

Effect of Antimicrobial Edible Coatings on Quality and Shelf Life of Minimal Processed Guava (*Psidium guajava*)

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ABSTRACT

The present study aimed to develop an antimicrobial edible coating and to evaluate their efficiencies in preserving guava slices. Guava slices were submitted to four treatments: without coating (control) A, coating with potato starch containing 0.25% or 0.5% chitosan, B and C treatments respectively, and coating with 3% potato starch, 1.5% ascorbic acid and 0.2% potassium sorbate, D treatment. All samples were stored in refrigerator at $4\pm 1^\circ\text{C}$ and 70-75% relative humidity for 18 days. The following characteristics were analyzed: weight loss, firmness, total soluble solids, total titratable acidity, vitamin C, polyphenol oxidase (PPO), catalase (CAT), microbial quality and sensory attributes. Treatments with edible coatings were effective in reducing weight loss and maintaining texture of guava slices. The coatings decreased the increase in total soluble solids and decreased the loss in total titratable acidity compared to the uncoated ones. The lowest activity values of enzymes (PPO and CAT) were recorded for coated samples. The use of edible coatings contributed in reducing microbial growth of guava slices and increasing their shelf-life.

Key words: Edible coating, guava slices, shelf-life .

INTRODUCTION

Guava fruit (*psidium guajava* L.) is a vital source of vitamins, minerals, antioxidants and dietary fibers (Hassimotto *et al.*, 2005, Adrees *et al.*, 2010). Highly perishable nature and proning towards chilling injury, diseases, insect-pests and physical damage are the main drawbacks of guava fruit after harvesting. However, during postharvest period, this fruit rapidly start senescence, a fact which prevents it from being stored for long period. This is a very serious problem, because it hinders or even prevents the commerce of this fruit to distant markets. Therefore, the application of postharvest technology is essential for guava fruit marketing (Lima *et al.*, 2002).

On the other hand, due to its climacteric nature, the fruit ripens rapidly and hence highly perishable, with a very short shelf life ranging from 2-3 days at room temperature (Bassetto *et al.*, 2005).

Minimal processing of fresh-cut fruits, which involves grading, washing, sorting, peeling, slicing and packaging, can affect the integrity of the fruits and cause biochemical changes and microbial spoilage that may result in degradation of the colour, texture and flavour of fruits (Watada & Qi, 1999). The removal of the natural protective skin of fruits caus-

es leakage of juices and sugars from the damaged tissue resulting in the fruits being highly susceptible to microbial spoilage (Oms-Oliu *et al.*, 2010). For such aspects the performance of packaging materials are quite important and depend on their efficiency to reduce transferring between foodstuff and the environment such as the transport of volatile compounds from food to the outer atmosphere or from the surrounding medium to the packaged product, and the adsorption of volatile compounds at the surface of the packaging (sorption or scalping) (Reineccius, 1991, Benet *et al.*, 1992, Blumental, 1997). An edible coating can be used as an alternative to modified atmosphere packaging to improve the shelf life of fresh-cut fruits (Rojas-Grau *et al.*, 2009). Moreover, coatings are effective barriers to water loss, provide good appearance and brightness, making fruits and vegetables more attractive to the market (Lemos *et al.*, 2007, Vila *et al.*, 2007).

Due to the strict regulation on chemical use in food production, the replacement of the traditional chemical fungicides by natural antimicrobial substances is a favorable factor for the acceptance of agricultural products in the international market (Raybaudi-Massilia *et al.*, 2007). Chitosan, which may be used as a biofilm, by itself or associated to other compounds, was very effective in inhibiting

microorganisms during the postharvest life of vegetables and fruits, and also in minimally processed food (Liu *et al.*, 2007). Chitosan is a biopolymer of high potential to be used as an edible coating and active package, because it is non-toxic, able to form biodegradable films and prevents antimicrobial activity. Among edible coatings, chitosan, also has long been known to protect perishable produce from deterioration by reducing transpiration, respiration and maintaining the textural quality. Chitosan {poly β -(1-4) *N*-acetyl-d-glucosamine}, a deacetylated form of chitin, is a natural compound obtained from crustacean shells (crabs, shrimp and crayfishes) either by chemical or microbiological processes and can be produced by some fungi (Tan *et al.*, 1996). Indeed, chitosan is often considered to be the ideal preservative coating for fresh fruits because of its excellent film-forming and biochemical properties (El-Ghaouth *et al.*, 2000).

In this context, the objectives of this work were to develop edible coatings based on potato starch and chitosan or potassium sorbate to be used on the surface of guava slices and to evaluate their preservation efficiencies through microbiological and physicochemical analyses. Shelf life and quality of guava slices were also taken into consideration in this respect.

