

Original Research Article

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## Mixing Xanthan Gum and Chitosan Nano Particles to Form New Coating for Maintaining Storage Life and Quality of Elmamoura Guava Fruits

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### ABSTRACT

Guava is an important fruit crops in tropical and subtropical regions. Edible coating used as alternative to waxes to enhance fruit quality and prolong storage and shelf life especially in soft fruits. Coating may cause off flavor results from anaerobic respiration. This investigation aims at prolong storage life and quality of guavas by enhance coating material properties through mixing xanthan with chitosan nanoparticles. This investigation carried out during 2015 and 2016 seasons on guava fruits (*Psidium guajava* L.) cv. Elmamoura, grown in a private orchard at El-Tahrer north district, El-Bihira Governorate, Egypt. Guava fruits coated with 1% xanthan gum, its mixture with 0.2 or 0.4% chitosan nano particles (CHNs) and control, to evaluate its effect on storage, shelf life and quality attributes for six weeks at  $8\pm 1^{\circ}\text{C}$  and RH~85-90%. Xanthan gum and its mix with 0.2% CHNs achieved the lowest decay percent, maintained fruit firmness and vitamin C. While, the high concentration of CHNs (0.4%) increased decay percent, loss of firmness and vitamin C. The mix of xanthan with the two concentrations of CHNs maintained good taste of fruits than xanthan gum. After shelf life, mixing xanthan with 0.2% CHNs showed a reduction in color change. CHNs coating treatments reduced total sugars than control and xanthan gum. All coating treatments reduced weight loss compared with control. The mix of xanthan gum with 0.2% CHNs coating enhanced overall quality of guava fruits during extended cold storage and shelf life periods. It is recommending mixing xanthan gum with 0.2% CHNs as a coating to guava fruit cv. Elmamoura to decrease decay, color change, maintain fruit firmness, good taste, vitamin C and reduce total sugars.

### Keywords

Guava, Xanthan gum, Chitosan nano particles, Coating, Weight loss, Fruit decay, Quality attributes, Storage life, Shelf life, Post-harvest.

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### Introduction

Guava (*Psidium guajava* L.) is an important fruit crop grown under a wide range of the tropical and subtropical regions in the world (Chopda and Barrett, 2001). Guava fruit is very perishable during storage at room temperature due to rapid ripening and deterioration, which decreases the potential for commercialization of the fruits (Samaan *et al.*, 2012). Cold storage is not enough to

preserve the quality. So, coating with thin layer film of natural materials extended fruit life under cold storage.

In many fruits, it used as alternative to natural waxes that removed during washing (Baldwin, 1994; Hagenmaier and Baker, 1994; Hagenmaier, 1998). Various compounds have been used as edible

coatings; most of them are based on proteins, lipids or polysaccharides (Bai *et al.*, 2003). Edible coatings are natural products and not chemically synthesized (Falcao-Rodrigues *et al.*, 2007). Chitosan is a natural polysaccharide with a polycationic nature which has numerous applications in agriculture (Bautista-Baños *et al.*, 2006), as an edible coating maintains fruits quality during the postharvest (Olivas and Barbosa-Ca´novas, 2005).

Chitosan forms a semi-permeable film that modifies the internal atmosphere, decreasing transpiration losses and preserves the fruits quality (El Ghaouth, Arul, and Ponnampalam, 1991; Olivas and Barbosa-Ca´novas, 2005).

Xanthan gum is a polysaccharide, it widely uses as a food additive and thickening agent. It produced by the fermentation of simple sugars; glucose, sucrose, or lactos (García-Ochoa *et al.*, 2000).

Off flavors, that reduces the fruit quality during storage results from anaerobic respiration. It may be one of the common problems of coating from ethanol and acetaldehyde (Chen and Nussinovitch, 2000).

Using different formulation of coating materials increased wax permeability and forms a homogenous coating especially when mixed with nanoparticles materials that improves the permeability and distribution on fruit surface (Zambrano-Zaragoza *et al.*, 2013).

The aim of this investigation is prolonging the storage life of guava fruits with high marketable quality suitable for exporting to Europe and other countries.

This may be achieved by enhancing coating material properties through mixing xanthan with chitosan nanoparticles and examine the

effects on guava fruits during cold storage and shelf life periods.

## **Materials and Methods**

### **Plant material**

This study was carried out during the 2015 and 2016 seasons on Elmamoura guava fruits (*Psidium guajava* L.). The fruits were harvested from a private orchard (the trees were 10-year-old, grown in sandy soil at 5×5 meters apart and received the standard horticultural practices adopted in this area) at El-Tahrer north district, El-Bihira Governorate, Egypt.

Fruits at mature-green or breaker stage were picked on the same date in mid-September in both seasons, using small clippers, packed in carton boxes, then transported directly to Post-Harvest Laboratory in Horticulture Department, Faculty of Agriculture, Zagazig University, Egypt. Chosen fruits sorted to be healthy, free of physiological and pathological disorders and uniform in color and size. Finally, it was washed using tap water then air-dried before treatment.

The experimental design was factorial (4 treatments × 5 weeks) in a complete randomized design, in three replicates, each replicate contained 30 fruits.

