + C (0.5%)	32.42 aBC	41.48 aABC	36.00 aB	29.22 aB	28.05 aB	27.16 aA
N (1%) + C (1%)	23.16 cC	56.64 aA	49.66 abAB	44.76 abAB	40.59 bAB	36.59 bcA

*Mean values followed by the same uppercase letters in a column and lowercase letters on a line do not differ statistically by Tukey's test at 5% probability. **N = neem; Q = chitosan

Table 5 - The effect of different concentrations of neem oil and chitosan on total extractable polyphenols (mg.100 g^{-1}) in the 'Paluma' guava stored at 24 ± 1 °C and 10 ± 1 °C, RH 85 ± 5 %

Treatment	Storage Time (24 ± 1 °C)					
	0	2	4	6	8	10
Control	58.71 abAB	62.82 aABC	62.17 aB	56.76 abBCD	53.07 bB	52.86 bAB
Neem (0.5%)	55.50 aABC	52.60 aD	52.57 aC	54.25 aD	53.05 aB	44.58 bCD
Neem (1%)	53.68 bBC	65.50 aA	71.31 aA	65.93 aA	57.73 bAB	45.28 cCD
Chitosan (0.5%)	62.10 bcA	64.02 bcAB	73.38 aA	67.78 abA	61.63 bcA	57.86 cA
Chitosan (1%)	58.38 abAB	58.14 abBCD	60.93 aB	62.07 aAB	53.04 bcB	51.27 cABC
N (0.5%) + C (0.5%)	49.05 bC	54.40 abD	59.84 aB	56.82 aBCD	53.56 abB	53.45 abAB
N (0.5%) + C (1%)	52.21 bBC	56.79 abCD	58.85 aBC	54.72 abCD	41.06 cC	44.13 cD
N (1%) + C (0.5%)	56.46 aAB	57.39 aBCD	58.40 aBC	61.54 aABC	57.29 aAB	47.28 bBCD
N (1%) + C (1%)	48.72 dC	63.25 bcABC	69.92 aA	65.50 abA	57.02 cAB	49.53 dBCD

Continued Table 5

Treatment	Storage Time (10 ± 1 °C)					
	0	4	8	12	16	20
Control	58.71 bAB	60.25 abCD	65.74 aBCD	63.97 abA	57.60 bA	58.32 bA
Neem (0.5%)	55.50 cABCD	64.83 abBCD	71.73 aAB	64.00 bA	58.96 bcA	53.48 cAB
Neem (1%)	53.68 cBCD	68.80 aAB	69.71 aABC	63.45 abA	57.70 bcA	52.89 cAB
Chitosan (0.5%)	62.10 bcA	72.45 aA	73.98 aA	64.79 bA	56.35 cdA	51.76 dAB
Chitosan (1%)	58.38 cAB	65.44 abABC	71.10 aAB	61.99 bcAB	56.60 cA	46.87 dB
N (0.5%) + C (0.5%)	49.05 cCD	64.06 abBCD	70.77 aABC	65.32 abA	60.63 bA	48.40 cB
N (0.5%) + C (1%)	52.21 cBCD	63.62 aBCD	61.23 abDE	54.97 bcB	54.37 bcA	54.23 cAB
N (1%) + C (0.5%)	56.46 bABC	63.13 abBCD	63.33 abCDE	66.97 aA	57.20 bA	37.62 cC
N (1%) + C (1%)	48.72 cD	57.74 abD	55.73 bE	63.37 aA	54.93 bcA	53.84 bcAB

*Mean values followed by the same uppercase letters in a column and lowercase letters on a line do not differ statistically by Tukey's test at 5% probability. **N = neem; Q = chitosan

Table 6 - The effect of different concentrations of neem oil and chitosan on total antioxidant activity (μg mL^{-1}) in the 'Paluma' guava stored at temperatures of 24 ± 1 °C and 10 ± 1 °C, RH 85 ± 5%