MATERIAL AND METHODS

Sample preparation

Guava fruit harvested at commercial maturity was obtained from the local market. Good quality fruit was selected for uniformity of size and colour. Fruit was washed and cut into slices. The guava slices were dipped in chlorinated water (150 ppm) for three min. and drained for 10 min. This was followed by air drying for 3min, and randomly divided into four groups, which corresponded to three coating treatments, and one water dipped control.

Coating materials

Potato starch was obtained from Leader Factory for chipsy, Tanta city. Preliminary treatment of potato starch (PS) was achieved using the method of Elabd (2013). Chitosan was purchased from Mifad Company, Badr city, Egypt. Ascorbic acid and potassium sorbate were purchased from El-Gomhoria Co. for chemical and drugs.

Coating treatments

Fruit slices groups were assigned to the follow-

ing coating treatments: A [non-coated (control)]. B [3% PS and 0.25% chitosan], C [3% PS and 0.5% chitosan], D [3% PS, 1.5% ascorbic acid and 0.2% potassium sorbate]. Glycerol was added as plasticizer (50% w/w) in all the previous coating solutions. Preparation of chitosan solution was done according to the method of Jiang & Li (2001), then was added to the solutions (B and C) and gradually heated to 70°C while stirring until gelatinization was attained. After gelatinization of coating solution (D), it was cooled and ascorbic acid and potassium sorbate were added (Nasution *et al.*, 2015). Experiment was conducted in three replications.

Coating procedure of fruit slices

Guava slices samples were dipped for 1 min in antibrowning solution composed of a mixture of 1% (w/v) L- ascorbic acid and 1% (w/v) citric acid. The excess liquid was gently removed by drying for 3 min using a fan to ensure dryness. This was followed by dipping in 2% (w/v) calcium chloride solution as solidifying agent. The slices were immersed in different coating solutions for 3 min and dried. The slices then were stored in refrigerator at 41± °C and relative humidity 70-75%, for 18 days. The guava slices were analyzed on day zero and every 3 days, during storage period of 18 days.

Physical and chemical analyses

- Weight loss was measured as percentage of weight loss from the initial weight of slices (AOAC 2000).
- Total soluble solid (TSS) was determined in the juices by the refractometric method at room temperature using a polish manual refractometer (R R 12, Nr 05116, 0-35% at 20 C) according to the method given in the AOAC (2000).
- Firmness of the fruit slices was measured using a hand dynamometer model FDP 1000 with a thump (2 mm) in gf (gram- force). The data were transformed into Newton units using (N) standard factor (1 gram- force = 0.00980665 Newton).
- Total titratable acidity (TTA) of the juices was determined as described in the AOAC (2000).
- Ascorbic acid (V.C) was determined using 2,6 dichlorophenolindophenol titrimetric method as described in the AOAC (2000). The results were expressed as mg ascorbic acid per 100 g of fruit.

- Polyphenol oxidase activity was determined according to the method described by Malik & Singh (1980) and Hafez (2010) using model UV-160A spectrophotometer (Shimadzu, Japan).
- Catalase activity (CAT) was determined spectrophotometrically according to Aebi (1984).

Microbial analysis

Total bacterial count (TBC) and count of yeasts and molds (Y&M) were determined according to the methods described in the DIFCO manual (DIFCO, 1977).

Sensory evaluation

It was carried out by trained panelists every three days, during storage period as described by Lawless & Heymann (1999).

Statistical analysis:

This was carried out according to SAS (2010).

RESULTS AND DISCUSSION

Weight loss

Application of all coating treatments significantly decreased the weight loss of guava slices

during storage period compared to the control. There was an added benefit to minimize of weight loss by increasing the concentration of chitosan from 0.25 to 0.5%. The lowest weight loss was found in C coated sample followed by B and D then the uncoated sample (control) (Fig. 1). Fresh-cut guava slices are prone to weight loss due to the removal of skin and pulp that exposes the fruit's internal tissues (Watada & Qi 1999). Loss weight in fresh fruit and vegetable is mainly due to the loss of water caused by transpiration and respiration processes (Zhu *et al.*, 2008). Chitosan coating forms a layer of semi-transparent to smooth the pericarp surface (Dong *et al.*, 2004). At the end of storage period, the lowest significant weight loss was recorded for C treatment (17.44%) followed by D coating treatment (18.26%).