The fruits were coated with 1% xanthan gum, mixing 1% xanthan gum with 0.2% chitosan nano particles (CHNs), mixing 1% xanthan gum with 0.4% CHNs and control fruits (without coating). 1% of tween 80 added to xanthan gum and CHNs as a surfactant. All fruits packed in perforated (0.06% of area) 20-micron thickness low-density polyethylene (LDPE) bags after coating treatments.

The fruits were stored for 2, 3, 4, 5 and 6 weeks at 8±1°C (RH~85-90%) in a cooler,

thereafter held at 20±1°C (RH~65-70%) for 6 days after cold storage to evaluate fruit marketability (shelf life).

### **CHNs preparation and measurement**

Chitosan nano crystallite powder was synthesized by high-energy ball milling. Powder mixture was conducted in a planetary ball mill to 40 h using ball to powder mass ratio of (8:1). The chitosan samples were examined by X-ray diffraction using a Philips model (PW-1729) diffract meter equipped with Cu K $\alpha$  radiation source ( $\lambda = 1.541178$  Å). Infrared spectra (FTIR) for the chitosan samples were carried out at room temperature by using a PERKIN-ELMER-1430 recording infrared spectra in the range 200 to 4000 cm<sup>-1</sup> (at Tanta University, Central lab).

The microstructure of the sintered samples examined using High Resolution Transmission Electron Microscope (HRTEM) model JOEL EM 2-100) according to Gad *et al.*, 2016.

Evaluation of cold storage period treatments and shelf life effects on guava fruits were carried out through the following quality attributes:

### **Fresh weight losses (FWL) percentage**

The fruits were weighed before cold storage to obtain the initial weight, and then weighed after each period of cold storage and shelf life. FWL were calculated as a percentage of the initial weight according to the following

equation: 
$$FWL \% = \frac{W_i - W_s}{W_i} \times 100$$

Where,

W<sub>i</sub> = fruit weight at initial period, W<sub>s</sub> = fruit weight at sampling period (Hazali *et al.*, 2013; Ibrahim and Gad, 2015).

### **Fruit decay percentage (FD %)**

It was determined as percentage of decayed fruits by using the following equation according to Ismail *et al.*, (2010)

$$Fruit\ decay\ \% = \frac{\text{number of decayed fruits}}{\text{initial number of stored fruits}} \times 100$$

### **Fruit pulp firmness (FPF)**

It was determined on five fruits per replicate on two opposite sides of the equatorial region of the fruit after peel removal by using a Push Pull dynamometer (Model FD 101).

The values were expressed as (g/cm<sup>2</sup>) (Ibrahim and Gad, 2015).

### **Peel color index (PCI)**

The color of each fruit was determined according to the following index: 0 = 100% green; 1 = 1-20% yellow; 2 = 21-40% yellow; 5 = 41-60% yellow; 8 = 61-80% yellow; 10 = 81-100% yellow (El-Hefnawi *et al.*, 2008).

### **Panel test index (PTI)**

Each replicate was judged by 5 persons who gave the score according to the following index: 4 = Excellent test; 3 = Very good test; 2 = Good test; 1 = Acceptable test; 0 = Bad test (El-Hefnawi *et al.*, 2008).

### **Juice total soluble solids content (TSS %)**

It was determined using a hand refractometer as Brix° (Hazali *et al.*, 2013 and Gad *et al.*, 2016).

### **Ascorbic acid (vitamin C) content**

It was determined by titration in presence of 2, 6 dichlorophenol-indophenol dye as

indicator against 2% oxalic acid solution as substrate.

Ascorbic acid was calculated as milligrams per 100 ml of juice (Kabasakalis *et al.*, 2000).

### **Juice titratable acidity**

It was determined by titrating an aliquot of juice against 0.1N NaOH in presence of phenolphthalein dye as indicator, the results were calculated as grams of citric acid per 100 ml fruit juice (AOAC, 1995).

### **Total sugars %**

It was determined by using the method of Smith *et al.*, (1956) and calculated as g glucose per 100 g fresh weight.

All chemicals used in this work obtained from El-Gomhouria for Trading Chemicals and Medical Appliances, El-Sawah, El-Amiria, Cairo, Egypt.

### **Statistical analysis**

Statistical analysis was conducted for the collected data using the Statistic 9 (2008) according to (Snedecor and Cochran, 1989). Data subjected to the ANOVA and a factorial in a complete randomized design, was used (Steel and Torrie, 1980). Means were tested using LSD test ( $P < 0.05$ ) to investigate the significant differences between coating treatments, weeks of storage and their interaction.

### **Results and Discussion**

In both seasons, fruit decay (%) was significantly decreased by coating fruit surface with mixing xanthan and CHNs compared to control (Tables 1 and 2). The same trend was obtained in fruit held for six days at 20°C after cold storage. No decay observed till the fourth week of storage, then

appeared at the fifth week and reached maximum after six weeks of storage. Similar results were obtained in fruit held for six days at 20°C. Control fruits after six weeks of cold storage gave the highest percentage of decayed fruits as well as after six days at 20°C.