Treatment	Storage Time (24 ± 1°C)					
	0	2	4	6	8	10
Control	4.47 abCD	4.54 abC	5.08 aA	3.71 bcAB	2.48 cB	3.20 cDE
Neem (0.5%)	5.28 bCD	7.09 aAB	4.89 bAB	4.66 bA	4.30 bA	5.05 bAB
Neem (1%)	5.76 bBC	7.50 aAB	3.64 dB	3.35 dAB	4.14 cdA	5.15 bcA
Chitosan (0.5%)	5.45 abBC	6.19 aB	4.25 bcAB	3.49 cAB	3.61 cAB	3.72 cBCDE
Chitosan (1%)	3.99 bD	7.94 aA	4.01 bAB	4.05 bAB	3.68 bAB	4.53 bABCD
N (0.5%) + C (0.5%)	7.22 aA	7.33 aAB	4.52 bcAB	4.63 bA	3.31 cdAB	2.89 dE
N (0.5%) + C (1%)	6.81 aAB	6.22 aB	4.15 bAB	4.58 bA	4.59 bA	4.37 bABCD
N (1%) + C (0.5%)	4.87 aCD	4.21 abC	3.88 abAB	4.29 abAB	4.25 abA	3.42 bCDE
N (1%) + C (1%)	4.01 abD	4.46 aC	4.53 aAB	3.17 bB	3.63 abAB	4.56 aABC
Treatment	Storage Time (10 ± 1°C)					
	0	4	8	12	16	20
Control	4.47 abDE	4.37 abcBC	4.23 abcCD	4.67 aA	3.90 cB	3.99 bcC
Neem (0.5%)	5.28 aBC	3.70 cD	4.62 bBCD	4.89 abA	4.41 bAB	4.76 abAB
Neem (1%)	5.76 aB	4.33 bC	4.42 bBCD	4.60 bAB	4.40 bAB	4.46 bABC
Chitosan (0.5%)	5.45 aBC	4.94 abAB	4.04 cD	4.74 bA	4.57 bcA	4.64 bAB
Chitosan (1%)	3.99 bE	4.72 aABC	4.96 aAB	4.02 bBC	4.04 bAB	4.73 aAB
N (0.5%) + C (0.5%)	7.22 aA	4.46 bcBC	4.37 bcBCD	4.81 bA	3.92 cB	4.78 bAB
N (0.5%) + C (1%)	6.81 aA	4.33 bC	4.64 bBC	4.64 bA	4.59 bA	4.30 bBC
N (1%) + C (0.5%)	4.87 abCD	5.13 aA	4.66 abBC	4.89 abA	4.41 bAB	4.93 abA
N (1%) + C (1%)	4.01 cE	4.77 bABC	5.38 aA	3.61 cC	3.94 cB	3.92 cC

*Mean values followed by the same uppercase letters in a column and lowercase letters on a line do not differ statistically by Tukey's test at 5% probability. **N = neem; Q = chitosan

storage, followed by a small increase at the end. The highest value for antioxidant activity (4.59 g sample/g of DPPH, also not differing from the other treatments) was obtained on the 8th day of storage in guava treated with neem (0.5%) + chitosan (1%).

Low total antioxidant activity was seen for storage at 10 ± 1 °C compared to 24 ± 1 °C. Guava treated with neem (0.5%) + chitosan (1%) showed the highest values (4.59 g sample/g of DPPH) on the 16 th day of storage in relation to those in the control treatment (3.90 g sample/g of DPPH). Significant differences were found between treatments and storage times.

Studying the importance of the maturation stage and the use of refrigeration in conserving the 'Kumagai' guava stored under ambient conditions (21 °C and 85% RH) and at 10 °C (85% RH), and evaluated periodically, Morgado *et al*. (2010), found that there was an increase in total antioxidant activity in almost ripe guava stored at 10 °C, which does not corroborate the data from the present study at either temperature.

The peroxidase enzyme showed a significant difference between treatments and storage times. Peroxidase enzyme activity at a temperature of 24 ± 1 °C was higher on the 6 th day of storage in fruit from the control treatment, at around 51.20 UEA/min/g (Table 7).

There was a decrease in enzyme activity from the 6 th day of storage in all treatments; however, the control treatment maintained high activity (50.49 UEA.g^{-1}) compared to the fruit treated with neem (0, 5%) + chitosan (1%) (34.21 UEA.g^{-1}) on the 8 th day of storage.