Total soluble solids

The control sample presented a decrease in the TSS values during the experiment, while the coated treatments were significantly more effective in controlling the sugar content until the end of storage period. Fig (2) indicated that the TSS% increased gradually throughout the storage periods. It reached the highest percentage at the end of storage period. However, no significant differences were detected

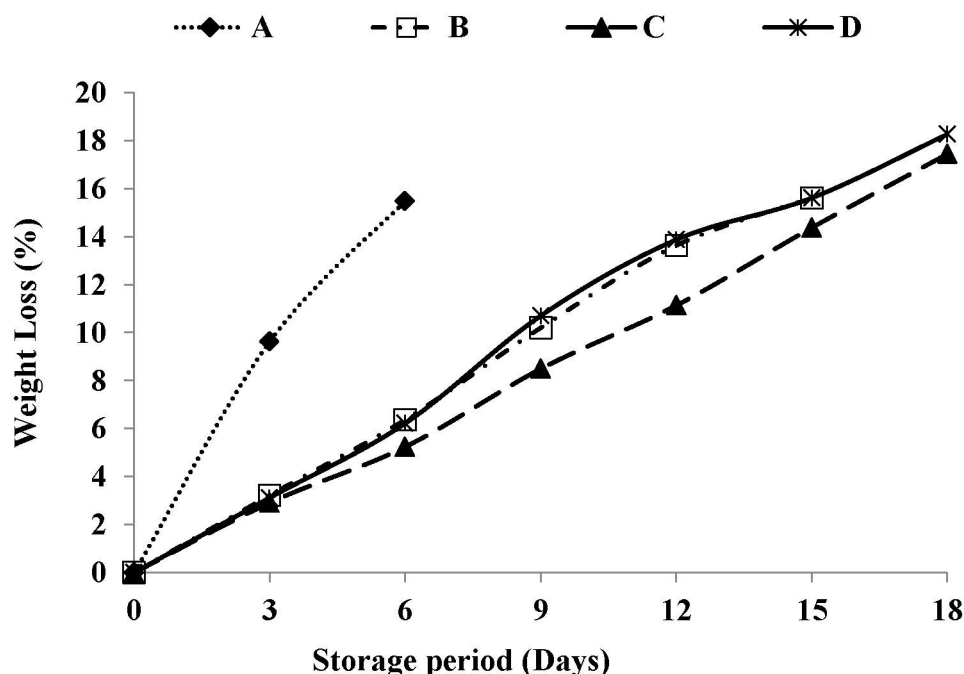


Fig. 1: Effect of coatings on weight loss (%) of guava slices during storage at $4\pm 1^\circ\text{C}$. A: Control, B: sample treated with 3% potato starch and 0.25% chitosan, C: sample treated with 3% potato starch and 0.5% chitosan, D: sample treated with 3% potato starch, 1.5% ascorbic acid and 0.2% potassium sorbate

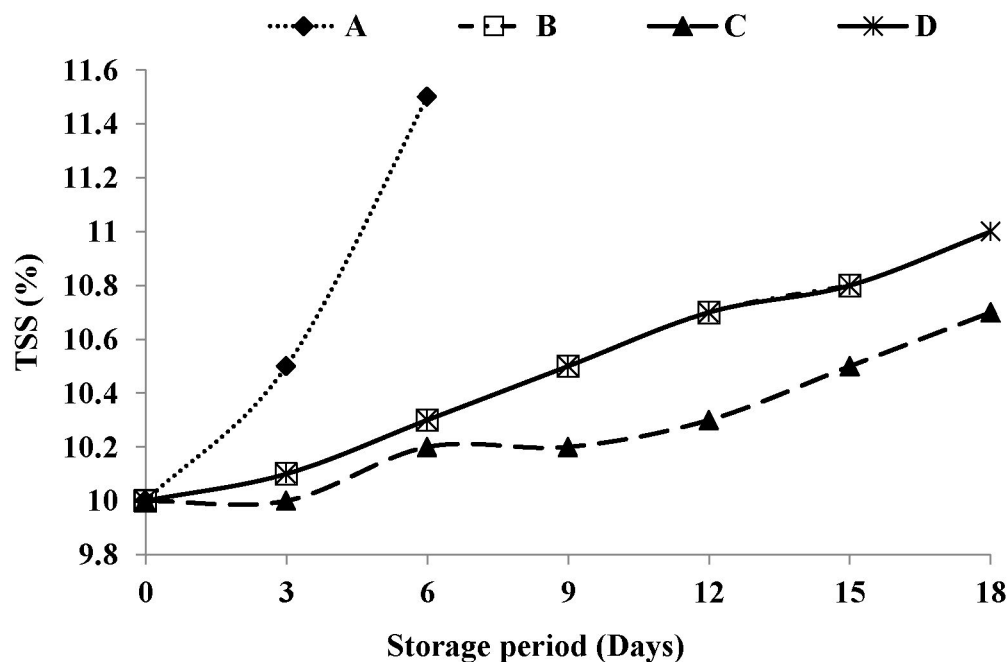


Fig. 2: Effect of coatings on TSS (%) of guava slices during storage at $4\pm 1^{\circ}\text{C}$. A, B, C and D as in Fig. (1)

between all coated treatments during the storage period. The control treatment gave the highest value of TSS, while the least value was recorded by C coated sample (10.7%) at the end of the storage period followed by D coated sample (11.0%).

