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Application of Chitosan Edible Coating for Soursop (Annona muricata) Storage

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Abstract.

Soursop (*Annona muricata*) is one of the exotic fruits prized for its very pleasant, sub-acid, aromatic and juicy flesh which consists of edible white pulp and a core of indigestible black seeds. However it's a highly perishable fruit and easily damaged, softens very rapidly during ripening, and becomes mushy and difficult to consume fresh. Application of chitosan as edible coating could be considered as a useful approach to maintain its product quality during preservation. Ojective of the present study focused on the effect of chitosan coating on some physicochemical, microbial and sensory characteristics of soursop during preservation. Optimal results showed that weight loss, pH, total soluble solids, titratable acidity and ascorbic acid; total plate count; sensory characteristics could be maintained at appropriate levels by coating soursop with 0.75% chitosan.

Keywords: Soursop, chitosan, coating, preservation, physicochemical, microbial, sensory

I. INTRODUCTION

Soursop fruit (Annona muricata) is becoming more popular due to its highly aromatic juicy and distinctive flavour (Quek et al. 2013) and also reported to be a major source of antioxidants (Umme et al. 1996). The plant possess the major pharmacological activities includes cytotoxic, antileishmanial, wound healing, antimicrobial activity. It also has the anticarcinogenic and genotoxic effect. Phytochemical analysis of the plant revealed the presence of tannins, steroids and cardiac glycosides which are the major phytochemical compounds (Gajalakshmi S et al., 2012). Soursop also has numberous activities, including anticancer, anticonvulsant, anti-arthritic, antiparasitic, antimalarial, hepatoprotective and antidiabetic, analgesic hypotensive, antiinflammatory, and immune enhancing effects (Singh D.R. et al., 2014; Sejal Patel, Jayvadan K Patel, 2016).

It is a major source of income for many farmers who cultivate them for fresh produce markets. It also provides a source of nutrients and play important role in the diet of many people living in the tropics by raising its nutritional value through the provision of essential minerals and vitamins and addition of flavors, colors and variety to the diet and equally by contributing to the proteins and calories contents of the diet (Raphael N Okigbo, Omokaro Obire, 2009).

The fruit of the soursop is considered suitable for both processed such as powder (Adela M. Ceballos Peñaloza et al., 2013; Lee Sin Chang et al., 2018), nectar (Mary Peters et al., 2001; Luis M. Anaya-Esparza et al., 2018), puree (Umme, A et al., 1996), juice (Quek, M.C., et al., 2013; Terhemba Theophilus Iombor et al., 2014; Prasetyorini Djarot, Moerfiah Badar, 2016; Stephanie Clara Akpeji et al., 2017), Emmanuel S. Abbo et al., 2006;

Nwachukwu, E. and Ezeigbo, C. G., 2013; Ajayi AA et al., 2015), vinegar (Chin Wai Ho et al., 2017), wine (Raphael N Okigbo, Omokaro Obire, 2009; Okafor D. C. et al., 2014) and fresh local consumption (Espinosa et al., 2013; Nguyen Phuoc Minh, 2017; José Orlando Jiménez-Zurita et al., 2017)

The soursop fruits, when harvested and ripened at room temperature, may reach consumer maturity up to 7 days depending on the maturation stage in which they were harvested. A service life of between 4 and 8 days for soursop fruit ripened at 25°C has been reported. Soursop fruit harvested and stored at 16°C with the application of 1-methylcyclopropene (1- MCP) require between 8 and 9 days to ripen (Espinosa et al., 2013).

The physicochemical composition of harvested soursop (Annona muricata L.) fruits during open-air storage was determined. The results showed that soursop fruits had high moisture content (73.1% - 82.1%), low titratable acidity (0.10 - 1.25% ca), low crude fat (0.42 mg/100 g-fw), moderate ash content (0.87 mg/100 g-fw) and crude fibre content (6.09 mg/100 g-fw), high ascorbic acid content (34.0 - 19.7 mg/100 g-fw), high total sugars content (34.3% – 45.3%), reducing sugar content (18.9% – 39.2%) and sucrose content (15.5% - 30.0%). Of the macroelements Na, Ca and K, the average content were 895.6, 870.3 and 367.5 mg/100 g-fw respectively. Heavy metals (Fe, Zn, Cu, Pb and Cd) content was very low in the soursop fruits, ranging between <0.0015 mg/100 g-fw for Cd and 0.82 mg/100 g-fw for Fe. During storage, the moisture content, titratable acidity level and sugars content in the fruit were all increasing whereas the ascorbic acid content was decreasing. There were no significant changes during storage for levels of crude fat, fiber, ash, mineral elements and heavy metals (Othman Chande Othman et al., 2014).