The guava stored at a temperature of 10 ± 1 °C and submitted to the treatment with neem (0.5%) + chitosan (1%) showed less peroxidase activity (21.21 UEA.g^{-1}) on the 16 th day of storage compared to the fruit submitted to

Table 7 - The effect of different concentrations of neem oil and chitosan on peroxidase activity (UEA.g^{-1}) in the 'Paluma' guava stored at temperatures of 24 ± 1 °C and 10 ± 1 °C, RH 85 ± 5%

Treatment	Storage Time (24 ± 1 °C)					
	0	2	4	6	8	10
Control	30.78 cBCD	41.81 bBC	47.62 aAB	51.20 aA	50.49 aA	31.78 cD
Neem (0.5%)	36.73 dA	42.44 bcB	46.85 aAB	44.70 abBC	39.47 cdBC	35.49 dBCD
Neem (1%)	28.09 dD	48.90 aA	48.68 aA	38.57 bD	35.13 bcCD	32.36 cdD
Chitosan (0.5%)	34.56 cAB	41.79 abBC	43.61 aB	44.29 aBC	42.72 aB	37.53 bcABC
Chitosan (1%)	28.62 cCD	33.78 bDE	43.07 aB	43.61 aC	42.92 aB	41.74 aA
N (0.5%) + C (0.5%)	31.33 bBCD	30.36 bE	30.90 bC	37.25 aD	36.93 aCD	32.95 abCD
N (0.5%) + C (1%)	26.59 bD	32.30 aE	34.90 aC	34.33 aD	34.21 aD	32.16 aD
N (1%) + C (0.5%)	27.04 dD	44.22 bcAB	50.31 aA	48.53 abAB	42.46 cB	39.88 cAB
N (1%) + C (1%)	32.95 dABC	37.46 cCD	48.87 aA	44.97 abBC	41.83 bB	36.50 cdBCD
Treatment	Storage Time (10 ± 1 °C)					
	0	4	8	12	16	20
Control	30.78 bBCDE	40.19 aA	44.10 aAB	31.79 bBCDE	31.61 bA	23.12 cAB
Neem (0.5%)	36.73 bA	41.51 aA	42.31 aB	43.40 aA	27.27 cAB	23.77 cAB
Neem (1%)	28.09 bDE	33.66 aB	34.87 aCD	34.36 aBCD	25.41 bBC	20.53 cB
Chitosan (0.5%)	34.56 aAB	32.52 aBC	36.70 aC	36.31 aB	27.91 bAB	26.89 bA
Chitosan (1%)	28.62 abCDE	31.22 aBC	31.36 aDE	30.10 aDE	25.74 bcBC	22.79 cAB
N (0.5%) + C (0.5%)	31.33 cBCD	39.99 bA	47.77 aA	30.84 cCDE	27.46 cAB	22.35 dAB
N (0.5%) + C (1%)	26.59 cdE	42.78 aA	32.23 bCDE	28.16 bcE	21.21 eC	23.60 deAB
N (1%) + C (0.5%)	27.04 dDE	38.65 bA	44.34 aAB	32.60 cBCDE	25.00 deBC	21.68 eB
N (1%) + C (1%)	32.95 abABC	28.67 bcC	29.01 bcE	34.87 aBC	25.83 cdBC	22.44 dAB

*Mean values followed by the same uppercase letters in a column and lowercase letters on a line do not differ statistically by Tukey's test at 5% probability. **N = neem; Q = chitosan

the control treatment, which had the greatest peroxidase activity (31.61 UEA.g^{-1}).

Evaluating the effects of the chitosan coating on the quality and post-harvest life of the guava (*Psidium guajava* L.) during cold storage, Hong *et al*. (2012), found that peroxidase activity in the guava decreased for 3 days and then increased during the final period of storage, demonstrating that treatment with a chitosan coating induced an increase in peroxidase activity.

The reduction in peroxidase activity for fruit coated with neem (0.5%) + chitosan (1%) and stored under refrigeration was more marked than in fruit stored at room temperature, possibly due to the low storage temperature (10 °C).

CONCLUSIONS

1. The application of neem oil (0.5%) + chitosan (1%) during pre-harvest in the 'Paluma' guava was efficient in preserving fruit quality at temperatures of 24 ± 1 °C and 10 ± 1 °C;
2. The use of a neem (0.5%) + chitosan (1%) coating reduced weight loss, helped to maintain the levels of ascorbic acid and had the lowest levels of polyphenols, greater antioxidant potential and low peroxidase enzyme activity, showing it to be an effective treatment in preserving the 'Paluma' guava stored for 8 and 16 days at temperatures of 24 ± 1 °C and 10 ± 1 °C respectively.

REFERENCES

BRAND-WILLIAMS, W.; CUVELIER, M. E.; BERSET, C. Use of a free radical method to evaluate antioxidant activity. **Lebensmittel-Wissenschaft & Technologie**, v. 28, n. 1, p. 25-30, 1995.

CANUTO, G. A. B. *et al*. Caracterização físico-química de polpas de frutos da Amazônia e sua correlação com a atividade anti-radical livre. **Revista Brasileira de Fruticultura**, v. 32, n. 4, p. 1196-1205, 2010.