Firmness of guava slices

A considerable decrease in the firmness was observed during storage period of guava slices irrespective of the treatment. Antimicrobial coatings delayed slices softening and exhibited significant differences during storage. Significantly more firmness was retained by C coating followed by B & D and the least firmness was recorded for the uncoated sample (Fig. 3). The maintenance of firmness in the coated samples with antimicrobial coatings could be due to covering of the cuticle and lenticels and their higher antifungal activity thereby reducing respiration, other ripening processes and infection during storage (Krishna & Sudhakar 2014). The retardation of fruit softening in response to chitosan treatment was previously reported in fruits such as Indian jujube fruit (Zhong & Xia 2007) and papaya (Raqeab *et al.*, 2008).

Total titratable acidity (TTA%)

Citric acid is the major organic acid in ripe guava fruit. The TTA% was relatively high at zero time in guava slices and then it decreased during storage period which is a natural phenomenon (Fig.

4). This might be due to rapid utilization of acids in guava fruits during the respiration process as a substrate (Gupta *et al.*, 1979). However, there was no significant difference in TTA among all coated treatments throughout the storage period which are in favour with titratable acidity in the guava slices given by Augustin & Christe (1989). Han *et al.* (2004) reported that the chitosan coating slowed down the changes in TTA of strawberry and raspberry and effectively delayed fruit ripening. The antimicrobial coating was probably able to modify the internal atmosphere of the fruit to prevent the decrease in TTA values. Therefore, the antimicrobial coatings produced a small change in TTA throughout storage. Han *et al.* (2004) also observed lower acidity loss during storage in strawberry, peach, tomato and litchi coated with chitosan.

Vitamin C content:

Guava contains four times more ascorbic acid as compared to citrus (Adrees *et al.*, 2010). Vitamin C in guava slices gradually decreased during storage, and this reduction was effectively inhibited by edible coatings. As shown in Fig. (5), slices treated with coatings delayed the loss of vitamin C compared with the control and significant differences were recorded between them. No significant differences between all coated samples during storage until 6th days and significant differences between them were found at 9th to 15th days of stor-

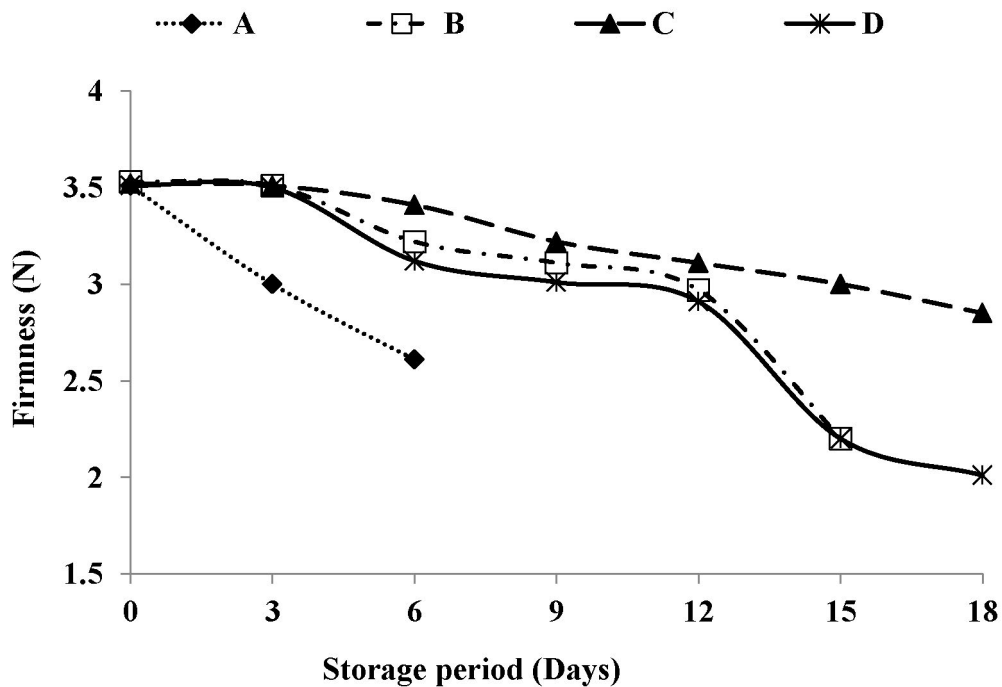


Fig. 3: Effect of coatings on firmness (N) of guava slices during storage at 4±1°C. A, B, C and D as in Fig. 1