José Orlando Jiménez-Zurita et al. (2017) evaluated the maturity of two selections of soursop (G1 and G2) from Nayarit, Mexico under environmental conditions at 22°C and refrigeration at 15°C stored for 6 and 8 days, respectively. Maximum CO2 and ethylene values were present on the fifth and sixth day. The fruits exposed at 15°C had a significantly lower weight loss (5%) and showed no chilling injury. The firmness of two selections decreased more than 90%. The concentration of TSS increased to 5.3 to 15°Brix, and the titratable acidity was higher for fruit stored at 22°C. The highest concentration of phenols was recorded on the fourth day of storage at 22°C. The enzymatic activity of PPO was increased from physiological ripening to consumption ripening for both treatments. The two selections stored at 22°C registered the highest level of PME activity at ripeness. Shelf life was increased by up to 8 days (4 days at 15°C plus 4 days at 22°C) without causing chilling injury or alterations in the ripening process of the fruits.

There was little research mentioned to edible coating for soursop fruit. Alginate contributed significantly different effects to weight loss, firmness, color, chlorophyl, total acidity and vitamin C content after 30 days of preservation at 1.5% alginate (Nguyen Phuoc Minh, 2017). Soursop is a tropical fruit that undergoes postharvest deterioration rapidly. Application of chitosan as edible coating could be considered as a useful approach to maintain its product quality during preservation. Ojective of the present study focused on the effect of chitosan coating on some physicochemical, microbial and sensory characteristics of soursop during preservation.

II. MATERIALS AND METHOD

2.1 Material

We collected soursop fruit in Da Nang province, Vietnam. They must be cultivated following VietGAP to ensure food safety. After harvesting, they must be conveyed to laboratory within 8 hours for experiments. Fruits were thoroughly rolled to remove dirt, dust and adhered unwanted material. Besides soursop fruits we also used other materials during the research such as chitosan, lactic acid, NaOH, 2,6-dichlorophenolindophenol, Petrifilm -3M, Tween 80, glycerol, PVC bag. Lab utensils and equipments included colony counter, refrigerator, pH meter, refractometer, digital balance, grinder, centrifugator.



Figure 1. Soursop (Annona muricata)

2.2 Researching procedure

2.2.1 Preparation of coat forming solution

The coating solution was prepared by dissolving 0, 2.5, 5, 7.5, 10 g of chitosan powder in 1000 ml of lactic acid for

10 h at 20°C to dissolve chitosan to prepare 1 L of 0%, 0.25%, 0.5%, 0.75%, and 1% chitosan solutions. Then, Tween 80 and glycerol were added in the chitosan solution (Vieira et al., 2016).

2.2.2 Coating application

The surface of the fruits were disinfected with 3% peracetic acid for 2 min and gently rinsed with distilled water, then air-dried. Fruits were separated into three groups in triplicate; each group of the fruits was quoted as control (without treatment) 0% and 0.25%, 0.5%, 0.75%, and 1% chitosan coating. Each group of soursop was divided into 20 batches in triplicate (60 batches) each containing 100±5g of whole soursop. They were dipped in the chitosan coating solution of 0%, 0.25%, 0.5%, 0.75%, and 1% for 1 min and the samples were air dried for 15 min at room temperature (about 25°C). The coated fruits were packed in PVC wrap and kept at 4°C in a refrigerated condition for a period of 30 days to study the shelf life and physicochemical and microbial parameters.

2.2.3 Determination of weight loss

Three batches of soursop containing 1000±5g of whole soursop were taken at an interval of three days for total storage period. The soursop fruits were weighed regularly to determine weight loss, which was calculated cumulatively by comparing the weights of the sample with the electronic weighing balance at an interval of 3 days for the total 30 days storage period and the results were expressed as percentages.

2.2.4 Measurement of pH, total soluble solids, titratable acidity and ascorbic acid

5 g soursop pulp was homogenized in 25 ml of distilled water. Then the mixture was filtered using muslin cloth. An aliquot of 25 ml was used to measure pH with a pH meter. The TSS was measured directly from the filtered residue using a hand refractometer and expressed as brix. The titratable acidity was determined with 0.1 N NaOH. Soursop pulp (5g) from fruit was homogenized using a grinder and then centrifuged at 4000 rpm for 5 minutes; the supernatant phase was collected and analyzed to determine ascorbic acid content by 2,6-dichlorophenolindophenol titration.

2.2.5 Measurement of microorganism load

The total colony forming units (CFU) was enumerated during the storage period by Petrifilm - 3M.

2.2.6 Sensory evaluation

The acceptability of the samples was evaluated through the standard sensory evaluation techniques. The sensory attributes such as visual appearance, color, taste, flavor and acceptability was carried out by selected panel of judges (9 members) rated on a five point hedonic scale.