CERQUEIRA, T. S. *et al*. Recobrimento de goiabas com filmes proteicos e de quitosana. **Bragantia**, v. 70, n. 1, 2011.

CHITARRA, M. I. F.; CHITARRA, A. B. Pós-colheita de frutas e hortaliças: fisiologia e manuseio. 2. ed. Lavras, MG: UFV, 2005.

DIAS, K. B. *et al*. Chitin and chitosan characteristics, uses and production current perspectives. **Journal of Biotechnology**, v. 4, n. 3, p. 184-191, 2013.

FONSECA, M. J. O. *et al*. Uso de revestimento comestível para extensão da vida útil da goiaba 'Pedro Sato'. **Revista Engenharia na Agricultura**, v. 24, n. 2, p. 101-110, 2016.

HONG, K. *et al*. Effects of chitosan coating on postharvest life and quality of guava (*Psidium guajava* L.) fruit during cold storage. **Scientia Horticulturae**, v. 144, p. 172-178, 2012.

INSTITUTO ADOLFO LUTZ. Métodos físico-químicos para análise de alimentos. 4 ed. São Paulo: Instituto Adolfo Lutz, 2008. v. 1, p. 1020.

IBGE. Produção agrícola anual. 2018. Disponível em: https://sidra.ibge.gov.br/tabela/5457#resultado. Acesso em: 25 jul. 2018.

LARRAURI, J. A.; RUPÉREZ, P.; SAURA-CALIXTO, F. Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. **Journal of Agricultural and Food Chemistry**, v. 45, n. 4, p. 1390-1393, 1997.

MATSUNO, H.; URITANI, I. Physiological behavior of peroxidase isozymes in sweet potato root tissue injured by cutting or with black root. **Plant and Cell Physiology**, v. 13, n. 6, p. 1091-1101, 1972.

MELO, E. A. *et al.* Teor de fenólicos totais e capacidade antioxidante de polpas congeladas de frutas. **Alimentos e Nutrição**, v. 19, n. 1, p. 67-72, 2008.

MORGADO, C. M. A. *et al*. Postharvest conservation of 'Kumagai' guavas in two maturation stages under two storage temperatures. **Revista Brasileira de Fruticultura**, v. 32, n. 4, p. 1001-1008, 2010.

NASCIMENTO, R. J.; ARAÚJO, C. R.; MELO, E. A. Atividade antioxidante de extratos de resíduo agroindustrial de goiaba (*Psidium guajava* L.) Antioxidant from agri-industrial wastes of the guava fruits (*Psidium guajava* L.). **Alimentos e Nutrição Araraquara**, v. 21, n. 2, p. 209-216, 2010.

PEARSON, D. **Técnicas de laboratório para el análisis de alimentos**. Zaragoza, España, 1976.

RUFINO, M. S. M. *et al*. Metodologia cientifica: determinação da atividade antioxidante total em frutas pela captura do radical livre DPPH. Fortaleza: Embrapa Agroindústria Tropical, 2007. 4 p. (Comunicado Técnico, 127).

SCHLESENER, D. C. H. *et al*. Effects of neem on tetranychus urticae Koch (Acari: *Tetranychidae*) and the predators *Phytoseiulus macropilis* (Banks) and *Neoseiulus Californicus* (Mcgregor) (Acari: *phytoseiidae*). **Revista Brasileira de Fruticultura**, v. 35, n. 1, p. 59-66, 2013.

SITORUS, R. F.; KARO, T.; LUBIS, Z. Pengaruh konsentrasi kitosan sebagai edible coating dan lama penyimpanan terhadap mutu buah jambu biji merah. **Jurnal Rekayasa Pangan danPertanian**, v. 2, n. 1, p. 37-46, 2014.

SOUZA, A. S.; SOUZA, J. B. Uso e ocupação do solo no município de Mamanguape-PB, interfaces: histórico, geográfico e ambiental. **Revista de Biologia e Ciências da Terra**, v. 10, n. 2, 2010.

VIEIRA, L. M. *et al*. Fenólicos totais e capacidade antioxidante in vitro de polpas de frutos tropicais. **Revista Brasileira de Fruticultura**, n. 33, p. 888-897, 2011.

WISSEMANN, K. W.; LEE, C. Y. Polyphenoloxidase activity during grape maturation and wine production. **American Journal of Enology and Viticulture**, v. 31, n. 3, p. 206-211, 1980.