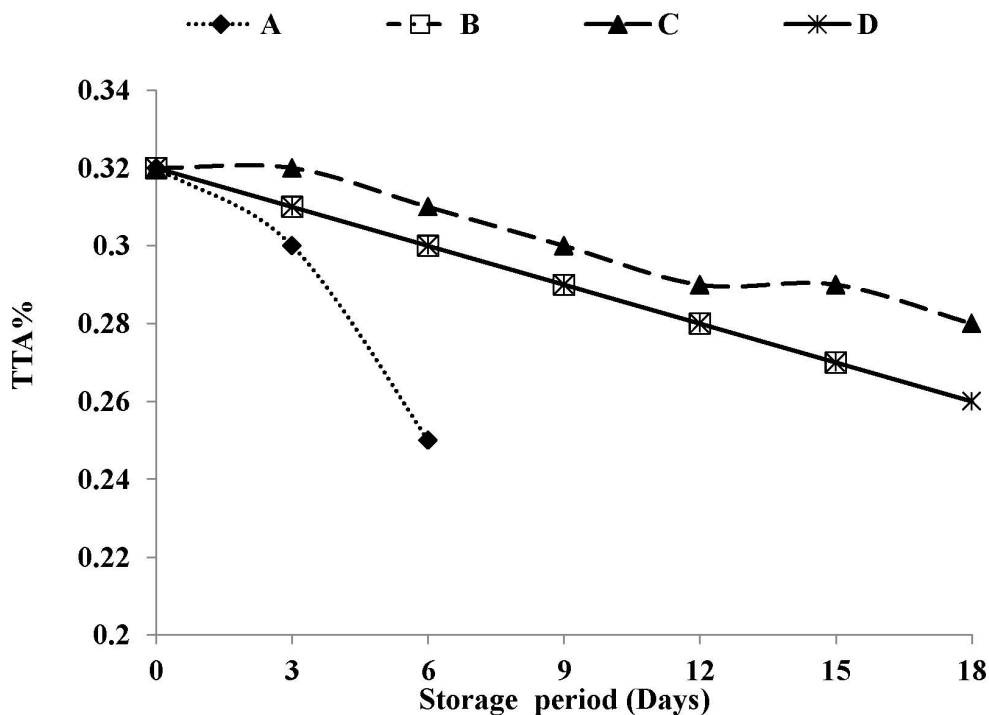


Fig. 4: Effect of coating on TTA (%) of guava slices during storage at 4±1°C. A, B, C and D as in Fig. 1

age. Meanwhile, C treatment showed significant effect on vitamin C content at the same period of storage in comparison with the others. However, there was no significant effect on vitamin C content of guava slices due to C and D coated samples

at the end of storage period. Similar results were reported by Ayranci & Tunc (2004) who suggested that the modified atmosphere created by chitosan coating suppressed the loss of vitamin C.

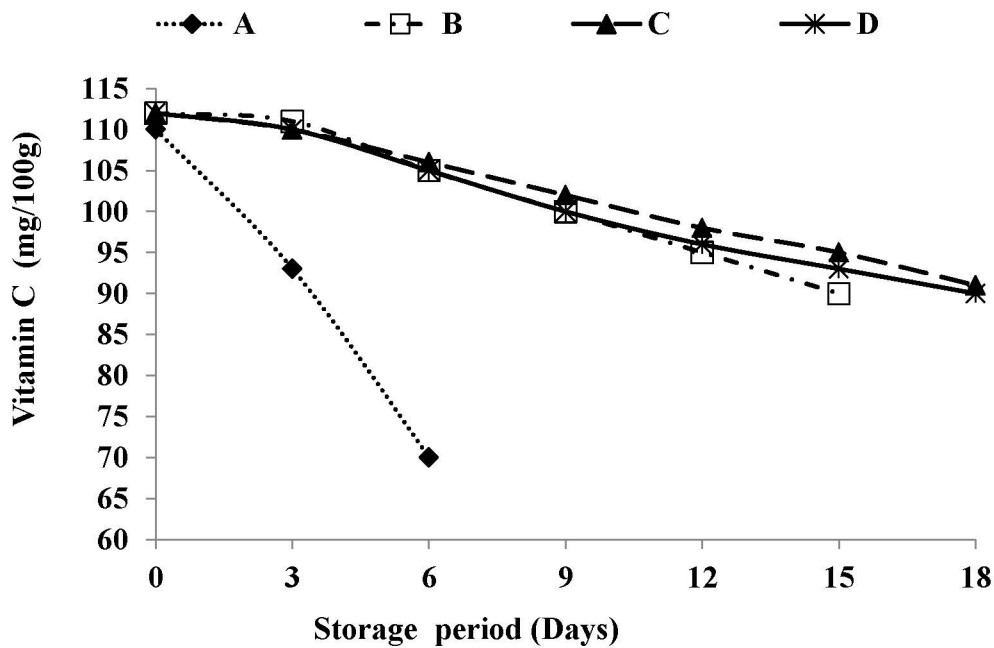


Fig. 5: Effect of coatings on vitamin C content (mg/100g) of guava slices during storage at 4 ± 1 °C. A, B, C and D as in Fig. 1

Activities of PPO and CAT

As shown in Fig. (6), PPO activity of guava slices gradually increased during storage period. However, C sample as the best in decreasing the increment during storage, and significant differences were noted between it and the other treatments. This was followed by B and D samples. Antimicrobial coatings generally minimized the increase in PPO activity.