2.3 Statistical analysis

The experiments were run in triplicate with three different lots of samples. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT). Statistical analysis was performed by the Statgraphics Centurion XVI.

III. RESULT & DISCUSSION

3.1 Nutritional composition in soursop fruit

Table 1. gives utritional composition in soursop fruit. René G. Degnon et al., (2003) found that the moisture content was ranged from 18.33 to 24.53%. The pH was between 4.1 and 4.8 with a mean acidity of 1.75%. The soursop pulps are rich in nutrients such as carbohydrates (23.05%), proteins (7.41%), ash (2.22%) and fiber (24.73%).

3.2 Effect of chitosan coating on weight loss of soursop

The weight loss of soursop observed in control was due to the shrinkage of fruits by loss of moisture which was not observed in the coated fruits. The chitosan coating prevented the evaporation of moisture from coated soursop fruits. There was a significant difference observed between the control and coated samples. Results were showed in table 2.

Composite coating with 1% chitosan and 1% phytic acid could decrease the weight loss rate and malondialdehyde (MDA) content of fresh-cutting lotus root, postpone browning, restrain the activities of peroxidase (POD), polyphenol oxidase (PPO) and phenylalanine ammonialysae (PAL), and maintain the content of vitamin C and polyphenol at relatively high level. At day 8, the weight loss rate of coated fresh cut lotus root was one half of control, MDA content was 18.8% lower, and L value was 51.2% higher than that of control sample. Composite coating with chitosan combined phytic acid is an effective method to preserve fresh cut lotus root, superior to single chitosan coating (Yu YW et al., 2012).

3.3 Effect of chitosan coating on pH, total soluble solids, titratable acidity and ascorbic acid of soursop

Chitosan coating could maintain the respiration at a minimal rate. Effect of chitosan coating on pH, total soluble solids, titratable acidity and ascorbic acid of soursop was clearly illustrated in table 3.

The heat treatment + chitosan treated fruit showed the lowest respiration rate, ethylene evolution, malondialdehyde and membrane leakage, and the highest firmness and consumer acceptance among the treatments. At the same time, this combined treatment could inhibit the lost of green color, titratable acidity and weight loss compared with heat treatment alone (Shao XF et al., 2012). **3.4 Effect of chitosan coating on total plate count (TPC)**

of soursop

The total plate count on soursop coated by chitosan showed no significant difference by preservation time. The chitosan coating on soursop effectively inhibited the growth of microorganisms. These findings were similarly to results in a study experimented by coating with chitosan (Sucharitha, K. V. et al., 2018).

Chitosan coating had certain preventive effect against microbes and can reduce decay (Yu Youwei1, and Ren Yinzhe, 2013). Chitosan nanoparticle coatings reduced microbial growth on fresh-cut apples while not affecting quality attributes (Lucimeire Pilon et al., 2014).

Table 1. Major nutrient compositions in pulp of soursop (Annona muricata)

Parameter	Moisture (g/100g)	Protein (g/100g)	Total soluble solid (^o Brix)	Fibre (g/100g)	Vitamin A (µg/100g)	Vitamin C (mg/100g)
Value	23.96±0.01	7.22±0.02	8.36±0.02	23.49±0.03	12.06±0.02	20.17±0.01

Preservation time	Carrageenan concentration					
(days)	0%	0.25%	0.5%	0.75%	1%	
0	0	0	0	0	0	
3	1.27±0.01 ^a	1.13±0.03 ^{ab}	1.02 ± 0.01^{b}	0.94 ± 0.01^{bc}	$0.74\pm0.02^{\circ}$	
6	3.29±0.03 ^a	3.15±0.01 ^{ab}	3.01±0.02 ^b	2.48 ± 0.04^{bc}	2.13±0.01 ^c	
9	4.68±0.01 ^a	4.32±0.02 ^{ab}	4.27±0.01 ^b	4.04 ± 0.02^{bc}	3.76±0.02 ^c	
12	6.36±0.02 ^a	6.11±0.01 ^{ab}	5.94 ± 0.02^{b}	5.48±0.03 ^{bc}	5.22±0.01 ^c	
15	7.55±0.01 ^a	7.38 ± 0.02^{ab}	7.11±0.01 ^b	6.95 ± 0.03^{bc}	6.88±0.02 ^c	
18	8.69±0.03 ^a	8.55±0.01 ^{ab}	8.44 ± 0.03^{b}	8.22 ± 0.00^{bc}	8.10±0.01 ^c	
21	9.39 ± 0.00^{a}	9.20±0.03 ^{ab}	9.11±0.02 ^b	9.03±0.00 ^{bc}	$8.92 \pm 0.02^{\circ}$	
24	10.04 ± 0.01^{a}	9.48 ± 0.02^{ab}	9.22 ± 0.01^{b}	9.11 ± 0.03^{bc}	9.02±0.01 ^c	
27	11.24±0.02 ^a	11.18±0.01 ^{ab}	11.04 ± 0.03^{b}	10.89±0.02 ^{bc}	10.76±0.01 ^c	
30	12.38±0.01 ^a	12.25 ± 0.02^{ab}	12.07±0.01 ^b	11.89±0.03 ^{bc}	11.70±0.02 ^c	

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant ($\alpha = 5\%$).