In the first season, fresh weight loss (FWL) was significantly decreased with increasing the concentrations of CHNs. While no significant differences between all treatments were detected in the second season and after six days at 20°C in both seasons. Almost no FWL through the first three weeks of cold storage was observed, and then increased to get the highest values at the end of storage period. Fruits held for six days at 20°C, was not clear any trend of FWL when the time advanced. Fruits of guava coated with 1% xanthan gave the highest in FWL at the end of cold storage in the first season. The interaction between treatments and periods was insignificantly effects on FWL in the second season and fruit held for six days at 20°C in both seasons.

The fruits coated with 1% xanthan and 1% xanthan + 0.2% CHNs maintained the highest fruit pulp firmness (FPF) in the first and second seasons. As well as, fruits held for six days at 20°C gave the same trend. The FPF decreased with the advance in cold storage period in both seasons after refrigeration and after six days at 20°C.

Control fruit samples had the lowest (FPF 547 g/cm<sup>2</sup>) at the end of sixth week of cold storage, while 1% xanthan after two weeks was the highest FPF (897 g/cm<sup>2</sup>) in the two seasons. While, the interaction between treatments and storage periods had insignificant effect on FPF either in cold storage or shelf life at 20°C for six days. Tables 3 and 4 present the variation between xanthan only and other treatments in panel test index (PTI). The lowest PTI was

observed in xanthan only compared with control and mixing with CHNs with different concentrations in the first and second seasons (2.9 and 3.0, respectively). As well as, xanthan or xanthan + 0.4% CHNs achieved the lowest PTI in fruit held for six days at 20°C, where no significant differences between them in both seasons. The PTI enhanced with the advance of cold storage period or in fruit held for six days at 20°C. Control fruits showed the highest in PTI (3.9) after six weeks of cold storage, while coating fruits with 1% xanthan showed the lowest value (2.3) after two weeks of cold storage in the first season only. The interaction between treatments and periods was insignificant in FWL after refrigerating in the second season and after six days at 20°C in both seasons.

Mixing CHNs with xanthan achieved the highest peel color index (PCI) (6.9 and 6.6, respectively) after refrigeration in the second season only. No significant differences were obtained in PCI between throw all treatments after refrigeration in the first season, as well as, between them after six days at 20°C in both seasons. PCI were increased with advancing cold storage period or in fruits held for six days at 20°C in both seasons. No significant differences in PCI observed in the interaction throw all treatments and cold storage periods and after six days at 20°C in the two seasons.

In the second season, xanthan gum at 1% + 0.2% CHNs gave the significant highest TSS compared with control. While, no significant differences were observed between all treatments in the first season and after six days at 20°C. The TSS gradually increased with advancing cold storage period or after six days at 20°C. No significant differences obtained between all treatments and cold storage periods and after six days at 20°C in both seasons. It is clear from Table 5 and 6 that, no significant differences were observed

in vitamin C between all treatments in both seasons. After six days at 20°C, the treatment 1% xanthan + 0.2% CHNs maintained the highest vitamin C compared to other treatments. While the lowest vitamin C came from higher concentration of CHNs 0.4%.

Vitamin C was decreased gradually with the advance of cold storage periods and after six days at 20°C in the two seasons.

In total acidity, no significant differences was observed between all treatments, with the advance in storage period and the interaction between them after refrigeration and after six days at 20°C in both seasons.

Control treatment had the highest value of total sugars (12.43 and 12.55 %), while 1% xanthan + 0.4% CHNs was the lowest (10.78 and 10.87%) respectively in the first and second seasons, respectively.

On the contrary, after six days at 20°C, 1% xanthan + 0.4% CHNs gave the highest total sugars (10.39 and 10.50%), while 1% xanthan was the lowest values (9.80 and 9.90%), in the first and second seasons, respectively.

Total sugars increased with the progress of storage period from the second to fifth weeks, and then decreased in the last week and after six days at 20°C in both seasons.

The particle size affects the distribution of coating material, and then effects on the different fruit characteristics during storage. The coating distribution was irregular with the large sizes of particle. On the contrary, the distribution of coating in nano form was more regular and stable (Shi *et al.*, 2011; Zambrano-Zaragoza *et al.*, 2013). Mixing xanthan gum and CHNs decreasing the particle size and increasing coalescence of the homogenization suspension (Noriega-Peláez *et al.*, 2011; Zambrano-Zaragoza *et al.*, 2013).



**Table.1** Effect of mixing xanthan gum and chitosan nano particles on fruit decay percentage, fresh weight losses and fruit pulp firmness of Elmamoura guava fruits during cold storage periods and their interaction in 2015 and 2016 seasons