CAT activity in all samples decreased during storage period as shown in Fig. (6). However, the coating samples delayed the decrease of CAT activity. At the end of storage period, there were 32.25 and 30.56 $U\ g^{-1}\ min^{-1}$ in CAT activity of samples treated with C and D coating, respectively. While the value of CAT activity in samples treated with B coating was 31.02 at 15th day of storage compared with the control sample which had only 33.16 at 6th

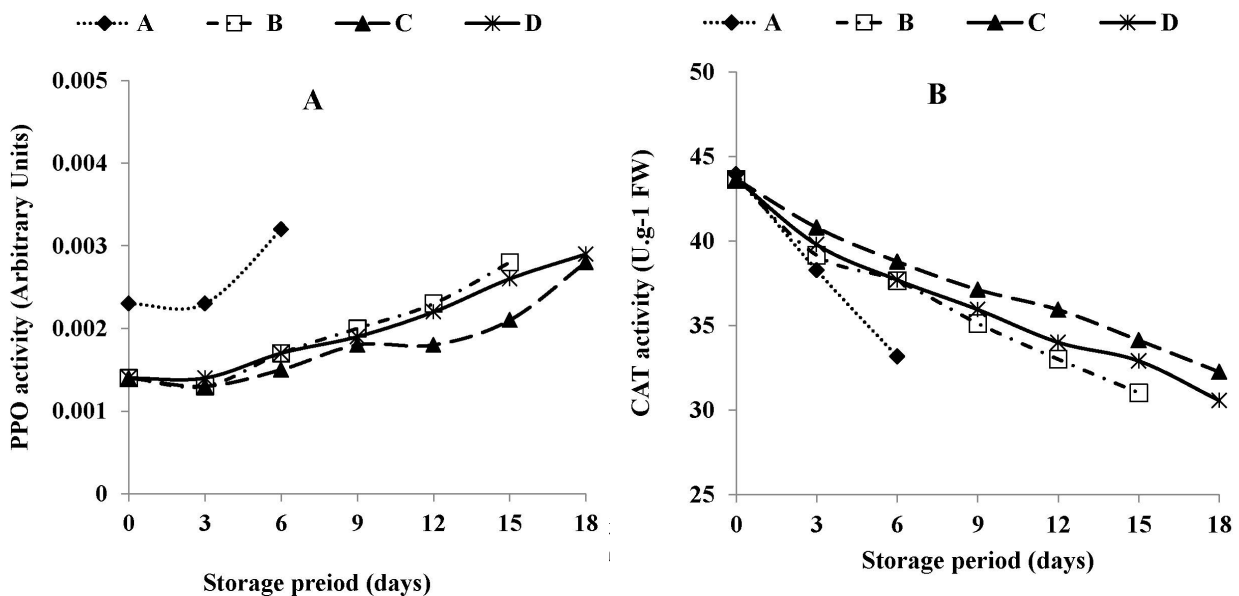


Fig. 6: Effect of coatings on polyphenol oxidase (A) and catalase (B) activities of guava slices during storage at 4 ± 1 °C. A, B, C and D as in Fig. 1

days of storage. There was also a significantly positive correlation among them. It was suggested that effect of chitosan on increase of antioxidant ability might be beneficial in delaying ripening process in guava fruit during cold storage (Hong *et al.* 2012). As an antioxidant enzyme, CAT plays an important role in oxidation resistant activity. Samples coated with chitosan had higher CAT activity than the control ones in the present study, indicating that the chitosan coating delayed the senescence of guava fruit.