Table 3. Effect of chitosan coating on pH, total soluble solids, titratable acidity and ascorbic acid of soursop stored at 4°C after
30 th day of preservation

	Parameters				
Soursop treated with	рН	Total soluble solids (o Brix)	Titratable acidity (%)	Ascorbic acid (mg/100g)	
0.75% chitosan before preservation	5.78±0.01 ^a	8.36±0.02 ^a	9.41±0.01 ^a	20.17±0.01 ^a	
0.75% chitosan after 30 th day of preservation	5.75±0.02 ^a	8.34±0.01 ^a	9.38±0.02 ^a	20.15±0.01 ^a	

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant ($\alpha = 5\%$).

Table 4. Effect of chitosan coating on sensory characteristics of soursop stored at 4°C after 30th day of preservation

Soursop treated with	Total plate count (TPC)		
0.75% chitosan before preservation	$1.2 \text{ x } 10^2 \pm 0.02^{\text{a}}$		
0.75% chitosan after 30 th day of preservation	$0.7 \text{ x } 10^1 \pm 0.01^b$		
Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant ($\alpha = 5\%$).			

Table 5. Effect of chitosan coating on sensory characteristics of soursop stored at 4°C after 30th day of preservation

Soursop treated with	Sensory score		
0.75% chitosan before preservation	7.34 ± 0.02^{a}		
0.75% chitosan after 30 th day of preservation	7.29±0.01 ^a		
Note: the values were expressed as the mean of three repetitions: the same characters (denoted above) the difference between them was not significant ($a = 50$)			

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant ($\alpha = 5\%$).

3.5 Effect of chitosan coating on sensory characteristics of soursop

Chitosan coating improved the sensory quality and extended the shelf life of soursop when compared to control. The soursop fruits coated with 0.75% chitosan were still commercially satisfactory in color, taste, flavor, appearance and overall acceptability after 30 days of preservation. These findings were similarly to results in a study experimented by coating with chitosan (Sucharitha, K. V. et al., 2018).

The effects of chitosan edible coating on microbiological and sensory quality of refrigerated broccoli were studied. The antimicrobial effects of chitosan on the native microflora (mesophilic, psychrotrophic, yeast and molds, lactic acid bacteria and coliforms) and on the survival of E. coli O157:H7 inoculated in broccoli were evaluated. Chitosan treatments resulted in a significant reduction in total mesophilic and psychrotrophic bacteria counts with respect to the control samples during the entire storage period. There was an immediate decontaminating activity of chitosan. At the end of the storage, yeast and molds was the most dominant flora representing the largest part of the total aerobic counts. Lactic acid bacteria (LAB) numbers remained relatively low during the whole storage in all samples. Chitosan coating inhibited the growth of total coliform throughout the storage time. Also, chitosan treatments resulted in а bactericidal effect on E. coli endogenous and a significant decreased in total E. coli counts (endogenous and O157:H7). The application of chitosan coating on fresh cut broccoli inhibited the yellowing and opening florets. The results of this experiment showed that the use of chitosan coating is a viable alternative in controlling the microorganisms present in minimally processed broccoli, improving its sensory quality (María del R.Moreira et al., 2011). Application of chitosan coating on chicken fillets could have a potential for preserving the microbiological quality and enhancing sensory attributes during chilled storage (Elsaid A. Eldaly et al., 2018).

IV. CONCLUSION

In recent years, the soursop fruit has received considerable attention from the public because numerous studies had indicated that soursop fruit is nutritionally high in carbohydrates and contains appreciable amounts of micronutrients and minerals. Soursop has many therapeutic properties; the juice is diuretic while the other parts have antibacterial, anticancerous, astringent, sedative, and other properties. The fruit has a very short shelf life and quickly becomes unacceptable for fresh consumption and further processing. Therefore, the fruit should be preserved for a long shelf life without using chemical substances. We have successfully optimized some physicochemical, microbial and sensory characteristics of soursop during preservation. By this study, there will be an alternative approach to prolong soursop shelf-life during post-harvest.

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