Storage period (Weeks)	Fruit decay (%)						Fresh weight losses (%)						Fruit pulp firmness (g/cm <sup>2</sup> )							
	0*	2	3	4	5	6	Treat. avg.	0*	2	3	4	5	6	Treat. avg.	0*	2	3	4	5	6
<b>Treatments</b>	<b>1<sup>st</sup> season</b>																			
Control	0.0	0.0	0.0	24.0	33.3	<b>11.5</b>	0.00	0.00	0.00	0.27	0.37	<b>0.13</b>	773	686	650	582	547	<b>648</b>		
Xanthan at 1%	0.0	0.0	0.0	16.0	19.0	<b>7.0</b>	0.00	0.03	0.00	0.23	0.40	<b>0.13</b>	897	763	666	570	555	<b>690</b>		
Xanthan at 1%+ 0.2% CHNs	0.0	0.0	0.0	16.0	22.0	<b>7.6</b>	0.0	0.00	0.00	0.00	0.00	<b>0.33</b>	910	815	725	710	680	<b>611</b>		
Xanthan at 1%+ 0.4% CHNs	0.0	0.0	0.0	20.0	24.3	<b>8.9</b>	0.00	0.00	0.00	0.00	0.27	<b>0.05</b>	660	64	62	610	569	<b>621</b>		
Period avg.	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>19.0</b>	<b>24.7</b>		<b>0.00</b>	<b>0.01</b>	<b>0.00</b>	<b>0.13</b>	<b>0.34</b>		<b>786</b>	<b>704</b>	<b>662</b>	<b>610</b>	<b>570</b>			
LSD at 5%	Treat.=0.8, period=0.3 and interaction=1.8						Treat.=0.04, period=0.045 and interaction=0.09						Treat.=38, period=43 and interaction=86							
<b>2<sup>nd</sup> season</b>																				
Control	0.0	0.0	0.0	20.0	35.0	<b>11.0</b>	0.0	0.0	0.2	0.3	0.2	<b>0.13</b>	615	556	545	459	445	<b>524</b>		
Xanthan at 1%	0.0	0.0	0.0	16.3	30.0	<b>9.3</b>	0.0	0.0	0.1	0.2	0.2	<b>0.12</b>	699	685	659	599	549	<b>638</b>		
Xanthan at 1%+ 0.2% CHNs	0.0	0.0	0.0	18.0	25.3	<b>8.7</b>	0.0	0.0	0.0	0.1	0.2	<b>0.1</b>	745	705	658	617	636	<b>605</b>		
Xanthan at 1%+ 0.4% CHNs	0.0	0.0	0.0	23.3	32.0	<b>11.1</b>	0.0	0.0	0.1	0.2	0.3	<b>0.12</b>	635	605	558	506	501	<b>561</b>		
Period avg.	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>19.4</b>	<b>30.6</b>		<b>0.00</b>	<b>0.00</b>	<b>0.11</b>	<b>0.23</b>	<b>0.22</b>		<b>664</b>	<b>626</b>	<b>595</b>	<b>550</b>	<b>524</b>			
LSD at 5%	Treat.=1.2, period=1.4 and interaction=2.7						Treat.=NS, period=0.07 and interaction=NS						Treat.=40, period=45 and interaction=NS							

CHNs= chitosan nano particles, treat. = treatments, avg.= average, NS= non-significant, \*=zero time

**Table.2** Effect of mixing xanthan gum and chitosan nano particles on fruit decay percentage, fresh weight losses and fruit pulp firmness of Elmamoura guava fruits during shelf life\* periods and their interaction in 2015 and 2016 seasons

Storage period (Weeks)	Fruit decay (%)						Fresh weight losses (%)						Fruit pulp firmness (g/cm <sup>2</sup> )							
	0*	2	3	4	5	6	Treat. avg.	0*	2	3	4	5	6	Treat. avg.	0*	2	3	4	5	6
<b>Treatments</b>	<b>1<sup>st</sup> season</b>																			
Control	0.0	0.0	0.0	26.3	35.3	<b>12.3</b>	0.1	0.1	0.1	0.1	0.3	<b>0.16</b>	610	560	540	534	512	<b>551</b>		
Xanthan at 1%	0.0	0.0	0.0	18.7	22.0	<b>8.1</b>	0.2	0.2	0.2	0.1	0.4	<b>0.21</b>	701	643	595	545	503	<b>598</b>		
Xanthan at 1%+ 0.2% CHNs	0.0	0.0	0.0	18.7	25.3	<b>8.8</b>	0.0	0.1	0.3	0.2	0.1	<b>0.2</b>	795	704	598	576	537	<b>494</b>		
Xanthan at 1%+ 0.4% CHNs	0.0	0.0	0.0	22.7	29.7	<b>10.5</b>	0.1	0.1	0.2	0.1	0.2	<b>0.15</b>	636	625	609	566	505	<b>588</b>		
Period avg.	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>21.6</b>	<b>28.1</b>		<b>0.13</b>	<b>0.18</b>	<b>0.16</b>	<b>0.13</b>	<b>0.27</b>		<b>663</b>	<b>606</b>	<b>580</b>	<b>546</b>	<b>504</b>			
LSD at 5%	Treat.=0.86, period=0.96 and interaction= 1.93						Treat.=NS, period=0.05 and interaction=NS						Treat.=28, period=31 and interaction=NS							
<b>2<sup>nd</sup> season</b>																				
Control	0.0	0.0	0.0	22.3	39.0	<b>12.3</b>	0.2	0.1	0.1	0.2	0.2	<b>0.17</b>	555	531	548	448	428	<b>502</b>		
Xanthan at 1%	0.0	0.0	0.0	20.0	32.3	<b>10.5</b>	0.3	0.3	0.2	0.1	0.1	<b>0.21</b>	660	629	583	524	492	<b>578</b>		
Xanthan at 1%+ 0.2% CHNs	0.0	0.0	0.0	20.7	27.7	<b>9.7</b>	0.0	0.3	0.3	0.2	0.1	<b>0.21</b>	720	696	647	573	573	<b>494</b>		
Xanthan at 1%+ 0.4% CHNs	0.0	0.0	0.0	27.0	34.7	<b>12.3</b>	0.3	0.3	0.2	0.2	0.2	<b>0.24</b>	600	564	563	522	452	<b>540</b>		
Period avg.	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>22.5</b>	<b>33.4</b>		<b>0.27</b>	<b>0.27</b>	<b>0.16</b>	<b>0.16</b>	<b>0.18</b>		<b>628</b>	<b>593</b>	<b>567</b>	<b>517</b>	<b>466</b>			
LSD at 5%	Treat.=1.4, period=1.5 and interaction=3.1						Treat.=NS, period=0.08 and interaction=NS						Treat.=35, period=39 and interaction=NS							