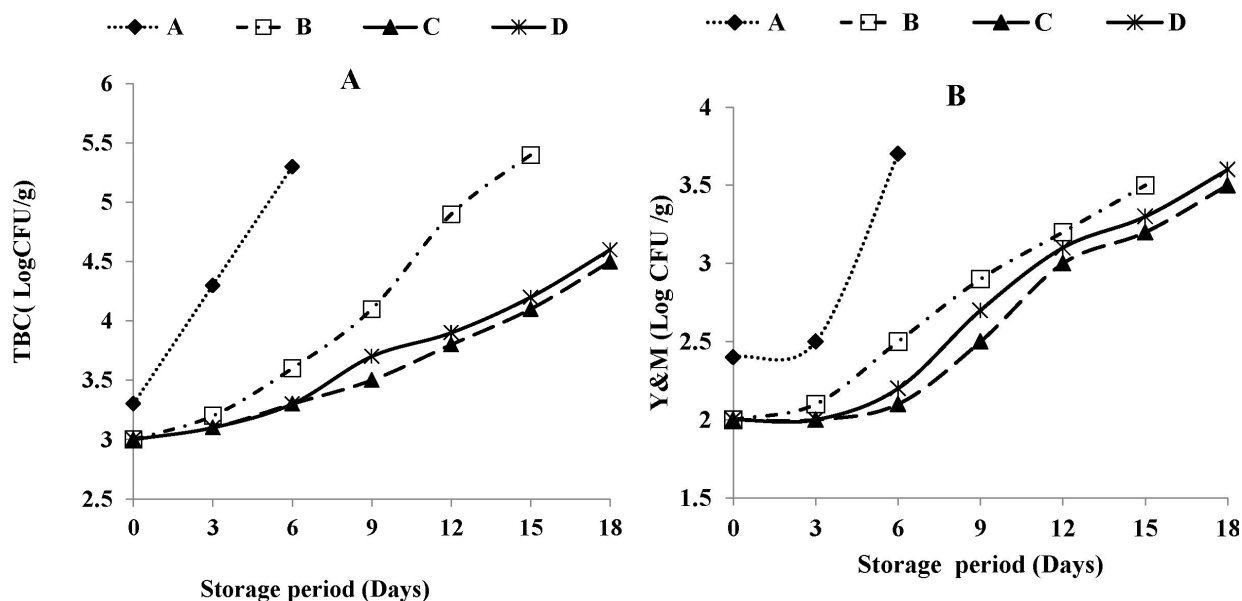


Fig. 7: Effect of coatings on TBC (A) and Y&M count (B) of guava slices during storage at 4±1°C. A, B, C and D as in Fig. 1

coated samples, however C and D coatings were the best among all coatings in reducing levels of TBC to 4.5 and 4.6 (Log CFU g⁻¹) and reducing Y&M count to 3.5 & 3.6 (Log CFU g⁻¹) respectively at the end of storage period. These results agreed with those reported by Soares *et al.* (2011).

Sensory evaluation

The data presented in Table (1) show the mean values of organoleptic scores of coated and uncoated guava slices during storage. The results indicated that no significant differences in organoleptic properties were recorded among uncoated and coated samples at zero time. After that, significant differences were noted between coated and uncoated slices. Uncoated samples (control) showed significant loss of quality at 6th days of storage, and then became unaccepted and discarded. Guava slices coated with different coatings had significant higher score than those of uncoated samples. No

Microbial growth

According to the analysis of variance, impact of coatings and storage time was significant ($P < 0.05$) on the total bacterial count and count of yeasts & moulds. Fig. (7) shows the statistical results for the counting of the TBC and Y&M (Log CFU g⁻¹ of guava slices). The results indicated that all coatings were significantly effective in maintaining low levels of TBC compared to the control. There were significant differences between all coated and un-

significant differences in scores of organoleptic qualities were noted between C and D coated samples for all attributes followed by B coated sample. At 15th days of storage, the samples coated with B coating were unaccepted and discarded, while samples coated with C and D coatings were still accepted until 18th day of storage with little changes in organoleptic qualities. Edible films can be used to protect perishable food products from deterioration by retarding dehydration, providing a selective barrier to moisture, oxygen and carbon dioxide, suppressing respiration, improving textural quality, helping retain volatile flavour compounds and reducing microbial growth (Lee *et al.*, 2003).

CONCLUSION

The use of antimicrobial edible coatings was efficient to extend guava slices shelf-life, keeping the physical, chemical properties, enzymatic activities and microbial quality. The results suggested that

Table 1: Effect of coatings on sensory evaluation of guava slices during storage at 4±1°C

| Storage period (Days) | Attributes | A | B | C | D * |
|-----------------------|------------|-------|-------|-------|--------|
| 0 | Colour | 9.5A | 9.8 A | 9.8 A | 9.8 A |
| | Taste | 9.5 A | 9.7 A | 9.7 A | 9.7 A |
| | Odour | 9.8 A | 9.9 A | 9.9 A | 9.9 A |
| | Texture | 9.9 A | 9.7 A | 9.7 A | 9.7 A |
| | Over all | 9.5 A | 9.7 A | 9.7 A | 9.7 A |
| 3 | Color | 7.5B | 9.5 A | 9.6 A | 9.5 A |
| | Taste | 7.7 B | 9.5 A | 9.5 A | 9.5 A |
| | Odor | 7.5 B | 9.7 A | 9.8 A | 9.8 A |
| | Texture | 7.0 B | 9.5 A | 9.6 A | 9.6 A |
| | Over all | 7.5 B | 9.5 A | 9.5 A | 9.5 A |
| 6 | Colour | 5.5 B | 9.0 A | 9.2 A | 9.1 A |
| | Taste | 6.2C | 9.0B | 9.2 A | 9.1 AB |
| | Odour | 5.5 C | 9.2 B | 9.5 A | 9.5 A |
| | Texture | 6.0 C | 9.0 B | 9.3 A | 9.3 A |
| | Over all | 5.5 C | 9.0 B | 9.4 A | 9.3 A |
| 9 | Colour | US | 8.5 B | 9.0 A | 9.0 A |
| | Taste | US | 8.0 B | 8.7 A | 8.7 A |
| | Odour | US | 8.0 B | 9.0 A | 9.0 A |
| | Texture | US | 8.5 B | 9.0 A | 9.0 A |
| | Over all | US | 8.0 B | 9.0 A | 9.0 A |
| 12 | Colour | US | 8.0 B | 8.6 A | 8.6 A |
| | Taste | US | 7.7 B | 8.5 A | 8.3 A |
| | Odour | US | 7.6 B | 8.7 A | 8.5 A |
| | Texture | US | 7.0 B | 8.6 A | 8.5 A |
| | Over all | US | 7.5 B | 8.8 A | 8.6 A |
| 15 | Colour | US | 7.8 B | 8.0 A | 8.0 A |
| | Taste | US | 7.5 B | 8.1 A | 8.1 A |
| | Odour | US | 7.2 B | 8.5 A | 8.5 A |
| | Texture | US | 6.7 B | 8.3 A | 8.1 A |
| | Over all | US | 6.5 B | 8.5 A | 8.4 A |
| 18 | Colour | US | US | 7.3 A | 7.2 A |
| | Taste | US | US | 7.8 A | 7.7 A |
| | Odour | US | US | 8.0 A | 8.0 A |
| | Texture | US | US | 7.5 A | 7.5A |
| | Over all | US | US | 7.8 A | 7.8A |