CHNs= chitosan nano particles, treat. = treatments, avg.= average, NS= non-significant, \*=zero time

\*six days at 20°C and 60-70% RH after cold storage.

**Table.3** Effect of mixing xanthan gum and chitosan nano particles on Panel test index, peel color index and Total soluble solids (TSS) of Elmamoura guava fruits during cold storage periods and their interaction in 2015 and 2016 seasons

Storage period (Weeks)	Panel test index						Peel color index						TSS %								
	0*	2	3	4	5	6	Treat. avg.	0*	2	3	4	5	6	Treat. avg.	0*	2	3	4	5	6	Treat. avg.
<b>Treatments</b>	<b>1<sup>st</sup> season</b>																				
Control																					
Xanthan at 1%																					
Xanthan at 1%+ 0.2% CHNs	2.3	2.4	2.7	3.1	3.5	3.7	3.1	4.0	5.3	6.0	6.0	6.0	9.4	6.5	10.0	12.0	12.3	12.0	12.2	12.5	12.2
Xanthan at 1%+ 0.4% CHNs																					
Period avg.	2.4	2.6	3.0	3.3	3.7	3.0	4.6	5.7	6.2	6.8	9.6	6.4	12.1	12.3	12.7	13.0	13.2	13.2	13.2	12.9	
LSD at 5%	Treat.=0.1, period=0.12 and interaction=0.23						Treat.=NS, period=0.5 and interaction=NS						Treat.=NS, period=0.6 and interaction=NS								
<b>2<sup>nd</sup> season</b>																					
Control																					
Xanthan at 1%																					
Xanthan at 1%+ 0.2% CHNs	2.2	2.5	2.9	3.2	3.6	3.9	3.2	4.0	5.8	6.2	6.3	6.4	9.6	6.9	9.0	11.5	12.0	13.5	12.5	14.0	12.7
Xanthan at 1%+ 0.4% CHNs																					
Period avg.	2.5	2.7	3.1	3.4	3.8	3.2	4.8	5.8	6.1	6.5	9.4	6.6	10.0	12.3	12.8	13.0	13.5	13.5	12.6	12.2	
LSD at 5%	Treat.=0.16, period=0.18 and interaction=NS						Treat.=0.3, period=0.4 and interaction=NS						Treat.=0.69, period=0.77 and interaction=NS								

CHNs= chitosan nano particles, treat. = treatments, avg.= average, NS= non-significant, \*=zero time

**Table.4** Effect of mixing xanthan gum and chitosan nano particles on Panel test index, peel color index and Total soluble solids (TSS) of Elmamoura guava fruits during shelf life\* periods and their interaction in 2015 and 2016 seasons

Storage period (Weeks)	Panel test index						Peel color index						TSS %								
	0*	2	3	4	5	6	Treat. avg.	0*	2	3	4	5	6	Treat. avg.	0*	2	3	4	5	6	Treat. avg.
<b>Treatments</b>	<b>1<sup>st</sup> season</b>																				
Control																					
Xanthan at 1%																					
Xanthan at 1%+ 0.2% CHNs	2.5	3.6	3.2	3.2	3.5	3.9	3.5	4.5	5.1	5.7	6.5	7.2	9.3	6.8	10.5	12.6	12.7	13.0	13.0	12.9	12.9
Xanthan at 1%+ 0.4% CHNs																					
Period avg.	3.1	3.1	3.3	3.4	3.8	3.2	5.4	6.2	6.8	7.1	9.4	7.2	12.4	13.0	12.7	13.0	12.9	12.8	12.7	12.7	
LSD at 5%	Treat.=0.16, period=0.18 and interaction=NS						Treat.=NS, period=0.4 and interaction=NS						Treat.=NS, period=NS and interaction=NS								
<b>2<sup>nd</sup> season</b>																					
Control																					
Xanthan at 1%																					
Xanthan at 1%+ 0.2% CHNs	2.4	3.7	3.4	3.5	3.7	4.0	3.7	4.2	4.2	4.6	5.2	6.5	8.7	5.83	10.0	12.0	12.0	12.0	12.6	13.0a	12.3
Xanthan at 1%+ 0.4% CHNs																					
Period avg.	3.24	3.20	3.39	3.60	3.91	3.3	5.07	5.88	6.36	7.19	8.89	7.11	11.6	12.6	12.4	12.6	12.9	12.4	12.4	12.4	
LSD at 5%	Treat.=0.15, period=0.17 and interaction=NS						Treat.=0.49, period=0.55 and interaction=NS						Treat.=NS, period=0.79 and interaction=NS								

CHNs= chitosan nano particles, treat. = treatments, avg.= average, NS= non-significant, \*=zero time

\*six days at 20°C and 60-70% RH after cold storage.

**Table.5** Effect of mixing xanthan gum and chitosan nano particles on vitamin C, total acidity and Total sugars of Elmamoura guava fruits during cold storage periods and their interaction in 2015 and 2016 seasons

Storage period (Weeks)	Vit. C (mg/ 100 cm <sup>3</sup> juice)						Total acidity %						Total sugars %								
	0*	2	3	4	5	6	Treat. avg.	0*	2	3	4	5	6	Treat. avg.	0*	2	3	4	5	6	Treat. avg.
<b>Treatments</b>	<b>1<sup>st</sup> season</b>																				
<b>Control</b>		109.6	106.0	101.0	94.0	92.0	<b>100.5</b>	1.280	1.253	1.216	1.216	1.152	<b>1.22</b>	12.06	12.26	12.69	12.78	12.37	<b>12.43</b>		
<b>Xanthan at 1%</b>		116.0	104.3	103.0	98.0	94.0	<b>103.1</b>	1.413	1.349	1.276	1.216	1.152	<b>1.28</b>	12.16	12.52	12.71	12.89	11.37	<b>12.33</b>		
<b>Xanthan at 1%+ 0.2% CHNs</b>	142.2	134.0	105.3	100.0	102.0	95.3	<b>107.3</b>	1.50	1.408	1.349	1.280	1.226	1.157	<b>1.28</b>	10.0	10.81	10.89	11.29	11.49	10.99	<b>11.10</b>
<b>Xanthan at 1%+ 0.4% CHNs</b>		124.0	114.0	102.0	91.3	88.3	<b>103.9</b>	1.349	1.275	1.280	1.221	1.157	<b>1.25</b>	10.38	10.59	10.97	11.34	10.59	<b>10.78</b>		
<b>Period avg.</b>		<b>120.9</b>	<b>107.4</b>	<b>101.5</b>	<b>96.3</b>	<b>92.4</b>		<b>1.362</b>	<b>1.306</b>	<b>1.263</b>	<b>1.220</b>	<b>1.155</b>		<b>11.35</b>	<b>11.57</b>	<b>11.92</b>	<b>12.13</b>	<b>11.33</b>			
<b>LSD at 5%</b>		Treat.=NS, period=5.5 and interaction=NS						Treat.=NS, period=NS and interaction=NS						Treat.=0.02, period=0.03 and interaction=0.05							
	<b>2<sup>nd</sup> season</b>																				
<b>Control</b>		106.0	98.0	96.0	88.0	86.0	<b>94.8</b>	1.23	1.22	1.18	1.26	1.22	<b>1.22</b>	12.15	12.33	12.81	12.82	12.64	<b>12.55</b>		
<b>Xanthan at 1%</b>		108.0	101.2	100.0	98.0	95.0	<b>100.4</b>	1.44	1.38	1.31	1.24	1.22	<b>1.32</b>	11.23	12.61	12.78	13.04	12.67	<b>12.47</b>		
<b>Xanthan at 1%+ 0.2% CHNs</b>	117.8	104.0	101.0	98.0	94.0	90.3	<b>97.5</b>	1.48	1.38	1.36	1.24	1.15	1.15	<b>1.26</b>	10.35	10.89	10.91	11.39	11.56	11.34	<b>11.22</b>
<b>Xanthan at 1%+ 0.4% CHNs</b>		108.0	106.0	98.0	94.3	88.7	<b>99.0</b>	1.31	1.27	1.21	1.18	1.12	<b>1.22</b>	10.50	11.67	10.76	11.44	11.00	<b>10.87</b>		
<b>Period avg.</b>		<b>106.5</b>	<b>101.6</b>	<b>98.0</b>	<b>93.6</b>	<b>90.0</b>		<b>1.34</b>	<b>1.31</b>	<b>1.24</b>	<b>1.21</b>	<b>1.18</b>		<b>11.19</b>	<b>11.63</b>	<b>11.94</b>	<b>12.22</b>	<b>11.91</b>			
<b>LSD at 5%</b>		Treat.=NS, period=5.2 and interaction=NS						Treat.=NS, period=NS and interaction=NS						Treat.=0.19, period=0.21 and interaction=0.42							

CHNs= chitosan nano particles, treat. = treatments, avg.= average, NS= non-significant, \*=zero time

**Table.6** Effect of mixing xanthan gum and chitosan nano particles on vitamin C, total acidity and Total sugars of Elmamoura guava fruits during shelf life\* periods and their interaction in 2015 and 2016 seasons