Averages with different superscripts in each row differed significantly ($P \leq 0.05$), US: unaccepted slices - * A, B, C and D as in Fig. (1)

coatings with potato starch and chitosan provided an antimicrobial effect on total bacterial count and yeast and moulds count. The use of an edible coating in association with natural antimicrobials is a good way to increase fruit slices shelf-life.

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تأثير الأغشية الطبيعية المضادة للميكروبات على جودة وفترة صلاحية الجوافة محدودة التجهيز

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الجوافة ثمار حساسة ومرغوبة لدى المستهلك نظراً لما تحتويه على نسبة عالية من فيتامين C، ومن سكريات ومواد فينولية و وكاروتينات وألياف ومعادن، ونظراً لأن عمرها التخزينى قصير جداً وحساسية عند التداول بالنسبة للمستهلك وسريعة الفساد والإصابة بالميكروبات لذلك تم استخدام الأغشية الغذائية لإطالة العمر التخزينى لها والمحافظة على الخواص الكيماوية والفيزيائية والجودة الميكروبية والحسية لأطول فترة ممكنه. تم استخدام نشا البطاطس كمادة تغطية طبيعية مع الشيتوزان كمضاد أكسدة ومضاد ميكروبى طبيعى وإستخدام حمض الإسكوريك وسوربات البوتاسيوم كمواد حافظة. تم تقسيم العينات إلى 4 مجموعات: A هى العينات غير المعاملة (الكنترول)، B العينات المعاملة بنشا البطاطس 3٪ مع الشيتوزان 25، ٪، C العينات المعاملة بنشا البطاطس 3٪ مع الشيتوزان 5، ٪، D العينات المعاملة بنشا البطاطس 3٪ مع حامض الإسكوريك 5، ٪ وسوربات البوتاسيوم 2، ٪. تم تخزين العينات المعاملة والكنترول فى الثلاجة على $4 \pm 1^{\circ}C$ و $70-75$ ٪ رطوبة نسبية. تم سحب عينات على فترات زمنية مختلفة لتقدير الفقد فى الوزن، الصلابة، المواد الصلبة الكلية الذائبة TSS، الحموضة الكلية التنقطية TTA، فيتامين C، نشاط إنزيم بولى فينول أو كسيديز PPO، نشاط إنزيم الكتاليز CAT، الحمل الميكروبى والخواص الحسية.

أظهرت النتائج أن الأغشية المضادة للميكروبات كان لها تأثيراً معنوياً على التركيب وصفات الجودة والحمل الميكروبى لشرائح ثمار الجوافة. وجد أن متوسط قيم الفقد فى الوزن، PPO، TSS، والحمل الميكروبى تزداد باستمرار مع تقدم فترات التخزين ولكن معدل الزيادة كان أقل منه فى العينات المغطاة مقارنة بالعينات غير المغطاة (الكنترول). كما أظهرت النتائج أن متوسط قيم الصلابة، TTA، فيتامين CAT، تقل بزيادة فترة التخزين وكان معدل الإنخفاض فى العينات المغطاة أقل منه فى عينات الكنترول. كما أوضحت النتائج أن أفضل المعاملات كانت عينات المعاملة C يليها D وأمتد تخزينها حتى 18 يوماً مع إحتفاظها بخواص حسية جيدة ثم العينات المعاملة B والتي أمتد تخزينها حتى 15 يوماً مقارنة بالعينات غير المغطاة (الكنترول) والتي وصل تخزينها حتى 6 أيام فقط ثم فسدت بعد ذلك.