Storage period (Weeks)	Vit. C (mg/ 100 cm <sup>3</sup> juice)						Titratable acidity %						Total sugars %								
	0*	2	3	4	5	6	Treat. avg.	0*	2	3	4	5	6	Treat. avg.	0*	2	3	4	5	6	Treat. avg.
<b>Treatments</b>	<b>1<sup>st</sup> season</b>																				
<b>Control</b>		102.0	94.0	96.0	91.0	87.7	<b>94.1</b>	1.18	1.20	1.20	1.13	1.13	<b>1.173</b>	9.75	9.94	10.09	10.09	9.88	<b>9.95</b>		
<b>Xanthan at 1%</b>		116.0	94.0	100.0	96.0	88.0	<b>98.8</b>	1.34	1.34	1.24	1.14	1.01	<b>1.219</b>	9.55	9.89	9.95	9.98	9.64	<b>9.80</b>		
<b>Xanthan at 1%+ 0.2% CHNs</b>	133.4	123.3	102.0	94.0	94.0	94.0	<b>101.5</b>	1.42	1.34	1.22	1.26	1.20	1.26	<b>1.261</b>	9.50	10.04	10.49	10.34	10.50	15.12	<b>10.30</b>
<b>Xanthan at 1%+ 0.4% CHNs</b>		98.0	86.0	88.0	92.0	84.3	<b>89.7</b>	1.22	1.26	1.15	1.15	1.12	<b>1.183</b>	10.20	10.39	10.44	10.65	10.28	<b>10.39</b>		
<b>Period avg.</b>		<b>109.8</b>	<b>94.0</b>	<b>94.5</b>	<b>93.3</b>	<b>88.5</b>		<b>1.27</b>	<b>1.260</b>	<b>1.220</b>	<b>1.158</b>	<b>1.133</b>		<b>9.89</b>	<b>10.18</b>	<b>10.21</b>	<b>10.31</b>	<b>9.98</b>			
<b>LSD at 5%</b>		Treat.=5.9, period=6.6 and interaction=NS						Treat.=NS, period=NS and interaction=NS						Treat.=0.047, period=0.052 and interaction=0.104							
	<b>2<sup>nd</sup> season</b>																				
<b>Control</b>		98.0	96.0	94.0	86.7	83.4	<b>91.6</b>	1.22	1.17	1.19	1.17	1.20	<b>1.19</b>	9.79	9.96	10.06	10.16	10.01	<b>9.99</b>		
<b>Xanthan at 1%</b>		95.4	96.0	94.0	94.0	90.3	<b>93.9</b>	1.31	1.31	1.31	1.11	1.09	<b>1.23</b>	9.60	9.96	10.10	10.06	9.761	<b>9.90</b>		
<b>Xanthan at 1%+ 0.2% CHNs</b>	107.3	98.0	96.0	93.0	90.0	87.7	<b>92.3</b>	1.35	1.28	1.22	1.31	1.17	1.22	<b>1.24</b>	9.50	10.14	10.33	10.43	10.61	10.22	<b>10.35</b>
<b>Xanthan at 1%+ 0.4% CHNs</b>		104.0	92.0	90.3	92.3	87.0	<b>93.1</b>	1.22	1.25	1.12	1.11	1.09	<b>1.16</b>	10.29	10.54	10.54	10.75	10.40	<b>10.50</b>		
<b>Period avg.</b>		<b>98.9</b>	<b>95.0</b>	<b>92.8</b>	<b>90.7</b>	<b>86.3</b>		<b>1.26</b>	<b>1.24</b>	<b>1.23</b>	<b>1.14</b>	<b>1.15</b>		<b>9.96</b>	<b>10.19</b>	<b>10.28</b>	<b>10.39</b>	<b>10.10</b>			
<b>LSD at 5%</b>		Treat.=NS, period=5.6 and interaction=NS						Treat.=NS, period=NS and interaction=NS						Treat.=0.023, period=0.026 and interaction=0.052							

CHNs= chitosan nano particles, treat. = treatments, avg.= average, NS= non-significant, \*=zero time

\*six days at 20°C and 60-70% RH after cold storage.



Xanthan gum mixed with two different concentrations of CHNs (0.2 and 0.4%) were tested on the different characteristics of guava fruits compared with xanthan gum and control fruits without any coating materials.

Xanthan and xanthan + 0.2% CHNs had the lowest decay percent may be due to the activation of fruit self-defense resistance to fungi, enhancing the activity of enzymes, which helps in killing fungi. Chitosan nano particles interfere with negatively charged residues of macromolecules exposed on the fungal cell surface that leads to the leakage of intracellular electrolytes and proteinaceous constituents. Chitosan nano particles able to bind with microbial DNA, leading to inhibition of the mRNA and protein synthesis. It forms gas-impermeable coating that inhibits fungal growth (Zakrzewska *et al.*, 2005; Bautista-Baños *et al.*, 2006; Meng *et al.*, 2008; Gad *et al.*, 2016; Khaliq *et al.*, 2017 and Romanazzi *et al.*, 2017). The effect of chitosan coatings that inhibited decay symptoms in various fruits, including peach (Ma *et al.*, 2013), strawberry (Gol *et al.*, 2013), table grapes (Gao *et al.*, 2013) mango (Khaliq *et al.*, 2017) and peach (Gad *et al.*, 2016).

Shriveling and dehydration is an indicator to water loss from fruit surface due to transpiration, which reduces fruit quality in the postharvest (Pérez-Gago *et al.*, 2010; Khaliq *et al.*, 2017; Romanazzi *et al.*, 2017). In this work, xanthan gum coating used to form a semipermeable fruit surface film, as a gas exchange barrier, which did not reduce the water transport, so that, mixing CHNs into the coating creates a lipophilic surface, which acting as a protective barrier against water (Khaliq *et al.*, 2017 and Romanazzi *et al.*, 2017). The limitation of water vapor exchange observed in these results with the increase of CHNs concentration. The hydrophilic nature of the gum, which absorbs

water and removes it through the ambient, may explain this effect (García-Ochoa *et al.*, 2000). The reduction in weight loss by using chitosan have been observed in some fruits as strawberry, litchi, dragon fruit, peach, mango and guava (Gol *et al.*, 2013; Lin *et al.*, 2011; Ali *et al.*, 2013; Gad *et al.*, 2016; Khaliq *et al.*, 2017; Romanazzi *et al.*, 2017).

Fruit softening positively related to ripening process. The activity of hydrolytic enzymes increased with ripening causes loss of fruit hardness (Razzaq *et al.*, 2014; Gad *et al.*, 2016). Xanthan and low concentration of CHNs maintained firmness more than control and high concentration of CHNs. Control treatment enhanced ripening compared to other treatments, so, it lose firmness. The deterioration in fruits treated with high concentration of CHNs caused reduction in firmness. These results are Compatible with Xing *et al.*, (2011) on sweet pepper; Zhou *et al.*, (2011) on pear; Ma *et al.*, (2013) and Gad *et al.*, (2016) on peach; Khaliq *et al.*, (2017) on mango and Romanazzi *et al.*, (2017) on guava.

It is clear from the results that mixing xanthan with CHNs enhanced PTI. The coating film created by chitosan inhibits CO<sub>2</sub> and ethylene production from the commodity (Gad *et al.*, 2016). Using wax and gum in coating may results anaerobic respiration, which results ethanol and acetaldehyde responsible of off flavor (Chen and Nussinovitch, 2000). Wax formulations with different materials may enhance coating distribution regularity on fruit surface (Zambrano-Zaragoza *et al.*, 2013), which maintains better taste for extended periods. It is associated with maturity, ripening and color change. After shelf life, mixing xanthan with 0.2% CHNs showed a reduction in color change. Delay in chlorophyll degradation is the main reason in delay color change. The delay in color change reported by Jain *et al.*, 2003 and Espinoza-

Zamora *et al.*, (2010). The deterioration in fruit surface resulted from high concentration of CHNs increased chlorophyll degradation by enhancing fruit ripening. The degradation in Vitamin C also associated with ripening and fruit deterioration, as the CHNs 0.2% maintained the highest vitamin C, while 0.4% was the lowest. This was in line with Gad *et al.*, 2016 on peach. The reduction in TSS in fruits coated with xanthan clarified by the elevation in respiration rate, which consumed sugars (Espinoza-Zamora *et al.*, 2010; Zambrano-Zaragoza *et al.*, 2013). The delay of ripening in CHNs treatments may be the main reason for the decrease of total sugars in these treatments compared with control and xanthan.

Coatings form a semi-permeable barrier to water vapor and gas exchange, leading to weight loss reduction, respiration rate modification, and senescence delay of coated produce (Hagenmaier and Baker, 1994; Nisperos-Carriedo, 1994; Olivas *et al.*, 2008).

However, the effect of the wax formulation on the fruit itself is often ignored (Petracek, Hagenmaier, and Dou, 1999). Well-recognized effects of waxing include - modified gas exchange of the peel, which often results in high levels of ethanol and acetaldehyde. At high levels, these volatiles are considered off-flavors and reduce fruit quality (Sinclair, 1984).

Wax permeability can be increased by using formulations with constituents that increase permeability or by promoting the development of small holes and cracks in the wax and puncturing the fruit (Petracek *et al.*, 1999). A recent study reported that the inclusion of xanthan gum, a non-gelling hydrocolloid, in a traditional wax coating creates disturbances in the ordered structure of the wax. Because of the “imperfect” coating, fruit respiration is less disturbed and

lower levels (relative to commercial coatings) of ethanol and acetaldehyde accumulate in the fruit (Chen and Nussinovitch, 2000).

Edible coatings generate a modified atmosphere by creating a semi-permeable barrier against O<sub>2</sub>, CO<sub>2</sub>, moisture and solute movement, thus reducing respiration, water loss and oxidation reaction rates (Martínez-Romero *et al.*, 2006).

Such coatings tend to overly restrict the exchange of O<sub>2</sub> and CO<sub>2</sub> between atmosphere and fruit to the extent that internal O<sub>2</sub> concentration becomes too low to sufficiently support aerobic respiration, resulting in high values of internal ethanol, acetaldehyde and internal CO<sub>2</sub>. Similar effects have also been observed with citrus fruit stored under controlled atmosphere conditions (Hatton and Spalding, 1990; Ke and Kader, 1990).

Mixing xanthan gum with 0.2% CHNs as a coating to guava fruit cv. ELmamura decreased decay, color change, maintained fruit firmness, good taste, vitamin C and reduced total sugars compared with xanthan gum or high concentration of CHNs. The mix of xanthan gum with 0.2% CHNs coating enhanced overall quality of guava fruits during extended cold storage and shelf life periods.

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